Chapter 1
An Overview of Antimicrobial Properties of Different Classes of Phytochemicals

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Abstract Plants produce a great diversity of phytochemicals, the beneficial properties of which have been used by humans for centuries since the advent of human civilization. With the discovery of effective and potent antimicrobial compounds, these synthetic antimicrobial compounds are widely used to prevent and cure microbial diseases. However, the development of antibiotic resistant strains of bacteria, reduced efficacy and safety of antimicrobials and the search of new antimicrobials against emerging incurable diseases by conventional antimicrobial agents have revived to explore phytochemicals as an alternative to synthetic antimicrobial compounds. Although numerous studies have been conducted *in vitro* and *in vivo* in the recent years on the efficacy of plant phytochemicals as antimicrobial agents, this chapter provides an overview of the antimicrobial properties of some major group of phytochemicals, namely, different phenolic compounds, alkaloids, saponins, iridoids and secoiridoids, polyacetylenes, glucosinolates, terpenoids, sulfinate, limonoids (tetranortepenoids) and anthranoids against pathogenic bacteria, fungi, viruses and commensal bacteria in the intestinal tracts of humans and animals. This chapter also discusses their antimicrobial mechanisms of action, the efficiency of different groups of phytochemicals against multiple-drug resistant bacteria, the effect of active dietary phytometabolites on the beneficial and pathogenic microbes of the gastrointestinal tracts and the outcomes of combination of phytofactors and drugs interactions.

Keywords Phytochemicals • Medicinal plants • Antimicrobial • Antiviral • Antifungal • Mechanism of action

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A.K. Patra (ed.), *Dietary Phytochemicals and Microbes*,
DOI 10.1007/978-94-007-3926-0_1,

1.1 Introduction

Plants contain a wide array of phytochemicals, which have traditionally been utilized for centuries in folk medicines or ethnomedicines. The earliest information on the medicinal use of plants comes from China in 5000 BC (Greathead 2003), from India (in Rigveda and Atharvaveda) in 2000 BC (Ramawat et al. 2008), from Mesopotamia in 2600 BC (Newman et al. 2000), and also from Egypt in about 1550 BC (Davidson and Naidu 2000). The natural medicines were widely used until the first half of the twentieth century, when a shift towards synthetic medicines that were more effective, patentable and highly profitable, occurred (Tyler 1999). However, there have been increasing interests towards use of natural chemicals in medicinal purposes in recent years. These ethnomedicines are encouraging for both the public and national health care institutions as alternatives to synthetic drugs due to relatively lower incidences of adverse reactions compared to modern conventional pharmaceuticals along with their reduced cost (Nair et al. 2005).

Recently, the growing occurrences of multi-drug resistant strains of bacteria and the appearance of strains with decreased susceptibility to antibiotics have led to a resurgence of research interests in the discovery of novel antimicrobial agents from natural sources for therapeutic and preventive purposes against microbial diseases, food preservatives and feed additives in the animal industry. The ethnopharmacologists, botanists, microbiologists and natural-product chemists are constantly in search of medicinal efficacy of plants and their phytochemicals, since the reported data so far available on plants are comparatively meager compared to the vast number of plant population. Plants produce a great diversity of compounds. The structures of close to 50,000 compounds have already been elucidated and there are perhaps hundreds of thousands of such compounds in plants (Pichersky and Gang 2000). Only a few of these are part of 'primary' metabolic pathways (those common to all organisms). The rest are secondary metabolites or phytochemicals whose biosynthesis is restricted to selected plant groups (Pichersky and Gang 2000). Phytochemicals can be divided into many major classes depending upon the chemical structures, botanical origins, biosynthesis pathways or biological properties. The most phytochemical classification scheme is based on chemical structures such as phenolics, alkaloids, saponins, terpenoids, limonoids, polyacetylenes and secoiridoids and so on. Numerous studies have been conducted *in vitro* and *in vivo* in the recent years on the efficacy of plant phytochemicals as antimicrobial agents. This paper presents the antimicrobial properties of some major group of phytochemicals against pathogenic bacteria, fungi and virus, and beneficial microbes of the gastrointestinal tracts and their mechanism of action.

1.2 Phenolic Compounds

Phenolic compounds are a group of phytochemicals, which have a phenol structure, i.e. an aromatic benzene ring bearing at least one hydroxyl substituent (Robbins 2003; Vermerris and Nicholson 2006). Phenolic compounds are commonly found

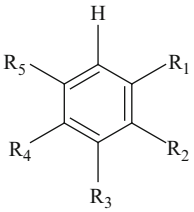
throughout the plant kingdom, where they protect the plants from microbial infections, ultraviolet radiation and chemical stressors. This large and diverse group of phytochemicals is classified into many subclasses depending upon chemical structures and occurrence in plants. The commonly categorized subclasses of phenolic compounds are simple phenolics (resorcinol and phloroglucinol), phenolic acids and aldehydes, coumarins, flavonoids, chalcones, aurones, benzophenones, xanthones, stilbenes, benzoquinones, naphthaquinones, anthraquinones, betacyanins, lignans, and polyphenols (proanthocyanidin, galloyl, hexahydroxydiphenyl ester, hydroxy cinnamic acid, and phloroglucinol derivatives) (Vermerris and Nicholson 2006; Handique and Baruah 2002). The detailed structures and chemistry of these phenolic compounds are presented elsewhere (Vermerris and Nicholson 2006). Foods containing phenolics are becoming an important part of diets due to their potential anti-oxidative properties. Besides, these compounds have also potent anti-microbial properties.

1.2.1 Phenolic Acids and Aldehydes

The phenolic acid and aldehyde group of phenolic compounds is characterized by the presence of a carboxylic acid or aldehyde group substituted on a phenol (Table 1.1; Vermerris and Nicholson 2006). The naturally occurring phenolic acids generally have two characteristic constitutive carbon frameworks: the hydroxycinnamic and hydroxybenzoic structures (Robbins 2003). Majority of cinnamic and benzoic acid derivatives in plants are linked through ester, ether or acetal bonds to structural components, polyphenols, organic acids (quinic, maleic, tartaric and shikimic acid), glucose and terpenes (Robbins 2003). Chlorogenic acid is an ester of quinic acid and caffeic acid. Some aldehyde analogues of phenols (e.g. vanillin) are also grouped with phenolic acids (Robbins 2003). The numbers and positions of the hydroxyl and other groups on the aromatic ring can produce a large number of compounds in this subclass (Robbins 2003; Vermerris and Nicholson 2006). Phenolic acids are present in a wide range of plants including in many common foods such as tea, coffee and berries. Besides, phenolic acids and aldehydes could be formed by the intestinal microbial biotransformation of other phenolic compounds in the intestine, where they may influence intestinal microbiota.

A number of simple phenols and phenolic acids possess antibacterial, antiviral and antifungal activities against a wide range of microbes, but at different concentrations. Gallic acid and *p*-hydroxybenzoic acid reduced the viability of *Camplylobacter jejuni* at concentrations as low as 1 mg/L (Ganan et al. 2009). Synaptic acid, vanillic acid, and caffeic acid were microbicidal at concentrations starting at 10 mg/L. Ferulic acid and cumaric acid were effective at a concentration of 100 mg/L (Ganan et al. 2009). Ozçelik et al. (2011) recently tested some phenolic acids such as gallic acid, caffeic acid, chlorogenic acid, and quinic acid for their *in vitro* antiviral, antibacterial, and antifungal activities. All these phenolic acids were inhibitory to herpes simplex virus type 1 (HSV-1), whereas gallic acid, chlorogenic acid and quinic acid showed potent antiviral effect against parainfluenza virus type 3 at the therapeutic range of 0.8–0.05 mg/L.

Table 1.1 Chemical structures of some phenolic acids found naturally in plants and foods (Robbins 2003; Vermerris and Nicholson 2006; Cueva et al. 2010)



Common name	R_1	R_2	R_3	R_4	R_5
Cinnamic acid	–CH.CH.COOH	H	H	H	H
p-Coumaric acid	–CH.CH.COOH	H	H	–OH	H
Ferulic acid	–CH.CH.COOH	H	$–OCH_3$	–OH	H
Sinapic acid	–CH.CH.COOH	H	$–OCH_3$	–OH	$–OCH_3$
Caffeic acid	–CH.CH.COOH	H	–OH	–OH	H
Benzoic acid	–COOH	H	H	H	H
Salicylic acid	–COOH	–OH	H	H	H
p-Hydroxybenzoic acid	–COOH	H	H	–OH	H
Vanillic acid	–COOH	H	$–OCH_3$	–OH	H
Syringic acid	–COOH	H	$–OCH_3$	H	$–OCH_3$
Protocatechuic acid	–COOH	H	–OH	–OH	H
Gentisic acid	–COOH	–OH	H	H	–OH
Gallic acid	–COOH	–OH	–OH	–OH	–OH
Veratric acid	–COOH	H	$–OCH_3$	$–OCH_3$	H
Syringealdehyde	–CHO	H	$–OCH_3$	–OH	$–OCH_3$
Vanillin	–CHO	H	$–OCH_3$	–OH	H
Phenylacetic acid	$–CH_2$.COOH	H	H	H	H
2-hydroxyphenylacetic acid	$–CH_2$.COOH	–OH	H	H	H
Phenylpropionic acid	$–CH_2.CH_2$.COOH	H	H	H	H
3-hydroxyphenylpropionic acid	$–CH_2.CH_2$.COOH	H	–OH	H	H
2-hydroxyacetophenone	–HCO	–OH	H	H	H

In general, antibacterial activity of phenolic acids is stronger against Gram-positive bacteria than Gram-negative bacteria (Merkl et al. 2010; Cueva et al. 2010). The outer membrane of Gram-negative bacteria provides them with a hydrophobic surface structure that is able to exclude certain hydrophilic molecules, making them inherently resistant to many antimicrobial agents including phenolic acids (Alakomi et al. 2007; Cueva et al. 2010). Gram-positive bacteria are enclosed in a plasma membrane covered by a thick peptidoglycan wall and lack an outer membrane (Alakomi et al. 2007; Cueva et al. 2010). Although, phenolic acids are effective against Gram-negative bacteria, their antimicrobial effect is strain dependent (e.g. different strains of *Escherichia coli*; Cueva et al. 2010).

