Antioxidant and Antimicrobial Potential of Polyphenols from Foods

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Abstract Change in the lifestyle is causing the overproduction of reactive oxygen species (ROS), free radicals, and decreasing the physiological antioxidant capacity. ROS can also cause protein and lipid oxidation, nucleic acid mutation, and further responsible for the development of various diseases including cancer, cardiovascular diseases, cataract, ageing, etc. Antioxidants can prevent the oxidation of biomolecules. Antioxidants present in food are now being preferred by consumers instead of synthetic antioxidants due to their non-toxic and non-carcinogenic effects. Antioxidants present in leafy vegetables, fruits, herbs, spices, seeds, alcoholic, and non-alcoholic beverages have the ability to reduce the damage caused by ROS. Polyphenols have redox potential high enough to scavenge or terminate ROS and also provide the environment favourable for inhibition of bacterial growth. Polyphenols also have metal ion chelating property which causes the deficiency of essential metal ions in the growth medium and ultimately responsible for antimicrobial effect. Binding of the polyphenols to the thiol groups at the active site of various microbial enzymes makes them inactive, thus inhibiting the growth of microbes. Polyphenols particularly phenolic acids and flavonoids have great potential as food additives with pharmaceutical, nutraceutical, and food preservative properties. The aim of this chapter is to present some valuable natural sources of polyphenols, structural characteristics, main classes of polyphenolic compounds, and extraction of polyphenols and also provide information on the most recent developments in the chemical investigation of polyphenols, emphasising their antioxidant and antimicrobial potential.

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1 Introduction

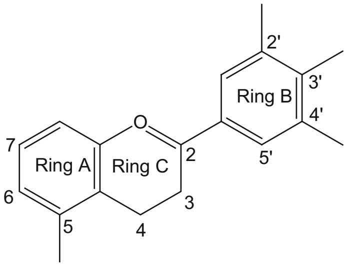
Phenolic compounds are secondary metabolites of plants, considered as important determinants in the nutritional and sensory quality of fruits, vegetables, other plant foods, and food preparations (Lapornik et al. 2005). These compounds possess an aromatic ring with one or more hydroxyl groups. The structures of phenolics from plants may range from a simple phenolic molecule to complex high-molecular mass polymer. As a large, most widely occurring group of bioactive phytochemicals, they have diverse physiological and biological functions. Phenolics may act as phytoalexins (Popa et al. 2008), antifeedants, attractants for pollinators, contributors to plant pigmentation, antioxidants, and protective agents against UV light (Naczk and Shahidi 2006). These bioactive properties made these compounds play an important role in plant growth and reproduction by providing an efficient protection against pathogens and predators (Popa et al. 2002). Phenolic compounds from plants have been reported to have excellent properties as food preservatives (Valenzuela et al. 1992). These compounds are reported to play an important role in the prevention and protection against a number of pathological disturbances, such as atherosclerosis, brain dysfunction, diabetes, and cancer (Gordon 1996). Phenolics from plants also contribute to the colour and sensory characteristics of fruits and vegetables (Alasalvar et al. 2001). These properties make them suitable for various industrial applications such as natural colourants and preservatives for foods or in the production of paints, paper, and cosmetics.

2 Structural Characteristics of Polyphenolic Compounds

Phenolic compounds include not only the polyphenols but also molecules with one phenol ring, e.g. phenolic acids and phenolic alcohols. Polyphenols are divided into several classes according to the number of phenol rings and based on the structural elements that bind these rings to one another. The main groups of polyphenols are flavonoids, phenolic acids, tannins, stilbenes, and lignans.

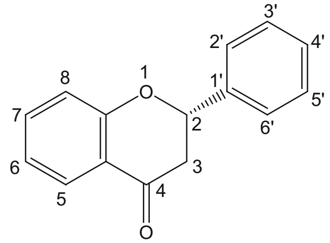
2.1 Flavonoids

Flavonoids are low-molecular-weight compounds, consisting of 15 carbon atoms, arranged in a C6–C3–C6 configuration. Essentially, the structure consists of two aromatic rings, A and B, joined by a three-carbon bridge, usually in the form of a heterocyclic ring, C. The aromatic ring A is derived from the acetate/malonate pathway, while ring B is derived from phenylalanine through the shikimate pathway (Merken and Beecher 2000).



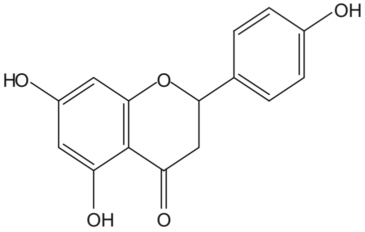
Variations in the substitution patterns of ring C result in the major flavonoid classes, i.e. flavonols, flavones, flavanones, flavanols (or catechins), isoflavones, flavanonols, and anthocyanidins, of which flavones and flavonols are the most widely occurring. Structural substitutions to rings A and B give rise to different compounds within each class of flavonoids. These substitutions may include oxygenation, alkylation, glycosylation, acylation, and sulphonation (Balasundram et al. 2006).

