Plant-derived antimicrobial compounds: alternatives to antibiotics

Dianella Savoia
Department of Clinical & Biological Sciences, University of Torino, Turin, Italy
Tel.: +39 0116705427
Fax: +39 0112365427 ■ dianella.savoia@unito.it

The increasing incidence of drug-resistant pathogens has drawn the attention of the pharmaceutical and scientific communities towards studies on the potential antimicrobial activity of plant-derived substances, an untapped source of antimicrobial chemotypes, which are used in traditional medicine in different countries. The aim of this review is to provide recent insights regarding the possibilities of the most important natural antimicrobial compounds derived from plant sources containing a wide variety of secondary metabolites, which are useful as alternative strategies to control infectious diseases. This review will focus on natural plant products as a useful source of antimicrobial molecules, active in particular, on bacteria and fungi. When considering that many of these compounds, which have been used for centuries, are a source of new drugs and that there are ever-increasing technical breakthroughs, it can be envisaged that in the next years some different molecules discovered by ingenious screening programs and obtained from different plant oils and extracts will become useful therapeutic tools.

The antibiotic era during the 20th century has substantially reduced the threat of infectious diseases. Nevertheless, over the years, there has been a decrease in microbial susceptibility to existing antimicrobial agents responsible for critical point drug resistance in hospitals and in communities. In fact, the theme of the World Health Day 2011 was ‘Antimicrobial resistance: no action today, no cure tomorrow.’ Recently different authors have reported the urgent need for new antimicrobial agents to replenish the arsenal of anti-infective agents [1–4]. However, owing to different, mostly financial, problems, a failure of antibiotic discovery was observed. In addition, for fungi and protozoa, current chemotherapeutic options are very limited and far from ideal, especially for undesirable side effects or toxicity [5]. Among the known plant species on earth (estimated at 250,000–500,000) only a small fraction have been investigated for the presence of antimicrobial compounds and only 1–10% of plants are used by humans [6–7]. However, a recent review reports the marked influence of natural products in the anti-infective area, accounting for the most part, in the antibacterial area [8].

In fact, during the last few years, medicinal plants have attracted the attention of pharmaceutical and scientific communities, and evidence has demonstrated the promising potential of antimicrobial plant-derived substances [9–14]. The antimicrobial effect of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies.

Plant materials are of wide use in traditional systems of medicine, and in several communities of the developing world, are the only resources available for the treatment of different infections. In some Asian and African countries, 80% of the population depends on traditional medicine for primary healthcare and more than 100 countries have regulations for herbal medicines [11]. Ginsburg and Deharo recently reported the rationale for further development of ethnic/traditional medicine, with specific consideration to antimalarial drugs [12]. Thus, innovative scientific methods for the discovery and validation of multicomponent botanical therapeutics are important for the development of medicine and both the standardization of extracts and the identification of the efficient chemical and/or biological compounds; therefore, emphasis must be placed on the preservation of plant populations to guarantee pharmacologically active sources of material for herbal medicine.

Ethnopharmacology, an area inside ethnobotany focused on the medicinal use of plants, is important in selecting raw materials for future drugs and studying bioactive chemical entities from natural sources. Plants are rich in several secondary metabolites and are a major source of chemical diversity; therefore, they are a potential source of new drugs for man whose use to control diseases is a centuries-old practice. Currently, numerous reviews have described
the importance of natural compounds to treat human diseases [13–15]. Among 109 new antibacterial drugs, approved in the period 1981–2006, 69% originated from natural products, and 21% of antifungal drugs were natural derivatives or compounds mimicking natural products [16].

The potential of different plant species to yield newer antibacterial agents was illustrated by Shahid et al., even if no toxicity tests were reported [17]. They divided the compounds in a first generation of plant drugs – simply botanicals – employed on empirical evidence by traditional societies from different parts of the world in more or less their crude form; a second generation, based on scientific processing of the plant extract to isolate their active constituents; and a third developed generation of phytotherapeutic agents assessed for detailed pharmacological/biochemical studies.