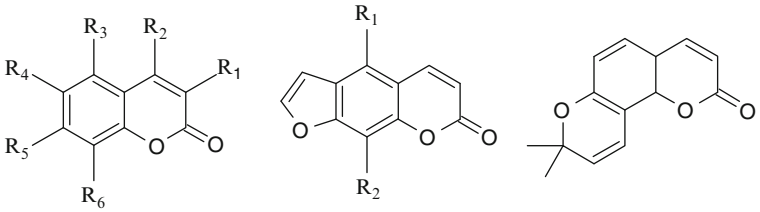
Phenolic compounds are usually poorly absorbed in the small intestine, and thus most of the dietary phenolics accumulate in the colon (Clifford 2004; van Duynhoven et al. 2011). Therefore, higher concentrations of phenolic acids may reach in the intestine than the concentrations in diets. Phenolics may selectively suppress or stimulate

the growth of certain members of intestinal microbiota, which may influence microbial population dynamics in the gastrointestinal tract (Tzounis et al. 2008). Chlorogenic, quinic and gallic acids stimulated growth of *Lactobacillus collinoides* relative to control cultures (no additive) up to concentrations of 1 g/L of tomato broth media. In contrast, growth of *Lactobacillus brevis* was little affected during early incubation, which has been suggested to be due to metabolism of these acids (Stead 1994).

From structure-activity relationship, phenols having different alkyl chain length with hydroxyl groups could be important for antimicrobial actions (Kubo et al. 1995). p-Hydroxybenzoic acid, protocatechuric, gentisic acid, vanillic acid, ferulic acid, caffeic acid and their methyl, ethyl, propyl and butyl esters were investigated for antibacterial action. It has been reported that the antimicrobial effect of phenolic acids derivatives increased with the increasing length of the alkyl chain (Merkl et al. 2010). The presence of hydroxyl groups on the phenol groups and oxidized status of phenol groups also determine the toxicity of microbes. The fluidity of the cell membrane could be disturbed with increasing hydrophobic alkyl chains. The phenolic acids could enter the molecular structure of the membrane with the polar hydroxyl group oriented into the aqueous phase by hydrogen bonding and nonpolar carbon chain aligned into the lipid phase by dispersion forces (Kubo et al. 1995). Thus, when the hydrophilic force exceeds hydrophobic one, the activity tends to disappear. Also, the number and position of substitutions in the benzene ring of the phenolic acids and the saturated side-chain length influenced the bacteriocidal effects of phenolic acids against the different microorganisms, but in different ways against Gram-positive and Gram-negative bacteria (Cueva et al. 2010). For example, Cueva et al. (2010) showed that for benzoic and phenylacetic acids, *E. coli* was inhibited in the following order of potency: non-substituted > 4-hydroxy-3-methoxy- > 3-hydroxy- > 4-hydroxy- > 3,4-dihydroxy-substituted acid. For phenylpropionic acids, the order differed slightly: nonsubstituted > 4-hydroxy- > 3-hydroxy- >3,4-dihydroxysubstituted acid. However, the potency of phenolic acids was in different order for *Lactobacillus* spp. For benzoic acids, the order of potency was: 4-hydroxy- > 3-hydroxy- > non-substituted > 4-hydroxy-3-methoxy- > 3,4-dihydroxy-substituted acids, except for *Lactobacillus coryniformis* CECT 5711 (4-hydroxy-> non-substituted > 3-hydroxy > 4-hydroxy-3-methoxy-substituted acids). For phenylacetic acids, growth inhibition of lactobacilli was on the order of non-substituted > 3-hydroxy- > 4-hydroxy- > 3,4-dihydroxy-substituted acids. For phenylpropionic acids, growth inhibition was as follows: non-substituted > 4-hydroxy- > 3-hydroxy > 3,4-dihydroxy-substituted acids, except for *Lactobacillus fermentum* CECT 5716 (3-hydroxy > non-substituted > 4-hydroxy- and 3,4-dihydroxy-substituted acids) and *Lactobacillus plantarum* LCH17 (non-substituted > 3-hydroxy- > 4-hydroxy-> 3,4-dihydroxy-substituted acids).

1.2.2 Coumarins

Coumarins are naturally found in many families of plants (Apiaceae, Asteraceae, Fabiaceae, Rosaceae, Rubiaceae, Rutaceae and Solanaceae) and microorganisms,



Coumarins with substituents in benzene or pyrone ring | Furocoumarins | Pyranocoumarins

Fig. 1.1 Basic chemical structures of different types of coumarins

and approximately 1,000 coumarins have been isolated from these sources (Weinmann 1997; Smyth et al. 2009). Coumarins can be classified into five groups depending upon the structure, i.e. coumarins with substituents in benzene ring, coumarins with substituents in pyrone ring, furocoumarins, pyranocoumarins, and coumarin dimmers (Fig. 1.1; Smyth et al. 2009).

Coumarins exhibit a broad diversity for antimicrobial activity. O-acetylcolumbianetin, edultin, cniforin A, columbianadin and imperatorin isolated from the fruits of *Cnidium monnieri* (L.) Cuss exerted a little to no appreciable growth-inhibition of Gram-positive and Gram-negative bacteria (Ng et al. 1996). An amino-coumarin – 7-amino-4-methylcoumarin showed broad-spectrum antibacterial and antifungal activities (Liu et al. 2008). Melliou et al. (2005) studied the antibacterial activity of pyranocoumarins using an agar disc diffusion method. Seselin, xanthyletin, 5-hydroxyseselin, and 7-hydroxyalloxanthyletin had no antibacterial effects. Coumarin derivatives such as 5-methoxyseselin and its brominated derivatives, alloxanthoxyletine, the acetylated derivatives, and dipetalolactone were active against all the tested bacteria. A seselin derivative, 3-bromo-4-benzoyloxyseselin showed moderate activity, while three coumarins containing acetoxy groups in pyrano ring were only active against the two Gram-positive bacteria. A new coumarin – cajanuslactone isolated from pigeon pea leaves showed anti-bacterial activity against *Staphylococcus aureus* (ATCC 6538), and the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were 0.031 and 0.125 mg/mL, respectively (Kong et al. 2010). Some seselin derivatives, including derivatives of 5-methoxyseselin, were found to be potent against human immunodeficiency virus (HIV) (Xie et al. 1999).

It has been suggested that the presence of oxygenated substituents in the ether or ester form usually enhances the antibacterial activity, while the presence of free hydroxyl group reduces the activity (Melliou et al. 2005). This fact could be at least partially attributed to the reduced lipophilicity of the hydroxyl derivatives, which hinders the penetration through the bacterial cell wall (Melliou et al. 2005).

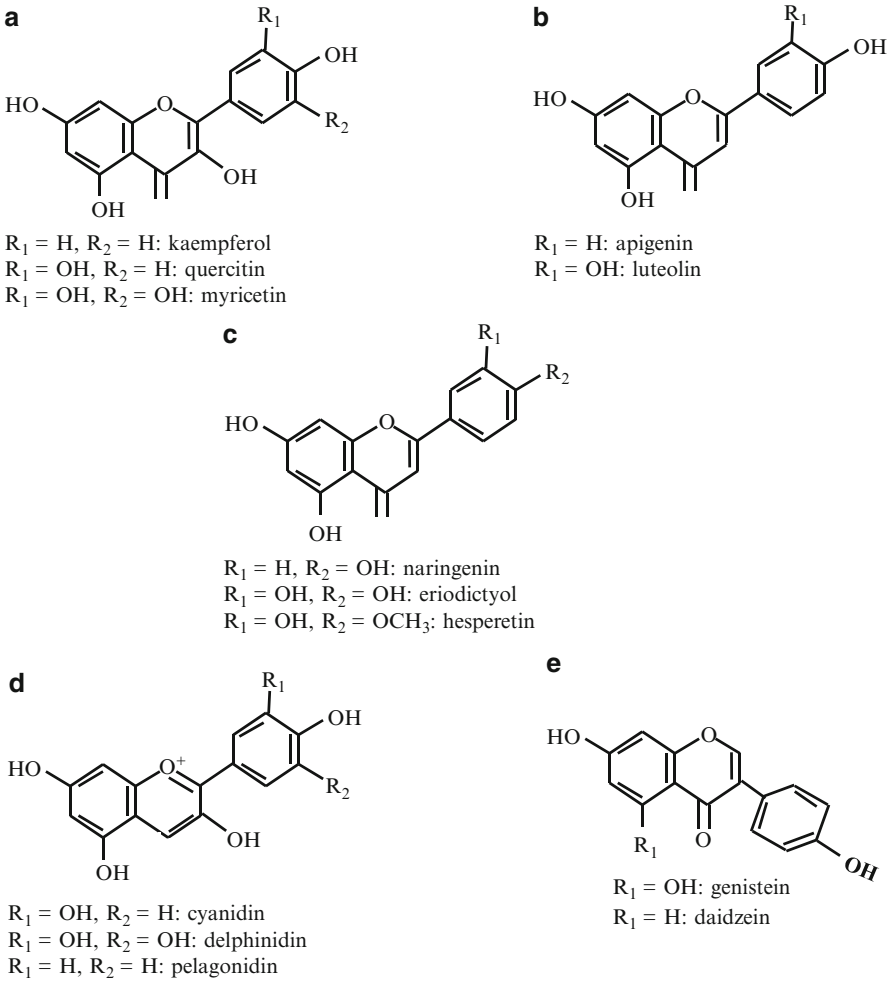


Fig. 1.2 The chemical structures of flavonoids; (**a**) flavonol, (**b**) flavone, (**c**) flavanone, (**d**) anthocyanidins and (**e**) isoflavone

