New Insights on the Use of Polyphenols as Natural Preservatives and Their Emerging Safety Concerns

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Polyphenols are natural high-valued secondary metabolites of plant that demonstrate strong antimicrobial potential as natural preservatives besides their well-established health promoting benefits. This review highlights the challenges, novel strategies to achieve industrial production of polyphenols and their safe use as potential alternative natural preservative. Since plant extraction presents considerable limitations of high cost due to excessive energy and solvent requirements, climate and long growth cycles, microbial biosynthesis using highly advanced omics techniques and metabolic engineering tools could provide superior alternative for commercial production of environmentally sustainable and cost-effective high-value metabolites in a short time. In spite of the many beneficial effects, some plant metabolites and polyphenol compounds at high dosage are found to be pro-oxidant or mutagenic with toxicity. Due to the controversial findings from sub-chronic and oral toxicity studies, more detailed safety and efficacy studies are needed to substantiate findings. Furthermore, extensive research efforts are required to ascertain optimal dosage for safe use in various foods to avoid potential harmful side effects.

Keywords: polyphenols, natural preservatives, microbial biosynthesis, high dose, safety

INTRODUCTION

Microbial contamination not only causes food loss and public health concerns but also affects the sensory characteristics and overall quality of food products. Spoilage and foodborne pathogenic microorganisms are one of the major causes of enormous economic losses despite several advances in food production technologies, distribution, hygiene standards, and consumer education. Food preservatives are defined as compounds that retard spoilage caused by microorganisms and other means such as oxidation. Several traditional food preservation technologies such as freezing, modified atmosphere packaging, thermal and non-thermal physical treatments (high hydrostatic pressure and pulsed electric fields) have been employed to control spoilage microorganisms and extend shelf life of products. More recently, synthetic chemical preservatives such as, nitrate, nitrite, citric acid, propionate, tartaric acid, sorbate, benzoate, sulfites, to mention a few, approved by regulatory agencies are widely used to control microbial growth (Russell, 1991; Manas and Pagán, 2005; Kuorwel et al., 2011; Silva and Lidon, 2016).

However, prolonged use of synthetic preservatives have been shown to pose severe health problems such as liver and kidney damage, gastrointestinal disorders, asthma, certain cancers, and many allergies (Varraso and Camargo, 2014; Etemadi et al., 2017).

Consumers are becoming increasingly conscious of their health and safety and the negative health impacts of chemically synthesized antimicrobials. Due to the increasing demand for minimally processed and healthier foods, the use of natural antimicrobials rather than synthetic preservatives for food preservation is highly sought after by both consumers and food manufacturers (Rico et al., 2007; Bouarab-Chibane et al., 2019a). Phenolic compounds are well documented for their antibacterial activities, besides their antioxidant, antidiabetic, anti-inflammatory, antihypertensive, anticancer, immune enhancing, and cognitive function that are beneficial for optimal health (Milner and Goldberg, 1994; Sun et al., 2017; Araya-Cloutier et al., 2018). A number of reviews on specific biological activities of polyphenols have been reported elsewhere and can be referred to for more details (Duthie and Brown, 1994; Pasinetti and Ho, 2010; Salehi et al., 2018).

Extracts of plant origin are rich sources of polyphenols. Numerous studies have elucidated the antimicrobial activities of various plant extracts, hence, their potential as target alternatives for synthetic preservatives. Owing to this growing interest, several governments and companies are investing heavily for the development of natural food preservatives (Carocho et al., 2014). Currently, commercial production of polyphenols is largely dependent on plant extraction and chemical synthesis (Figure 1). However, plant extraction has considerable limitations of high cost due to excessive energy and solvent requirements, climate and long growth cycles. Microbial biosynthesis using highly advanced omics techniques and system metabolic engineering tools could provide superior alternative for large scale production of environmentally sustainable and cost-effective high-value metabolites in a short time frame to address the increasing demand of the food and nutraceutical market (Schempp et al., 2017; Ong et al., 2018; Zhao and Li, 2018). In spite of the beneficial effects, some plant metabolites and polyphenol compounds at high dosage are found to be pro-oxidant or mutagenic with toxicity (Cory et al., 2018). Due to the controversial results from sub-chronic and oral toxicity studies, more detailed safety and efficacy studies are needed to substantiate findings. Moreover, extensive research efforts are required to ascertain optimal dosage for safe and beneficial use in various foods to avoid potential harmful side effects. This review highlights the current challenges, novel design and strategies to achieve commercial production of polyphenols using advanced systems metabolic engineering tools and their use as alternative natural food preservatives. Furthermore, the safety and potential health risks associated with high dosage polyphenol consumption in food applications are discussed.

CHEMISTRY AND CLASSIFICATION OF POLYPHENOLS

Plant-derived antimicrobials are promising natural preservatives considered safe, healthy and possess additional properties from their bioactivity and nutritional value. Traditionally, plant extracts have been consumed by mankind for centuries. Polyphenols are secondary plant metabolites derived from shikimate and or the polyketide pathway. They vary widely within different plants and play key roles in pigment formation, resisting environmental stresses including defense mechanisms against pathogens or ultraviolet radiation, and acting as chemical messengers. They consist of one or more aromatic rings with hydroxyl groups attached as shown in Figure 2 (Fantini et al., 2015; Lyu et al., 2019).

Polyphenols are highly diverse, numerous and widely distributed in plants. Their structure and content are greatly affected by environmental conditions and plant species. Over 10,000 phenolic compounds are presently identified, with flavonoids constituting the largest group. They are largely found in foods such as fruits, vegetables, whole grains, coffee, wine, tea, and chocolate. Polyphenols are generally categorized according to their source of origin, structural differences, and biological activity. Therefore, polyphenols can be grouped into phenolic acids, flavonoids, lignans, stilbenoids, coumarins, and tannin polymers (Table 1). These naturally occurring compounds exist as glycosides with various sugar molecules and their acylated forms attached at different positions in the carbon skeleton (Naczk and Shahidi, 2004; Tsao, 2010; Liu, 2013). Polyphenol classification and their sources are shown in Table 1.

