Send Orders of Reprints at reprints@benthamscience.net

932 *Current Medicinal Chemistry,* **2013,** *20,* **932-952**

Antimicrobial Plant Metabolites: Structural Diversity and Mechanism of Action

N.S. Radulović*,[1], P.D. Blagojević[1], Z.Z. Stojanović-Radić[2] and N.M. Stojanović[3]

[1]Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia; [2]Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia; [3]Faculty of Medicine, University of Niš, Zorana Đinđića 81, 18000 Niš, Serbia

Abstract: Microbial infectious diseases continue to be one of the leading causes of morbidity and mortality. It has been estimated that microbial species comprise about 60% of the Earth's biomass. This, together with the fact that their genetic, metabolic and physiological diversity is extraordinary, makes them a major threat to the health and development of populations across the world. Widespread antibiotic resistance, the emergence of new pathogens in addition to the resurgence of old ones, and the lack of effective new therapeutics exacerbate the problems. Thus, the need to discover and develop new antimicrobial agents is critical to improve mankind's future health. Plant secondary metabolites (PSMs) offer particular promise in this sense. Plant Kingdom could be considered a rich source of the most diverse structures (e.g. there are more than 12,000 known alkaloids, more than 8,000 phenolic compounds and over 25,000 different terpenoids), many of which were proven to possess strong antimicrobial properties (e.g. thymol, eurabienol, etc.). In many instances, PSMs can be easily isolated from the plant matrix, either in pure state or in the form of mixtures of chemically related compounds. What is also important is that the development of bacterial resistance toward natural plant products (that are generally regarded as eco-friendly) has been thus far documented in a very limited number of cases (e.g. for reserpine). Having all of the mentioned advantages of PSMs as potential antimicrobials in mind, a major question arises: why is it that there are still no commercially available or commonly used antibiotics of plant origin? This review tries to give a critical answer to this question by considering potential mechanisms of antimicrobial action of PSMs (inhibition of cell wall or protein synthesis, inducing leakage from the cells by tampering with the function of the membranes, interfering with intermediary metabolisms or DNA/RNA synthesis/function), as well as their physical and chemical properties (e.g. hydrophilicity/lipophilicity, chemical stability). To address the possible synergistic/antagonistic effects between PSMs and with standard antibiotics, special attention has been given to the antimicrobial activity of PSM-mixtures (e.g. essential oils, plant extracts). Moreover, possible ways of overcoming some of PSMs molecular limitations in respect to their usage as potential antibiotics were also discussed (e.g. derivatization that would enable fine tuning of certain molecular characteristics).

Keywords: Plant metabolites, antimicrobials, resistance, molecular properties, mechanisms of antimicrobial action.

1. INTRODUCTION

It has been estimated that microbial species (MS), found in almost every habitat present in nature, comprise about 60% of the Earth's biomass [1]. This, together with their extraordinary genetic, metabolic and physiological diversity, makes them a major threat to the health and development of populations across the world. According to the latest published data in 2012, infectious (including parasitic) diseases were altogether responsible for the death of more than 8.7 million people worldwide in 2008 [2-4]. The majority of these deaths were of poor people living in low and middle income countries, with many of the deaths occurring in children under five years of age. Given the sketchy data, misdiagnosis and underdetection that are typical of health systems in impoverished areas, these numbers are almost certainly underestimated. However, the severity of the microorganism-caused infections is not connected only to the high mortality and morbidity rates. Corresponding number of cases could sometimes be poor indication of the burden of diseases. Some infectious diseases could have low mortality rates, but could result in a heavy loss of healthy years of life [2-4]. WHO (World Health Organization) data show that 1 billion people worldwide are directly affected by one or more infectious diseases [2-4].

The health problems related to MS infections are seriously exacerbated by the widespread antibiotic resistance, and the lack of effective new therapeutics. For example, tuberculosis (TB) is known to cause more than 10% of pediatric hospital admissions and deaths [4]. In 2009, 9.4 million new cases of TB were reported and 1.7 million people died of the disease. Meanwhile, the number of cases of multidrug-resistant tuberculosis (MDR-TB) is rising steadily. Each year, more than 400,000 people develop MDR-TB, which can spread from one person to another. Only in 2008, 440,000 cases of MDR-TB and 150,000 deaths were reported [4]. In some TB hotspots, up to 30% of patients are infected with drug-resistant strains. Extensively drug-resistant TB (XDR-TB), highlighted as a global threat to public health in 2006, is resistant to all of the most effective anti-TB drugs [4]. Meticillin-resistant *Staphylococcus aureus* (MRSA) that was detected for the first time in 1961 (Britain) is nowadays a "common" bacterium and it spread around the world very quickly. Everyday medical practitioners also face significant worldwide resistance problems with pathogens such as *Pseudomonas aeruginosa*, which causes the hospital-acquired pneumonia and complicated skin and soft tissue infections (cSSTI), *Escherichia coli*, the causer of urinary tract- (urethritis, cystitis, pyelonephritis; cUTI) and intra-abdominal infections (IAI), and other extended spectrum β-lactamase-producing Enterobacteriaceae, as well as *Klebsiella* species [5, 6]. These are just a few of the many examples, showing that the discovery and development of new effective agents against bacteria-borne infections is critical to improving mankind's future health. However, these new drugs should not only be effective, but also readily available, especially to those from the most vulnerable communities.

