Chapter

10

ANTIMICROBIAL COMPOUNDS FROM PLANTS

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10.1. INTRODUCTION

The increased spread of drug-resistant microorganisms, due to the indiscriminate and irrational use of antibiotics, has become a current threat in the therapy of microbial diseases that these days has led to the selection of new resistant strains of bacteria, genetically changed. It is known that antibiotic resistance is generally caused by spontaneous mutations in specific genes, but bacterial drug resistance mechanisms are more complex than they appear. In many cases, the effectiveness of common antibiotics is lost after a period of a few years. The World Health Organisation (WHO) reports that there are internationally high levels of antimicrobial resistance (AMR) in common bacteria alongside limited understanding and uncoordinated surveillance of AMR [1]. In the last decades there is a more prevalent resistance in cases of bacterial infections such as respiratory tract infections, diarrhea, meningitis, syphilis, gonorrhea and tuberculosis [2]. *Staphylococcus aureus* isolated from clinical samples are now showing resistance to more than three drugs and are considered as multiple-drug resistant bacteria [2] Antibiotic resistance is a natural phenomenon, but the abuse and overdose have caused numerous resistance problems. The rise of multidrug resistance (MDR) pathogens seems to be caused by the continuous selective pressure, and the emergence of new survival strategies of bacteria are in response to the new classes of antibiotics [3]. In addition, the incorrect and inappropriate use of antibiotics has increased the prevalence of resistant bacteria. Also, for fungi and protozoa, current chemotherapeutic options are very limited and characterized by side effects or toxicity.

If the situation remains unchanged, the number of deaths attributable to MDR will be more than 10 million in 2050, higher than the number of cancer-associated deaths (8.2 million *per annum*) [3]. On average, the cost of producing a new antibiotic is higher than the cost in the 1990s by up to 60 % [3].

Consequently, researchers are currently looking for new sources of antibiotics with a broad spectrum of action against Gram-negative and Gram-positive bacterial strains, with as few as possible side effects [2]. The search for new therapeutics related to the urgent development of new, more effective drugs and to the eradication of MDR pathogens that can cause deadly infections represents a major problem for present and future.

A renewable source of antibacterial, antifungal and antiparasitic compounds is represented by plants, belonging to the most different genera. Plants are characterized by a large structural and functional diversity of their compounds synthesized in all morphological parts as secondary metabolites, with various functions in plant survival.

Many of the contemporary pharmaceutics, cosmetics, and food industries are founded on the knowledge of the properties of medicinal plants. The antimicrobial compounds extracted from plants can be used for applications in treating infectious, systematic and inflammatory diseases, in food preservation and agriculture, against phytopathogens [4].

New plant compounds with diverse chemical structure and mechanisms of action have been extensively studied by ethnopharmacologists, botanists, microbiologists and chemists. These phytochemicals are considered to be effective, safe, and natural compounds, with no or lower side effects. During the growing stages, plants synthesize a wide variety of secondary metabolites, such as flavonoids, alkaloids, tannins, terpenoids, peptides and others, which have been found *in vitro* to have antimicrobial properties. The antimicrobial activity of plant-derived extracts is correlated with the plant species, local climatic and environmental conditions, harvesting conditions, and extraction technology [5].

In food industry, the antimicrobial properties of many aromatic plants were used for the control of spoilage and harmful pathogenic bacteria, along with chemical preservatives, whose use is limited due to their toxicity, carcinogenity, teratogenicity and environmental problems. The public opinion about chemical antimicrobial additives has become unfavorable and generated interest in the use of natural compounds [6].

10.2. HISTORY

The importance of medicinal plants was acknowledged at least 50,000 years ago, as evidenced by numerous archaeological excavations. The oldest medical document — the Ebers papyrus — dated to the 15th century BC, contains 800 recipes using herbs and different extracts. In a copy of the book *Pen-tsao*, a document describing medicinal plants used in China and the Far East, dated 7th century AD, nearly 400 medicinal herbs used in juices, infusions and ointments are described [7].

In India, the traditional Ayurvedic concept mentioned in the ancient Vedas was developed between 2500 and 500 BC and represents the 'science of life' and the 'science of longevity'. The Ayurvedic system promotes the use of medicinal herbs and extracts, and other special diets, exercise and lifestyle recommendations.

In the culture of Chinese, Indians, Egyptians, Romans, and Arabs, herbalism was integrated as a philosophical principle. In ancient Europe, herbal medicine was discovered later, in Greece, and was mentioned by Hippocrates of Kos. The document *Corpus Hippocratium* presents the beneficial effects of more than 400 species of plants. Another greek philosopher, Theophrastus of Eresos,

considered to be the father of botany, studied more than 500 plants. In 40–90 AD, Dioscorides wrote *De Materia Medica* containing a series of herbal treatments. In ancient Rome, Claudius Galenus (130–200 AD) studied 450 plants and the formulations of plant medication. After the fall of the Roman Empire, the Arabs introduced new medicinal plants and flavoring agents such as cloves, vanilla, camphor and nutmeg. Avicenna, the physician and philosopher, wrote nearly 500 books containing more than 700 herbal treatments. Paracelsus (1493–1541) claimed that specific substances extracted from plants are involved in the treatment of disease [7].

In the Middle Ages the cultivation of medicinal plants in abbeys and monasteries facilitated the study of therapeutic properties. In the period of the Ottoman Empire hospitals were built near Orthodox monasteries, where the medicinal plants were used in the treatment of disease.

The methods used for extraction allowed the preparation of many different drugs, and, to this day, a lot of research has been done concerning the separation of different compounds from plants. At the same time, the synthesis of plant substances was studied. The obtaining of salicylic acid from willow is considered now as the beginning of pharmaceutical industry.

With the development of medicine, plant extracts have begun to be replaced with synthetic drugs, considered more effective and easier to produce. Nowadays, the use of medicinal plants has been re-examined and the active principles and action mechanisms have been researched in numerous studies. The current tendency to use, in addition to chemical substances of synthesis, herbal preparations is increasing, and this is explained by their antimicrobial, antioxidant, anticancer and other functional properties [4].

10.3. CLASSIFICATION OF ANTIMICROBIAL COMPOUNDS

In addition to the primary metabolites (proteins, carbohydrates, fats), that play a major role in maintenance of plant viability, a series of compounds including terpenes, polyphenols, quinones, alkaloids, and peptides, which belong to the secondary metabolism, are also synthesized. In fact, the separation between primary and secondary metabolism is uncertain, as many of the primary metabolic intermediates perform similar roles in secondary metabolism. Secondary metabolites are present only in certain species, often exhibiting organ or tissue specificity, and can only be identified at a certain stage of growth and development within a species or may be activated only during periods of stress caused for example by the attack of microorganisms or nutrient depletion.

Their synthesis (Figure 1) seems to have no direct meaning for the plant cell, but it can be decisive for the development and functioning of the plant organism as a whole. As a result, given their conservation during the evolution

of the plant kingdom, it is highly plausible to suggest that secondary metabolites offer a selective advantage to species.

Plants remain the only sources of extraction of some compounds, since many of the secondary metabolites cannot be synthesized chemically, being complex stereostructures with many chiral centers that may be essential for biological activity. Out of the secondary metabolites, up to 12,000 were isolated, representing less than 10 % [8].