1.2.3 Flavonoids

Flavonoids are one of the largest groups of secondary metabolites that are distributed in various plant species. They have significant antioxidant properties, which are beneficial for health. These polyphenolic compounds are constructed basically with an A and C ring of benzo-1-pyran-4-quinone and a B ring. The main classes of flavonoids (Fig. 1.2) are (1) flavones (basic structures), e.g. luteolin, apigenin, diosmetin, chrysoeriol, tangeretin, sinensetin, gardenin, vitexin and baicalein; (2) flavonols

(having a hydroxyl group at the 3-position), e.g. kaempferol, quercetin, galangin, datiscetin, morin, robinetin, isorhamnetin, tamarixetin, quercetagetin and myricetin; (3) flavanones (2–3 bond saturated), e.g. hesperetin, taxifolin, eriodictyol and naringenin; (4) flavan-3-ol, e.g. catechin and epicatechin; (5) isoflavone, e.g. genistein, daidzein and coumestrol; (6) anthocyanidins: cyanidin, delphinidin, pelargonidin and peonidin (Crozier et al. 2006). The majority of flavonoids commonly remain conjugated with sugars as glycosides.

Numerous flavonoid derivatives showed antiviral activity against a wide range of viruses such as HSV, HIV, coxsackie B virus, coronavirus, cytomegalovirus, poliomyelitis virus, rhinovirus, rotavirus, poliovirus, sindbis virus, and rabies virus (De Bruyne et al. 1999; Evers et al. 2005; Nowakowska 2007). Ozçelik et al. (2011) investigated the effects of quercitin, apigenin, genistein, naringin, silymarin and silibinin against HSV-1 and PI-3 virus. All flavonoids inhibited HSV-1 activity, but only genistein inhibited parainfluenza type-1 (PI-1) activity. Of the three flavonoids (baicalin, rutin and naringin) examined by Ng et al. 1996, baicalin was found to be the most potent in inhibiting the growth of *S. aureus*: 11 of the 16 strains tested were inhibited at 128 mg/L. However, no inhibitory activity of rutin and naringin against *S. aureus* was observed at 128 mg/L. At this concentration, naringin and baicalin inhibited two strains and rutin inhibited one strain of the eight *P. aeruginosa* strains tested. The flavonoids compounds display different mode of antiviral action. For instance, baicalein probably block human cytomegalovirus infection at entry level while the primary mechanism of action for genistein may be to block immediate-early protein functioning off human cytomegalovirus (Evers et al. 2005). Both these flavoinoids did not inhibit the virus replication (Evers et al. 2005).

Puupponen-Pimia et al. (2001) investigated 13 falovonoid compounds (apigenin, (+)-catechin, chlorogenic acid, cyanidin chloride, delphinidin chloride, isoquercitrin, kaempferol, cyanidin-3-glucoside (kuromanin), luteolin, myricetin, pelargonidin chloride, quercetin dehydrate and rutin trihydrate), and 4 phenolic acids (caffeic acid, 3-coumaric acid, ferulic acid, trans-cinnamic acid) on 7 Gram-positive lactic acid bacteria of intestines, Gram-negative *E. coli* CM 871 and *Salmonella*. Myrecetin strongly inhibited the growth of *Lactobacillus* as well as *E. coli*, but did not affect *Salmonella*. Luteolin was weakly inhibitory to Gram-positive lactic acid bacteria but not to Gram-negative bacteria. The anthocyanidins pelargonidin, delphinidin and cyanidin, as well as cyanidin-3-glucoside, only inhibited growth of *E. coli* and had no effect on other bacterial strains (Puupponen-Pimia et al. 2001). However, phenolic acids did not inhibit lactic acid bacteria, but inhibited Gram-negative *E. coli* and *Salmonella* sp.

Hatano et al. (2005) discussed that some prenylated flavonoids such as licoricidin (an isoflavan) effectively suppressed the antibiotic resistance of methicillin-resistant *S. aureus* (MRSA) compared to other flavonoids. The addition of 4 μg/mL of licoricidin shifted the MIC of oxacillin from 128–256 to 8–16 μg/mL, and 8 μg/mL of licoricidin reduced it to less than 0.5 μg/mL. The requirement for dimethylallyl or equivalent substituents suggests the importance of affinity for the bacterial cell membrane.

Phenolic acids show greater antimicrobial potency than their corresponding flavonoids precursors such as the monomers (+)-catechin and (−)-epicatechin (Ganan et al. 2009; Cueva et al. 2010). Therefore, microbial transformations of dietary flavonoid compounds in the intestine could lead to more potent microbial-inhibitory compounds (phenolic acids) and could reach greater concentrations in the intestine. This may selectively influence intestinal bacteria species, and therefore could affect the diversity and metabolic activity of the intestinal microbiota, including the transformation of phenolics in the gut (Cueva et al. 2010).

Epigallocatechin gallate exerted strong antibacterial growth against Gram-positive bacteria than against Gram-negative bacteria (Yoda et al. 2004; Engels et al. 2009). It has been stated that Gram-positive bacteria absorb more epigallocatechin gallate into their peptidoglycan cell wall and aggregate its presence, while Gram-negative bacteria do not aggregate and absorb less epigallocatechin gallate (Ikigai et al. 1993; Engels et al. 2009) because of the repulsive negative charge of lipopolysaccharides on the surfaces of Gram-negative bacteria. The binding of epigallocatechin gallate to peptidoglycan disrupts its function in osmotic protection, cell division, and cell wall biosynthesis (Yoda et al. 2004). Detailed information of antimicrobial activities of flavonoids has been discussed elsewhere in this book (Chap. 2).

1.2.4 Polyphenols

Some phenolic acids (ellagic and gallic acids) or flavonoids (flavan-3-ol, flavan-3-4-diol or flavan-4-ol) in plants are esterified or polymerized into dimeric, oligomeric or polymeric compounds. Most abundantly present polyphenolic compounds in plants are tannins, which are usually of two types: hydrolysable tannins (HT) and condensed tannins (CT). The HT are complex molecules with a polyol as a central core such as glucose, glucitol, quinic acids, quercitol and shikimic acid that is partially or totally esterified with a phenolic group, i.e. gallic acid (3,4,5-trihydroxy benzoic acid; gallotannins) or gallic acid dimmer hexahydroxydiphenic acid (ellagitannins) (Haslam 1989). The CT (proanthocyanidins) are mainly polymers of the flavan-3-ols (epi)catechin and (epi)gallocatechin units, which are linked by C4-C8 and C4-C6 interflavonoid linkages (Ferreira et al. 1999; Hagerman and Butler 1989).

The polyphenols also exert a wide range of antibacterial and antifungal activities. Ellagitannin extracts inhibited a range of pathogenic organisms including *Vibrio cholerae, Shigella dysenteriae* and *Campylobacter* spp. (Silva et al. 1997; Puupponen-Pimia et al. 2002). Puupponen-Pimia et al. (2005) reported that berry extracts exhibit selective inhibitory properties against intestinal bacteria such as *Staphylococcus, Salmonella, Listeria* and *Lactobacillus* strains, and the selective inhibitory actions varied with berry extracts. In general, pathogenic *Staphylococcus* and *Salmonella* were sensitive to various berry extracts and ellagitannins fractions, while pathogenic *Listeria* and beneficial *Lactobacillus* were not inhibited.

Rauha et al. (2000) studied antimicrobial effects of some berry extracts against food spoilage and poisoning bacteria. The widest antibacterial activity was present in berries belonging to the genus *Rubus* (cloudberry and raspberry) that are rich in ellagitannins. Ellagic acid has been reported to exhibit a dose-dependent inhibitory effect (IC50 = 1 mM) on *Helicobacter pylori* isolated from peptic ulcer patients (Chung 1998). Tannins isolated from *Dichrostachys cinerea* roots exerted antimicrobial effects against *S. aureus*, *E. coli*, *Shiegella* spp. and *P. aeruginosa* with MIC of the tannins ranging between 4.0 and 5.5 mg/mL, while the MBC ranging between 4.5 and 6.0 mg/mL (Banso and Adeyemo 2007). Gallotannins extracted from the mango seed kernel inhibited the growth of Gram-positive food spoilage bacteria and decreased the growth of Gram-negative *E. coli*, but did not affect lactic acid bacteria (Engels et al. 2009). The antibacterial properties of cranberry juice with inhibition of *E. coli* adherence to mucosal surfaces by cranberry juice is reported to be associated with the presence of proanthocyanidins (Howell et al. 1998).

Many polyphenols have antiviral activities against different types of viruses (De Bruyne et al. 1999; Cheng et al. 2002). It has been suggested that prodelphinidin B-2 3′-*O*-gallate (a proanthocyanidin gallate isolated from green tea leaf) showed anti-HSV-2 properties with the mechanism of inhibiting the attachment and penetration between cells and viruses possibly through the instability of viral glycoproteins (Cheng et al. 2002). The structure and functional groups of the polyphenol compounds may determine the effectiveness of the antiviral activities (De Bruyne et al. 1999).