The search for new antimicrobials became the main goal of many research groups oriented toward medicinal chemistry and pharmacology. Many of them focused their work on the Plant Kingdom (*Plantae*) [7-17]. Since antiquity and up to modern age, different plant species are used in the treatment of common infectious diseases. For example, bearberry (*Arctostaphylos uva-ursi* (L.) Spreng) and cranberry (*Vaccinium macrocarpon* Ait.) are used to treat urinary tract infections [18], while tee tree (*Melaleuca alternifolia* (Maiden & Betche) Cheel) essential oil is a common therapeutic tool to treat acne and other infectious troubles of the skin [19-23]. An interesting statistics is that approximately 10-30% of all higher plants known are used in a therapeutic context and are regarded as medicinal [24-27], depending on the region and cultural diversity. Many medicinal plants, as extracts of a single species or within herbal mixtures, are now registered and commercially available as molecular mixtures under the label of botanical drugs. Only

*Address correspondence to this author at the Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia; Tel: +381628049210; Fax: +38118533014; E-mail: nikoradulovic@yahoo.com

1875-533X/13 $58.00+.00

in China, more than 100,000 formulae are currently documented [28, 29]. Even the contemporary Western pharmacopoeias list medicinal plant combinations that are as ancient as Dioscurides *De Materia Medica* [30]. Even in well developed countries, where pharmaceutical monosubstances (mono-constituent substance is a substance, defined by its quantitative composition, in which one main constituent is present to at least 80% (w/w)) [31] are easily available, plant-based preparations are prescribed frequently. This is the case with Japan, UK and US, where the number of visits to providers of Complementary Alternative Medicine in the last decades has exceeded the number of visits to all primary care physicians [32-34]. It is estimated that more than two thirds of the world population still rely on traditional medical remedies, mainly plants and plant derived agents, because of limited availability or affordability of pharmaceutical medicines [32-24]. There are a number of recent reviews disclosing the importance of natural compounds not only in direct treatment of human diseases, but also as lead compounds in drug design. Among 109 new antibacterial drugs approved by the U.S. Food and Drug Administration in the period 1981-2006, 69% originated from natural products of microbial origin, while 21% of the antifungal drugs approved were also microbial natural derivatives or compounds mimicking natural products [35].

The Plant Kingdom represents an enormous reservoir of the most structurally diverse compounds, many of which are proven to be active against a number of microbial species [10-17, 36]. Plant secondary metabolites (PSMs) (alkaloids, flavonoids, terpenoids, tannins, and many others) are particularly interesting in this sense [36]. While primary metabolism mainly leads to a relatively small number of target biomolecules, with one specific pathway leading to only a small number of primary metabolites, secondary metabolism is diversity oriented. In his excellent recent review article, Jurg Gertsch pointed out that biosynthetic molecular promiscuity, just like polypharmacology, seems to be a hallmark of molecular evolution [7]. The number of studies dealing with antimicrobial properties of PSMs is constantly rising [16] and the obtained results are often very encouraging. For example, according to the SciFinder search of the CAS data base, there are more than 3250 different studies published from 2000 to present (the SciFinder was last accessed on July 12, 2012; key words used: essential oil, antimicrobial activity), dealing with antimicrobial properties of essential oils alone (complex mixtures of volatile plant secondary metabolites, mainly mono- and sesquiterpenoids and phenylpropanoids). Before that, only 534 papers on the same topic were available. What is also important is that the development of bacterial resistance toward natural plant products (that are generally regarded as eco-friendly) has been reported in only several cases thus far [37-39]. Having all of the mentioned positive characteristics of PSMs as potential antimicrobials in mind, a major question arises: why is it that there are still no commercially available or commonly used antibiotics of plant origin? Although reviews covering some aspects of the problem [40-45] already exist, this review is aimed at giving a critical answer to this question by considering the problem as a whole, i.e. from a number of different stand points.

2. *PLANTAE* – RICH SOURCE OF THE MOST DIVERSE STRUCTURES

Just a quick look at the surrounding world is more than sufficient for anyone to become aware of the extraordinary diversity of the Plant Kingdom. That diversity does not end with the number of different species, their colors, shapes and odors, but is even more pronounced if we go down to the molecular level. All the beauty, versatility and "creativity" of the nature are reflected and could be seen in action in the large group of the most amazing and interesting plant products: the secondary metabolites (PSMs). The great majority of compounds from this heterogeneous (from both biosynthetic and structural points of view) group do not appear to participate directly in plant growth and development [36]. Instead, they have been shown to have important adaptive significance in protection against herbivores and microbial infection, as attractants for pollinators and seed-dispersing animals, and as allelopathic agents (allelochemicals are those secondary metabolites that enable interspecies communication) [7, 36]. Plant secondary metabolites were for a very long time, considered as biologically insignificant, unfairly neglected and received little attention from most plant biologists. On the contrary, organic chemists were quite amazed with the structural diversity of these novel phytochemicals. Extensive studies of their chemical properties started in the mid 19th century and are still going on [36, 46-51]. In this way, organic chemistry contributed significantly to the collection of knowledge on PSMs that was necessary for the better understanding of their biological importance, but also the studies of PSMs were crucial in shaping modern organic chemistry, by stimulating development of the separation and spectroscopic techniques and advanced synthetic methodologies that now constitute the core of contemporary organic chemistry [36].

One could recognize several different levels of PSM molecular diversity Fig. (**1**). C-atom framework, characteristic of a given (sub)class of natural products, would be the first one. Biosynthetically speaking, PSMs can be divided into three major groups: the terpenoids, the alkaloids, and the phenylpropanoids and allied phenolic compounds [36]. Some representatives of the mentioned classes of compounds of our choice appear in (Table **1**). Compiling a comprehensive list of all up to now known PSMs would be a formidable task. For example, there are more than 12,000 known alkaloids, more than 8,000 phenolic compounds and over 25,000 different terpenoids (excluding primary metabolites) [36]. Additionally, all mentioned classes could be further subdivided. For instance, all terpenoids are derived from the five-carbon precursor isopentenyl diphosphate (IPP), and based on the number of IPP units they are recognized as mono-, sesqui-, diterpenoids, etc. According to the Dictionary of Natural Products (DNP) [52], there are 147 different sesquiterpene skeletal types, and 118 different diterpene subclasses. In addition to that, some of the most interesting secondary metabolites do not originate from only one biosynthetic pathway [53]. And finally, nowadays, the border between secondary and primary metabolism is taken as quite blurred [36].