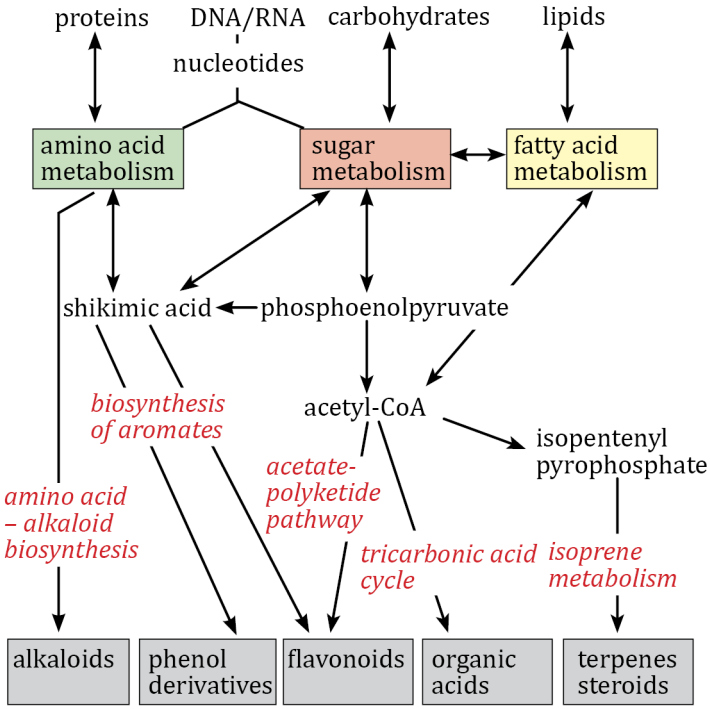
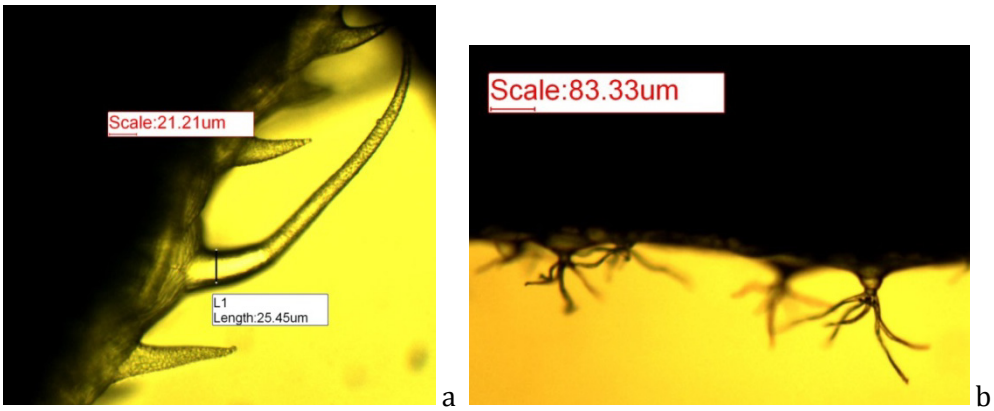


Figure 1. General scheme of biosynthetic pathways and precursors for the major classes of secondary metabolites [9]

Many plant secondary metabolites with a defensive role are secreted externally by epidermal cells or epidermal formations such as glandular hairs, that may be short or long, single or pluricelular. Many multicellular hairs (trichomes) secrete a variety of compounds, such as volatile oils, alkaloids, oleoresins, resins, and balms.

In *Mentha piperita* (*Lamiaceae* family), glandular hairs are usually formed from several secretory cells, on a short pedicel inserted into an epidermal intrusion (Figure 2).



a b

Figure 2. Glandular hairs in a) *Mentha piperita* and b) *Artemisia dracunculus*

In plants, the functional metabolites are grouped into different classes based on chemical structure and similar properties.

10.3.1. Polyphenols

Polyphenols are one of the most important and at the same time the most numerous of the secondary metabolite groups, omnipresent in the plant kingdom. At present, over 8,000 phenolic structures have been identified in a wide variety of forms, of which more than 4,000 belong to the flavonoid class, and of these, several hundred are present in edible plants [8]. Of polyphenols, a series of pigments with the quinonic structure are responsible for the color of fruits and flowers (alizarin, purpurin, benzoquinone, juglone).

Phenolic compounds are very important for plants and can have multiple functions. These molecules are generally involved in the defense against ultraviolet radiation, oxidizing agents or the aggression of some phytopathogenic agents [8], and they have a role in adapting to biotic and abiotic stress.

Polyphenols can be classified into different groups according to the number of phenolic rings they contain and the structural elements linking these rings. They are classified in phenolic acids, flavonoids (flavonones, flavones, xanthones and catechins, anthocyanins, anthocyanidins), lignans, stilbens and other polyphenols with non-flavonoid structure.

Phenolic acids are phenols possessing a carboxylic functional group, varying due to the hydroxylation or methoxylation of aromatic nucleous. Gallic acid, which is also part of the composition of hydrolysable tannins, and vanillic acid are present in almost all plants. Caffeic acid (Figure 3) is considered to be the most common phenolic compound distributed in the plant kingdom, followed by chlorogenic acid, which is known to cause allergic dermatitis. Phenols are essentially a series of natural antioxidants, used as nutraceuticals. However, it is assumed that the total content of polyphenols in plants is underestimated and many of the phenolic compounds and their derivatives have not yet been identified due to limitations of the methods used and techniques of analysis [2,3,7,10]. Phenolic compounds possessing a C3 side chain at a lower level of oxidation and oxygen-free levels are classified as essential oils and also have antimicrobial action. Eugenol in clove oil is well known as having an inhibiting action against bacteria and fungi.

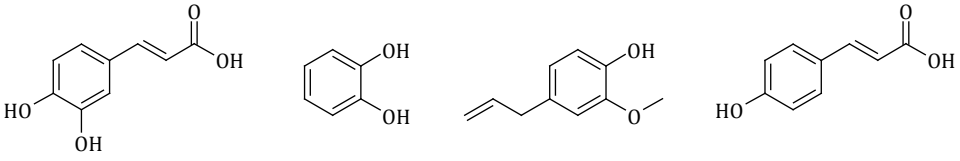
Catechol and pyrogallol are hydroxylated phenols, toxic to microorganisms. Catechol (Figure 3) has two OH groups and pyrogallol has three. The mechanisms of action responsible for phenol toxicity against microorganisms refer mainly to the inhibition of enzymes by oxidative compounds, possibly by reaction with sulfhydryl groups or by several other non-protein interactions [8]. In 2006 Kocacaliskan *et al.* investigated the antimicrobial activity of catechol and pyrogallol against three bacterial species, namely *Pseudomonas putida, P. pyocyanea*, and *Corynebacterium xerosis* and two fungal species, *Fusarium oxysporum* and *Penicillium italicum*, phytopathogenic species. Using the disc diffusion method, the authors demonstrated that bacteria were inhibited at 5 mM concentration of catechol and pyrogallol, but only catechol had an effect against the tested fungi [11]. Methanolic extract of *Diospyros kaki* Thunb. roots, containing catechol, inhibits the growth of *Clostridium difficile*, *C. perfringes* (significant inhibition for a dose of 5.0 mg/disc), *Escherichia coli* (moderate inhibition), but did not inhibit *Bifidobacterium breve*, *B. longum* and *Lactobacillus casei.* The authors claimed that *D. kaki* root-isolated catechol and its derivatives (4-nitrocatechol, 4-tert-butylcatechol, tetrabromocatechol) could be useful as preventive agents against diseases caused by harmful intestinal bacteria [12].

