FOOD AND NUTRITION SECURITY: UNDERUTILIZED PLANT AND ANIMAL-BASED FOODS

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FOOD AND NUTRITION SECURITY: UNDERUTILIZED PLANT AND ANIMAL-BASED FOODS

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EDITORIAL
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Editorial: Food and Nutrition Security: Underutilized Plant and Animal-Based Foods

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Editorial on the Research Topic

Food and Nutrition Security: Underutilized Plant and Animal-Based Foods

There is an urgent need to address the issue of climate change. A transdisciplinary approach integrating the sciences and humanities is required to manage such a global challenge. The development of sustainable food systems is a vital component of climate change management. The current global food system is not viable in terms of health, affordability, or the environment—in other words, it is not sustainable. The triple burden of malnutrition is leaving billions of people undernourished worldwide. Paradoxically, there are also billions who are over-nourished, yet micronutrient deficient, due to the repeated consumption of energy dense, nutrient poor foods. There is a clear relationship between malnutrition and reliance on a few staple crops or low dietary diversity. Lack of dietary diversity has also been associated with inadequate intake and risks of deficiencies of essential micronutrients, including vitamin A, folate, iron, and zinc. Typically, this is seen in low-income households who subsist on staple-based diets, often produced through unsustainable food systems.

Phytochemicals in plant foods, otherwise known as non-nutritive compounds, are associated with health-promoting properties due to their essential function in biological mechanisms that maintain health and well-being. The growing understanding of the chemo-preventive properties of foods of plant origin has led to discussions on their potential contribution to daily diets or the inclusion of their constituents into dietary patterns for the prevention of non-communicable diseases.

A radical transformation of the food system as we know it is needed to enact positive changes globally and to impact food security. This Research Topic focuses on the investigation of alternate foods and sustainable food systems—in particular, underutilized plant and animal-based foods. The alternate foods and systems presented could lead to increased equity in food availability, affordability, and nutrition, especially for more vulnerable populations. Underutilized edible plants and animal-based foods used by Indigenous populations globally could play a key role in the development of such alternate food systems. These underutilized foods can be rich sources of macro- and micronutrients; some examples include legumes such as lentils and wattles which are high in protein, dietary fiber, and trace elements. They can be well-adapted to grow in arid and semi-arid conditions and are able to fix atmospheric nitrogen, which enriches soil fertility resulting in more sustainable agricultural and food systems. Similarly, there are many examples of underutilized roots and tubers, fruits and vegetables, and oil seeds that have been consumed by Indigenous communities for thousands of years. These have significant potential to contribute to

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dietary diversity and could be included in the diet of future generations. The sustainability of these systems is also strengthened by the inherent cultural practices of these communities with respect to land and water management, leading to sustainable food production.

Seventeen quality papers, 11 research articles, five reviews, and one perspective article, are published in this Special Issue. The topics that were researched and/or reviewed are: neglected and underutilized crop species: the key to improving dietary diversity and fighting hunger and malnutrition in Asia and the Pacific; the nutritional value of edible Crickets; consumer acceptance of insect-based protein; potential of farming Shipworms as a sustainable, nutritious, and affordable food source; seed composition and amino acid profiles for Quinoa grown in Washington State; diversity of essential oil profiles in Cardamom accessions from Southern India; bioactive components and radical scavenging activity in salt-tolerant Amaranth; metabolite fingerprinting of Kersting's Groundnut seeds; nutritional potential of the native Australian Green Plum; promising nutritional attributes of Samphire, an underutilized Australian indigenous edible halophyte; assessment of dietary bioactive phenolic compounds and agricultural sustainability of *Corchorus olitorius* L., an African leafy vegetable; interactions between phytochemicals and minerals in *Terminalia ferdinandiana* and implications for mineral bioavailability; potential of Bambara Groundnut for global food security and nutrition; the effect of irrigation, postharvest processing, and different household cooking techniques on the nutritional quality of African Nightshade and Chinese Cabbage; traditional food systems and indigenous food consumption in selected local Indian communities and existing challenges.

We hope that this Special Issue will further promote the interest in underutilized plant and animal-based foods and their crucial role in a diverse, sustainable, and healthy diet.

AUTHOR CONTRIBUTIONS

MN prepared the original draft which was reviewed and edited by OW, DS, and YS. All authors approved the submitted version.

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ORIGINAL RESEARCH
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Seed Composition and Amino Acid Profiles for Quinoa Grown in Washington State

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Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal celebrated for its excellent nutritional quality and potential to improve global food security, especially in marginal environments. However, minimal information is available on how genotype influences seed composition, and thus, nutritional quality. This study aimed to provide a baseline for nutritional quality of Washington grown quinoa and test the hypothesis that these samples contain adequate amounts of essential amino acids to meet daily requirements set by the World Health Organization (WHO). One hundred samples, representing commercial varieties and advanced breeding lines adapted to Washington State, were analyzed for content of 23 amino acids, as well as crude protein, ash, moisture, and crude fat. Mean essential amino acid values for Washington grown quinoa met the daily requirements for all age groups for all essential amino acids, except for the amount of leucine required by infants. We found that only nine genotypes met the leucine requirements for all age groups. A total of 52 and 94 samples met the lysine and tryptophan requirements for all age groups, respectively. Mean values for isoleucine, leucine, lysine, tryptophan, valine, and the sulfur and aromatic amino acids are higher for Washington grown samples than those reported previously reported in the literature. Our results show that not all Washington grown quinoa samples meet daily requirements of essential amino acids, and we identify limiting amino acids for the germplasm and environments investigated. This study provides the first report of leucine as a limiting amino acid in quinoa. Additional research is needed to better understand variation in quinoa nutritional composition, identify varieties that meet daily requirements, and explore how genotype, environment, and management interactions influence nutritional quality.

Keywords: quinoa, essential amino acid, limiting, complete protein, protein quality

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INTRODUCTION

Andean farmers have domesticated, adapted, diversified, and conserved quinoa genetic resources for the last 7,000 years, and until recently quinoa has been regarded as a neglected and underutilized species (NUS) (1, 2). Quinoa is a gynomonecious allotetraploid and a facultative autogamous annual species in the Amaranthaceae family, with a base chromosome number of x = 9 (2n = 4x = 36) and outcrossing from 0.5 to 17.36% (2–5). Due to domestication along latitudinal and elevational gradients, quinoa exhibits a large amount of genetic diversity and adaptive capacity (6). Two germplasm pools exist representing major centers of diversity, one in the Andean highlands, and the other in central and southern Chilean coastal lowlands (2, 7, 8). Quinoa is grown in a

wide range of environments, is resilient to agro-ecological extremes, and is tolerant to several abiotic stressors (e.g., drought, salinity, frost) (9–11). The broad genetic variability and adaptability of quinoa to diverse climates has produced a gene pool that supports the strategic development of germplasm with varying morphological (12) and physiological (13, 14) characteristics, and end-uses (15–18) suitable for adoption in novel agroecological climates worldwide. However, the germplasm pool currently available to facilitate quinoa expansion and adoption in novel production regions is narrow and represents only a small portion of quinoa's genetic diversity. The germplasm pool is primarily constrained by physiological issues (e.g., grain filling) associated with day length sensitivity (19).

Quinoa has the potential to improve the functional properties and nutritional quality of a diverse range of dishes and food products, from traditional and non-traditional applications to industrial innovations. These include soups, desserts, pastries, hot and fermented drinks (traditional), cereals, granolas, snack bars, cakes, chocolates (non-traditional), and extruded, puffed and expanded products (industrial innovations). Quinoa can also be used to produce almost all products of the milling industry (20–24). Moreover, quinoa flour has a major advantage in the flour industry since it can meet the increasing international demand for gluten free products (25–27). Quinoa protein, oil, and starch fractions can be isolated for specific value-added applications. Quinoa has good freeze-thaw capabilities and the gelatinized starch is opaque, which makes quinoa useful in prepared frozen foods and as an emulsifier, respectively (28, 29). The wide range of quinoa starch physicochemical properties provides for diverse applications in food and non-food innovations (16, 30, 31).

Quinoa starch is more viscous and has better water holding and expansion properties than wheat and barley, and starch gelatinization occurs at higher temperatures, making quinoa perform better as a thickening agent and in baby foods (12, 26). López de Romaña and others have used quinoa in two studies with Peruvian children recovering from malnutrition. In both studies, a lower digestibility of quinoa was observed compared to potato and wheat diets and casein, which contributed to decreased protein and fat utilization; however, milling improved both parameters (32, 33). Processing methods and quinoa variety both contribute to variability in end-use quality (16, 30). Additional research is needed to characterize nutritional quality and functional properties depending on variety and processing, to ensure successful utilization of quinoa in appropriate end-uses.

The superior nutritive potential of quinoa has relatively recently garnered international interest in the expansion of quinoa (19). It is beyond the scope of this article to provide a comprehensive review of quinoa nutritional composition, since several already exist (34–39). Quinoa protein content can be highly variable, and is often comparable to most cereals, ranging from 8 to 22% (37, 40). The quality of quinoa protein is noteworthy. Quinoa is reported to be a complete protein, because it contains all nine of the essential amino acids (35, 37, 41); however, it is better described as "nearly complete," because of limiting essential amino acid content. In a review of quinoa data reported as edible portion, which allows for comparison to food composition databases, Nowak et al. (37) had to relax the data selection criteria for amino acids and minerals because of a lack of information. Although the authors claimed that quinoa provides a sufficient amount of essential amino acids, even at the lower ends of the ranges to meet adult daily requirements, this is based on a miniscule number of data points ($n = 37$) (37). Furthermore, the authors neglected to evaluate the ability of quinoa to meet the daily essential amino acid requirements of younger age groups. Far too much has been inferred about quinoa nutrition composition from the limited number of peer-reviewed studies available.

By declaring 2013 as the "International Year of Quinoa," the United Nations recognized the emerging potential of quinoa to contribute to global food security, especially when grown on marginal lands that cannot currently support major crops (1). It has been reported that quinoa has been tested or cultivated in 95 countries, a doubling since the declaration in 2013, and it appears that this trend will continue (19, 42). However, the large amount of genetic diversity, resiliency to agroecological extremes, and diversity of morphological and physiological characteristics is not necessarily represented in the germplasm pool currently supporting the global expansion of quinoa (42, 43). Furthermore, and perhaps most importantly, the nutritional quality of quinoa produced in novel environments is assumed to be comparable to the quality of quinoa produced in Bolivia and Peru, which represents roughly 80% of global production (19). Thus, this study aims to provide baseline information on quinoa grown in western North America (i.e., Washington state), representing the first report that provides a baseline for the protein quality of quinoa produced in this novel production region. We also test the hypothesis that Washington grown quinoa contains adequate amounts of essential amino acids to meet daily requirements set by the World Health Organization (WHO) for all age groups. Comparisons are made to not only adult requirements, but also to the requirements for infants and all other age groups. It is estimated that between 25 and 33% of children below the age of five experience stunting worldwide, possibly due to insufficient protein intake (44, 45). For example, Semba et al. (46) found that 62% of the children in their study in rural Malawi were stunted, and that these children had lower serum concentrations of all nine essential amino acids compared to non-stunted children ($p < 0.01$). Furthermore, the stunted children had significantly lower serum concentrations of conditionally and non-essential amino acids (46). Therefore, providing adequate nutrition to children under the age of five, especially regarding sufficient intake of amino acids, is critically important for reducing the risk of stunting and effects on growth and development (47, 48).

METHODS

Study Region and Field Trials

Raw quinoa seed sent for analysis was grown in 2016 and 2017 in western Washington as part of two separate experimental designs (13, 49). Site characteristics for all locations are summarized in **Table 1**. In 2016, F5:F6 advanced breeding lines and control varieties were planted on three organic farms in Chimacum (Finnriver Farm; 48°0'29"N 122°46'12"W), Quilcene (Dharma

TABLE 1 | Site characteristics for each year and location that samples were randomly selected from for chemical analysis. Raw quinoa seed sent for analysis was grown in 2016 and 2017 in western Washington as part of two separate experimental designs (13, 49).

	2016			2017
	Chimacum	**Quilcene**	**Sequim**	**Mount Vernon**
Elevation (m)	37.8	68	31.1	7
Average Annual Precipitation (mm)	711	1397	432	841
Planting Date	April 8, 2016	April 7, 2016	May 5, 2016	May 18, 2017
Soil Type	Gravelly sandy loam	Silty Clay Loam	Silt Loam	Silt Loam
pH	6.1	6.9	6.5	6.6
Phosphorus (mg kg^{-1})	124	12	31	7
Potassium (mg kg^{-1})	762	290	114	260
Ammonium (mg kg^{-1})	1.1	5.7	4.5	1.2
Nitrate (mg kg^{-1})	21.5	16.6	3.8	25[†]
Organic Matter (%)	12.1	3.2	3.3	2.9
Previous Crop	Barley	Vegetable Crops	Pasture	Rye Cover Crop

Mount Vernon plants were planted in the greenhouse 1 month before being transplanted on the planting date listed.
[†] *Reported as nitrate + nitrite.*

Ridge Organic Farm; 47°55'04.0"N 122°53'23.2"W) and Sequim (Nash's Organic Produce; 48°08'31"N 123°07'19"W) on the Olympic Peninsula. Control varieties included Cherry Vanilla (Wild Garden Seed, Philomath, OR, US), CO407Dave (PI 596293, USDA Plant Introduction, Ames, Iowa) and Kaslaea (Ames 13745, USDA Plant Introduction, Ames, Iowa). At each location, advanced breeding lines and control varieties were planted in single hand-sown plots that measured 4.9 m in length and 40.64 cm from center and were seeded at a rate of 4 g row m^{-1} in an augmented randomized complete block design (ARCBD). An ARCBD uses control varieties to account for field variation by replicating control varieties across blocks; control varieties can be used as covariates to make spatial adjustments across blocks. This design is useful for evaluating advanced breeding lines when seed quantity is low, land and other resources are limited, and when many advanced breeding lines must be evaluated.

In 2017, quinoa seeds were planted in a greenhouse, and after 1 month the seedlings were transplanted in the field on 19 May at the WSU Northwest Research and Extension Center in Mount Vernon, WA (NWREC; 48°26'24"N 122°23'24" W). The experimental design in 2017 consisted of a split-plot randomized complete block design with irrigation factor (irrigated, non-irrigated) as the main-plot and genotype as the sub-plot. Each plot had a distance of 30 cm between plants, with 30 plants in each plot. Following harvest each year, seed was dried at 32°C and cleaned using metal screens and a seed blower (Seed Processing Holland Inc., Salinas, CA).

Germplasm and Sample Selection

The F5:F6 advanced breeding lines were generated from single plant selections made on six bi-parental populations through an evolutionary participatory breeding (EPB) method (49). Crossing events in 2012 produced the six, original bi-parental populations (50) (**Table 2**). Germplasm included in the 2017 trial represents commercially available varieties and landraces. A summary of the germplasm is included in **Table 3**.

TABLE 2 | Female and pollen parents listed for each bi-parental population, with the number of samples from each population included in the study.

Population	Female parent	Pollen parent	Number of samples
102	CO407 Dave	QQ74	21
104	Kaslaea	QQ74	26
105	QQ065	QQ74	3
106	QQ065	Black	5
107	QQ74	Black	10
108	QQ74	Cherry Vanilla	20

Protein values were predicted for a representative selection of field-grown material ($n = 194$), including samples from the two aforementioned experimental designs, using a DA7250 NIR analyzer (Perten Instruments, Springfield, IL) with a default quinoa calibration. Predicted values were normally distributed, and samples included in the study were randomly selected across the distribution for wet chemistry analyses ($n = 100$), with a greater number of samples selected within one standard deviation of the mean to better represent this dense region of the distribution.

Chemical Laboratory Analysis

Samples were sent to the (AESCL) for determination of seed composition via proximate analysis (crude protein, crude fat, moisture, ash, and carbohydrates) and determination of the complete amino acid profiles ($n = 23$) [AOAC Official Method 982.30 E(a,b,c), chp. 45.3.05]. AESCL is an American Association for Laboratory Accreditation (A2LA) accredited proficiency testing provider in accordance with the international standard 17043:2010. This accreditation demonstrates technical competence for a defined scope and the operation of a quality management system.

The complete amino acid profile included essential amino acids (leucine, lysine, valine, isoleucine, phenylalanine, threonine, histidine, methionine, and tryptophan) and non-essential amino acids (glutamic acid, aspartic acid, arginine, glycine, alanine, proline, serine, tyrosine, cysteine, taurine, hydroxyproline, hydroxylysine, ornithine, and lanthionine). Crude protein was determined by combustion analysis (LECO), and the calculation of total nitrogen × 6.25 [AOAC Official Method 990.03 (51)]. Crude fat was determined by ether extraction [AOAC Official Method 920.39 (A)]. Moisture was determined by vacuum oven [AOAC Official Method 934.01 (51)] and ash was determined by sample ignition (AOAC Official Method 942.05). Total carbohydrates were determined by difference calculation [100—(Crude Protein + Crude Fat + Ash + Moisture)]. Proximate values are reported as g/100 g sample, and amino acids are reported as g/100 g crude protein, unless otherwise noted.

TABLE 3 | Summary of samples from both years, each location, and the identity of the advanced breeding lines (ABL) and control varieties (CV).

2016 ($n = 91$)						2017 ($n = 9$)
Ch ($n = 24$)		Qu ($n = 37$)		Sq ($n = 30$)		MV ($n = 9$)
ABL	CV	ABL	ABL	ABL	CV	Accessions
102.04	Cherry Vanilla•	102.05	107.03	102.08•	Cherry Vanilla•	17GR (Ames 13735)††
102.08•		102.09	107.07	102.17•		Japanese Strain (PI 677100)††
102.13	CO407 Dave•	102.12	107.50	102.24	CO407 Dave•	QQ74 (PI 614886)††
102.31	Kaslaea•	102.17•	107.65•	102.36•	Kaslaea•	Baer (PI 634918)†
102.52•		102.23	107.72	102.52•		3 UISE (Ames 13756)††
102.76••		102.25	107.84	102.76••		
104.01		102.36•	108.18	104.20••		
104.02		102.40	108.51•	104.21•		
104.20••		102.76••	108.56	104.27•		
104.27•		104.20••	108.66	104.28•		
104.60		104.21•	108.70	104.45		
104.73•		104.28•	108.86•	104.59•		
105.43		104.30	108.90	104.71		
107.67		104.38		104.80		
107.78•		104.53		104.88		
108.33		104.59•		105.92•		
108.34		104.73•		106.37•		
108.39		104.75		106.49•		
108.46		104.77		107.65•		
108.51•		104.87		107.78•		
108.54•		105.92•		108.11		
		106.37•		108.26		
		106.49•		108.42		
		106.85		108.54•		
				108.69		
				108.81		
				108.86•		

ABL are denoted by the population number and selection number separated by a period. In 2016, ABL and CV were grown at Chimacum (Ch), Quilcene (Qu), and Sequim (Sq). Samples were randomly selected from the 2016 experimental design; certain ABL were selected from two (•) or three (••) locations and none of the CV were selected from Quilcene by chance. Samples were also randomly selected from the Mount Vernon (MV) experimental design, from either the non-irrigated treatment (†) or both the non-irrigated and irrigated treatments (††).

Daily Requirements and Scoring Patterns

FAO/WHO/UNU (52) scoring patterns should be based on amino acid requirement values divided by the mean protein requirement, and are presented as g/100 g protein (**Table 4**). Scoring patterns are calculated as the age-related amino acid requirement levels divided by the safe level of protein intake (52).

Data Analysis

Data were analyzed and figures were generated using Microsoft Excel 2010 (Seattle, WA), and RStudio Version 1.2.1335 (53). Data was tested for normality using the *shapiro.test* function in the *stats* R package. An overall rank score was assigned to each sample by summing individual ranks for each nutritional attribute (**Table 4**). For example, the samples with the highest and lowest essential amino acid content received a rank of 1 and 100, respectively. Spearman rank correlation coefficients were calculated using the *corstars* function and *Hmisc* R package, with significant correlations ($p < 0.05$) plotted using the *corrplot* package and diverging palette "RdBu" from the *RColorBrewer* package (54–56). Principal component analysis was conducted using the *prcomp* function in the *stats* package, and biplots were generated using the *ggplot2* package (53, 57). The *tabular* function in the *tables* package provided summary statistics (58).

RESULTS

Seed Composition (Proximates) Profile

Quinoa grown in Chimacum ($n = 24$; advanced breeding lines and control varieties; **Table 3**) had the highest mean total amino acid, crude protein and moisture content, and the lowest mean

TABLE 4 | Daily requirements for essential amino acids presented as scoring patterns (amino acid requirements/protein requirements for the selected age groups) for all age groups considered.

	Scoring pattern (g/100 g protein requirement)								
Age (years)	**His**	**Ile**	**Leu**	**Lys**	**SAA**	**AAA**	**Thr**	**Trp**	**Val**
0.5	2	3.2	6.6	5.7	2.8	5.2	3.1	0.85	4.3
1–2	1.8	3.1	6.3	5.2	2.6	4.6	2.7	0.74	4.2
3–10	1.6	3.1	6.1	4.8	2.4	4.1	2.5	0.66	4
11–14	1.6	3	6	4.8	2.3	4.1	2.5	0.65	4
15–18	1.6	3	6	4.7	2.3	4	2.4	0.63	4
>18	1.5	3	5.9	4.5	2.2	3.8	2.3	0.6	3.9

Amino acids are abbreviated with standard three letter codes. The sulfur amino acids (SAA) include methionine and cysteine and the aromatic amino acids (AAA) include phenylalanine and tyrosine. This table is adapted from the World Health Organization/Food and Agriculture Organization/United Nations University suggested indispensable amino acid requirements for all age groups (present estimates; 2007).

crude fat content (**Table 5**). Quinoa grown in Mount Vernon ($n = 9$; accessions; **Table 3**) had the highest mean ash content, and the lowest mean total amino acid, crude protein, moisture, and total carbohydrate content. Quinoa grown in Quilcene ($n = 37$; advanced breeding lines; **Table 3**) had the highest mean total carbohydrate content, whereas quinoa grown in Sequim had the highest mean crude fat content and the lowest mean ash content (**Table 5**). Our sample of Washington grown quinoa seeds is primarily composed of total carbohydrates (69.56–74.00 g/100 g sample) followed by crude protein (10.04–13.68 g/100 g sample), moisture (6.41–7.37 g/100 g sample), crude fat (4.56–7.19 g/100 g sample), and ash (2.70–5.00 g/100 g sample) (**Table 5**).

Control varieties grown in Chimacum and Quilcene in 2016 (i.e., CTRL seed source) had the highest mean total amino acid and crude protein content (**Table 5**). Population 102 had the highest mean ash content. Samples from Population 105 had the highest total carbohydrate content and the lowest total amino acid, crude protein, and moisture. Population 106 had the lowest ash content. Population 107 had the lowest mean crude fat content. Samples from Population 108 had the highest mean crude fat content, and the lowest total carbohydrate content.

Amino Acid Profile

The most abundant essential amino acids ($n = 9$), from highest to lowest mean content, were leucine, lysine, valine, isoleucine, phenylalanine, threonine, histidine, methionine, and tryptophan (**Table 5**). The most abundant non-essential amino acids ($n = 14$), from highest to lowest mean content, were glutamic acid, aspartic acid, arginine, glycine, alanine, proline, serine, tyrosine, cysteine, taurine, hydroxyproline, hydroxylysine, ornithine, and lanthionine (not reported). Lanthionine was measured at 0.00 mg/100 g protein for all samples (**Table 5**).

Essential amino acid content varied by location and population, with particular locations and populations having higher content on average; however, we were not able to test for significant differences between groups (i.e., locations and populations).

Samples from Chimacum had the highest mean total essential amino acid, aromatic amino acid (AAA), leucine, valine, and histidine content (**Table 5**). Mount Vernon samples had the lowest mean total essential amino acid, leucine, lysine, sulfur amino acid (SAA), isoleucine, threonine, histidine, and tryptophan content. Samples from Quilcene had the highest mean lysine, SAA, and threonine content. Samples from Sequim ($n = 30$; breeding lines and control varieties; **Table 3**) had the highest mean tryptophan content, and the lowest mean AAA and valine content (**Table 5**).

Essential Amino Acid Content by Seed Source

The "Controls" seed source had the highest mean total essential amino acid, AAA, valine, SAA, and tryptophan content (**Table 5**). Population 102 had the highest mean lysine and threonine content. Population 104 had the lowest mean AAA, leucine, valine, isoleucine, and histidine content. Population 106 had the highest mean leucine, isoleucine, and histidine content, and the lowest mean tryptophan content. Population 107 had the lowest mean total essential amino acid, lysine, SAA, and threonine content (**Table 5**).

Satisfaction of Essential Amino Acid Daily Requirements

Mean values for histidine, isoleucine, lysine, sulfur amino acids, aromatic amino acids, threonine, tryptophan, and valine content met the daily requirements for these amino acids for all age groups (**Figure 1**, **Table 6**). The mean leucine content for locations and populations did not meet the requirements for all groups (**Table 5**); however, the mean value for leucine content did meet the daily requirements of the 3–10, 11–14, 15–18, >18 year-old age groups, although the infant (0.5 year) and 1–2 year-old daily requirements were not met (**Tables 4**, **6**). Only 9% of samples met the leucine requirements for all age groups. These samples include Kaslaea (Chimacum), 102.52 (Chimacum), CO407Dave (Chimacum), 107.84 (Quilcene), 108.18 (Quilcene), 106.37 (Quilcene), 102.08 (Sequim), 102.23 (Quilcene), and 102.17 (Quilcene). Furthermore, 8% of samples failed to meet the leucine requirements for any of the age groups. These samples include 17GR (Mount Vernon; non-irrigated), 102.17 (Sequim), 102.52 (Sequim), 102.76 (Chimacum), QQ74 (Mount Vernon;

TABLE 5 | Mean and standard deviation (sd) values for the nutritional components (NC) analyzed are reported for all samples ($n = 100$), and the samples grouped by location and population (i.e., seed source).

			Location				Population						
		All	**Ch**	**MV**	**Qu**	**Sq**	**CV**	**102**	**104**	**105**	**106**	**107**	**108**
NC	*n*	100	24	9	37	30	6	21	26	3	5	10	20
Carb	mean	72.27	71.83	72.07	72.74	72.09	71.68	72.55	72.48	72.17	71.85	71.90	72.27
	sd	0.87	0.84	0.89	0.73	0.80	0.80	0.64	0.88	0.69	1.74	0.74	0.80
CP	mean	11.77	12.25	11.26	11.46	11.91	12.09	11.70	11.75	12.23	12.01	11.84	11.84
	sd	0.70	0.73	0.87	0.54	0.50	0.81	0.55	0.72	0.70	0.85	0.71	0.63
Moist	mean	6.97	7.08	6.60	7.03	6.91	7.04	6.96	7.01	6.99	7.11	7.04	6.98
	sd	0.20	0.08	0.17	0.13	0.20	0.09	0.16	0.16	0.29	0.15	0.19	0.16
Fat	mean	5.89	5.73	5.82	5.79	6.16	6.19	5.89	5.79	5.56	5.87	6.17	5.88
	sd	0.50	0.35	0.72	0.47	0.48	0.59	0.34	0.46	0.23	0.96	0.70	0.26
Ash	mean	3.11	3.12	4.25	2.97	2.93	3.00	2.90	2.98	3.05	3.15	3.06	3.04
	sd	0.41	0.18	0.35	0.17	0.12	0.14	0.08	0.16	0.10	0.29	0.26	0.16
TAA	mean	87.11	88.41	84.49	87.71	86.10	89.09	87.28	86.61	89.47	88.52	86.83	87.60
	sd	3.61	3.87	3.48	3.16	3.46	4.92	4.67	2.89	1.01	2.37	3.20	3.15
TEAA	mean	34.13	34.53	32.79	34.47	33.78	34.92	34.37	33.97	34.68	34.50	33.93	34.36
	sd	1.42	1.56	1.11	1.27	1.29	1.93	1.84	1.13	0.15	0.95	1.37	1.17
His	mean	2.66	2.72	2.53	2.65	2.66	2.74	2.65	2.64	2.75	2.79	2.65	2.68
	sd	0.14	0.15	0.12	0.12	0.13	0.15	0.16	0.11	0.04	0.06	0.12	0.12
Ile	mean	4.00	4.09	3.90	4.00	3.96	4.07	4.00	3.95	4.14	4.16	4.03	4.01
	sd	0.18	0.19	0.16	0.16	0.16	0.22	0.23	0.14	0.08	0.05	0.18	0.16
Leu	mean	6.25	6.35	6.04	6.31	6.16	6.38	6.28	6.18	6.41	6.41	6.27	6.28
	sd	0.26	0.27	0.21	0.24	0.23	0.33	0.34	0.21	0.05	0.21	0.25	0.21
Lys	mean	5.72	5.64	5.33	5.92	5.65	5.82	5.84	5.76	5.62	5.65	5.60	5.79
	sd	0.33	0.32	0.27	0.26	0.26	0.37	0.38	0.25	0.12	0.32	0.31	0.28
SAA	mean	4.02	4.05	3.87	4.06	4.00	4.17	3.97	4.05	4.12	3.95	3.94	4.09
	sd	0.19	0.18	0.23	0.17	0.19	0.23	0.23	0.15	0.26	0.09	0.10	0.12
Met	mean	2.17	2.20	1.97	2.21	2.14	2.26	2.15	2.20	2.24	2.15	2.12	2.22
	sd	0.13	0.11	0.15	0.11	0.10	0.17	0.12	0.10	0.15	0.07	0.10	0.08
AAA	mean	6.65	6.74	6.55	6.70	6.54	6.81	6.67	6.56	6.81	6.65	6.66	6.68
	sd	0.28	0.29	0.28	0.26	0.27	0.39	0.36	0.21	0.12	0.33	0.30	0.23
Phe	mean	3.90	3.96	3.82	3.94	3.84	3.99	3.92	3.86	4.03	3.98	3.92	3.92
	sd	0.16	0.18	0.14	0.15	0.15	0.21	0.21	0.13	0.05	0.14	0.16	0.14
Thr	mean	3.57	3.58	3.47	3.64	3.50	3.62	3.63	3.55	3.57	3.55	3.53	3.58
	sd	0.16	0.16	0.13	0.15	0.15	0.19	0.22	0.14	0.06	0.18	0.13	0.13
Trp	mean	1.08	1.14	1.00	1.01	1.15	1.15	1.12	1.11	1.04	0.93	1.03	1.09
	sd	0.14	0.11	0.07	0.16	0.10	0.09	0.16	0.14	0.03	0.22	0.10	0.12
Val	mean	4.78	4.85	4.73	4.79	4.72	4.89	4.79	4.72	4.88	4.87	4.79	4.81
	sd	0.20	0.22	0.18	0.19	0.20	0.26	0.27	0.17	0.05	0.14	0.22	0.16
TNAA	mean	52.98	53.88	51.70	53.24	52.32	54.16	52.91	52.64	54.79	54.02	52.90	53.24
	sd	2.26	2.33	2.57	1.94	2.22	3.03	2.87	1.79	0.86	1.44	1.87	2.04
Glu	mean	13.22	13.50	13.02	13.08	13.23	13.46	13.14	13.11	13.81	13.77	13.22	13.24
	sd	0.78	0.68	1.07	0.71	0.82	0.99	0.91	0.62	0.36	0.42	0.66	0.78
Asp	mean	8.00	8.09	7.80	8.12	7.84	8.14	8.01	7.93	8.32	8.32	8.07	7.97
	sd	0.35	0.35	0.29	0.30	0.34	0.43	0.45	0.30	0.06	0.22	0.32	0.27
Arg	mean	7.64	7.80	7.23	7.65	7.64	7.93	7.54	7.64	8.04	7.97	7.62	7.72
	sd	0.43	0.45	0.63	0.35	0.39	0.45	0.46	0.35	0.17	0.24	0.35	0.36
Gly	mean	5.54	5.49	5.47	5.63	5.49	5.63	5.56	5.51	5.61	5.63	5.48	5.57
	sd	0.24	0.27	0.33	0.19	0.23	0.35	0.29	0.21	0.05	0.22	0.22	0.21
Ala	mean	4.30	4.35	4.12	4.37	4.22	4.38	4.36	4.28	4.33	4.29	4.28	4.32

(Continued)

TABLE 5 | Continued

			Location				Population						
		All	Ch	MV	Qu	Sq	CV	102	104	105	106	107	108
	sd	0.20	0.19	0.16	0.18	0.17	0.23	0.26	0.16	0.03	0.20	0.19	0.15
Pro	mean	3.85	4.04	3.69	3.85	3.75	3.96	3.80	3.82	4.21	3.90	3.89	3.91
	sd	0.24	0.21	0.13	0.21	0.22	0.28	0.26	0.22	0.25	0.22	0.22	0.22
Ser	mean	3.76	3.74	3.68	3.83	3.69	3.77	3.80	3.74	3.85	3.85	3.77	3.71
	sd	0.17	0.18	0.18	0.15	0.17	0.19	0.21	0.17	0.12	0.25	0.14	0.13
Tyr	mean	2.74	2.78	2.72	2.75	2.70	2.82	2.76	2.71	2.78	2.67	2.75	2.76
	sd	0.13	0.13	0.15	0.12	0.14	0.18	0.15	0.10	0.07	0.21	0.14	0.11
Cys	mean	1.85	1.84	1.89	1.85	1.85	1.92	1.82	1.85	1.89	1.80	1.82	1.87
	sd	0.09	0.09	0.13	0.08	0.10	0.07	0.12	0.08	0.11	0.07	0.03	0.06
Tau	mean	1.39	1.54	1.14	1.48	1.25	1.44	1.45	1.44	1.36	1.23	1.33	1.45
	sd	0.19	0.16	0.12	0.13	0.11	0.25	0.16	0.14	0.17	0.24	0.19	0.18
Hpro	mean	0.47	0.45	0.62	0.47	0.45	0.51	0.45	0.43	0.39	0.45	0.48	0.49
	sd	0.10	0.09	0.11	0.08	0.09	0.10	0.07	0.08	0.14	0.07	0.09	0.08
Hlys	mean	0.13	0.17	0.22	0.08	0.12	0.12	0.13	0.11	0.13	0.08	0.12	0.13
	sd	0.06	0.05	0.04	0.02	0.05	0.06	0.06	0.04	0.05	0.06	0.04	0.07
Orn	mean	0.09	0.08	0.10	0.09	0.09	0.08	0.09	0.09	0.08	0.08	0.08	0.09
	sd	0.01	0.01	0.03	0.01	0.02	0.01	0.01	0.01	0.00	0.01	0.01	0.02
Lan	mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	sd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

The control varieties (CV) included were only selected from the Chimacum (Ch) and Sequim (Sq) locations by chance, while advanced breeding lines from the populations (102, 104, 105, 106, 107, and 108) were randomly selected from Ch, Sq, and Quilcene (Qu). Data from the Mount Vernon samples (n = 9) are only included under the "MV" location and "All" column. The first five NC, total carbohydrates (Carb), crude protein (CP), moisture (Moist), crude fat (Fat), and ash are reported as grams per 100 g sample. All amino acids are abbreviated using standard letter codes, except for total amino acid content (TAA), total essential amino acid content (TEAA) sulfur amino acids (SAA; methionine and cysteine), aromatic amino acids (AAA; phenylalanine and tyrosine); total non-essential amino acids (TNAA), taurine (Tau), hydroxyproline (Hpro), hydroxylysine (Hlys), ornithine (Orn), and lanthionine (Lan). All amino acid data are reported as grams per 100 g protein.

non-irrigated), 104.59 (Sequim), 108.39 (Chimacum), 104.53 (Quilcene) (**Table 6**; **Supplementary Table 1**).

Although the mean value for lysine content met the requirements of all age groups, the mean value for lysine content of certain locations and populations failed to meet daily requirements for certain age groups (**Table 5**). The mean value for lysine content for Chimacum, Mount Vernon, Sequim, Population 105, Population 106, and Population 107 failed to meet the requirements of infants, but met the requirements for all other age groups (**Tables 4**, **6**). The lysine daily requirement for all age groups was met by 52 samples, while 42 samples and 6 samples failed to meet the lysine daily requirement for infants and children 1–2 years old, respectively. The tryptophan daily requirement for infants and children 1–2 years old was not met by 3 samples for each age group. Ninety-four samples met the daily requirement of tryptophan for all age groups (**Table 6**; **Supplementary Table 1**).

Comparison to Nowak et al. (37) Mean Values

Mean values for isoleucine, leucine, lysine, sulfur amino acids, aromatic amino acids, tryptophan and valine are higher for Washington grown samples than those reported in the review by Nowak et al. (37) (**Figure 1**). For histidine and threonine, the Nowak et al. (37) mean value is within one standard deviation of the mean value for the Washington grown samples. However, we were not able to test for significant differences or report measures of dispersion around the population mean for the Nowak et al. (37) samples because the raw data was not available. Differences among groups were not statistically significant. The mean value reported by Nowak et al. (37) for isoleucine, leucine, and lysine does not meet the infant daily requirement, and the mean value reported for valine does not meet the adult or infant daily requirement (**Figure 1**).

Samples With the Highest and Lowest Values for Nutritional Components

The five highest and five lowest samples are reported for overall rank (total content of all nutritional components), total amino acid content, total essential amino acid content, crude protein, crude fat, and ash (**Table 7**).

Samples 104.27 (Chimacum), 106.49 (Sequim), 108.54 (Chimacum), 104.20 (Chimacum), and CO407Dave (Sequim) had the highest crude protein content, while 102.09 (Quilcene), Baer (Mount Vernon), QQ74 (Mount Vernon), 107.07 (Quilcene), and QQ74 (Mount Vernon) had the lowest crude protein content.

Overall rank was highest for CO407Dave (Chimacum), 104.52 (Chimacum), Kaslaea (Chimacum), 108.08 (Sequim), and 102.17 (Quilcene) (**Table 7**). Samples 102.52 (Sequim) and

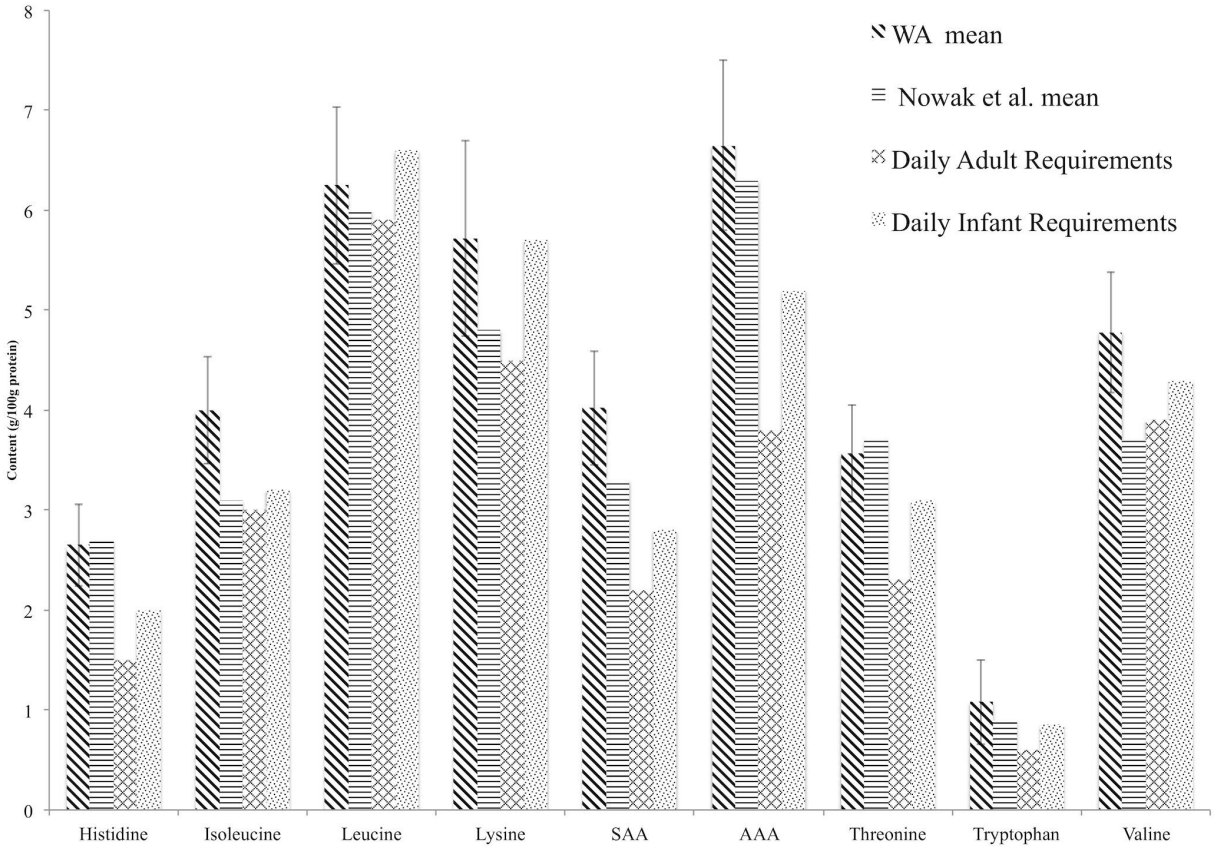

FIGURE 1 | Comparison of essential amino acid mean values from the Washington grown samples (WA Mean) reported with error bars representing one standard deviation, mean values reported in the review by Nowak et al. (37), and daily adult and infant essential amino acid requirements established by the World Health Organization (52). Differences among groups are not statistically significant. SAA, sulfur amino acids; AAA, aromatic amino acids.

TABLE 6 | Number of samples ($n = 100$) that fail to meet the daily requirements for each essential amino acid within each age group, and the number of samples that meet all age group requirements for each amino acid (i.e., all met).

	Number of samples that fail to meet requirements		
Age (years)	**Leu**	**Lys**	**Trp**
0.5	31	42	3
1-2	35	6	3
3-10	9	0	0
11-14	8†	0	0
15-18	8†	0	0
>18	8	0	0
All met	9	52	94

All samples meet the histidine, isoleucine, sulfur amino acid (methionine and cysteine), aromatic amino acid (phenylalanine and tyrosine), threonine, and valine requirements for all age groups.
† *Same samples.*

102.17 (Sequim) were the two lowest ranked samples overall for total content of all nutritional components. Additionally, 17GR (Mount Vernon), 104.59 (Sequim), and 102.76 (Chimacum) were among the five lowest ranked samples for total content of all nutritional components.

These same samples were similarly ranked for total amino acid content and total essential amino acid content, although the order differed slightly. Total amino acid content was highest for 102.17 (Quilcene), CO704Dave (Chimacum), 102.52 (Chimacum), Kaslaea (Chimacum), and 102.08 (Sequim) while total amino acid content was lowest for 102.76 (Chimacum), 17GR (Mount Vernon), 102.52 (Sequim), QQ74 (Mount Vernon), and 120.17 (Sequim). Total essential amino acid content was highest for 102.17 (Quilcene), 102.52 (Chimacum), CO407Dave (Chimacum), Kaslaea (Chimacum), and 102.08 (Sequim); total essential amino acid content was lowest for QQ74 (Mount Vernon), 102.76 (Chimacum), 102.52 (Sequim), 102.17 (Sequim), and 17GR (Mount Vernon).

Correlations Between Nutritional Components

Total carbohydrate content is negatively correlated with crude protein and ash content (**Supplementary Table 2**). Crude protein content is positively correlated with moisture and ash content.

TABLE 7 | The five highest and lowest ranking samples are presented for overall rank (all nutritional components combined), total amino acids (AA), total essential AA (TEAA), crude protein, crude fat, and ash.

Rank	Overall	TAA	TEAA	Crude Protein	Crude Fat	Ash
1	C4D•	102.17t	102.17t	104.27•	107.78†	3UISEΦ
2	102.52•	C4D•	102.52•	106.49†	106.49t	JSΦ
3	Kaslaea•	102.52•	C4D•	108.54•	106.49†	QQ74Φ
4	102.08†	Kaslaea•	Kaslaea•	104.20•	Cherry Vanilla†	17GRΦ
5	102.17t	102.08†	102.08†	C4D†	107.07t	BaerΦ
96	17GRΦ	102.76•	QQ74Φ	102.09t	JSΦ	102.52†
97	104.59†	17GRΦ	102.76•	BaerΦ	106.37†	106.37t
98	102.76•	102.52†	102.52†	QQ74Φ	3UISEΦ	107.07t
99	102.52†	QQ74Φ	102.17†	107.07t	107.84t	108.42†
100	102.17†	102.17†	17GRΦ	QQ74Φ	104.38t	104.77t

A rank closer to zero corresponds to higher rank; rank 1 and 100 correspond to the highest and lowest ranks, respectively. Samples were either grown in 2016 in Chimacum (•), Sequim (†), Quilcene (t), or Mount Vernon (Φ). The variety CO407Dave is abbreviated as C4d and the accession Japanese Strain is abbreviated as JS.

Total non-essential amino acid content is positively correlated with total essential amino acid content, crude protein, moisture and ash content, and negatively correlated with total carbohydrate content. Each of the non-essential amino acids are positively correlated with total essential and non-essential amino acid content, except for hydroxyproline, hydroxylysine, and ornithine content. Ash content is positively correlated with hydroxyproline, hydroxylysine, aspartic acid, glutamic acid, proline, cysteine, valine, isoleucine, leucine, phenylalanine, and histidine content (**Supplementary Table 2**).

Total essential amino acid content is positively correlated with crude protein, and moisture, and negatively correlated with total carbohydrate content. Each of the essential amino acids is positively correlated with total essential amino acid content and crude protein content (**Supplementary Table 2**).

Principal Component Analysis

Principal component analysis (PCA) of essential amino acid data identified nine principal components, with the first two principal components explaining 92.1% of the cumulative variance (**Figure 2**; **Supplementary Table 3**). Threonine, valine, isoleucine, leucine, lysine, histidine, the SAA, and the AAA have large negative loadings on principal component one, and tryptophan has a large negative loading on principal component two (**Figure 2**; **Supplementary Table 4**). The Chimacum and Sequim samples appear to cluster near the loadings, in quadrats two and three. The Quilcene and Mount Vernon samples appear to cluster on the opposite side of the biplot, in quadrats one and two.

A biplot of seed composition components further illustrates results reported in **Table 5**; the Mount Vernon samples have lower total essential amino acid content and lower content of each of the essential amino acids compared to samples from the other locations. Furthermore, the Mount Vernon samples cluster separately on the biplot of seed composition components (**Supplementary Figure 1**).

DISCUSSION

The quinoa germplasm pool adapted to cultivation in Washington is extremely narrow, and is primarily limited by day-length sensitivity (i.e., photoperiod sensitivity) (59). Therefore, the samples in this study represent a fraction of the over 16,000 quinoa accessions maintained in *in situ* and *ex situ* collections (6). The limited amount of germplasm adapted to Washington agroecosystems is reflected by the shared parents within the pedigrees of the breeding line populations, the small number of accessions selected and grown at Mount Vernon, and the commercial varieties included as controls at Chimacum, Quilcene, and Sequim (**Table 3**). However, analysis of this germplasm pool greatly enhances the data available for amino acid content in quinoa below the species level. Moreover, these samples provide a baseline for the nutritional quality of Washington grown quinoa, especially with respect to identification of limiting amino acids when compared to human health requirements. Prior to this study, little information was available regarding seed composition components and amino acid content of quinoa grown in North America. This information should inform breeding efforts and strategies, research objectives, and crop production as they relate to understanding and enhancing the nutritional quality of quinoa.

Ash Content

Analysis of proximate content revealed considerable variation in ash content. Mean ash values were remarkably higher for samples grown at the Mount Vernon location in 2017 (4.25/100 g sample) compared to the other locations, and the mean value calculated for all samples (3.11/100 g sample) (**Table 5**). This may be due to the influence of genotypes (i.e., commercially available varieties), site-specific environmental characteristics, or possibly year effects (i.e., 2017 compared to 2016). Overall, mean ash content for the samples included in this study is comparable to the value reported by Navruz-Varli and Sanlier (36) (3.4%). In the review by Nowak et al. (37), the authors report ash content between 2.0 and 7.7/100 g sample, with a mean value of 3.3/100 g sample.

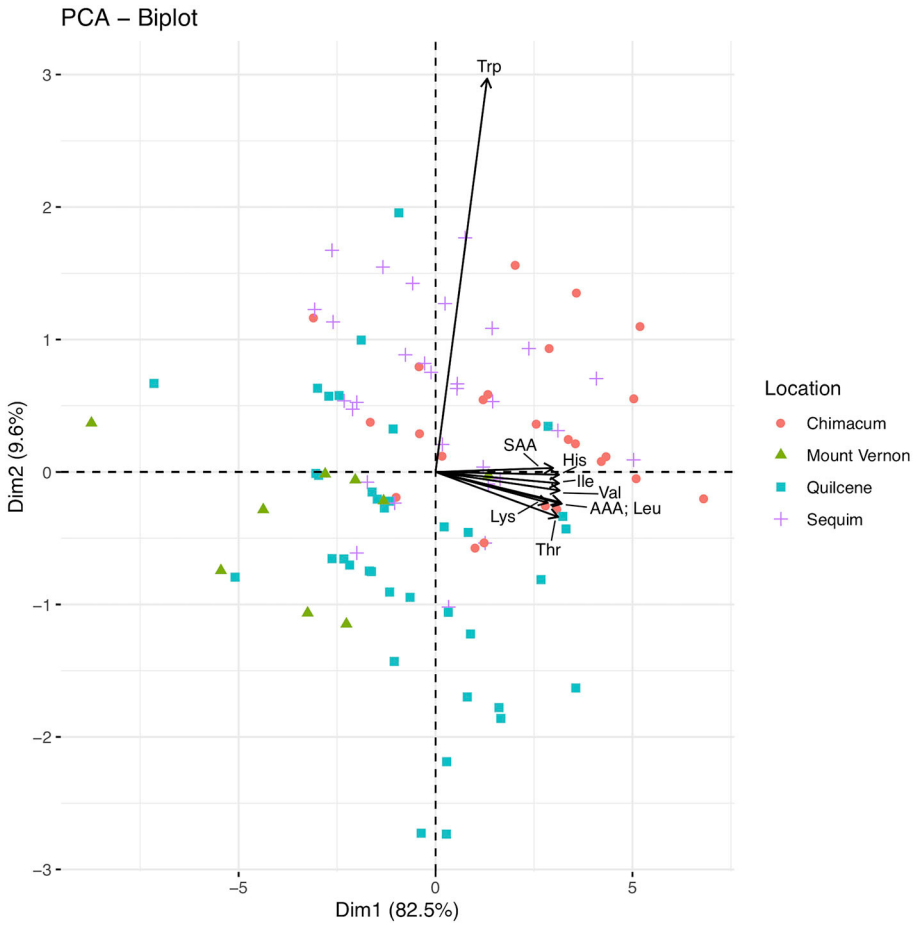

FIGURE 2 | Biplot for principal component analysis (PCA) of the essential amino acid dataset ($n = 100$). Principal component one (i.e., Dim1; 82.5% of variance) and two (i.e., Dim2; 9.6% of variance) are plotted as the x- and y- axis, respectively. Essential amino acids are abbreviated using standard three letter abbreviations. The sulfur and amino acids (methionine and cysteine) aromatic amino acids (phenylalanine and tyrosine) are abbreviated as SAA and AAA, respectively. Points are color and shape coded by locations as shown in the figure legend.

They suggest that differences are likely due to the interactions of several factors (e.g., varieties/cultivars, analytical methods, and environmental conditions). Reguera et al. (60) observed varying concentrations of minerals in quinoa varieties grown in three different agroecological environments and hypothesize that soil composition is responsible for these differences. Similar results supporting this hypothesis are reported by Miranda et al. (61) and Prado et al. (62).

Relationships Between Nutritional Components

Strong positive correlations exist between each essential amino acid and crude protein content. These results are supported by Gonzalez et al. (63), where they report positive correlations between amino acid content and protein content. Correlation coefficients for leucine, phenylalanine, isoleucine, tyrosine, valine, and tryptophan were significantly higher for the Bolivia/Argentina site compared to the Northwest Argentina site, while the correlation coefficients for lysine, methionine, and threonine did not significantly differ. The authors interpret these results as evidence of better adaptation of the cultivars to the Andean highland environment, because of relatively higher correlation coefficients and no significant difference in protein content found between the two agroecological regions. For researchers that do not have the capability to characterize the amino acid profile, measuring crude protein content and relying on the fact that an increase in protein content corresponds to a linear increase in essential amino acid content may suffice; however, Gonzalez et al. (63) claim that their results could indicate that the essential amino acid composition is independent of the amount of protein in the seed.

Factors That Influence Quinoa Nutritional Protein Quantity and Quality

It should be noted that the quinoa seeds in this study were not processed prior to analysis. Processing can impact protein content, and typically consists of washing, polishing, or pearling

to remove bitter saponins from the seeds before consumption. For example, Stikic et al. (64) manually dehulled seeds using a mortar to remove the pericarp and a sieve to separate the hulls, and then washed the dehulled seeds until "purified" (i.e., no foaming in rinse water from rubbing and washing seeds to remove saponins). They report a mean protein content of 17.41% for whole quinoa seeds, 15.69% for dehulled seeds, and 15.16% for purified seeds. Furthermore, they report a reduction in ash content following dehulling and sieving, from 7.06 to 3.59%, and an additional reduction to 2.24% following washing and rubbing (i.e., "purified"). Aluwi et al. (15) report significantly lower protein content for degermed Bolivian Royal, and higher protein content for scarified Cherry Vanilla. Based on the literature, the protein content reported in this study would change depending on the processing method performed on the raw quinoa before nutritional analysis.

Quinoa is often reported as having high protein content (35, 60, 64); however, quinoa protein content can be highly variable, and is often comparable to most cereals (37, 40, 65–67). Across all samples, mean protein content was 11.77/100 g sample, and ranged from 10.04 to 13.68/100 g sample (**Table 5**). This is comparable to values reported by Nowak et al. (37) (range = 9.1–15.7/100 g edible portion; mean = 13.1/100 g edible portion), Gonzalez et al. (63) (9.2%-15.4%), Dini et al. (68) (12.5%) and Miranda et al. (69) (11.3–16.1%), and lower than values reported by Reguera et al. (60) (14.8–17.5%), Vidueiros et al. (70) (14.5–18.2%), Koziol (67) (16.5%), Wright et al. (71) (16.7%), Bruin (72) (15.6%), Bhargava et al. (73) (12.5–21.0%), and Mota et al. (74) (12.2-16.3%). Rojas et al. (6) provide a summary of the nutritional value of a germplasm collection maintained in Bolivia, and report protein content from 10.21 to 18.39%.

In quinoa, the effects of genotype, environment, management practices, and their interactions (G × E; G × E × M) on nutritional parameters have not been completely elucidated. Protein quantity is influenced by factors such as soil fertility, environment, and genotype (6). Several studies support the hypothesis that environmental and agroecological conditions can influence quinoa protein content. In a study of three quinoa cultivars (Regalona, Salvedo, Titicaca) grown in three different agroecological zones (Spain, Peru, Chile), Reguera et al. (60) found no differences in protein content among the varieties within a location; however, protein content significantly differed between locations. Präger et al. (75) show that environmental conditions can modulate protein content in a genotype dependent manner. For example, the cultivars Jessie and Titicaca showed significant differences across years for protein content, while protein content values for Zeno and Puno remained stable across the 2 years at the one location studied. To this point, results from Gonzalez et al. (63) showed both significant inter- and intra-cultivar differences in protein content, and no differences for particular cultivars, although minimum, maximum, and mean values for both sites were practically the same in a study of 10 cultivars grown at two different agroecological sites. The authors suggest that complex underlying environmental and/or G × E interactions contribute to the significant changes in protein content in quinoa seeds from different agroecological regions for certain genotypes, but not others. Miranda et al. (61) found no difference in protein content values in a study of two cultivars (Regalona and Villarica) grown in two contrasting environments in Chile, perhaps because of broad adaptability to contrasting environmental conditions. Genotype dependent susceptibility to the effect of various factors (e.g., agro-environmental conditions) on nutritional protein quality should be further investigated and considered in the context of germplasm expansion to novel regions outside the environment the germplasm was developed in.

This study did not explicitly test for the influence of G × E interactions on quinoa nutritional parameters. However, our results provide insight into possible G × E interactions and their influence on Washington grown quinoa essential amino acid content. **Two** breeding lines in particular (102.17-Quilcene and 102.52-Chimacum) ranked in the top five among all samples in terms of overall content of nutritional parameters, total amino acid content, and essential amino acid content; however, these same two breeding lines ranked in the bottom five among all samples when grown at the Sequim location (**Table 7**). Soil analysis of the field locations (**Table 1**) reveals distinct differences with respect to environmental (e.g., annual precipitation) and soil quality characteristics that may be contributing to variation in the content of nutritional attributes. The Sequim location can be characterized by lower annual precipitation, although the farmer-collaborator did irrigate the field. Furthermore, the Sequim location had the lowest soil nitrate levels (3.8 mg kg^{-1}) compared to Quilcene (16.6 mg kg^{-1}) and Chimacum (21.5 mg kg^{-1}). It is possible that G×E interactions contributed to the stark differences in nutritional quality observed among replicated genotypes, and that perhaps soil nitrate levels are a driver of these interactions.

Gomaa (76) found that increased nitrogen application increased quinoa protein content, and Gonzalez et al. (63) showed that protein content and essential amino acid content are positively correlated in quinoa. We expect soil nitrate levels to influence protein content, and consequently essential amino acid content, but were not able to adequately test this with the present study. The Chimacum location had the highest soil nitrate levels, and samples grown at this location had the highest mean protein content (12.25/100 g sample), highest total amino acid content (10.82/100 g crude protein), and highest total essential amino acid content (34.53/100 g crude protein), albeit within one standard deviation of the mean values for the other locations (**Table 5**). One sample from Sequim (102.08) ranked fourth overall in terms on nutritional composition, fifth for total amino acid content, and fifth for essential amino acid content. This sample belongs to Population 102, which had two other breeding lines ranked among the top five samples for the aforementioned traits. This population has CO407Dave as the female parent; a sample of CO407Dave from Chimacum was ranked number one overall, second for total amino acid content, third for total essential amino acid content, and CO407Dave from Sequim is ranked fifth overall for crude protein content (**Table 7**). It's possible that these specific breeding lines are capable of efficient nitrogen uptake for protein and amino acid synthesis, regardless of the location, perhaps because of the presence of CO407Dave

in their pedigree. A lack of sufficient replication prohibits us from explicitly testing this hypothesis and making a definite conclusion. Studies are currently underway to better understand these relationships.

Perhaps more important than protein content is protein quality, or the composition with regards to the proportion of essential amino acid content (35). Quinoa is often regarded as having high lysine, leucine and sulfur amino acid content, especially in comparison to cereal crops (35, 38, 77–79). Leucine, lysine, and valine had the highest mean values, which is a finding supported by previous studies (37, 74, 75). For the non-essential amino acids, glutamic acid, aspartic acid, and arginine were the most abundant, which is also supported by other studies (64, 69, 75). Total essential amino acid content ranged from 30.78 to 37.32/100 g crude protein, which is similar to the total essential amino acid values reported by Miranda et al. (69) (34.1–35.9/100 g crude protein). Our values fall within a narrower range than those reported by Gonzalez et al. (63) (8.0–37.5/100 g crude protein), and are higher than the values reported by Präger et al. (75) (20.4–30.0/100 g) (**Supplementary Table 5**).

The aforementioned factors (e.g., genotype, environment, management, and their interactions) that can influence quinoa protein quantity also have the potential to influence quinoa protein quality (i.e., amino acid content and composition) (6). However, the primary factors responsible for influencing amino acid content in quinoa are not definitively agreed upon in the literature. For example, Miranda et al. (69) provide evidence in support of Wright et al. (71) and claim that the genetic characteristic of the quinoa genotypes decisively influences amino acid content. In a study of three Chilean landraces exposed to two levels of salinity under controlled conditions, Aloisi et al. (80) present results that support strong genotype-dependent responses to salinity with respect to essential amino acid content. Conversely, Gonzalez et al. (63) propose that amino acid content is higher among germplasm when grown in the environment to which it is adapted, based on the idea that cultivars growing in the geographic area of origin would exhibit better gene expression related to amino acid synthesis. Moreover, they state that both environmental and climatic factors influence the amino acid composition of quinoa cultivars growing in different agroecological zones. They report significant differences in total amino acid content and total essential amino acid content between the two agroecological sites examined for nine out of ten cultivars. The findings of Reguera et al. (60) and Präger et al. (75) support Gonzalez et al. (63) and the case for complex G × E interactions influencing essential amino acid content, as both studies found considerable variation in essential amino acid content depending on the variety and area of cultivation.

Identification of Limiting Amino Acids

Quinoa is often referred to as a "complete protein" because it contains all the essential amino acids; however, it is better described as "nearly complete" because of limiting amino acid content. Our study provides data on essential amino acid profiles for 100 distinct samples, representing 92 unique commercial varieties, landraces, and advanced breeding lines adapted to cultivation in Washington State, and evaluates the nutritional protein quality of each sample compared to the requirements of all age groups (52). We identify samples that fail to meet the daily requirements of all age groups for leucine, lysine, and tryptophan. Of the samples analyzed, only nine met the leucine requirements for all age groups. These samples include 7 advanced breeding lines (102.52, 107.84, 108.18. 106.37, 102.08, 102.23, and 102.17) and two commercial varieties (Kaslaea, CO407Dave); five samples were grown at Quilcene, three samples were grown at Chimacum, and one sample was grown at Sequim. Moreover, 52 samples and 94 samples met the lysine and tryptophan requirements for all age groups (**Supplementary Table 1**). These results provide the first report of leucine as a limiting amino acid in quinoa.

Quinoa protein quality is often compared to that of casein, a milk protein, because of similar values for protein digestibility and essential amino acid content (33, 67, 81, 82). In a joint report, the FAO/WHO/UNU Expert Consultation on Protein Quality Evaluation recommended the use of the protein digestibility-corrected amino acid score (PDCAAS) method for evaluating protein quality. Casein PDCAAS values are typically close to, or exceeding, the 1.0 truncation value (83). Reports of quinoa PDCAAS values and protein digestibility are sparse in the literature; however, 82 report recalculated PDCAAS values of 0.85 (raw quinoa) and 1.00 (washed quinoa) for the 1–2 year-old age group, and 0.89 (raw) and 1.09 (washed) for the 3–10 year age group based on data from Ruales and Nair (41). Quinoa protein digestibility varies depending on genotype, processing, and evaluation method, although saponin removal and cooking generally improves digestibility (34, 65, 81, 84–87). We calculated PDCAAS values using an apparent protein digestibility value of 84.3% based on fecal protein losses in rats as reported by Ranhotra et al. (82) for the variety "Colorado D407." Values ranged from 0.74 to 0.90 and 0.78 to 0.95 for the 1–2 and 3–10 year-old age groups, respectively (**Supplementary Tables 6**, **7**).

The vast majority of studies that report quinoa essential amino acid content, and compare these values to daily human health requirements, either make comparisons to outdated daily requirements, or only consider requirements of the adult age group. For example, Ruales and Nair (41) report the aromatic amino acids, threonine, and lysine as the first, second, and third limiting amino acids, respectively, while Boye et al. (83) identified valine and lysine, and lysine as limited amino acids for the 1–2 and 3–10 year-old age groups using values from Ruales and Nair (41) and current WHO/UNU/FAO (52) requirements. Mahoney et al. (81) analyzed a single quinoa variety (Sajama; Patacamaya Agricultural Experiment Station, Lapaz, Bolivia) and identified methionine as the first limiting amino acid, followed by tryptophan. Gonzalez et al. (63) report lysine, threonine, and methionine content in relatively adequate amounts for both human and animal feeding. They also claim that content for the aromatic amino acids, isoleucine, threonine, and valine were sufficient for 10–12 year-old children. The authors identify lysine, tyrosine, and tryptophan as limiting

amino acids for 2–5 year-old children. In addition to lysine and tryptophan being limiting amino acids for 2–5 year-old children, leucine is also a limiting acid for this age group for our samples. The comparisons made be Gonzalez et al. (63) are made to the FAO/WHO guidelines published in 1990, even though updated guidelines were published in 2007. Repo-Carrasco et al. (88) cite studies conducted at the Universidad Nacional Agraria La Molina (UNALM) that found that one cultivar of quinoa, Amarilla de Marangani, does not have any limiting amino acids, although no information is provided concerning the age group or requirements that form the basis for this comparison.

The potential for quinoa to adapt to diverse agroecological conditions and contribute to food and nutritional security is generating worldwide interest and contributing to the current global expansion of quinoa. Recent studies have evaluated the potential of quinoa in novel production environments, with a focus on agronomic traits and nutritional quality, especially essential amino acid content. Indirect evaluation of quinoa nutritional protein quality, for studies that do not make a direct comparison, can be accomplished by comparing published values for essential amino acid content to daily essential amino acid requirements. For example, the mean value of the four cultivars (Zeno, Jessie, Puno, Titicaca), and each individual cultivar, reported by Präger et al. (75) for a single location and 2 years, fail to meet leucine, lysine, and histidine requirements for all age groups for the first year of their study, while only one cultivar (Puno) met the requirements for isoleucine, the sulfur and aromatic amino acids, and threonine (**Supplementary Table 5**). In the second year of the study, the mean value of the four cultivars, and each individual cultivar, failed to meet the leucine, lysine, sulfur and aromatic amino acid requirements; Puno met the isoleucine requirement within one standard error of the mean, although the mean value was lower than the requirement. All cultivars and the mean value for all four cultivars met the tryptophan requirements for both years (**Supplementary Table 5**). The amino acid values reported by Miranda et al. (69) for six genotypes and one genotype failed to meet the lysine and leucine requirements for all age groups, respectively. The ability of quinoa to meet global health challenges depends in part on the ability to meet essential amino acid requirements for all age groups.

CONCLUSIONS

This study provides a baseline analysis of the nutritional quality of quinoa grown in Washington state. For the germplasm tested, protein content is lower than values reported in the literature; however, essential amino acid content is generally higher. Mean essential amino acid values meet the daily requirements for infants and adults, except for the amount of leucine required by infants. This is the first report of leucine as a limiting quinoa amino acid. We identify 9% of the samples that meet the leucine requirements for all age groups, and 8% of the samples that fail to meet the requirements for any age group. Lysine requirements for the <0.5 and 1–2 year-old age groups were not met by 31 and 35 genotypes, respectively. Tryptophan requirements for the <0.5 and 1–2 year-old age groups were each not met by three genotypes. This study greatly augments the amount of data available for quinoa nutritional quality below the species level, and provides the first in-depth report of the protein quality of quinoa grown in North America. The information reported in this study will be useful for guiding research objectives and breeding strategies, in pursuit of supporting the global expansion of quinoa and the potential for quinoa to contribute to addressing global public health challenges. Effective breeding strategies for improving quinoa protein quality should focus on identifying limiting amino acids, the factors that influence amino acid content, and increasing the content of limiting amino acids to improve PDCAAS. Moreover, increased lysine and sulfur amino acid content are important targets, because these amino acids are limiting in most common cereals (e.g., wheat and maize), in addition to leucine content. Future work must be context specific with respect to germplasm adapted to the target production environment and culture.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/**Supplementary Material**. The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

KM conceived the project and provided edits to the manuscript. EC performed data collection and analysis and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.00126/full#supplementary-material

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CORRECTION
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Corrigendum: Seed Composition and Amino Acid Profiles for Quinoa Grown in Washington State

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Keywords: quinoa, essential amino acid, limiting, complete protein, protein quality

A Corrigendum on

Seed Composition and Amino Acid Profiles for Quinoa Grown in Washington State
by Craine, E. B., and Murphy, K. M. (2020). Front. Nutr. 7:126. doi: 10.3389/fnut.2020.00126

In the original article, there was a mistake in the legend for **Table 1** as published: important citations were not included for the referenced studies. The correct legend appears below.

Table 1 | Site characteristics for each year and location that samples were randomly selected from for chemical analysis. Raw quinoa seed sent for analysis was grown in 2016 and 2017 in western Washington as part of two separate experimental designs (13, 49).

In the original article ***(Hinojosa et al., 2019; Kellogg and Murphy 2019)*** was not cited in the article. The citation has now been inserted in ****Methods Section****, ****Study Region and Field Trials****, ****Paragraph one**** and should read:

****Raw quinoa seed sent for analysis was grown in 2016 and 2017 in western Washington as part of two separate experimental designs (13, 49). Site characteristics for all locations are summarized in Table 1. In 2016, F5:F6 advanced breeding lines and control varieties were planted on three organic farms in Chimacum (Finnriver Farm; 48°0'29"N 122°46'12"W), Quilcene (Dharma Ridge Organic Farm; 47°55'04.0"N 122°53'23.2"W) and Sequim (Nash's Organic Produce; 48°08'31"N 123°07'19"W) on the Olympic Peninsula. Control varieties included Cherry Vanilla (Wild Garden Seed, Philomath, OR, US), CO407Dave (PI 596293, USDA Plant Introduction, Ames, Iowa) and Kaslaea (Ames 13745, USDA Plant Introduction, Ames, Iowa). At each location, advanced breeding lines and control varieties were planted in single hand-sown plots that measured 4.9 m in length and 40.64 cm from center and were seeded at a rate of 4 g row m^{-1} in an augmented randomized complete block design (ARCBD). An ARCBD uses control varieties to account for field variation by replicating control varieties across blocks; control varieties can be used as covariates to make spatial adjustments across blocks. This design is useful for evaluating advanced breeding lines when seed quantity is low, land and other resources are limited, and when many advanced breeding lines must be evaluated.****

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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From Pest to Profit—The Potential of Shipworms for Sustainable Aquaculture

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We face a food crisis. Suboptimal diet is the biggest cause of death worldwide, food production the biggest greenhouse gas emitting sector, and by 2050 an extra 2.5 billion people need affordable nutrition. Current farming systems will fail to tackle this crisis, and there is an urgent need to diversify global food production and find effective solutions in currently underexploited food sectors. Shipworms, or shell-less *Teredo* clams, could prove a highly valuable component of such solutions. Historically viewed as a marine pest, they have unique physiological characteristics which make them an ideal food source, including exceptionally fast growth rates, the ability to feed on waste wood or sustainable microalgae, and a high protein and omega 3 content. Today only a select few traditional cultures in the Philippines consume shipworms, but there is considerable opportunity to develop mechanisms to farm shipworms and provide a sustainable, nutrient rich, affordable food source. This will require significant challenges to be overcome, ranging from fundamental research to industry development to food processing and marketing. Leveraging new innovations in breeding, aquaculture feeds, growth systems, food processing methodologies and consumer engagement can however offer powerful solutions, and could help turn what was once a maritime villain into a nutritional savior.

Keywords: aquaculture, shipworm, *Teredo navalis*, sustainable, nutrition, clam, food security, bivalve

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INTRODUCTION

Our current food production system is failing us. Suboptimal diet is already the biggest cause of death worldwide and the global food system the single largest greenhouse-gas emitting sector. Yet by 2050 a further 2.5 billion people need access to affordable nutrition (Rockström et al., 2020). Farming of conventional crops including wheat, rice, and maize has received extensive investment over the last century, with productivity growing 2–3-fold between 1950 and 1990 (FAO, 2020a). However, yields are now beginning to stagnate, and the overreliance on this small variety of crops is a major explanation as to why 30% of humanity are now micronutrient deficient worldwide (Willett et al., 2019; FAO, 2020a). Other more nutrient rich food sources have received considerably lower research attention (**Figure 1**). In order to meet planetary health goals, there is an urgent need to diversify global food production and to seek productivity improvements in currently underexploited food sectors that can provide sustainable, nutrient rich, affordable food solutions (Willett et al., 2019; Rockström et al., 2020).

There are major environmental and human health wins that could be made from research and industry investment in the farming of underexploited aquaculture species. Aquaculture is already the world's fastest growing food sector, growing 8.2% pa between 1970 and 2010 (FAO, 2020a,b). Growth has however been focussed on species such as salmon and shrimp, which are expensive to consumers and unsustainable to farm due to a reliance upon foods comprising predominantly of fish meal and oil from wild-caught fish (Willer and Aldridge, 2019a). The farming of bivalve shellfish (clams, mussels, oysters) is a highly attractive alternative. Bivalves have a higher protein content than beef, are a rich source of essential fatty acids and micronutrients, have a lower environmental footprint than all other meats and many plant crops, and are cheap to produce (Willer and Aldridge, 2019a). There is outstanding potential for growth of bivalve aquaculture; developing just 1% of the suitable coastline worldwide could fulfill the protein requirements of over one billion people (Gentry et al., 2017; United States Department of Agriculture Agricultural Research Service, 2020). Yet the bivalve industry remains small scale and artisanal. There are bivalve species with high farming potential such as shipworms which could play a key role in sustainable aquaculture, where further research and industry attention is required in order to realize food production potential (Waite et al., 2014).

Shipworms, or *Teredo* clams, are a saltwater bivalve species with unique life-history characteristics that could be leveraged to provide significant economic, sustainability, and nutritional benefits to human society. Shipworms grow exceptionally fast relative to other aquaculture species, reaching 30 cm (or ∼250 g wet weight) within 6 months, compared to shelled bivalves which typically take 2–3 years to reach a harvestable size of around 7 cm (Helm and Bourne, 2004; Paalvast and van der Velde, 2011a), presenting a highly productive and efficient possibility for producing animal protein. Shipworms lack a shell for protection, instead burrowing into and feeding on submerged wood. This wood digesting ability has historically made shipworms a pest in the maritime industry, where they have damaged traditional wooden hulls and marine piling; an issue now reduced with modern engineering materials (Paalvast and van der Velde, 2011a). Whilst *Teredo* clams can survive on wood alone, obtaining additional nitrogen via symbiotic nitrogen fixing bacteria in their gills, they can also filter feed on marine detritus like other bivalves (Paalvast and van der Velde, 2013), thus presenting numerous options for low-environmental impact feeding in aquaculture production. Shipworms are rich in protein and essential omega-3 fatty acids, and like other bivalves can provide a highly valuable source of quality nutrition to humans (Paalvast and van der Velde, 2013; Sinyo et al., 2019; United States Department of Agriculture Agricultural Research Service, 2020).

Today shipworm harvest and consumption as a food is limited to a few select regions of southeast Asia, notably the Philippines and Thailand. Shipworms grow on decaying wood in the mangroves and are harvested by local populations. In some areas such as Bakhawan Eco Park (New Buswang, Panay, Philippines), the coastlines have been seeded with dead wood as part of mangrove restoration projects which in turn has increased the supply of shipworms for harvest, although there remain no formalized farming approaches (Aklan Government, 2020). Shipworms are sold raw in wet markets, and are particularly popular on Palawan island, Philippines where they are known as tamilok and in Trat Province, Thailand where they are known as priyang talay (Slow Food Foundation, 2020). Filipinos typically eat Tamilok raw and dipped in salt, chili and vinegar in a dish known as kinilaw, whilst the Thais eat priyang talay in curries or braised with fish paste and bananas in a stew (Sinyo et al., 2019; Aklan Government, 2020; Slow Food Foundation, 2020)). Over the last decade increasing popularity of shipworms as a food for tourists and increasing ecological threats to mangrove swamps has put pressure on shipworm supply, and socioeconomic benefits could be yielded from increased mangrove protection or improved shipworm culture practices (Sinyo et al., 2019; Aklan Government, 2020). Outside of southeast Asia, shipworms are still viewed as a pest. In Europe and the Americas climatic change is leading to increasing coastal salinity and temperature, making conditions more favorable to shipworms (Paalvast and van der Velde, 2011b). This could lead to the re-emergence of shipworms as a maritime nuisance, or alternatively with research and industry innovation and efforts to change consumer perception, could present a new opportunity to establish shipworm aquaculture.

APPROACH

Several approaches were leveraged to build a synthesis of the potential of shipworms as a food source and the key challenges and opportunities to establish aquaculture. Scopus was used (Elsevier, 2020) (**Figure 1**, legend) to count the percentage of all research articles published corresponding to key food types across each decade since 1990, and highlight the relatively low research investment in bivalve aquaculture. We spoke to stakeholders across the aquaculture and food industry to gain an insight on the potential obstacles in creating a new food type and methods which could be effective in providing a solution. This provided direction for a thorough literature review. We searched Scopus (Elsevier, 2020) and Google Scholar (Google, 2020) for studies on shipworms, aquaculture, and the development of unexploited food sectors. We used several key terms when covering the field: "shipworm," "*Teredo*," "bivalve," "aquaculture," "invasive species," "sustainable nutrition," "food safety," "food processing," "food marketing"; and included all relevant research up to and including 20th June 2020. Prior to our study, no studies had built a synthesis on the potential of shipworms for aquaculture.

CHALLENGES AND OPPORTUNITIES

Production of shipworms could offer major advantages over other forms of aquaculture for food production. Farming would be significantly more sustainable than finfish aquaculture, with shipworms requiring waste wood or natural or sustainably grown algae rather than fish as a feed source, helping contribute toward circular economies in food production (Paalvast and van der Velde, 2013; Waite et al., 2014). The disadvantages of

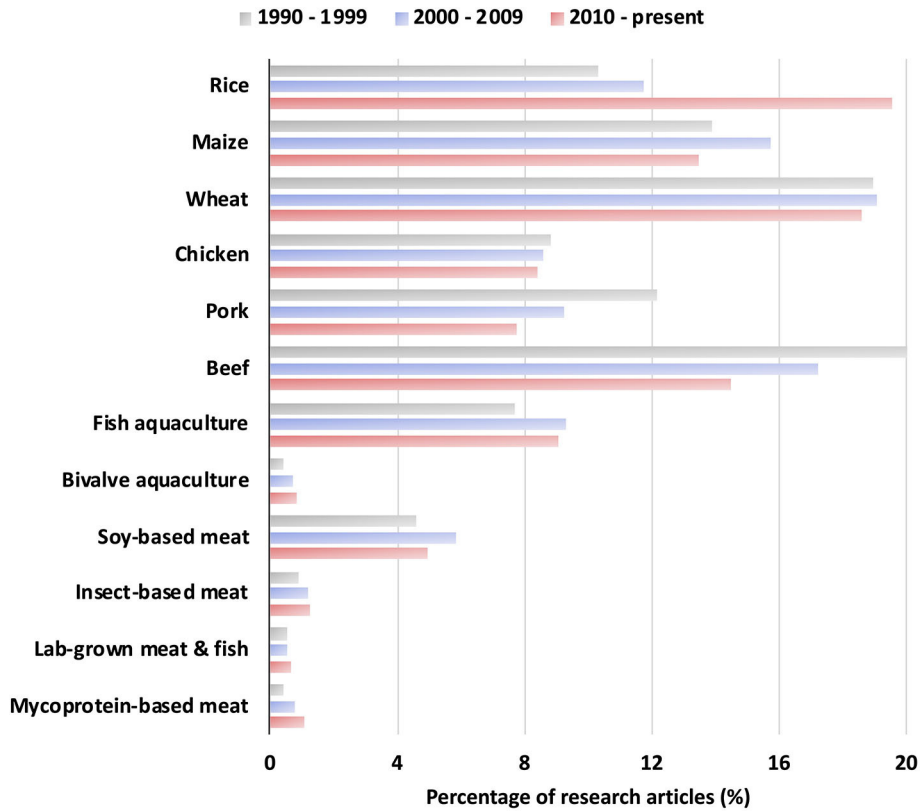

FIGURE 1 | Percentage of all research articles published corresponding to key food types across each decade. Many food sources with a high sustainability and nutritional potential (bivalve aquaculture and below) continue to receive relatively low levels of research investment. Scopus (Elsevier, 2020) was used to count the total number of peer-reviewed publications corresponding to each food type between the dates 1990–1999, 2000–2009, and 2010–12/05/2020. Five most relevant search phrases were selected for each food type. To be counted articles had to include any one of the search phrases in the abstract, title or keywords. Search phrases were as follows. Rice: "rice farm," "rice production," "rice industry," "rice food," "rice field." Maize: "maize farm," "maize production," "maize industry," "maize food," "maize field." Wheat: "wheat farm," "wheat production," "wheat industry," "wheat food," "wheat field." Chicken: "poultry farm," "poultry production," "chicken farm," "chicken production," "broiler farm." Pork: "pork farm," "pig farm," "pork production," "pig production," "swine farm." Beef: "beef farm," "cattle farm," "cow farm," "cattle production," "beef production." Fish aquaculture: "fish farm," "fish aquaculture," "pisciculture," "fish mariculture," "fish hatchery." Bivalve aquaculture: "bivalve aquaculture," "bivalve farm," "clam farm," "mussel farm," "oyster farm." Soy-based meat: "tofu," "tempeh," "soy-based meat," "soy protein," "miso." Insect-based meat: "insect meat," "insect flour," "insect food product," "insect snack," "insect burger." Lab-grown meat and fish: "lab-grown meat," "cultured artificial meat," "lab-grown fish," "*in vitro* meat," "cell-based meat." Mycoprotein-based meat: "mycoprotein," "quorn," "fusarium venenatum," "fungiculture protein," "fungal protein food."

conventional bivalve aquaculture—notably the slow growth rates and energy investment in a shell which cannot be used as food—would be avoided. Shipworms would provide a valuable source of protein and essential fatty acids to replace less sustainable and more expensive meat and fish products. In order for this potential to be realized there are key challenges that must be overcome, ranging from fundamental research to industry development to food processing and marketing. Novel technologies and new application of innovations from other industries can however provide exciting opportunities for success (**Figure 2**).

FUNDAMENTAL RESEARCH

Fundamental research is required in several key areas to allow the exploration and development of shipworm aquaculture. There are ∼65 species of shipworm worldwide and we do not yet know which would be most appropriate for efficient farming and human nutrition (Horton et al., 2020). The only small-scale attempts at culture to date have involved floating logs in coastal waters or seeding mangroves with dead wood to encourage *Teredo* proliferation (Sinyo et al., 2019). When an appropriate species is identified, there will also be a need to develop scientific methodologies for trait selection and large-scale breeding of shipworm juveniles, which is challenging in bivalves due to high fecundity, self-fertilization, genetic load and segregation distortion (Hollenbeck and Johnston, 2018). The nutritional profile of shipworms also needs to be formally analyzed—whilst they are recognized to have a similar profile to other bivalves no quantitative data has been published in peer-reviewed literature (Paalvast and van der Velde, 2013; Sinyo et al., 2019). The most appropriate abiotic conditions for farming

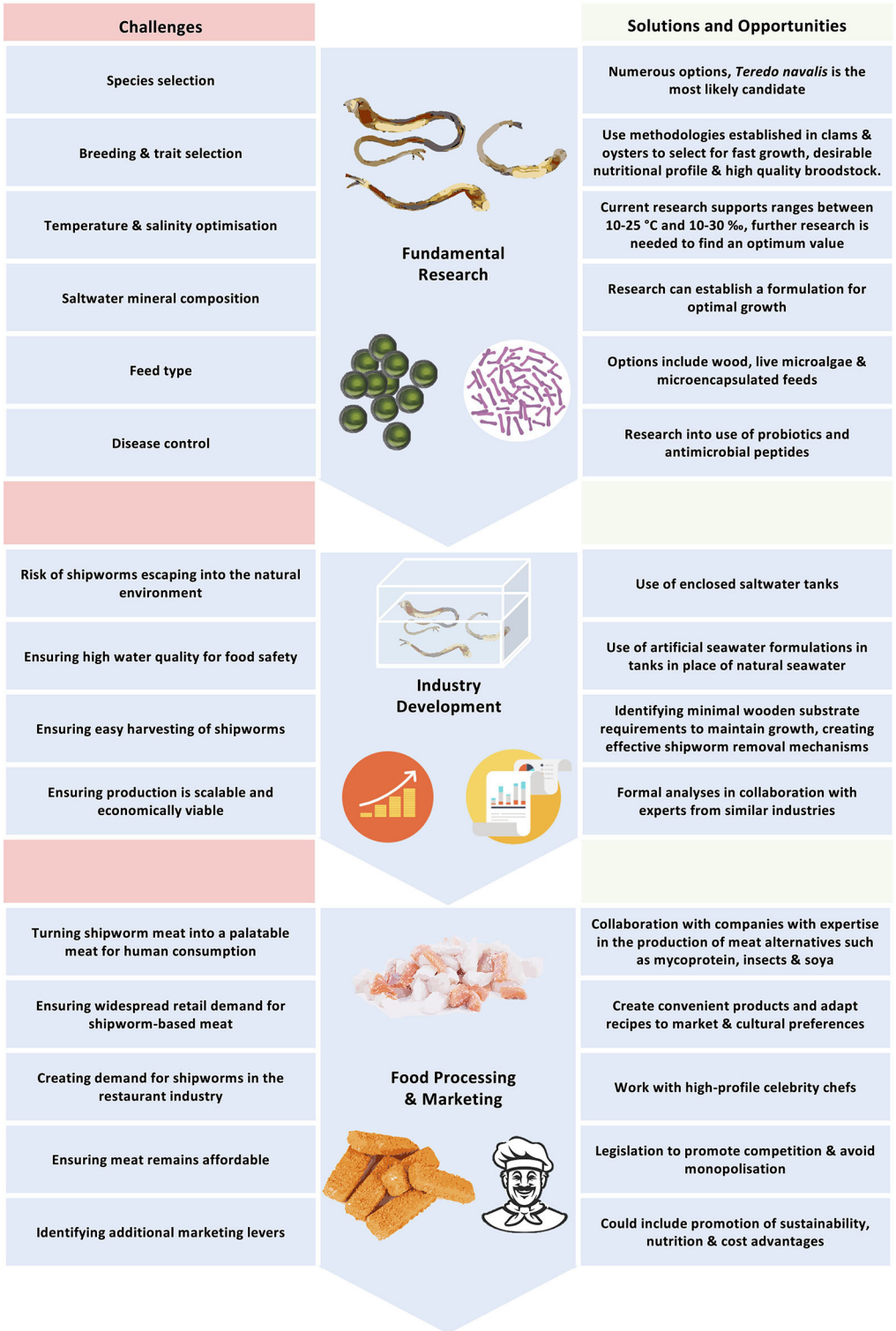

FIGURE 2 | Key challenges, solutions and opportunities in the development of shipworm aquaculture.

shipworms also need to be identified, including temperature, salinity, and saltwater mineral composition. We must also develop a greater understanding as to how climatic change may affect these values, as this may affect site selection for farms without facilities to control temperature and salinity (Filgueira et al., 2016). Crucial to successful shipworm aquaculture will be

the selection and development of appropriate feeds. Research indicates that filter feeding is in fact the preferred source of dietary nutrition for shipworms, with wood drilling performed primarily to provide shelter (Paalvast and van der Velde, 2013), but we do not yet know what the most appropriate feed would be for shipworm farming. Infection control must also be considered. Disease is a major concern in aquaculture, costing the industry over US$6 billion annually, yet there is an urgent need to reduce the widespread use of antibiotics which is driving bacterial resistance and increasing threat to human health (Stentiford et al., 2017).

Knowledge and methodologies gained from research in conventional agriculture and aquaculture can help tackle challenges in shipworm aquaculture. Current scientific literature suggests *Teredo navalis* may be a promising shipworm candidate for aquaculture, the same species as consumed by Philippine societies at present (Sinyo et al., 2019). The development of whole genome sequences and genetic linkage maps, as has been done in other bivalve aquaculture (Hollenbeck and Johnston, 2018) could be used to select for desirable traits such as faster growth, greater broodstock quality, and an improved profile regarding nutrition and palatability. Optimum temperatures and salinities for farming are likely to be between 10–25°C and 10–30%, respectively (Paalvast and van der Velde, 2011a); further research can refine these values, make predictions as to which coastal locations might become more ideal under climate change, and make use of new innovations in artificial salt formulations (e.g., Homarsel, produced by Zoutman, Belgium). Regarding feed for shipworm aquaculture, waste wood, live algae, and artificial or microencapsulated feeds are all viable options. The use of wood may allow the recycling of waste, and the use of microencapsulated feeds (Willer and Aldridge, 2017, 2019b) would avoid major challenges faced by conventional algal feed including quality inconsistencies, contamination, and poor shelf life (Willer and Aldridge, 2019a). Research would allow identification as to whether a combination of these feeds or a single feed is most optimal for growth. There are also emerging new opportunities for more sustainable disease control, including the use of probiotics and antimicrobial peptides (Destoumieux-Garzón et al., 2016; Hoseinifar et al., 2018), and it would be advisable to quantify the efficacy of their use in shipworms.

INDUSTRY DEVELOPMENT

Industry will play the central role in the establishment of shipworm aquaculture, and will need to overcome challenges regarding facility design, safety and economics in order to develop and expand successfully. The practical aspects of setting up shipworm farms pose an initial hurdle. The invasive nature of shipworms means that they can represent a hazard if they escape into the natural marine environment (Paalvast and van der Velde, 2011a) and farming systems and infrastructure need to be designed to nullify this risk. Overzealous farming of other bivalves such as *Magallana gigas* has already been shown to displace important native species and modify marine ecosystems (Herbert et al., 2016). Food safety is also a major concern when farming filter feeding bivalves, and in polluted waters there is high potential for hazardous substances such as heavy metals, microplastics and toxic cyanobacteria to accumulate in bivalves—with oysters farmed in the South China Sea for example now containing ∼11 microplastic particles per individual (Elston and Ford, 2011; Eriksen et al., 2014; Hossen et al., 2014; Willer and Aldridge, 2020a). Ocean water quality is likely to further decline as the human population expands and shipworm farms should be designed to cope with these changes (Häder et al., 2020). Coastal areas will undergo further urbanization and development, which could both restrict and provide new opportunities as to where shipworm farms could be located. The industry will also need to develop automated mechanisms to efficiently monitor shipworms and at harvest remove them from the woody substrates they burrow in. In conventional bivalve production the shucking (de-shelling) process in manufacturing packaged bivalve foods such as tinned clams is highly labor intensive and a major contributor to food costs (Hackney and Rippen, 2000), and while shipworms lack a shell it will be important to find cost-effective processing systems. Finally and crucially, a thorough economic assessment of the entire proposed production value chain will be required in order to ensure shipworm aquaculture is scalable and financially viable. Inadequate assessment could lead to expensive bottlenecks in production (Willer and Aldridge, 2019a), or at worst could result in complete failure of shipworm aquaculture (Shang, 1985).

Lessons learned from the commercial success of other forms of aquaculture could help ensure effective development of the shipworm industry. To mitigate risk of shipworms escaping into the open sea, farming of shipworms could involve the use of enclosed saltwater tanks, either semi-submerged on the coastline or onshore. The installation of flood protection structures and nearshore or offshore wind farms as a means to mitigate climatic change and its impacts could provide a new opportunity for mounting shipworm growth tanks. Larger enclosed areas could also be constructed within mangrove restoration projects, in parallel improving water quality and coastal protection (Aklan Government, 2020). There may also be an option to setup enclosed tanks in urban areas away from the sea as is seen in finfish aquaculture (Zohar et al., 2005), which would further reduce the risk of escape due to the lack of surrounding saltwater. Enclosed tanks would enable optimization of temperature and salinity to improve growth rates, and also the use of artificial seawater or clean depurated seawater which would dramatically increase food safety (Zohar et al., 2005; Willer and Aldridge, 2020b). Given that consumer fears regarding safety are one of the biggest barriers to increased bivalve consumption, the use of enclosed tanks could be the underpinning factor in the commercial success of shipworms as a food source (Birch and Lawley, 2012). Automation during growth and at the point of harvest through the use of optical sensors, machine vision systems and low-cost robotics could help avoid excessive labor costs in shipworm production, with this approach already yielding great benefits in finfish aquaculture (Saberioon et al., 2017). Identifying the minimal requirements and optimal arrangement of woody substrates for shipworm

farming would further increase operating efficiency during growth and harvest. There is also the opportunity to use circular economies to divert side-streams of shipworm production back into the system for example for feed or wood production. To ensure commercial viability of shipworm farming, bioeconomic modeling methods that have recently been tried and tested across salmon, tilapia and shrimp aquaculture can be applied. Compared to using simple cost-benefit analyses alone, the use of scenario simulations and algorithm-based approaches can ensure that farming solutions identified are more resilient to changes in external economic and environmental factors (Llorente and Luna, 2016).

FOOD PROCESSING AND MARKETING

Research and industry development will provide the foundation for shipworm aquaculture, but unless challenges in food processing and marketing are tackled these efforts will be in vain. The first major challenge will be in turning shipworm meat into a product that is palatable for human consumption. Invertebrate-based foods such as bivalves and insects are viewed with fear and disgust by many western societies, and innovations in food manufacturing are being developed to overcome this hurdle (Dobermann et al., 2017). Molluscs also have a different muscle structure (obliquely striated, cross-striated, and smooth) to the striated muscles of fish and mammals, which may pose additional food processing challenges (Millman, 1967). To ensure widespread retail demand, it will also be essential to ensure that products developed fit a wide range of consumer tastes and cultural preferences (Apostolidis and McLeay, 2016), and this will be of equal importance in the restaurant and hospitality market alongside supermarkets. Products and dishes will also need to remain affordable to consumers. This requires measures to ensure production is efficient as well as approaches to avoid market monopolization which could drive up prices and reducing industry innovation, as has unfortunately been seen in the agricultural and mycoprotein industries (Howard, 2016). Paramount to the overall strategy will be for researchers and industry to work together to identify the most effective methods to market shipworm food products and promote consumer uptake.

There are a wide array of effective solutions that can be applied to the discussed challenges in the processing and marketing of shipworm-based foods. Innovations in food manufacturing made during the development of mycoprotein and insect-based foods can be applied to shipworm meat. These range from simple dehydration, powdering, and reconstitution, to thermoplastic extrusion and fiber spinning of meat proteins into a completely new form, which could help with the potential challenge of processing the different molluscan muscle types (Featherstone, 2015; Apostolidis and McLeay, 2016). This could enable the development of food products with a wide range of forms to fit a range of cultures, from burgers and fish fingers to meat-style pieces to use in traditional stews, and is an approach that has led to great commercial success for products including Quorn (USA), Beyond Meat (USA), and Eat Grub (UK) (Deroy et al., 2015). Working with high profile chefs and finding methods to explicitly present shipworms undisguised in a highly palatable form on a plate will also play a key role. Historically in Asia and increasingly in the West this strategy of including insects in premium dishes has been central to establishing a high level of cultural acceptance for insect-based foods (Deroy et al., 2015; Dobermann et al., 2017). Marinated shipworm kinilaw is already popular with both locals and tourists in the Philippines, and gaining further insight from Filipino culinary practices could help such dishes become a global delicacy (Sinyo et al., 2019). To ensure shipworm-based foods are easily affordable to consumers several mechanisms could be deployed. These include governmental and private investment to allow the industry to scale rapidly and achieve lower operating costs, subsidies to encourage purchase of nutritious shipworm-based food in place of less sustainable meat products (An, 2013), and enforcement of industry regulations to promote competition in shipworm production and food processing (Sodano and Verneau, 2014). Marketing will also play a pivotal role in encouraging consumer selection and purchase of bivalve based foods. Mass surveys, consumer co-creation activities, and domestic trials can help identify the most effective marketing levers to pull. A promotion strategy in which shipworms are double framed as an environmental solution to excessive meat consumption and as a protein packed superfood, an approach already being used in the seafood and insect industry (Schiemer et al., 2018), may prove one of the most effective mechanisms.

CONCLUSION

As the world's population grows by 200,000 people every day, we have a global responsibility to find new ways to feed everyone without further depleting the planet's already stretched natural resources. Finding viable, sustainable, affordable solutions will mean thinking outside-the-box and considering the previously inconsiderable. Shipworm aquaculture has outstanding potential to become a component of a broader global solution. Challenges need to be overcome, ranging from fundamental research and viability assessments to industry development to food processing and marketing. Yet there is an opportunity to provide our global community with a fast growing, sustainable, nutritious food source that could help remediate the catastrophic damage current food productions system are causing to environmental and human health. Policy changes will play a key role in stimulating research and industry efforts. Financial incentives that support sustainable shipworm research and aquaculture in place of less sustainable meat production, legislations that support mangrove restoration, coastal and urban aquaculture, and tax breaks on the production and retail of sustainable nutrient-dense foods are all options. Changing industry practice and consumer behavior will not be easy, but rewards are seldom reaped without new ways of thinking. Shifting our perception of shipworms as a pest and giving them a place on our plates is one change in our thinking that could yield great rewards.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/supplementary material.

AUTHOR CONTRIBUTIONS

DW led the investigation and wrote the manuscript. DA contributed to design, interpretation, and reviewed the manuscript. All authors gave final approval for publication.

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Consumers' Willingness to Consume Insect-Based Protein Depends on Descriptive Social Norms

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Eating of insects has been discussed as a more sustainable source of animal protein, but consumer research about uptake behavior of Western consumers is still scarce. Based on previous psychological research highlighting the role of social norms, the present research shows that even subtle cues about descriptive social norms affect Westerners' willingness to eat unprocessed insects. In a series of four studies, we demonstrate that adherence to descriptive social norms underlies eating intention and behavior. Study 1 shows that individual beliefs about the descriptive social norm correlates with the willingness to eat an unprocessed insect, an effect which is replicated in an experiment showing the causal direction from norm beliefs to eating behavior (Study 2). Study 3 establishes that even in the absence of concrete information about social norms, consumers construe norms based on other options. Manipulating the perceived eating-contingent financial rewards for other people from the same population, un-incentivized participants are more readily willing to eat when they believe that others receive a higher incentive, an effect that is mediated by beliefs about the eating frequency of these participants. Study 4, finally, shows that manipulating beliefs about the norms provides the causal explanation as the effect of the incentive disappears when norm information is explicitly given. Taken together, the studies show that descriptive social norms partially underlie Westerns willingness (or reluctance) to consume insects and that behavioral change initiative could focus on the importance of using norms to increase reliance on non-standard sources of animal protein.

Keywords: entomophagy, social norms, consumer psychology, sustainability, behavioral economics

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INTRODUCTION

The ecological burden of human food consumption poses a major challenge for climate change mitigation. According to the Intergovernmental Panel on Climate Change (IPCC) fifth assessment report, food production contributes to about a quarter of global anthropogenic greenhouse gas emissions and increasing food demand will exacerbate this trend (e.g., Pelletier and Tyedmers, 2010). Quite crucially, the production of meat and dairy products accounts for half of these food emissions (Eshel et al., 2014; IPCC, 2014). Given the problematically high level of meat consumption worldwide, interest in alternative protein sources, such as insect-based foods, has therefore increased remarkably in recent years. In fact, insect-containing food products have not only been suggested as a more sustainable source of protein (e.g., less greenhouse gas emissions and less land/water required for production, Oonincx and de Boer, 2012), but have also been associated

with health benefits (e.g., rich in proteins, fats, vitamins, and minerals, Rumpold and Schlüter, 2013; van Huis, 2013).

Entomophagy, which is the consumption of edible insects, has also attracted increasing public attention due to recent progress in agricultural technology and food safety: Automation, reduction in microbial contamination by personnel, and increased space utilization have finally made insects a viable option for industrial and private production alike (Parker, 2005; van Huis, 2013). Moreover, deregulation allowing for the commercial production and marketing of edible insects (e.g., in Switzerland in 2017 or as provided by the new regulation of the European Union 2015/2283 on novel foods which entered into force by 2018) have allowed innovators from companies and top-level cuisine to enter the market for insect-based foods (Van Raamsdonk et al., 2017; Berger et al., 2019). However, despite these favorable developments and obvious ecological advantages of insect consumption, Westerners' willingness to eat insect-containing foods is still very low (Deroy et al., 2015; Hartmann et al., 2015) and, up to this date, scientific knowledge about the factors underlying this aversion is relatively scarce. For example, Verbeke (2015) provides an attempt to profile potential customers willing to eat insects by showing the correlational pattern with various demographic or psychological factors. Accordingly, younger males with a weak attachment to meat, who are more open to trying novel foods, and who are interested in the environmental impact of their food choices are more likely to become early adopters of edible insects. Other results addressing situational factors of insect-consumption point to effects of insect processing (i.e., insect visibility, Hartmann et al., 2015; Jensen and Lieberoth, 2019), advertising content (e.g., hedonic framing, Berger et al., 2018) and cultural variation (Tan et al., 2015). Finally, previous research suggests that one main reason for individuals' aversion toward insect-based foods lies in the disgust they evoke (Hartmann and Siegrist, 2016; La Barbera et al., 2018; Berger et al., 2019), which allegedly results from Westerners' association of insects with decaying matter and feces (Looy et al., 2014).

However, the fact that Westerners have always eaten other types of food strongly associated with decay (e.g., mold cheese or fungi) and that entomophagy is widespread across several Asian and African countries (van Huis et al., 2013), indicates that food-evoked disgust is primarily culturally learned (Rozin and Haidt, 2013; Looy et al., 2014). Thus, Westerners' reluctance to consume insect-containing products may stem from perceived cultural or social norms rather than a genuine fear of eating contaminated food. And, indeed, there is ample evidence showing that food choice and food intake are strongly influenced by the social environment (e.g., Robinson et al., 2013b; Cruwys et al., 2015; Higgs, 2015). In fact, such norms can be transmitted directly via cultural practices and rules (e.g., by the use of certain foods—or the lack thereof—in Western cuisine), observed reactions in a given situation (e.g., disgust responses to eating insects), or more subtly via environmental cues (e.g., portion sizes) (Higgs, 2015). For example, studies have documented the strong influence of portion size norms on how much people eat (Herman et al., 2003), which is so strong, that even food-deprived individuals adjust their amount of food intake to others who eat very little (Goldman et al., 1991). Research further highlights the role of social influence on *what* we eat (see Robinson et al., 2013a, 2014 for reviews). More specifically, studies found that perceived perception of peers' attitudes to and intake of certain foods predict healthy (e.g., fruits, vegetables) as well as unhealthy (e.g., fast food, soft drinks) food choices (Ball et al., 2010; Lally et al., 2011).

Notably, recent studies have also shown that the influence of social norms on food-related behaviors also translates into the context of entomophagy. Jensen and Lieberoth (2019), for example, found that subjective insect eating norms significantly predicted individuals' tasting behavior of food products containing visible as well as invisible mealworms. In the study of Berger et al. (2019), individuals were exposed to different peer and expert ratings about mealworm-based food products. The authors found that these social norm manipulations affected participants' acceptance of mealworm-based burgers and nutrition bars. However, it is unclear whether these effects also apply to unprocessed insects and, more generally, causal evidence for the role of social norms in acceptance of insects as foods remains scarce. The goal of the present research is thus to contribute to this open spot in research landscape by addressing the question of whether and how normative influence causally affects people's willingness to consume unprocessed insects. To do so, four studies were designed to show an association of beliefs about social norms and subsequent eating behavior that takes the form of tasting an entirely unprocessed, visible insect in all studies. Throughout the manuscript, we conceptualize norms as descriptive, which is the belief about the share in the population engaging in a certain behavior.

OVERVIEW OF STUDIES

In total, four psychological studies show the impact of descriptive norms on participants' willingness to consume unprocessed insects. Study 1 was designed as an initial test demonstrating a correlation between a participant's belief about the share of other people consuming an unprocessed insect in the course of the study and his or her own willingness to consume. Study 2 re-examines this effect and, additionally, demonstrates a causal relationship by manipulating social beliefs experimentally while observing subsequent consumption behavior. Study 3 shows that in principle non-normative information may be used to infer descriptive norms. More specifically, it manipulates participants' beliefs about a consumption-contingent payment for other participants, but not the target participant, and shows that this information not only affects their beliefs about the share of other participants consuming the insect, but that this belief translates into a higher probability to consume themselves. Finally, Study 4 experimentally manipulates the mediator found in Study 3 and shows that the effect of consumption-contingent payments only transfer into eating intentions in the absence of more concrete norm information. As consumption of insect-based products is legally possible and insects are readily available in supermarkets, the studies did not require additional ethical approval at the university where the studies were conducted. All studies strictly

followed the Declaration of Helsinki and all participants gave informed consent and were debriefed at the end of the respective study. In order to avoid selection effects, in none of the studies were participants recruited for an "insect-eating" study. Rather they were always approached to a consumer study involving the opportunity to sample or judge novel foods, but learned in the information that the study would involve the opportunity to eat insects.

Study 1

Participants and Procedure

Thirty-five participants (16 males, 19 females; $M_{age} = 22.34$ $SD_{age} = 2.50$) were recruited on the campus of a Swiss university. Participants were directly approached within a central building and asked to participate in a consumer study in exchange for a chocolate or candy bar. After agreeing to participate, the researchers guided the participants to the laboratory room. First, they disbursed theinformed consent sheet, after which the study began. In the information sheet, participants learnt that they could not take part in the study if they reported at least one allergic reaction to food (e.g., seafood, gluten, nuts, etc.) or were presently pregnant and that opting-out at this stage would equally lead to their compensation. None of the participants reported a food allergy or pregnancy. The laboratory seated up to eight participants in a single session, but the number of participants varied in each session.

In the study documents, participants learnt that the study involved the opportunity to eat a freeze-dried locust (*locusta migratoria,* see **Figure 1** for an image of the insect as well as the original packaging). First, they were asked to work through the questionnaire. The initial question assessed the subjective belief about how many (out of 100) participants would be willing to consume the insect. Subsequently, we assessed their taste expectation ("What do you expect regarding the taste?") using a 7-point scale ranging from "1" (unpalatable) to "7" (delicious). Finally, participants had the opportunity to consume a whole insect. Our central dependent variable is dichotomous taking on the value "1" if the insect was eaten or "0" otherwise. After the consumption decision, participants completed a brief questionnaire including an assessment of their demographics (age, gender) as well as some additional information such as being vegan/vegetarian. Finally, participants were thanked, debriefed and dismissed from the study.

FIGURE 1 | Images of insect and products. It displays the image of the insect as displayed to participants (top panel) as well as the original packages (left side) sourced from the Swiss firm Essento Food AG (bottom panel).

Results

Out of 35 participants, 23 decided to eat the insect, while 12 decided not to eat. As our central result, there was a highly significant point-biserial correlation between a participant's social belief about how many other people would eat the insect and his or her own willingness-to-eat, $r = 0.45$, $p = 0.006$. In order to test the robustness of this correlation, we used a probit-regression accounting for the dichotomous nature of the dependent variable and controlled for several variables typically associated with the choice of eating insects, such as gender and vegetarianism. **Table 1** displays the regression results. Importantly, the main correlational pattern associating social beliefs and eating behavior remains significant even after controlling for these variables. In addition, as the laboratory included the opportunity that more than one participant was present at each individual session, we report the regressions with clustered standard errors at the session level.

To sum up, Study 1 provided initial evidence that social beliefs about the descriptive norm in place associates to the willingness-to-eat an unprocessed insect.

Study 2

Showing a simple correlational pattern does not imply any causal direction suggesting that social beliefs about descriptive norms in fact *cause* participants to eat insects. Thus, it is necessary to experimentally induce descriptive norms in order to demonstrate their causal role on insect-eating. Study 2 attempts to do that by using a highly established tool to manipulate the perception of descriptive norms, scale-manipulations. Especially in behavioral economics, such manipulations are frequently used to experimentally vary the subjective experience about locally

TABLE 1 | Probit regression analysis including control variables (Study 1).

Dependent variable: eating behavior (1 if yes)	Model 1	Model 2
Norm beliefs	0.03689**	0.0322***
	(0.0155)	(0.0111)
Gender (0 = male respondent, 1 female respondent)		−1.570**
		(0.6475)
Age		0.0579
		(0.0967)
Vegetarian (0 = no, 1 = yes)		0.6645
		(8.326)
Constant	−0.4481	−0.7556
	(0.3598)	(2.1532)
Observations	35	35
(Pseudo) R-squared	0.19	0.007

*Robust standard errors in parentheses, clustered at session level accounting for the fact that in some sessions more than one individual was present in the lab (28 clusters), Dependent variable: eating behavior (0 = no, 1 = yes). ***p < 0.01, **p < 0.05, *p < 0.1.*

present social norms (e.g., Ockenfels and Werner, 2014; Feldhaus et al., 2018).

Participants and Procedure

Study 2 followed the identical protocol as Study 1, with the exception that it did not openly ask about the share of other people eating an insect, but restricted answers to this question to a scale that was manipulated to either induce high or low social beliefs. One hundred and fifty nine participants (67 males, 92 females; $M_{age} = 22.60$ $SD_{age} = 4.14$) were recruited on the campus from the same Swiss university, but we did not allow participants to enter who had already participated in Study 1. As in Study 1, participants were directly approached within a central building and asked to participate in a consumer study in exchange for a chocolate or candy bar. After agreeing to participate, the researchers guided the participants to the laboratory room. Participants were randomly assigned to one of only two experimental condition, the "low beliefs" condition or the "high beliefs" condition. The progression through the study was the following. First, the researchers disbursed an informed consent sheet. After giving informed consent, the study began. In an information sheet, participants learnt that they could not take part in the study if they reported at least one allergic reaction to food (e.g., seafood, gluten, nuts, etc.) or were presently pregnant and that opting-out at this stage would equally lead to their compensation. None of the participants reported a food allergy or pregnancy. The laboratory seated up to eight participants in a single session.

In the study documents, participants learnt that the study involved the opportunity to eat a freeze-dried locust. First, they were asked to work through the questionnaire. The initial question assessed the subjective belief about how many (out of 100) participants would be willing to consume the insect. Participants in the "low beliefs" condition were asked to indicate their belief using a five-point scale anchored at the points <10, 15, 20, 25, or >30%. Participants in the "high beliefs" condition were asked to indicate their belief the same five-point scale, but anchored at substantially higher values, which were <60, 65, 70, 75, or >80%. Subsequently, we assessed their taste expectation ("What do you expect regarding the taste?") using the same 7-point scale ranging from "1" (unpalatable) to "7" (delicious) as in Study 1. Finally, participants had again the opportunity to consume a whole insect, leading to the same dichotomous dependent variable as in Study 1. After the consumption decision, participants again completed a brief questionnaire assessing their demographics (age, gender) as well as some additional information such as being a vegan or vegetarian. Finally, participants were thanked, debriefed, and dismissed from the study.

TABLE 2 | Probit regression on non-vegetarians ($n = 133$) including control variables (Study 2).

Dependent variable: eating behavior (1 if yes)	
High norm induction (1 = high scale, 0 = low scale)	0.3899**
	(0.1870)
Gender (0 = male respondent, 1 female respondent)	−0.5237*
	(0.6475)
Observations	133
(Pseudo) R-squared	0.044

*Robust standard errors in parentheses, clustered at session level accounting for the fact that in some sessions more than one individual was present in the lab (51 clusters), Dependent variable: Eating behavior (0 = no, 1 = yes). ***p < 0.01, **p < 0.05, *p < 0.1.*

Results

Out of 159 participants, 84 (52.83%) decided to eat the insect, while 75 (47.17%) did not decide to eat. As a central analysis, we compare the eating rate across conditions. In the high condition, the eating rate was 55.56%, whereas the eating rate in the low condition was 50.00%. This difference failed to reach statistical significance in a χ^2-test ($p = 0.483$). This apparent non-result, however, seems entirely explained by an oversampling of females and vegetarians into the high beliefs treatment. Unfortunately, there was a stark over-representation of females in one experimental condition that makes it necessary to control for gender effects. Therefore, a probit-regression using session-clustered standard errors was used to estimate the causal effect of social norms, controlling for the strong gender difference in insect-eating. **Table 2** displays the regression results on non-vegetarian respondents ($n = 133$). Importantly, the main correlational pattern associating social beliefs and eating behavior established in Study 1 emerges when social beliefs are causally manipulated in Study 2. Controlling for gender, the causal effect of social norms on eating emerges as significant ($p = 0.0037$). To sum up, Study 2 provided additional indication that social norms may partially underlie the uptake of entomophagy in Western cultures. Although the scale manipulation did not provide a large and robust effect on participants' willingness to eat unprocessed insects, regression analysis controlling for potential confounders such as gender provided evidence that norms underlie eating behavior.

Study 3

Study 3 was designed to replicate the effect and to show that information that does not necessarily in itself carry norm-information may be used to construct social beliefs. In particular, the experimental manipulation was adopted from behavioral economic work on the normative role of incentives (Ambühl, 2018). In this line of reasoning, we use a study design that manipulates seemingly non-normative information in an effort to nevertheless affect people's belief about what others will do. We hypothesize that high incentives create a belief that more people will eat an insect compared to a situation where they are offered a smaller incentive. Thus, we are able to show that non-normative information (monetary compensation) is used to infer social norms, which is strongly indicative that people's eating preferences in fact depend on the inferred normative environment.

Participants and Procedure

In Study 3, 120 participants (60.8% females, $M_{age} = 22.9$, $SD_{age} = 4.93$) were recruited at a Swiss university. After agreeing to take part, participants were guided to the laboratory and given an information pamphlet starting with an informed-consent form. In the information sheet, participants learnt that they could not take part in the study if they reported at least one allergic reaction to food (e.g., seafood, gluten, nuts, etc.) or were presently pregnant and that opting-out at this stage would equally lead to their compensation. None of the participants reported a food allergy or pregnancy. Next, they learnt that in Switzerland it is possible to consume edible insects as at the time of the study, the corresponding legislation was just passed. We informed them that we are planning a set of studies in which we are paying other participants a financial compensation in order to take part in a study, which is contingent on actually sampling an insect (*locusta migratoria*, as in Studies 1 and 2). We randomly enrolled participants in one of only two conditions. They were made to believe that in our planned studies, participants would receive either CHF3 or CHF30 as a financial compensation for eating an insect. Next, participants were asked to report their belief about the share of people actually tasting the insect. After they have made their judgment, they were invited to taste a locust without any financial compensation being offered. Next, they completed a brief questionnaire involving demographic information such as age and gender. Finally, participants were thanked, debriefed and dismissed from the study.

Results and Discussion

Participants in the 30 CHF condition estimated the consumption rate to be significantly higher than in the 3 CHF condition (44.1 vs. 26.4%, $t = 3.9512$ $p < 0.001$). In a regression model using clustered standard errors at the session level, the same effect emerged ($p < 0.001$, see **Table 3**, Model 1). Next, we analyzed the effect of the experimental manipulation on the actual eating behavior. Whereas, in the 3 CHF condition, 28.81% eat the insect, this value rises to 44.26% in the 30 CHF condition, an effect that is marginally significant ($p = 0.079$, based on a χ^2-test). In a regression using clustered standard errors at the session level, this result emerges as well when not controlling for gender ($p = 0.064$) as well as when controlling for gender ($p = 0.063$, see **Table 3**, Models 2 and 3).