The content of small-molecular phenolic compounds have greater influence on the antibacterial activity of extracts than tannins (Nazaruk et al. 2008). Thus, polyphenols could be cleaved by bacterial enzymes to form a number of phenolic acids in the intestine, where they may influence the microbial populations (Bock and Ternes 2010). Engels et al. (2009) recently studied the effects of gallotannins with different galloyl units from mango seed kernel on various Gram-positive and Gram-negative bacteria. Gallotannins showed antibacterial activities with MICs ranging from 0.1 g/L for *S. aureus* to 3.3 g/L for *Pediococcus acidilactici*. They also observed that degree of galloylation did not affect the growth of bacteria. It has been suggested that the antibacterial activities of gallotannins are due to their strong affinity for iron and the inactivation of membrane-bound proteins (Engels et al. 2009). It has also been shown that gallotannins changed the morphology of *Bacillus subtilis*, which has been hypothesized due to inhibition of cell division by binding of gallotannins to the cell wall or inhibition of enzymes involved in cell separation (Engels et al. 2009).

1.2.5 Naphthoquinones

Naphthoquinones are widely distributed in plants, fungi, and some animals. Lapachol, plumbagone, juglone and lawsone are naturally occurring naphthoquinones

of plant origin that have antimicrobial effects against various pathogenic bacteria and fungi. Adeniyi et al. (2000) reported that two dimeric naphthoquinones, diospyrin and isodiospyrin, isolated from the root of *Diospyros piscatoria* (Gurke), a common ingredient in several folk medicines, exhibited a broad spectrum of antibacterial activity against *S. pyogenes* and *S. pneumoniae* (MICs of diospyrin ranged from 1.56 to 50 μg/mL) *Salmonella choleraesuis* serotype *typhi* (*S. typhi*) and *Mycobacterium chelonae* (MICs of diospyrin were between 25 and 100 μg/mL). Isodiospyrin was more active than its racemic isomer diospyrin (MICs against Gram-positive bacteria ranged from 0.78 to 50 μg/mL, while those against *Pseudomonas aeruginosa* and *S. typhi* ranged from 50 to 100 μg/mL). Another naphthoquinones, lapachol and β-lapachone, found in species of *Tabebuia,* had relevant effects against *Candida albicans, Candida tropicalis,* and *Cryptococcus neoformans,* and were more active than the reference standard, ketoconazole. Lapachone showed strong antimicrobial activity than lapachol against the fungi (Guiraud et al. 1994). Methanol extract from the dried inner bark of *Tabebuia impetiginosa* exhibited potent antibacterial activity against *H. pylori* which contained lapachol and anthraquinones (Park et al. 2006).

1.3 Alkaloids

Alkaloids have been defined as N-heterocyclic basic metabolites, although the definition does not clearly separate from other N-containing compounds. Alkaloids have been classified in many ways depending upon biogenic precursors or carbon skeleton characteristics. They have a great structural diversity compared with other classes of phytochemicals. Alkaloids are generally known according to their carbon skeleton structures. Pyridine (e.g. piperine), piperidine, quinoline, indole, pyrrolidine, quinazoline, isoquinoline, glyoxaline, lupinane, tropan, phenanthridine, imidazoline, alkaloidal amines and terpenoid types of alkaloids are commonly found in plants (Hegnauer 1988).

Alkaloid fractions isolated from *Strychnos potatorum* L.f. (Loganiaceae) seeds, which were of indole type, were tested for their antimicrobial properties against some pathogenic Gram-positive, Gram-negative and acid-fast bacteria and fungi. These fractions had shown considerable antimicrobial activity against both bacteria and fungi at the tested concentrations (100 and 200 μg/mL). Further, the growth of *Proteus vulgaris*, *S. aureus, Salmonella typhimurium, Vibrio cholerae, Mycobacterium tuberculosis, Aspergillus niger* and *C. albicans* were significantly inhibited (Mallikharjuna and Seetharam 2009). Similarly, two benzophenanthridine alkaloids, dihydrochelerythrine and dihydrosanguinarinealkaloid constituents of *Bocconia arborea* showed considerable antimicrobial activity against Gram-positive and Gram-negative bacteria and *C. albicans* (Navarro and Delgado 1999).

Sensitivity of DNA and RNA viruses to alkaloids may differ. Ozçelik et al. (2011) investigated various alkaloids namely yohimbine and vincamine (indole-type), scopolamine and atropine (tropane-type), colchicine (tropolone-type), allantoin

(imidazolidine-type), trigonelline (pyridine-type) as well as octopamine, synephrine, and capsaicin (exocyclic amine-type) for their antiviral activities against DNA virus herpes simplex (HSV-1) type 1 and RNA virus parainfluenza type-3 (PI-3). All the alkaloids were effective against HSV-1 at 0.05–1.6 mg/L, but atropin and octopamine showed potent antiviral activities against PI-3 at 0.05–0.8 mg/L (Ozçelik et al. 2011). Antibacterial alkaloids from *Chelidonium majus* Linn, i.e. benzo[c] phenanthridine-type alkaloids, 8-hydroxydihydrosanguinarine, 8-hydroxydihydrochelerythrine were potently active against MRSA strains with MICs/MBCs ranged from 0.49 to 15.63 and 1.95 to 62.50 μg/mL, respectively (Zuo et al. 2008).

1.4 Organosulphur Compounds

There are two rich sources of organosulphur compounds from plants; (1) Alliaceae family containing alliin-alliinase system and (2) Cruciferae (Brassicacae) family e.g. *Brassica juncea*, *Wasabia japonica* (wasabi), *Armoracia rusticana* (horseradish) and *Brassica oleracea* (cauliflower) containing glucosinolate-myrosinase (Mithen 2006). A number of sulphur-containing compounds can be derived from these plants through the action of myrosinase and alliinase enzymes.

1.4.1 Thiosulfinate

The primary sulphur-containing constituents in *Alliums* spp. (e.g. *A. sativum* (garlic), *A. cepa* (onion), *A. porrum* (leek)) and *Brassica* spp. (e.g. cabbage, kale, cauliflower and turnip) are *S*-alk(en)yl-L-cysteine sulphoxides and γ-glutamyl-*S*-alk(en)yl-L-cysteine sulphoxides (Block et al. 1992; Ross and Milner 2007; Fig. 1.3). The content of S-alk(en)yl-L-cysteine sulphoxides in garlic may range from 0.53% to 1.3% of fresh weight with *S*-allyl-L-cysteine sulphoxide (alliin) being the largest contributor. By the action of alliinase enzyme present inside the cells, these compounds are converted into thiosulfinate (a functional group consisting of the linkage R-S(=O)-S-R'), which are then spontaneously and enzymatically converted into a large array of volatile compounds, e.g. diallyl disulphide, diallyl trisulphide, allyl methyl disulphide and dipropyl and disulphide (Mithen 2006).

Antimicrobial activities of garlic and onion against a wide range of Gram-positive and Gram-negative bacteria, virus and fungi are known for many years (Ankri and Mirelman 1999). The antifungal activities of garlic oils appear to be more than the antibacterial activity (Avato et al. 2000). Extracts of garlic exhibit the most potent antibacterial activity, followed by onion, and *Brassica* including cabbage (Kyung and Lee 2001). The principal antimicrobial compounds of *Allium* and *Brassica* are allicin (*S*-allyl-L-propene thiosulfinate) and methyl methanethiosulfinate, respectively (Kyung and Lee 2001). These compounds are derived from *S*-allyl and *S*-methyl derivatives of L-cysteine sulfoxide, respectively. Avato et al. (2000)

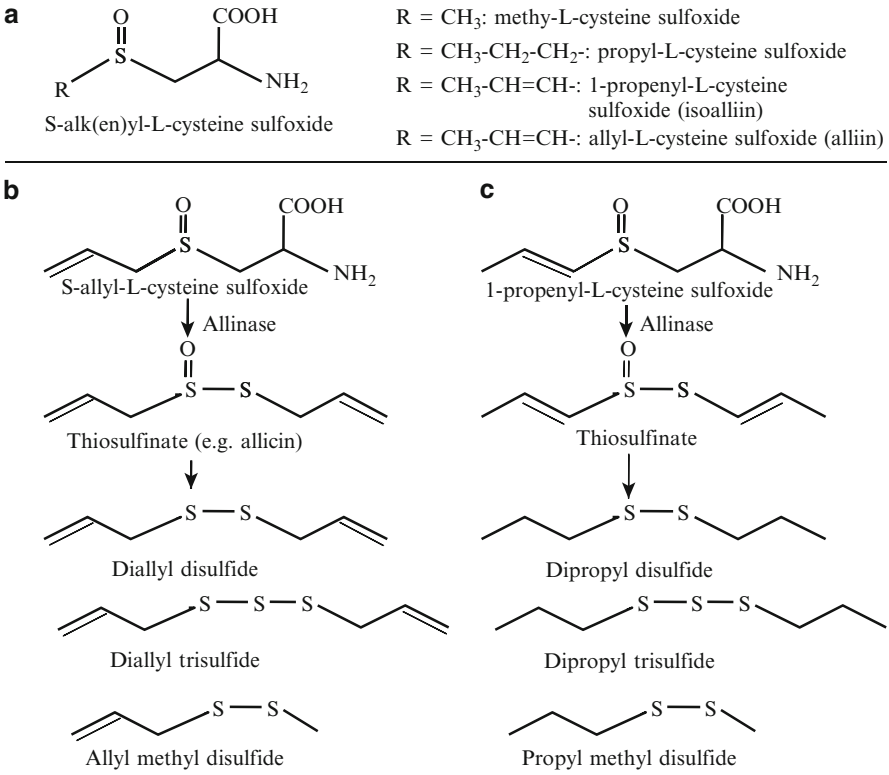


Fig. 1.3 Major thiosulfinate compounds found in Alliaceae family: (**a**) organosulfur compounds in intact plants, (**b**) compounds Produced from allyal cystein sulfoxide (in garlic) and (**c**) 1-propenyl cystein sulfoxide (in onion) by aliinase