Flavanones are the flavonoids having a saturated three-carbon chain and an oxygen atom attached to C_4. They are generally glycosylated by a monosaccharide/ disaccharide at C_7. Flavanones are present in high concentrations in citrus fruits, tomatoes, and some aromatic plants such as mint. The main aglycone in grapefruit is naringenin, a bitter tasting antioxidant, in oranges is hesperetin, and in lemons is eriodicitrin. Flavanones are compounds that give many plants colour, as well as affect their taste. Various foods and juices, as well as bee pollen, include such materials; in general, these can aid in the body's response to viruses, allergens, and even carcinogenic substances.



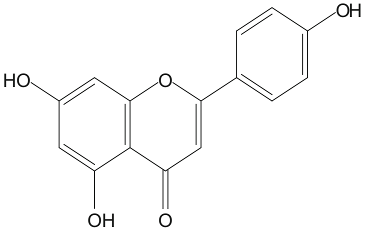
Isoflavones have hydroxyl groups in the C_7 and C_4 positions like estradiol molecule. They are phytochemicals that are found in many plants and plant-derived foods in both native ('aglycone') and acetyl- or malonyl-β-glucoside forms. Some physiological effects are attributed to their structural similarities to β-estradiols, and they are occasionally referred to as 'phytoestrogens' (Klejdus et al. 2007). With important health effects attributed to them, it has been suggested that they should be used for the prevention or cure of prevalent diseases such as atherosclerosis or cancer.

Flavanone



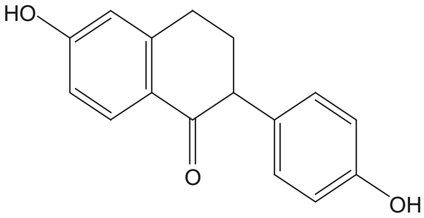
Naringenin

Flavone



Apigenin

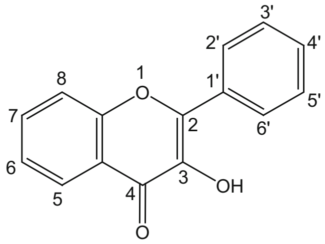
Isoflavones



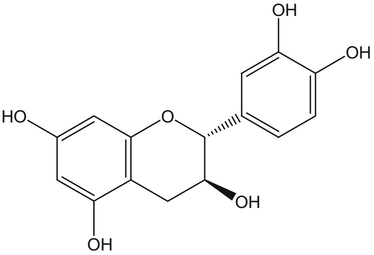
Daidzein

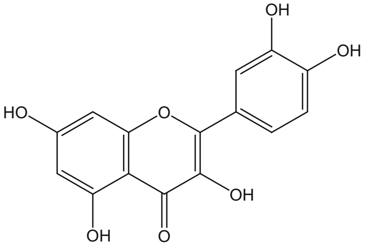
Flavonols are a class of flavonoids that have the 3-hydroxyflavone backbone. Their diversity stems from the different positions of the phenolic –OH groups. Flavonols are a class of flavonoids commonly found in many fruits and vegetables, their content varying widely, depending on environmental factors such as growing conditions, climate, storage, and cooking conditions (Caridi et al. 2007). The phenomenon of dual fluorescence (due to excited state intramolecular proton transfer or ESIPT) is induced by tautomerism of flavonols (and glucosides) and could contribute to plant UV protection and flower colour.

Flavonol

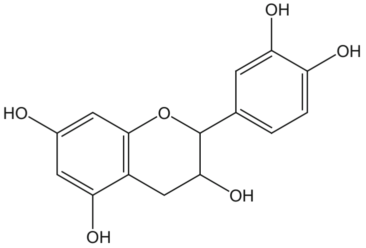


Flavanol





Quercetin



Catechin

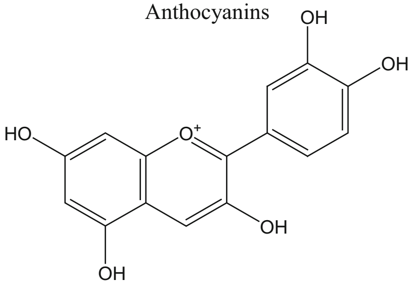
Anthocyanidins, the basic structures of the anthocyanidins, consist of an aromatic ring A bonded to an heterocyclic ring C that contains oxygen, which is also bonded by a carbon–carbon bond to a third aromatic ring B. When the anthocyanidins are found in their glycoside form (bonded to a sugar moiety), they are known as anthocyanins. These are water-soluble vacuolar pigments that may appear as red, purple, or blue depending on pH. These are synthesised via the phenylpropanoid pathway. Anthocyanins have been reported in all plant tissues, including leaves, stems, roots, flowers, and fruits. Pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin are six anthocyanidins most frequently found in plants. The sugars commonly linked to anthocyanidins are monosaccharides: glucose, galactose, rhamnose, and arabinose and di- or tri-saccharides formed by combination of these monosaccharides (Bureau et al. 2009).