However, the key interest is in whether the effect of the (arbitrary) experimental manipulation is mediated by the perception of the social norms and, therefore, we tested the mediation hypothesis, according to which the financial compensation affects beliefs, which in turn should translate into eating behavior. Importantly, methodological research highlights that a significant total effect is not a necessary pre-condition (i.e., "a gate-keeper") to test a mediation hypothesis and make a statistical conclusion about an indirect effect (Shrout and Bolger, 2002; Hayes, 2009). Without a statistical significant effect or with just a marginally significant effect (as is the case here) of the independent variable (X) on the dependent variable (Y), one can still observe a mediation effect of the mediator (M) on the XY relationship. We therefore used a bootstrapping method with 5,000 resamples to estimate the indirect effect using the SGMEDIATE command in Stata. For our analysis, the low incentive condition served as reference category. The 95% bootstrap confidence interval of the indirect effect excluded 0 (0.16, $SE = 0.26$ [0.07, 0.30]) showing a significant mediation of the effect of the incentive on eating behavior via norm beliefs. In sum, Study 3 showed that even in in-principle non-normative cues are used to derive norm information, which in turn affects the willingness to eat unprocessed insects.

Study 4

Study 4 re-examines the effect of Study 3 and shows that norm-beliefs are in fact causal in this relationship. To do so, we replicate the effect while addressing various supplementary aspects. Most importantly, we experimentally manipulate the mediator by using the scale manipulation established in Study 2 to assess the norm-belief after confronting the participants with either the low or high incentive condition. Thus, we fully cross the design in an effort to show that manipulating norms in fact affects eating preferences and in-principle non-normative information (i.e., information about compensation) is just used in the absence of such normative information. Furthermore, as Studies 1–3 have all been conducted in a university context in Switzerland, we extend the external validity by using a broader sample from the United States. As Studies 1–3 established that actual eating behavior is affected by norm-manipulations, we do not replicate this effect once again, but rather rely on Amazon Mechanical Turk to assess norm beliefs at the benefit of getting access to a broader participant population. Amazon Mechanical Turk is an online labor market that is frequently used by behavioral scientists to run online-studies. A major advantage for using Amazon Mechanical Turk is that the sample of recruited subjects has been shown to be more diverse and more nationally representative than the typical college student sample at major research universities (e.g., Buhrmester et al., 2011).

Participants and Procedure

A total of 213 participants (46% females, $M_{age} = 38.05$, $SD_{age} = 11.68$) were recruited using Amazon Mechanical Turk in exchange for a small monetary compensation that was paid upon successful completion of a brief survey. After giving informed

TABLE 3 | Regression models (Study 3, Model 1: OLS; Models 2/3: Probit).

Dependent variable: norm beliefs	Model 1 (beliefs)	Model 2 (eating behavior)	Model 3 (eating behavior)
Experimental condition (1 = high, 0 = low)	17.675***	0.415*	0.409*
	(4.160)	(0.224)	(0.220)
Gender (0 = male respondent, 1 female respondent)			−0.817*** (0.269)
Constant	26.407	−0.559***	−0.080
	(0.360)	(0.173)	(0.214)
Observations	120	120	120
(Pseudo) R-squared	0.12	0.064	0.09

*Robust standard errors in parentheses, clustered at session level accounting for the fact that in some sessions more than one individual was present in the lab (28 clusters), Dependent variable: Norm beliefs (0–100; Model 1), Eating behavior (0 = no, 1 = yes, Model 2). ***p < 0.01, **p < 0.05, *p < 0.1.*

consent, participants learnt that as part of a consumer study in Switzerland, the researchers are trying to gain insights into estimates about how many participants (out of 100) are willing to eat a freeze-dried insect as displayed in **Figure 1**. Participants were randomly enrolled into an experimental condition of a 2 × 2 design. The key experimental manipulations were thus 2-fold: first, the experimental remuneration that participants of the would-be study in Switzerland receive in exchange for eating the insect (3 vs. 30 CHF [1 CHF ∼ 1 USD at the time of the study]); second, whether or not the answer included a first guess using a scale (yes vs. no). The scale anchored the beliefs around the midpoint 31–35% (i.e., low beliefs). If the experimental manipulation of the announced remuneration in fact transports norm information, the effect should uniquely emerge when the scale is not present. The key dependent variables was the belief about the percentage of prospective participants eating the insect. Participants could therefore enter any number between 0 and 100. Afterwards, we gathered participants' age and gender and they were dismissed from the study by entering their completion word that triggered their payment.

Results and Discussion

The general pattern that emerged in Study 3 also replicated using a sample recruited online, importantly only when the answering option did not include a scale that more explicitly transports norm information. ANOVA shows a marginally significant interaction effect ($p = 0.081$), in addition to a significant effect of the CHF condition ($p < 0.01$) and a marginally significant effect of the scale presence ($p = 0.106$). As predicted, planned contrasts using a Tuckey-HSD correction show that the difference in the 3 vs. 30 CHF condition uniquely emerges when no scale is used between displaying the information and the assessment of the norm belief ($p < 0.001$; Tuckey 95%-CI ranging from 4.20 to 27.97, excluding zero). In a supplementary regression using gender and age as control variables, the interaction effect of the two experimental conditions remains at the identical significance level ($p = 0.064$). Neither age nor gender significantly predicted the belief assessment. The regression results are displayed in **Table 4**.

Thus, Study 4 showed causally that providing normative information renders a manipulation that previously transmitted norm information (3 vs. 30 CHF as offered compensation) insignificant, suggesting that participants actively use minimal cues to infer normative information about eating insects, which have in the previous studies been consistently linked to participants own eating behavior.

TABLE 4 | Regression model (Study 4).

Dependent variable: norm beliefs	Beliefs
CHF condition (1 = high, 0 = low)	16.193***
	(4.584)
Scale condition (0 = no, 1 = yes)	0.520
	(4.430)
Interaction effect	−11.922*
	(6.39)
Constant	26.407
	(0.360)
Observations	120
Gender & Age controls	YES
(Pseudo) R-squared	0.12

*Robust standard errors in parentheses, Dependent variable: Norm beliefs (0–100). ***p < 0.01, **p < 0.05, *p < 0.1.*

GENERAL DISCUSSION

The present research demonstrated a link between perceptions of descriptive social norms and the willingness to consume unprocessed insects. Based on four studies utilizing freeze-dried locusts, we find that norms affect eating intentions and behavior. Up to date, only little research investigates Westerners' willingness to eat such unprocessed insects following norm-manipulations. The present research therefore contributes to an emerging field investigating the consumer psychology of entomophagy. **Table 5** displays the design and the results of each study in plain language as a summary.

Future research can take several directions. First, it can augment the ecological validity by running experiments in more naturalistic decision environments (e.g., with the use of

TABLE 5 | Summary of results.

Study	Type of study	Independent variable	Dependent variable	Main result
Study 1	Correlational	Belief about eating behavior	Eating of an insect (yes vs. no)	Beliefs about eating and eating behavior correlate with each other
Study 2	Experimental	Exposure to high vs. low belief	Eating of an insect (yes vs. no)	Exposure to high beliefs may increase probability to eat
Study 3	Experimental	Exposure to high vs. low incentives that other people face	Eating of an insect (yes vs. no)	Exposure to information that other people receive high may affect probability to eat
Study 4	Experimental	Exposure to high vs. low incentives that other people face Exposure to low belief vs. no exposure	Belief about eating rate	Effect of Study 3 uniquely emerges when participants do not receive explicit norm-information (i.e., the low beliefs scale)

field experiments) or in-store samplings. Especially research in behavioral economics has established elegant tools and strategies to test effects of social norms in the field where real people make real decisions without being aware of being monitored (e.g., Alcott and Mullainathan, 2010 in the domain of energy behavior). Whereas, our research has uniquely relied on laboratory work as well as Amazon Mechanical Turk using one kind of insect, future research could transfer these findings also to other insect species that are suitable for human consumption and to other (Western) markets. Second, laboratory work always comes at a certain degree of artificiality. It is unclear whether the results and effects would emerge equally (e.g., in terms of effect size) in real-world contexts, although it is also imaginable that true social norms (e.g., observations of peers and opinion leaders) actually amplify the established effects.

Third, our research is essentially mute on potential moderators such as individual differences. For instance, people with a strong inclination to follow norms could be particular prone to the established effects, whereas consumers with a more individualist approach to life may actually prefer insects so long others do not share that preference. An alternative individual difference measure could be novelty seeking. Corresponding research showed that novelty seeking, i.e., "the sheer 'strangeness' and 'novelty' of other landscapes, lifeways and cultures that satisfy tourists' desires, and which cannot be satisfied at home" (Ji et al., 2016, p. 389) is positively related to novel food consumption (in foreign countries). In fact, strong normative information could actually decrease the impact of novelty seeking on insect-eating behavior as strong norm information may suggest that eating insects is not very special. However, one can expect that personality differences or other individual differences are important variables affecting the results and consumer research should continue to address the presented effects on various customer segments to gain a better understanding.

To summarize, our research provided more evidence that insect products may be promoted using social norms, which complements recent research showing that social influence factors are associated with insect eating (Berger et al., 2019; Jensen and Lieberoth, 2019). As humans are a particular social species, leveraging the social nature may prove particularly useful. Other domains of combating climate change show similar results that coordinated action is strongly influenced by social motives. For example, in cooperation games where foregoing individual benefits in order to secure social gains is necessary, coordinated reciprocal actions have been shown to strongly influence sustainable behavior (e.g., MacKay et al., 2015). Thus, making norms transparent (e.g., giving people feedback about how frequent consumption is) can actually not only take the fear of novel foods away, but lead to collective action in favor for a more sustainable way of consuming animal proteins (Oonincx and de Boer, 2012; van Huis, 2013; van Huis and Oonincx, 2017) from traditionally uncommon sources such as insects in the Western world. Quite clearly, social scientific research offers many routes to influence the uptake of more sustainable diets and social norms are one of many variables affecting humans' decision about what to eat.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SB designed and performed the experiments, analyzed the data, and drafted the manuscript. AW provided critical feedback on the analysis and contributed to the final manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Phytochemical and Nutritional Quality Changes During Irrigation and Postharvest Processing of the Underutilized Vegetable African Nightshade

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Underutilized or traditional leafy vegetables are grown in the wild and cultivated. They are consumed as nutritional accompaniments to staples, either raw (fresh), cooked, or in a dried form, through custom, habit, and tradition. These traditional leafy vegetables are natural rich sources of phytochemicals and nutritional compounds. Over time, the keenness for consumption of traditional vegetables has become less popular. Poor nutrient diets are the main cause of mortality and morbidity, especially in developing countries, where the problem is predominant due to poverty. Consumption of traditional vegetables can assist in the prevention of chronic disease development, as they contain various bioactive compounds that exhibit multiple health benefits. Traditional leafy vegetables play a vital role in combatting hunger, food insecurity, and malnutrition, and most are suitable for food intervention programs. African nightshade (*Solanum* family) is one such commonly consumed traditional leafy vegetable. During dry seasons, communities often face shortages of vegetables; thus, the preservation of edible leaves is one strategy to help overcome this problem. The adoption of solar drying and fermentation are traditional methods to extend the availability of African nightshade vegetables. Additionally, the agronomy practices and postharvest processing methods affect the phytochemicals and nutritional compounds of African nightshade accessions. This mini-review provides information on changes in phytochemicals, nutrition, and antinutritive compounds with different postharvest processing methods and irrigation. The review provides the justification to promote the cultivation for consumption, by identifying the potential African nightshade accessions that are rich in phytonutritional compounds. This mini-review summarizes and discusses the major information on (i) the micro- and macronutrients present in *Solanum retroflexum*, the most commonly consumed nightshade species compared with other traditional vegetables in Southern Africa, (ii) the composition of phytochemical compounds present in different nightshade accessions, (iii) the impact of irrigation on phytochemical composition

in different nightshade species, and (iv) the impact of postharvest processing on phytochemicals and antinutritive compounds in *S. retroflexum*. Inclusion of African nightshade, especially *S. retroflexum*, with the main staple foods can improve protein, iron, and calcium levels in daily diets, which will help to improve people's health and well-being.

Keywords: traditional leafy vegetables, polyphenols, antioxidants, minerals, postharvest processing

INTRODUCTION

Consumer preference for the intake of fruit and vegetables in the daily diet is increasing, and the World Health Organization (1, 2) recommends a minimum of 400 g of fruit and vegetables, or five portions, per day, excluding starchy tubers. The United States Department of Agriculture (USDA) guidelines (2011) (3) state that an individual must consume at least one cup (∼237 g) of raw or cooked vegetables or two cups of raw leafy greens daily. In developing countries, particularly Africa and Asia, consumers need to meet the minimal requirement of caloric values, because micronutrient deficiency, referred to as hidden hunger, is prevalent (4). Micronutrient deficiency is due to a lack of dietary intake of calcium (Ca), iron (Fe), zinc (Zn), potassium (K), magnesium (Mg), iodine (I), copper (Cu), and selenium (Se). Additionally, vitamin A deficiency remains a common health-associated problem in South Asia and sub-Saharan Africa (5). Women of reproductive age (≥15–49 years) in Ethiopia, Kenya, Nigeria, and South Africa suffer from anemia (18–51%), iron deficiency (9–18%), and iron deficiency anemia (10%), as well as vitamin A (4–22%), iodine (22–55%), zinc (34%), and folate (46%) deficiency (6). Consequently, it is worth including underutilized African leafy vegetables in a diet diversification strategy for the sub-Saharan African population to fight against hidden hunger (7). The most commonly grown traditional African leafy vegetables in the sub-Saharan African region are of the *Amaranthus* species: wild mustard (*Brassica* spp.), African nightshade (*Solanum* spp.), sweet potatoes (*Ipomoea batatas*), spider flower (*Cleome gynandra*), Jew's mallow (*Corchorus olitorius* and *Corchorus tridens*), cowpeas (*Vigna unguiculata*), pumpkins (*Cucurbita pepo*, *Cucurbita maxima*, and *Cucurbita moschata*), melons (*Citrullus lanatus* and *Cucumis melo*), and balsam pear (*Momordica balsamina*). Of these, wild mustard and African nightshade are widely consumed (7). Once cooked, the leaves are an accompaniment to the staple starch-based maize meal and tomato relish; sometimes the leaves are fermented with milk. These food preparation methods help reduce the bitter-tasting compounds in the leaves derived from anti-nutrients, such as solanaceous glycoalkaloids (8).

Nightshade plants, propagated *via* the seeds (9), are an annual plant growing to almost 75 cm in height (9), with a simple leaf morphology—alternate margins with blunt teeth and slightly hairy. *Solanum retroflexum* (**Figure 1**), which is endemic to South Africa, belongs to the Solanaceae family and known as Black nightshade, or nastergal, umsobo, and muxe in the region; it is a popular leafy vegetable consumed in the Southern and Eastern parts of Africa. Other nightshade vegetables consumed in the sub-Saharan region are *S. retroflexum*, *Solanum americanum*, *Solanum nigrum*, *Solanum scabrum*, and *Solanum villosum* (8, 10); *S. scabrum* Mill (mnavu), a broad-leafed type of nightshade, is popular in West, Central, and East Africa (11).

In Southern Africa, subsistence farmers cultivate nightshade vegetables on a small scale and market them to generate income and improve their livelihoods. African nightshade does not need extensive fertilizer application, thrives in drought, and is less prone to pest attack; therefore, it is cost effective to produce and environmental-friendly when compared with commercial leafy vegetables (12). Currently, there are efforts to increase production, and linking these vegetables to agro-processing and the supply chain will alleviate hunger and improve nutrition and the rural economy. Unfortunately, postharvest losses of vegetables are high during marketing, primarily due to lack of cost-effective cold chain infrastructure; therefore, the traditional ways to preserve and reduce food loss are by adopting drying or fermentation technologies. Traditionally, drying was by the sun or shade drying; however, in Asian and African countries, community cooperatives have recently had cost-effective solar dryers erected. The two agro-processing technologies, solar drying, and fermentation, play a major role in facilitating the food available and sustaining food security in rural regions. These technologies are considered as recommended strategies to improve the bioavailability of micronutrients, and sometimes they can reduce the antinutritive compounds (13, 14). Considering the aforementioned, this review summarizes the research-based information on phytochemical nutritional properties of African nightshade species and the changes in phytonutritional components during irrigation or postharvest processing. In addition, this review discusses the antinutritive components in *S. retroflexum* species and changes of these compounds during postharvest processing and safety for consumption.

MICRO- AND MACRONUTRIENTS IN SELECTED NIGHTSHADE SPECIES COMPARED WITH OTHER COMMONLY CONSUMED TRADITIONAL VEGETABLES IN SOUTHERN AFRICA AND COMMERCIAL VEGETABLES

Micronutrients

Figure 2 shows the iron (Fe) content in African nightshade species *S. retroflexum* (per 100 g fresh weight, FW) compared with other traditional vegetables in the Southern African region. The iron content in African nightshade was higher than that

FIGURE 1 | *Solanum retroflexum* (African nightshade) leafy vegetable.

in other traditional vegetables in the Southern African region. A 100 g (FW) portion of African nightshade *S. retroflexum* contained 7.2 mg of iron, whereas other traditionally southern African vegetables, such as *C. tridens* (wild jute), *Amaranthus cruentus* (pigweed), *C. olitorius* (Jew's mallow), *V. unguiculata* (cowpea), *C. lanatus* (tsamma melon leaves), *C. gynandra* (spider flower), *Amaranthus hybridus* (cockscomb), and *Bidens pilosa* (black jack), contained 6.3, 5.1, 3.6, 4.7, 6.4, 2.1, 4.1, and 2.0 mg, respectively, in 100 g (FW) portion (7, 15).

Iron is regarded as an essential trace element for many bodily functions, such as biosynthesis of hemoglobin and activity of the central nervous system, and it also participates in the oxidation of macronutrients (e.g., carbohydrates, proteins, and fats) (16). The recommended daily intake (RDI) of iron is 8 mg day^{-1} for adults (17). Leaves of *S. villosum* were reported to contain 12 mg 100 g^{-1} iron on dry weight (DW) basis (15, 18). *S. villosum* contained higher iron levels than *C. gynandra* (spider plant) (48.6 mg 100 g^{-1}) and *A. cruentus* (Madiira AM 38) (52.66 mg 100 g^{-1}) at level-three maturity stages (7, 15, 18). This information suggests the best harvesting time for optimal Fe levels to the consumer.

The calcium content in a 100 DW portion of *S. villosum* is 442 mg, which was higher than the amount reported in *S. retroflexum* (199 mg), *C. maxima* (pumpkin leaves) (177 mg), and *B. pilosa* (black jack) (162 mg). Calcium is necessary for the maintenance of strong bones and teeth; 99% of calcium in the human body is used for this function (19). The RDI of calcium is 1,200 mg for adults (20).

The magnesium content in a 100 DW portion of *S. retroflexum* (92 mg) was reportedly higher than that of wild jute (80.9 mg), Jew's mallow (87 mg), cowpea (62 mg), pumpkin leaves (67 mg), tsamma melon leaves (59 mg), spider flower (76 mg), and black jack (79 mg) (7, 15, 21). Manganese content in *S. retroflexum* (2,080 μg 100 g^{-1}) was higher than that found in spider flower (580 μg 100 g^{-1}), tsamma melon leaves (760 μg 100 g^{-1}), pumpkin leaves (540 μg 100 g^{-1}), Jew's mallow (790 μg 100 g^{-1}), cockscomb (4.1 μg 100 g^{-1}), and black jack (2.5 μg 100 g^{-1}) (21). Magnesium functions as a cofactor for more than 300 enzymatic reactions, and the RDI of magnesium is 420 mg for adult males and 320 mg for adult females (22).

The copper content in *S. retroflexum* (0.16 mg 100 g^{-1}) was higher than that found in cowpea (0.14 mg 100 g^{-1}). Copper is an essential mineral needed for growth and multiple functions, such as cardiovascular integrity, lung elasticity, neovascularization, neuroendocrine activity, and iron metabolism (23). The WHO guidelines advise 30 μg kg^{-1} of body weight per day^{-1}, which is about 2 mg per day^{-1} for the average adult (23).

When compared with the commonly consumed exotic leafy vegetables, spinach, lettuce, kale, mustard green, rapini (broccoli

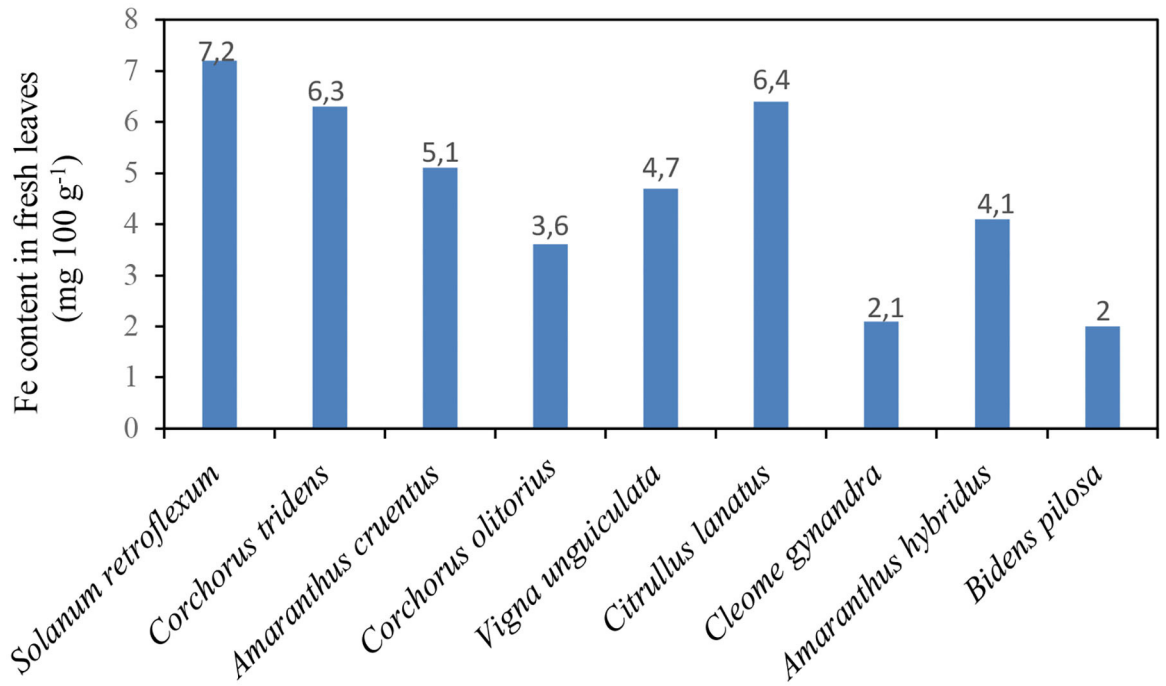

FIGURE 2 | Iron content in *Solanum retroflexum* leaves compared with the other traditional leafy vegetables Source (15).

TABLE 1 | Minerals and protein in African nightshade (per 100 g fresh weight) compared with other commercial vegetables.

	Protein (g)	K (mg)	P (mg)	Ca (mg)	Mg (mg)	Fe (mg)	Cu (mg)	Zn (mg)
S. retroflexum	6	257	36	199	92	7.2	0.16	0.56
S. villosum	4.6			442		12		
Spinach	2.86	558	49	99	79	2.71	0.13	0.53
Lettuce	0.9	141	20	18	7	0.41	0.025	0.15
Kale	2.92	348	55	254	33	1.6	0.053	0.39
Mustard green	2.35			118		1.69		
Rapini (broccoli raab)	3.17	196	73	108	22	2.14	0.042	0.77
Swiss chard greens	1.8	379	46	51	81	1.8	0.366	0.36

Source: (8).

raab), and Swiss chard greens (3), *S. retroflexum* (15) revealed higher levels of calcium, magnesium, iron, and copper (**Table 1**).

Macronutrients

African nightshade *S. retroflexum* contained higher amounts of proteins (6%) than other African traditional vegetables (**Table 1**), such as *C. maxima* (pumpkin leaves) (4.24%), *A. cruentus* (pigweed) (3.49%), *C. olitorius* (Jew's mallow) (5.19%), *V. unguiculata* (cowpea) (4.7%), *C. maxima* (pumpkin leaves) (2.9%), *C. lanatus* (tsamma melon leaves) (3.5%), *C. gynandra* (spider flower) (5%), *B. pilosa* (black jack) (6%), and *A. hybridus* (cockscomb) (5%) (7, 15). Conversely, *S. villosum* showed lower levels of proteins (4.6%) than *S. retroflexum*, but similar levels to pumpkin and cowpea leaves (7, 15, 18).

In addition, a 100 g portion of *S. retroflexum* and *S. villosum* leaves had a sugar content of 1.02 and 1.15 g, respectively. For fat content, *S. retroflexum* contained monounsaturated fatty acids and omega 3 and 6 fatty acids at concentrations of 2.61, 0.33, and 0.63 g in a 100 g portion, respectively (24). Most importantly, due to its higher levels of proteins and iron, African nightshade is an important nutritional source for African people (11).

COMPOSITION OF PHYTOCHEMICAL COMPONENTS IN DIFFERENT AFRICAN NIGHTSHADE SPECIES

Phytochemicals, plant-based non-nutritive compounds that contribute toward biological activity, aid in protecting the body against non-communicable diseases (25). An ∼120 g portion of fruits or vegetables provides 100 different phytochemicals (26), among which phenolic compounds are the most

TABLE 2 | Concentration of different kaempferol derivatives present in *Solanum scabrum* and *Solanum retroflexum* species.

	Kaempferol-3-diglucoside	Kaempferol-3-glucosylrhamnogalactoside	Kaempferol-3-rhamnosyl-rhamnogalactoside (isomer 1)	Kaempferol-3-rhamnosyl-rhamnogalactoside (isomer 2)	Total amount of kaempferol derivatives
			mg g^{-1}		
Solanum scabrum	0.057	0.043	0.083	0.040	0.223
			mg g^{-1}		
	Kaempferol-sophoroside-hexoside	Kaempferol-3-*O*-hydroxyferuloyl-trihexoside	Kaempferol-3-*O*-hydroxyferuloyl diglucoside	Kaempferol-dihexoside	
Solanum retroflexum	0.051	0.0041	0.028	0.020	0.098

Source: (28, 29).

abundant functional compounds. Aerial parts, especially the leaves, of different nightshade plants, *S. nigrum*, *S. scabrum*, *S. americanum*, *S. villosum*, and *S. retroflexum*, predominantly contain chlorogenic acid (caffeoylquinic acid), which belongs to the group of hydroxycinnamic acids (8) and is an ester of caffeic and quinic acids. Chlorogenic acid is a pronounced phenolic compound in *Amaranthus* leaves, including the red (*Amaranthus tricolor*) and green (*Amaranthus lividus*) genotypes (27), and a major phenolic compound in lettuce. Caffeoylmalate was detected in *S. scabrum* and *S. retroflexum* (27, 28). Kaempferol glycoside derivatives in *S. scabrum* leaves were mainly kaempferol-3-diglucoside, kaempferol-3-glucosylrhamnogalactoside, and kaempferol-3-rhamnosyl-rhamnogalactoside (isomers) (28) (**Table 2**). It was found that *S. retroflexum* leaves contained the following kaempferol derivatives: kaempferol-3-*O*-sinapoyldihexoside-hexoside, kaempferol-3-*O*-rutinoside, and kaempferol-dihexoside (28) (**Table 2**). The concentration of kaempferol derivatives in fresh *S. retroflexum* was at lower concentrations than that in *S. scabrum* (**Table 2**). Isorhamnetin-*O*-hexoside and rutin were found in *S. retroflexum* leaves (28). *S. scabrum* leaves contained mainly non-acylated quercetin glycosides, such as quercetin-3-neohesperidoside-7-glucosylrhamnoside (isomers), quercetin-3-rutinoside-7-rhamnosylglucoside, quercetin-3-galactorhamnoside, quercetin-3-rhamnosylgalactoside, quercetin-3-pentosylglucoside, and quercetin-3-pentosylrutinoside (28); conversely, quercetin-3-*O*-xylosyl-rutinoside was only detected in *S. retroflexum* leaves (28). Among the 11 phenolic compounds found in *S. retroflexum* leaves, rutin was the predominant compound.

Vitamin E content in *S. nigrum* and *S. scabrum* varied from 92.0 to 229.7 μg g^{-1} and from 90.4 to 192.5 μg g^{-1} on DW basis, respectively (8). *S. nigrum* (PI 312110, USDA) contained the highest amount of vitamin E of all African nightshade species (8), and *S. scabrum* (SS 04.2, World Vegetable Center [WAC] East and Southern Africa, Arusha, Tanzania) contained the lowest (8). In *S. americanum* (PI 268152, USDA) and *S. villosum* (Grif 16939, USDA), the vitamin E content was 145.5 and 114.3 μg g^{-1}, respectively (8).

Total carotenoid content in *S. scabrum* leaves ranged from 586 to 691 μg g^{-1} on DW basis and 0.733 μg g^{-1} in *S. retroflexum* leaves on FW basis (8, 30, 31). The β-carotene content in the leaves of *S. nigrum* and *S. scabrum* species differed from 28.1 to 141.7 μg g^{-1} DW and from 55.1 to 96.0 μg g^{-1} DW, respectively (8). *S. villosum* (Grif 16939, USDA) reportedly contained the highest total carotenoids of 138.1 μg g^{-1} DW, whereas the lowest amount of 65.2 μg g^{-1} DW was found in *S. scabrum* (SS 04.2, WAC) (8).

African traditional leafy vegetables are rich in vitamin A and meet more than 75% of the recommended dietary allowance (RDA) (15). The vitamin A content in African nightshade (422 μg retinol activity equivalent, RAE) is greater than that in Jew's mallow (329 μg RAE), pumpkin leaves (325 μg RAE), and tsamma melon leaves (375 μg RAE) (15, 32).

Based on Yuan's et al. (8) findings, vitamin E, and total phenols contributed toward the antioxidant property [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS] of the accessions of African nightshade *S. nigrum* PI 312110 and PI 381290, obtained from the USDA collection. *S. scabrum* (BG 16, Nduruma, WAC) showed the highest ABTS activity of 25 Trolox equivalent antioxidant capacity (TEAC) mg g^{-1} DW (8). Different African nightshade species from the USDA collection showed ABTS activity, on DW basis, in the following order: *S. scabrum* (25.00 TEAC mg g^{-1}; BG 16, Nduruma), >*S. americanum* (24.81 TEAC mg g^{-1}; PI 268152, USDA), >*S. nigrum* (23.93 TEAC mg g^{-1}; PI 381290, USDA), ≥*S. nigrum* (23.45 TEAC mg g^{-1}; PI 312110, USDA), >*S. scabrum* (22.46 TEAC mg g^{-1}; SS 49 Olevolosi, WVC), *S. scabrum* (21.36 TEAC mg g^{-1}; SS 52, WVC), ≥*S. scabrum* (21.26 TEAC mg g^{-1}; Grif 14198, USDA), >*S. scabrum* (19.14 TEAC mg g^{-1}; BG-29, WVC), >*S. nigrum* (18.34 TEAC mg g^{-1}; PI 381289, USDA), >*S. scabrum* (17.92 TEAC mg g^{-1}; Ex Hai, WVC), >*S. scabrum* (16.22 TEAC mg g^{-1}; PI 643126, USDA), and >*S. nigrum* (15.49 TEAC mg g^{-1}; PI 306400, USDA) (8).

Phenolic compounds positively correlated with antioxidant activity (33). Phenolic compounds participate in the antioxidant activity due to their redox properties, predominantly adsorbing, and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (33). Current consumer trend is to replace synthetic antioxidants with natural dietary antioxidants for health benefits. Vitamin E (tocopherols) is important for disease prevention by preventing the breakdown of polyunsaturated fatty acids in membrane lipids and alleviating the oxidative stress (34). Lipophilic antioxidant ABTS activity

demonstrated a strong correlation with tocopherols (35). However, correlations need to be established between the antioxidant activity and total phenolic content in different accessions of African nightshade.

IMPACT OF IRRIGATION ON MINERALS AND PHYTOCHEMICALS IN AFRICAN NIGHTSHADE

Minerals, such as Ca, Mg, K, and Mn, increased during severe stress in irrigation treatment at 30% field capacity, whereas P, Fe, and Zn content was the highest at 90% field capacity (28). Many developing countries adopt intercropping systems for effective use of the land, improving the productivity. The intercropping approach is popular among smallholder farmers (36). It can modify the nutritional composition in the plant parts, including the leaves; however, it did not significantly favor the accumulation of minerals in leaves in *S. scabrum* (28). The hydroxycinnamic acid derivatives, 3-caffeoylquinic acid, 5-caffeoylquinic acid, 4-caffeoylquinic acid, and caffeoylmalate, were significantly affected by the irrigation levels but not by the intercropping with *Brassica carinata* leaves (28); likewise, sinapoylmalate and sinapic acid were affected by irrigation. The irrigation treatments significantly affected the concentration of kaempferol-3-diglucoside in leaves (28). Ngwene et al. (28) confirmed that agronomy practices, such as intercropping, late-season drought, or irrigation management, can be adopted as a strategy to boost the levels of some health-related phytochemicals to benefit rural people and food manufacturers. The concentration of chlorogenic acid in *S. retroflexum* leaves (1.04 $\mu g\ g^{-1}$) was less than that in pak choi (2.03 $\mu g/g$), salad spinach (1.59 $\mu g\ g^{-1}$), red amaranth (9.06 $\mu g\ g^{-1}$), and green amaranth (15.34 $\mu g\ g^{-1}$) (37). Chlorogenic acid was mostly detected in *S. nigrum*, *S. scabrum*, and *S. villosum*; however, in some cases, chlorogenic acid was either detected in trace amounts or not detected, depending on the location (8). Quercetin-glucosyl-rhamnosyl-galactoside was detected at higher intensities in *S. nigrum*, *S. scabrum*, and *S. americanum*, but again not in all locations (8).

The other important phytochemicals in leafy vegetables are carotenoids. Low irrigation favored an increase in carotenoid accumulation in intercropped *S. scabrum.* The β-carotene and lutein contents in *S. scabrum* leaves increased when the irrigation treatment simulated drought treatment (30% water holding capacity, WHC) (28). The temperature variation during cold winter months and hot summer months did not show significant variation in total carotenoid content in *S. retroflexum* leaves (27).

IMPACT OF POSTHARVEST PROCESSING AND CHANGES IN PHYTOCHEMICALS IN AFRICAN NIGHTSHADE

Postharvest processing (e.g., drying) and food preparation methods (e.g., cooking or fermentation) have a significant influence on the maintenance of phytochemical content of vegetables (38). Methods of postharvest processing can modify the composition of functional compounds in green leafy vegetables (29). Postharvest drying increased the income generation from traditional vegetable functional food. Global functional foods' market size is rising and expected to increase in 2025 to US$ 275.77 due to the increasing consumer appeal for nutritional and fortifying food additives (39). Traditionally adopted drying methods do not meet the requirement of homogenous quality standards; therefore, the installation of cost-effective solar dryers enables subsistence farmers to perform the postharvest drying at 50°C (30); this is a controlled drying method to prevent the depletion of vitamins and functional compounds.

Solar drying of *S. retroflexum* leaves at 50°C increased the total carotenoid content by 40%, compared with the raw leaves. Similarly, phenolic metabolites, caffeoylmalic acid, rutin, and kaempferol-3-*O*-rutinoside revealed a remarkable increase in solar dried leaves. Chlorogenic and neochlorogenic acids substantially increased in solar dried *S. retroflexum* leaves (**Figure 3**), and antioxidant activity (FRAP) was enhanced compared with raw leaves (30). However, the temperature during the drying process plays a vital role in the chemical transformation of biochemical metabolites. Higher temperatures accelerate oxidation reactions, which can negatively affect the concentration of phenolic compounds (40). Loss of total polyphenols due to hot air drying have been reported previously (20).

The solar dried functional powder of *S. retroflexum* leaves contains 17.50 g carbohydrate in a 100 g portion; a lower carbohydrate content correlated with a lower calorific content 1,118.67 kJ 100 g^{-1} (267.36 cal) (**Table 3**) (30), the protein content is 32.91 g 100 g^{-1}, slightly higher than the solar dried cowpea leafy vegetable (*V. unguiculata* L.) (29.40 g 100 g^{-1}) and moringa leaves (*Moringa oleifera*) (28.09–28.99 g 100 g^{-1}) (30, 41), and total dietary fiber is 28.81 g 100 g^{-1} (30). The solar dried *S. retroflexum* leaf powder is low in sodium, but the 100 g portion meets the daily requirement of potassium, calcium, and zinc intake; the iron and magnesium contents were ∼8- and 2-fold higher than the required amount per day, respectively (**Table 3**) (30).

In developing a low calorie, meal replacement product, solar dried functional powder of *S. retroflexum* leaves were utilized as the main ingredient. The final product contained 32.8 g of protein, 12.9 g of dietary fiber, 40 g of total sugar, 40.8 g of carbohydrate, 5.1 g of fat, and 1.4 g monounsaturated fatty acids (**Table 4**) with 369 cal in a 100 g portion (24). The protein content of African nightshade protein shake meets approximately half the daily requirement, but the available carbohydrate levels are much higher than those prescribed for daily intake (24). Low sodium content intake is preferred by those who suffer from high blood pressure and kidney problems; however, sodium is an important intracellular and extracellular cation that facilitates the regulation of plasma volume and acid–base balance during nerve and muscle contraction (42).

Phenylalanine is an essential amino acid, which acts as a precursor of the amino acid tyrosine. Phenylalanine content in African nightshade powder was 308.7 mg g^{-1} (**Table 4**). It is generally recognized as "safe" by the Food and Drug Administration FDA (43). There are unlikely side effects reported at supplement doses of 50–100 mg per kg of body weight

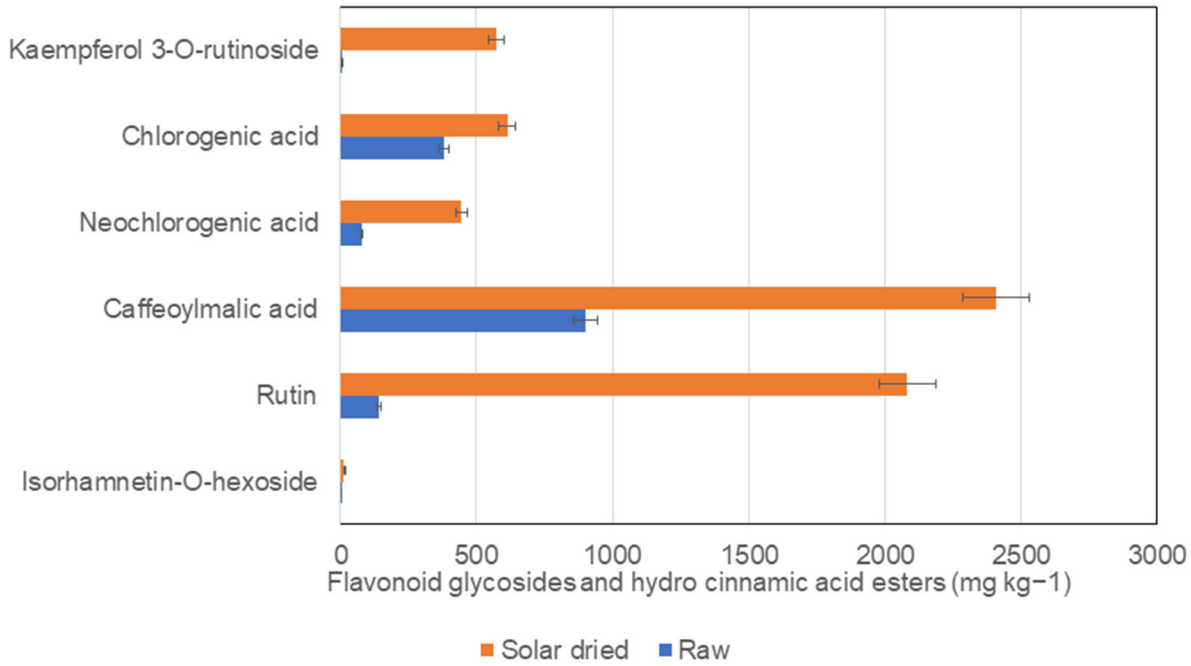

FIGURE 3 | Comparison of phenolic components in solar dried and raw *S. retroflexum* leaves from Venda, Limpopo, South Africa. Source (24).

TABLE 3 | Minerals in African nightshade (*S. retroflexum*) powder.

Minerals	Quantity	Nutrition information
Sodium (Na)	0.063 g 100 g^{-1}	1.3 g day^{-1} AI [16]
Potassium (K)	4.9 g 100 g^{-1}	4.7 g day^{-1} AI [16]
Iron (Fe)	60.6 mg 100 g^{-1}	8 mg day^{-1} RDA [16]
Calcium (Ca)	1.400 mg 100 g^{-1}	1,200 mg day^{-1} AI [16]
Magnesium (Mg)	556 mg 100 g^{-1}	350 mg day^{-1} EAR [16]
Zinc (Zn)	14 mg 100 g^{-1}	11 mg day^{-1} RDA [16]

Source: (24).
RDA, recommended dietary allowance; AI, adequate intake; EAR, estimated average requirement.

(44); therefore, it is possible for patients with amino acid metabolism disorder (phenylketonuria) to avoid the intake of high phenylalanine-containing meals (45). The African nightshade protein shake powder also contains aspartic acid (53.3 mg g^{-1}), a non-essential amino acid used in building proteins; other plant sources of aspartic acid are avocado and asparagus. A serving size of 0.83 g of avocado provides 220 mg of aspartic acid, whereas a comparative portion of the protein shake powder contains 44 mg. Similarly, asparagus contains 5.08 mg g^{-1} of aspartic acid, which is much lower than the amount present in African nightshade powder (53.3 mg g^{-1}; **Table 5**) (3). Another important non-essential amino acid in African nightshade powder is glycine, a powerful antioxidant with many health benefits.

Processing African nightshade leaves into powder is a preliminary step in the formulation of instant soups or meals. Evaluations indicated that this product will provide an instant, easy to handle and prepare meal, high in nutritional and sensory quality (46, 47).

TABLE 4 | Proximate analysis of the African nightshade (*S. retroflexum*) protein shake powder.

Proximate composition	Quantity per 100 g DW	% daily intake* per serving (100 g powder)
Energy	1,544 kJ 369 cal	18
Protein	32.8 g	66
Total fat	5.1 g	7
Saturated fatty acids	1.3 g	5
Monounsaturated fatty acids	1.4 g	
Polyunsaturated fatty acids	2.4 g	
Trans fatty acids	<0.01 g	
Total sugar	40 g	44
Available carbohydrate	40.8 g	13
Total dietary fiber	12.9 g	43
Ash	3.4 g	n/a
Moisture content	5.0 g	n/a

Source: (24).
**Percentage daily intakes are based on an average adult diet of 8,700 kJ. Your daily intakes may be higher or lower depending on your energy needs; n/a, not applicable.*

Fermentation—A Traditional Food Processing Technique

Fermentation is a traditional food processing technique, adopted as a preservation technique in Africa, performed with the

TABLE 5 | Amino acid profile of the African nightshade (*S. retroflexum*) protein shake powder.

Amino acid components	Amount ($-H_2O$; mg g^{-1})* Aver.	Amount (mg g^{-1})** Aver.
Histidine	14.1 ± 0.3	17.0 ± 0.3
Serine	23.4 ± 0.6	26.1 ± 0.7
Arginine	10.2 ± 0.2	13.4 ± 0.2
Glycine	30.2 ± 1.1	34.9 ± 1.3
Aspartic acid	46.8 ± 1.5	53.3 ± 1.7
Glutamic acid	10.3 ± 0.2	12.2 ± 0.2
Threonine	10.7 ± 0.3	13.5 ± 0.3
Alanine	13.6 ± 0.3	16.1 ± 0.4
Proline	19.9 ± 0.7	22.7 ± 0.8
Lysine	8.4 ± 0.5	9.4 ± 0.5
Tyrosine	2.7 ± 0.3	3.1 ± 0.3
Methionine	14.3 ± 0.2	16.9 ± 0.3
Valine	13.6 ± 0.2	15.8 ± 0.3
Isoleucine	24.5 ± 0.5	28.4 ± 0.5
Leucine	16.3 ± 0.3	18.3 ± 0.3
Phenylalanine	265.9 ± 7.1	308.7 ± 8.2
Total	278.7	325.3

Source: (24).
**Calculation based on amino acid residue mass in protein (molecular weight minus H_2O).*
***Calculation based on free amino acid molecular weight.*

objective of increasing food safety and make food more edible and appealing in terms of sensory properties, by improving flavor and aromas (32, 48). A frequently reported main effect of lactic fermentation is the improvement of the bioavailability of nutritional components. A popular fermented food on the African continent is *Kawal*, especially in Sudan, produced by spontaneous fermentation of leguminous leaves (49–51). *Bacillus* spp. and *Lactobacillus plantarum* are involved in fermentation, but most antinutritional factors, especially phytic acid content, decreased during the process. Fermentation of African nightshade (*S. scabrum*) and cowpea leaves, using *L. plantarum* and *Leuconostoc mesenteroides* ssp. *mesenteroides*, for 48 h reduced the pH and inhibited the growth of foodborne pathogens, such as *Listeria monocytogenes* and *Salmonella enterica* Enteritidis. Likewise, fermentation of African kale leaves (*B. carinata*) with *L. plantarum* BFE 5092 and *Lactobacillus fermentum* BFE 6620 starter strains inhibited the growth of *L. monocytogenes*, *S.* Enteritidis, and other enterobacteria, while maintaining appreciably the concentration of vitamin C (35 mg 100 g^{-1}) in the fermented product (32). Oguntoyinbo et al. (32) demonstrated that controlled fermentation is a promising method to reduce food spoilage and extend shelf life and food safety.

Fermentation of African nightshade *S. scabrum*, using 3% salt–sugar solution with *L. plantarum* BFE 5092 and *L. fermentum* BFE 6620 as starter cultures, had a greater impact on the microbial profile of the fermented product due to the rapid and stable decline of pH and production of lactic acid (52). The fermented product retained substantial levels of vitamins B1, B2, and C, which are sufficient to supplement the RDI and improve the sensory attributes, color, taste, and aroma (52).

L. plantarum 75 enhanced the functional potential of nightshade leaves and improved the bioavailability of phenolic compounds including phenolic acids (gallic, vanillic, 2,5-dihydroxybenzoic, coumaric, ferulic, and ellagic acids) and flavonoids (catechin, quercetin, and luteolin) in the fermented product of *S. retroflexum* (**Figure 4**) (53). However, caffeic and ferulic acids were not detected in African nightshade (*S. scabrum*) after fermenting with *L. plantarum* 75, possibly due to the different types of microbial metabolism and transformation of the phenolic compounds, which are predominantly determined by the different enzyme systems involved, irrespective of the species (53). Simultaneously, ferulic acid could have been reduced to dihydroferulic acid, whereas caffeic acid possibly metabolized to vinylcatechol, ethylcatechol, or dihydrocaffeic acid (54). Degrain et al. (53) concluded that *L. plantarum* 75 fermentation improved the extraction of phytochemical components in nightshade leaves and reduced carbohydrate content and calculated energy of the final product (53).

As with solar drying, fermentation of *S. retroflexum* with *L. plantarum* 75 improved the antioxidant activity compared with raw leaves (53); flavonoids containing multiple hydroxyl groups mostly have higher antioxidant activities. Many studies have underlined the beneficial health effect of antioxidant-rich foods, such as reducing the risk of non-communicable diseases and premature aging (55). Similarly, *S. scabrum* leaves fermented with *L. plantarum* BFE 5092 and BFE 6620 in 2.1 L of a 2.5% brine solution (containing 3% salt and 3% sugar) at 25°C for 144 h revealed an increase in the concentration of caffeoylquinic acid isomers, sinapoylmalate, kaempferol-3-diglucoside, quercetin-3-pentosylrutinoside, sinapic acid, quercetin-3-rutinoside, and caffeoylmalate. The total polyphenols increased in concentration compared with the raw leaves, whereas quercetin-3-glucosyl-rhamnogalactoside and quercetin-3-rhamnosyl-rhamnogalactoside slightly reduced in the fermented leaves compared with the raw leaves (38). This is possibly due to the action of glycosyl hydrolases generated from the fermentation activity of lactobacillus strains on the conversion of flavonoid glycosides to the corresponding aglycones, which shows the higher enhancement of antioxidant activity and the biological bioactivity and benefit to the consumers (56). Coumaric acid levels after fermentation also reduced in *S. scabrum* leaves (38), possibly due to the coumaric acid acting as an external acceptor of electrons to gain one extra mole of ATP (57).

Proximate analysis of fermented African nightshade (*S. retroflexum*) leaves with *L. plantarum* 75 showed 2.51 g carbohydrate content in a 100 g serving portion. Dietary fiber, protein, fat, and sugar contents in a 100 g serving portion of fermented African nightshade vegetable product were 2.52, 3.82, 0.23, and <0.50 g, respectively. However, sodium content (231 g) was higher in the fermented nightshade vegetable product than in the solar dried powder of the same product (53). Sodium content is of great concern, and the daily limit of 2.4 g per day is due to an increase in the prevalence of chronic diseases, such

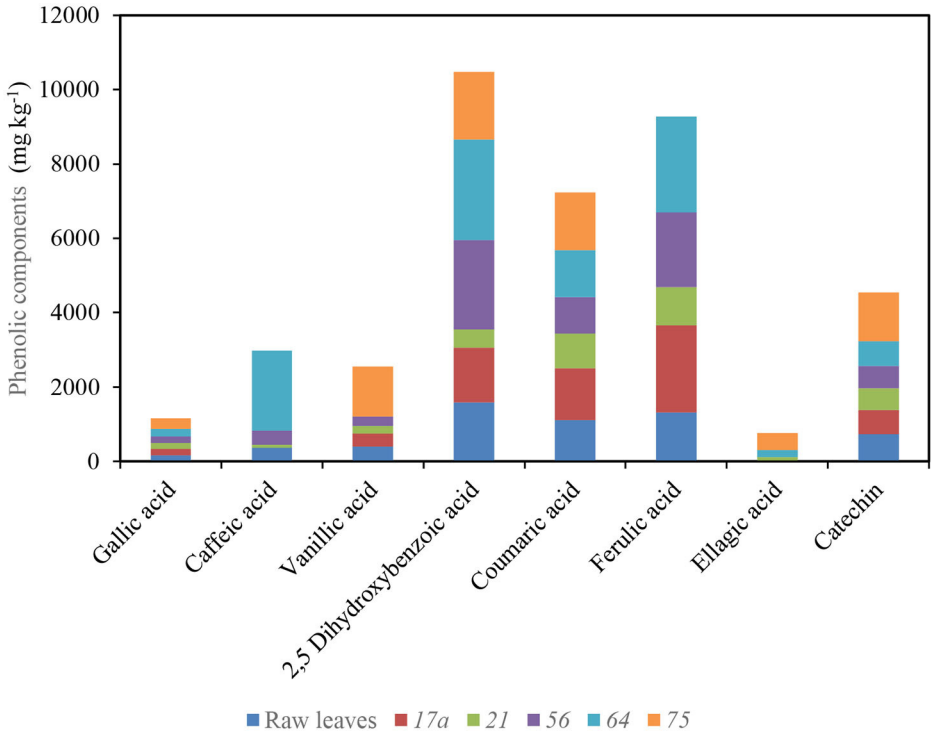

FIGURE 4 | Impact of fermentation with different lactic acid bacterium strains on different phenolic components in African nightshade (*S. retroflexum*) leaves (75 *L. plantarum*, 21 and 64 *Weissella cibaria*, and 56 *Leuconostoc pseudomesenteroides*). Source (53).

as high blood pressure, which positively correlate to high salt intake (54).

Household Cooking

Traditionally, the consumption of African nightshade leaves is after cooking, and the adoption of different cooking methods, such as a boiling and steaming, is to improve palatability and sensory properties. Cooking can enhance the bioavailability of phenolic components and the antioxidant activity (58); however, it can have deleterious effects on the nutrient composition and functional compounds and their bioavailability in different vegetables (59).

Traditional food preparation methods, including blanching, are widely adopted to improve the palatability and reduce the bitterness of African nightshade leaves. Blanching treatments (steam or cook in hot water using plain water or lemon juice) at 95°C for 5 min increased the concentrations of hydroxycinnamic acid derivatives (chlorogenic, neochlorogenic, and cryptochlorogenic acids) and caffeoylmalic acid (60). However, steam blanching in either water or lemon juice at 95°C for 5 min significantly improved the concentration of caffeoylmalic acid. In addition, cooking improved the antioxidant capacity of vegetables (58). The increase of chlorogenic acid concentration in *S. retroflexum* leaves during blanching treatments could be due to the formation of different caffeoylquinic acid isomers or the hydrolysis of dicaffeoylquinic acid (59). A similar significant increase in caffeoylquinic acid was reported in fried artichokes compared with raw and other cooking methods (60). Transesterification of caffeoylquinic acid is dependent on the pH of the food matrix, as well as temperature and time.

S. scabrum leaves boiled in water demonstrated an increasing trend in the levels of 3-caffeoylquinic acid, 5-caffeoylquinic acid, and 4-caffeoylquinic acid compared with the raw leaves. Boiling also reduced the levels of caffeoylmalate compared with the raw leaves and remarkably reduced the levels of quercetin-3-glucosylrhamnogalactoside, coumaric acid, quercetin-3-rhamnogalactoside, quercetin-3-rhamnosyl-rhamnogalactoside isomers, kaempferol-3-rhamnosyl-rhamnogalactoside, quercetin-3-pentosylrutinoside, and quercetin-3-rutinoside; sinapoylmalate, sinapic acid, kaempferol-3-diglucoside, and kaempferol-3-rhamnosyl-rhamnogalactoside were not detected in the cooked *S. scabrum* leaves (38). Non-acylated kaempferol diglucosides in broccoli demonstrated higher loss after boiling and minor loss after steaming, but during higher temperature heat treatments, it was expected that kaempferol-3-diglucoside would degrade to its monoglucoside or kaempferol; additionally, 4-O-position had a higher stability against deglucosilation than 3-O-position (58).

CHANGES IN ANTINUTRITIVE COMPOUNDS IN AFRICAN NIGHTSHADE DURING POSTHARVEST PROCESSING

Some accessions of African nightshade leaves contain glycoalkaloids, which can cause health concerns. Among the five accessions of African nightshade, *S. nigrum* reportedly contains solasodine glycosides, including solamargine and solasonine, as in other plants belonging to the family Solanaceae, such as potatoes, tomatoes, and eggplants. Yuan et al. (8) reported the absence of glycoalkaloids in methanol leaf extracts of *S. scabrum* and *S. villosum*.

Based on previous reports (8), safe consumption of eggplants was allowed at glycoalkaloid levels ranging from 6.25 to 20.5 mg 100 g^{-1} FW. Higher levels of glycoalkaloids were detected in commonly consumed African nightshade leaves, which were confirmed as safe for consumption (8).

Steroidal saponins, mass of tigogeninas, were detected in *Solanum* spp. Tigogenin-5G is detected in most of the African nightshades spp. Dehydrodiosgenin-G-G-Rha-Rha and diosgenin-G-G-Rha-Rha are detected only in *S. nigrum* from the USDA collection PI 312110. Tigogenin-G-G-Rha-Xyl-Xyl is detectable in all *S. nigrum* (Kenya) from the USDA collection PI 306400, PI 381289, and PI 381290; *S. scabrum* SS 5, Ex Ha, SS 49, Olevolosi SS 04.2, BG 16, Nduruma BG-29, Grif 14198, and PI 643126; *S. americanum*; and *S. villosum*. Tigogenin-G-G-G is detected mainly in *S. nigrum* obtained from Kenya, USDA collection PI 30640, and in *S. villosum* (8). Tigogenin-3G-Xyl-G, tigogenin-5G, and tigogenin-GG-Rha-Xyl-Xyl are detected in *S. retroflexum* (30). The raw leaves contained 0.45 mg kg^{-1} of tigogenin and 0.56 mg kg^{-1} of tigogenin-GG-Rham-Xyl-Xyl, and solar drying increased the levels of tigogenin and tigogenin-GG-Rham-Xyl-Xyl to 70.54 mg kg^{-1} and 73.92 mg kg^{-1}, respectively (30). Similarly, steam blanching, in water or lemon juice at 95°C for 5 min, increased the peak responses of the tigogenin-5-G, tigogenin-3G-Xyl-G, and tigogenin-GG-Rha-Xyl-Xyl, but the effect was greater in tigogenin-5-G (30). Tigogenin is an important raw material for pharmaceutical use and the synthesis of steroid drugs, demonstrating anti-inflammatory, and anti-diabetic activities (type 2 diabetics) (61).

The raw leaves of *S. retroflexum* contain other antinutritive compounds, such as tannins (55.4 mg 100 g^{-1}), phytates (88 mg 100 g^{-1}), and oxalates (87.5 mg 100 g^{-1}). Hot water blanching and steam blanching treatments during food preparation help to significantly reduce the levels of these compounds (61).

Tannins, which are polyphenols, can prevent the availability of protein for absorption by forming complexes with proteins (61). Oxalates also prevent the absorption of dietary calcium by binding with Ca^{2+} (61, 62); furthermore, the insoluble calcium oxalates are stored in the kidney, causing "kidney stones." The increased oxalate:calcium ratio >9:4 can affect Ca absorption negatively (63). Phytic acid chelates with Zn or phytates, binding with proteins making them unavailable for absorption (62).

FUNCTIONAL POTENTIAL FOR CONSUMERS' DIET

Inclusion of African nightshade, especially *S. retroflexum*, with the main staple food can improve protein, iron, and calcium levels, which will help improve people's health and well-being. It is also possible to use African nightshades as food and medicinal ingredients. The increased polyphenol compounds can contribute to antioxidant activity, and an increased intake of chlorogenic acid correlates with the reduced risk of type 2 diabetes mellitus (64). Available literature suggests that the chlorogenic acid suppresses postprandial hyperglycemia by inhibiting α-glucosidase similar to the α-glucosidase inhibitors, such as acarbose, miglitol, and voglibose (64). At the same time, chlorogenic acid modulates the glucose and lipid metabolism *via* the activation of adenosine monophosphate-activated protein kinase and stimulates glucose uptake in the skeletal muscle, which shows similar activity as anti-diabetic agents (65).

Solar dried or blanched African nightshade *S. retroflexum* leaves can be included as a functional ingredient or a functional food to manage type 2 diabetes in rural regions and as a promising potential for the food industry and food manufacturers. For health claims, and to popularize use as an ethnic food, future research on the biological activities of African nightshade leaves on anti-diabetic, anti-proliferative, or anti-inflammatory effects is necessary. Furthermore, *in vitro* results revealed the chemo-preventive properties in terms of anti-genotoxicity against the liver carcinogen aflatoxin B1 (AFB1) and antioxidant potential, at non-toxic concentrations, of the leaf extract of *S. scabrum* (38). The authors concluded that although the food preparation and processing methods affected the concentration of phytochemicals, the compositional changes could have acted positively in the observed antioxidant activity and chemo-preventive properties (38).

CONCLUSIONS

African nightshade vegetables are rich in minerals and phytochemicals, and the adoption of different food processing or preparation methods can prevent postharvest losses during the supply chain to contribute to food security and reduce hidden hunger. Food processing or preparation methods improved the phytochemicals (functional compounds) in the African nightshade vegetables, and agronomy practices affected the nutritional properties. For all the USDA and WAC African nightshade collections, it is important to profile the mineral composition to select the best accession to benefit consumers. Based on the available literature regarding the antioxidant activity, the recommendation for growers is to use *S. scabrum* (BG 16, Nduruma, WAC); however, all the accessions need further testing to correlate with the biological actives in order to identify the suitable accession for commercial production and marketing.

AUTHOR CONTRIBUTIONS

DS obtained the funding for the program and conceptualized the research. AP formatted and validated the data. YS under the Australia–Africa partnership program, collaborated with data generation. RS research collaborator, proofread and edited the review. FR research collaborator under the SA-France bilateral program, assisted with the generation of some data and editing of the review. All authors contributed to the article and approved the submitted version.

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Neglected and Underutilized Crop Species: The Key to Improving Dietary Diversity and Fighting Hunger and Malnutrition in Asia and the Pacific

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Asia continues to suffer from a high prevalence of malnutrition. Persistent malnutrition can be attributed to low dietary diversity, together with low production diversity. Dietary diversity represents a more healthy, balanced, and diverse diet, which ensures nutrient adequacy. The principle of dietary diversity is affirmed in all national food-based dietary guidelines. Food-based approaches that address malnutrition, especially micronutrient deficiencies, are embedded in evidence-based healthy diet patterns; however, they are disconnected from the current agricultural production system. Promising neglected and underutilized species (NUS) that are nutrient-dense, climate-resilient, profitable, and locally available/adaptable are fundamental to improving dietary and production diversity. The Future Smart Food Initiative, led by FAO's Regional Initiative on Zero Hunger, aims to harness the enormous benefits of NUS in the fight against hunger and malnutrition. Recognizing that NUS covers crops, livestock, fisheries and aquaculture, and forests, the FAO has set crops as an entry point for NUS to address hunger and malnutrition.

Keywords: Asia pacific region, food security and nutrition, future smart food, neglected and underutilized species, malnutrition, dietary diversity, healthy diet, sustainable development goals

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ISSUE: HIGH PREVALENCE OF MALNUTRITION IN ASIA

The 17 sustainable development goals (SDGs) adopted by the General Assembly of the United Nations in 2015 cover a broad range of global issues (1). There is a strong call to end hunger and malnutrition by 2030, especially in the second SDG2. Other related SDGs include SDG3 (good health and well-being), SDG12 (responsible consumption and production), and SDG15 (life on land). Although substantial advances have been made, ending hunger and malnutrition remain a major concern in the Asia Pacific region. In 2018, the region had an estimated 479 million undernourished people, being 58% of the worldwide total (2). Countries in the region are still facing a high prevalence of undernutrition, especially chronic undernutrition. Chronic undernutrition is measured by stunting (low height for age) and is due to the persistent inability to meet minimum micronutrient and macronutrient requirements, or the frequent recurrence of acute malnutrition episodes, or a combination of both. Stunting, wasting, and underweight are important indicators of child undernutrition. The region has a high prevalence of stunting and wasting, with an estimated 77.2 million children under 5 years of age suffering from stunting and 32.5 million suffering from

wasting in 2018 (2). In South Asia, an estimated 58 million children suffer from stunted growth; the prevalence of stunting is medium to very high (over 40%) in most developing countries in the region [apart from China, Fiji, Iran (Islamic Republic of), Mongolia, Samoa, and Tonga] (**Figure 1**) (2).

There is an increasing incidence of adult obesity throughout the Asia Pacific region (**Figure 2**) (2). Overweight and obesity are significant risk factors for all age groups for many diseases, including non-communicable diseases (NCDs), such as diabetes, hypertension, cardiovascular diseases, certain cancers, obstructive sleep apnea, osteoarthritis, respiratory diseases, and diabetes. Worldwide, NCDs are the leading cause of death; in the Asia Pacific region, premature NCD deaths (death before age 70) are high (2). The prevalence of obesity-related diseases, including NCDs, has increased in many countries in the region, particularly the Pacific Islands (2).

High rates of micronutrient deficiency are being observed in many countries in the region. For instance, the prevalence of anemia (iron deficiency) in most ASEAN countries is alarming, amounting to more than 40% for children under 5 years. Countries suffering from severe micronutrient deficiencies (HHI $>$ 25) include Nepal, India, Bangladesh, Bhutan, and Lao PDR (3). In Myanmar, the prevalence of anemia in children under 5 years and reproductive and pregnant women is 57.4, 46.6, and 54.0%, respectively. The prevalence of anemia in Cambodia, Lao PDR, and Nepal ranges from 40–55%.

WHY IS MALNUTRITION HIGH?

Low Dietary Diversity

Why is malnutrition so prevalent in Asia when most countries are increasing food production to feed the growing population? Malnutrition is generally the result of an inadequate diet, with insufficient nutrients, minerals, and vitamins for the growth and maintenance of a healthy body. The inadequate consumption of food (quantity and quality) or inadequate childcare and feeding practices cause childhood stunting, wasting, and overweight (2).

Dietary diversity in children is positively correlated with the mean micronutrient adequacy of the diet, that is, adequate nutrients for growth and development (4). Minimum dietary diversity (MDD) is a measure of the dietary quality and feeding practices of children (5). An infant or young child is considered to have reached MDD if he/she has consumed food from five of the eight food groups in the previous 24 h. The eight food groups are: (1) grains, roots, and tubers; (2) legumes and nuts; (3) dairy products; (4) flesh foods, including meat, poultry, and fish; (5) eggs; (6) vitamin A-rich fruits and vegetables; (7) other fruits and vegetables; (8) breastmilk (2). Low dietary diversity usually comprises a high consumption of cereals, mainly rice, and relatively low consumption of vegetables, fruits, and pulses.

In the Asia Pacific region, dietary quality and diversity are suboptimal, particularly among infants and young children, with fewer than 50% of children fulfilling the MDD in 15 of the 20

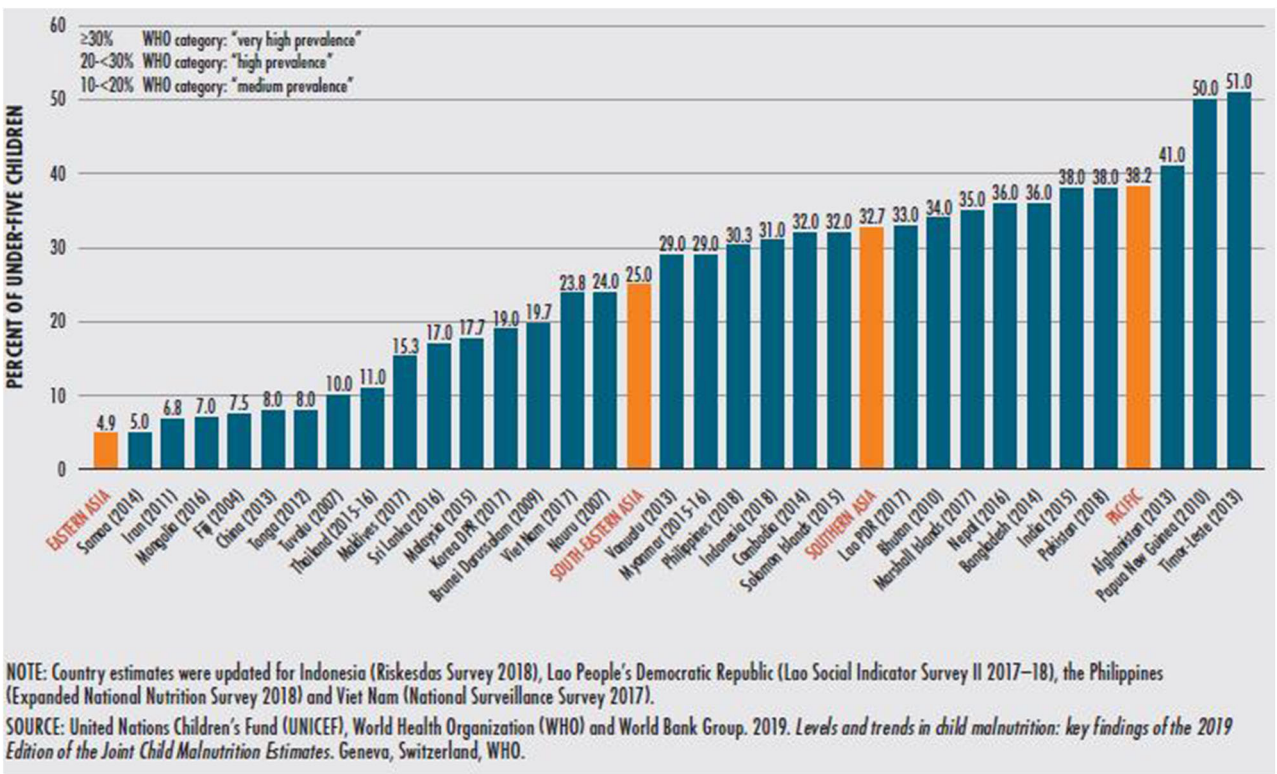


FIGURE 1 | Prevalence of stunting in children under 5 years of age in the Asia Pacific region, by country, latest available year.

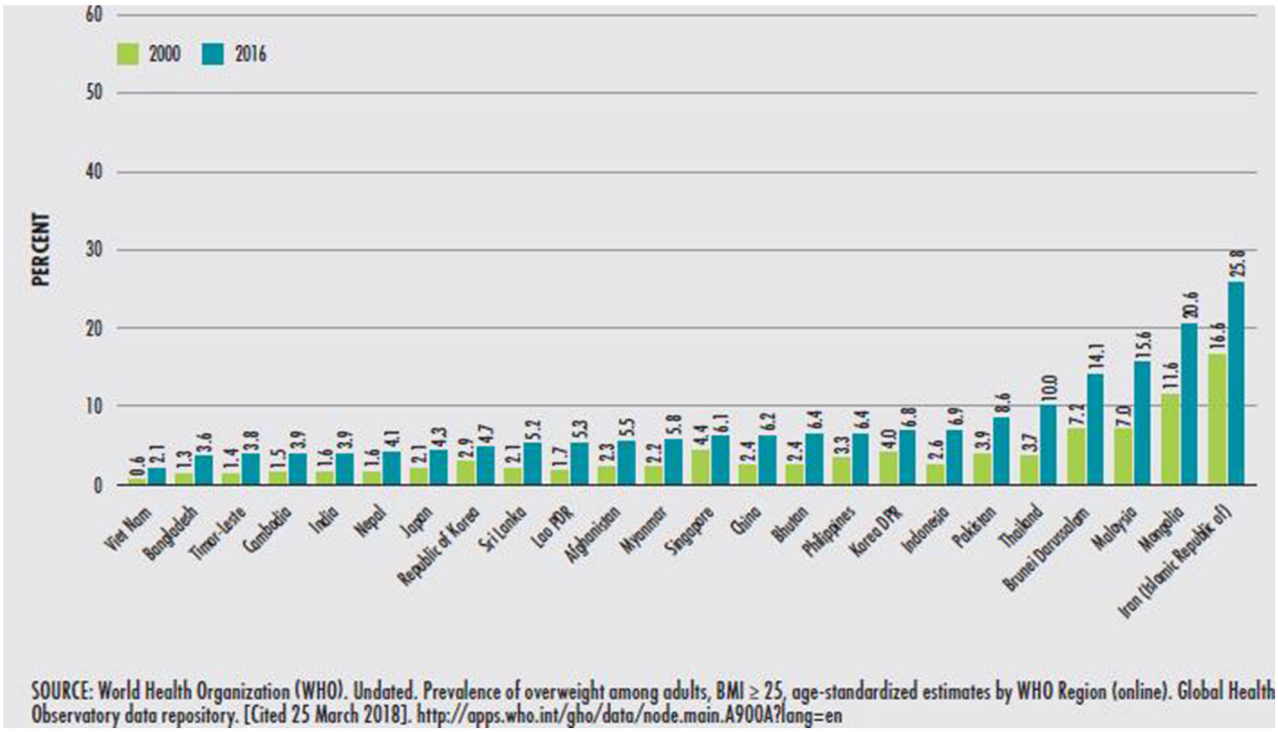


FIGURE 2 | Trends in the prevalence of adult obesity in Asia, by country, 2000 and 2016.

countries. Only 20 and 21% of children achieve MDD in India and Myanmar, respectively. In most countries in the region, less than half of all very young children (aged 6–23 months) meet the minimum standards of dietary diversity; hence, the high prevalence of stunting and wasting among children under five years of age (2).

Evidence

What is the relationship between malnutrition and dietary pattern? The evidence suggests that high rice consumption areas tend to have high levels of stunting and underweight, particularly in rural areas. In Laos, rice consumption is positively correlated with stunting (**Figure 3**). The Phongsaly and Huaphanh provinces—where rice constitutes 43 and 52.2% of the diet, respectively—have high levels of all three malnutrition indicators (stunting, wasting, and underweight) (6).

In Myanmar and sub-regions, strong negative relationships exist between stunting and dietary energy consumption and micronutrient intakes. With high levels of dietary energy consumption, stunting levels are low. Similar relationships exist for protein and fat intakes, indicating that stunting is highly correlated with dietary quantity and quality. A reduction in stunting rates requires increased dietary energy consumption, accompanied by more diversified food products.

Anemia has a strong negative correlation with dietary energy consumption and macronutrient intake. Low dietary energy consumption is linked with high anemia among urban populations. Protein-rich foods also help to reduce anemia levels among children. Foods rich in proteins and fats, coupled with an overall increase in consumption, will help combat diseases and improve children's health.

How to Ensure Dietary Diversity?

Agricultural diversification is a formidable tool for achieving Zero Hunger (7). However, current agriculture and food systems have (1) have limited production diversity, resulting in unbalanced diets and, thus, malnutrition. In the Asia Pacific region, only a few staple crops are grown, mainly rice, which form the bulk of people's diets—the lack of dietary diversity fails to deliver wholesome nutrition, as per the recommended nutrition intake (8). The prevalence of rice cultivation in the region is associated with multiple factors: national policies in favor of rice production and consumption; rice varieties from earlier breeding activities that focused on yield resulting in increased energy content and low nutrient density with little consideration of the higher nutritional profiles of indigenous rice varieties; established agronomic practices and environmental conditions for rice cultivation; cultural preference and social recognition for rice as rich people's food; poverty not allowing for diversifying food procurement and thus using the most available; and (2) high-input requirements, such that farming is vulnerable to environmental stresses (9). According to the FAO (10), the reliance on only a few crops negatively affects ecosystems, food diversity, and health. Food monotony increases the risk of micronutrient deficiencies (10). Dietary diversity is a cost-effective, affordable and sustainable way to minimize hunger

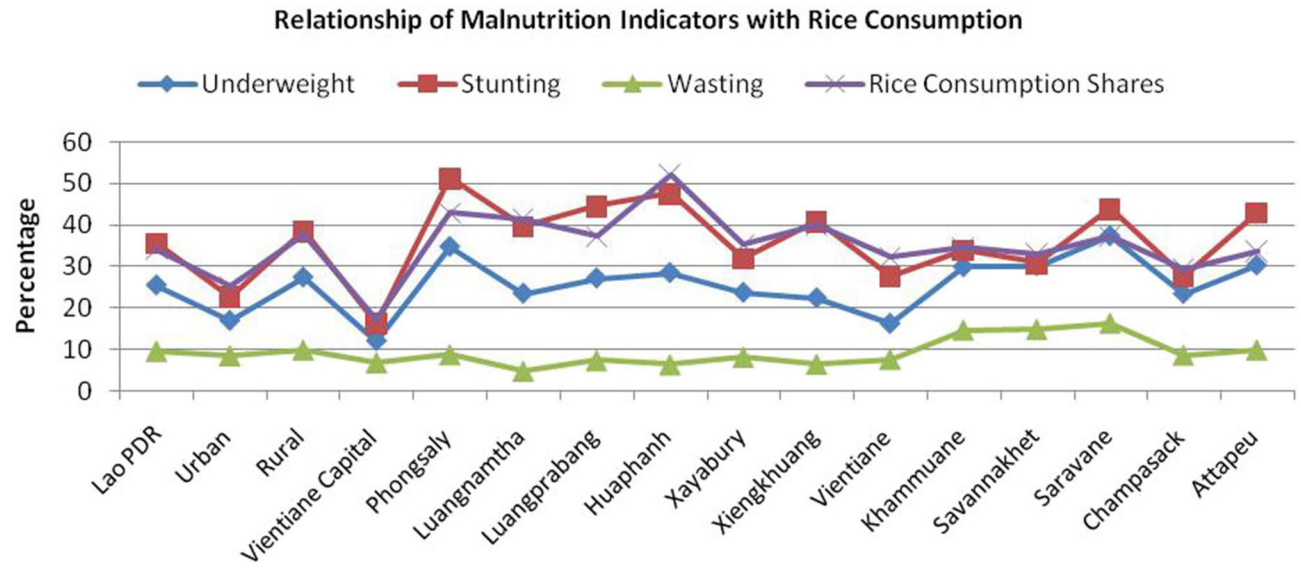


FIGURE 3 | Relationship between rice consumption and malnutrition indicators for Laos and sub-regions 2014.

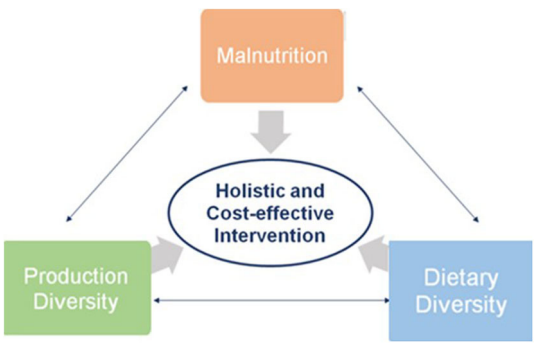


FIGURE 4 | Features of agriculture and food systems Source: Li and Siddique (6).

and malnutrition, and production diversity facilitates the supply of nutritious and diversified food and addresses the effects of climate change (**Figure 4**) (6).

ADDRESSING MALNUTRITION: A HEALTHY DIET

What Is a Healthy Diet?

Healthy diets have an optimal caloric intake comprising a diversity of plant-based foods, low amounts of animal source foods, unsaturated rather than saturated fats, and limited amounts of refined grains, highly processed foods, and added sugars (11). Generally, a healthy diet provides the right nutrients (energy, protein, fats, fiber, and essential nutrients such as carbohydrates, amino acids, fatty acids, vitamins, minerals, and fluids) in the right balance, with sufficient diversity for healthy growth and reducing the risk of diet-related diseases (2). While humans are omnivores, not herbivores, a healthy diet is often largely plant-based and includes modest amounts of fish, meat, and dairy (11–14).

What Is the Benefit of a Healthy Diet?

Micronutrient deficiency mostly affects children and women, particularly those of reproductive age. While the most common indicators for malnutrition in children under 5 years of age are stunting, underweight, and wasting, those for women and children (>5 years) are anemia and vitamin A deficiency. Of the world's estimated 7,000 million people, 500 million suffer from protein-energy malnutrition, >1,600 million suffer from iron deficiency, and >200 million suffer from vitamin A insufficiency (15, 16). More than 400,000 children under 5 years are estimated to die each year from zinc deficiency (17). Diet is one of the most important contributors to health but also disease. Inadequate diets have a direct negative impact on the health of individuals, leading to high NCDs and even death. An unhealthy diet is a significant contributor to most NCDs (18). A systematic evaluation of dietary consumption patterns across 195 countries suggested that dietary improvements could prevent one in every five premature deaths globally (19). The WHO estimates that diets low in fruits and vegetables cause 2.7 million deaths each year and about 19% of gastrointestinal cancer, 31% of ischemic heart disease, and 11% of strokes (20). That is, diet-related NCDs are a leading preventable cause of death worldwide. Dietary modifications toward healthy diets are expected to result in significant health benefits, including preventing 19–24% of total deaths among adults (11).

Healthy Diets in the Real World

The Mediterranean diet and the Japanese diet are two examples of a healthy diet. The Mediterranean diet—mainly incorporating legumes, cereals, fruits and vegetables, olive oil,

fish, and moderate consumption of dairy products (mostly cheese and yogurt)—is the traditional way of eating around the Mediterranean basin. The Mediterranean diet emphasizes the consumption of plant-based foods, including fruits, vegetables, beans, nuts, cereals, and other seeds, olive oil as the main source of dietary fat, red meat in moderation, and herbs and spices instead of salt to flavor food. Compared to the "modern Western" diet, the Mediterranean diet contains much higher quantities of unprocessed foods, uses much less red meat, and has a much higher proportion of unsaturated fats (21). The Japanese diet emphasizes the consumption of fish as a major source of protein, vegetables (including daikon radish and sea vegetables), rice, soy (tofu, miso, soy sauce), noodles, fruit, and tea (preferably green). Fish features prominently in Japanese cuisine: Japanese account for only 2% of the global population, but they collectively consume 12% of the world's fish. With its high popularity, Japanese cuisine is often associated with *sushi* (raw fish and rice served with pickled ginger) and *sashimi* (fresh raw seafood that is dipped in soy sauce and *wasabi*) (21). While *sushi* and *sashimi* are originally "made in Japan," Japanese cuisine has had strong external influences: around 300 BC, the Japanese learned how to cultivate rice from China, as well as the preparation of soy sauce and tofu (important sources of plant protein). The other external influence was Buddhism: a ban on eating meat was promulgated with the arrival of Buddhism in the seventh century. The popularity of *sushi* came about as a result of this ban. While not always strictly observed, for many centuries, eating meat, particularly beef, was unthinkable; the beef-eating habit returned to Japan only in the late nineteenth century.

These two examples show healthy diets developed in vastly different cultural, climatic, and geographic settings. Both use diverse ingredients linked to people and cultures as much as to their natural environment. Consequently, the Mediterranean and Japanese diets are on UNESCO's World's Intangible Cultural Heritage list (22, 23). Japan and the Mediterranean countries can demonstrate the health effects of their respective healthy diets. Medical research has shown that the Japanese diet has the lowest prevalence of obesity among developed countries—and other chronic diseases, such as osteoporosis, heart ailments, and some cancers (24). Following the Mediterranean diet for several years reduces the risk of developing heart disease, cancer, hypertension, Type 2 diabetes, Parkinson's disease, and Alzheimer's disease (25).

Indeed, the Japanese have one of the longest average life expectancy in the world-—87.45 years for women and 81.41 years for men in 2019—according to the Japanese Ministry of Health, Labor, and Welfare (26). Japanese women outstrip all competitors in life expectancy, including their American counterparts, who can expect to live up to 81 years (76 years for American men). The same holds for developed countries in the Mediterranean: women in Italy and Spain have a life expectancy of 85 years, while the figure is 83 years in Germany. Both Italian and Japanese men have a life expectancy of 80 years (27).

Food-Based Dietary Guidelines

A healthy diet is the pillar of well-being throughout a person's life. Policies that aim to prevent malnutrition, primarily by ensuring healthy diets for children to prevent stunting and obesity, are more effective than those aiming to reduce malnutrition (2). Unfortunately, there is no one-size-fits-all healthy diet. A healthy diet must be affordable, based on locally available foodstuffs, and meet cultural preferences (28). Since the First International Conference on Nutrition held in 1992, the FAO together with WHO has worked with governments on national food-based dietary guidelines: short, science-based, positive messages on national healthy eating and lifestyles. National governments use similar approaches. For example, the US Government regularly publishes *Dietary Guidelines for Americans* to show how individuals can have a healthy diet following updated scientific evidence. It is the role of governments and public agencies—rather than special interest groups—to provide unbiased information on what constitutes a healthy diet. The WHO Global Strategy on Diet, Physical Activity and Health (29) and the Commission on Ending Childhood Obesity (30) provide strategies for improving diets and physical activity patterns at the population level (2).

NUS: THE KEY TO ENSURING HEALTHY DIETS AND FOOD SECURITY AND NUTRITION

What Is NUS?

Agrobiodiversity is essential to sustainable agriculture, of which NUS are key elements. About 30,000 edible plant species have been identified worldwide; of these, more than 7,000 crop species have been cultivated for food (31, 32). Currently, fewer than 150 crop species are commercially cultivated; 103 deliver up to 90% of the calories in the human diet, and only four (rice, wheat, maize, and potato) provide 60% of the human energy supply (33). Thus, tens of thousands of edible plant species are relatively "underutilized" and could be used to increase the world's food requirements (34).

Crops can be divided into two main categories (staple and underutilized). Underutilized crops (also called neglected, minor, orphan, promising, or little-used) are mostly wild or semi-domesticated species adapted to local environments (6). These crops were used as traditional foods for centuries but became increasingly neglected when more productive crops became available in farming systems. NUS face multidimensional challenges ranging from agro-technical, socioeconomic, policy, and institutional perspectives that have resulted in their underutilization. Agricultural modernization, widespread monoculture, and the promotion of high-yielding varieties have marginalized NUS, which play a minor role in current farming and food systems. Culturally, NUS has been stigmatized by the perception as "food of the poor," creating a disincentive for their production and consumption (6). Politically, governments tend to give priority to rice production, such as the rice self-sufficiency policy. In short, the lack of an environment conducive to the production, processing, marketing, distribution, and consumption of NUS prevented them from being included in current diets.

What Value Does NUS Bring?

Neglected and underutilized species offer immense opportunities to fight poverty, hunger, and malnutrition, and their incorporation into farming systems could lead to nutrient-dense, climate-resilient, and sustainable agriculture. Neglected and underutilized species have high nutritional value and are a good source of micronutrients, protein, energy, and fiber. Many NUS crops can also be grown on marginal land, intercropped or rotated with staple crops, and easily fit with integrated practices (35, 36). Many NUS can tolerate various stresses, which will not only make production systems more diverse but more sustainable and climate-resilient (6).

Reduce Malnutrition

"Hidden hunger"—having enough calories but insufficient vitamins and minerals—is a killer factor affecting both developed and developing countries (37). Hidden hunger is partly due to the reliance on only a few staple crops (38). Since the Green Revolution in the 1960s, agricultural research has focused on increasing crop yields to deliver sufficient food for the world's growing population. Nutritional quality has been less of a concern, despite many people suffering from hunger. While people's standard of living has improved, the effect of diets deficient in essential vitamins and minerals has become apparent in many parts of the world. Neglected and underutilized species have the potential to reverse the trend in hidden hunger. They are often richer in nutrients than their more popular staple crop cousins, with high levels of essential micronutrients (minerals, vitamins) and phytochemicals (such as flavonoids) and good macronutrient profiles (energy, fat, protein, carbohydrates) (39). For example, quinoa is a highly nutritious NUS that came into the limelight in 2014 when the United Nations General Assembly endorsed the International Year of Quinoa. Quinoa has twice as much protein, five times more dietary fiber, four times more iron, and 23 times more folate than rice (**Figure 5**) (40).

Neglected and underutilized species have outstanding health benefits. For example, lentils are rich in micronutrients, with the potential to provide adequate dietary amounts, especially for iron (Fe), zinc (Zn), and selenium (Se) (41). An empirical study by Wijesuriya et al. (42) revealed the impact of an Fe-rich lentil diet on Fe-deficient anemic children in Sri Lanka. The pilot study, involving 33 mildly anemic children (hemoglobin levels $= 11 \pm 0.8$ g/dl), showed a significant improvement in Fe status in the group fed 50 g of red lentils per day for 2 months (**Figure 6**) (42). These findings indicate that introducing lentils into children's meals, even in the short-term, helps to reduce the prevalence of anemia among children in Sri Lanka by improving their Fe nutritional status and thus has potential in other populations (41). In India, areas that grow lentils had lower anemia rates than those that did not grow lentils. The Indian Government recognized the key role of dietary diversity for preventing nutritional anemia and used food-based approaches to attain adequate dietary iron by encouraging the consumption of micronutrient-rich foods, such as dark green leafy vegetables, lentils, and vitamin-C-rich NUS fruits, which are often available but underutilized by the nutrient-deficient population (6, 43).

Millets also have superior nutritional and health benefits; they are often referred to as "high-energy" cereals, with higher protein, vitamin A and oil contents than maize (44). Vitamin A is often deficient in staple diets, making millets a suitable crop for tackling the nutritional challenges faced by mountain communities (34, 45). **Table 1** compares the nutritional value of selected millets and staple crops (46) and shows, for instance, that pearl millet has higher calcium, iron, zinc, riboflavin, and folic acid contents than rice or maize and higher micronutrient contents (excluding calcium) than wheat (47).

Fight Food Insecurity

From a food security perspective, the world's current food system is vulnerable as it relies on a limited range of food items (16). Current farming systems favor monocultures that require high inputs, which facilitate operations but threaten food security. Basing our diet on such a small number of staple crops has serious implications for food security and nutrition (48). The major cultivated crops lack genetic diversity within their gene pools, which leaves agricultural systems exposed to pests and diseases and abiotic stresses (49).

The Great Irish Potato Famine provides an alarming lesson from history. It began in 1845 and lasted for 6 years, killing about two-fifths of the population (over a million people) in Ireland and causing another million to flee the country. The famine was caused by potato blight, a disease that ravaged potato crops throughout Europe. The impact in Ireland was disproportionate, as one-third of the population depended almost entirely on potato for food (50). The marginalized Irish smallholders had cultivated the potato as a staple food since the eighteenth century, as potato yields were much higher per acre than cereals. However, potatoes grown in Ireland were mostly of a single variety, the Irish Lumper. When the disease spread, the lack of genetic variability among the potato plants in Ireland led to devastating effects, while elsewhere in Europe, with more diversity in the varieties of potato being cultivated and/or reliance on a broader range of crops, the effects were much less severe.

Rediscovering neglected crops could reduce the risk of over-reliance on a few major crops. Agricultural sustainability relies on a healthy interaction between agriculture and nature involving three hierarchical levels of genetic diversity: agroecosystems, interspecific diversity (among species), and intraspecific diversity (within species) (51, 52). Marginalizing NUS endangers agrobiodiversity and threatens food system sustainability. Neglected and underutilized species can increase agricultural sustainability by reducing the need for external inputs, such as inorganic fertilizers and pesticides (53). Introducing NUS in a farming system can reduce pest and disease buildup when grown in rotation with main crops. Depending on their characteristics, NUS can also increase soil fertility, prevent soil erosion, reduce evaporation, and suppress weed growth (54).

Neglected and underutilized species are often less demanding of the environment, more resilient to climate change, and more resistant to biotic stresses, thus providing more reliable harvests under unfavorable climatic conditions or on depleted soils

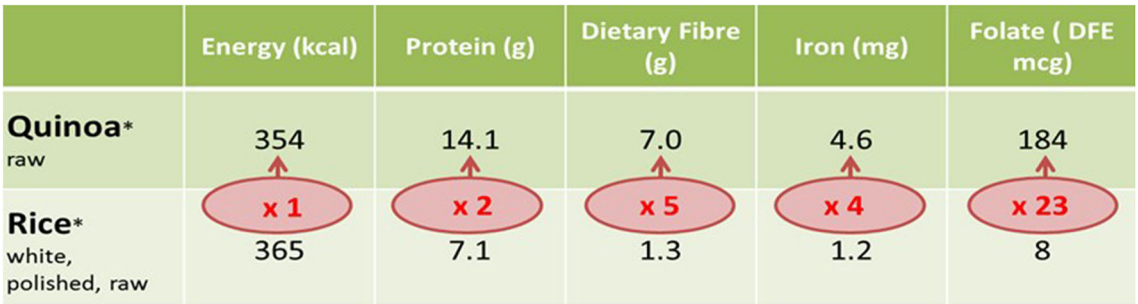


FIGURE 5 | Nutritional comparison of quinoa and rice.

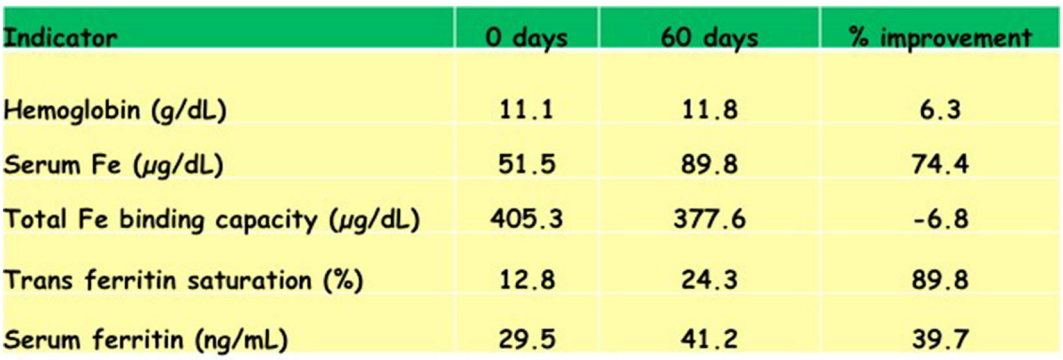


FIGURE 6 | Percent improvement in mildly anemic children ($n = 33$) in Sri Lanka after a 60-day red lentil feeding trial.

TABLE 1 | Comparison of nutritional values of selected millets and staple crops.

Nutrient	Selected millets (/100 g)							Staple foods (/100 g)		
	Pearl millet	Sorghum	Finger millet	Foxtail millet	Proso millet	Barnyard millet	Kodo millet	Rice (milled)	Maize	Wheat flour
Energy (kcal)	361	349	328	331	341	397	309	345	342	346
Protein (g)	11.6	10.4	7.3	12.3	7.7	6.2	8.3	6.8	11.1	12.1
Fat (g)	5.0	1.9	1.3	4.3	4.7	2.2	1.4	0.4	3.6	1.7
Calcium (mg)	42.0	25.0	344	31.0	17.0	20.0	27.0	10.0	10.0	48.0
Iron (mg)	8.0	4.1	3.9	2.8	9.3	5.0	0.5	3.2	2.3	4.9
Zinc (mg)	3.1	1.6	2.3	2.4	3.7	3.0	0.7	1.4	2.8	2.2
Thiamine (mg)	0.33	0.37	0.42	0.59	0.21	0.33	0.33	0.06	0.42	0.49
Riboflavin (mg)	0.25	0.13	0.19	0.11	0.01	0.10	0.09	0.06	0.10	0.17
Folic acid (mg)	45.5	20	18.3	15.0	9.0	-	23.1	8.0	20	36.6
Fiber (g)	1.2	1.6	3.6	8.0	7.6	9.8	9.0	0.2	2.7	1.2

Source: NIN (1989).

(55). For instance, Canahua, an underutilized Andean grain, is remarkably frost tolerant, a key trait of many NUS. Frost-tolerant NUS crops can be grown where high-input major staples fail and are generally more resistant to local pests and diseases. Thus, NUS provide a safety net when the weather turns bad, or external inputs become undesirable as they damage the environment, become unavailable during disasters and emergencies, or become unaffordable due to high prices (6).

Neglected and underutilized crops offer more options for building temporal and spatial diversity into cropping systems. Some NUS have considerable commercial value, such as vegetables and fruits, which can improve household income. Being locally available/adaptable, NUS are accessible and affordable for the local population and therefore contribute to food security and nutrition, livelihood improvements, and cultural diversity (56).

In sum, NUS crops offer superior nutritional value for improving micronutrient deficiencies and addressing NCDs for millions of people. Their resistance to climate change implies that NUS can provide food when other crops fail.

FAO's REGIONAL INITIATIVE ON ZERO HUNGER ON FUTURE SMART FOOD

Given the multidimensional benefits that NUS offer, and considering that not all NUS are nutrient-dense or climate-resilient, the FAO, in collaboration with national and international partners, under its Regional Initiative on Zero Hunger (RI-ZH), launched a Future Smart Food (FSF) Initiative to support countries in the identification of NUS with high potential to be integrated into agricultural and food systems. The FSF initiative's scope does not include invasive plants and weed species and is focused on crops and their products. Future Smart Food is defined as NUS that are nutrient-dense, climate-resilient, economically viable, and locally available or adaptable (6). Only NUS that met four criteria qualify as FSF, being:

a) nutrient-dense (nutrients)
b) climate-resilient (e.g., require low inputs, promote climate change resiliency, environmentally friendly by reducing runoff and erosion)
c) economically viable (generate income and reduce female drudgery)
d) locally available or adaptable (6).

A regional priority-setting exercise for scoping and prioritizing, led by the FAO, supported countries in identifying and prioritizing NUS that qualify as FSF. Considering that NUS covers crop, livestock, fisheries and aquaculture, and forest, FAO started with crop among NUS in the FSF initiative. The FSF initiative started with an interdisciplinary priority-setting exercise comprising three phases in eight countries in Asia: Bhutan, Bangladesh, Cambodia, India (West Bengal), Lao PDR, Myanmar, Nepal, and Vietnam (6):

1) Stage 1: Scoping and identification of NUS (prior to Regional Expert Consultation)
   - Preliminary scoping report on the availability of NUS at the national level
   - Circulation of a preliminary scoping report
   - Review of a preliminary scoping report by international experts designated independently by partner institutions.
2) Stage 2: Validation and prioritization of NUS (during Regional Expert Consultation)
   - Joint validation of preliminary scoping reports from the selected countries
   - Ranking of high-potential NUS according to the four prioritization criteria (i.e., nutrient-dense, climate-resilient, economically viable, and locally available or adaptable)
   - Prioritization of 5–6 NUS crops per country.
3) Stage 3: Mapping
   - Mapping of selected NUS according to their geographical availability/prominence using geographic information system
   - Preparation of GIS reports on selected crops by country.

The regional priority-setting exercise targeted the following food crops groups: (a) cereals, (b) roots and tubers, (c) nuts and pulses, (d) horticulture, and (e) others. Those NUS present in the national gene banks were considered for the exercise. The four FSF prioritization criteria were adapted as (a) nutritional benefits, (b) agricultural sustainability, (c) ecological sustainability, and (d) socioeconomic sustainability, with each criterion further broken down into a series of parameters (e.g., water requirement, drought tolerance, area under cultivation), requiring experts to provide an aggregated dataset on NUS related to each criterion (6). The FSF initiative also established the principle of country ownership. The NUS scoping and prioritization results are owned by the participating country. Considering that NUS is contingent on the local context of each country, a species considered as NUS in one country may not be in another country (6).

Applying the same methodology as above, each country nominated national experts by the Ministry of Agriculture and prepared the national scoping reports on promising NUS based on the expertise required as per the guidelines for preparing a national report by the FAO. The NUS scoping and prioritization exercises were entirely country-driven, and the resulting NUS priority lists were determined by a multidisciplinary review of scientific data and specific conditions of the participating country. At the end of the exercise, 39 FSFs were selected and prioritized by the eight countries (**Figure 7**). All chosen FSFs have the potential to transform current conventional agricultural practices into more sustainable, nutrient-sensitive, and climate-resilient agriculture systems (6).

The integration of FSFs into farming systems has promise for transforming the current agricultural and food system into a more sustainable, nutrient-sensitive, and climate-resilient system (6). While over-dependency on rice with less nutritional properties in comparison to FSF such as quinoa and pulses leads to insufficient intake of nutrient-rich foods (57–60), noting rice-based diet is dominated in Asia culturally, it is culturally more acceptable, technically sound, environmentally mutual beneficial to *integrate* FSF into rice-based production system in Asia, rather than *replace* FSF with rice. The selected FSFs are adapted to different farming systems and agro-ecological zones in the region. **Table 2** lists some examples of prioritized FSFs that could be integrated into mountain agriculture and food systems (61).

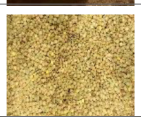
WAY FORWARD

Future smart foods can play a key role in transforming agriculture and food systems into diversified, nutrition-sensitive, and climate-resilient if they are mainstreamed into farming systems. Prioritization of NUS as FSFs is the first step. Moving forward, the FSF value chain must be promoted from production, post-harvest and processing, marketing to consumers, and all stages of the food system are connected

Cereals	Roots & Tubers	Pulses	Fruits & Vegetables	Nuts, Seeds & Spices
Buckwheat	Taro	Grass pea	Drumstick	Linseed
Tartary buckwheat	Swamp taro	Faba bean	Chayote	Walnut
Foxtail millet	Purple yam	Cow pea	Fenugreek	Nepali butter tree
Proso millet	Fancy yam	Mung bean	Snake gourd	Perilla
Finger millet	Elephant's foot yam	Black gram	Pumpkin	Nepali pepper
Sorghum	Sweet potato	Rice bean	Roselle	
Amaranth		Lentil	Indian gooseberry	
Grain amaranth		Horse gram	Jack fruit	
Quinoa		Soybean	Wood apple	
Specialty rice				

FIGURE 7 | Selected future smart foods in eight countries in Asia Source: Li and Siddique (6).

TABLE 2 | FSF examples for mountain areas.

FSF	Image	Nutritional and climate-resilient traits	Country
Lentil		• Second-highest ratio of protein • Huge potential to be grown as a winter crop in warm temperate and subtropical zones	Bhutan, India
Buckwheat		• Rich in iron and zinc—deficiencies of which are a major cause of hidden hunger • Cultivated from alpine to subtropical regions	Bhutan
Mung bean		• High in protein, resistant starch, and dietary fiber • Short growing cycle, increased adaptability, drought tolerant	Bangladesh, Nepal, and Viet Nam
Taro		• Rich in carbohydrates and high levels of calcium and vitamin A • Cultivatable in a wide range of areas; multipurpose vegetable with high market value	Bangladesh, Cambodia, Lao PDR, Nepal, Viet Nam, and India
Drumstick		• Rich in calcium, potassium, vitamin A, vitamin C, and protein • Popular vegetable with medicinal value • Powerful anti-inflammatory and antioxidant properties • Fast-growing, drought-resistant	Bhutan, Cambodia, Myanmar, Nepal, Viet Nam, Bhutan, India, Lao PDR
Quinoa		• Rich in fiber, antioxidants, protein, iron, and zinc • Climate-resilient; adapts well to various altitudes	Bhutan, Nepal and Lao DPR
Foxtail millet		• Helps to control blood sugar levels and reduces the risk of heart attack • Climate-resilient crop; grows in a wide range of agro-climatic conditions • Suitable for cultivation in marginal soils of char land	Bangladesh and India

Adapted from Li and Siddique (6).

to minimize transaction costs (61). Future smart foods need to be produced and marketed in large quantities to guarantee economies of scale and access to upmarket outlets (groceries, supermarkets, export markets). It involves addressing many constraints: from better management of genetic resources, production, processing, and marketing of crops to

FIGURE 8 | Development stages of food systems for future smart foods Source: FAO (62).

educating consumers on the nutritional and healthy benefits of FSF.

A holistic food systems approach for FSF has been developed as follows (see also **Figure 8**):

1) Prioritization: identify and prioritize NUS as potential FSFs.
2) Production: increase the production of targeted mountain FSFs in farming systems adaptable to various agro-ecological zones.
3) Processing: improve the efficiency of post-harvest and processing of FSFs.
4) Marketing: promote the distribution and marketing of FSFs.
5) Consumption: increase the demand for FSFs among consumers by increasing awareness and knowledge on their multidimensional benefits, including nutritional value (61).

Improving value chains for FSFs is critical, which will contribute to increased farmer income and agricultural diversification. Recent examples for the re-discovery of NUS include the growing markets for quinoa, growing sales of farro (an ancient wheat variety, often translated as spelt or emmer) in Italy, and the domestication of tropical fruit trees, such as *Dacryodes edulis* (sometimes called bush butter tree), in West Africa.

Creating an enabling environment for promoting FSF production, marketing, and consumption is crucial. Governments need to tap into the immense potential of alternative NUS crops. Governments can create incentives or remove disincentives, upgrade basic infrastructure to facilitate market access and reduce transaction costs, and improve licensing and legal frameworks to promote FSF and encourage agricultural diversification. Public policies promoting FSF as components of sustainable diets could encourage their use. Incentives can support farmers to grow and conserve NUS on-farm and *ex-situ*. Governments should mainstream FSF best practices, methods, and tools into routine operations, particularly for the rural poor, who suffer the most from production and nutrition gaps, shocks, and uncertainties (63). Traditional food systems in Asia have developed over hundreds of years, featuring an abundance of nutritionally dense and climate-resilient foods: promoting these alternative options offers increased yield potential and an opportunity to diversify dietary patterns and generate income for the rural poor (6).

CONCLUSION

While substantial progress has been made, countries in the Asia Pacific region face a high prevalence of hunger and all forms of malnutrition. Why? One of the main causes of malnutrition is an inadequate diet, with insufficient nutrients, minerals, and vitamins to grow and maintain a healthy body. Poor dietary diversity leads to an inadequate diet in terms of quality and hence malnutrition. Dietary diversity is alarmingly low among young children in the Asia Pacific region. Policies aimed at preventing malnutrition through healthy diets are most effective. Unfortunately, there is no one-size-fits-all healthy diet—healthy diets must be affordable, based on locally available foodstuffs, and meet cultural preferences.

Neglected and underutilized species offer enormous opportunities for fighting poverty, hunger, and malnutrition. Many NUS have superior nutritional values for improving micronutrient deficiencies. Many NUS can tolerate various stresses, which would not only make production systems more diverse but also more sustainable and climate-resilient. Their resistance to climate change implies that NUS can provide food when other crops fail. To identify NUS that are nutrient-dense, climate-resilient, economically viable, and locally available or adaptable, the FAO launched a Future Smart Food Initiative to support countries in identifying NUS with high potential for integration into agricultural systems. Consequently, 39 FSFs were selected and prioritized by the countries as the first step, all of which could transform current conventional agricultural practices into more sustainable, nutrition-sensitive, and climate-resilient agriculture systems. Moving forward, future endeavors should promote FSFs in terms of production, post-harvest and processing, marketing, and consumption.

AUTHOR CONTRIBUTIONS

XL and KS conceived the idea and returned the first draft. All authors contributed subsequent revisions of manuscript.

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Bioactive Components and Radical Scavenging Activity in Selected Advance Lines of Salt-Tolerant Vegetable Amaranth

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Four selected advance lines of salt-tolerant vegetable amaranth were evaluated for proximate, nutraceuticals, pigments, phytochemicals, and antioxidants components antioxidants activity in completely randomized block design (RCBD) design in three replicates. Salt-tolerant vegetable amaranth contained adequate carbohydrates, protein, moisture, and dietary fiber. The remarkable contents of iron, manganese, copper, zinc, sodium, molybdenum, boron, potassium, calcium, magnesium, phosphorus, sulfur, betacyanins, betalains, betaxanthins, chlorophylls, ascorbic acid, polyphenols, flavonoids, and antioxidant potentiality were found in salt-tolerant vegetable amaranth. The genotypes LS7 and LS9 had abundant proximate, nutraceuticals, pigments, phytochemicals, and antioxidants compared to the genotypes LS3 and LS5. Salt-tolerant vegetable amaranth demonstrated high content of flavonoid compounds including flavonols such as rutin, kaempferol, isoquercetin, myricetin, hyperoside, and quercetin; flavanol, such as catechin; flavone such as apigenin; and flavanone, such as naringenin. For the first time, we identified one flavonol such as myricetin; one flavanol, such as catechin; one flavone such as apigenin; and one flavanone, such as naringenin in salt-tolerant vegetable amaranth. Across six flavonols, rutin and quercetin were identified as the most prominent compounds followed by isoquercetin and myricetin in selected salt-tolerant vegetable amaranths. Across the genotypes, LS7 exhibited the highest flavonols such as rutin, kaempferol, isoquercetin, myricetin, hyperoside, and quercetin as well as the highest flavanols, such as catechin; flavones such as apigenin; and flavanones, such as naringenin. It revealed from the correlation study that antioxidant components of salt-tolerant vegetable amaranth genotypes exhibited good radical quenching capacity of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl equivalent to Trolox. The two genotypes LS7 and LS9 of vegetable amaranth containing excellent sources of proximate, nutraceuticals, pigments, phytochemicals, and antioxidants components could be used as potent antioxidants to attaining nutrients and antioxidant sufficiency in the saline prone area of the globe. We can extract colorful juice from the genotypes LS7 and LS9 as drink purposes for

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consuming the nutraceuticals and antioxidant deficient community in the saline prone area around the world. However, further detail experimentation is required to confirm the standardization and stabilization of functional components of vegetable amaranth for extraction of juice as drinks.

Keywords: nutraceuticals, pigments, polyphenols and flavonoids, vitamin C, antioxidant activity, HPLC, LC-MS-ESI, abiotic stress-tolerant underutilized vegetables

INTRODUCTION

Foods' acceptability mostly depends on color, flavor, and taste. For this reason, recently coloring food products have been put forward as they considerably accepted the common interest of the people around the globe. These products interested the consumers in the safety, nutritional, and aesthetic aspects of foods. These products also increase the consumption of natural pigments including betacyanins, betaxanthins, betalains, anthocyanin, amaranthine, chlorophylls, and carotenoids. Vegetable amaranth is a unique source of betalains (betaxanthins and betacyanins) that has important free radical-scavenging activity (1). Betalains could be used as a food colorant in low-acid foods and it has higher pH stability than anthocyanins (2). Amaranthine, a major pigment of betacyanins in vegetable amaranth had very strong antioxidant potentials. It could be used as a substitute source for the well-known betanins from red beets in the food colorants and natural antioxidants (1). Vegetable amaranth has wide adaptability to different abiotic stresses like drought (3–6) and salinity (7–9).

Amaranth (belongs to the family *Amaranthaceae*) is C_4 and a fast-growing plant with versatile uses such as for ornamental plants, vegetables, and grains. It has wider acclimatization and distributed in America, Africa, Australia, Asia, and Europe. Edible stems and leaves of vegetable amaranth are low-cost vegetables and have abundant protein with important amino acids including methionine and lysine, dietary fiber, carotenoids, vitamin C, minerals, such as calcium, magnesium, potassium, phosphorus, iron, zinc, copper, and manganese (10–15). This genus has many traditional medicinal uses, especially as antiviral, antimalarial, antidiabetic, antibacterial, antihelminthic, and snake antidote (16, 17). It has also abundant antioxidant pigments, such as betacyanins, anthocyanin, betaxanthins, betalains, carotenoids, and chlorophylls (18, 19); and antioxidant phytochemicals, such as vitamin C, phenolic acids, and flavonoids (20). These natural compounds have a remarkable contribution to the industry of food as they scavenge reactive oxygen species (ROS) in the human body and remedy several diseases like cardiovascular diseases, cancer, cataracts, atherosclerosis, retinopathy, arthritis, emphysema, and neurodegenerative diseases (21–24).

Morphologically amaranth is red and green in color (25). Red color amaranth has more pigments like amaranthine, betacyanins, anthocyanin, betaxanthins, carotenoids, and betalains than green color amaranth. Vegetable amaranth (*Amaranthus gangeticus*) has great variability and phenotypic diversity in Asia including Bangladesh and India (26) and has multipurpose uses. The selected genotypes are bright red-violet and maroon in color because of the presence of abundant betalains. It is popular and the cheapest leafy vegetables in Bangladesh and Asia. Its nutritional value, taste, and attractive leaf color has attracted people as a very popular vegetable in the Asian continent and elsewhere. In comparison to lettuce, Amaranth contains 18 times more vitamin A, 13 times more vitamin C, 20 times more calcium and 7 times more iron (27). Salt-tolerant vegetable amaranth leaves contain higher zinc and iron content than cassava leaves (28) and beach pea (29). Jimenez-Aguiar and Grusak (30) reported that potassium, calcium, magnesium, phosphorus, sulfur, manganese, iron, zinc, and copper in the leaves of amaranth were more pronounced than black nightshade, spinach, spider flower, black nightshade, and kale. In Bangladesh and India, Vegetable amaranth is grown year-round and even in the gaps of foliage crops between winter and hot summer (10, 11). Vegetable amaranth leaves inhibit the proliferation of colon (Caco-2) and breast (MCF-7) cancer cell lines and liver (HepG2) exhibit anticancer potential (31).

Recently, we have been exploring salt-tolerant vegetable amaranth genotypes containing high pigments, nutraceuticals, antioxidant phytochemicals, and phenolics of interest for making drinks for the sustainable health benefit of the consumers in the salinity-prone and coastal belt area of the globe. For this purpose, previously, we evaluated germplasms based on salt-tolerance, high yields, and antioxidant potential and four selected advance lines of salt-tolerant vegetable amaranths. It is the first attempt to study the pigments, nutraceuticals, antioxidant phytochemicals, phenolic and flavonoids, and antioxidant capacity in salt-tolerant vegetable amaranth. We ultimately study the possibility of the salt-tolerant genotypes for extracting colorful juice as drink purposes containing abundant pigments, nutraceuticals, antioxidant phytochemicals, phenolics, antioxidant capacity, and flavonoids.

MATERIALS AND METHODS

Experimental Materials

This is the first report on phenolic profiles, antioxidant compositions, and antioxidant capacity in salt-tolerant vegetable amaranth. We previously evaluated several genotypes based on salt tolerance, antioxidant, and yield potentiality to select the best four high-yielding and antioxidant-enriched salt-tolerant genotypes for this experiment.

Design and Layout

We executed the experiment in three replicates following a completely randomized block design (RCBD) at Bangabandhu Sheikh Mujibur Rahman Agricultural University. Each genotype

was grown in a 1 m^2 experimental plot following 20 cm and 5 cm distances between rows and plants, respectively.

Intercultural Practices

Recommended compost doses, fertilizer, and appropriate cultural practices were maintained (32). For maintaining the exact spacing of plants in a row, proper thinning was executed. Weeds of experimental plots were regularly removed through proper weeding and hoeing. We provided regular irrigation in the experimental plots for maintaining the proper growth of vegetable amaranth. Leaves from 35-day-old plants were sampled for all biochemical analyses.

Solvent and Reagents

Solvent: Acetone, hexane, and methanol. Reagents: dithiothreitol (DTT), cesium chloride, $HClO_4$, HNO_3, H_2SO_4, ascorbic acid, standard compounds of pure Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid), Folin-Ciocalteu reagent, gallic acid, DPPH, rutin, $ABTS^+$, 2, 2-dipyridyl, aluminum chloride hexahydrate, potassium acetate, sodium carbonate, and potassium persulfate.

Estimation of Proximate Composition

ASAE standards were followed to estimate moisture content (3). In triplicates, the fresh samples of vegetable amaranth leaves were oven-dried for 72 h at 103°C. Then the samples were transferred to a desiccator and allowed to stand at room temperature for cooling. A Denver digital balance (USA) was used to record the weights of the samples. AOAC method described by Sarker and Oba (3) was followed to estimate the ash, crude fat, fiber, crude protein contents, and gross energy. The weight of leaf samples was recorded before and after heat treatment (550°C for 12 h) to estimate ash content. Crude fat content was determined according to AOAC method 960.39. Crude protein was assessed by the micro-Kjeldahl method described by Sarker and Oba (3). Finally, nitrogen was multiplied by 6.25 to measure crude protein (AOAC method 976.05). ISO method described by Sarker and Oba (3) was followed to determine fiber content. Powdered leaf samples were boiled for 30 min adding 0.255 M sulfuric acid. The insoluble residue was filtered again, washed, and boiled in 0.313 M sodium hydroxide. After filtering and washing the sample, it was dried at 130 ± 2°C for 2 h. At 350 ± 25°C temperature, the loss of weight was measured. Fiber content was expressed as fresh weight (FW). The total moisture, crude protein, ash, and crude fat (%) were subtracted from 100 for calculating carbohydrate (g 100 g^{-1} FW). A bomb calorimeter was used to measure gross energy according to ISO method 9831 method described by Sarker and Oba (3).