ANTIMICROBIAL ACTIVITIES OF POLYPHENOLS

Globally, antibiotic resistance has been one of the serious health problems over the past few decades. Harvey et al. (2015) disclosed that more than 70% of pathogenic bacteria have shown resistance to antibiotic treatment. This has untold public health impact and together with other findings has necessitated the urgent search for novel antimicrobial compounds. Since time immemorial, plants have long been used as food and medicine for humans (Duthie and Brown, 1994). Numerous studies have demonstrated antimicrobial activities from several plants and their extracts (Savoia, 2012). Naturally occurring compounds including phenolic secondary metabolites (polyphenols), terpenoids, alkaloids, and peptides have been reported to exhibit antimicrobial properties (Cowan, 1999; Gibbons, 2008; Obied, 2013; Reichling, 2018). These plant metabolites have been found to elicit both direct and indirect inhibitory activities against efflux pump, biofilm formation, and or quorum sensing (Savoia, 2012).

Recently, (Bouarab-Chibane et al., 2019b) designed a quantitative model to establish the structure-activity relationship between polyphenols and pathogenic or food spoilage bacteria. These authors predicted the mechanisms of toxic action of 35 polyphenols (1g L^{-1}) against Gram-positive (*Staphylococcus aureus, Bacillus subtilis, and Listeria monocytogenes*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, and Salmonella enteritidis*) bacteria. Their findings revealed a strong interaction between bacterial cell surface and polyphenols in the exertion of antibacterial activity. This was mainly attributed to the lipophilicity, electronic and charge properties of the polyphenols. Furthermore, they also observed a strain and dose-dependent effect of polyphenols.

FIGURE 1 | Current techniques for the commercial production of polyphenols.
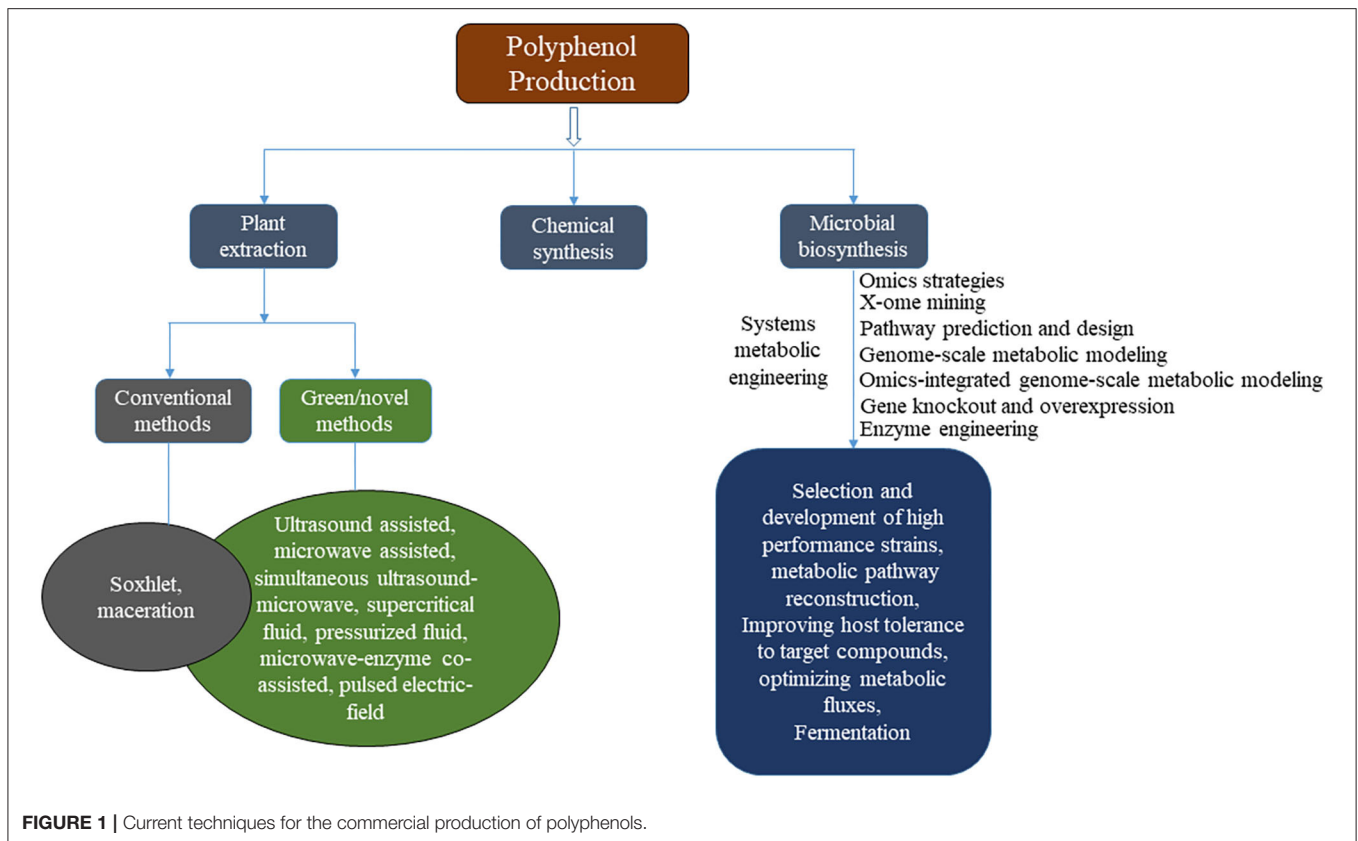
They found that *L. monocytogenes* was sensitive to polyphenols whereas *P. aeruginosa* was not. Strong antibacterial effect was demonstrated by 5,8-dihydroxy-1,4-naphthoquinone and butyl gallate against five out of the six bacterial strains. However, further investigations on surface properties of bacteria with other techniques besides adhesion of microorganisms to solvents are needed. Generally, extracts have shown stronger antimicrobial potency than individual polyphenols. In addition, the antimicrobial properties of plant extracts also depend on extraction solvent polarity, composition, concentration, and the type of pathogen used. Different research groups have reported the antimicrobial properties of olive leaf polyphenol extracts (Markin et al., 2003; Pereira et al., 2007; Sudjana et al., 2009; Lee and Lee, 2010; Karygianni et al., 2014; Lim et al., 2016). Wine polyphenols were found to exhibit antibacterial effect against pathogenic *E. coli* and *L. monocytogenes* in fish and could find application in the preservation of fish (Rodríguez-Vaquero et al., 2013). Recently, Aziz and Karboune (2018) extensively reviewed the application of natural antimicrobials from plant (phenolic extracts and essential oils), animal (peptides and lactoperoxidase system), and microbial origin (chitosan, bacteriocins, bacteriophages, fermented ingredients) for prolonging the shelf life of fruits, vegetables, and meat products. Flavonoid rich green tea and grape seed extracts containing flavanols such as epicatechin, catechin, epicatechin gallate, procyanidin oligomers, and epigallocatechin were reported to control the growth of pathogenic bacteria in meat products. Cooked beef samples treated with 1% grape seed