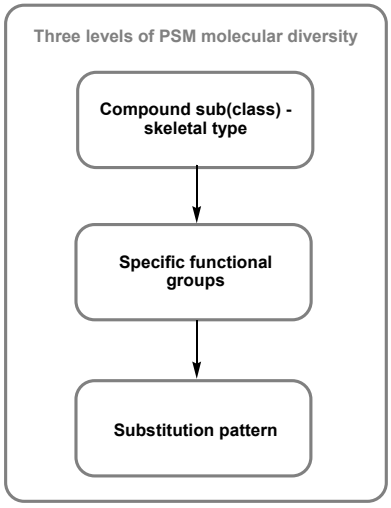
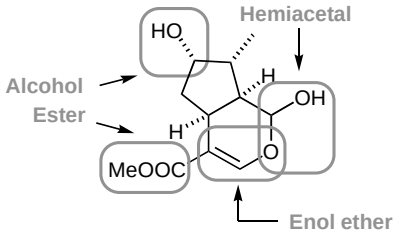
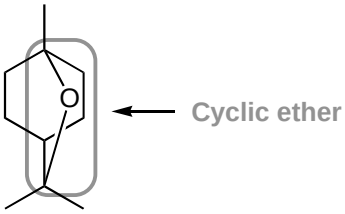


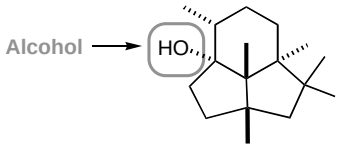
Fig. (1). Scaffold, functional group and substitution pattern based molecular diversity of PSMs.

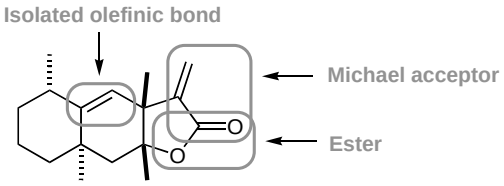
Presence/absence of some specific functional groups further diversifies the compounds belonging to the same biosynthetic (sub)class, Fig. (**1**). Compounds from (Table **1**) exemplify the great diversity of natural compounds as "carriers" of different chemical characters.

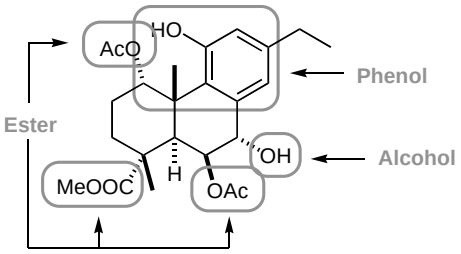
Table 1. Plant Secondary Metabolites: Insight into the Diversity of Skeletal Types, Functional Groups and Substitution Patterns

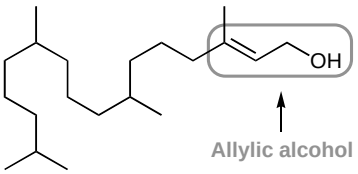
<table>
<tr>
<td> 

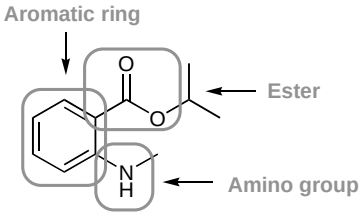
Loganetin (monoterpene-iridoid)</td>
<td> 

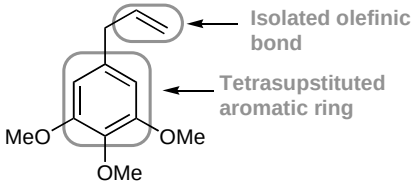
1,8-Cineole (Monoterpene)</td>
</tr>
<tr>
<td> 

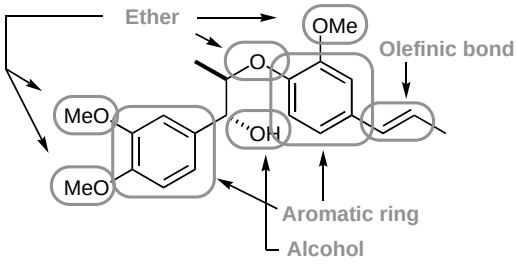
epi-Presilphiperfolan-1-ol (Sesquiterpene)</td>
<td> 

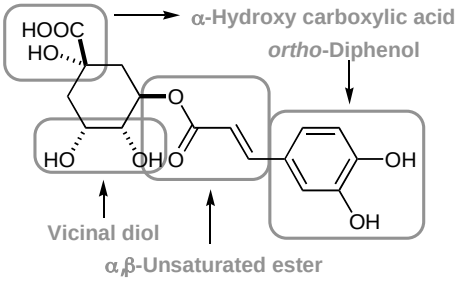
Alantolactone (Sesquiterpene lactone)</td>
</tr>
<tr>
<td> 

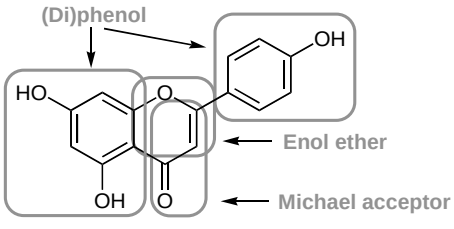
Eurabienol (Diterpene)</td>
<td> 

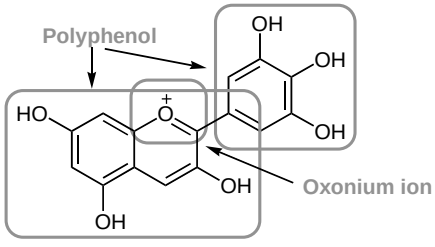
(E)-Phytol (Diterpene)</td>
</tr>
<tr>
<td> 