The large spectrum of activity of natural resins derived mostly from plants and their secondary metabolites suggests their potential application as antimicrobial agents, in particular the main natural resins that belong to the family of Pinaceae, Cupressaceae, Apiaceae, Burseraceae, Anacardiaceae, Palmaceae, Euphorbiaceae, Dracenaceae and Fabaceae. Natural gums and extracts of the whole resins, as well as specific extracts, fractions, essential oils and isolated compounds from the above resins revealed antifungal, antibacterial and antiprotozoal activity [18,19].

Different species of the plant genus Hypericum, used in traditional medicine, contained several compounds including hyperenone A, hypercalin B and hyperphorin, responsible for antibacterial activity on resistant Staphylococcus aureus and also on Mycobacterium tuberculosis [6,20]. In particular, hyperenone A inhibited the ATP-dependent MurE ligase of M. tuberculosis, a crucial enzyme in the cytoplasmic steps of peptidoglycan biosynthesis.

Numerous extracts and compounds derived from Cameroonian plants have recently been tested for antimicrobial activity [21]; it was shown that phenolics and alkaloids were the most bioactive compounds revealing significant activity (MIC <10 μg/ml).

Termentzi et al. also discussed the effectiveness of propolis, this is an apicultural term characterizing all the resins that honey bees collect from various plants and mix with wax, and exudates from their salivary glands [18]. The chemical texture of propolis varies according to the geographical region and the type of vegetal resins that bees use. Whilst assessing two propolis specimens, our group demonstrated that the extract richer in the flavonoids pinocembrin and galangin, as shown by using high-performance liquid chromatography, was more active against Streptococcus pyogenes strains [22]. The antimicrobial effect of Korean propolis was studied against Streptococcus mutans suggesting the use of this product for the prevention of dental caries [23].

Using a multidisciplinary approach Wangchuk et al. effected a scientific validation of selected Bhutanese medicinal plants establishing the major classes of phytochemicals responsible for the broad range of biological activities [24]. They selected plants of different families containing tannins, alkaloids and flavonoids, plants ethnopharmacologically indicated apt to treat bacterial, fungal, malarial and Trypanosoma brucei rhodesiense diseases. Their study demonstrated the significant scientific evidence of the ethnodirected biorational approach. Also, caper (Capparis sp.) – a shrub and aromatic plant with a large natural distribution, in particular in the Mediterranean Sea Basin – have been used in traditional medicines to cure some diseases [25]. Phytochemical studies indicated the presence of different compounds, such as spermidine, rutin, quercetin, kaempferol, stigmasterol, campesterol, tocoferol and carotenoids, responsible for antimicrobial, anti-oxidative, anti-inflammatory, immunomodulatory and antiviral properties. Seed extracts of Capparis decidua in particular showed antibacterial, antifungal and antileishmanial activity probably due to quaternary ammonium and glucosinolate.

Essential oils, derived from aromatic medicinal plants (e.g., fennel, peppermint, thyme and lavender) and containing mixtures of volatile substances, such as monoterpenes, sesquiterpenes and/or phenylpropanoids, have been reported to be active on Gram-positive and Gram-negative bacteria and on yeasts, fungi and viruses [3,5,17,26–31]. Helicobacter pylori was susceptible to different essential oils, in particular carrot (Daucus carota) seed oil and Mycoplasma pneumoniae to a tea tree (Melaleuca alternifolia) oil, which seems to affect the intracellular cytoskeleton structure [24]. Essential oil mixtures, primarily composed of terpenoids and aromatic and aliphatic constituents, have been studied for antimicrobial activity also against caries-related bacteria. Some components act as inhibitors of bacterial growth, some as inhibitors of exopolysaccharide synthesis and others inhibit bacterial adherence [32,33].

The aim of this review is to report, based on the consultation of the up-to-date literature, the antimicrobial activity of natural products derived from plants, focusing on the mechanisms
of action of the compounds contained may constitute a reservoir of substances that can be used against bacteria and fungi.

**Antibacterial activity**

Among the new botanical antibacterial therapeutics and strategies, we will consider the main antibacterial phytochemicals; plant extracts with efflux inhibitory activity against Gram-negative bacteria; plant extracts with bacterial quorum-sensing inhibitory activity; and plant extracts with biofilm inhibitory activity.