Their stability is affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, and the presence of enzymes, flavonoids, proteins, and metal ions (Castaneda-Ovando et al. 2009). Anthocyanins possess well-known pharmacological properties and strong biological functions such as anti-inflammatory and antioxidant activities. Their antioxidant potential is dependent on the number and arrangement of the hydroxyl groups and the extent of structural conjugation, as well as the presence of electron-donating and electron-withdrawing substituents in the ring structure (Lapornik et al. 2005).

In recent years, synthetic food dyes have been banned in many countries because of their toxicity and carcinogenicity. Anthocyanins, coloured natural compounds easily obtained from fruits and vegetables, can be considered potential substitutes for the banned food dyes: they have, in fact, bright attractive colours, while their high solubility in water allows their easy incorporation into aqueous food systems. Moreover, the proved antioxidant activity of anthocyanins, related to the prevention of a number of degenerative diseases, provides additional benefits to the food supplemented with these natural substances (Bleve et al. 2008; Scalbert et al. 2005).

Flavonoids are especially important antioxidants due to their high redox potential, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelating potential. Flavonoids help to protect the plant against UV light, fungal parasites, herbivores, pathogens, and oxidative cell injury. Clinical trials indicate that when consumed regularly by humans, flavonoids have been associated with a reduction in the incidence of diseases such as cancer and heart disease (Scalbert et al. 2005; Liu et al. 2008). There is currently great interest in flavonoid research due to the possibility of improved public health through diet, where preventative health care can be promoted through the consumption of fruit and vegetables.

Anthocyanins



Cyanidin

2.2 Phenolic Acids

Phenolic acids or phenolcarboxylic acids are a type of aromatic acid compounds. Included in that class are substances containing a phenolic ring and an organic carboxylic acid function (C_6–C_1 skeleton). Phenolic acids consist of two subgroups: the hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids include gallic, p-hydroxybenzoic, protocatechuic, vanillic, and syringic acids, which have in common the C_6–C_1 structure. Hydroxycinnamic acids are the aromatic compounds with a three-carbon side chain (C_6–C_3) represented by caffeic, ferulic, p-coumaric, and sinapic acids. About one-third of the dietary phenols are present as phenolic acids, which may be present in free and bound forms. Bound phenolics may be linked to other biomolecules through ester, ether, or acetal bonds (Zadernowski et al. 2009). Phenolic compounds, including anthocyanins, flavonoids, and phenolic acids, are known to be responsible for antioxidant capacities in fruits, the fruits with higher phenolic contents generally showing stronger antioxidant capacities (Fang et al. 2009).

2.3 Tannins

The third important group of phenolics is tannins. These are the high-molecular-weight compounds which may be hydrolysable or non-hydrolysable condensed tannins. Hydrolysable tannins are derivatives of gallic acid (3,4,5-trihydroxybenzoic acid). Gallic acid is esterified to a core polyol, and the galloyl groups may be further esterified or oxidatively cross-linked to yield more complex hydrolysable tannins. Condensed tannins are polymeric flavonoids. The biosynthetic pathways for flavonoid synthesis are well understood, but the steps leading to condensation and polymerisation have not been elucidated. Condensed tannins are based on flavan-3-ols (−)-epicatechin and (+)-catechin and are most widely studied. Tannins being potential metal ion chelators, protein precipitating agents, and biological antioxidants have diverse effects on biological systems. Tannins have great structural variation and can play varied biological roles, and therefore it has been difficult to predict structure function relationship for tannins.

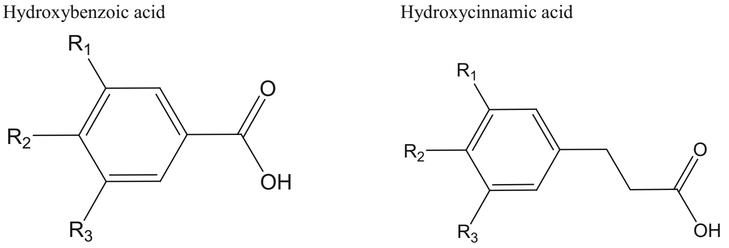
2.4 Stilbenes and Lignans

Stilbenes are 1,2-diarylethenes. Ring A usually carries two hydroxyl groups in the m-position, while ring B is substituted by hydroxy and methoxy groups in the o-, m-, and/or p-position. They are synthesised from cinnamic acid derivatives, and the substitution pattern of the cinnamic acid determines that of ring B of the stilbene.