10.3.2. Resveratrol

A very studied compound in the last decades is resveratrol, especially known for its antioxidant properties. Resveratrol (3,5,4′-trihydroxystilbene) is a small polyphenol with 228 g mol^{-1} molecular weight, in the same time a stilbenoid compound (hydroxylated stilbene derivatives) that was first isolated in the root of white hellebore, *Veratrum grandiflorum* by M. Takaoka in 1939 [22]. Resveratrol production is stimulated by pathogens, ultraviolet (UV)-irradiation, and exposure to ozone, being considered a phytoalexin [44]. This

compound has shown antibacterial, antiviral and antifungal activity and seems to possess a significant role in regulation of immune systems, chemoprevention, neuroprotection, cardioprotection, diabetes prevention and lipid regulation. This antimicrobial compound is produced in more than 70 particular families of plants including peanuts, grapevines, pines, different kinds of berries, legumes and grasses, Scots pine (*Pinus sylvestris*), and other plants [13].

Resveratrol has antiviral activity against hepatitis C virus, respiratory syncytial virus, herpes simplex virus, varicella zoster virus, influenza virus, human immunodeficiency virus *etc.* The antiviral effect is explained by the inhibition of viral replication, nucleic acid synthesis, protein synthesis, and gene expression. The antifungal effect of resveratrol is demonstrated against pathogenic fungi like *Pyricularia oryzae*, *Plasmopara viticola*, *Cladosporium cuccumerinum*, and *Sphaeropsis sapinea* [13,14], for which the mechanism of action in unknown. In *Candida albicans*, resveratrol could penetrate the membrane and induce apoptosis through activated metacaspase and promoted cytochrome c release [15]. The antibacterial effects of resveratrol have been demonstrated in *E. coli* O157:H7 [16], *Salmonella typhimurium* [17], *Listeria monocytogenes* [18], *S. aureus*, *Vibrio cholerae* [17], *Pseudomonas aeruginosa* [19], *Campylobacter jejuni*, *Mycobacterium tuberculosis* [20], methicillin-resistant *S. aureus* (MRSA) [21], vancomycin-resistant *Enterococcus* (VRE) [20], *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and others. Resveratrol has bacteriostatic activity rather than bactericidal activity [22].



caffeic acid catechol eugenol oxybenzoic acid; B-coumaric acid

Figure 3. Chemical structures of representatives of phenolic derivatives

10.3.3. Flavones, flavonoids, and flavonols

Flavonoids are phenolic compounds found in land plants like bryophytes (hornworts, liverworts, mosses) and vascular plants (ferns, gymnosperms, angiosperms). They are synthesized in the cytoplasm of the plant cell and then accumulate in vacuoles that fuse with the central vacuole of epidermis and cortex cells. Flavonoids have a defensive function against insects, fungi and viruses, as well as against invading invertebrates.

The biological action of flavonoids is due to their ability to complex soluble and extracellular proteins and to bind to the bacterial cell wall. It has been shown that several lipophilic flavonoids may disrupt cell membranes [23,24]

Flavones (Figure 4) are flavonoids having an unsaturated 3-C chain with a C2-C3 double bond, such as flavonols, which differ in the absence of a hydroxyl group at position 3. This minor difference in structure has major consequences in biosynthesis and the physiological role of the flavones in the cell. Flavones are found especially in vascular plants in which they form glycosides and aglycones.

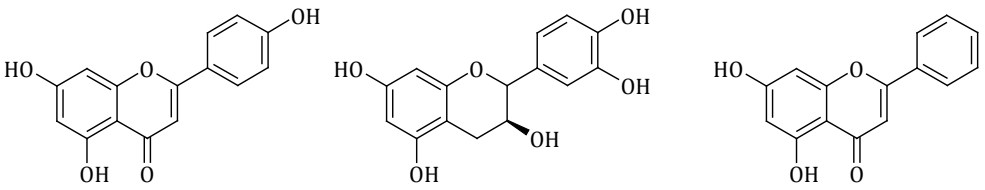


Figure 4. Chemical structures of some representative flavones and flavonoids

10.3.4. Catechins

Catechins are the most reduced form of the C3 unit in flavonoid compounds and have been extensively researched due to their presence in oolong tea and other types of tea. These compounds have been shown to have antimicrobial action against *Streptococcus mutans* [25], *Shigella* [26], *Vibrio* [27] and other pathogenic bacteria [8].

10.3.5. Quinones

Quinones are composed of aromatic rings with two ketone substitutions, divided into four classes, namely benzoquinones, naphtoquinones, phenanthrenequinones, and anthraquinones, according to the number of benzene rings in their structure (Figure 5).

Quinones are widespread in plants and mainly exist in higher plants such as *Polygonaceae*, *Rubiaceaae*, *Leguminosae, Rhamnaceae, Labiatae*, and *Boraginaceae* families. A large number of quinones are synthesized in plants *via* the shikimate or polyketide pathways [8].

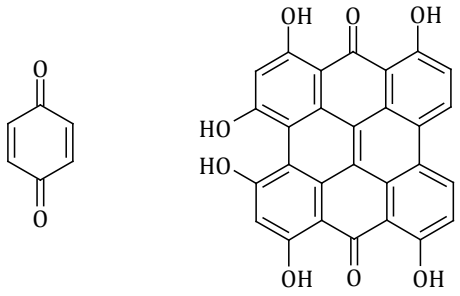
A large number of quinones possess significant biological activities, such as the antibacterial and anticancer activities of juglone and plumbagin isolated from *Juglans* and *Plumbago* [28] species. Juglone showed antimicrobial effect against

S. aureus by reducing cell wall formation and increasing membrane permeability [29].

S. Inouye demonstrated the antimicrobial activity of five terpenoid quinones from *Monarda fistulosa* against *Trichophyton mentagrophytes*, one of the major dermatophytes causing tinea infections in humans [30].

Quinones are very reactive due to their structure. They are colorful compounds responsible for browning reactions in fruits and vegetables. They are intermediaries in the synthesis of melanin in the skin [8]. In addition to antimicrobial activity, these compounds exhibit numerous other biological activities such as neurological, antiplasmodial, antioxidant, trypanocidal, antitumor, and anti-HIV. In particular, anthraquinones have a broad spectrum of antibacterial activities (including antimycobacterial) based on the inactivation and loss of bacterial protein functions, such as adhesins, cell wall polypeptides, and membrane enzymes [31]. Habbal *et al.*, in 2011, reported the presence of quinine in *Lawsonia inermis* used for henna extract, with antimicrobial activity against *P. aeruginosa* [32]. Hypericin, an anthraquinone from *Hypericum perforatum*, possess antimicrobial properties against methicillin-resistant and methicillin-sensitive *Staphylococcus* [46].

The increased activity and reactivity of the quinones are due to the redox properties of the carbonyl groups. The reversible transformation between diphenol (or hydroquinone) and diketone (or quinone) easily results in redox reactions. The electrophilic nature of quinones is one of the causes of quinone toxicity for microbial cells. In addition, in biological systems, quinones can cause toxicity through the formation of reactive oxygen species (ROS). Superoxide anion radicals (O_2^-) can trigger spontaneous reactions or enzymatic reactions and generate hydroxyl radicals that alter the structure of proteins, by oxidizing nucleophilic amino acids such as cysteine. Also, these oxidation reactions can affect membrane lipids by forming lipid hydroperoxides. They give characteristic precipitation reactions with numerous reagents (potassium iodide, platinum chloride, picric acid, sulfuric acid, *etc.*).



quinone hypericin

Figure 5. Chemical structures of representative quinones, quinone and hypericin

10.3.6. Alkaloids

Alkaloids exert considerable physiological effects on humans and animals and are used in therapeutics as narcotics and have a calming effect. Some alkaloids are strong poisons, for example, curare, which has the ability to paralyze the nervous system. Alkaloids are considered to be an insecticide defense means in plants. Some alkaloids, for example nicotine, play a role in enzymatic oxidation-reduction processes.

