



# Nutritional and Functional Properties of Wild Leafy Vegetables for Improving Food Security in Southern Angola

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In Southern Angola, numerous non-woody forest products are sold at local markets, namely in Lubango (Huíla Province). Such is the case of herbaceous wild plants, locally known as *lombi*, which are sold fresh throughout the year and cooked as a vegetable. Although these wild leafy vegetables are commercialized and widely used in local food, there is still a lack of scientific knowledge about their properties. Thus, this study aimed to identify and characterize the species sold, and to determine their nutritional and functional properties. Our results revealed that three species—*Amaranthus hybridus*, *Bidens pilosa*, and *Galinsoga parviflora*—are usually sold at Lubango markets and consumed by local populations. These are annual exotic plants, native to Southern America, and usually occur spontaneously in croplands or disturbed areas, but can also be cultivated, particularly *A. hybridus*. Physico-chemical analyses of *lombi* species and mixtures sold at the markets included measurements of moisture, protein, lipid, and mineral content, as well as total phenolic content, antioxidant activity, and levels of heavy metal contaminants. The results revealed that *lombi* contain a significant amount of protein (20–28 g/100 g, dry basis), high values of macronutrients and micronutrients, as well as of phenolic compounds (10–40 mg GAE/g) and a good antioxidant capacity. Given the availability of *lombi* throughout the year, our study demonstrated the importance of wild edible plants in Angola, both as a valuable natural resources and as a complementary food sources, as well as additional sources of income for many families.

**Keywords:** ethnobotany, nutritional composition, Southern Africa, traditional leafy vegetables, wild edible plants, bioactive properties

## INTRODUCTION

Wild edible plants (WEP) and mushrooms are important resources commonly used by rural communities around the world, and often traded in urban markets, particularly in Africa (Ambrose-Oji, 2009; Uusiku et al., 2010; Cernansky, 2015). With generally affordable prices and good acceptance by consumers, they allow a diversification of food sources, contribute to food

security of the populations, and help to fight poverty (Weinberger and Pichop, 2009; Yang and Keding, 2009).

Wild edible plants are important in rural communities as they are cheap and easy to obtain by families, allowing a more diversified diet and representing a source of sufficient macro- and micronutrients, thus reducing vulnerability to diseases (Shackleton and Shackleton, 2004; Shackleton et al., 2007; Garekae and Shackleton, 2020). Also, these plants can be very resistant to environmental changes and are an alternative for local communities during droughts or food shortages (Ohiokpehai, 2003). According to FAO (2017), indigenous food systems have characteristics that make them particularly attractive, including the use of both cultivated crops and gathered wild edible plants, synergies with the natural environment and biodiversity, close adaptation to local conditions, a high level of diversification, a light carbon footprint, fewer negative externalities and reduced use of external inputs.

African ecosystems support a rich plant biodiversity (White, 1983), among which many naturalized species (Maundu et al., 2009). The terms African Indigenous Vegetables or Traditional African Vegetables refer those vegetables originating from the African continent and those with a long history of cultivation and domestication adapted to African conditions, due to their frequent and traditional use (Maundu et al., 2009).

About 1,000 species are used as vegetables across the African continent, of which 80% are Wild Leafy Vegetables (WLV) or African Leafy Vegetables, the rest consisting of fruits, seeds, roots and tubers, stems, and flowers (Maundu et al., 2009). Leafy vegetables are dominated by plant families such as the Amaranthaceae, Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae, Solanaceae, and Tiliaceae (e.g., Njume et al., 2014). Most of these belong to herbaceous genera (e.g., *Amaranthus*, *Bidens*, *Solanum*, *Brassica*, *Phaseolus*, and *Vigna*), but there are also leafy vegetables from tree genera such as *Adansonia*, *Bombax*, *Ficus*, *Sterculia*, and *Moringa* (Maundu et al., 1999; Catarino et al., 2019a; Bancessi et al., 2020; Fernandes et al., 2021).

An interesting group of plants that stands out and has been very useful for indigenous communities are ruderal species, that thrive in environments strongly disturbed by human actions. They are able to disseminate rapidly in natural environments and gardens, and grow either on productive soils such as cropland or on poor soils such as fallows (e.g., Damalas, 2008). This capacity helped people to increasingly know them, and the easy access and low cost promoted a greater use of this resource over time (Ambrose-Oji, 2009). Due to the cultural diversity, the communities adopted different ways of use, domestication, storage and culinary preparation of wild edible plants (Oluoch et al., 2009; Yang and Keding, 2009).

Over the last decades, a large amount of work was done on the importance of nutritional and medicinal uses of African plants (Addis et al., 2013; Catarino et al., 2016, 2021a). These plants already contribute to food security of local populations, are often harvested and traded in town markets, and some are also used in traditional medicine (Shackleton et al., 2007, 2009; Cernansky, 2015; Charrua et al., 2021).

With globalization, important socio-economic changes took place in Africa, influencing people's dietary habits in both rural and urban areas. Today, most people prefer introduced crop plants to traditional foods, including plant foods whose consumption is widely regarded as a primitive culture manifesting poor lifestyles (Uusiku et al., 2010). As in other African countries, the Angolan population concentrated in urban centers after the civil war (1975–2002) and grew exponentially (Raleigh and Hegre, 2009). The social asymmetries are visible; low income and increased unemployment in a population living in a state of war until recently has contributed to it (Da Rocha, 2016). This population growth and increasing urbanization in Angola led to an increase in the consumption of woody and non-woody natural resources in the areas surrounding the cities (Romeiras et al., 2014).

In Angola, a large number of plant species is traditionally used by rural communities for food, medicinal purposes (Catarino et al., 2019b), or both, as reported by Urso et al. (2016) and Kissanga (2016) for southern Angola, and Heinze et al. (2017) for northern Angola. Many forest products are also sold in markets and on roadsides, representing a source of income for the families that harvest and sell them (Heinze et al., 2019). However, the available information about them is scarce and a lot more research is needed to inventory and evaluate the nutritional properties and socio-economic potential of wild edible plants and mushrooms across the country.

In the markets of Lubango, capital of Huíla Province, in southern Angola, a large number of non-wood forest products are sold, both from this municipality and from the surrounding ones. Among those marketed products, three major types can be considered: (i) wild fruits, sold throughout the year according to their ripening seasons; (ii) fresh leafy vegetables, sold throughout the year and cooked as pot herbs, known locally as *lombi*, and (iii) mushrooms, sold fresh at the time of ripening of the fruiting bodies, and dried throughout the year.