tested different mixtures of garlic distilled oils containing diallyl disulfide (DDS) and diallyl trisulfide (DTS), ranging from 1% to 51% and 88% to 38%, respectively, against yeasts (*C. albicans, C. tropicalis* and *B. capitatus*), Gram-positive bacteria (*S. aureus* and *B. subtilis*) and Gram-negative bacteria (*P. aeruginosa* and *E. coli*). Incubation of garlic extracts made up of 1% DDS and 88% DTS did not show growth inhibition against all the tested microorganisms, whereas garlic oils with higher quantities of DDS showed significant inhibitory activity, increasing with the increase of DDS amount, thus implicating the DDS as the active antimicrobial agent (Avato et al. 2000). It has been reported that allicin (MIC, 6 μg/mL; MBC, 6 μg/mL) was more potent than DDS (MIC range, 100–200 μg/mL; MBC range, 100–200 μg/mL), its corresponding sulfide, but of a strength similar to that of diallyl tetrasulfide (MIC range, 3–6 μg/mL; MBC range, 3–6 μg/mL) against *H. pylori* (O'Gara et al. 2000). Kyung and Fleming (1997) investigated the different S-compounds found in cabbages on the growth of 15 bacteria and 4 fungi. *S*-Methyl-L-cysteine sulfoxide, sinigrin, and dimethyl sulfide at 500 ppm did not inhibit the

growth of any of the bacteria and yeasts. Dimethyl disulfide at 500 ppm retarded the growth of some bacteria, but was not bactericidal to any of the test microorganisms. Dimethyl trisulfide, methyl methanethiosulfinate and methyl methanethiosulfonate had MICs of 200 ppm, between 50 and 200 ppm, and between 20 and 100 ppm, respectively for bacteria, and 20 ppm, between 6 and 10 ppm and between 50 and 500 ppm for yeasts, respectively (Kyung and Fleming 1997).

There are numerous reports showing the effectiveness of garlic or allicin as antimicrobial agents in comparison to antibiotics (Fujisawa et al. 2009; Cai et al. 2007). Also, allicin with antibiotics may synergistically augment the antimicrobial actions (Cai et al. 2007; An et al. 2009). Besides, thiosulfinates and their derivatives show promising activity against multidrug resistant bacteria including MRSA (Ankri and Mirelman 1999; Fujisawa et al. 2009). The main mode of action of thiosulfinate derivatives have been proposed to be due to its chemical reaction with the thiol groups of various enzymes (Ankri and Mirelman 1999) and thus antimicrobial properties of allicin may be abolished by cysteine, coenzyme A and glutathione (Fujisawa et al. 2009). Antimicrobial activity of the diallyl sulfides has been reported to increase with the number of sulfur atoms (O'Gara et al. 2000).

1.4.2 Glucosinolates

Glucosinolates are the sulphur-containing metabolites found in large number of edible plants. Over 120 glucosinolates are present in 16 families of dicotyledonous angiosperms, most of which are clustered within the Brassicaceae and Capparaceae (Fahey et al. 2001). Allyl (sinigrin) and 3-butenyl (gluconapin) glucosinolate are found in brown mustard, *p*-hydroxybenzyl glucosinolate in white mustard, allyl and other glucosinolate in horseradish and wasabi, methylthiopropyl in cabbage and 2-hydroxy 3-butenyl glucosinolate in rapeseed (Fig. 1.4; Fahey et al. 2001; Mithen 2006).

The antibacterial and antifungal properties of glucosinolates are known for a long time (Fahey et al. 2001). Intact glucosinolates do not show antimicrobial action, but the hydrolysis products of glucosinolates are active against various microorganisms (Manici et al. 1997; Tierens et al. 2001). Aires et al. (2009a) observed that the *in vitro* growth inhibition and the sensitivities of the individual bacteria are influenced by the structure of glucosinolates and their hydrolysis products. The most effective glucosinolate hydrolysis products were the isothiocyanates; sulforaphane and benzyl isothiocyanate were the strongest inhibitory against the growth of human pathogenic bacteria. Regarding action of glucosinolates products on the type of bacteria, 4-methyl sulfinyl butylisothiocyanate exhibited antibacterial activity against a larger range of bacteria. Indole-3-carbinol had some inhibitory effects against the Gram-positive bacteria, but had no effect against the Gram-negative bacteria. Indole-3-acetonitrile had some inhibitory activity against the Gram-negative bacteria. Glucosinolates, nitriles and amines were ineffective at the doses up to 3 μmol (Aires et al. 2009b). Saavedra et al. (2010) evaluated the *in vitro* antibacterial actions of

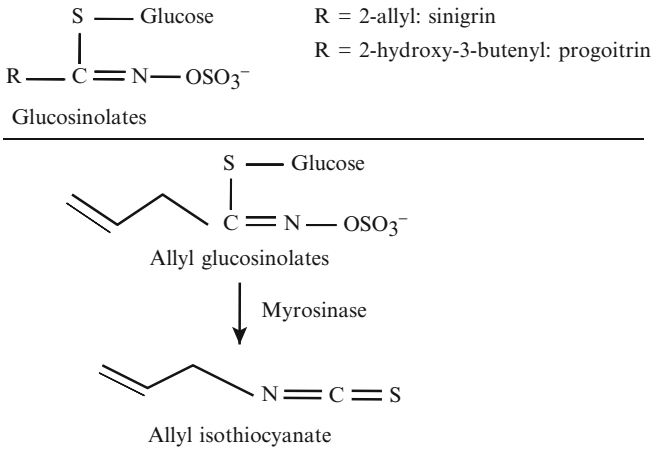


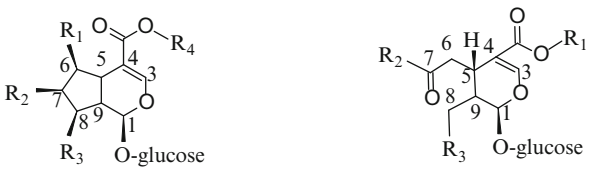
Fig. 1.4 Common glucosinolates found in Brassicaceae family: (**a**) in intact plants and (**b**) enzymatic conversion of allyl glucosinolate to allyl isothiocyanate, a potent antibacterial compound

different classes of common dietary phytochemicals, i.e. simple phenolics – tyrosol, gallic acid, caffeic acid, ferulic acid, and chlorogenic acid; chalcone – phloridzin; flavan-3-ol – (−) epicatechin; secoiridoid – oleuropein glucoside; glucosinolate hydrolysis products – allyl isothiocyanate, benzyl isothiocyanate and 2-phenylethyl isothiocyanate) against four pathogenic microbes. All of the isothiocyanates had significant antimicrobial activities, while the phenolics were much less efficient. No antimicrobial activity was observed with phloridzin. Allyl isothiocyanate from cabbage had an MIC between 50 and 500 ppm for bacteria and between 1 and 4 ppm for yeasts (Kyung and Fleming 1997).

1.5 Iridoids and Secoiridoids

Iridoids is a group of cyclic monoterpenoids having iridane skeleton (cis-2-oxabicyclo-(4.3.0)-nonane), which mostly remain as glycosides (Fig. 1.5; Perez et al. 2005). Secoiridoids derive from iridoids by the elimination of the link 7–8 to yield the basic structure (Perez et al. 2005). This group of phytochemicals is found in a number of folk medicinal plants and many of them possess significant biological and pharmacological activities (Dinda et al. 2009).

A number of iridoids and secoiridoids (nepetalactones from Serbian *Nepeta* species**,** Nestorović et al. 2010; plumericin and isoplumericin from the stem-cut latex of *Himatanthus sucuuba*, Silva et al. 2010; Cantleyoside dimethyl acetal from the aerial parts of *Pterocephalus perennis*; Graikou et al. 2002**)** from different plants (Chinese medicinal plant *Cymbaria mongolica*, Dai et al. 2002; aerial parts of the



Iridoids

Loganin,

R_1=H, R_2=OH, R_3=CH_3, R_4=CH_3

Loganic acid,

R_1=H, R_2=OH, R_3=H, R_4=H

Secoiridoids

Secologanin,

R_1=CH_3, R_2=H, R_3=CH_2

Fig. 1.5 Structures of iridoids and secoiridoids

Argentinean plant *Caiophora coronata,* Khera et al. 2003; aerial parts of *Verbena littoralis* (Verbenaceae), Castro-Gamboa and Castro 2004; roots of *Patrinia rupestris,* Yang et al. 2006b) have been reported to have antibacterial and antifungal properties. Three iridoids, phloyoside1, phlomiol, and pulchelloside 1, isolated from the rhizomes of the Iranian flora *Eremostachys laciniata* (Lamiaceae) had low to moderate levels of antibacterial activity (MIC = 0.05–0.50 mg/mL) against five bacterial strains, *Bacillus cereus*, *Citrobactor freundii*, *Proteus mirabilis*, *P. aeruginosa*, *S. aureus* (Modaressi et al. 2009). Out of these three compounds, pulchelloside 1 showed highest antibacterial activity against *B. cereus*, penicillin- resistant *E. coli*, *P. mirabilis* and *S. aureus* with an MIC value of 0.05 mg/mL.