extracts (GSE) significantly reduced *E. coli* O157:H7 and *Salmonella typhimurium* counts and controlled the growth of *L. monocytogenes* and *Aeromonas hydrophila* (Ahn et al., 2007). With minimum inhibitory concentrations (MICs) below 10 μg phenol/mL and 15%, cranberry extract with proanthocyanidins as its main antimicrobial compound showed strong inhibitions against several pathogenic bacteria including *E. coli* O157:H7 EDL 933, *E. coli* ATCC 25922, *L. monocytogenes* HPB 2812, *P. aeruginosa* ATCC 15442, *Klebsiella pneumoniae, S. Typhimurium* SL1344, *Enterococcus faecium, Enterobacter aerogenes*, and *S. aureus* ATCC 29213 (Sagdica et al., 2006; Côté et al., 2011).

Akhtar et al. (2019) investigated the antimicrobial properties of phenolic extracts of different spices and herbs in minced beef during refrigeration storage (4°C). The methanolic extracts of ginger and coriander showed the highest total phenolic content (TPC) of 70.8 and 69.8 mg GAE/100 g, respectively. They found that ginger and coriander extracts at a concentration of 6% significantly inhibited the growth of *S. aureus* (0.24; 0.32 log10 CFU/g, repectively) and *E. coli* (0.23; 0.36 log10 CFU/g, respectively) compared to the antibiotic, gentamycin (0.60; 0.77 log10 CFU/g, respectively) in minced beef stored at 4°C for 9 days and could be developed as natural preservatives. Ghosia et al. (2019) illustrated the antioxidant and antibacterial properties of the leaves, roots and bark of *daphne mucronata* plant extracts. The antibacterial activity of methanolic extracts of roots, bark and leaves was stronger compared to other solvents. The methanolic, hexane, chloroform and ethyl acetate extracts of the roots against *Acinetobacter baumannii* was 86.95, 78.26, 60.86, and 69.56%,
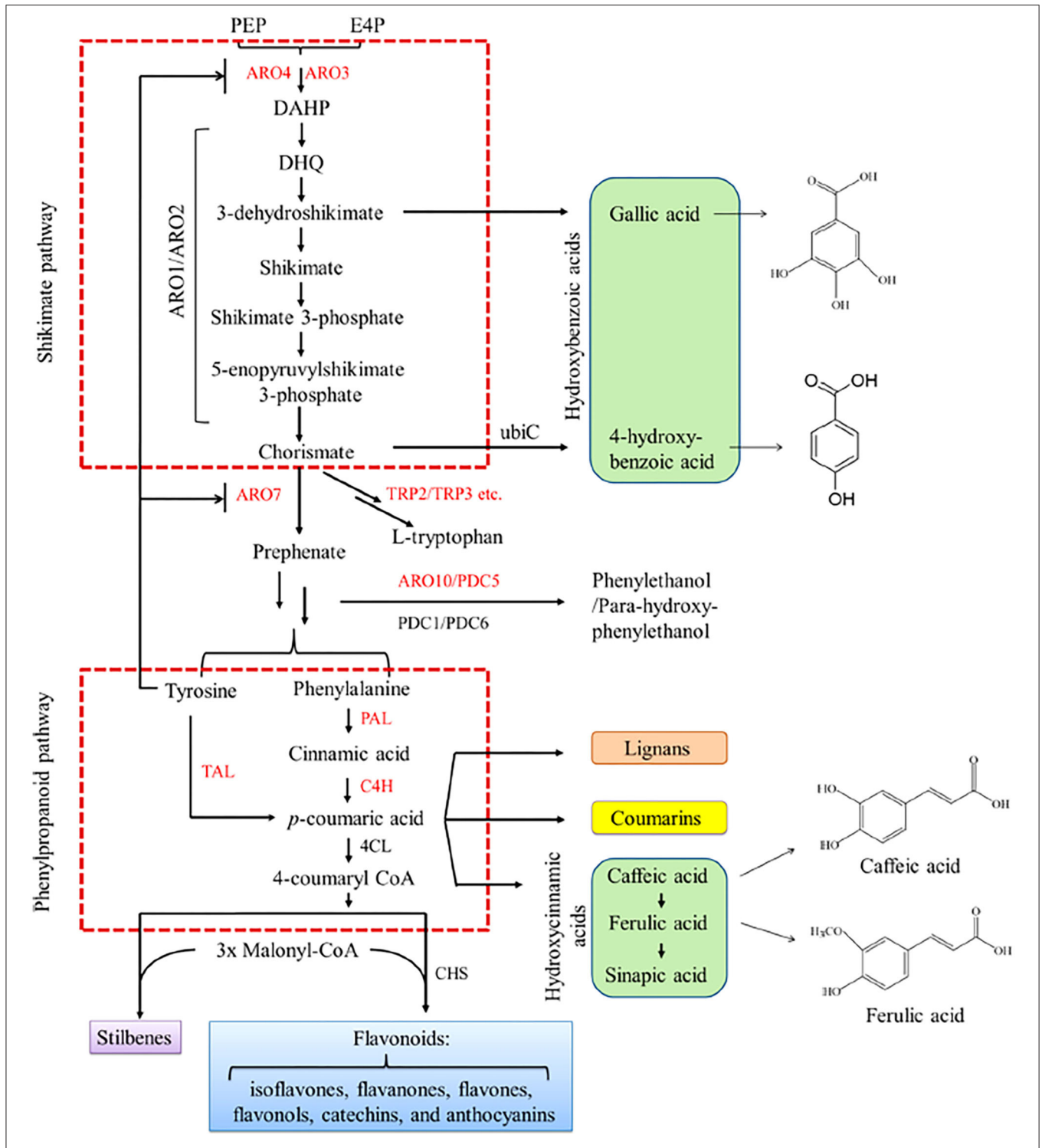
FIGURE 2 | Schematic representation of biosynthetic pathway of polyphenols. Green, pink, yellow, purple, and blue areas depicts phenolic acids, lignans, coumarins, stilbenes, and flavonoids, respectively [Adopted with permission from Lyu et al. (2019)]. Copyright 2019 American Chemical Society. Intermediates: PEP, phosphoenolpyruvate; E4P, erythrose4-phosphate; DAHP, 3-deoxy-D-arabino-heptulosonate-7-phosphate; DHQ, 3-dehydroquinate. Enzymes: ARO4/ARO3, DAHP synthase; ARO1, pentafunctional arom protein; ARO2, bifunctional chorismate synthase and flavin reductase; ARO7, chorismate mutase; TRP2, anthranilate synthase;TRP3, indole-3-glycerol-phosphate synthase; ARO10, phenylpyruvate decarboxylase; PDC5, minor isoform of pyruvate decarboxylase; TAL, tyrosine ammonia lyase; PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, p-coumaric acid; CoA ligase; CHS, chalcone synthase. Key enzymes are marked in red.