This study focused on the *lombi*, edible leaves and young shoots of plant species that grow spontaneously in fallows, abandoned land and home gardens, some of them also cultivated. Several cultivated species of *lombi* that are locally grown are cabbage, spinach, common beans, pumpkin, amaranth, and *Hibiscus* spp. The spontaneous species of *lombi* are included among the wild leafy vegetables, which are often characterized as weeds for agriculture but, being edible, abundant and easy to obtain, are an important resource that must be known and valued. Also, these traditional leafy vegetables are commonly used in the gastronomy of central and southern Angola and can contribute to the food security for both rural and urban communities. Wild *lombi* are usually consumed as a component of traditional meals, as side dishes for “*pirão*” (corn paste), or “*funje*” (cassava paste), accompanying a meat or fish preparation.

The main objective of this work was to identify the species of wild leafy vegetables sold at Lubango markets, their prices and their socio-economic importance in southern Angola, as well as to determine their bio-ecological characteristics and to evaluate the nutritional composition and functional properties of the main species and the mixtures sold.

## MATERIALS AND METHODS

### Study Site and Field Data Collection

The socio-economic part of the study took place from June to November 2018, involving the municipalities of Lubango and Humpata, from Huíla Province (Figure 1). The wild leafy vegetables on sale in the markets of Mutundo, Rio Nangombe, and Hoque (Lubango municipality), and Humpata (Humpata municipality) were initially prospected, followed by semi-structured interviews with vendors about the prices, origin and characteristics of the *lombi* sold. The interviews were preceded by explanation of the study objectives and by informed consent from the respondents.

The *lombi* samples for analysis were obtained in October 2019, at Mutundo market, the one with higher availability of *lombi* year-round. Samples of the piles of wild leafy vegetables were acquired at the beginning of the month and weighed on site with a portable scale (Figure 2a). The samples were separated according to different species, and the proportion of each one in the mixtures sold was quantified, and then dried (species' samples). By the end of October, samples of the mixture were acquired from a different seller at the same market (mixture sample) and dried.

Herbarium vouchers of the species were made for later identified by comparison with specimens already identified and using the available bibliography, namely Figueiredo and Smith (2008) as well as the African Plant Database (2021). The herbarium vouchers are deposited at LISC (IICT/University of Lisbon) and LUBA (Instituto Superior de Ciências da Educação da Huíla, Lubango, Angola) herbaria.

After identifying the species of *lombi*, a literature search was conducted to document their origin and distribution, bio-ecological characteristics and food and phytochemical properties. The websites JSTOR Global Plants (2021) (<https://plants.jstor.org/>), PlantUse English contributors (2021) (<https://uses.plantnet-project.org/en/>), and Useful Tropical Plants (<http://tropical.theferns.info/>) were initially searched, and then a targeted literature search on the identified species was conducted.

### Sample Preparation

At the LUBA herbarium facilities, the fresh samples of the three species (*Amaranthus hybridus* L., *Bidens pilosa* L., and *Galinsoga parviflora* Cav) as well fresh samples of the mixture sold at the markets were weighed fresh, subsequently dried at 45°C for 72 h in a household electrical food dehydrator (SilverCrest SDA 350 A1, Hoyer Handel GmbH, Hamburg, Germany) and re-weighed after drying. Then, the samples were packed in paper bags and transported to the ISA (School of Agriculture, University of Lisbon) laboratory in Lisbon, where the physico-chemical analyses were performed.

### Physico-Chemical Characterization of *lombi*

The *lombi* were characterized for: moisture, protein, lipids, minerals, total phenolic content and antioxidant activity (DPPH, FRAP, and ABTS), as well as metallic contaminants. Physico-chemical determinations were conducted in triplicate.

### Moisture, Protein, and Lipid Contents

The moisture content was determined by drying the samples at 105°C until constant weight (Bi et al., 2018). Protein analysis was performed by the Kjeldhal method (Jimoh et al., 2018); the total nitrogen content was multiplied by 6.38 to determine total (crude, total N 6.38) protein, expressed in g/100 g of dry weight. Protein determination was performed in triplicate. The total lipid content was determined by the Soxhlet method. Extraction was done with hexane for about 90 min.

### Mineral Content

The mineral content was evaluated by inductively coupled plasma spectrometry (ICP) [iCAP Spectrometer equipped with ASX-520 AutoSampler (Thermo Scientific, Waltham, MA, USA)]. Samples (0.25 g) were digested in aqua regia (9 mL of HCl and 3 mL of HNO<sub>3</sub>). Digestion took place in several stages: (1) 30 min/40°C, (2) 30 min/80°C, and (3) 90 min/105°C in a SCP Science equipment (DigiPREP MS, Baie d'Urfe, QC, Canada). After cooling, 50 mL of distilled water was added and waited to decant. The cleared supernatant was used in inductively coupled plasma to determine macronutrients (potassium, calcium, magnesium, phosphorus, and sulfur); micronutrients (sodium, iron, copper, zinc, manganese, and boron) and contaminants (lead, chromium, nickel, and cadmium).