Estimation of Mineral Composition

The fresh leaf samples of salt-tolerant vegetable amaranth were dried in an oven at 70°C for 24 h. Dried samples were ground in a mill. We determined calcium, potassium, magnesium, phosphorus, sulfur, iron, manganese, copper, zinc, sodium, molybdenum, and boron from powdered leaves following the nitric-perchloric acid digestion method (3). For this digestion, in the presence of carborundum beads, 40 ml $HClO_4$ (70%), 400 ml HNO_3 (65%), and 10 ml H_2SO_4 (96%) were added to 0.5 g dried leaf sample. After digestion, the ascorbic acid method was followed to measure P through dilution of the solution appropriately in triplicate. We added ascorbic acid and antimony to the yellow-colored complex solution for converting it to a blue-colored phosphomolybdenum complex. The method of Sarker and Oba (3) was followed to read the absorbance by atomic absorption spectrophotometry (AAS) (Hitachi, Tokyo, Japan) at a wavelength of 285.2 nm (magnesium), 76 6.5 nm (potassium), 880 nm (phosphorus), 258.056 nm (sulfur), 248.3 nm (iron), 422.7 nm (calcium), 279.5 nm (manganese), 213.9 nm (zinc), 324.8 nm (copper), 589 nm (sodium), 313.3 nm (molybdenum), and 430 nm (boron).

Determination of Chlorophylls

Chlorophyll *ab*, chlorophyll *b*, and chlorophyll *a* were calculated by extracting the fresh leaves in acetone (80%) (3). A spectrophotometer (Hitachi, U-1800, Tokyo, Japan) was used to measure the absorbance at 646 nm for chlorophyll *b* and 663 nm for chlorophyll *a*, respectively. Chlorophylls were calculated as micrograms per gram of FW.

Betacyanins and Betaxanthins Content Measurement

The fresh leaves were extracted in 80% methyl alcohol having 50 mM ascorbate to measure betacyanins and betaxanthins according to the method of Sarker and Oba (33, 34). A spectrophotometer (Hitachi, U-1800, Tokyo, Japan) was used to measure the absorbance at 540 nm for betacyanins and 475 nm for betaxanthins, respectively. The data were calculated as the ng betanin equivalent per g of FW for betacyanins and ng indicaxanthin equivalent per gram of FW for betaxanthins.

Estimation of Ascorbic Acid

A Hitachi spectrophotometer (U-1800, Tokyo, Japan) was utilized to estimate ascorbic acid (AsA) and dehydroascorbic acid (DHA) from the fresh leaves. Dithiothreitol (DTT) was used for the sample pre-incubation and reduction of dehydroascorbic acid into ascorbic acid. Ascorbic acid reduced ferric ion to ferrous ion. Reduced ferrous ion forms complexes with 2, 2-dipyridyl (35, 36). We read the absorbance of Fe^{2+} complexes with 2, 2-dipyridyl at 525 nm for estimation of vitamin C through the spectrophotometer (Hitachi, U-1800, Tokyo, Japan). We calculated vitamin C in mg 100 g^{-1} FW.

Estimation of Total Polyphenols

Extraction of total polyphenols was carried out according to Jimenez-Aguilar and Grusak (30) using 25 mg of fresh sample in 2.5 mL of 1.2 M HCl containing methanol (90%) at 90°C for 2 h in a water bath. With readjusting the volume (2.5 mL), the leaf extract was centrifuged at 7,500 rpm for 20 min. The leaf extracts (100 μL) were added to the Folin-Ciocalteau reagent (2 N, 50 μL); after 5 min, 2 N Na_2CO_3 (400 μL) and water (1 mL). The leaf extracts were incubated for 90 min at 37°C. Finally, it was removed to a microplate (flat bottom). In a microplate reader,

the absorbance was detected at 740 nm using gallic acid (GAE) as standard $\mu g\ g^{-1}$ of FW.

Estimation of Total Flavonoids

Total flavonoids were extracted and quantfied according to the method described by Jimenez-Aguilar and Grusak (30). Dry leaf samples (100 mg) were mixed with 5 mL methanol (50%) in water and placed for 1 h with ultrasound. The leaf extracts were centrifuged for 10 min at 13,000 g (4°C). The supernatants were then recovered. Flavonoid extracts (400 μL) were homogenized with water (500 μL), 5% $NaNO_2$ (60 μL), 10% AlCl3 (140 μL). After 10 min, 1 mM NaOH (400 μL) was added. The leaf extracts were incubated for 10 min at a normal temperature. Finally, it was removed to a flat bottom microplate. The absorbance was read at 500 nm in a microplate reader. Results are expressed in μg of rutin equivalents (RE) per gram of sample DW.

Radical Quenching Capacity Assay

Fresh leaves were harvested from 35-day-old plants. For the antioxidant capacity assay, the leaves were dried in the air in a shade; 40 ml aqueous methanol (90%) was utilized to extract ground dried leaves (1 g) from each cultivar in a capped bottle (100 ml). A Thomastant T-N22S (Thomas Kagaku Co. Ltd., Japan) shaking water bath was utilized to extract leaf samples for 1 h. An exactly 0.45 μm filter (MILLEX-HV syringe filter, Millipore Corporation, Bedford, MA, USA) was used to filter the homogenized mixture. After centrifugation for 15 min at 10,000 × *g*, the antioxidant capacity was estimated from the filtered extract.

Diphenyl-picrylhydrazyl (DPPH) radical degradation method (37, 38) was used to estimate the antioxidant activity. We added 1 ml DPPH solution (250 μM) to 10 μl extract (in triplicate) in a test tube. After adding 4 ml distilled water the extract was placed in the dark for 30 min. A Hitachi U1800 spectrophotometer (Hitachi, Tokyo, Japan) was used to measure the absorbance at 517 nm. The method of Khanam et al. (39) was followed for $ABTS^+$ assay. To prepare two stock solutions separately an $ABTS^+$ solution of 7.4 mM and potassium persulfate of 2.6 mM was used. We mixed both solutions in equal proportion to prepare the working solution at room temperature. The working solution was allowed to react in the dark for 12 h; a 150-μl extract was added to 2.85 ml of $ABTS^+$ solution and allowed to react in the dark for 2 h. For the preparation of the solution, 1 ml of $ABTS^+$ solution was mixed with 60 ml of methanol. A Hitachi spectrophotometer (U1800, Tokyo, Japan) was utilized to take the absorbance against methanol at 734 nm. The inhibition (%) of DPPH and $ABTS^+$ corresponding with control was used to determine antioxidant capacity using the equation as follows:

$$\text{Antioxidant activity}(\%) = (\text{Abs. blank} - \text{Abs. sample}/\text{Abs. blank}) \times 100$$

Where, Abs. blank is the absorbance of the control reaction [10 μl methanol for TAC (DPPH), 150 μl methanol for TAC ($ABTS^+$) instead of leaf extract] and Abs. sample is the absorbance of the test compound. Trolox was used as the reference standard, and the results were expressed as μg Trolox equivalent g^{-1} DW.

Samples Extraction for HPLC and LC-MS Analysis

The fresh leaf samples were extracted by adding 10 ml methanol (80%) containing acetic acid (1%) in 1 g leaves. The mixture was thoroughly homogenized. Then the mixture was kept in a test tube (50 ml) and capped tightly. The test tube was shaken in a shaker (Scientific Industries Inc., USA) for 15 h at 400 rpm. An exactly 0.45 μm filter (MILLEX®-HV syringe filter, Millipore Corporation, Bedford, MA, USA) was used to filter the homogenized mixture. We centrifuged the mixture at 10,000 × *g* for 15 min. The flavonols, flavanols, flavones, and flavanones were analyzed from the final filtrate. We performed all extractions in triplicate independent samples.

Flavonols, Flavanols, Flavones, and Flavanones Analysis Through HPLC

The method previously described by Sarker and oba (4, 33) was followed to determine flavonols, flavanols, flavones, and flavanones in the fresh leaf sample using HPLC. We equipped the Shimadzu SCL10Avp (Kyoto, Japan) HPLC with a binary pump (LC-10Avp), DGU-14A degasser, and a Shimadzu SPD-10Avp UV–vis detector. A CTO-10AC (STR ODS-II, 150 × 4.6 mm I.D. (Shinwa Chemical Industries, Ltd., Kyoto, Japan) column was used for the separation of flavonols, flavanols, flavones, and flavanones. The binary mobile phase was pumped with solvent A [6% (v/v) acetic acid] in water and solvent B (acetonitrile) at the flow rate of 1 ml/min for 70 min. HPLC system was run using a gradient program with 0–15% acetonitrile for 45 min, 15–30% for 15 min, 30–50% for 5 min, and 50–100% for 5 min; 35°C temperature in the column was maintained with a 10 μl volume of injection (29). We set the detector at 360, 370, and 280 nm, respectively, for continuous monitoring of flavonols, flavanols, flavones, and flavanones. For identification of the compound, we compared the retention time and UV–vis spectra with their respective standards. We confirmed the flavonols, flavanols, flavones, and flavanones through the mass spectrometry assay method. We estimated phenolic compounds as mg kg^{-1} FW. A mass spectrometer (AccuTOF JMS-T100LP, JEOL Ltd., Tokyo, Japan) was fitted with an Agilent 1100 Series HPLC system and a UV–vis detector coupled on-line with an ElectroSpray Ionization (ESI) source to analyze the mass spectrometry with negative ion mode with the column elutes in the range of m/z 0–1,000 and needle voltage at −2000 V. Extract constituents were identified by LC-MS-ESI analysis.

Quantification of Phenolic Compounds

We used the respective standards of calibration curves to quantify each flavonols, flavanols, flavones, and flavanones. We dissolved 9 flavonol, flavanol, flavone, and flavanone compounds in 80% methanol as stock solutions to the final concentration of 100 mg/ml. Respective standard curves (10, 20, 40, 60, 80, and 100 mg/ml) were used to quantify the individual flavonols, flavanols, flavones, and flavanones compounds with external standards. UV spectral characteristics, retention times, and co-chromatography of samples spiked with commercially available

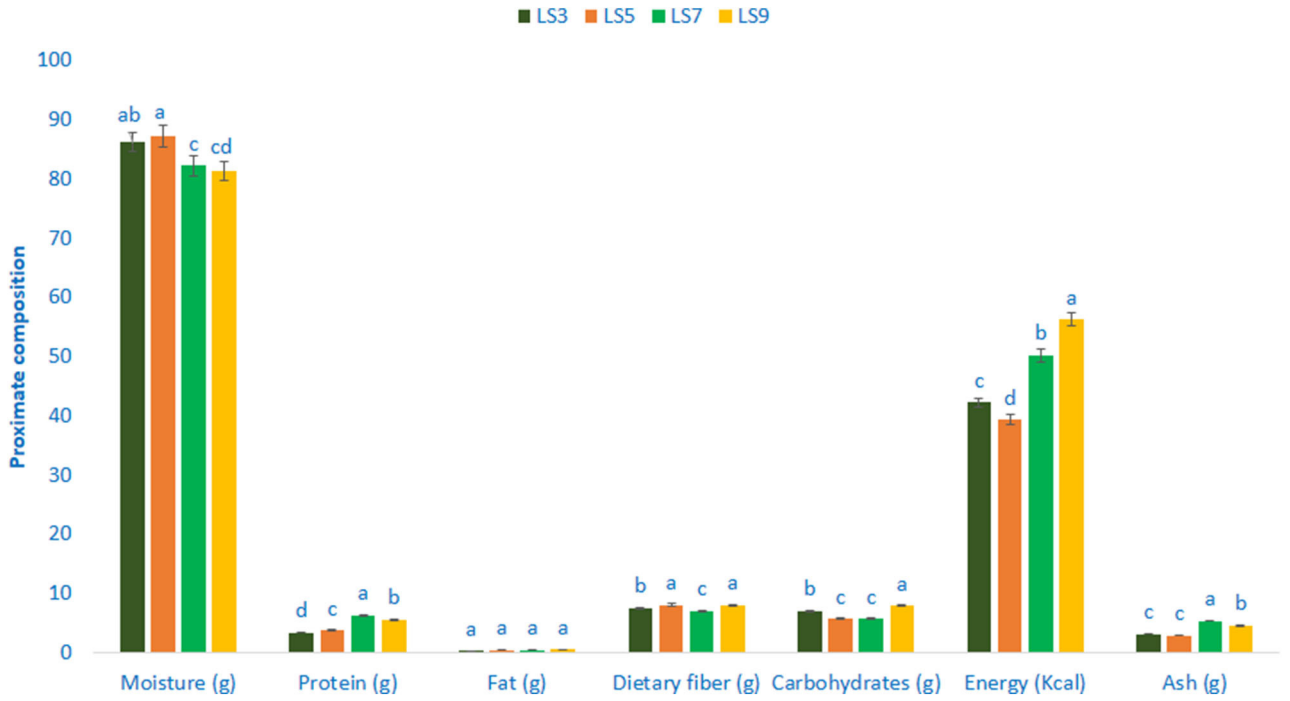

FIGURE 1 | Proximate compositions (g 100 g^{-1} FW) in four selected salt-tolerant vegetable amaranths; different letters are differed significantly by Duncan multiple range test ($P < 0.01$), ($n = 3$).

standards were utilized for identification and match the flavonols, flavanols, flavones, and flavanones.

Statistical Analysis

The Statistix 8 software was used to analyze the data for analysis of variance (ANOVA) (40, 41). Duncan's Multiple Range Test (DMRT) at 1% level of probability was used to compare the means. The results were reported as the mean ± SD of three separate replicates.

RESULTS

The analysis of variance revealed a wide range of variability of the studied traits regarding selected drought-tolerant leafy vegetable amaranths.

Composition of Proximate

The composition of the proximate of salt-tolerant vegetable amaranth is shown in **Figure 1**. The moisture content ranged from 81.35 to 87.24 g 100 g^{-1} FW. The highest moisture content was recorded in LS5 (87.24 g 100 g^{-1} FW), while the lowest moisture content was found in LS9 (81.35 g 100 g^{-1} FW). Salt-tolerant vegetable amaranth leaves exerted significant and very much noticeable variations in protein content. The genotype LS7 showed the highest protein content (6.34 g 100 g^{-1}) followed by LS9, whereas, the genotype LS3 had the lowest protein content (3.35 g 100 g^{-1}). There were no significant variations in fat content in terms of four selected salt-tolerant vegetable amaranths. The range of fat content was 0.33–0.57 g 100 g^{-1} FW. The genotype LS9 had the highest carbohydrates content (7.93 g 100 g^{-1} FW) followed by LS3, while the carbohydrates content was the lowest in LS5 and LS7 (5.73 and 5.64 g 100 g^{-1} FW, respectively). The genotype LS9 had the highest energy (56.28 kcal 100 g^{-1} FW) followed by LS7, while the lowest energy was obtained from the genotype LS5 (39.28 kcal 100 g^{-1} FW). Ash content was the highest in LS7 (5.36 g 100 g^{-1} FW) followed by LS9, while the lowest ash content was noted in LS5 and LS3 (2.88 and 3.13 g 100 g^{-1} FW). Content of digestible fiber exhibited the least variations in four selected salt-tolerant vegetable amaranths studied. The accession LS5 and LS9 showed the highest dietary fiber content (8.06 and 7.95 g 100 g^{-1} FW) followed by LS3, whereas dietary fiber content was the lowest in LS7 (6.98 g 100 g^{-1} FW).

Mineral Composition (Macroelements)

Mineral composition (macroelements) of salt-tolerant vegetable amaranth is shown in **Figure 2**. In this study, the range of potassium content was 4.66 mg g^{-1}-7.54 mg g^{-1} FW. The genotypes LS7 had the highest potassium content, while genotype LS5 had the lowest potassium content. Calcium content ranged from 1.68 to 3.25 mg g^{-1} FW. The genotypes LS7 showed the highest calcium content, while the genotype LS9 had the lowest calcium content. Magnesium content was the highest in LS3 (3.59 mg g^{-1} FW) followed by LS5 and LS9. In contrast, the lowest magnesium was recorded in LS7 (2.49 mg g^{-1} FW). Phosphorus and sulfur content of vegetable amaranth leaves ranged from

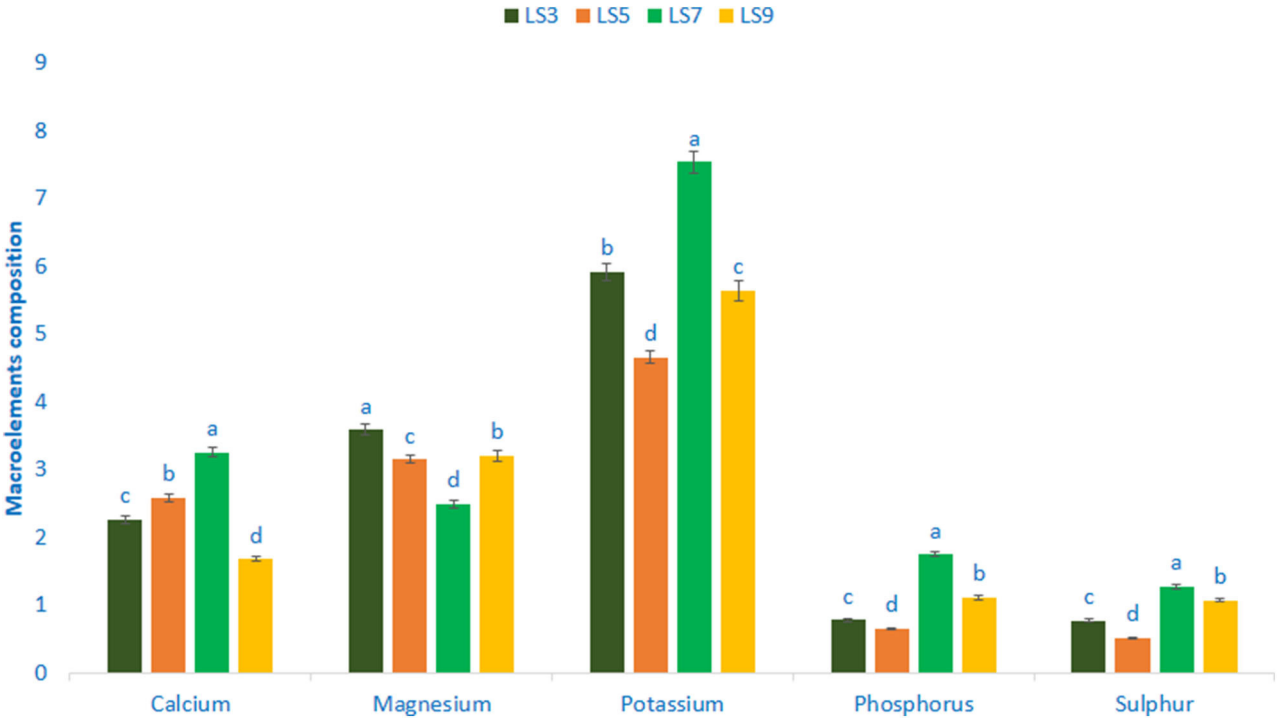

FIGURE 2 | Mineral compositions (Macroelements mg g^{-1} FW,) in four selected salt-tolerant vegetable amaranths; different letters are differed significantly by Duncan multiple range test ($P < 0.01$), ($n = 3$).

0.65 to 1.75 and 0.51 to 1.27 mg g^{-1} FW. The genotype LS7 exhibited the highest phosphorus and sulfur content, while the genotype LS5 showed the lowest phosphorus and sulfur content.

Mineral Composition (Microelements)

Minerals composition (Microelements) of salt-tolerant vegetable amaranth is shown in **Figure 3**. Salt-tolerant vegetable amaranth showed remarkable iron and manganese content. The genotype LS9 had the highest iron content (17.35 μg g^{-1} FW) followed by LS7 and LS5, whereas the genotype LS3 showed the lowest iron content (12.99 μg g^{-1} FW). In this study, the range of manganese content was 12.25 μg g^{-1} FW and 16.77 μg g^{-1} FW. The genotype LS7 had the highest manganese content; however, the genotype LS3 had the lowest manganese content. The significant and notable variations of copper content were reported in salt-tolerant vegetable amaranth genotypes (1.27–2.26 μg g^{-1} FW). The copper content was the highest in LS7, followed by LS9; whereas the lowest copper content was obtained from the genotype LS3 and LS5, respectively. Salt-tolerant vegetable amaranth showed remarkable zinc, sodium, and boron content. Zinc, sodium, and boron content ranged from 11.33 to 14.61, 72.24 to 84.29, and 5.27 to 7.36 μg g^{-1} fresh weight, respectively. The genotypes LS7 had the highest zinc, sodium, and boron content, while LS3 showed the lowest zinc and sodium, and LS5 had the lowest boron content. Molybdenum content ranged from 0.26 to 0.57 μg g^{-1} FW. The genotypes LS7 had the highest molybdenum content, while LS3 showed the lowest molybdenum content.

Pigments Composition

Pigments of four selected salt-tolerant vegetable amaranths are shown in **Figure 4**. Betalains, betaxanthins, and betacyanins varied significantly and remarkably with the genotypes. Betalains, betaxanthins, and betacyanins ranged from 542.35 to 1,029.12, 355.98 to 517.12, and 185.02 to 512.06 ng g^{-1} FW, respectively. The genotype LS7 exhibited the highest betacyanins content, followed by LS9. Conversely, the genotype LS3 had the lowest betacyanins content. Among genotypes, considerable and significant variations were observed in betaxanthins content. Betalains and betaxanthins content were the highest in genotype LS7 followed by LS9. In contrast, genotype LS3 showed the lowest betaxanthins and betalains. The significant and notable variations were noticed for chlorophyll *a* content (156.09–545.06 μg g^{-1} FW). The genotype LS7 had the highest chlorophyll *a* content (545.06 μg g^{-1} FW), whereas the lowest chlorophyll *a* was recorded in LS3 (156.09 μg g^{-1} FW). Similar to chlorophyll *a*, significant and marked differences in chlorophyll *b* content were noted in vegetable amaranth genotypes (64.90–394.35 μg g^{-1} FW). LS9 had the highest chlorophyll *b* content (394.35 μg g^{-1} FW), followed by LS7. Conversely, LS3 had the lowest chlorophyll *b* (64.90 μg g^{-1} FW). Total chlorophyll content showed significant and noticeable variation (221.62–879.42 μg g^{-1} FW). LS7 and LS9 exhibited abundant total chlorophyll

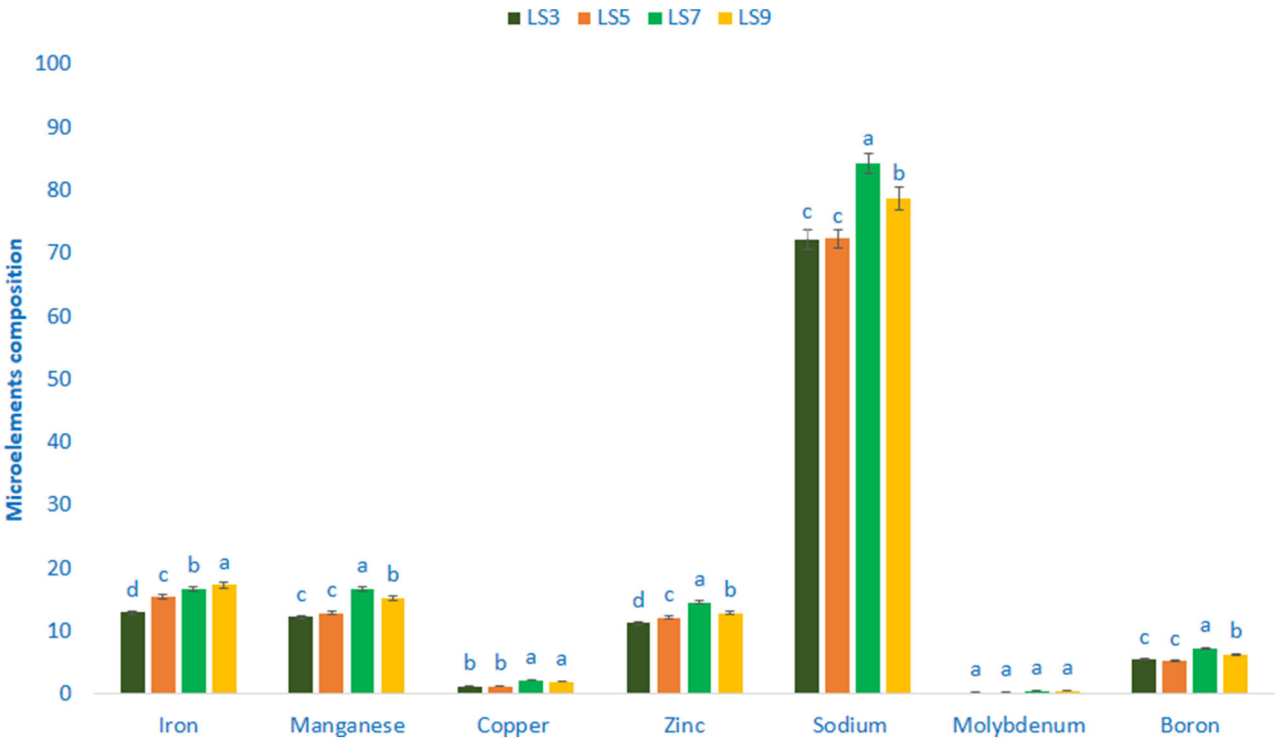

FIGURE 3 | Mineral compositions (Microelements μg g^{-1} FW) in four selected salt-tolerant vegetable amaranths, different letters are differed significantly by Duncan multiple range test ($P < 0.01$), ($n = 3$).

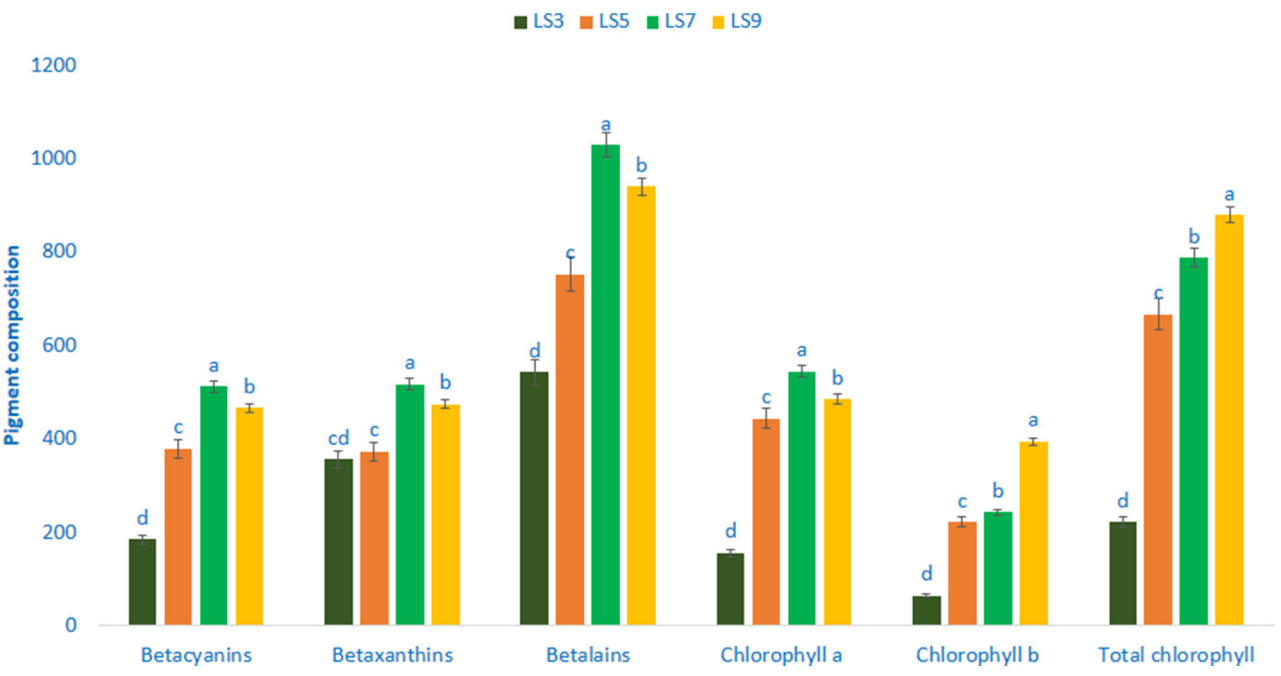

FIGURE 4 | Pigment composition in four selected salt-tolerant vegetable amaranths, betacyanins (ng g^{-1} FW), chlorophyll *a* (μg g^{-1} FW), betaxanthins (ng g^{-1} FW), chlorophyll *b* (μg g^{-1} FW), betalains (ng g^{-1} FW), total chlorophyll (μg g^{-1} FW); different letters in the bar are differed significantly by Duncan multiple range test ($P < 0.01$), ($n = 3$).

content, whereas, the lowest total chlorophyll content was obtained from LS3 (221.62 $\mu g\ g^{-1}$ FW).

Phytochemicals and Antioxidant Capacity

Polyphenols, flavonoids, ascorbic acid, and antioxidant capacity (AC) varied significantly among the studied salt-tolerant genotypes (**Figure 5**). Ascorbic acid content ranged from 72.45 mg 100 g^{-1} FW in the genotype LS3 to 152.96 mg 100 g^{-1} FW in the genotype LS7. Polyphenols ranged from 92.26 GAE $\mu g\ g^{-1}$ FW (LS3) to 184.76 GAE $\mu g\ g^{-1}$ FW (LS7). The genotype LS7 had the highest polyphenols followed by LS9. Flavonoids exhibited much noticeable variation in terms of genotypes, which ranged from 158.34 RE $\mu g\ g^{-1}$ DW in the genotype LS5 to 282.87 RE $\mu g\ g^{-1}$ DW in the genotype LS7. AC (DPPH) ranged from 13.35 TEAC $\mu g\ g^{-1}$ DW (LS3) to 35.36 TEAC $\mu g\ g^{-1}$ DW (LS7). The highest AC (DPPH) was recorded in the genotype LS7 followed by LS9 and LS5. In contrast, LS3 had the lowest AC (DPPH). AC ($ABTS^+$) ranged from 27.62 TEAC $\mu g\ g^{-1}$ DW to 70.24 TEAC $\mu g\ g^{-1}$ DW. The salt-tolerant vegetable amaranth genotype LS7 had the highest AC ($ABTS^+$) followed by LS9. In contrast, AC ($ABTS^+$) was the lowest in LS3.

Flavonols, Flavanols, Flavones, and Flavanones

Table 1 shows the data on main fragment ions in MS^2, identified compounds, the molecular ion, λ_{max}, and retention time. The liquid chromatography separated values of flavonols, flavanols, flavones, and flavanones compounds from four salt-tolerant leafy vegetable amaranths (LS3, LS5, LS7, and LS9) were compared with standard masses of flavonols, flavanols, flavones, and flavanones compounds through the respective peaks of the compounds. Nine flavonoids compounds were determined in salt-tolerant vegetable amaranth including six flavonols, such as rutin, kaempferol, isoquercetin, myricetin, hyperoside, and quercetin, one flavanol, such as catechin, one flavone such as apigenin, and one flavanone, such as naringenin. For the first time, we identified one flavonols such as myricetin, one flavanol, such as catechin, one flavone such as apigenin, and one flavanone, such as naringenin in salt-tolerant vegetable amaranth. **Figure 6** showed the identified flavonols compounds and **Figure 7** showed the identified flavanols, flavones, and flavanones compounds of leaves of four selected salt-tolerant vegetable amaranths. Across four principal groups of compounds, the most identified pronounced compounds in four selected salt-tolerant vegetable amaranths were observed in the following order: flavavanones > flavones > flavanols (**Figures 6**, **7**). Across six flavonols, rutin and quercetin were identified as the most prominent compounds followed by isoquercetin and myricetin in selected salt-tolerant vegetable amaranths. Across the genotypes, LS7 exhibited the highest flavonols such as rutin, kaempferol, isoquercetin, myricetin, hyperoside, and quercetin. LS5 contained high total flavonols which were statistically similar to LS3, while LS9 demonstrated the lowest flavonols. Rutin, kaempferol, isoquercetin, myricetin, hyperoside, and quercetin of selected salt-tolerant vegetable amaranths varied from 6.75 to 9.62, 2.42 to 4.88, 3.45 to 6.58, 3.28 to 5.68, 1.25 to 2.58, and 3.55 to 6.62 mg kg^{-1} FW, respectively (**Figure 6**). LS7 exhibited the highest flavanols, such as catechin, flavones such as apigenin, and flavanones, such as naringenin followed by LS9 (**Figure 7**). In contrast, LS3 showed the minimum flavanols, such as catechin. LS5 showed the minimum flavones such as apigenin which was statistically similar to LS3. Similarly, LS3 showed the minimum flavanones, such as naringenin which as statistically similar to LS5 and LS9 (**Figure 7**).

Correlation Coefficient Analysis

Correlation of antioxidant pigments and phytochemicals of salt-tolerant vegetable amaranth are shown in **Table 2**. Highly significant positive associations of betalains, betaxanthins, betacyanins, total chlorophyll, chlorophyll *b*, and chlorophyll *a* were exhibited among pigments and with AC ($ABTS^+$), AC (DPPH), ascorbic acid, polyphenols, and flavonoids. Ascorbic acid exerted significant associations with all traits along with AC ($ABTS^+$) and AC (DPPH). The significant associations of polyphenols and flavonoids were observed with AC ($ABTS^+$) and AC (DPPH). Similarly, a significant relationship of AC ($ABTS^+$) with AC (DPPH) validated antioxidant activity measurement of different methods in salt-tolerant vegetable amaranth.

DISCUSSION

The analysis of variance revealed a wide range of variability of the studied traits regarding selected drought-tolerant leafy vegetable amaranths. A wide range of variability was also reported in red and green color amaranth (33), rice (42–56), maize (57–59), and coconut (60, 61). The lowest moisture content was noted in the salt-tolerant vegetable amaranth genotypes LS9 and LS7. As leaf higher dry matter obtained from lower moisture contents, two genotypes (19–18% dry matter) had considerable dry biomass. The maturity is directly interrelated to the moisture content of leaves. The results obtained in this study were corroborated to the reports of *A. tricolor* and sweet potato leaves by Sarker and Oba (3) and Sun et al. (62), respectively. Salt-tolerant vegetable amaranth leaves exerted significant and very much noticeable variations in protein content. Poor people and vegetarians of the developing countries mainly depend on vegetable amaranth for their protein source. The protein content of salt-tolerant vegetable amaranth was much higher as compared to *A. tricolor* (1.26%) in our earlier study (11). There were no significant variations in fat content in terms of four selected salt-tolerant vegetable amaranths. Our results were corroborated with the results of Sarker and Oba (3) and Sun et al. (62) in *A. tricolor* and leaves of sweet potato, respectively. They reported that fat influences the cell function, covering the organs of the body, and upholding the temperature of the body. Fats have abundant omega-6 and omega-3 fatty acids. Fats play a significant role in digestion, absorption, and transport of vitamins E, D, A, and K that are soluble in fats.

The salt-tolerant vegetable amaranth genotypes showed considerable dietary fiber. Dietary fiber remarkably contributed to the cure of constipation, increment of digestibility, and palatability (13). It revealed from our results that leaves of salt-tolerant vegetable amaranth have abundant protein, moisture, carbohydrates, and digestible fiber. The results of this study

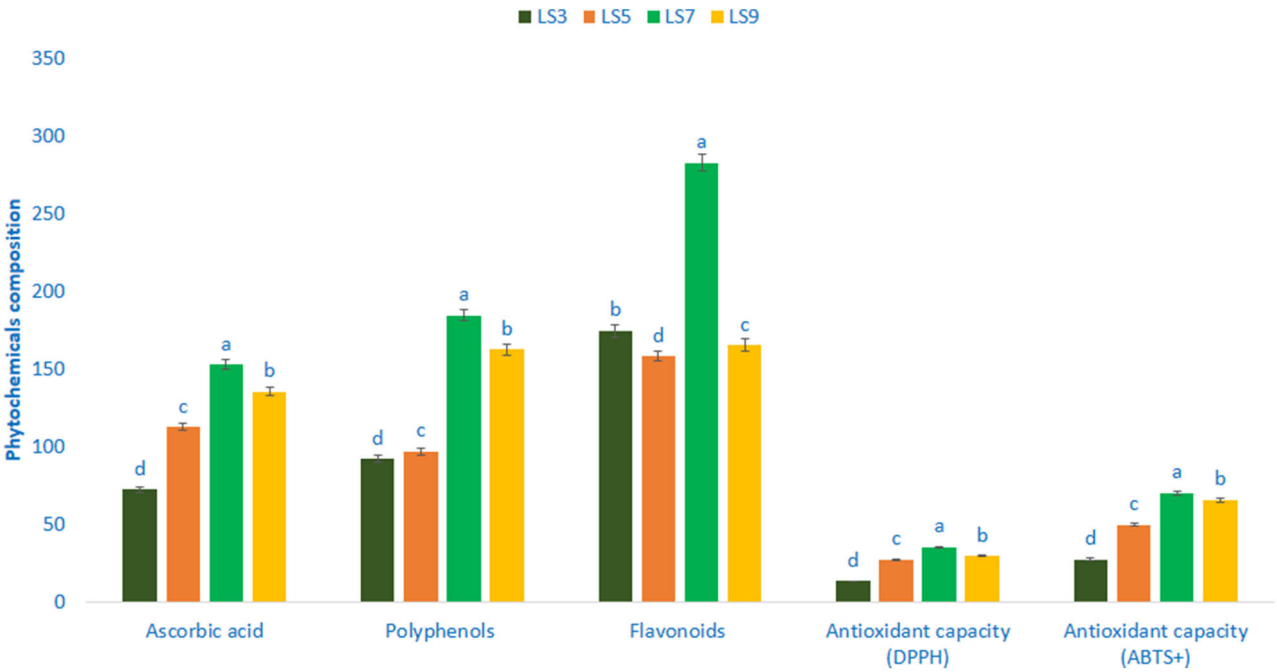

FIGURE 5 | Phytochemical composition and free radical scavenging capacity in four selected salt-tolerant vegetable amaranths, ascorbic acid (mg 100 g^{-1} FW), polyphenols (GAE μg g^{-1} FW), flavonoids (RE μg g^{-1} DW), antioxidant capacity (DPPH) (TEAC μg g^{-1} DW), antioxidant capacity ($ABTS^{+}$) (TEAC μg g^{-1} DW); different letters in the bar are differed significantly by Duncan multiple range test ($P < 0.01$), ($n = 3$).

TABLE 1 | Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data and tentative identification of flavonols, flavanols, flavones, and flavanones in four selected salt-tolerant vegetable amaranths.

Peak no	Rt (min)	λ_{max} (nm)	Molecular ion $[M–H]^-$ (m/z)	MS^2 (m/z)	Identity of tentative compounds
1	4.58	370	626.1882	626.2714	Myricetin-3-*O*-rutinoside
2	7.55	370	301.0426	301.0421	2-(3,4-dihydroxy phenyl)-3,5,7-trihydroxychromene-4-one
3	15.47	370	270.3432	270.3324	4′,5,7-Trihydroxyflavone, 5,7-Dihydroxy-2-(4-hydroxyphenyl)-4-benzopyrone
4	17.84	370	593.5312	593.3412	kaempferol-3-*O*-rutinoside
5	23.91	280	290.2287	290.2175	(2R-3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2-chromene-3,5,7-triol
6	26.74	280	271.0812	271.1621	Naringenin
7	54.36	360	463.3215	463.3287	Quercetin-3-*O*-glucoside
8	53.35	360	463.4621	463.5325	Quercetin-3-*O*-galactoside
9	53.36	360	609.3698	609.3574	Quercetin-3-*O*-rutinoside

corroborated with the results of our earlier study (3). The digestible fiber and carbohydrates contents obtained from the advance lines were corroborated with our previous studies of red morph amaranth (63), weedy amaranth (64), green morph amaranth (65), stem amaranth (66), and *A. blitum* (67). However, dry matter contents of these advance lines were greater than the dry matter contents of red morph amaranth (63), weedy amaranth (64), green morph amaranth (65), stem amaranth (66), and *A. blitum* (67). Except for weedy amaranth, protein contents of these advance lines were greater than the protein contents of red morph amaranth (63), green morph amaranth (65), stem amaranth (66), and *A. blitum* (67).

In our present study, we found remarkable potassium (7.54 mg g^{-1}), calcium (3.25 mg g^{-1}) magnesium (3.59 mg g^{-1}) phosphorus (1.75 mg g^{-1}), and sulfur (1.27 mg g^{-1}) in salt-tolerant vegetable amaranth. Chakrabarty et al. (15) in *A. lividus* and Sarker and Oba (3) in *A. tricolor* also observed similar results. Jimenez-Aguiar and Grusak (30) noted abundant potassium, calcium, magnesium, phosphorus, and sulfur in different amaranths. They also noticed that amaranth potassium, calcium, magnesium, phosphorus, and sulfur were much pronounced than black nightshade, spinach, spider flower, and kale. Salt-tolerant vegetable amaranth leaves contained higher zinc and iron content than the cassava leaves (28) and beach pea (29). In this study, we found remarkable iron (17.35 μg g^{-1}), manganese (16.77 μg g^{-1}), copper (2.26 μg g^{-1}), zinc (14.61 μg g^{-1}), sodium (84.29 μg g^{-1}), molybdenum (0.57 μg g^{-1}), and boron (7.36 μg g^{-1}) in salt-tolerant vegetable

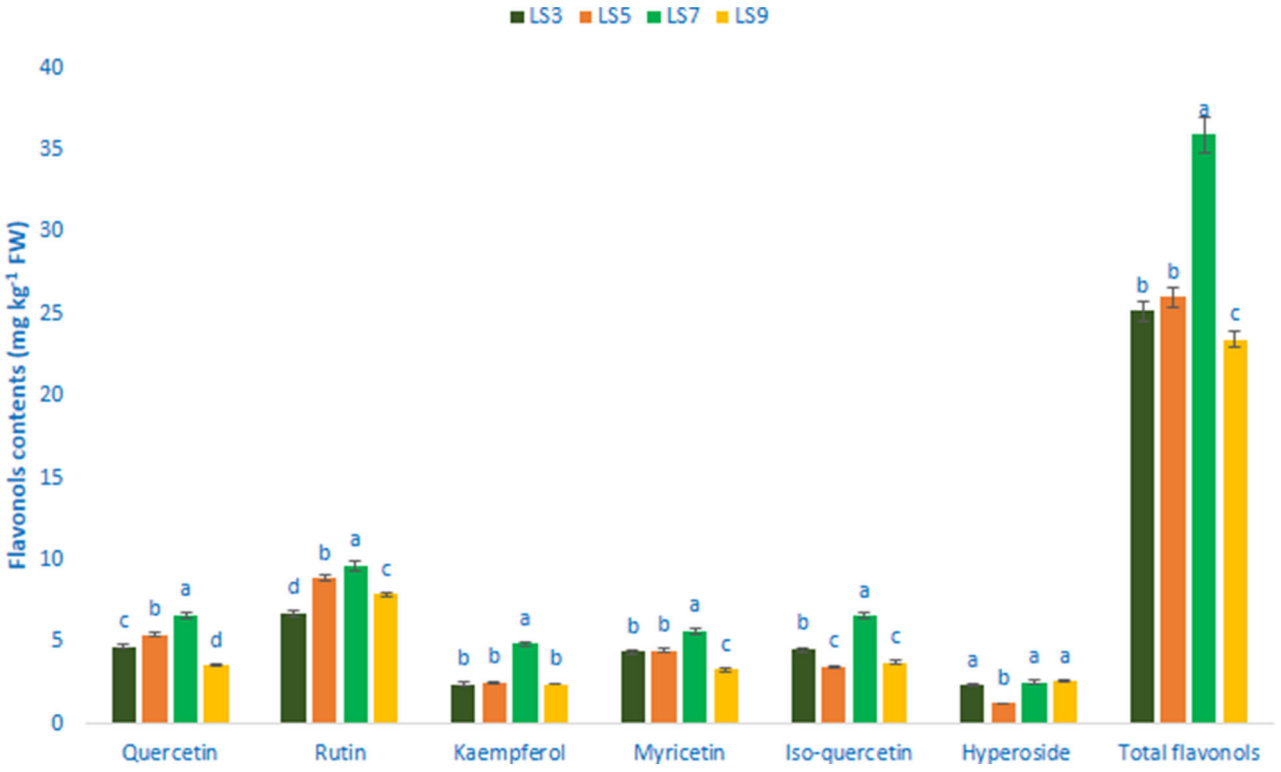

FIGURE 6 | Flavonol contents (mg kg^{-1} FW) in four selected salt-tolerant vegetable amaranths; different letters in the bar are differed significantly by Duncan multiple range test ($P < 0.01$), ($n = 3$).

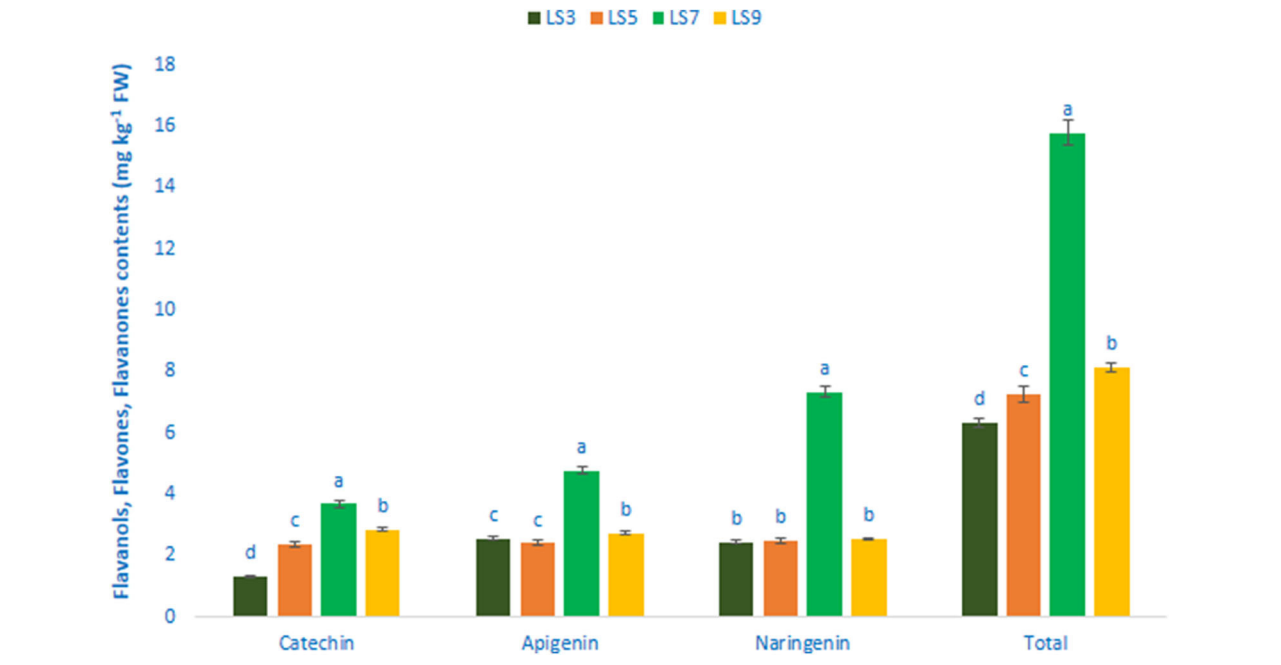

FIGURE 7 | Flavanol, flavone, and flavanone content (mg kg^{-1} FW) in four selected salt-tolerant vegetable amaranths; different letters in the bar are differed significantly by Duncan multiple range test ($P < 0.01$), ($n = 3$).

TABLE 2 | The correlation coefficient for pigments, phytochemicals, and antioxidant capacity in four selected salt-tolerant vegetable amaranths.

	Betaxanthins (ng g^{-1})	Betalains (ng g^{-1})	Chl *a* (μg g^{-1})	Chl *b* (μg g^{-1})	T chl (μg g^{-1})	Ascorbic acid (mg 100 g^{-1})	Polyphenols (GAE μg g^{-1} FW)	Flavonoids (RE μg g^{-1} DW)	AC (DPPH)	AC ($ABTS^+$)
Betacyanins	0.96**	0.94**	0.97**	0.95**	0.98**	0.78**	0.88**	0.96*	0.88*	0.89*
Betaxanthins		0.96**	0.92**	0.94**	0.97**	0.76**	0.81**	0.95*	0.91*	0.93*
Betalains			0.94**	0.86**	0.94**	0.78**	0.89**	0.93*	0.96*	0.98**
Chl *a*				0.96**	0.97**	0.75**	0.88**	0.96*	0.82**	0.95**
Chl *b*					0.97**	0.84**	0.84**	0.98**	0.93*	0.92**
T Chl						0.82**	0.87**	0.87**	0.89*	0.94*
Ascorbic acid							0.88**	0.95**	0.97**	0.88**
Polyphenols								0.91**	0.88**	0.87**
Flavonoids									0.88*	0.96*
AC (DPPH)										0.97**

*Chl a, Chlorophyll a; Chl b, Chlorophyll b; T chl, Total chlorophyll; Polyphenols (GAE μg g^{-1} FW); Flavonoids (RE μg g^{-1} DW); AC (DPPH), Antioxidant capacity (DPPH) (TEAC μg g^{-1} DW); AC ($ABTS^+$), Antioxidant capacity ($ABTS^+$) (TEAC μg g^{-1} DW); *significant at 5% level, **significant at 1% level, (n = 3).*

amaranth. Jimenez-Aguiar and Grusak (30) noted abundant iron, manganese, copper, zinc sodium, molybdenum, and boron in different amaranths. They also noticed that manganese, iron, zinc, and copper in the leaves of amaranth were pronounced than black nightshade, spinach, spider flower, black nightshade, and kale. Potassium contents obtained from these advance lines were corroborated with our previous studies of green morph amaranth (65), while calcium contents recorded in these advance lines were greater than red morph amaranth (63), stem amaranth (66), and *A. blitum* (67). We observed high phosphorus and sodium in the current investigation than our weedy amaranth (64). Similarly, iron, zinc, and magnesium obtained from the current investigation were much pronounced than our earlier investigation in red morph amaranth (63), green morph amaranth (65), stem amaranth (66), and *A. blitum* (67). We obtained high copper than our earlier study of green morph amaranth (65) and high manganese than weedy amaranth (64), green morph amaranth (65). Hence, these selected salt-tolerant advance lines could contribute as high minerals enriched genotypes than our previously tested amaranth genotypes.

In this study, we found remarkable total chlorophyll (879.45 μg g^{-1} FW), chlorophyll *a* (545.06 μg g^{-1} FW), and chlorophyll *b* (394.35 μg g^{-1} FW) in salt-tolerant vegetable amaranth, whereas, Khanam and Oba (68) observed comparatively lower chlorophyll content in *A. tricolor*. On the other hand, Khanam and Oba (68) observed a more or less similar trend in betacyanin, betalain, chlorophyll, and betaxanthin content of red and green amaranth. The genotype LS7 and LS9 had abundant betacyanin, betalain, chlorophyll, and betaxanthin content indicating the presence of the high antioxidant activity. The genotype LS7 and LS9 had abundant betacyanins, betalains, chlorophylls, and betaxanthins among leafy vegetables that have important free radical-scavenging activity (1). Presence of high betacyanins, betalains, chlorophylls, and betaxanthins in vegetable amaranth genotype LS7 and LS9 is an important parameter for consumers, having an essential role in detoxification of ROS in the human body and preventing many degenerative human diseases and antiaging (22, 24). Total chlorophyll, chlorophyll *a*, chlorophyll *b*, betacyanins, betalains, and betaxanthins content obtained from these advance lines were greater than red morph amaranth (63), green morph amaranth (65), stem amaranth (66), weedy amaranth (64), and *A. blitum* (67). Hence, these selected salt-tolerant advance lines could contribute as high antioxidant pigments enriched genotypes than our previously tested amaranth genotypes.

Salt-tolerant vegetable amaranth genotype LS7 and LS9 had high polyphenols, flavonoids, ascorbic acid, and antioxidant capacity (AC). Our results were corroborated with the results of Khanam and Oba (68) where they observed higher polyphenols, flavonoids, and AC content in the red amaranth genotype compared to green amaranth. Salt-tolerant vegetable amaranth LS7 and LS9 contained higher ascorbic acid, polyphenols, flavonoids, and AC compared to the genotype LS3 and LS5. Hence, these antioxidant phytochemicals of salt-tolerant vegetable amaranth genotypes could be an important parameter for consumers, playing a crucial role in detoxification of ROS in the human body and preventing antiaging and many degenerative human diseases (22, 24). Our result showed that salt-tolerant vegetable amaranth genotypes contained antioxidant phytochemicals such as ascorbic acid, polyphenols, flavonoids, and AC among leafy vegetables that have the important scavenging activity of free radicals (1). The ascorbic acid and total polyphenols obtained from these advance lines were greater than our previous studies of green morph amaranth (65) and weedy amaranth (64), while total flavonoids recorded in these advance lines were greater than our previous studies of green morph amaranth (65) and red morph amaranth (63). Antioxidant capacity in DPPH obtained from these advance lines was greater than our previous studies of red morph amaranth (63) and antioxidant capacity in $ABTS^+$ obtained from these advance lines was greater than our previous studies of red morph amaranth (63), green morph amaranth (65), and stem amaranth (66). Hence, these selected salt-tolerant advance lines could contribute as high vitamin C, polyphenols, flavonoids, and antioxidants enriched genotypes than our previously tested amaranth genotypes.

We observed considerable pigments including betacyanins, betalains, betaxanthins, chlorophylls, and antioxidant phytochemicals such as ascorbic acid, polyphenols, antioxidant potentiality, and flavonoids in salt-tolerant vegetable amaranth genotypes. Our results were fully in agreement to the results of Khanam and Oba (68) where they observed higher AC, betacyanins, flavonoids, betalains, betaxanthins, and polyphenols content in the red amaranth genotype compared to green amaranth. Pigments such as betalains (1,029.12 ng g^{-1}), betacyanins (512.06 ng g^{-1}), betaxanthins (517.12 ng g^{-1}), total chlorophyll (879.45 μg g^{-1} FW), chlorophyll *a* (545.06 μg g^{-1} FW), chlorophyll *b* (394.35 μg g^{-1} FW), and antioxidant phytochemicals such as AC ($ABTS^+$) (70.24 TEAC μg g^{-1} DW), flavonoids (282.87 RE μg g^{-1} DW), and AC (DPPH) (35.36 TEAC μg g^{-1} DW) obtained in this study, more or less similar to the findings in *A. tricolor* of Khanam et al. (39), whereas polyphenols obtained in our study were much prominent than the findings in *A. tricolor* of Khanam et al. (39). The genotypes LS7 and LS9 had high pigments such as betacyanins, betalains, betaxanthins, chlorophylls, and antioxidant phytochemicals such as ascorbic acid, polyphenols, flavonoids, and AC.

In this study, we found plentiful protein, carbohydrates, nutraceuticals, and digestible fiber, moisture, remarkable pigments profile such as betacyanins, betalains, betaxanthins, chlorophylls, antioxidant phytochemicals such as ascorbic acid, polyphenols, flavonoids, and antioxidant potential in salt-tolerant vegetable amaranth genotypes. We obtained corroborative results compared to the results of Khanam et al. (39) where they observed higher AC, betacyanin, flavonoid, betalain, betaxanthin, and polyphenols content in the red amaranth. The genotypes LS7 and LS9 had abundant carbohydrates, protein, moisture, and dietary fiber, nutraceuticals, pigments, antioxidant phytochemicals, flavonoids, and antioxidant potentials. The genotypes LS7 and LS9 could be used as antioxidant profile enriched high-yielding varieties as drink purposes. It revealed from the investigation that these two genotypes contained adequate polyphenols, flavonoids, ascorbic acid, pigments, and antioxidant potentials that have prospects for extracting colorful juice for drinking purposes as well as for consuming the nutraceuticals and antioxidant-deficient community in the saline prone area of the world.

Salinity stress induces the ROS accumulation in plants that alters the biosynthesis of flavonoids (69) to mitigate the damaging effects of ROS and to adjust unfavorable stress conditions (70). These flavonoid compounds act as non-enzymatic antioxidants to alleviate the negative effect of ROS in plants through quenching these free radicals (71). Abiotic stress facilitates flavonoid biosynthesis in higher concentration to adjust oxidative stress in plants because of their high antioxidant potentiality (72). Furthermore, salinity stress highly accelerates the activity of biosynthesis pathway genes such as *TT3, TT4, TT5, TT6, TT7, TT8, TT9, TT18, FLS,* and *F60H1* that are involved in the biosynthesis of flavanone, flavone, flavonol, anthocyanin, and its derivatives (73–75). In addition, the regulatory gene *TT8* stimulates many flavonoid biosynthesis pathways genes and facilitates the accumulation of more antioxidant flavonoid compounds in plants (76). The data of foxtail millet roots showed that 17 flavonoid biosynthesis genes were significantly up-regulated 2–11-fold under salinity. In keeping with gene expressions, the over-accumulation of 27 flavonoids was obtained under salt tolerance (77).

Nine flavonoid compounds were determined in salt-tolerant vegetable amaranth including six flavonols, such as rutin, kaempferol, isoquercetin, myricetin, hyperoside, and quercetin; one flavanol, such as catechin; one flavone such as apigenin; and one flavanone, such as naringenin. For the first time, we identified one flavonol such as myricetin; one flavanol, such as catechin; one flavone such as apigenin; and one flavanone, such as naringenin in salt-tolerant vegetable amaranth. Khanam et al. (39) and Khanam and Oba (68) noticed three flavonols such as isoquercetin, rutin, and hyperoside in red and green amaranth. In the leaf, stalks, flowers, sprouts, and the seed of *A. cruentus*, *A. caudatus*, and *A. hypochondriacus*, Li et al. (78) observed three flavonols, such as kaempferol, rutin, and quercetin. Three flavonoids including isovitexin, vitexin, and rutin were reported in the seeds and sprouts of *A. cruentus* (79). Across four principal groups of compounds, the most identified pronounced compounds in four selected salt-tolerant vegetable amaranths were observed in the following order: flavonols > flavanones > flavones > flavanols. Across six flavonols, rutin and quercetin were identified as the most prominent compounds followed by isoquercetin and myricetin in selected salt-tolerant vegetable amaranths. Across the genotypes, LS7 exhibited the highest flavonols such as rutin, kaempferol, isoquercetin, myricetin, hyperoside, and quercetin. LS5 contained high total flavonols which were statistically similar to LS3, while LS9 demonstrated the lowest flavonols. Quercetin and hyperoside of our selected salt-tolerant leafy vegetable amaranths were higher than the content of quercetin and hyperoside reported by Khanam et al. (39) in *A. tricolor* genotypes. The varietal differences, and differential geographic locations, climatic and edaphic conditions, and cultural managements may have played a major contribution in securing higher quercetin and hyperoside in our salt-tolerant vegetable amaranth genotypes in comparison with the results of Khanam et al. (39). LS7 exhibited the highest flavanols, such as catechin; flavones such as apigenin; and flavanones, such as naringenin followed by LS9.

Highly significant positive associations of betalains, betaxanthins, betacyanins, total chlorophyll, chlorophyll *b*, and chlorophyll *a* were exhibited among pigments and with AC ($ABTS^+$), AC (DPPH), ascorbic acid, polyphenols, and flavonoids. Pigments of salt-tolerant vegetable amaranth (betalains, betaxanthins, chlorophylls, and betacyanins) showed strong antioxidant activity as all the pigments exhibited significant associations with AC ($ABTS^+$) and AC (DPPH). Ascorbic acid exerted significant associations with all traits along with AC ($ABTS^+$) and AC (DPPH). The significant positive associations of ascorbic acid with AC ($ABTS^+$) and AC (DPPH) also suggested a strong antioxidant activity. The significant associations of polyphenols and flavonoids were observed with AC ($ABTS^+$) and AC (DPPH) indicating the strong antioxidant capacity of phenolics and flavonoids in salt-tolerant vegetable amaranth. The TPC, TFC, and TAC of salt-induced purslane

and amaranth corroborated with the results of the present investigation (80, 81). Similarly, the significant relationship of AC ($ABTS^+$) with AC (DPPH) validated the antioxidant activity measurement of different methods in salt-tolerant vegetable amaranth.

CONCLUSION

Salt-tolerant vegetable amaranth genotypes contained ample proximate, pigments, nutraceuticals, and phytochemicals such as protein, carbohydrates, moisture, dietary fiber, polyphenols, minerals, betaxanthins, flavonoids, betacyanins, betalains, and chlorophylls. Salt-tolerant vegetable amaranth genotypes LS7 and LS9 had greater proximate, nutraceuticals, pigments, antioxidant phytochemicals, and antioxidant activity compared to the genotype LS3 and LS5. Nine flavonoids compounds were determined in salt-tolerant vegetable amaranth including six flavonols, such as rutin, kaempferol, isoquercetin, myricetin, hyperoside, and quercetin; one flavanol, such as catechin; one flavone such as apigenin; and one flavanone, such as naringenin. For the first time, we identified one flavonol such as myricetin; one flavanol, such as catechin; one flavone such as apigenin; and one flavanone, such as naringenin in salt-tolerant vegetable amaranth. Across six flavonols, rutin and quercetin were identified as the most prominent compounds followed by isoquercetin and myricetin in selected salt-tolerant vegetable amaranths. Across the genotypes, LS7 exhibited the highest flavonols such as rutin, kaempferol, isoquercetin, myricetin, hyperoside, and quercetin as well as the highest flavanols, such as catechin; flavones such as apigenin; and flavanones, such as naringenin. The correlation study revealed that all antioxidant constituents of salt-tolerant vegetable amaranth had strong antioxidant activity. It revealed from the study that salt-tolerant vegetable amaranth genotypes LS7 and LS9 exhibited excellent sources of proximate, nutraceuticals, pigments, antioxidant phytochemicals, and antioxidant activity that offered huge prospects for nutritional and health-boosting effects. We can extract colorful juice from the genotypes LS7 and LS9 as drink purposes for consuming the nutraceuticals and antioxidant deficient community in the saline prone area around the world. However, further details experimentation is required to confirm the standardization and stabilization of functional components of vegetable amaranth for extraction of juice as drinks.

DATA AVAILABILITY STATEMENT

All the data supporting the conclusions of this article is provided within the article.

AUTHOR CONTRIBUTIONS

US initiated the research work, conceived the study, performed biochemical analysis, statistical analysis, drafted, edited, interpreted data, and prepared the manuscript. US, MH, and MI performed the experiments. SO edited the manuscript, provided valuable suggestions during the experiment. All authors contributed to the article and approved the submitted version.

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Interactions Between Phytochemicals and Minerals in *Terminalia ferdinandiana* and Implications for Mineral Bioavailability

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Oxalic and phytic acid are phytochemicals considered to be anti-nutritional factors as they are predominantly found as oxalates and phytates bound to minerals like calcium and potassium. Studies have associated excessive oxalate consumption with increased urinary excretion of oxalate (hyperoxaluria) and calcium oxalate kidney stone formation, and excessive phytate consumption with decreased bioaccessibility and bioavailability of certain minerals and reduced utilization of dietary protein. However, other studies suggest that dietary consumption of phytate may be beneficial and inhibit formation of calcium oxalate kidney stones. In light of these conflicting reports, dietary intake of oxalate and phytate enriched plants should be considered in relation to potential health outcomes following consumption. *Terminalia ferdinandiana* is one such plant and is investigated here with respect to oxalate, phytate, and mineral contents. Assessment of oxalate and phytate contents in *T. ferdinandiana* fruit, leaf, and seedcoat tissues through hydrolysis into acid forms revealed oxalic acid contents ranging from 327 to 1,420 mg/100 g on a dry weight (DW) basis whilst phytic acid contents ranged from 8.44 to 121.72 mg/100 g DW. Calcium content in the different tissues ranged from 131 to 1,343 mg/100 g. There was no correlation between oxalic acid and calcium, however a significant, positive correlation was observed between phytic acid and calcium ($r = 0.9917$; $p < 0.001$), indicating that tissues rich in phytic acid also contain higher levels of calcium. The high content of phytic acid in comparison to oxalic acid in *T. ferdinandiana* fruit found in this study and the dietary significance of this in terms of calcium bioavailability, needs to be investigated further.

Keywords: *Terminalia ferdinandiana*, Kakadu plum, phytate, oxalate, ascorbic acid, mineral

INTRODUCTION

Terminalia ferdinandiana (Kakadu plum) is a native Australian fruit consumed by Indigenous communities for centuries. In recent years, *T. ferdinandiana* has been established as a rich source of antioxidants and other biologically active compounds (1–3). Commercial demand for *T. ferdinandiana* fruit continues to increase because of its high vitamin C content (2). A variety of *T. ferdinandiana* food products are available in the market such as juices, sauces, jam and whey product isolates. Moreover, a number of *T. ferdinandiana* nutraceutical products such as energy bar (with quandong), probiotic (with manuka honey), probiotic punch (with cranberry), analgesic spray, dietary supplements (tablet and liquid format) and freeze dried powder are also available. However, a report on the high levels of oxalate (2) present in *T. ferdinandiana* fruits has raised concerns regarding the potential risk of kidney stone formation following fruit consumption. High dietary intake of oxalate is considered by some studies as the primary risk factor in the formation of calcium oxalate stones (4), however other studies have found only a modest positive correlation between stone formation and dietary intake of oxalate (5). Bioavailability of oxalate may also be influenced by the presence of *Oxalobactor formigenes* in the gastrointestinal tract (6). *O. formigenes* is one of the intestinal bacteria responsible for the degradation of oxalate in the intestine with higher levels of urinary oxalate excretion possibly due to the absence or low levels of this bacteria (6). Normal dietary intake is reported to be in the range of 50–200 mg oxalate per day but can exceed 1,000 mg per day if foods rich in oxalate are consumed.

Dietary oxalate is associated with increased urinary oxalate concentrations accounting for 50% of urinary oxalate excretion, however oxalate can also be metabolically produced by the body (7). Epidemiological evidence and short term experiments with human subjects have indicated that ascorbic acid ingestion can also serve as a risk factor for calcium oxalate stone disease and increased urinary oxalate excretion (8). Ascorbic acid (AA) and its oxidation product dehydroascorbic acid (DHAA) can form diketogulonic acid which is unstable and breaks down to oxalate (8). However, the amount of AA and DHAA that is metabolized in cells and tissues, and the amount of AA and DHAA that is converted to diketogulonic acid and ultimately oxalate, is not yet known (8). Previous studies have suggested that compromised renal function coupled with excessive AA ingestion can result in oxalate nephropathy in susceptible individuals (8).

Plant phytochemicals can also affect the bioavailability of minerals. For instance, a high phytate content is associated with poor bioavailability of minerals (9). Phytate can form complexes with endogenous minerals in the intestine making them unavailable for absorption. High amounts of calcium may inhibit zinc absorption by forming insoluble calcium-zinc-phytate complexes in the intestine. However, phytic acid has also been claimed to have beneficial effects such as antioxidant properties (10) and as an inhibitor of calcium oxalate kidney stone formation (11). As a further complication, phytate can also bind proteins and reduce protein utilization (12, 13). **Figure 1** illustrates the chemical structures of ascorbic acid (AA), phytic acid and oxalic acid.

Analyses of oxalate and phytate contents of *T. ferdinandiana* are necessary for informing current consumers of *T. ferdinandiana* fruit who may be at risk of kidney stone formation. Therefore, it is important to investigate and provide insights into the safety of *T. ferdinandiana* fruit products for such consumers.

This study aims to investigate certain nutritional and non-nutritional compounds in *T. ferdinandiana* fruits to provide information for determining the safe dietary intake of this fruit. For comparison, the fruits and underutilized parts, like leaves and seedcoats of *T. ferdinandiana,* are included in this study. Oxalate, phytate, and mineral contents in *T. ferdinandiana* have been evaluated along with predicted mineral bioavailability calculated as the molar ratios of phytate/oxalate/minerals (14, 15). A proximate analysis is also reported as limited studies are available on the nutrient and chemical compositions of *T. ferdinandiana* plant parts.

MATERIALS AND METHODS

Sample Collection and Preparation

The collection of fruits has been described by Akter et al. (16). The collected fruits were sorted, washed and processed in Sunshine Tropical Fruit Products, Nambour, Queensland, Australia, to provide a seedless puree. The seeds were also collected as byproducts and were stored at −80°C until further analysis. The puree was freeze-dried (ScanVac CoolSafe Superior Touch, LabGear Australia, QLD, Australia) and ball-milled (Retsch MM400, Metrohm Australia Pty Ltd., NSW, Australia) to provide a uniform powder and stored at −20°C and used throughout this study. The frozen seeds were thawed and washed with distilled water to remove the pulp and peel residue. The seeds were then oven-dried for 48 h at 40°C. After drying, the seeds were individually cracked using an Engineers vice size 125 (DAWN tools and Vices Pty Ltd., Heidelberg West, Victoria, Australia) to release the kernels from the seedcoats. The kernels were processed and analyzed separately in a previous study (17). The separated seedcoats were hammer milled and used for this study. Mature leaves were collected from ten trees (ten leaves from each tree) from the same region during the same fruit harvest and were freeze-dried and milled together to prepare a composite leaves samples. The milled freeze-dried powders of leaves were used throughout this study.

Proximate Composition Analysis

A complete proximate analysis was performed in a National Association of Testing Authorities (NATA) accredited laboratory that complies with ISO/IEC17025:2005, Symbio Alliance (Eight Mile Plains, Queensland, Australia) using AOAC standard methods (18). Moisture was measured (AOAC 925.10) by air oven with a measurement of uncertainty (MU) of ±15%, ash (AOAC 923.03), crude protein (AOAC 990.03) by Dumas combustion with a MU of ±10%, dietary fiber by (AOAC 985.29) with a MU of ±15%, crude fiber by (AOAC 962.09), crude fat (AOAC 991.36) with a MU of ±15%, and saturated, mono-unsaturated, poly-unsaturated and trans fat by in house method CFH068.2. Carbohydrate and energy by calculation

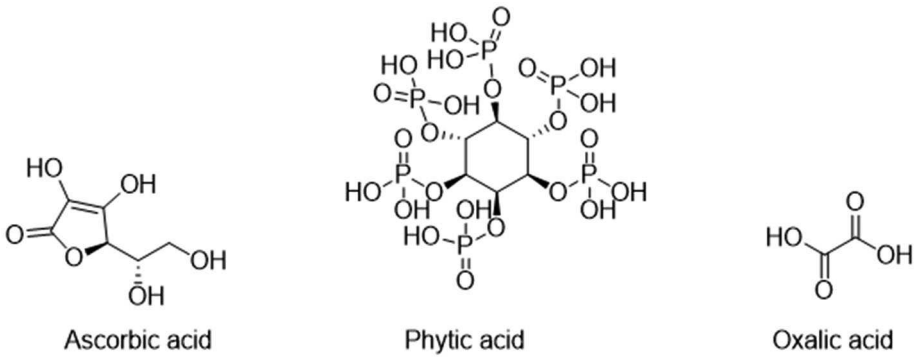

FIGURE 1 | Structures of ascorbic acid (L-AA), phytic acid, and oxalic acid.

using information from the Food Standards Australia New Zealand (FSANZ) Code.

Mineral and Trace Element Composition Analysis

Mineral and trace element levels of *T. ferdinandiana* fruit, leaf and seedcoat were determined based on the methods described previously (17) and expressed on a dry weight basis. Briefly, samples (ca. 0.3 g) were subjected to overnight slow digestion at room temperature and followed by microwave digestion (MarsXpress, CEM, Matthews, NC, USA) at a gradual increase in temperature. The levels of minerals were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES, 700 Series, Agilent, VIC, Australia) and the trace elements were analyzed using ICP-MS for greater sensitivity on a 7700 instrument (Agilent, Tokyo, Japan). Operating conditions and parameters of ICP-OES were recorded as follows: operating power 1.20 KW, plasma gas flow rate 15.0 L/min, auxiliary gas flow rate 1.50 L/min, nebuliser gas flow rate 0.90 L/min, sample uptake 50 s, pump rate 25 rpm and rinse time 30 s. The elements were recorded at the following wavelengths (nm): Ca 370.602 (nm), K 766.491 (nm), Mg 383.829 (nm), Na 568.821 (nm) and P 185.878 (nm). Operating conditions of the ICP-MS were recorded as follows: radio frequency power 1,350 W, carrier gas 0.8 L/min (argon) and gas flow rate 4.5 mL/min (helium reaction cell). Standard reference materials were used for the quality control and were treated similarly to the samples (19).

Phytate Content Determination

T. ferdinandiana plant parts were analyzed for phytic acid content by a previously described method with slight modification (20). Samples of fruits, leaves and seedcoat powder (0.5–1.0 g) were weighed and mixed with 2.4% HCl (20 mL). The samples were vortexed and placed in a rotary mixer for 16 h at room temperature at low speed. The sample mixtures were centrifuged at 5,000 rpm for 10 min at 10°C (Eppendorf centrifuge 5810R). The supernatants were transferred to 50 mL centrifuge tubes containing 1 g of NaCl and rotary mixed for 20 min at room temperature. The tubes were allowed to settle at 4°C for 1 h and then centrifuged at 5,000 rpm for 20 min at 10°C. Aliquots of these supernatants (100 μL) were diluted with 1,900 μL of RO water in 75 mm culture tubes. Phytic acid sodium salt hydrate purchased from Sigma-Aldrich (St. Louis, MI, USA) (0–50 μg/mL) was used as a standard dissolved in RO water. An aliquot (900 μL) of both standards and diluted supernatants of the samples were then mixed with 300 μL of Wade's reagent (0.03% $FeCl_3.6H_2O$ + 0.3% Sulfosalicylic acid) in 2 mL microfuge tubes and vortexed. The absorbance was recorded at 500 nm (21) using a DU 530 UV-Vis spectrophotometer (Beckman Coulter Inc. Brea, CA, USA). An external calibration curve was prepared from standard phytic acid sodium salt hydrate to calculate the phytic acid concentrations in the samples. The results were multiplied by 0.282 (molar ratio of phytate—phosphorous in a molecule of phytate) in order to express the phytate content in the sample as mg/100 g of phytate on a dry matter basis (22).

Oxalate Content Determination

Extraction and quantification of water-soluble and total oxalate were performed according to a previously described method (2). Oxalate compounds were converted to oxalic acid using hydrochloric acid and analyzed on a Phenomenex HPLC system (Phenomenex, Lane Cove West, NSW, Australia) equipped with a Gilson UV/VIS 151 multiwavelength detector (Gilson, Middleton, WI, USA). A Phenomenex reversed phase column (Synergi 4 μm Hydro-RP 80A, 250 × 4.60 mm) (Phenomenex, Lane Cove West, NSW, Australia) was used with an isocratic mobile phase of 20 mM phosphate buffer (pH 2.4) and a flow rate of 0.5 ml/min. The injection volume was 20 μL and detection was carried out at 210 nm. Sodium oxalate was obtained from Sigma-Aldrich (St. Louis, MI, USA). Sodium oxalate at the concentrations of 1, 2, 3, 4, and 8% in 20 mM phosphate buffer (pH 2.4) was used to prepare an external calibration curve to quantify the levels of total and water-soluble oxalate in *T. ferdinandiana* tissue samples.

Ascorbic Acid Determination

Vitamin C extraction was performed by following previously described method (23, 24) with modification. Briefly, 0.5 g

powders of *T. ferdinandiana* fruit, leaves and seedcoat was added to 5 ml of freshly prepared extraction solution composed of 3% metaphosphoric acid (MPA) (Merck, Darmstadt, Germany), 8% acetic acid (Merck, Darmstadt, Germany) and 1 mM ethylenediaminetetraacetic acid tetrasodium salt (EDTA) (Merck, Darmstadt, Germany). The homogenate was thoroughly vortexed and then centrifuged at 10,000 rpm (refrigerated at 2–4°C) for 10 min. The supernatant was collected, the extraction was repeated twice and supernatants were pooled together. Samples for ascorbic acid (AA) analysis were prepared according to a previously described method (23). Briefly, 500 μL of extracted solution was added to 500 μL of freshly prepared 0.5 M Trizma buffer (Merck, Darmstadt, Germany). An aliquot (100 μL) of formic acid (Sigma-Aldrich, Castle Hill, NSW, Australia) was added to the mixture. In an UPLC vial, 900 μL of milli-Q water and 100 μL of the sample mixture was added. Samples for dehydroascorbic acid (DHAA) analysis were prepared by adding 500 μL of extracted solution to 500 μL of 40 mM DTT (DL-Dithiothreitol) (Sigma-Aldrich, Castle Hill, NSW, Australia) in Trizma buffer. The mixture was vortexed and left in the dark at room temperature for 30 min. In an UPLC vial, 900 μL of milli-Q water and 100 μL of the sample mixture was added. The DHAA content of the samples were calculated by subtracting the initial AA content from the total vitamin C content, after reduction with DTT. Oxidation of AA from standard solutions and samples were prevented by performing the experiment under reduced light, using amber flasks and vials, minimal oxygen exposure and in low temperature. DHAA was quantified by the difference between the total AA content (after DHAA reduction to AA) and the AA contents before the DHAA conversion (23). A standard stock solution of AA (Sigma-Aldrich, Castle Hill, NSW, Australia) (ca. 2 mg/10 mL) in milli-Q water was prepared fresh on each day of analysis and was stored in amber flasks at 4°C prior to chromatographic analysis. Solutions of variable concentrations were prepared by diluting the standard stock solution with milli-Q water. An Acquity UPLC system (Waters Corp., Milford, MA, USA), equipped with a Waters Acquity UPLC photodiode array (PDA) detection system was used to carry out the analysis. Empower™ software (Waters Corp., Milford, MA, USA) was used to process and quantify peaks after recording the signals. An Acquity HSS T3 analytical column (100 × 2.1 mm, 1.8 μm particle size) (Waters Corp., Milford, MA, USA), using an isocratic mobile phase 0.1% aqueous (v/v) formic acid at a flow rate of 250 μL/min was used with an injection volume of 2 μL. The absorbance was measured at room temperature at 245 nm using PDA.

Estimation of the Potential Bioavailability of Minerals

The molar ratios have been calculated by using the following formula (25):

$$\text{Phytate to mineral molar ration} = \frac{\text{Phytate (mg)/atomic weight of phytate}}{\text{Mineral (mg)/atomic weight of minerals}}.$$

TABLE 1 | Proximate composition of *T. ferdinandiana*.

		T. ferdinandiana plant parts		
		Fruits	**Leaves**	**Seedcoats**
Protein	%	4.7	7.1	2.3
Fat	%	0.9	6.5	0.6
Saturated Fat	%	0.3	2.5	0.1
Mono-unsaturated Fat	%	<0.1	0.4	0.2
Poly-unsaturated Fat	%	0.6	3.7	0.4
Trans Fat	%	<0.01	<0.01	<0.01
Ash	%	5.5	5.9	0.6
Moisture	%	6.0	3.0	2.2
Dietary Fiber (Total)	%	45.9	44.0	90.2
Energy	KJ/100 g	1,110	1,283	853
Total Sugar	g/100 g	2.3	2.5	<0.10
Available Carbohydrate	%	37.0	33.4	4.0

Results are expressed as mean of triplicate experiments on a dry weight basis. Means are not significantly different ($p = 0.96$).

Statistical Analysis

GraphPad Prism version 8 (GraphPad Software, San Diego, California, USA) was used to analyse the data. One-way ANOVA was performed with Tukey's multiple comparison test to evaluate the significance of differences between groups and to compare between groups. *P*-values ≤ 0.05 was considered as statistically significant. Regression analyses were performed to determine the correlation (pearson R^2) between oxalate vs. phytate, oxalate vs. calcium and phytate vs. oxalate contents of the plant parts. Data are reported as mean ± SD of three measurements unless otherwise specified.

RESULTS AND DISCUSSION

Proximate Composition

The results of the proximate analysis are summarized in **Table 1**. Protein, fat, ash and energy content were highest in leaves followed by fruits and seedcoats. Seedcoats contains the highest amount of dietary (90.2%) fiber. Moisture content of fruits is higher than leaves and seedcoats. Study on the proximate composition of the kernels of *T. ferdinandiana* showed that kernels contain 32% protein, 35% fat and is a good source of minerals and nutrients (17). Studies on the proximate composition of *T. ferdinandiana* fruits, leaves and seedcoats are scarce and hence no values are available for comparison. The high dietary fiber content in seedcoats could potentially be a functional food ingredient to improve dietary fiber intake and help reduce hypercholesterolemia, gallstone, constipation, diabetes, coronary heart disease, and various digestive disorders (26).

TABLE 2 | Mineral composition of *T. ferdinandiana*.

	Fruits	Leaves	Seedcoats	Dietary reference intakes	
	mg/100 g DW	mg/100 g DW	mg/100 g DW		Units
Major elements					
Ca	295 ± 0.6	1,343 ± 14	131 ± 19	1,200 AI (27)	mg/person/day
K	2,718 ± 99	1,179 ± 22	265 ± 0.4	4.7 AI (27)	g/person/day
Mg	204 ± 0.7	403 ± 2	24 ± 4	350 EAR (27)	mg/person/day
Na	212 ± 43	202 ± 80	96 ± 11	1.3 AI (27)	g/person/day
P	73 ± 2	64 ± 2	20 ± 4	700 RDA (27)	mg/person/day
Trace elements					
Fe	1.7 ± 0.0	3.4 ± 0.1	3.9 ± 0.7	8 RDA (27)	mg/person/day
Zn	2.2 ± 1.6	2.0 ± 0.1	0.3 ± 0.0	11 RDA (27)	mg/person/day
Co	0.01 ± 0.0	0.09 ± 0.0	<0.01	0.12AI (27)	μg/person/day
Ni	0.5 ± 0.0	0.1 ± 0.0	0.04 ± 0.0	1.0 UL (27)	mg/person/day
Cu	1.4 ± 0.0	0.6 ± 0.0	1.0 ± 0.1	700 RDA (27)	μg/person/day
Cr	0.07 ± 0.0	0.02 ± 0.0	0.02 ± 0.0	35 AI (27)	μg/person/day
Mn	5.0 ± 0.0	25.5 ± 0.7	1.3 ± 0.0	2.3 AI (27)	mg/person/day
Sr	4.0 ± 0.0	13.0 ± 0.0	1.4 ± 1.4	1–5 RDA (27)	mg/person/day
Mo	0.04 ± 0.0	0.01 ± 0.0	<0.1	34 EAR (27)	μg/person/day
Se	<0.01	<0.01	<0.1	45 EAR (27)	μg/person/day
B	2.0 ± 0.1	3.5 ± 0.0	0.4 ± 0.1	20 UL (27)	mg/person/day
Ba	2.3 ± 0.0	2.8 ± 0.0	0.7 ± 0.0	0.02 UL (28)	mg/kg BW
Heavy metals					
As	<0.01	<0.01	<0.1	12.5–25 UL (29, 30)	μg/kg BW/week
Cd	<0.01	<0.01	<0.1	2.5 UL (30, 31)	μg/kg BW/Week
Hg	<0.01	<0.01	<0.1	5 UL (30, 32)	μg/kg BW/week
Pb	<0.01	<0.01	0.04 ± 0.0	25 UL (30, 32)	μg/kg BW/week

Data are presented as mean ± SD of duplicate determinations. Means are not significantly different ($p = 0.46$). RDA, recommended dietary allowance; AI, adequate Intake; UL, tolerable upper intake level; EAR, Estimated average requirement; BW, body weight.

Mineral Composition

The levels of major minerals and trace elements in *T. ferdinandiana* are presented in **Table 2**. Calcium content was found to be higher in leaves [1,343 mg/100 g on a dry weight (DW) basis] compared to fruits (295 mg/100 g DW) and seedcoats (131 mg/100 g DW). Seedcoats were found to contain low levels of all major minerals compared to fruits and leaves consistent with the ash content of *T. ferdinandiana* plant parts included in **Table 1**. The findings here are similar to previous studies that report the calcium content of freeze dried *T. ferdinandiana* fruits to be 282 mg/100 g DW (33) with an average content of individual whole fruit to be 243 mg/100 g DW (2). Potassium content in *T. ferdinandiana* fruits was found to be 2,718 mg/100 g DW in this study and is more than 30% higher than previously reported levels (1,906 mg/100 g DW) (33). Phosphorus content of the fruits was found to be 73 mg/100 g DW and is also higher than a previous study (52.5 mg/100 g DW) (33).

The contribution of *T. ferdinandiana* to daily intakes of minerals and trace elements can be assessed based on the guideline on daily requirements of essential elements as recommended for adults. The average daily adult requirements for trace elements (in men and women aged 19–70 years) are as follows: Fe 8–18 mg/day; Zn 8–14 mg/day; Cu 1.2–1.7 mg/day; and Mn 5–5.5 mg/day (34). *T. ferdinandiana* fruits contain 1.7 mg/100 g DW of Fe that is much lower than some grains such as sorghum 3.7 mg/100 g DW, soybean 7.3 mg/100 g DW, and mung bean 7.2 mg/100 g DW (35). Zinc content of *T. ferdinandiana* fruit is 2.2 mg/100 g DW and is higher than a value (0.6 mg/100 g DW) previously reported (33). *T. ferdinandiana* fruits have lower levels of Zn compared with other common legumes and grains such as soybean (3.6 mg/100 g DW), mung bean (2.8 mg/100 g DW), rice (2.9 mg/100 g DW), and millet (3.7 mg/100 g DW) (35). Manganese levels in *T. ferdinandiana* leaves are much higher (25.5 mg/100 g DW) compared to fruits (5.1 mg/100 g DW) and seedcoats (1.3 mg/100 g DW).

The heavy metal content of *T. ferdinandiana* (**Table 2**) are <0.1 mg/100 g DW for all metals tested except for Pb content in the seedcoats that was found to be 0.04 mg/100 g DW. From our present study, it can be inferred that *T. ferdinandiana* fruit presents no known risk for heavy metal toxicity if 1 g freeze dried powder is consumed per day. It is important to note that differences in geographical variables such as soil fertility, mineral uptake efficiency of plants, and growth conditions may cause variation in the heavy metal concentrations in *T. ferdinandiana* (33, 36).

Phytate Content

Phytate contents, measured as phytic acid following hydrolysis of phytate, in *T. ferdinandiana* plant parts are presented in **Figure 2**. Leaves were found to contain the highest amount of phytate (121.72 mg/100 g DW) compared to fruits (37.6 mg/100 g DW) and seedcoats (8.44 mg/100 g DW). Typical values for phytic acid content in cereal foods are in the range of 0.5–1% by weight (37). The values obtained in the present study are lower than plants and grains previously reported to contain high phytate levels. For example, phytate levels range from 1,000 to 2,200 mg/100 g DW in soybeans and from 590 to 1,100 mg/100 g DW in mung beans (35). The phytate levels of some seeds and grains were also reported to be high such as rice 1,084, cowpea 559, maize 908, sorghum 925, and soybean 878 mg/100 g DW (35). Globally, daily intake of phytic acid varies largely according to diet from ∼0.2–4.6 g. Vegetarian diets generally contains higher amounts of phytic acid compared to mixed diets (37). Several methods have been developed so far to reduce the phytic acid content in food owing to the anti-nutritional effect and to improve the nutritional value of the food. Several pre-treatment methods such as fermentation, soaking, germination and enzymatic treatment of grains with phytase enzyme can be applied besides genetic improvement (9). On the other hand, researchers have also suggested that soy foods and soybeans can be advantageous for kidney patients due to the inhibition of calcium kidney stone formation with high concentrations of phytate (38). The Phytate content determination by spectroscopic method can sometimes cause overestimation if the plant material also contains oxalate. Krome et al. have shown that determining phytate contents by using photometric method require correction due to the fact that the Wade-reagent interacts with oxalate and phytate present in plant material (39). Extensive literature search in support of the existing issue of overestimation has showed us that overestimation of phytate has been documented before (36, 37).

In the spectroscopic method, the pink color of the Wade reagent is due to the reaction between the ferric ion and the sulfosalicylic acid. In the presence of phytic acid, the ferric ion binds to the phosphate ester and is unavailable to react with the sulfosalicylic acid, resulting in a decrease of the pink colors intensity. Determination of the phytic acid level by the spectrophotometric method can show higher phytic acid levels (27% in some cases) than the chromatographic methods (40). The researchers have shown that the cause of phytate overestimation with methods involving the Wade reagent lies in other anions present in the samples predominantly oxalate. They have suggested that the magnitude of the error of a phytate determination involving the Wade-reagent therefore depends on the concentration of these compounds in the respective plant material. However, they have suggested that this method should only be applied if the concentration and their degree of influence on the analysis is known (39). To overcome the ongoing issue of overestimation of phytate content by using colorimetric assay, an appropriate anion-exchange chromatography combined with colorimetric method was suggested by researchers (40, 41). Considering the limitation of the colorimetric assay, future work has been designed to determine the phytate levels in *T. ferdinandiana* plant parts by employing an anion-exchange chromatography combined with colorimetric assay. A comparative study will also be performed on the levels of phytic acid in the *T. ferdinandiana* plant parts obtained from colorimetric assay and anion-exchange chromatography combined with colorimetric assay.

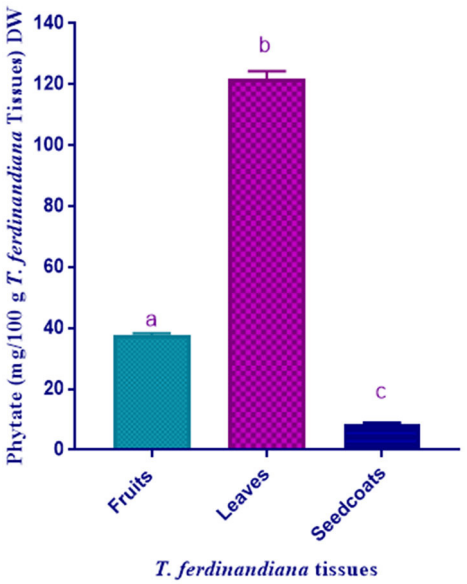

FIGURE 2 | Phytate content of *T. ferdinandiana*. Results are shown as the mean of triplicate experiments ± SD. Different lower case letters indicate significant differences ($P \leq 0.05$).

Oxalate Content

Oxalate contents (measured as oxalic acid following hydrolysis of total and soluble oxalate) in *T. ferdinandiana* plant parts are presented in **Table 3**. Among the three parts analyzed, fruits were found to contain the highest levels of oxalate followed by leaves. Total oxalate content in seedcoats was significantly lower than leaves and fruits. The content of total and soluble oxalate varied widely between the three different parts with variations more evident when soluble oxalate was expressed as a percentage of total oxalate (**Table 3**). The greatest proportion of soluble oxalate was in seedcoats (96%) followed by fruits (79%) and leaves (32%). Previous reports of oxalate levels in *T. ferdinandiana* fruits and leaves were 2,717 mg/100 g DW and 1,636 mg/100 g DW and are much higher than the values obtained in our present study (2). Variables including, but not limited to, geographical location, soil fertility, growth conditions, methodological aspects and extraction techniques may cause variation in the oxalate concentrations compared with previous reports.

Foods are considered high in oxalate when levels are >50 mg/100 g DW (42). The American Dietetic Association recommends that patients with kidney stones should restrict dietary intake of oxalate to <40–50 mg per day (42). High oxalate containing foods include spinach (400–900 mg/100 g FW), legumes, rhubarb raw (275–1,336 mg/100 g FW), cereals, beets (roots and leaves), chocolate, some tree nuts, star fruit raw

(80–730 mg/100 g FW), black teas (not green or herbal), and bran concentrates (42–44). The results of the present study indicate that the serving suggestion of 1 g of freeze-dried powder of *T. ferdinandiana* per day may provide 14.2 mg total oxalate. This value is lower than the recommended dietary intake of oxalate for patients with kidney stones (42). The recommended serving size for green leafy vegetables, such as spinach is 1 metric cup (ca. 30 g) (45) that contains at least 270 mg total oxalate calculated from published data (42). However, caution should be taken by kidney stone patients, and patients with increased risk of kidney disease, when consuming any foods high in oxalate.

There is no significant correlation between calcium and oxalate (**Figure 3**) or between oxalate and phytate, however there is a significant correlation between phytate and calcium ($r = 0.9917$; $p < 0.001$) across all parts of the plant.

Vitamin C Content

The contents of AA, DHAA, and total vitamin C in freeze dried *T. ferdinandiana* fruits was 16.4 ± 0.7, 4.7 ± 2.8, and 21.1 ± 2.2 g/100 g DW, respectively. The total vitamin C content (AA and DHAA together) in food products is used as an index of quality because it is very sensitive to degradation during processing and storage (23). Therefore, simultaneous analysis of AA and DHAA is necessary in food analysis to determine the total vitamin C content, as DHAA exhibits equivalent biological activity to AA (23). In a previous report, total vitamin C levels in whole fruit was 18.16 g/100 g DW (2) and in composite fruit extracts it was 14.04 g/100 g DW (46). This variation could be due to wild harvesting of fruits occurring in different geographical locations, climatic and soil conditions and seasonality. However, it is also important to determine the accurate content of vitamin C in foods to better understand the relationship of dietary intake and human health (47). National Health and Medical Research Council Australia has recommended the Recommended Dietary Intake (RDI) of vitamin C for healthy adult males and females as 45 mg/day and a upper level of intake (UL) is not possible to establish with certainty for supplementary vitamin C however a 1,000 mg/day can be a prudent limit (48). Based on the result of this study, having 1 g of freeze dried *T. ferdinandiana* powder everyday would fulfill the RDI (100%) for Vitamin C and will be within the 1,000 mg/day prudent limit.

TABLE 3 | Oxalate levels in *T. ferdinandiana*.

***T. ferdinandiana* plant parts**	**Fruits**	**Leaves**	**Seedcoats**
Total oxalate (mg/100 g DW)	1,420 ± 105[a]	1,133 ± 131[b]	327 ± 15[c]
Water-soluble oxalate (mg/100 g DW)	1,120 ± 141[a]	360 ± 20[b]	313 ± 35[b]
Soluble as % of Total	79	32	96

Results are presented as mean ± SD of triplicate determinations. Numbers with different superscripts[a,b,c] in the same row are significantly different ($p \leq 0.05$) as determined by one-way ANOVA and Tukey's multiple comparison test.

Molar Ratios to Predict the Potential Bioavailability of Minerals

From a nutritional point of view, bioavailability can be defined as the fraction of an ingested component available for utilization in normal physiological functions (49). Calcium bioavailability can be influenced by the presence of phytic acid through the formation of calcium-phytate complexes that limit the number of free calcium able to bind with oxalate (potentially forming calcium oxalate kidney stones). Many techniques have been employed to determine the bioavailability of minerals from foods to assess mineral utilization in the human body (50). Measuring the molar ratio of phytate/minerals and oxalate/minerals can predict the bioavailability of minerals (25) and the result obtained from this method is only relative.

Critical molar ratio values are used as guideline to assess mineral bioavailability. The critical values for phytate:calcium is >0.24 (26), phytate:iron >1 (15), phytate:zinc >15 (51). If molar ratios are close to these values, a normal homeostasis can be maintained. However, if molar ratios are higher than these values, the presence of phytate can alter the bioavailability of minerals. Molar ratios of phytate to minerals >0.24 and oxalate to minerals >2 have been reported as unsafe (15). Oxalate to mineral ratio

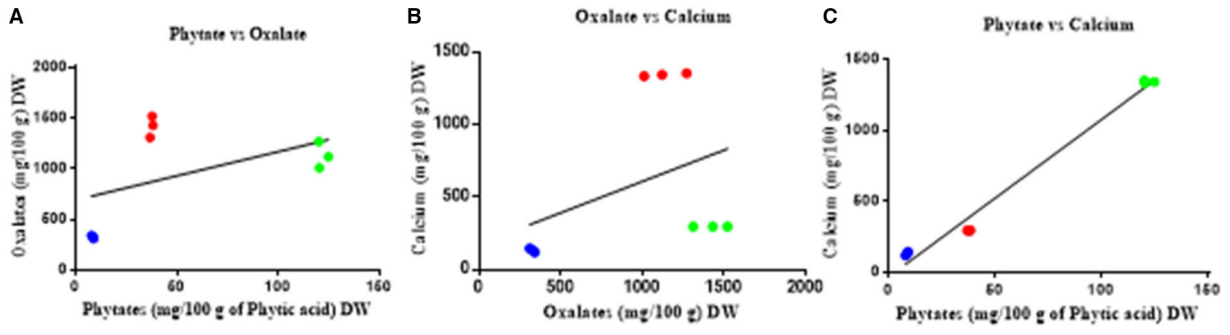

FIGURE 3 | Correlation between oxalate, phytate, and calcium of *T. ferdinandiana*. **(A)** Phytate vs. oxalate, **(B)** oxalate vs. calcium, and **(C)** phytate vs. calcium in fruits ●, leaves ●, seedcoats ●. No significant associations were found except with phytate vs. calcium (pearson $r = 0.9917$; $p < 0.001$).

TABLE 4 | Molar ratios of phytate, mineral, and oxalate in *T. ferdinandiana*.

	Fruits	Leaves	Seedcoats
Phytate: Iron	1.87	3.03	0.18
Phytate: Zinc	1.69	6.03	2.79
Phytate: Calcium	0.01	0.01	<0.01
Phytate: Magnesium	0.01	0.01	0.01
Phytate: Potassium	<0.01	0.11	0.02
Oxalate: Calcium	1.51	0.26	0.78
Oxalate: Magnesium	1.32	0.53	2.59
Oxalate: Potassium	0.16	5.40	4.99
Phytate: Oxalate	0.01	0.02	0.01

>2 indicates that excess oxalate is bioavailable and could limit mineral absorption (15).

The molar ratio of phytate: calcium present in the fruits and leaves is 0.01 and in seedcoats <0.01 (**Table 4**). The results suggest that phytate level in *T. ferdinandiana* are within the safe level and it can be suggested that the presence of phytate would not decrease the availability of calcium in *T. ferdinandiana*. According to some previous reports, the molar ratio of phytate: calcium for some commonly consumed food items such as kidney beans is 0.53, white bean 0.76, wheat bran 8.89, honey coated cereal 0.01, cooked white rice 1.65 and wheat flour 3.07 that are comparable to the phytate: calcium ratio of *T. Ferdianadiana* (15, 51). The molar ratio of phytate: magnesium in the fruit, leaves and seedcoats is 0.01 which is also within the safe level (**Table 4**). The ratios of phytate: iron in the *T. ferdinandiana* fruit is 1.87, leaves 3.03 and seedcoats 0.18, respectively. Phytate: zinc molar ratios >15 is indicative of poor zinc bioavailability (26). The phytate: zinc molar ratios of *T. ferdinandiana* (1.69–6.03) are within the safe level. Based on the results, it can be stated that phytate would not impact the bioavailability of zinc in the *T. ferdinandiana*. Moreover, the phytate: zinc molar ratios of soybean is 23.5, cowpea 15.8, sorghum 62.8, and maize 40.6 (35) which are much higher than the ratio of *T. ferdinandiana*.

The molar ratio of oxalate: calcium present in the fruits is 1.51, seedcoats 0.26 and leaves 0.78 in **Table 4**. The results suggest that oxalate level in *T. ferdinandiana* are within the safe level as oxalate to mineral molar ratio of >2.0 indicates unsafe (15). Similarly, the molar ratio of oxalate: magnesium is within the safe level for fruit (1.32) and leaves (0.53) and slightly high in seedcoats (2.59) (**Table 4**). The molar ratio of oxalate:calcium for some commonly consumed food items such as oat bran is 3.14, barley bran 3.14, red kidney bean 0.95 and white bean 0.28, respectively (15).

CONCLUSION

Recent research on the *T. ferdinandiana* have revealed information about the biological activities of phytochemicals present in *T. ferdinandiana* as well as the functions of *T. ferdinandiana* in food formulations and applications. In the present study, the proximate and mineral composition, vitamin C, phytate and oxalate contents of *T. ferdinandiana* have been investigated. This is the first study to report the phytate levels in *T. ferdinandiana* as well as in providing a detailed report on the nutritional and anti-nutritional compositions of *T. ferdinandiana*. The findings of this study will potentially increase consumer knowledge on the safe use of *T. ferdinandiana*. Phytate is considered anti-nutritional due to the chelating action of essential minerals. Results of the present study suggests that the phytate present in *T. ferdinandiana* would not have an impact on the availability of essential minerals, especially calcium. The oxalate calcium molar ratio is also within a safe level. However, predictive bioavailability determinations need to be confirmed by various *in vitro* and *in vivo* studies before making any claim on the safety of these anti-nutritional compounds. Future investigations into the *in vitro* bioaccessibility and bioavailability of oxalate, phytate, and calcium in *T. ferdinandiana* should be performed to better understand the implications of the anti-nutritional factors for health.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

SA, MN, MF, and YS conceived and designed the study. SA performed the experiments, analyzed data, and wrote the manuscript. UT helped in analyzing the mineral contents. MN, UT, SO, MF, and YS critically revised and edited the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Bambara Groundnut: An Underutilized Leguminous Crop for Global Food Security and Nutrition

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Rapid population growth, climate change, intensive monoculture farming, and resource depletion are among the challenges that threaten the increasingly vulnerable global agri-food system. Heavy reliance on a few major crops is also linked to a monotonous diet, poor dietary habits, and micronutrient deficiencies, which are often associated with diet-related diseases. Diversification—of both agricultural production systems and diet—is a practical and sustainable approach to address these challenges and to improve global food and nutritional security. This strategy is aligned with the recommendations from the EAT-Lancet report, which highlighted the urgent need for increased consumption of plant-based foods to sustain population and planetary health. Bambara groundnut (*Vigna subterranea* (L.) Verdc.), an underutilized African legume, has the potential to contribute to improved food and nutrition security, while providing solutions for environmental sustainability and equity in food availability and affordability. This paper discusses the potential role of Bambara groundnut in diversifying agri-food systems and contributing to enhanced dietary and planetary sustainability, with emphasis on areas that span the value chain: from genetics, agroecology, nutrition, processing, and utilization, through to its socioeconomic potential. Bambara groundnut is a sustainable, low-cost source of complex carbohydrates, plant-based protein, unsaturated fatty acids, and essential minerals (magnesium, iron, zinc, and potassium), especially for those living in arid and semi-arid regions. As a legume, Bambara groundnut fixes atmospheric nitrogen to improve soil fertility. It is resilient to adverse environmental conditions and can yield on poor soil. Despite its impressive nutritional and agroecological profile, the potential of Bambara groundnut in improving the global food system is undermined by several factors, including resource limitation, knowledge gap, social stigma, and lack of policy

incentives. Multiple research efforts to address these hurdles have led to a more promising outlook for Bambara groundnut; however, there is an urgent need to continue research to realize its full potential.

Keywords: Bambara groundnut, legume, food security, nutrition, underutilized crops, dietary diversification, food systems

INTRODUCTION

Eliminating hunger requires an adequate intake of energy and nutrients. Providing a healthy diet requires a food-based approach to improving diet and nourishing individuals. Despite the rich agrobiodiversity on Earth, humanity has evolved to rely on a few crops for nourishment. The last few decades have seen a global increase in the supply of dietary energy, through increased yield and production worldwide (1). However, this does not translate to the nutritional quality of the food we consume, nor does it ensure availability, accessibility, and affordability of food to vulnerable populations. The recent decades have seen an increase in prevalence of hunger, childhood overweight, and adult obesity (2). Should we continue with our current production and consumption patterns, we are unlikely to achieve the UN Sustainable Development Goal (SDG) of Zero Hunger by 2030 (2). Factors such as population growth, urbanization, and changes in dietary pattern toward resource-intensive foods are driving the demand for increased food production (3). Pest and disease outbreaks, resource depletion, regional conflicts, and climate change are set to further undermine the capacity of the food system and exacerbate the situation (2, 3). To meet the SDG of zero hunger by 2030 and to end malnutrition in all its forms, the target is to increase the availability and accessibility to nutrients, not just calories. Adoption of a diversified healthy diet, with emphasis on affordable nutrient-rich plant-based foods such as fruits, vegetables, whole grains, and legumes can contribute to sustainable food and nutrition security (2, 4) and to the achievement of SDG2.

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is a legume indigenous to Africa and is cultivated across the semi-arid sub-Saharan Africa region (5). It is a hardy crop and has been recognized as an important nutritious food source when food is scarce (6). This could be attributed to its climate-smart features, including its ability to fix nitrogen, and to grow under adverse environmental conditions such as poor soils and drought (7, 8). This nutrient-dense legume is sometimes termed a "complete food" due to its balanced macronutrient composition. Bambara groundnut contains ∼64.4% carbohydrate, 23.6% protein, 6.5% fat, and 5.5% fiber and is rich in minerals (9). It is relatively underutilized compared with major cash crops and has often been associated with small-scale, subsistence farming, with women being the major producers and processors (6, 10). The utilization constraints of Bambara groundnut include the knowledge gap in improved seed system, agronomic practices, processing, and utilization. Genetics, agronomy, and nutritional aspects of Bambara groundnut and its food uses have recently been reviewed by other authors (7, 9, 11, 12). This paper gives an overview of the value chain and discusses the potential role of Bambara groundnut in closing the gaps in the food system to ensure sustainability of food and nutritional security.

CLOSING THE FOOD SUPPLY GAP THROUGH IMPROVED PRODUCTION OF BAMBARA GROUNDNUT

Bambara groundnut is thought to have its center of origin somewhere between West and Central Africa (13). It is grown widely in sub-Saharan Africa and is also present at low levels in Thailand, Malaysia, and Indonesia (7). Higher preference for Bambara groundnut has been observed in dry regions prone to drought (14). This is possibly linked to its ability to produce reasonable yields under such conditions, hence acting as a safety net for farmers. Bambara groundnut production in Africa is reported to be ∼0.3 million tons annually with an average of 0.85 t/ha, although the yield potential is reported to be over 3 t/ha (5, 15). Nigeria is regarded as the largest producer of Bambara groundnut with a mean production of 0.1 million tons, followed by Burkina Faso 44,712 tons, and Niger 30,000 tons (5).

Genetic Diversity and Implications—Traditional Landraces vs. Modern Varieties

Most germplasm planted by famers is in the form of landraces with high genetic variability. This is reflected by the wide variations in morphological (16) and nutritional (9) traits across Bambara groundnut landraces. Genetic variability can act as a form of insurance for farmers as some members of the landrace population can provide local adaptation, stress tolerance, and yield stability (17), thus giving farmers a higher chance of obtaining some form of seed yield in times of drought or other stresses.

High genetic variability observed in landraces also lends itself to high potential for crop improvement in Bambara groundnut. Most of the currently grown improved varieties of Bambara groundnut are generally landraces selected for improved yield, seed and flour quality, and drought tolerance (18). Bambara groundnut is usually sown as a minor crop, intercropped with other staples, by small holder African farmers for household consumption (5). For this reason, coupled with low current market demand for the crop, yield stability is seen to be a more important aspect for landrace improvement than grain yield in order to ensure food security. Besides, the genetic diversity preserved in the

gene pool allows some of these accessions to be developed into high protein and high oil cultivars (9), suggesting its potential in contributing to nutritional security in the region. Breeding improvement efforts of the promising landraces could consequently lead to improved profitability of the crop as well as adoption of the crop in diverse Bambara groundnut growing regions.

Productivity Traits and Agroecological Adaptation

Variation in Bambara groundnut productivity has been attributed to agroecological factors, such as climate (19), soil fertility (20), water availability (21), and daylength (22). Nonetheless, it has been shown to exhibit adaptability across different regions under diverse growing conditions. For instance, the crop exhibits tolerance to soil acidity and low soil fertility (23), as well as adaptability to the tropical degraded acidic soils (24). Despite being classified as a facultative short-day crop for pod set (22), many landraces have adapted to regions with a broad range of daylengths. Physiological experiments have also revealed good recovery qualities when the crop is subjected to water stress (25). Its yield is reported to be well above those of chickpea and similar to groundnut cultivars under comparable drought stress conditions (26, 27). This indicates that selection for drought tolerance is key considering that the crop is generally cultivated in arid to semi-arid regions of sub-Saharan Africa. Limited studies indicated that, although field drought conditions reduce the seed yield in Bambara groundnut, there is no effect on the nutritional quality of the seed (28). This trend has been observed in limited landraces and also in common bean (*Phaseolus vulgaris* L.) (29), but further studies on Bambara groundnut would be required to confirm this hypothesis.

Some areas where Bambara groundnut is grown have poor soils that are lacking in nitrogen. Most farmers in those regions do not apply synthetic fertilizers to their crops because the costs are often prohibitive (30). Bambara groundnut, as other nodulating legumes, can fix atmospheric nitrogen to replenish soil nitrogen, hence making it a potential companion crop for intercropping and rotational systems. It is often intercropped with cereals and root crops that can provide a significant amount of the calorie intake (31). Its incorporation into crop rotation cycles can help to maintain soil fertility and break the cycles of pests and diseases, which is advantageous to resource poor farmers who might generally be unable to afford fertilizers and pesticides (32). Incorporation of Bambara groundnut in intercropping system with maize has been shown to increase the productivity of maize (33), indicating its potential contribution toward agrobiodiversity and subsequently food security. Varying rates and amount of nitrogen fixation have been observed for different Bambara groundnut accessions (24), and the enhancement of symbiotic nitrogen fixation was indicated to potentially increase its yield (34). The variability of nitrogen fixing capacity among Bambara groundnut landraces offers room for cultivar improvement and a positive correlation with yield would be an ideal scenario for the breeder.

CLOSING THE NUTRIENT GAP THROUGH ENHANCED UTILIZATION OF BAMBARA GROUNDNUT

Bambara Groundnut as a "Complete Food"

There is a growing trend toward increased consumption of plant-based diets, resulting in a need for more plant-based protein foods. Bambara groundnut is the obvious crop to consider. It serves as an important source of essential nutrients in areas where animal protein is scarce (35). The nutritional composition of Bambara groundnut has earned it the reputation of being a complete food, and this will be explored further in subsequent sections.

Balanced Macronutrient Composition

Carbohydrates

Carbohydrates are the most abundant macronutrient in Bambara groundnut, accounting for up to 64.4% of the total dry weight of the seed (9). The majority of the carbohydrate fraction is complex oligosaccharides and polysaccharides, of which starch accounts for up to 49.5% of the total carbohydrates. The reported starch content of Bambara groundnut seeds varies considerably (22 to 49.5% of dry seed weight), depending on genetic and environmental factors, stage of maturation, and method of analysis (12). Amylose represents 19.6–35.1% of the total starch content, while the rest of the constituents consist primarily of amylopectin and a small quantity (1–2%) of protein, lipid, and ash (12). Raw Bambara groundnut has a higher proportion of slowly digestible starch (SDS) and resistant starch (RS) than rapidly digestible starch (RDS) (36), implicating poor digestibility. Nonetheless, cooking can substantially increase the RDS fraction (37), thereby improving digestibility and carbohydrate availability.

Protein

The protein content of Bambara groundnut ranges from 9.6 to 40% (11), with an average value of 23.6% (9). This variation is also attributed to differences in genetic background, growing conditions, and analytical techniques used for estimation (e.g., nitrogen conversion factor) (10, 35). Storage proteins are the predominant protein fractions in Bambara groundnut, of which vicilin (7S) is reported to be the major constituent, followed by legumin (11S) (38).

High protein content is a desirable trait in foods, but the importance of protein quality, which is determined by both amino acid composition and protein digestibility, should not be overlooked. Variability in amino acid profile between cultivars of Bambara groundnut is evident. In general, most studies report glutamic acid to be the most abundant amino acid in Bambara groundnut, suggesting its potential to be isolated for use as a flavoring agent. Out of the essential amino acids, leucine and lysine are present at a higher concentration while methionine is the lowest (39–41). Phenylalanine, valine, histidine, and isoleucine were also reported to be present in high concentrations, while tryptophan has been found to be the limiting amino acid (37, 39). Its lysine-rich, methionine-poor composition makes Bambara groundnut a good complementary

protein source to cereals, which are often deficient in lysine but rich in methionine (35). The *in vitro* protein digestibility (IVPD) of raw and cooked Bambara groundnut varies between 70 and 81% and 82 and 87.5%, respectively (37, 42). The increase of IVPD after cooking is attributed to the destruction of heat labile antinutritional factors (ANFs) and fragmentation of native proteins into smaller polypeptides, subsequently improving enzyme accessibility and protein bioavailability.

Lipids

There is considerable variation (1.4 and 9.7%) in the reported values of lipid content in Bambara groundnut (38, 39). The majority of fatty acids in Bambara groundnut are unsaturated, predominated by oleic and linoleic acids (omega-6) (39, 43). Palmitic acid is the third most abundant fatty acid, and linolenic acid (omega-3) is present at a low concentration. While having high unsaturated fatty acid content is appealing from a consumer health perspective, it increases the susceptibility of fats to oxidation and rancidity. Therefore, the end uses should be taken into consideration when selecting the desirable trait of lipid composition.

Rich in Essential Micronutrients

Minerals

The most abundant minerals in Bambara groundnut are potassium, magnesium, phosphorus, zinc, and iron (37, 41, 44). Halimi et al. (9) reported that the levels of these minerals were higher than those found in commonly consumed legumes such as chickpea and mung bean, but they vary by cultivar and growing conditions. The presence of ANFs in the seeds can adversely affect the bioavailability of the minerals. Gwala et al. (45) reported that the concentration and bioaccessibility of calcium, magnesium, iron, and zinc in Bambara groundnut seeds were influenced by factors such as storage period, processing method, location of mineral in the seeds (testa or cotyledons), and the degree and strength of mineral chelation. Despite being a relatively good source of these minerals, it is unlikely that the dietary needs of individuals can be met through consumption of Bambara groundnut alone.

Phytochemicals

Bambara groundnut seeds contain phytochemicals such as flavonoids and tannins. These compounds are usually found in the seed coats and are more abundant in seeds with dark or red-colored seed coats. A positive correlation between darkness of seed coat and total phenolic compounds has been established (46). Mubaiwa et al. (47) reported an abundance of the flavonoids epicatechin and catechin in raw and cooked red seed, respectively. Catechin and epicatechin can polymerize to form proanthocyanidins, also known as condensed tannins, which have been associated with nutraceutical properties, such as antioxidant, cardioprotective, antitumor, and neuroprotective properties (48). Antioxidant properties have been reported in brown and red Bambara groundnut seeds, levels of which were comparable with commonly consumed legumes, but inferior to the powerful antioxidant ascorbic acid (49, 50). Despite the positive health outcomes associated with consumption of phytochemical compounds, their antinutritional implications should not be overlooked.

Other Important Functional Properties

Dietary Fiber

Bambara groundnut contains appreciable levels of dietary fiber in the form of RS and non-starch polysaccharides. The concentration and composition of dietary fiber are influenced by maturity stage and processing methods (39). Total dietary fiber content of Bambara groundnut ranges from 1.4 to 10.3%, of which insoluble fiber represents a higher fraction than soluble fiber (9). The relatively high proportions of SDS, RS, and dietary fiber in Bambara groundnut reduce the rate of digestion and lower the postprandial glycemic response, rendering Bambara groundnut a low glycemic index (GI) food (36). From one point of view, it is advantageous to encourage the consumption of low GI foods as these confer numerous health benefits, e.g., lowering postprandial blood glucose and insulin levels, regulating appetite, and reducing the risks of obesity and other non-communicable diseases. Conversely, the increased consumption of flatus-causing non-starch polysaccharides has been associated with irritable bowel (51). More importantly, from a nutritional security point of view, non-digestible dietary fibers can bind to minerals and and form a physical barrier to digestive enzymes, thus reducing the bioavailability of essential minerals (52).