Nestorović et al. (2010) investigated the nepetalactones content in the methanol extracts of the shoot cultures of three endemic Serbian Nepeta species: *Nepeta rtanjensis*, *N. sibirica* and *N. nervosa*, and evaluated the antimicrobial activity of these extracts against eight bacterial strains *E. coli*, *P. aeruginosa*, *S. typhimurium*, *Listeria monocytogenes*, *Enterobacter cloacae* (human isolate), *B. cereus* (clinical isolate), *Micrococcus flavus* and *S. aureus,* and eight fungal species: *Aspargillus flavus*, *Aspargillus fumigatus*, *Aspargillus niger*, *Fusarium sporotrichoides*, *Fulvia fulvum, Penicillium funiculosum, P. ochrochloron* and *Trichoderma viride*. *Trans, cis*-nepetalactone was present in shoots of *N. rtanjensis*, while *cis,trans*-nepetalactone stereoisomer was present in *N. sibirica*. No nepetalactone was observed in shoots of *N. nervosa*. All these extracts had significant antibacterial and antifungal activities against all the tested species. *N. rtanjensis* extract showed the strongest antibacterial activity with MIC of 50 μg/mL. *N. nervosa* and *N. sibirica* extracts showed antibacterial activities with MIC of 50–100 and 100 μg/mL, respectively. Similarly, *N. rtanjensis, N. nervosa* and *N. sibirica* extracts showed MIC of 25–5, 50–100 and 25–100 μg/mL, respectively. The presence of trans-nepetalactone in *N. rtanjensis* extract was probably responsible for strongest activity against bacteria and fungi, while cis-nepetalactone in *N. sibirica* extract showed higher antibacterial and antifungal activity than that of *N. nervosa* extract.

Iridoids compounds also exhibit potent antiviral action. A number of swerilactones, which are secoiridoids, isolated from endemic Chinese herb *Swertia mileensi* exhibited significant *in vitro* anti-hepatitis B virus activity on the Hep G 2.2.15 cell line with IC_{50} values ranging from 1.53 to 5.34 μM (Geng et al. 2009a, b, 2011). Iridoid aglycone moieties, but not its glycosides, exhibit the antiviral activities. Zhang et al. (2009) studied an anti-hepatitis C virus pseudoparticles (HCVpp) entry essay on both aqueous and methanol extracts of the flowering tops of *Lamium album*. Iridoid glucoside lamalbid isolated from the methanol extract was inactive against HCVpp, whereas its aglycone, and epimers named lamiridosins A and B present as major constituents in the aqueous extract significantly inhibited *in vitro* HCV entry (IC50 value of 2.31 μM). These were nontoxic to the Hep G2 2.2 cells at a concentration of 50 μg/mL. They also demonstrated that the parent iridoid glycosides did not show anti-HCV entry activity, but the aglycones of shanzhiside methyl ester, loganin, loganic acid, verbenalin, eurostoside and picroside II exhibited significant anti-HCV entry and anti-infectivity activities.

1.6 Saponins

Chemically, saponins are a group of high molecular-weight glycosides, in which saccharide chain units (1–8 residues) are linked to a triterpene (triterpene saponins) or steroidal (steroid saponins) aglycone moiety, i.e. sapogenin (Fig. 1.6). They occur in a wide variety of plants with triterpene saponins (in soybean, alfalfa, quillaja, and guar), and are more widely distributed in nature than steroidal (in yucca, tomato, and oats) saponins (Hostettmann and Marston 1995). The steroidal saponins may possess furostanol or spirostanol (e.g. smilagenin and sarsapogenin) moiety. The saccharide chains are commonly attached at the C3 position (monodesmosidic), but some sapogenins contain two saccharide chains (bidesmosidic) attached at the C3 and C17 (via C28) position (Vincken et al. 2007). A large number of saponins could be possible depending upon the modifications of the ring structure of aglycone moieties and number of sugars added to it, and in turn producing different biological properties.

Many plant extracts containing saponins from various plants and purified saponins show antimicrobial activities at different concentrations (Sen et al. 1998; Avato et al. 2006). However, the types of saponins exhibit different spectra of antimicrobial effects. Oleanolic acid isolated from the root bark of *Newbouldia laevis* have broad-spectrum antimicrobial activity against 6 Gram-positive, 12 Gram-negative bacterial species and three *Candida* species (Kuete et al. 2007). β-sitosterol-3-*O*-β-d-glucopyranoside isolated from this plant also showed antibacterial effects on three Gram-positive, six Gram-negative bacterial species and three *Candida* species. A saponin fraction from the stem of *Y. schidigera* exhibited potent growth-inhibitory activity with MIC ranging from 31.3 to 125 μg/mL against certain food-deteriorating yeasts (*C. albicans*), film-forming yeasts (*Debaryomyces hansenii, Pichia nakazawae, Zygosaccharomyces rouxii*), dermatophytic yeasts

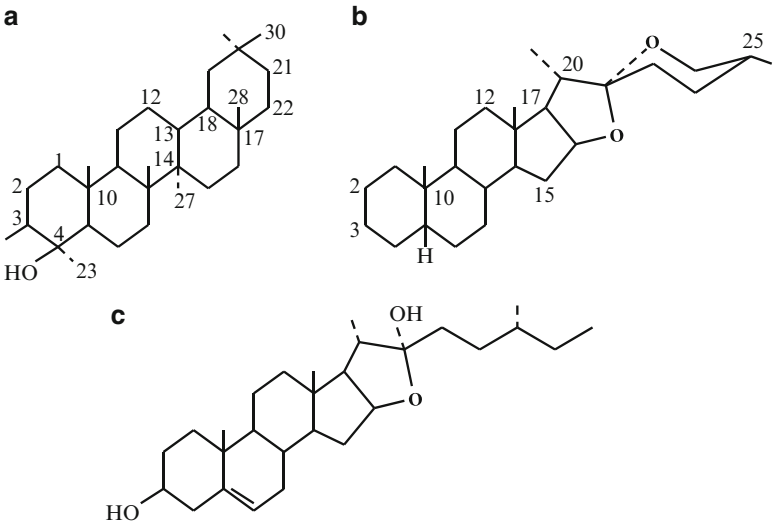


Fig. 1.6 Chemical structures of sapogenins: triterpenoid, for example, oleanane (**a**); steroids, for example, spirostanol (**b**) and furostanol (**c**)

(*Candida famata, Hansenula anomala, Pichia carsonii*), and against brewer's yeast (*Saccharomyces cerevisiae*) (Miyakoshi et al. 2000). Different saponins, i.e. tigogenin from *Tribulus terrestris*, dioscin from the rhizomes of *Smilacina atropurpurea*, minutosides from bulb of *Allium leucanthum* were very active against different fungal strains such as *C. albicans*, *C. glabrata* and *Cryptococcus neoformans* (Zhang et al. 2006a, b; Barile et al. 2007). Saponins appear to have stronger activities against fungi, and act by disrupting the membrane integrity of fungal cells.

Different extraction procedures and storage may affect the antimicrobial action of saponins probably due to chemical transformation of saponins (Guclu-Ustundag and Mazza 2007). Commercially produced quillaja (*Quillaja saponaria*) and yucca (*Yucca schidigera*) saponins showed different antibacterial activities against *E. coli*, suggesting that saponins from various commercial sources differ in their biological activities (Sen et al. 1998). In this study, commercial saponin-rich quillaja and yucca extracts exhibited antibacterial activity against *S. aureus* and *E. coli* at different concentrations. The antimicrobial activity of saponins may also be modified by the pH of media. The tea saponins exhibited greater antimicrobial activities against Gram-positive *S. aureus* (MIC <0.006 vs. >0.2), Gram-negative *E. coli* (MIC 0.003 vs. >0.2) and *C. albicans* (MIC <0.006 vs. >0.2) at low pH 4 than high pH 8.5 (Li et al. 2009).

Some saponins, in general, exhibit stronger antimicrobial activity against Gram-positive bacteria than against Gram-negative bacteria (Avato et al. 2006). Saponins fraction from soapnut pericarps (*Sapindus mukurossi*, Tanaka et al. (1996) and guar (*Cyamopsis tetragonoloba*, Hassan et al. 2010a, b) showed greater antibacterial activity against Gram-positive bacteria than against Gram-negative

bacteria. Conversely, saponins isolated from orchid tree (*Bauhinia variegata* L.) bark exhibited greater antibacterial activity for Gram-negative bacteria than Gram-positive bacteria at concentrations ranging from 2.5 to 10 mg/mL (Morrissey and Osbourn 1999).

The relationships between saponin structures and antimicrobial activity are strongly noted. The structure of sapogenin moiety, chain length and composition of sugars influences the antimicrobial activities. The *Y. schidigera* saponin fraction possessing a trisaccharide chain without any oxygen functionalities at C-2 and/or C-12 of the aglycone exhibited potent anti-yeast activity, while saponins with 2b-OH or 12-keto groups showed very weak or no activity. Low activity was observed for saponins with a disaccharide chain and no activity was observed for the aglycones obtained after acid hydrolysis (Miyakoshi et al. 2000). Yang et al. (2006a) noted that no activity was observed in the hecogenin saponins when its sugar moiety was less than four monosaccharide units. Pentaglycoside was more active than tetraglycoside and shows extended antifungal spectrum against *A. fumigatus*. In the diosgenin saponin series, saponins with only triglycosides are active against *C. albicans* and *C. glabrata*, while the diosgenin saponins with monoglycoside and diglycoside did not show any activity. Again, within the group of tigogenin saponins, their antifungal capacity was slightly influenced by the composition of the sugar moiety. The replacement of a glucosyl unit with a xylosyl unit showed enhanced activity against *A. fumigatus*. Avato et al. (2006) suggested that the sugar moiety is not important for the antimicrobial efficacy from their study since antibacterial activity increased from the saponin extracts to the sapogenin samples.