**Main antibacterial phytochemicals**

Plant-derived compounds of therapeutic value are mostly secondary plant metabolites traditionally used for medicinal purposes. They have a wide activity range, according to the species, the topography and climate of the country of origin, and may contain different categories of active principles [34–36]. Variations in the chemical composition modifies their antimicrobial activity.

Some main categories of phytochemicals extracted from medicinal plants are examined to evaluate their pharmacological activity.

**Flavonoids**

Flavonoids, previously called bioflavonoids and included in aromatic compounds, are phenolic structures ubiquitous in photosynthesizing cells and are commonly found in fruit, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey. For centuries, preparations containing these compounds as the principal physiologically active constituents have been used to treat human diseases. The basic structural feature of flavonoid compounds is the 2-phenylbenzopyrane or flavane nucleus, consisting of two benzene rings linked through a heterocyclic pyrane ring.

In total, there are 14 classes of flavonoids, differentiated on the basis of the chemical nature and position of substituents on the different rings. The antibacterial properties of flavonoids are thought to come from the ability to form complexes with both extracellular and soluble proteins, as well as with bacterial membranes [37,38].

Kuete demonstrated that among the flavonoids hydroxylating the prenyl groups of stipulin, the compounds obtained, angusticorin B and bartercin A, had a superior antimicrobial activity [21]. Thus, the prenyl group plays an important role in the activity of chalcones. Recently two flavonoids (6-hydroxy-7-methoxyxanthone and the xanthone 8-carboxymethyl-1,5,6-trihydroxy-3-methoxyxanthone) extracted from the leaves of *Leiothrix spiralis*, a South American plant belonging to the Eriocaulaceae family, showed a promising activity on *Escherichia coli* and *Pseudomonas aeruginosa* [39]. Some flavonoids also revealed activity against *M. tuberculosis* [40].

A synergy has been demonstrated between active flavonoids as well as between flavonoids and existing chemotherapeutics, even if the reports of activity in the field of antibacterial flavonoid research are widely conflicting, probably owing to inter- and intra-assay variation in susceptibility testing [41]. Future optimization of these compounds through structural alteration may allow the development of a pharmacologically acceptable antimicrobial agent or group of agents. Existing structure–activity data suggest that it might be possible, for example, to prepare a potent antibacterial flavanone by synthesizing a compound with halogenation of the B ring as well as lavandulyl or geranyl substitution of the A ring. Also, it is worth noting that by elucidating flavonoid biosynthetic pathways it would be possible to produce structural analogs of active flavonoids through genetic manipulation.

Numerous research groups have sought to elucidate the antibacterial mechanisms of action of selected flavonoids; the activity of quercetin has been at least partially attributed to the inhibition of DNA gyrase, whereas sophoraflavone G and (-)-epigallocatechin gallate inhibit cytoplasmic membrane function, and licochalcones A and C inhibit energy metabolism.

**Alkaloids**

Alkaloids are heterocyclic nitrogen compounds characterized by different antimicrobial activities. The analysis of the leaf extracts of *Gymnema montanum* and of ethanol extract of *Tabernaemontana catharinensis* root bark revealed an antimicrobial activity [42,43] in the first case due to an activity depending upon the chemical composition of the extracts and membrane permeability of the microbes, and in the second case linked to indole alkaloids responsible for the observed antibacterial and antidermatophytic activity. Diterpene alkaloids, commonly isolated from the plants of the Ranunculaceae group, had antimicrobial properties [44]. Berberine, an isoquinoline alkaloid, present in roots and stem-bark of Berberis species, is a hydrophobic cation widely used in traditional medicine owing to its activity against bacteria, fungi, protozoa and viruses [45]. It accumulates in cells driven by the membrane potential and is an excellent DNA intercalator [46] active on several microorganisms.
with a target on RNA polymerase, gyrase and topoisomerase IV and on nucleic acid [47].

**Terpenes**

Terpenes compounds are also referred to as isopenoids and their derivatives containing additional elements, usually oxygen, are called terpenoids. The antibacterial activity of some monoterpenes (C10), diterpenoids, sesquiterpenes (C15), triterpenoids and their derivatives was recently reviewed [48]. The results obtained illustrate the strong structure–function influence of the antibacterial potential of terpenes. Diterpenoids, such as sesquiterpenes, isolated from different plants exhibited bactericidal activity against Gram-positive bacteria and inhibited the growth of *M. tuberculosis* [40,48]. The mechanism of action of terpenoids is not fully understood, but is speculated to involve membrane disruption by the lipophilic compounds [38].