The alkaloid-producing plants are dicotyledons, and to a lesser extent monocotyledons and cryptogamas. Generally, a plant contains several alkaloids. The alkaloid content depends on the age of the plant, region, climate and season. Algae and bryophytes do not produce alkaloids. They are rare in mushrooms (ergotamine), in pteridophytes (nicotine in *Equisetum*, coniine in *Lycopodium* spores) and in gymnosperms (ephedrine). In the angiosperms, some families are reputed by their high contents in alkaloids: *Solanaceae* (solanine, nicotine, hyoscyamine, atropine), *Rubiaceae* (caffeine, quinine), and *Papaveraceae* (morphine, papaverine, codeine, narceine, *etc.*). The most important alkaloids are: coniine, nicotine, tropane, atropine, cocaine, quinine, papaverine, morphine, codeine, strychnine, caffeine, and others [3,8].

Alkaloids have a great structural diversity and are synthesized mainly in *Solanaceae* and *Fabaceae*. For example, pepper (*Capsicum annuum*), contains alkamide alkaloids such as affinin (spilanthol) and capsaicin [33], potato (*Solanum tuberosum*) contains several toxic and teratogenic glycoalkaloids [34], and lupin (*Lupinus angustifolius*) contains a large number of quinolizidine alkaloids, notably 13 α-hydroxy lupanine and lupanine. Highly unsaturated planar quaternary alkaloids possess the ability to intercalate with DNA [8] and modify the nucleotide sequences [10].

Tamarindus indica (Fabaceae) aqueous pulp extract was found to have antimicrobial effect against *E. coli*, *S. aureus*, and *P. aeruginosa*, but not against *Salmonella typhi* [35]. *Zapoteca portoricensis* leaf extract has antibacterial action against Gram-positive bacteria *S. aureus*, *E. coli*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, and Gram-negative *P. aeruginosa* [35].

10.3.7. Lignans

Lignans are a group of dimeric phenylpropanoids discovered in 1948 by Howarth. Lignans are widespreaded throughout plants in pterydophytes, gymnosperms and angiosperms and are considered one of the earliest forms of defense in vascular plants. Lignans are found in six families of the Coniferae order of Gymnosperms [36]. Cupressaceae and Pinaceae contain the largest variety of compounds. Lignans are also found in Taxaceae, Ephedraceae and Ginkgoaceae [37].

Styraxjaponoside C isolated from *Styrax japonica* (Figure 6) presented an antifungal effect against the human pathogenic yeast *C. albicans,* with membrane-active mechanisms [38]. Lignans isolated from *Pseudolarix kaempferi* showed antimicrobial activity against *C. albicans* and *S. aureus* [39]. Dibenzocyclooctadiene lignin isolated from *Schissandra chinensis* was reported to inhibit *Chlamydia trachomatis* and *C. pneumoniae* [40].

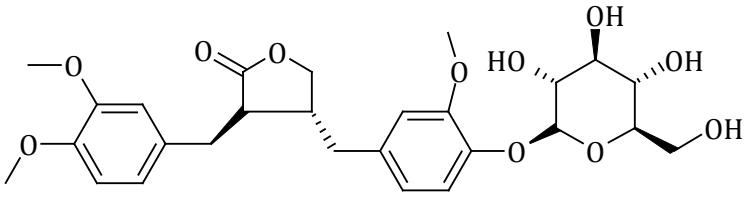


Figure 6. Chemical structure of styraxjaponoside

10.3.8. Glycosides

Glycosides are organic compounds in which a sugar is bound to another functional group through a glycosidic bond (Figure 7). According to the type of aglycone, glycosides are classified in phenolic glycosides and cyanogenic glycosides (aglycone represented by a cyanide group). In many plants chemicals are in the form of inactive glycosides, which can be activated by enzyme hydrolysis. An important group of glycosides are the glucosinolates, the precursors of isothiocyanates, found in 16 dicotyledonous families [10].

Glucosinolates are secondary metabolites that contain sulphur and nitrogen, mainly found in the *Brassicaceae* family. Sinigrin is present in broccoli, mustard and Brussels sprouts and is reported to have antifungal, antimicrobial, anticancer, antioxidant and anti-inflammatory activity. *Lobularia libyca* produces glucosinolates such as glucoiberverine, glucoiberin and glucoerucin with antimicrobial activity against *C. albicans* and *P. aeruginosa* [2].

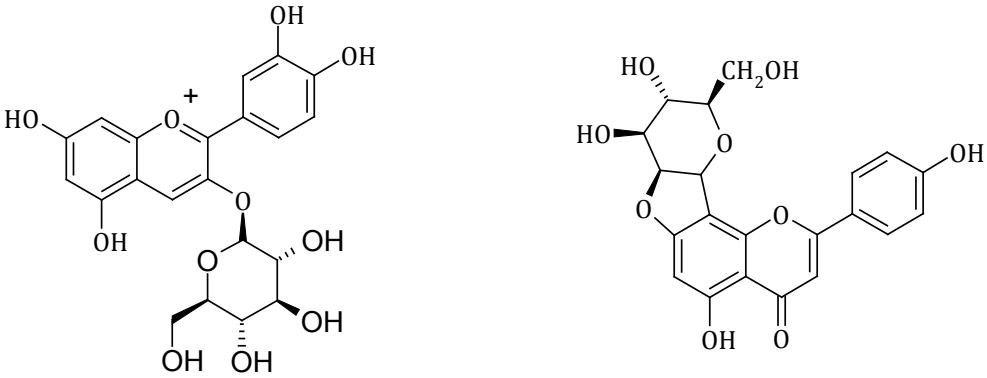


Figure 7. Cyanidin O-galactoside and flavone C-glycoside

10.3.9. Saponins

Saponins are compounds derived from steroids or triterpenoid glycosides, which occur in many plants and act on microbial cells by permeabilization of the membrane. Saponins are found in *Solanum* [41], shoots of oats (*Avena sativa*) [42], seeds of *C. annuum* [43], and *Medicago* sp. [44]. These saponins have been tested against several Gram-positive and Gram-negative bacteria, yeast, and fungi, and many of them exhibited a weak growth inhibition against the microorganisms. Saponins from *Yucca* (Figure 8) exhibit antimicrobial activity against Gram-positive bacteria but do not affect Gram-negative bacteria [10].

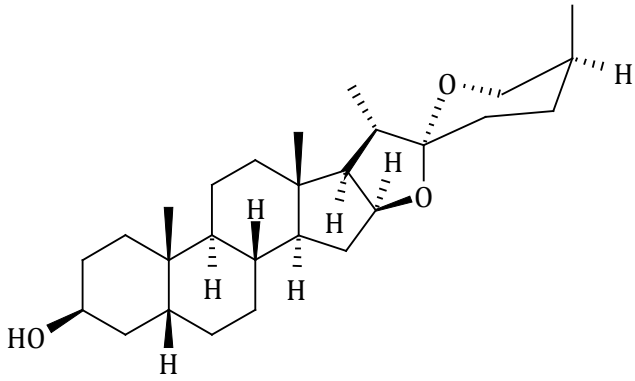


Figure 8. Sarsapogenins from *Yucca* [10]

10.3.10. Tannins

Tannins are polymeric phenolic compounds, water-soluble, astringent, with molecular weight ranging from 500–3000, with different biological activities.