Processing of Bambara Groundnut to Increase Nutritive Value and Utilization

Antinutritional Factors

In common with other legumes, several ANFs have been identified in Bambara groundnut. Their presence can negatively affect the digestion and bioavailability of essential nutrients. The commonly reported ANFs in Bambara groundnut include condensed tannins, phytic acid, and trypsin inhibitor. Condensed tannins are mainly located in the testa and are more abundant in the darker-colored seeds (53). Despite having an antioxidant capacity, these polyphenolic compounds can form indigestible complexes with dietary minerals, starch, and proteins, thereby reducing their bioavailability (51, 54). Binding with proteins can inhibit the activity of digestive enzymes. Tannin compounds can also impart bitterness and astringency to the food (48), thereby affecting palatability. Phytic acid is more abundant in the seed cotyledon, where it serves as a phosphorus reserve for the plant (52). At physiological pH, the highly charged phosphate groups have a high tendency to chelate to mineral cations and form stable, indigestible complexes (55). Phytic acid can also crosslink with dietary proteins, starch, and digestive enzymes, thus impairing the bioavailability of nutrients (51, 52). However, it is worth noting that phytic acid has been reported to exhibit antioxidant and anticancer properties, suggesting its potential health-promoting properties (51). The major enzyme inhibitor reported for Bambara groundnut is trypsin inhibitor (55). Inhibition of protease can negatively affect protein digestion and subsequently impede its absorption. Furthermore, low trypsin level can result in increased pancreatic secretory activity, thereby causing pancreatic hypertrophy (43). The reported levels of

ANFs among different Bambara groundnut cultivars vary widely (condensed tannins, 0.0011–18.61 mg/g; phytic acid, 1.10–15.11 mg/g; trypsin inhibitor, 0.06–73.40 TI mg/g). These differences are attributed to genetic and environmental factors, as well as extraction and analytical methods (54, 55).

Some forms of dietary fibers are also considered to have antinutritional properties. Pectins can bind to metal cations such as calcium, zinc, and iron, which, not only reduces mineral bioavailability, but affects the cookability of the legume (52). Raffinose and stachyose, the flatus-causing alpha-oligosaccharides, are also present in Bambara groundnut (43, 55). Other ANFs such as oxalate, hydrogen cyanide, and saponins have also been detected in Bambara groundnut (43, 46, 56).

Certain food processing methods are effective at lowering the ANFs, and this will be discussed in the following section. It is possible that the inherent levels of ANFs present in raw beans could be reduced by plant breeding (57), which would be advantageous for improved utilization of the legumes and in their contribution to enhanced nutritional security. However, gains made in improving the nutritional value through reduction of the antinutritional compounds, may be lost through increased susceptibility to pests and diseases during production and subsequent storage of the seeds. This is because these components are plant secondary metabolites that provide some resistance to stress, pests, and pathogens, therefore, reducing the levels may result in a compromised defense system (57).

Traditional Processing Methods

If not eaten fresh, Bambara groundnut is dried postharvest for long-term storage. Drying is an effective food preservation technique to prolong the storage period and ensure food availability during food shortages (58). Prior to consumption, the dry seeds are either rehydrated by soaking in water or milled in the dry form into flour. Most of the pretreatments or processing have an impact on the nutritional, sensory, and functional properties of the seeds. Traditional processing of Bambara groundnut involves basic equipment and can be carried out at the household level. Some of these processes have the potential to be mechanized and industrialized to improve the cost effectiveness, process efficiency, and product uniformity, while creating employment opportunities and providing income for rural people (58). The following section describes some of the traditional, often essential, processing stages of Bambara groundnut, and the impact on nutritional, processing, and eating quality.

Dehulling

The seed coat, or testa, is sometimes separated from the cotyledons before further processing. Since a high proportion of the antinutritional components are present in the testa, dehulling can improve the digestibility and nutritional value of the seeds, in particular through increased mineral and protein availability (40, 59). Removal of the testa also reduces the dietary fiber content (45, 52), which can have both negative and positive implications, depending upon the nutritional status of the consumer. With respect to sensory attributes, removal of the highly pigmented seed coats, which are rich in tannins and fibers, has been shown to improve the appearance, texture, and taste of Bambara groundnut products (53). Other implications of dehulling include increased leaching of minerals during soaking and cooking, which negatively impacts on the nutritional quality, and shortened fermentation time which is advantageous from a utilization point of view (40).

Milling

Dried Bambara groundnut can be ground into flour to improve its versatility (11). However, at the small scale, milling is laborious and time consuming due to a phenomenon described as "hard-to-mill" (10). The disruption of cell wall structure, through milling or other abrasive processing activity, can increase the availability and digestibility of nutrients, starch, and protein in particular (60). Increased interaction between starch, protein, and cell wall materials also results in structural and functionality changes (61). From a negative point of view, milling could increase interactions between minerals and ANFs, thus reducing their bioavailability (62). In terms of food security, the hard-to-mill attribute is advantageous in that it allows dried seeds to be stored for very long periods as they are impervious to water and resistant to pest and insect attack. However, from the utilization aspect, these inherent difficulties incur increased energy costs for processing and may deter potential end users from choosing Bambara groundnut as a raw material, despite its nutritional and agronomic advantages.

Soaking

After drying, Bambara groundnut seeds are typically rehydrated by soaking in water for 12–24 h before cooking. Soaking also has positive and negative impacts on the nutritional value of the seeds, primarily through leaching of nutrients and ANFs into the soaking water (42, 52). The rate and degree of leaching are influenced by the binding strength of the biomolecules to the intracellular matrix (45), which can be manipulated by the temperature and pH of the soaking liquid. Studies reported higher loss of trypsin inhibitor and tannins in Bambara groundnut during hot water soaking, but the reverse was observed for phytate and oxalate (63, 64). Soaking also facilitates the subsequent processing of Bambara groundnut. Increasing soaking temperature (up to 60°C) improved water absorption rate and dehulling efficiency and reduced dehulling loss (61). Regarding functional properties, presoaking Bambara seeds before milling results in flour with higher foam capacity and improved pasting properties (10, 64). From a food and nutritional security perspective, soaking is a low-cost, low-energy processing stage that can significantly improve the utilization of Bambara groundnut.

Germination/Malting

The nutritional value of Bambara groundnut seeds can be manipulated by germination. The process of soaking followed by sprouting for up to 72 h (65) reduces the carbohydrate and lipid content of the sprouts (66), while at the same time enhancing the protein content, amino acid profile, and IVPD (43, 67). Reduction of ANFs, such as tannins, trypsin inhibitor, oxalate, oligosaccharides, and saponin, is due to leaching during soaking

(43, 66). Sprouting is also beneficial to the dehulling process (63) as the seed coat splits open during sprouting.

Fermentation

Fermentation is another traditional, low-technology processing option that can be used to enhance the nutritional value of Bambara groundnut. Typically, the process involves soaking of the whole seeds, followed by dehulling, cooking, and wrapping in banana leaves before fermenting for about 4 days (40). Starter cultures may sometimes be added (44) to enhance the fermentation. The positive impacts of fermentation are a breakdown of the flatus-forming non-digestible oligosaccharides and polysaccharides into digestible simpler carbohydrates and a reduction in ANFs and phenolic content (40). The production of yogurt from extracted Bambara groundnut milk is reported to improve protein content and digestibility while lowering the phytate content (68).

Boiling

Bambara seeds are often cooked in excessive water for variable periods of time until the desired texture is attained. In common with the other treatments, genetic variability, physicochemical properties, age of the seed, and storage conditions can affect the time taken to reach the desired end point. In the presence of water, thermal treatment leads to starch swelling and gelatinization, protein denaturation, solubilization of water-soluble pectins, and eventually cell separation (60). The effects of boiling on nutritional quality of Bambara groundnut vary with cultivar, pretreatment applied, and the length of cooking time. However, most studies report that boiling has a positive impact on nutritional quality through the destruction of ANFs (53, 56, 64) and improved *in vitro* digestibility of starch and protein (37, 42). It is likely that improved digestibility of protein is due to destruction of ANFs, which are more susceptible to wet-heat than dry-heat, thereby releasing protein bound to them; whereas improved starch digestibility is due to the disruption of starch granules, consequently increasing amylolytic attack, and hydrolysis.

The various processing stages applied to Bambara groundnut, at small and larger scale, have positive and negative implications for its food and nutritional security. The presence of various ANFs and the indigestible nature of the raw seeds mean that processing is an essential stage for all legumes. From a food and nutritional security point of view, it is important to select a combination of processes that enhance the digestibility of the macronutrients, while at the same time minimizing losses of both macronutrients and minerals. When selecting or recommending particular processing methods, it is essential to consider the energy costs of each one, as these can be prohibitive to the utilization of the crop by resource-poor end users.

Traditional Food Uses

Bambara groundnut is commonly consumed as snack food after roasting or boiling (14, 69). The seeds and the flour have also been used to produce a myriad of traditional foods in different parts of Africa (**Figure 1**) (6, 14, 53, 56, 70). During the preparation of local delicacies, Bambara groundnut is often paired with cereals such as maize and millet (6, 69), which is beneficial in improving protein quality. In Nigeria, the popular *"okpa"* (steamed Bambara groundnut pudding), which is made from Bambara groundnut flour and red palm oil, plays an important role in contributing to dietary protein and vitamin A intake among school children (71).

Advanced Processing Technologies and Potential Food Uses of Bambara Groundnut

Advanced Processing Technologies

In addition to the traditional low-cost processing technologies, which are essentially used to make the seeds edible and fit for consumption, more advanced forms of processing can be employed to improve nutritional quality, modify the physicochemical characteristics, and expand the range of value-added products for increased utilization. Irradiation, infrared heating, and autoclaving are among these more advanced techniques. Unlike traditional processing techniques, which can be employed at household level, these technologies require more sophisticated equipment and therefore are carried out on a larger scale.

Despite being a non-thermal processing method, gamma-irradiation causes starch degradation or pregelatinization in Bambara groundnut, thus reducing the cooking time of the seeds (72). Shorter cooking time could also be due to increased cell wall permeability to water and/or heat as a result of damaged microstructure (73). Irradiation also affects protein structure and conformation, thus altering the functionality of the flour (72).

Infrared heating (micronization) can also result in reduction in cooking time of Bambara groundnut (74), which not only helps to save energy and water but also has knock-on positive impacts on the retention of nutrients. This instant heating process, which causes starch gelatinization and protein denaturation, could enhance the utilization of Bambara groundnut seeds and flour for production of a diverse range of convenience products such as partially cooked seeds or instant flour (75).

The use of high-pressure heating, via autoclaving, is useful for improving the nutritional value of the cooked seeds. The application of heat under high pressure is effective at deactivating ANFs, thus increasing the digestibility of proteins and starch (43). The high-temperature process can reduce the functionality of proteins, as measured by a reduction in foaming and emulsifying properties (64), which will limit the use of Bambara groundnut in food applications where emulsification and/or the ability to form a stable foam are essential attributes. From a nutritional security point of view however, any process that can improve the nutritional quality through improved digestibility and removal of ANFs, at the same time as reducing the cooking time and energy requirements, is a beneficial process.

Bambara Groundnut in Processed Foods

Advanced processing technologies and enhanced insights into food science, coupled with increased consumer demand for convenience food have led to increased availability and accessibility to processed foods at lower prices (76). While processed foods offer numerous advantages to consumers such

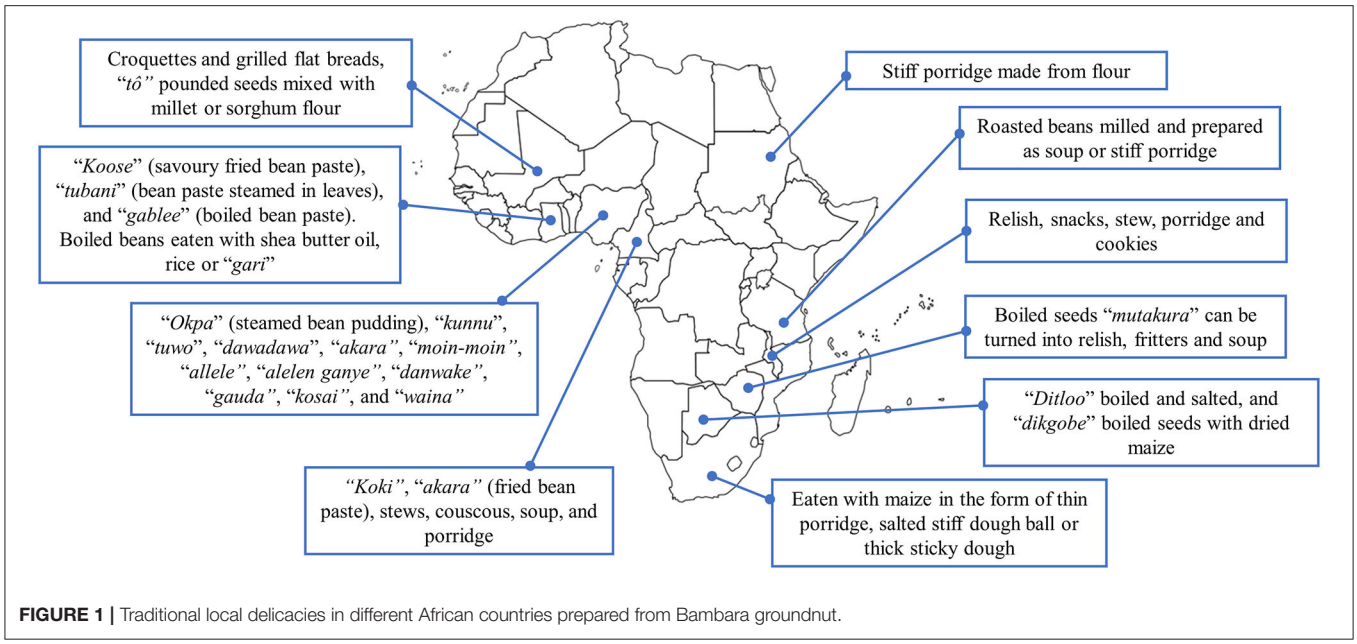

FIGURE 1 | Traditional local delicacies in different African countries prepared from Bambara groundnut.

as convenience, diverse food choices, and stability of food supply (76), the ultra-processed foods are often stripped of nutrients and are energy dense. Excessive consumption of empty calories can have negative impacts on health. To replace the micronutrients that are removed by processing, public health initiatives make the fortification of some food commodities with vitamins and minerals mandatory in most countries. Increasingly, alternative ingredients are being used to fortify staple foods, rather than relying on premix vitamins and minerals. This approach confers additional benefits to the consumer and producer—it offers a more holistic approach to food fortification or enrichment, utilizing otherwise underutilized plant species, and diversifying the diet with more than just the specific mineral or vitamin. Bambara groundnut has the potential to serve as an ingredient for food fortification due to its affordability, versatility, nutritional quality, and sensory acceptability.

Nwadi et al. (11) recently reviewed studies on the application of Bambara groundnut flour in a number of products including snacks and pastries, breakfast cereal and pasta, traditional foods, composite flour, complementary food, and milk and yogurt. To summarize, most studies (77–79) reported that the protein content of the Bambara groundnut flour-incorporated products increased with the supplementation level, but sensory attributes were negatively affected. This paper discusses a few points that were not covered by Nwadi et al. (11) review.

Staple Foods: Breads and Wheat noodles

Bread is one of the staple foods consumed on a global scale. It is commonly made from wheat flour, which is deficient in lysine. Complementing with legume flour or extracted legume protein can therefore improve its nutritional quality. However, consumers have come to expect leavened bread to have an airy texture, which is the result of gas being trapped inside a gluten network. Like other legumes, Bambara groundnut flour lacks gluten. Inclusion of Bambara groundnut flour into bread dough therefore has an adverse impact on the texture of the bread and this will limit the amount that can be used to substitute wheat flour. Reports on the use of Bambara groundnut flour in bread highlight impacts on protein weakening, increased dough development time, decreased dough consistency, stability and extensibility, water absorption, and loaf volume (80, 81). These observations were attributed to an impaired development of the gluten network due to dilution of gluten. Nonetheless, the dough rheology and physical characteristics of Bambara groundnut flour-incorporated dough can be improved by addition of active surfactants such as pectin and emulsifiers (78), thereby improving the organoleptic properties of breads.

Wheat noodles are staple in many Asian countries and are gaining popularity in other parts of the world. Gluten is also important in the production of noodles but not as critical as for a leavened bread. Wheat-based noodles are increasingly being fortified with ingredients such as spinach, pumpkin, and sweet potato to improve the nutritional value. Bambara groundnut flour has been included in wheat-based noodles with varying degrees of success (41, 82).

In general, addition of Bambara groundnut flour into bread and noodles improved the protein quality, reduced ANFs, and increased mineral contents (41, 77, 81). The level of substitution of wheat flour could be the key to consumer acceptability. An appropriate substitution level can help to ensure improved nutrition without compromising consumer acceptability (41, 81), thus making these products a promising food vehicle to combat nutrient deficiency. An alternative, more promising option for developing this area is to change consumer expectations, by marketing Bambara groundnut-enriched loaf/noodle as a novel and nutritious alternative product (41).

Snacks: Crackers/Biscuits/Extruded Products

Popular snack foods, such as crackers, biscuits, and extruded products, are typically made from wheat, rice, or maize. They are potential foods for fortification programs targeting school children and adult alike. Studies on the inclusion of Bambara groundnut flour in these products showed variable results in terms of their physical and functional properties (83–85). These variations could be due to different formulations and production methods used, which might also explain the differences in nutritional and sensory qualities among these products. In order to maximize the potential of Bambara groundnut-enriched snacks in improving nutritional security of the population, local consumer preferences should be taken into consideration during product development.

Complementary/Weaning Foods

Traditional weaning foods in Africa are often prepared from low-cost but highly accessible ingredients such as cereals, roots, tubers, and legumes (86, 87). They are often nutrient poor, characterized by high levels of starch, fiber, and antinutrients, but with inadequate levels of essential amino acids and micronutrients. Inappropriate processing methods also lead to poor texture and nutrient bioavailability (86). Several studies have successfully developed complementary foods containing Bambara groundnut and reported enhanced nutritional quality: Bambara groundnut-enriched maize "*ogi*" showed increased protein, ash and fat contents and high consumer acceptability (88); banana and fermented Bambara groundnut flour mix at 60:40 ratio showed comparable nutritional quality with commercial infant formula (89); and maize-Bambara groundnut complementary food fortified with micronutrients showed acceptable micronutrient levels to meet infant daily requirements (90). To conclude, its low-cost, nutrient-dense features allow Bambara groundnut to be a viable alternative ingredient in enriching infant food products.

Beverages: Milk and Fermented Drink

Inaccessibility of dairy milk in some countries, specific health-related dietary requirements and the trend toward plant-based diets are factors that are driving the surge in demand for vegetable milk (91, 92). Bambara groundnut has the potential for the production of vegetable milk and yogurt [reviewed by Murevanhema and Jideani (69)]. The development of shelf-stable spray-dried milk powder with acceptable hydration properties has been reported (93). Several reports refer to the nutritional and/or sensory properties of Bambara groundnut milk and yogurt (68, 92, 94). Overall, it can be concluded that despite its high nutrient content, there remains works to be done to optimize the sensory and physicochemical properties of Bambara groundnut milk to gain wider consumer acceptance.

Amahewu, or *mahewu*, is a popular fermented drink traditionally made by fermenting sorghum or maize flour. It is non-alcoholic and is sometimes used as a weaning food (44). However, it is nutrient poor and is often characterized as lacking essential amino acids (67). Substituting the conventional ingredients with Bambara groundnut reduced phytate content while improving its protein quality and sensory acceptability (44, 67).

Bambara Groundnut as a Functional Ingredient

In addition to providing essential nutrients, both the starch and protein of Bambara groundnut have functional properties, which may find wide application for food and non-food uses. Understanding and improving the functional properties of Bambara groundnut starch and protein isolate may increase the potential application and end-use of the crop, which *may* translate into an increased demand for the crop and benefits to the producer. However, it is prudent to point out that the relationship between increased utilization and producer benefits is far from simple—it is complex, fraught with issues of sovereignty and equity, and with no guarantee of improved livelihoods for the producer.

Native Starch

Starch is one of the most widely used and adaptable polysaccharides; in addition to providing energy, within the food industry, starch is used variously as a thickener, gelling agent, stabilizer, and humectant; and for non-food uses such as a replacement for polystyrene and plastic in disposable packaging material, plates, and cutlery, to name but a few. To fully maximize the potential application of Bambara groundnut starch, it is essential to understand the structure and physicochemical properties of the native starch.

The composition, physicochemical properties, and modification of starch has been reviewed by Oyeyinka and Oyeyinka (12). Bambara groundnut starch granule is characterized by spherical, polygonal, irregular, or oval-shaped granules with smooth surface and a diameter from 6 to 35 μm (12, 95). Both the major starch constituents, the amylose and amylopectin fractions, influence its physicochemical and functional properties, which in turn affect its application.

Bambara groundnut starch has poor swelling capability compared with conventional starches such as potato, corn, and cereal starches (12). This could be attributed to its relatively high amylose content, which results in a more rigid granular structure and therefore restricted swelling. The swelling power of Bambara groundnut starch increases with temperature, peaking at 80–90°C, and decreasing thereafter (96). This temperature range corresponds to its gelatinization temperature range, beyond which the starch granules rupture and the contents leach out, leading to inhibition of water uptake and swelling ability (97). The peak gelatinization temperature and enthalpy of gelatinization of Bambara groundnut starch are higher than most of those reported for cereal and tuber starches (98), indicating its thermal stability. Bambara groundnut starch also exhibits a relatively high pasting temperature that is comparable with other legumes (97), while its pasting viscosities show huge variations (12). This could be due to differences in cultivar, experimental condition, for instance starch concentration and purity, and the analytical equipment used, thus making it difficult to compare the results (99).

Its poor functionality, e.g., low swelling capacity and poor pasting properties gives Bambara groundnut native starch limited

applications as a functional ingredient. Possible application may include products in which restricted swelling and high thermal stability are desirable.

Modified Starch

Native Bambara groundnut starch can be modified to improve and diversify its behavioral characteristics. Modifications can be made physically or chemically. Physical modification, which is considered a safer modification approach, is associated with alteration of the starch granules by heat application (12, 98). Heat treatment leads to intragranular molecular reorganization of starch, thus leading to variable effects such as increased gelatinization and pasting temperatures, and reduced swelling power, solubility, and pasting viscosity (12). On the other hand, chemical modifications alter Bambara groundnut starch structure through introduction or formation of new functional groups (98), thereby affecting its physicochemical properties. Different modification methods (oxidation, acetylation, and carboxymethylation) can have variable effects on the solubility, swelling capacity, pasting properties, and water and oil absorption capacities of Bambara groundnut starch (100, 101). Bambara groundnut starch can also be modified by forming complexes with other components, such as lipids and cyclodextrin. This leads to increased thermal stability, and the resultant starch paste displays higher ability to withstand shear stress with a lower tendency to retrograde (96, 102).

These studies indicate that, with proper selection of modification techniques, Bambara groundnut starch has a great potential to be used as a functional ingredient in food applications such as improving viscosity, mouthfeel, adhesion, and freeze-thaw stability (98). Reduced digestibility of lipid-modified starch (102), which could be due to formation of type-4-resistant starch (103), could provide important nutritional functionality for diabetic patients or in weight management program.

Bambara Groundnut Protein Isolate

Bambara groundnut protein can be extracted and used as a functional ingredient in a number of foods. The reported protein content in Bambara groundnut protein isolate (BGPI) ranged from 81.4 to 92.8% (95, 104). Its solubility is pH dependent and has been shown to be higher than mung bean and black bean protein isolates (104). Besides, BGPI displays high thermal stability, which is comparable with mung bean, black bean, and soy protein isolates (38, 104). Studies reported variable results on the physicochemical properties (water and oil absorption capacities, gelation capacity, and foaming and emulsifying properties) of BGPI (105–107). The differences in functional properties of BGPI can be explained by several factors, including the amino acid composition (104), extraction method (38), extraction condition (108), and drying condition (109).

Since the physicochemical properties of BGPI can be altered and improved by various methods, BGPI has the potential to be a useful functional ingredient, especially for those avoiding animal-based products. BGPI exhibits high trypsin inhibitor activity (104), which is undesirable from a nutritional quality point of view, but can be exploited as a preservative. BGPI can act as protease inhibitor to lower proteolysis and improve the gel properties of surimi when applied at an appropriate level (110). The hydrolysates of BGPI have been shown to exhibit potent antioxidant activities, which may find application in food preservation or as a functional food (111). The bioactive peptides were also found to inhibit renin and angiotensin-converting enzyme (ACE), two components known to be associated with hypertension (112).

Enhanced Production and Consumption of Bambara Groundnut as Food: Challenges and Recommendations

The utilization of Bambara groundnut is challenged by several factors, as summarized in **Figure 2**. This section focuses on three of the constraints, namely the cookability, bottlenecks in the value chain, and safety issues, and discusses the possible solutions to these challenges.

Hard-to-Cook Phenomenon

The hard-to-cook (HTC) trait in Bambara groundnut is one of the major hurdles to its utilization [reviewed by Mubaiwa et al. (113)]. The HTC feature is associated with legume cotyledon resistance to softening during cooking, resulting in a prolonged cooking time to attain a desirable texture. Development of the phenomenon can be hereditary (114), and it can also be induced by extended storage under elevated temperature (>25°C) and humidity (>65%), that is, the ambient storage conditions in humid tropic areas (115). The pectin-cation-phytate model is the most widely accepted postulated mechanism for the development of HTC (114, 116): Pectin is present in the middle lamella where it acts as intercellular cement. Phytic acid, being a powerful chelator for cations, is usually bound to divalent cations especially Ca^{2+} and Mg^{2+}. During storage, phytase hydrolyzes the phytate-cation complex, hence liberating the divalent cations; meanwhile, pectin methylesterase removes the methyl esters of pectin, thus producing free carboxyl groups ($-COO^{-}$), which favor binding to the free divalent cations. The formation of insoluble Mg- or Ca-pectate complexes strengthens cell wall structure and cell-cell adhesion, thereby restricting cell separation and bean softening during cooking (117). Mubaiwa et al. (47) also suggested the involvement of phenolic compounds (tannins and hydroxycinnamic acid) in binding to pectin to form insoluble pectate, thereby reinforcing cell adhesion. Another popular hypothesis is cell lignification, which postulates that protein degradation may occur during storage, and the crosslinking between the freed aromatic amino acids and free phenolic compounds in the cell leads to lignin synthesis and causes cell wall toughening (114, 117). Besides, other mechanisms, e.g., lipid peroxidation (118), protein solubilization (119), cell membrane damage, and ion redistribution (120, 121) might also occur during storage, soaking, and cooking. The synergism of these reactions may amplify the effects, eventually leading to HTC phenomenon (116).

HTC phenomenon is reported to have negative impacts on the nutritional quality of Bambara groundnut. Gwala et al. (45) found that aging decreased *in vitro* bioaccessibility of calcium and magnesium in Bambara groundnut seeds. Prolonged cooking

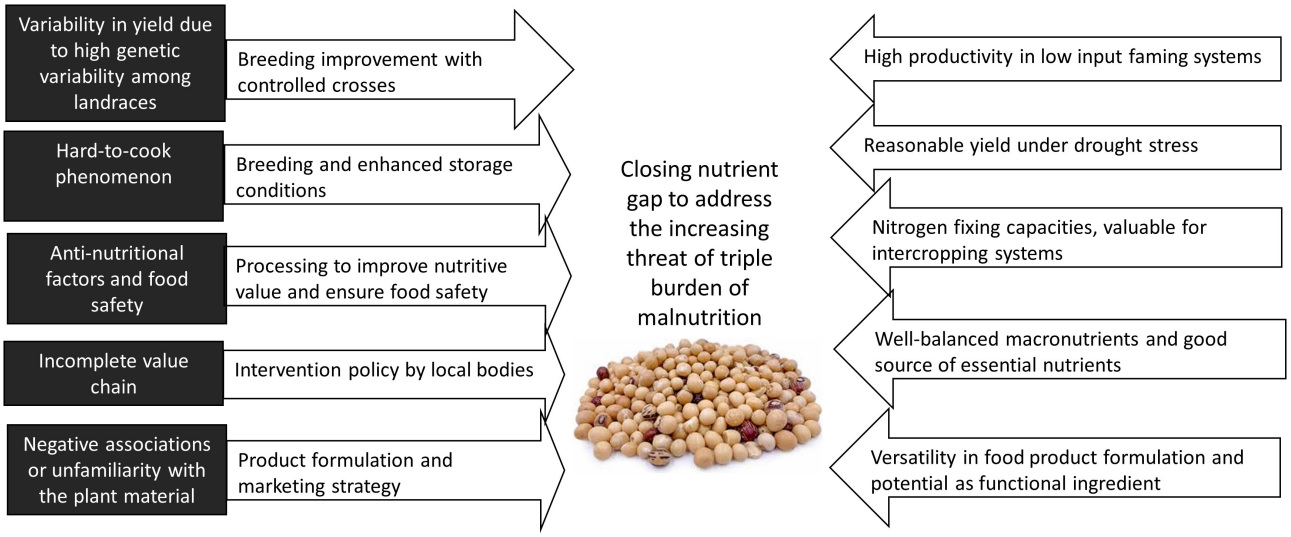

FIGURE 2 | An overview of the challenges and opportunities of utilizing Bambara groundnut in addressing food security and nutrition.

of aged seeds also reduced the levels of minerals [Mg, Fe, and Zn; (45)] and protein quality (122). The level of HTC and degree of cooking also influenced starch digestibility of HTC Bambara groundnut (115). Additionally, hardening of beans not only reduces the eating quality but also increases fuel and water consumption during cooking (14). This becomes a major problem especially for areas where firewood is used for fuel or water resources are limited. Consequently, the HTC phenomenon is not only inconvenient, it increases the cooking cost and also poses challenges for sustainability, which may limit its potential uptake on a larger scale (115).

Possible solutions to address the HTC defect include improving the storage conditions and breeding cultivars that are less prone to HTC (114). The former may require energy and incur additional costs, which may be infeasible for resource-poor areas; while the latter may take several generations of cross breeding. Simple low-cost processing techniques, such as soaking hard seeds in salt solution, has been demonstrated to reduce the cooking time (47, 123, 124). Sodium chloride, sodium bicarbonate, and alkaline rock salt are among the commonly used salts. Salts are believed to reduce cooking time through several actions: increased pectin solubilization by displacing divalent cations bound to pectin; increased protein solubility through modifying the pH of soaking medium; and enhanced water uptake and thermal penetration by improving bean porosity (116). These actions are influenced by the type, concentration, and monovalent-to-divalent ratio of salt (123, 124).

Further research is required to gain a better understanding of the fundamental mechanisms that lead to the development of HTC traits, and to elicit a viable, long-term solution to the problem.

Value Chain Constraints

Throughout Africa, Bambara groundnut is regarded by many as an important form of food security to be relied on when food is scarce (6, 66). Its tasty, nutritious properties are also recognized and valued by consumers, creating demand for the fresh seeds (125). Additionally, various cultural and religious beliefs are associated with consumption of the crop (126, 127). Despite the importance attached to the crop, there remain several obstacles to promoting local utilization of Bambara groundnut in the region. Inadequate (94) and inconsistent supply (128) are among the major barriers to wider consumption. Low market availability of the crop could be due to inefficient farming (129), poor weather and soil (127), competition and displacement by cash-crop farming (130), pests and diseases (5), lack of resources including access to high-quality seeds, land and storage, labor, capital and extension services (125), and societal norms and beliefs that restrict cultivation, retail, and consumption of Bambara groundnut (126). Difficulty to process and prepare due to its HTC and hard-to-mill properties are also reported to be one of the major concerns by consumers (10, 94). Besides, large-scale postharvest processing is further limited by lack of capital, processing facilities, and low product quality (6). Other factors such as disliked by consumers and flatus-causing attribute also limit its local adoption and consumption (125). Local demand and price may fluctuate over the year (6), which might in turn discourage cultivation of the crop.

Seed selection criteria, production constraints, and socioeconomic challenges vary across regions. Therefore, local bodies may play a significant role in developing strategies and policies to effectively pinpoint and address the local challenges. Community biodiversity management through provision of training and education, and enhanced seed access, has been proven to successfully improve adoption of Bambara groundnut in a few regions in Mali (130). Tackling other value chain bottlenecks such as infrastructure, processing unit and market access, and raising awareness about the contribution of Bambara groundnut to agri-food systems, population health, and community welfare, may prove crucial in encouraging

more widespread local uptake of Bambara groundnut (130). A complete value chain can help to ensure constant supply and reduced wastage, thereby preventing price fluctuation and enhancing utilization.

There is very little evidence of Bambara groundnut trading outside Africa (5), suggesting that it is relatively unknown by the rest of the world. Promoting the use of Bambara groundnut at global levels through effective promotion strategies may drive demand for the crop, which in turn could encourage cultivation and intervention policies by local and national bodies. In order to increase its popularity worldwide, it would be sensible to first understand consumers' demand and preferences. Several studies indicated sensory attributes being the major determinant of consumer acceptance to novel Bambara groundnut products (41, 67, 94). Certain pretreatments, e.g., dehulling, roasting, germination, and fermentation have been shown to result in higher sensory scores (53, 67). Besides, product formulation is imperative in improving consumer acceptability. Correct formulations and substitution levels could sometimes yield products that are more desirable than conventional products (67, 81). Lack of familiarity with the ingredient could deter consumers, but this aspect could be remedied by incorporating traditional ingredients into product formulation (88, 94). Additionally, pricing of the products should be taken into consideration to ensure they are affordable for most people. Lastly, marketing strategy of these products should be specialized for targeted consumer groups. Innovation, nutritional quality, and agroecological and health-promoting features are among the factors motivating consumers to consume Bambara groundnut products (41, 131).

Food Safety and Allergenicity

It is essential that any undesirable, and potentially toxic, attributes of Bambara groundnut are addressed to improve utilization. The presence of *Aspergillus flavus* and its aflatoxin has been detected in Bambara groundnut (132). The study reported that uncontrolled fermentation resulted in the worst proliferation of the fungus, and that although roasting eliminated aflatoxin before storage, it could not suppress fungal growth during storage. This raised concerns about implications of quality control during post-harvest processing, food production and storage, especially in humid tropical areas and regions with limited resources. There is also a need for quality assurance by accredited food testing facilities to ensure compliance with food safety regulations before the product reaches consumers.

Another area of concern is the possible presence of allergenic proteins in Bambara groundnut (133). There is very little reported research specific to Bambara groundnut, but it is essential to determine the magnitude of the problem among the various cultivars and landraces if it is to be adopted and promoted widely as an alternative food or ingredient.

The Roles of Bambara Groundnut in Closing Nutrient Gap

The nutrition transition in Africa, characterized by a move away from the production and consumption of traditional staple foods, rich in starch and dietary fiber, to more palatable staples and cheap processed food, is one of the drivers of the decline in consumption of Bambara groundnut. Other typical impacts of the nutrition transition include a decrease in plant protein sources, such as legumes, and increased availability and consumption of energy-dense snack foods, carbonated sweetened beverages, added sugar, fats, and oils in food preparation (134). Such changes in dietary pattern are propelled by economic and social development, urbanization, and acculturation, and they affect people of all socioeconomic status. For the rich and those with increased disposable income, the shift to highly palatable refined carbohydrates and animal sourced protein fulfills an aspirational goal. For others, the choice of food is dictated by circumstances. Trade liberalization and increased availability and affordability of ultra-processed food, table sugar, and cooking oil have elevated energy-dense nutrient-free food to be the "food of necessity." These foods are affordable, palatable, and easy to prepare. Increases in fuel and electricity prices have hampered food preparation and forced households to resort to less nutritious processed food that requires little preparation.

Nutrition transition is associated with increases in non-communicable diseases (NCDs) in developing countries (135). As a result, many of the developing countries, including the poorest, face the multiple burden of malnutrition. Hunger and under-nutrition, of especially energy and several micronutrient deficiencies, have not been successfully addressed in Africa while the epidemiologic transition is seen in the increased prevalence of obesity and other NCDs in many African countries (136, 137). The nutrition transition in developed countries presents well-identified features that help to predict consumption changes in low- and middle-income countries (LMICs) as they go through socioeconomic changes. Taking lessons from intensive monoculture and heavy reliance on major crops in developed economies, as well as their impacts on agricultural sustainability and nutritional status, future policy development in LMICs should place emphasis on diversification of national food supplies, and the access to and affordability of diverse and quality diets.

Countries at different stages of structural transformation should employ different strategies to enhance the contribution of agriculture to diet quality and nutrition (138). Pingali et al. (138) suggested that low-productive agricultural systems should focus on yield enhancement, while maintaining production diversity and ensuring equitable conditions for working women. Diversification of food supplies has been shown to be negatively associated with indicators of the prevalence of undernutrition (139). Climate and soil fertility are also critical to semi-subsistence agricultural production and human welfare in LMICs. As crops are grown for both household consumption and for income, there are multiple connections between these two factors and poverty and the health of the families that work the land. Bambara groundnut, being a climate-resilient and nitrogen-fixing legume, is an ideal candidate crop for diversifying local food production system while improving the nutritional status of the community.

The elevation of Bambara groundnut, from under-utilized to more mainstream crop, would theoretically be less challenging if industry led. By promoting its use at a commercial level,

smallholder farmers, while producing enough to feed the family, can sell the surplus to industrial producers for monetary returns that can be then used to improve the quality of life and nutritional status of the family. To increase utilization at the household level, it is important to address the HTC phenomenon. As evidenced by the health benefits detailed above, fortification or substitution of commercial products with Bambara groundnut can enhance the nutrient density of the products. There are numerous potential opportunities that deserve more research before they can be fully exploited. From a commercial point of view, demand for these products has to be created. Changing consumers' perceptions requires creative and effective marketing strategies, as is the case of quinoa (140). Sustainability and equity aspects should be considered and integrated into the entire value chain.

CONCLUSION

Despite being a minor crop, Bambara groundnut has the potential to play a role in combating food insecurity and malnutrition at household, national, and global levels. It has high adaptability in various growing conditions and can produce reasonable yield under environmental stresses. Not only does Bambara groundnut fix nitrogen to improve soil health, it also increases crop yield when incorporated into intercropping system. These positive attributes address the supply chain gap by ensuring supply stability and sustainability in the face of climate variability and resource depletion. The genetic diversity across Bambara groundnut landraces allows crop improvement program to select for desirable agronomic, nutritional, and processing traits that are advantageous to improved food and nutritional security.

Knowledge of the detailed nutritional quality and physicochemical attributes of Bambara groundnut will enable the wider use and application of the crop in numerous food products, for instance as alternative flour, modified starch, or protein isolate. The nutritional values, sensory attributes, and functional characteristics of food products can be modified through appropriate processing techniques.

The hard-to-cook phenomenon, value chain bottlenecks, and food safety remain the major constraints to increasing the widespread utilization of Bambara groundnut. These obstacles could be addressed by different players across the value chain through increased collaborative and interdisciplinary approaches in order to realize the full potential of Bambara groundnut's contribution toward food and nutrition security.

AUTHOR CONTRIBUTIONS

XT and FM: conceptualization. XT, EG, HC, MM, TM, and SuA-A: methodology and original draft preparation. FM and SM: funding from the University of Nottingham Future Food Beacon of Excellence. All authors: review and editing.

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Metabolite Fingerprinting of Kersting's Groundnut [*Macrotyloma geocarpum* (Harms) Maréchal & Baudet] Seeds Using UPLC-qTOF-MS Reveals the Nutraceutical and Antioxidant Potentials of the Orphan Legume

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The identification and subsequent quantification of phenolic compounds in plants is the first step toward harnessing their associated nutritional and health benefits. Due to their diverse phenolic compound compositions, grain legumes are known for their high nutritional and health values. The aim of this study was to assess the inter-variations in chemical composition, phytochemical content, and antioxidant capacity of seed extracts from eight Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Marechal & Baudet] landraces. The chemical profiles were evaluated using UPLC-qTOF-MS. Total phenolics and flavonoids content were determined by the Folin-Ciocalteu and aluminum chloride methods, respectively. The antioxidant capacities in the forms of DPPH and ABTS were evaluated using spectrophotometric methods. Principal component analysis was used to define similarities/differences between the landraces. Based on untargeted metabolomics analysis, 57 metabolites were identified, with phenolics, triterpenes, fatty acids, and sphingolipids being the most predominant. The results showed that the black seeded KG1 (Puffeun) had the highest total phenolic (9.44 mg GAE/g) and flavonoid (3.01 mg QE/g) contents, as well as antioxidant capacity (9.17 μg/mL and 18.44 μg/mL based on DDPH and ABTS assays, respectively). The concentrations of ferulic acid hexoside, procyanidin B2, eryodictyiol-7-rutinoside and quercetin pentoside ranged from 51.78–441.31, 1.86–18.25, 3.26–13.95 to 5.44–63.85 μg/mg, respectively. This study presents a useful report on the phytochemical characterization of Kersting's groundnuts and shows that the grains can be used as a source of nutraceuticals for human consumption.

Keywords: underutilized legumes, *Macrotyloma geocarpum*, UPLC-qTOF-MS, antioxidants, phenolic compounds, flavonoids, fatty acids, sphingolipids

INTRODUCTION

Legumes have long been known for their nutritional and health benefits through the provision of proteins and mineral elements in human diets (1–3). The nutritional benefits of grain legumes may be attributed to their phytochemical compositions that often confer biological activities of interest (4, 5). For example, most legumes are known to exhibit a wide range of seed pigmentation due to differences in the relative concentrations of anthocyanins, a flavonoid with multiple benefits for plant protection and human health (6–8). Moreover, legume seeds contain a diverse range of phenolics which may be classified according to their chemical structures as flavonoids (anthocyanins, flavones, and flavanols), phenolic acids, stilbenes, and tannins (9). In the plant system, phenolic compounds act as phytoalexins, antifeedants, attractants for pollinators, growth regulators, contributors to plant pigmentation, antioxidants, and protective agents against UV light (10, 11). They are also known to modulate several pathophysiological processes in humans, including inflammation, oxidative stress, blood pressure, microbial growth, mutagenic processes, as well as the reduction in the risk of chronic metabolic and degenerative diseases (12). Phenolic compounds also contribute to the bitterness, astringency, color, flavor, odor, and oxidative stability of food products (5, 13). Aside the nutraceutical benefits of phenolic and flavonoid compounds, they are also employed during the ensuing processes that lead to nodulation in the legume-rhizobia symbiosis (14, 15).

Legume seeds have been found to exhibit higher antioxidant activity among selected crops and have significant total phenolic content (16–18). Since antioxidant capacity in various legume seeds is directly linked to their total phenolic, flavonoid, and anthocyanin contents (17), the consumption of such legume products could contribute to the management and/or prevention of several chronic and degenerative diseases, in addition to their traditional role in the prevention of protein-calorie malnutrition (2). Like other phytochemicals, fatty acids, and sphingolipids also present various health related benefits in humans and are known to play an important role in complex metabolic processes. However, whereas saturated fatty acids often pose adverse health effects, the unsaturated fatty acids have protective roles (19). Due to their wide range of functions and biological properties, sphingolipids play vital roles in human systems, including the management of diseases such as cancer, obesity, and atherosclerosis (20).

In addition to the pressure on agricultural systems to produce enough food for the growing human population, the high demand for healthy foods has also led to increased efforts to develop or bio-prospect for crop genotypes with increased phenolic concentrations (21, 22). For example, recent breeding efforts have focused on incorporating biochemical pathways involved in the biosynthesis of desired phytochemicals in cultivated crops (23). However, the identification of crop genotypes showing a wide variation in phytochemical compositions offers an opportunity for their incorporation into other species. For this reason, there is renewed interest in the search for useful biological traits such as increased levels of biologically desired phytochemicals among underutilized legumes for improved human nutrition and health (24–27).

Kersting's groundnut (KG) is an under-utilized grain legume indigenous to sub-Saharan Africa (28). Grown for its seeds at the subsistence level, KG is known to possess high nutritional, medicinal and cultural values. In some parts of Africa, water from the boiled KG seeds is used for the treatment of diarrhea (29). Given its nutritional and medicinal attributes as well as the variable seed coat pigmentation, KG could be a source of natural antioxidants for daily consumption (8). In addition to its nutritional value, KG is capable of forming root nodules when in symbiosis with rhizobia, leading to the reduction of atmospheric N_2 to NH_3 for the benefit of cropping systems (30–32). There is however little information on the metabolite profile of KG seeds and their bioactivities, an aspect much needed to tap the nutraceutical benefits of this underutilized legume.

The aim of this study was to identify and characterize the metabolites in the edible seeds of eight KG landraces exhibiting variable seed coat pigmentation. The antioxidant capacity of the seed extracts was estimated using two independent assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS). The differences in the chemical profiles of the test landraces was assessed by means of principal component analysis (PCA). This study reports the phytochemical composition and the antioxidant activities of KG seeds and highlights their potential utilization as nutraceuticals.

MATERIALS AND METHODS

Chemicals and Reagents

Formic acid and all solvents used were of the LC-MS grade. Ultrapure water (resistivity of 18.2 MΩ cm^{-1} at 25°C) obtained from a Millipore water purification system was used. Standards of phenolic acids (gallic acid, caffeic acid, quinic acid, *p*-coumaric acid, ferulic acid, and sinapic acid) and flavonoids (catechin, epicatechin, rutin, naringin, gallocatechin, and eryodictyol-7-rutinoside) were obtained from Sigma Chemicals Co. (Germany). Sodium carbonate, potassium ferric cyanide, iron (II) sulfate, aluminum chloride, and hydrochloric acid were obtained from Merck (South Africa), while 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's reagent, gallic acid, 2,5,7,8-tetramethylchroman carboxylic acid (Trolox), potassium persulfate, glacial acetic acid, ascorbic acid, methanol, and ethanol were sourced from Sigma-Aldrich (St. Louis, MO, USA). All other solvents and chemicals used were of the reagent grade and purchased from Sigma-Aldrich (Germany).

Sample Materials

The eight (8) KG landraces (**Table 1**, **Supplementary Figure 1**) used in this study were sourced from the University for Development Studies (Nyankpala, Ghana). The landraces were previously collected from communities across the Upper West region of Ghana and described

TABLE 1 | Geographic origin and characteristics of the Kersting's groundnut (KG) landraces reported in this study.

Sample code	Local names	Seed weight[a]	Seed phenotypes	Origin	Geographic coordinates	
–	–	(g/100 seeds)	(color)	District	Longitude	Latitude
KG1	Puffeun	11.2 ± 0.74	Black	Lawra	10.6459	−2.8827
KG2	Boli	15.1 ± 0.01	White	Wa	10.0601	−2.5099
KG3	Funsi	14.3 ± 0.38	Brown mottled	Wa East	9.9858	−1.9099
KG4	Sigiri	16.1 ± 0.64	Brown mottled	Jirapa	10.5238	−2.7034
KG5	Nakori	17.0 ± 0.38	Brown mottled	Wa	10.0601	−2.5099
KG6	Heng Milk Mottled	16.5 ± 1.04	Brown mottled	Nandom	10.8526	−2.7606
KG7	Dowie	16.5 ± 0.89	Brown mottled	Sisala West	10.5229	−2.0665
KG8	Belane Mottled	13.6 ± 0.93	Brown mottled	Nadowli	10.3669	−2.6636

[a]*Seed weight is expressed as the mean ± standard deviation of triplicate weights of 100 seeds. Seed photos are provided in* ***Supplementary Figure 1****.*

according to their morphological and genetic traits (32, 33). The identification of the plants was done by examining the morphological characteristics of their flowers, leaves and seed structures. Voucher specimens (TUTMG1401-TUTMG1408), corresponding to KG1–KG8, respectively, were assigned to the seeds and deposited at the Herbarium of the Department of Crop Sciences, Faculty of Science, Tshwane University of Technology, Pretoria, South Africa.

To obtain fresh seeds for this study, each landrace was sown in three replicate plots measuring 2.4 m × 2 m at a spacing of 60 cm between rows and 20 cm between plants. Planting was done in open field experiments in June 2014 at Nyankpala in the Northern Region of Ghana. At maturity (120 days after sowing), plants were harvested, and the pods collected. Pods were sun-dried and threshed to obtain the seeds, which were then dried to 13% moisture content prior to determination of phenolic compounds in the laboratory. For each landrace, three biological samples were analyzed separately.

Sample Extraction

Extracts were obtained from the seeds of KG landraces harvested from the field experiments in Ghana. The seeds were ground using a coffee grinder (Cuisinart, model DCG-20N series). Approximately 30 g of ground KG were extracted thrice by maceration with 200 mL of 1% trifluoroacetic acid (TFA) in CH_3OH/H_2O (80:20). The mixture was then sonicated twice for 2 h and left at room temperature (20°C) for 24 h under constant agitation on a shaker. The extract was centrifuged for 15 min at 4,000 rpm and the supernatant collected. The resulting solutions were combined and evaporated to dryness under vacuum using a Buchi rotavapor R-100 (Buchi Labortechnik, Flawil, Switzerland) at 40°C and stored at 4°C prior to use within 24 h (34). One milligram of dried extracts was re-dissolved in 1 mL of CH_3OH/H_2O (1:1, v/v), filtered through a 0.22 μm syringe filter and transferred directly into HPLC vials (2 mL) before being analyzed by ultra-performance liquid chromatography.

To assess biological variance, 3 biological replicates for each sample were extracted and analyzed in parallel under identical conditions.

Identification of Phenolic Compounds in Seeds of Kersting's Groundnut

Qualitative Analysis Using UPLC-qTOF-MS

Chromatographic analysis was performed on a Waters Acquity Ultra Performance Liquid Chromatographic system with PDA detector (USA) coupled to MS detector (Waters Corporation, H-Class Bio System, Milford, USA). Separation was achieved on an Acquity UPLC BEH C18 column (150 mm × 2.1 mm, i.d., 1.7 μm particle size, Waters) maintained at 40°C. Preliminary tests were done prior to setting of the chromatographic conditions to obtain chromatograms with better resolution and short analysis time. The mobile phase consisted of 0.1% trifluoroacetic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 0.3 mL/min. The gradient elution was executed as follow: initial ratio was 90% A: 10% B, keeping for 1 min., changed to 25% A: 75% B in 10 min., to 5% A: 95% B in 5 min., keeping for 1 min and back to initial ratio in 0.5 min., with the equilibration of the system for 1.5 min. The total running time was 18 min. with an injection volume of 2.0 μL (full-loop injection). The positive and negative ion modes were examined, and the negative ion mode found to yield results with more information and higher sensitivity. Thus, the mass spectrometry was operating in a negative ion electrospray mode and nitrogen (N_2) was used as the desolvation gas. Data were acquired between 50 and 1,200 *m/z*. The following parameters were then set: capillary voltages of 3,000 V, sampling cone voltages of 45 V, extraction cone of 4, source temperature of 100°C; desolvation temperature of 350°C and desolvation gas flow of 400 L/h. The chromatographic software MarkerLynx (Version 4.1, Waters, Milford, MA, USA) was used to process and obtain all the chromatographic data. The proposed identification of compounds was based on both the UV-Vis spectra (indicating the different classes of phenolic compounds), mass spectra (including molecular formulas of the parent ions, the related fragments and the lost moieties), comparison with pure standards when available, and with previous literature and reference data from the PubChem database (http://pubchem.ncbi.nlm.nih.gov). Molecular formulas were considered when the mass error was below 4 ppm.

Quantitative Analysis Using UPLC-DAD

For metabolites quantification, chromatographic analysis was carried out on a Shimadzu® LC-20AD (Japan) UPLC system

equipped with a LC-20AD UPLC pump, a SIL-20AD auto sampler, a CTO-20AD thermostatted column compartment and an SPD-20AD photodiode array detector with a wavelength range of 190–700 nm. The analytical column was an ODS-Ultra Aqua C18 column (100 mm × 2.1 mm i.d., 3 μm particle size, Restek® (USA) protected with a Guard Cartridge (Restek®, Bellefonte, PA, USA). The column was maintained at 25°C. The mobile phase consisted of water solution of 0.1% trifluoroacetic (solvent A) and acetonitrile (solvent B). A linear gradient program at a flow rate of 0.200 was used: 0–5 min., 5% B; 5–25 min., 5–15% B; 25–35 min., 15–35% B; 35–45 min, 35–100% B; 45–48 min, 100–10% B; 48–50 min. Calibration curves were constructed for each standard using six concentrations with linearity range from 0 to 1,000 ($R^2 >$ 0.99). The concentrations of ferulic acid hexoside, procyanidin B2, eryodictyol-7-rutinoside and quercetin pentoside in samples were, respectively, calculated from the calibration curves constructed from pure ferulic acid, catechin, eryodictyol-7-rutinoside and quercetin standards (**Supplementary Table 1**). The assumption made for the quantification of ferulic acid hexoside was that its molar absorptivity is similar to that of its aglycone. The concentration of secondary metabolites in samples were expressed in μg/mg of dry weight.

Quantification of Phenolic Compounds

Total Phenolic Contents (TPC)

Total phenolic content of the samples was estimated using Folin-Ciocalteu's reagent according to the modified procedure of Singleton and Rossi (35) with slight modifications. A 20 μL of the extract solution (prepared by re-dissolving 1 mg of extract in 1 mL of methanol) was mixed with 100 μL of Folin-Ciocalteu's reagent and incubated at room temperature (20°C) for 5 min. Following the addition of 300 μL of saturated aqueous sodium to the mixture, total phenolic contents were determined after 2 h of incubation in the dark at room temperature (20°C). The absorbance of the resulting blue solution was measured at 765 nm with a Jenway 7300 UV-vis spectrophotometer (United Kingdom). Quantification was done using the standard curve of gallic acid (**Supplementary Table 1**), and the results expressed as milligrams of gallic acid equivalent (GAE) per gram of dry weight.

Total Flavonoid Contents (TFC)

Analysis of total flavonoid content was carried out as described by Woisky and Salatino (34). A standard curve ($R^2 > 0.99$) was constructed using six concentrations of quercetin (0, 25, 100, 500, 750, and 1,000 μg. mL^{-1}) for use in extrapolating the concentration of flavonoids in samples. For this, 5 mg of quercetin was dissolved in 5 mL of 50% ethanol and then diluted to 12.5, 25, 50, 100, and 200 μg. mL^{-1}. The standard solutions (2 mL) were separately mixed with 20 mL of 99% methanol, 1 mL of 5% aluminum chloride (wt/vol), 1 mL of 1 M potassium acetate, and the total volume was made up to 50 mL with distilled water. After incubation at room temperature (20°C) for 30 min, the absorbance of the reaction mixture was measured at 425 nm with a Jenway 7300 UV-vis spectrophotometer. An amount of 5% aluminum chloride was substituted by the same amount of distilled water in the blank. For the analysis of extracts from seeds of the different landraces, the procedure described above for the standard solutions were applied to 1 mL of each methanolic extracts (0.1 g/mL). The results were expressed as milligrams of quercetin equivalent (QUE) per gram of dry weight of plant material.

Antioxidant Capacity (AC)

Free Radical-Scavenging Ability Using Stable DPPH Radical

DPPH free radical scavenging activity was determined according to the method described previously by Kim et al. (36). Briefly, 0.2 mM of methanolic DPPH solution was prepared and diluted with methanol until the absorbance reached 0.9 at 517 nm. One sixty microliter of the resulting solution was added to 40 μL sample extracts at different concentrations (1.95–250 μg/mL) in each of 96 well plates. After incubation in the dark at ambient temperature (20°C) for 30 min, the absorbance was measured at 517 nm using a micro-plate reader.

Free Radical-Scavenging Ability Using Stable ABTS Radical

ABTS free radical scavenging activity was evaluated by ABTS radical cation decoloration following the procedure described by Nenadis et al. (37). For this, 7 mM of ABTS ammonium was dissolved in water and treated with 2.45 mM of potassium persulfate, and the mixture was then allowed to stand at room temperature (20°) for 12–16 h to obtain a dark blue solution. This solution was diluted with methanol until the absorbance reached 0.7 at 734 nm. A 160 μL of the resulting solution was mixed with 40 μL methanolic extracts of the samples at different concentrations (1.95–250 μg/mL) in each well of 96 well plates. After incubation in the dark at ambient temperature (20°C) for 5 min, the absorbance was measured at 734 nm using micro plate reader.

For both the DPPH and ABTS assays, varying concentrations of Trolox and ascorbic acid were used as standards to construct the calibration curves. The % scavenging effect was calculated according to the following equation:

$$\%\ \text{scavenging} = [1 - (\text{Absorbance of sample}/\text{Absorbance of control})] \times 100.$$

Percent of scavenging effect was plotted against log concentration (μg/mL). The antioxidant activity of KG seed extracts was expressed as half-inhibitory concentration (IC_{50}), which was defined as the concentration in μg/mL.

Data Analysis

Statistical analyses were performed using Statistica version 10 software package. Data were analyzed using one-way ANOVA statistical model. Duncan's multiple range test (DMRT) was employed for mean comparisons among landraces. The difference was considered significant at $p \leq 0.05$. All the experiments were performed in triplicates and data presented as mean ± standard error (SE).

Peak detection, alignment and the filtering of raw data were carried out using MarkerLynx v4.1. The parameters used

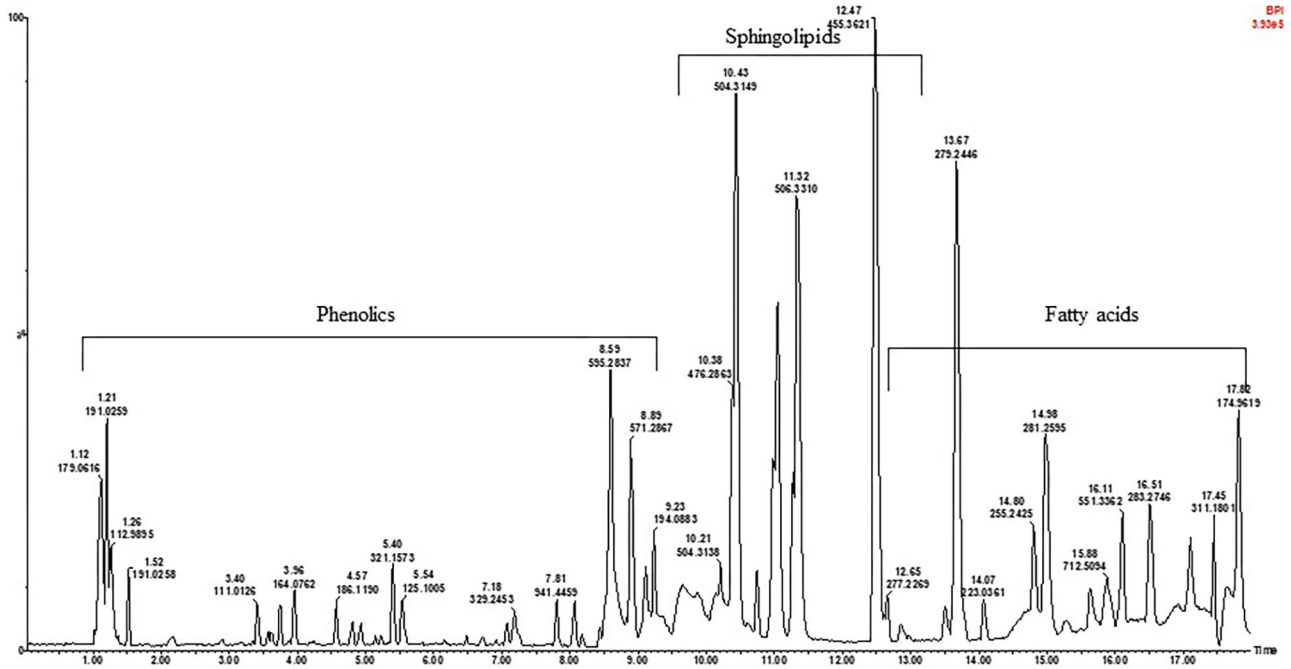

FIGURE 1 | A representative UPLC-qTOF-MS fingerprint analysis of the black seeded Kersting's groundnut [Puffeun (KG1)] seed extract in negative ionization mode. Each peak represents individual compound identified, with details provided in **Table 2**.

included a retention time range of 1–18 min, a mass range of 100–1,000 Da, and a mass tolerance of 50 mDa. Noise elimination level was set at 1.00, the intensity threshold (counts) of collection parameters was set at 500; retention time tolerance was set at 0.4 min. The retention time and m/z data pair for each peak was determined by the software. Thereafter, the data was exported to PAST under the 3.06 environment which was used to construct various chemometric models and the relationships between the datasets determined. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were used to arrange unsupervised and supervised large data sets. PCA model was constructed to investigate clustering patterns and to obtain a general overview of the variance of KG metabolites among the landraces.

RESULTS

Metabolites Identification Using UPLC-qTOF-MS Analysis

To assess the metabolite composition of the different KG seeds, a non-targeted metabolites profile of the extracts was conducted *via* high resolution UPLC-qTOF-MS. As an example, the TIC (Total ion chromatogram) profile of the black seeded KG1 in the negative mode is presented in **Figure 1**. The chromatograms for the white and brown mottled landraces are shown in **Supplementary Figure 2**. A total of 57 compounds belonging to the sub-classes of phenolic acids, flavonoids, saponins, sphingolipids, and fatty acids were identified in the seeds tested (**Table 2**).

The compounds detected from each chromatographic peak were tentatively characterized according to the corresponding spectral characteristics by comparison of their retention times and UV-vis spectra with those of available pure standards and confirmed by analysis of the mass spectra recorded for each peak. Other metabolites for which commercial standards were not available were identified based on their UV and MS spectra in comparison with previous reports on other grain legume sources of polyphenols.

Identification of Hydroxybenzoic and Hydroxycinnamic Derivatives

In this study, 15 phenolic acid derivatives were successfully identified based on UPLC-qTOF-MS analysis and MS/MS fragmentation patterns. Of these phenolic acids, hydroxycinnamic acids, with maximum UV-Vis absorbance (λ_{max}) within the range of 320 and 330 nm were the most represented. Peaks 3, 6, 15, 28, and 34 exhibited molecular ions $[M\text{-}H]^-$ at *m/z* 355.1007, 341.0934, 385.0896, 329.2181, and *m/z* 325.1961 corresponding to the elemental formulas $C_{16}H_{19}O_9$, $C_{15}H_{17}O_9$, $C_{17}H_{21}O_{10}$, $C_{14}H_{11}O_9$, and $C_{15}H_{17}O_8$, respectively (**Figure 1**; **Table 2**). The fragment ions at *m/z* 193 ($C_{10}H_{10}O_4$), *m/z* 179 ($C_9H_8O_4$), *m/z* 223 ($C_{11}H_{12}O_5$), *m/z* 267 ($C_8H_8O_4$), and *m/z* 163. ($C_9H_8O_3$) were indicative of a loss of an hexosyl unit (162 amu; $C_6H_{10}O_5$). Peaks 3, 6, 15, 28, and 34 were putatively identified as ferulic acid-hexoside, caffeic acid-hexoside, sinapic acid-hexoside, vanillic acid-hexoside and *p*-coumaroyl hexoside, respectively. Peak 26 presented a UV spectra similar to that of gallic acid but with different retention

TABLE 2 | Peak assignments of metabolites in acidified methanolic extracts of Kersting's groundnut (KG) seed analyzed using UPLC-qTOF-MS in negative ionization mode.

Peaks	R_t (min)	Tentative assignment of identified compounds	UV (nm)	$[M-H]^-$ (m/z)	Molecular formula	Error (mDA)	MS-MS fragmentation
1	1.00	Gallic acid[a]	272	169.0142	$C_7H_5O_5^-$	−2.2	125
2	1.13	Quercetin malonyl hexoside	256, 354	549.1440	$C_{24}H_{21}O_{15}^-$	1.2	505, 300, 301
3	1.15	Ferulic acid hexoside	291, 329	355.1007	$C_{16}H_{19}O_9^-$	−0.6	193, 175
4	1.16	Caffeic acid[a]	283, 320	179.0616	$C_9H_7O_4^-$	0.8	135
5	1.17	*p*-coumaroylquininc acid	265, 317	471.0544	$C_{22}H_{31}O_{11}^-$	−0.7	337, 191
6	1.19	Caffeic acid hexoside	287, 328	341.0934	$C_{15}H_{17}O_9^-$	−0.6	193, 179
7	1.21	Quinic acid I[a]	nd	191.0111	$C_7H_{11}O_6^-$	−2.0	nd
8	1.52	Quinic acid II	nd	191.0095	$C_7H_{11}O_6^-$	−2.0	nd
9	3.20	Catechin hexoside	279	451.1066	$C_{21}H_{23}O_{11}^-$	−0.6	162, 289
10	3.23	Catechin[a]	277	289.0598	$C_{15}H_{13}O_6^-$	1.2	245, 179
11	3.28	Syringic acid	275, 328	197.0341	$C_9H_9O_5^-$	0.9	182, 153, 135
12	3.43	*p*-coumaric acid[a]	229,312	163.0322	$C_9H_7O_3^-$	0.8	119
13	3.64	Ferulic acid[a]	281, 323	193.0400	$C_{10}H_9O_4^-$	0.9	178, 149
14	3.75	Sinapic acid[a]	238, 326	223.0495	$C_{11}H_{11}O_5^-$	1.1	208, 179
15	3.80	Sinapic acid hexoside	279, 329	385.0896	$C_{17}H_{21}O_{10}^-$	−0.8	233, 205
16	3.88	Epicatechin[a]	280	289.0597	$C_{15}H_{13}O_6^-$	1.2	245, 179
17	3.98	Rutin[a]	254, 350	609.1842	$C_{27}H_{29}O_{16}^-$	−0.9	343, 301, 300
18	4.09	Procyanidin dimer B1	232, 278	577.1085	$C_{30}H_{25}O_{12}^-$	2.8	559, 425, 289
19	4.41	Procyanidin dimer B2	278	577.1074	$C_{30}H_{25}O_{12}^-$	3.1	451, 425, 407, 289, 125
20	4.59	Procyanidin trimer B	279	865.1539	$C_{45}H_{39}O_{18}^-$	2.9	713, 695, 577,289, 287
21	4.12	Naringin[a]	225, 280	579.1148	$C_{27}H_{31}O_{14}^-$	2.9	459, 271
22	4.62	Gallocatechin Dimer	281	609.1154	$C_{30}H_{25}O_{14}^-$	3.2	441, 423
23	4.81	Gallocatechin[a]	279	305.0630	$C_{15}H_{13}O_7^-$	1.5	287, 179
24	4.84	Kaempherol-O-rutinoside	263, 340	593.1190	$C_{27}H_{29}O_{15}^-$	0.7	285, 447
25	4.94	Quercetin dihexoside	255, 372	625.1478	$C_{27}H_{29}O_{17}^-$	−0.6	300, 301
26	5.40	Digallic acid	272	321.1573	$C_{14}H_9O_9^-$	0.9	169
27	5.54	Pyrogallic acid	270	125.1005	$C_6H_5O_3^-$	0.9	nd
28	7.26	Vanillic acid hexoside[a]	254, 293	329.2181	$C_{14}H_{17}O_9^-$	0.3	267
29	7.88	Dihydroxy-olean-enoic acid dihexosyl rhamnoside	nd	941.5104	$C_{48}H_{77}O_{18}^-$	−5.4	915, 883, 795
30	8.43	Hydroxy oleanolic acid dirhamnosyl hexouronide	nd	939.4414	$C_{48}H_{75}O_{18}^-$	0.6	913, 881
31	8.78	Dihydroxy-octadecadienoic acid	nd	311.2098	$C_{18}H_{31}O_4^-$	5.5	275, 263
32	8.82	Apigenin dihexoside	267, 338	593.2672	$C_{27}H_{29}O_{15}^-$	1.3	225, 269
33	9.33	Eryodictyol 7-O-rutinoside[a]	287	595.2837	$C_{27}H_{31}O_{15}^-$	−1.1	287
34	9.64	*p*-coumaroyl hexoside	312	325.1961	$C_{15}H_{17}O_8^-$	−0.7	163
35	10.21	LysoPE (20:2)	nd	504.3149	$C_{25}H_{47}NO_7P^-$	8.9	301
36	10.33	Quercetin pentoside	373	433.2163	$C_{20}H_{17}O_{11}^-$	2.8	301
37	10.38	LysoPE (18:1)	nd	476.2863	$C_{23}H_{43}NO_7P^-$	7.8	279
38	10.42	LysoPC (18:1/2:0)	nd	564.3290	$C_{27}H_{51}NO_9P^-$	5.3	279
39	10.65	Trihydroxy-9,14-octadecatrienoic acid	nd	325.1973	$C_{18}H_{29}O_5^-$	2.7	nd
40	10.95	LysoPE (16:0)	nd	452.2878	$C_{21}H_{43}NO_7P^-$	6.1	nd
41	11.05	LysoPE (18:0)	nd	480.3171	$C_{23}H_{47}NO_7P^-$	6.5	nd
42	11.28	LysoPE (18:2)	nd	478.3016	$C_{23}H_{45}NO_7P^-$	4.9	184
43	11.32	LysoPE (20:1)	nd	506.3310	$C_{25}H_{49}NO_7P^-$	7.1	281
44	11.35	LysoPC (18:0/2:0)	nd	566.3447	$C_{27}H_{53}NO_9P^-$	5.2	184
45	12.53	LysoPA (20:4)	nd	456.3652	$C_{21}H_{47}NO_7P^-$	7.4	nd
46	12.61	Oleanolic acid	nd	455.3304	$C_{30}H_{47}O_3^-$	−0.5	nd
47	12.64	Linolenic acid	nd	277.2269	$C_{18}H_{29}O_2^-$	−3.0	211
48	13.19	Hydroxy-palmitic acid	nd	271.2151	$C_{16}H_{31}O_3^-$	1.9	180
49	13.50	Palmitoleic acid	nd	253.2257	$C_{16}H_{29}O_2^-$	−3.2	113

(Continued)

TABLE 2 | Continued

Peaks	R_t (min)	Tentative assignment of identified compounds	UV (nm)	$[M-H]^-$ (m/z)	Molecular formula	Error (mDA)	MS-MS fragmentation
50	13.88	Linoleic acid II	nd	279.2207	$C_{18}H_{31}O_2^-$	−1.4	211
51	14.07	Linoleic acid I	nd	279.2439	$C_{18}H_{31}O_2^-$	−1.7	211
52	14.80	Palmitic acid	nd	255.2203	$C_{16}H_{31}O_2^-$	−2.5	113
53	14.98	Oleic acid I	nd	281.2747	$C_{18}H_{33}O_2^-$	−1.6	183
54	15.48	Oleic acid II	nd	281.2598	$C_{18}H_{33}O_2^-$	−1.6	183
55	15.62	Phenyl lactic acid	270	165.0454	$C_9H_9O_3^-$	−2.3	nd
56	16.51	Stearic acid	nd	283.2746	$C_{18}H_{35}O_2^-$	−1.7	nd
57	17.45	Dihydroxy octadecadienoic acid	nd	311.2211	$C_{18}H_{31}O_4^-$	5.5	275, 263

Rt, retention time; LPE, lysophosphatidylethanolamine; PLC, lysophosphatidylcholine; LPA, Lysophosphatidic acid. I, II, III stand for isomers. [a]*Confirmed using available standards; all the other compounds were identified based on MS/MS data; nd, not detected.*

times. The MS presented the fragment $[M-H]^-$ at *m/z* 321.1573 corresponding to 2 galloyl units. Based on the MS data and previous literature (38), this compound was assigned to the hydrolysable tannin, digallic acid. The above analytical approach also led to the proposed identification of peaks 27 and 55 as pyrogallic acid and phenyl lactic acid, respectively.

Identification of Flavonoids

Compounds 9, 10, 16, and 18–20 showed UV spectra with shape and maximum wavelength which were similar to that of catechin. Peak 20 had molecular ion $[M–H]^-$ with *m/z* 865.1539 and λ_{max} of 279 nm and was identified as procyanidin trimer with B-type linkage. Compounds 22 and 23 presented molecular ions $[M-H]^-$ at *m/z* 609.1154 and 305.0630, respectively, and were tentatively identified as gallocatechin dimer and gallocatechin, even if the fragment ions from their MS/MS fragmentations could not be obtained. The UV spectra of compounds 2, 25 and 36 were similar to that of quercetin. The MS (ESI) analysis presented their peaks at m/z 549.1440, 625.1478, and 433.2163, respectively. The fragmentation pattern analysis of peaks 25 and 36 revealed a loss of two hexose units and pentose, respectively. The resulting fragments at *m/z* 301 ($C_{15}H_{10}O_7$) for both peaks could be attributed to quercetin, suggesting that in these compounds, quercetin is linked to two hexose units and one pentose, respectively. Peak 2 ($\lambda_{max} = 354$ nm) had a molecular ion $[M-H]^-$ at *m/z* 549.1440 and showed MS/MS fragment at *m/z* 301.0348, corresponding to a loss of 248 amu. This indicated a loss of 162 amu for hexose plus 86 amu for malonic acid. Apigenin dihexoside is proposed for peak 32 with molecular ion $[M-H]^-$ at *m/z* 593.2479. In the MS/MS spectra, the loss of two hexose (322 amu) moieties gave a fragment ion at *m/z* 270, which corresponds to the structure of apigenin. Following the above analytical approach, peaks 17, 21, 24, and 33 were proposedly identified as rutin, naringin, kaempherol 7-rutinoside, and eriodictyol 7-rutinoside, respectively.

Identification of Fatty Acids and Sphingolipids

In the second half of the chromatogram, peaks corresponding to fatty acids (saturated and unsaturated) and sphingolipids were identified. Several signals of unsaturated fatty acids were assigned as oleic acid (53), linoleic acid (50), linolenic acid (47), and palmitoleic acid (49). This was evident from the high-resolution masses at *m/z* 281.2747, 279.2439, 277.2269, and 253.2257 corresponding to the molecular formula $C_{18}H_{33}O_2^-$, $C_{18}H_{31}O_2^-$, $C_{18}H_{29}O_2^-$, and $C_{16}H_{29}O_2^-$ (39, 40). Other signals were attributed to saturated fatty acids, i.e., stearic acid (56) and palmitic acid (52) as evident from the high-resolution mass at *m/z* 283.2746 and 255.2203; with predicted molecular formulae of $C_{18}H_{35}O_2^-$ and $C_{16}H_{31}O_2^-$, respectively.

In this same half of the chromatogram, several intense peaks which presented an even molecular mass of 452.2878, 456.3652, 476.2863, 478.3016, 480.3171, 504.3149, 506.3310, 564.3290, and 566.3447 with predicted molecular formula of $C_{21}H_{43}NO_7P^-$, $C_{21}H_{47}NO_7P^-$, $C_{23}H_{43}NO_7P^-$, $C_{23}H_{45}NO_7P^-$, $C_{23}H_{47}NO_7P^-$, $C_{25}H_{47}NO_7P$, $C_{25}H_{49}NO_7P^-$, $C_{27}H_{51}NO_9P^-$, and $C_{27}H_{53}NO_9P^-$, respectively, were observed. The difference of 2 amu between these masses is indicative of the presence of unsaturation (41).

Identification of Saponins

The seeds of the KG landraces contained 3 triterpenoid saponins of oleanane series which were detected in peaks 29, 30, and 46, containing 3-hydroxy-12-olean-28-oic acid (*m/z* 455) aglycones based on the report by Frang et al. (41).

Total Phenolic and Flavonoid Contents, and Antioxidant Activity of KG Seed Extracts

The ability of the methanolic extracts prepared from KG seeds to scavenge free radicals using DPPH and ABTS assays in comparison to trolox and ascorbic acid used as positive controls were evaluated (**Table 3**), with low IC_{50} values indicating a strong capacity to quench free radicals and *vice versa*. With respect to DPPH assay, the tested landraces recorded significantly ($p < 0.05$) higher IC_{50} values compared to trolox and ascorbic acid used as positive controls. The IC_{50} values ranged from 5.12 for KG3 to 95.21 µg/mL for KG8 (**Table 3**). The ABTS results revealed marked variations in the IC_{50} values of landraces, with values ranging between 11.77 for KG3 to 63.65 µg/mL for KG8. The landrace KG3 recorded similar IC_{50} value as

TABLE 3 | Total phenolic, flavonoid and anthocyanin contents as well as the antioxidant capacity of Kersting's groundnut (KG) seeds.

Landraces	TPC[a] (mg GAE/g)	TFC[b] (mg QUE/g)	DPPH IC_{50} (μg/mL)	ABTS IC_{50} (μg/mL)
KG1	9.44 ± 0.03a	3.01 ± 0.3a	9.17 ± 0.12bc	18.44 ± 0.30ef
KG2	1.73 ± 0.05h	0.54 ± 0.03c	11.17 ± 0.10b	23.08 ± 0.29d
KG3	6.45 ± 0.16c	2.78 ± 0.30a	5.12 ± 0.10d	11.77 ± 0.14g
KG4	5.16 ± 0.01e	2.49 ± 0.18ab	7.70 ± 1.35cd	19.91 ± 0.55e
KG5	7.96 ± 0.15b	2.82 ± 0.15a	9.10 ± 0.92bc	29.85 ± 1.69b
KG6	5.57 ± 0.19d	2.11 ± 0.23b	6.57 ± 1.44cd	16.83 ± 0.52f
KG7	3.61 ± 0.07g	2.75 ± 0.11a	6.62 ± 0.62cd	26.91 ± 1.90c
KG8	4.07 ± 0.02f	2.89 ± 0.08a	95.21 ± 1.62a	63.65 ± 0.43a
Trolox	–	–	2.38 ± 0.08e	10.91 ± 0.39g
Ascorbic acid	–	–	2.15 ± 0.40e	3.43 ± 0.78h
F statistics	516.8***	16.8***	996.0***	330.1***

[a] *Total phenolic content is expressed as milligrams of gallic equivalent (GAE) concentration per gram of dry seeds.* [b] *Total flavonoid content is expressed as milligrams of quercetin equivalent (QUE) concentration per gram of dry seeds. Values (means ± SE) with dissimilar letters in a column are significantly different at ***$p < 0.001$.*

the trolox control, and together with the remaining landraces had lower values when compared to the ascorbic acid control (**Table 3**). Among the landraces, KG3 appears to exhibit the most antioxidant activity as evidenced by its lower IC_{50} values in both assays. Conversely, the markedly higher IC_{50} values of KG8 with respect to DPPH and ABTS assays indicates least antioxidant activity of the landrace compared to the others (**Table 3**).

Furthermore, TPC and TFC were determined using colorimetric methods, and results were expressed as mg of gallic acid and quercetin equivalents, respectively, per gram of seed dry weight. The total phenolic content showed significant ($p < 0.05$) variation among the landraces and ranged from 1.73 to 9.44 mg GAE/g[1] of dry weight (**Table 3**). The highest and lowest TPC values were observed in the black seeded KG1 (9.44 mg GAE/g) and white seeded KG2 (1.73 mg GAE/g), respectively (**Table 3**). Total phenolic content decreased in the order KG1 > KG5 > KG3 > KG6 > KG4 > KG8 > KG7 > KG2. Clearly, the KG seeds in this study exhibited marked variations in flavonoid contents (**Table 3**). The white seeded KG2 recorded the least total flavonoid content (0.53 mg QUE/g) compared to the remaining landraces which had higher but similar total flavonoid contents, ranging from 2.11 to 3.01 mg QUE/g of dry weight (**Table 3**). Whereas, the white pigmented KG2 showed the least total flavonoid content, the variation in total flavonoid content was not significant ($p > 0.05$) among the remaining dark-seeded landraces in this study.

To elucidate the relationship among the different KG landraces, the $1/IC_{50}$ values were correlated to the TPC, TFC, ferulic acid hexoside, procyanidin B2, eryodictyiol-7-rutinoside and quercetin pentoside contents of the eight samples using Pearson's correlation analysis, and several meaningful correlations were observed (**Supplementary Table 2**). Firstly, the ABTS correlated with DPPH, TPC, TFC, and all individual phenolics, except for quercetin pentoside. A significant correlation between TPC and antioxidant capacity (ABTS and DPPH) was also observed ($r = 0.79$, $p < 0.05$ and 0.67, $p < 0.05$, respectively) while a similar trend was also noticed between TFC and antioxidant activity based on ABTS ($r = 0.87$, $p < 0.01$) and DPPH ($r = 0.83$, $p < 0.05$) assays (**Supplementary Table 2**).

Multivariate HCA and PCA Analysis of UPLC-qTOF-MS Data

Due to the complexity of the data acquired, as reflected on the chromatograms, chemometric analysis of the biochemical profiles derived from the eight KG landraces was used to evaluate the contribution of the major phenolic compounds to their diversity and relationships. Differences in the chemical composition and concentrations of individual compounds were evident from the UPLC profiles of the tested landraces with varying susceptibility. The extracts from the samples were analyzed in both positive and negative ionization modes, and the negative mode appeared to be more sensitive. From the 24 samples, 5,232 and 4,680 mass signals were extracted by MarkerLynx software from the UPLC-qTOF-MS data set acquired in negative and positive modes, respectively. Since the PCA results obtained from both positive and negative modes were similar, the results derived from the negative mode are presented herein. Triplicate measurements from the same sample were found to be reproducible, as the scores of replicate measurements were close and/or superimposed (**Figure 2A**). Considering the 57 variables as analytical data, the PCA was able to discriminate among landraces. Five principal components (PCs) were required to capture 99% of the total variance. The main PC that differentiated the KG samples was PC1, which accounted for 66% of the variance, while PC2 explained 21% of the variation captured. Landraces KG2, KG6, KG7, and KG8 were positioned on the right side (positive PC1 values) whereas landraces KG1, KG3, KG4, and KG5 were located on the left side of the vertical line (representing negative PC1 values) with KG7 being the most distant from the others. The PC1/PC2 scores plot (**Figure 2A**) revealed the existence of three distinct clusters distributed over three regions. The segregation observed in the PCA score plot can be explained in terms of the identified compounds using the loadings plot that revealed the compounds having significant effect on the principal component (PC1). Examination of the loadings plot (**Figure 2B**) showed that the variables refer to signals with retention/mass ratio ($r_t/m/z$) of 1.15/355, 4.09/577, 9.33/595, 10.33/433, 12.61/455, and 14.07/279, respectively corresponding to procyanidin B2, eriodictyol-7-O-rutinoside, oleanolic acid, quercetin pentoside, ferulic acid hexoside, linoleic acid, and dihydroxy-octadecadienoic acid, and were the most involved in discriminating among the KG landraces. Dihydroxy-octadecadienoic acid, quercetin pentoside, and eriodictyol-7-O-rutinoside contributed positively to PC1 while ferulic acid hexoside, oleanic acid, and procyanidin B2 contributed negatively.

HCA was also used as an additional tool to explain the segregation between the different landraces. From the

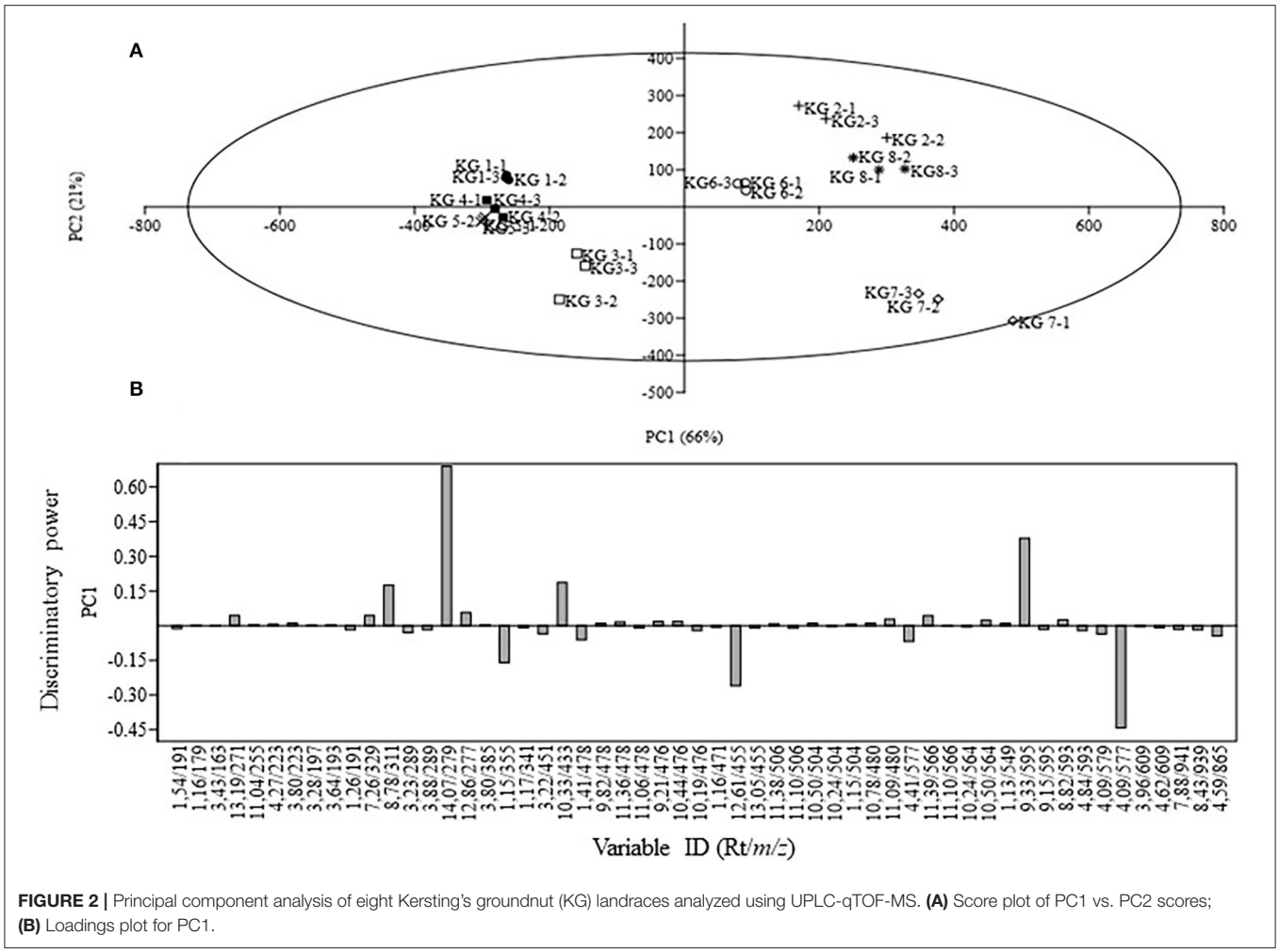

FIGURE 2 | Principal component analysis of eight Kersting's groundnut (KG) landraces analyzed using UPLC-qTOF-MS. **(A)** Score plot of PC1 vs. PC2 scores; **(B)** Loadings plot for PC1.

dendrogram (**Figure 3**), two distinct clusters with 4 landraces were observed. Examination of Cluster **A** showed that KG3 is more related to KG4 toward KG5 and KG1. In Cluster **B**, KG7 was the most distant landrace in comparison to KG2, KG6, and KG8, while all the other landraces grouped in one separate sub-cluster. The clustering of landraces mirrored the patterns of their total phenolic contents. For example, the samples in Cluster **A** had greater phenolic contents and antioxidant properties compared to those in Cluster **B**.

Metabolites showing differential accumulation among the seeds were subjected to absolute quantification using pure standards (**Table 4**). In agreement with the PCA and HCA results, the highest concentrations of ferulic acid hexoside, procyanidin B2 were recorded in landraces KG1, KG3, KG4, and KG5. As shown in **Table 4**, ferulic acid hexoside was the most predominant compound in the KG seeds, with the highest concentration detected in KG1 with a mean value of 441.31 ± 55.80 μg/g DW, while KG7 recorded a much lower concentration (51.78 ± 11.39 μg/g DW). Procyanidin was found in traces in the white seeded KG2 while eriodictyol-7-*O*-rutinoside was not detected in KG7.

DISCUSSION

Phenolic Acids Composition of Kersting's Groundnut (KG) Seeds

Although KG seeds have been reported to have medicinal value, not much information exists on the crop's metabolite composition. To explore the potential of KG as a source of nutraceuticals, this study screened for the presence and relative concentrations of metabolites in the seeds of different landraces of this orphan legume. The findings revealed the presence of diverse classes of secondary metabolites in the seeds tested, some of which exhibited variable abundance among the landraces. For example, several phenolic acid derivatives such as ferulic acid-hexoside, caffeic acid-hexoside, sinapic acid-hexoside, vanillic acid-hexoside, and *p*-coumaroyl hexoside were detected in the seeds tested. A previous study by Nyau et al. (21) identified similar compounds in seeds of Bambara groundnuts, another underutilized legume indigenous to Africa and known

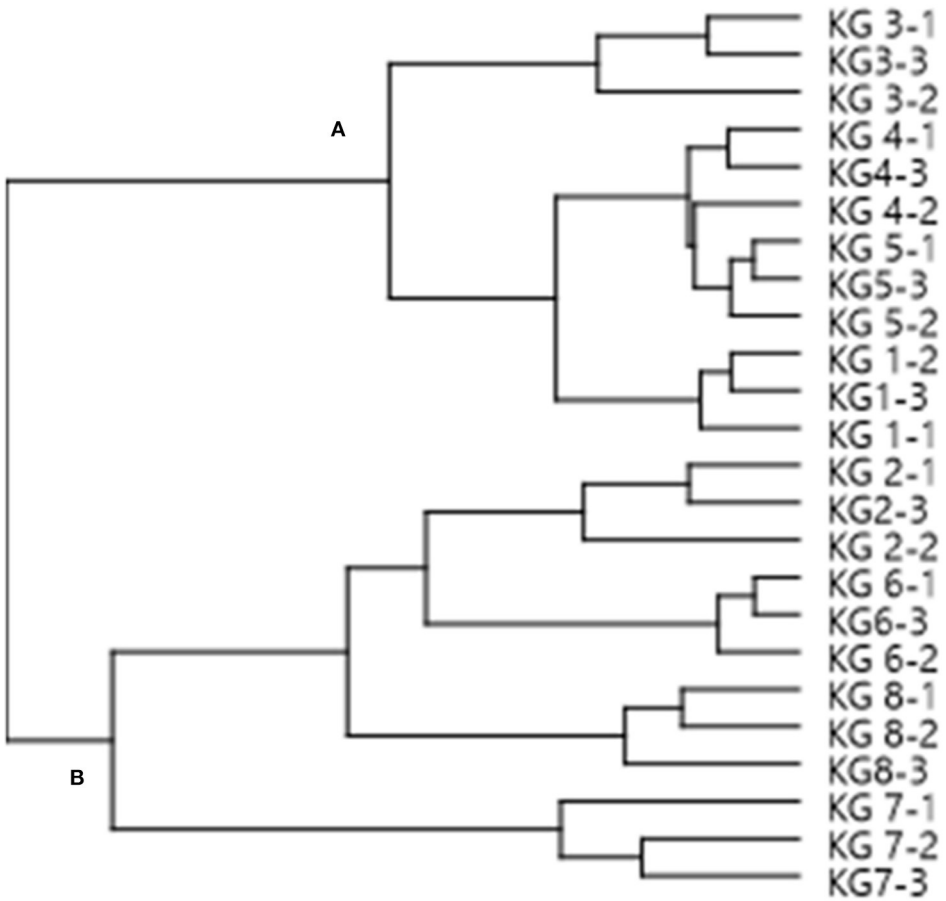

FIGURE 3 | A dendrogram of Kersting's groundnut (KG) landraces based on group cluster analysis, using MS chemical profiles as analytical data. The MS chemical profiles of three replicate samples per landrace were used for the analysis. The name of each landrace is followed by a hyphen (-), and then the replicate number 1, 2 or 3.

for its nutritional values. In this study, the identity of other hydroxycinnamic and benzoic acids was deduced from the fragmentation patterns of their respective mass spectra, which corresponded to gallic acid, caffeic acid, *p*-coumaroylquinic acid, quinic acid, syringic acid, *p*-coumaric acid, ferulic acid, and sinapic acid. These compounds are ubiquitous in the seeds of many grain legumes such as cowpea, pea, common bean and horse gram (42–45). Even though the UV spectra of peak 26 was similar to that of gallic acid, the retention times were different; nevertheless, the compound was identified as the hydrolysable tannin, digallic acid, based on the MS data previously reported by El Sissi et al. (38). The presence of these phenolic acids in plants is linked to diverse functions, including nutrient uptake, protein synthesis, enzymatic, and photosynthetic activities (46). For example, gallic acid identified in seeds of the test KG landraces is reported to act as an antioxidant and allochemical, and is also known for its anticancer, antiviral and astringent properties (46). Quinic acid on the other hand, is involved in DNA repair, a reduction in the influence of lifestyle factors on the risk to disease as well as the inhibition of cell proliferation (47). The observed phenolic profile of the tested KG seeds highlights the potential health-promoting benefits of the crop when included in diets.

Flavonoids in Seeds of the Orphan Kersting's Groundnut

Along with the phenolic acids, several classes of flavonoids including catechin and procyanidin B dimers were detected in seeds of the KG landraces tested. For example, compounds 18 and 19 had a similar UV spectrum as that of procyanidin oligomers, and had molecular ions $[M\text{-}H]^-$ at *m/z* 577.1085 and 577.1074 corresponding to procyanidin type B dimers (48). Since procyanidins B are mostly catechin dimers, these compounds, previously identified in the seed and seed coats of lentil, pardina lentil, pea and common bean were assigned as procyanidin B1 and procyanidin B2 because of the MS^2 fragment ion $[M\text{-}H]^-$ at *m/z* 289 which is characteristic of catechin monomer (49). The presence of procyanidin type B dimers has been described in seeds of most grain legumes, and their synthesis may depend on the legume's phenotype (50, 51). Moreover, B-type procyanidin trimers which were found in the test landraces were earlier

TABLE 4 | Quantitative analyses of metabolites showing differential accumulation in seeds of Kersting's groundnut (KG) landraces.

Peak no.	Name	mol. ion [m/z]	KG1	KG2	KG3	KG4	KG5	KG6	KG7	KG8	μg/mg
3	Ferulic acid hexoside	355	441.31	138.57	287.85	354.30	287.78	229.36	51.78	155.59	Average
			55.80	11.87	26.13	5.57	32.87	12.93	11.39	18.59	Std dev
19	Procyanidin B2	577	11.24.	trace	18.25	16.67	18.12	8.39	11.92	1.86	Average
			0.27	nd	3.69	0.11	0.48	0.11	0.26	0.32	Std dev
33	Eryodictyol-7-rutinoside	595	7.49	10.22	6.83	7.58	5.97	13.95	3.26	nd	Average
			0.36	2.60	0.02	0.76	1.34	0.28	0.25	nd	Std dev
36	Quercetin pentoside	433	7.94	11.55	11.80	6.06	5.44	15.20	63.85	14.14	Average
			0.86	1.97	0.33	0,27	0.30	0,18	10.45	2.57	Std dev

Trace, detected in trace amount; nd, not detected.

detected in the seeds of cowpea, adzuki bean, pea and lentil (4, 49). Of the flavonoids detected in this study, gallocatechin is reported to improve lipid metabolism and contributes to the prevention of metabolic syndrome, and was earlier reported in seeds of pea and lentil (51, 52). The presence of quercetin dihexoside and quercetin pentoside in the test KG landraces was previously reported in the seeds of cowpea (53). In diabetic rats, quercetin was found to exert antidiabetic properties by increasing the regeneration of pancreatic islets and the release of insulin (54). Acyl groups such as acetyl or malonyl usually occur as 6″-*O*-acetylglucoside or 6″-*O*-malonylglucoside (43). Thus, peak 2 was tentatively identified as quercetin-3-(6″-malonoyl)-glucoside. Flavonoids containing 6″-malonoyl-glucoside groups have been reported in the seeds of cowpea and soybean (36, 53). This study further identified compounds such as apigenin (peak 32), rutin (17), naringin (21), kaempherol 7-rutinoside (24), and eriodictyol 7-rutinoside (33) which were previously reported in seeds of pardina lentil (55). In addition to the diverse roles of these classes of flavonoids in the signaling processes during the legume-rhizobium symbiosis (14), they are also linked to reduced incidence of cancers and cardiovascular diseases in humans (56). Aside their antioxidant activities, flavonoids have also been implicated in the regulation of metabolic functions of the gut microbiota in favor of human health (57).

Fatty Acids and Sphingolipids Detected in the Seeds of Kersting's Groundnut

From the chromatogram in this study (**Figure 1**), peaks corresponding to unsaturated fatty acids (e.g. oleic, linoleic, linolenic, and palmitoleic acids) and saturated acids (e.g., stearic acid and palmitic acid) were identified in the seeds tested. Fatty acids are metabolites that take part in complex metabolic pathways and play major biological roles in organisms. Whereas, saturated fatty acids are associated with adverse health effects, unsaturated fatty acids are thought to be protective (19). The chromatogram also presented several other intense peaks corresponding to sphingolipid conjugates which are known for their roles in a wide range of biological processes and functions in human systems, including the management of several diseases such as cancer, obesity and atherosclerosis (20).

Antioxidant Activities and Relative Concentrations of Total Phenolic Compounds in Seeds of Kersting's Groundnut

Legumes are natural sources of antioxidants that can be used to manage neurodegenerative diseases. In the management of these diseases, scavenging reactive oxygenated species is an important mechanism of antioxidant action (58). The methanolic extracts from seeds of the test KGs exhibited significant variations in antioxidant activities, with the brown mottle seeded KG3 showing the highest activity than the other landraces based on DPPH and ABTS assays. For example, the DPPH assay revealed markedly higher ($p < 0.05$) IC_{50} values in the landraces when compared to trolox and ascorbic acid used as positive controls, with values ranging from 5.12 μg/mL for the extract from KG3 to 95.21 μg/mL for the extract from KG8. The landrace KG8 was found to show the least antioxidant activity based on its greater IC_{50} values in both DPPH and ABTS assays (**Table 3**).

In this study, the total phenolic content in seeds decreased in the order KG1 > KG5 > KG3 . KG6 > KG4 > KG8 > KG7 > KG2, a finding consistent with earlier reports which showed that legumes with light seed coat colors tend to have lower phenolic content when compared to those with dark-pigmented seed coats (59, 60). Consequently, the total flavonoid content also ranged from a low 0.53 mg QUE/g in the white seeded KG2 to a high 2.11–3.01 mg QUE/g in the brown and black seeded landraces. Ojwang et al. (53) also reported greater accumulation of flavonols in red seeded cowpea when compared to black, green and white seed coat phenotypes. The total flavonoid contents observed in seeds of the test KG landraces were within the range reported for cowpea (61).

The antioxidant activity of the KG seeds studied can partly be attributed to their phenolic composition, especially the ability of polyphenolic compounds to quench free radicals. The positive correlation between total phenolic content, total flavonoid content and the individual phenolic compounds with antioxidant capacity indicated that phenols and flavonoids in KG seeds improved its antioxidant properties. These findings confirm that seed polyphenolic content can be considered as a predictor of the antioxidant activity (*in vitro*) as indicated by earlier studies on grain legumes (4, 62). Nevertheless, in

evaluating the contribution of polyphenolics to antioxidant activity, Cardador-Martinez et al. (63) found that 40–71% of the free scavenging is linked to total phenolic content, and that flavonoids were responsible for only 20–30% of the antioxidant activity. Despite the relatively lower total phenolic and flavonoid contents of the white seeded KG2 in this study, the extracts of the landrace were among those with higher antioxidant properties based on ABTS assays. Thus, although total phenolic and flavonoid contents were directly correlated with potential antioxidant activities in this study, the few discrepancies may be due to the reported contribution of non-phenolic compounds to the total antioxidant activities of seed extracts (64, 65). Considering the marked differences observed in the phytochemical profiles and antioxidant activities of the seeds tested, and although not addressed here, such observations can be attributed to the impact of edaphoclimatic conditions, such as light exposure, temperature, and soil properties, which have been increasingly recognized as having a direct impact on both chemical composition and biological activity (66). Moreover, Cheng et al. (67) also reported a marked effect of plant processing and extraction methods on the phytochemical and antioxidant properties of grape residues. However, the effect of these factors on the phytochemical composition and antioxidant properties of KG are yet to be determined.

A PCA analysis of the metabolites identified revealed marked differences in the eight KG landraces, with KG7 being the most distant landrace due to its relatively unique metabolite composition. The PCA grouped the test landraces into three major clusters, with procyanidin B2, eriodictyol-7-*O*-rutinoside, oleanolic acid, quercetin pentoside, ferulic acid hexoside, linoleic acid, and dihydroxy-octadecadienoic acid showing a greater contribution to the discrimination among the landraces. However, the HCA analysis grouped the landraces in two major clusters which mirrored the patterns in their phenolic contents, with samples in Cluster **A** exhibiting greater phenolic contents than their counterparts in Cluster **B** (**Figure 3**).

CONCLUSION

By means of UPLC-qTOF-MS and UPLC-DAD, the chemical profiles of eight KG landraces were deciphered. A total of 57 secondary metabolites were putatively identified and some of them quantified. The chemical fingerprints of all the analyzed landraces were dominated by phenolics, sphingolipids and fatty acids. The results from this study provide a useful documentation on the chemical profile of KG seeds using untargeted large-scale metabolite analysis. The variability in phenolic content among the different landraces could provide an interesting and valuable source of information for breeding programs to improve the cultivation and utilization of KGs. Our findings therefore highlight the potential of this under-utilized crop as a source of health-promoting foods due to the presence of diverse classes of nutraceutical compounds. These results have implications for the role of compounds identified in the dietary intake by malnourished African populations, and also highlight the potential of KG as an alternative subsistence crop that can be used to combat malnutrition in sub-Saharan Africa. The compounds detected have been variously reported for their biological functions and can be exploited for improved human health and nutrition subject to more research that should include cell-based assays of seed extracts or it's other formulations.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

ATT designed and performed the experiments, interpreted the results, and drafted the manuscript. MM collected the plant materials, analyzed the data, interpreted the results, and took part in drafting the manuscript. FDD provided the funding, conceived the idea, supervised the work, edited, and approved the final version of the paper. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.593436/full#supplementary-material

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REVIEW
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The Nutritional Potential of the Native Australian Green Plum (*Buchanania obovata*) Compared to Other Anacardiaceae Fruit and Nuts

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The native Australian green plum (*Buchanania obovata*) is a small fruit that grows in the northern parts of the Northern Territory and Western Australia. The fruit belongs to the family Anacardiaceae, which includes the other agriculturally important fruit mangoes, pistachios and cashew nuts. The green plum is a favored species of fruit for the Aboriginal communities and an important bush food in the Northern Territory. To date, only minimal scientific studies have been performed on the green plum as a food. This review is about plant foods in the family Anacardiaceae and the key nutritional compounds that occur in these fruit and nuts. It looks at the more traditional nutrient profiles, some key health metabolites, allergens and anti-nutrients that occur, and the role these foods play in the health of populations. This provides a guide for future studies of the green plum to show what nutritional and anti-nutritional properties and compounds should be analyzed and if there are areas where future studies should focus. This review includes an update on studies and analysis of the green plum and how its nutritional properties give it potential as a food for diet diversification in Australia.

Keywords: Anacardiaceae, green plum, *Buchanania obovata*, nutrition, mango, cashew, pistachio, family

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INTRODUCTION

The family Anacardiaceae is a member of the flowering plant order Sapindales and contains about 80 genera. There are ∼870 species in the family characterized as deciduous or evergreen trees, shrubs and woody vines which contain resin ducts in the bark and that exude resins and gums (1). The fruits of this family are drupes that are fleshy (1). The Anacardiaceae family contains a number of plants that produce foods, some are globally important economically and others are important in smaller communities. They provide nutritional properties and diet diversification to people all throughout the world. The family includes one of the most well-known fruit in the world, the mango (*Mangifera indica*), and the equally well-known cashew nut (*Anacardium occidentale*) and pistachio nut (*Pistacia vera).* Other foods in the family that are not globally available but are important in the countries they grow in include the fruit of

Pistacia lentiscus, the marula fruit (*Sclerocarya birrea*), the sumac of the genus Rhus and in particular *Rhus corriaria,* the yellow mombin (*Spondias mombin*), and the chironji (*Buchanania lanzan*).

A less well-known fruit of the family Anacardiaceae is the green plum, the fruit of the tree *Buchanania obovata*. It is a small green/yellow fruit that grows as a drupe and it is a favored species of fruit and an important bush food for Aboriginal people in the Northern Territory and Western Australia where it grows (**Figures 1**, **2**). They are eaten raw from the tree and the individual fruit are also eaten dried or reconstituted (2).

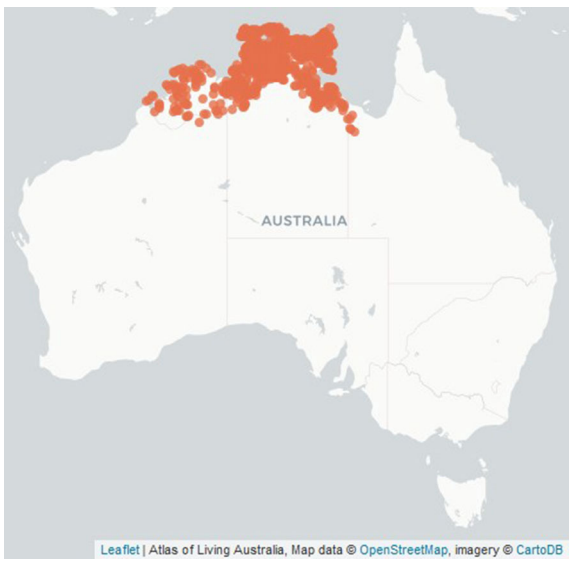

FIGURE 1 | Location of botanically identified *Buchanania obovata* trees in Australia (Atlas of Living Australia).

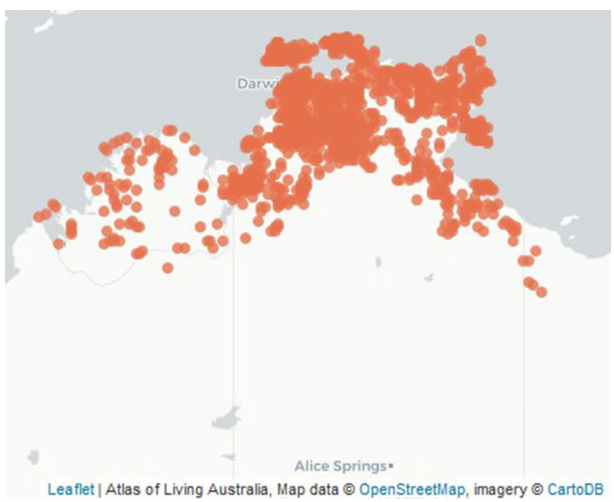

FIGURE 2 | Location of *Buchanania obovata* trees botanically identified in the northern Western Australia and Northern Territory (Atlas of Living Australia).

Green plums were prepared and stored by Aboriginal people so they could be eaten at a later time, the fruit and seed were pounded into a pulp or paste and sun-dried then stored in sheets of paperbark (2, 3). The accepted name of the plant that grows the green plum fruit is *Buchanania obovata* Engl. (4), it was first described in 1883 (5) and the plant taxonomy is shown in **Table 1**.

There are two published studies on the green plum as a food, however, both of these studies were done on underripe green plums. One study looked at the nutritional properties of the green plum flesh and seed as both parts are eaten. It found the flesh was high in protein [12.8 g/100 g dry weight (DW)], potassium (2274.7 mg/100 g DW) and was a good source of magnesium, calcium and phosphorous. The seed was found to be high in iron (8.15 mg/100 g DW). Both the flesh and seed were found to be high in dietary fiber. There were also high levels of folate found, with the flesh containing 752.4 μg/100 g DW as pterolmonoglutamic acid equivalents (7).

The second study was on the functional properties and phytochemicals of the same underripe green plums. This study found that the flesh had antimicrobial activity against Gram-negative *Escherichia coli* 9001—NCTC and Gram positive *Staphylococcus aurerus* 6571—NCTC bacteria and caused cell wall disintegration and cytoplasmic leakage. Antioxidant testing using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay showed the flesh had high radical scavenging activity (106.3 μM Trolox equivalent/g DW in methanol). Extract of the seed had a pink color and contains a delphinidin-based anthocyanin. Polyphenols found in the flesh and seed include gallic acid, ellagic acid, p-coumaric acid, quercetin, kaempferol, and trans-ferulic acid (8).

These preliminary results show that the green plum has promising nutritional properties and should be further studied, especially when it is at its ripe and edible stage. This review aims to understand how best to study the green plum as a food by analyzing the nutritional properties and importance of other foods from the Anacardiaceae family. It does this by looking at how underutilized plant food can be used as nutrition. Then it looks at foods that are produced by plants in the family Anacardiaceae and how they are used as foods. Then the key nutritional properties of these foods are tabled and reviewed. Next, the known and potential roles of these foods as functional

TABLE 1 | Taxonomy of *Buchanania obovata* (6).

Class	Green plum classification
Kingdom	Plantae
Phylum	Charophyta
Class	Equisetopsida
Subclass	Magnoliidae
Superorder	Rosanae
Order	Sapindales
Family	Anacardiaceae
Genus	*Buchanania*
Species	*Buchanania obovata*

foods and nutraceuticals is reviewed. The properties that could prevent these foods from playing a role in nutrition is looked at, especially the allergenic causing compounds. Then the role that these foods play in nutrition of populations is described, in doing this the role of these foods in nutrition for individuals and populations are both reviewed. Finally, it concludes with how the nutritional properties of foods in the Anacardiaceae family can give insight and understanding for studying the green plum and discovering its nutritional potential.

UNDERUTILIZED PLANT FOODS AS NUTRITION

Foods are fundamental to the existence of humans and the nutritional value of them has a direct impact on the health of populations (9). Therefore, nutritional components are important to study so the health benefit of these foods can be understood. Fruit and vegetables, or plant foods, are an important part of a healthy diet because they provide valuable carbohydrates, dietary fiber, vitamins, minerals, and trace elements.

Malnutrition can be caused by overnutrition, undernutrition and micronutrient deficiencies. It is estimated that over 2 billion people in the world suffer from micronutrient deficiencies from insufficient intake of vitamins and minerals in the diet (10). There are an estimated 821 million people who are undernourished (11). Malnutrition from food insecurity can be due to insufficient quantity of food, insufficient quality of food and insufficient variety of foods in the diet (11). Across the world 60% of dietary calories comes from the food staples rice, wheat, maize, potato, and soybean, and 90% of dietary calories comes from just 103 of the 30,000 plant species that are edible (12). Adding more variety of plant foods into the diet can increase the micronutrient intake and increase nutrition and health of consumers. Understanding the nutrition composition of Australian native fruit is important to be able to promote them as healthy foods and to add variety to the diets of Indigenous and non-Indigenous people across Australia. There is the possibility of using native Australian fruit for diet diversification in Indigenous and non-Indigenous Australians, to increase nutrition intake and health (7).

At least 37 native plant foods are available commercially in Australia, including 13 fruit. There are many plant nurseries selling bush food plants that can be bought and grown for food. A few of these foods have been studied in detail, but most have had very little modern scientific analysis. The native Australian fruit that has been studied the most intensely is the Kakadu plum from the tree *Terminalia ferdinandiana* which grows in the Northern Territory and Western Australia. The Kakadu plum has extremely high levels of vitamin C with up to 32% of dry weight (322.2 ± 2.1 mg/g DW) being recorded (13). Even the kernels of the Kakadu plum can be used for their nutritional value due to their content of dietary fiber (21.2% DW), energy (2,065 kJ/100 g DW), potassium (6,693 mg/kg DW), calcium (5,385 mg/kg DW), zinc (60 mg/kg), and iron (61 mg/kg DW). The kernels also contain high levels of protein (32.0% DW) and fat (35.1% DW) and contain linoleic, palmitic, and oleic acids (14). In Australia, native fruit that have been traditionally eaten by Indigenous Australians are allowed to be commercialized as they are classed by the Food Standards Australia New Zealand (FSANZ) as traditional foods with a history of consumption in Australia and are not considered novel (15).

FOODS OF THE FAMILY ANACARDIACEAE

The tree *Mangifera indica* is an evergreen tree that produces the major fruit crop mango (16). Many different mango cultivars are eaten and they vary in size, shape, color, flavor, and fiber quantity (16). Mangoes can be oval, round, heart-shaped, kidney-shaped, or slender and long, can be as small as plums or weigh up to 2.3 kg and can vary in color between red, yellow, and green (17). Mango is eaten as a fresh fruit with the pulp and occasionally the skin consumed. Processed mango food products are made from the flesh and consumed throughout the world including juice beverages, candies and fruit bars, jams, jellies, pickles, and powder mixes (18).

The cashew tree (*Anacardium occidentale*) grows the cashew apple and cashew nut that are eaten across the world. The kernel from the seed is known and eaten as the cashew nut and the swollen pedicel of the flower stalk is eaten as fresh cashew apple and as a juice (19). The tree is believed to be native to Brazil and was moved to other parts of the world in the sixteenth century by the Portuguese (19). The apples are edible but are often not used or eaten, in Northeast Brazil about 90% of the apple is left in the field during harvest (20). The kernel is mostly consumed roasted and salted and is used in the food industry, particularly in chocolate, pastry, and biscuit factories. The oil from the kernel is used in food and cosmetic products (21). The juice from the apple can be extracted and used to produce vinegar, syrup, and alcohol (21) or can be fermented and used to produce added value products, such as lactic acid, dextran, mannitol, and oligosaccharides (20). The shell and peel can be used as fuel for the drying and cooking processes of the nut and the peel is used as food for livestock. The oil from the cashew nut shell is extracted and purified and used in the manufacturing of chemical products (21).

The pistachio nut grows on the tree *Pistacia vera* and is actually a kernel that grows inside the seed pod and flesh of the fruit. The pistachio tree is thought to be indigenous to Iran and is grown in California in the US, and across from Afghanistan to the Mediterranean region (22). The kernel is often eaten fresh or roasted, sometimes with salt or flavoring, they are used in sweets and desserts and for their yellow-green color (22, 23). The shell around the pistachio kernel splits naturally before it is harvested which allows it to be sold in-shell (23).