respectively. The hexane and chloroform root extracts did not show any activity against *E. coli*. However, methanolic and ethyl acetate root extracts showed 85.18 and 62.96% activity, respectively, against *E. coli*. Moreover, *S. aureus* was strongly inhibited by methanolic (84.61%), chloroform (61.53%) and ethyl acetate root extracts (69.23%). Good activity of methanolic leave extracts was exhibited against *A. bumanni* (78.26%), *E. coli* (77.78%) and *S. aureus* (73.07%). They also found moderate activity of methanolic bark extracts against *A. bumanni* (65.21%) and *E. coli* (62.96%). Furthermore, a good antioxidant activity with EC_{50} values ranging from 157.82 to 361.61 μg/ml was obtained for the extracts. It is worthy to note the antimicrobial activities of *Azadirachta indica* (*A. indica*) phenolic extracts evaluated by Ouerfelli et al. (2019) on shelf-life stability of raw beef patties during storage at 4°C for 11 days. *A. indica* leaf extracts was found to inhibit microbial growth and retard lipid oxidation of chilled beef patties. *A. indica* extracts showed no activity on *Listeria* and *B. cereus* strains. However, strong antimicrobial activity was observed against *E. coli* and *S. aureus* with an inhibition zone of 21 and 19 mm, respectively. Moderate activity was obtained against *Micrococcus luteus* and *Salmonella paratyphi* with inhibition zones of 12 and 10 mm, respectively. Beef samples treated with 0.7% (w/w) fresh powdered *A. indica* leaves retarded the growth of mesophilic bacteria ($<10^4$ CFU/g) compared to the control without treatment (4.2×10^4 CFU/g) after 11 days refrigeration storage (4°C). Findings from these studies can be used to assess the safety of these extracts in different food applications to ascertain their beneficial use and consumer safety.

POLYPHENOL EXTRACTION FROM PLANT SOURCES

As mentioned earlier, plants represent an abundant resource for polyphenol-rich metabolites synthesized during their growth and under stressful environmental conditions. Literature is replete with numerous studies on the extraction of polyphenols from diverse plant materials. Generally, these phytochemicals are extracted from either fresh or dried plant sources prior to their utilization as food ingredients or in nutraceutical, pharmaceutical, and cosmetic products. Because of the diverse composition and structural differences of polyphenols in various plant materials and the varying nature of plant matrix, the establishment of a standard protocol for extracting all target metabolites from plant sources remains a huge challenge. The type of solvent, plant to solvent ratio, extraction time, temperature and agitation rate are among several parameters or conditions that affect the efficiency of the extraction process (Jovanović et al., 2017; Asofiei et al., 2019). Recent years has seen the development of more efficient extraction techniques, owing to the low efficiency of conventional methods such as maceration, Soxhlet extraction and percolation with long extraction times, large solvent volume, high temperatures and polyphenol degradation. These extraction methods include ultrasound and microwave assisted, simultaneous ultrasound-microwave assisted, supercritical and pressurized fluid, microwave and enzyme co-assisted, and pulsed electric-field. Compared to
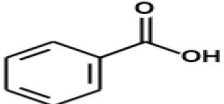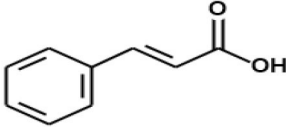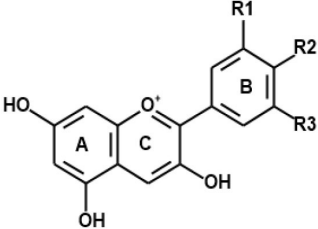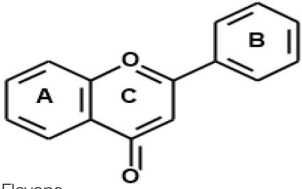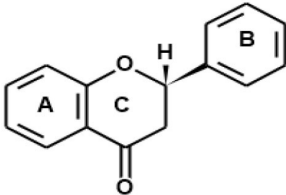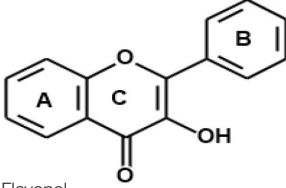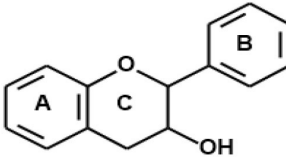
the conventional methods, these novel methods have the advantages of a shorter extraction time, reduced solvent usage, reduced thermolabile polyphenolic compound degradation, less laborious, environmentally friendly, and reduced energy and operational cost (Ameer et al., 2017; Jovanović et al., 2017; Asofiei et al., 2019). Asofiei et al. (2019) developed an efficient semi-continuous microwave assisted extraction of polyphenols from sea buckthorn leaves using a modified standard reactor. The total phenolic content (TPC) increased from 144.66 to 176.66 mg GAE/g using microwave assisted extraction at a solvent flow rate of 6 ml/min. TPC was found to increase with increasing stirring rate using larger magnetic stirrer. In addition, pre-heating of solvent (50% ethanol) at 60°C increased polyphenol extraction efficiency by 80%. Comparatively, the antioxidant capacity of sea buckthorn leaves from microwave assisted extracts (405.88 mg TE/g) was higher than the conventional extracts (354.56 mg TE/g). Furthermore, the composition of phytochemicals was not affected by the optimized semi-continuous microwave assisted process. Compared with the conventional extraction, the developed semi-continuous microwave assisted extraction resulted in a significantly lower energy consumption. However, the optimization of extraction kinetic parameters or conditions, taking into consideration plant matrices and the composition and structure of compounds of interest are some bottlenecks for industrial scale up. In addition to the aforementioned, the impact of climate change and long growth cycles poses significant limitations to polyphenol extraction from plants for commercialization.