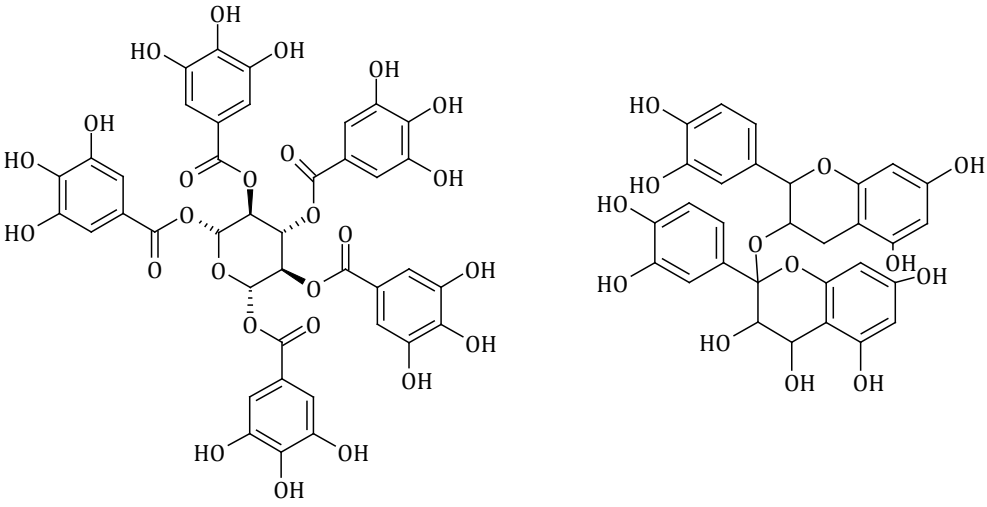
Vegetable tannins are substances well known for their many practical applications, especially in traditional medicine and the tanning industry. The tanning operation of animal skin with plant tannins has been known since antiquity and was done using the bark of different trees, especially oak species [8].

The determination of tannins' molecular structure allowed differentiation in the practical use between different types of tannins. Tannins (Figure 9) are classified as 1) hydrolysable tannins and 2) condensed tannins. Hydrolysable tannins are based on gallic acid and contain esters of gallic acid with D-glucose. By acid hydrolysis or in the presence of tannases, these tannins release a sugar, usually glucose, and gallic acid (gallotannins) or a compound related to gallic acid such as m-digallic acid or ellagic acid (ellagotannins).

Condensed tannins (proanthocyanidins) are substances derived from flavonoids. The biosynthesis of these metabolites occurs either by condensation of flavan derivatives or by polymerization of quinones units. They seem to play a role in plant protection against insects and ruminant animals [4,8].

Tannins are synthesized in all organs of the plant, especially in leaves, roots and stems. They are found in large quantities in bark, wood and oak leaves, alder, spruce, poplar, walnut, blueberries, mangroves and in the fruits of some species like *Terminalia chebula, Caesalpinia brevifolia*, *etc.* [45]. In plants, tannins play an important biochemical role. They increase the resistance of plants to viruses and microorganisms. Consumption of tannins from plant products may have an impact on physiological activities: tannins can stimulate phagocytic cell activity, have host-mediated antitumor activity, have antibacterial and antifungal activity in some infectious diseases.

Their mechanism of action can be explained by forming complex proteins through physical bonds and hydrophobic effects, but they can also form covalent bonds with proteins. In this way, tannins can inactivate functional cell proteins such as adhesins, enzymes, and transport proteins in the cell envelope [8,34].



pentagallylglucose procyanidin

Figure 9. Structures of representative tannins

10.3.11. Terpenes and essential oils

A very interesting and studied group of antimicrobial compounds in plants are essential oils. Essential oils are natural volatile liquids found in flowers, roots, barks, leaves, seeds, fruits, and wood [46]. The International Organization for Standardization (ISO) has defined essential oils as a 'product obtained from a natural raw material of plant origin, by steam distillation, by mechanical processes from the epicarp of citrus fruits, or by dry distillation, after separation of the aqueous phase if any by physical processes' [47].

Essential oils (Figure 10) are aromatic compounds insoluble in water but soluble in organic solvents. The composition of essential oils varies depending on plant species and other climatic factors and has a significant role in plant defense and pollination. Essential oils are extracted by hydrodistillation, extraction in organic solvents or in supercritical fluids such as supercritical CO_2 [25].

The antimicrobial activity of essential oils can be explained by their composition: about 90–95 % are monoterpenes and sesquiterpene hydrocarbons and their oxygenated derivates, aldehydes, alcohols and esters. In the rest of the non-volatile part there are hydrocarbons, fatty acids, sterols, carotenoids, waxes, cumarines and flavonoids. In this mixture of compounds in essential oils, the most active against bacteria and fungi are: terpens (*e.g.*, p-cymene, limonene), terpenoids (*e.g.*, thymol, carvacrol), phenylpropenes (*e.g.*, eugenol, vanillin) and other compounds such as allicin or isothiocyanates [48].

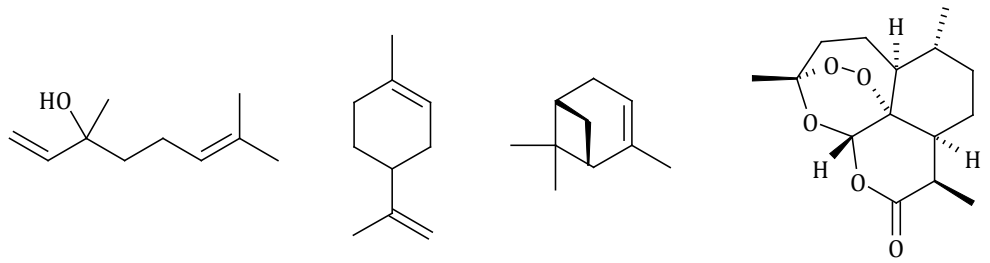


Figure 10. Chemical structure of a) linalool; b) limonene; c) *α*-pinene; d) artemisin

The main mechanisms of action for essential oils are presented in Figure 11 [46]. Essential oils interact with the lipids in a cell membrane and can pass easily in the cytoplasm, due to their lipophilic character. The simpler structure of cell wall in Gram-positive bacteria allows the interaction with the compounds of essential oils, while the more complex structure of cell envelope in Gram-negative bacteria is like a barrier through which the essential oils pass with more difficulty [49].