In the same genus as the pistachio is *Pistacia lentiscus,* the mastic or lentisk tree, which grows in the Mediterannean basin and produces the lentisc fruit with an edible oil that is used in the daily diet of Tunisians (24). In Tunisia it is used in salads and pastries and served as a condiment, there is interest in furthering its use as a vegetable oil (24). Another food product is collected

by incisions made into the side of the tree and the resin that drips out is used as a chewing gum known as mastic gum (25).

The *Sclerocarya birrea* tree grows in Africa and produces marula fruit. It grows in the African triangle from Cape Verde, to the Horn of Africa, to the Cape of Good Hope (26). Marula is a pale yellow fruit and has a juicy mucilageinous flesh that is eaten fresh or is fermented to make beer and other alcoholic beverages (27, 28). The flesh is tart, sweet, and refreshing with a slight turpentine aroma (28). The flesh of the fruit is used for making the South African liquor Amarula Cream (28). The seed is opened to obtain and eat the macadamia-like kernel inside (26, 29).

The name of the fruit sumac is used for about 35 species in the genus *Rhus*. One of the most common species of sumac is *Rhus corriaria* which is in Turkey, Syria, and throughout the Arab world (30, 31). It is used as a spice, condiment, appetizer, and a souring agent (32).

Spondias mombin, whose fruit is known as yellow mombin, grows in the tropical parts of America, Asia, and Africa (33). In Brazil it is harvested wild and its flesh is eaten and used in other food products (33). *Spondias purpurea* are native to Ecuador and the fruit, known as ovo, is sweeter and more aromatic then the yellow mombin and usually eaten fresh or in jams, ice-creams, or beverages (34).

The *Buchanania lanzan* tree produces seeds that are edible and are known as chironji, char, achar, or the cuddapah almond. It occurs in the wild in India where it is eaten and used in cooking and where the tree is used as a medicinal plant (35). The tree is also found in Myanmar, Sri Lanka, Malaysia, Burma, and Nepal (35, 36). To obtain the kernel, the fruit are harvested, the skin allowed to blacken in storage then removed, the seed is washed and dried and then the seed shell is removed from the kernel (37). The kernel of the seed is eaten either raw or roasted, it can be used as a substitute for almonds and is used in manufacturing sweetmeats and confectionery (37–39). Its use as a cooking spice is also starting to become more well-known outside of India. The fruit are a small drupe, growing to 12 mm and they turn from green to reddish to black, the fruit weight is made up of about 16–18% kernel (35, 39).

Like *B. obovata, Buchanania arborescens* is also native to Australia and its fruit have also been eaten by Aboriginal Australians. It also has fruit that are a small globular drupe, growing to 10 mm in diameter. The fruit turn reddish-purple to purple-black when it ripens and its fruiting time is the same as that of the green plum (2). The fruit are eaten raw and taste sweet and pulpy and have a thin rind (40). The tree is known as the little gooseberry tree and the satinwood tree. It grows across the northern parts of the Northern Territory and Queensland in Australia and in East Timor, West Papua, and Papua New Guinea (6).

The Burdekin plum or gambozia (*Pleiogynium timoriense*) is a dark purple fruit with a thin layer of flesh which is sour and astringent when it falls from the tree but becomes palatable a few days after harvesting (41, 42). It grows in Queensland in Australia, and in the area between Queensland, the Philippines, Indonesia, and the Cook Islands (41, 43) and is cultivated as an ornamental plant in Egypt (42). The fruit are eaten by Indigenous Australians and the flesh has been used to make jam (41).

There are some other species in the Anacardiaceae family that are found throughout the world and are used as foods, but which have not have as much in-depth nutritional study. The fruit from *Pistacia palaestina* are used in the Middle East as a component of the "Zaatar" a blend eaten daily with bread, olive oil and tea (44). Likewise with terebinth, *Pistacia terebinthus,* which is an important spice plant in Turkey and is eaten as an appetizer (30). The fruit of the tree *Schinus molle* is known throughout the world as pink pepper and sometimes as Peruvian pepper. It is not related botanically to true black pepper (*Piper nigrum*). Fruit from other *Mangifera* species are used in similar ways to the closely related mango in parts of Asia including *M. caesia*, *M. foetida*, and *M. parvifolia* (45).

NUTRITIONAL PROPERTIES OF THE FAMILY ANACARDIACEAE

Nutritional analysis on some of the foods from the Anacardiaceae family has been published. The mango, pistachio nut, and cashew nut have been studied in detail as they are widely eaten foods. Other foods have had some nutritional studies done but not the in-depth analysis of the more widely eaten foods, while others, such as the green plum have had very little. The foods of the Anacardiaceae family provide valuable nutrients and health benefits to the individuals who eat them and add diversity of nutrients into the diet.

To understand the nutritional potential that the green plum might have, the nutrition properties of the foods in the Anacardiaceae are described in detail to see if there are trends or key nutrients that occur in this family. This review first looks at the more traditional nutrients then later in the review looks at phytonutrient compounds in them that effect health and may be able to be used as functional foods or nutraceuticals. The traditional nutrients include the proximate analysis and the key minerals present. Then the fatty acids and lipids are reviewed as this group of foods contains a number of edible kernels. Folates and vitamin C are essential vitamins that are important to health and obtained through diet, and folate was found to be high in the initial study of the green plum (7).

There are large databases of food nutrients that give nutritional profiles of some of these foods. Of these databases, mango, pistachio and cashew can be found in the Australian Food Composition Database (46), the FoodData Central (47), Ciqual the French food composition table (48), and the McCance and Widdowson's composition of foods integrated dataset (49). It was decided that for these three well-studied foods only the USDA data would be included for review to keep the data pertinant.

The nutrients and phytonutrients of mango, pistachio, and cashew have been previously looked at individually in other reviews and book chapters, but not as a plant family together. These reviews include the constituents of cold pressed pistachio oil (50), the bioactive compounds and the functional effects of pistachio green hull (51), cancer preventive and anticancer therapeutic potential of mango and its phytochemicals (52),

TABLE 2 | Key proximate and mineral levels of fruit flesh of the Anacardiaceae family, results as fresh weight.

	Green plum	Mango	Marula	Sumac	Terebinth	Yellow mombin	Ovo	Burdekin Plum
Plant or fruit part	Underripe flesh	Flesh	Flesh	Flesh	Flesh	Flesh	Flesh	Flesh
References	(7)	(60)	(61)	(30)	(30)	(33)	(34)	(62)
Moisture g/100 g	79	83.46	86.4	6.0	4.0	83.66	77.6	72.7
Protein g/100 g	2.69	0.82	–	2.3	6.4	1.06	0.7	1.3
Fat g/100 g	0.53	0.38	0.54	17.4	42.4	0.62	0.2	1.3
Fiber g/100 g	11.6	1.6	–	–	–	1.87	0.5	18.4
Carbohydrate g/100 g	4.5	14.98	–	–	–	13.90	19.1	25.0
Potassium mg/100 g	478	168	355	526	762	288	250	458
Iron mg/100 g	0.79	0.16	9.9	18.1	10.7	0.3	0.72	0.9
Phosphorous mg/100 g	46	14	18	–	–	33	42	–
Calcium mg/100 g	89	11	30	133	310	11	17	241
Magnesium mg/100 g	120	10	16	77	97	15	–	32
Zinc mg/100 g	0.5	0.09	5.8	2.6	3.9	–	0.02	1.1

– means a reported result was not available.

how the levels of lupeol, mangiferin, and phenolic acids can be regulated and improved in mangoes (53), the potential for the fat fraction of mango kernels to be used as a healthy food ingredients and cocoa butter alternatives (54), the ethnomedical and pharmacological activity of compounds in mango (55), the major polyphenols that are in mango and their potential health benefits (56) the efficacy of cashew nut consumption on lipid profile and blood pressure (57), the effect of cashew nut consumption on lipid profile (58), and the effect of cashew nuts on cardiovascular risk factors and blood pressure (59).

Proximates and Minerals

The proximate and some mineral components of the Anacardiaceae fruit flesh are in **Table 2**. The trends seen across the flesh of these fruit are that the mango, marula, Burdekin plum, yellow mombin, and ovo contain large amounts of moisture and carbohydrate. The sumac and terebinth are comparably very low in moisture and much higher in fat. Potassium is the most abundant mineral in all of the fruit flesh, but calcium is found in considerably higher levels in sumac and terebinth then the other fruit.

The results in **Table 3** show the trends across the kernels. They do not contain much water, but are very high in fat and in protein. The kernels are also high in potassium and phosphorous, and good sources of calcium and magnesium. The cashew nut kernel is made up mostly of fat, carbohydrate, and protein (**Table 2**) (60). Analysis of cashew nut kernels from six different parts of India as well as the Ivory Coast, Brazil, Vietnam, Mozambique, and Kenya show consistent protein results with a mean and standard deviation of 21.3 ± 0.8 g/100 g, consistent carbohydrate of 20.5 ± 1.5 g/100 g and consistent energy levels 2,525 ± 35.8 kJ/100 g (64).

The USDA data (**Table 3**) shows potassium is the most abundant mineral present in cashew nut kernels followed by phosphorous and magnesium which is consistent with the results of Rico et al. (64) (622, 503, and 249 mg/100 g). The marula kernel has much higher levels of phosphorous than the other kernels and higher levels of magnesium, but it has lower levels of potassium. The high levels of phosphorous in the kernels were confirmed in a study of marula from Kenya which found levels of 782 and 741 mg/100 g DW in the kernel (61). As sources of potassium, calcium, and magnesium, the Anacardiaceae kernels provide important health benefits. Potassium is critical for muscle function and nerve transmission and involved in energy metabolism glycogenesis and cellular growth and division (65). Phosphorous is critically important and is mostly found in bone with some in soft tissue and in the phospholipids of erythrocytes and plasma lipoproteins (65). Magnesium is in bones, soft tissue and in all compartments of cells performing many cellular reactions and involved in at least 300 enzymatic steps in metabolism (65). Analysis of underripe green plums show the flesh has similarly high levels of potassium, calcium, and magnesium to sumac and terebinth. The green plum seed that was analyzed was whole including the seedcoat, thus analysis of the kernel to compare with nutritional properties of other kernels in the Anacardiaceae family could determine if it has a similar profile (7).

Fatty Acids and Lipids

The kernels and some of the fruit of the Anacardiaceae family have high levels of fat which indicates they are a good source of energy and good for membrane health (66). **Table 4** shows the total lipids and fatty acid concentrations in some of the flesh and kernels of Anacardiaceae fruit. It is significant that the kernels and even some of the flesh have high lipid contents. All of them show similar fatty acid profiles, with the highest fatty acid levels of the unsaturated 18:1, then the polyunsaturated 18:2 and the saturated 16:0, then the saturated 18:0 and with low or trace levels of some other fatty acids also present.

TABLE 3 | Key proximate and mineral levels of seed and kernels of the Anacardiaceae family, results as fresh weight unless stated.

	Green Plum	Chironji	Cashew nut	Pistachio	Marula
Plant or fruit part	Underripe seeds	Kernel	Kernel	Kernel	Kernel (dry weight)
Reference	(7)	(63)	(60)	(60)	(29)
Moisture g/100 g	35	3.6	5.2	4.37	–
Protein g/100 g	2.0	43.24	18.22	20.16	36.4
Fat g/100 g	1.2	38	43.85	45.32	47.0
Fiber g/100 g	57.0	18.5	3.3	10.6	–
Carbohydrate g/100 g	4.4	12.96	30.19	27.17	–
Potassium mg/100 g	185	–	660	1025	364
Iron mg/100 g	5.3	4.8	6.68	3.92	2.77
Phosphorous mg/100 g	30	593	593	490	1040
Calcium mg/100 g	30	70	37	105	154
Magnesium mg/100 g	32	275	292	121	421
Zinc mg/100 g	0.24	3.32	5.78	2.2	6.24

– means a reported result was not available.

Chemotaxonomic analysis of seed oils from other plant families show similarities in fatty acid profiles within families and differences between families. The principal fatty acids in the family Ribes are 18:2 and 18:3 with lower percent composition of 18:1 and 16:0, the family Boraginaceae have predominantly 18:1, 18:2, and 18:3 unsaturated fatty acids with some 16:0 also present, the family Ranunculaceae predominantly have 18:2, 18:3, and 16:0 with lower levels of 18:1, the family Onagraceae is mostly 18:2 with a small amount of 16:0 and the family Scrophulariaceae are predominantly 18:2 with lower levels of 18:1 and 16:0 and a few members of this family with 18:3 (68). The family Proteaceae which contains the macadamia nut (genus *Macadami*a with four species) has higher levels of 16:1 unsaturated then the other families, as well as 18:1 and lower levels of 16:0, 18:2, and 20:1 (69).

The different fatty acids have different roles in health and well-being, so the seeds and their oils from different plant families give diverse benefits nutritionally. The fatty acid profile common to the family Anacardiaceae has 18:1 oleic acid as the most abundant fatty acid, which is stable to oxidation and able to enhance the activity of antioxidants and antipolymerization agents (70). Higher intakes of oleic acid and limited intakes of saturated fats are believed to have beneficial health effects and may help prevent cardiovascular disease (70). The palmitic acid, 16:0 present in the Anacardiaceae family plays a number of important physiological roles including being a part of normal pulmonary surfactant in the lungs essential for breathing, and is present in membrane phospholipids and adipose triacylglycerols (71).

A fatty acid profile of the green plum could confirm if it contains a similar profile to the other fruit and kernels of the Anacardiaceae family and to understand the energy and nutrition that it provides to the people who eat it.

Folates

Folate is an important vitamin group for health that is not synthesized by the human body so must be consumed in food or supplements. It is used by the body in the synthesis of adenosine, guanosine, thymidine and in many methylation reactions (72). Folates measured on six varieties of mango from India had total folate ranging from 60 up to 138 μg/100 g FW (73). Five varieties of mango bought in Germany contained total folate between 55.8 and 74.5 μg/100 g and the highest folate type present was 5-CH_3-H_4folate (5-methyltetrahydrofolate) (74). These findings show mango is a good source of natural folate vitamers (74). The USDA nutrition reports of foods in the Anacardiaceae family as raw cashew nuts 25 μg/100 g, mango 43 μg/100 g, and pistachio nuts as 51 μg/100 g (47). The Vadu mango is a very small mango and was tested in its unripe form as this is how it is eaten. It has the highest level of folate of the mangos studied at 138 μg/100 g fresh weight (FW) (73). The initial study of underripe green plums gave an even higher total folate content of 161 μg/100 g FW (7). Further analysis on folates of green plums as a ripe fruit would give beneficial nutritional information, and understanding the folate levels as it matures could be of interest as a comparison to the underripe Vadu mango.

Vitamin C

Cashew apple juice has been found to have high levels of vitamin C at 203.5 mg/100 mL which was more than four times higher than the juices of orange (54.7 mg/100 mL), grape (45.0 mg/100 mL), lemon (33.7 mg/100 mL), mango (30.9 mg/100 mL), and pineapple (14.7 mg/100 mL) (75). When mixed with these other juices it boosts the nutrition quality by increasing the vitamin C content, while the other fruits improved the taste and flavor of the cashew apple juice (75). The USDA reported level of vitamin C in mango flesh at 36.4 mg/100 g (2019). Ovo has a similar level of vitamin C at 49 mg/100 g (34). Marula fruit has been found to have high levels of vitamin C with levels up to 2,118 mg/100 g DW (76).

Amino Acids

Amino acids play important roles in human health and well-being as substrates for protein synthesis, regulators of enzyme activity and protein turnover and many of them have individual roles in tissue and organ functions (77). The amino acid profiles analyzed from Anacardiaceae foods are in **Table 5**. All of these foods have glutamic acid as their most abundant amino acid. Glutamic acid is used for protein synthesis, used in muscle, it controls the acid-base balance, scavenges ammonia, is used as a nitrogen donor and for nitrogen transport, is a substrate for hepatic ureagenesis and gluconeogenesis and a fuel for intestinal enterocytes and generation of cytotoxic products in immunocompetent cells (77).

The other amino acids most abundant in the kernels are arginine, aspartic acid, glycine, leucine, serine and valine.

TABLE 4 | Fatty acid content of Anacardiaceae seeds showing total fatty acid groups and those with highest presence; all in g/100 g fresh weight except for marula kernel and lentisc flesh which are in dry weight.

Fatty acid	Fatty acid common name	Mango	Lentisc (dry weight)	Pink Pepper	Sumac	Terebinth	Cashew Nut	Pistachio Nut	Chironji	Marula (dry weight)
Fruit part		Flesh	Flesh	Flesh	Flesh	Flesh	Kernel	Kernel	Kernel	Kernel
Reference		(60)	(24)	(67)	(30)	(30)	(60)	(60)	(38)	(29)
Total lipids		0.38	42.54	5.35	17.4	42.4	43.85	45.32	50	47
Total saturated fatty acids	Butyric acid	0.092	10.2	1.55	4.66	10.34	7.783	5.907	19.05	13.78
4:0	Caproic acid	0	–	–	–	–	0	0	–	–
6:0	Caprylic acid	0	–	–	–	–	0	0.012	–	–
8:0	Capric acid	0	–	–	–	–	0.015	0	–	–
10:0	Lauric acid	0	–	–	–	–	0.015	0.004	–	–
12:0	Tridecylic acid	0.001	–	0.004	–	–	0.015	0	–	–
14:0	Pentadecylic acid	0.013	–	0.031	–	–	0.015	0.019	0.1	0.05
16:0	Margaric acid	0.072	9.9	1.196	3.67	9.20	3.916	5.265	15.15	7.35
17:0	Stearic acid	–	–	0.047	–	–	0.046	0.009	–	–
18:0	Arachidic acid	0.004	0.48	0.115	0.82	1.06	3.223	0.478	3.8	5.22
20:0	Behenic acid	–	0.003	0.004	0.12	0.08	0.266	0.046	–	0.59
22:0	Lignoceric acid	–	–	0.039	0.05	0	0.173	0.04	–	0.18
24:0		–	–	0.113	–	–	0.101	0	–	0.39
Total monounsaturated fatty acids	Myristoleic acid	0.14	22.5	1.098	6.71	15.56	23.797	23.257	27.9	30.79
14:1	Palmitoleic acid	–	–	0.012	–	–	0	0	–	–
16:1	Oleic, Vaccenic and Elaidic acid	0.067	0.545	0.107	–	–	0.136	0.495	–	0.11
18:1	Gondoic acid	0.075	22.00	0.954	6.71	15.56	23.523	22.674	27.9	30.12
20:1	Erucic acid	0	0.077	0.007	–	–	0.138	0.089	–	0.25
22:1	Nervonic acid	0	–	0.018	0	0	–	0	–	0.31
Total polyunsaturated fatty acids		0.071	9.4	2.702	4.77	7.55	7.845	14.38	3.05	2.45
18:2	Linoleic acid Linolelaidic acid	0.019	9.3	2.416	4.77	7.55	7.782	14.091	3.05	2.45
18:3	Linolenic acid	0.051	–	0.219	–	–	0.062	0.289	–	–
18:4	Stearidonic acid	0	–	–	–	–	0	0	–	–
20:2		–	–	–	–	–	0	0	–	–
20:3	Dihomo-y-linolenic and Mead acid	–	–	0.041	–	–	0	0	–	–
20:4	Arachidonic acid and Docosatetraenoic acid	0	–	0.027	0	0.13	0	0	–	–
20:5	Eicosapentaenoic acid	0	–	–	–	–	0	0	–	–
22:5		0	–	–	–	–	0	0	–	–
22:6	Cervonic acid	0	–	–	–	–	0	0	–	–
Total trans fatty acids		0	–	–	–	–	–	0	–	–

– means a result is not available.

Arginine is a precursor for urea and nitric oxide synthesis, aspartic acid is a nitrogen donor and transfers it to urea, glycine is a donor of methylene groups, leucine and valine are nitrogen donors and metabolic fuel and serine is a donor of hydroxymethylene groups (77).

The cashew nut kernel amino acid profile in **Table 5** is consistent with those from cashew nuts kernels from the geographically dispersed Vietnam, India, Brazil, and Ivory Coast (64, 78, 79).

NUTRITION AND PHYTONUTRIENTS AND THEIR POTENTIAL AS FUNCTIONAL FOODS AND NUTRACEUTICALS

Plant foods contain many phytonutrients that have a positive effect on human health. Some of these are already used as or are being studied to understand their content in foods and if they can be used as functional foods or nutraceuticals. Functional foods are foods that exert a specific health benefit

TABLE 5 | Amino acid content of foods from the Anacardiaceae family g/100 g fresh weight except for marula kernel which is dry weight.

Amino acid	Mango	Cashew nut	Pistachio nut	Marula (dry weight)
Fruit part	Flesh	Kernel	Kernel	Kernel
Reference	(60)	(60)	(60)	(29)
Alanine	0.082	0.84	0.97	0.90
Arginine	0.031	2.12	2.13	5.23
Aspartic acid	0.068	1.80	1.88	4.62
Cystine	–	0.39	0.29	0.89
Glutamic acid	0.096	4.51	4.3	10.97
Glycine	0.034	0.94	1.01	1.29
Histidine	0.019	0.46	0.51	0.92
Isoleucine	0.029	0.79	0.92	1.20
Leucine	0.05	1.47	1.60	1.74
Lysine	0.066	0.93	1.14	0.73
Methionine	0.008	0.36	0.36	0.59
Phenylalanine	0.027	0.95	1.09	1.32
Proline	0.029	0.81	0.94	1.00
Serine	0.035	1.08	1.28	1.49
Threonine	0.031	0.69	0.68	0.87
Tryptophan	0.013	0.29	0.25	0.54
Tyrosine	0.016	0.51	0.51	0.74
Valine	0.042	1.09	1.25	1.43

– means a reported result is not available.

effect when they are consumed regularly (80). Nutraceuticals are food phytochemicals processed and made into pharmaceutical forms and dietary supplements, such as tablets, capsules, powders, and solutions, etc. (80). Phytochemicals from food that are available as nutraceuticals include anthocyanins, flavonols, hydroxycinnamate, and ellagic acid (80). Some foods from the Anacardiaceae family are used as or have the potential to be used as functional foods or nutraceuticals for their nutrient and phytonutrient content.

Dietary fiber is found in plants and includes carbohydrates and lignin with different properties and physiological effects. Plant fiber can beneficially affect health as functional foods through laxation and the delay of nutrient loss, attenuating blood glucose, normalizing serum cholesterol levels, reducing the risk of cardiovascular disease, may reduce breast cancer risk by altering sex hormone levels and may prevent colon cancer (81). Dietary fiber can enhance satiety and inhibit appetite which may enable it to be used in weight management to reduce calorie intake (81, 82).

Mango contains a relative abundance levels of phytonutrients in its pulp including lupeol, mangiferin and phenolic acids (53). Mango has anti-diabetic, anti-oxidant, anti-viral and anti-inflammatory properties as well as compounds with other health benefits (55). Mango peel and the flesh that attaches has the possibility of being used as a functional food (83) as it is a rich source of soluble dietary fiber (12.8–23.0%), insoluble dietary fiber (27.8–49.5%) and total dietary fiber (40.6–72.5%) and contains galactose, glucose and arabinose as well as bound polyphenols and flavonoids (84). It has a high water absorption capacity of 7 mL/g and swelling volume of 21 mL/g which could lead to its inclusion in food ingredients as a dietary fiber source and a functional food (85). These water absorption and swelling capacity are higher in mango peel fiber then from mango fiber concentrate made from combined peel and pulp which has a water holding capacity of 6.4 g/g, swelling capacity of 4.6 mL/g and oil holding capacity of 1.6 g/g (83).

Analysis of the effect of mango peel extracted with methanol on 3T3-L1 pre-adipocyte cell line show that some mango cultivars can inhibit adipogenesis, inhibiting mitotic clonal expansion formation of fat cells (adipocytes) and could potentially be a source of nutraceuticals to be used for obesity and to prevent an increase in fat mass (86). The gallotannin derivatives from mango can work in part through the AMO-activated protein kinase pathway to suppress adipogenesis in adipocytes (87). Clinical trials show that daily consumption of mango for 6 weeks can significantly increase systemic exposure to gallotannin-metabolites with implications for gallotannin-derived health benefits and gut microbial composition with body mass index associated differences of effect on them (88). Mango seed has antiplatelet aggregation effects with 72% inhibition that may be due to the mangiferin it contains (89). Mango peel phenolic compounds are able to be encapsulated and stabilized (90) which could lead to their use as a nutraceutical.

Clinical studies on the beneficial effects of cashew apple juice and the health benefits obtained by supplementation with it show a number of promising properties. Supplementation with cashew apple juice of men performing regular exercise gave lower carbohydrates and higher fat oxidation rates than those on a placebo (91). Daily consumption of the juice for 12 weeks gave an improved oxidative stress status shown by a decrease in malondialdehyde levels, an increase in plasma glutathione peroxidase and enhanced physical performance (92). There was enhanced physical performance shown by increased endurance and strength in cyclists who consumed the cashew apple juice for 4 weeks (93), and improvement in immunological mechanisms occurred as seen by increases in resting neutrophil counts and exercise-induced leukocyte counts (94). The high levels of vitamin C in the juice gives increased resting vitamin C levels in people who supplemented daily (91). Cashew nut consumption may be able to reduce systolic blood pressure (57).

Clinical trials of pistachio nuts suggest they may have a beneficial effect on the blood lipid profile and therefore cardiovascular health when they replace other calories, due to their unsaturated fatty acids, phytosterols, dietary fiber, protein, and magnesium (95). A 24 weeks study of patients with metabolic syndrome who ate pistachios as 20% of their total energy showed an improvement in lipids and a decrease in waist circumference (96). Patients with mild dyslipidemia who ate 40 g of pistachio's a day for 3 months had an increase in high-density lipoprotein cholesterol and decrease in low-density lipoprotein cholesterol, a decrease in total cholesterol and a decrease in fasting blood sugar (97). Obese mice with regular consumption of pistachio nuts have improved inflammation that could be related to positive modulation of the gut microbiota (79). Obese mice on a pistachio diet have had significant reduction in serum

triglycerides and cholesterol and are able to reduce metabolic and cellular dysfunctions in the brain which may be useful in preventing obesity-related neurodegeneration (98). Diabetic mice who consumed a diet of pistachio nuts have increased gut populations of lactobacilli and bifidobacteria and normalized microbial flora was restored in them (99).

Pistachio nuts have anti-mutagenic potential and cytoprotective capacity (78). Pistachio kernel extracts have been shown to cause a significant decrease in cell viability and cell death of MCF-7 breast cancer cells (100). Pistachio nuts contain melatonin which has antioxidant capacity that can be protective against reactive oxygen and nitrogen species and sphingolipids which modulate cell health (101). The antioxidant capacity of pistachio nuts can be increased with the use of regulated deficit irrigation which may increase the production of secondary metabolites (78). Xylan has been isolated from pistachio nuts and can be used to produce a prebiotic mixture of the xylooligosaccharides xylobiose and xylotriose that may be suitable for functional or pharmaceutical use (102). Pistachio powder has been used to produce fortified bread that is enriched with lysine (103). Pistachio green hull contains a range of metabolites that benefit human health with antioxidant, photoprotective, cytoprotective, anti-inflammatory, anti-melanogenic, and anti-mutagenic activity (51).

The seeds of the Burdekin plum have been shown to have antihyperglycaemica and antihyperlipidemic effects on rats by significantly reducing the levels of blood glucose, total cholesterol, total triglycerides, and low density lipoprotein cholesterol. The seeds contain the phenolic compounds catechin, gallic acid, paramethoxybenzaldehyde, and pyrogallol (104). The fruit have also shown a cytotoxic effect against breast adenocarcinoma and laryngeal carcinoma human tumor cells and a moderate cytotoxic effect on human hepatoma cells (105).

ALLERGENS AND ANTI-NUTRITIONAL COMPOUNDS

The family Anacardiaceae contains plants that are known to cause allergic reactions (106). Approximately 32 genera in the Anacardiaceae family have been found to contain compounds that cause dermatitis upon contact (107). Plants in the Anacardiaceae family that are known to cause contact allergies include poison ivy (*Toxicodendron radicans*), poison oak (*Toxicodendron toxicarium*), poison sumac (*Toxicodendron vernix*), Chinese lacquer tree (*Toxicodendron vernicifluum*), African poison ivy (*Smodingium argutum*), cashew nut, pistachio nut, and mango (106, 108). Chironji has also been shown to have allergenic potential in both mice and humans (109).

The main polyphenol compounds that cause the allergies in this family are the 3-alkyl and 3-alkenyl catechols, particularly the C_{15}-Catechols and C_{17}-Catechols, which are sometimes known as urushiols, and the C_{15}-Resorcinols and C_{17}-Resorcinols (108).

Oxidation of these catechols turns them to highly reactive ortho-quinones which react with proteins in the skin to form antigens (108). The catechols that occur in the *Toxicodendron* genus are very strong contact allergens (108). The response appears to be an Immonoglobulin-E (IgE) mediated response after sensitization (110). The protein that causes chironji allergies leads to an increase in allergenic mediators, such as IgE, IgG1, and histamine levels and increased release of mast cell degranulation mediators (111).

Allergies to these plants and the foods they produce can be life threatening (106). The cashew nut shell liquid is used in the chemical products industry and is considered dangerous and cannot be handled with bare hands (21). It has been shown to be effective as a toxin and molluscicidal against golden apple snails (*Pomacea canaliculata*) which are a pest in Thailand that destroy rice crops (112).

The allergic reaction caused by mango includes skin irritation and erythematous lesions (113), pruritic erythema and periorvital edema (114) which can become severe anaphylactic reactions including urticaria, deep-tissue swelling, difficulty breathing, dyspnea, rhinorrhea, and cardiopulmonary symptoms (115, 116). The allergic reaction to these plants may be immediate or delayed and can appear up to 2 weeks after the contact (108, 116). The known allergen plants poison ivy and poison oak are also in the Anacardiaceae family and the clinical features of allergic dermatitis from these plants and mango are very similar (113, 117). Allergic rashes to mango have occurred in people with no previous exposure to it but who have been previously exposed to poison ivy or poison oak (117), and patients who have known allergies to these plants have also had mango dermatitis (113). Other cross-sensitivities between Anacardiaceae plants have also been detected (118).

Another anti-nutritional factor in the Anacardiaceae family is the contamination of pistachio nuts with aflatoxins (119). Fungal contamination of pistachios can occur in the field, during harvest, in post-harvest operations or in storage and the *Aspergillus* fungus produces aflatoxins in the nut (119). Aflatoxins can cause acute intoxication resulting in death (119) and contain carcinogenic compounds (120) and are among the most potent mutagenic substances known (121). Fungal contamination is prevented in the pistachio nut industry by controlling the moisture levels, insect activity, and rodent activity in the crop (121). Maximum legal limits of aflatoxin content are used to prevent contaminated pistachio nuts from entering the food supply (119).

There may be no known allergies to green plums at present, as they are currently only eaten by Indigenous populations in Australia where there are not many of the other known Anacardiaceae allergen plants growing. However, the fruit should be studied for the resorcinol and catechol compounds so that it is known if people already sensitized to these compounds could have an allergic reaction to green plums. This would particularly be the case if they were to be exported to countries where sensitization and allergies to poison ivy and poison oak are common.

NUTRITIONAL IMPORTANCE FOR POPULATIONS

Fruit and kernels of some of the Anacardiaceae family play major roles in nutrition in the world as they are major food crops. They are grown and distributed on enormous scales

and provide valuable nutrients to many individuals and to many populations. Some of them are eaten by small isolated communities and others are grown and exported to cities and countries across the world. Regardless of how widely spread their availability, they affect the health of individuals who eat them, and in turn their availability plays a role in population health. Although they are not considered staple foods, they add significant diversification into the diet and therefore add essential components of nutrition. One way to estimate the impact they might have on population health is by looking at the economic impact they have, which shows their widespread availability and therefore the large number of people in whose health and nutrition they play a role. The economic growth of these foods is also a measure of nutritional importance. Production and trade occurs as a result and response to consumption and the level of consumption shows the important roles that these foods play in diet and nutrition. The Anacardiaceae family contains some species that are economically important including nuts, fruit, ornamentals, oils, resins, lacquers, and tannins (1). The foods with the highest level of economic importance and therefore the highest global impact on nutrition are the mango, the cashew nut and the pistachio nut.

Mangoes are grown in over 90 countries and are distributed throughout these countries and internationally to many more (122). Mango is the predominant tropical fruit produced globally with an estimated 39.1 million tons produced globally in 2018 which accounted for more than half of total global fruit production of major tropical fruits that year (123). The global market size of processed mango products in 2018 was estimated to be $16.55 billion USD (18). The consumption of mango continues to rise and it has been forecast that the global compound annual growth rate of processed mango products will increase by 6.4% from 2019 to 2025 (18).

The cashew nut is economically important around the world in many countries including Brazil, India, Nigeria, and Vietnam (19). It is cultivated in Vietnam, India, Guinea-Bissau, Ivory Coast, Tanzania, Brazil, Benen, and other parts of Central Africa and South East Asia (124). The global production of cashews for 2019/20 was 790,000 metric tons (MT) (kernel basis) with India producing 170,000–195,000 MT, the Ivory Coast 149,000 MT, Vietnam 82,000 MT, and Tanzania 53,000 MT (124).

Pistachio nuts are a major tree nut produced in the world. In the 2019/2020 production year 694,068 MT were produced with 331,538 MT from the United States, 205,000 MT from Iran, 85,000 MT from Turkey and 55,000 MT from Syria. The US exported about 200,000 MT and Iran about 125,000 MT. The biggest importers of pistachio nuts were China who imported 100,000 MT and the European Union who imported 95,000 MT (125).

The chironji nuts are economically important as they provides income to tribal people in north, west, and central India because of the high value placed on the seed kernel (35, 63). Chironji adds nutrition to the diet of tribal people in India who harvest it from the wild and eat it (35). Similarly, in Brazil the yellow mombin are harvested wild and then sold at local markets or frozen as a pulp and sold commercially throughout the country (33).

The marula fruit is eaten throughout Africa and is considered a good food-security resource providing food during the "hungry season" when grain stocks are low and other crops are not yet ready for harvesting (26, 29). The fruit are eaten fresh or cooked and used for juice which can be boiled to a syrup (26). If the seeds are clean, dry and completely ripe they can be stored for months without deterioration and used as emergency food caches (26). They can be used to flavor dishes or pounded into flour or used like a nut in baking (26). The edible kernel of the marula is eaten as a snack food by children in Niger and could be used as a food supplement by the larger population providing essential energy in the diet (29). For children in these communities who mostly eat millet and other grains the marula kernel plays an important role in bringing diversity to the diet and providing critical nutrients (29).

Many Australian Aboriginal communities commonly eat the green plum as food and it is a favorite with children (126, 127). Finding ways of value-adding to native foods or being able to use them as value-add ingredients may increase the economic returns for the community, and could increase the ecological cultural and social benefits (128). The native Australian fruit are nutritionally important and could add to the global food supply and be used for diet diversification providing valuable nutrients and micronutrients to the supply chain and giving better nutrition and health outcomes and help to combat dietary deficiencies.

FUTURE STUDIES OF THE GREEN PLUM

As a food of the Anacardeaceae family, further studies on the green plum should be done as the family contains a number of important foods that provide nutrition to individuals and populations. The initial study of its nutritional properties was on underripe green plums which yielded promising results (7) but further analysis on ripe green plums should be done to confirm these. Proximate and metals, minerals and trace elements could show if the ripe green plum flesh has high levels moisture and carbohydrate, and if it is also a good source of potassium. Analysis of the fat content and fatty acid profile of the flesh and the kernel could tell if it is consistent with other foods of the Anacardiaceae family and if it also contains the high levels of fat and the 18:1 oleic acid fatty acid that are seen in this family, particularly in the kernels. The underripe green plums have high folate levels (7) and a further study of the folate in the fruit as it matures and ripens could give valuable information about folate in fruit, particularly in this family, and as a comparison to the folate levels seen in the small underripe mangos (3). These nutritional studies could enhance the role that green plum already plays in providing nutrients to the Aboriginal communities of northern Australia and the potential role they could play in providing diet diversification to the larger population of Australia or globally, as other economically important fruit and kernels in this family are already doing.

Studies on the mango, cashew apple, pistachio nut and Burdekin plum show that they have potential as functional foods or nutraceuticals due to their nutritional content and the compounds they contain, thus, the green plum should be

characterized to demonstrate if it has similar properties and potential for future use. The anti-nutritional properties in the family and particularly the allergy causing compounds found in some of the plants and fruit indicate that the green plum should be analyzed to find out if it also contains these compounds and if sensitization can occur from it. If storage trials indicated that fungal contamination could be a problem for green plums then aflatoxin and other toxin assays should be done to understand their contamination and risk.

CONCLUSION

The fruit of the Anacardiaceae family provide many individual health benefits from the nutrients they contain. Some of them are major food crops that are grown and distributed across the world, providing essential nutrition to many populations. The fruit are good sources of carbohydrates, potassium and folate. Of some concern are the allergenic compounds that many Anacardiaceae fruit contain, and testing for these could be beneficial. The Anacardiaceae family has a number of nutritionally important kernels, providing high levels of protein, fat, potassium, phosphorous, and amino acids. The kernels are all high in oleic acid, linoleic acid, palmitic acid, and stearic acid. The green plum is a very small fruit and the kernel in the seed is very small, however, it could be of interest to study the green plum kernel to see if it also contains the valuable nutrients seen in these other kernels. Some Anacardiaceae fruit are being used as functional foods and if the green plum industry were to grow it could be of interest to understand the health functions they can play on the human body. As an Anacardiaceae fruit, the green plum has potential as a source of nutrition and diet diversification and further studies on it as a food are justified.

AUTHOR CONTRIBUTIONS

SF, HES, HJS, and YS planned the paper. SF researched and wrote the manuscript. SF, HES, HJS, MR, and YS edited the manuscript. HES, HJS, MR, and YS supervise the project and Ph.D. All authors contributed to the article and approved the submitted version.

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Impact of Household Cooking Techniques on African Nightshade and Chinese Cabbage on Phenolic Compounds, Antinutrients, *in vitro* Antioxidant, and β-Glucosidase Activity

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Different household cooking techniques (boiling, steaming, stir frying, and microwave) were tested on the changes of targeted phenolic compounds, antioxidant property (ferric reducing-antioxidant power (FRAP) activity), α-glucosidase activity, antinutritive compounds, and sensory properties in commonly consumed traditional leafy vegetables in Southern Africa, the non-heading Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*) and African nightshade (*Solanum retroflexum* Dun). Stir frying increased kaempferol-3-*O*-hydroxyferuloyl-trihexoside, kaempferol-dihexoside, sinapoyl malate, rutin, and isorhamnetin-*O*-dihexoside in Chinese cabbage leaves, followed by steaming. Similarly, stir frying increased kaempferol-3-*O*-rutinoside, chlorogenic acid, caffeoylmalic acid, and quercetin-3-*O*-xylosyl-rutinoside in nightshade, followed by steaming. Biomarkers, sinapoyl malate (Chinese cabbage) and caffeoylmalic acid (nightshade), separated the stir frying from the other cooking techniques. Steaming and stir-frying techniques significantly increased the FRAP activity; whereas boiling and microwaving reduced the tannin, oxalate, and phytate contents in both leafy vegetables and steroidal saponins in nightshade. Stir-fried nightshade leaf extract showed the most effective inhibition against α-glucosidase activity, with an IC_{50} of 26.4 μg ml^{-1}, which was higher than acarbose, a synthetic compound (positive control; IC_{50} 69.83 μg ml^{-1}). Sensory panelists preferred the stir-fried Chinese cabbage and nightshade leaves, followed by steamed, microwaved, and boiled vegetables.

Keywords: indigenous leafy vegetables, oxalates, β-glucosidase activity, kaempferol derivatives, chlorogenic acid, FRAP activity

INTRODUCTION

Consumer preference for the intake of fruit and vegetables in the daily diet is increasing, and the World Health Organization (1) recommends a minimum of 400 g of fruit and vegetables, or five portions, per day excluding the starchy tubers. The United States Department of Agriculture (USDA) (2) guidelines state that an individual must consume one cup (∼237 g) of raw or cooked vegetables or two cups of raw leafy greens. These recommendations help in the prevention of non-communicable diseases and micronutrient deficiencies. The number of people affected with type 2 diabetes in Africa was projected to increase to 41.5 million in 2035, and it will be more prevalent in middle aged (40–59) people (3). The crop diversification for sustainable diets, nutrition, and income generation helped to recognize the importance of traditional indigenous vegetables for smallholder crop production and to sustain food and nutrition security (4).

Traditional vegetables, African nightshade (*Solanum retroflexum* Dun) and non-headed Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*), are popularly consumed in the Southern African region. Nightshade (Ca 199 mg 100 g^{-1}, Fe 12.8 mg 100 g^{-1}) and Chinese cabbage (Ca 27–31 mg 100 g^{-1}, Fe 0.5–3.5 mg 100 g^{-1}) (5) contain higher levels of Ca and Fe than raw spinach (5, 6). Traditionally, these vegetables are consumed in cooked form. Various cooking methods, such as boiling and steaming, are adopted to improve their palatability and sensory properties (stir frying in hot oil has become a popular cooking method of vegetables due to its convenience, taste preference, and fresh cooking pattern) (7).

Cooking improved the availability of phenolic compounds and antioxidant capacity of vegetables (8). Moreover, the dietary phenolic compounds demonstrated inactivation of carbohydrate digestive enzymes, α-amylase and β-glucosidase, and acted as appropriate anti-hypoglycemic agents (9). Thus, one way to manage type 2 diabetes is *via* encouraging consumption of food rich in anti-hypoglycemic agents. However, types of cooking technique, temperature, and the duration affect the extent of the loss of nutrients (10). Although vegetables are generally cooked in households based on convenience and taste preference, the type of cooking method adopted should be standardized.

In leafy vegetables, tannins, oxalates, and phytates are known as common antinutritive compounds (11). Tannins (polyphenols) form complexes with proteins and make them unavailable for absorption (11). Oxalates bind with dietary calcium and prevent them from being absorbed (11, 12); besides the insoluble calcium, oxalates stored in the kidney manifest as a health-related condition known as "kidney stones." The negatively charged phosphate groups in phytic acid chelate with Zn or phytates binding with proteins make them unavailable for absorption (11). Different cooking techniques, particularly blanching, reduced the contents of antinutritional factors (11).

Thus, the objective of this study was to investigate the influence of different household cooking methods, such as boiling, steaming, stir frying, and microwaving, on the changes in (i) phenolic components, (ii) antioxidant properties, (iii) antinutritive compounds, (iv) sensory properties, and (v) α-glucosidase activity of Chinese cabbage and nightshade leafy vegetables.

MATERIALS AND METHODS

Materials

Chinese cabbage and nightshade were planted in winter. The leaves (5 kg) were selectively harvested by avoiding leaves that were infected with fungi or infested with pest and washed as described by Managa et al. (13). Thereafter, the leaves were manually chopped into small pieces to mimic the typical domestic preparation and mixed well for homogeneity. Leaf samples (100 g) were selected for four different household techniques as described below. Raw leaf samples (100 g) were freeze-dried (Telstar Lyoquest Freeze Dryer, model 61644 at −55°C) for biochemical analysis.

Cooking Techniques

The time taken for each household cooking technique was concluded based on interviews and literature-based evidence (14).

Boiling

Nightshade and Chinese cabbage leaves (100 g) were boiled in 150 ml of water at 98°C in a covered stainless steel pot on a moderate flame for 15 min, mimicking the traditional method of cooking, drained.

Steaming

Nightshade and Chinese cabbage leaves (100 g) were steamed in 250 ml of boiling water in a stainless steel steamer pot (98°C) for 15 min.

Microwave Cooking

Nightshade and Chinese cabbage leaves (100 g) of vegetables were placed in a glass dish with 12 ml of water for 15 min in a microwave oven (Defy) (household) working at 2,450 MHz−900 W for 5 min. Afterwards, the vegetables were drained.

Stir Frying

10 ml of virgin olive oil was placed onto a preheated pan, and then 100 g of vegetables was placed in the pan and stir fried for 1–2 min. The oil temperature was ranging from 125 to 140°C. The temperature of the vegetables was 100°C after stir frying.

The samples were cooled rapidly on ice-cold water after each of the above-mentioned household cooking technique to stop further post-cooking biochemical changes.

Chemicals

The analytical standards, chlorogenic acid, catechin, luteolin, epicatechin, and rutin (purity >95%), and other chemicals were purchased from Sigma Aldrich, Johannesburg, South Africa.

Targeted Phenolic Metabolites

An ultra-high-pressure liquid chromatography (UHPLC) system equipped with quadrupole time-of-flight (QTOF) mass spectrometer (MS) (Waters, Milford, MA, United States) was employed to identify and quantify the predominant

polyphenolic metabolites as described by Managa et al. (13) and Ndou et al. (15) without any modifications. Briefly, phenolic compounds were extracted from 50 mg freeze-dried sample of nightshade and Chinese cabbage leaves subjected to different cooking techniques, using 70% aqueous ethanol coupled with ultra-sonication. The identification and quantification of the phenolic components were carried out using a cocktail standard solution comprising chlorogenic acid ($y = 1.6315x - 1.8800$, $r^2 = 0.99$, LOQ = 0.52 ppm), catechin ($y = 0.0006x + 0.0006$, $r^2 = 0.99$, LOQ = 0.92 ppm), luteolin ($y = 0.0005x - 0.0005$, $r^2 = 0.96$, LOQ = 0.45 ppm), epicatechin ($y = 0.0008x + 0.0008$, $r^2 = 0.99$, LOQ = 0.54 ppm), and rutin ($y = 0.0007x + 0.0007$, $r^2 = 0.99$, LOQ = 0.42 ppm) due to the unavailability of commercial standards for all the studied compounds. Working range solution from 1 to 1,500 ng ml^{-1} and UV spectra were monitored over a range of 200–400 nm. The cocktail standard solution was prepared in 50% aqueous methanol containing 1% formic acid, and the concentration of phenolic compounds was expressed as mg kg^{-1}. Data processing using the TargetLynx software was conducted as described previously by Managa et al. (13) and Ndou et al. (15).

In vitro Antioxidant Activity Using FRAP Assay

The ferric reducing-antioxidant power (FRAP) assay was performed according to the method described by Managa et al. (13) and Mpai et al. (16) without any modifications. Raw and cooked nightshade and Chinese cabbage leaf freeze-dried samples (0.2 g) were extracted with sodium acetate buffer (pH 3.6). The reaction mixture consisted of leaf extract (15 µl) and 220 µl of FRAP reagent solution (10 mmol L^{-1} 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) and 20 mmol L^{-1} $FeCl_3$). Subsequently, absorbance was read at 593 nm using a microplate reader (CLARIOstar Plus BMG Labtec; Lasec, Cape Town, South Africa). The reducing antioxidant power was estimated using an external standard curve of Trolox and expressed as µmol TEAC 100 g^{-1}.

Antinutritive Compounds

Tannin Content

Freeze-dried leaf samples (0.2 g) mixed with 10 ml 1% HCl. The reaction mixture included 100 µl aliquot of the sample extract and 50 µl vanillin–HCl in methanol (5 ml of 8% HCl in methanol and 5 ml of 1% vanillin in methanol) according to the method described by Price et al. (17) and Managa et al. (18) without any modifications. Tannin content was expressed as mg 100 g^{-1}.

Phytate Content

100 ml of 2.4% HCl was added to the freeze-dried leaf samples (0.5 mg) to extract the phytates. The quantification was performed using Wade reagent (0.03 g monohydrate ferric chloride and 0.3 g sulfosalicylic acid in 100 ml distilled water) as described previously by Latta and Eskin (19) and Managa et al. (18) without any modifications. Phytate content was expressed as percentage.

Oxalate Content

Freeze-dried leaf samples (0.1 g) were homogenized with 30 ml 2 mol L^{-1} HCl to extract the insoluble oxalates. Soluble oxalates were extracted with distilled water using leaf samples (0.1 g) according to the standard Association of Official Analytical Chemists (AOAC) method (20) and Managa et al. (18). The CaC_2O_4 was precipitated by adding 5% $CaCl_2$, and the pellets were collected and washed three times with 0.35 M NH_4OH and afterwards dissolved in 0.5 M H_2SO_4. The resulting solution (combined soluble and insoluble oxalates) was titrated against 0.1 M of $KMnO_4$ at 60°C until an extremely faint pale pink color persisted for 15 s. Oxalate concentration was expressed as mg 100 g^{-1}.

Sensory Analysis

Sensory analysis for the Chinese cabbage and nightshade leaves, subjected to different cooking treatments, was performed using a 9-point hedonic scale as described by Managa et al. (18). Color, taste, and aroma were evaluated using an overall acceptance of a 9-point hedonic scale (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely). Untrained sensory panelists ($n = 16$, men and women) within the age range of 20–60, who are familiar with Chinese cabbage and nightshade leaves and consumed these vegetables at least twice a month, voluntarily participated to evaluate the sensory properties of the cooked leaf samples.

In vitro α-Glucosidase Inhibitory Activity

Leaf extract (5 µl) of nightshade and Chinese cabbage leaves prepared at concentrations of 50–250 µg/ml was mixed with 20 µl α-glucosidase solution (50 µg ml^{-1}) into a 96-well plate. α-Glucosidase inhibitory effect was measured according to Sagbo et al. (21) without any modifications. Briefly, 60 µl potassium phosphate buffer (pH 6.8; 67 mM) was added to the mixture and incubated at 35°C for 5 min. Subsequently, 10 µl of 10 mM ρ-nitrophenyl-α-D-glucoside solution (PNPGLUC) was added, and the incubation at 35°C was extended for an additional 20 min. Following this, 25 µl of 100 mM Na_2CO_3 was added, and the absorbance was read at 405 nm using a microplate reader. The absorbance was measured for both the leaf extracts, acarbose, and the blank control (without α-glucosidase). The enzyme inhibitory activity was expressed as the percentage of α-glucosidase inhibition. The IC_{50} value (i.e., the concentration of nightshade and Chinese cabbage leaf extracts from different household cooking methods that resulted in 50% inhibition of maximal activity) was determined.

Statistical Analysis

A completely randomized design was adopted with 10 replicates per cooking technique with the experiments repeated twice. One-way analysis of variance (ANOVA) was used to test the significant differences between the different cooking treatments. Means were compared among the treatments by the least significant difference (LSD) test, with $p < 005$, using the Genstat statistical

program for Windows 13th Edition (2010) (VSN International Hempstead, UK). The obtained UHPLC-QTOF/MS data were exported for unsupervised principle component analysis (PCA). Following this, supervised orthogonal projections to latent structures discriminant analysis (OPLS-DA) was executed as described by Managa et al. (18).

RESULTS AND DISCUSSION

Multivariate Analysis and Tentative Identification of Biomarker Metabolites

Supplementary Figures 1A–C presents the total ion chromatograms scanned in negative ESI-mode for the Chinese cabbage and nightshade leaves subjected to different household cooking techniques. **Supplementary Tables 1A,B** illustrates the tentative identification of predominant phenolic compounds in both leafy vegetables. Stir-fried Chinese cabbage and nightshade samples contained the highest concentration of total polyphenol content. Chinese cabbage raw leaves contained kaempferol derivatives: kaempferol-sophoroside-hexoside, kaempferol-3-*O*-hydroxyferuloyl-trihexoside, kaempferol-3-*O*-hydroxyferuloyl-diglucoside, kaempferol-dihexoside, kaempferol-3-*O*-sinapoyl-dihexoside-hexoside, isorhamnetin-*O*-dihexoside, isorhamnetin-*O*-hexoside, and sinapoyl malate (**Supplementary Table 1A**). Raw nightshade leaves contained kaempferol-3-*O*-rutinoside, isorhamnetin-*O*-hexoside, rutin, chlorogenic acid, and its isomer neochlorogenic acid, caffeoylmalic acid, and quercetin-3-*O*-xylosyl-rutinoside (**Supplementary Table 1B**). The PCA plots indicated two clear groupings, separating the stir frying from the other cooking techniques for both Chinese cabbage and nightshade leaves (**Supplementary Figures 1A,B**). From the *S*-plots, while sinapoyl malate, a biomarker, separated the stir-fried Chinese cabbage leaves from the other cooking techniques (**Figure 1A**), rutin separated the stir-fried nightshade leaves from the other cooking techniques (**Figure 1B**).

Changes in Phenolic Compounds During Domestic Cooking

The significant influence of four different household cooking techniques on the targeted phenolic compounds in these two vegetables is shown in **Tables 1A**,**B**. There was no significant change in the concentration of kaempferol-sophoroside-hexoside between the raw and stir-fried Chinese cabbage leaves (**Table 1A**). However, a severe decline in the amount of this corresponding compound (∼50%) was observed in the microwaved and boiled leaves compared with the raw, stir-fried, and steamed leaves (**Table 1A**). The concentration of kaempferol-3-*O*-hydroxyferuloyl-trihexoside, kaempferol-dihexoside, isorhamnetin-*O*-dihexoside, isorhamnetin-*O*-hexoside, sinapoyl malate, and rutin was significantly highest ($p < 0.05$) in the stir-fried leaves. Kaempferol-3-*O*-hydroxyferuloyl-diglucoside was significantly ($p < 0.05$) highest in the raw leaves, and a lower concentration was found in the stir-fried leaves. On the contrary, kaempferol-3-*O*-hydroxyferuloyl-diglucoside was completely disappeared in the boiled, microwaved, and steamed leaves (**Table 1A**). Sinapoyl malate significantly ($p < 0.05$) increased during stir frying, and a relatively low amount was detected after steaming. Sinapoyl malate was not found in the boiled and microwaved leaves.

Boiled and microwaved methods caused the complete loss of kaempferol-3-*O*-hydroxyferuloyl-trihexoside and kaempferol-3-*O*-hydroxyferuloyl-diglucoside, with the latter significantly ($p < 0.05$) decreased after stir frying compared with the raw leaves. Kaempferol-3-*O*-sinapoyl-dihexoside-hexoside was not detected after the four different adopted cooking techniques, which could be due to the relatively low concentration of this compound in raw material (0.45 mg kg^{-1}; **Table 1A**). Generally, among the studied household cooking techniques, stir frying maintained or even increased the major kaempferol derivatives in Chinese cabbage leaves, probably due to the cooking time related to the different techniques, the stability of kaempferol-3-*O*-hydroxyferuloyl-trihexoside and kaempferol-3-*O*-hydroxyferuloyl-diglucoside was affected. The longer duration of cooking time in this study severely affected the kaempferol derivatives compared with moist cooking (e.g., blanching in hot water at 95°C for 5 min) (22). During domestic food preparation, acylated kaempferol tri-, or tetra-glycosides showed thermal resistance (23). In addition, non-acylated kaempferol diglucosides demonstrated higher loss after boiling and minor loss after steaming broccoli (23), and the degree of loss of these compounds also depends on differences in the texture of different food matrices, such as broccoli florets and Chinese cabbage leaves. The increase of non-acylated kaempferol-dihexoside during stir-frying Chinese cabbage leaves was probably due to transformation of acylated kaempferol-3-*O*-sinapoyl-dihexoside-hexoside through the loss of sinapic acid and further the deglycosylation of a hexoside moiety. It has been reported that acylated kaempferol tri- or tetra-glycosides were more thermally resistant during domestic food preparation (23) than kaempferol-3-*O*-hydroxyferuloyl-trihexoside during domestic cooking. During higher temperature heat treatments, kaempferol-3-*O*-hydroxyferuloyl-diglucoside was expected to degrade to its monoglucoside form; however, either kaempferol-3-*O*-hydroxyferuloyl-monoglucoside or kaempferol aglycone was undetectable. A similar observation was reported during transformation of quercetin-3,4′-*O*-diglucoside in roasted onions (24). The monoglucoside compounds could possibly have been transformed after deglycosylation of kaempferol-3-*O*-hydroxyferuloyl-trihexoside. In addition, the 4′-*O*-glycoside position had a higher thermal stability against deglycosylation than the 3-*O*-glycoside position (24). However, in the present investigation, kaempferol aglycone was not detected at higher temperatures (stir frying). Also, isorhamnetin-*O*-dihexoside could have transformed to isorhamnetin-*O*-hexoside by losing a glucose molecule. Cooking in water (aqueous conditions) at higher temperatures degraded the aglycone compounds into several other simple compounds. In addition, phenolic compounds, such as sinapoyl malate and kaempferol-3-*O*-hydroxyferuloyl-trihexoside, could have leached into the water with higher extents during boiling and microwaving than steaming and stir frying.

In nightshade leaves, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-sinapoyl-dihexoside-hexoside, rutin, quercetin-3-*O*-xylosyl-rutinoside, and chlorogenic and caffeoylmalic acids increased for all household cooking techniques when compared with the raw leaves (**Table 1B**). Kaempferol-dihexoside was not detected after cooking. However, kaempferol-3-*O*-rutinoside, quercetin 3-*O*-xylosyl-rutinoside, and chlorogenic and caffeoylmalic acids increased significantly ($p < 0.05$) during stir frying, followed by steaming. Rutin content was detectable at a similar concentration in the steamed and stir-fried nightshade leaves. An increase in rutin and chlorogenic acid contents was also reported during home cooking of sofrito tomato sauce (25). Frying technique at 180°C has been known to increase the availability of chlorogenic acid in Mediterranean vegetables (26). The increase of chlorogenic acid concentration (∼88%) observed in the nightshade leaves could be due to the formation of different caffeoylquinic acid isomers during cooking or due to the hydrolysis of dicaffeoylquinic acid (27) as this compound was detected in the raw nightshade leaves (**Table 2B**). A new compound, neochlorogenic acid, that was not present in the raw leaves, was found in all cooking techniques, and its highest concentration was obtained in the stir-fried nightshade

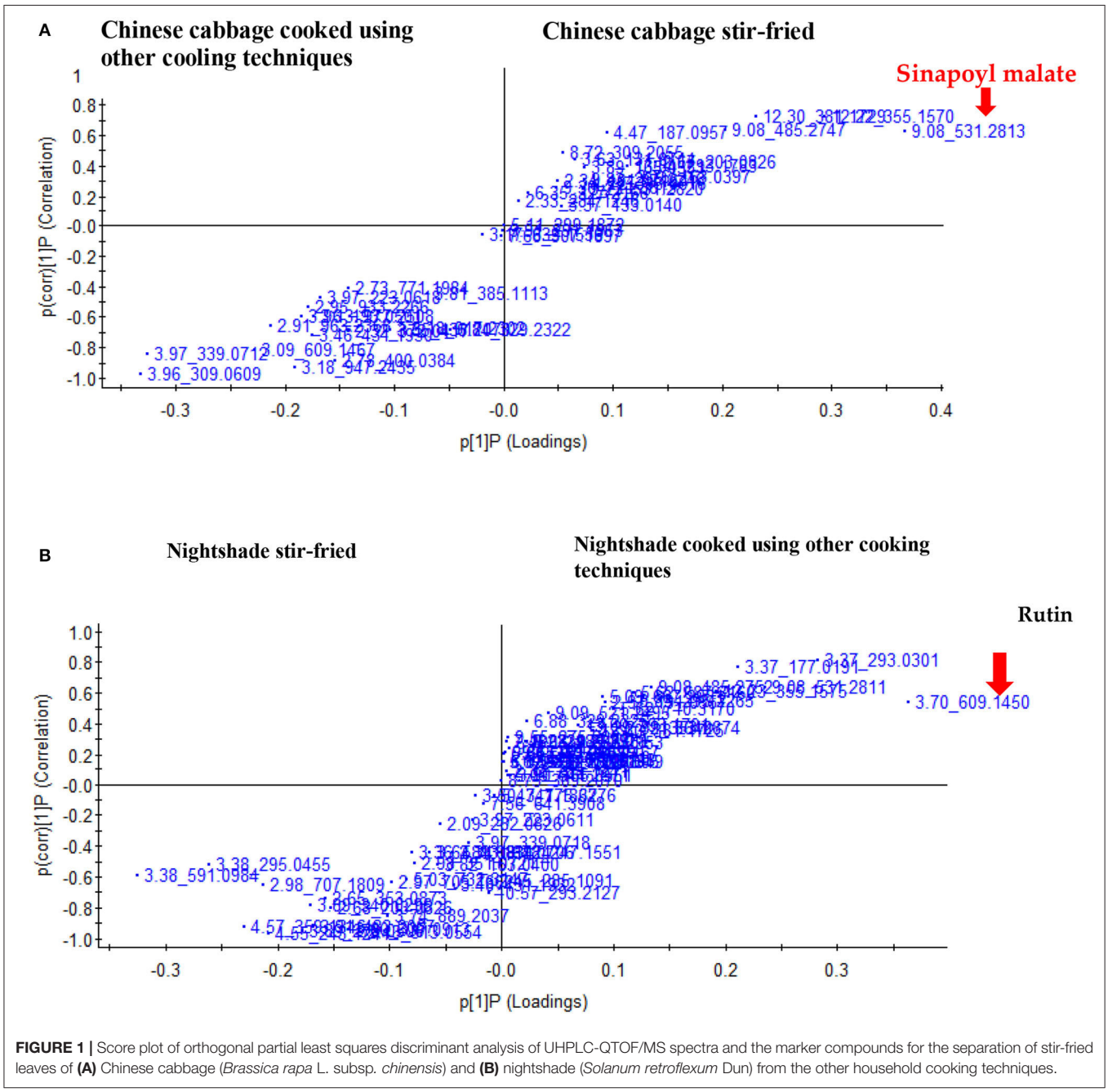

FIGURE 1 | Score plot of orthogonal partial least squares discriminant analysis of UHPLC-QTOF/MS spectra and the marker compounds for the separation of stir-fried leaves of **(A)** Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*) and **(B)** nightshade (*Solanum retroflexum* Dun) from the other household cooking techniques.

TABLE 1A | Changes in targeted phenolic compounds in Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*).

Phenolic compounds (mg kg^{-1})	Raw	Boiling	Steaming	Microwaving	Stir frying
Kaempferol-sophoroside-hexoside	50.4 ± 0.01^{*a}	23.77 ± 4.78^{c}	42.67 ± 3.03^{b}	19.87 ± 3.24^{d}	57.98 ± 5.90^{a}
Kaempferol-3-*O*-hydroxyferuloyl-trihexoside	4.10 ± 2.77^{c}	nd	7.45 ± 4.72^{b}	nd	33.44 ± 2.93^{a}
Kaempferol-3-*O*-hydroxyferuloyl-diglucoside	27.7 ± 0.05^{a}	nd	nd	nd	2.50 ± 0.23^{b}
Kaempferol-dihexoside	20.3 ± 0.06^{b}	4.84 ± 0.75^{d}	16.13 ± 0.25^{b}	9.11 ± 4.11^{c}	22.69 ± 0.08^{a}
Isorhamnetin-*O*-dihexoside	0.13 ± 0.60^{d}	0.219 ± 0.0^{c}	2.61 ± 0.47^{b}	2.41 ± 0.55^{b}	6.26 ± 0.0^{a}
Isorhamnetin-*O*-hexoside	2.34 ± 0.02^{d}	17.39 ± 0.52^{b}	33.01 ± 2.59^{a}	13.57 ± 5.29^{c}	31.65 ± 1.58^{a}
Sinapoyl malate	0.04 ± 0.07^{c}	nd	21.13 ± 0.10^{b}	nd	58.13 ± 48.8^{a}
Rutin	7.20 ± 2.77^{d}	11.11 ± 5.05^{c}	18.68 ± 0.56^{b}	18.87 ± 5.75^{c}	25.99 ± 3.87^{a}
Total polyphenols	112.21	57.329	141.68	63.84	238.64

Rows with similar alphabetic letter are not significantly different at $p < 0.05$ according to Fisher's LSD test.
*nd, not detected, *standard deviation (n = 3, cumulated sample of 10 makes 1 n replicate).*

TABLE 1B | Changes in targeted phenolic compounds in nightshade (*Solanum retroflexum* Dun) during household cooking.

Phenolic compounds (mg kg^{-1})	Raw	Boiling	Steaming	Microwaving	Stir frying
Kaempferol-3-*O*-sinapoyl-dihexoside-hexoside	0.67d ± 0.1^{b}	14.72 ± 0.00^{b}	nd	11.06 ± 4.59^{c}	26.46 ± 3.40^{a}
Kaempferol-3-*O*-rutinoside	5.60 ± 0.54^{e}	63.97 ± 1.39^{d}	81.29 ± 5.50^{b}	68.70 ± 6.21^{c}	101.18 ± 1.35^{a}
Kaempferol-dihexoside	1.63 ± 0.46	nd	nd	nd	nd
Isorhamnetin-*O*-hexoside	0.96 ± 0.23^{a}	nd	1.55 ± 0.11^{a}	nd	1.29 ± 0.00^{a}
Rutin	250 ± 0.23^{d}	394.42 ± 1.52^{c}	428.04 ± 1.88^{ab}	421.68 ± 16.57^{b}	440.36 ± 0.12^{a}
Neochlorogenic acid	nd	14.72 ± 139^{c}	30.23 ± 1.74^{b}	10.63 ± 1.04^{d}	37.96 ± 2.14^{a}
Chlorogenic acid	1.04 ± 0.33^{e}	43.03 ± 1.37^{c}	86.41 ± 1.09^{b}	35.91 ± 3.16^{d}	92.60 ± 1.80^{a}
Caffeoylmalic acid	3.44 ± 1.81^{e}	102.75 ± 1.88^{c}	251.97 ± 1.31^{b}	87.15 ± 13.75^{d}	440.04 ± 1.50^{a}
Dicaffeoylquinic acid	35.0 ± 0.21	nd	nd	nd	nd
Quercetin-3-*O*-xylosyl-rutinoside	4.00 ± 1.50^{c}	58.28 ± 1.26^{b}	76.5 ± 1.70^{b}	52.0 ± 0.34^{b}	102.46 ± 1.41^{a}
Total polyphenols	302.34	691.92	704.03	687.13	1,242.35

Rows with similar alphabetic letter are not significantly different at $p < 0.05$ according to Fisher's LSD test. nd, not detected, standard deviation (n = 3, cumulated sample of 10 makes 1 n replicate).

leaves. Intramolecular transesterification of 5-*O*-caffeoylquinic acid had produced neochlorogenic acid, a new compound that was not detected in the raw leaves, and it significantly increased after all cooking process and showed the highest concentration in the stir-fried leaves, followed by the steamed leaves. A similarly significant increase in caffeoylquinic acid was reported in fried artichokes compared with the raw and other cooking methods adopted (28). Transesterification of caffeoylquinic acid is dependent on the pH of the food matrix and the temperature and time as well. Neochlorogenic, chlorogenic, and caffeoylmalic acids and rutin were detected after moist cooking (blanching) in our previous investigation and Managa et al. (18); however, the concentrations of the above-mentioned compounds during blanching were much higher than those obtained during domestic cooking (**Table 1B**). Additionally, the lower pH of the food matrix also exhibited a major role in obtaining higher concentrations of these phenolic compounds after blanching, based on Managa et al. (18). Isorhamnetin-*O*-hexoside was detected in the raw, steamed, and stir-fried leaves without any significant changes ($p > 0.05$) and could possibly have been lost during microwaving and boiling. Additionally, using olive oil for stir frying has been reported to further increase the flavonoids in asparagus spears (29).

Antioxidant Activity and Different Cooking Techniques

The antioxidant property (FRAP activity) of Chinese cabbage and nightshade leaves after different types of domestic cooking techniques is shown in **Figures 2A,B**. All tested household cooking techniques showed higher antioxidant activity than the raw vegetables. Steaming demonstrated a significant ($p < 0.05$) increase in FRAP activity, followed by stir frying, which could be due to polymerization of phenols during cooking increasing the antioxidant activity. Polymerization of procyanidins was reported to increase the antioxidant activity (28). Furthermore, some other phenolic compounds that could not be identified by the UHPLC-QTOF/MS due to the unavailability of the commercial standards might also be involved and contributed to the increase of FRAP activity. Similarly, steamed artichokes showed the highest antioxidant

TABLE 2A | Effect of different cooking treatments on antinutritive compounds in Chinese cabbage leaves.

Treatments	Tannins (mg 100 g^{-1})	Oxalates (mg 100 g^{-1})	Phytates (%)
Raw leaves	57.63 ± 0.66^{a}	101 ± 0.58^{a}	90.69 ± 2.85^{a}
Boiling	29.99 ± 0.17^{c}	17.35 ± 0.21^{e}	17.02 ± 0.34^{e}
Microwave	29.97 ± 0.19^{c}	24.2 ± 0.00^{d}	23.59 ± 0.39^{d}
Stir frying	51.01 ± 0.17^{b}	34.1 ± 0.45^{c}	31.87 ± 0.69^{c}
Steaming	51.24 ± 0.24^{b}	44.33 ± 0.09^{b}	75.17 ± 0.67^{b}

Means followed by the same letter within the column are not significantly different at $p < 0.05$ level; standard deviation ($n = 3$, cumulated sample of 10 makes 1 n replicate).

TABLE 2B | Effect of different cooking treatments on antinutritive compounds in nightshade leaves.

Treatments	Tannins (mg 100 g^{-1})	Oxalates (mg 100 g^{-1})	Phytates (%)	Tigogenin-5G (mg kg^{-1})	Tigogenin-GG-Rham-Xyl-Xyl (mg kg^{-1})
Raw leaves	55.63 ± 0.38^{a}	88 ± 0.00^{a}	88 ± 0.00a	nd	0.04 ± 0.00^{e}
Boiling	28.58 ± 0.12^{e}	55 ± 0.00^{d}	22.1 ± 0.06^{e}	9.18 ± 1.79^{d}	11.89 ± 2.42^{c}
Microwave	50.21 ± 0.02^{b}	65.95 ± 0.68^{c}	67.14 ± 0.11^{b}	9.30 ± 0.87^{c}	9.52 ± 0.28^{d}
Stir frying	37.93 ± 0.71^{d}	77.73 ± 0.73^{b}	40 ± 0.11^{d}	9.78 ± 1.90^{b}	19.44 ± 1.79a
Steaming	46.24 ± 0.62^{c}	22.23 ± 0.12^{e}	44 ± 0.58^{c}	10.29 ± 1.46^{a}	15.65 ± 0.94^{b}

Means followed by the same letter within the column are not significantly different at $p < 0.05$ level; standard deviation ($n = 3$, cumulated sample of 10 makes 1 n replicate).

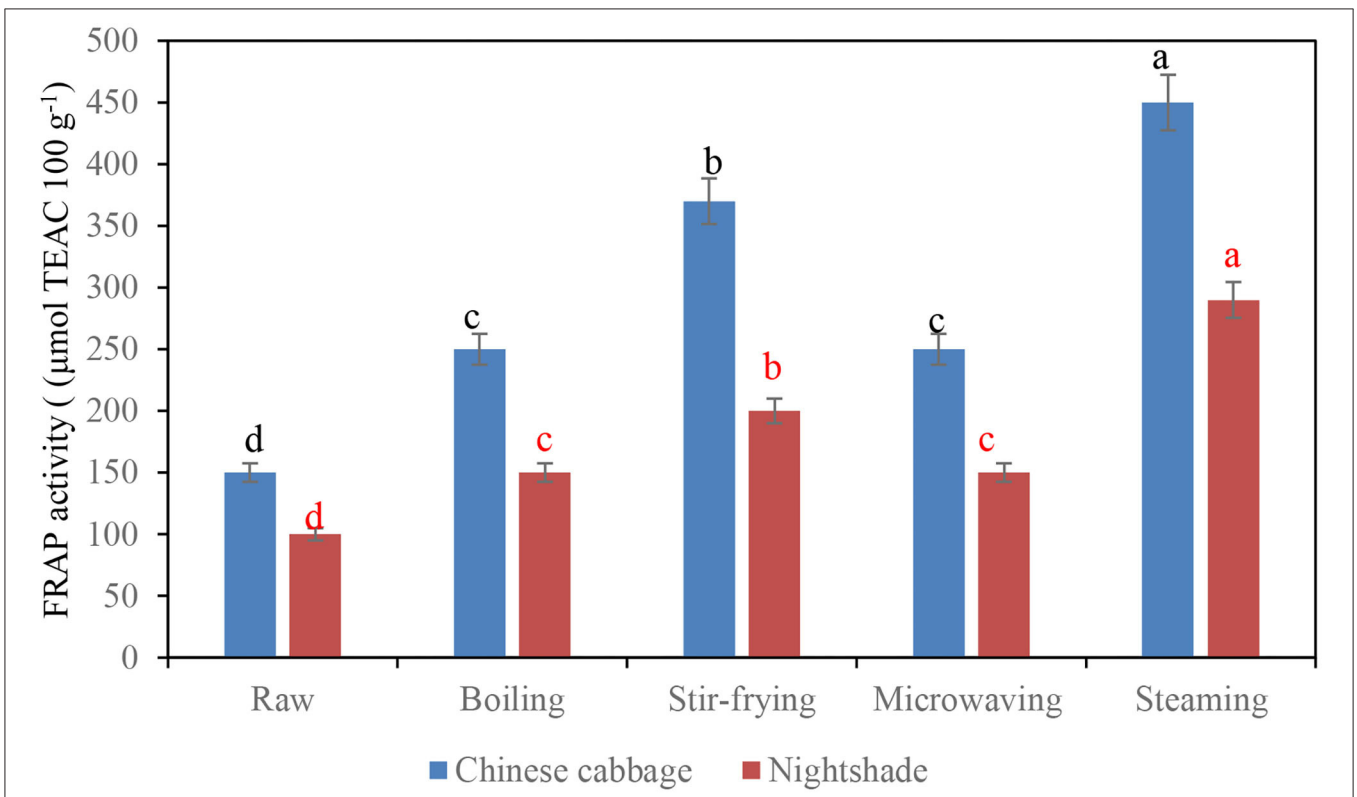

FIGURE 2 | Influence of household techniques on antioxidant property (FRAP) activity of Chinese cabbage and nightshade leaves. Bars with similar alphabetic letter for a particular leafy vegetable are not significantly different at $p < 0.05$ according to Fisher's LSD test.

activity (FRAP) compared with the stir-fried samples (28). Conversely, stir frying increased the FRAP activity in raw Pak Choi, a dark green Chinese cabbage (7). The lowest ($p < 0.05$) FRAP activity was demonstrated in both microwaved Chinese cabbage and nightshade leaves (**Figures 3A,B**); similarly, Boari et al. (30) revealed lower antioxidant properties of microwaved

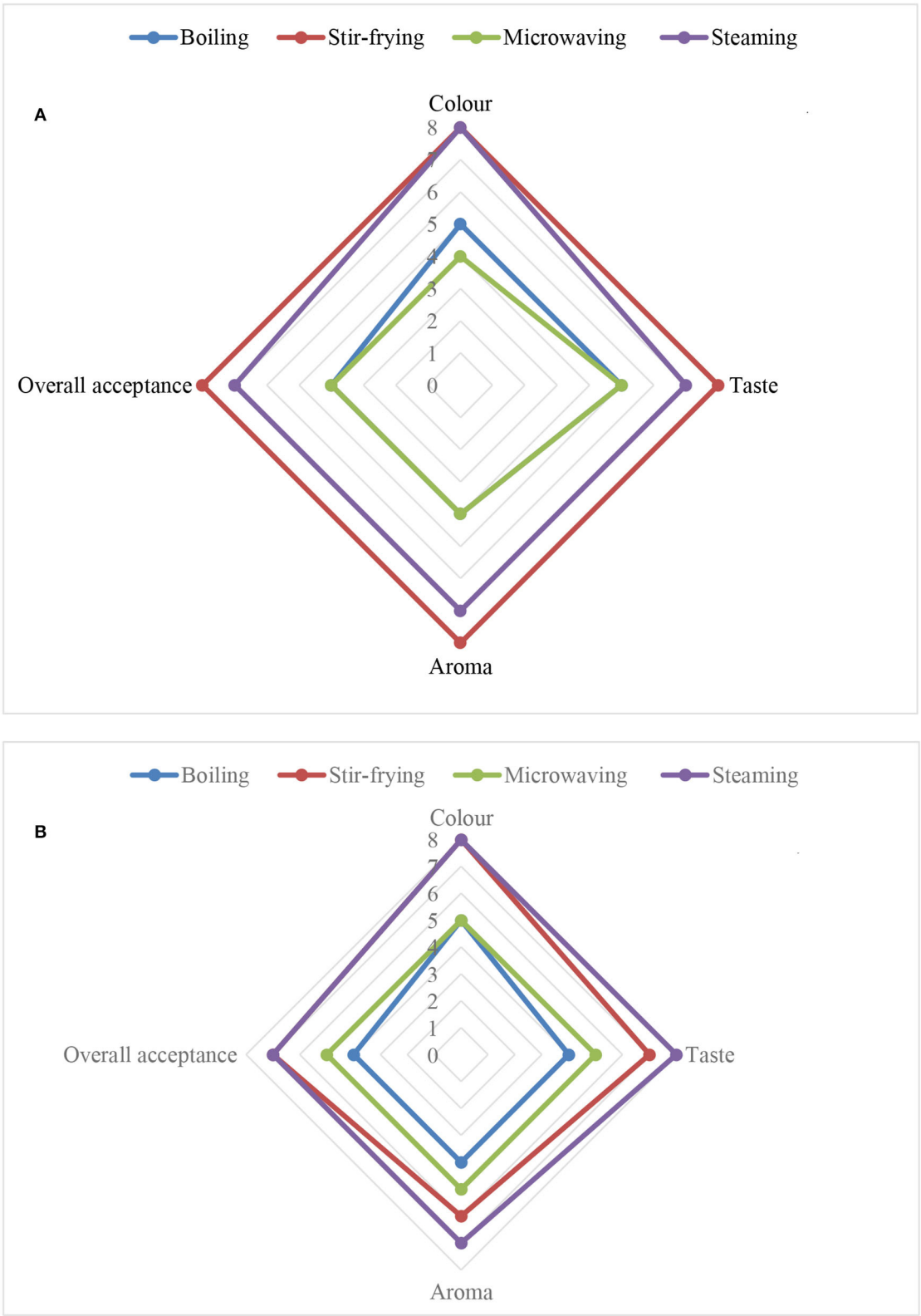

FIGURE 3 | Influence of different household cooking techniques on sensory properties of **(A)** Chinese cabbage and **(B)** nightshade leaves.

asparagus. Although individual antioxidants respond differently to the different types of cooking methods, the changes in chemical structure of polyphenols mediated during cooking (thermal treatment) can significantly influence the antioxidant property of traditional vegetables. Glycosylation of flavonoids reduces the antioxidant activity of their corresponding aglycones; however, the effectiveness of the antioxidant property depends on the configuration of hydroxyl groups attached to the flavonoid B and C rings (31). In addition, the number of hydroxyl groups in polyphenol molecular structure determines the effectiveness of the antioxidant property of the molecule (31). During cooking, the release of sugar moieties from the deglycosylation of phenolic triglycosides, resulting in the formation of diglycoside and monoglycoside compounds (due to cellular structural changes), could have contributed toward the antioxidant property (31). Boiling and microwaving in water always induced the highest decrease in phenolic acids and flavonoids, mainly due to greater losses derived from leaching effects (32).

In addition, the interference of tissue matrix could have played a role especially during the extraction of the samples that underwent steaming or stir frying. According to the literature, phenolic compounds and antioxidant activity declined during different cooking treatments with different vegetables (33). However, several studies demonstrated the enhancement of phenolic content and antioxidant activity of different vegetables *via* different cooking techniques (14, 34, 35).

Sensory Properties and Different Cooking Techniques

Sensory analysis data shown in **Figures 3A,B** indicated that the panelists "very much liked" the color of the steamed or stir-fried Chinese cabbage or nightshade leaves. They "moderately liked" the steamed Chinese cabbage and nightshade leaves and liked the aroma of the stir-fried Chinese cabbage "very much," but nightshade leaves "moderately." The panelists "very much liked" the taste of stir-fried Chinese cabbage and nightshade leaves, but "moderately liked" the steamed leaves. Overall acceptance (liked very much) was highest by the panelists for stir-fried Chinese cabbage leaves, and stir-fried nightshade leaves were accepted moderately, compared with the boiled and steamed samples. The light green color of the boiled and microwaved leaves could be due to the degradation of chlorophyll (18). Panelists' preference was high for stir-fried Chinese cabbage and nightshade leaves probably due to an increase in aroma from the release of volatile compounds associated with cooking temperature. Increased volatile compounds could have been due to the oxidization of fatty acids by lipoxygenase *via* a series of enzyme-like reactions; this requires further investigation. A similar increase in aroma and volatile compounds was reported in Bok Choy (Chinese cabbage, *Brassica chinensis* L; Shanghai Qing) (7).

Antinutritive Compounds and Different Cooking Techniques

Antinutritive compounds, including tannins, oxalates, and phytates, were found at the highest concentrations in raw (uncooked) nightshade and Chinese cabbage leaves (**Tables 2A**,**B**). The concentrations of tannins, oxalates, and phytates decreased with different cooking techniques. Boiling significantly ($p < 0.05$) reduced the tannin, oxalate, and phytate content in Chinese cabbage and nightshade leaves, whereas microwaving significantly ($p < 0.05$) reduced the tannin content in Chinese cabbage.

Phytates are thermostable, and the thermal inactivation of phytates takes place at temperatures above 60°C (36); 40% residual activity was reported at 95°C (37). The temperature of four different cooking techniques adopted in this study exceeded 60°C. The decrease in phytate content during cooking could be due to the formation of insoluble complexes between proteins or minerals, and during boiling and microwaving in water, these compounds can leach into the water (38); a similar explanation can be applicable to the loss of tannins.

Oxalate reduction during boiling could be due to its solubility in boiling water, facilitated by the breakage of cells to leak soluble oxalates into the cooking water. Boiling was reported to remarkably lower the soluble oxalate content by 30–87% than steaming (5–53%) in red and green Swiss chard leaves, spinach, and rhubarb stalks (39). Boiling in water also reduced total oxalates in Thai vegetables significantly by 16–79%, also in Mexican vegetables (40) and in chard (*Beta vulgaris*), watercress (*Nasturtium nasturtium-aquaticum*), spinach (*Spinacia oleracea*), and purslane (*Portulaca oleracea* L.) (38). Our data confirmed that boiling was most effective in reducing the soluble oxalate content of Chinese cabbage and nightshade leaves compared with steaming. Therefore, consumption of boiled Chinese cabbage and nightshade leaves may account a lower risk because soluble oxalate levels were markedly reduced during boiled cooking technique. On the other hand, the oxalate content in Chinese cabbage and nightshade leaves was much lower than that in green and red Swiss chad (964–1,167 mg 100 g^{-1} on fresh weight basis) and spinach (1,145 mg 100 g^{-1} on fresh weight basis) (41). It is also important to consider the content of soluble oxalate and the methods used for cooking vegetables when making dietary recommendations for individuals predisposed to kidney stones. Oxalate in foods can affect the bioavailability of minerals, such as Ca. Noonan and Savage (41) had classified foods into three groups based on oxalate:calcium ratio. Vegetables with oxalate:calcium ratio above 2 have high oxalate content and show the ability to bind with Ca from the other foods consumed at the same time. Spinach leaves showed oxalate:calcium ratio >2, indicating higher risk of binding with Ca. Green and red amaranth leaves showed oxalate:calcium ratio <2, suggesting the limited capacity to bind to available Ca from other foods. However, total oxalate/total calcium (mEq) needs to be estimated for Chinese cabbage and nightshade leaves in the future for dietary recommendations.

Steroidal saponins, tigogenin-G-G-G-G-G (5 glucose units attached) and tigogenin-G-G-Rham-Xyl-Xyl (2 glucose + rhamnose + 2 xylose units), were only detected in nightshade leaves (18). The changes in tigogenin-5G and tigogenin-GG-Rham-Xyl-Xyl during cooking are shown

in **Supplementary Figure 1B** and **Table 2B**. Raw samples contained significantly lower levels of saponins, after which increased significantly during cooking with different techniques. Among the different types of cooking techniques, boiling and steaming demonstrated a reduced degree of accumulation of these two saponins; steaming and stir frying significantly favored the accumulation of these saponins, and steamed leaves contained the highest levels of tigogenin-5G and tigogenin-GG-Rham-Xyl-Xyl. This could be probably due to reduced leaching of steroidal saponins into water compared with boiling and microwaving. Similarly, tigogenin-5G and tigogenin-GG-Rham-Xyl-Xyl were detected in hot water bath after blanching and steaming for 5 min; however, it was not quantified in our previous study (18). The changes in tigogenin-5G and tigogenin-GG-Rham-Xyl-Xyl during cooking are shown in **Supplementary Figure 1B** and **Table 2B**. Raw samples contained significantly lower levels of saponins, and it increased significantly during cooking with different techniques. Among the different types of cooking techniques, boiling and microwaving showed a reduced degree of accumulation of tigogenin-5G and tigogenin-GG-Rham-Xyl-Xyl. Steaming and stir frying significantly enhanced the accumulation of these two saponins. Steamed leaves contained the highest levels of tigogenin-5G, whereas stir-fried leaves showed the highest concentration of tigogenin-GG-Rham-Xyl-Xyl. This could be probably due to reduced leaching of steroidal saponins into water compared with boiling and microwaving. Similarly, tigogenin-5G and tigogenin-GG-Rham-Xyl-Xyl were detected in hot water bath blanching and steaming for 5 min; however, it was not quantified in our previous study (18).

Moreover, the solubility of tigogenin-5G and tigogenin-GG-Rham-Xyl-Xyl is affected by temperature and pH (42). It was stated that the solubility of saponins increased in water with temperature, probably due to rupture of cell wall and release of compounds (43). More solubility of saponins has been reported during heating (43). In addition, the tigogenin-GG-Rham-Xyl-Xyl had been extracted more from the stir-fried leaf. However, the longer duration of cooking time can include molecular transformation and could reduce the concentration of tigogenin-5G and tigogenin-GG-Rham-Xyl-Xyl. Leafy vegetables cannot be cooked for a longer duration, such as yams or tubers, and the sensory properties can be affected negatively.

α-Glucosidase Activity and Different Cooking Techniques

The effectiveness of the inhibitory effect of stir-fried or steamed Chinese cabbage and nightshade leaf extracts on α-glucosidase activity was compared on the basis of their resulting IC_{50} values (**Table 3**). Stir-fried nightshade leaf extract showed the most effective inhibitory effect on α-glucosidase activity with an IC_{50} of 26.4 μg ml^{-1} (**Table 3**), whereas stir-fried Chinese cabbage extract with an IC_{50} value of 36.5 mg ml^{-1} showed the second most active of the treatments tested. Steamed Chinese cabbage with an IC_{50} value of 40.6 mg ml^{-1} and nightshade with an IC_{50} value of 38.9 mg ml^{-1} were less active. Acarbose, the positive control used in this study, inhibited the activity of α-glucosidase with an IC_{50} value estimated at 69.83 mg ml^{-1} (**Table 3**). Our finding is consistent with literature reports, where acarbose was reported to show less inhibition on α-glucosidase activity. Shai et al. (44) and Shettar and Vedamurthy (45) had reported similar observation previously, and Anam et al. (46) had reported the absence of α-glucosidase inhibition by acarbose. Stir-fried nightshade was the most active cooking technique that showed the highest inhibitory effect on α-glucosidase activity.

α-Glucosidase is one of the most important carbohydrate digestion enzymes located on the brush-border surface membrane of intestinal cells (46). α-Glucosidase facilitates the production of glucose for intestinal absorption by hydrolyzing the disaccharides and oligosaccharides present in the intestine (lumen) (47). Inhibition of α-glucosidase, a carbohydrate digestive enzyme, is reportedly one of the most important approaches in managing obesity and diabetes (48). Therefore, consumption of steamed or stir-fried Chinese cabbage and nightshade leaves can be beneficial in reducing the risk of type 2 diabetes (48).

TABLE 3 | Antidiabetic activity of stir-fried or steamed Chinese cabbage and nightshade leaf extracts as determined by α-glucosidase inhibition assay.

Sample	IC_{50} α-glucosidase (μg ml^{-1})
Acarbose (positive control)	69.83 ± 0.02^{b}
Chinese cabbage	
Stir fried	36.50 ± 0.06^{b}
Steamed	40.60 ± 0.14^{c}
Nightshade	
Stir fried	26.38 ± 0.20^{b}
Steamed	38.90 ± 0.18^{a}

Each value is expressed as mean ± SD in triplicate experiments. Values are mean ± SD (n = 3). Mean values with different letters are significantly different at $p < 0.05$ level. The IC_{50} values were measured as the concentration of the test sample required to inhibit the activity by 50% under assayed condition.

TABLE 4 | Pearson's correlation coefficients between targeted phenolic components and *in vitro* antioxidant property (FRAP activity) and α-glucosidase activity from stir-fried or steamed Chinese cabbage and nightshade leaves.

Targeted phenolic components	FRAP activity	α-Glucosidase activity
Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*)		
Kaempferol-dihexoside	0.75	0.67
Kaempferol-sophoroside-hexoside	0.81	0.74
Isorhamnetin-*O*-dihexoside	0.65	0.62
Nightshade (*Solanum retroflexum* Dun)		
Chlorogenic acid		0.92
Neochlorogenic acid		0.80
Quercetin 3-O-xylosyl-rutinoside		0.88

The presence of –OH groups in positions 3 (ring C), 7 (ring A), and 4 and 5 (ring B) in the polyphenol molecular structure or C-4 ketonic functional group or C-2–C-3 double bond plays a vital role in the inhibitory effects of the α-glucosidase by binding to the active sites of the enzyme (49). Reportedly, methylation and acetylation of hydroxyl groups reduce the *in vitro* antioxidant and anti-diabetic properties of the flavonoids (50).

Correlations Between Bioactive Compounds and Associated Functional Properties

Kaempferol-dihexoside, kaempferol-sophoroside-hexoside, chlorogenic, and neochlorogenic acids, and quercetin-3-*O*-xylosyl-rutinoside demonstrated a positively strong correlation with the FRAP activity (**Table 4**). It is also evident that there is a positive correlation between α-glucosidase activity and bioactive compounds, such as kaempferol-sophoroside-hexoside or kaempferol-dihexoside in the steamed or stir-fried Chinese cabbage leaves (**Table 4**). Likewise, steamed or stir-fried nightshade leaves also demonstrated a positive correlation between β-glucosidase activity and rutin or chlorogenic acid, caffeoylmalic acid, or quercetin-3-*O*-xylosyl-rutinoside (**Table 4**). Both steroidal saponins demonstrated a positive correlation with α-glucosidase activity when maltose was used as substrate (**Table 4**). The hypoglycemic effect of saponins was reported in the root bark of *Berberis vulgaris* Linn (51). Thus, steaming and stir frying improved the inhibitory effect of nightshade and Chinese cabbage leaves against α-glucosidase; it is possible that the synergistic effect of different phenolic compounds and their varying concentrations played a vital role.

CONCLUSIONS

It is evident from the study that the dietary phenolic compounds, antinutrients, and associated bioactivities of Chinese cabbage and nightshade leaves are significantly altered by different household cooking techniques. The changes in phenolic compounds involved multiple transformation processes including deacylation, deglycosylation, and hydrolysis during household cooking of Chinese cabbage and nightshade leaves. Stir-fried Chinese cabbage and nightshade leaves showed potent antidiabetic activity and can be used as nutraceuticals to control diabetes by preventing the adsorption of glucose in the lumen of the intestine. In addition, further cell toxicity studies must be performed to recommend frequent consumption. Based on our investigation, among the tested cooking techniques, the recommendation is stir frying for traditional African cuisine to maintain optimal health benefits of consumers. This information is valuable for food manufacturers and chefs. However, further studies with more samples from different seasons are recommended to substantiate the results of the present study.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

MM performed the experiment, generated the data, and wrote the first draft of this manuscript. AT visualized and validated the data for phenolic compounds and also provided editorial support. JS was responsible for the antidiabetic activity and data. YS was the research collaborator involved in planning and conceptualizing the research. DS was a grant holder and conceptualized the research, supervised the MM, and provided editorial support. All authors contributed to the article and approved the submitted version.

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REVIEW
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Edible Crickets (Orthoptera) Around the World: Distribution, Nutritional Value, and Other Benefits—A Review

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Edible crickets are among the praised insects that are gaining recognition as human food and livestock feed with a potential of contributing to food security and reduction of malnutrition. Globally, the sustainable use of crickets as food or feed is undermined by lack of information on the number of the edible crickets, the country where they are consumed, and the developmental stages consumed. Furthermore, lack of data on their nutritional content and the potential risks to potential consumers limits their consumption or inclusion into other food sources. We reviewed published literature on edible cricket species, countries where they are consumed, and the stage at which they are consumed. We further reviewed information on their nutritional content, the safety of cricket consumption, and the sensory qualities of the edible crickets. We also looked at other benefits derived from the crickets, which include ethnomedicine, livestock feed, pest management strategies, contribution to economic development, and livelihood improvement, particularly in terms of use as food preservatives and use within music, sports, and cultural entomology. Lastly, we reviewed information on the farming of edible crickets. In this review, we report over 60 cricket species that are consumed in 49 countries globally. Nutritionally, crickets are reported to be rich in proteins, ranging from 55 to 73%, and lipids, which range from 4.30 to 33.44% of dry matter. The reported amount of polyunsaturated fatty acids (PUFA) is 58% of the total fatty acids. Edible crickets contain an appreciable amount of macro- and micro-mineral elements such as calcium, potassium, magnesium, phosphorus, sodium, iron, zinc, manganese, and copper. Also, the crickets are rich in the required amount of vitamins such as B group vitamins and vitamins A, C, D, E, and K. Overall, the cricket species examined in this review are safe to be consumed, and they display high proximate content that can replace plant and livestock products. The crickets play valuable roles in contributing to the economies of many countries and livelihoods, and they have medicinal and social benefits. This review is expected to promote greater recognition of crickets as a source of food, feed, and other benefits in the world and encourage up-scaling by farming them for sustainable utilization.

Keywords: edible crickets, food, food security, distribution, nutritional value, medicine, cultural entomology

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INTRODUCTION

The rapid day-to-day global population increase is predicted to reach 9.74 billion people by the year 2050 (1). This population growth requires an urgent intervention to increase food production to keep it in tandem with the expanding demand (2). As it is, food production may not meet demand because of the increasing scarcity of the necessary arable land. This situation is exacerbated by climate change, lack of water, and poverty (3). This therefore calls for a shift toward alternative and novel food production systems that are cheap, environmentally friendly, adaptable to climate change, and sustainable. One of the promising options is entomophagy, which is defined as the practice of eating insects (4–8). Entomophagy is a common practice in many parts of the world, and there are 2,100 species of insects that are consumed as food in over 110 countries (9). Out of this number, 500 insect species are consumed in Africa (10–13), 324 insect species are consumed in China (14–22), 255 insect species are consumed in India (23, 24), and over 164 species of insects are consumed in Thailand (25, 26). The commonly consumed insects include the orders Coleoptera, Lepidoptera, Hymenoptera, Orthoptera, and Hemiptera, respectively (27). Among the Orthopterans, crickets stand as the most-consumed insects across the globe (28–30) (**Figure 1**). Both the nymph and adult stages of crickets are consumed as food (27, 31). The most common species usually reported include *Brachytrupes membranaceus* (**Figure 2**), *Gryllus similis* (**Figure 3**), *Gryllus bimaculatus* (**Figure 4**), *Gryllotalpa orientalis* (**Figure 5**) and *Acheta domesticus* (**Figure 6**) (29, 32–36). However, this may not be representative of the exhaustive number of crickets that are edible globally. Moreover, a more recent discovery of a new edible cricket *Scapsipedus icipe* (**Figure 7**) (37, 38) in Africa makes us conclude there may be other edible crickets that have not yet been documented, and this forms a basis for our review (**Figure 2**).

Jongema (9) documented edible insects around the globe. The database contains several species and covers several orders of edible insects, including edible crickets. Such a database provides valuable information that could further be improved upon with additional data on the nutritional content of these edible insects, their sensory attributes, and the potential risks to potential consumers. Information on whether these insects can be farmed and the overall benefits to consumers are crucial.

Crickets have been consumed as food in Asia, Latin America, and Africa as far back as prehistoric times. In Biblical scriptures, cricket consumption is recommended to the Israelites by God to be fit for their consumption: "these you may eat any kind of locust, katydid, cricket or grasshopper" (Leviticus 11: 22). In China, crickets have been consumed as food for over 2,000 years (14, 39). In Africa, crickets have formed a daunted cuisine and a valuable complement of food enrichment for many years (32, 40–43). In recent years, consumption of edible crickets has become more appreciated in Europe, America, and Australia with the recognition of its nutritional benefits and food security (44–47).

The high nutritional content with the presence of protein, essential amino acids, lipids, the monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), mineral elements, carbohydrates, energy, and the ease of farming make crickets promising as a sustainable food source (29, 48). The rearing of crickets as mini livestock seems to be more ecofriendly because of their low emission of greenhouse gases, low water and feed intake, and the small land requirement for their production as compared to livestock (29, 30, 49). Crickets also show higher feed conversion efficiency when compared to mammalian livestock. For instance, van Huis et al. (50) reported the feed conversion efficiency of *A. domesticus* to be two-fold as compared to that of broiler chickens and pigs, four-fold compared to that of sheep, and more than 12-fold compared to that of cattle. Moreover, crickets may be produced on locally available food substrates such as agro byproducts and weeds, and they thus aid in cleaning the environment (28, 29, 51). In recent years, research on using crickets as human food and feed has increased with the recognition of cricket nutritional benefits and their potential of ensuring food security (27, 44, 45). Globally, the most frequently consumed cricket family is Gryllidae followed by the Gryllotalpa family (9, 52). House cricket *Acheta domesticus* Linnaeus forms the most-consumed cricket species worldwide.

While edible crickets are found to be rich sources of proteins and other nutrients (5, 53), there remain challenges and scientific knowledge gaps that need to be filled. One of the challenges for promoting edible crickets for human food is the lack of knowledge of the particular species that are edible and where they are found in the globe. The overall goal of this review is consequently to offer exact information concerning the number of crickets that are edible in the world, their nutrition content, sensory attributes, the possibility of being farmed, the safety of consumers and other benefits one can draw from them.

THE GLOBAL DISTRIBUTION OF EDIBLE CRICKETS

Crickets are non-wood wild products found in natural resources all around the globe apart from cooler regions at latitudes 55° and beyond; the greatest species abundance is found in the tropics where temperatures are warm and suitable for their faster development compared to cold regions (54). Crickets occur in the various habitats that include grassland, bushes, forests, trees, marshes, beaches, caves, underground, and in buildings (55). The edible crickets in this review belong to the infraorder Gryllidea that comprise the families Gryllotalpidae, Trigonidiidae, Gryllidae, and Phalangopsidae. Although more than 6,000 Gryllidea have been described (56), the actual number of crickets that are edible in this group is not known. In this review, we report 62 cricket species that are consumed as human food or used as livestock feed in different parts of the world (**Supplementary Table 1**). The consumption of the crickets depends on their distribution and the cultural appropriateness of cricket consumption to people in a particular country. The distribution of these edible crickets in five continents is as follows: Asia (41 species), Africa (26 species), America (five species), Europe (four species), and Australia (four species) (**Supplementary Table 1**). Africa tops the list with 25 countries that consume various crickets, followed by Asia (13 countries), America (five countries), Europe (four countries), and Australia

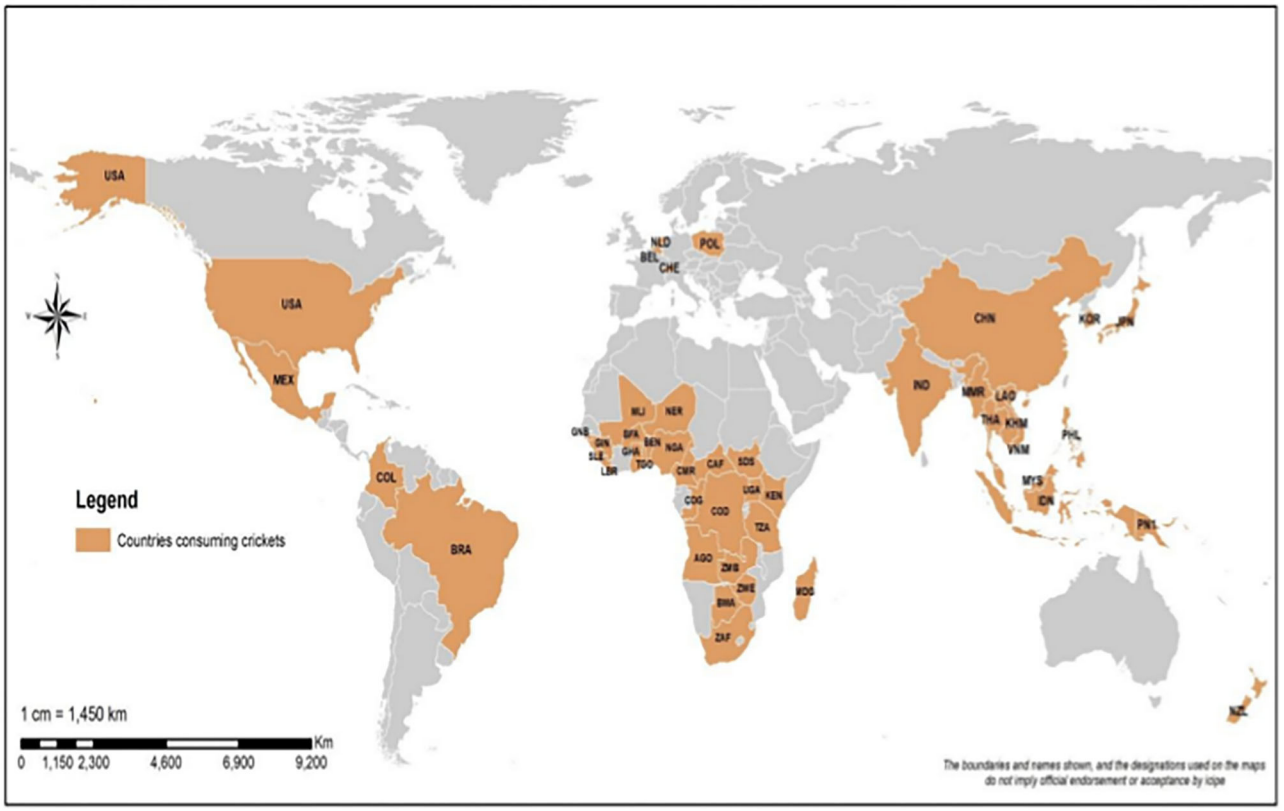

FIGURE 1 | A world map showing countries where crickets are consumed as food. USA (United State of America), Mex (Mexico), Col (Columbia), Bra (Brazil), KEN (Kenya), ZMB (Zambia), GNB (Guinea Bissau), SLE (Sierre Leone), GIN (Guinée), LBR (Liberia), BEN (Benin), TGO (Togo), NGA (Nigeria), COD (Democratic Republic of Congo), SDS (South Sudan), UGA (Uganda), ZWE (Zimbabwe), TZA (Tanzania), NER (Niger), AGO (Angola), COG (Congo/Congo Brazzaville), BWA (Botswana), ZAF (South Africa), MLI (Mali), GHA (Ghana), CAF (Central African Republic), BFA (Burkina Faso), CMR (Cameroon), MDG (Madagascar), PNG (Papua New Guinea), NZL (New Zealand), NLD (Netherlands), BEL (Belgium), CHE (Switzerland), POL (Poland), THA (Thailand), PHL (Philippines), VNM (Viet Nam), IND (India), IDN (Indonesia), LAO (Laos People's Democratic Republic), KOR (South Korea), KHM (Cambodia), MYS (Malaysia), JPN (Japan), PNI (Sabah), MMR (Myanmar), CHN (China).

(two countries) (**Supplementary Table 1**). Our review shows that crickets are more consumed in developing countries that are experiencing food insecurity than in developed countries. However, the consumption of crickets as food and feed is starting to take off in western countries despite the early stigma that has depicted insect consumption as a poor man's food in developing countries. This trend is changing rapidly as legislations have been put in place in some western countries, recognizing edible crickets as novel resources to mitigate food insecurity and malnutrition (44).

THE NUTRITIONAL COMPOSITION OF DIFFERENT SPECIES OF EDIBLE CRICKETS

Edible crickets are excellent sources of proteins, lipids, carbohydrates, mineral salts, and vitamins (**Table 1**). However, the nutritional composition of these crickets varies across the different species (29, 66, 78). The nutritional content can also vary within the same species of cricket influenced by the stage of development, habitat, climate, sex, and the food substrate fed on by the cricket (71, 79). The nutritional value may also be influenced by the method in which the crickets are processed (i.e.,

FIGURE 2 | *Brachytrupes membranaceus*. Source: Authors.

FIGURE 3 | *Gryllus similis* male. Source: Anankware et al. [(101), p. 36].

FIGURE 4 | *Gryllus bimaculatus*. Source: Orinda [(29), p. 15].

FIGURE 5 | *Gryllotalpa orientalis*. 15, February, 2014. Photograph by Michael Kesil.

FIGURE 6 | *Acheta domesticus*. 29 August, 2010. Courtesy of Aiwok.

drying, cooking, smoking, deep-frying, roasting, and toasting) before consumption (50, 80). Most of the edible crickets supply adequate energy and proteins to the consumer diet, at the same time meeting the amino acid requirements. Crickets also possess a high value of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) (59, 67, 68). Besides, these insects are rich in micro-nutrient elements such as calcium, potassium, magnesium, phosphorus, Sodium, Iron, zinc, manganese, and copper as well as vitamins like folic acid, pantothenic acid, riboflavin, and biotin, which are the most deficient nutrients in humans (5, 29, 66). This therefore implies that crickets are a good source of various nutrients required by humans for proper growth and development. The following subsections provide details of the specific nutritional composition of edible crickets.

Protein Composition of Different Species of Edible Crickets

Previous studies have reported protein valuea for 14 edible cricket species, ranging from 18.6 to 71.1% in dry weight (29, 57, 66, 81) (**Table 1**). The protein content of the different cricket species is within the range of the reported protein for other edible insects, including other Orthopterans (5). The variation in protein content observed in the crickets could be due to the influence of the species, diet, habitat, and the stage of development of the examined cricket. Compared to the protein content of the common meat sources listed in **Table 1**, most of the edible crickets have a higher protein content than that of the roasted goat, broiler chicken, and pork. The protein digestibility of some crickets was also investigated in a review and was 50.2%

for *Brachytrupes* sp. and 83.9% for *A. domesticus* (82, 83). These protein digestibility values for the crickets are slightly lower compared to values for eggs (95%), beef (98%), and cow milk (95%) (84). On the other hand, the protein digestibility values for the crickets are higher than those of many plant proteins, such as sorghum (46%), maize (73%), wheat (81%), and rice (66%) (85).

FIGURE 7 | *Scapsipedus icipe*. Source: Magara et al. [(28), p.2].

The amounts of nitrogenous substances in crickets may, however, be higher than their actual protein content since some nitrogen is also bound in the exoskeleton (29, 86).

Lipids Composition of Different Species of Edible Crickets

Edible crickets contain, on average, 4.30–33.44% of lipids in dry matter basis (**Table 1**). In some cricket species, the lipids content are higher in the nymphal stages than in adults (65, 87), while in other species they are lower in nymphs compared to the adult stage (29, 58). *Gryllus bimaculatus and A. domesticus* are among those cricket species with the highest lipid content.

Edible crickets have two different forms of lipids, namely, triglycerols and phospholipids. Triglycerols are ∼80% of lipids. They store energy that is required for activities that require high energy intensity in the cricket, such as longer flight and hopping. This energy can be available for humans after feeding on crickets. Phospholipids are the second most dominant group of lipids. Their value in cricket lipids is usually <20%, but their variation is dependent on the stage of development of the cricket and cricket species (88, 89). The role of phospholipids in the structure of cell membranes has been studied (88). Crickets are richer in

TABLE 1 | The nutritional composition of different species of edible crickets and selected animal tissues.

Cricket species	Stage	Protein (g/100 g dry weight)	Lipid (g/100 g dry weight)	Fiber (g/100 g dry weight)	Ash (g/100 g dry weight)	Carbohydrates (g/100 g dry weight)	Energy value (kcal/100 g dry matter)	References
Acheta domesticus	Nymph and Adult	62.41–71.09 NR	9.80–22.8 19.20–29.58	10.20 NR	5.10–9.10 NR	NR NR	455.19 NR	(5, 29, 57, 58)
Gryllus assimilis	Adult Nymph	56.00 ± 3.10 55.60 ± 1.10 65.52 ± 1.39 71.04 ± 0.01 56.4	21.80 ± 2.65 11.90 ± 0.50 7.00 ± 0.12 NR 34.00	8.28 8.00 7.00 NR NR	6.40 NR NR NR 4.08 ± 0.43	12.46 ± 0.16 8.60 ± 1.49 NR NR NR	397.00 ± 1.69 NR NR NR 537.50	(59–65)
Gryllus bimaculatus	Adult	57.49–70.10	14.93–33.44	9.53 ± 0.46	NR	NR	120.00	(29, 66)
Brachytrupes spp	Adult	65.35 ± 0.36	11.76 ± 0.63	13.29 ± 1.61	4.88 ± 0.23	2.50 ± 0.85	536.42 ± 0.47	(67)
Gryllus testaceus	Adult	58.30 ± 0.91	10.30 ± 0.31	10.40	2.96 ± 0.09	NR	18.10	(51, 68, 69)
Tarbinskiellus portentosus	Adult	58.00 ± 0.05	23.70 ± 0.05	1.16 ± 1.01	7.93 ± 0.04	NR	460.82	(70)
Gryllodes sigillatus	Nymph	56.00	NR	NR	NR	NR		(60, 61)
Teleogryllus emma	Adult	55.65 ± 0.28	25.14 ± 0.11	10.37 ± 0.19	10.37 ± 0.19	NR		(66)
Brachytrupes membranaceus	Adult Nymph	53.4 ± 0.19	15.80 ± 0.23	6.30 ± 0.14 5.0 ± 0.30	6.00 ± 0.12 3.23 ± 0.01	15.10 ± 0.22	454.7 ± 2.25	(17, 71, 72)
Brachytrupes portentosus	Adult	48.69 ± 0.25 NR	20.60 ± 0.60 NR	11.61 ± 0.20 0.5–8.3	5.40–20.50 9.36 ± 0.34	NR NR	90.06–134.0 NR	(73, 74)
Gryllotalpa africana	Adult	22.0 ± 0.86	10.80 ± 1.24	7.4 ± 0.24	12.60 ± 0.97	47.20 ± 0.32	362.3 ± 2.34	(71)
Acheta testacea	Adult	18.6	6.00	NR	NR	NR	133.00	(75, 76)
Acheta confirmata	Adult	NR	21.14	NR	NR	NR	NR	(61)
Animal tissue								
Goat, roasted		27	3	0	NR	0	143	(77)
Broiler		24	14	0	NR	0	165	(77)
Pork		27	6.00	0	NR	1.5	242	(77)

lipid content when compared to goat, chicken, and pork meats (**Table 1**).

Ash Composition of Different Species of Edible Crickets

Edible crickets possess a significant amount of ash, ranging from 2.96 to 20.50 mg/100 g dry weight (**Table 1**). The lowest ash content was reported by Wang et al. (68) for *G. testaceus*. The low ash content of *G. testaceus* implies low mineral content. On the other hand, the highest ash content was reported by McDonald et al. (74) for *B. portentosus*. The higher the ash content the higher the value of the mineral elements for human health. Crickets have a higher content of ash when compared to goat, broiler, and pork meat (**Table 1**).

Fiber Composition of Different Species of Edible Crickets

Crickets contain a significant quantity of fiber that ranges between 0.5 and 13.4% (**Table 1**). The insoluble chitin in the exoskeleton of the edible crickets forms a major part of fiber (50, 90). In commercially farmed crickets, the chitin ranges from 2.7 to 49.8 mg per kg of fresh weight and 11.6 to 137.2 mg per kg of dry weight (91). People from tropical countries can digest chitin by the help of a bioactive chitinase enzyme, which has developed in their gastric juices as a result of consuming edible crickets in their regular diet unlike people from outside the tropics (92). To enable people from outside the tropics to consume crickets without any complication, the chitin must be removed (91).

The chitin plays a significant role in protecting crickets against some parasitic attacks and allergic states (91, 93). Lee et al. (94) also reported that chitin is antivirally active against tumor formation. Chitin and its associated derivative chitosan have a functional role that could improve retinol and 6.8–8.2 μg of β-carotene per 100 g of dry weight. The chitin of the crickets has also been shown to influence the gut microflora, which plays an important role in the health of human beings and other animals, such as dogs (95, 96). Crickets are richer in fiber compared to the other meat sources (**Table 1**).

Carbohydrate Composition of Different Species of Edible Crickets

Carbohydrate is a major source of energy, though crickets do not require it for their growth since they can synthesize it from amino acids and lipids in their bodies (97). The carbohydrate content of edible crickets reported in the previous studies ranged from 2.50–47.20 g/100 g of dry weight (**Table 1**). The carbohydrate content in crickets is greatly influenced by the diet they consume (98). Crickets store their carbohydrates in the fat body, mainly in the form of glycogen, which can be later rapidly hydrolyzed into a readily useable form of energy: trehalose. The utilization of carbohydrates as a source of energy in insects is mostly relevant during metamorphosis due to metabolic interconversions (98) as well as during male stridulation in crickets (99, 100). By feeding on the crickets or their byproducts, we can obtain and make use of these carbohydrates. The highest amount of carbohydrates is reported in the mole cricket *Gryllotalpa africana* while the lowest carbohydrate content is found in *Brachtrupes* sp. Edible crickets are a good source of carbohydrates when compared to goat, broiler, or pork meat (**Table 1**).

Energy Content of Different Species of Edibles Crickets

Different studies have reported the caloric energy content of 12 cricket species, which ranges from 18 to 536 kilocalories (kcal) per 100 g of dry weight (**Table 1**). The energy value of the crickets is influenced by the species, lipid content, and their stage of development. *Gryllus assimilis* and *Brachytrupes* sp. have the highest energy content while *Gryllus testaceus* has the lowest. Furthermore, the review shows that the *Gryllus assimilis* nymphal stage has more energy than the adult stage (60, 61) (**Table 1**). On one hand, the calorific energy content for six cricket species (61, 62, 67, 70) is within the range of 293 and 762 kcal per 100 g of the dry weight of the other edible insect species (83). On the other hand, the calorific energy content of the five other crickets (**Table 1**) was low (65, 68) compared to the description obtained by Ramos-Elorduy et al. (83) for the other edible insect species. This data varied—most probably because of the variation of the cricket species and the method of analyzing their nutritional content. When the crickets are compared with the goat, broiler, or pork meat, most of the crickets have a higher energy content than these other meat sources (**Table 1**). This finding shows that the crickets are an energy-rich food source for humans.