Ternanthranin (C_6C_1 Shikimate metabolite)</td>
<td> 

Elemicine (Phenylpropanoid)</td>
</tr>
<tr>
<td> 

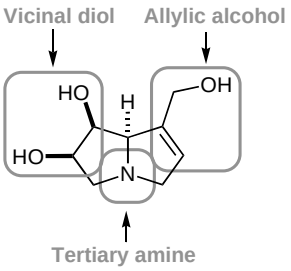
Virolin (Lignan)</td>
<td> 

Chlorogenic acid (Phenylpropanoid)</td>
</tr>
<tr>
<td> 

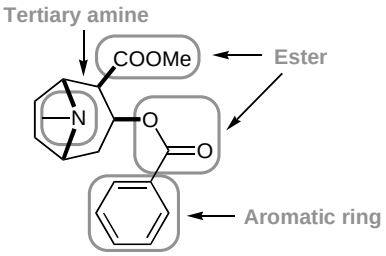
Apigenin (Flavone)</td>
<td> 

Delphinidin (Anthocyanin)</td>
</tr>
</table>

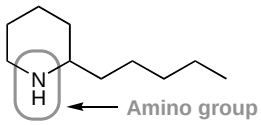
(Table 1) contd…



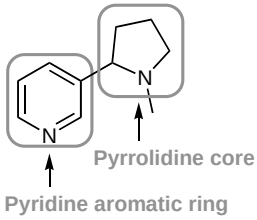
Crotanecine (Pyrrolizidine alkaloid)



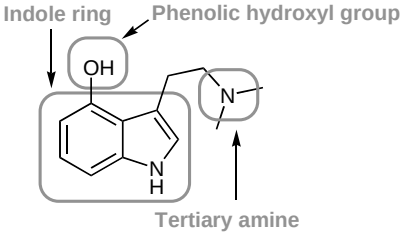
Cocaine (Tropane alkaloid)



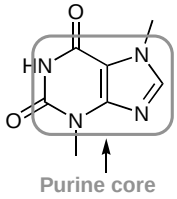
Conmaculatin (Piperidine alkaloid)



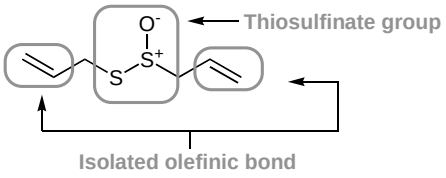
Nicotine (Pyridine alkaloid)



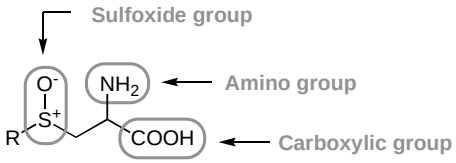
Bufotenin (Indole alkaloid)



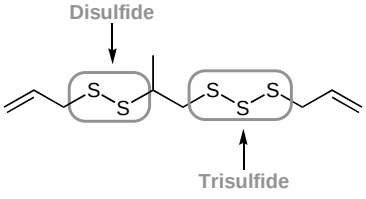
Theobromine (Xanthine i.e. purine alkaloid)



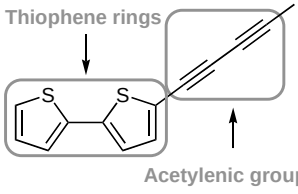
Allicin



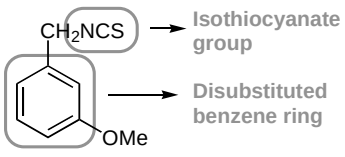
***S*-alk(en)yl cysteine sulfoxides**



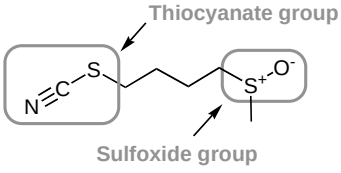
8-Methyl-4,5,6,9,10-pentathiatrideca-1,12-diene



5-(Penta-1,3-diynyl)-2,2'-bithiophene (Polyacetylene)



3-Methoxybenzyl isothiocyanate



1-(Methylsulfinyl)-4-thiocyanatobutane

Loganetin (loganin aglycone), iridoid monoterpene identified in the diethyl ether extract of *Lonicera fragrantissima* Lindl. & Paxton (Caprifoliaceae) [49], could serve as an excellent example of this. Although its skeleton is comprised of only 11 carbon atoms, this compound has 4 chiral centers and 4 different functional groups. Two of these (hemiacetal and enol ether) are in fact masked carbonyl groups, regarded to be at the very heart of organic chemistry.

Examples of strong antimicrobial PSMs possessing a Michael acceptor, the α,β-unsaturated lactone ring, are the eudesmane type sesquiterpenes alantolactone and its two regioisomers (diplophyllin and isoalantolactone) which are the main constituents of *Inula helenium* L. root essential oil [54]. Apigenin and chlorogenic acid are further examples of such molecules. Although Michael acceptors are traditionally shunned in modern drug discovery, trapping of thiols by covalent coupling represents an important mechanism of bioactivity, and many biologically relevant and druggable pathways are targeted by thiol-reactive compounds [55]. Ternanthranin, a new alkaloid found in the essential oil of *Choisya ternata* Kunth. (Rutaceae), is not only structurally (*N*-methyl derivative of anthranilic acid) but also pharmacologically similar to aspirin (acetylsalicylic acid) [51]. Other compounds listed in (Table **1**) also possess a number of interesting structural attributes: aromatic rings that could be involved in non-covalent π-π bonding interactions with target biomolecules, H-donors/acceptors, ionizable groups, electrophilic centers or nucleophilic groups that could be involved in the corresponding substitution/addition reactions [56].

In addition to being characterized by a high diversity of complex scaffolds and presence of different functional groups, the substitution pattern of the parent carbon skeleton of PSMs is often very specific and in some instances hardly obtainable in laboratory in satisfactory yields, especially using traditional synthetic methods. All of this appears to hold great promise for future drug discovery [36, 52, 53]. As an illustration of such diversity within a single subclass, one can use the structures of 10 different (but relatively common) and simple PSMs from the large family of monoterpenes having a *p*-menthane skeleton, Fig. (**2**). Although some being only mutually isomeric, differing in the position of double bonds, and others bearing a different functionality/hetero atom, the differences between these molecules, no matter how insignificant these might be at first glance, may be of great importance when speaking of biological properties of these compounds. Such activity is strongly related to compound's molecular structure and there is a number of examples showing that slight changes lead to either fine tuning, enhancing or complete loss of activity [56]. The already mentioned isomeric eudesmane type sesquiterpene lactones alantolactone, isoalantolactone and diplophyllin can provide an example of this. Even though differing only in the position of the isolated double bond, the composition-activity relationships analyses revealed that the antimicrobial potential of diplophyllin is significantly higher than that of the other two isomers [54].