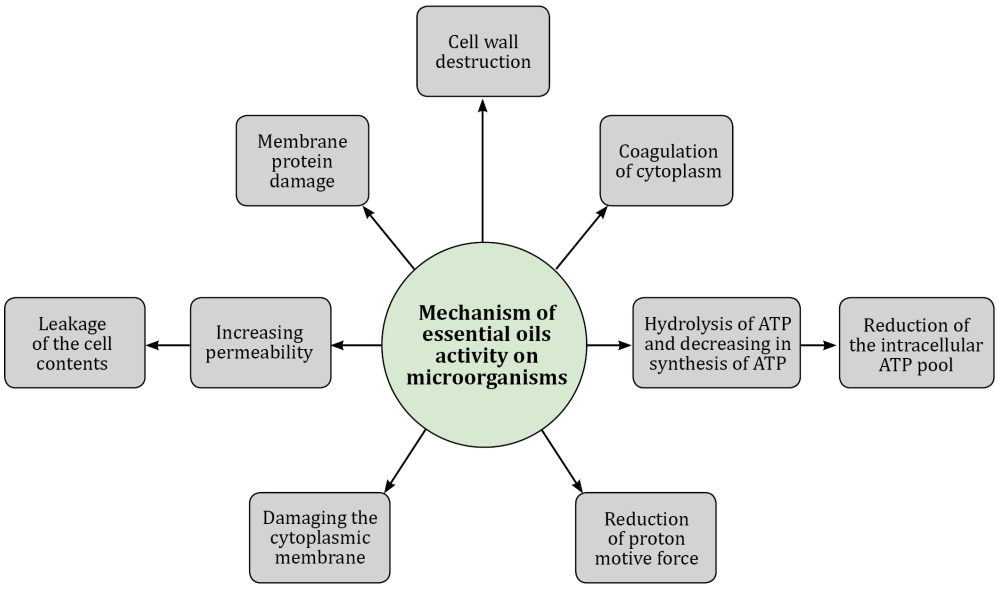


Figure 11. Mechanisms of antimicrobial activity of essential oils [46]

The antimicrobial activity of essential oils is based on the hydrophobicity, disturbance of the cytoplasmic membrane, disruption of the electron flow, active transport, and coagulation of cell contents. Other mechanisms include disturbances of the pH gradient and the electric potential of the proton-motive force [46]. The great effect of essential oils is due to the lipophilic nature of the hydrocarbon skeleton and hydrophilic nature of functional groups. The most active molecules are the phenolic compounds, following in order by aldehydes, ketones, alcohols, ethers and hydrocarbons [50], that interfere with the cell membrane and with the enzymes involved in energy production. The shape of the bacteria may influence the effect of essential oils, and it has been demonstrated that the rod-shaped cells are more susceptible than coccus-shape [4,49].

The volatile oils of black pepper (*Piper nigrum* L.), clove (*Syzygium aromaticum* (L.), geranium (*Pelargonium graveolens* L'Herit), nutmeg (*Myristica fragrans* Houtt.), oregano (*Origanum vulgare* ssp. *hirtum* (Link) Letsw.) and thyme (*Thymus vulgaris* L.) were screened for antimicrobial activity against 25 microorganisms of significant importance. The volatile oils exhibited considerable inhibitory effects against all the microorganisms, the oil with the widest spectrum of activity being the extract of *T. vulgaris* [51].

Sakkas *et al.*, in 2017 [4], studied the correlation between the composition and the antimicrobial efficacy for basil, oregano, and thyme oil and demonstrated that their activity is attributed to different compounds. In basil antibacterial activity of essential oil is due to its high content in linalool and estragole, whereas the antimicrobial spectrum is restricted to specific bacteria (*Staphylococcus* spp., *Enterococcus* spp., *E. coli*, *P. aeruginosa*, *A. baumannii*, *A. hydrophila*, *B. cereus*, *B. subtilis*, *Enterobacter* spp., *Listeria* spp., *Proteus* spp., *Salmonella* spp., *Serratia marcescens*, and *Y. enterocolitica*) and fungi (*Candida* spp., *Rhodotorula* spp., and *S. cerevisae*). The antimicrobial effect of oregano oil is accredited to carvacrol and thymol, and its antimicrobial spectrum is broad, including among others several species of harmful bacteria (methicillin-resistant *S. aureus*, *Listeria innocua*, *L. monocytogenes*, *A. baumannii*, *K. pneumoniae*). The antimicrobial effect of thyme oil is also attributed to carvacrol and thymol.

The effect of essential oil from *Mentha piperita* on the hyphal cell wall and sporulation in fungi is visible on the optical microscope (Figure 12).

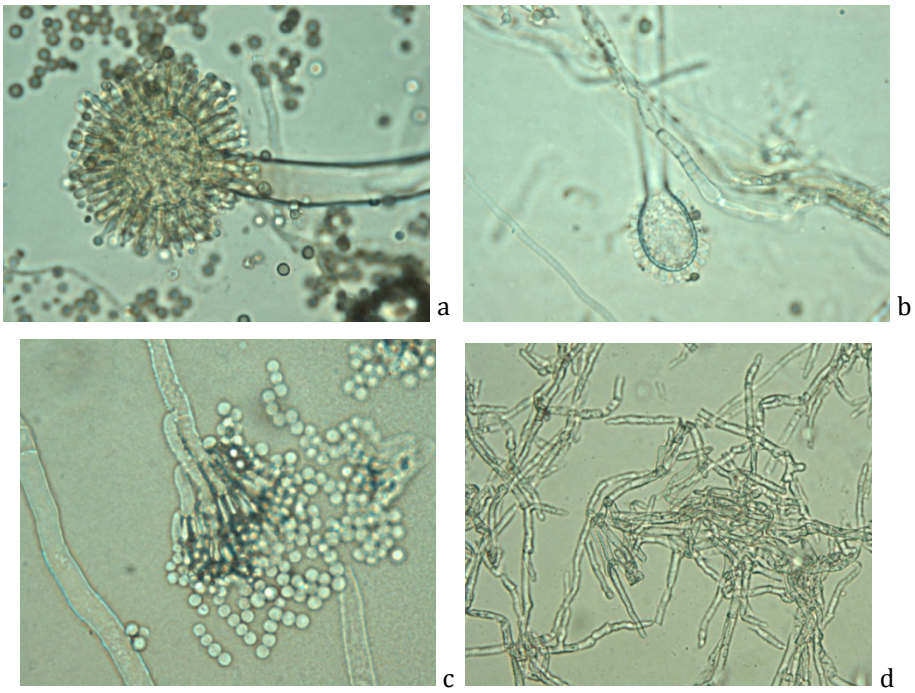


Figure 12. Microscopic appearance of *Aspergillus niger* and *Penicillium hirsutum* in control colonies a), c) (respectively) and after growing in Petri dishes containing 20 µL mint essential oil b), d) (Ferdes, 2017) [52]

10.3.12. Peptides

In their defense against pathogens, plants synthesize both proteins with an enzymatic role (glucanases, proteinases, amylases, oxydases) as primary metabolites, but also peptides with a lower molecular weight of about 10 kDa, known as antimicrobial peptides (AMPs). AMPs have been isolated from a large number of plants and were classified into several categories according to the secondary structure and the three-dimensional conformation (Figure 13). These groups are: 1) linear α-helical peptides; 2) cyclic peptides with β-sheet structures and disulfide bonds; 3) α-helix combined with β-sheet, linked through disulfide bonds; 4) peptides with β-hairpin or looped arrangement with disulfide bonds; 5) linear peptides containing predominant amino acid residues as proline, glycine, tryptophan and histidine; and 6) small peptides with coil or undefined side structures [53].

A large number of AMPs are characterized by a large number of specific amino acids, such as cysteine, which allow the stabilization of peptide structures by disulfide bridges (S–S). Such examples are defensins and snakins [54]. Small-sized molecules, positive charge and high hydrophobic zones give AMPs specific properties and a characteristic structure with distinct hydrophobic

portions and positively charged amino acids and allow the formation of an amphiphilic conformation.