### Total Phenolic Content

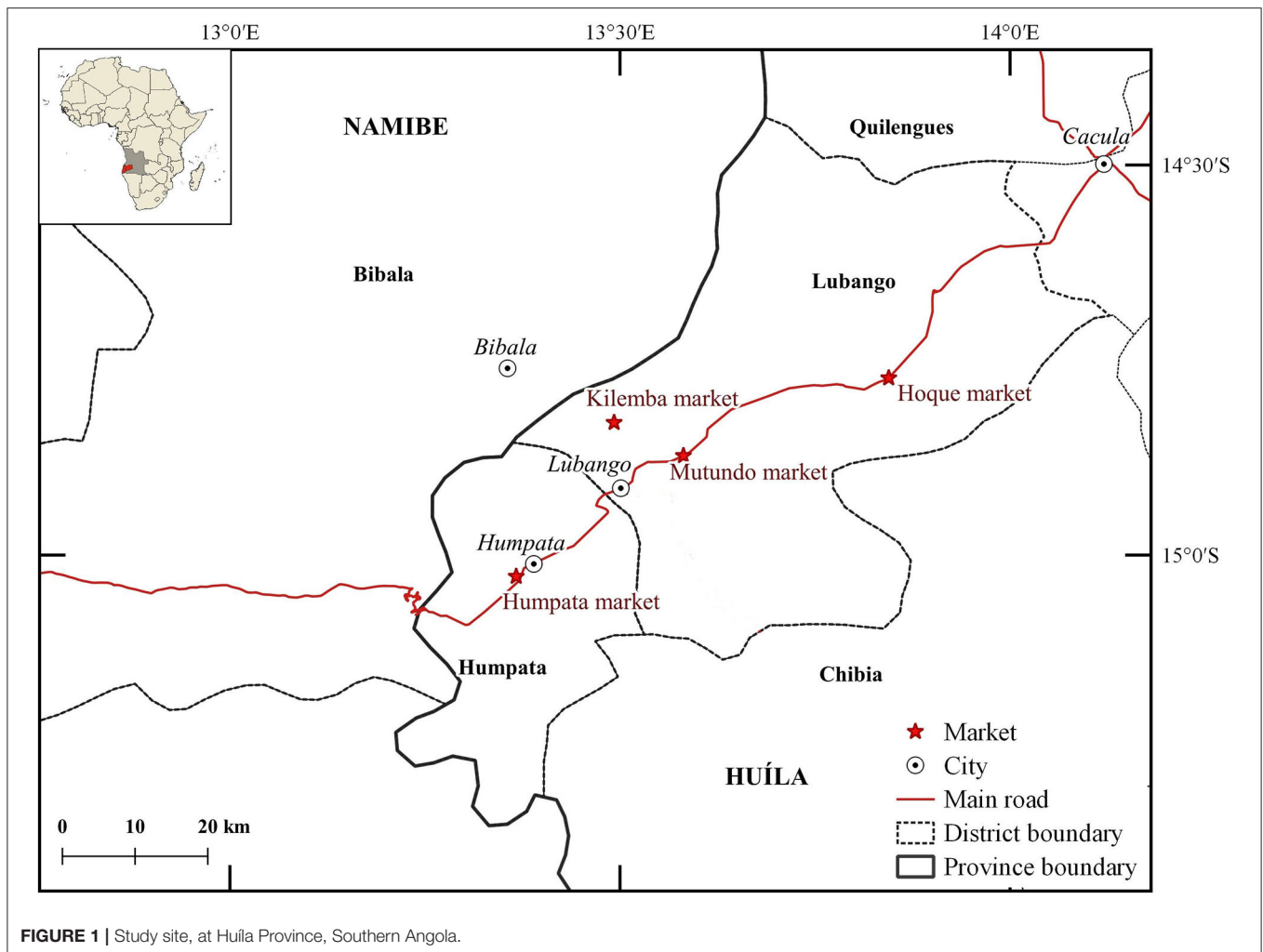
The total phenolic content (TPC) of the samples was determined according Heredia and Cisneros-Zevallos (2009), and Swain and Hillis (1959). Extract aliquots (150 µL) were diluted with 2,400 µL nanopure water, mixed with 150 µL Folin-Ciocalteu reagent (Panreac AppliChem, Germany; 0.25 mol.L<sup>-1</sup>). The reaction was interrupted by adding 300 µL sodium carbonate (1 mol.L<sup>-1</sup>) and the mixture was incubated (2 h) in darkness. The samples were read at 725 nm in spectrophotometer (UNICAM UV/Vis Spectrometer). The total phenolic content was determined by a standard curve of equivalent of gallic acid (GAE) and expressed as mg GAE.g<sup>-1</sup> dry weight; the extracts were analyzed in triplicate and the average was used for each condition.

### Antioxidant Activity (DPPH, FRAP, and ABTS)

The antioxidant capacity by DPPH (2,2-diphenyl-1-picrylhydrazyl) method was applied following the procedure of Brand-Williams et al. (1995). The DPPH solution was prepared with DPPH reagent (Sigma-Aldrich, Germany) diluted in methanol until reaching 1.1 units of absorbance at 515 nm. The supernatant (100 µL) was added with a DPPH solution (3.9 mL). The reaction occurred for 40 min. The samples were read at 515 nm, using a spectrophotometer (Agilent Technologies, Cary 100 UV/Vis, Santa Clara, USA).

The FRAP (Ferric Reducing Antioxidant Power) test was performed according to Benzie and Strain (1996). The reaction initiated by mixing the FRAP solution (2.7 mL), 270 µL nanopure water with the extract samples (90 µL), and afterwards warmed in a water bath at 37°C for 30 min. The colored product (ferrous tripyridyltriazine complex) was read at 595 nm.

Antioxidant activity was measured using the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) method as



described by Re et al. (1999) and Rufino et al. (2007). The reaction was performed by mixing 2970  $\mu\text{L}$  ABTS solution (Sigma-Aldrich, Germany) with sample aliquots (30  $\mu\text{L}$ ) during 6 min, and the absorbance was read at 734 nm. The antioxidant activity (DPPH, FRAP and ABTS) was determined using Trolox (Acrós Organics, Belgium), and the results were expressed by Trolox Equivalent Antioxidant Capacity [TEAC ( $\mu\text{mol Trolox}\cdot\text{g}^{-1}$  dry matter)]. For each antioxidant determination method (DPPH, FRAP and ABTS) the extracts were analyzed in triplicate and the average was used for each condition.

### Statistical Analysis

Significant differences were determined by one-way analysis of variance (ANOVA) followed by Tukey's test. Statistically significant differences ( $p < 0.05$ ) between samples were defined using Tukey's honestly significant difference test. Statistical analyses were carried out using the data analysis software system STATISTICA TM version 8.0 (StatSoft Inc., Tulsa, OK, USA, 2007).

## RESULTS

### The Trade and Use of Wild Leafy Vegetables at Huila

In the present study, the communes (sub-municipality administrative rank) of Hoque, Quilemba, Cacula, and Humpata were found to be the main suppliers of wild leafy vegetables to Lubango's markets (Figure 1). Wild leafy vegetables are sold in the markets exclusively by women, who also sell other fresh food products. A total of 22 sellers were interviewed, 6 at Mutundo market, 10 at Hoque market, and 3 at Quilemba and Humpata markets. From the interviewed sellers, 12 were young adults under 30 years-old, eight were adults between 30 and 50 years-old, and the remaining were two seniors over 50 years-old.

During the dialogue with the vendors, we learned that the *lombi*, traditionally, are herbaceous plants that have always been highly appreciated by the majority of the rural population at the peripheral area of the city of Lubango and that, currently, there is a greater demand from customers in the city center.

The *lombi* are sold in piles of about 1 kg (see Figure 2a), at a price of 50 Angolan Kwanzas (AKZ) each (c. 0.10



**FIGURE 2** | Species of traditional leafy vegetables sold at Lubango markets. **(a)** Mixture in piles, at the market; **(b)** *Amaranthus hybridus*; **(c)** *Bidens pilosa* and **(d)** *Galinsoga parviflora*. Photos by Ruth Francisco **(a)** and F. Lages **(b–d)**.

USD), but the quantities sold per day per seller are difficult to know because most of them also sell other agricultural products. The found *lombi* species grow spontaneously, and are harvested by the peasants from several types of environments, both in rural and peri-urban areas, such as home gardens, fallows and in croplands, where they grow as adventive species.