BIOSYNTHESIS AND METABOLIC ENGINEERING FOR PRODUCTION OF POLYPHENOLS

Literature on the biosynthesis of polyphenols has been thoroughly discussed by other authors (Knaggs, 2001; Tsao, 2010; Lyu et al., 2019) and because of that only a summary will be given in this work. In plants and microbes, the shikimate and phenylpropanoid metabolic pathways represent the main routes for polyphenol biosynthesis (**Figure 2**). The central carbon metabolism is linked to aromatic amino acids biosynthesis via the shikimate pathway. Shikimate intermediates such as chorismate are key precursors for the biosynthesis of various aromatic compounds including phenylalanine, tryptophan, tyrosine and polyphenolic compounds (Herrmann, 1995). Intermediates from the phenylpropanoid pathway such as 4-coumaroyl CoA serves as good precursors for the biosynthesis of complex polyphenols such as flavonoids and stilbenes (Lyu et al., 2019). Hence, gaining a complete understanding of polyphenol biosynthesis pathways and developing efficient strategies for their production is extremely important for the *de novo* synthesis of diverse high value metabolites for industrial applications.
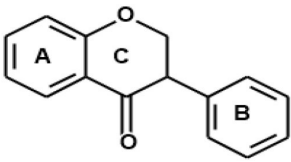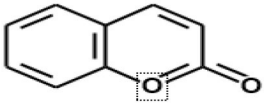
Metabolic engineering is a technique used to manipulate and optimize genetic and regulatory processes in living organisms to increase the production of target metabolites. Metabolically engineered microbes have gained increasing attention in pharmaceutical, nutraceutical and food applications. In recent years, the development of high-performance strains

TABLE 1 | Classification of polyphenols and their sources [adapted and modified from Papuc et al. (2017)].

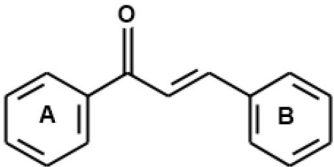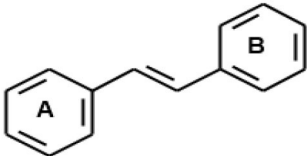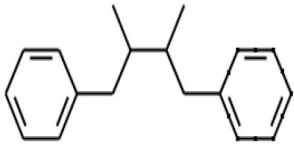| Class name and backbone structure	Examples	Sources	References
1. Phenolic acids			
Benzoic acid	Gallic acid Syringic acid Vanillic acid Protocatechuic acid	Fruits, vegetables and cereals	Rice-Evans et al., 1996; Manach et al., 2004
Cinnamic acid	*p*-coumaric acid Caffeic acid Ferulic acid Chlorogenic acid Sinapic acid	Fruits, vegetables, cereals, coffee	Manach et al., 2004; Ofosu et al., 2020
2. Flavonoids			
Anthocyanin	Cyanidin Pelargonidin Petunidin Malvidin Delphinidin Peonidin	Fruits, vegetables, cereals, red and blue flower petals	Mazza and Francis, 1995; Bravo, 1998; Pietta, 2000
Flavone	Luteolin Apigenin Chrysin Baicalein	Alfalfa Indian trumpet flower	Kawaii et al., 1999; Chen et al., 2003; El-Shafey and Abdelgawad, 2012; Mishima et al., 2015; Kim et al., 2016
Flavanone	Hesperetin Naringenin	Citrus and grapefruit peels	Lien et al., 2008; Mullen et al., 2008; Tomas-Navarro et al., 2014
Flavonol	Quercetin Kaempferol Galangin Fisetin Myricetin Morin	Propolis, honey, fruits, vegetables, cereals	Chan et al., 2007; Bertelli et al., 2012; Kurtagić et al., 2013
Flavan-3-ol	(+)-Catechin (−)-Epicatechin (−)-Epigallocatechin (−)-Epicatechin-3-gallate (−)-Epigallogatechin-3-gallate	Green tea, black tea, cocoa, cereals	Zaveri, 2006; Subhashini et al., 2010; Gadkari and Balaraman, 2015

(Continued)

TABLE 1 | Continued

Class name and backbone structure	Examples	Sources	References
Isoflavone	Genistein Genistin Daidzein Daidzin Biochanin A Formononetin	Legumes, red clovers	Wang and Murphy, 1994; Mazur et al., 1998; Dixon and Ferreira, 2002; Tsao et al., 2003; Kim et al., 2012; Kuligowski et al., 2017
Chalcone	Isoliquiritigenin Flavokawain A Flavokawain B Flavokawain C Gymnogrammene	Apples, flowers, hop, beer	Tsao et al., 2003; Tsao and McCallum, 2009; Aksöz and Ertan, 2012; Suwito et al., 2014
3. Stilbenoids	*trans*-Resveratrol *trans*-Piceatannol *trans*-Piceid *trans*-Pterostilbene Cajanotone Cajanamide	Red grapes, wine, blueberries, peanuts, dark chocolate, *Cajanus cajan*, sorghum	Sanders et al., 2000; Burns et al., 2002; Lyons et al., 2003; Counet et al., 2006; BröHan et al., 2011; Zhang et al., 2012
4. Lignan	Sesamin Matairesinol Pinoresinol Medioresinol	Flax seed, sesame seed, fruits, vegetables, legumes and many grains	Mazur et al., 1998; Milder et al., 2005; Peñalvo et al., 2005; Smeds et al., 2007, 2012
5. Coumarins	Ostruthin Ammoresinol Anthogenol Felamidin Agasyllin	Fruits, flowers, seeds	Basile et al., 2009; Rosselli et al., 2009; Chakthong et al., 2012

has seen tremendous acceleration with the emergence of systems metabolic engineering. This interdisciplinary field employs new tools and strategies from systems biology, synthetic biology and evolutionary engineering with conventional metabolic engineering (Choi et al., 2019). Recent reviews have focused on sustainable biosynthesis of polyphenols in bioengineered yeast; advances in tools and strategies for systems metabolic engineering with significant potential for industrial applications (Choi et al., 2019; Lyu et al., 2019).