1.7 Terpenoids/Essential Oils

Terpenoid compounds derive from a basic structure of C5 isoprene units. They are classified according to the number of isoprene unit involved for their synthesis, i.e. monoterpenoid (C10), sesquiterpenoids (C15), diterpenoids (C20), sesterterpenoids (C25) and triterpenoids (C30). They can be acyclic (myrcene and geraniol), monocyclic (cymene and carvacrol), bicyclic (pinene) and tricyclic with different groups (alcohol, phenol, and aldehyde). The most commonly occurring essential oils (EO) are included in two chemical groups (Fig. 1.7): terpenoids (monoterpenoids and sesquiterpenoids) and phenylpropanoids, which are synthesized through mevalonate and shikimic acid metabolic pathways, respectively (Gershenzon and Croteau 1991; Calsamiglia et al. 2007). Among these two classes, terpenoids are the more diversified group of plant bioactives abundantly found in many herbs and spices (Gershenzon and Croteau 1991). Within terpenoids, the most important components of EO of the majority of plants belong to the monoterpenoids and sesquiterpenoids (Gershenzon and Croteau 1991; Calsamiglia et al. 2007). Phenylpropanoids have a side chain of three carbons bound to an aromatic ring of C6 (Calsamiglia et al. 2007). Phenylpropanoids are less abundant compounds of EO compared with terpenoid family, but some plants contain in significant proportions. The EO are a group of

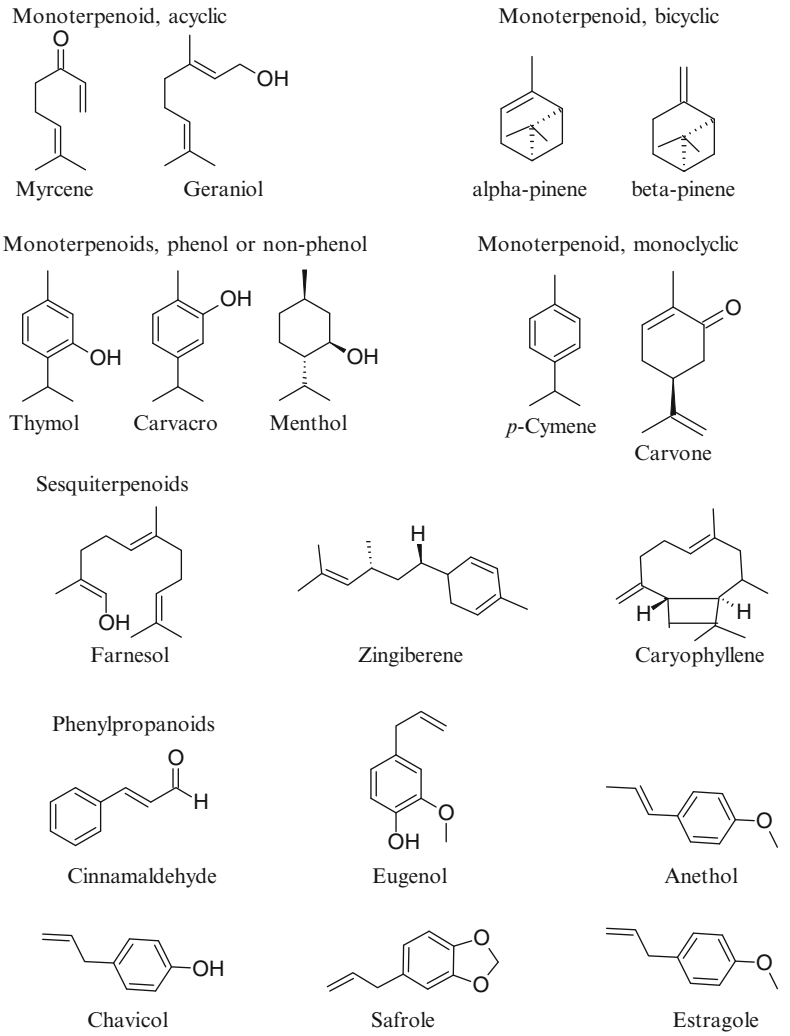


Fig. 1.7 Chemical structures of different components of essential oils

secondary plant metabolites obtained from volatile fractions of plants by steam distillation process (Gershenzon and Croteau 1991). The EO are used traditionally by humans, for many centuries, which provide characteristic flavor and aroma specific to many plants, and are used as antimicrobial agents and preservatives. The EO have diverse chemical composition, nature and biological properties. The EO can be obtained from flowers, petals, leaves, stems, fruits, roots and barks and the concentrations of EO in these parts depends upon the stage of growth, environmental conditions (Hart et al. 2008).

A number of EO are known for their strong anti-microbial activities against many pathogenic and non-pathogenic bacteria and fungi. Curcumin and its derivatives,

the phenylpropanoids, are the principal compounds in rhizome of *Curcuma longa* (turmeric), which exhibit antibacterial properties against different bacteria and fungi. Essential oil fractions of turmeric inhibited the growth of pathogenic Gram-positive (*S. aureus* and *Staphylococcus epidermidis*) and Gram-negative (*E. coli*, *P. aeruginosa* and *S. typhimurium*) bacteria (Singh et al. 2002). The EO fraction was more effective against Gram-positive compared to Gram-negative strains, and was comparable to standard antibiotics gentamycin, ampicillin, doxycycline and erythromycin in these strains (Singh et al. 2002). A recent study by De et al. (2009) demonstrated that curcumin inhibited the growth of different clinical isolates of *H. pylori* with MICs ranging from 5 to 50 μg/mL. The gingerols, another phynylpropanoids from *Zingiber officinalis* (zinger), possess antifungal and antibacterial properties (Park et al. 2008). Ginger extract containing gingerol inhibited the growth of *H. pylori* with MICs ranging from 0.8 to 12.5 μg/mL (Mahady et al. 2003).

Constituents of EO differ in their antimicrobial activity against bacteria and fungi. Investigating the antimicrobial properties (18 bacterial species and 12 fungi) of five EO constituents (cineole, citral, geraniol, linalool and menthol), Pattnaik et al. (1997) showed that linalool had the most antibacterial activity and inhibited 17 bacteria, followed by cineole, geraniol (each of which inhibited 16 bacteria), menthol and citral aromatic compounds, which inhibited 15 and 14 bacteria, respectively. However, the antifungal activities of these EO constituents did not follow the pattern of antibacterial activities. Citral and geraniol oils were the most effective against fungi (inhibiting all 12 fungi), followed by linalool (inhibiting 10 fungi), cineole and menthol (each of which inhibited 7 fungi) compounds (Pattnaik et al. 1997).

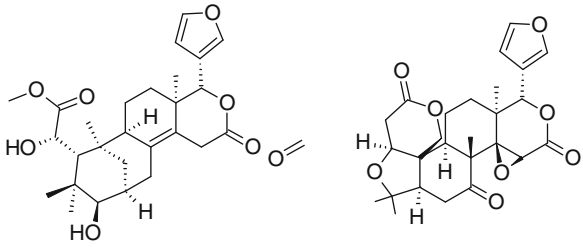
It has been suggested that the pH of EO in culture media may modify antimicrobial properties. For example, anise oil had higher antifungal activity at pH 4.8 than at 6.8, while the oil of *Cedrus deudorawas* was most active at pH 9 (Janssen et al. 1987). The structure and stereochemistry of the essential oils have profound influences on the antimicrobial activities. Alkenyl substituents incorporated into nonphenolic ring structures of essential oils such limonene showed increased antibacterial activities compared with alkyl substituents such as *p*-cymene with alkylation showing more inhibitory effect on Gram-negative bacteria (Dorman and Deans 2000). From stereochemistry of EO, it has been reported that α-isomers such as α-pinene are less active relative to β-isomers such as geraniol and nerol; *cis*-isomers are inactive contrary to *trans*-isomers; compounds with methyl-isopropyl cyclohexane rings are the most active; or unsaturation of the cyclohexane ring further increases the antibacterial activity, e.g. terpinolene, terpineol and terpineolene (Hinou et al. 1989; Dorman and Deans 2000). However, Griffin et al. (1999) reported that the specificity and level of antimicrobial activity of terpenoids were not always characterized by the functional groups, but were associated with hydrogen-bonding parameters, and for Gram-negative organisms a combination of hydrogen-bonding parameters and molecular size parameters. The antimicrobial properties of EO from different sources have been discussed in details elsewhere (Chap. 5).

1.8 Limonoids (Tetranortepenoids)

Chemically, limonoids are unique secondary metabolites, characterized by a tetranortriterpenoid skeleton with a furan ring (Fig. 1.8). They are commonly isolated from Citrous and Maliaceae plants (Hallur et al. 2002; Rahman et al. 2009; Vikram et al. 2010). Besides their health promoting effects, various limonoids have been shown to possess antibacterial, antifungal and antiviral effects (Govindachari et al. 2000; Battinelli et al. 2003; Atawodi and Atawodi 2009).

Various limonoid compounds such as mahmoodin, azadirone, epoxyazadiradione, nimbin, gedunin, azadiradione, deacetylnimbin and 17-hydroxyazadiradione, isolated from various parts of *Azadirachta indica* (Meliaceae family) have been reported to have antimicrobial activities (Siddiqui et al. 1992; Govindachari et al. 2000; Atawodi and Atawodi 2009). Rahman et al. (2009) tested two limonoids isolated from the seeds of *Swietenia mahagoni* (Meliaceae family), swietenolide and 2-hydroxy-3-O-tigloylswietenolide against various multiple-drug-resistant bacterial strains including Gram-positive (*S. aureus, S. pneumoniae* and *Haemophilus influenzae*) and Gram-negative (*E. coli, Klebsiella pneumoniae, Salmonella typhi,* and *Salmonella paratyphi*) strains. The most potent activity of swietenolide was observed against *H. influenzae*, *S. typhi*, and *S. paratyphi*, whereas 2-hydroxy-3-O-tigloylswietenolide was most active against *S. pneumoniae, S. typhi,* and *S. paratyphi*. The lowest activity was observed against *K. pneumoniae* for both compounds. The limonoids compounds may exhibit antibacterial properties against pathogenic bacteria by disrupting the quorum sensing system and biofilm production. Vikram et al. (2010) demonstrated limonin, nomilin, obacunone, deacetyl nomilin and limonin 17-O-β-D-glucopyranoside purified from seeds of grapefruits to possess the anti-quorum sensing activity and inhibitory effect on biofilm formation of pathogenic *E. coli* O157:H7 with obacunone exhibiting strong antagonistic activity.