**Phenolics & polyphenols**

Phenolic compounds are widely distributed in plants, where they protect the plants from microbial infections. They have potential antioxidative properties but are also potent anti-infectives [49]. They are a large group of aromatic compounds, consisting of flavones, flavanoids and flavonols containing one carbonyl group, quinones with two carbonyl groups, tannins, polymeric phenolic substances, and coumarins, phenolic compounds with fused benzene and pyrone groups [37,48].

Flavones and their derivatives represent an antibacterial therapeutic possibility to disrupt bacterial envelopes [50]. The catechins are included among the flavan-3-ols or flavanols, present in different plants, particularly in tea plant *Camelia sinensis*, where they form complexes with the bacterial cell wall and are active on intestinal microorganisms [51]. Biological assays indicated the inactivation of specific bacterial enzymes by several of these compounds. Moreover significant synergy was also observed between theaflavin and epicatechin against important nosocomial Gram-negative pathogens [52].

Quinones (aromatic rings with two ketone substitutions), ubiquitous in nature, are another significant group of secondary metabolites with potential antimicrobial properties. They provide a source of stable free radicals and irreversibly complex with nucleophilic amino acids in microbial proteins determining loss of their function [49]. Anthraquinones in particular had a large spectrum of antibacterial (also antimycobacterial) activity, based on inactivation and loss of function of bacterial proteins, such as adhesins, cell wall polypeptides and membrane-bound enzymes [48], consequently leading to the death of the pathogens.

Tannins are a group of polymeric phenolic substances found in almost every plant part characterized by antibacterial activity owing to inactivation of bacterial adhesins, enzymes, cell envelope and transport proteins. Recently, gallotannin-rich plant extracts demonstrated inhibitory activities on different bacteria attributable to their strong affinity for iron and to the inactivation of membrane-bound proteins [53].

Hydrolysable and condensed tannins, derived from flavanols, and called proanthocyanidins, exert antimicrobial activity by antiperoxidation properties inhibiting in particular the growth of uropathogenic *E. coli* [54,55]. Anthocyanin synthesis occurs in plants on the cytoplasmic leaflet of the endoplasmic reticulum and then accumulates in the large central vacuole [56]; in many plants, anthocyanidins might occur in oligomeric form and in this case they are called proanthocyanidins. Depending on the type of bond between the oligomer-forming anthocyanidin molecules, two general types (A and B) of proanthocyanidins are distinguished. In less common A-type proanthocyanidins, two bonds are formed between 2β-7 and 4β-8 carbon of oligomer-forming molecules; in B-type, only one 4β-8 bond is formed. The beneficial effects of anthocyanins on human health have been known at least from the 16th century, when blackberry juice was used in the treatment of mouth and eye infections. However, only few studies have focused on the antimicrobial activity of these compounds. Recently, Cisowska et al. described the anthocyanin profile of action of different fruits, mainly berries, but also red grapes and, by consequence, red wine, also containing stilbenoid resveratrol, indicating a superior activity against Gram-positive bacteria [56].

**Coumarins**

One known coumarin, scopoletin, and two chalcones were isolated as antitubercular constituents of the whole plant *Fatoua pilosa* [40].

Also, spices and aromatic plants have an antimicrobial effectiveness that depends on the kind of plant, its composition and concentration of essential oils, often rich in monoterpens and sesquiterpenes [57–60]. Studies analyzing the antimicrobial activity of essential oil of *Allium sphaerocephalon* inflorescences [61] revealed the accordance with the popular use of plants
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belonging to the *Allium* genus in traditional medicine, indicating the importance of aroma precursors (cysteine sulfoxides) for a potent biologic activity.

**Plant extracts with efflux pump inhibitory activity**

Multidrug resistance due to the expression by bacteria of an efflux pump is an increasing clinical problem. Therefore an interesting approach to the therapy of many infections would be one based on the identification of molecules interfering with the process of efflux.