Several microrganisms have been engineered to synthesize various polyphenols. The advantages of using bioengineered microorganisms over enzymatic and chemical syntheses for production of natural products include but are not limited to inexpensive cofactor requirement, and specific regioselectivity and stereoselectivity. *E. coli, Saccharomyces cerevisiae* and *Corynebacterium glutamicum* with GRAS (generally regarded as safe) status have been widely used as microbial cell factories to produce diverse high-value compounds. Nevertheless, industrial application of polyphenols produced using metabolic engineered microbes still faces quite a number of challenges. The limitation of low productivity or yield, high toxicity to cell growth or low host tolerance to target compounds and expensive feedstock supply poses significant difficulty for their commercial production (Lyu et al., 2019). The development of highly advanced omics techniques and systems metabolic engineering tools is expected to unveil and provide a complete understanding of polyphenol biosynthesis pathways needed to circumvent microbial biosynthesis productivity challenges. Choi et al.

(2019) provides a thorough description of the selection and improvement of high performance strains using novel design and strategies in genetic and genomic engineering, *in silico* metabolic simulation and high-throughput screening to reconstruct metabolic pathways, increase host tolerance and optimize metabolic fluxes for enhanced target compound production. Chung et al. (2017) used plant aromatic aldehyde synthase (AAS) and uridine diphosphate-dependent glycosyltransferases (UGTs) to synthesize polyphenols in *E. coli*. Using engineered *E. coli* harboring *AAS* and 12 UGTs, tyrosol, hydroxytyrosol and salidroside were synthesized at 531, 208, and 288 mg/L, respectively. When glucose was used as carbon source, naringenin was produced in *E. coli* at a titer of 29 mg/L (Santos et al., 2011). Other studies have reported the production of flavonoids; kaempferol, quercetin and fisetin using *p*-coumaric acid and L-tyrosine as precursor in *E. coli*, respectively (Leonard et al., 2006; Stahlhut et al., 2015). Rodriguez et al. (2017) reported the synthesis of the flavonoids; naringenin, liquiritigenin, kaempferol, resokaempferol, quercetin, and fisetin in an engineered *S. cerevisiae* directly from glucose. Generally, extracts have shown stronger antimicrobial potency than individual polyphenols. Compared to pure flavonoids and phenolic acids, phenolic compounds (naringenin, phloretic acid, phenylacetaldehyde, and homogentisic acid) from engineered *S. cerevisiae* strain N2 was found to exert more potent antibacterial activity against pathogenic *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 (Ng et al., 2019). N2 extracts of concentrations up to 400 μg showed inhibition zones of 4 and 8.33 mm against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213, respectively. On the contrary, pure naringenin did not show any inhibition against either strains, while 6-prenylnaringenin and 8-prenylnaringenin demonstrated inhibition zones of 4 and 7.67 mm against *S. aureus*, respectively. It is therefore imperative to show the safety of these extracts in human trials to prove their viability as food preservatives. Using engineered *C. glutamicum,* Kallscheuer et al. (2016) produced resveratrol at titers of 5 mg/L independent from endogenous aromatic amino acid pathway. Using a different strategy which avoided ammonia lyase activity, a rate limiting step in microbial synthesis, the authors reversed the β-oxidative phenylpropanoid degradation pathway and could produce polyphenols from cheap benzoic acids. A more recent review by Kogure and Inui (2018) summarizes the potential of metabolically engineered *C. glutamicum* for commercial production of several valuable aromatic and natural products including plant polyphenols at high titer from renewable feedstocks.

POLYPHENOL SAFETY EVALUATION AND FUTURE PERSPECTIVES

It is a common believe that compounds from natural sources are safe, even at high doses. Thus, the limited evidence in literature regarding toxicity of natural plant extracts. Moreover, the safety aspects on the long term consumption of high doses of polyphenols either as dietary supplements or food additives is not clearly known in humans and needs to be thoroughly investigated. Although numerous animal studies have confirmed the beneficial role of polyphenols, findings from several sub-chronic and oral toxicity studies, still remain controversial (**Table 2**). Therefore, assessing the safe levels of polyphenols and other natural compounds for use as food preservatives is crucial for determining potential cytotoxicity. Findings from Yamakoshi et al. (2002) showed a lack acute and subchronic toxicity of proanthocyanin–rich grape seed extracts adminsistered orally in rats at high doses of 2 and 4 g/kg. They found the lethal dose to be higher than 4 g/kg in the acute study on the 14th day of clinical observation. However, the no-observed-adverse effect level (NOAEL) during 90 days subchronic toxicity study was found to be 2 g/kg of GSE (about 240 times the estimated daily proanthocyanidin intake by humans). Grape seed polyphenolic extract (GSPE) has shown high tolerability and found to be safe in animal models fed with chow and extract in a 90 days subchronic toxicity study. No detectable adverse effects were observed after treatment with doses from 200 to 2150 mg/kg/day (Bentivegna and Whitney, 2002; Wang et al., 2008). Moreover, treatment of pre-hypertensive people with GSPE 300 mg/day showed a significant reduction in blood pressure with no observed adverse effects (Sivaprakasapillai et al., 2009). This evidence suggests the safety and tolerability of GSPE for treatment in humans. In addition, Charradi et al. (2018) have reported the safety of high repeated dosing of GSPE (8 and 16 g/kg bw) fed to healthy rats for 2 months. Their findings showed no toxicity and support the potential applications in different foods for biotic or abiotic stress-induced multi-organ dysfunction. European Food Safety Authority (EFSA) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has established the safety and efficacy of dry grape extract used as feed flavoring for all animals and categories except dogs at a maximum level of 100 mg/kg complete feed. The panel emphasized no safety concerns for consumers at this level in feeds (EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016). Despite these results, more detailed safety and efficacy human studies are needed to substantiate findings for other plant extracts.