The structures of several well known synthetic broad-spectrum antibacterial drugs of the (fluoro)quinolone class, with an important role in treatment of serious bacterial infections, are given in Fig. (**3**), and should demonstrate another potential of PSMs [57]. Although their basic pharmacophore is the quinoline ring system, each substitution introduces a specific feature, increasing their potency against certain bacterial infections [57]. Similarly, if we start with a PSM compound, that need not have very strong antimicrobial properties, by means of an appropriate chemical modification, we could arrive at much more active compounds. Modification of functional groups, addition/elimination of some specific substituents, or changing the substitution pattern could result in a broad spectrum of structures with finely tuned biological/pharmacological properties (both activity and selectivity). Even if PSMs are only poorly active, they could be, as candidates for the SOSA approach (selective optimization of side activities of drug molecules), excellent starting points in drug discovery. SOSA is an intelligent approach for the generation of new biological activities: only a limited number of highly diverse and well characterized (bioavailability and toxicity) drug molecules are screened and only positive hits are used as the starting point for a drug discovery program. Using the traditional medicinal chemistry as well as parallel synthesis, the initial "side activity" is transformed into the "main activity" and, conversely, the initial "main activity" is significantly reduced or abolished [58]. In the course of a study having a goal to identify the naturally occurring antimicrobial volatile glucosinolate autolysis products from *Hornunqia petraea* (L.) Rchb. (Brassicaceae), a series of possible glucosinolate breakdown products was synthesized: benzylic isothiocyanates and thiocyanates and phenylacetonitriles, bearing methoxy- and hydroxyl-groups at different positions of the benzene ring [46]. The antimicrobial activity (expressed as MIC and MBC/MFC values (minimal inhibitory/ bactericidal/fungicidal concentrations)) of these structurally closely related products varied from 0.001 to 1.25 mg/ml against several common human pathogens (bacteria and fungi), corroborating once again all of the previously mentioned [46].

At present, when data on biological properties of numerous natural products are available, the main interest concerning PSMs is aimed at the search for new drugs. The mentioned structural assortment provided by *Plantae*, makes them rich mines of biologically valuable molecules, with finely tuned activity. Hypothetically speaking, if one class of compounds "doesn't work" in a given situation, there are a vast variety of others that might be suitable. Also within a class, two compounds differing only in the functional group type, or just in their mutual position, may have completely different activity or selectivity. Thus, it seems that the main task of phytochemistry, ethnopharmacology and related disciplines is to recognize (identify) and pick (isolate) appropriate PSMs from a rich pool provided by the Plant Kingdom. The fact that one of the reasons plants biosynthesize these compounds is the defense against microorganisms [7, 36] justifies the efforts of many researchers aimed towards finding PSMs with antimicrobial activity. Complexity of their structures (carbon skeleton, number of different functional groups and stereo centers) makes their synthesis very challenging and costly, and leaves the isolation from plant material as the best option. However then, new questions arise: how easily can we obtain a PSM from a plant matrix? Do we need the pure compound, or can a mixture suffice? We will try to systematically answer these and many other related questions in the following sections.

3. ANTIMICROBIAL POTENTIAL OF PSMs

If one wants to study or use whatever natural compound for any reason, one must have satisfactory amount of it. Usually, in the case of plant metabolites, that means that the compound has to be isolated from a plant matrix. So, where to begin? Isolation of any natural product generally follows the procedure given in Fig. (**4**). The first step is always the preparation of a plant crude extract (PCE) [59]. This can be a solvent extract, an essential oil (obtained by steam distillation or hydrodistillation) or a super-critical extract. Crude extracts usually represent highly complex mixtures (of both secondary and primary metabolites), belonging to different biosynthetic and chemical classes that share some general mutual characteristic, such as polarity and/or volatility. The choice of the extraction procedure directly depends on the type of compounds we wish to investigate. For (semi-)volatile compounds, hydrodistillation or steam-distillation is certainly appropriate. In that case we end up with an essential oil sample. Diethyl-ether will extract a large number of structurally different PSMs, but of similar polarity, etc. [59]. The number of PSMs that can be found in a crude extract vary from several tens to several hundreds [59]. Common separation techniques (most usually different types of liquid chromatography) enable some sort of PCE simplification, i.e. its partitioning to compositionally more coherent semi-pure mixtures, in the best case comprised of 5-10 different compounds [59]. With some additional effort and a little bit of good fortune, these mixtures might be further purified to yield a single compound in the end.

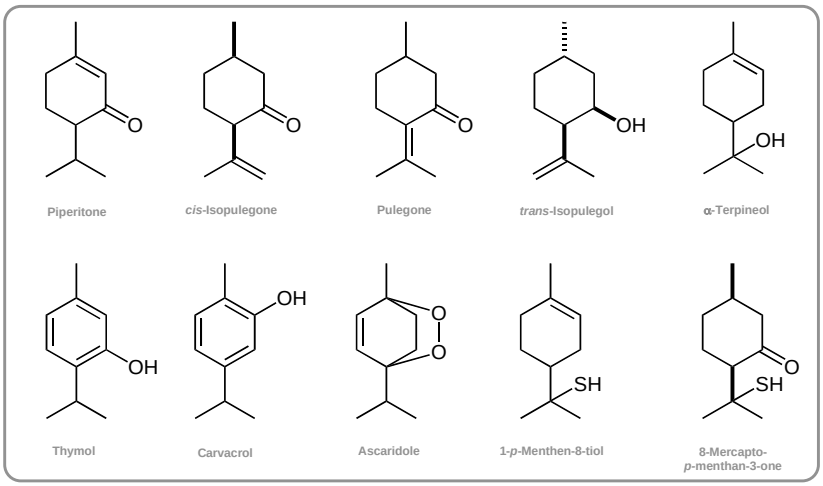


Fig. (2). p-Menthane type monoterpenoids.