In 1998, it was shown that plant-derived compounds are active against Gram-positive bacteria, in particular *Staphylococcus aureus* [62]; successively numerous phytochemicals were shown to act as potential efflux pump inhibitors (EPIs) with antimicrobials for Gram-positive bacteria [63–65]. Gram-negative bacteria have innate multidrug resistance to many antimicrobial compounds owing to the presence of efflux pumps, in particular, the AcrAB-TolC efflux system [66], and some authors suggested that plants may not produce molecules active on these organisms [67].

However, the chemical diversity between plants and microorganisms represents an ecological possibility to identify EPIs from natural sources. Reviewing the literature concerning bacterial resistance modulators from natural plants, Stavri *et al.* described different bacterial EPIs, such as the plant alkaloid reserpine, berberine and methoxylated flavones and isoflavones, that revealed putative interfering activity on efflux [68]. Moreover, the level of accumulation of berberine in the cells was increased in presence of 5'-methoxyhydnocarpin, a multidrug pump inhibitor, reported as a minor component of chaulmoogra oil, used in traditional therapy for leprosy [69]. Recent data indicate that the AcrAB-TolC (in Enterobacteriaceae) and MexAB-OptM (in *P. aeruginosa*) efflux pumps are involved in the resistance of Gram-negative bacteria to most of the natural products [70]. In the presence of the EPI phenylalanine arginine β-naphthyamide (PAβN), the activities of some natural products belonging to the phenolics, in particular to the naphthoquinones (plumbagin), and flavonoids (4-hydroxyloncharpin), showed a significant increase in activity, whereas terpenoids are not active, probably due to difficulty in passing through the bacterial membrane barrier. The natural products exhibiting the best antibacterial activities have the same pharmacophore; plumbagin, which revealed significant antibacterial activity in the absence of an EPI, is the minimal scaffold required for activity. The other functional groups may modulate the susceptibility of the molecule to bacterial resistance mechanisms. Moreover, extracts from plants, and in particular an extract of an essential oil from a Corsican plant, *Helichrysum italicum*, containing geraniol, was able to synergize with chloramphenicol against different Gram-negative bacteria [71]. Garvey *et al.* indicated that extracts of different plants that are used as herbal medicinal products contain inhibitors of efflux in Gram-negative bacteria [72]; the most active compound, falcarindiol, extracted from *Levisticum officinale*, revealed a synergistic activity with ciprofloxacin. By adding EPI PAβN to Dichrostachys glomerata extracts, an increase of the activity on *E. coli*, Klebsiella pneumoniate and Providencia stuartii resistant strains was shown; moreover, a synergistic effect was noted by associating *D. glomerata* extracts with some antibiotics [73].

Since the antimicrobial effectiveness of flavonoids comes from the ability to form complexes with both extracellular and soluble proteins and bacterial membranes, penetration of, and maintaining its position in, a microorganism is a critical point. Thus, the presence of EPIs is essential for flavonoid antimicrobial activity. Recently Fowler *et al.* used the natural flavonoid scaffold to synthesize non-natural flavanone molecules with functional groups responsible for activity against bacteria and fungi with minimal toxicity to human cells [74].