On the contrary, certain studies have delineated the pro-oxidant and adverse effect of plant metabolites and polyphenol compounds (Galati et al., 2002; Shao et al., 2003). Thus, it is imperative to mention that natural compounds may not be safe as previously thought at high concentrations and cause deleterious effects to humans (Li et al., 2008). Higher doses of GSPE (100 or 500 μg/mL) showed pro-oxidant effects and toxicity to cells (Shao et al., 2003). According to a recent systematic review on toxicological studies on green tea extracts (rich in catechin phenolic profiles) by Oketch-Rabah et al. (2020), adverse effects of hepatotoxicity was associated with (−)-epigallocatechin-3-gallate (EGCG) consumption from 140 mg to 1,000 mg/day. The authors also concluded that individual genetic susceptibility and underlying liver health could contribute to these effects and that product label should include caution of usage.

Therefore, extensive research efforts in humans are required to ascertain optimal dosage for safe use to avoid potential harmful side effects. Likewise, recent advances in omics tools and strategies, together with more carefully designed animal

TABLE 2 | *In-vivo* and *in-vitro* studies on the safety and toxicity potential of polyphenol consumption.

Plant source	Major polyphenol	Duration of intervention/Cell lines	Purpose of the study	Clinical outcomes	References
Grape seed extracts (GSE)	Proanthocyanidin	90 days	Examined GSE for acute and subchronic oral toxicity in Fischer 344 rats and their mutagenicity potential.	No evidence of mutagenicity and acute oral toxicity at doses of 2 and 4 g/kg. 2% (w/w) GSE was the NOAEL in subchronic toxicity study. Lack of toxicity of GSE.	Yamakoshi et al., 2002
Unripe Apple polyphenol extract	Procyanidins, epicatechin, catechin, chlorogenic acid, phloridzin	90 days	Evaluated the mutagenicity, genotoxicity, the acute oral and subchronic toxicity of Applephenon in Sprague-Dawley rats.	Lethal dose of >2 g/kg in acute oral toxicity in both male and female rats. No abnormal hematological, clinical, histopathological or urinary effects at a dose of 2 g/kg in subchronic oral toxicity study.	Shoji et al., 2004
Capparis spinosa L. leave extracts	Gallic acid, caffeic acid, coumaric acid, ferulic acid, chlorogenic acid, rutin, quercetin	3 days	Investigated the oral acute toxicity of polyphenolic extract of *Capparis spinosa* L. leaves in female rats and *in vitro* antibacterial effect.	No adverse effect or mortality at doses of 100 mg/kg. The extract showed antibacterial activity against *S. aureus* (12 mm), *B. subtilus* (11 mm), *E. coli* (12 mm), *P. aeruginosa* (12 mm).	Oudah et al., 2019
Seventeen freshly prepared plant extracts, two commercial plant extracts, naringin, kaempferol and resveratrol.	Not provided	HepG2, Caco-2, A549, HMEC-1, and 3T3 cell lines.	Screened the toxicity potential of 22 polyphenol-rich compounds on mitochondrial membrane potential, cell membrane integrity and nuclear size using high content analysis.	Buckthorn bark, walnut husk and hollyhock extracts showed high cytotoxicity to mitochondrion and cell membrane but not the nucleus. On the contrary, spent hop and kale leaf extracts showed low cytotoxicity to mitochondrial membrane, cell membrane integrity and nuclear area. Kaempferol exerted strong toxicity on mitochondrial and nuclear compartments whiles naringin showed least cytotoxicity.	Boncler et al., 2017
Green tea	Epigallocatechin gallate (EGCG)	30 days	Examined the safety and pharmacokinetics of chronic green tea polyphenol or polyphenon E consumption in healthy men and women with Fitzpatric skin type II or III.	Mild effects of excess gas, stomach upsets, heartburn, abdominal pain, headache, dizziness and muscle pain at doses of 800 mg EGCG once/day or 400 mg EGCG twice/day in treated group. Daily dose of 800 mg EGCG was safe and well-tolerated and resulted in >60% increase in systemic EGCG but did not provide protection against UV-induced erythema.	Chow et al., 2003
Green tea	Epigallocatechin-3- gallate (EGCG), propyl gallate, epicatechin-3-gallate, epigallocatechin, epicatechin, gallic acid	1 day/rats hepatocytes	Examined the cytotoxicities of major tea phenolics on isolated rat hepatocytes and its hepatotoxicity in mice administered intraperitoneally.	Tea phenolics showed cytotoxicity on hepatocytes. Epigallocatechin-3- gallate exerted strong toxicity on mitochondrial membrane collapse and inducing ROS formation. Tea phenolics at doses of 50–800 mg/kg caused liver injury in mice after 24 h.	Galati et al., 2006
Green tea extract	Epigallocatechin gallate	13 weeks	Evaluated the dermal, acute and short-term safety studies of EGCG in rats and dogs.	Lethal dose of 2 g/kg, no oral toxicity at a dose of 0.2 g/kg in rats. The NOAEL was 0.5 g/kg/day for subchronic toxicity study.	Isbrucker et al., 2006
Herbal mixture	Gallic acid, Cinnamic acid, Coumarin, Benzoic acid, Paoniflorin, Albiflorin, Amygdalin, Oxypaeoniflorin	13 weeks	Assessed the safety of oral administration of traditional herbal formula of *Gyejibokryeong-hwan* in male and female Sprague-Dawley (SD) rats.	At a dose of 5 g/kg/day, ulcers and dilated stomach glands were observed in males, whiles squamous cell hyperplasia and epithelial atrophy in stomach were observed in both male and female rats. Hematological examinations showed a significant increase in white blood cells, neutrophils and fibrinogen at 5 g/kg/day. The NOAEL was 2 g/kg/day.	Jin et al., 2018
Grape seed and skin extracts	Proanthocyanidin	3 months	Investigated the subchronic oral toxicity of grape seed extracts (GSE) and grape skin extracts GSKE) in SD rats.	No significant histological changes were observed in all tissues. The NOAEL for male rats was 1.78 g/kg/day GSE or GSKE and 2.15 g/kg/day GSE or GSKE in female rats.	Bentivegna and Whitney, 2002