Parent compounds of common stilbenes

Parent compound	Stilbene
Cinnamic acid	Pinosylvin
p-Coumaric acid	Resveratrol
Caffeic acid	Piceatannol
Isoferulic acid	Rhapontigenin

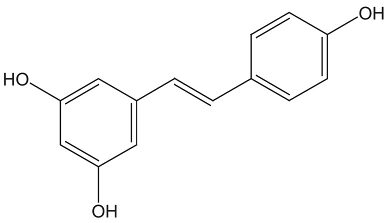
Low quantities of stilbenes are present in the human diet and the main representative is resveratrol that exists in both cis and trans isomeric forms, mostly in glycosylated forms (Delmas et al. 2006). It is produced by plants in response to infection by pathogens or to a variety of stress conditions (Bavaresco 2003). It has been detected in more than 70 plant species, including grapes, berries, and peanuts. Lignans are defined as compounds possessing 1,4-diarylbutane structure. Lignans are produced by oxidative dimerisation of two phenylpropane units; they are mostly present in nature in the free form, while their glycoside derivatives are only a minor form. These are widely distributed as minor constituents of some plant species. Several plants contain high concentrations of lignans; for example, flax seed is the richest identified source of the precursor seco-isolariciresinol. The interest in lignans and their synthetic derivatives is growing because of potential applications in cancer chemotherapy and various other pharmacological effects (Saleem et al. 2005).


Hydroxybenzoic acid

Hydroxycinnamic acid

R1=R2=R3=OH;Gallic acid
R1=R2=OH;Protocatechuic acid

R1=OH; R2 = R3 = H; Coumaric acid
R1=R2=OH; R3 = H; Caeffic acid



Resveratrol

Chemical structures of phenolic compounds

3 Extraction and Identification of Polyphenolics

Extraction is an important step in the isolation, identification, and use of phenolic compounds. Different extraction media and procedures are used for isolation and identification of polyphenols depending upon the characteristics of sources. Two most commonly used techniques for the isolation of phenolic compounds are solvent extraction (Bucić-Kojić et al. 2007) and extraction with supercritical fluid (Bleve et al. 2008). The phenolic compounds have been extracted by grinding, drying, or lyophilising fruits, vegetables, and herbs or only by soaking fresh plants with subsequent solvent extraction (Merken and Beecher 2000). Organic solvents such as ethanol, methanol, acetone, ethyl acetate, and their aqueous mixtures are commonly used for the extraction of polyphenols from plant material. These methodologies imply the co-extraction of non-phenolic substances, such as sugars, organic acids, and proteins, requiring subsequent purification processes (Castaneda-Ovando et al. 2009). Based upon the type and status of the source, solvent extraction may be liquid–liquid extraction or solid–liquid extraction. Conventional extraction procedures such as heating, boiling, or refluxing are used to extract natural phenolic compounds; however, these procedures have some disadvantages like the long extraction time, the loss of polyphenols due to ionisation, hydrolysis, and oxidation during extraction. To overcome these losses during extraction, various novel extraction techniques have been developed in recent years, including ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, and high hydrostatic pressure extraction (HHP) (Wang and Weller 2006).

A number of spectrophotometric methods have been developed for the quantification of plant phenolics. These assays are based on the principles to determine different structural groups present in phenolic compounds. The Folin–Ciocalteu assay (Lapornik et al. 2005) is widely used for determining total phenolics. Total flavonoid content can also be determined using a colorimetric method based on the complexation of the phenolic compounds with Al(III) (Huang et al. 2009; Naczk and Shahidi 2006).

The spectrophotometric assays cannot be used to separate or to analyse individual phenolic compound quantitatively. Chromatographic techniques are used to isolate and quantify polyphenols. Amongst the different chromatographic methods, for the separation, identification, and quantification of polyphenolics in fruits and herbs, HPLC is preferred. The chromatographic conditions of the HPLC methods include the use of, almost exclusively, a reversed-phase C18 column, UV–Vis diode array detector, and a binary solvent system containing acidified water (solvent A) and a polar organic solvent (solvent B). Reverse phase (RP) HPLC has become a dominating analytical tool for the separation and determination of polyphenols with different detection systems, such as diode array detector (DAD), mass spectrometry, or tandem mass spectrometry. Sakakibara et al. (2003) determined all polyphenols in foodstuffs simultaneously with HPLC–DAD and constructed a library comprising respective calibration curves for 100 standard chemicals.

Lower molecular mass polyphenols can be analysed by HPLC on reversed-phase or normal phase columns. However, these techniques are time-consuming and can have poor resolution as the polymer chain length and structural diversity increase. Counter current chromatography (CCC) uses a biphasic liquid system to separate the components of a mixture. The high advantage of the technique in preparative separation is the dual-mode capability of CCC. The role of the phases can be switched during a run. The mobile phase becomes stationary and vice versa. Then no injected material can be left in the machine. Combinations of these techniques are tried to get best output. Cao et al. (2009) applied two methods of separation and purification of polyphenols from apple pomace extract by combining gel chromatography with high-speed counter current chromatography (HSCCC) and solvent extraction with HSCCC, respectively.