**Plant extracts with bacterial quorum sensing inhibitory activity**

It is now well recognized that populations of bacteria from many Gram-positive and Gram-negative species cooperate and communicate to perform diverse social behaviors, including swarming, toxin production and biofilm formation. Communication among bacterial cells involves the production and detection of diffusible signal molecules and has become commonly known as quorum sensing (QS), a density-dependent system that regulates the bacterial expression of specific genes, whose products modify the local host environment favoring the invasion and persistence of the pathogen [75,76]. The discovery that many pathogenic bacteria employ QS to regulate their virulence makes this system interesting as a target for antimicrobial therapy [77]. Therefore, the ability to interfere with QS interrupting bacterial communication opens new therapeutic prospects. The ideal
QS inhibitor (QSI) would be a low-molecular-mass molecule able to reduce the expression of QS-controlled genes; in order to avoid toxic side effects, the inhibitor should exhibit a high degree of specificity for the target QS-related molecule. Finally, the QSI agent should be chemically stable and resistant to the metabolic and disposal processes of the host organism. The study of a strategy to interfere with bacterial QS is the classical pharmacological approach to receptor antagonism. In particular, halogenated furanones, a class of natural products isolated from the marine red algae Delisea pulchra, have an effect on bacterial QS. Zang et al. showed that the mechanism of action is the modification and inactivation of LuxS (S-ribosylhomocysteine lyase), the enzyme which produces autoinducer-2, that mediates interspecies QS among many bacteria, but is absent in humans [78]. Moreover, a number of plant extracts and natural compounds inhibiting P. aeruginosa QS have been identified by Rasmussen et al., including bean sprout, chamomile, carrot, garlic, habanero (Capsicum chinensis), propolis, water lily, yellow pepper, and two products of Penicillium fungi, patulin and penicillic acid [79,80]. The authors further investigated the effects of garlic extract, which contains at least three different QS inhibitors and was able to inhibit QS in a concentration-dependent manner and with a structure–activity relationship hypothesizing competitive binding. In fact, GeneChip® analysis revealed that garlic extract had a profound effect on QS-regulated virulence genes, significantly reduced P. aeruginosa biofilm tolerance to tobramycin and lowered the pathogenicity of P. aeruginosa in a Caenorhabditis elegans nematode model. Docherty et al. showed that the phytoalexin resveratrol (3,5,4’-trihydroxystilbene), an antifungal agent found in grapes and other plants, has direct antibacterial activity against Neisseria gonorrhoeae and Neisseria meningitidis, but not against P. aeruginosa [81]. However, we observed that resveratrol can inhibit P. aeruginosa QS in vitro [82]. Also, solenopsin A, a venom alkaloid from the fire ant Solenopsis invicta, has been shown to be able to interfere with P. aeruginosa QS, probably by targeting the C6-HSL-dependent rhl system [83]. Solenopsin A reduced biofilm production in P. aeruginosa in a dose-dependent manner, indicating a QS signaling suppression mechanism [84]. An inhibition of QS-controlled virulence factors, such as LasA protease, LasB elastase, pyoverdin and biofilm production, in the same microorganism by extracts from different south Florida (USA) plants was also reported [85]. Recently, some traditional Chinese medicine herbs, in particular Areca catechu, which are a rich source of compounds which inhibit QS-controlled molecules, were selected [86]. Several QSI of natural origin, in particular the isothiocyanate iberin from horseradish, and ajoene, a sulfur rich molecule from garlic that inhibits P. aeruginosa genes controlled by QS, were identified [87,88]. Both ajoene and horseradish juice extract, in combination with tobramycin, have a synergistic antibacterial efficacy [89]. A natural nonpeptide compound isolated from the bark of Hamamelis virginiana, hamamelitannin (2’5-di-O-galloyl-d-hamamelo), was found to inhibit QS in S. aureus and S. epidermidis, inhibiting the production of RNAIII and ð hemolysin in vitro [90].

### Plant extracts with biofilm inhibitory activity

Biofilms are the default mode-of-life for many bacterial species and biofilm-based infections cause harm to millions of humans annually. The difficulty of eradicating biofilm bacteria with classic systemic antibiotic treatments is a prime concern of medicine. In particular, the ability of staphylococci to adhere on both eukaryotic cells and abiotic surfaces and to form biofilm is an important virulence factor in chronic infections associated with implanted biomaterials, which are particularly difficult to eradicate. Recently, Artini et al., assessing four compounds (derived from aerial and root parts of Krameria lappacea, Ascleucus hippocastanum, Chelidonium majus and Macleaya cordata) that contained several alkaloids and flavonoids, revealed a potentially interesting activity on staphylococci, clinically significant microorganisms also for the emergence of methicillin-resistant variants [91]. Two compounds in particular, proAc (proanthocyanidin A2-phosphatidylcholine) isolated from A. hippocastanum and CH (chelerythrine) purified from Macleaya cordata, exhibited an inhibition of ‘de novo’ biofilm formation without bactericidal activity. The treatment of bacteria with these alkaloids downregulates some important proteins belonging to different pathways. In particular, proAc acts on the iron-binding protein (determining the impairment of the uptake of iron, an essential micronutrient for microorganisms), blocking the switch process from planktonic to sessile state of bacteria and ablating autolysin (penicillin-binding protein), thus inhibiting biofilm formation. The treatment with sanguinarine and CH acts on some bacterial proteins involved in heat shock response, 