As the *lombi* are sold year-round and each species has a different phenology, namely concerning the harvest period, the proportion of each species in the mixture can largely vary along the year, and even from seller to seller. Also, since *lombi* are typically collected from fallows, home gardens and disturbed

places, their species composition can vary from one place to another.

The proportion of each species in the mixtures sold showed some variability, probably related with the availability and growth cycle of each of the species composing it. In the five samples analyzed, *Bidens pilosa* was the most abundant species, corresponding to about 60% of the fresh weight, followed by *Galinsoga parviflora* with 30% and *Amaranthus hybridus* with 10% (Table 1).

It is also possible that there are residual amounts of some other species that are also used as *lombi*, such as *Amaranthus spinosus*, *A. caudatus*, *A. viridis*, *Chenopodium quinoa*, *Cleome*

**TABLE 1** | Weights in grams, percentage of each species by pile ( $n = 5$ ), and moisture content by species.

	Average	StDev	Min-max	Moisture content
<b>Pile weight (g)</b>	<b>999.6</b>	65.0	887–1,090	
<i>Amaranthus hybridus</i> (%)	<b>10.2</b>	2.1	6.0–11.9	81.2
<i>Bidens pilosa</i> (%)	<b>59.6</b>	16.1	40.0–75.0	86.5
<i>Galinsoga parviflora</i> (%)	<b>30.1</b>	17.6	13.9–54.0	88.1

*ginandra*, and *Portulaca oleracea*. All species showed high moisture content, which ranged from 81 to 88 percent of fresh weight (Table 1). Similar values were found by Catarino et al. (2019a) for wild leafy vegetables in Guinea-Bissau.

The wild edible plants are eaten in Huila as pot vegetables or side-dishes, and included in several recipes. After washing, the mixture of leafy vegetables is usually cooked for 15–20 min, depending on the cook. Some people remove the water from the first boil, claiming that this reduces the initial bitter taste. A paste is then prepared by sautéing onions, garlic, and tomatoes in vegetable oil, which is mixed with the cooked vegetables.

During fieldwork, it was verified that a pile of fresh *lombi* is usually used to prepare a family meal for four adult people, which represents about 250 g of fresh matter per person and meal. Using a water content of c. 86% as a reference, this individual dose corresponds to a dry matter of 35 g per person per meal, which can be considered the average edible portion.

## Characterization of the Studied Wild Leafy Vegetables

In terms of distribution and ecology, the three studied species are exotic, annual plants of South American origin, and occur spontaneously in cultivated fields, house gardens, recent fallows and other disturbed places. They can also be cultivated for consumption, namely *A. hybridus*.

*Amaranthus hybridus* L., Amaranthaceae (R Kissanga 619, LISC, LUBA; Figure 2b).

Local names: jimboa, mboa; olombwa. English name: amaranth.

An annual, erect, branched herb that can reach 50–200 cm in height. It can be grown for its leaves and seeds. Originally from tropical America, it is now naturalized in all tropical regions and in Europe. In Southern Angola it is a ruderal species, growing in cultivated fields, fallow land, roadsides and other disturbed places, and often cultivated.

The leaves and young branches are eaten fresh or cooked, as an accompaniment to various dishes and can also be used in stews and soups. The seeds, *in natura* or cooked, are eaten as a cereal. *A. hybridus* is also used for various medicinal and phytochemicals purposes. It is frequently cultivated and can be consumed by livestock (Burkill, 1985). It is a high-productivity species with a C4 photosynthetic mechanism that can produce 30–60 tons of biomass annually and is widely cultivated in other Southern African countries (Van Wyk and Gericke, 2017). According to several authors, the seeds and leaves of various *Amaranthus* species are quite rich in nutrients, with high protein content and

good amounts of other nutrients, including mineral salts, and can be considered a high quality food (Van Wyk and Gericke, 2017; Fern, 2021).

*Bidens pilosa* L., Asteraceae (R Kissanga 620, LISC, LUBA; Figure 2c).

Local names: jisongo-jia-ngoma, olokoso, osungua. English name: black jack.

An erect annual herb, branched, fast growing, that can reach a height of 60–80 cm. It is a heliophilous plant that grows easily in various climates and soil types and is considered a weed in crop fields. It is usually obtained from spontaneous growth but can be cultivated for its leaves and young branches, which can be repeatedly cut. Native to South and Central America, this species has now a cosmopolitan distribution. In Angola it is a ruderal plant, growing in cultivated fields, fallow land and disturbed places, including in urban environments.

The leaves and young branches are eaten after cooking and are often used in sauces and as a side dish. They can also be dehydrated, ground and preserved for later use. *B. pilosa* is an aromatic plant, rich in secondary metabolites, also used for medicinal purposes in many regions of Africa, as well as Asia and tropical America. Roots, leaves and seeds are reported to possess antibacterial, antidysenteric, anti-inflammatory, antimicrobial, antimalarial, diuretic, hepatoprotective and hypotensive activities (Burkill, 1986; Mvere, 2004; Van Wyk and Gericke, 2017). However the roots, leaves and flowers are considered phototoxic and harmful to the skin (Fern, 2021).

*Galinsoga parvifolia* Cav., Asteraceae (R Kissanga 624, LISC, LUBA; Figure 2d).

Local names: felisberto, lume, okalume, otulume. English name: gallant soldier.