Supercritical fluid chromatography (SFC) is a relatively recent chromatographic technique used in the separation and identification of phenolic compounds, where supercritical fluid is used as the mobile phase. Supercritical fluid chromatography is more versatile, more cost-efficient, user friendly, with higher output, better resolution, and faster analysis times than general liquid chromatographic methods. Capillary electrophoresis (CE) is especially suitable for the separation and quantification of low- to medium-molecular-weight polar and charged compounds. This is faster and more efficient than the corresponding HPLC separations (Caridi et al. 2007). Low sensitivity of CE as compared to GC or HPLC is the limitation of this technique (Liu et al. 2008). Nuclear magnetic resonance (NMR), mass spectroscopy (MS), and near-infrared (NIR) spectroscopy are some powerful, fast, accurate, and non-destructive analytical tools used for elucidating the chemical structures of polyphenolic compounds (Ignat et al. 2011).

4 Antioxidant Activity Measurement Methods

Antioxidants include a wide range of phytochemicals, which have redox potential intermediate to reduce ROS and get reduced by reducing power like glutathione in biological systems. Scavenging free radicals such as hydroxyl or superoxide radicals, terminating chain reaction, chelating metal ions, inhibiting ROS production, and donating electrons or hydrogen to terminate chain reactions are some of the ways by which antioxidants reduce oxidation (Schafer et al. 2003). The mode of action of natural antioxidants may be varied and could involve multiple mechanisms of action. Phenols act as primary antioxidants, while ascorbic acid may reductively regenerate oxidised primary antioxidants. The antioxidant activity of a natural source is generally related to either of these activities or as a synergist. Synergism between various antioxidants has been reported (Hudson and Lewis 1983). DPPH is a stable free radical which can absorb an electron or hydrogen to become a stable diamagnetic molecule. Scavenging of these free radicals by the antioxidants is observed as a decrease in optical density of the reaction mixture. Scavenging of free radicals generated by ABTS (2,2′-azino-bis-(3ethylbenzoline-6-

sulfonic acid)) diammonium salt with potassium persulfate is recorded at 645 nm (Matanjun et al. 2008). Superoxide scavenging activity in the reaction mixture containing NBT (Nitroblue tetrazolium), NADH, and PMS (Phenazine methosulphate) is recorded as a decrease in absorbance at 560 nm (Ani et al. 2006). Metal ions such as iron and copper can induce oxidation of lipids leading to the production of peroxy radicals, which in turn propagate chain reaction and accelerate lipid oxidation. Malonaldehyde produced by metal-induced oxidation of lecithin is determined as thiobarbituric acid reactive substances (Dua et al. 2013a, b, c, d). Reducing power is also estimated as the ferric ion reducing capacity (Ghorab et al. 2010). Oxidative stress generated by Fenton's reaction can cause breaks in calf thymus DNA and can uncoil the supercoiled DNA. Incubation of DNA with $FeSO_4$ and ascorbate has caused damage to DNA and damaged DNA moves to a greater extent in the gel. Protection of DNA against damage and protection of ribose oxidation is also used as an index of antioxidant activity (Ani et al. 2006).

5 Natural Source of Polyphenols

Polyphenols are widely distributed in plants such as fruits, vegetables, tea, olive oil, tobacco, herbs, medicinal plants, and others. The plant kingdom offers a wide range of natural antioxidants including polyphenols. Consequently, antioxidants have become an essential part of the preservation technology and contemporary health care. The potential toxicity of some synthetic antioxidants, however, has intensified research efforts to discover and utilise antioxidants from natural sources, such as fruits and vegetables (Zhang et al. 2009).

The most common sources of plant phenolics are presented in Table 1 (Pérez-Jiménez et al. 2010)

5.1 Spices and Herbs

Although spices and herbs have been added to foods since ancient times to improve or modify their flavours, the polyphenols present in them impart antioxidant, antimicrobial, and preservative properties to them. Cloves contain 10–13 % tannins. Eugenin and ellagitannin are the major phenolics present in conjugated form. Biflovin and isobiflovin are two flavonoids found in pyranoside form (Milind and Khanna 2011). Nakatani and Inatani (1984) have identified carnosol, rosmanol, and epirosmanol in rosemary and sage extracts. Baroty et al. (2010) have identified cinnamyl aldehyde, cinnamyl alcohol, eugenol, and ethylcinnamate as the major phenolics in cinnamon bark and leaves. Wojdylo et al. (2007) have analysed the extracts of 32 herbs for the presence of polyphenols and identified caffeic, coumaric, ferulic, and neochlorogenic acid as major phenolic acids, whereas