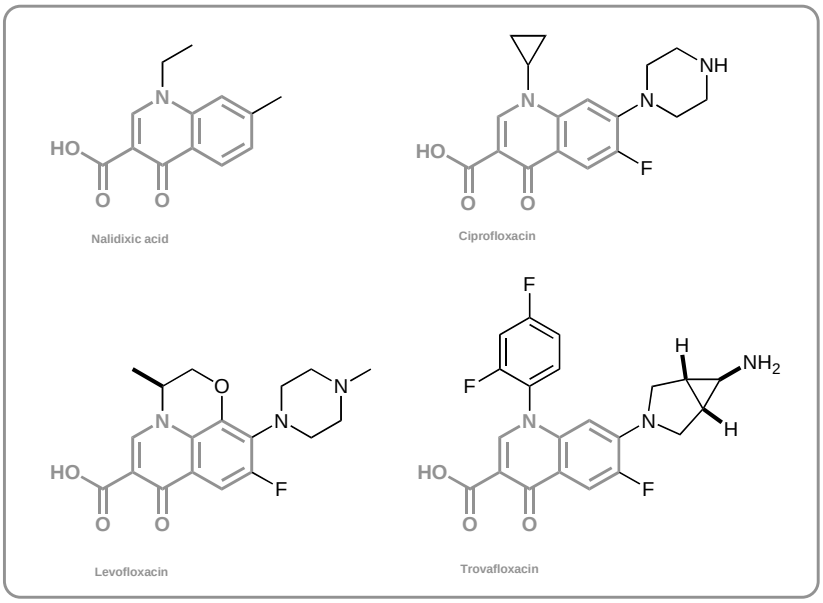


Fig. (3). Structures of (fluoro)quinolone antibiotics.

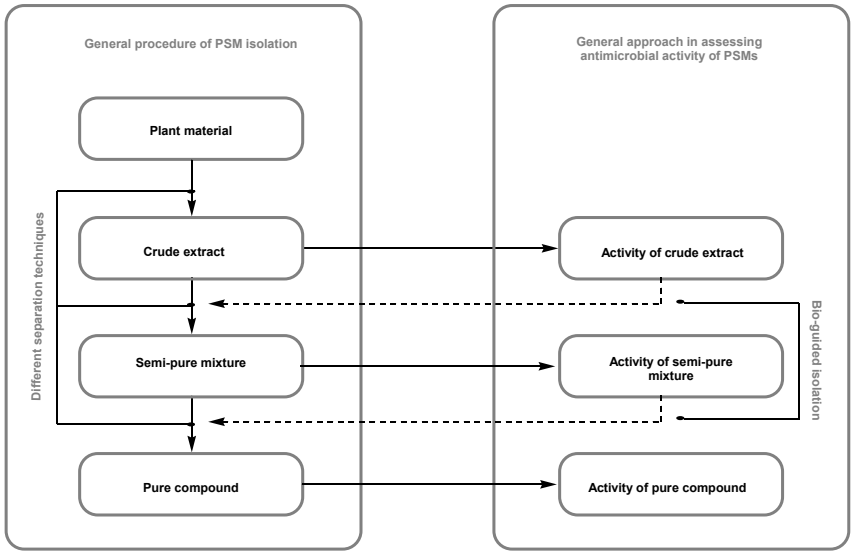


Fig. (4). "Links" between PSM isolation and the assessment of antimicrobial activity.

The general approach in assessing antimicrobial activity of plant products correlates very closely to the described procedure of PSMs isolation/purification, Fig. (**4**). There are three different general levels of investigation of antimicrobial activity of plant metabolites depending on the type of PSM-biological sample being tested (level 1: antimicrobial activity of PCE; level 2: antimicrobial activity of semi-purified mixtures; level 3: antimicrobial activity of pure PSM). Probably the most frequent study is that focused on the antimicrobial properties of different PCE [7, 9-15, 46, 47]. The selection of taxa to be investigated in this way usually strongly relies on their potential usage in ethnopharmacology [7, 9, 51, 60-67]. There are two general reasons why such studies are gaining popularity. Firstly, this represents the easiest way (time saving) to obtain a PSM-sample for biological assays. Secondly, such mixtures correspond to those usually directly employed in folk medicine. Since traditional healing practices employ crude extracts, and not purified molecules, one cannot ignore the possible interactions between the various constituents and different species, and these level 1 studies do not exclude these interactions. Researchers working in the field of ethnopharmacology often try to set their methodologies as to be as coherent as possible with the real-life environment in a traditional setting [7, 9].

The results obtained by PCE-antimicrobial screening sometimes serve as a starting point for more in depth studies. Positive results in PCE-antimicrobial assays could motivate further work, focused on the location, bio-guided isolation and chemical characterization of the active principle(s), Fig. (**4**). As an example, the results of Rios *et al.*, and Recio *et al.* could be used [16, 60, 61]. They have preliminary screened 140 medicinal plants (two extracts of each) used in the Mediterranean region as anti-infection agents, and then selected one (*Helichrysum stoechas* (L.) Moench) of them, as the most promising one, to study comprehensively. At the end, they managed to locate, isolate and identify 10 active principles, four of which exhibited activity (MIC) in the range of 3–25 μg/ml against Gram-positive bacteria [16, 60, 61].