#### References

surface exposed lipids and methoxy–mycolic acid synthase, until protein synthesis disappearance. Both sanguinarine and CH also act on some elements of the bacterial cytoskeleton, structural compartment recognized as a potential target for antimicrobial therapy; therefore, inhibitors of cytoskeletal proteins may function as lead compounds for the development of novel antimicrobials. Hamamelitannin, a polyphenol extracted from the bark of Hamamelis virginiana belonging to the family of tannins, significantly reduces biofilm metabolic activity of different microorganisms [92]. Carvacrol, a monoterpenic phenol natural biocide, had an effect on dual-species biofilms formed by S. aureus and Salmonella enterica serovar typhimurium [93]. Nonbiocidal concentrations of this molecule disrupted normal development of biofilm, preventing the build up of protein mass and arresting at the microcolony stage. This component, together with thymol, is the principal phenolic component that determined the antimicrobial activity of oregano oil on staphylococci [94]. These molecules, characterized by a hydrophobic nature, interact with the lipid bilayer of cytoplasmic membranes causing considerable effects on its structural and functional properties and loss of integrity of bacterial cell. Moreover, these compounds may diffuse through the polysaccharide matrix of the biofilm thus destabilizing it.

A compound (1-deoxynojirymycin) purified from Morus alba inhibited biofilm formation of S. mutans, a major causal organism of dental caries, reducing bacterial extracellular polysaccharide secretion [95]. Similarly a new naphthalene compound from Trachypermum annum seeds exhibited the same effect indicating great potential as a therapeutic agent against caries [96]. Moreover, another novel strategy to reduce development of dental caries may be the use of plant lectins, proteins that recognize the glycoconjugates present on the surface of S. mutans; in particular glucose/mannose-specific lectin altered the adhesion of bacteria on saliva-coated surfaces [97]. Also, Propionibacterium acnes, microorganism responsible for acne vulgaris and able to form biofilm, resulted susceptible to plant extracts containing icariin, resveratrol and salidroside, compounds able to reduce biofilm formation [98].

For the treatment of urinary tract infections, Melia dubia, a plant from Meliaceae family present in the Indian subcontinent, has been used in folk medicine. Ravichandiran et al., examining the antiviral potentiality of this plant, evaluated the principles antagonizing the quorum sensing systems of uropathogenic E. coli (99). They found few compounds which can curtail the bacterial biofilm formation and virulence factor by controlling their quorum sensing.

### Antifungal activity

Human fungal infections, particularly in immunocompromised persons (AIDS, cancer and transplant patients), are a very challenging problem because the therapeutic options are hampered by serious drawbacks, such as the development of drug resistance and toxic side effects [5]. Thus, there is a clear demand for new therapeutic approaches based on molecules found in plants that may be used directly or considered as a model for developing better molecules. Before 2009, more than 600 plants have been reported for their antifungal properties, but few were examined for the active molecules [100]. Recently the use of the natural product tea tree oil in antifungal therapy has been proposed. This compound appears to be effective in vitro against multidrug-resistant Candida and in vivo against mucosal candidiasis [101]. Moreover, it has also been documented that terpinen-4-ol rather than 1,8-cineole is the most likely mediator of tea tree oil activity or, at least, a main contributor to anti-Candida activity. The genus Paeonia is one of the most important sources of drugs in traditional Chinese medicine. Picerno et al. observed that its extracts and some of their compounds inhibited C. albicans growth [102]. Different authors [103–112] recently demonstrated the antifungal properties of essential oils obtained from different aromatic plants, in particular from Mentha suaveolens [105], whose main microbicidal components were pulegone and piperitone oxide. A strong antifungal activity of essential oils obtained from other plants was demonstrated [28–30,103,104]. In particular, in Bidens tripartite L. roots [106], the main components are α-pinene, β-bisabolene, p-cymene, hexanal and linalool; in Coriandrum sativum extracts, the effect is fungicidal and responsible for a marked reduction of germ tube formation [107]. From several parts (flower, leaf and stem containing different compounds) of Aloysia triphylla, Gypsophila bicolor, Lavandula viridis, Erigeron acris and annuus, and also from star anise (Illicium verum) an activity, linked to trans-anethole, was observed [108–112]. Coumarin and phytoalexins, which are hydroxylated derivatives of coumarins, revealed a certain antifungal activity [113]. The antifungal activity of dill (Anethum graveolens) oil results from its ability to disrupt the permeability barrier of
the plasma membrane and from mitochondrial dysfunction-induced reactive oxygen species accumulation in Aspergillus flavus.