Table 1 Common sources of plant phenolics

Type of food	Food	Total polyphenols	Polyphenols as aglycone equivalents
Spice/herb	Cloves	15,188	16,047
	Peppermint, dried	11,960	980
	Mexican oregano, dried	2319	—
	Celery seed	2094	—
	Common sage, dried	1207	2920
	Rosemary, dried	1018	2519
	Common thyme, dried	878	1815
	Sweet basil, dried	322	4317
	Curry, powder	285	1075
	Ginger, dried	202	473
	Cumin	55	2038
	Ceylon cinnamon	27	9070
	Marjoram, dried	23	3846
	Curry, powder	285	285
Fruit/vegetable	Black chokeberry	1756	1432
	Black elderberry	1359	804
	Lowbush blueberry	836	496
	Blackcurrant	758	464
	Black olive	569	320
	Plum	377	285
	Green olive	346	233
	Sweet basil, dried	322	166
	Sweet cherry	274	145
	Globe artichoke heads	260	154
	Blackberry	260	180
	Strawberry	235	205
	Red chicory	235	131
	Red raspberry	215	107
	Black grape	169	124
	Red onion	168	99
	Green chicory	166	117
	Spinach	119	68
	Shallot	113	67
	Peach	59	54
	Broccoli	45	21
	Redcurrant	43	23
Non-alcoholic beverages	Cocoa powder	3448	3294
	Coffee, filter	214	110
	Black tea	102	90
	Green tea	89	82
	Pure apple juice	68	61

(continued)

Table 1 (continued)

Type of food	Food	Total polyphenols	Polyphenols as aglycone equivalents
	Pure pomegranate juice	66	37
	Pure blood orange juice	56	28
	Pure grapefruit juice	53	23
	Pure lemon juice	42	20
	Chocolate beverage with milk	21	21
	Soy milk	18	11
	Pure pummelo juice	18	7.9
Alcoholic beverages	Red wine	101	91
	White wine	10	8.6
	Rosé wine	10	7.8

quercetin, luteolin, apigenin, kaempferol, and isorhamnetin are the major flavonoids. Shan et al. (2005) have reported that phenolic acids, phenolic diterpenes, and flavonoids are the main phenolic constituents of extracts of 26 spices screened. Clove, cinnamon, and oregano were found to have high phenolic content and antioxidant activity. Zheng and Wang (2001) have reported a direct correlation between the total phenolic content and antioxidant activity of 27 culinary and 12 medicinal herbs. Rosmarinic acid, quercetin glycosides, and kaempferol glycosides have been found to be the major phenolic compounds. Ani et al. (2006) have also identified gallic acid, protocatechuic acid, caffeic acid, ellagic acid, ferulic acid, quercetin, and kaempferol in methanolic extract of cumin (*Cuminum nigrum*) after glycosidic bond breakage by HCl hydrolysis. The potential medicinal benefits of spices and herbs including possible roles in lowering the risk for atherosclerosis, cardiovascular diseases, cancer, and diabetes are well documented (Srinivasan 2005; Milind and Khanna 2011).

The recent studies done by Dua and coworkers indicated that polyphenol-rich methanolic extract had efficient free radical scavenging and metal chelating activity to protect the biomolecules (proteins, lipids, DNA) against oxidative stress (Table 2).

5.2 Agro-industrial By-Products

Along with fruits, vegetables, and different herbs, agricultural and industrial residues are also rich in natural antioxidants. By-products and residues after processing fruits and vegetables in the food processing industry still contain a huge amount of phenolic compounds, which could be potential sources of antioxidants. One of the richest sources are berry skins, which remain as husks during wine and juice making and are usually used to make compost (Lapornik et al. 2005). The olive

Table 2 Polyphenolic content of some important spices

S. No.	Spice	Type of extract	Polyphenolic content (mg gallic acid equivalent/g dry seeds)	Reference
1	Cumin	Methanolic extract	7.45 ± 0.10	Dua et al. (2012)
2	Fennel	Methanolic extract	16.506 ± 0.32	Dua et al. (2013a)
3	Fenugreek	Methanolic extract	9.47 ± 0.10	Dua et al. (2013b)
4	Coriander	Methanolic extract	18.696 ± 0.12	Dua et al. (2014a)

mill wastes and brassica seed meal are also a major potential source of phenolics. The phenolic content of the olive mill wastewater (OMWW) is reported to fluctuate between 1.0 % and 1.8 % depending on various factors and processing effects. Hydroxytyrosol, tyrosol, oleuropein, and a variety of hydroxycinnamic acids are the major components of OMWW (Obied et al. 2005). Olive leaves, another by-product of the industry, are also rich source of phenolics (Benavente-Garcia et al. 2000). Extract of mustard seed meal in 80 % methanol had comparatively higher amount of polyphenols (15.726 ± 0.15 mg GAE/g dry weight of seeds) (Dua et al. 2014c).