Amino Acids Composition of Different Species of Edible Crickets

Edible crickets are rich in amino acids, which vary across the cricket species (**Table 2**). Glutamic acid is the most abundant amino acid in *T. portentosus, G. assimilis, G. testaceus, A. testacea, G. bimaculatus*, and *A. domesticus*, while leucine is the most abundant amino acid in *G. sigillatus*. The most abundant essential amino acids in these crickets are valine, ranging from 1.07 to 11.45 g/100 g of dry matter, leucine, ranging from 3.97 to 9.75 g/100 g of dry matter, and lysine, ranging from 2.42 to 7.90 g of dry matter (**Table 2**). The extensive variation of the amino acids amongst the edible crickets could be due to the variation in the diet they consume, stage of development, species, sex, habitat, and measuring methods (110, 111). Compared to amino acids from livestock meats in **Table 2**, crickets such as *T. portentosus*, *G. sigillatus*, and *G. assimilis* have more valine amino acid than pork and broiler chicken and similar content of all other amino acids (108). On the other hand, *T. portentosus*, *G. sigillatus, G.* testaceus, and *A. domesticus* have a higher content of phenylalanine than chicken (112) but similar content to that of pork (106).

Some crickets possess high values of lysine, tryptophan, and threonine, which are lacking in some of the cereal proteins that are major parts of the daily diets of many households. For instance, in Africa, where malnutrition is rampant, the consumption of crickets such as *A. domesticus*, *G. bimaculatus*, and *G. assimilis* can help mitigate deficiencies in the required amino acids (66, 102, 104). In Australia, the people of Papua

TABLE 2 | Amino acid composition (g/100 g protein) of different species of edible crickets and selected animal tissue (g/100g protein) and amino acid score.

Amino acid	Cricket species							Animal tissue		Amino acid Score	
	Tarbinskiellus portentosus	*Gryllodes sigillatus* nymph	*Gryllus assimilis*	*Gryllus testaceus*	*Acheta testacea*	*Gryllus bimaculatus*	*Acheta domesticus*	Pork loin muscle	Broiler	Infants	Children and adult
Essential Amino acids											
Valine	11.45 ± 0.98	5.20	4.62 ± 0.59	4.42 ± 0.00	3.44	3.20 ± 0.03	1.07	3.36–3.62	3.36–4.58	1.0–1.1	1.3–1.4
Isoleucine	3.03 ± 0.19	3.70	2.12 ± 0.73	3.09 ± 0.00	2.98	2.16 ± 0.04	4.45 ± 0.21	3.69–4.80	3.09–4.23	0.85–0.89	1.4–1.5
Leucine	ND	6.90	7.74 ± 0.64	5.521 ± 0.13	6.09	3.97 ± 0.05	9.75 ± 0.35	6.50–7.36	5.12–6.88	0.84–0.92	1.2–1.3
Lysine	6.10 ± 0.07	5.30	7.90 ± 0.64	4.79 ± 0.10	4.61	2.42 ± 0.01	5.40 ± 0.00	7.80–8.78	5.81–7.77	0.78–1.0	1.0–1.2
Threonine	3.81 ± 0.21	3.50	3.55 ± 0.63	2.75 ± 0.12	2.90	2.00 ± 0.04	3.60 ± 0.00	3.37–5.11	2.78–3.66	0.90–0.98	1.3–1.4
Phenylalanine	2.59 ± 0.13	3.10	0.72 ± 0.20	2.86 ± 0.06	NR	1.83 ± 0.01	3.00 ± 0.28	2.83–3.98	2.33–2.49	NR	NR
Methionine	2.42 ± 0.09	1.60	0.63 ± 0.20	1.93 ± 0.06	NR	0.27 ± 0.01	1.40 ± 0.14	2.36–2.86	1.40–2.08	NR	NR
Histidine	ND	2.20	1.32 ± 0.37	1.94 ± 0.01	1.54	2.50 ± 0.08	2.25 ± 0.07	3.46–3.63	2.47–4.44	1.1–1.2	1.2–1.3
Trypatophan	1.35 ± 0.23	0.90	0.95 ± 0.20	NR	2.44	NR	0.55 ± 0.07	0.82–1.33	1.05–1.11	0.40–0.57	0.8–1.1
Methionine and Cystine	NR	NR	NR	NR	3.09	NR	NR	3.35–4.00	2.22–3.36	0.70–0.84	0.86–1.0
Phenylalanine and Tyrosine	NR	NR	NR	NR	6.24	NR	NR	4.63–5.89	4.28–6.01	0.90–1.1	1.6–2.0
Non-essential amino acids											
Tyrosine	4.73 ± 0.13	4.20	5.44 ± 0.66	3.94 ± 0.02	NR	2.73 ± 0.02	1.00	1.80–1.91	1.95–3.52	NR	NR
Arginine	0.32 ± 0.31	5.70	3.02 ± 1.36	3.68 ± 0.12	4.51	3.60 ± 0.04	6.10 ± 0.00	4.60–6.62	3.76–7.08	NR	NR
Aspartic acid	6.99 ± 0.97	7NR	8.64 ± 0.63	3.72 ± 0.07	6.92	3.60 ± 0.04	7.75 ± 0.92	7.26–8.09	5.96–7.89	NR	NR
Glutamic acid	19.24 ± 1.32	NR	2.41 ± 0.14	9.07 ± 0.31	9.68	6.39 ± 0.07	10.45 ± 0.07	12.9–13.3	9.35–11.03	NR	NR
Serine	3.17 ± 0.69	NR	0.61 ± 0.20	3.72 ± 0.07	3.59	2.73 ± 0.01	1.02	3.11–3.30	2.58–3.06	NR	NR
Aspargine	3.27 ± 0.52	NR	NR	6.290 ± 0.2	NR	NR	NR	NR	NR	NR	NR
Glycine	NR	NR	0.36 ± 0.73	3.62 ± 0.11	4.72	3.32 ± 0.01	1.04	2.99–3,14	3.44–3.75	NR	NR
Alanine	0.14 ± 0.02	NR	4.02 ± 0.63	5.55 ± 0.09	7.80	5.64 ± 0.01	8.85 ± 0.07	3.93–4.21	3.79–4.91	NR	NR
Cysteine	NR	0.90	0.74 ± 0.14	1.01 ± 0.02	NR	5.10 ± 0.00	0.8 ± 0.00	0.99–1.14	0.82–1.28	NR	NR
Proline	1.44 ± 0.39	NR	1.26 ± 0.73	4.50 ± 0.08	4.52	1.99 ± 0.01	1.15	2.99–3.14	1.94–1.98	NR	NR
Taurine	1.25 ± 0.43	NR	NR	NR	NR	NR	141.00	NR	NR	NR	NR
Ornithine	3.10 ± 2.96	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
EAA	35.48	NR	NR	32.25	NR	21.08	NR	NR	NR	NR	NR
NEAA	66.16	NR	NR	36.42	NR	32.75	NR	NR	NR	NR	NR
EAA/NEAA	0.54	NR	NR	0.89	NR	0.64	NR	NR	NR	NR	NR
Total amino acids	101.64	43.20	56.49	68.67	NR	53.83	NR	NR	NR	NR	NR
References	(70)	(60, 61)	(102)	(68, 103)	(76)	(66)	(104, 105)	(106, 107)	(107, 108)	(107, 109)	(107, 109)

NR, not reported; nd, not detected; EAA, Essential Amino Acids; NEAA, Non Essential Amino Acids.

New Guinea consume tubers as food, which contain low values of the lysine and leucine. The resulting nutritional deficiency can, therefore, be solved through consuming nymphs and adults of the mole cricket *Gryllotalpa* species and *A. domesticus* and *G. bimaculatus* as food (66, 104, 113) with high quantities of lysine. On the other hand, tubers contain a high proportion of tryptophan and aromatic amino acids, which are available in small quantities in the nymphs and adult crickets. It is therefore advisable to consume a mixed diet of tubers and crickets to have a balance in the required amino acid (50, 114). When the protein of about 100 edible insect species was analyzed, the content of essential amino acids was found to be ranging between 46 and 96 g/100 g dry matter of the total amount of amino acids (87). This implies

that the crickets in this review are rich sources of amino acid for humans.

Fatty Acids Composition of Different Species of Edible Crickets

The edible crickets in this review possess higher contents of oleic, linoleic, linolenic, stearic (C18 fatty acids), and palmitic acid (C16 fatty acid) as compared to other fatty acids (66, 68, 73, 115), (**Table 3**). Linoleic acid, ranging from 4.15 to 41.39 g/100 g of dry matter, is the most abundant fatty acid in *T. portentosus, G. testaceus, G. assimilis, A. domesticus, G. bimaculatus, T. emma,* and *A. confirmata*. On the other hand, oleic (38.27 g/100 g of dry matter) and arachidonic acid (50.43 g/100 g of dry matter) are the most abundant fatty acids in *Brachytrupes* sp. and *B. portentosus*, respectively (**Table 3**). The second and third most abundant fatty acid in various crickets is as follows: in *T. portentosus*, we have pentadecanoic and myristic acid; in *G. testaceus* we have oleic and palmitic acid, in *G. assimilis* we have palmitic and oleic acid, in *A. domesticus* we have palmitic and oleic acid, in *G. bimaculatus* we have oleic and palmitic acid, in T. emma we have oleic and palmitic acid, in *A. confirmata* we have oleic and myristic acid, in *Brachytrupes* sp. we have linoleic and palmitic acid, and in *B. portentosus* we have stearic and Eicosatrienoic acid. The variation in fatty acid values in the crickets can be attributed to the difference in species, stage of development, diet, and environmental conditions in different localities (29, 58, 114).

This review demonstrates that the different cricket lipids are highly unsaturated, with either linoleic and oleic or linoleic and pentadecanoic acid or arachidonic and eicosatrienoic acid being the most abundant unsaturated fatty acids and palmitic, myristic, and stearic acids being the most abundant saturated fatty acids. Linoleic, oleic, myristic, Pentadecanoic, stearic, and palmitic acid are also predominant in other edible insects, including other Orthopterans (5, 107). These fatty acids are also the most abundant in livestock meat, including chicken and pork (106, 107, 112). *Tarbinskiellus portentosus, G. testaceus, G. assimilis, A. domesticus, A. confirmata, Brachytrupes* sp., and *B. portentosus* have higher content of polyunsaturated fatty acids (PUFA) compared to pork and broiler chicken meat (**Table 3**). *Gryllus bimaculatus* and *T. emma*, on the other hand, have lower content of polyunsaturated fatty acids (PUFA) compared to pork and broiler chicken meat. Most of the crickets in our review, except for *G. bimaculatus* and *T. emma*, have more essential fatty acids than the pork and broiler chicken. Crickets generally have more unsaturated fatty acids (UFA) compared to saturated fatty acids (SFA) (59, 61). A notable exception occurs in *T. portentosus*, which has more SFA compared to UFA (70). This difference could be a result of unreported oleic acid, which has been reported in other crickets. The majority of the non-communicable diseases, such as type 2 *Diabetes Mellitus*, obesity, cardiovascular disease (thrombosis, atherosclerosis, and high blood pressure), and some cancers affecting human beings, are due to the consuming of SFA (22). Consumption a high PUFA and MUFA in crickets is therefore capable of reducing the detrimental effects of high-SFA diets (22).

Mineral Composition of Different Species of Edible Crickets

Edible crickets are a good source of mineral elements such as phosphorus, sodium, potassium, calcium, magnesium, iron, and zinc. Based on dry matter, edible crickets have phosphorus ranging from 0.80 to 1169.60 mg/100 g; potassium ranging from 28.28 to 1079.90 mg/100 g, and sodium ranging from 0.99 to 452.99 mg/100 g as the most abundant mineral macro mineral elements. The differences in the macro-mineral elements could be due to the diet the crickets feed on in different parts of the globe. The differences could also be due to the age of the cricket, species, contaminants, especially heavy metal during the time of processing the crickets, and the measuring methods. In this review, the majority of the crickets have higher macro-mineral elements than those found in beef, pork, and broiler chicken, although some have similar content, and a few crickets exhibit a low content of macro-mineral elements (**Table 4**). Edible crickets lack a mineralized skeleton and hence have very little calcium, ranging from 4.98 to 240.17 mg/100 g dry weight; however, when we compare the calcium content of the edible crickets in this review, it is higher than that of beef, chicken, and pork (66, 118). This is because the bones that have calcium reserves do not form part of the muscle tissue that is subjected to analysis. Edible cricket species in this study possess calcium that ranges between 4.98 and 240.22 mg/100 g dry weight. *Gryllus bimaculatus* cricket contains the highest amount while *Brachytrupes sp*. contains the least amount of calcium (**Table 4**). This finding contradicts the finding by (122) that showed that crickets contain calcium of 33–341 mg/100 g dry matter. The sodium level in edible crickets is higher compared to the one recorded in other Orthopterans and other edible insects (5, 66, 107). The micro-mineral elements such as zinc, manganese, iron, copper, cobalt and aluminum in edible crickets are higher in content compared to micro-mineral elements in beef, chicken, and pork (123, 124) (**Table 4**). Most of the edible crickets have higher iron content than livestock meats, although we currently have scant information concerning the iron bioavailability of crickets (125, 126). A rare study of iron bioavailability found that consuming the *G. bimaculatus* cricket can enable you to meet a high percentage of your recommended daily iron intake (127). In this case, a child must consume 120.08 mg of the G. bimaculatus to acquire the 11.60 mg/100 g recommended daily iron intake. An adult human is supposed to consume 283.64 mg of cricket to meet the recommended daily intake of iron (27.40 mg/100 g).

Vitamins Composition of Different Species of Edible Crickets

Edible crickets are an excellent source of a wide range of water-soluble vitamins and lipophilic vitamins, including thiamine, riboflavin, niacin, and vitamin B12 (62, 105) (**Table 5**). House cricket, *A. domesticus,* contains 0.4 mg of thiamine per 100 g of dry weight, which is within the range of 0.1 to 4 mg per 100 g of dry matter thiamine content reported in other edible insects (72).

TABLE 3 | Fatty acid composition (g/100 g DM) of different species of edible crickets.

Fatty acid	Cricket species										Animal tissue	
	Tarbinskiellus portentosus	*Gryllus testaceus*	*Gryllus assimilis*	*Acheta domesticus*	*Gryllus bimaculatus*	*Teleogryllus emma*	*Brachytrupes* sp.	*Brachytrupes portentosus*	*Acheta testacea*	*Acheta confirmata*	Pork loin	Broiler
Lauric acid (C12:0)	1.16 ± 0.16	0.54 ± 0.04	0.12 ± 0.00	0.10 ± 0.00	0.04	0.02	NR	NR	NR	NR	0.21	NR
Tridecanoic acid (C13:0)	NR	NR	0.02 ± 0.01	NR	0	0	NR	NR	NR	NR	NR	NR
Myristic acid (C14:0)	6.74 ± 0.47	0.39 ± 0.02	1.28 ± 0.01	0.44 ± 0.00	0.05	0.18	0.96 ± 0.01	Nd	NR	26.10	1.3–1.4	0.45–0.69
Pentadecanoic acid (C15:0)	16.74 ± 1.33	NR	0.37 ± 0.01	0.11 ± 0.00	0.01	0.02	NR	Nd	NR	NR	4.1–4.7	NR
Palmitic acid (C16:0)	NR	10.18 ± 0.20	25.85 ± 0.06	22.65 ± 0.37	2.16	3.06	21.31 ± 0.49	1.61 ± 0.05	NR	5.50	23.2–27.3	23.8–24.9
Heptadecanoic acid (C17:0)	NR	NR	0.57 ± 0.01	0.12 ± 0.00	0.03	0.04	NR	0.13 ± 0.02	NR	NR	0.2–0.3	NR
Stearic acid (C18:0)	NR	2.63 ± 0.09	14.07 ± 0.03	8.54 ± 0.00	0.76	0.07	12.24 ± 0.24	35.79 ± 0.02	NR	1.20	12.2–16.1	5.7–5.9
Arachidic acid (C20:0)	NR	NR	0.56 ± 0.01		0.12	0.09	0.49 ± 0.01	Nd	NR	NR	NR	NR
Heneicosanoic acid (C21:0)	NR	NR	0.03 ± 0.00	0.24 ± 0.00	0.04	0.04	NR	NR	NR	NR	NR	NR
Behenic acid (C22:0)	2.34 ± 0.27	NR	0.57 ± 0.00		0.03	0.01	NR	NR	NR	NR	NR	NR
Tricosanoic acid (C23:0)	NR	NR	0.22 ± 0.01	0.02 ± 0.04	0	0.07	NR	NR	NR	NR	NR	NR
Lignoceric acid (C24:0)	NR	NR			0.01	0.01	NR	NR	NR	NR	NR	NR
Myristoleic acid (C14:1)	NR	NR	0.06 ± 0.01	0.44 ± 0.00	0	0.02	NR	NR	NR	NR	NR	NR
Palmitoleic acid (C16:1)	NR	3.11 ± 0.10	1.92 ± 0.01	0.34 ± 0.00	0.17	0.91	0.96 ± 0.00	0.71 ± 0.03	NR	2.40	2.1–2.8	7.1–7.4
Heptadecenoic acid (C17:1)	NR	NR	0.19 ± 0.00	0.24 ± 0.00	0.01	0.03	NR	NR	NR	NR	NR	NR
cis Oleic acid (C18:1n-9)	NR	29.58 ± 0.20	25.03 ± 0.11	20.18 ± 0.02	2.91	6.98	38.27 ± 0.67	3.4 ± 0.03	NR	31.10	32.8–43.7	40.3–40.9
Eicosenoic acid (C20:1)	NR	NR	0.24 ± 0.00	NR	0.03	0.04	NR	NR	NR	NR	NR	NR
Erucic acid (C22:1n-9)	NR	NR	0.05 ± 0.01	0.52 ± 0.01	0.01	0.04	NR	NR	NR	NR	NR	NR

(Continued)

TABLE 3 | Continued

Fatty acid	Cricket species										Animal tissue	
	Tarbinskiellus portentosus	*Gryllus testaceus*	*Gryllus assimilis*	*Acheta domesticus*	*Gryllus bimaculatus*	*Teleogryllus emma*	*Brachytrupes* sp.	*Brachytrupes portentosus*	*Acheta testacea*	*Acheta confirmata*	Pork loin	Broiler
cis Linoleic acid (C18:2n-6)	18.94 ± 0.02	37.82 ± 0.20	26.13 ± 0.18	41.39 ± 0.29	4.15	9.61	22.14 ± 0.59	NR	NR	32.20	10.7–14.2	16.2–17.5
Eicosadienoic acid (C20:2)	NR	NR	1.60 ± 0.01	0.00	0.04	0.02	NR	NR	NR	NR	NR	NR
Eicosatrienoic (C20:3n-3)	NR	NR	0.01 ± 0.00	NR	NR	NR	NR	NR	NR	NR	NR	NR
Eicosatetraenoic (C20:4n-3)	NR	NR	0.21 ± 0.02	NR	NR	NR	NR	NR	NR	NR	NR	NR
Docosadienoic acid (C22:2n-6)	NR	NR	0.03 ± 0.02	0.11 ± 0.01	0.02	0.01	NR	NR	NR	NR	NR	NR
Linolenic acid (C18:3n-6)	NR	10.12 ± 0.10	NR	1.11 ± 0.00	0.01	0	2.55 ± 0.18	NR	NR	NR	NR	NR
Alpha-linolenic acid (C18:3n-3)	NR	NR	NR	NR	0.08	0.22	NR	Nd	NR	1.70	1.0–1.1	0.77–0.85
Eicosatrienoic acid (C20:3n-6)	NR	NR	NR	0.01 ± 0.02	0.02	0.01	NR	7.94 ± 0.04	NR	NR	NR	NR
Arachidonic acid (C20:4n-6)	0.55 ± 0.28	NR	NR	0.01 ± 0.02	0.01	0.27	NR	50.43 ± 0.55	NR	NR	0.1–0.2	0.76-0.97
Eicosapentaenoic (C20:5n-3)	NR	NR	NR	0.01 ± 0.02	0	0.01	NR	Nd	NR	NR	0.2-0.4	0.05–0.07
SFA	50.58	13.74	43.72	32.22	3.25	3.61	34.99 ± 0.24	37.54 ± 0.08	36.5	32.80	40.7	30.9–32.2
MUFA	28.98	32.69	27.49	21.72	3.13	8.02	39.23 ± 0.66	4.11 ± 0.06	30.1	33.50	47.2	48.0–49.1
PUFA	20.32	47.94	28.80	42.64	4.33	10.15	24.68 ± 0.77	58.37 ± 0.59	31.1	33.90	11.7	19.1–20.4
TUFA	49.30	80.63	56.29	64.36	7.46	18.17	63.91	62.48	61.20	67.40	58.90	67.10–69.50
PUFA/SFA ratio	0.40	3.49	0.66	1.32	1.33	2.81	0.71	1.55	0.86	1.03	0.6	0.61–0.66
n-3	NR	NR	1.99	0.01	0.08	0.23	NR	NR	NR	NR	1.2–1.5	0.82–0.93
n-6	19.49	47.94	26.81	42.63	4.25	9.92	24.69	58.37	NR	NR	10.8–14.4	17.8–19.1
EFA	18.94 ± 0.02	37.82 ± 0.20	26.13 ± 0.18	41.39 ± 0.29	4.23	9.83	22.14 ± 0.59	NR	NR	33.90	11.70–15.30	16.20–18.35
References	(70)	(68)	(59)	(115)	(66)	(66)	(67)	(73)	(76)	(61)	(106)	(112)

NR, not reported; nd, not detected; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acids; EFA, essential fatty acids.

TABLE 4 | Mineral-Nutrient elements composition (mg/100 g DM) of different species of edible crickets and selected animal tissues (mg/100 g DM).

Cricket species	Mineral element											
	Phosphorus	**Potassium**	**Sodium**	**Calcium**	**Magnesium**	**Zinc**	**Manganese**	**Iron**	**Copper**	**Cobolt**	**Aluminum**	**References**
Gryllus bimaculatus	1169.60	1079.90	452.99	240.17	143.65	22.43	10.36	9.66	4.55	NR	NR	(66)
Teleogryllus ema	1085.4	895.50	278.23	193.54	152.48	18.47	5.86	10.75	2.19	NR	NR	(66)
Acheta domesticus	832.9	126.62	435.06	171.07	94.71	20.22	3.35	8.75	1.43	NR	NR	(105)
Tarbinskiellus portentosus	506.1 ± 2.33	1240.89 ± 1.05	370.81 ± 0.82	26.00 ± 0.02	10.50 ± 0.06	7.0 ± 0.00	NR	122.5 ± 0.00	4.50 ± 0.01	NR	NR	(70)
Brachytrupes membranaceus	126.9	NR	NR	9.21	0.13	NR	NR	0.68	NR	NR	NR	(116)
Brachytrupes spp	38.50 ± 1.91	877.26 ± 41.39	150.22 ± 28.23	4.98 ± 0.58	NR	23.02 ± 0.06	NR	33.60 ± 3.26	NR	NR	NR	(66, 67)
Gymnogryllus lucens	NR	28.28 ± 17.88	15.63 ± 5.30	NR	153.88 ± 27.47	25.66± 28.70	NR	51.90 ± 44.5	6.91 ± 0.70	0.21 ± 0.70	NR	(117)
Gryllus assimilis	0.80	NR	0.99	45.30 ± 4.45	2.19 ± 3.46	5.22 ± 0.27	1.42 ± 0.09	2.78 ± 0.28	0.68 ± 0.01	NR	4.21 ± 2.51	(63, 118)
Animal tissue												
Beef	NR	NR	NR	5.43	49.33	5.53	0.04	3.31	0.45	NR	NR	(76)
Broiler chicken	407.00	248.00	46.00	5.80	29.00	0.70–1.30	NR	0.40–0.70	0.04–0.10	NR	NR	(107)
Pork	223.00–320.00	370.00–400.00	45.00–87.00	4.30–6.00	21.00–26.10	2.40–6.90	NR	1.40–21.00	0.10–2.70	NR	NR	(107)
Recommended nutrient intake (mg/day)												
Children	100.00			300.00	26.00	2.80	0.003	11.60				(77, 119–121)
Adults	700.00	2000.00	500.00	1300.00	260.00	7.20	2.30	27.40	1.50			(77, 119–121)

NR, Not Reported.

Riboflavin in edible crickets ranges from 0.23 to 3.41 mg/100 g. *Gryllus assimilis* cricket is richer in vitamin B12 with a content of 5 mg per 100 g compared to *A. dometicus* (62). Retinol (vitamin A) and β-carotene were detected in *A. domesticus,* while only Retinol was detected in *G. assimilis. A. domesticus* possess a retinol content of up to 67 g/100 g dry weight and a β-carotene of up to 0.02 g/100 g dry weight (105). *Gryllus assimilis*, on the other hand, has a retinol content of 2.90 mg/100 g of dry matter (62). Vitamin E is found in both *A. domesticus and G. assimilis* (**Table 5**). This review shows that the edible crickets are a good source of riboflavin, pantothenic acid, biotin, vitamin A, vitamin C, niacin, and thiamine. This is in line with the findings of Rumpold and Schlüter (5), who reported that insects consumed as food and feed are usually rich in riboflavin, pantothenic acid, and biotin. On the other hand, however, they are poor sources of vitamin A, vitamin C, niacin, and in most cases thiamine. The number of vitamins in edible insects collected from the wild is seasonal and influenced by the meal the insect consumes. This problem of seasonal availability of the vitamin can be overcome through the rearing of the crickets on farms using diets rich in vitamins. The review shows that edible crickets can meet the recommended daily intake of most of the vitamins. This can be achieved by either increasing the number of mg/ 100 g for those vitamins that are in low supply in crickets or by reducing the mg/100 g consumed where the vitamins contained in the edible cricket is high.

SENSORY QUALITIES OF EDIBLE CRICKETS

Crickets captured from the wild or the one raised in the farms must be processed before they are consumed. During processing, the crickets are starved for 1–3 days before they are killed humanely by scalding them using hot water (129). After killing them, they are then cooked, smoked, fried, toasted, dried, or processed into cricket products, such as crackers, to improve their taste and palatability (50, 129, 130). The method of preparation of crickets and their products play an important role in influencing a person's willingness to sample and consume them. Before consumption, the consumer will employ their sensory organs, such as smell, touch, sight, and sound, to choose whether or they will eat it.

Sensory attributes as they relate to the processed crickets and their products therefore influence cricket consumption. Processed crickets and their products have diverse taste, color, and flavor. The flavor of the cricket depends on their surface odor (130). The flavor of crickets also depends on the diet they eat. Diet choices for crickets can also be adapted depending on how we want to the crickets to tase. During cooking, crickets tend to take the flavor of the additives.

The exoskeleton of crickets has a high impact on the texture of the cricket, i.e., its crunchiness. Crunchy crickets or their products tend to produce an accompanying sound like that of crackers or pretzels while being eaten (130). Nymphs of about 6–7 weeks are the stages of crickets when they are most consumed, as they contain a low quantity of chitin. The reductions of the chitin make these crickets less crispy during their consumption and increase their digestibility. A pleasant color does not always indicate the deliciousness of the cricket but only influence the consumer to accept the cricket. During cooking, the cricket color may change from the initial shades of gray or brown to red, or its color may be retained, especially if the cricket is black (130). Crickets containing a considerable amount of oxidized fat, or improperly dried crickets, may be black, which is a color that may discourage consumers. Properly dried crickets are golden or brown and can be easily crushed by the fingers (129).

OTHER BENEFITS DERIVED FROM CRICKETS

Crickets possess benefits other than being consumed as food by human beings. These benefits include the following.

Crickets as a Source of Medicine

Humans have used crickets and their products for therapeutic functions since ancient times (47, 131–134). Recent studies have shown that crickets can be utilized as a traditional remedy for fever and high blood pressure (135). The cricket legs are ground into a powder and mixed with water and then taken as a drink to relieve dropsy (oedema) (134, 136–138). In Nigeria, the intestinal content of mole crickets (*Gryllotalpa africana* Beauvois) is applied to patients suffering from athlete's foot for treatment (134, 139). In some places, *Brachytrupes* sp. crickets are also consumed as food supplements for healthy mental development and pre- and pro-natal care purposes (134, 140). In China, edible Chinese mole crickets are sun-dried to make a herb called China Gryllotalpa, which is then used as a decoction or is made into a tincture to enhance bodily functions (141).

Research has been conducted on the utilization of crickets as a new supplementary diet to fight deficiency diseases, such as Marasmus and Kwashiorkor, in schoolchildren (36, 142). The findings are interesting in that the incorporation of cricket powder in diets of the schools optimized the growth and learning of the children (142). Moreover, the presence of essential amino acids, including valine, lysine, threonine, and methionine, in edible crickets help in breaking down of saturated fatty acids, which are implicated in lifestyle conditions like obesity, hypertension, type 2 diabetes, and cancer in human beings (143). Previous studies have also shown that the cricket powder is rich in most of the mineral-nutrient elements deficient in human beings, such as calcium, potassium, magnesium, iron, and copper. One can thus obtain these minerals that are important in fighting various diseases, such as osteoporosis, malfunction of the nervous system, and anemia, by consuming the edible crickets. Direct consumption of crickets has also been shown to decrease ethanol levels in the blood by the help of enzymes such as alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH), which stimulates the liver mitochondria to break down alcohol that may damage the liver (144). Glycosaminoglycan (GAG), which mediates anti-atherosclerotic and antilipemic effects, have been confirmed in crickets, and one can attain this compound by consuming the

TABLE 5 | Vitamin composition of different species of edible crickets.

Vitamin	Cricket species		Recommended daily intake		
	Acheta domesticus mg/100 g	*Gryllus assimilis* mg/100 g	Children mg/day	Adult female mg/day	Adult male mg/day
Retinol (Vitamin A)	<67.00	2.90 ± 0.05	6	15	15
β carotene	<0.02	NR	NR	NR	NR
Thiamine (Vitamin B1)	0.04	NR	0.4	1.1	1.2
Riboflavin (Vitamin B2)	3.41	0.23 ± 0.08	0.3	1.1	1.3
Niacin (Vitamin B3)	3.84	NR	2	14	16
Pantothenic acid (Vitamin B5)	2.30	NR	1.7	5	5
Pyridoxine (Vitamin B6)	0.23	NR	0.0001	0.0013	0.0013
Biotin (Vitamin B7)	0.02	NR	0.005	0.03	0.03
Folic acid (Vitamin B9)	0.15	NR	0.65	0.40	0.40
Vitamin B_{12}	0.01	10.00 ± 0.00	0.004	0.0024	0.0024
Vitamin C	3.00	1.01 ± 0.63	15	65	75
Vitamin D	<17.15	NR	5	5	5
Vitamin E	1.32	30.00 ± 0.01	6	15	15
Vitamin K	NR	40.00 ± 0.00	0.03	0.065	0.065
Choline	151.90	NR	125	425	550
References	(105)	(62)	(128)	(121, 128)	(121, 128)

NR, Not Reported.

crickets (145). Besides, cricket extracts have been studied as a therapeutic agent for inflammatory diseases, such as chronic arthritis and gut inflammation (95, 135, 144).

Crickets as Livestock Diets

The high nutritional content of edible crickets, the small space requirement for their production, and the effect they have on the environment make them valuable as animal feed. Moreover, crickets have an added advantage since they have already been in use as an ingredient in animal feed (146). Crickets can be given to the animals as feed either whole after killing them or can be crushed into a powder and then used to formulate livestock diets. Livestock feeds formulated by incorporating crickets are cheaper compared to the cost of commercial feeds, which currently account for 70% of the cost of livestock production (147). The most promising crickets for production of livestock feed are *A. domesticus*, *G. veletis*, *G. bimaculatus*, *G. sigillatus*, *T. mitratus, G. mitratus*, *T. emma, B. portentosus*, and *G. assimilis* (50, 146, 148–152). Recent studies have shown that cricket meal can partially replace broiler mash, especially the protein part. Cricket meal can replace 5–15% of fish meal or soy meal without any negative effects on broiler feed intake, weight gain, or feed conversion efficiency (153). Also, replacing the protein composition with *Gryllus testaceus* cricket meal in a broiler chicken diet did increase the essential amino acids in the chicken (150). In addition to the nutritional value, the insect-based feed could have a further advantage in improving the taste of final meat products (154).

Another report has demonstrated that African catfish (*Clarias gariepinus*) diet containing 100, 75, 50, 25, and 0% fish meal can be successfully changed to contain 0, 25, 50, 75, and 100% *G. bimaculatus* crickets, respectively (151). Furthermore, growth performance and body composition could be improved when African catfish were provided with a diet containing 50–100% crickets (151, 152). As compared with commercial fish-based meals, a cricket-based meal significantly increased the body weight, resistance to diseases, protein efficiency ratio, and specific growth rate of the catfish (152). The study also found the fish provided with 100% cricket meal contained a significantly lower feed conversion ratio compared to the lower inclusion level. The findings further revealed the whole-body crude protein value was higher in catfish fed with a meal containing 50–100% cricket meal.

In summary, existing studies have clearly demonstrated that crickets are a promising protein source for animal meals and can meet the increasing global requirement (5). Before the mass-production of such cricket-based meals, however, governments and companies should ensure health and safety concerns relating to edible crickets, such as the presence of anti-nutrient properties and legislation of use of these crickets, are addressed (57).

Cricket Harvesting as a Strategy of Pest Control

In recent years, there has been a prevalence of crickets in warm areas around the world, which has caused a remarkable loss of field crops and other plants. Moreover, when the

crickets get into domestic houses they become a significant nuisance by destroying household properties. The capturing and consumption of edible crickets therefore not only ensures nutritional availability to people and livestock but also protects the plants and household properties from unexpected insects infestation (http://www.entomoljournal.com/archives/2016/vol4issue6/PartA/4-5-101-553.pdf; https://www.bbc.com/news/av/world-middle-east-52991180/pakistan-locust-plague-locals-collect-insects-for-chicken-feed). Gathering crickets from farms and consuming them as food can also help in reducing pesticide use in controlling these cricket pests. This, in return, can protect the environment from pollution, minimizing the killing of other useful insects and poisoning of consumers (78). An excellent example of a place where the gathering of crickets for human consumption or feeding chickens is Mexico, where this has contributed to a reduction in the cricket population of farms, a reduction in the amount of pesticides used in crops, and a decreased financial burden on farmers (155, 156).

Cricket Contribution to Economic Development

Collection and rearing of crickets provide employment and cash income to people both at the household level and at the level of industrial production. For example, in Africa, Asia, and Latin America, there is a demand for edible crickets, and this makes it easier to bring them into the market for sale (157–159). The crickets are sold at local markets while alive or after being killed. Live crickets are packed into various weights for various buyers and then brought to the streets of towns and the markets for sale. Alternatively, the crickets are killed and are either fried or are processed into cricket products as human food or chicken and fish feed and then brought to the market for sale (32–34, 159, 160). The cricket businesses that occur in many countries are usually influenced by the demand of the local people or immigrant communities because of the development of a specific market that sells cricket products. Cricket collection and farming have also led to further opening up for international trade, such as border trade in edible crickets, which is commonly practiced in the Southeast part of Asia and Central Africa (159).

Crickets and Livelihood Improvement

Edible crickets trapped from the wild or domesticated in farms play a role in livelihood improvement by providing an improved diet in terms of nutritional content and diversity and as a supplement to the dietary needs of low-income families. These crickets also provide food at times of famine for people living in developing countries and Western countries. The resource-poor people in the society, including women and landless people living in urban centers and rural areas, can actively involve themselves in the collection, rearing, processing, and selling of the surplus of crickets and their products in the streets of towns. These ventures can significantly change their quality of life through the generation of income, which, in return, they can use to purchase the basic needs they are lacking. Crickets could be directly and easily gathered from the wild or reared on the farm with a little technology and by involving much capital in procuring basic rearing and harvesting equipment (161). Rearing crickets requires a small portion of land and minimal market introduction efforts, as crickets already form part of some local food cultures (32, 162). Malnutrition is a widespread issue affecting many disadvantaged members of society, especially during times of social conflict and natural disasters. Since crickets are nutritionally rich and easily accessible, having simple rearing techniques and rapid growth rates, they can offer a cheap and efficient chance to mitigate food insecurity. The edible crickets and their products can be provided for hunger-stricken people as a relief food and thus improve their livelihoods. Furthermore, cricket flour and powder can be used to fortify the traditional food in different communities before feeding vulnerable persons in society to improve their livelihood (36, 142).

Crickets as a Food Preservative

Chitosan from the chitin of the edible cricket species exoskeleton has been identified to be a possible intelligent and biodegradable bio-based polymeric material for packaging of various foods. Such natural packaging using the "exoskeleton" of crickets can change the internal conditions of the food product, thereby protecting the food product from spoiling and micro-organisms. This is possible because it has been proved that chitosan from crickets stores antioxidants and has antimicrobial activity against yeasts, molds, and bacteria (163–165). However, the chitosan polymeric material can be compromised when it gets into contact with moisture and may therefore not be utilized in true natural form but may be synthesized further into chitosan film for a positive impact to be achieved (165).

Singing Crickets as a Source of Music

Rearing of crickets as pets has existed since prehistoric time in Asia some Western countries. For instance, crickets have been mentioned in an adage dating back to 600 BCE found in Ancient Greece: a young girl and her dying pet cricket. Since then, some poetry work has been scripted on the songs of crickets (166). In the People's Republic of China, singing crickets have been household pets for more than 2,000 years. During the Tang Dynasty (618–906 CE), Chinese people reared crickets in small cages for their songs. Whenever the autumn arrived, the ladies of the palace trapped crickets and placed them in small golden cages, which they placed near their pillows to listen to their songs when night fell. This tradition was also embraced by ordinary people (167). Some South American crickets have been implicated to have beautiful songs that made Amerindians people rear them (168, 169). In the Luo community in Kenya, there is a traditional belief that eating crickets improves the vocal prowess of musicians. As such, during music festivals, children who are members of the school choir would hunt and eat crickets to improve their vocal ability (32).

Cricket Fighting as a Sport

Edible crickets can be used as sporting activity for recreation purposes. In China, Cricket fighting has existed from the time of the Song Dynasty (960–1278 CE). This practice of allowing crickets to fight was later declared illegal during the Qing Dynasty (1644–1911 CE). Currently, however, cricket fighting is legal, and

it has become a common sporting activity in many Chinese cities, such as Beijing, Guangzhou, Huwan, Hong Kong, Shanghai, and Tianjin, where cricket fighting clubs and societies have been formed (170). Cricket fighting has spread to other parts of the world, such as New York and Philadelphia (171) as a result of the migration of Chinese people to these places. During the sport, people gather together in social halls with their crickets to get entertained as the crickets fight. The best example of the fighting cricket is the Chinese fighting cricket *T. mitratus*.

Promotion of Cultural Entomology

Crickets have contributed a lot to the shaping of literature, art, and doctrine in societies around the world—referred to as cultural entomology (172). Contributions from this discipline have assisted in highlighting the different roles the crickets have undertaken in literature, especially in children's books, movies, and visual art, as collected artifacts, decoration, and especially as inspiration for innovative expression.

The crickets have also played different roles in folklore and superstition in different parts of the globe. In this perspective, some communities hold a lot of esteem for crickets since they believe that once you hear the song of the cricket it spells good fortune, although others say it is a bad omen when a cricket makes noise around you. In China, for instance, the crickets have been implicated to foretell the coming of rain, death, or the returning of a lover who has been away (173). Moreover, the people of China keep crickets in a small cage to have good luck (174). In Barbados, when a singing cricket enters the house, it spells the fortune of money into the family, and no person is therefore allowed to kill or chase away the visiting cricket. On the other hand, when a quiet or less noisy cricket gets into the house, it forecasts sickness or a pending death in the family (175). Omens concerning crickets are also found in Brazilian folklore where they bear different meanings to various events. For instance, when a black cricket gets into a house of someone, it indicates that a person in that house will be sick while a gray cricket is a sign of money coming to the household (176). A cricket also foretells the pending death of a member of the family, and, therefore, whenever a cricket sings in the house, it is captured and killed immediately to avert the death (177). In the other parts of Brazil, the cricket spells the pregnancy in a member of the household when it sings non-stop. If it sings and breaks, it then spells a windfall of money to that home (178). A singing cricket also directs people to the source of drinking water during droughts. In the case of a cricket aboard a sailing ship, singing foretells the proximity of land to the captain and sailors (176).

A mole cricket (genera *Scapteriscus* and *Neocurtilla*, Gryllotalpidae) that enters into the house of a Brazilian brings both good luck and rainfall (178). When it digs tunnels in the soil, loosening it, people usually interpret this behavior as a sign that rainfall is imminent (178). It is said that the appearance of a mole cricket on the surface of the ground is an indicator that the soils are waterlogged after a heavy downpour or they are ready to disperse to occupy new areas (179). In Zambia, there is a belief that whoever comes across an African mole cricket will have luck (180). Zambians therefore keep mole crickets to retain luck.

RISKS OF CRICKET CONSUMPTION

Consumption of crickets is generally safe. However, it could expose one to various risks that must be taken into consideration. For instance, (44) has published various risk profiles related to the consumption of crickets. Gathering a large number of crickets from the wild for consumption or sale could cause a serious imbalance to the ecosystem (181). To overcome such an effect on biodiversity, it is advisable to rear crickets at a farm level under controlled and defined conditions for consumption for food and marketing. This means that farm rearing of crickets must be done with appropriate and safe substrates to guarantee the health and safety of consumers. The wrong choice of cricket diet may pose a health hazard to consumers. For instance, the result of analyses carried out from 2003 to 2010 indicated possible risks of consuming heavy metals due to the nature of the bran used as a substrate (182). Additionally, consuming crickets reared in inappropriate organic waste is discouraged. Furthermore, some crickets can also contain naturally poisonous compounds such as cyanogenic glycosides (183). Cyanogenic glycosides are natural plant toxins that are present in several plants, most of which are consumed by crickets.

Consumption of crickets containing cyanogenic glycosides may cause acute poisoning, leading to growth retardation and neurological symptoms due to a damaged central nervous system (CNS) (184). The other likely risks of consuming edible crickets include poor handling and delish treatment. According to (185), consuming crickets with their feet can cause intestinal discomfort based on the amount ingested. Eating crickets can also cause allergies to those persons sensitive to insect chitins. Some individuals have such a small amount of chitosan enzyme that the eating of crickets can cause an allergic reaction to them (44). Some crickets have a tough exoskeleton formed of chitin, which is difficult to digest for humans.

The risk of contracting zoonotic diseases from some cricket species must also be taken into consideration. The intestinal flora of crickets could be a predisposing agent for the growth of unwanted microorganisms. Klunder et al. (86) evaluated the microbial content of fresh, processed, and stored edible crickets *A. domesticus* and *Brachytrupes*. The results showed that various types of Enterobacteriaceae and sporulating bacteria can be identified and subsequently seperated from raw crickets entering them most likely during contact with the soil (186). Fasting, heat treatment, and appropriate storage conditions are paramount to dangerous disease-causing pathogens in crickets and other edible insects (155, 187).

CRICKET FARMING AROUND THE WORLD

The high potential of crickets as food and feed has led to the development of rearing systems and establishment of subsistence and commercial cricket farms in several countries in Asia, Europe, America, Australia, and, recently, Africa. Asia is the

leading continent in cricket farming in countries such as Thailand, Indonesia, Cambodia, Myanmar, and Lao Peoples' Democratic Republic (PDR) (35, 188, 189). Examples of edible cricket species that have been farmed successfully in Asia for food and feed include the house cricket *A. domesticus*, *G. bimaculatus*, *T. occipitalis*, *T. mitratus*, *G. testaceus,* Jerman cricket (*Gryllus* sp.), the short-tail cricket *B. portentosus,* and *T. portentosus* (35, 54, 146, 189–191). *Acheta domesticus* is the preferred cricket species for large-scale production for most parts of the globe (192).

Cricket farming in Thailand, which is said to be the hot spot of cricket consumption, has an established cricket industry with over 20,000 farms producing cricket products such as adult crickets, eggs, and biofertilizer from cricket waste for commercial purposes (35, 146, 193, 194). Farmers in Thailand rear two cricket species: *A. domesticus*, *G. bimaculatus.* They, however, prefer rearing *G. bimaculatus,* which form a greater portion of the Thai production since *G. bimaculatus* has a short lifecycle and is stronger and hardy, though less popular than *A, domestica* (146). Thai farmers initially used to rear their crickets in small concrete cylinders, plastic boxes, wood, and other types of containers, but of late they farm crickets in large pens with concrete walls (193). These pens are easy to clean, cheap to build, and durable. Several egg trays supply the living section of the pen as hiding places for the crickets. Predators that may kill the crickets are kept off the rearing pens and the farm by use of mosquito nets. The cricket eggs used to start a colon are either purchased or tapped from crickets in the wild by supplying bowls containing clean egg-laying substrates, such as rice bran, wheat bran, ash, or fine sand soil, for the females to lay the eggs. Eggs take 7 to 10 days to hatch. Each harvesting cycle is between 28 and 35 days. Cricket farming in Thailand follows a family-owned business model which produces about 3,000–7,000 tons of crickets per year (49). A medium-scale farm can yield 500–750 kg of mature crickets per 45-days harvesting cycle, which generates a revenue of about 2,000–2,500 USD (193).

In Indonesia, cricket farming is extensively practiced in several cities of the Java islands for livestock feed, home consumption, and business purposes. The crickets are farmed in Java cities, including Cirebon, Bekasi Demak, Kudus, Purwodadi, and Yogyakarta and in East Java in Tulungagung, Kediri, and Porong (54). Cricket production in Cirebon is 200 kg of young crickets and 8 kg eggs per day. However, some small-scale cricket farmers have been reported in some villages, where farmers rear crickets to feed their poultry or as an ingredient for medicines (149). The crickets farmed in Indonesia are *G. bimaculatus*, *G. testaceus, G. mitratus,* and the German cricket *Gryllus* sp.

Cricket farming has just been initiated in South Korea with the support of the Korean government, which has already established legal measures to support the cricket industry. Currently, several research projects are being carried out in South Korea under the guidance of the Rural Development Administration. This has led to an increased number of *G. bimaculatus* farms in South Korea (195). This has further led to a Korean company using an automated farming system for large-scale production of the crickets (196).

In Cambodia, cricket farming is of small-scale production aimed for home consumption (193). This is as a result of the farmers in Cambodia being resource poor. They therefore rear crickets in small farms using small plastic containers, wood, and other types of containers. The Cambodian farmers use plastic bags instead of egg trays as a living area for the crickets. Whereas, these bags are said to be cheaper, they pose a risk to the crickets which will consume the particles of the bags. Farmers use ash in the water containers to avoid small crickets drowning. It is unclear if this is a good method. Sometimes farmers spraying small particles of water on the floor of the rearing pens for the crickets to drink but this may be safe for the crickets since the water may generate too much humidity in the rearing pen. Recently, some farmers are enrolled in training programs to get equipped with better information on how to rear healthy crickets. There are breeders of crickets in Myanmar, but no farmers have been reported. Likewise, there are a few cricket farmers in Malaysia (197). The Philippines collect their edible crickets from the wild (193, 198).

Cricket farming in western countries is a new trend that is about 10 years old. The house cricket (*A. domesticus*), brown field cricket (*G. assimilis*), and two-spotted field cricket (*G. bimaculatus*) are the common crickets bred in Europe and for industrial processing (44, 148). The western follows a farming model that aims at optimizing breeding activities by reducing human labor during the production of the crickets. Their model aims at raising crickets on a large-scale basis for industrial processing unlike for the Asian model that aims at producing enough for subsistence use. Due to the tough conditions of EU regulations, there are a few farms rearing crickets for food in Europe in countries such as Belgium, France, Finland, and the Netherlands (44). The largest cricket farms for food in the EU are run by a company called Kreka, which is based in the Netherlands. In Finland, the Nordic Insect Economy used to be a major cricket producer, but production as decreased (193). Finland is also the home of Entocube, a startup that began by rearing crickets in containers placed in urban areas but which has now progressed to a new 250,000 Euro project of farming crickets in a 60-years-old mine, taking advantage of the 28°C temperatures emanating from the geothermal station. Cricket farming in North America is practiced in Canada and the US. In Canada, cricket farming is carried out by private companies such as Entomo Farms, Third Millenium, and next Millenium. These companies rear crickets to sell as dry insects and/or processed cricket powder and flour to most of the edible cricket startups in the US, where there are only a few cricket farms (199, 200). In the USA, cricket farming is undertaken by Aspire Food Group, which started its cricket flour processing with the Aketta brand, has expanded its market activities by purchasing the cricket energy bar brand Exo in 2018. The cricket farmed in the US is *A. domesticus.*

In Africa, cricket farming is at its infancy stage in countries such as Kenya, Uganda, Mali, and Madagascar (32, 201, 202). Cricket-rearing technology has been disseminated to farmers in these countries through mass media (televisions, radios,

and print materials) and training of students and farmers. The institutions of training include Jaramogi Oginga Odinga University of Science and Technology, Jomo Kenyatta University of Agriculture and Technology and Makerere universities, and the International Center for Insect Physiology and Ecology (*icipe*). A grant from the Danish government facilitates the technology transfer of cricket farming through GREENiNSECT project that supports "the rearing and eating crickets as a delicious, affordable and healthy solution for malnutrition." The project has accelerated, leading to the establishment of small-scale cricket farms in L. Victoria region, which was the initial point of introduction in Kenya and Uganda. From this point of introduction, cricket farming has spread to other regions, such as central Kenya and the coast of Kenya. Three cricket species, *S. icipe, A. domesticus,* and *G. bimaculatus* are reared (28, 29, 203, 204). In most cases, these cricket species are reared in the same farm by one farmer; however, in some instances, they are reared in separate farms by different farmers. The cricket species reared by the farmer depends on which species is more appealing to him or her. *Scapsipedus icipe* and *A. domesticus* are most popular among farmers because they are softer than *G. bimaculatus.* In Kenya, there are about 300 cricket farmers who produce 28,800 kg of crickets per year (205). The cricket farm capacity can produce about 160 kg of crickets/ one harvesting cycle of 60 days. According to the field survey by Ayieko et al. (32), the largest production volume of farmed crickets is at Bondo and Kisumu counties in the Nyanza region. Most farmers use rectangular plastic containers while others rear the crickets in cylindrical plastic containers that have ventilation covered with plastic netting (28, 29),. The yield is low at 4 kg per cage at the harvesting stage.

Farming of crickets requires varying degrees of labor input during the rearing cycle (29). Each day one person is involved in the transfer of the egg containers from the main enclosures to the empty egg-laying enclosures and for daily feeding of crickets. This requires one person for 1 h of work. But where large-scale farming of crickets is practiced, such as in Thailand, more people are required to work in the cricket farms (146). Cricket farming in Kenya has picked up since cricket requires a small starting and maintenance capital and it is easy to set up farms for crickets. Farmers must explore the idea of adding value to their crickets by processing them. Moreover, rearing of crickets will ensure there are enough crickets for consumption and to stop depending on wild-collected crickets.

CONCLUSION

The current study has shown that consuming crickets as food by human beings is traditionally practiced in 49 countries around the world. Over 60 cricket species are known to be edible. Crickets are a highly nutritious food resource and may therefore be included in the list of the common diet of global consumers in the future. These crickets could also be used as nutritional supplements for special diets for schoolchildren, sick people, and athletes. Inclusion of potentially suitable species of crickets into the normal diet requires defined and standardized conditions of their rearing as well as the detailed monitoring of their composition, including biologically active compounds. Though the EFSA and *icipe* have already assessed hygienic and toxicological and microbial risks related to edible crickets, more research on their composition and nutrient profile should be carried out to fully implement edible crickets as food into the global legislation documents. Currently, there are only a few cricket species that are farmed. The farmers must be encouraged to start rearing other species of crickets that have not yet been confined. Also, animal breeders should try to find out whether it is possible to crossbreed the crickets with a long lifecycle with the ones with a short lifecycle.

CONTRIBUTION TO THE FIELD

Edible crickets have become popular in the past few years not only in the scientific literature but in other platforms as well. One of the major advantages of eating crickets is their impressive nutritional composition. Many sources report that crickets have better nutritional characteristics than traditional protein sources. In our research, we aimed to give a complete picture of edible crickets in the world, their nutritional profile and other benefits. The materials we used are published results of different authors from the past few years. The list of crickets provided by various authors' shows that there are 66 crickets that are consumed as food and feed in the world and crickets generally have a better nutritional profile than other meats. Based on our findings, crickets have a promising nutritional profile in terms of energy, protein, lipids and important fatty acids, mineral elements vitamins, carbohydrate and medicinal elements and may become part of many food products in future. As an enterprise, cricket farming, can mitigate food insecurity, act as a source of income when sold and a source of employment. The present review provides comprehensive information on the diversity of crickets, their nutritional values and their potential to contribute to the livelihood of mankind.

AUTHOR CONTRIBUTIONS

HM, SN, MA, MM, SE, JE, EK, JO, SH, KF, MO, NR, and CT: conceptualization, writing—original draft, and writing—review and editing. HM, SN, EK, and SH: data curation. HM, SN, EK, SE, and MM: formal analysis. HM, SN, MA, MM, SH, NR, SE, JE, EK, and KF: methodology. HM, SN, and EK: software. SN, MM, JE, SE, SH, CT, and KF: validation. HM: investigation. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.537915/full#supplementary-material

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Traditional Food Environment and Factors Affecting Indigenous Food Consumption in Munda Tribal Community of Jharkhand, India

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Indigenous food (IF) systems, derived from natural ecosystems are perceived to be sustainable and nutritionally adequate. Mundas, an indigenous tribal community in Jharkhand India, are surrounded by rich agroforestry resources, yet display high levels of malnutrition. Our study explored the food environment of Munda community, different IFs they accessed, levels of utilization of IFs in routine diets, their nutritional attributes and factors influencing IF consumption. A cross-sectional mixed-methods study was conducted in nine villages of Murhu and Torpa blocks in Khunti district, Jharkhand. Using focus group discussions and key informant interviews, we did free-listing of IFs known to the community. This was followed by enumerating preferred and little used/historically consumed IFs, along with reasons. Qualitative enquiries were recorded and transcribed verbatim; data were coded and analyzed using thematic framework approach. The listed IFs were identified through common names and photographs, and verified by ethnobotanist in the team. The nutritive values of identified IFs were searched in literature or nutritional analysis of specific plant based foods were undertaken in an accredited laboratory. The community demonstrated traditional ecological knowledge of several IFs ($n = 194$), which are accessed from wild, cultivated and built food environments. Taxonomic classification was available for 80% ($n = 156$) IFs, out of which 60 foods had nutritive values in secondary literature and 42 foods were analyzed in laboratory. Many IFs were rich in micronutrients like calcium, iron, folate, vitamin A and C. Among the listed IFs, only 45% were commonly consumed, while rest were little used/historically consumed. Factors like desirable taste, satiety, perceived nutrition benefits, adaptability to climate variability, traditional practice of food preservation and their cultural importance promoted IF consumption. However, local climatic impacts on agroforestry systems, easy access to foods bought from markets or distributed under government food security schemes, and promotion of hybrid seeds by local agricultural organizations, emerged as

potential barriers. Thus, reinforcement of traditional ecological knowledge and informal food literacy, along with promotion of climate resilient attributes of IFs, can contribute to sustainable food systems in Munda community.

Keywords: indigenous foods, traditional food environment, underutilized indigenous foods, nutritive value, micronutrients, factors affecting indigenous food consumption, Munda tribes

INTRODUCTION

Food systems comprise of elements and activities that relate to the way in which the food is produced, processed, distributed, prepared, and consumed (1). The interface where people interact with the wider food system to acquire and consume foods is defined as the food environment (2, 3). Depending on geographical location, people interface with wild, cultivated and built (i.e., market) food environments (4). The attributes of foods within these environments influence peoples' food choices and has the potential to affect their nutritional status (5). Sustainable Development Goal (SDG) 2 promises to end hunger, achieve food and nutrition security, and promote sustainable agriculture among all populations, especially nutritionally vulnerable people (6). However, achieving this goal is riddled with uncertainty because of the way in which the world currently produces and consumes foods. Globally, the diets we consume and the food systems that produce them, are neither healthy nor sustainable, which has implications for achieving SDG 2. Despite being reasonably safe and consistent in food supplies (quantity), current global food systems are struggling to meet the nutritional needs of the growing population and have placed significant strain on land, water, soil, air, and other natural resources (7, 8). In this context, the concept of sustainable food systems and healthy diets are receiving renewed attention (9).

Sustainable food systems are those that aim at achieving food and nutrition security while limiting negative environmental impacts and improving socio-economic welfare of all, including poor and marginalized populations (7). These sustainable food systems are derived from sustainable cultures and ecosystems, and are accessible, affordable, safe and healthy, while simultaneously promoting environmental stability (8, 10). Indigenous foods, accessed as part of traditional food systems and consumed by indigenous people throughout the world, are also derived from natural ecosystems, and are hence, perceived to be sustainable (11). These food systems are reservoirs of unique traditional ecological knowledge, incorporated in both cultivated and wild foods derived from plants, animals, and fungi species that are available from local natural resources. Moreover, indigenous food systems are better adapted to local conditions, more resistant to drought, altitude, flooding, or other extreme conditions, are low resource intense, have low carbon footprints and use environmentally sensitive technologies (12). Several indigenous foods accessed as part of these food systems are known to be nutrient rich and may have potential in alleviating hunger and malnutrition (10, 13). The indigenous food systems are grounded in historical legacy and spirituality that acknowledge the inextricable link of people with their sustainably managed resources, and thus can be utilized to provide sustainable diets and can play a crucial role in achieving SDG 2 (7, 8).

Indigenous people, despite their vast knowledge of the world's territories and guardianship of 80% of global species diversity, are nutritionally vulnerable and experience significant disparities in health outcomes, grounded in poverty and marginalization (14). This contributes to their inability to realize the potential of their traditional food systems in providing sustainable solutions to the existing nutrition insecurity within the population (15). Factors like declining traditional knowledge, opportunity cost in access, displacement of traditional crop species by a few major crops and shifting diets and food cultures have substantially influenced their food systems (16) and led to underutilization of many diverse indigenous food resources (17, 18). Nonetheless, several factors have been documented in the scientific literature to promote these food systems such as local accessibility, cost effectiveness, existence of traditional knowledge, sustainable utilization of the natural environment from farming or wild harvesting and socio-ecological resilience (19–22).

The tribal communities in India are a good example of indigenous populations, having their own rich cultural and social traditions and unique indigenous food systems (23). Jharkhand, a central eastern Indian state known for its rich biodiverse agroforestry (24, 25), is home to several indigenous tribal communities that constitute 26.2% of the state's population (26). Mundas, the third most populous tribal community of Jharkhand, are the inhabitants of Chota Nagpur region in the state (27). This community lives in geographical locations that are surrounded by natural resources and have largely retained their traditional ecological knowledge (TEK) of gathering and preparing foods from the natural sources in traditional ways (28). They are subsistence farmers (29) and are also known to use local wild plants, fruits and tubers which could help to augment their food and nutrition security (28, 30). Despite access to a rich agroforestry, factors like geographical isolation, poverty, lack of formal education and poor access to health services contribute to poor nutrition outcomes in Munda community (31). Although limited literature is available on the nutritional status of Mundas, a few studies have documented high prevalence of malnutrition among women and children (32, 33). In a cross-sectional study conducted among Munda children, more than half the children (56%) were found to be underweight, with 29% children as severely underweight (32). The nutrient intakes among Munda women were found to be below recommended levels of Indian women, especially for nutrients like protein, calcium, vitamin A and C, thus indicating consumption of poor quality diets (33). Hence, the aims of this study were to explore the traditional food environment of the Munda tribal community of Jharkhand, examine their TEK and nutritive

value of indigenous foods and the factors that influence their consumption.

MATERIALS AND METHODS

Study Locale and Population

This study was conducted in Khunti district of Jharkhand, India. The total area of Khunti district is about 2,535 sq. km (34) and 40% of it is covered by forests and uneven landscapes (28). The total population in the district is 531,885, out of which 61% is comprised of the Munda tribal community (34). Two geographically distinct blocks of Khunti, namely, Murhu and Torpa were purposively selected for the study (**Figure 1**). Both these blocks comprise of hilly terrain with forest cover and plain lands, and have a predominantly higher population of Munda tribe, in comparison to other blocks. Using probability proportional to size sampling, a total of eleven villages from Murhu and Torpa blocks were selected for the study, details of which are reported elsewhere (35). Out of these villages, qualitative enquiries were conducted in nine villages until theoretical saturation was achieved (i.e. no additional information relevant to the research question was generated) (36).

Study Design

A cross-sectional mixed-method study was conducted using both qualitative enquiries and quantitative estimates, to explore the traditional food environment of the Munda tribal community, including the types of food environments (wild, cultivated, and built) that they interface with, the types of indigenous foods (IFs) that are accessed, their nutritional attributes as well as the factors influencing the consumption of these foods. This work was part of a larger study documenting the role of IFs consumed by tribal groups of Jharkhand and assessing their contribution to food security and dietary diversity among women and children (35). The data collection was conducted between June 2019 and January 2020; multiple visits were conducted to capture the diversity of foods that were consumed during different seasons.

Study Procedures

The study included both qualitative and quantitative methods of data collection. The mixed methods approach facilitated the triangulation of both qualitative and quantitative data to increase the data integrity and provide rich contextual information (37). A detailed flow chart on the methodological approach used in the study is provided in **Figure 2**.

Qualitative enquires were conducted using focus group discussions (FGDs) and key informant interviews. A total of nine FGDs and six interviews were conducted in nine study villages using FGD and interview guides that were pretested in three villages (other than the study villages). The FGD guides were used to do free-listing of all IFs known to the community, and gain information on their traditional farming methods, food collection from natural sources like forests and water bodies, access to market, usual eating patterns and cultural use of IFs. The impact of climate change on local food systems was also assessed by adapting questions from the tool developed by Bioversity International and the Institute of Development Studies (38). After developing a free list of all the IFs known to the community, participants were further probed in order to develop the list of popular and preferred IFs vs. those that were little used or historically consumed. A pairwise ranking method (39) was used to elicit further information on the preference toward specific IFs in a food group category among the list of preferred foods in a specific season. The perceptions on reasons why

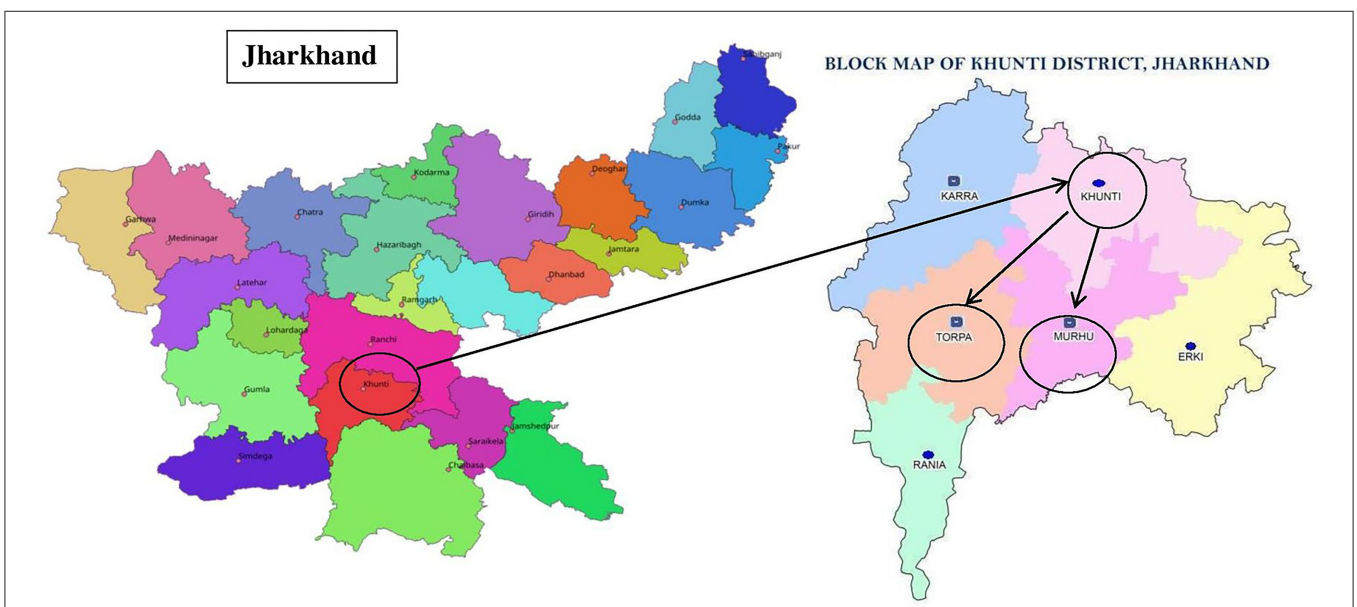

FIGURE 1 | Selection of Murhu and Topra blocks from Khunti district of Jharkhand, India.

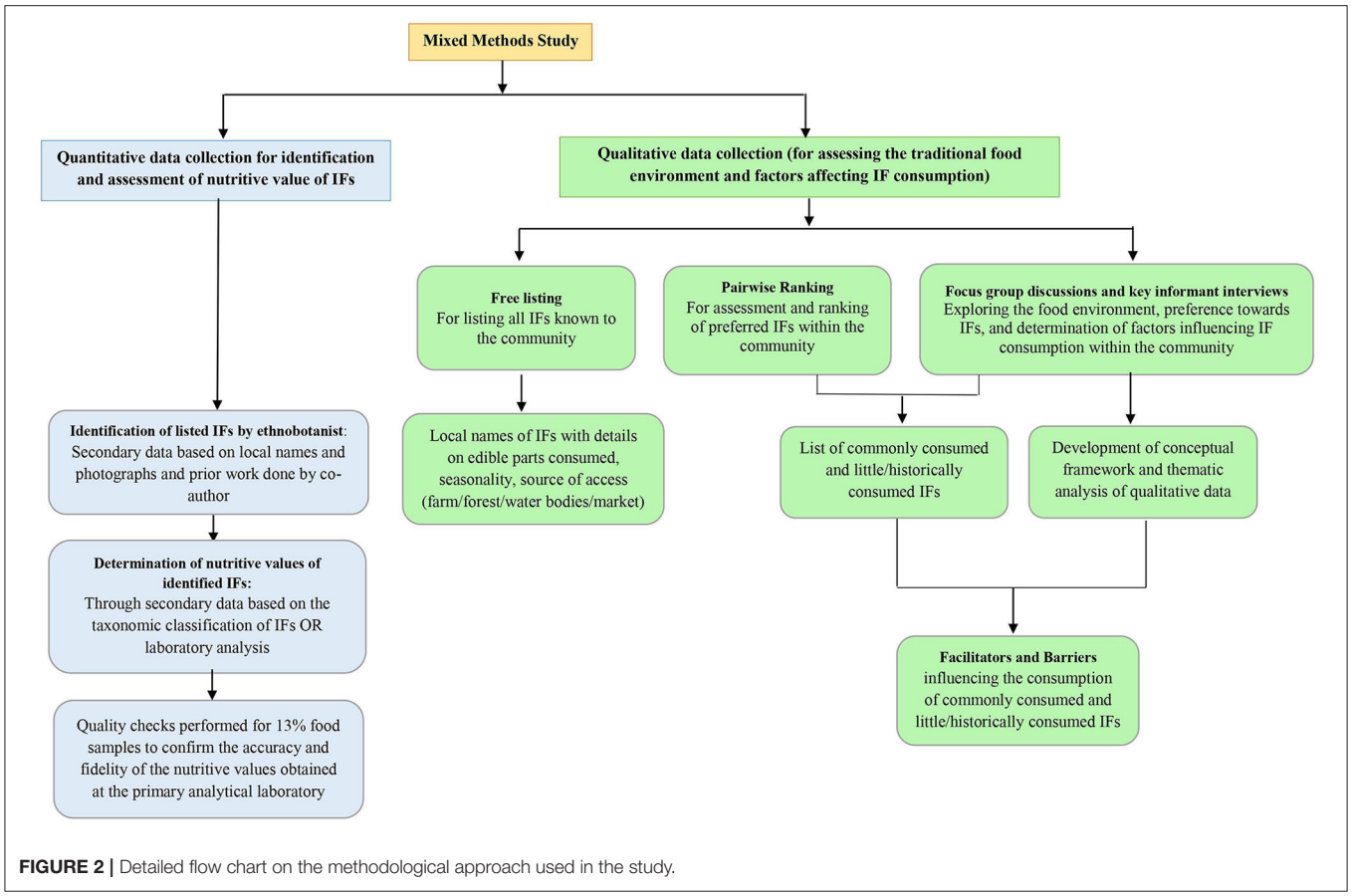

FIGURE 2 | Detailed flow chart on the methodological approach used in the study.

specific IFs were preferred or infrequently used (e.g., availability, access/production, and/or taste) and other factors that influenced IF consumption were also explored. Based on certain queries and information gaps from FGD, additionally six key-informant interviews were conducted to substantiate and cross-compare the findings.

The listed IFs were identified through their common names as well as photographs and verified by an ethnobotanist in the team, who has extensively worked on taxonomic classification of Munda tribal foods (28, 30, 40–47). The nutritive value of these identified IFs were searched in the Indian food composition database and other secondary literature (23, 40, 42–45) and were collated. IFs with no secondary data on nutritional values, were collected from field sites and sent for analysis to a food testing laboratory accredited with National Accreditation Board for Testing and Calibration Laboratories (NABL). The food sample collection was carried out following the standard protocol developed as part of the larger study (35) and nutrient analysis was conducted according to standard reference procedures in consultation with the NABL accredited laboratory. The parameters analyzed included energy, protein, carbohydrate, fat, dietary fiber, vitamin A (as beta-carotene), vitamin C, vitamin B1, B2, iron, calcium, zinc, folate, and phosphorous. The analyte values were reported per 100 g of edible weight. The details of the methods used for specific nutrients and the limit of quantification are provided in **Supplementary Table 1**.

The entire data collection was conducted and supervised by the core research team. In addition to the core research team, the study team also included well-trained local field workers fluent in the native *Mundari* dialect who assisted the team in facilitating the qualitative enquiries and collection of food samples for identification and nutritional analysis purposes. Two local field workers were given a two-day training prior to the FGDs and interviews wherein they were briefed about the study objectives and their role as translators during the qualitative enquiries. Three workers were also trained regarding the steps of collection, packaging and transportation of food samples from the field to the ethnobotanist's laboratory and food analysis laboratory.

Study Participants

The FGDs were attended by village members from different age groups such as young adults as well as elderly men and women, along with community health and nutrition workers (*Anganwadi* worker and Accredited Social Health Activist, ASHAs) and community leaders. Village wise details on age and gender characteristics of study respondents are listed in **Table 1.** The community health workers and village heads were contacted prior to the FGDs and were asked to inform the village members about the scheduled discussion. Sample selection was based on snowball sampling method (48); wherein study participants were asked to identify other potential community members who had

TABLE 1 | Characteristics of study participants in villages of Murhu and Torpa blocks, Khunti district, Jharkhand.

Block	Study Village	Group size	Gender and age group of respondents
Block 1: Murhu	Charid	10	10 adults (women)
	Gangina	6	1 elderly (woman), 5 adults (4 men and 1 woman)
	Burju	7	1 elderly man, 6 adult men
	Kurki	11	1 elderly woman, 10 adult women
Block 2: Torpa	Urikel*	13	13 adults (7 men and 6 women)
		9	2 elderly men, 7 adult men
	Tati	17	15 adults (8 men and 7 women), 2 elderly women
	Nichitpur	24	5 elderly (2 men and 3 women), 19 adults (10 men and 9 women)
	Jibilong	14	1 elderly man, 13 adults (6 men and 7 women)

**Two separate FGDs were conducted in two different Tolas (hamlets) of the village.*

traditional knowledge on indigenous foods, their accessibility, use and other related information. Key informant interviews were conducted with community leaders and/or health and nutrition workers in the villages in order to substantiate and fill some critical information gaps on IF preferences and reasons for consumption /non-consumption gathered during the FGDs.

Data Analysis

All the FGDs and interviews were recorded and transcribed from *Mundari* to Hindi and then translated to English. The transcripts were used to generate a list of all the IFs known to the community. Further, based on the inputs from the participants, the listed foods were categorized into commonly consumed and little consumed/historically consumed IFs. The information from pairwise ranking was used to assess preference toward specific IFs in the preferred food list category. This was done by creating comparisons and scoring between IFs under each food group. The nutritive value of IFs were documented and utilized to prepare a list of IFs that are "good" and "rich" nutrient sources. The foods with at least one nutrient level between 10 and 19% and higher than 20% of recommended dietary allowances (RDA) for Indian adults per serve, were considered as "good" and "rich" sources of nutrients, respectively (49–51). This was done for both commonly consumed and little used/historically consumed IFs. Atlas.ti version 8 was used for coding the content of the transcripts, which was analyzed further using thematic analysis (52). The data were coded using deductive approach; similar codes were identified and merged to produce main themes and sub-themes related to factors affecting IF consumption. A conceptual framework was then developed based on the analysis of qualitative enquiries and seven main themes were generated which highlighted factors that directly or indirectly influenced consumption of IFs within the community.

Ethics Approval

The study was conducted according to guidelines laid down in Declaration of Helsinki (53) and all procedures involving humans in this study were approved by the Institutional Ethics Committee at Indian Institute of Public Health-Delhi, Public Health Foundation of India, and All India Institute of Medical Sciences, New Delhi. Administrative approvals from authorities at district level and cluster level consent from the village leaders were obtained before conducting FGDs. Written informed consent was obtained from literate respondents and third-party witnessed verbal consents were sought from illiterate respondents. All respondents were informed that the FGDs and interviews were being recorded.

RESULTS

Traditional Food Environment of Munda Tribal Community

The community reported a predominantly rice based habitual diet with most of the households consuming two to three main meals a day. The usual meals consist of rice along with sautéed or curried green leafy vegetables (GLVs) or roots and tubers or sometimes pulses and flesh foods (meat, poultry, egg, or fish). Both indigenous as well as non-indigenous varieties of foods are consumed, which comprise of different varieties of rice, pulses, fruits, GLVs, roots and tubers, vegetables, and flesh foods (detailed information on individual food items are added in later sections). Milk and milk products are rarely consumed by the community. The community practices smallholder subsistence agriculture and accesses foods from cultivated (agricultural lands, backyard gardens and raising livestocks), wild (surrounding forests, pastures, roadsides, wastelands and local water bodies), and built food environments (local informal markets and food entitlements under government's food security programmes). The agroforestry and livestock produce is mainly utilized for household consumption, while the surplus is sold in local markets for income generation. We have described the food environments as well as the specific IFs accessed within them in additional detail below.

Cultivated Food Environment

The Munda community mainly practices settled agriculture at three levels of farm lands, namely-*Loyong* (low level lands with highest water requirement for crops), *Badi* (middle level lands with relatively low water requirement for crops), and *Godha* (dry stony plain lands with least water requirement). The usual size of farmlands varies between 1.5 to 3 Bigha (traditional unit of land measurement, equivalent to 0.25 Hectares). The crops that are commonly cultivated include both indigenous as well as hybrid varieties of rice, millets, and pulses (details given in

later sections). However, the proportion of land use reported for cultivation of hybrid varieties is substantially larger than indigenous varieties. In addition to their farm lands, they also grow vegetables, roots and tubers in their backyard kitchen gardens, locally known as *Bakdi*. The community also raises livestock such as goat, pig, and poultry to produce commodities such as meat and eggs.

Wild Food Environment

The community accesses the wild food environment, including forests and water bodies and natural vegetation like fields and pastures within the village. Most of the study villages reported accessing local forests for gathering forest foods as well as firewood. Different varieties of indigenous leafy vegetables, fruits, roots and tubers, and mushrooms (details discussed in later sections) are collected for the purpose of both household consumption and sale in local markets, while collected firewood is used as a household cooking fuel. The practice of hunting was also reported, wherein all men in the village gather once in a year and collectively hunt the wild animals for consumption. Local rivers, lakes and ponds are accessed, especially during monsoons, for collection of indigenous fishes, crabs, and snails. In addition to these, weeds grown in the field, pastures and wastelands are also collected for consumption.

Built Food Environment

The community frequently accesses weekly local informal markets or "*Hatiya,*" which are situated within a range of 5–10 kilometers from the villages. These local markets are mainly accessed for procurement of cooking oil, spices, packaged foods, and freshly prepared sweets and savories. Apart from this, the local markets also provide the community with indigenous as well as non-indigenous varieties of pulses, fruits, vegetables, and roots and tubers, meat and fish (details discussed in later sections). Specific varieties of indigenous foods accessed from local markets include pulses [Munmuna ("*Baturi dal*": *Vicia hirsuta),* Horse gram ("*Kulthi*": *Macrotyloma uniflorum)*], vegetables [Cowpea, white ("*Bodi*": *Vigna unguiculata*), Field beans ("*Simbi*": *Lablab purpureus)*] and roots and tubers [Pechki ("*Toti*": *Colocasia esculenta), "Jat sanga*" (*Dioscorea alata)*].

In addition to the weekly markets, households in all study villages receive subsidized food commodities (rice, sugar, salt etc.) under the Public Distribution System (PDS) – a government food security scheme (54). The children under 6 years, receive supplementary nutrition, mostly in the form of hot cooked meals and take home ration from *Anganwadi* centers (maternal and child health and nutrition center) under Integrated Child Development Service (ICDS) (55), while school children receive cooked meals under the Mid-Day Meal (MDM) program (56).

Indigenous Foods of Munda Tribal Community

The community was asked to list out all IFs known. This was followed by identifying IFs that are routinely consumed and those that are little used or historically consumed.

Types of IFs Based on Free Listing

The FGDs revealed a rich TEK within the community which resulted in a diverse list of 194 IFs, comprising of 34 cereals (17.5%), 7 pulses (3.6%), 57 green leafy vegetables (GLVs) (29.2%), 11 other vegetables (5.7%), 9 roots and tubers (4.7%), 15 fruits (7.8%), 23 mushrooms (12%), 37 flesh foods (19%), and honey. **Supplementary Table 2** provides a list of all the IFs mentioned by the study participants with details of the parts consumed (in case of plants), their primary sources from where they are harvested, gathered, or collected and the season in which they are available.

Classification of IFs Based on Preference

Out of 194 IFs listed, 87 (45%) were identified as commonly consumed and 107 IFs (55%) as little used or historically consumed. Pairwise ranking method, based on criterion such as taste, availability, ease of production or collection by the community, further revealed comparative food group wise preference toward specific IFs from the preferred food list category for a specific season. **Figure 3** provides some examples of scoring and ranking of indigenous rice and GLVs. To elaborate, the FGD participants were asked to identify 4 to 5 most preferred indigenous rice varieties which were then tabulated as a matrix on a flip chart. Participants were asked to compare the first rice variety in the row with various others in the column one by one. This step was repeated for the subsequent rice varieties listed in the columns. A score was provided based on the number of times a specific rice variety was selected, and then the varieties were ranked. The rice variety with the highest scores ("*Laldhan*": *Oryza sativa*) was ranked first and the successive scores in descending order provided information on comparative preferences toward other varieties. This helped in identifying the popular IFs among the commonly consumed IFs under different food groups. Detailed list of the preferred and little used/ historically consumed IFs are provided in **Supplementary Table 2.**

Rice is the primary cereal consumed, though other cereals like Maize ("*Jondra*": *Zea mays)*, Finger millet ("*Kodde*": *Eleusine coracana*), Pearl millet ("*Gaangi*": *Pennisetum glaucum)*, Sorghum ("*Jowar*": *Sorghum bicolor),* and Little millet ("*Gondli*": *Panicum Miliare)* were also mentioned during the FGDs. About 29 varieties of indigenous rice were mentioned, but only 14 were reported to be routinely consumed. Millets are presently little used or historically consumed. Out of 57 GLVs reported, only about half are routinely consumed. There were several mushrooms ($n = 23$) listed, however, only 12 varieties are routinely consumed. Similarly, only about 50% of roots and tubers (5 out 9) and fruits (7 out of 15) are routinely consumed at present. Only a third of the reported 37 animal foods are included in the routine diets.

A systematic documentation of total number of identified IFs (preferred and little used/historically consumed), the status of their taxonomic classification and assessment of their nutritive values are provided in **Figure 4.**

Taxonomic Classification

A total of 156 IFs (80%) were identified with taxonomic classification, that comprised of 80 (51%) commonly

FGD1

Rank	Indigenous varieties of rice	Scores
1	*Karnidhan*	3
2	*Arababa/Laldhan*	2
2	*Anthanawbeydhan*	2
2	*Jhillidhan*	2
3	*Songalpuridhan*	1

Rank	Indigenous varieties of GLVs	Scores
1	Beng saag *(Chokke ara)*	4
2	Drumstick leaves (*Munga ara*)	3
3	Koinaar leaves *(Sing ara)*	2
4	Amaranth leaves *(Leped ara)*	1
5	Katai saag (*Sarla ara)*	0

FGD2

Rank	Indigenous varieties of rice	Scores
1	*Arababa/Laldhan*	4
2	*Hesalsar*	2
2	*Pundi baba*	2
3	*Songalpuridhan*	1
3	*Anthanawbeydhan*	1

Rank	Indigenous varieties of GLVs	Scores
1	Koinaar leaves *(Sing ara)*	4
2	Water spinach *(Kalmi ara)*	3
3	Ponnaganni *(Garundi ara)*	2
4	Amaranth leaves *(Leped ara)*	1
5	Kauwa leaves *(Kauwa ara)*	0

FGD3

Rank	Indigenous varieties of rice	Scores
1	*Arababa/Laldhan*	4
2	*Lalhat*	3
3	*Munadhan*	2
4	*Godadhan*	1
5	*Karnidhan*	0

Rank	Indigenous varieties of GLVs	Scores
1	Beng saag *(Chokke ara)*	4
2	Phutkal leaves *(Phutkal ara)*	3
3	Koinaar leaves *(Sing ara)*	2
4	Ponnaganni *(Garundi ara)*	1
5	Kauwa leaves *(Kauwa ara)*	0

FIGURE 3 | Pairwise ranking and scores to indigenous foods under different food groups. Note: GLVs, Green Leafy Vegetables.

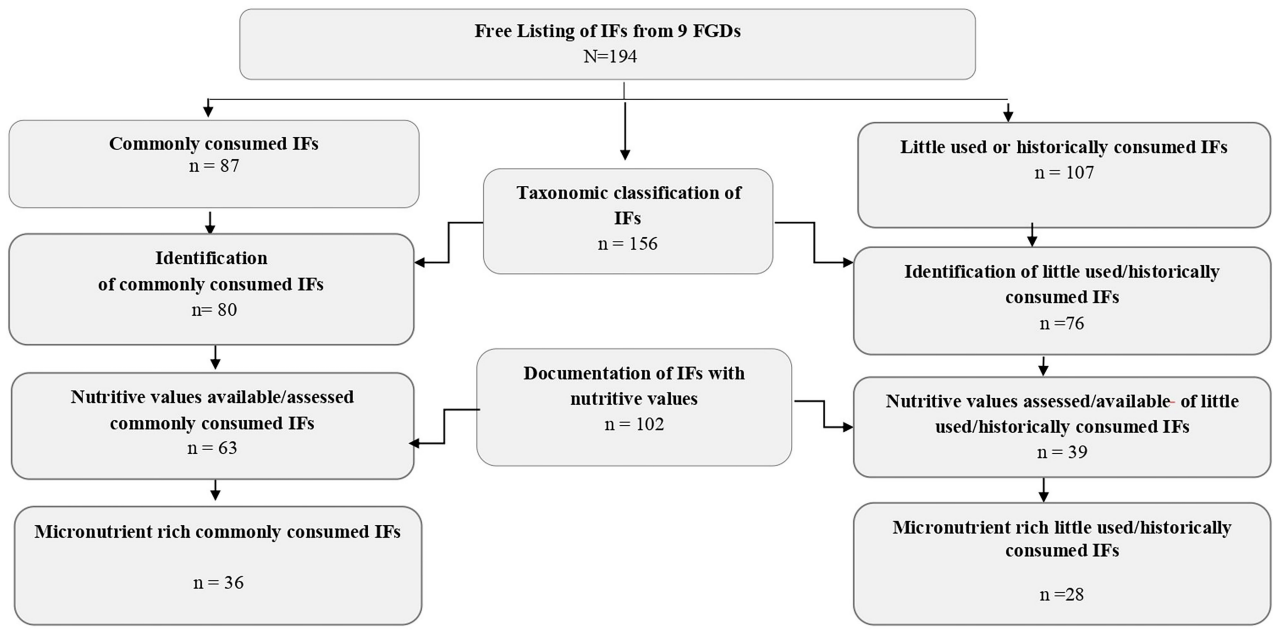

FIGURE 4 | The systematic approach adopted to classify IFs available in the community.

consumed foods and 76 (49%) little used/historically consumed foods. Details on taxonomic classification are provided in **Supplementary Table 2**.

Nutritive Values of IFs

Based on the taxonomic classification of IFs, the nutritive values of 102 IFs were documented (63 commonly consumed IFs and 39 little used/historically consumed). Out of these, nutritive values were available for 60 IFs from secondary sources while the rest were assessed in laboratory as the part of the study (n = 42), **Supplementary Table 3**.

Many IFs were found to be good sources of micronutrients (**Table 2**). Among indigenous cereal varieties, Finger millet (*Kodde*), a little consumed IF, was found to be a good source of calcium (364 mg/100 g). Indigenous pulses were not only found to have good levels of protein (range 21.3–28.2 g/100 g), but were also rich in several micronutrients. For instance, Horse gram (*Kulthi*), a commonly consumed pulse, was found to be a good source of calcium (269 mg/100 g) as well as folate

TABLE 2 | **Indigenous foods with high micronutrient content.

S. No.	Food item (Mundari Name)	Common name (English/ Hindi)	Scientific name[β]	Energy (Kcal/ 100 g)	Protein (g/100 g)	Carbohydrate (g/100 g)	Fat (g/100g)	Dietary fiber (g/100g)	β-Carotene/ Retinol (μg/100g)[a]	Vit C (mg/100g)	Vit B1 (mg/100g)	Vit B2 (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)	Calcium (mg/100g)	Total folate (μg/100g)	Phosphorus (mg/100g)
Commonly consumed Indigenous foods																	
1	*Pundigoda*[¥]	Rice	*Oryza sativa* L.	351	4.28	80.7	1.26	5.19	ND	ND	0.44	0.12	0.4	0.1	20.6	3	37.6
2	*Kulthi*[*]	Horse Gram, whole	*Macrotyloma uniflorum* (Lam.) Verdc.[€]	321	22	57.2	0.5	NA	59	NA	0.32	0.24	8.8	2.7	269	163	298
3	*Garundi/ Gundri ara*[*]	Ponnaganni	*Alternanthera sessilis* (L.) R.Br. ex DC.	51	5.3	5.17	0.71	6.74	5288	103	0.02	0.1	3.9	1	388	48	53.2
4	*Jiri ba*[¥]	Sanai phool	*Crotalaria juncea* L.	120	2.9	27.04	ND	7.43	1113	1.77	3.09	ND	7.6	0.15	320.2	ND	537.2
5	*Ber*[*]	Zizyphus	*Zizyphus jujuba* Mill.	49	1.3	9.4	0.35	3.73	2	60.9	0.01	0.02	0.4	0.1	46.6	6	32.3
6	*Dahu*[¥]	Barhar	*Artocarpus lakoocha* Wall. Ex Roxb.	121	2.88	27.33	ND	7.2	1843	8.9	0.32	1.32	1.8	0.1	54.7	ND	ND
7	*Kusum/ Baru*[¥]	Kusum fruit	*Schleichera* oleosa (Lour.) Merr.	144	6.31	29.72	ND	14.89	6238	3.1	ND	ND	44.2	0.8	135	ND	51.3
8	*Rugra/ Putuh*[¥]	Mushroom	*Geastrum*	138	4.86	29.53	0.06	7.37	ND	ND	0.58	0.37	6.8	3.1	193	0.2	30.2
9	*Gitilud*[¥]	Mushroom	-	67	3.52	13.17	ND	6.89	9	ND	0.19	0.17	10.8	0.6	10	3	24.3
10	*Loa suti*[$]	Snail	*Pila globosa*	97	10.5	12.4	0.6	NA	NA	NA	NA	NA	NA	NA	870	NA	116
11	*Setua/ Keyosuti*[$]	Mussel	*Margaritifera margaritifera*	81	14.5	2.1	1.6	NA	NA	NA	NA	NA	NA	NA	592	NA	NA
12	*Demta/Hau anda*[$]	Eggs of red ants	*Oceophylla smaragdina*	131	13.4	9.1	4.6	NA	NA	NA	NA	NA	NA	NA	104	NA	107
Little used/historically consumed Indigenous foods																	
1	*Kodde* [*]	Finger millet	*Eleusine coracana* (L.) Gaertn.	321	7.2	66.82	1.92	11.18	2	NA	0.37	0.17	4.6	2.5	364	35	210
2	*Bodi*[*]	Cow pea, white	*Vigna unguiculata* (L.) Walp.[€]	320	21.3	53.77	1.14	11.7	8	NA	0.33	0.09	5.0	3.6	84.1	249	378
3	*Sutri*[$]	Rice bean	*Vigna umbellata* (Thumb.) Ohwi & H. Ohashi[€]	332	21.5	60.9	0.3	NA	NA	NA	NA	NA	NA	NA	302	NA	297
4	*Baturi dal*[¥]	Munmuna	*Vicia hirsuta* (L.) Gray	341	27	55.54	ND	8.79	178	12.45	0.22	1.15	17.1	3.8	77.5	ND	83.3
5	*Kantha ara*[#]	Kantha leaves	*Dentella repens* (L.) J.R. Forst. & G. Forst.	46	3.5	8	NA	7.1	11680	9	3.07	NA	81.1	1.0	425	7	NA
6	*Lupu ara*[^]	Chhaya/ Kapurijari/ Gorakhbuti	*Aerva lanata L.* Juss. Ex Schult.	56	4.6	9.5	ND	5.9	21760	12	ND	ND	22.1	0.7	202	41	ND
7	*Kecho ara*[¥]	Punarnava	*Boerhavia procumbens* Banks ex Roxb.[€]	52	4.37	8.67	ND	2.47	2257	0.7	0.42	1.31	9.4	1.7	877.9	0.4	8.7
8	*Burju Baha*[¥]	Kachnar flower	*Bauhinia variegata* L.	83	2.98	17.7	ND	8.49	416	2.4	ND	0.3	3.4	0.7	404.9	ND	447.7

(Continued)

TABLE 2 | Continued

S. No.	Food item (Mundari Name)	Common name (English/Hindi)	Scientific name[β]	Energy (Kcal/ 100 g)	Protein (g/100 g)	Carbohydrate (g/100 g)	Fat (g/100g)	Dietary fiber (g/100g)	β-Carotene/ Retinol (μg/100g)[a]	Vit C (mg/100g)	Vit B1 (mg/100g)	Vit B2 (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)	Calcium (mg/100g)	Total folate (μg/100g)	Phosphorus (mg/100g)
9	*Adel sanga*[*]	Tapioca	*Manihot esculenta* Crantz.	80	1.03	17.81	0.2	4.61	NA	17.6	0.07	0.02	0.8	0.1	25.9	26	42.6
10	*Haseaar sanga*[¥]	-	*Dioscorea quartiniana* A. Rich.[€]	72	4.4	13.49	ND	1.34	ND	3.1	1.09	ND	55.9	0.6	33.2	ND	26.4
11	*Soso*[$]	Marking nut (kernel)/ Bhelwa	*Semecarpus anacardium* L.f.	587	26.4	28.4	36.4	NA	NA	NA	NA	NA	6.1	NA	295	NA	NA
12	*Baadi*[ß]	Banyan fruit	*Ficus benghalensis* L.	72	1.7	11.8	2	NA	NA	NA	NA	NA	NA	NA	364	NA	NA
13	*Redhayi/ Mangri*[$]	Walking catfish	*Clarias batrachus*	86	15	4.2	1	NA	NA	NA	NA	NA	0.7	NA	210	NA	290

Text in Italics represents Mundari names.

NA, Not available; ND, Not detected; [¥] Laboratory analyzed; [a] Retinol expressed in μg/100g for animal foods.

Secondary data on nutrient analysis:[*] *(43);* [^] *(45);* [#] *(23);* [$] *(44).*

[ß] Scientific name cited from secondary sources (28, 30, 40–45) and [€] additionally verified from (46, 47).

***All the foods listed in the table have at least one nutrient content per serve between 10 and 19% or >20% of RDA for Indian adults (49–51).*

(163 μg/100 g). Little consumed pulses were also found to be good source of micronutrients; for example, Rice bean ("*Sutri*": *Vigna umbellata*) had high levels of calcium (302 mg/100 g), Cow pea, white (*Bodi*) had high levels of folate (249 μg/100 g) and Munmuna (*Baturi dal*) was rich in iron (17.1 mg/100 g). Most of the GLVs were found to be rich sources of vitamin A (range: 1015 to 21760 μg/100 g), with the highest content observed in Kapurijari ("*Lupu ara*": *Aerva lanata*), an under-utilized GLV. The commonly eaten Ponnaganni leaves ("*Garundi ara*": *Alternanthera sessilis*) were found to have remarkably high levels of vitamin C (103 mg/100g). Maximum calcium content among GLVs (877.9 mg/100 g) was seen in Punarnava ("*Kecho ara*": *Boerhavia procumbens*), while highest iron content (81.1 mg/100 g) was seen in Kantha leaves ("*Kantha ara*": *Dentella repens*), both little consumed within the community. Some GLVs were found to have ideal calcium to phosphorous ratio of around 2:1, which is important for bone health. Flowers of Kachnar ("*Burju Baha*": *Bauhinia variegate*) (little consumed) and Sanai ("*Jiri ba*": *Crotalaria juncea)* (frequently consumed), which are eaten as vegetables, were found to be rich in calcium (404.9 mg/100 g) and vitamin A (1113 μg/100 g), respectively. Among roots and tubers, under-utilized varieties of Tapioca ("*Adel sanga*": *Manihot esculents*) was found to be rich in vitamin C (17.6 mg/100 g), while "*Haseaar sanga*" *(Dioscorea quartiniana)* was found to be iron-rich (55.9 mg/100 g). Little consumed fruits such as Marking nut ("*Soso*": *Semecarpus anacardium*) and Banyan fruit ("*Baadi*": *Ficus benghalensis*) were found to have high calcium content (295–364 mg/100 g). Marking nut was also found to be exceptionally rich in protein (26.4 g/100 g). Fruit of Kusum ("*Baru*": *Schleichera* oleosa) which is frequently consumed in summers, was found to be rich in vitamin A (6238 μg/100 g), calcium (134.8 mg/100 g), and iron (44.2 mg/100 g). Highest value of vitamin C (60.9 mg/100 g) was observed in Zizphus ("*Godaari*": *Zizyphus jujuba*), which is frequently consumed during winters. Commonly consumed mushrooms such as, "*Rugra/Putuh*" *(Geastrum),* was found to be a rich source of calcium (193.4 mg/100 g), while *Gitilud* (classification NA) was found to be rich in iron (10.8 mg/100 g). Most of the commonly consumed flesh foods were found to have high content of calcium (range 104 to 870 mg/100 g). Among little consumed flesh foods, Walking catfish ("*Redhayi*": *Clarias batrachus*) was found to be a good source of calcium (210 mg/100 g). Pictures of some micronutrient rich indigenous foods are provided in **Figure 5**.

Qualitative Enquires to Assess Factors Affecting IFs Consumption

A large number of IFs (n = 194) were reported during the free listing exercise, however, more than 50% of these foods were found to be either little used or historically consumed. Based on the themes identified from the qualitative enquires, a conceptual framework was developed which revealed a list of factors that directly or indirectly influenced the IF consumption in the community (**Figure 6**). The factors, which promoted the IF consumption were classified as facilitators, while those factors that hindered the consumption of IFs were classified as barriers.

FIGURE 5 | Some micronutrient rich indigenous foods of Munda tribal community. Picture courtesy: ˚Indigenous Foods project, DBT/Wellcome Trust India Alliance.

Facilitators of Indigenous Food Consumption in Munda Tribal Community

The FGDs revealed four key themes that facilitate the consumption of IFs, which included, (i) desirable taste, satiety and perceived nutrition benefits of IFs, (ii) adaptability and resilience to climatic variability resulting in improved productivity and availability, (iii) the traditional practices of IF preservation and conservation which promote

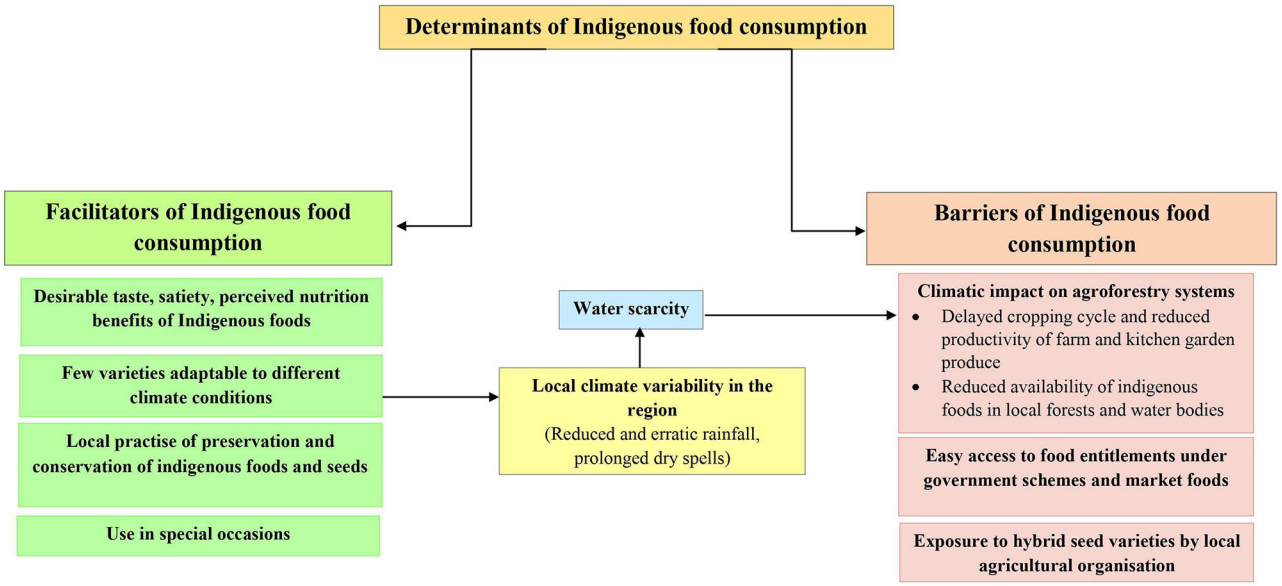

FIGURE 6 | Factors influencing indigenous food consumption in Munda tribal community of Jharkhand, India.

their incorporation in daily diets, and (iv) the cultural importance associated with IFs which facilitates their use in special occasions.

Desirable Organoleptic Properties and Perceived Nutritional Benefits of IFs

One of the main facilitators of IF consumption is the associated desirable taste and satiety giving properties of these foods. As one respondent said: *"Indigenous foods taste better and have more nutrition than hybrid varieties"* (Respondent number 5, male, study village 3, Torpa block, 24th June, 2019). Many indigenous varieties of rice (such as *Laldhan, Karnidhan, Reici baba, Karangadhan)*, GLVs (Koinaar leaves ("*Sing ara*": *Bauhinia purpurea)*, Amaranth leaves ("*Leped ara*": *Amaranth spinosus),* Bengal gram leaves ("*Boot ara*": *Cicer arietinum),* Water spinach ("*Kalmi ara*": *Ipomoea aquatica)* etc.) and flesh foods (like Puti fish ("*Pothi*": *Barbus* spp.), Bele fish ("*Bale*": *Glossogobius giuris),* Spotted snakehead fish ("*Chodha Machli*": *Channa punctatus),* Snails ("*Loa suti": Pila globosa*), Mussels ("*Setua*": *Margaritifera margaritifera),* Wild pig ("*Jungli suar": Sus scrofa),* Wild hen *("Jungli murgi": Galloanseres* sp*)* etc.) are locally known for their relishing flavor and are frequently consumed by the community members. For instance, one respondent stated*: "We grow indigenous rice varieties that taste good. If we eat indigenous food, we feel full for longer time duration*" (Respondent number 2, male, study village 4, Murhu block, 22nd June, 2019).

In addition to their desirable organoleptic properties, the community perceived the IFs to be nutritious, especially in comparison to hybrid varieties of crops. Certain varieties of indigenous rice (such as *Laldhan* and *Karnidhan*) and GLVs (Amaranth leaves/*Leped ara*) are particularly perceived as a good source of nutrition. As shared by respondents: *"We get lot of strength when we consume indigenous foods and they help us to improve the blood production in our body*" (Respondent number 1, male, study village 1, Torpa block, 21st June, 2019).

Resilience of IFs Amidst Climatic Variability

Apart from having desirable flavor and nutritional characteristics, the IFs are also perceived to be adaptable to changing climate conditions, topography and soil. The community reported the cultivation and consumption of different IFs that provide assured yield, irrespective of the climate situations. These included drought-resistant, indigenous rice varieties (*Laldhan, Reicibaba, Mansoridhan, Gitil baba, Dhusridhan,* and *Karangadhan*), which require less water. Certain indigenous varieties of pulses, such as Black gram ("*Rambada/Urad*"*: Vigna mungo*) and Horse gram (*Kulthi*), are reported to be cost-effective as their cultivation require minimal manpower (labor) or supplementary inputs (chemical fertilizers). For these reasons, these pulse crops are grown by almost every household in the community and are frequently incorporated in the main meals. The seeds of some indigenous rice and pulses are also reported to be insect-resistant. The community also reported accessing certain IFs that are available in forests and surrounding areas amidst adverse climatic conditions. These include specific varieties of indigenous roots and tubers like *Jat sanga* and GLVs like Amaranth leaves (*Leped ara),* Katai leaves ("*Sarla ara*": *Meyna pubescens*), Mata leaves ("*Mattha ara*": *Antidesm aacidum*), Phutkal leaves ("*Phutkal ara*": *Ficus virens)*, Pot Cassia *("Chakod ara": Senna obtusifolia),* Garkha ("*Sirgiti ara*"*: Celosia argentea) and* Hurhura *("Charmani ara": Cleome monophylla).*

Local Practice of Food and Seed Preservation and Conservation Within the Community

Due to the desirable taste and perceived nutritional benefits of IFs, the community reported a common practice of preservation

and conservation of these foods. This local practice mainly involves the preservation of flavorsome varieties of GLVs, fruits and vegetables that have limited seasonal availability throughout the year. In case of GLVs, various indigenous varieties such as Phutkal leaves (*Phutkal ara*), Koinaar leaves (*Sing ara*), Beng leaves (*Chokke ara)*, Sweet potato leaves ("*Sanga ara*": *Ipomoea batatas*), Garlic leaves ("*Lehsun ara*": *Allium sativum*) and many others, are preserved using traditional methods of sun-drying. These sun-dried GLVs are reconstituted and cooked with rice water (*Maad*) and eaten as a curry along with rice. On the other hand, indigenous fruits like "*Dahu" (Artocarpus lakoocha)*, Ambada *("Amda*": *Spondias pinnata*) and Zizyphus (*Godaari)* are preserved using pickling methods. These fruit pickles are usually consumed alongside main meals, to add flavor.

In addition to IF preservation, the community also preserves the indigenous seeds of rice and pulses through a sun-drying technique. These sun-dried seeds are often wrapped in *Neem* leaves and stored in sacks till their use in the next sowing cycle.

Cultural Practices of Using IFs in Festivals and Special Occasions

Another factor that promotes the inclusion of IFs is their cultural value and importance among the Munda community. Mundas are intricately connected to their local cultures and traditions, some of which involve the use and consumption of different varieties of IFs during festivals and special occasions. For example, in the local festival of "*Sohrai,*" the community consumes various rice-based delicacies that are prepared using indigenous rice varieties. During special occasions like "*Annaprashan,*" [celebrated to mark the initiation of complementary feeding when a baby turns 6 months old) the baby is usually served a local dish "*Khichdi,*" which is prepared using indigenous rice, black gram (*Rambada/Urad)*, along with chopped leafy vegetables such as Koinaar leaves (*Sing ara*) and Amaranth leaves (*Leped ara*)]. Apart from these occasions, a local community tradition called "*Puwal*" (practiced after rice harvesting) also promotes the IF consumption. During "*Puwal,*" the entire village hosts a feast in which, mainly indigenous rice, pulses, vegetables and animal meats are served and consumed.

Barriers to Indigenous Food Consumption

Despite several facilitators associated with IFs consumption, a large number of IFs (55%) were reportedly under-utilized in the community. Based on our conceptual framework, we identified three main barriers toward the consumption of IFs. These include: (i) local climatic impact on agroforestry systems leading to reduced IF production, availability and consumption, (ii) easy access to foods purchased from local markets and/or distributed under government food security programmes, and (iii) promotion of high-yielding hybrid varieties by local agricultural organizations for food security.

Local Manifestation of Climate Variability on Agroforestry Leading to Poor IF Production, Access and Consumption

Local climate variability emerged as one of the main barriers for IF production and consumption. The Munda community reported erratic rainfall pattern, which includes a short rainy season followed by long periods of dry season. These climate-change induced events have significantly influenced the farming patterns of the community, which is mostly rain-fed. Due to low rainfall, water scarcity has become a major crisis in the region, leading to acute water shortage for crop irrigation. This has resulted in delayed farming cycles along with reduced productivity of both farm and kitchen garden produce. As shared by one respondent: *"Our farming is totally dependent on rain water. If rainfall is sufficient, then our farm produce is sufficient. If rainfall is inadequate, then our farming suffers*" (Respondent number 2, male, study village 1, Torpa block, 21st June, 2019).

Water scarcity has also impacted the crop diversity, with many people switching from multi-cropping to mono-cropping pattern: *"Earlier we used to cultivate a lot of crops in our farms and kitchen gardens, but now we cultivate less crops because of water shortage "*(Respondent number 4, male, study village 1, Torpa block, 21st June, 2019). Since the last two decades, the community has been mainly engaged in paddy cultivation, in contrast to earlier times, when different varieties of indigenous crops such as millets, GLVs and vegetables were cultivated in farms and kitchen gardens. One respondent stated: *"Due to less rainfall, we are not able to cultivate crops at the right time. Last year, due to shortage of water for crop irrigation, we couldn't grow many crops on our farm lands. The yield of the cultivated crop (paddy) was also very less"* (Respondent number 1, female, study village 4, Murhu block, 22nd June, 2019).

The local forests, that were once home to rich vegetation and biodiversity, have also been affected. The reduced and infrequent patterns of rainfall are gradually leading to forest degradation, because of which, there is reportedly a declining availability of several wild foods in the region. Some of the foods which are not consumed any more due to the loss of biodiversity include indigenous varieties of fruits such as Banyan (*Baadi)*, Piar ("*Tarom*": *Buchanania lanazan), "Loa" (Ficus racemosa),* Bhui-gular *("Aanri*": *Ficus semicordata*), vegetables like Kachnar flower (*Burju Baha)*, Jirhul ("*Hutarba*": *Indigofera cassioides*), GLVs like Hirmicha leaves ("*Muchdi ara*": *Enhydra fluctuans),* Karchul leaves ("*Lundi ara*": *Butomopsis latifolia)*, Kantha leaves (*Kantha ara),* Punarnava (*Kecho ara)* etc., roots and tubers like Tapioca (*Adel sanga), Hasaer sanga, Koolerumpa, Maisaranga* (classification NA), mushrooms like *Patkaud, Kurthiud, Bunumud* (classification NA) etc. and wild birds and animals like Porcupine ("*Jikki":Erethizon dorsatum),* Spotted Dove *("Putam":Streptopelia chinensis), Duhur, Sursuri and Askal* (classification NA). The local water bodies have mostly dried up, resulting in poor availability of indigenous fishes such as Gangetic mud eel ("*Noya*": *Monopterus cuchia), Binghayi* and *Linda* (classification NA). All these factors have thus markedly reduced the IF production and access within the community, collectively leading to reduced consumption and use of diverse IFs in the local diets. For instance, one respondent said: *"Now, since it rains less, the crop production is less. Due to this, the availability of food decreases at home*" (Respondent number 3, male, study village 2, Murhu block, 20th June, 2019). While another respondent said: *"We eat very less amount of local (indigenous) pulses and green leafy vegetables. Now everyone eats potato along with rice, Because of this, the*

situation of food consumption is becoming worse" (Respondent number 1, female, study village 4, Murhu block, 22nd June, 2019).

Easy Access to Non-indigenous Foods From Markets and Food Security Schemes

Due to climate impacts on agroforestry, many community members are unable to utilize the farm and forest produce for household consumption as well as income generation. In view of this, the community has started adopting alternative sources of livelihood such as wage laboring, working in factories, shops, hotels etc. The income generated from these jobs is mostly utilized for purchasing foods from local markets, which mainly comprise of non-indigenous pulses (green gram and lentils), vegetables (brinjal, cabbage, cauliflower, tomato, onion), GLVs (spinach and bathua leaves), and roots and tubers (potato). Moreover, the tribal community has access to food distributed under PDS, which supplies them with non-indigenous rice (and other food commodities such as wheat, sugar and salt) at highly subsidized rates. With diminished access to forest foods and increasing dependence on foods procured from markets and food security schemes, many people are failing to include IFs in their daily food basket. As one respondent said: *"Since the farming yield has reduced, we need to do labor work to earn money so that we can buy food from market and eat"* (Respondent number 2, male, study village 2, Torpa block, 23rd June, 2019).

Increasing Exposure to Hybrid Crop Varieties

The low crop yields associated with indigenous seeds and increased emphasis over modern farming methods by the local agricultural organizations, have led to changes in the traditional subsistence farming practices of Munda community. As a consequence, local farmers are utilizing the high-yielding hybrid seeds and chemical fertilizers for better farm productivity. For instance, one respondent said: *"…. Mainly hybrid paddy is being cultivated as compared to indigenous varieties because it gives twice or thrice more yields than indigenous varieties"* (Respondent number 2, male, study village 2, Murhu block, 20th June, 2019).

Since hybrid seeds provide better yield than indigenous seeds, the production of many indigenous crops have drastically reduced and/or ceased. For example, certain indigenous varieties of rice such as *Raajdhan, Daanidhan, Kanaaudhan, Raasdhan, Minjri, Jolpo baba, Dondo baba, Chorayagoda, Hengdahgoda, Hathipanjardhan, Jhilli baba, Heselsar, and Pasoda baba*, pulses like Munmuna (*Baturi Dal*) and millets like Finger millet (*Kodde*), Pearl millet (*Gaangi)*, Little millet (*Gondli),* Sorghum (*Jowar)* which are known for their nutritional benefits, are no longer cultivated and consumed by the community, due to their reportedly low yield in comparison to hybrid crop varieties. All these factors are contributing to declining IF production, which further translates into their reduced consumption within the community. As shared by one of the respondents: *"…..Yes, there is change in our consumption patterns. Earlier we used to eat local (indigenous) grains, but now we eat hybrid grains*" (Respondent number 5, male, study village 4, Torpa block, 22nd June, 2019).

DISCUSSION

TEK regarding a diverse range of IFs was observed within the Munda tribal community. These included several varieties of indigenous rice, GLVs, flesh foods, mushrooms, fruits, other vegetables, roots and tubers and pulses. The community accessed these IFs mostly from the cultivated and wild food environments i.e., farmlands, kitchen gardens, open fields, roadsides, wastelands, local water bodies, and forests. Despite awareness about several IFs, less than half of these were routinely consumed. Nonetheless, several of the routinely consumed as well as little used foods were found to be rich sources of micronutrients. Enquiries on factors favoring the consumption of IFs and probable barriers revealed specific socio-cultural and environmental factors.

Presently, rice is predominantly cultivated and consumed as a staple in the villages inhabited by Mundas. The community however, reported the historical cultivation and consumption of pearl millet, finger millet, little millet, and sorghum in the region. A similar trend on consumption of limited variety of cereals and diminishing consumption of diverse coarse cereals is observed in other tribal communities of Jharkhand as well as across India (23, 40, 57). Loss of coarse cereals like millets from the habitual Indian diet have been reported to have substantially reduced iron intake in the population, particularly in states where rice has replaced coarse cereals (58). Though our study community continued to grow several varieties of indigenous rice, hybrid varieties contributed to a major part of their produce as well as consumption. This practice of cultivation of high-yielding hybrid varieties of rice in this tribal community is consistent with the overall pattern of paddy cultivation among tribal groups, and, in general, in the state of Jharkhand. The available data from the state shows that only around 13 % of the total production of rice is contributed by traditional indigenous varieties (59). In this context, it is important to recognize that traditional pure lines are required to be preserved even for developing hybrid varieties with desirable traits. Due to the agricultural modernization throughout the country, the state of Jharkhand is also witnessing a fast decline of traditional varieties, which may have implications on the traditional cultivation systems of tribal communities (including Mundas), leading to the erosion of rich genetic diversity as well as the ancestral knowledge of preserving the seeds of traditional varieties (23, 57, 60–62). This may also compromise the socio-ecological resilience of indigenous communities (20). Further, studies from this region (including the present study) have also documented higher nutritional value of these indigenous rice varieties (63–65). Thus, there is an urgent need to conserve the indigenous varieties that are still cultivated, albeit, in lesser proportion of lands, by this tribal community. This community needs to be supported and empowered with knowledge and technology to promote and revive the cultivation of indigenous varieties with enhanced and assured yields.

Apart from cereals, the community also reported routine consumption of indigenous GLVs, roots and tubers, pulses and/or flesh foods; similar dietary patterns have been observed

in other tribal communities of Jharkhand (40, 45, 57, 66). Many of the IFs reported in the study were found to be rich sources of proteins, vitamins and minerals especially iron, calcium, vitamin A, vitamin C and folic acid. The rich nutrient content of IFs consumed by Indian tribal communities have also been documented in other studies (23, 40, 45, 67, 68). A study on IFs of the Munda tribal community has documented consumption of some of the nutrient rich IFs in their routine diets. These included GLVs like Amaranth (*Amaranthus* spp.), Malabar spinach (*Basella alba*), Kangkong (*Ipomoea aquatica*), and Chenopodium (*Chenopodium album*), Moringa (*Moringa oleifera*), *Bauhinia* spp. and *Hibiscus sabdariffa* (69) which were also identified in the present study.

Despite a rich TEK of several IFs sourced from the natural food environment, a large proportion of these were infrequently used or historically consumed by the Mundas. Similar paradoxical findings on awareness about IFs and yet their poor consumption have been reported in studies conducted on Munda as well as other tribal communities of Jharkhand (23, 30, 57, 60).