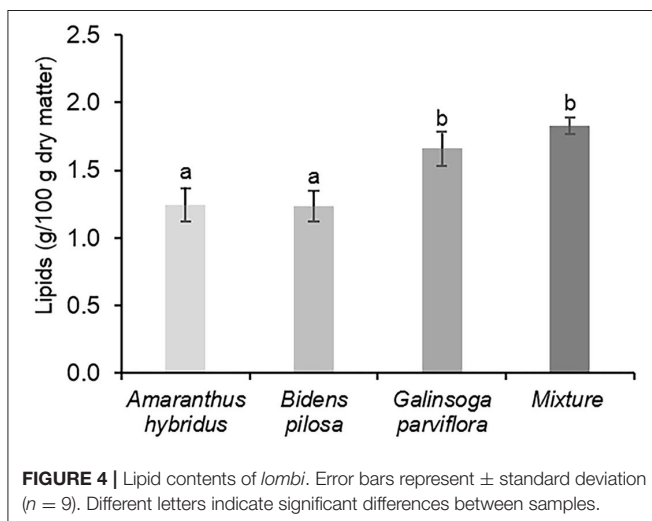
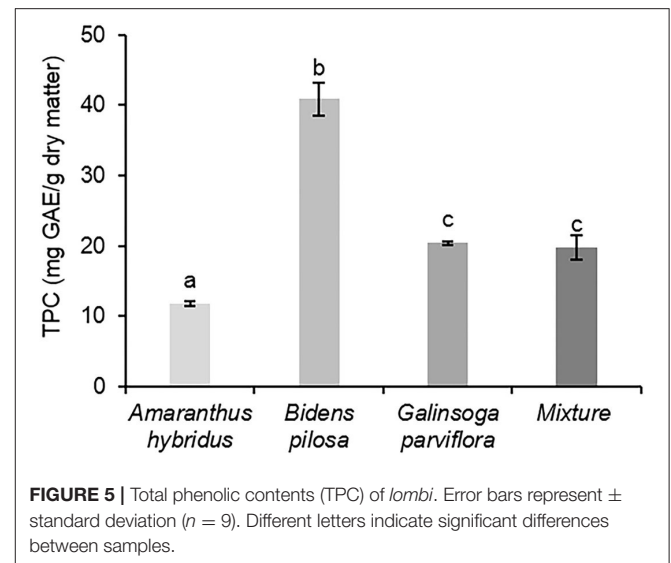
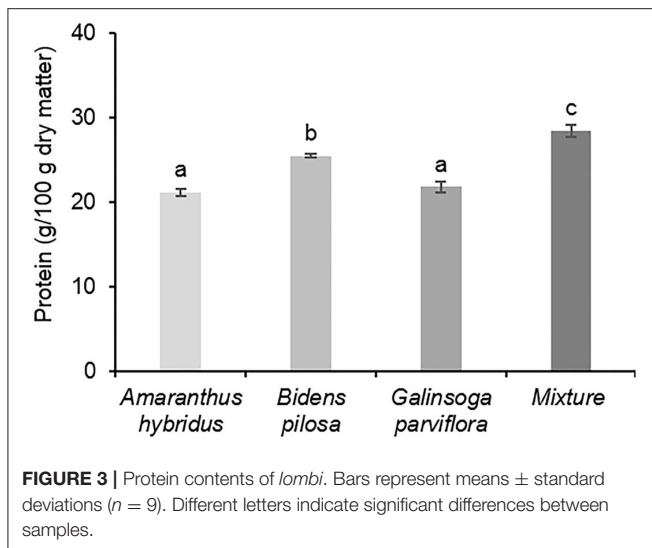
Annual, erect herb with a branched stem that can reach a height of 40–80 cm. It can be cultivated for food (leaves and seeds). Native to Central America, this species is now naturalized in all tropical regions and in Europe. It is a ruderal plant, growing in cultivated fields, fallow land, disturbed places, and home gardens.

The leaves and young branches are cooked as a vegetable and eaten as an accompaniment to traditional dishes. The dehydrated leaves are sometimes used as a condiment in some dishes (Schippers, 2004). *G. parviflora* is also used in traditional medicine for skin inflammations and wounds, as well as for fodder and in veterinary medicine (Burkill, 1985). An analysis of the composition per 100 g of fresh matter showed a water content of almost 90% and moderate contents of protein, carbohydrates and minerals (Wehmeyer and Rose, 1983).

## Physico-Chemical Characterization of *lombi*

### Protein Content

The protein content of the three analyzed wild leafy vegetables is presented in Figure 3. The samples had a protein content higher than 20%, between around 21% for *A. hybridus* and 28% for the mixture, which is considered high for vegetable products. Among the individual species' samples, *B. pilosa* showed the



highest protein content (around 25%). These values are quite high and the slightly higher values obtained for the mixture can be attributed to compositional differences related with the date of acquisition of samples, and probably also because they were from different sellers. On the other hand, the values obtained in this study for protein content were higher than those reported by Chatepa and Masamba (2020) for a similar set of species, and by Akubugwo et al. (2007) for *A. hybridus*.

### Lipid Content

The lipid content of each of the three species can be considered low (Figure 4), ranging from about 1.3 g/100 g of dry matter in *A. hybridus* and *B. pilosa* to c. 1.6 g/100 g in *G. parvifolia*.

These values are moderate if compared with other African leafy vegetables (e.g., Catarino et al., 2019a). The slightly higher values obtained for the mixture can be attributed to compositional differences between the samples used for separated species analysis.

### Mineral Content

The macro and micronutrient contents of the three analyzed species is presented in Tables 2, 3, respectively.

Concerning the macronutrients, among the individual samples, *A. hybridus* showed a significantly higher amount of potassium ( $44,551 \pm 4,082 \text{ mg.kg}^{-1}$ ), calcium ( $19,498 \pm 158 \text{ mg.kg}^{-1}$ ), and magnesium ( $6,964 \pm 13 \text{ mg.kg}^{-1}$ ), while *B. pilosa* stands out for its significantly higher content of phosphorous ( $5,262 \pm 42 \text{ mg.kg}^{-1}$ ). Regarding micronutrients, the individual samples of *A. hybridus* showed the highest amount of sodium ( $494.8 \pm 38.9 \text{ mg.kg}^{-1}$ ) and zinc ( $37.3 \pm 0.6 \text{ mg.kg}^{-1}$ ), *G. parviflora* had the highest contents of iron ( $466.7 \pm 19.6 \text{ mg.kg}^{-1}$ ) and copper ( $10.9 \pm 0.2 \text{ mg.kg}^{-1}$ ), and *B. pilosa* presented the highest values for manganese ( $50.6 \pm 0.5 \text{ mg.kg}^{-1}$ ) and boron ( $25.7 \pm 0.3 \text{ mg.kg}^{-1}$ ).

The mineral content values of the mixture were not always within the range determined for individual species: calcium ( $12,481 \pm 234 \text{ mg.kg}^{-1}$ ), sulfur ( $3,070 \pm 54 \text{ mg.kg}^{-1}$ ), iron ( $291.5 \pm 7.9 \text{ mg.kg}^{-1}$ ), copper ( $12.9 \pm 0.4 \text{ mg.kg}^{-1}$ ), and manganese ( $28.8 \pm 0.4 \text{ mg.kg}^{-1}$ ). This might be due to the variability of the amount of each species in *lombi* mixtures, to different vegetative stages of the plants and the location of harvest. Still, the values for the mixture were in the same order of magnitude of those reported for individual species.

### Total Phenolic Content (TPC) and Antioxidant Activity (DPPH, FRAP, and ABTS)

The results for total phenolic content are presented in Figure 5.

*B. pilosa* presented a TPC around two times and four times higher than that of *G. parviflora* and *A. hybridus*, respectively. The mixture presented a TPC value around 20 mg EGA/dry matter, to which the major contribution is probably from *B. pilosa*.

The antioxidant activity values quantified *in vitro* by three different methods and expressed as TEAC (Trolox Equivalent Antioxidant Capacity) are presented in Figure 6.