The citrus industry produces large quantities of peel and seed residues, which may account for up to 50 % of the total fruit weight. Peels of the citrus fruits have been found to contain higher amounts of total phenolics even as compared to the edible portions (Balasundram et al. 2006).

Citrus industry by-products, if utilised optimally, could be major sources of phenolic compounds. By-products obtained after artichoke, cauliflower, carrot, celery, and onion processing were investigated by Larrosa et al. (2002).

The peels of several other fruits have also been found to be rich in phenolics than the edible fleshy parts. Apple peels were found to contain up to 3300 mg/100 g dry mass of phenolics (Wolfe and Liu 2003), whereas 118 mg/g of phenolics could be recovered from apple pomace by lyophilisation (Schieber et al. 2003). The peels and seeds of tomatoes have been also found to be richer sources of phenolic compounds than the fleshy pulp. The efficiency of extraction is influenced by various process parameters such as solvent type and feed pre-treatment (crushing, removal of stems) (Lapornik et al. 2005).

5.3 *Beverages*

Beverages such as fruit juices, tea, and wines are important sources of phenolics in the human diet. Important dietary polyphenolic antioxidants include a large variety of both flavonoid (flavonol, flavan-3-ol, and anthocyanin) and non-flavonoid compounds (phenolic acids, phenolic alcohols, stilbene, hydroxycinnamic acid) (Makris et al. 2007).

Fruit juices like grapefruit, orange, and apple are also abundant sources of natural phenolic compounds. Commercial or natural fruit juices contain vitamin C and an abundance of phytonutrients with antioxidant properties. Most of the data available on the phenolic contents of commonly consumed juices are for commercial samples.

Tea in different forms of non-fermented (green), semi-fermented (Oolong), and fermented (black) is widely used around the world. In addition, extracts of tea have become commercially available as antioxidants to control deterioration of lipids in foods. Fresh green leaves contain about 36 % of polyphenols on dry weight basis. The main phenolic compounds present in tea are catechins. Epigallocatechins (9–12 %) and epicatechin gallate (9–12 %) followed by epicatechin (5–7 %), catechin (0.3–0.6 %), and gallic acid (0.3–0.5 %) are identified in green tea leaves (Shahidi 2000). Green tea has been subjected to many scientific and medical studies to determine the extent of its long-purported health benefits, with some evidence suggesting that regular green tea drinkers may have lower chances of developing heart disease and certain types of cancer. Although the content of phenolics is quite diversified depending on the type of technology of its preservation, generally, green tea contains more phenolics than black or red tea and shows higher antioxidant activity (Sikora et al. 2008).

Red wine has been found to be more protective on health than other alcoholic beverages (Alen-Ruiz et al. 2009). Red wine helps in preventing oxidative stress-related diseases, possibly because of the polyphenols it contains. The polyphenolic profile of red wines is usually different from that of white wines due to differences in the composition of red and white grapes and also due to those in the vinification technology used (Alen-Ruiz et al. 2009).

Coffee also provides a significant source of dietary antioxidants. Roasted coffee contains phenolic compounds up to 8 % of the weight. Chlorogenic acid is the dominant phenolic in coffee, which is about 28 mg/g in this drink (Sikora et al. 2008). Klatsky et al. (2006) studied the interrelation between the consumption of coffee as a dietary source of polyphenolic compounds and the apparent reductions in the risks of Alzheimer's disease, Parkinson's disease, heart disease, diabetes mellitus type 2, and liver cirrhosis.

6 Measurement of Antimicrobial Activity

Different antimicrobial susceptibility testing methods are employed to confirm the antimicrobial activity of a chosen antimicrobial agent. The most widely used testing methods include:

- Tube dilution test
- Disc diffusion test

- Agar well diffusion test

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation.

Contamination of food due to microorganisms occurs during production, processing, sale, and distribution (Deak and Beuchat 1996), which ultimately results in microbial food-borne diseases (Mead et al. 1999) It is, therefore, necessary to emphasise the use of antimicrobial agents (Sagdıc and Ozcan 2003) to prevent food spoilage. The naturally occurring polyphenols present in vegetables and fruits have been shown to possess antibacterial activity and thus act as a source of antibacterial agents against food spoilage bacteria.

The antibacterial activity of different vegetable extracts such as broccoli, Brussels sprouts, and white cabbage was measured against *Listeria monocytogenes*, *Enterococcus faecalis*, *Salmonella abony*, and *Pseudomonas aeruginosa*, and extracts were effective against these microorganisms (Jaiswal et al. 2011a, b).

The *Listeria monocytogenes* growth is inhibited by the grape wine (Rodriguez-Vaquero et al. 2007) and the inhibition increased with the increase in concentration of polyphenols. Pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* are inhibited due to the antimicrobial activity exhibited by the extracts of alcohol-free red and white wine (Papadopoulou et al. 2005). A considerable number of white and red wine phenolic extracts inhibited the growth of *C. albicans*. However, in all tests, the diameter of the inhibition zone for *C. albicans* was smaller than the diameter measured for *S. aureus* and *E. coli* inhibition zones.