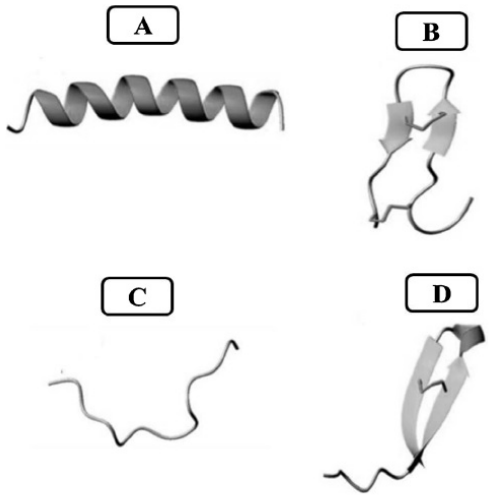


Figure 13. Secondary structures of AMPs: A) α-helical, B) β-turn/sheet, C) random coil, D) mixed α/β [55]

AMPs have been classified according to their specific properties in several groups: defensins, knottin-like, 2S albumins, cyclotides, lipid transfer proteins (LTPs), heveins and snakins.

10.3.12.1. Defensins

Defensins were the first studied AMPs, isolated from wheat and barley and named γ-thionins. Defensins have a low molecular weight of 5 kDa [53], a primary structure of 45–54 amino acids and have been isolated mainly from seeds, but also from other organs of the plant (leaves, stem, root, flowers). α- and β-thionins were discovered later. All three types of defensins have two or three disulfide bonds in the molecule, although their structures are different. Disulfide bonds play a role in stabilizing the structure of these peptides, consisting of a β-sheet conformation and an α-helical segment, so that the defensin molecules are particularly resistant to extreme temperature, pH and enzyme action.

Pelegrini *et al.*, in 2008, isolated from *Petunia hybrida* two defensins, which they named PhD1 and PhD2, with different structures. Other defensins were found in *Raphanus sativus*, *Vigna unguiculata*, *Fagopyrum esculentum* Moench,

and *Phyllostachys pubescences*, that exhibit antibacterial and antifungal activity [53,55].

Plant defensins have demonstrated antifungal and antibacterial activity but can also act as enzyme inhibitors.

10.3.12.2. Knottin-like peptides

Knottin-like peptides were discovered in 1990 by Nguyen *et al.*, in *Ecballium elaterium* seeds, and were named knottins. Knottins contain about 40 amino acids and three disulfide bonds whose arrangement provide the structure with an extraordinary proteolytic, thermal and chemical stability [56]. Knottins possess cytotoxic, antimicrobial, insecticidal, anti-HIV and hormone-like activity. These compounds were isolated from *Mirabilis jalapa* and exhibit antifungal activity against 13 phytopathogenic fungi (*Botrytis cinerea*, *Alteraria brassicola*, *Fusarium oxysporum* and others) and antibacterial action against two Gram-positive bacteria (*Bacillus megaterium* and *Sarcina lutea*) [57]. Other knottin-like peptides were isolated from *Phytolacca americana* and had inhibitory action on *Alternaria tenuis*, *Fusarium graminearum*, *F. oxysporum* and *Trichoderma viridae* [58].

10.3.12.3. 2S Albumins

2S albumins are storage proteins with important plant survival functions, which also have a defensive role. They are water-soluble peptides, rich in basic amino acid glutamine, with a sedimentation coefficient of about 2 Svedberg units. They have a wide spread in monocotyledonous and dicotyledonous plants and were first discovered in castor seeds. They appear to be synthesized by post-translational modification processes by enzymatic cleavage. 2S albumins have different functions in the cell: protein storage, emulsion stabilization, acting as inhibitors of proteolytic enzymes and having antimicrobial activity. It has been shown that 2S albumins from *Arachis hypogaea* have antifungal action against *Aspergillus flavus* by inhibiting conidiospore germination. 2S albumins from *Passiflora edulis* had inhibitory action on the mold species of *Trichoderma harzianum*, *Fusarium oxysporum*, *Aspergillus fumigatus.* It was found that *Sesamum indicum* produces 2S albumines with antibacterial activity against *Klebsiella* sp. [53].

10.3.12.4. Cyclotides

Cyclotides are cyclic peptides formed consisting of 28–37 amino acids, found in some plants of the families *Rubiaceae, Violaceae, Poaceae* and *Fabaceae*. Cyclotides are synthesized by excisions from precursor polypeptides and cyclization. They are molecules with high resistance to extreme temperatures and chemical agents and have antimicrobial, antitumor, nematicidal, insecticidal, anti-HIV activity. Cyclotides synthesized in *Viola abissynica*, *V.*

odorata and *Oldenlandia affinis* exhibit antibacterial activity against *E. coli*, *Salmonella enterica* and *S. aureus* [53].

10.3.12.5. Lipid transfer proteins (LPTs)

LPTs are small molecules of cationic peptides of between 7 and 10 kDa and have the ability to transfer lipids by reversibly binding and transporting them. LPTs have in the molecule eight cysteine residues with four disulfide bonds. These chemical bonds have the role of stabilizing the tertiary structure of four *α*-helices, which have a hydrophobic site where lipid molecules can bind. LPT classification is based on the differences between helix structures and their arrangement. It seems that LPTs play an important role in plant signaling, plant defense, and antimicrobial activities. LPTs have been found in *Allium cepa*, *Helianthus anuus*, *Capsicum annuum*, *Raphanus sativus, Hordeum* sp. and other plants [53,55].

10.3.12.6. Heveins

Heveins have been isolated from the first time by Archer in 1960 from the *Hevea brasiliensis* rubber tree. Heveins belong to the class of lectins and have the ability to chemically bind *N*-acetyl-glucosamine from the fungal cell wall. This peptide contains 43 amino acids and possesses similar structures to other lectins in plants. The hevein structure is composed of an anti-parallel *β*-sheet and a few short *α*-helices and is stabilized by three to five disulfide bonds. Due to their structure, heveins can bind chitin in the hyphal cell wall and thus stop the apical growth of the fungi. However, it has been observed that heveins can inhibit the growth of chitin-free microorganisms such as oomycetes and bacteria [53]. Heveins have been isolated from *Pharbitis nil* and *Triticum kiharae*.

10.3.12.7. Snakin / Gasa peptides

Snakins are peptide molecules rich in cysteine (19 %), with two to five disulfide bonds, composed of about 63 amino acid residues [53]. The primary structure of snakins is similar to the snake venom hemotoxic protein. To date, no tertiary structure has been discovered for these peptides. Porto *et al.* proposed a structure containing six disulfide bonds formed from three helices.

A quite different class of peptides are the short non-disulfide peptides, called so because they are cysteine-free or present very low cysteine content. An example of this group of peptides is ginkbilobin from *Ginkgo biloba*, consisting of 40 amino acids, with antimicrobial effect against the bacterial species of *S. aureus*, *P. aeruginosa* and *E. coli* and several fungi. Two cysteine-free AMPs were isolated from *Capsella bursa-pastoris* and were named shepherin I, and shepherin II that were active against *E. coli*, *Pseudomonas putida*, *P. syringae* and *Serratia* sp. and against the fungi *C. albicans, Cryptococcus neoformans* and *Saccharomyces cerevisiae*, respectively [51].

Other peptides in plants with antimicrobial activity are myrosinase binding protein, glycine-rich protein, α-hairpinins, α β-trumpet, and others.

Because the AMPs in plants can only be obtained in small amounts, the isolation of AMPs from a natural source is not economically sustainable. Thus, it is necessary to find different methods for producing AMPs. These methods refer to chemical synthesis or DNA recombinant technology. In the chemical synthesis the costs are also high for the production of sequences longer than ten amino acid residues, and costs are rising more for the production of specific disulfide bridges. Recombinant DNA technology facilitates the production of AMPs on a large scale by cloning the genes of interest in vectors for expression in host cells [53].