**TABLE 2** | Macronutrients' contents of *lombi* (mg.kg<sup>-1</sup> dry matter).

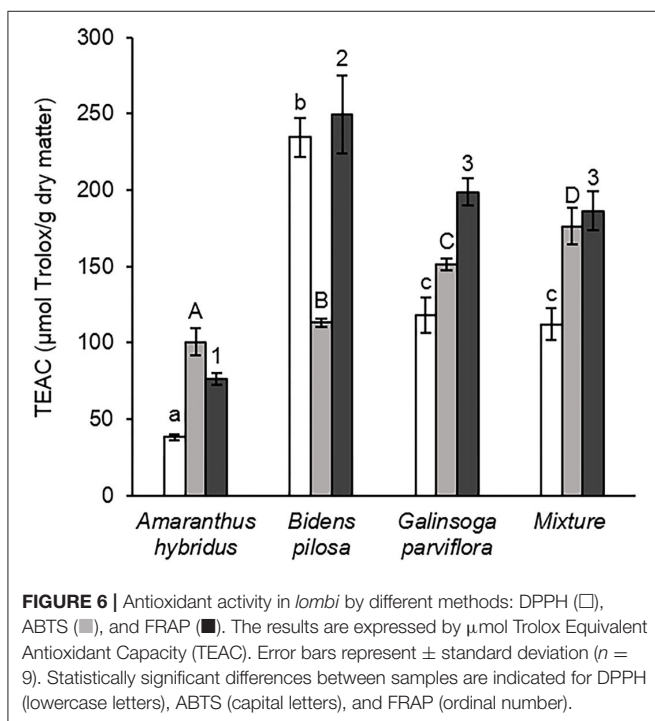
Products/species	K	Ca	Mg	P	S
<i>Amaranthus hybridus</i>	44,551 ± 4,082 <sup>a</sup>	19,498 ± 158 <sup>a</sup>	6,964 ± 13 <sup>a</sup>	3,553 ± 25 <sup>a</sup>	2,775 ± 2 <sup>a</sup>
<i>Bidens pilosa</i>	28,750 ± 444 <sup>b</sup>	9,832 ± 194 <sup>b</sup>	3,251 ± 84 <sup>b</sup>	5,262 ± 42 <sup>b</sup>	2,438 ± 15 <sup>b</sup>
<i>Galinsoga parviflora</i>	30,534 ± 331 <sup>b</sup>	16,059 ± 89 <sup>bc</sup>	4,091 ± 38 <sup>cd</sup>	3,792 ± 34 <sup>a</sup>	2,888 ± 20 <sup>a</sup>
Mixture	28,743 ± 307 <sup>a</sup>	12,481 ± 234 <sup>bc</sup>	5,961 ± 57 <sup>cd</sup>	3,921 ± 55 <sup>a</sup>	3,070 ± 54 <sup>a</sup>

Mean values ± standard deviations (n = 9). Different letters in a column indicate significant differences between samples.

**TABLE 3** | Micronutrients' contents of *lombi* (mg/kg dry matter).

Sample	Na	Fe	Cu	Zn	Mn	B
<i>Amaranthus hybridus</i>	494.8 ± 38.9 <sup>a</sup>	460.0 ± 8.4 <sup>a</sup>	6.0 ± 0.1 <sup>a</sup>	37.3 ± 0.6 <sup>a</sup>	49.8 ± 0.5 <sup>a</sup>	18.2 ± 0.8 <sup>a</sup>
<i>Bidens pilosa</i>	274.1 ± 11.6 <sup>b</sup>	329.8 ± 7.9 <sup>b</sup>	9.5 ± 0.1 <sup>b</sup>	44.0 ± 0.2 <sup>b</sup>	50.6 ± 0.5 <sup>a</sup>	25.7 ± 0.3 <sup>b</sup>
<i>Galinsoga parviflora</i>	440.6 ± 28.4 <sup>a</sup>	466.7 ± 19.6 <sup>a</sup>	10.9 ± 0.2 <sup>b</sup>	33.1 ± 0.2 <sup>c</sup>	33.8 ± 0.7 <sup>b</sup>	16.5 ± 0.2 <sup>a</sup>
Mixture	367.5 ± 27.7 <sup>c</sup>	291.5 ± 7.9 <sup>c</sup>	12.9 ± 0.4 <sup>c</sup>	43.6 ± 0.5 <sup>b</sup>	28.8 ± 0.4 <sup>c</sup>	18.1 ± 0.1 <sup>a</sup>

Mean values ± standard deviations (n = 9). Different letters in a column indicate significant differences between samples.



**FIGURE 6** | Antioxidant activity in *lombi* by different methods: DPPH (□), ABTS (■), and FRAP (■). The results are expressed by µmol Trolox Equivalent Antioxidant Capacity (TEAC). Error bars represent ± standard deviation (n = 9). Statistically significant differences between samples are indicated for DPPH (lowercase letters), ABTS (capital letters), and FRAP (ordinal number).

TEAC results indicate that all the analyzed species present a good antioxidant capacity. When applying the DPPH and FRAP methods, the antioxidant activity follows the same trend as TPC. It is significantly higher for *B. pilosa* than in the other species and in the mixture. These results are attributed to the bioactivity generally observed for phenolic compounds in terms of antioxidant activity (Stagos, 2020). However, the same correlation between TEAC and TPC values is not perceived when the ABTS method is used. This might be related to the complexity

of the chemical composition of plant extracts, which also include compounds with different reactivities to *in vitro* methods.

### Metallic Contaminants

The levels of lead, chromium, nickel, and cadmium in the samples of the three species and the mixture are shown in **Table 4**, as well as the maximum limits according to EU Commission and Nagajyoti et al. (2010). The indicated limit values for lead and cadmium are presented on a fresh matter basis.

The lead contents of the analyzed samples (0.52–0.69 mg/kg dry matter) are apparently out of range, but when expressed on a fresh matter basis (0.07–0.08 mg/kg fresh matter) they are below the maximum limits.

The values obtained for chromium (1.31–2.28 mg/kg dry matter) are clearly beyond the limits and can be concern. The cause of such high values of chromium is not evident, and it could not be ascertained whether it is predominantly hexavalent or trivalent chromium. Still, these plants tend to grow in disturbed places, with a high potential to present a non-negligible degree of soil contamination with domestic and industrial residues (e.g., batteries and metallic packages).

## DISCUSSION

### Socioeconomic Analysis

Available year-round and easy to obtain in home gardens and to buy at low price in the markets, *lombi* and other wild leafy vegetables are very convenient food products. The wild food plants, in particular the three species of herbaceous plants analyzed in this study, can contribute to the food and nutritional security of populations and be additional sources of income for families. Thus, the characterization of their composition and functional characteristics, as well as of their bioecology, is important for the knowledge and appreciation of these traditional food products.