3.1. Mechanism of PSM Antimicrobial Action

Plant secondary metabolites can affect the microbial cell in several different ways as schematically depicted in Fig. (**5**). These include the disruption of membrane function and structure (including the efflux system), interruption of DNA/RNA synthesis and function, interference with intermediary metabolism, induction of coagulation of cytoplasmic constituents and interruption of normal cell communication (quorum sensing, QS) [68-82]. This antibacterial action usually includes the following sequence of events: PSM interaction with the cell membrane, diffusion through the membrane (i.e. PSM penetration into the interior of the cell), PSM interaction with intracellular constituents/processes [83]. When trying to elucidate a mechanism or mechanisms of antimicrobial action of a compound, one should bear in mind that all antibiotics that have been successfully employed for decades as monotherapies in the treatment of bacterial infections rely on mechanisms of bacterial growth inhibition which are by far more complex than inhibition of a single enzyme [84]. Some authors even speculate that nature itself has evolved the concept of so-called "dirty" or promiscuous agents, to achieve pharmacological potency and plasticity by polypharmacology [7]. Similarly is true for plant natural products. Thymol, an aromatic *p*-menthane type monoterpene phenol and one of the plant's most active secondary metabolites provides a good example of such a type of PSM. This compound is thought to interact with both outer and inner cytoplasmic cell membranes, by integrating at the polar head group region of the lipid bilayer. This alternates the cell membrane and leads to its increased permeability/disintegration [85-87]. However, thymol could also take part in the up- or down regulation of genes involved in outer membrane protein synthesis, inhibition of enzymes involved in protection against thermal stress, synthesis of ATP, citric metabolic pathways, etc. [88, 89]. A shikimate metabolite *trans*-cinnamaldehyde causes inhibition of the fungal cell-wall synthesizing enzymes by functioning as a non-competitive inhibitor of β-(1,3)-glucan synthase, as well as a mixed inhibitor of chitin synthase isozymes [90]. Also, a study on *Saccharomyces cerevisiae* demonstrated that *trans*-cinnamaldehyde caused a partial collapse of the integrity of the cytoplasmic membrane, leading to excessive leakage of metabolites and enzymes from the cell and final loss of viability [72]. According to Hyldgaard *et al.* [91], at least three processes occur during the cinnamaldehyde antimicrobial action: at subinhibitory concentrations, enzymes involved in cytokinesis are affected, while higher concentrations cause inhibition of the enzyme ATPase; lethal concentration induces perturbation of the cell membrane [72, 77, 85, 92-94]. It seems that another phenylpropanoid, vanillin, mainly functions as a membrane active compound, but has intracellular targets as well [91, 95, 96]. Since a great number of PSMs function in this way, in the following text, further examples of antimicrobial polypharmacology of individual PSMs will be described.

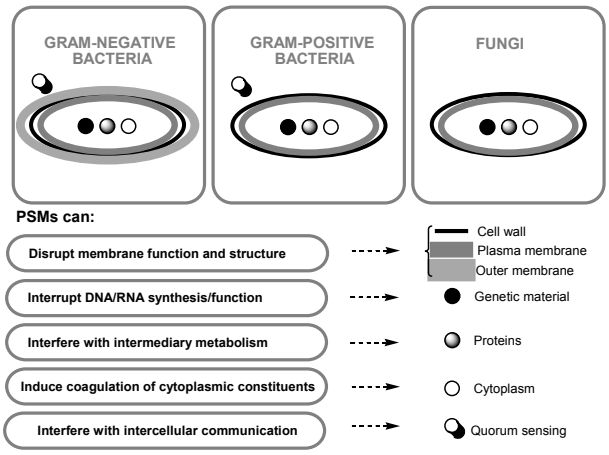


Fig. (5). Possible targets of PSMs.

The situation with "locating" targets of PSM-based drugs becomes even more complicated when dealing with botanical mixtures (e.g. essential oils) that contain hundreds of potentially bioactive natural products. A major problem in botanical drug research is the identification of molecular targets of all bioavailable compounds within the extract (i.e. the overall mode of action). Biochemical methodologies, commonly applied in elucidating the mode of action of a drug, are often useless in the case of complex PSM mixtures [7, 97]. Antimicrobial potential and mechanism of action of different PSMs can be influenced and is highly dependent on several factors such as the features of target cells (bacterial/fungal cell, Gram-positive/Gram-negative bacteria), and also the environment where the antimicrobial action should be exhibited. Environmental conditions - hydrophilicity (i.e. solubility in water), concentration, temperature and pH are very important for the final effect of a PSM/PSM-mixture [98]. Here, it could be useful to state that the effect of natural compounds is expected to be very similar for Gram-positive bacteria and fungal organisms, where the main target is the cell envelope, whose disintegration or changes in permeability are followed by an efflux of the intracellular compounds and coagulation of cytoplasm [99].

Available literature data on the antimicrobial action of PSMs points to the fact that their primary target site is the cytoplasmic membrane. Natural products can affect its structure and integrity, permeability or functionality in more than one way. For example, some antifungal agents interact with ergosterol, which is the main sterol of the fungal membrane involved in processes such as main-

taining the fluidity and integrity of the membrane and regulation of enzymes necessary for the growth and division of fungal cells [100, 101]. Saponins (e.g. avenacins A-1, B-1, A-2 and B-2, a family of four structurally related compounds, containing a common esterified trisaccharide moiety), many of which have potent antimicrobial activity, could be used as an example of such compounds [79]. The antifungal properties of saponins are generally ascribed to the ability of these molecules to complex with sterols in fungal membranes, so causing pore formation and loss of membrane integrity [79]. Experiments with planar lipid bilayers have confirmed that avenacin A-1 induces permeabilization in a sterol-dependent manner and that it also affects membrane fluidity. The presence of an intact sugar chain attached to the C-3 position is critical for these effects on artificial membranes and also for effective antifungal activity [79]. The sugar chains may mediate the aggregation of saponin–sterol complexes in the membrane, so facilitating membrane disruption [79]. Removal of a single D-glucose molecule from the trisaccharide chain results in a substantial reduction in biological activity [79].