10.4. MECHANISM OF ANTIMICROBIAL ACTION

Different modes of functions have been proposed for the antimicrobial compounds as a whole. It is obvious that one solitary mechanism has a weaker effect against a microbial cell, but an assembly of mechanisms of antimicrobial functions have a strong impact on cellular structures. In general, plants synthesize complexes of related or chemically different secondary metabolites, the action of which could occur synergistically. In addition, the effect of plant metabolites can be emphasized in the presence of traditional antibiotics [60].

Several major mechanisms (Table 1) of antimicrobial activities of plant extract are described by Djilani and Dicko [61]:

1) Alteration of cytoplasmic membrane structures.

2) Interaction with extracellular proteins (for example ATPase).

3) Disturbance and inactivation of the function of the outer membrane in Gram-negative bacteria by modifying lipopolysaccharides.

4) Fluctuation of the proton engine force of the cells with permeance of ions.

5) Coagulation of cytoplasmic contents.

6) Prevention of enzyme generation.

Table 1 Mechanisms of plant antimicrobial compounds [8]

Class	Subclass	Examples	Mechanism
Phenolics	Simple phenols	Catechol	Substrate deprivation
		Epicatechin	Membrane disruption
	Phenolic acids	Cinnamic acid	–
	Quinones	Hypericin	Bind to adhesins, complex with cell wall, inactivate enzymes
	Flavonoids	Chrysin	Bind to adhesins
	Flavones	Abyssinin	Complex with cell wall, inactivate enzymes, inhibit HIV reverse transcriptase
	Flavonols	Totarol	Not identified
	Tannins	Ellagitannin	Bind to proteins, bind to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption, metal ion complexation
	Coumarins	Warfarin	Interaction with eucariotic DNA (antiviral activity)
Terpenoids, essential oils		Capsaicin	Membrane disruption
Alkaloids		Berberine Piperine	Intercalate into cell wall and/or DNA
Lectins and polypeptides		Mannose-specific agglutinin Fabatin	Block viral fusion or adsorption Form disulfide bridges
Polyacetylenes		8S-Heptadeca-2(Z),9(Z)-diene-4,6-diyne-1,8-diol	–

The study on the interactions of these compounds with the microbial cell demonstrated that the major cellular targets include (Figure 14) [59]:

1. The biomembrane;
2. Proteins (cell receptors, ion channels, enzymes, transport systems, regulatory proteins, structure proteins, transcription factors);
3. Nucleic acids (DNA and RNA).

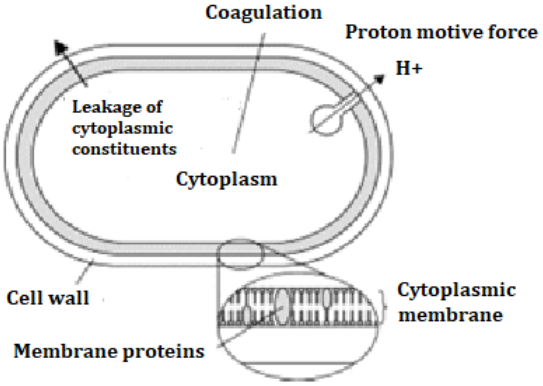


Figure 14. Target bacterial structures for antimicrobial compounds [59]

1. The biomembrane structure is deteriorated by lipophilic or amphiphilic molecules present mainly in terpenoids. These hydrophobic compounds alter the double layer of phospholipids in membranes forming transient pores that modify the permeability of the cell envelope and allow the diffusion or transport of harmful compounds. Other secondary metabolites can interact with the extramembrane or transmembrane proteins (ion channels, transporters, receptors), blocking their function of signaling and transport. This effect can be transient or permanent and leading to cell death.

2. Antimicrobial metabolites can interact with cell proteins, thus altering the secondary and tertiary structure of these important molecules. When the three-dimensional conformation of proteins is changed, their structural and metabolic functions are modified with dramatic consequences in cell life. The conformational changes can either activate or inactivate a protein. The secondary metabolites can also modify the conformation of the catalytic center of enzymes causing the loss of catalytic function. The correct conformation is indispensable for the recognition of the substrates, ligands and other substances. Some highly reactive antimicrobial compounds can make covalent bonds with proteins, and others may form physical bonds such as hydrogen bonds, dipol-dipol or others. The most reactive metabolites belong to the classes of aldehydes, epoxides, sulfhydryls, exocyclic methylenes or cyclopropanes [59]. The hydroxyl groups of phenolic compounds can dissociate under physiological conditions into negatively charged O-groups. These negative groups can react with the basic amino acid residues in proteins thus modifying the three-dimensional conformation.

3. Nucleic acids. Secondary metabolites containing alkyl groups or molecules similar to nitrogenous bases in DNA or RNA can modify nucleic acids by alkylation and intercalation. These changes can lead to point mutations, and finally the primary sequences of amino acids in proteins are modified, if the

mutations are not repaired. In fact, antimicrobial metabolites can affect the whole replication, transcription and translation systems [59].

10.5. SYNERGISTIC ACTION OF PLANT METABOLITES AND ANTIBIOTICS

A relatively new trend in the development of new sources of antibiotics is the study of the combination between natural plant derivates and standard antibiotics in order to enhance their activity through bactericidal synergism. For example, the pluripotent activity of phytochemicals may stimulate the antimicrobial activity of aminoglycosides, quinolones, macrolides, and tetracyclines. In multidrug therapy, the obtained effects may be insignificant (when the effect of two compounds is the same), additive (as the sum of effects), synergistic, or antagonistic. In the case of a synergistic effect, the activity of the combination of compounds is higher than the sum of the effects of individual compounds [3].

It was demonstrated that extracts of *Punica granatum* in combination with chloramphenicol, ampicillin, gentamicin, tetracycline, and oxacillin have a synergistic effect on MRSA bacteria. The berberine alkaloid berberine exhibits a highly synergistic activity with β-lactam antibiotics against MRSA [3].

10.6. CONCLUSIONS

Since ancient times, plants have traditionally been used for the prevention and treatment of various diseases. Plants represent a promising alternative to treating medically challenging pathogens and to combat the growing number of bacteria that have become resistant to conventional antibiotics. In addition, with the increased negative attitudes of consumers to chemical preservatives, the use of antimicrobial plant extracts has become an especially interesting alternative.

Plant cells produce a variety of phytochemicals, especially secondary metabolites for defense mechanisms against microorganisms, parasites, and herbivores. These bioactive compounds are present in all plant material (roots, stems, leaves, flowers, fruits, seeds) and are responsible for the medicinal properties and health benefits of herbs. These metabolites belong to a large number of classes of chemical compounds: phenols, alkaloids, quinones, terpenes, essential oils, tannins, saponins, glycosides, lignans, and peptides, whose identification and isolation is still a challenge.

The structure of antimicrobial secondary metabolites explains the variety of mechanisms by which these compounds act against microbial cells, different cellular architectures and target functional groups. Studies on the interactions of these compounds with the microbial cell have demonstrated that the major cellular targets include the biomembrane, proteins (cell receptors, ion channels, enzymes) and nucleic acids (DNA and RNA). Several mechanisms of action of antimicrobial metabolites in plants consist of alteration of membrane structures, interaction with extracellular proteins, fluctuation of the proton engine force of the cells, and coagulation of cytoplasmic contents.

Antimicrobial compounds in plants as a whole have a broad spectrum of action that can exceed the limited specificity of antibiotics. In this context, a relatively new trend in the development of new sources of antibiotics is the study of combination between natural plant derivates and standard antibiotics, in order to enhance the activity of latter through bactericidal synergism.

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