Also, it is important to investigate the antinutritional and toxicological properties of the wild edible plants (Brilhante et al., 2021). Despite their use as ingredients in food, their toxicity for humans and animals is still inadequately and insufficiently studied (Damalas, 2008; Frida et al., 2008; Bartolome et al., 2013), and soil contamination with heavy metals can be a cause of concern (Atayese et al., 2008; Chia et al., 2019; Amadi et al., 2020).

Climate changes and difficulties in agricultural production contribute to change families' diets, reducing their quality and diversity due to income reductions and high food prices, promoting an increase in the consumption of imported processed products (FAO, 2018). The consumption of wild leafy vegetables varies and usually decreases with urbanization in many sub-Saharan African populations (Uusiku et al., 2010). Also, changes in diet and environmental factors, particularly among urban dwellers, have caused a significant increase in lifestyle diseases (Walker et al., 2002).

Angola has a high floristic diversity and endemism, resulting from a great diversity of ecogeographical regions, and the effective *in situ* and *ex situ* conservation of Angolan wild food plants is both a national and a global priority (Catarino et al., 2021b). Nevertheless, information about wild leafy vegetables is still very scarce, namely about their diversity, distribution and conservation, as well as about their consumption and methods of preparation.

Little is known about the actual quantities of traditional dishes and wild leafy vegetables consumption, but it seems that many families, particularly from urban and peri-urban areas, resort to the traditional diet at least twice a week, on weekends (Yang and Keding, 2009). In Angola, the *lombi* are mainly used as side-dishes to accompany traditional dishes made of cassava and maize and fish or meat. Although our study provides more information about wild leafy vegetables, the availability, prices, and consumption per person should be important to address in further studies, as well as the contribution of *lombi* to the food security of local communities.

In a comparative study of 10 countries in sub-Saharan Africa, Ruel et al. (2005) demonstrated that the vegetable consumption per capita may range from 19.6 to 88.3 kg/person/year, and that the price ranges from 0.13 to 0.57 USD/kg in those countries. In the present study, wild leafy vegetables were sold at 50 AKZ/kg, which corresponds to c. 0.10 USD (average exchange rate for 2019: 1 USD = 500 AKZ, National Bank of Angola) and ranks them among the cheapest vegetables in sub-Saharan Africa.

## Physico-Chemical Analyses

### Protein and Lipids

The protein contents found in this work is higher than those reported by Chatepa and Masamba (2020) for the same species in Malawi (around 18, 19, and 16% for *Amaranthus* spp., *B. pilosa* and *G. parviflora*, respectively). As in the present study, the species with the highest protein content is *B. pilosa*. However, other studies report much higher values for *B. pilosa* (from 20% up to 42%), as well as for *G. parviflora* (36%) (Uusiku et al., 2010). This difference may be due to differences in the agro-climatic conditions between these studies. In addition, when compared with other African leafy plants, the species of *lombi*

show an intermediate protein content. A lower protein content is reported for *Adansonia digitata* (10.1%), *Bombax costatum* (10.8%), *Sesamum radiatum* (13.3%), and *Hibiscus sabdaria* (13.7%) in Guinea-Bissau (Catarino et al., 2019a), whereas higher protein content values are presented for *Portulaca oleracea* (43%), *Vigna unguiculata* (36%), and *Senna occidentalis* (30%) (Uusiku et al., 2010). Protein contents from 11.3% (*Vernonia amygdalina*) to 30.6% (*Telfairia occidentalis*) were found in vegetable leaves from markets in Southern Nigeria (Dan et al., 2013), and from 13.2% (*Sonchus asper*) to 26.4% (*Chenopodium album*) when studying wild leafy vegetables in South Africa (Afolayan and Jimoh, 2009).

Lipid contents measured in this study ranged from 1.3% in *A. hybridus* and *B. pilosa* to c. 1.65% for *G. parvifolia*. For the same species in Malawi, Chatepa and Masamba (2020) reported much higher values (13.1, 9.0, and 9.0%, respectively). The values were also lower than those of wild leafy vegetables in South Africa (*Chenopodium album*, *Sonchus asper*, *Solanum nigrum*, and *Urtica urens*) which present values ranging from 4.2 to 8.5% (Afolayan and Jimoh, 2009).

### Macro- and Micronutrients

Dietary minerals play an important role in human health, such as in the control of blood pressure (sodium/potassium ratio), growth and maintenance of bones, teeth, and muscles (calcium and phosphorous), and control of anemia (iron). Indigenous leafy vegetables have been reported to be valuable sources of macro and micronutrients (Odhav et al., 2007; Uusiku et al., 2010; Njume et al., 2014), and this is in agreement with the results of the present study. Dietary minerals contents depend substantially, not only on the type of plant, but also on the location and moment of harvest. To understand the dietary impact of the consumption of the wild plants targeted in the present study, the percentages of Daily Recommended Doses (DRDs; NIH, 2019) for some elements provided by *lombi* were estimated. A daily intake of 35 g of dry matter per person provides about 90% of the recommended daily dose, so it can be regarded as an excellent source of iron. In addition, 55 and 45% of DRDs of Mg and Ca are also expected, though a lower intake was estimated for Zn and P (15 and 20% of DRDs, respectively). The results indicate that *lombi* have a high potential to substantially contribute for enriching the mineral diet of local consumers.

### Metallic Contaminants

Of the metallic contaminants analyzed (Pb, Cr, Ni, and Cd), the one raising major concerns is Cr, as all *lombi* species present contents above the limit referred by Nagajyoti et al. (2010). Chromium oxidation states range from  $-2$  to  $+6$ , with  $+3$  and  $+6$  being the most often studied in relation to human health (EFSA, 2014). The high energy needed to oxidize Cr(III) to Cr(VI) results in the fact that oxidation does not occur in biological systems. Some authors refer a positive role of Cr(III) in human physiology (e.g., Mandiwana et al., 2011). However, according to EFSA, the mechanisms for these roles and the essential function of Cr(III) in metabolism have not been substantiated and there is no evidence of beneficial effects associated with chromium intake in healthy individuals (EFSA,