Limonoids also have significant antiviral activity. Limonin and nomilin showed inhibitory effects on HIV-1 replication in peripheral blood mononuclear cells and monocytes/macrophages, which was not cytotoxic at the active concentrations (Battinelli et al. 2003). The antiviral activity was not much influenced by structural differences by limonin and nomilin in this study (Battinelli et al. 2003).



Swietenolide Limonin

Fig. 1.8 Chemical structures of limonoid compounds

Parida et al. (2002) demonstrated in an *in vivo* study that azadirachtin obtained from *A. indica* inhibited dengue virus type-2 replication as confirmed by the absence of dengue-related clinical symptoms in sucking mice and absence of virus specific 511 bp amplicon.

1.9 Polyacetylenes

More than 700 polyacetylene compounds have been characterized from plants, which are mainly prominent in the Asteraceae, Apiaceae and Campanulaceae including many medicinal plants from various parts of the world (Hudson 1989). Food plants of the Apiaceae plant family such as carrots, celery, parsley, fennel and parsnip contain a group of bioactive aliphatic C17-polyacetylenes including falcarinol, falcarindiol, panaxydiol, and polyacetylene 8-O-methylfalcarindiol (Zidorn et al. 2005; Christensen and Brandt 2006).

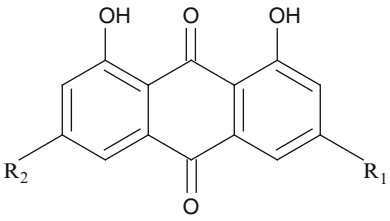
Avato et al. (1997) investigated the different polyacetylene compounds from the aerial organs of *Bellis perennis* L. Of the major constituents, methyl deca-4,6-diynoate and deca-4,6-diynoic acid, and their structural analogues, i.e. deca-4,6-diyne, dimethyl octa-3,5-diyne-1,8-dioate and deca-4,6-diyne-1,10-dioic acid, deca-4,6-diynoic acid and deca-4,6-diyne-1,10-dioic acid showed antimicrobial activity against Gram-positive and Gram-negative bacteria, respectively.

Polyacetylene carboxylic acids, 13(*E*),17-octadecadiene-9,11-diynoic acid (13,14-dihydrooropheic acid, and the known 17-octadecene-9,11,13-triynoic acid (oropheic acid, isolated from the stem bark of *Mitrephora celebica* demonstrated significant activity against MRSA and *Mycobacterium smegmatis* (Zgoda et al. 2001). Similarly, pentayne diol, a polyacetylene which was isolated from *Bidens pilosa* (a traditional medicinal herbs) showed highly potent and extensive inhibitory activities against several Gram-positive and Gram-negative pathogenic bacterial species, including MRSA, and vancomycin-resistant *Enterococcus faecalis* and *C. albicans* (Tobinaga et al. 2009). In a recent finding, a polyacetylene compound from *Carlina acaulis*, i.e. carlina oxide exhibited strong antibacterial activity against two MRSA strains, *Streptococcus pyogenes, P. aeruginosa, C. albicans*, and *C. glabrata* with less toxicity to human HeLa cells (Herrmann et al. 2011).

1.10 Anthranoids

Anthranoid compounds are widely distributed in various plants particularly in *Aloe*, *Cassia, Rheum*, *Cassia* and *Frangula*, which are traditionally used in ethnomedicine for laxative and cathartic action (Paneitz and Westendorf 1999). Naturally occurring anthranoids can be chemically described as dihydroxyanthraquinones, -dianthrones and -anthrones, often present in plants as glycones (Table 1.2; Paneitz

Table 1.2 Chemical structures of some anthranoids (Paneitz and Westendorf 1999)



Anthranoids	R1	R2
Emodin	CH_3	OH
Rhein	COOH	H
Aloe-emodin	CH_2OH	H
Physcion	CH_3	OCH_3
Chrysophanol	CH_3	H

and Westendorf 1999). Different anthranoids such as aloe-emodin, rhein, emodin, physcion and chrysophanol occur in *Rheum* species.

Anthranoids have shown antimicrobial properties in different studies. The anthranoid compounds from the rhizome of *Rheum emodi* exhibited antibacterial and antifungal activities (Babu et al. 2003). The antimicrobial effects of the three anthraquinones on *S. aureus* found to be in the order of rhein > emodin > 1,8-dihydroxyanthraquinone (Wu et al. 2006). Similarly, Wang et al. (2010) demonstrated that the sequence of antimicrobial activity against *Bifidobacterium adolescentis* of the five hydroxyanthraquinones was rhein > emodin > aloe-emodin > chrysophanol > physician. They also suggested the influence of substituent groups on phenyl ring in hydroxyanthraquinones against *B. adolescentis* activity might be related with the polarity and the sequence was carboxyl > hydroxyl > hydroxylmethyl > methyl and methoxyl. Prenylated anthranoids from leaves of *Harungana madagascariensis* have shown to inhibit *Bacillus megaterum* (Kouam et al. 2007). Additionally, the effect of emodin with antibiotics (ampicillin and oxacillin) was found to be synergistic or partially synergistic against MRSA, where emodin reduced the MICs of the antibiotics (Lee et al. 2010). However, some of the anthranoids have potent mutagenic effect (Paneitz and Westendorf 1999), which is required to consider when evaluating the antimicrobial properties of these compounds.

1.11 Conclusions and Future Prospects

There is considerable evidence that a number of phytochemicals have potential to become useful antimicrobial agents that could be employed as preventative or treatment therapies against microbial and viral diseases. Although, there are some encouraging effects *in vivo* to inhibit pathogenic microbes without affecting beneficial bacteria in the gastrointestinal tracts, more studies would be required for the

safety and efficacy of these phytochemicals to establish whether they could offer therapeutic benefits over conventional therapies.

Besides, the combination of some antimicrobial drugs and phytochemicals may act as better antimicrobial agents than antimicrobial drugs alone. For example, the application of dual combinations demonstrated synergy between streptomycin and gallic acid, ferulic acid, chlorogenic acid, allylisothiocyanate and 2-phenylethylisothiocyanate against the Gram-negative bacteria. Moreover, they can act synergistically with less efficient antibiotics to control bacterial growth (Saavedra et al. 2010). 3,4-dihydroxyphenylacetic acid and 3-hydroxyphenylacetic acid increased the susceptibility of *S. enterica* subsp. *enterica* serovar Typhimurium strains for novobiocin. In addition, organic acids present in berries, such as malic acid, sorbic acid, and benzoic acid, were shown to be efficient permeabilizers of *Salmonella* as shown by an increase in the 1-N-phenylnaphthylamine uptake assay and by lipopolrsaccharide release (Alakomi et al. 2007). Cinnamon essential oil and its major component (trans-cinnamaldehyde) enhanced the antibacterial activity of clindamycin against a toxicogenic strain of *Clostridium difficile* (Shahverdi et al. 2007). In addition, the enhancement activity of different essential oils (*Mentha longifolia* L. and *Mentha spicata* L.) and different monoterpenes (piperitone, carvone and menthone) on the antibacterial activity of nitrofurantoin has been reported (Rafii and Shahverdi 2007; Shahverdi et al. 2004). The antibacterial activity of cefixime, cephotaxime, vancomycin and tetracycline was also increased by curcumin (Moghaddam et al. 2009). Allicin has a synergistic effect with amphotericin B against *C. albicans* via enhancing the phospholipid peroxidation reaction *in vitro* and *in vivo*, which suggests that allicin could reduce the amphotericin B dose to lessen side effects (An et al. 2009). Due to the growing use of phytochemicals and other dietary phytochemical-rich supplements, it is required to understand whether problems might arise from using these preparations in combination with conventional drugs. There is lack of comprehensive studies that can establish the consequences of phytochemicals-drug interactions. However, all these evidence also suggest that intake of phytochemicals rich foods could be considered in future research while antimicrobial agents are applied to the body.

Plant genomes contain 20,000–60,000 genes, and about 15–25% of these genes encode enzymes for secondary metabolism (Bevan et al. 1998; Somerville and Somerville 1999). The genome of a plant species encodes only a small fraction of all the enzymes that are required to synthesize the entire set of secondary metabolites found throughout the plant kingdom (Pichersky and Gang 2000). Identification of particular genes for target phytochemicals and the genetic engineering techniques could allow expressing the biosynthetic pathways of some phytochemical synthesis in organisms such as *E. coli*, *B. subtilis* or *S. cerevisiae*. For example, Miyahisa et al. (2006) reported that introduction of four genes for a phenylalanine ammonia-lyase, cinnamate/coumarate:CoA ligase, chalcone synthase, and chalcone isomerase, in addition to the acetyl-CoA carboxylase, in *E. coli* cells resulted in efficient production of (2S)-naringenin from tyrosine and (2S)-pinocembrin from phenylalanine. Finally, the possibility of using phytochemicals as antimicrobial compounds would be a paradigm shift towards the potential health benefits and safety overcoming the problem of microbial resistance to drugs.

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