The informal food literacy acquired from traditional knowledge associated with food, including agro-ecological knowledge (where and what type of food is produced), cultivation and production knowledge (how food is produced), and processing and consumption knowledge (how food is prepared and distributed) derived informally from people's everyday practices in home and community environments (70) can play a vital role in the maintenance and revival of traditional food systems. The Munda community was aware of the superior taste, nutritional, cultural, and agro ecological attributes of their IFs. Therefore, it is crucial to systematically explore and document these factors, and reinforce the informal food literacy with structured curriculum-based formal learning environments, which can facilitate value addition and resulting production and consumption of these foods by the coming generations. The community was aware of the climate resilient indigenous varieties of crops that they were cultivating. Literature suggests that indigenous farmers and local people perceive the impacts of climate change in their own ways and prepare for it through various adaptation practices (71). It is well documented that the adaptation of modern day agriculture to climate change would depend on the conservation and introduction of crop's wild relatives from the rich "native"/indigenous bio-diverse stocks that are managed by these indigenous people globally (72). In India, many indigenous communities, e.g., the Kondhs (inhabiting the forest villages in Koraput, Rayagada, Kandhamal, and Kalahandi districts of Odisha), who are unaware about the scientific premise of indigenous farming methods for their invaluable contribution in creating a climate resilient food systems, are effectively resisting climate change and maintaining the quality of their soil while protecting biodiversity in their local regions (73). In Arunachal Pradesh, the Adi tribe accesses several indigenous plant and animal species from diverse ecosystems, based on their sound knowledge of local biodiversity, and apply traditional agronomic, cultural, and harvest strategies to conserve and sustain their natural resources against abrupt weather anomalies (20, 74–78).

The changing agrarian practice of mono-cropping pattern along with diminished cultivation and production of indigenous cereals owing to water scarcity that reportedly resulted from impacts of local climate change in the region was a barrier to IF consumption. The climate change has also affected the availability of wild foods from forests and other natural environments. The declining availability of IFs is also being observed in other regions of Jharkhand (79, 80), which is worrisome from both nutritional and ecological point of view. Promotion of high yield varieties of paddy by agricultural organizations and reliance on non-indigenous commercial foods available in open market and mono-diets distributed under the government food security program were further identified as other barriers. Studies have documented the impact of climate uncertainties on indigenous farming practices that go beyond reductions in yield and influence how farmers make choices about the timing of planting, soil management, and the use and spatial distribution of narrow range of crop varieties (81, 82). Furthermore, household processing techniques to preserve and detoxify native foods rely on key environmental and climatic resources, which may be vulnerable to climatic shifts (81). Studies have also documented the role of agricultural organizations in limiting IF production as well as consumption (80, 83). Apart from changing agricultural production, it is well documented that improved access to market has led to consumption of non-indigenous market foods (usually rich in sugar, fat, and salt) as part of the daily diets of tribal communities in Jharkhand (84). The shift from consumption of nutrient-dense IFs to energy dense foods is indicative of the ongoing nutrition transition, a trend that has also been reported among other indigenous communities of India as well as globally (85–87). Changing preferences toward consumption of market foods, overdependence on PDS, along with shift toward mono-cropping patterns, may not only result in reduced consumption of nutrient dense IFs, but may also collectively impact the local, resilient food systems of these communities.

STUDY LIMITATIONS

Although we analyzed and compiled nutritive values for 102 IFs of Munda community, owing to limitations around seasonal availability and accessibility of some foods, taxonomic classification could not be performed for all IFs, as a result of which, their nutritive values could not be documented. Moreover, though several IFs were found to be rich sources of nutrients, it is important to assess the anti-nutritional components and toxicity levels in these foods. Nonetheless, these gaps can be addressed by conducting future research in the study community.

CONCLUSION

The Munda community reported a diverse food system, demonstrated TEK about several IFs, yet had underutilization of IFs in their daily diets. The perceived socio-cultural value assigned to IFs was an important facilitator to their

consumption while environmental factors like climatic shifts and the resulting influences on the agroforestry systems emerged as potential barriers. The food systems of Munda tribes can potentially contribute to biodiversity conservation with low energy inputs and climate change mitigation. This may prove to be a time tested model contributing to maintenance and propagation of modern sustainable agriculture systems, which may be key to achieving SDG 2 targets of eliminating hunger and malnutrition and improving sustainability of food systems (6). Relevant measures are required to gear the transformation toward more localized, nutritious and climate-resilient food and production systems. Supportive policies and the grassroots developmental and agricultural extension initiatives can play an instrumental role in ensuring improved IF production, access and utilization, through a range of activities. Nutrient-rich IFs need to be incorporated in government-run supplementary nutrition programmes (MDM, ICDS, and PDS) for increasing IF consumption, which will not only address the nutritional vulnerability of the tribal populations, but will also create more demand for locally produced foods. Creation of community seed banks for indigenous seeds distribution, education on sustainable farming methods for conservation of local landraces as well as drought-resistant varieties, supporting communities for establishing home gardens and conducting nutrition education sessions that reinforce TEK and raise interest about IFs and their nutritional significance should be encouraged (7, 78). All these actions are necessary elements for preserving resilient, nutritious and sustainable food systems.

In conclusion, our findings indicate the importance of retaining and reinforcing TEK and informal food literacy about IFs among Munda community while promoting and supporting climate resilient attributes of their IF systems.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

All procedures involving humans in this study were approved by the Institutional Ethics Committee at Indian Institute of Public Health-Delhi, Public Health Foundation of India and All India Institute of Medical Sciences, New Delhi. Administrative approvals from authorities at district level was also taken. Written informed consent was obtained from all participants who were literate. Third-party witnessed verbal consents were obtained from illiterate participants.

AUTHOR CONTRIBUTIONS

SG-J and AS conceived and designed the study with overall supervision from JF. SG-J, RK, SB, and AS supervised the entire data collection process. GS, RK, and SB did the data analysis. SG-J, RK, and SB prepared the first draft of the manuscript. AS, GS, SD, and JF critiqued and modified the draft. SG-J had final responsibility for the decision to submit for publication. All authors read and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.600470/full#supplementary-material

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Tecticornia sp. (Samphire)—A Promising Underutilized Australian Indigenous Edible Halophyte

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Salinization is gradually increasing over cropping soils and is challenging Governments in many countries, including Australia. There has been a high demand for utilizing arid and semi-arid land for sustainable food production. Currently, the main crops and forage plants are salt sensitive, while halophytes can tolerate a wide range of salinities. Samphire is an Australian indigenous edible halophyte and belongs to the genus *Tecticornia*. It is an underutilized, succulent plant growing on arid or semi-arid land. Most samphire species have a long history of use as food, but also as non-food (fodder and medicine), among indigenous communities in Australia, while scientific information is limited on their nutritional composition and potential bioactivity. The present study reports, for the first time, the nutritional composition, bioactive compounds (phytochemicals) and antioxidant capacity of six Australian grown samphire from different locations. The results showed that celosianin II and isocelosianin II could be identified as the predominant betalains (phytochemicals) in pigmented samphire species. Proximates and fiber varied significantly ($p < 0.05$) between the samphire species with a highest value of fiber of 46.8 g/100 g dry weight (DW). Furthermore, samphire could be identified as a valuable source of essential minerals and trace elements, such as iron (41.5 mg/100 g DW), magnesium (1.2 g/100 g DW) and sodium (16.7 g/100 g DW). The fatty acid profile, mainly palmitic, stearic, oleic, linoleic and α-linolenic acid, was similar among the studied species. Total phenolic content and DPPH-radical scavenging capacity were different ($p < 0.05$) between the six samphire samples. These initial results are very promising and indicate that Australian grown samphire may have the potential to be utilized as a functional food ingredient.

Keywords: halophyte, samphire, Australian indigenous, salinization, nutritional composition, phytochemicals, food

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INTRODUCTION

Throughout history, humans have attempted to utilize various natural produce for food solely to satisfy hunger (an essential need) at first; later they had choices to select in times of surplus, and they learnt how to produce food in terms of cooking and/or preservation through evolution (1, 2). Plants or plant-based products were gathered by First Peoples

with various objectives such as food, folk medicine, clothes and rituals. Until agriculture was invented, humans were identified as hunter-gatherers during the paleolithic era. They mostly had a vast knowledge about native edible plants, varieties and variety of applications in the then modernized civilization. Unfortunately, this knowledge has gradually declined over time after a few generations of acculturation in Indigenous or Aboriginal Communities in various parts of the world (3). Today, most native edible plants are underutilized even though they have immense nutritional value for the community, while they may be still consumed in other parts of the world.

Underutilization of natural resources, the salinity of soil and water along with food insecurity, has become a major concern worldwide. It has been reported that 20% of the total farming land (45 million ha) are salt affected worldwide (4). It was estimated that salinity affects ∼52.7 million ha (Mha) in Asia, 14.8 Mha in Africa, and 0.9 Mha in Australia (5). According to the National Land and Water Resources Audit (2001), ∼1.8 Mha corresponding to 10% of the total farmed area is affected by dryland salinity in South-Western Australia, and another 6 Mha is at risk (6). Therefore, there is a high potential for utilizing saline lands for sustainable food production in the future.

Genus *Tecticornia*, Australian indigenous edible halophytes, commonly recognized as samphires, comprise 44 species endemic to Australia (7–9). *Tecticornia* sp. is an underutilized, succulent plant distributed in arid or semi-arid lands belonging to the subfamily Salicornioideae of the family Amaranthaceae. This family includes several striking economically important food crops such as spinach, beets, chard and quinoa (10). Remarkably, the subfamily Salicornioideae encompasses 110 species in 11 genera including *Sarcocornia* and *Salicornia* (8) with a broad range of climatic adaptations. Notably, *Tecticornia* sp. also expresses high salinity tolerance and thrives in flood, saline and drought conditions like other halophytes.

Halophytes consist of 1% of the world's flora that can tolerate salinity stressed environments through various mechanisms, while 99% of other plant species are supressed (11). For instance, *Tecticornia* sp. possess a variety of adaptations such as formation of compatible solutes (glycinebetaine), adventitious root, accumulation of salt in the tissues and selectivity in shoots for K^+ over Na^+ (12–14) to survive in environments with higher degrees of salinity. Unfortunately, studies which have investigated *Tecticornia* sp. have been largely limited to salinity tolerance (12, 15, 16) and their nutritional profile and potential bioactivity have not been explored to the same extent as other samphire species (*Salicornia* and *Sarcocornia* sp.).

The fleshy leaves and young green shoots of samphire are crunchy in texture with fresh sea-salt flavor. It is still consumed by indigenous people as part of a meal or complimentary meal without any processing, or quickly blanched and tossed with olive oil, vinegar or lemon, added to a meal as a salt substitute or served with seafood. Though *Tecticornia* species have been utilized by the Indigenous Australians for centuries as food, animal feed and as conventional medicine, this is no longer reported as physical evidence. However, a few studies have explored its medicinal properties. Bhanuvalli et al. (17) have recently examined the diuretic, analgesic, and anti-inflammatory properties of *Tecticornia* species (formerly *Halosarcia* sp.), collected from India. Later, *Halosarcia indica* was utilized in the production of low salt dried fish with value addition (18).

Remarkably, a few important chemical compounds are limited to halophytes with high potential to be used as functional ingredients (19, 20), which make them even more appealing to investigate further on nutritional properties. Besides the ecological importance of *Tecticornia* sp., investigations regarding its potential as functional food or functional (food) ingredient are very limited. Therefore, to bridge this gap, the present study provides the first comprehensive analysis of the nutritional composition, bioactive compounds (phytochemicals) and antioxidant capacity of six Australian grown samphire collected from different sublocations in the Kimberley Region of Western Australia.

MATERIALS AND METHODS

Materials

Fresh samples of *Tecticornia* sp. (**Figure 1**) were supplied by the Indigenous Community of Twin Lakes Cultural Park (Kimberley, WA) in 2019. The leaves and young twigs were freeze-dried and ground into a fine powder by using a MM 400 Retsch Mixer Mill (Retsch, Haan, Germany) and stored in airtight containers at −35°C for further analysis. All samples were identified and reference voucher numbers were given by the Queensland Herbarium, Botanic Gardens Mt Coot-tha, Brisbane, QLD, Australia (**Table 1**).

Reagents

Phenolic standards including gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (HPLC grade) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Betanin (>99% purity) was purchased from Adooq Bioscience LLC (Irvine, CA, USA).

Proximate and Mineral/Trace Element Analysis

Proximate analysis was conducted (on the lyophilized powder) at the School of Agriculture and Food Sciences, The University of Queensland, St. Lucia, QLD, Australia. The analysis were conducted according to the Association of Official Analytical Chemists (21) methods as follows: dry matter (method 925.10), crude protein (method 990.03), crude fat by Soxhlet extraction (method 960.39), crude ash (method 923.03), and neutral detergent fiber (method 962.09). Soluble carbohydrates (glucose) and starch were measured using an enzymatic method (22, 23). Minerals, trace elements and heavy metals were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (24). The results of proximate composition and minerals were expressed as a percentage (%) on dry weight (DW) basis while trace elements and heavy metals were expressed as mg/kg DW.

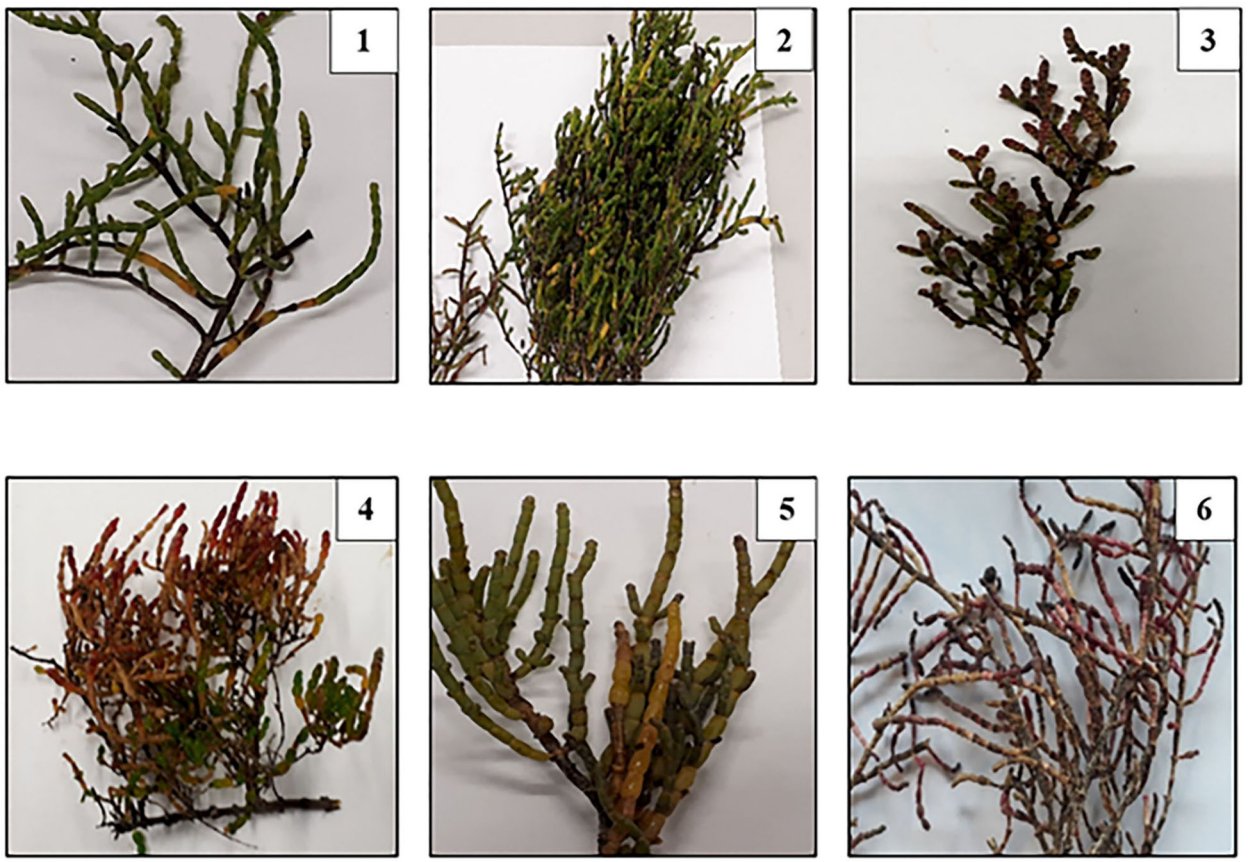

FIGURE 1 | *Tecticornia* species collected from different sub-locations. 1: *T. halocnemoides*; 2: *T. halocnemoides*; 3: *T. halocnemoides*; 4: *T. halocnemoides*; 5: *T. indica*; 6: *T. halocnemoides*.

Fatty Acid Analysis

Lipid Extraction

The extraction of lipids followed Bligh and Dyer (25) and Ryckebosch et al. (26), with modifications. Briefly, ∼0.2 g of dried sample material was mixed with methanol for 5 s using a vortex mixer (Fisher Biotech, Perth, WA, Australia). Then the samples were placed in a sonication bath (ELMA ultrasonic bath, Techspan, Brisbane, QLD, Australia) for 15 min at room temperature (RT). Chloroform ($CHCl_3$) and Milli Q water were added to the sample and vortexed for 5 s. The sample was then centrifuged at 800 rpm for 5 min at RT (Eppendorf Centrifuge 5804, Eppendorf, Hamburg, Germany). The upper layer was removed, and the lower layer was collected into pre-weighed containers using a Pasteur pipette. The remaining pellet was re-extracted with chloroform and methanol mixture (1:1, v/v). The mixture was centrifuged at 800 rpm for 5 min at RT and the supernatant was collected and combined with the previously collected top layer. Finally, the combined solution was evaporated at 45°C under nitrogen flow. The extraction was performed in triplicate.

Fatty Acid Methyl Ester Analysis

The lipids and free fatty acids extracted from *Tecticornia* sp. were derivatized to the corresponding fatty acid methyl esters (FAME) and analyzed according to Chua et al. (27), with some modifications. In brief, 40 μL of the resuspended lipid solution (10 mg/mL in chloroform) was mixed together with 40 μL of heneicosanoic acid (C21) (2 mg/mL in isooctane) and 500 μL of 5% acetyl chloride in methanol in a thermal vial. Then the samples were heated at 95°C for 1 h and subsequently cooled to RT. After cooling, 500 μL of 0.9% NaCl solution and 500 μL of isooctane were added and vortexed for 5 s. Finally, 180 μL of the top (isooctane) layer was collected and transferred to a GC vial. Methyl nonadecanoate (C19) (20 μL, 1 mg/mL in isooctane) was added into the vials as an internal standard before injecting into a Shimadzu GCMS-TQ8040 system (Shimadzu

TABLE 1 | Identified botanical specimens by Queensland Herbarium.

Sample code	Identified name	Voucher reference no.
UQ19DAM1	*Tecticornia halocnemoides*	AQ952399
UQ19DAM2	*Tecticornia halocnemoides*	AQ952394
UQ19DAM3	*Tecticornia halocnemoides*	AQ952395
UQ19DAM4	*Tecticornia halocnemoides*	AQ952396
UQ19DAM5	*Tecticornia indica*	AQ952401
UQ19DAM6	*Tecticornia halocnemoides*	AQ952424

Scientific Instruments, Sydney, NSW, Australia) using an Agilent DB-23 fused silica capillary column (60 m × 0.25 mm diameter *i.d*, 0.15 μm film thickness; Agilent Technologies, Santa Clara, CA, USA). Helium was used as a carrier gas at a constant linear velocity of 42.7 cm/s. The temperature of the injection port was set at 230°C, and 0.2 μL of the sample was injected in split mode with a split ratio of 10. The gradient temperature program was as follows: 70°C for 1 min, then increased to 170°C at 30°C/min, and an increase to 230°C at 3°C/min. The ion source and interface temperatures of the mass spectrometer were set at 200 and 230°C, respectively. The analysis was set to Q3 full-scan mode with a mass range of 89–400 m/z. The fatty acids were identified using a Supelco 37-component FAME mix standard (Sigma-Aldrich) and verified using the National Institute of Standards and Technology (NIST14) library.

Determination of Total Phenolic Content and DPPH Radical Scavenging Capacity

Extraction

The extraction of the samples was performed as described by Hong et al. (28). Briefly, 0.5 g of dried powder of *Tecticornia* sp. was vortexed with 3 mL of 80% aqueous methanol containing 0.1 M HCl. Then the mixture was shaken using a reciprocating shaker (RP1812, Paton Scientific, Victor Harbor, SA, Australia) for 10 min at 200 rpm and centrifuged (Eppendorf Centrifuge 5804) at 3,900 rpm for 10 min at 4°C. The supernatant was collected and the residue was re-extracted with the extracting solvent, followed by ultra-sonication at 4°C, shaking and centrifugation as described above until the supernatant was colorless. Finally, the supernatants were combined and filtered through a 0.2 μm PP membrane filter prior the determination of the total phenolic content and DPPH radical scavenging capacity. All extractions were performed in triplicate.

Total Phenolic Content (TPC)

TPC was determined employing the Folin-Ciocalteu assay as described previously by Phan et al. (29), using a micro-plate absorbance reader (Sunrise, Tecan, Mannedorf, Switzerland) at 700 nm. TPC was expressed as milligrams of gallic acid equivalents per 100 grams of sample (mg GAE/100 g), based on an external gallic acid standard curve (0–105 mg/L).

DPPH Radical Scavenging Capacity

The methanolic sample extract was evaporated at 40°C, using a miVac sample Duo concentrator (Genevac Inc., Gardiner, NY, USA). The dried extract was re-dissolved in absolute methanol and further diluted to different concentrations for the DPPH assay.

The DPPH radical scavenging capacity was determined as previously described by Moore and Yu (30) with slight modifications using a microplate absorbance reader (Sunrise, Tecan) at 517 nm. The radical scavenging capacity was expressed as μM Trolox equivalents (TE) per g dry weight extract, based on an external Trolox standard curve (5–35 μM).

Determination of Betalains

Extraction of Betalains

Powder samples (0.5 g) were vortexed with 3 mL of extractant (80% aqueous methanol containing 50 mM sodium ascorbate, pH 6.5) as reported previously by Schliemann et al. (31), with slight modifications. The sample mixture was shaken for 10 min at 200 rpm/min by using a RP 1812 reciprocating shaker (Paton Scientific, Victor Harbor, SA, Australia) followed by centrifugation (Eppendorf Centrifuge 5804) at 3,900 rpm for 10 min at 4°C. The supernatant was collected and the residue was re-extracted twice with 3 mL of the extractant. Supernatants were combined and filtered through a 0.2 μm PP membrane filter prior to betalain analysis. All extractions were performed in triplicate.

Analysis of Betalains

Compound separation and chromatographic analysis were performed on an Agilent 1290 Infinity ultra-high-performance liquid chromatography (UHPLC) system (Agilent Technologies, Waldbronn, Germany) equipped with a 1290 Infinity Diode Array Detector (DAD) and a reverse-phase Acquity UPLC BEH C18 column (150 × 2.1 mm i.d., 1.7 μm; Waters, Dublin, Ireland) maintained at 40°C. Mobile phases including A (96% MQ water, 3% acetonitrile, 1% formic acid v/v) and B (1% formic acid in acetonitrile v/v) eluted the compounds at a flow rate of 0.3 mL/min. The injection volume was 2 μL. The elution gradient was performed with 100% of mobile phase A for 1 min as an initial isocratic hold, then 96% A in 11 min and 84.8% A in 5 min, and from 84.8 to 10.9% in the next of 2 min. Then, an isocratic condition was used for 2 min, conditioning 1 min and re-equilibration for 5 min with 100% mobile phase A. DAD spectrum was scanned from 190 to 600 nm. The detection signal was recorded and quantified at 535 nm using betanin as an external standard.

A DIONEX Ultimate 3000 UHPLC system equipped with a UV/Vis detector and a Q Exactive high resolution Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific Australia Pty Ltd., Melbourne, VIC, Australia) was used to confirm the identity of the eluted betalains. The Q-Exactive mass spectrometer was operated in positive mode with full MS and all-ion-fragmentation (AIF) scans at a resolving power of 70,000 full width half maximum, at collation energy of 35 eV. A scan range of 100–1,000 m/z and 80–1,000 m/z was applied for the full MS and AIF scans, respectively. The automatic gain control (AGC) was set at 3e6. Chromatography and elution program were the same as those used for the UHPLC-DAD analysis described above. Peak identities were based on data regarding mass spectrum, fragmentations, calculated accurate mass, and retention time of betalain compounds and compared to previously reported literature. The Thermo Xcalibur™ software (Thermo Fisher Scientific) was used for data acquisition.

Analysis of Vitamin C

Ascorbic acid (L-AA) extraction and analysis was conducted as previously described by Phan et al. (32). Briefly, 0.5 g dried powder of *Tecticornia* sp. was extracted with 3% meta-phosphoric acid containing 8% acetic acid and 1 mM ethylenediamine-tetraacetic acid (EDTA). The reduction of

dehydroascorbic acid (DHAA), which was also present in the extracts/samples, to L-AA was performed prior to UPLC-PDA analysis. Total vitamin C (L-AA + DHAA) was determined using a Waters UPLC-PDA system (Waters, Milford, MA, USA) and a Waters HSS-T3 column (Waters, Rydalmere, NSW, Australia) (100 × 2.1 mm i.d; 1.8 μm; 25°C), with aqueous 0.1% formic acid as the mobile phase (0.25 mL/min) and isocratic elution. An external calibration curve of L-AA was used for quantification.

Determination of Anti-nutritional Components

Hydrolysable Tannins

The hydrolysable tannins in the *Tecticornia* sp. were determined using the potassium iodate assay previously described by Hoang et al. (33). Briefly, 50 μL extract was added to a 96-well plate with 150 μL of 2.5% w/v potassium iodate. Absorbance was measured after 15 min, using a Tecan microplate absorbance reader (Tecan Infinite M200, Mannedorf, Switzerland) at 550 nm. Tannic acid was used as a standard and results were expressed as mg tannic acid equivalents (TAE)/g DW.

Total Saponins

Extraction and quantification of saponins followed the spectrophotometric method described by Phan et al. (34) with modifications. Approximately 0.5 g dried powder of *Tecticornia* sp. was extracted with 10 mL of 80% methanol. Then the mixture was shaken using a reciprocating shaker (RP1812, Paton Scientific) for 1 hr at 200 rpm followed by ultra-sonication at RT and centrifuged (Eppendorf Centrifuge 5804, Eppendorf, Hamburg, Germany) at 3,900 rpm for 5 min at RT. The supernatant was collected and the residue was re-extracted with the extracting solvent while shaking on the reciprocating shaker at 150 rpm overnight. The supernatant was collected after centrifugation (3,900 rpm, 10 min), while the residue was re-extracted twice with 80% methanol (for 15 min). The supernatants were combined and evaporated until dryness at

TABLE 2 | Proximate composition, minerals and trace elements of *Tecticornia* species from different sub-locations.

Plant species	*T. halocnemoides* 1	*T. halocnemoides* 2	*T. halocnemoides* 3	*T. halocnemoides* 4	*T. indica* 5	*T. halocnemoides* 6	Nutritional information
Proximate composition (g/100 g DW)							
Protein	8.6 ± 0.0^{b}	12.6 ± 0.1^{d}	12.5 ± 0.1^{d}	8.9 ± 0.1^{c}	8.7 ± 0.1^{b}	7.6 ± 0.1^{a}	50 g**
Fat	1.76 ± 0.0^{c}	1.77 ± 0.0^{d}	1.86 ± 0.1^{e}	1.84 ± 0.1^{e}	1.07 ± 0.1^{a}	1.45 ± 0.0^{b}	70 g**
Glucose	5.1 ± 0.0^{e}	5.6 ± 0.1^{f}	2.7 ± 0.1^{a}	4.6 ± 0.1^{d}	4.0 ± 0.1^{c}	3.5 ± 0.1^{b}	90 g**
Starch	0.08 ± 0.1^{c}	0.03 ± 0.0^{ab}	0.09 ± 0.0^{d}	0.2 ± 0.0^{e}	0.04 ± 0.0^{b}	0.03 ± 0.0^{a}	310 g**
Fiber	35.1 ± 0.5^{e}	30.8 ± 0.09^{d}	30.1 ± 0.2^{c}	27.8 ± 0.1^{b}	46.8 ± 0.1^{f}	26.4 ± 0.2^{a}	30 g**
Moisture	$59.0 \pm 0.4^{b*}$	$77.0 \pm 0.5^{de*}$	$71.7 \pm 0.6^{c*}$	$78.1 \pm 0.5^{e*}$	$75.5 \pm 0.4^{d*}$	$28.9 \pm 2.1^{a*}$	-
Ash	1.0 ± 0.1^{a}	1.2 ± 0.0^{b}	2.6 ± 0.0^{f}	2.0 ± 0.0^{d}	2.4 ± 0.0^{e}	1.5 ± 0.0^{c}	-
Minerals (g/100 g DW)							
Ca	0.51 ± 0.0^{e}	0.41 ± 0.0^{c}	0.41 ± 0.0^{c}	0.35 ± 0.0^{a}	0.38 ± 0.0^{b}	0.48 ± 0.0^{d}	1.2 g/day AI✓
Mg	0.8 ± 0.1^{c}	0.9 ± 0.0^{d}	0.59 ± 0.0^{a}	1.2 ± 0.0^{f}	0.63 ± 0.0^{b}	1.1 ± 0.0^{e}	0.35 g/day EAR✓
Na	11.8 ± 0.0^{c}	11.6 ± 0.0^{b}	13.3 ± 0.1^{d}	16.3 ± 0.1^{e}	8.8 ± 0.0^{a}	16.7 ± 0.1^{f}	0.46–1.3 g/day AI✓✓
K	1.1 ± 0.1^{b}	1.7 ± 0.1^{e}	1.5 ± 0.0^{d}	1.5 ± 0.0^{d}	0.3 ± 0.0^{a}	1.3 ± 0.0^{c}	4.7 g/day AI✓
P	0.12 ± 0.0^{b}	0.13 ± 0.0^{c}	0.2 ± 0.0^{d}	0.12 ± 0.0^{b}	0.12 ± 0.0^{b}	0.07 ± 0.0^{a}	0.7 g/day AI✓
S	0.5 ± 0.1^{b}	0.67 ± 0.0^{d}	0.69 ± 0.0^{e}	0.8 ± 0.0^{f}	0.3 ± 0.0^{a}	0.6 ± 0.0^{c}	-
Trace elements (mg/kg DW)							
Fe	162.3 ± 3.0^{c}	107.6 ± 4.2^{ab}	112.9 ± 2.3^{b}	160.9 ± 2.9^{c}	101.4 ± 3.7^{a}	414.6 ± 8.0^{d}	8 mg/day RDA✓
Zn	4.9 ± 0.1^{b}	6.3 ± 0.2^{c}	9.8 ± 0.3^{d}	16.9 ± 0.3^{e}	4.2 ± 0.4^{a}	6.5 ± 0.3^{c}	11 mg/day RDA✓
Mn	11.3 ± 0.1^{c}	15.3 ± 0.4^{d}	10.8 ± 0.3^{c}	44.1 ± 0.4^{e}	4.9 ± 0.2^{a}	9.7 ± 0.3^{b}	2.3 mg/day AI✓
Cu	2.9 ± 0.1^{a}	7.0 ± 0.5^{d}	10.5 ± 0.7^{e}	6.9 ± 0.2^{d}	3.7 ± 0.2^{b}	5.4 ± 0.1^{c}	900 μg/day AI✓
Ni	0.03 ± 0.1^{a}	1.5 ± 0.2^{e}	0.6 ± 0.1^{c}	0.9 ± 0.1^{d}	0.8 ± 0.1^{cd}	0.3 ± 0.0^{b}	-
Mo	2.3 ± 0.1^{d}	0.8 ± 0.1^{a}	1.2 ± 0.1^{c}	1.0 ± 0.1^{b}	0.8 ± 0.1^{a}	1.2 ± 0.1^{c}	45 μg/day AI✓
Se	0.1 ± 0.1^{a}	0.1 ± 0.0^{a}	0.33 ± 0.0^{bc}	0.27 ± 0.0^{b}	0.27 ± 0.1^{b}	0.35 ± 0.1^{c}	55 μg/day AI✓
Sr	51.5 ± 0.1^{d}	44.9 ± 1.4^{c}	41.8 ± 1.5^{b}	45.6 ± 0.9^{c}	30.7 ± 1.5^{a}	53.2 ± 0.9^{d}	-
B	36.8 ± 0.1^{a}	49.9 ± 1.6^{d}	40.4 ± 0.8^{b}	46.7 ± 2.1^{cd}	79.0 ± 3.3^{e}	44.2 ± 1.5^{c}	-

Values are means ± SD (n = 3); Means not sharing a common superscript (a–f) within a row are significantly ($p < 0.05$) different.

**-(g/100 g FW); **Daily Intake (38); ✓Nutritional information (39); (-) not available; RDA, recommended dietary allowance; AI, adequate intake; EAR, estimated average requirement; ✓✓Nutritional Information (40).*

40°C in a miVac sample Duo concentrator. The dried extract was redissolved in water and successively extracted with diethyl-ether to remove the pigments, followed by extraction of saponins with saturated n-butanol. The n-butanol extracts were combined and dried under reduced pressure using a rotary evaporator (Buchi Rotavapor R-100, BÜCHI Labortechnik AG, Flawil, Switzerland). The dried extract was redissolved in aqueous methanol 80% (v/v) and subjected to the Vanillin-H_2SO_4 assay using a microplate reader (Sunrise, Tecan) at 544 nm. Oleanolic acid (0–1.0 g/L) was used to prepare an external calibration curve. Total saponins were expressed as mg of oleanolic acid equivalents (OE) per 100 g of sample.

TABLE 3 | Heavy metals in *Tecticornia* species from different sub-locations.

Plant species	*T. halocnemoides* 1	*T. halocnemoides* 2	*T. halocnemoides* 3	*T. halocnemoides* 4	*T. indica* 5	*T. halocnemoides* 6	Nutritional Information
Heavy metals (mg/kg DW)							
Al	108.3 ± 0.0^{d}	56.2 ± 1.5^{bc}	57.0 ± 1.6^{c}	51.3 ± 0.8^{a}	52.3 ± 0.3^{ab}	130.5 ± 5.3^{e}	1.0 mg/kg BW/week TWI*
As	0.1 ± 0.0^{a}	0.2 ± 0.1^{b}	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	0.3 ± 0.1^{c}	0.2 ± 0.0^{b}	128 μg/week for a 60 kg BW TWI**
Cd	0.15 ± 0.0^{b}	0.15 ± 0.0^{b}	0.23 ± 0.0^{d}	0.19 ± 0.0^{c}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	2.5 μg/kg BW/week TWI√
Cr	0.93 ± 0.0^{f}	0.5 ± 0.0^{d}	0.05 ± 0.0^{a}	0.12 ± 0.0^{b}	0.4 ± 0.0^{c}	0.87 ± 0.1^{e}	35 μg/day AI^
Pb	0.6 ± 0.0^{b}	0.4 ± 0.1^{a}	0.8 ± 0.1^{c}	1.0 ± 0.1^{c}	0.9 ± 0.1^{c}	0.9 ± 0.2^{c}	25 μg/kg BW/week TWI¥

Values are means ± SD (n = 3); Means not sharing a common superscript (a–f) within a row are significantly ($p < 0.05$) different.
**European Food Safety Authority (EFSA) (45); **Leblanc et al. (46); √EFSA (47); ^Otten et al. (39); ¥Cheung Chung et al. (48); BW, body weight; TWI, tolerable weekly intake; AI, adequate intake.*

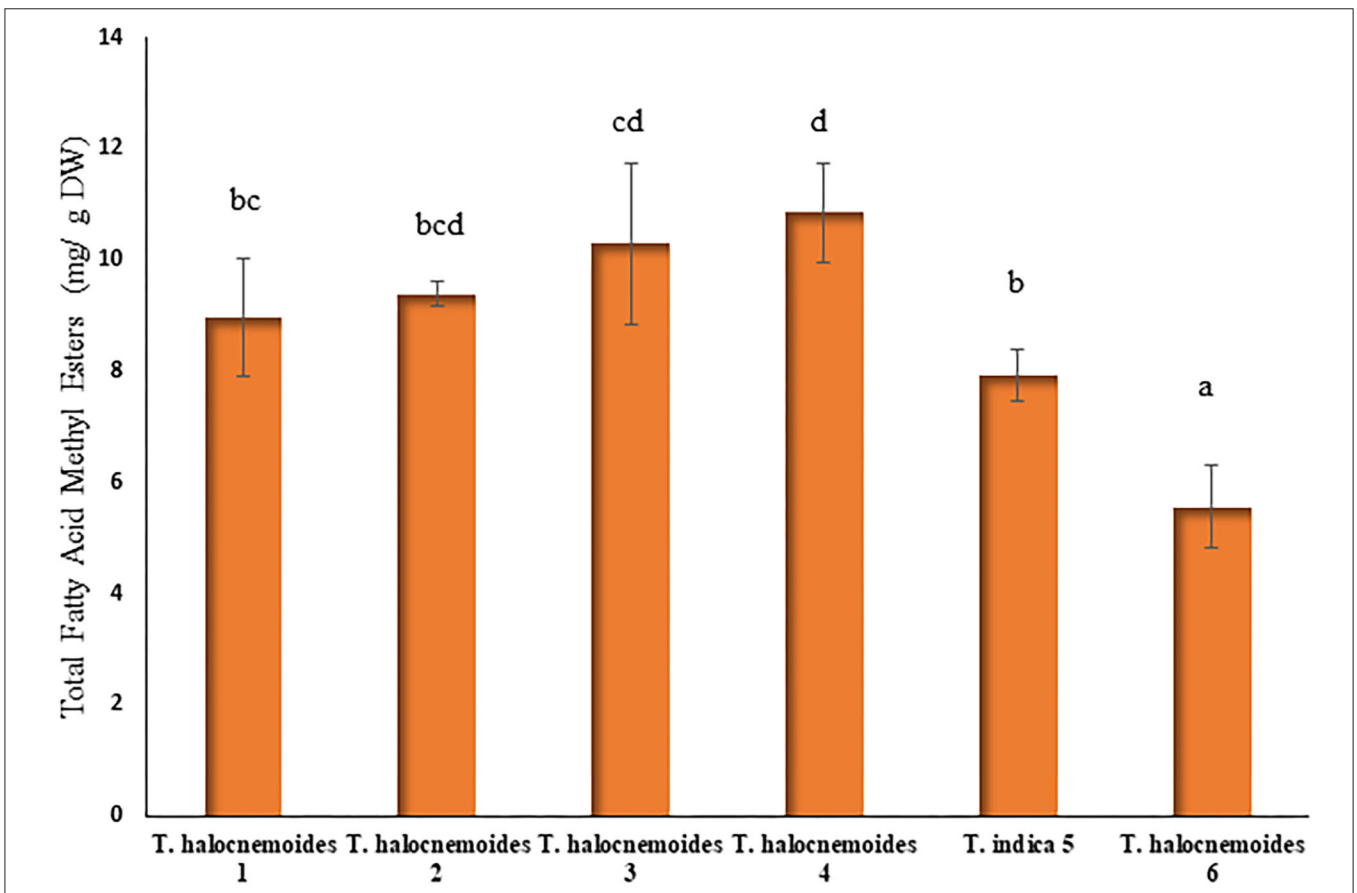

FIGURE 2 | Total fatty acid methyl esters (FAME) concentration (mg/g DW) of *Tecticornia* species from different sub-locations. Data present means ± SD ($n = 3$). Different letters indicate significant differences ($p < 0.05$) in total fatty acid methyl esters among the samples tested.

Phytate Content

Total phytate content was determined according to Joshi-Saha and Reddy (35). Approximately 0.5 g dried powder of *Tecticornia* sp. was added to 10 mL of 2.4% HCl in a centrifuge tube and shaken for 1 h. The mixture was then centrifuged at 10,000 × g at 10°C for 20 min. The supernatant obtained was mixed with 0.5 g NaCl in another centrifuge tube and vortexed for 60 s. The tube was kept in a freezer (−20 C) for 20 min, centrifuged at 10,000 × g at 10°C for 20 min and the clear supernatant obtained was used for the total phytate content assay. Briefly, 1 mL of the extract was mixed with 300 μL of Wade's reagent (0.03% $FeCl_3.6H_2O$ + 0.3% sulfosalicylic acid). The mixture was centrifuged at 4,000 × g at 10°C for 5 min, and the absorbance was read using a microplate absorbance reader (Tecan Infinite M200) at 500 nm. Phytic acid (Inositol hexaphosphoric acid) dodecasodium salt (0–120 μg/mL) was used as a standard and results were expressed as mg/g of phytic acid (PA) on a dry matter basis (36).

Trypsin Inhibitor Activity Assay

The extraction and determination of trypsin inhibitor activity (TIA) was carried out using the method of Liu (37) with some modifications. Approximately 0.5 g dried powder of *Tecticornia* sp. was extracted with 25 mL NaOH. Then the mixture was shaken using a reciprocating shaker (RP1812, Paton Scientific) for 1 hr at 200 rpm. The mixture was allowed to settle for 10 min and the extract was carefully decanted without centrifugation for TIA estimation. For the TIA assay, an inhibitor assay buffer was prepared which contained 20 mM $CaCl_2$ and 50 mM Tris-HCl at pH 8.2. The N-benzoyl-D-L- arginine- p-nitroanilide (DL-BAPA) substrate (0.4 mg/mL) was prepared fresh on the same day in the assay buffer that contained 1% dimethyl sulfoxide solution and pre-warmed at 37°C. An aliquot (2.5 mL) of the DL-BAPA substrate was added to 1 mL of the diluted extract after which 1.0 mL bovine trypsin (20 μg/mL in 1 mM HCl solution containing 5 mM $CaCl_2$) was added and immediately mixed. The whole assay was conducted in a water bath at 37°C. Following incubation for 10 min at 37°C, the color reaction was terminated by addition of 0.5 mL of 30% acetic acid solution. The mixture was centrifuged at 3,000 × g for 10 min and the absorbance for the sample reading (A410S) at 410 nm was a measure of the trypsin activity in the presence of the sample inhibitors. The absorbance was read using a spectro-photo meter (Thermo Fisher Scientific Genesys 20, Melbourne, VIC, Australia) at 410 nm. Concurrently, the reaction was also run in the absence of inhibitors by replacing the sample extract with an equal amount of reverse osmosis (RO) water and reference reading was also recorded as A410R. Furthermore, sample blanks (A410SB) and reference blank (A410RB) were also run by adding the acetic acid solution before the trypsin solution. A trypsin unit is defined as an increase of 0.02 absorbance at 410 nm. The TIA is expressed in trypsin units inhibited (TUI) per mg sample and calculated as follows:

$$\frac{TUI}{mg} = \frac{(CR - CS)\ x\ 100\ x\ mL\ diluted\ extract}{mg\ sample\ per\ mL\ diluted\ extract\ used\ for\ the\ assay\ x\ 2}$$

Where CS (corrected sample reading) = A410S-A410SB.
CR (corrected reference reading) = A410R-A410RB.

Statistical Analysis

The results were expressed as mean ± standard deviations (SD) and analyzed using a multi-variate general linear model (IBM SPSS statistics 26; IBM, Sydney, NSW, Australia). Pearson's correlation coefficient (R) and the coefficient of determination (R^2) was calculated for testing the correlation between the DPPH radical scavenging capacity and TPC. The means were compared using ANOVA and Duncan's multiple range test, and probability was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Proximate, Minerals, and Trace Elements

The results of the proximate composition, minerals and trace elements of the *Tecticornia* species collected from different sub-locations are presented in **Table 2**.

Moisture

The moisture content of the samples from sub-location 6 (28.9 g/100 g) was found to be lowest among the samples tested while the samples from sub-location 4 had the highest value (28.9 vs. 78.1 g/100 g, $p < 0.05$). Moisture content is crucial in terms of the physicochemical properties of processed foods, since low moisture content decreases the susceptibility for

TABLE 4 | Fatty acid profile, expressed as % of total fatty acids on dry weight basis, of *Tecticornia* species from different sub-locations.

FA (%)	Common Name	*T. halocnemoides* 1	*T. halocnemoides* 2	*T. halocnemoides* 3	*T. halocnemoides* 4	*T. indica* 5	*T. halocnemoides* 6
C16:0	Palmitic acid	29.5 ± 0.5^{d}	28.2 ± 0.5^{c}	27.4 ± 0.2^{b}	29.5 ± 0.3^{d}	24.7 ± 0.1^{a}	35.5 ± 0.6^{e}
C18:0	Stearic acid	7.6 ± 0.9^{b}	5.3 ± 0.7^{a}	5.9 ± 1.3^{a}	6.8 ± 0.5^{ab}	7.8 ± 0.2^{b}	11.0 ± 1.3^{c}
∑ SFA		37.1	33.5	33.3	36.3	32.5	46.5
C18:1 (*n*-9)	Oleic acid	20.3 ± 1.9^{b}	15.0 ± 0.9^{a}	16.8 ± 1.9^{a}	20.1 ± 0.5^{b}	19.6 ± 0.3^{b}	23.5 ± 1.8^{c}
∑ MUFA		20.3	15.0	16.8	20.1	19.6	23.5
C18:2 (*n*-6)	Linoleic acid	15.5 ± 1.5^{a}	22.2 ± 1.7^{b}	29.3 ± 2.8^{c}	22.2 ± 1.0^{b}	25.7 ± 0.6^{b}	18.6 ± 2.7^{a}
C18:3 (*n*-3)	α-linolenic acid	27.0 ± 0.8^{d}	29.2 ± 0.4^{e}	20.7 ± 0.3^{b}	21.4 ± 0.4^{bc}	22.2 ± 1.2^{c}	11.4 ± 0.2^{a}
∑ PUFA		42.5	51.4	50.0	43.6	47.9	30.0
∑ PUFA/∑ SFA		1.1	1.5	1.5	1.2	1.5	0.6
n-6/*n*-3		0.6	0.8	1.4	1.0	1.1	1.6

Values are means ± SD (n = 3); Means not sharing a common superscript (a–e) within a row are significantly ($p < 0.05$) different.

microbial growth and undesirable biochemical changes (41). The moisture content of 28.9% in the *Tecticornia halocnemoides* sp. is associated with minimum risk for microbial growth and shelf-life stability during storage.

Protein and Fat

The protein content of the studied *Tecticornia* species (samphire) ranged from 7.6 to 12.6 g/100 g DW. Since we report for the first time on the nutritional composition of *Tecticornia* sp., comparison with literature values for the same species could not be presented. However, when compared with other plants belonging to the subfamily Salicornioideae, the studied *Tecticornia* sp. had higher protein levels than *Salicornia ramosissima* (5.2 g/100 g DW), but lower levels than *Salicornia herbacea* (22.1 g/100 g DW) and comparable levels with *Salicornia bigelovii* (10.2 g/100 g DW) (42–44). A 100 g serve of *T. halocnemoides* sp. contributes to 15–25% of the daily intake of protein for adults [Food Standards Australia New Zealand (FSANZ)] (38). Regarding the fat content, *T. halocnemoides* collected from different sub-locations showed relatively similar values (1.5–1.9 g/100 g DW), and these were significantly ($p < 0.05$) different from *Tecticornia indica* (1.1 g/100 g DW). However, the fat content was similar to that previously reported for *S. ramosissima* (1.9 g/100 g DW) (42).

Ash and Fiber

The ash content of the samples collected from the 6 different sub-locations was different (1.0–2.6 g/100 g DW), reflecting the impact of the growing conditions. In contrast, the ash content of the genus *Tecticornia* was lower than that of *S. ramosissima* (29.2 g/100 g DW), *S. bigelovii* (52.7 g/100 g DW) and *S. herbacea* (8.1 g/100 g DW) (42–44). Interestingly, *Tecticornia* species are rich sources of fiber irrespective of their growing locations. The fiber content of the samples in the present study ranged from 26.4 to 46.8 g/100 g DW. *T. indica* (46.8 g/100 g DW) had a significantly ($p < 0.05$) higher fiber content than *T. halocnemoides* (26.4–35.1 g/100 g DW), collected from different sub-locations. The fiber content of the genus *Tecticornia* was surprisingly higher than that of the genus *Salicornia*. Although *S. ramosissima* (22.5 g/100 g DW) had slightly lower fiber, it can still be considered as a rich source of fiber according to Barreira et al. (42). As reported by FSANZ (38), the AI values of dietary fiber for males and females are 30 and 25 g per day, respectively. Thus, it is no surprise that consuming samphire with other meal components would be beneficial, since the high content of fiber would aid in improving gut health and digestion beyond the food's nutritional "standard" value. Overall, the studied samphire species have the potential to be used as low-energy foods since they are low in fat and carbohydrates, but high in dietary fiber and protein.

Minerals and Trace Elements

The most abundant minerals were sodium (Na), iron (Fe), magnesium (Mg), molybdenum (Mo) and manganese (Mn) in the genus *Tecticornia* (**Table 2**). The sodium content in 100 grams of the *Teticornia* species is substantially higher than

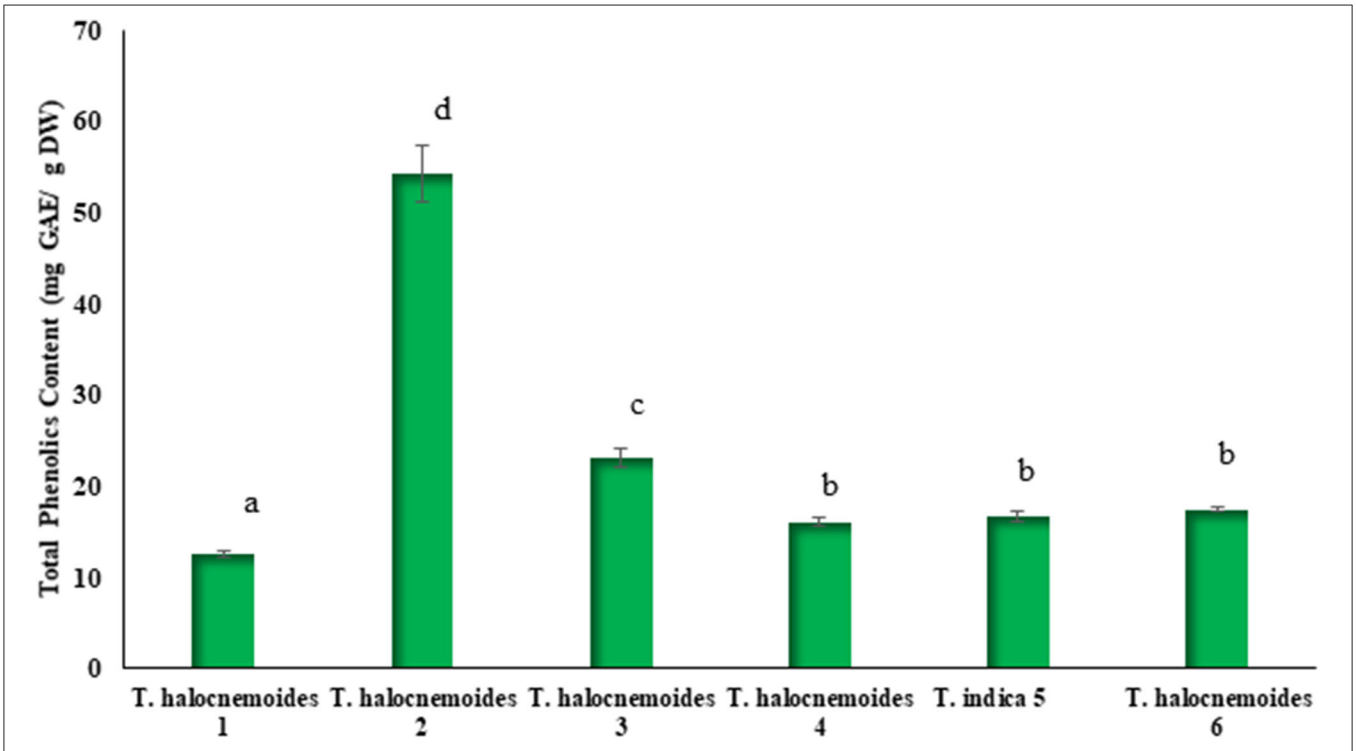

FIGURE 3 | TPC of *Tecticornia* species from different sub-locations. Data present means ± SD ($n = 3$). Different letters indicate significant differences ($p < 0.05$) in TPC between the samples tested.

the recommendations for daily sodium intake internationally (0.46–1.3 g per day) (40). Among the *Tecticornia* sp., *T. indica* (8.8 g/100 g DW) had the lowest amount of sodium, while *T. halocnemoides* ranged from 11.6 to 16.7 g/100 g DW, which could be related to the different growing conditions (sub-locations). Fe was also lowest in *T. indica* (101.4 mg/kg DW). In addition, the minerals in *Tecticornia* sp. were found at comparable levels with that reported for *S. ramosissima* (42). However, certain minerals were higher in *Tecticornia* sp., particularly calcium (Ca) and potassium (K). In general, the studied Australian indigenous edible halophytes are valuable sources of important minerals and trace elements. The high content of minerals present in halophytes is a result of the environment where they grow (high salinity) as well as their potential to "absorb" and accumulate these compounds as postulated by Díaz et al. (44).

Heavy Metals

Concerning the presence of heavy metals, the samples studied contained low concentrations of these elements (**Table 3**). This is important, as heavy metal intake can cause significant health complications (39, 49). Our findings are in agreement with the results reported by Barreira et al. (42). Nutritional information on heavy metals has been added to **Table 3** for extrapolation. However, when plants like halophytes grow in polluted areas, they may accumulate heavy metals at higher concentrations, specifically in their roots.

Fatty Acids and Methyl Esters

The total lipid content (**Figure 2**) ranged from 5.6 to 10.8 mg/g DW which is comparable with the results (1.0–7.27 mg/g DW) reported for other halophytes (50). *Tecticornia halocnemoides* (10.8 mg/g DW; sub-location 4) had the highest lipid content, whilst *T. halocnemoides* (5.6 mg/g DW; sub-location 6) the lowest.

The saturated fatty acids (SFA) in *Tecticornia* species from different sub-locations ranged from 32.5% (*T. indica*) to 46.5% [*T. halocnemoides* (sub-location 6)] of the total fatty acids (**Table 4**). It is interesting that almost all the species had a SFA content <40% of the total lipid content, except *T. halocnemoides* from sub-location 6. The most abundant SFA was palmitic acid (C16:0), which was found to be one of the prevalent fatty acids in other halophytes (50, 51). Besides palmitic acid, stearic acid was also found in those plant species studied. Furthermore, the fraction of monounsaturated fatty acids (MUFA) was significantly ($p < 0.05$) lower than that of SFA and polyunsaturated fatty acids (PUFA) in the studied *Tecticornia* species.

MUFA contributed 15% [*T. halocnemoides* (sub-location 2)] to 23.5% [*T. halocnemoides* (sub-location 6)] to the total fatty acids and oleic acid was found to be the only MUFA in the studied species (**Table 4**). Similar results were reported for *Carpobrotus edulis*, *Arthrocnemum macrostachyum,* and *S. maritima* (50, 52, 53). A significant ($p < 0.05$) variation in PUFA between the *Tecticornia* sp. could also be observed, with

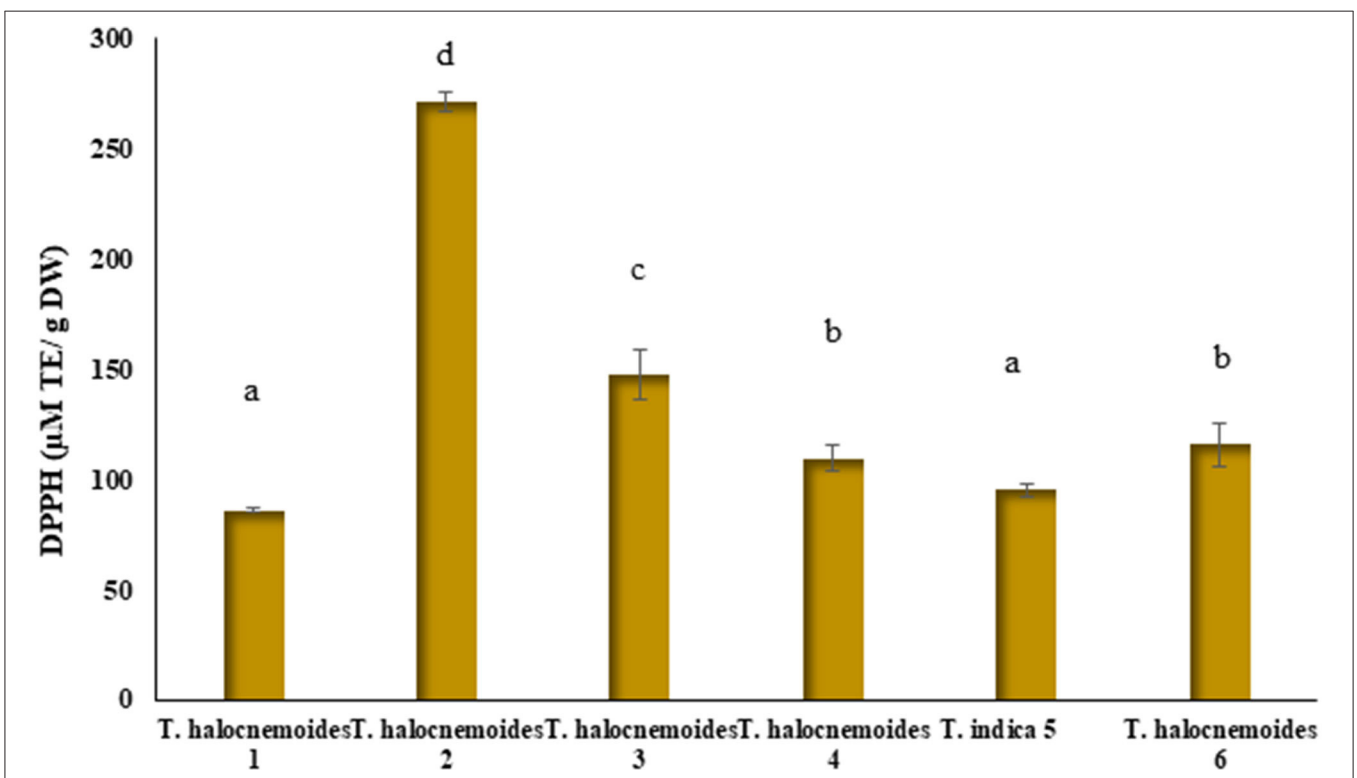

FIGURE 4 | DPPH radical scavenging capacity of *Tecticornia* species from different sub-locations. Data present means ± SD ($n = 3$). Different letters indicate significant differences ($p < 0.05$) in DPPH radical scavenging capacity among the samples tested.

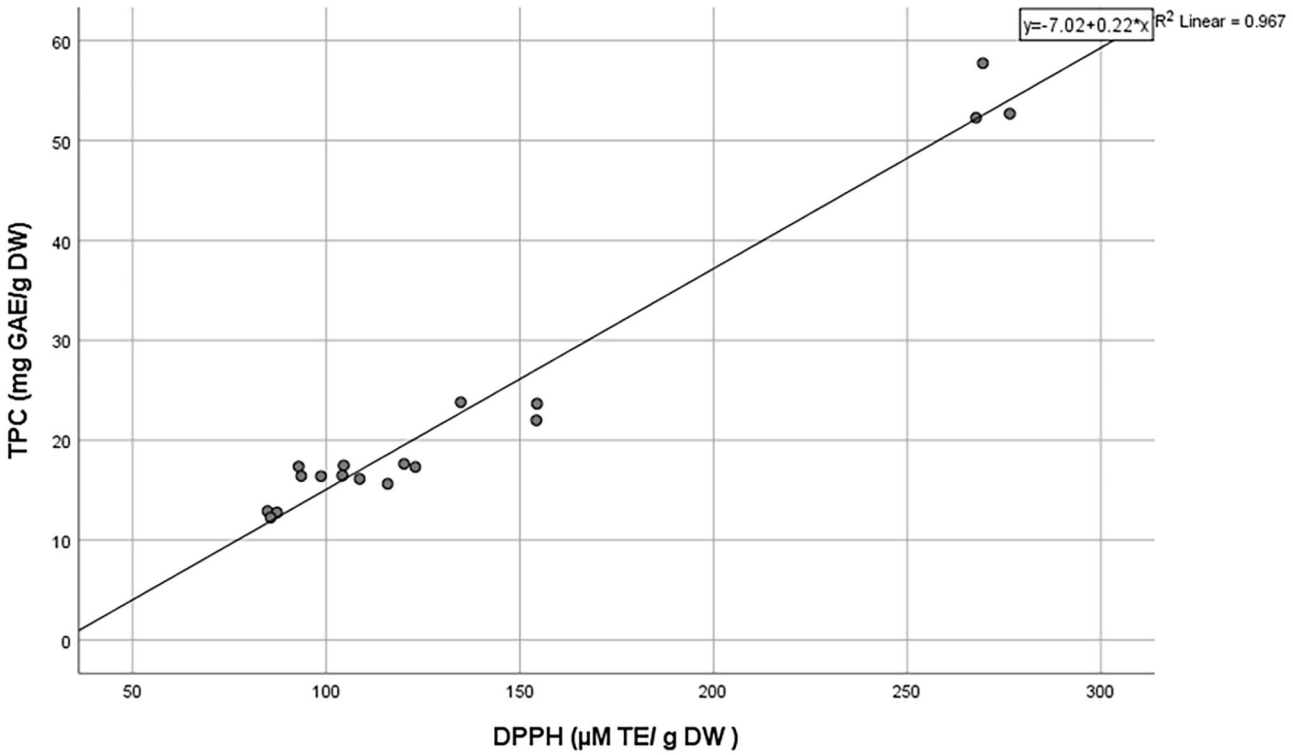

FIGURE 5 | Correlation between TPC and DPPH radical scavenging capacity of *Tecticornia* species collected from different sub-locations.

T. halocnemoides from sub-location 2 having the highest (51.4%) and *T. halocnemoides* (sub-location 6) the lowest (30%) content (**Table 4**).

Except *T. halocnemoides* (sub-location 6), all other samples contained linoleic acid, α-linolenic acid, and palmitic acid as major fatty acids. This was in agreement with previous studies on other halophytes such as C*rithmum maritimum* (51). The highest proportion (29.3%) of linoleic acid was found in *T. halocnemoides* (sub-location 3) and the lowest (15.5%) in *T. halocnemoides* (sub-location 1), whereas *T. halocnemoides* (sub-location 2) had the highest content of α-linolenic acid (29.2%).

Since vertebrates are not able to synthesize linoleic acid and α-linolenic acid, these PUFAs must be delivered through the diet (54). Furthermore, it is recognized that PUFAs provide health benefits, including anti-inflammatory activity, protection of the nervous system, delaying the onset of chronic diseases and are important for the proper functioning of proteins, enzymes and certain receptors (55–57). Particularly, linoleic acid was found to possess antimicrobial activity against fungi and potential preventative effects against specific cancers and atherosclerosis (58). All studied *Tecticornia* species had ratios of PUFA/SFA > 1 [except *T. halocnemoides* (sub-location 6)] with *T. halocnemoides* (sub-locations 2 and 3) and *T. indica* having the highest PUFA/SFA ratio of 1.5 (**Table 4**). It is suggested that the ratio of n-6/n-3 PUFA in foods should be <4 (59) for optimum health. The n-6/n-3 PUFA ratios found in this study are within the values suggested.

Total Phenolic Content

Figure 3 shows significant ($p < 0.05$) differences in TPC of the studied *Tecticornia* species. The TPC ranged from 12.6 to 54.2 mg GAE/g DW, with *T. halocnemoides* (sub-location 2) and *T. halocnemoides* (sub-location 1) having the highest and lowest values, respectively. *Tecticornia halocnemoides* (sub-locations 4 and 6) and *T. indica* had similar values while large variations in TPC content were observed among the same species of *T. halocnemoides* collected from different sub-locations (1, 2, and 3). The TPC in the *Tecticornia* sp. investigated in the present study was comparable with that of *S. ramosissima* and *S. fruticosa* (42, 60).

DPPH Radical Scavenging Capacity

Figure 4 shows significant ($p < 0.05$) differences in the DPPH radical scavenging capacity of the studied *Tecticornia* species. The highest radical scavenging capacity was determined in *T. halocnemoides* (sub-location 2) (271.3 µM TE/g DW) and the lowest in *T. halocnemoides* (sub-location 1) (85.9 µM TE/g DW), respectively. There was also a strong positive correlation between TPC and the DPPH values ($R^2 = 0.967$; **Figure 5**). This finding is a strong indication that phenolic compounds are most likely the main antioxidants in the studied *Tecticornia* sp. However, the present results need to be interpreted with caution at this stage since only TPC data (spectro-photometrical assay) were determined.

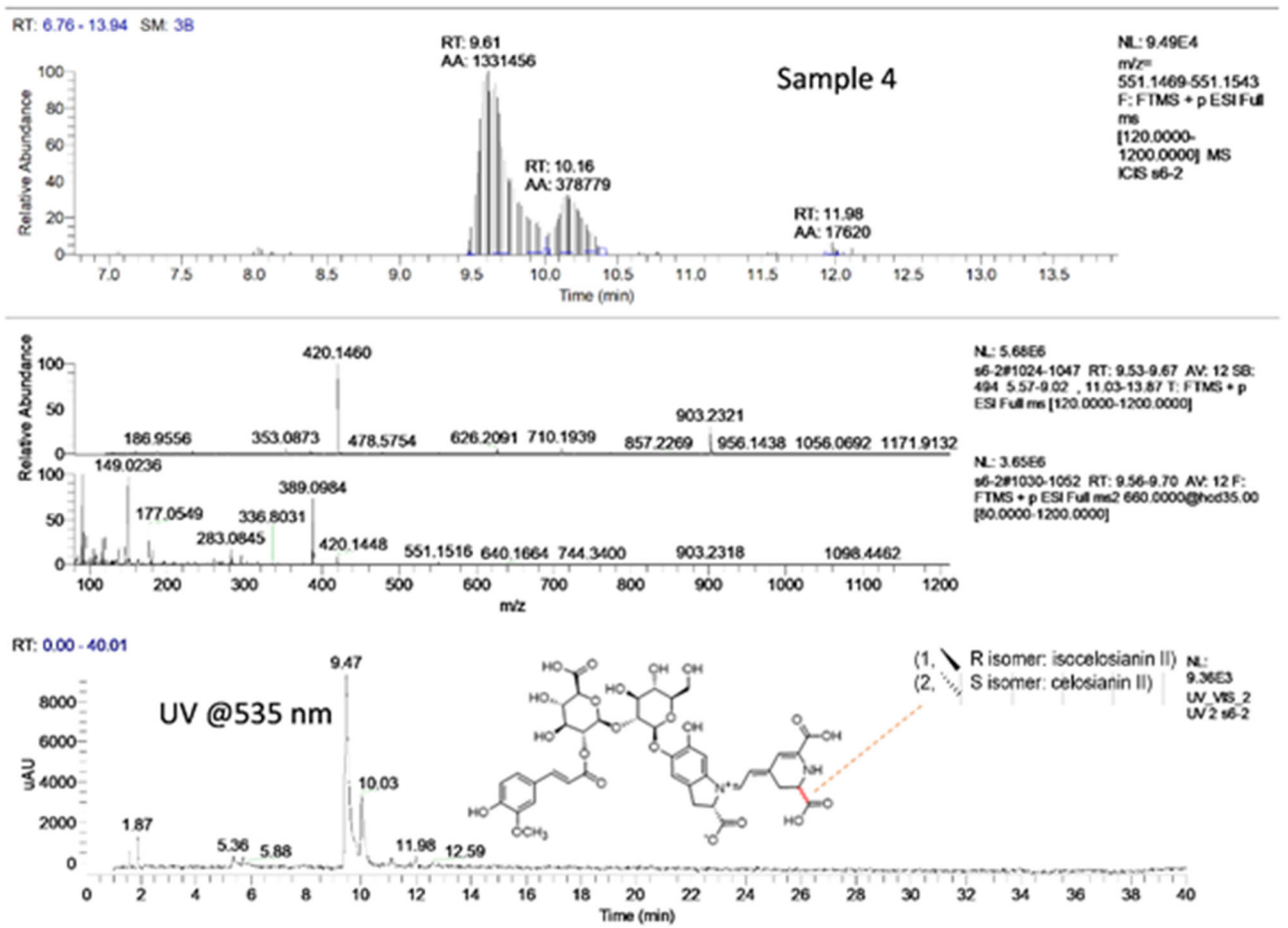

FIGURE 6 | UHPLC-UV-MS/MS chromatograms of pigmented *T. halocnemoides* (sub-location 4) at 535 nm. Peak 1: celosianin II at 9.47 min and peak 2: isocelosianin II at 10.03 min.

Betalains

Identification of Betalains

The identification of betalains was based on their UV spectrum and their molecular masses determined by ultra-high-performance liquid chromatography with diode array detection and electrospray ionization-mass spectrometry (UHPLC-DAD-ESI-MS). Two main betalains, celosianin II and isocelosianin II (**Figure 6**) were identified in the pigmented *Tecticornia* sp.

An identical 2-peak profile was found in *T. halocnemoides* collected from sub-locations 2, 4, and 6. Peaks were identified by spiking experiments with betanin (commercial standard) and comparison with previously reported data (61, 62) (**Table 5**). As shown in **Table 5**, the identity (tentatively) of the two betalains in *T. halocnemoides* species were confirmed by using Q-Exactive high-performance quadrupole-Orbitrap high-resolution mass spectrometry. The MS spectra of peak 1 and 2 m/z 903.2321 $[M+H]^+$ (calculated for $C_{40}H_{42}O_{22}N_2$, 903.2321) and its MS^2 fragment patterns showed that the respective ion at m/z 903.2321 was fragmented to two product ions at 389.0984 and 551.1515, respectively, corresponding to betanidin (calculated for $C_{18}H_{16}O_8N_2$) and betanin (calculated for $C_{24}H_{26}O_{13}N_2$). Therefore, peak 1 and 2 are the signals of celosianin II and isocelosianin II, respectively. Interestingly, celosianin II was found in *Celosia cristata* (63), the plant having a similar color as the *T. halocnemoides* species. This study reported for the first time the presence of celosianin II and isocelosianin II as the main pigments in *T. halocnemoides*.

Marchesini et al. (64) reported the pigment profile of *T. indica* and *T. auriculata* and also identified betalains as the main pigments/phytochemicals which is in agreement with the present study. It is interesting to note that the pigment concentrations in *Tecticornia* were more sensitive to seasonal changes (64). The large variation in the pigment profile of *Tecticornia* sp. probably reflects the differences in cultivars, locations and growing conditions. Therefore, further studies investigating the impact of these "parameters/variables" on the betalain profile and composition in Australian grown halophytes are necessary.

Betalain Content

The betalain content of the studied pigmented *Tecticornia* sp. ranged from 1.9 to 13.3 mg BE/100 g DW (**Figure 7**). Among

TABLE 5 | Identification of betalain compounds in *Tecticornia* sp. using UHPLC-DAD-ESI-MS.

Peak number	Retention time (min)	UV Max	HR-M^+ ion	Formula	Identification	Plant
1	9.61	535	903.23	$C_{40}H_{42}O_{22}N_2$	betanidin 5-O-(2″-O-E-feruloyl)-β-glucuronosyl-glucoside	*T. halocnemoides*
2	10.16	535	903.23	$C_{40}H_{42}O_{22}N_2$	isobetanidin 5-O-(2″-O-E-feruloyl)-β-glucuronosyl-glucoside	*T. halocnemoides*

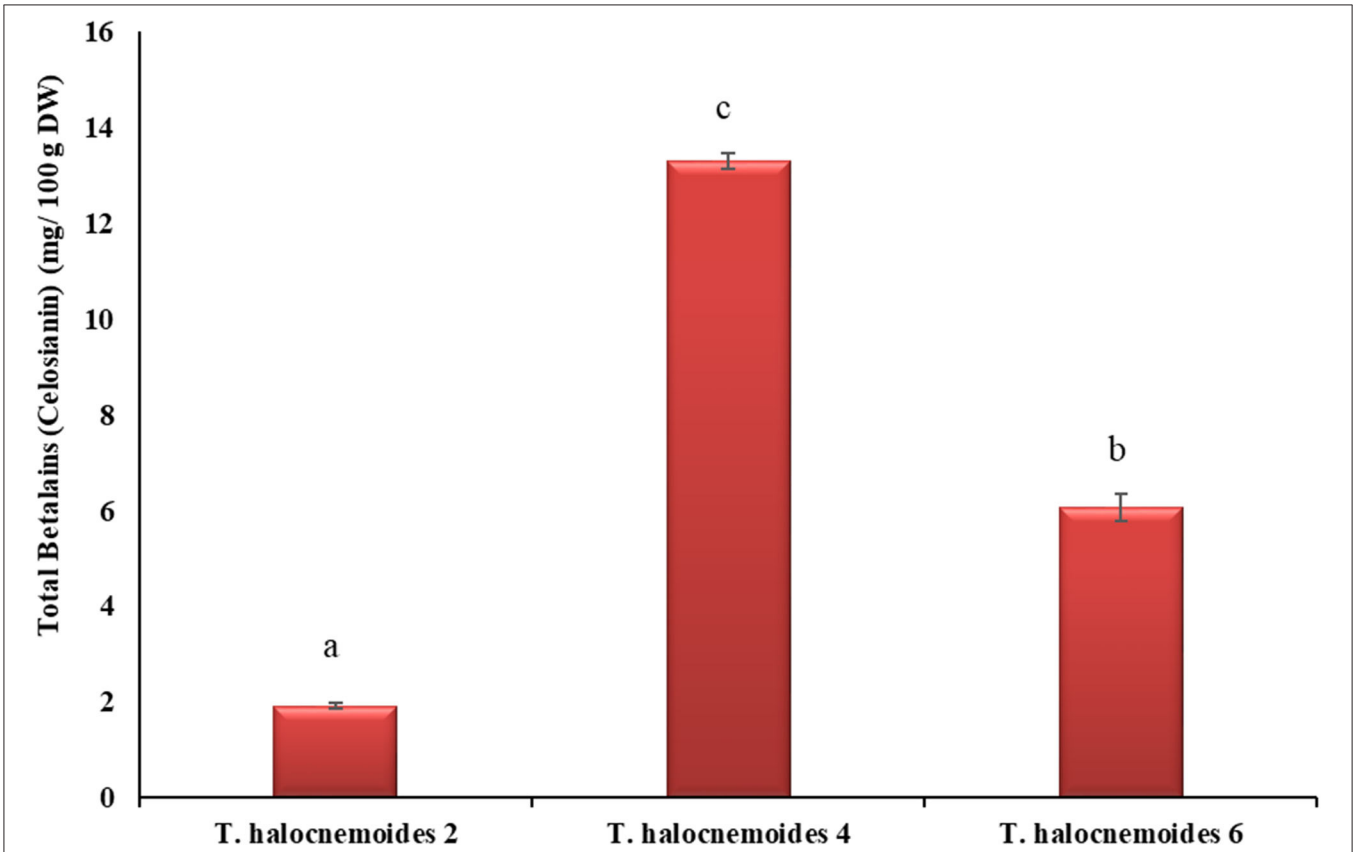

FIGURE 7 | Betalains in pigmented *Tecticornia* species from different sub-locations (calculated as betanin equivalents (BE). Data present means ± SD ($n = 3$). Different letters indicate significant differences ($p < 0.05$) in total betalains among the samples tested.

the three *Tecticornia* sp. investigated, *T. halocnemoides* (4) had the highest ($p < 0.05$) betalain content (13.3 mg BE/100g DW) which is most likely caused by its reddish colored leaves and stems. These findings are comparable with previously reported concentrations for betalains in *T. auriculata* (20 mg/100 g DW), but lower than in *T. indica* (30 mg/100 g DW) (64). The observed differences to literature data may be caused by differences in harvest season and maturity stage as well as soil composition and growing conditions. It should also be noted that the betalain content can increase during stress induced by salinity or seasonal shifts (64).

Among the three pigmented *Tecticornia* species, celosianin II was the predominant betalain, ranging from 66.1 to 74.8% of the total peak area (**Figure 8**). The resulting concentrations of celosianin II, expressed as betanin equivalents (BE), in *T. halocnemoides* (sub-location 2), *T. halocnemoides* (sub-location 4) and *T. halocnemoides* (sub-location 6) were 1.4, 9.9 and 4.0 mg BE/100 g DW, respectively. Isocelosianin II was found in lower concentrations: 0.5 mg BE/100 g DW in *T. halocnemoides* (sub-location 2), 3.4 mg BE/100 g DW in *T. halocnemoides* (sub-location 4) and 2.1 mg BE/100 g DW in *T. halocnemoides* (sub-location 6).

Overall, the findings obtained in this study are consistent with what was reported by Marchesini et al. (64), regarding betalains in *Tecticornia* sp. It should be noted that betalains received significant attention due to their use as natural food colorants and their antioxidant and radical scavenging properties (65–67). Other biological activities such as inhibition of lipid peroxidation and LDL oxidation, prevention of DNA-damage, induction of antioxidant and phase II detoxifying enzymes,

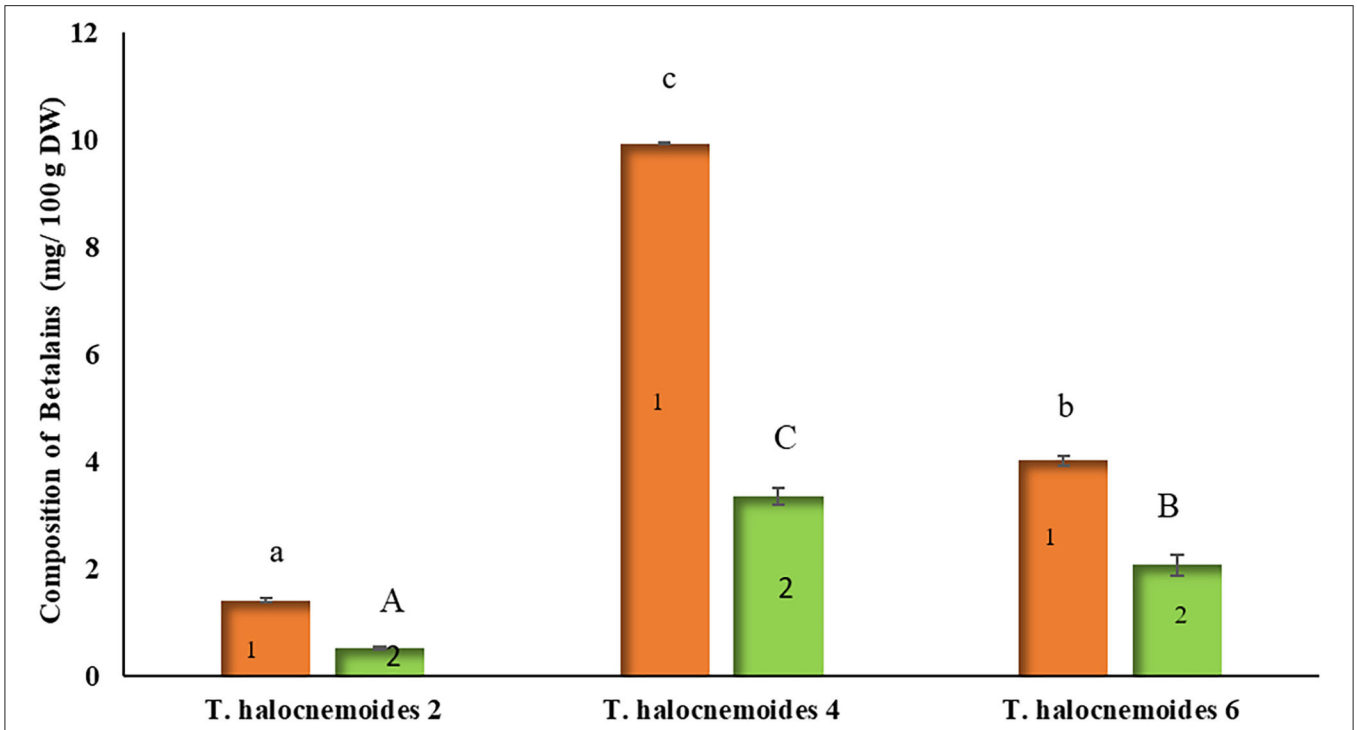

FIGURE 8 | Individual betalains in pigmented *Tecticornia* sp. from different sub-locations (calculated as BE): (1) celosianin II; (2) isocelosianin II. Data present means ± SD ($n = 3$). Different letters indicate significant differences ($p < 0.05$) in composition of betalains among the samples tested.

TABLE 6 | Anti-nutrients in the studied *Tecticornia* species.

Plant species	*T. halocnemoides* 1	*T. halocnemoides* 2	*T. halocnemoides* 3	*T. halocnemoides* 4	*T. indica* 5	*T. halocnemoides* 6	*Spinach*
Hydrolysable Tannin (mg TAE/g DW)	1.2 ± 0.3^{a}	7.4 ± 0.5^{c}	4.0 ± 0.9^{b}	1.4 ± 0.3^{a}	3.8 ± 0.4^{b}	3.5 ± 0.5^{b}	1.5 ± 0.6^{a}
Trypsin Inhibitor (TUI/mg DW)	0.3 ± 0.0^{a}	1.3 ± 0.0^{e}	0.9 ± 0.1^{d}	0.3 ± 0.0^{a}	0.6 ± 0.0^{c}	0.4 ± 0.0^{b}	3.5 ± 0.3^{f}
Total Saponin Content (mg OE/100 g DW)	496.7 ± 41.3^{bc}	571.5 ± 63.0^{c}	158.7 ± 38.1^{a}	336.0 ± 27.8^{ab}	1035.2 ± 102.1^{d}	340.6 ± 24.4^{ab}	2245.7 ± 148.4^{e}
Phytate Content (mg PA/g DW)	15.4 ± 0.1^{b}	6.0 ± 0.2^{a}	46.2 ± 1.4^{c}	6.0 ± 0.2^{a}	14.9 ± 0.5^{b}	6.0 ± 0.1^{a}	46.0 ± 1.1^{c}

Values are means ± SD ($n = 3$); Means not sharing a common superscript (a–f) within a row are significantly ($p < 0.05$) different.

gene regulatory activity, anti-inflammatory, antiproliferative and antimicrobial activities have also been attributed to betalains and betalain-rich foods (66, 67). As for many other bioactive compounds, most of these studies are based on *in vitro* cell culture experiments and animal models, whereas human clinical trials as the "Gold-Standard" are still lacking. Furthermore, betanin was also reported to preserve the quality of frozen and refrigerated foods due to its capacity of preventing lipid oxidation (68). However, the use of *Tecticornia* betalains as natural food colorants, their possible synergistic or antagonistic interactions with other food components as well as their potential health benefits need to be investigated in more detail in future studies.

Vitamin C

Vitamin C was found between 20.5 mg/100 g DW (*T. halocnemoides* 4) and 55.2 mg/100 g DW (*T. halocnemoides* 2), which was in the same range as reported for green tea leaf powder [60 mg/100 g DW] (69). Furthermore, a 200 g serve of fresh *T. halocnemoides* 2 (taking the moisture content of 77% into account) would deliver 56% of the recommended dietary intake (RDI) for vitamin C, which is 45 mg/day for adults in Australia (38).

Anti-nutrients

The determined anti-nutrients were all lower than those in spinach, except for hydrolysable tannins in *T. halocnemoides* 2, 3, 5, and 6, and phytate in *T. halocnemoides* 3 (**Table 6**). Furthermore, all *Tecticornia* species had considerably lower levels of saponins compared to Gumby Gumby [*Pittosporum angustifolium*; 1,590-3,645 mg OE/100 g DW; (34)], another plant with functional properties endemic to Australia.

CONCLUSIONS

To the best of our knowledge, this is the first comprehensive evaluation of the nutritional composition of Australian indigenous edible halophytes, grown in different (sub)locations in the Kimberly Region, Western Australia. The results demonstrated the nutritional potential of these *Tecticornia* species in terms of fiber, favorable fatty acid profile (PUFA/SFA ratio), natural pigments and antioxidant capacity. However, future studies are warranted to elucidate the complete nutritional profile, including vitamins (except vitamin C), individual polyphenols and other non-betalain phytochemicals. Furthermore, *in vitro* bioaccessibility and *in vivo* bioavailability studies, together with sensory trials and product development are crucial, to fully understand the nutritional value of these "unique" edible plants for consumers and industry.

DATA AVAILABILITY STATEMENT

The original contributions generated in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

All authors contributed extensively to the manuscript and gave final approval for publication.

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Essential Oil Profile Diversity in Cardamom Accessions From Southern India

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The essential oil of cardamom capsules is a high-value ingredient in foods, beverages, perfumery, and traditional medicines. It is responsible for the characteristic aroma of cardamom. The present study aimed to evaluate essential oil yield and chemical constituents of 22 diverse accessions of cardamom. A total of 20 g of the cured capsules were hydrodistilled in a Clevenger apparatus for 3 h in three replications. The amount of essential oil yield ranged from 4.5 to 9.5%, indicating a substantial variation in this feature among the accessions. The GC/MS analysis results discovered 24 constituents that constituted 98.1–100% of total essential oil. The main fractions were found to be oxygenated monoterpenes (40.7–66.7%), monoterpene hydrocarbons (23.1–58.6%), and sesquiterpenes (0.1–2.0%). Among the monoterpenoids, the predominant constituents were α-terpinyl acetate (29.9–61.3%) followed by 1,8-cineole (15.2–49.4%), α-terpineol (0.83–13.2%), β-linalool (0.44–11.0%), and sabinene (1.9–4.9%). Two sesquiterpene constituents, cardinen and nerolidol and p-cresol (a phenol derivative) were also identified. The compositional data were subjected to euclidean-distance-based similarity analysis, which showed two major clusters. The major constituents of cardamom essential oil (CEO) are 1,8-cineole, α-terpinyl acetate, sabinene, and β-linalool that can be used in food, aroma, and pharmaceutical applications.

Keywords: cardamom, essential oil, GC/MS analysis, 1,8-cineole, α-terpinyl acetate

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INTRODUCTION

Elettaria cardamomum (L.) Maton, commonly known as small cardamom, Indian cardamom, green cardamom, or true cardamom, is a herbaceous perennial plant that belongs to the Zingiberaceae family. It is also called the "Queen of spices" as it is the third most valuable spice after vanilla and saffron. Cardamom is grown mainly in India, Guatemala, Sri Lanka, Nepal, and can also be found in Tanzania, Indonesia, Vietnam, Thailand, Papua New Guinea, and El Salvador (Garg et al., 2016).

Middle Eastern countries constitute major global consumers of cardamom. The wide variety of national dishes that use cardamom as a major component ingredient and the age-old practice of using cardamom spices in medicine clarifies the spice's popularity. According to data collected,

Saudi Arabia was the largest importer of cardamom in 2017, accounting for 19.3% of the global market. UAE, Syria, Jordan, India, Bangladesh, and Singapore are the next countries (UN Comtrade, 2017). Cardamom is widely used as a flavoring ingredient in whole and ground form in the Middle East, especially Saudi Arabia. It is used extensively in the preparation of "kahwa"–a drink that is a sign of hospitality in any home. It gives a lingering flavor in most Asian cuisines. Cardamom is used in baked goods and confectioneries in Scandinavian countries. Curry powder and some sausage products in Europe and North America contain it (https://cardamomassociation.com/report/cardamom/).

Despite cardamom as a spice and in the pharmaceutical and cosmetic industries, the European market remains limited. The crushed cardamom capsules are boiled with tea and water in south India to add a good fragrance to tea, which is popularly known as "*Elakkai tea*" and has been used to alleviate tiredness and depression (Ashokkumar et al., 2020a). Some people believe that excessive cardamom capsules' excessive use could cause impotence in humans (Nair, 2011). However, to date, there is no scientific evidence reported that the daily consumption limit of cardamom. The cardamom preparation, "Eladigana chooranm" is commonly used to cure arthritis, congestion, and itching in south Indian Ayurvedic medicine (Nair, 2011). Cardamom capsule extracts and cardamom essential oil (CEO) have numerous potential therapeutic activities. The use of this plant as a source of various natural products has a great interest in many parts of the world. According to Hamzaa and Osman (2012) and Khan et al. (2011), cardamom capsules have been used in traditional medicine for controlling asthma, nausea, diarrhea, cataracts, teeth, and gum infections, digestive, kidney, and cardiac disorders. Besides folk medicinal uses, potential applications in modern medicine have been explored (Saeed et al., 2014; Elguindy et al., 2018).

Cardamom capsules are a storehouse of several bioactive metabolites like flavonoids, carotenoids, and terpenes, etc. (Ashokkumar et al., 2019, 2020a,b). The primary bioactive metabolites of CEO contribute to its characteristic strong aromatic aroma. The CEO's concentration in cardamom capsules ranges from 6 to 14%, depending upon the extraction and processing methods (Nirmala Menon, 2000). The CEO's composition could rely on the origin of the sample, varieties, and parts used (Ashokkumar et al., 2020a). The CEO is rich in monoterpene constituents like α-terpinyl acetate, 1,8-cineole (28.94–34.91%), α-terpineol (12.47–14.89%), sabinene (11.17–13.50%), nerol (3.69–6.10%), β-linalool (1.43–2.97%), and α-pinene (1.15–2.42%) (Murugan et al., 2005, 2019; Yashin et al., 2017; Ashokkumar et al., 2019). These predominant constituents have potential pharmacological and therapeutic properties such as antioxidant, anti-inflammatory, antidiabetic, anticancerous, antimicrobial, antiviral, and gastroprotective activities (Hamzaa and Osman, 2012; Winarsi et al., 2014).

Several studies showed substantial variation in the essential oil from cardamom capsules; however, these samples' origin has generally not been sufficiently defined. In several cases, commercial samples from unknown original habitat and bulk samples obtained from the mixture of various cardamom genotypes, which do not truly represent the individual chemotypes. Besides, the CEO's major constituents can be affected by several factors like origin, soil types, seasonal influence, storage, processing conditions, and extraction methods. Hence, most of the studies have not adequately elucidated the true chemo-diversity of CEO. The variability in essential oil constituents among cardamom types is very high (Murugan et al., 2005; Ashokkumar et al., 2019). The chemo-profiling of elite cardamom types has been done for the first time. Thus far, there has been limited information on the chemical composition of essential oils extracted from south Indian *E. cardamomum* accession. Identifying cardamom accessions with higher essential oil may offer future breeding activities. In this context, the present study's objective was to evaluate essential oil yield and chemical constituents of 22 diverse accessions of *E. cardamomum,* which will aid the selection of suitable accessions to address consumer and manufacture demand.

MATERIALS AND METHODS

Plant Materials

The Cardamom Research Station, Pampadumpara, Idukki, Kerala, India located at 9°45′ N latitude, 77°10′ E longitude, and altitude is 1,100 m above mean sea level. This station is maintained 187 germplasm collections. Among them, 22 cardamom accessions were grown under uniform field conditions, and each accession had 12 plants. The plants were planted at spacing 2.5 × 2.5 m, and fertilizer was applied 100:100:250 Kg NPK ha^{-1}, yr^{-1} in two splits before (May–June) and after (September–October) the first primary season. The selected 22 accessions were chosen based on the observation made by the previous year's high yield, pest, and disease resistance potential. The matured capsules were harvested from 5-year-old plants during June 2019 and cured as per the standard procedure of Kerala Agricultural University (KAU, 2011) to evaluate CEO composition. Sufficient quantities (100 g) of cured capsules of each accession were stored at room temperature (24°C). The diagrammatic representation of usable parts of cardamom is presented in **Figure 1**.

Extraction of Essential Oils

Cured capsules collected from each accession were ground individually into a fine powder (20 μ). Twenty grams of powdered sample was placed in a 500 ml distillation flask separately, to which 250 ml deionized water was added. Several studies from cardamom capsules have been utilized a similar sample weight of 20 g and produced 0.8–1.5 ml of essential oil (Murugan et al., 2005, 2019; Ashokkumar et al., 2019). A hydro distillation run time of 3 h was used to obtain the optimum yield (Ashokkumar et al., 2019). Oil yield was estimated with an average of three replications. The obtained essential oil was dried over anhydrous sodium sulfate, weighed, and then stored at 4°C in the dark until use. The essential oil yield was calculated as a volume by weight basis using the following formulae: Essential oil (%, v/w) = volume of oil collected (ml)/weight of the sample (g) × 100 (AOAC, 2000).

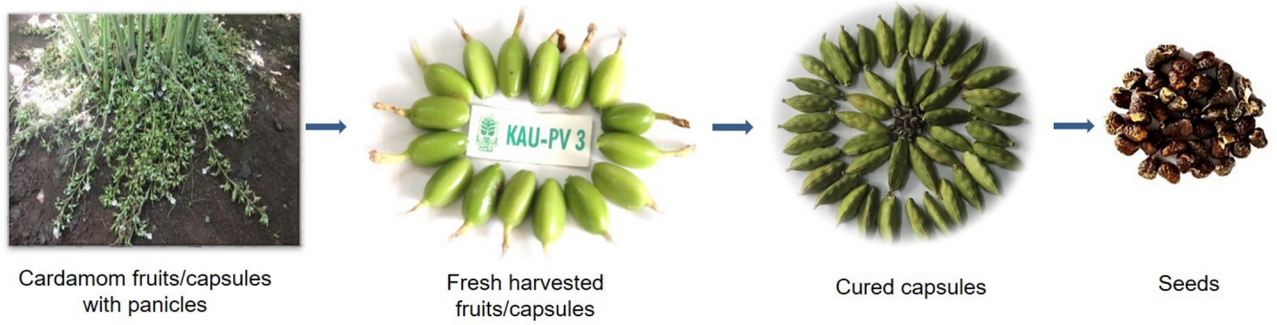

FIGURE 1 | Diagrammatic representation of usable of parts of cardamom.

TABLE 1 | Minimum and maximum range, retention time, retention index of essential oil compounds in 22 cardamom accessions.

S. No.	Compound name	RT[a]	RI[b]	RI[c]	Area %		
					Minimum	Maximum	Mean
1.	α-Thujene	8.3	924	930	0.1	1.0	0.2
2.	α-Pinene	8.6	948	943	0.6	1.5	1.0
3.	Sabinene	9.6	969	975	1.9	4.9	3.5
4.	β-Pinene	9.8	974	979	0.2	0.5	0.3
5.	β-Myrcene	10.0	988	990	0.9	1.9	1.4
6.	3-Carene	10.6	1,008	1,011	0.1	0.7	0.1
7.	α-Terpinolene	10.7	1,022	1,022	0.1	1.9	0.3
8.	Limonene	11.1	1,024	1,029	0.9	9.4	2.3
9.	1,8-Cineole	11.2	1,026	1,031	15.2	49.4	34.5
10.	β-Cymene	11.3	1,042	1,030	0.1	0.7	0.2
11.	γ-Terpinene	11.9	1,054	1,059	0.2	1.4	0.4
12.	β-Linalool	13.0	1,082	1,087	0.4	11.0	2.0
13.	Terpinen-4-ol	15.2	1,137	1,137	0.4	3.2	1.8
14.	α-Terpineol	15.6	1,143	1,140	0.8	13.2	3.4
15.	β-Terpineol	15.7	1,158	1,159	0.3	2.7	0.8
16.	β-Citral	15.8	1,174	1,174	0.1	0.5	0.2
17.	Nerol	16.8	1,228	1,229	0.2	1.1	0.7
18.	Linalyl acetate	16.9	1,231	1,231	0.2	4.4	1.2
19.	α-Citral	17.3	1,264	1,267	0.1	0.6	0.2
20.	α-Terpinyl acetate	19.4	1,333	1,300	29.9	61.3	43.5
21.	Geranyl acetate	19.9	1,379	1,381	0.1	2.3	0.9
22.	p-Cresol	22.9	1,382	1,385	0.2	9.0	1.0
23.	γ-Cadinene	23.3	1,513	1,513	0.3	0.4	0.1
24.	Nerolidol	24.2	1,564	1,563	0.1	2.0	0.7

[a]RT, Retention time; [b]RI, Retention index (experimental) on Rxi®-5 Sil MS column; [c]RI, Retention index in literature; Monoterpene hydrocarbons, 1–8 and 11; Oxygenated monoterpenes,9,12–21; Sesquiterpene hydrocarbon, 23; Oxygenated sesquiterpene, 24; Phenylpropanoid, 10; phenol, 22.

Analysis of Essential Oils

The qualitative analysis of CEO was carried out through gas chromatography (GC) coupled with a mass spectrometer (MS) (GC-MS—QP2020 NX SHIMADZU). The GC was equipped with a fused silica capillary column, Rxi®-5 Sil MS (20 m, 0.18 mm ID), with a film thickness of 0.18 μM. The EO was injected by split mode (1:20). The helium gas flow rate was constantly maintained at 1 ml/min (Ashokkumar et al., 2019). The oven temperature was programmed at 70°C for 15 min and then gradually increased at 6°C/min to 200°C and then 30°C/min to 280°C (10 min). The detector and injector temperature was maintained at 290°C. The MS conditions were electron energy 70 eV, electron impact (EI) ion source temperature 260°C, and transmission line temperature 280°C. The mass scan range (m/z) was 50–650 amu, data acquired at full scan mode with solvent delay for 3 min. The qualitative analysis of volatile oil

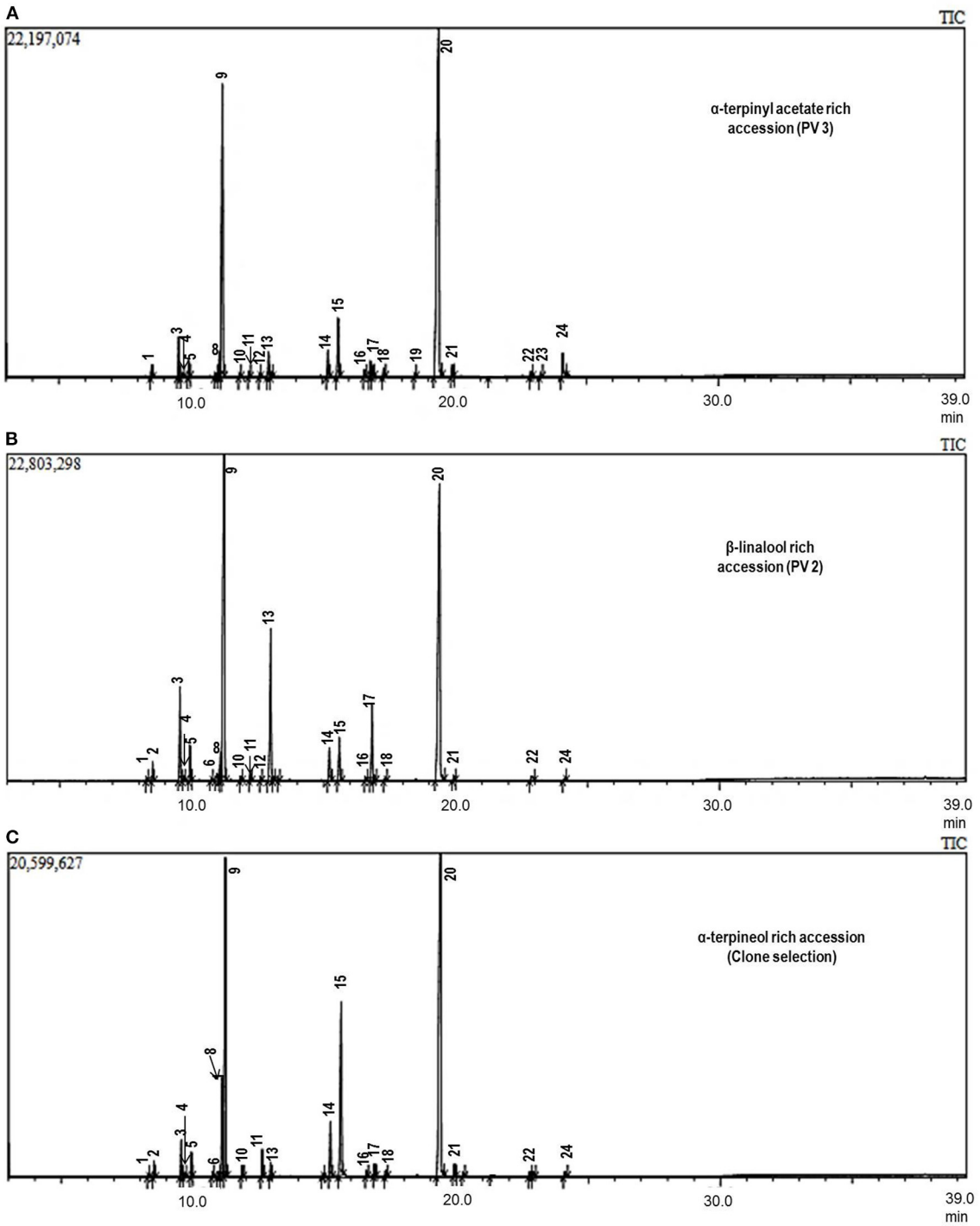

FIGURE 2 | Essential oil chromatogram of chemovariants of *Elettaria cardamomum* (L.) Maton: **(A)** α-terpinyl acetate-rich chemotype, **(B)** β-linalool rich chemotype, and **(C)** α-terpineol rich chemotype.

was carried using Shimadzu GC/MS solution™ Ver.4 software. The CEO constituents were identified by comparing retention indices (RI) under programmed similar oven temperature conditions for homologous series of *n*-alkanes (C8–C24). Identification of individual essential oil constituents was based on comparing mass spectra with those present in NIST and

TABLE 2 | Essential oil composition in 22 promising cardamom accessions.

Sr. no.	Compound name	PV1	PV2	PV3	PV4	PV5	PV8	PV34	HY9	HY12	HY13	HY14	HY15	Pinkbase	PS1 x GGType1	GG	Elarajan	Clone selection	SAM5	HY3	HY11	Kaniparamban	Minipink
1.	α-Thujene	0.1	0.1	0.9	0.2	0.2	–	–	0.1	0.1	0.1	0.1	0.2	0.12	0.8	0.2	0.1	0.1	0.1	0.1	–	–	–
2.	α-Pinene	1.0	0.9	–	1.3	1.1	0.7	0.7	1.2	1.3	1.2	1.1	1.4	1.3	1.5	1.4	1.1	0.9	1.3	1.2	0.6	0.8	1.0
3.	Sabinene	4.9	4.9	3.1	4.5	3.2	2.3	2.1	3.6	3.5	3.3	2.9	3.9	2.8	4.2	4.3	3.6	1.9	4.1	3.7	2.9	4.00	2.6
4.	β-Pinene	0.4	0.3	0.3	0.4	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.4	0.4	0.5	0.4	0.3	0.2	0.4	0.3	0.3	0.3	0.3
5.	β-Myrcene	1.8	1.9	1.2	1.7	1.3	1.0	0.9	1.5	1.4	1.2	1.1	1.	1.2	1.6	1.8	1.4	1.2	1.6	1.4	1.2	1.6	1.0
6.	3-Carene	–	0.1	–	0.2	0.2	–	–	0.2	0.2	–	–	–	–	0.1	*t*	0.2	0.3	*t*	–	*t*	0.7	*t*
7.	α-Terpinolene	0.4	–	–	*t*	*t*	–	–	*t*	*t*	–	–	0.1	0.1	0.2	0.2	0.2	–	0.5	1.9	–	0.5	0.1
8.	Limonene	1.8	1.8	0.9	1.9	2.0	2.0	2.4	2.1	2.2	1.4	2.3	1.6	1.9	2.1	2.1	2.0	9.4	1.9	–	*t*	2.0	1.4
9.	1,8-Cineole	29.3	33.6	15.2	35.0	36.1	25.8	29.8	36.6	41.0	42.7	41.1	49.4	46.6	39.7	32.5	33.8	24.4	33.3	41.4	29.1	27.4	39.5
10.	β-Cymene	0.1	0.6	*t*	*t*	*t*	0.3	0.4	*t*	*t*	0.2	0.4	–	*t*	–	–	–	0.4	–	0.2	0.3	0.7	–
11.	γ-Terpinene	0.4	0.2	1.4	0.5	0.4	0.3	*t*	0.4	0.3	0.2	0.2	0.3	0.3	0.4	0.4	0.3	0.6	0.3	0.3	0.3	0.2	0.3
12.	β-Linalool	0.6	10.9	1.2	0.7	1.2	1.7	1.7	0.5	0.9	0.6	1.0	0.5	4.4	0.7	0.4	3.2	0.8	0.7	0.5	1.0	1.4	1.6
13.	Terpinen-4-ol	2.2	2.0.	0.4	2.0	2.0	1.9	1.4	1.9	1.7	1.6	2.3	1.3	1.4	1.8	1.8	1.6	3.2	1.7	2.0	1.7	2.3	1.8
14.	α-Terpineol	3.0	2.8	0.8	2.1	2.3	4.4	3.7	2.9	2.7	2.3	3.4	1.3	2.1	2.0	2.0	3.0	13.8	1.6	1.8	3.3	4.4	2.0
15.	β-Terpineol	0.4	0.4	0.5	0.5	0.4	2.7	0.4	0.3	0.5	1.2	0.4	1.2	0.7	0.9	0.4	0.4	1.5	0.6	0.4	0.7	0.8	0.9
16.	β-Citral	0.1	0.2	–	–	0.2	0.5	0.2	0.2	0.5	0.2	0.3	0.2	0.4	–	*t*	0.2	0.4	0.3	0.3	0.3	0.4	0.2
17.	Nerol	0.6	0.4	0.6	0.6	1.0	0.8	0.9	0.8	0.6	0.5	0.5	0.2	0.2	0.7	0.8	0.8	0.4	0.9	0.7	0.7	1.1	0.8
18.	Linalyl acetate	0.4	4.4	0.6	0.9	1.4	1.0	0.8	0.5	0.9	1.0	0.2	0.8	3.8	0.7	0.8	1.6	0.8	0.8	0.6	0.6	0.9	1.4
19.	α-Citral	0.2	0.2	0.2	–	0.2	0.6	0.3	0.2	0.2	0.2	0.3	0.2	0.2	–	*t*	0.1	*t*	0.4	0.4	0.4	0.6	0.3
20.	α-Terpinyl acetate acetate	50.0	33.1	61.3	44.4	43.6	52.4	49.8	44.7	39.6	40.6	40.7	34.8	29.9	40.7	47.2	43.4	38.3	46.7	41.3	52.1	46.2	41.4
21.	Geranyl acetate	1.6	0.3	0.8	1.4	0.8	0.8	1.0	0.8	0.5	0.3	0.4	0.1	1.0	1.1	1.0	1.3	0.7	0.6	0.4	1.6	2.2	0.7
22.	p-Cresol	0.3	0.5	9.0	0.3	0.4	0.3	0.4	0.3	0.3	0.4	0.3	0.2	0.3	0.3	–	0.2	0.4	0.4	0.3	0.3	–	0.2
23.	γ-Cadinene	–	–	0.3	–	–	0.3	0.3	–	–	–	–	–	–	–	–	–	–	–	–	0.3	0.4	–
24.	Nnerolidol	*t*	0.1	0.1	0.5	1.4	1.9	1.6	0.3	0.7	0.7	0.3	0.2	0.4	0.5	0.7	0.6	0.4	1.2	0.5	0.7	1.0	2.0
	Volatile oil (%)	6.1	6.0	6.1	6.7	7.5	6.0	6.0	6.1	6.1	5.5	5.7	6.1	5.4	5.4	6.0	6.7	5.4	9.3	9.6	4.5	5.4	5.4
	Monoterpene hydrocarbons	10.9	10.2	7.9	10.5	8.6	6.5	6.2	9.5	9.4	7.7	8.0	9.2	8.2	10.8	10.7	9.3	14.5	10.1	9.0	5.2	10.1	6.8
	Oxygenated monoterpenes	88.2	88.2	81.6	87.5	89.0	92.5	89.9	89.5	89.2	91.1	90.6	90.1	90.7	88.2	86.9	89.5	83.6	87.4	89.8	91.5	87.7	90.3
	Sesquiterpene hydrocarbons	–	–	0.3	–	–	0.4	0.3	–	–	–	–	–	–	–	–	–	–	–	–	0.3	0.4	–
	Oxygenated sesquiterpenes	–	0.11	0.1	0.5	1.4	1.9	1.5	0.3	0.7	0.7	0.3	0.2	0.4	0.5	0.7	0.6	0.4	1.2	0.5	0.7	1.0	1.9
	Phenylpropanoid + Phenol	0.4	1.14	9.0	0.3	0.4	0.6	0.8	0.3	0.3	0.5	0.7	0.2	0.3	0.3	-	0.3	0.7	0.4	0.5	0.6	0.7	0.2
	Total compounds (%)	99.5	99.6	98.9	98.8	99.4	99.8	98.7	99.6	99.5	99.9	99.6	99.7	99.6	99.7	98.2	99.6	99.2	99.1	99.8	98.2	99.9	99.3

t, Traces (<0.1%); (–), no detected.

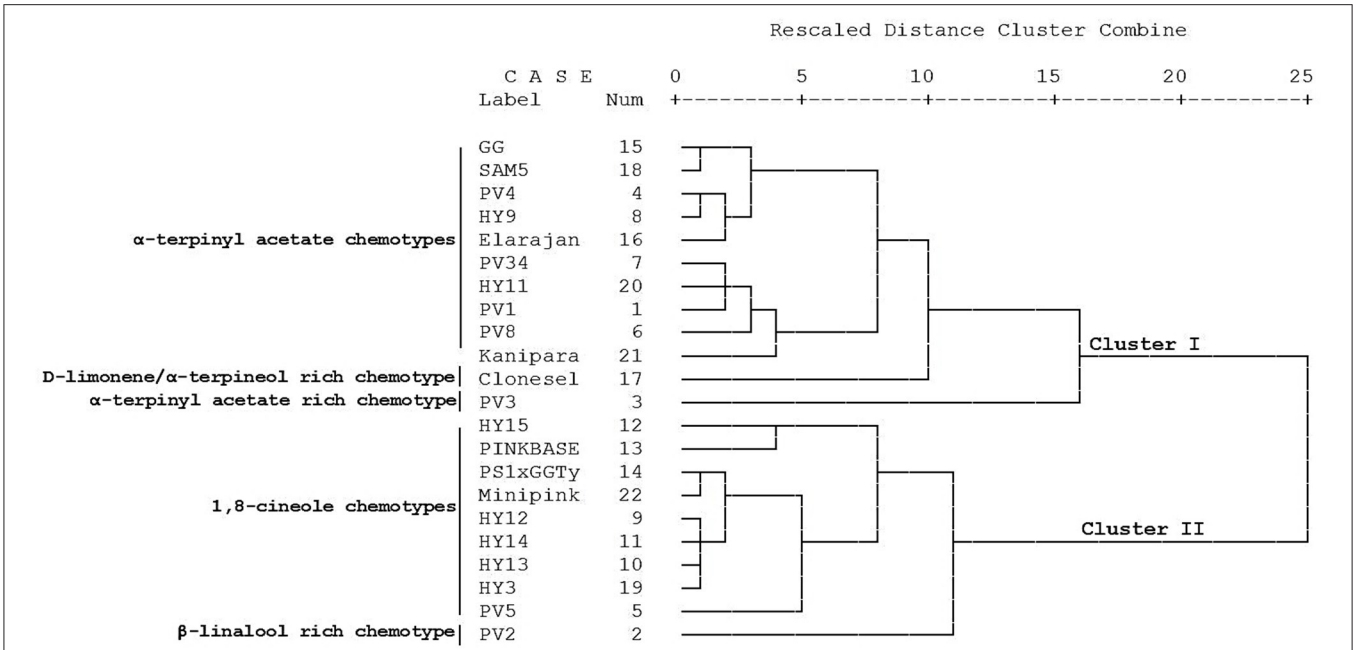

FIGURE 3 | Clustering (nearest-neighbor method using the Euclidean distance) based on oil constituents for 22 cardamom accessions.

Wiley libraries and literature data (Adams, 2007). Identification of certain compounds (1,8-cineole, α-terpineol, β-linalool, α-pinene, β-pinene, sabinene, α-terpinyl acetate, α-citral, nerol, and geranyl acetate) was further confirmed by co-injection of their authentic standards (Sigma-Aldrich, Mumbai, India) under same chromatographic conditions mentioned above.

Cluster Analysis

Hierarchical clustering was used to understand the relationship between the cardamom accessions based on essential oil composition and determine the chemotypes. Euclidean distance was selected to measure the similarity and the nearest-neighbor method used for cluster definition (Gwari et al., 2016). SPSS software (version 24.0) used for cluster analysis (Cor, 2016) (IBM Corp 2016).

RESULTS AND DISCUSSION

The extraction of CEO was performed by hydrodistillation method, and an average yield of three separate analyses was ranged from 4.5 to 9.6%. Among the accessions, the highest essential oil content was observed in accession HY 3 (9.6%) followed by SAM 5 (9.3%), which were higher than previously reported in four different cardamom types viz., *malabar, mysore, vazhukka,* and *guatemala* (7.9–8.8%) (Padmakumari et al., 2010). CEO varied with various extraction methods, varieties, and plant parts (Ashokkumar et al., 2020b). GC/MS examined the CEO extracted from these 22 accessions. The list of constituents identified among the accessions is presented in **Table 1**. The constituents found in the greatest quantity were 1,8-cineole, α-terpinyl acetate, α-terpineol, sabinene, and β-linalool. The typical CEO chromatograms of major bioactive constituent-rich chemotypes are shown in **Figure 2**.

The chemical profile of 22 cardamom diverse accessions essential oil showed the presence of 24 volatile constituents, which comprised about 98.1–99.9% of the total essential oil. Oxygenated monoterpenes dominated oil composition with 81.6–92.5%, followed by monoterpene hydrocarbons (6.2–14.5%), oxygenated sesquiterpenes (0.1–2.0%), and sesquiterpene hydrocarbon (0.3–0.4%) (**Table 1**). Among the monoterpenes, α-terpinyl acetate (29.9–61.3%) is the predominant constituent and was found in CEO of all the cardamom accessions evaluated. Similar results were observed in earlier studies (Ashokkumar et al., 2019), based on that highest α-terpinyl acetate (61.3%) was observed in accession PV3. The second most major monoterpene constituent, 1,8-cineole (15.2–49.4%), also presents all the cardamom accession's essential oil. According to a recent study conducted by Alagupalamuthirsolai et al. (2019), cardamom variety Appangala-1 had a 1,8-cineole concentration of 41.8% under 75% shade condition. In our research, the accession HY15 had recorded the highest 1,8-cineole (49.4%) compared with previous reports. This higher concentration could depend on varietal difference and environmental effects (Ashokkumar et al., 2020a). However, the present study shows that a higher concentration of α-terpinyl acetate in some cardamom accessions had a lesser concentration of 1,8-cineole and vice versa.