**TABLE 4** | Metallic contaminants' contents of *lombi* (mg/kg dry matter).

Products/species	Pb	Cr	Ni	Cd
<i>Amaranthus hybridus</i>	0.69 ± 0.05 <sup>a</sup>	2.28 ± 0.05 <sup>a</sup>	0.58 ± 0.06 <sup>a</sup>	0.09 ± 0.01 <sup>a</sup>
<i>Bidens pilosa</i>	0.52 ± 0.02 <sup>b</sup>	1.31 ± 0.04 <sup>b</sup>	0.37 ± 0.07 <sup>b</sup>	0.09 ± 0.01 <sup>a</sup>
<i>Galinsoga parviflora</i>	0.68 ± 0.01 <sup>a</sup>	2.25 ± 0.07 <sup>a</sup>	0.57 ± 0.11 <sup>a</sup>	0.16 ± 0.01 <sup>b</sup>
Mixture	0.44 ± 0.03 <sup>b</sup>	1.57 ± 0.03 <sup>b</sup>	0.58 ± 0.07 <sup>a</sup>	0.11 ± 0.01 <sup>a</sup>
Maximum limits	0.30 mg kg <sup>-1</sup> fresh matter (a)	0.2–1.0 mg kg <sup>-1</sup> dry matter (b)	1.0 mg kg <sup>-1</sup> dry matter (b)	0.20 mg kg <sup>-1</sup> fresh matter (c)

Mean values ± standard deviations (n = 9). Different letters in a column indicate significant differences between samples.

(a) Commission Regulation (EU) 1005/2015 (2015); (b) Nagajyoti et al. (2010); (c) Commission Regulation (EU) 488/2014 (2014).

2014). In terms of chromium maximum intake, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) derived a tolerable intake of 300 µg Cr(III)/kg body weight per day from the lowest No Observed Adverse Effect Level (NOAEL) identified in a chronic oral toxicity study in rats (EFSA, 2014).

However, Cr(VI) is toxic and carcinogenic to humans (Ebdon et al., 2001). Data presented in the current work refers to total Cr, like in most literature. Still, speciation studies have been carried out in the determination of Cr(VI) in plants, showing that the amount of Cr(VI) was only 8 and 6% of total Cr for plants from Russia and South Africa, the latter harvested from soil with chromitite (Panichev et al., 2005).

Taking into account the Cr toxic threshold of 0.2 mg person<sup>-1</sup> day<sup>-1</sup> considered by Marini et al. (2021), and the consumption levels observed, the risk of toxic or negative health effects by consuming *lombi* seems to be negligible. Also, the practice of discarding the boiling water during initial stages of cooking can help to reduce the ingestion of this contaminant. The same happens for Cd considering the provisional tolerable intake of 2.5 µg person<sup>-1</sup> week<sup>-1</sup> (EFSA, 2011), for Pb considering the benchmark dose level of 1.5 µg person<sup>-1</sup> day<sup>-1</sup> (EFSA, 2012), and for Ni, taking into account the tolerable intake of 13 µg person<sup>-1</sup> day<sup>-1</sup> (EFSA, 2020). For negative health effects to happen, an amount much higher than 30 kg of fresh plants should be ingested per day, considering a body weight of 60 kg.

### TPC and Antioxidant Activity

The *lombi* species present an important content of phenolic compounds, *B. pilosa* showing the highest value (around 41 mg GAE/g dry matter), followed by *G. parviflora* and the mixture (around 20 mg GAE/g dry matter) and *A. hybridus* with around 12 mg GAE/g dry matter. These values are within the range presented by Catarino et al. (2019a) for several leafy vegetables from West Africa (from 13.1 mg GAE/g dry matter for *Hibiscus sabdariffa* to 40.3 mg GAE/g dry matter for *Sesamum radiatum*). Regarding the antioxidant activity, the *lombi* species used in the present study showed DPPH values from 32.3 to 234.6 µmol Trolox/g dry matter, lower than the values referred for leafy vegetables from West Africa, from 111.5 to 681.9 µmol Trolox/g dry matter (Catarino et al., 2019a). Anyway, the TPC and the antioxidant capacity values measured by DPPH, FRAP, and ABTS methods are an important indication of the potential health benefits that may arise from the regular consumption of *lombi*.

### Comparative Phytochemical Analyses

As demonstrated above, *B. pilosa* is the predominant component of the *lombi* mixtures sold at Lubango markets. This species attracted the attention of many leading researchers for its potential nutraceutical use in both functional and medicinal foods. Chiang et al. (2004) demonstrated that, adding to antioxidant properties, the extract of *B. pilosa* exhibited significant inhibitory effect on NO production (an inflammatory mediator) in macrophages. An essential oil extracted from *B. pilosa* var. *radiata* proved to have antibacterial and antifungal activities (Deba et al., 2008). A study reported the effect of *B. pilosa* leaf extract in uterine muscle in labor (Frida et al., 2008). Polyenes, flavonoids, phenylpropanoids, fatty acids, and phenolics are the main bioactive compounds of *B. pilosa* and they have been reported effective to treat different diseases, namely: tumors, diabetes, viral diseases, gastrointestinal diseases, hypertension, or cardiovascular diseases (Bartolome et al., 2013). Some harmful effects of this species have also been reported, such as phototoxicity and a possible carcinogenic effect of leaf consumption (e.g., Mvere, 2004). However, these effects were not mentioned in more recent studies (e.g., Xuan and Khanh, 2016; Kuo et al., 2020). An interesting finding about the phytochemical properties of the three analyzed species of *lombi* is that the most abundant species in the mixtures sold (*B. pilosa*) is the one with the highest contents of protein, P, Cu, B, and total phenolics. In addition, *B. pilosa* also presented a significantly lower concentration of some of the metallic contaminants (Pb and Cr).

### CONCLUSION

The wild leafy vegetables are important food resources for both rural and urban populations in many parts of the world. In southern Angola they are called *lombi* and are important components of peoples' diet, being collected and traded in the local markets, and thus contributing to the income of many families.

Our study demonstrated the socio-economic importance of *Amaranthus hybridus*, *Bidens pilosa*, and *Galinsoga parviflora*, which are abundant and grow spontaneously in southern Angola. As stated above, these species are sold in local markets, and

the species composition of the mixtures depends on the local availability and abundance along the year.

Although, they are good alternative sources of nutrients for both rural and urban populations, namely of protein, iron, magnesium or calcium, the contents of some heavy metals, particularly chromium, can be a matter of concern and deserves further analyses. Thus, more field surveys are needed in various areas of Angola, namely to identify the plants and their proportions used in the mixtures traded in the local markets, as well as to carry out more chemical analyses (e.g., functional, nutritional, and antinutritional properties) of individual species and mixtures, currently consumed in Angola.

## DATA AVAILABILITY STATEMENT

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

## AUTHOR CONTRIBUTIONS

RK: methodology, fieldwork, writing—original draft, and writing—review and editing. JS: methodology and laboratory analyses. MM: methodology, resources, supervision, and writing—review and editing. VA: methodology, laboratory

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