Green tea (*Camellia sinensis* var. *sinensis*) has a high content of polyphenol particularly flavonoids. Catechins are the main flavonoids found in green tea (Cabrera et al. 2006). Green tea also contains gallic acid (GA), chlorogenic acid, caffeic acid, and flavonols such as kaempferol, myricetin, and quercetin. Cho et al. (2007, 2008) demonstrated the antibacterial effect of tea polyphenols (TPP) on *E. coli* as well as methicillin-resistant *Staphylococcus aureus*. Starting with an inoculum of approximately 10^7 bacteria/ml, there were no *E. coli* CFUs after 30 h in the presence of 5000 μg/ml or after 18 h at 10,000 μg/ml TPP, whereas 50–180 μg/ml concentration of TPP was found to be minimal inhibitory for 30 clinical isolates of *Staphylococcus aureus*.

Fattouch et al. (2008) used aqueous acetone extracts of two apple cultivars, ‘Golden Delicious’ and ‘Red Delicious’; a pear ‘Williams’ cultivar; and a local quince cultivar (*Cydonia oblonga* Miller) for the determination of antimicrobial activities against a wide range of microorganisms including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* sp. strain. The MIC value of each phenolic extract ranged from 10^2 to 10^4 μg/ml among different tested microorganisms, but in case of *Salmonella*, a moderate antimicrobial activity was shown by ‘Red Delicious’ peel extract.

The antimicrobial activities of polyphenolic extracts of three wild red berry fruit species, namely, European cornel (*Cornus mass*), blackthorn (*Prunus spinosa*), and wild blackberry (*Rubus fruticosus*), were assessed by Radovanovic et al. (2013) using disc diffusion method. European cornel extract was found to contain maximum amount of total phenolic contents and the zone of inhibition was significant for the extracts against both the Gram-positive (*Clostridium perfringens*, *Bacillus subtilis*, *Staphylococcus aureus*, *Listeria inocula*, *Sarcina lutea*, *Micrococcus flavus*) and the Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Shigella sonnei*, *Klebsiella pneumonia*, *Proteus vulgaris*) tested bacterial strains.

Different solvent extracts of *Passiflora ligularis* fruit also exhibited antimicrobial activities against *Streptococcus fecalis*, *S. pyogenes*, *Bacillus subtilis*, *Klebsiella pneumonia*e, *Salmonella paratyphi*, and fungi *Candida albicans* and *Aspergillus niger* (Shanmugam and Thangaraj 2014), and the acetone extract of the fruit pulp showed maximum inhibitory effect.

Rahman et al. (2011) studied the antimicrobial effect of six crude plant extracts (*Allium sativum*, *Zingiber officinale*, *Allium cepa*, *Coriandrum sativum*, *Piper nigrum*, and *Citrus aurantifolia*) against five *Escherichia coli* isolated from potable water sources and observed that the aqueous extracts of *Allium cepa*, *Piper nigrum*, and *Coriandrum sativum* seeds alone did not exhibit any in vitro antibacterial effect; however, the combination of *Allium cepa* + *Allium sativum* (1:1) and *Citrus aurantifolia* + *Zingiber officinale* + *Allium sativum* (1:1:1) showed inhibition zones.

Antibacterial activities of methanolic extract of dried spice seeds were determined against pathogenic bacteria by determining cell damage, growth inhibition zone diameter, and minimum inhibitory concentration. Incubation of bacterial cultures with these spice extracts caused damage to their cell membranes and release of intracellular nucleotides and proteinaceous materials from the cells (Table 3).

Table 3 MIC value of some important spices against bacteria

S. No.	Spice	Type of extract	MIC value against bacteria equivalent to mg dry weight of spice/ml of extract	Reference
1	Cumin	Methanolic extract	*E. coli*—12.5 *P. aeruginosa*—6.25 *S. aureus*—25.0 *B. pumilus*—6.25	Dua et al. (2013d)
2	Fennel	Methanolic extract	*B. pumilus*—8.33 *S. aureus*—8.33	Dua et al. (2013c)
3	Coriander	Methanolic extract	*E. coli*—4.16 *P. aeruginosa*—4.16 *S. aureus*—4.16 *B. pumilus*—4.16	Dua et al. (2014b)

7 Conclusion

The studies carried out in the last few decades significantly demonstrate the antioxidant and antimicrobial role of polyphenols. The vegetable and fruit extracts can be utilised in food products for enhancing the quality and nutritive value of foods. The polyphenolic compounds including flavonoids, flavonols, as well as phenolic acids have a variety of bioactivities including antioxidant and antimicrobial activities against a wide range of microorganisms. The phenolic compounds could be utilised as preservatives and thus are better applicable in food preservation and also contribute as antioxidant supplements and antimicrobial agents for food.

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