The amount of several monoterpene compounds accumulated substantially *viz*., sabinene (1.9–4.9%), β-terpineol (0.3–2.7%), β-linalool (0.4–11.0%), terpinen-4-ol (0.4–3.2%), α-terpineol (0.8–13.2%), geranyl acetate (0.1–2.3%), linalyl

acetate (2.2–4.4%), D-limonene (0.9–9.4%), and nerol (0.2–1.1%) have varied among the accessions. Furthermore, the higher concentration of sabinene (4.9%), β-linalool (11.0%), α-terpineol (13.2%), and nerol (1.1%) was present in accessions PV1, PV2, Clone selection, and Kaniparamban, respectively (**Table 2**).

Two sesquiterpene constituents, cardinen and nerolidol, and a phenol derivative of p-cresol were also identified (**Table 2**). Different chemotypes have been reported from CEO richness of 1,8-cineole, α-terpinyl acetate, α-terpineol, β-terpineol, β-myrcene, sabinene, β-linalool, α-terpinyl acetate, geranyl acetate, limonene, nerol, and linalyl acetate was reported by several workers (Miniraj et al., 2000; Murugan et al., 2002, 2005; Kumar et al., 2005; Kaskoos et al., 2006; Goudarzvand Chegini and Abbasipour, 2017; Alagupalamuthirsolai et al., 2019; Ashokkumar et al., 2019). To find out the similarity of oil composition among the 22 cardamom accessions, a hierarchical cluster analysis was carried out based on the composition of its major constituents (**Figure 3**). Two major clusters were identified, namely, clusters 1 and 2. Cluster 1 is composed of 12 accessions, while cluster 2 is composed of 10 accessions. Cluster 1 formed four sub-clusters: Green gold (GG), SAM5, PV4, HY9, and Elarajan; PV34, HY11, PV1, PV8, and Kaniparamban; Clone selection and PV3. Cluster 2 formed four sub-clusters, namely HY15 and Pink base; PS1 x GG Type-1, Minipink, HY12, HY14, HY13, and HY3; PV5; and PV2. Accessions in the same sub-cluster were similar in essential oil composition. In brief, accessions in cluster-1 had higher α-terpinyl acetate concentration, and cluster 2 accessions had higher 1,8-cineole concentration. Accessions having the highest α-terpinyl acetate concentration (61.3%) were in cluster 1, and accessions with the highest 1,8-cineole (49.4%) concentration were in cluster 2. Furthermore, Clone selection had the highest D-limonene concentration (9.4%), and PV3 had the highest p-cresol concentration (9.0%) and is separately sub clustered in cluster 1. Accession PV2 had the greatest concentration of β-linalool (11.0%) and sub-clustered in cluster 2.

These chemotypes offer unlimited opportunity to produce cardamom to meet the market supplies of essential oil and individual constituents. Further, the present study revealed new essential oils/chemotypes (γ-cadinene) of the cardamom (PV3, PV8, PV34, HY11, and Kaniparamban), which were not described before from southern India. Also, new essential oils/chemotypes (3-carene) of the Cardamom were not described in this region. The results of this research will be useful for cardamom breeders to choose accessions for developing new varieties with greater CEO concentration and a higher concentration of specific pharmaceutically desired CEO constituents.

CONCLUSION

The present study investigated the essential oil yield and chemical composition from 22 promising cardamom accessions from south India. This research demonstrated substantial variation in essential oil concentration and its composition of available cardamom accessions. Since all accessions were grown under the same environmental conditions, the effects of the environmental factors such as soil type, shade, and location have been excluded from the compositional variation of CEO. In this study, we have identified that cardamom accessions had predominantly oxygenated monoterpene compounds followed by monoterpene hydrocarbons, and sesquiterpenes. The CEO compositional data were subjected to similarity analysis, which showed two major clusters. Cluster 1 and 2 is composed of 12 and 10 accessions, respectively. In overall observation, Cluster-1 accessions had higher α-terpinyl acetate concentration, and cluster 2 accessions had higher 1,8-cineole concentration. The accession PV 3 had the highest α-terpinyl acetate (61.3%), and accession HY 15 had the highest 1, 8-cineole (49.4%) were in clusters 1 and 2, respectively. These outcomes implied that there is a large potential for domestication, cultivation, and selective breeding programs. Also, these results could be used as a database for trading and pharmaceutical sectors engaged with cardamom processing. Farmers will also benefit from the study results by adopting suitable varieties with superior quality for cultivation. Furthermore, the two major constituents of CEO were α-terpinyl acetate, and 1,8-cineole can serve as a new potential natural source, which can be used in the food, aroma, cosmetics, and pharmaceutical domains.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

KA and MM conceptualized the study. KA, SV, GA, and MA performed the experiment. SA, MKD, and AK collected the samples and performed the data analyses. KA, TW, and AK drafted the manuscript. All authors contributed to the article and approved the submitted version.

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Assessment of Dietary Bioactive Phenolic Compounds and Agricultural Sustainability of an African Leafy Vegetable *Corchorus olitorius* L.

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Corchorus olitorius L. is an African leafy vegetable of high nutritional interest. To assess its agricultural suitability to sustainable cultivation conditions and its potential benefits for human nutrition, its phytochemical content in response to conservation agriculture practices [i.e., no-tillage (NT) and cover crop maintenance] and low water regime were evaluated and compared with response under conventional agriculture management. Hydric stress and NT did not affect the content of antioxidant metabolites, compared to conventional agricultural practices. In both conditions, leaves were found to be a great source of phenolic compounds. The effect of these phenolic fractions was assessed on two colon cell phenotypes to evaluate putative nutraceutical properties. Polyphenol-enriched extracts (PEEs) displayed selective cytotoxic activities against tumor Caco-2 cells but not on the healthy CCD841 line. PEEs were able to trigger oxidative stress and to inhibit the activity of glutathione-independent antioxidant enzymes on Caco-2 cells. *C. olitorius* showed to be a promising crop for improving both agricultural sustainability and health benefits due to the great amount of antioxidant compounds in leaves, whose occurrence is not altered by stressful farming conditions. Given its high adaptability, the cultivation of this crop is therefore recommendable also in the Mediterranean Basin.

Keywords: indigenous vegetables, antioxidants, phenolic compounds, bioactivity, agronomic practices

INTRODUCTION

The UN Sustainable Development Goals (i.e., SDG 2) highlight the need of identifying sustainable ways to produce food for human mankind as one of the major challenges of the Anthropocene era. As a matter of fact, hunger is still on the rise and many people consume low-quality food causing micronutrient deficiencies (1). Also in developed countries, the population is inadequately nourished and many environmental systems and processes are pushed beyond safe boundaries by food production. In this context, the scientific targets for healthy diets and sustainable food systems are integrated into a common framework of "one health"; therefore, the innovation of the agri-food system in the modern society allows to merge the issue of malnutrition and sustainability.

Moreover, diet is not only aimed at guaranteeing a suitable source of macronutrients but also at ensuring a balanced intake of micronutrients (2), including bioactive compounds that can promote human health and solve global nutritional issues, such as hidden hunger, stunting and famine. Not only vitamins and minerals, but also dietary phytochemicals such as plant antioxidant, anti-inflammatory, and anti-aging compounds originating from leafy vegetables have been proved to contrast the outbreak of non-communicable diseases (NCDs), as well as metabolic disorders (e.g., obesity), so that their uptake is recommended by institutions such as the World Health Organization (WHO) (3–5). Leafy vegetables are sources of nutritional components, including proteins (6), dietary fibers (4), vitamins (7), and minerals (8), as well as natural antioxidants, including phytochemicals (9, 10).

In African rural communities, there is a wide range of indigenous vegetables cultivated by households for self-consumption that represent a valuable source of micronutrients and phytochemicals (2). The dietary diversification through the introduction of these food items in feeding programs is recommendable to reduce the risk of malnutrition and hidden hunger-related pathologies (6). However, the importance of identifying or resuming new species of agricultural interest does not deal only with nutritional and healthy needs, but also with environmental and sustainability concerns. This could be achieved by fostering the adoption of low-impact practices at the environmental level (11), such as conservation agriculture which is able to guarantee energetic, hydric and emissions savings (12, 13). In this work, we evaluated the healthy properties of *Corchorus olitorius* L., an indigenous African leafy vegetable known as jute mallow. This plant is largely diffused in the Middle East and in African countries (14–16) and is mainly cultivated in smallholding farms for self-consumption. It is made into a common mucilaginous soup or sauce in some West African cooking traditions (17). It has a high content of fiber, vitamins and antioxidant compounds (18), it is highly resistant to environmental stressors such as heat and water deficiency (2), and requires low fertilization to grow (8). We hypothesized that similarly to other leafy vegetables, stressful cultivation conditions, such as hydric stress, could trigger variations in the *C. olitorius* metabolic profile leading to higher polyphenolic and antioxidant compounds contents (19, 20). This may also have an impact on the nutraceutical properties of leaves.

The aims of the study are (i) to unravel the metabolic composition of jute mallow phenolic fractions obtained from leaves cultivated under different tillage and watering regimes in order to identify the most suitable and sustainable agronomic condition to enhance the leafy antioxidant content of this species, and (ii) to evaluate the bioactivity of the leafy phenolic fraction on human colon cell lines to unravel if the consumption of this leafy vegetable could be related to a reduced risk of gut malignancies, as already shown for other vegetables (21). This hypothesis is derived from a previous study (22), which highlighted a selective cytotoxic activity of jute mallow leaves against a liver cancer cell line (HepG2), with no effects on healthy FL83B cells. However, no information has been reported so far about the effect of such green leafy items on gut cancer and, more generally, on their potential role in enhancing human well-being.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Seeds of *C. olitorius* (accession ID. SUD-2) were obtained from the seed bank of the Word Vegetation Center (AVRDC, Tanzania). Plants were cultivated with two different agricultural approaches (conventional vs. conservation agriculture practices) following the same experimental design described in (13) through a randomized complete block design (RCBD) with two factors (i.e., tillage and irrigation with two levels each). Briefly, between May and August 2018, the trial took place in a long-term experimental field in San Bonico, Piacenza, Italy (45°00'18.05"N, 9°42'15.285"E; 68 m above sea level). Environmental temperatures ranged between 15 and 40°C and humidity was between 35 and 85%. A total of eight plots (5 × 3 m) were set up. Four of these were managed following the principles of conservation agriculture [i.e., 8 years of no-tillage (NT) and cover crops maintenance during the resting season of the field], while the other four were conventionally managed [i.e., conventional tillage (CT) and absence of cover crops]. Before sowing, all plots were fertilized at the same rate [50 kg nitrogen (N) ha^{-1}], by applying urea (*N*-46%). The main physicochemical properties at the beginning of the experiment were pH 6.8, soil organic C 12.8 g kg^{-1}, total N 1.2 g kg^{-1}, available P 32 mg kg^{-1}, exchangeable K 294 mg kg^{-1} and cation-exchange capacity 30 cmol kg^{-1}. High phosphorus (P) and potassium (K) concentrations in the 0–30 cm soil layer [available P (Olsen): 32 mg kg^{-1}; exchangeable K: 294 mg kg^{-1}] suggested that P and K fertilization processes would not have been necessary according to the plant requirements (23). The soil is a fine, mixed, mesic Udertic Haplustalf (24) with a silty clay loamy texture (sand 122, silt 462, and clay 416 g kg^{-1}) in the upper layer (0–30 cm), well-drained and non-saline. Plants were seeded at a distance of ∼10–15 cm from each other with a total density of 40 individuals per plot. To test the ability of *C. olitorius* to resist against water stress, two separated subplots for each growing condition were defined (20 plants per subplot): one subplot was fully watered (FW), while the other was half-watered (HW) and protected from rain by applying greenhouses only during rainfalls (in order to induce stress without modifying the growth conditions of not-irrigated plants). Moreover, to avoid water percolation, the HW subplots were delimited by using steel gilders planted into the soil up to a depth of 30 cm. Specifically, the HW subplots received only 46% (113 mm) of the total water used for the irrigation of the FW subplots (245 mm). Stressful watering conditions for this species are joined with ∼160–180 mm per growth season according to Maseko et al. (25). Just before flowering, leaves were manually collected and stored at−20°C before analyses.

Phytoextraction

Pooled mixtures of leaves obtained for each subplot were processed in triplicate independently. In particular, leaves were

washed and boiled in water at 100°C for 15 min in order to mimic African traditional culinary habits (17, 18, 26, 27). Leaves were dried in an oven at 50°C overnight and then grinded and stored at −20°C until analysis. Each pool of leaves was extracted according to the protocol of (28). Briefly, 1 g of powdered leaves was suspended in 4.8 mL of methanol 67% and 4.8 mL of chloroform. The powder was extracted on an orbital shaker (VDRL 711+, Asal, Italy) for 15 min at a constant rotation (400 rpm). Samples were extracted for a second time by adding a further 2.4 mL of the abovementioned extraction solution. The alcoholic portion of the extract was evaporated by using a rotary evaporator (40°C, 120 rpm, 1,000 bar); then, the remaining aqueous fraction was freeze-dried. Finally, samples were resuspended in water in order to obtain a concentration of extract equal to 6.7 mg/mL. All chemicals were purchased from Sigma-Aldrich Merck, Germany.

Total Phenol Content Evaluation

The total phenol content of each extract was estimated by the Folin-Ciocalteau assay as described in (29) with minor modifications. Each sample was diluted to a concentration equal to 0.2 mg/mL and a calibration curve was made up by using increasing concentrations of gallic acid (from 0 to 100 μg/mL). The analysis was performed by adding in a quartz cell 400 μL of ultrapure milli-Q water, 80 μL of sample or standard, 40 μL of Folin-Ciocalteau reagent and 480 μL of 10.75% Na_2CO_3 solution. After an incubation of 30 min at room temperature, samples were read against the blank at a wavelength of 760 nm. Results are expressed as mg GAE (gallic acid equivalent) per gram of leaves. All chemicals were purchased from Sigma-Aldrich Merck, Germany.

DPPH Radical Scavenging Activity

The radical scavenging activity of jute mallow total extracts was evaluated through the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as described in (29) with minor modifications. In brief, 50 μL of samples at a concentration of 0.2 mg/mL was added to 950 μL of 0.1 mM DPPH.

A calibration curve was made up by using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as a standard reference in a range of concentrations between 0 and 500 μM. After an incubation of 30 min at room temperature, samples were read against the blank at a wavelength of 515 nm. Results are expressed as mg TE (Trolox Equivalent) per gram of leaves. All chemicals were purchased from Sigma-Aldrich Merck, Germany.

Solid-Phase Extraction and UHPLC-DAD-MS Analysis

In order to enrich the polyphenolic fraction of jute mallow extracts, a SPE-based purification procedure was performed. Samples were loaded, washed (3 mL H_2O), eluted (4.5 mL MeOH pH 2) and finally evaporated in a speed vacuum centrifuge (Eppendorf, Germany). Dried samples were resuspended (0.3 mg/mL) for UHPLC-DAD-MS analysis (ThermoFisher Scientific, Italy). Chromatographic separation was performed through a Kinetex Biphenyl column (50 × 2.1 mm, 2.6 μm, Phenomenex, USA) at a flow rate of 0.3 mL/min by gradient elution. Two wavelengths (280 and 330 nm) were employed for the detection and quantification of target analytes. ESI HRMS analysis, both in positive and in negative ion modes, was used for the characterization of the main phenolic compounds in the polyphenol-enriched extracts (PEEs). The ESI parameters and product ions scans were optimized to obtain a good response of polyphenols and their characteristic fragments. Detected compounds were tentatively identified as described in (30). A calibration external standard method was used to quantify the main phenolic compounds in PEEs. A mixture of eight standards—neochlorogenic acid (NCA, LOD: 1.178 μg/g; LOQ: 3.927 μg/g); cryptochlorogenic acid (CCA, LOD: 0.508 μg/g; LOQ: 1.738 μg/g); chlorogenic acid (CA; LOD: 0.354 μg/g; LOQ: 1.21 μg/g); quercetin-3-*O*-galactoside (Q3Gal, LOD: 2.12 μg/g; LOQ: 7.11 μg/g), isoquercitrin (Q3Gly, LOD: 1.2 μg/g; LOQ: 3.9 μg/g), quercetin-3-*O*-malonyl-glycoside (Q3MGly, LOD: 5.33 μg/g; LOQ: 17.73 μg/g), 3,5-dicaffeoylquinic acid (3,5-DCQA, LOD: 0.28 μg/g; LOQ: 0.964 μg/g), and kaempferol-3-O-glycoside (K3Gly, LOD: 4.87 μg/g; LOQ: 16.25 μg/g)—was used to produce a calibration curve (in a range between 1 and 100 μg/mL).

Bioactivity Analysis

The eight most concentrated PEEs of the 16 plots were evaluated for their bioactive effects on healthy and colorectal cancer cell lines. The effect of each extract on the colon cells was evaluated by using CCD841 (ATCC® CRL-1790™) human healthy intestinal mucosa cell line and Caco-2 (ATCC® HTB-37™) human colorectal cancer cell line by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assays and through oxidative stress analyses [reactive oxygen species (ROS) and antioxidant enzymes assessment]. Cells were grown in EMEM supplemented with heat-inactivated 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1% nonessential amino acids, 100 U/mL penicillin, and 100 μg/mL streptomycin. All cell lines were maintained at 37°C in a humidified 5% CO_2 incubator. ATCC cell lines were validated by short tandem repeat profiles that are generated by simultaneous amplification of multiple short tandem repeat loci and amelogenin (for gender identification). All the reagents for cell cultures were supplied by Lonza (Lonza Group, Basel, Switzerland).

Viability Assay

Cell viability assay was investigated using MTT-based *in vitro* toxicology assay kit (Sigma, St. Louis, MO, United States), according to the manufacturer's protocols.

The cell lines were seeded in 96-well microtiter plates at a density of 1×10^4 cells/well, cultured in complete medium and treated after 24 h with 100 μg/mL of PEEs. After 48 h at 37°C, the medium was replaced with 100 μL of complete medium without phenol red containing 10 μL of 5 mg/mL MTT. After 4 h of further incubation for CCD841 and 2 h for CRC cell line, formazan crystals were solubilized with 10% Triton X-100 and 0.1 N HCl in isopropanol, and absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage against untreated cell lines used as controls.

Oxidative Stress Assay

The intracellular ROS and reactive nitrogen species (RNS) were detected by the oxidation of 2′,7′-dichlorofluorescin diacetate (H2DCFDA) (Sigma Chemical Co., St. Louis, MO). Caco-2 cell line was seeded in 96-well black microtiter plates at a density of 1 x 10^4 cells/well, cultured in complete medium, and incubated after 24 h with 5 μM H2DCFDA in PBS for 30 min in the dark at 37°C. After two washes in PBS, cells were treated with 100 μg/mL of extract for 2 h or 1 mM H_2O_2 for 1 h for positive control. The fluorescence (λem = 485 nm/ λex = 535 nm) was measured at 37°C using a fluorescence microtiter plate reader (VICTOR X3, PerkinElmer).

Antioxidant Enzyme Activity Assessment

To evaluate the effect of PEEs on enzymatic activities, Caco-2 cell line was seeded at 1 × 10^6 cells/100 mm dish and treated for 48 h with PEE at 100 μg/mL. Cells were rinsed with ice-cold PBS and lysed in 50 mM Tris–HCl pH 7.4, 150 mM NaCl, 5 mM EDTA, 10% glycerol, and 1% NP-40, containing protease inhibitors (1 μM leupeptin, 2 μg/mL aprotinin, 1 μg/mL pepstatin and 1 mM PMSF). Homogenates were obtained by passing five times through a blunt 20-gauge needle fitted to a syringe and then centrifuged at 15,000 × *g* for 30 min at 4°C. Supernatants were used to measure the enzymatic activities: Glutathione S-transferase (GST) was assayed as previously described by the method in the study by (31); glutathione reductase (GR) was assayed according to the method described in the study by (32); glutathione peroxidase (GPox) was assayed as reported in the study by (33); superoxide dismutase (SOD) was assayed as previously described in the study by (34); catalase (CAT) was assayed according to the method described in (35). All the experiments were normalized against an untreated control (CTRL). Enzymatic activities were expressed in international units and referred to protein concentration as determined by the Bradford method. All assays were performed in triplicate at 25°C in a Jasco V-550 Spectrophotometer.

Statistical Analyses

All the data were analyzed using R version 3.3.3. Data from Folin-Ciocalteau and DPPH assays were analyzed by a Generalized Linear Mixed-Effect Model (GLMM) assuming a beta-binomial distribution of the response variable. Data from the MTT assay were analyzed again by a GLMM considering cell viability as a response variable and the cell line as an independent variable. The random effect was the plot which plants were grown in, and the fixed effect was the growth treatment. MTT data from single plots were analyzed by a Generalized Linear Model (GLM) with a quasi-binomial distribution of the response variable (% cell viability). The fixed effect was the cell line. To test the impact of the agronomic treatments on the metabolic profile of *C. olitorius*, data from HRMS were analyzed by comparing the intensity of the peaks both in positive and in negative mode through one-way ANOVA. Packages used were TMB, glmmTMB, and ggplot2. Data from enzymatic activity assays and ROS produced were analyzed by one-way ANOVA and Dunnett's *post-hoc* test through the multcomp package.

RESULTS

Antioxidant Composition of *C. olitorius* Grown Under Different Agronomic Conditions

Figures 1A,B show the total phenol content and total antioxidant composition of leaves harvested from plants grown under the different agronomic management. Data suggested that the total phenol content of boiled leaves of *C. olitorius* is around 0.25% of the total weight of leaves. No significant differences were detected among cultivation treatments ($\chi^2 = 0.38$, $p = 0.944$, **Figure 1A**). The radical scavenging activity followed the same trend identified for the total phenol content and, in particular, is comparable among treatments with values ranging between 2.5 and 3.2 mg TE per gram of leaves. Also in this case, none of the treatments was found to significantly affect the radical scavenging activity of the extracts ($\chi^2 = 0.3$, $p = 0.9599$; see **Figure 1B**).

Qualitative and Quantitative Profiles of Jute Mallow Extract by UHPLC-DAD-HRMS^n

In order to estimate the chemical composition of main compounds occurring in leafy extracts and to identify the compounds responsible for the abovementioned antioxidant activity (see **Figure 1B**), a HRMS untargeted screening was performed on the extract from each experimental growth

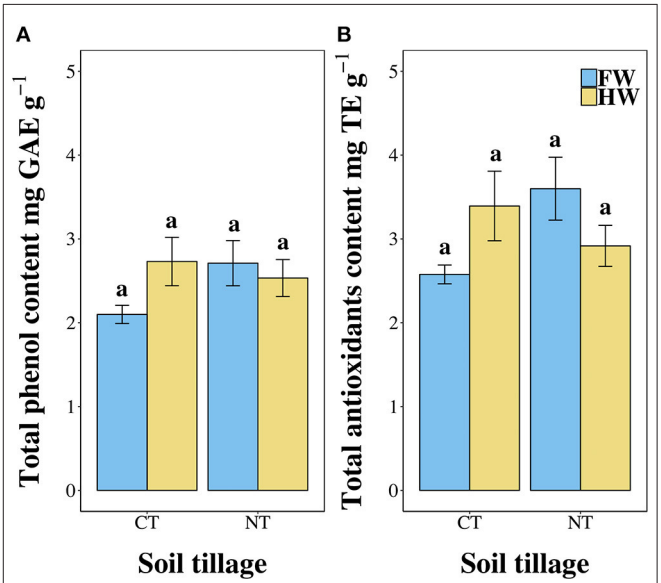

FIGURE 1 | (A) Total phenol content (expressed as gallic acid equivalents per gram of dry leaves), **(B)** total antioxidants content (expressed as Trolox equivalents per gram of dry leaves) of jute mallow plants grown under the four different agronomic treatments. Data are expressed as the mean ± SEM. CT, conventionally tilled; NT, not-tilled; FW, fully watered; HW, half-watered.

condition. The leafy extracts were analyzed by UHPLC-DAD-HRMS^n to investigate their qualitative phenolic profile (**Supplementary Table 1**). Metabolite assignments were made by comparing retention times, UV/Vis spectra, and MS data (accurate mass and MS^n fragment ions) of the detected compounds with standards, whenever available, and jute mallow compounds reported in the literature and databases. The most abundant phenolic compounds are listed in **Supplementary Table 1** with the main information (name, retention times, UV and MS data) for each component. The main compounds were quercetin and isomers of kaempferol-(malonyl-)-hexosides, chlorogenic acids, dicaffeoylquinic acids, and feruloyl-quinic acids. Results of HRMS untarget analysis did not reveal statistically significant differences between agricultural treatments (see **Supplementary Table 1**). Quantitative data are reported in **Table 1**, whereas **Supplementary Figure 1** shows the phenolic profile of the main compounds both in standard solution and in PEEs at 280 (a) and 330 nm (b). A total of 11–20 peaks per sample were detected and eight of these peaks were identified. PEEs showed a total phenolic content calculated as the sum of individual phenolic compounds ranging between 74 and 99 μg/mL (with a range between 1,794.4 and 2,612.5 μg per gram of dry leaves). In particular, Q-3-O-MG was the most abundant phenolic compound in all the analyzed samples (32–39 μg/mL, with a range between 695.7 and 1,026.9 μg per gram of dry leaves). Conversely, the content of chlorogenic acid derivatives in all the treatments was very low, ranging between 0.18 and 0.65 μg/mL (4.32–53.7 μg per gram of dry leaves) as well as the level of K-3-O-Gly (1.9-3.3 μg/mL with a range between 34.3 and 86.2 μg per gram of dry leaves). In the case of these latter compounds, values display a high variability due to the fact that the presented data (**Table 1**) come from plants grown in different plots, although subjected to the same agronomic treatments. However, the results of the quantitative analysis did not show statistically significant differences among the investigated agronomic treatments.

TABLE 1 | Concentration (μg/g) of the main phenolic species occurring within PEEs.

Compounds (μg/g)	NTFW	CTFW	NTHW	CTHW
NCA	7.6 ± 7.6	7.2 ± 7.2	<LOQ	4.9 ± 4.9
CCA	16.5 ± 10.3	11.8 ± 7.7	<LOQ	6.3 ± 6.3
CA	29.6 ± 15.5	13.9 ± 4.5	4.32 ± 4.32	15.0 ± 6.2
3,5-DCQA	77.5 ± 19.9	54.9 ± 32.4	50.4 ± 6.7	56.5 ± 0.7
Q-3-O-Gal	630.4 ± 69.7	525 ± 178.1	488.4 ± 40.3	768.2 ± 123.6
Q-3-O-Gly	574.8 ± 123.5	595.4 ± 43.5	398.1 ± 90.5	648.5 ± 69.4
Q-3-O-MG	819.6 ± 188.8	695.7 ± 188.4	805.5 ± 25.1	1,026.9 ± 205.0
K-3-O-Gly	67.9 ± 16.5	34.3 ± 29.3	47.7 ± 11.1	86.2 ± 6.0
Total	2,223.9	1,938.2	1,794.4	2,612.5

Data represent the concentration of each compound occurring in 1 g dry leaves and are expressed as the mean ± SEM. For each cultivation condition, the quantification of the specific analytes was performed considering three biological replicates (i.e., leaves coming from plants cultivated in different plots). NTFW, not-tilled and fully watered plots; CTFW, conventionally tilled and fully watered plots; NTHW, not-tilled and half-watered plots; CTHW, conventionally tilled and half-watered plots. <LOQ: values not quantifiable since below the limit of quantification.

MTT Viability Assay

PEEs from the different experimental conditions were evaluated for their bioactive properties on CCD841, a healthy colon cell line used as a reference and Caco-2, a colorectal cancer cell line.

MTT assay performed on cells treated with PEEs showed significant effects ($p < 0.001$) on Caco-2 cells at a concentration of 100 μg/mL with an average viability reduction equal to 39%, compared with the healthy cell line, which maintained a percentage of viability higher than 80%; therefore, it was not negatively affected by the treatment with PEEs of *C. olitorius*.

The analysis performed on single plots (**Figure 2**) highlighted a stronger effect on Caco-2 from plants grown under NT management. Three of the four plots analyzed showed a higher viability reduction in Caco-2 cell line than in healthy CCD841 cells. Only one plot from conventional agriculture management (CT) was found to be significantly more cytotoxic against Caco-2 cells compared with the healthy control. No significant effects were found to be driven by the drought treatment.

Oxidative Stress Analysis

An oxidative stress analysis was performed to better clarify the action mechanism of jute mallow PEEs on cancer cells. The fluorescence analyses performed on Caco-2 cells revealed a significant increase in the production of ROS compared to the control ($F = 38.53$, $p < 0.001$) after 2-h treatment. Each tested sample induced the same effect with quantitative differences expressed in **Figure 3A**.

The intracellular detoxification systems were also evaluated to clarify the cellular response after PEE treatments in the Caco-2 cell line.

SOD activity was found to be significantly affected by PEE treatments ($F = 11.54$, $p < 0.001$; **Figure 3B**). In particular, SOD activity was negatively affected by the treatment with PEEs from tilled plots, both FW ($p = 0.01$) and HW ($p = 0.023$) compared to the control, while no significant effects were elicited by the treatment with PEEs from not-tilled plots, both FW ($p = 0.562$) and HW ($p = 0.127$).

CAT was found to be the most affected enzyme ($F = 13.13$, $p < 0.001$; **Figure 3C**). Almost all the conditions induced a significant reduction in its enzymatic activity, more marked from PEEs coming from the FW condition, both from CT ($p < 0.001$) and from NT ($p < 0.001$) treatment. PEEs from HW plots displayed a weaker effect, significant for those obtained from NT plots ($p = 0.04$) and only marginally significant concerning those derived from CT conditions ($p = 0.058$).

GST activity was found to be slightly increased by PEE treatment (**Figure 3D**). However, the only experimental group showing a significant difference ($t = 2.833$, $p = 0.037$) compared with the untreated control was CTFW, which was the PEE group showing the least ability to discriminate with the healthy cell line (**Figure 2**). Conversely, the other groups showed a not significant difference in the GST activation compared to the control. Furthermore, despite some fluctuations among treatments, GR activity did not show any significant variation compared to the

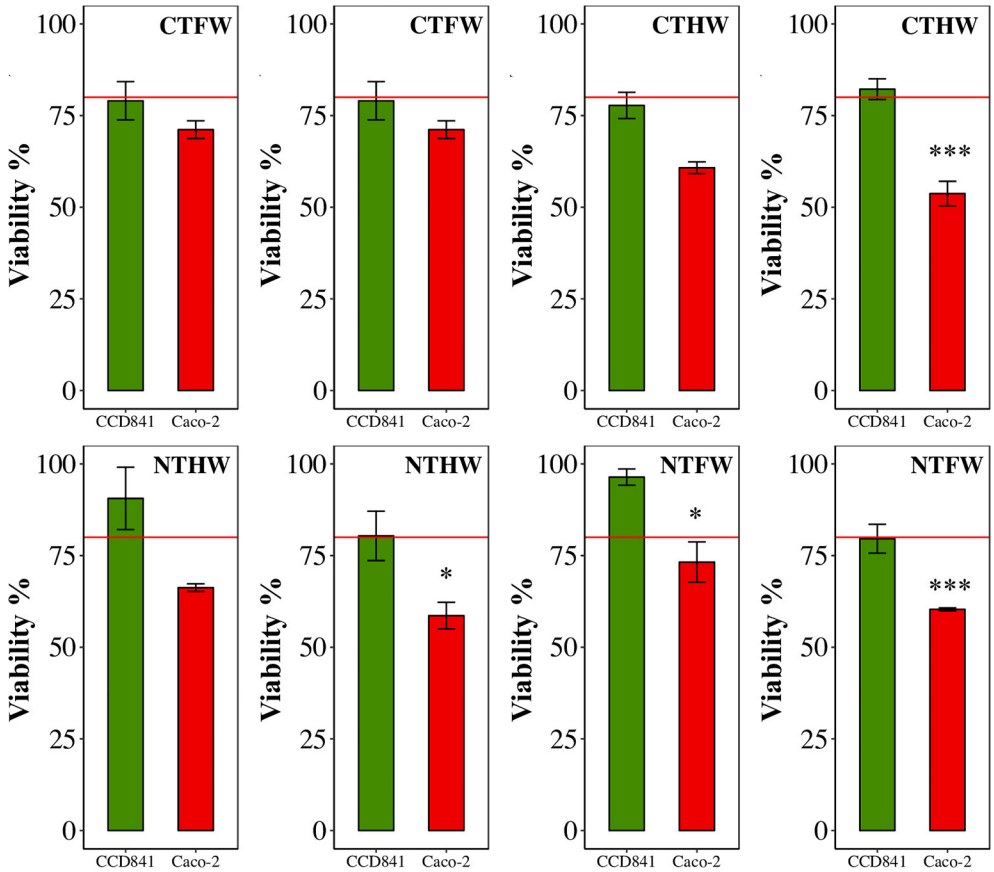

FIGURE 2 | MTT viability assay on two human: cell lines: CCD841 (green, healthy line) and Caco-2 (red, colorectal cancer cell line). All cell lines were treated with 100 μg/mL GAE in the medium. Data are mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. CTFW, conventionally tilled and fully watered plots; CTHW, conventionally tilled and half-watered plots; NTHW, not-tilled and half-watered plots; NTFW, not-tilled and fully watered plots.

control in all treated samples ($F = 0.67$, $p = 0.622$; **Figure 3E**). Therefore, GR did not appear to be a candidate to explain the decrease in viability of Caco-2 cells treated with PEEs. Also, GPox activity was found not to be influenced by extracts obtained from plants deriving from the different growth treatments ($F = 1.331$, $p = 0.304$; **Figure 3F**). Overall, enzymes involved in glutathione metabolism did not display significant variations under PEE treatment compared to the control.

DISCUSSION

Responses of *C. olitorius* Antioxidant Metabolites to Conservation Agriculture Management

According to FAO (36), conservation agriculture is one of the most promising management strategies for worldwide agroecosystems, since it allows to improve and sustain productivity, to increase profits and food security while preserving and enhancing environmental resources (37). In terms of agricultural sustainability in Mediterranean countries, NT is reported to enhance water soil content in response to climate change (13) and to preserve soil from erosion and leaching (38). In our work, we highlighted that *C. olitorius* is rich in several polyphenols and that their amount and composition are not modified by stressful cultivation conditions, such as NT and the reduction of water irrigation. Generally, environmental stressors, such as osmotic stress raised from water scarcity, may be able to elicit the production of secondary metabolites in leaves, as it happens in the case of vegetable amaranth (19), *A. tricolor* (20) and *C. olitorius* itself, although this species was found to greatly vary in its response depending on the genotype (14). A dedicated screening of these cultivars for their suitability to be grown in the Mediterranean context could also be performed to select the most promising accession in terms of the production of secondary metabolites.

The total antioxidant activity is associated with the concentration of plant endogenous antioxidants, such as glutathione, provitamin A, ascorbic acid, different types of pigments, and secondary compounds, including chlorophylls, carotenoids, xanthophylls, and polyphenols (30, 39). In accordance with the variation in the phenolic content of *C. olitorius* leaves, their total antioxidant activity was found not to significantly differ based on the cultivation treatments.

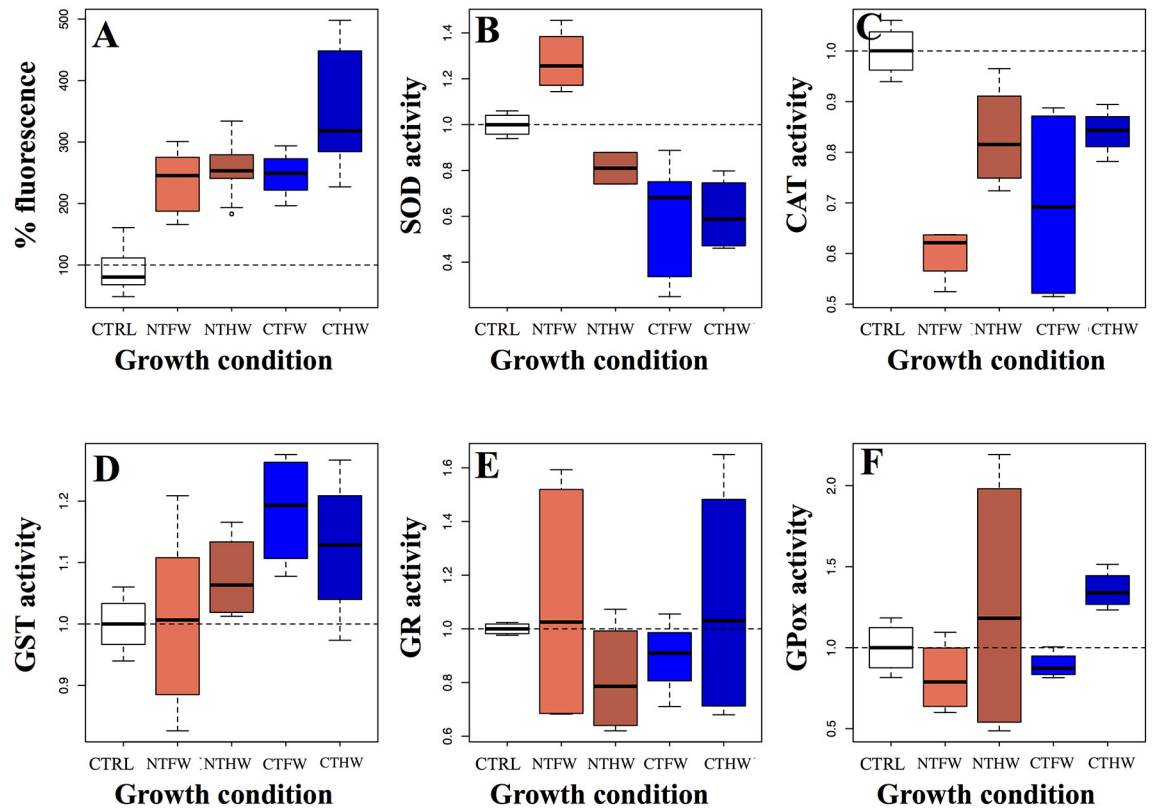

FIGURE 3 | Oxidative stress detection **(A)** and enzymatic activity assays expressed as fold units compared to a non-treated control (CTRL) of five antioxidant cellular enzymes **(B)** superoxide dismutase, **(C)** catalase, **(D)** glutathione S-transferase, **(E)** glutathione reductase, **(F)** glutathione peroxidase performed on Caco-2 cells treated with 100 μg/mL GAE in the culture medium. CTFW, conventionally tilled and fully watered plots; CTHW, conventionally tilled and half-watered plots; NTHW, not-tilled and half-watered plots; NTFW, not-tilled and fully watered plots.

Concerning the nutritional properties of *C. olitorius* leaves, the main critical element is represented by the consumption habits that involve boiling the leaves. In the case of *C. olitorius*, recent data showed that the total polyphenols content of fresh leaves ranges from 5.41 to 7.78 mg GAE/g DW depending on the genotype (40). Considering these reference values, our data suggest that boiling reduced the amount of polyphenols by at least 55–65%. This is in accordance with a similar research conducted on other leafy vegetables (41). Currently, there are no indications of *C. olitorius* leaves consumption alternative to boiling. However, innovative technologies could be applied to identify the methods of preparation and consumption able to preserve antioxidant metabolites, such as microwave cooking processes that were found to be the suitable strategies to preserve (or also to ameliorate) the amount of phenolic compounds and the antioxidant capacity of several green leafy vegetables (9).

Healthy Properties of *C. olitorius*

In this study, the phenolic fractions occurring in the leaves of *C. olitorius* were found to induce a significant reduction in the viability of Caco-2 cancer cells without any detrimental effect on the healthy cell line. These properties have been observed despite the preliminary boiling and make *C. olitorius* a promising candidate species in the field of nutritional prevention. A similar effect in terms of selectivity in the reduction of HepG2 liver cancer cell line viability was shown in a previous study performed by (22), which found that the mechanism of action promoted by jute mallow extracts is the mitochondria-dependent apoptosis pathway. In our study, the selective cytotoxic activity triggered by *C. olitorius* PEEs appeared to be mediated by a sudden increase in the ROS level, still high after 2-h treatment and through a successive decrease in the activity of glutathione-independent antioxidant enzymes. This activity may appear as a paradox because phenolic compounds are known to be among the best antioxidant phytochemicals. However, it is important to note that the tumor environment shows different redox status compared to healthy tissues and this study confirms previous observations highlighting that—despite their well-known antioxidant properties on healthy tissues—these secondary compounds can induce pro-oxidant responses on tumor cells, triggering programmed cell death mechanisms such as apoptosis (42, 43).

Actually, many flavonoids were identified within PEEs, mostly belonging to the family of quercetin derivatives (**Table 1**; **Supplementary Figure 1**). Similarly, other studies

showed the occurrence of these metabolites in leafy vegetables, as documented, for instance, in drought-tolerant vegetable amaranth (22, 23) and also in *C. olitorius* leaves (20). As an example, Q-3-O-Gal accounted for 20–30% of the total phenolic content of PEEs. It is likely that this compound (together with the other quercetin derivatives) may be responsible for the selective cytotoxicity against the Caco-2 cell line with no detrimental effects on the healthy one. The anti-inflammatory and antioxidant properties of this compound extracted from jute mallow leaves have been already documented by (44).

Another noteworthy secondary metabolite identified in PEEs is the flavonoid astragalin (K-3-O-Gly), which was found in different amounts in approximately all PEEs. This compound has been reported to modulate inflammatory responses by regulating the expression of NF-κB, iNOS as well as cytokines and chemokines (COX-2, TNF-α, IL-10, and IL-6). Astragalin is also known to be an inhibitor of ERK-1/2 and Akt signaling; therefore, it is a significant compound against the proliferation of cancer cells (45).

Finally, CA and its isomers have also been identified in the majority of the analyzed PEEs and are probably involved in the selective cytotoxicity exerted by *C. olitorius* phenolic fractions, as previously shown by (46).

The last part of this study is focused on evaluating if cultivation conditions may impact on jute mallow cancer preventive abilities. Our results suggested that samples obtained from conservation agriculture plots are endowed with a higher selectivity (in terms of cytotoxicity) toward the cancer cell line compared to the healthy one. Considering that no significant differences in the polyphenolic composition among different field growing conditions were identified, we hypothesize that other unidentified compounds could play a synergic role in reducing the viability of cancer cells by acting on multiple cellular pathways. It is likely that other antioxidant compounds, displaying selective cytotoxic properties against cancer cell lines, have been elicited in response to the growth conditions (47).

The new trends in cancer prevention and therapy are directed at identifying combinations of different phytochemicals, acting simultaneously on several pathways to contrast cancer progression and to overcome drug resistance mechanisms typical of chemotherapy (48, 49). In this regard, deeper differential analyses will better clarify which compounds vary between the tested growth conditions leading to differential bioactive responses.

CONCLUSION

In this study, we showed that *C. olitorius* is an African indigenous vegetable whose phenolic profile is not affected by stressful cultivation conditions such as conservation management and low hydric regime. *C. olitorius* leaves are a source of antioxidant compounds such as flavonoids and chlorogenic acids that can trigger a selective cytotoxicity against colorectal cancer cells, but not on healthy cells. Further investigations may be performed in order to unravel if other chemicals acting as antioxidants, such as carotenoids, vitamins and minerals, may be elicited by conservation management and hydric stress conditions. Overall, *C. olitorius* fully meets the interests of modern food sciences in terms of sustainability and health. Furthermore, this plant constitutes an important resource not only for developing countries, but also for the Mediterranean context, where its cultivation may be recommended in order to improve the nutritional status of both African and Western diets.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

LG and ML designed the experiment. LG, DP, NT, and AF performed the field cultivation experiment. LG, LC, and MU performed the phytoextractions. LG, DP, LC, and MU chemically characterized the extracts. GS, MF, and PF performed the cellular assays. LG analyzed data. ML provided the financial support to the experiment. All authors significantly contributed to the writing of the manuscript.

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SUPPLEMENTARY MATERIAL

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Mainstreaming Local Food Species for Nutritional and Livelihood Security: Insights From Traditional Food Systems of *Adi* Community of Arunachal Pradesh, India

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This study brings out the critical role of lesser-known local plant species in the food, nutrition and livelihood security of *Adi* community in Arunachal Pradesh, India. Considering women as a major custodian in knowledge and practices on foods, a total of 90 *Adi* women and 60 key knowledgeable community members (thus a total of 150 participants) were selected from East Siang and Upper Siang districts of Arunachal Pradesh. Data were collected using combination of methods including recipe contest, focus group discussion, personal interviews and laboratory analyses. The results indicated that *Adi* women were able to identify 39 bioculturally important species from a range of locally available plant species. Used alone or with other foods, these plants remain central to the *Adi* people's cultural identity and livelihood security. In addition to improving food and nutritional security, these species accessed from different land use systems, are also sold on the local markets to generate decent incomes. Of the species identified by *Adi* women, 28 were culturally shared and used frequently in food and ethnomedicine. Laboratory analyses of the selected 22 species revealed exceptionally high levels of minerals and other nutrients, such as proteins and anti-oxidants, supporting their traditional use for health benefits. Our study results provide valuable insights to the researchers to explore the vast hidden potential of these and other similar species for improving nutritional well-being of local communities in marginal areas. Adequate policy support is needed to enable *Adi* and other such marginalized communities to cope with challenges being posed to traditional food systems.

Keywords: wild edible plants, biocultural knowledge, nutritional values, ethnomedicine, recipe contest, food and nutritional policies, participatory learning

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INTRODUCTION

The social-ecological knowledge accumulated orally over generations has a great influence on traditional food systems and Indigenous biodiversity (1, 2). The Indigenous biodiversity, consisting of natural and domesticated local species nurtured and augmented over a certain geographical area (3), plays a pivotal role in the livelihood security of the local communities (4). There exists an intricate linkage between the foods consumed by Indigenous peoples and the local

social-ecological conditions (5). Such locally available foods represent an easily accessible and affordable means to nutritional security (6, 7). The fact that women often play a critical role in sustaining and promoting the traditional foods, knowledge systems and livelihood security across the social-ecological systems is widely acknowledged (8–10). As biochemical constituents of locally consumed foods mostly remain unexplored, local people employ the traditional knowledge to understand the health benefits of different species used as food (11). Based on this knowledge, the local communities prioritize the conservation and management plans for local natural resources including agro-biodiversity (12). Of late, socio-economic and cultural shifts are increasingly altering the food preferences such that many traditional foods are being eclipsed by commercial foods (13), putting the traditional food systems and associated knowledge at risk (14). Conservation and promotion of local food practices are critical to global efforts toward safeguarding the culturally important foods and associated knowledge (1, 15).

Women of the tribal communities of Arunachal Pradesh (Ar P), India, are the real custodians of the local biodiversity (16) and traditional food systems (17, 18). They still rely on lesser-known local plant species for the household food and nutritional security (19). Many such species have exceptionally high levels of bio-active compounds displaying nutraceutical properties and contributing to overall health and well-being (20). However, their knowledge and participation are often little valued in the formal programs and policies aimed at strengthening family and community well-being (21). Therefore, validating this knowledge is particularly important (6, 22). The objectives of this study were: (i) to explore commonly used food plant species, and (ii) assess their nutritional, ethnomedicinal and cultural values identified by *Adi* women to better understand the intricate relationship with livelihoods.

CONCEPTUAL FRAMEWORK

Connecting cultural diversity with landscapes and ecosystems is critically important for sustaining the social-ecological resilience and traditional food systems of tribal and Indigenous peoples (23, 24). Understanding of such interactions can help to learn how a community has evolved its food system and add value to them. A better knowledge of such interactions is vital to learn the community's preferences in tune with livelihood needs and local conditions (7, 25, 26). In this study, we explored in collaboration with local *Adi* women, some of their locally valued food species that are still either semi-domesticated or collected from the wild. We also documented how the traditional knowledge embedded with these edible plants relates to their cultural significance, conservation, livelihood support and sustainability (8, 11, 27).

Indigenous and tribal communities of remote locations have evolved distinctive life-ways and cultural practices to meet their nutritional requirements (8, 28, 29). Drawing insights from Pretty (18), Kuhnlein et al. (6) and Kuhnlein (8), we tried to understand traditional ways and means employed by the *Adi* women to ensure nutritional and livelihood security. The complex interrelationships between food and culture of Indigenous people, like the *Adi,* are not readily apparent. Following Kuhnlein and Receveur (22), we enlisted the local food plants also being used as ethnomedicine by the *Adi*. The tribal communities, who are often marginalized, live in harsh ecological conditions (30), have evolved integrated strategies for accessing and using foods from diverse ecosystems (31). Following Kuhnlein (8), we documented some of these practices of *Adi* women relating to food and nutritional security that enrich the cultural diversity as well. We followed Shanley's approach (32) for devising a new methodology–the "recipe contest"–to mobilize the *Adi* community for exploring the plant food resources of high cultural and nutritional significance. This approach also helped us in rapport building with other stakeholders including community leaders and study participants. Drawing insights from Davidson-Hunt et al. (29), we collaborated with *Adi* women and leading knowledge holders over a long period (2006–2014) to gain deeper insights on current practices and concerns regarding traditional foods and to enhance the future opportunities. Through this approach, we were able to learn from *Adi* women as well as to support them through our research. For example, we undertook nutritional analyses of 22 important local food plant species. This was necessary to establish a scientific basis for the nutritional value of these species so that developmental agencies can devise appropriate future action plans for their multiple use and conservation. Our efforts in this direction led to positive results in terms of enhanced recognition of *Adi* women's traditional knowledge and linking these traditional foods with potential markets (33).

RESEARCH METHODOLOGY

The Study Area

Arunachal Pradesh (Ar P) is the largest state in northeastern India, covering a geographical area of 83, 743 km^2. It lies between 26° 28′ to 29° 30′ N latitude and 91° 30′ to 97° 30′ E longitude. It has a hilly terrain, with mountains up to 7,090 m high. It is considered one of the most biodiverse regions of India, supporting extensive forests rich in plant and animal resources (34). There are 27 tribes and 110 ethnic groups in the state, most residing in close proximity to natural areas, on which they depend greatly for their livelihoods. The *Adi* are a major collective tribe living mainly in East Siang, Upper Siang, Upper and Lower Dibang Valley districts of Ar P (35). In both East Siang and Upper Siang (study districts), *jhum* cultivation (upland slash and burn agriculture) is a major socio-economic adaptive practice for *Adi*. Home gardens and community forests also provide considerable subsistence support to the *Adi*. In a nutshell, these integrated land use systems remain vital to food, nutritional and livelihood security of *Adis* (16, 18). Day-to-day interactions of *Adi* women with these local land use systems and resources over generations have shaped their distinct food preferences, practices and beliefs. While *Adi* women play a lead role in conserving and accessing the food resources for household livelihood security (16, 18), hunting of wild games and drudging tasks including

slash and burn activities (*jhum* cultivation) are the major responsibilities of men.

Sampling Design

Districts and Villages

Based on the people's ethnicity, relative dependency on natural resources and remoteness, three circles (administrative units) namely Pasighat and Mebo from East Siang and Mariyang from Upper Siang district were selected. Further, in consultation with Block Development Officers, we selected five villages randomly from each of these circles. Mirasm, Balek, Napit, Sibut and Yagrung villages were selected from Pasighat, while Ayeng, Kiyit, Borguli, Namsing and Sibuk from Mebo circle of East Siang district. Similarly, Mariyang H.Q, Damroh- Gingkong, Adi Pasi-Bine, Milang-Karket and Peki-Modi villages were selected from Mariyang circle of Upper Siang district.

Pilot Study and Duration: Key Insights for Methodology Enrichment

The studies involving human/animals were reviewed and approved by the Research Advisory Committee headed by the Dean, College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh. Initially, we conducted a pilot study in two villages (December 2006 and January 2007) with six key knowledgeable women (3 from each village) after consultation with village *Gaon Bura* (GB) - customary chief and co-chiefs (Co-GB). The purpose was to generate key insights for the research methodology to be adopted for this study (36). Subsequently, different field activities and data collection exercises were carried out from 2008 to 2009. Building upon the knowledge of these activities, the initial plant samples were collected during August to October 2008 and 2009 for nutritional analysis. Further, a repeat sampling was done in 2012 and 2014 in the same months, to validate the results obtained in the previous analysis. Based on stability in results, data from these samplings were used as replicates in analysis.

Recipe Contests: Selection of Respondents

As recipe contest is considered a major participatory approach to record the women's knowledge and practices on food plants (9, 10), we organized recipe contests in each of the selected villages to record the diversity in traditional foods of plant and animal origin. Considering *Adi* women as more knowledgeable than men on food resources (16, 18), we requested them for free listing of food resources, with the objective of rapidly assessing food diversity and recording consensus among community members on shared cultural knowledge (37, 38). These contests were organized in each village, with the help of the village elders, *GB* and *Co-GB*- heading village *Kebang* (indigenous institution), and members of Village Panchayat (democratic institution). *Adi* women were informed about the recipe contests 15 days in advance. Interested women were invited to these recipe contests to demonstrate their traditional culinary skills at the village community hall (*mosup*) and to display dried and stored plant and animal samples used in the food preparation. The number of women participating in these contests varied from 20 to 35 (∼28) per village. These women displayed 18–39 (∼24) local plants used as ingredients in the traditional foods. The panel of judges for each contest consisted of a *GB* and Co-*GB*, two elderly women (who were not participants), and 2–3 (∼2) research scientists. Thus, a total of 60 resource persons from 15 villages served as judges in the recipe contests and subsequent activities, as discussed later. The plant samples brought by the women contestants were collected, photographed and prepared as herbarium specimens for identification at the Botanical Survey of India (BSI) herbarium, Itanagar, Arunachal Pradesh. The author citations and botanical nomenclature were matched with IPNI checklist, PIC (Kew garden) of world flora, and identified by the name in current use (NCU) as per ICBN rules (St. Louis Code 2000) (39).

In each village, the traditional foods prepared and displayed by the *Adi* women during contests were evaluated by an expert panel using the Hedonic scale with slight modifications. The taste, flavor, texture, ethnicity, consumer preference, and overall community acceptance (38) were the scoring criteria for selecting the dishes and the contest winners. In case of any ambiguity in results, the decision of the *GB* and *Co-GB* (participating in the judge panel) was considered final. Each criterion was assigned a score between a maximum of "3" and a minimum of "0." Thus, a particular food (using a local species) demonstrated by a woman could receive a maximum score of 18 and a minimum of 0. On the basis of mean values generated by the judges for each recipe and total number of foods presented by a woman, the overall recipe contest winners were identified. Six women from each study village (total 90) were conferred the awards in first, second, third and consolation (3 each) categories. These 90 women awardees were finally selected as the respondents of this study.

Methods of Data Collection

Personal Interviews

A semi-structured interview schedule was developed with open-ended questions. The respondents were interviewed in the *Adi* dialect in presence of local guides (37). This interview schedule included questions on women's knowledge about local food plant species, association of plants with animal species, land use types where species are found, economic value of species, local creativity in mixing plants with different food resources and cultural and livelihood dimensions of the foods (**Supplementary Material 1**). The schedule was pilot tested with 5 *Adi* women in the non-sampled areas to assess the effectiveness of the language and suitability of the questions.

Participatory Techniques: Combined Approaches With Personal Interviews

A combined methodology is considered to be very effective in conducting in-depth interviews and discussions for collecting the knowledge (data) on traditional food practices (40). In addition to personal interviews, participant observation was applied as a participatory tool with knowledge holders to study some key food practices in the real field situations (41, 42). This exercise, under the guidance of local resource persons, helped us in recording the local practices and methods for collecting the food plants, food preparation and processing. As informal visits enrich the data by supplementing the participant observations on traditional food

related knowledge and practices (43), we made several such visits during local festivals and cultural occasions (34 visits in total) to gain deeper insights into local food and cultural practices. Finally, random visits to the selected villages helped us in cross-checking and validating the local food-related knowledge documented during the previous visits/exercises. Transect walks (a participatory tool) are conducted in the presence of local knowledge holders to understand and verify the status and patterns of local resource use (44, 45). We conducted transect walks in each study village in consultation with village elders, *GB* and recipe contests winners to document the local plant and animal species used in the *Adi* foods. Focus group discussions were used to assess the preparation methodologies of certain dishes such as fermented foods and mixed foods (alcoholic beverages and plants mixed with fish and wild game).

Methods of Sampling and Nutritional Profiling of Selected Wild Vegetables

Based on the frequency of use, perceived nutritional importance, their shared cultural knowledge (37) and the high scores in recipe contests, 22 local food plant species were selected for their nutritional profiling. Samples were collected from study villages in each of four years (2008, 2009, 2012, and 2014), to account for variability in composition. For each species, about 2 kg of pooled sample was collected and composited. Each composite sample was analyzed in triplicate as analytical replicate to ensure repeatability and precision. ASFRM-6 (fish meal), ASFRM-14 (Rice flour) food reference standards obtained from Institute of Nutrition, Mahidol University, Thailand were used to ascertain recovery and accuracy of results. Results are presented as mean of means for four years data ($n = 4$) on per 100 gram fresh weight basis. The samples were processed using standard protocol and analyzed using official and standard methods. The moisture, ash, dietary fiber, protein, fat, starch and minerals contents were estimated using AOAC 934.01, AOAC 938.08, AOAC 985.29, AOAC 2001.11, AOAC 920.58, AOAC 996.11, and AOAC 999.11 methods (46), respectively. The total soluble sugars, ascorbic acid, total phenols and total flavonols were analyzed using Hedge and Hofreiter (47), Jagota and Dani (48), Singleton et al. (49), Quettier-Deleu et al. (50) methods, respectively. For details of analysis methods, see **Supplementary Material 2**.

Methods of Scoring the Selected Variables

To test the statistical significance of the correlation between land use type and access to different food species, we quantified these variables using a scoring technique: score of "1" was assigned to species conserved and accessed from *jhum* land (JL, based on topographical constraints); "2" to species from community forests (CF, based on limited conservation- allowing species to grow naturally) and "3" to those harvested from both JL and CF (cumulative weightage of both). Score "4" was assigned to the species accessed from both home gardens (HG) and JL, while a score "5" to those harvested from both HG and JL (cumulative weight of both). Seasonal availability of species was quantified by assigning a score of "1" to the species available for <3 months, "2" to those available for 3–6 months, "3" to availability for 6–9 months and "4" if available for >9 months. Frequencies of plant and animal species were accounted for analyzing diversity and similarity indices.

Statistical Analysis

Data triangulation technique was applied to synthesize the information obtained through qualitative and quantitative approaches (51). Qualitative data, including socio-cultural, economic and ecological variables, were analyzed using thematic techniques (51). This data-set was complemented with verbatim responses recorded in our research diary to support the quantitative observations and explain the processes and interrelated dynamics of traditional food species. Nutritional values of local food plants presented as mean ± standard deviation. A "*t*"-test was used to assess the differences in incomes gained from the species accessed from different land use types using STAR software (52). Correlations between land use type and access to food species in a particular month/season were analyzed using Spearman rank correlation. Diversity and similarity analyses of the same set of data, and plant and animal species being used on cultural occasions, were computed using Shannon-Weaver index in the PAST statistical software (version 4, 2020) (53). Other quantitative data were entered into spread-sheet and analyzed using frequency and percentage. Our key findings were shared with the knowledge holders through a follow-up village meeting to clarify and refine the responses; especially in case of ambiguity or misinterpretation.

RESULTS

Local Food Plant Resources

We recorded a total of 39 local plant species used by *Adi* women as traditional foods (**Table 1**). Out of these, 17 (43.59%) were domesticated and reared in different land use systems for food and cultural purposes. Fifteen (38.46%) were semi-domesticated (allowed to grow naturally), while 7 (17.95%) species were both semi-domesticated and domesticated. *Belang* (seeds of *Artocarpus heterophyllus*), *sirang* (*Castanopsis indica*), *taje* (*Amomum subulatum*), *angyat* (*Coix lacryma-jobi*) and *tasat* (*Arenga obtusifolia*) were the major food species consumed during droughts. Epidermal layer of *tasat*, though considered a delicacy among wealthier *Adi*, is also frequently consumed as bread during droughts by the poor. Selection of different parts of the local plants by *Adi* is mainly based on difference in taste in different seasons. For example, tender leaves of *ongin* (*Clerodendrum colebrookinanum*) are plucked during winter, while both older and tender leaves are chosen during rainy season.

There was a considerable diversity ($H = 3.44$) in land use types for accessing food species by *Adi* women. Overall, a total of 17 species were accessed from these varied land use types (**Figure 1**) during the rainy season. Home gardens were found to be more prominent across the seasons for accessing food (14 species) followed by 9 species collected from the *jhum* lands. Another 9 species were from two land use systems combined i.e., *jhum* lands and community forests, or *jhum* lands and home gardens. There was an appreciable diversity ($H = 3.32$) in availability of seasonal food species, and *Adi* women were using

TABLE 1 | List of 39 local plant species used as food by *Adi* and their habitat and seasonal availability.

Adi name	Scientific name	Family	Habitat	Season of availability	Types of plant	Plant part, and mode of use
Aksap	*Mussaenda roxburghii* Hook. f.	Rubiaceae	HG	September–November	SD	Leaf is boiled with other leafy vegetables
Adi-ori	*Eryngium foetidum* L.	Apiaceae	HG	(October–March)	D	Leaf is used as chutney, flavoring agent, and is boiled with meat, fish and other leafy vegetables
Payin	*Cucurbita moschata* Duchesne ex Poir.	Cucurbitaceae	HG	July–August	D	Tender leaves and flowers are used as vegetable and sometimes mixed with local fishes
Takang	*Diplazium esculentum* (Retz.) Sw.	Athyriaceae	HG	February–November	SD	Tender leaf is boiled with other leafy vegetables like *Spilanthes paniculata, Pouzolzia zeylanica, Fagopyrum esculentum,* etc.
Angyat	*Coix lacryma-jobi* L.	Poaceae	JL	October–December	D	Flour made from grain is used as food/beer preparation during lean period
Bangko	*Solanum spirale* Roxb.	Solanaceae	HG	August–November	D	Fruit is boiled with *Spilanthes paniculata, Zanthoxylum rhetsa* and *Allium hookerii* or with wet/dry fermented bamboo shoot. Tender leaves are used as vegetable
Belang	*Artocarpus heterophyllus* Lam.	Moraceae	JL	June–July	D	Ripen fruit is eaten; seed is boiled and eaten during lean period. Exchanged in barter also
Buluka	*Dendrocalamus giganteus* Wallich *ex* Munro.	Poaceae	JL and CF	September–October	SD + D	Culm-sheath of tender shoot is removed and tender shoot is chopped into fine size or cut into eatable size to boil as vegetable without fermentation
Dibang	*Bambusa tulda* Roxb.	Poaceae	CF	May–June	SD + D	Culm-sheath of tender shoot is removed and tender shoot is chopped or cut into eatable size (lengthwise) before cooking
Dilap	*Allium hookeri* Thwaites	Liliaceae	HG and JL	(November–February)	D	Bulb and leaf are used as flavoring agent, and cooked preferably with *Spilanthes paniculata, Zanthoxylum rhetsa* and *Solanum khasianum*.
Eng	*Dendrocalamus hamiltonii* Gamble	Poaceae	CF	September–October	SD + D	Culm-sheath of tender shoot is removed and tender shoot is chopped into fine size and kept in basket/bamboo container for fermentation or cut into eatable size to boil as vegetable without fermentation
Engin	*Dioscorea alata* L.	Dioscoreaceae	HG and JL	November–January	SD + D	Tuber is burnt under hot soil/boiled as food, basically used with tea as snack
Gham-oying	*Sauropus androgynus* (L) Merr.	Phyllanthaceae	HG and JL	Year round	D	Leaf is boiled with mixture of other leafy vegetables: *Spilanthes paniculata, Pouzolzia zeylanica, Fagopyrum esculentum,* etc.
Gubor-oying	*Amaranthus viridis* L.	Amaranthaceae	HG and JL	November–December	SD + D	Leaf is boiled with mixture of other leafy vegetables as above
Hipe-oyik	*Alternanthera philoxeroides* (Mart.) Griseb.	Amaranthaceae	HG	November–December	SD + D	Leaf is boiled with mixture of other leafy vegetables as above
Kekir	*Zingiber siangensis* Tatum and A K Das*	Zingiberaceae	HG	November–February	D	Used as major ingredients in many leafy vegetables. The rhizome, strongly aromatic and pungent, is a highly preferred local spice and taken while drinking local alcoholic beverages
Kodum	*Musa flaviflora* N. W. Simmonds	Musaceae	JL	September–October	SD	Ripe fruit is edible
Koppi	*Solanum khasianum* C. B Clarke	Solanaceae	HG	August–November	D	Fruit is boiled with *Spilanthes paniculata, Zanthoxylum rhetsa* and *Allium hookerii* or with wet/dry fermented bamboo shoot
Kuso-belo	*Ficus auriculala* Lour.	Moraceae	JL and CF	July–September	SD	Ripen fruit is eaten
Lori	*Piper pedicellatum* C. DC.	Piperaceae	HG	July–September	SD	Leaf is used as vegetable along with other leafy vegetables
Marsang	*Spilanthes paniculata* Wall. *ex* DC.	Asteraceae	HG	May–November	D	Leaf is steamed or boiled with other leafy vegetables like *Pouzolzia zeylanica*, *Clerodendrum colebrookinanum, Allium hookerii, Solanum kurzii, Solanaum torvum, Solanum khasianum*, *Gynura nepalensis,* etc.

(Continued)

TABLE 1 | Continued

Adi name	Scientific name	Family	Habitat	Season of availability	Types of plant	Plant part, and mode of use
Mirung	*Eleusine coracana* Gaertn.	Poaceae	JL	October–January	D	Powdered grain is used as food during lean periods and used in beer preparation during surplus periods
Nemar	*Piper mullesua* Buch. Ham. *ex* D. Don	Piparaceae	HG	September–December	SD	Steamed in bamboo with mixture of jungle meat and *Musa balbisiana* and *Musa flaviflora*
Ongen	*Gynura nepalensis* DC.	Asteraceae	HG and JL	September–December	D	Leaf is boiled with mixture of other leafy vegetables like *Spilanthes paniculata, Pouzolzia zeylanica, Fagopyrum esculentum,* etc.
Oko-mamang	*Solanum nigrum* L.	Solanaceae	HG and JL	July–November	D	Leaf is steamed with dry fermented bamboo shoot
Okung	*Fagopyrum esculentum* Moench.	Polygonaceae	HG and JL	March–November	D	Leaf is boiled with mixture of other leafy vegetables like *Spilanthes paniculata, Pouzolzia zeylanica, Fagopyrum esculentum,* etc.
Onger	*Zanthoxylum rhetsa* (Roxb.) DC.	Rutaceae	HG	August–October	SD	Leaf is steamed or boiled with other leafy/fruit vegetables such as *Pouzolzia zeylanica*, *Clerodendrum colebrookinanum, Gynura nepalensis, Allium hookerii, Solanaum torvum, Solanum khasianum*, etc.
Ongin	*Clerodendrum colebrookinanum* Walp.	Verbenaceae	HG	February–April	SD + D	Leaf is steamed or boiled preferably with other green leafy vegetables
Oyik	*Pouzolzia zeylanica* (L.) Benn. & R. Br.	Urticaceae	HG	October–November	SD	Leaf is boiled with fermented bamboo shoot and other leafy vegetables (*Pouzolzia zeylanica*, *Spilanthes paniculata, Zanthoxylum rhetsa,* etc.)
Pakte	*Musa balbisiana* Colla	Musaceae	HG and JL	Round the year	D	Ripe fruit is edible. Spadix is burned over fire and mixed with jungle meat (bird, squirrel, etc.). Leaves of *Piper pedicillatum* are steamed over fire and bamboo shoot is added to it
Paput	*Pseudognaphalium affine* (D. Don) Anderb.	Asteraceae	HG and JL	September–December	SD	Leaf is used as vegetable and mixed with other leafy vegetables
Poi	*Basella rubra* L.	Basellaceae	HG and JL	May–August	SD	Leaves consumed in boiled form and also mixed with meat and fish
Sirang	*Castanopsis indica* (Roxb. ex Lindl.) A. DC	Fagaceae	CF	October–December	SD	Epicarp is removed by lightly heating on pan over flame to get edible nut. Used as drought food also
Tali	*Amomum maximum* Roxb.	Zingiberaceae	JL	June–September	SD	Ripe fruit is plucked and epicarp is removed to eat mesocarp with seed (sweet in taste). Outer scape of young shoot is removed and inner tender scape is cooked with jungle meat/fish/leafy vegetables (*Pouzolzia zeylanica* and *Clerodendrum colebrookinanum*)
Tapir	*Phoebe cooperiana* P C Kanjilal and Das	Lauraceae	JL	August–October	SD	Epicarp and mesocarp are steamed in green bamboo before consumption
Tare	*Calamus erectus* Roxb.	Arecaceae	JL	May–July	SD	Epicarp is removed to eat mesocarp and nut
Taje	*Amomum subulatum* Roxb.	Zingiberaceae	HG and JL	April–August	D	Seeds are aromatic and pungent with pleasant taste. Raw part is boiled and used as vegetable after mixing with other leafy plants
Tasat	*Arenga obtusifolia* Griff.	Arecaceae	CF	October–March	SD	Epidermic layer is used as bread during drought, and also in making traditional alcoholic beverage *apong*
Titabaigan	*Solanum kurzii* Brace ex Prain	Solanaceae	HG	July–September	D	Fruits are taken as boiled vegetable, and mixed with a variety of fish and meat as well

**It is treated as synonym of Z. officinale but many pharmaceutical experts mention that it is distinct.*
HG, Home gardens; JL, Jhum lands; CF, Community forest.
Habitat data: D, Domesticated 17 species (43.59%); SD, Semi-domesticated but local 15 species (38.46%); D + SD, Domesticated and semi-domesticated 7 species (17.95%).

6 species round the year. Spearman rank correlation indicated a moderately significant correlation ($r = 0.524$, $p = 0.0004$) between seasons and access to local food species from varied land use types.

Income Generation From Local Food Plants

There were 39 local food plant species that helped generate subsistence income (**Table 2**). Community forests emerged as the

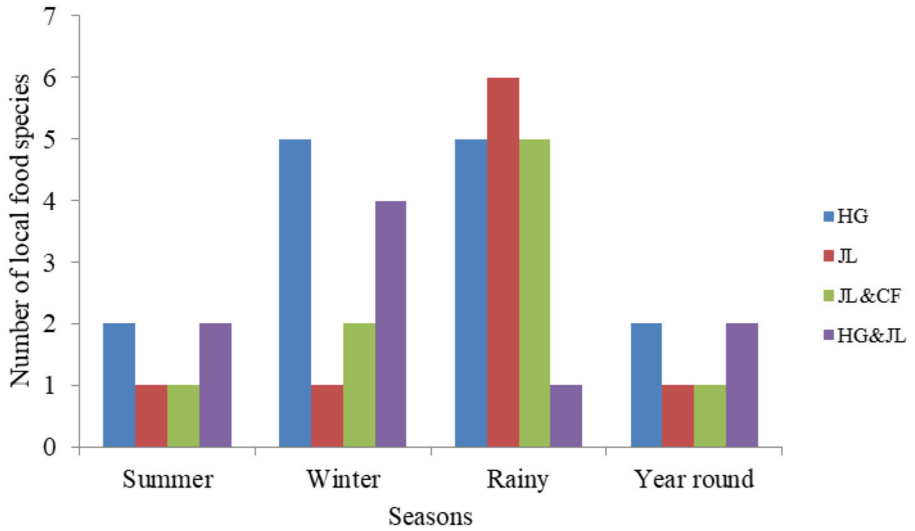

FIGURE 1 | Seasonal availability of local food species from diverse land use types.

TABLE 2 | Income generated by the sale of local food plants species accessed from different land use systems.

Land use types	Income in INR/kg*	Number of species	Comparison	"*t*" statistics	*p*-value
Home gardens (HG)	35.36 ± 4.12	13	HG vs. JL	2.39	0.011
Jhum lands (JL)	37.06 ± 4.09	17	HG vs. CF	0.75	0.230^{NS}
Community forests (CF)	73.33 ± 19.45	9	JL vs. CF	2.72	0.006

**The value with ± indicates standard error; NS, non-significant.*

major land use type, with mean income of INR 73.33 ± 19.45 per kg followed by *jhum* lands (INR 37.06 ± 4.09 per kg). With regard to income generation, there were significant differences between HG and JL (13 and 17 species, respectively; "t" = 2.39 p = 0.011) and between *jhum* lands and community forests (15 and 9 species, respectively; "t" = 2.72 p = 0.006) (**Table 2**). Despite a lower species count than other land use systems, food species conserved in community forests had a very high economic value.

Ethnomedicinally Important Food Species

A total of 28 local food plant species were also considered important for ethno-medicinal value (**Supplementary Table 1** in online resources 2). These were used for relief from various health problems and diseases including diabetes (*migom-koppi- Solanum torvum*), malaria (*bangko- Solanum spirale*), snake bites (*kekir- Zingiber siangensis*), high blood pressure (*ongin- Clerodendrum colebrookianum*) and fever (*nemar- Piper mullesua*), among others. Foods believed to improve the digestive system included *talap* (*Allium chinense*) and *kekir*. Foods made from unripe *koppi* and *kopi* (*Solanum kurzii*) were used as laxatives. Sometimes, a few species were mixed together with wild meat and fish to further improve their nutritional value. For example, *paput* (*Pseudognaphalium affine*), *mamang* (*Physalis minima*), *sayong* (*Polygonum* sp.), and *onger* (*Zanthoxylum rhetsa*) were used as flavor-enhancers in meat dishes. Similarly, *nayang* (*Erigeron canadensis*), *tangum* (*Bidens pilosa*) and *gende* (*Gynura nepalensis*) were boiled together with wild game or local fish- *ngopi* (*Labeo dero* F. Hamilton) and given to the sick and lactating mothers as energy boosters.

Beliefs relating to food restrictions were also recorded, since they may aggravate particular aliments or disorders. For example, *Adi* women believed that lactating mothers should not consume *ongin* and *marshang* (*Spilanthes paniculata*) based foods, as their breast-fed babies may suffer from diarrhea and lactation may cease early (93.5 and 78.4%, response, respectively) (**Supplementary Table 2** in online resources 2). Similarly, 76.9% of the respondents believed that consumption of bamboo shoots by a malaria patient was likely to further deteriorate his/her health condition.

Nutritional Significance of Selected Local Food Plants

Leafy Vegetables

Among the key local food plants evaluated for nutritional composition, 16 were leafy vegetables (11 herbs and 5 shrubs) (**Table 3A**). Among these leafy vegetables, edible portions ranged from 51 to 92 %; minimum in *takang* (*Diplazium esculentum*) and maximum in *Adi-ori* (*Eryngium foetidum*). *Gham-oying* (*Sauropus androgynus*) had the lowest moisture content, but was highest in crude protein (9.14%), crude fat (3.69%), ash (3.31%), iron (27.3 mg/100 g), zinc (25.7 mg/100 g) and manganese (13.4

TABLE 3A | Estimated values on various nutritional parameters of local food plants used by the *Adi*.

Adi name	Botanical name	Nutritional parameters[c]							
	Leafy vegetables	**Edible %**	**Moisture %**	**Ash %**	**Crude fat %**	**Crude Protein %**	**Dietary fiber %**	**Total sugar %**	**Total starch %**
Adi-ori	*Eryngium foetidum* L.	92.5 ± 0.95	72.0 ± 1.56	0.87 ± 0.05	1.34 ± 0.20	2.77 ± 0.10	9.65 ± 0.27	0.84 ± 0.06	1.29 ± 0.06
Akshap	*Mussaenda roxburghii* Hook. f.	64.4 ± 9.24	84.7 ± 4.24	1.47 ± 0.47	1.34 ± 0.27	3.75 ± 0.14	10.3 ± 0.52	0.57 ± 0.12	1.19 ± 0.08
Bangko	*Solanum spirale* Roxb.	73.6 ± 3.1	76.3 ± 2.4	4.05 ± 0.25	0.93 ± 0.10	1.27 ± 0.10	8.84 ± 0.19	0.874 ± 0.09	2.13 ± 0.11
Takanga	*Diplazium esculentum* (Retz.) Sw.	51.4 ± 2.1	84.7 ± 1.5	1.91 ± 0.54	0.76 ± 0.10	4.79 ± 0.57	9.09 ± 0.17	0.941 ± 0.07	2.16 ± 0.12
Dilap	*Allium hookeri* Thwaites	89.3 ± 3.6	76.1 ± 2.1	3.96 0.26	0.83 ± 0.18	2.69 ± 0.11	10.6 ± 0.34	1.42 ± 0.08	3.01 ± 0.26
Gham-oying	*Sauropus androgynus* (L) Merr.	67.9 ± 5.1	71.4 ± 1.6	3.31 ± 0.16	3.69 ± 0.69	9.14 ± 0.90	9.20 ± 0.78	0.799 ± 0.06	2.51 ± 0.06
Lori	*Piper pedicellatum* C. DC.	74.9 ± 1.5	82.2 ± 1.6	2.74 ± 0.13	1.13 ± 0.75	4.14 ± 0.38	10.3 ± 2.36	0.831 ± 0.05	1.49 ± 0.17
Marsang	*Spilanthes paniculata* Wall. *ex* DC.	69.9 ± 10.9	85.9 ± 1.4	2.04 ± 0.16	1.25 ± 0.13	3.38 ± 0.58	6.22 ± 0.69	0.259 ± 0.02	0.93 ± 0.01
Obul	*Mackaya neesiana (*Wall.) Das	90.3 ± 1.1	85.7 ± 1.0	1.94 ± 0.05	0.79 ± 0.13	2.58 ± 0.16	6.13 ± 0.19	0.345 ± 0.03	1.89 ± 0.22
Ongen[b]	*Gynura nepalensis* DC.	64.7 ± 5.6	82.4 ± 1.3	1.93 ± 0.14	1.68 ± 0.08	6.05 ± 0.73	7.45 ± 0.17	0.701 ± 0.03	1.25 ± 0.04
Onger	*Zanthoxylum rhetsa* (Roxb.) DC.	55.1 ± 6.0	81.8 ± 1.0	1.66 ± 0.24	1.96 ± 0.48	5.14 ± 0.36	7.97 ± 0.43	0.323 ± 0.04	0.55 ± 0.05
Ongin	*Clerodendrum colebrookinanum* Walp.	67.7 ± 6.6	79.6 ± 1.9	1.91 ± 0.12	1.67 ± 0.09	5.84 ± 1.11	8.46 ± 0.10	0.958 ± 0.07	1.14 ± 0.06
Oyik	*Pouzolzia zeylanica* (L.) Benn. & R. Br.	54.1 ± 1.8	79.0 ± 1.6	2.92 ± 0.24	1.05 ± 0.17	5.96 ± 0.08	9.92 ± 0.12	0.808 ± 0.07	0.95 ± 0.10
Poi	*Basella rubra* L.	75.3 ± 2.1	86.8 ± 1.5	2.60 ± 0.20	0.91 ± 0.05	4.75 ± 0.40	3.96 ± 0.28	0.862 ± 0.06	1.78 ± 0.09
Payin	*Cucurbita moschata* Duchesne ex Poir.	54.7 ± 1.5	82.6 ± 1.7	1.49 ± 0.13	1.09 ± 0.10	1.90 ± 0.14	8.08 ± 0.61	0.556 ± 0.03	1.12 ± 0.08
	Mean	69.7 ± 13	80.5 ± 4.9	2.37 ± 1.05	1.36 ± 0.74	4.24 ± 3.1	8.32 ± 1.9	0.739 ± 0.30	1.56 ± 0.67
	Fruit based vegetables								
Kopir	*Solanum indicum* L.	71.5 ± 1.4	73.0 ± 1.06	1.11 ± 0.67	2.09 ± 0.36	5.86 ± 0.47	15.6 ± 1.18	1.21 ± 0.09	1.73 ± 0.09
Migom-koppi	*Solanum torvum* Sw.	87.0 ± 2.7	77.3 ± 4.8	1.09 ± 0.47	1.18 ± 0.25	2.55 ± 0.53	14.4 ± 0.70	0.463 ± 0.08	1.24 ± 0.02
Bangko	*Solanum spirale* Roxb.	73.6 ± 4.4	80.0 ± 5.60	1.06 ± 0.06	1.89 ± 0.15	4.62 ± 0.37	8.77 ± 0.44	0.689 ± 0.11	1.31 ± 0.02
Kopi	*Solanum kurzii* Brace ex Prain	77.4 ± 4.7	76.5 ± 1.98	1.89 ± 0.62	1.52 ± 0.18	5.09 ± 1.07	6.89 ± 0.48	0.86 ± 0.13	2.31 ± 0.17
	Mean	77.4 ± 6.9	76.2 ± 3.1	1.79 ± 1.4	1.67 ± 0.40	4.53 ± 1.4	11.4 ± 4.2	0.806 ± 0.31	1.65 ± 0.49
	Rhizomatous vegetables								
Kekir	*Zingiber siangensis* Tatum and A K Das	82.8 ± 1.4	80.7 ± 1.1	1.29 ± 0.14	2.36 ± 0.19	2.48 ± 0.34	12.2 ± 0.87	0.42 ± 0.08	0.97 ± 0.11
Adi ginger	*Zingiber officinale* Roscoe	83.4 ± 0.8	72.4 ± 1.3	0.50 ± 0.04	1.35 ± 0.15	2.86 ± 0.45	14.0 ± 0.14	1.46 ± 0.12	2.58 ± 0.21

[a] *Also known as Dhekia saag.*

[b] *Known as gende also.*

[c] *Each composite sample was analyzed in triplicate. Results are presented as mean of means of four years data (n = 4), and mean value for each year is derived from three analytical replicates.*

TABLE 3B | Estimated values on various nutritional parameters of local food plants used by the *Adi*.

***Adi* name**	**Botanical name**	**Nutritional parameters[c]**										
		Ascorbic acid	**Phenol**	**Flavanol**	**Na**	**K**	**Ca**	**Mg**	**Fe**	**Zn**	**Mn**	**Co**
	Leafy vegetables	**mg/100 g**	**mg/100 g**	**mg/100 g**	**mg/100 g**	**mg/100 g**	**mg/100 g**	**mg/100 g**	**mg/100 g**	**mg/100 g**	**mg/100 g**	**μg/100 g**
Adi-ori	*Eryngium foetidum* L.	17.3 ± 0.97	130 ± 9.10	18.4 ± 0.92	78.6 ± 3.9	118 ± 8.3	143 ± 8.6	44.4 ± 2.9	8.47 ± 0.33	1.55 ± 0.09	2.61 ± 0.14	1.56 ± 0.26
Aksap	*Mussaenda roxburghii* Hook. f.	12.1 ± 0.29	383 ± 27	40.9 ± 2.9	33.7 ± 1.7	86.7 ± 6.1	476 ± 29	47.2 ± 1.9	3.79 ± 0.19	3.89 ± 0.23	4.53 ± 0.16	5.30 ± 0.42
Bangko	*Solanum spirale* Roxb.	23.2 ± 1.8	374 ± 23	38.7 ± 1.4	90.3 ± 2.7	476 ± 33	279 ± 11	62.5 ± 1.8	3.45 ± 0.78	1.22 ± 0.10	2.13 ± 0.11	3.62 ± 0.41
Takang[a]	*Diplazium esculentum* (Retz.) Sw.	19.2 ± 1.6	213 ± 18	23.1 ± 1.3	35.2 ± 1.7	381 ± 27	67.4 ± 4.0	48.4 ± 2.0	8.33 ± 0.31	3.76 ± 0.31	2.17 ± 0.11	3.15 ± 0.33
Dilap	*Allium hookeri* Thwaites	23.1 ± 2.1	134 ± 17	48.2 ± 2.7	97.5 ± 3.2	270 ± 16	158 ± 22	33.3 ± 2.4	11.8 ± 0.48	0.716 ± 0.08	0.934 ± 0.10	3.84 ± 0.35
Gham-oying	*Sauropus androgynus* (L) Merr.	15.8 ± 2.6	591 ± 27	67.7 ± 1.3	131 ± 6.6	269 ± 19	791 ± 47	72.3 ± 1.4	27.3 ± 1.1	25.7 ± 1.5	13.4 ± 0.46	0.95 ± 0.17
Lori	*Piper pedicellatum* C. DC.	51.3 ± 1.9	385 ± 25	63.9 ± 7.2	54.0 ± 2.7	133 ± 9.3	293 ± 18	86.9 ± 1.7	10.3 ± 0.41	4.86 ± 0.29	2.10 ± 0.13	5.96 ± 0.51
Marsang	*Spilanthes paniculata* Wall. *ex* DC.	12.1 ± 0.9	185 ± 8.8	19.0 ± 1.03	52.2 ± 2.6	134 ± 9.3	281 ± 17	34.0 ± 1.7	2.26 ± 0.08	6.19 ± 0.37	1.68 ± 0.09	3.78 ± 0.28
Obul	*Mackaya neesiana (*Wall.) Das	17.5 ± 0.77	118 ± 20	20.8 ± 1.04	31.0 ± 1.5	90.4 ± 6.3	358 ± 22	44.9 ± 0.93	2.10 ± 0.11	1.39 ± 0.08	0.81 ± 0.12	3.52 ± 0.28
Ongen[b]	*Gynura nepalensis* DC.	26.6 ± 2.5	338 ± 15	50.0 ± 2.1	472 ± 23	776 ± 54	456 ± 27	57.3 ± 1.1	5.31 ± 0.23	7.03 ± 0.42	6.73 ± 0.23	8.43 ± 0.67
Onger	*Zanthoxylum rhetsa* (Roxb.) DC.	14.8 ± 1.3	678 ± 18	41.8 ± 1.3	56.3 ± 2.8	129 ± 9.0	679 ± 41	45.2 ± 1.8	8.12 ± 0.32	6.67 ± 0.40	4.01 ± 0.11	7.36 ± 0.59
Ongin	*Clerodendrum colebrookinanum* Walp.	28.9 ± 2.3	214 ± 16	33.9 ± 1.7	185 ± 9.3	341 ± 23	513 ± 31	42.1 ± 1.7	6.35 ± 0.32	5.13 ± 0.33	3.92 ± 0.09	5.94 ± 0.51
Oyik	*Pouzolzia zeylanica* (L.) Benn. and R. Br.	18.0 ± 1.2	190 ± 8.0	33.0 ± 1.1	318 ± 21	514 ± 49	850 ± 69	60.6 ± 1.2	6.39 ± 0.29	3.53 ± 0.21	2.65 ± 0.13	7.60 ± 0.58
Poi	*Basella rubra* L.	81.0 ± 2.4	167 ± 12	26.4 ± 2.1	79.2 ± 3.9	192 ± 13	165 ± 9.9	198 ± 4.0	4.65 ± 0.24	1.64 ± 0.10	0.952 ± 0.03	1.46 ± 0.22
Payin	*Cucurbita moschata* Duchesne ex Poir.	31.80 ± 1.91	127 ± 5.8	35.6 ± 1.8	36.4 ± 1.8	412 ± 29	63.2 ± 3.8	52.4 ± 1.1	5.80 ± 0.23	2.34 ± 0.14	1.89 ± 0.06	4.54 ± 0.20
	Mean	26.2 ± 18	282 ± 173	37.4 ± 15	117 ± 124	288 ± 197	372 ± 252	62.0 ± 40	7.63 ± 6.1	5.04 ± 6.1	3.37 ± 3.2	4.47 ± 2.3
	Fruit based vegetables											
Koppir	*Solanum indicum* L.	20.0 ± 1.1	413 ± 31	67.5 ± 3.4	153 ± 7.7	323 ± 23	717 ± 43	69.8 ± 1.4	10.3 ± 0.41	5.12 ± 0.35	3.81 ± 0.11	11.9 ± 1.8
Migom Koppi	*Solanum torvum* Sw.	19.4 ± 0.72	221 ± 26	8.93 ± 1.1	88.5 ± 4.4	169 ± 12	92.4 ± 5.5	50.2 ± 1.0	4.73 ± 0.2	8.54 ± 1.9	1.67 ± 0.08	7.28 ± 0.63
Bangko	*Solanum spirale* Roxb.	26.70 ± 0.53	195 ± 18	53.7 ± 3.2	211 ± 11	357 ± 23	203 ± 12	33.1 ± 1.7	3.76 ± 0.15	3.29 ± 0.20	1.63 ± 0.05	3.76 ± 0.30
Kopi	*Solanum kurzii* Brace ex Prain	24.6 ± 0.48	336 ± 39	23.4 ± 3.7	56.1 ± 4.8	268 ± 18	137 ± 16	41.6 ± 2.8	3.55 ± 0.21	2.70 ± 0.15	0.912 ± 0.28	4.84 ± 0.33
	Mean	22.7 ± 3.5	291 ± 102	38.4 ± 27	127 ± 69	279 ± 82	287 ± 290	48.7 ± 16	5.59 ± 3.2	4.91 ± 2.6	2.01 ± 1.3	6.95 ± 3.6
	Rhizomatous vegetables	14.1 ± 0.71	306 ± 21	97.4 ± 6.8	58.3 ± 2.9	357 ± 25	103 ± 6.2	33.1 ± 2.7	3.76 ± 0.22	3.29 ± 0.50	0.632 ± 0.09	1.38 ± 0.17
Kekir	*Zingiber siangensis* Tatum and A K Das											
Adi ginger	*Zingiber officinale* Roscoe	12.6 ± 0.59	221 ± 9.2	78.3 ± 4.7	23.3 ± 1.2	368 ± 26	26.2 ± 1.6	74.8 ± 1.5	3.89 ± 0.23	1.15 ± 1.7	4.32 ± 0.14	1.26 ± 0.15

[a] *Also known as Dhekia saag.*

[b] *Known as gende also.*

[c] *Each composite sample was analyzed in triplicate. Results are presented as mean of means of four years data (n = 4), and mean value for each year is derived from three analytical replicates.*

Na, Sodium, K, Potassium; Ca, Calcium; Mg, Magnesium; Fe, Iron; Zn, Zinc; Mn, Manganese; Co, Cobalt.

mg/100 g). It also had high content of phenols (591 mg/100 g gallic acid equivalent GAE), flavonols (67.7 mg/100 g quercetin equivalent QE), calcium (791 mg/100 g) and magnesium (72.3 mg/100 g) (**Tables 3A**,**B**).

The *piper* leaves displayed the highest dietary fiber (10.3%), ascorbic acid (51.3 mg/100 g) as well as high flavonol (63.9 mg/100 g QE) and ash (2.74%) content. However, piper leaves had moderate amounts of total phenols (385 mg/100 g GAE) (**Tables 3A**,**B**). Leaves of *dilap* had the highest ash content (3.96%), total sugars (1.42%), total starch (3.01%) and high iron (11.8 mg/100 g). *Onger* (*Zanthoxylum rhetsa*) leaves showed high amounts of crude fat (1.96%), total phenols (678 mg/100 g GAE) and calcium (679 mg/100 g). *Oyik* (*Pouzolzia zeylanica*) and *ongen* (*Gynura nepalensis*) were considered highly nutritious among the *Adi* peoples. An *Adi* proverb aptly signifies this perception:

> "*Oyik doboname reyik reyik, ongen doboname regen regen.*"
> ["Those who eat *oyik* are handsome and beautiful and those who eat *ogen* have good health and physique." Terms *reyik-reyik* and *regen-regen* connote a healthy child].

Comparative assessment revealed that *ongen* was particularly rich in protein (6.05%), total ash (1.93%), crude fat (1.68) ascorbic acid (26.6 mg/100 g), phenols (338 mg/100 g) and flavanol (50.0 mg/100 g QE). It also displayed appreciable amounts of sodium (472 mg/100 g), potassium (776 mg/100 g), zinc (7.03 mg/100 g), manganese (6.73 mg/100 g) and cobalt (8.43 μg/100 g). Similarly, *oyik* had very high calcium content (850 mg/100 g), and was moderately rich in dietary fiber (9.92%), protein (5.96%), total ash (2.92%), sodium (318 mg/100 g), potassium (514 mg/100 g), and cobalt (7.60 μg/100 g) (**Tables 3A**,**B**).

Further, *poi* (*Basella rubra*) had the highest moisture (86.8%), ascorbic acid (81 mg/100 g) and magnesium (198 mg/100 g) levels. It also had moderate amounts of ash (2.60%) and starch (1.78%). Tender leaves of *payin* (*Cucurbita moschata*) showed below average values for most of the nutrients except for ascorbic acid, potassium and cobalt (**Tables 3A**,**B**). *Gham-oying* was found to be the best among all leafy vegetables in terms of several nutrients. Interestingly, local people also refer it as "multi-vitamin plant."

Fruit Based Food

Results indicated that there were four Solanaceous fruit-based foods. These included *koppir* (*Solanum indicum*), *migom-koppi* (*S. torvum*), *bangko* (*S. spirale*) and *kopi* (*S. kurzii*). Despite low water content and lower levels of total sugars and starches, these four species had much higher amounts of other nutrients as compared to commonly consumed eggplant (*Solanum melongena)* fruit (protein 1.48%, ash 0.70%, fat 0.32%, dietary fiber 3.98%, and available carbohydrate 3.52%) Longvah et al. (54). *Koppir* was found to be higher in fat content (2.09%), protein (5.86%), dietary fiber (15.6%), total sugars (1.21%), total phenols (413 mg/100 g), and total flavanols (67.5 mg/100 g). This also displayed higher calcium (717 mg/100 g), magnesium (69.8 mg/100 g), iron (10.3 mg/100 g), manganese (3.81 mg/100 g), and cobalt (11.9 μg/100 g) as compared to the other three Solanaceous fruit vegetables.

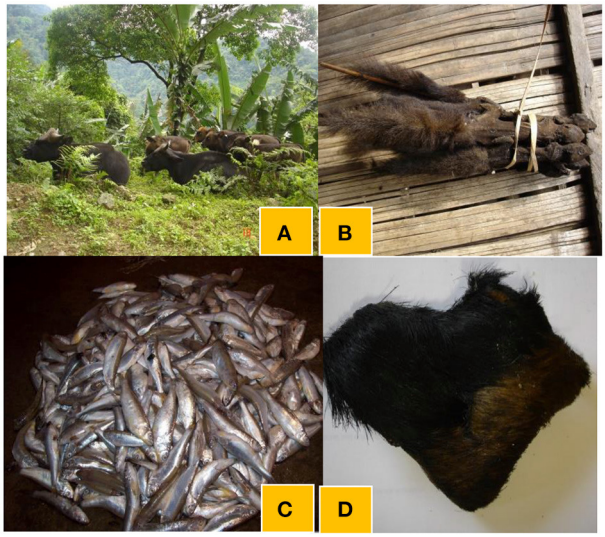

FIGURE 2 | Local food plants and animal resources used in traditional foods of *Adi* community. **(A)** *Mithun* (*Bos frontalis*)- a culturally important semi-wild animal used in meat with plants; **(B)** *Kebung* (*Ratufa bicolor*)- a culturally important wild animal used for meat and gifted in festivals and marriages; **(C)** *Ngopi* fish (*Labeo dero*), used in fresh, fermented and dried form; **(D)** Dried deer meat. All the photos by Ranjay K Singh with consent from study participants.

Rhizome Based Food

Results revealed that there were two Zingiberale plants being used as traditional foods. In comparison to commonly cultivated ginger (*Z. officinale*), *kekir* (*Zingiber siangensis*) rhizome had a higher juice content, low sugars and starches. This species displayed a high content of ash, fat, total phenols, total flavanols, sodium, calcium and zinc.

Cultural Dynamics of Local Food Plant Species

Adi women have developed traditional knowledge of local food plants over the course of time, demonstrating their culinary creativity in sustaining the cultural diversity. The following sections elaborate on the relationship between traditional knowledge of food and cultural diversity.

Culture and Biodiversity: Intricate Relations

There were certain traditional foods prepared using locally available resources such as fish (e.g., *ngopi* and *tasum jhhinga machh*), insects (*tari-pug- Aspongopus najus*; *eri-pug- Samia cynthia*; citrus red ant-*Oecophylla smaragdina*, bamboo worm-*Omphisa fuscidentalis*, etc.) meat of *mithun* (*Bos frontalis*), chicken and wild animals. The *Adi* women uses leafy and other vegetables in conjunction with meat-based dishes such as fish, insect and wild games for enhanced taste and perceived health

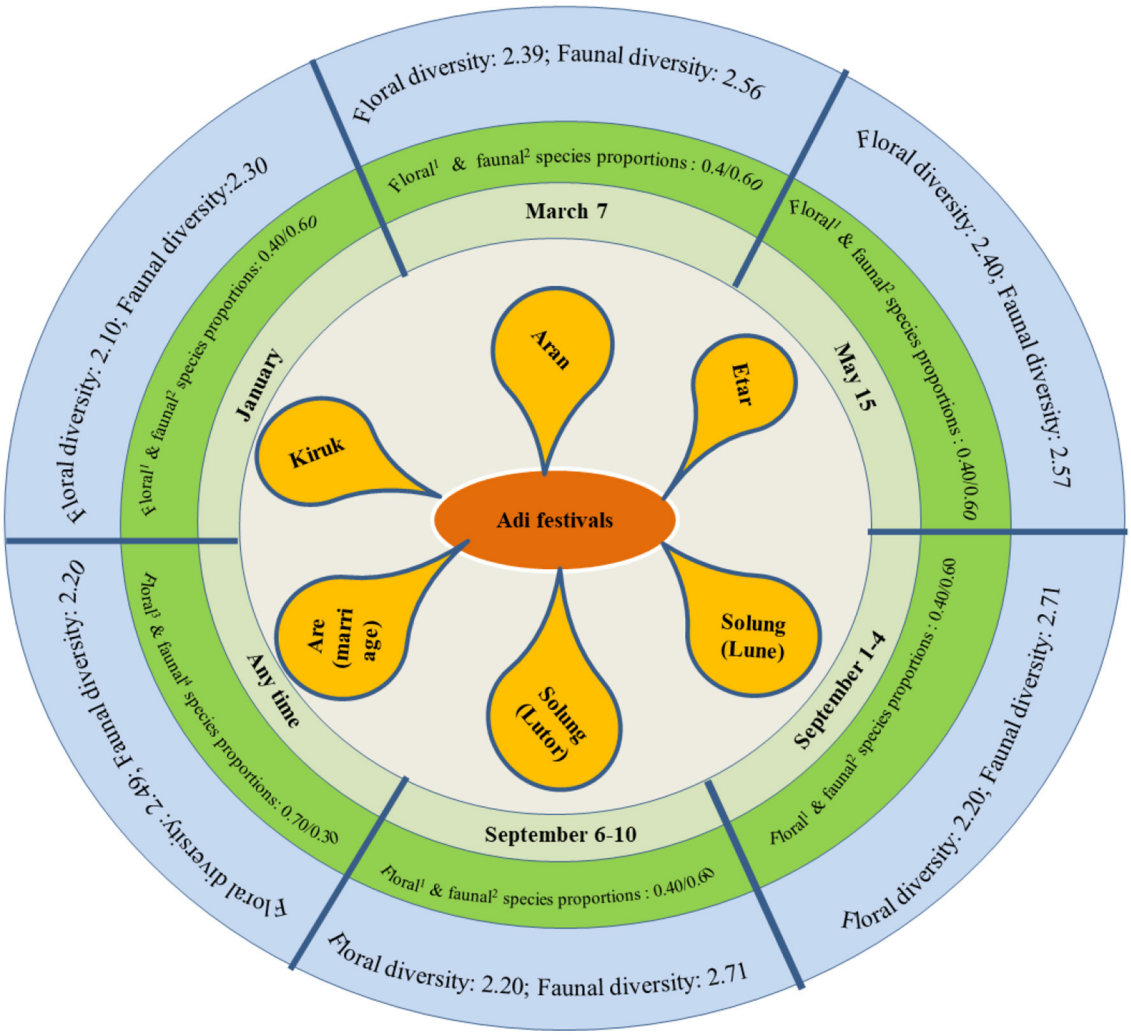

FIGURE 3 | Major *Adi* festivals and their relations with local food plants and animal species as traditional foods. Diversity and similarity indices are based on Shannon-Weaver diversity analysis. [1]Major local food plant species include: *kekir*, *takeng* (local ginger black in color), *onger, ongin, gam oying, koppi, koppir*, *paput, gende,* ikung and local paddy (*amkel*, *deku* and *jajung*) for food, and *apong* (fermented traditional beverage). The number of plant species may vary according to seasons. [2]Major animal species used in food include: squirrels, *mithun*, wild rates, porcupine, deer, boar, local fishes, insects.

benefits. There are at least six major *Adi* festivals reflecting strong association between the culture and local dishes. Some local plants such as *amkel* rice*, kekir, takeng, onger, ongin, gham-oying, kopi, koppir, Adi-ori,* bamboo shoot*, namsing peron* are used as bulk ingredients in traditional foods along with foods prepared from domesticated animals (chicken and pork), fish, wild games [(*mithun, kebungs, nagopi* fish, deer (**Figures 2A–D**), boar, silkworm, bamboo worms*, tari-pug*, birds, etc.)] (**Figure 3**). During each *Adi* festival, celebrated in a particular month, these local food plants and meat/wild games are cooked together in different proportions (plant vs. animal: 60: 40) to make them delicious. Shannon-Weaver index revealed that despite changing proportions and combinations of food plants with animal species, considerable diversity was demonstrated through such cultural foods during the festivals. For example, during the *aran* festival, diverse local plants are cooked with such animals (**Figure 3**). Similarly, in other major festivals, such as *etor* and *solungs*, these foods are prepared and consumed. A team of *Adi* men and women collect dried wild games (dried rats, squirrel, *mithun* meat, etc.), rice grains, plant products (powder of *kopi, koppir*, *Adi-ori*) and fresh local vegetables from each household. Later on, these ingredients are cooked in the community hall (*mosup*), and are served along with cooked leafy vegetables to the community members, especially the elders, as a symbol of equitable sharing of the locally available nutritious food. These are supplemented by *apong,* a traditional (alcoholic) beverage prepared from *amkel* rice and or finger millet. During these celebrations, the *miris* (*Adi*'s cultural priest) perform a ritual dance and other *Adi* dancers sing folksongs signifying the cultural values of *jhum* lands, community forests, rivers and mountains.

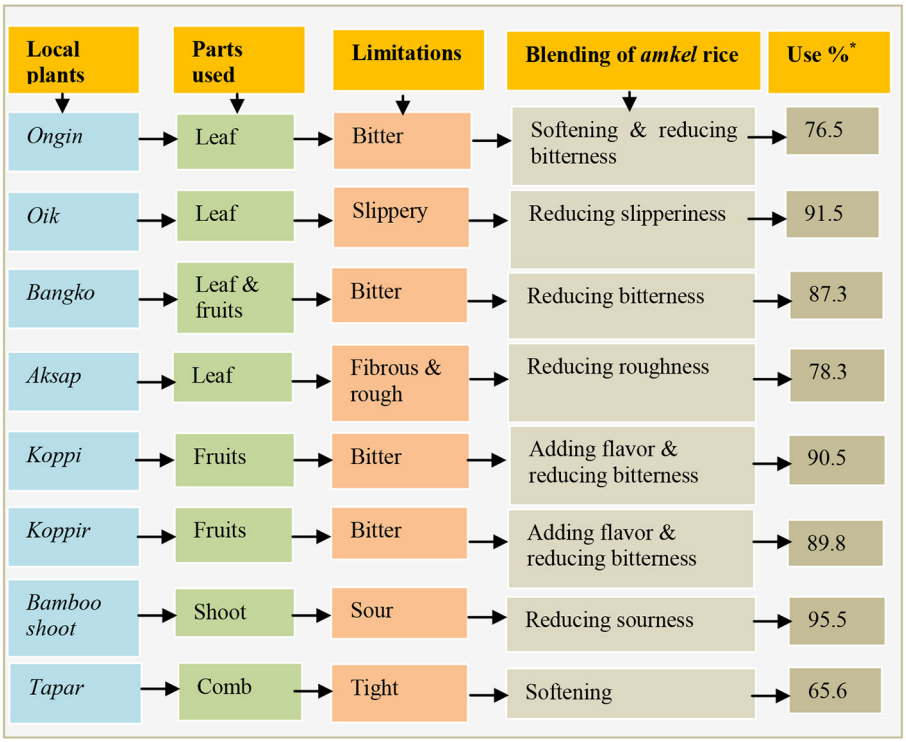

FIGURE 4 | *Amkel* rice blended with some common ethnobotanicals to enhance food tastes. *Multiple percentage.

Local Creativity in Making Culturally Rich Foods

Ongin, koppi, koppir and *bangko* were the four most preferred local food plants by adults and elders, but were less preferred by children due to their "bitterness," "roughness," and "slippery" nature. To reduce the odd taste and enhance the softness, *Adi* women select tender parts of leaves or fruits and mix them with broken *amkel* rice (**Figure 4**). Similar practices were adopted for improving the palatability of "sour" plant foods. *Adi* women crush the soft corn grains between two stones to flatten the kernels. *Ngopi* fish is then boiled in water for 15–20 min, after which the bones are removed. The flesh is boiled again after being mixed with *luktir* (dried mixture of a local chili-*ritsar*, fruits of *bangko*, leaves of *onger* and dried bamboo shoots), along with salt and the flattened corn. The mixture is stirred well for 30 min, then water is added and is left to sit for a while. Thereafter, it is served with *namsing-peron* chutney made from fermented soybean (*Glycine max* L.) seeds, chili, ginger, garlic and salt.

Another food, called *khamti aamin/ambin*, is prepared with *amkel* rice soaked in cold water for 10 min and then made into a paste. Handful of *hilsha* (*Tenualosa ilisha* F. Hamilton) or the local fish (*ngopi*) are boiled (15 min) and deboned. Subsequently, the flesh is mixed with *amkel* rice paste together with salt, *chili*, *hopsi* (*Schizophyllum commune*) and *eepe* (dried powder of fermented bamboo shoots). The mixture is further cooked over a high heat for about 15–20 min, while being stirred continuously till it reaches to a ready-to-serve semi-liquid form. The fresh peel of *champa* fruits (*Dillenia indica* L.) are mixed with small fish like *ngopi,* to enhance the taste and keep the flesh firm during cooking.

Local beer (*apong*) is a specialty of *Adi* beverage prepared from time immemorial. Its quality mainly depends on the quality of rice grains used (*amkel* rice is most preferred) and on the yeast tablet (*siye*). *Adi* people make yeast tablets locally using some wild plants. These enhance the strength and storage quality of the beer. For this purpose, they use the green leaves of *rugzi* [*Pteridium aquilinum* (L.) Kuhn], *belang*, *rayil* [*Litsea cubeba* (Lour.) Pers.], *kopi* and tender shoots of sugarcane, blended together along with yeast culture from a previously fermented local beer lot.

DISCUSSION

Food and Livelihood Security

The *Adis* live in remote locations of Ar P, and are recognized as marginalized in the context of India's developmental process (55). For these reasons, they have not had access to food grown in the plains and lowlands of India or to fully adopt scientific farming practices (56). Their very remoteness, however, has enabled them to evolve their own culturally rich food systems. This study demonstrated that *Adi* women are particularly knowledgeable about local plant species accessed from varied land use systems for food security. We documented a total of 39 local plant food species, used in dishes mixed with wild game. Gender-specific differences in food knowledge and practices have previously been

reported (57). Accessing these food species provides a sustainable base for food and income to the *Adi* community. Indigenous tribal people living in remote locations adopt combined strategies and explore a variety of ecosystems in harvesting and preparing a large number of local species to ensure their nutritional security (58) and reduce the livelihood risks (59).

Adi women living in remote and fragile ecosystems face a set of interrelated food security and environmental challenges (60). They often attempt to solve these challenges by applying their knowledge holistically. For instance, an *Adi* woman might value her home garden and *jhum* land not just as a source of common herbs, but as a treasure trove of food, ethnomedicine and marketable products (61). *Adi* women's decisions about the use of a particular food might be affected by the knowledge acquired in past, perceived constraints on accessing it and the preference for local food habits (12). This was true with use of plants like *oyik* and *ogen* which were of high nutritional and cultural significance to *Adi*. The high calcium content along with other nutrients in *oyik* can be considered as a supportive evidence of their historical traditional knowledge; for example, in increasing the mothers' lactation (62). Therefore, these two local food species would have played important role in providing essential minerals (sodium, potassium, calcium, other nutrients as well as protein) given that milk and dairy product consumption is negligible among the *Adi,* and the common salt was scarce prior to the 1980s. Such relationships between a woman's choices with regard to food species, food habits and the reasoning behind use decisions, influenced by social-ecological factors (63), might not be easily discernible to an outsider but certainly affect the sustainability of local food resources (20, 41). As a result, it may undermine the potential future role of the local food species in meeting dietary diversity and food security (63). To address these issues, community-based prioritization and promotion of some potential local food plants through their enhancement, added to a scientific package of practices, can halt the erosion of food practices and associated biodiversity. Those concerned with ensuring people's food security and conserving local biodiversity (64) might investigate regions like Ar P, where high cultural diversity of foods is still evident (8, 65). Such regions and their local communities, if integrated, can have synergistic relations with policies contributing to better nutrition and health (e.g., Public Distribution System) and ecosystem integrity (66, 67).

Nutritional and Health Security

Some of the local food species analyzed in our study were particularly nutritious. Globally, it is well-recognized that many local food plants consumed by the Indigenous peoples have high nutritional and medicinal qualities and have been selected based on years of informal experimentation (27). Local food species derived from various less manipulated land use systems, are typically higher in calcium, iron, magnesium, and vitamin C than their cultivated counterparts (68, 69). Other inquiries on nutritional composition of wild plants indicate a high content of proteins (70), fatty acids and amino acids (71, 72), and of minerals, especially K, Na, Ca, P, Mg (73, 74). Kuhnlein and Receveur (27) reported that many Indigenous foods, prepared from local species, are rich in protein, vitamins, iron, zinc, copper, magnesium and potassium (69). These local species play multiple roles not only in health promotion, but also in sustaining cultural diversity and knowledge. Our study also documented *Adi* women's use of 28 food plants in ethnomedicine. Their creativity in selecting, combining and processing local food/ethnomedicine species contributes to better health outcomes for the entire communities (1, 75). The use of certain foods as ethnomedicines may also help in addressing some contemporary health problems (76).

Some of the traditional food species documented here are particularly important during the lean seasons and adverse weather conditions (77). Some like *namdung* (*Perilla frutescens*) are recommended by elderly women to their younger pregnant counterparts as they are considered to be good for the health of the mother as well as the baby. An earlier study suggests that *namdung* is rich in omega 3 fatty acids, essential amino acids, manganese and zinc (71): nutrients needed more during pregnancy. Some other food plants, such as *onger, gham-oying, lori* and *ongen* (traditionally highly preferred) have also shown nutraceutical potential (phenols and flavanols). These observations support *Adi* women's traditional knowledge in selecting local food plants to maintain the health (78).

Meticulous processing and well-thought-out combinations of the local food resources by *Adi* women not only make them tasty and culturally unique, but also nutritionally balanced (22). Such practices, for example, were true with Solanaceous fruit vegetables having varying degrees of bitterness and less liked by younger people. With low water and carbohydrate levels, these species were found nutrient rich as compared to the common brinjal and were processed by women to make them acceptable to family members across the age groups. Surprisingly, several of these species are little known to outsiders and thus are not acknowledged in the research and policy arenas. Guijit et al. (25) referred to such food species as "hidden harvests," and noted that they have the potential to enhance the overall well-being of marginalized communities. The *Adi* culture of using local food species, community-based cooking and sharing with others during cultural occasions are examples of equitability for those who seldom have access to such traditional foods (79). Significantly, most of the traditional food species (used alone or in combination with other plant and animal species) reported here are least known to other parts of India. In the remote and fragile ecosystems of Ar P where promotion of exotic food species is generally impractical, such species could potentially meet the current nutritional needs of marginalized people and thus enable them to adapt to environmental and livelihood challenges (69, 74).

CONCLUSION AND POLICY IMPLICATIONS

Adi women of Arunachal Pradesh, India have rich knowledge on culturally and nutritionally important foods. Notably, this knowledge of nutritional biodiversity is also applied to maintain health and secure the subsistence livelihoods. *Jhum* lands and home gardens contribute significantly to food and nutrition

security, together with community forests that provide species of high economic value. The *Adi* women adopt a meticulous strategy in using the local food plants with animals, fish and edible insects to fulfill their community's food and nutritional requirements. Many of the local food plant species (22) were nutritionally rich. In addition, 28 local plant species, including some from the 22 nutritionally assessed species, were also used simultaneously as ethnomedicines. Many of these species are rarely known to the rest of India. Some of them (22) are reported here for the first time for their nutritional value. These food resources (plants and animals) and related knowledge have a strong affinity with cultural diversity, Indigenous institutions and proximity to accessing local ecosystems. Based on the key findings of this study, the following points seem to be highly relevant to the future policy planning:

(i) Conservation plans for halting the genetic erosion of these valuable plant species need to be developed on priority. Further, package of practices for semi-domesticated and domesticated wild edible plants need to be developed to ensure their commercial production while ensuring sustainability and maintaining socio-ecological resilience of the traditional food systems.
(ii) Particular attention on the part of scientific and policy institutions is needed to create public awareness about the potential role and contributions of these traditional foods in health and nutrition, sustaining ecosystems and cultural knowledge.
(iii) Some of the plant-based foods with unique nutraceutical potential can be integrated with broader food and nutritional security programs (e.g., "Mid-Day-Meal" and PDS programs) in the study region and similar other geographical areas.
(iv) Several local vegetable species reported in this study (e.g., *oyik, ongen, gham-oying* and *dilap*) have high potential for introduction into similar other areas, as they would have high level of acceptance on account of taste and ease of integration with other foods.
(v) Local food plant resources can be mainstreamed by the state government to assist and support a "*bottom-up*" approach to development programs in promoting the better health, nutrition and women's empowerment. This can be pursued by leveraging the relevant provisions of the micro-entrepreneurship and skill development policies of India.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human/animals were reviewed and approved by the Research Advisory Committee headed by the Dean, College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh.

AUTHOR CONTRIBUTIONS

RS: substantial contributions to the conception and design of the work. RS, RB, TP, and AnaS: recipe contest and field-based activities, data collection. RB, AR, LW, and SU nutritional analysis and their interpretation. TP, SU, and AnsS: economic and cultural interpretation of relevant data. RS and RB: drafting the work and questions relating to results may be asked. RS and AnsS: revising it critically for important intellectual content. All authors final approval of the version to be published.

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SUPPLEMENTARY MATERIAL

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