(Continued)

TABLE 2 | Continued

Plant source	Major polyphenol	Duration of intervention/Cell lines	Purpose of the study	Clinical outcomes	References
Grape seed powder (GSP)	Procyanidins, catechin, epicatechin	2 months	Evaluated the antioxidant, anti-inflammatory effects and the subchronic toxicity of GSP administered orally in Wistar rats.	No adverse effects at the high dose of 16 g/kg. GSP reduced lipoperoxidation, increased antioxidant enzyme activities (CAT, GPx and SOD), reduced plasma IL17 A and CRP, increased IL10 and adiponectin, improved heart and renal microcirculation.	Charradi et al., 2018
Morus nigra L. leaves	Quercetin, caffeic acid	28 days	Examined the phytochemical composition of ethanolic extracts of *Morus nigra* L. leaves, their oral acute and subacute toxicities in male and female Wistar rats.	Lethal dose of 2 g/kg was observed in renal and hepatic organs in acute studies. The NOAEL in male rats was 0.75 g and 1 g/kg and 1 g/kg in female rats.	Figueredo et al., 2018
Grape seed extract	Proanthocyanidin	Chick cardiomyo-cytes.	Investigated the effects of grape seed proanthocyanidin extract (GSPE) on ROS generation, cell survival, LDH and caspase-3 activity using chick cardiomyocytes.	Higher doses (100 or 500 μg/mL) of GSPE increased ROS generation, LDH release and caused cell death. At 500 μg/mL, caspase-3 activity was significantly increased.	Shao et al., 2003
Vernonia mespilifolia Less.	Not provided	28 days	Evaluated the acute and subacute toxicity of aqueous extract of *Vernonia mespilifolia* Less in male and female Wistar rats.	Lethality dose was > 5 g/kg in acute toxicity (single dose) study. No evidence of heart, hepatic and renal toxicity at doses up to 0.6 g/kg in subacute study.	Unuofin et al., 2018
Olea europaea L. (olive tree)	Oleuropein, hydroxytyrosol, luteolin-7-glucoside, apigenin-7-glucoside, verbascoside	90 days	Investigated the genotoxicity and repeated-dose oral toxicity of water-soluble extract of olive tree leaves in male and female Wistar rats.	No evidence of mutagenicity and genotoxicity in mice micronucleus up to doses of 2 g/kg/day. No toxic effects or mortality in both male and female rats at high dose of 1 g/kg/day in subchronic study.	Clewell et al., 2016
Olea europaea L.	Oleuropein, Verbascoside, hydroxytyrosol, Rutin, Oleanolic acid	28 days	Investigated the acute and subacute oral toxicities of olive leaves ethanolic extracts in male and female Wistar rats.	No adverse effects or mortality was observed at a single dose of 2 g/kg. Subacute repeated doses up to 0.4 g/kg showed no toxicities in both male and female rats.	Guex et al., 2018
Olea europaea L.	Oleuropein, Verbascoside, hydroxytyrosol, Rutin, Oleanolic acid	6 weeks	Evaluated the effect of olive leaf extracts (OLE) on liver and kidney toxicity in Wistar albino rats.	Dosage of 0.9% OLE showed hepatocellular and renal abnormalities.	Omer et al., 2012
Olea europaea L.	Oleuropein, Verbascoside, hydroxytyrosol, Rutin, Ligstroside	5 weeks	Assessed the effect of OLE administered orally on busulfan (BU) induced damages in rat testes and the safety profile.	OLE at doses of 0.25, 0.5 and 0.75 g/kg repaired defects in rats testes. 0.25 and 0.5 g/kg OLE significantly reduced apoptotic spermatogonia cells. However, OLE at a dose of 0.75 g/kg increased markers of liver damages (ALP and AST).	Hakemi et al., 2019
Campomanesia guazumifolia (Cambess.) O. Berg.	quercetin pentose, quercetin deoxyhexoside, myricetin deoxy-hexoside, quinic acid	28 days	Evaluated the toxicity and anti-inflammatory activities of leave extracts of *C. guazumifolia* in female mice.	Lethal dose of > 5 g/kg in acute oral toxicity. Doses of up to 1 g/kg did not show any histological and hematological toxicity in subacute study. Doses of 0.3 g and 0.7 g/kg showed anti-inflammatory activity by reducing mechanical hyperalgesia, leukocyte migration and extravasation protein in pleural cavity.	Catelan et al., 2018

LDH, Lactate dehydrogenase; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase; NOAEL, No Observed Adverse Effect Level; IL17A, interleukin-17A; IL10, Interleukin-10; ROS, Reactive oxygen species; AST, Aspartate aminotransferase; ALP, alkaline phosphatase.

studies will contribute immensely to provide clearer insight into emerging safety concerns. Further works on understanding structure-activity relationship of natural antimicrobials and their mechanisms of action may help facilitate their use in different food systems, thus mitigating antibiotic resistance. Finally, in food applications regulatory authorities should assess and ensure acceptable levels for long term use to guarantee consumer safety.

CONCLUSION

Polyphenols are natural high-valued secondary metabolites with well-established benefits in human health. This had led to an increased intake of dietary polyphenol supplements. Moreover, due to the surge in demand for minimally processed and healthier foods, polyphenols have gained tremendous interest as promising alternative natural antimicrobial preservatives for inactivating spoilage and pathogenic microorganisms and to enhance microbial safety. Therefore, gaining a complete understanding of polyphenol biosynthesis pathways using highly advanced omics techniques and systems metabolic engineering tools could help circumvent microbial biosynthesis productivity challenges and provide superior alternative to plant

extraction and chemical synthesis for large scale production of environmentally sustainable and cost-effective high-value metabolites in a short time frame to address the increasing demand of the food and nutraceutical market. Thus, phenolic compounds producing GRAS microbes could be promising source as natural food preservatives. Hence, direct or indirect incorporation of these antimicrobial extracts into foods or their packaging materials should be done with caution to ensure consumer safety since safe levels are not fully known. However, the controversial findings from sub-chronic and oral toxicity animal studies require that more detailed safety and efficacy human studies be conducted. Furthermore, extensive research efforts are required to ascertain optimal dosage for safe use in various foods that are beneficial to avoid potential harmful side effects.

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