Promising activity against C. albicans biofilm formation was displayed by eugenol and cinnamaldehyde, molecules belonging to the phenolic group of essential oil compounds [114], which also showed synergy with fluconazole in vitro.

The essential oils of different Curcuma spp., containing caryophyllene as major compound, displayed varying degrees of antimicrobial activity, in particular against Cryptococcus neoformans [115]. A protective effect of an oral natural phytonutrient was observed in recurrent vulvovaginal candidiasis [116], and promising alternatives were revealed by several terpenic derivatives for the topic treatment of oral candidiasis and denture stomatitis [117]. Some antidermatophytic compounds, that have been long used as Chinese medicines to treat various ailments such as dermatomycosis, were obtained from extracts of Fructus psoraleae and Folium eucalypti globuli [118] and also from Achillea millefolium extracts [119]. Moreover, flavonoids isolated from mango (Mangifera indica) leaves revealed antifungal activity on different species, in particular Aspergillus sp. [120], and schinol and a new biphenyl compound were active on Paracoccidioides brasiliensis [121]. Moreover, metronidazole showed a potentiation of its antifungal effect when combined with plant extracts [122,123], as did fluconazole with other phytocomponents [114]. Using genetic and biochemical approaches, Xu et al. showed the antifungal activity of a plant-derived acetylenic acid by interfering with the fatty acid homeostasis pathway [124].

**Conclusion**

The spread of drug-resistant microorganisms is a big threat to successful therapy of microbial diseases. Therefore there is an urgent need to search new compounds characterized by diverse chemical structures and mechanisms of action. The use of different plant natural compounds as antibacterial and antifungal agents is an interesting strategy for discovering bioactive products. Resurgence of interest of ethnopharmacologists, botanists, microbiologists and natural-product chemists in the study of phytochemicals, and increasing consumer demand for effective, safe, natural products, means that quantitative data on this activity and ingenious screening programs are required. Plants are rich in a wide variety of secondary metabolites, such as flavonoids, alkaloids, tannins and terpenoids, which have been found in vitro to have antimicrobial properties. There is a relationship between the chemical components of plant-derived extracts and oils and the antimicrobial activity, even if the comparison of the different literature data may be problematic, owing to the composition of plant oils and extracts varying according to local climatic and environmental conditions, and to the production of particular chemical compounds from different plant species. These studies represent a good basis to select particular molecules belonging to the indicated categories that in the near future will become useful therapeutic tools.

**Future perspective**

The spread of drug-resistant microorganisms is a big threat to successful therapy of microbial diseases. Therefore there is an urgent need to search new compounds characterized by diverse chemical structures and mechanisms of action. The use of different plant natural compounds as antibacterial and antifungal agents is an interesting strategy for discovering bioactive products that in the next years could become useful therapeutic tools.

**Financial & competing interests disclosure**

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.
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Papers of special note have been highlighted as:

- of interest
- of considerable interest


- Updates the research on the utility of natural products as sources of novel therapeutic tools.


- Summarizes the current status of botanical screening efforts to evaluate antimicrobial activities.

Review

Savoia

**Owing to the emergence of new cases and the increased incidence of multidrug-resistant strains of *Mycobacterium tuberculosis*, the authors studied different sources and chemical type natural compounds as antimicrobial leads.**

**The authors studied the activity of flavonoids as antibacterial agents evaluating the structure–activity relationship.**


Nazaruk J, Karna E, Wieczorek P, Sacha P, Tryniszewska E. *In vitro* antiproliferative and


- The authors observed that some phytocompounds had promising antibiofilm activity on *Candida albicans* and also synergy with fluconazole *in vitro*.