Carvacrol, a compound isomeric to thymol, interacts with the cytoplasmic membrane by inserting between acyl chains of phospholipids [72, 33, 102]. The mentioned process leads to disturbance (increase) of the membrane fluidity and its higher permeability as a consequence. Increased permeability results with an efflux of ions and ATP, and with a disturbed membrane potential and pH gradient [102, 103]. Also, by measuring the release of lipopolysaccharides (present in the outer membrane), it was proved that it also affects the outer membrane, a structure responsible for higher resistance of Gram-negative bacteria [85, 86, 104]. Moreover, although it was found that it affects both the outer and inner membrane, studies showed that its main site of action is the cytoplasmic membrane, where the effect of this compound is significantly enhanced by its hydroxyl group which functions as a transmembrane carrier of monovalent cations, leading to the disturbance of the membrane potential [105, 106]. A recent study showed that this group is not essential for the antimicrobial activity of carvacrol, but significantly enhances its action [107].

Eugenol, a phenylpropanoid found in many plant species, shows a lytic effect on bacterial cells [108] and its mode of action is a non-specific membrane permeabilization [91], demonstrated in several studies by the efflux of potassium and ATP [97, 93]. It is thought that eugenol binds to membrane proteins, inhibiting and changing their functions [91]. Evident from the change in the fatty acid composition of the eugenol-treated cells, the membrane fluidity seems to be also affected that is a well known bacterial adaptive mechanism, developed in order to maintain optimal membrane features [102, 72, 77].

Permeabilization of the membrane and subsequent processes are induced by a number of other plant metabolites frequently present in antimicrobial plant extracts: linalool [109], linalyl acetate [110], menthol [110], and citral [111]. On the other hand, limonene changes cell morphology and the membrane fluidity [72, 77, 112].

Numerous studies have shown that many essential oils, extracted from different plant species, induce changes in the cell membrane permeability/integrity. Examples of such species from a number of plant families include: *Ocimum gratissimum* L. [113], *Cinnamomum verum* J. Presl [86], *Origanum vulgare* L. [86, 108, 114], *Syzygium aromaticum* (L.) Merrill & Perry [108], *Cinnamomum cassia* (L.) Presl [115], *Cymbopogon citratus* (DC.) Stapf [113], *Rosmarinus officinalis* L. [116], *Corydothymus capitatus* (L.) Reichb [116], *Satureja montana* L. [115], *Thymus eriocalix* (Ronniger) Jalas [117], *Thymus x-porlock* [117], *Kaempferia pandurata* Roxb. [118], *Origanum compactum* L. [119], *Sinapis alba* L. [120], *Thymus vulgaris* L. [88], *Coriandrum sativum* L. [121-123], *Mentha longifolia* (L.) Huds. [124], *Inula helenium* [54], *Carlina acanthifolia* All. [125], *Cuminum cyminum* L. [126], *Trachyspermum ammi* Sprague [127] and *Gnaphalium affine* D. Don [128]. One of the first studies dealing with the mode of action of an essential oil sample was the research of Takaisi-Kikuni *et al*. [129], which investigated the effect of *Cymbopogon densiflorus* on metabolic activity, growth and morphology of *S. aureus*. The results pointed to the decreased metabolism and lysis of the treated cells. The most detailed studies on essential oils' antimicrobial action were done for *Melaleuca alternifolia* essential oil, which has an exceptional antimicrobial activity, owing to its mainly monoterpene composition with terpinene-4-ol as the major constituent [19, 20, 22, 23, 73, 130, 131]. The study of Cox *et al.* [73] demonstrated inhibition of respiration, together with increased permeability of *E. coli* cell membrane, providing evidence of a lethal effect resulting from membrane damage. Cells of *E. coli* visualized by electron microscopy after exposure to tea tree oil showed a loss of cellular electron dense material and coagulation of cytoplasmic constituents, although it was apparent that these effects were secondary events that occurred after cell death [130]. Another study [22] on *E. coli, S. aureus* and *Candida albicans* (Gram-positive and Gram-negative bacteria and a yeast, respectively) gave similar results: cell death was the result of increased permeability of bacterial and yeast membranes, with notable differences in the susceptibility of the tested organisms. The conclusion of the mentioned study was that the ability of tea tree oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control is the most likely source of its lethal action at minimum inhibitory levels. Studies that followed confirmed the proposed mode of action, but expanded the knowledge about the processes occurring in the treated cells before its death – formation of mesosomes [23], decreased tolerance to high concentrations of NaCl [20], leakage of various cell materials, that all, once again, pointed to the cell membrane as the main target place of the microbial cells. The essential oil of *M. alternifolia* also possesses a remarkable activity against fungal cells, which prompted the study of Hammer *et al*. [131], where its mode of action was investigated against yeasts *Candida albicans*, *Candida glabrata* and *S. cerevisiae*. The mentioned study showed that tea tree oil changes the fluidity and, thus, permeability of the fungal membrane.

It has been proved that the effectiveness of PSM antibacterial agents generally increases with their increasing lipophilicity, which is in a way in direct relationship with their ability to interact with the cell membrane [83]. In most of cases, the different effects PSMs have on membrane are mutually highly dependent and one is followed by another. Plant secondary metabolites affect structure and stability of the phospholipid bilayer, which leads to the disturbance of membrane integrity and consequently increase of its permeability for ions. Consequentially, the membrane electrochemical potential will change, as well as the activities of membrane enzymes. Certain PSMs can interfere with the translocation of protons across the membrane, which affects the primary energetic metabolism by interrupting ATP synthesis. Loss of ATP leads to decreased active transport, and as additional consequences, inhibition of respiration, together with emerging inhibited anabolism/catabolism. Terpenoids with aldehyde or alcoholic moieties can interact with membrane incorporated proteins, by changing their conformation and, thus, their functionality. Gram-negative bacteria have innate multidrug resistance to many antimicrobial compounds owing to the presence of efflux pumps [132]. Recently Garvey *et al*. [82] indicated that extracts of different plants, used as herbal medicinal products, contain inhibitors of efflux in Gram-negative bacteria (the polyacetylene falcarindiol, for example). This is a very important finding, as it gives hope that PSMs could be truly useful in fighting multidrug resistant strains.

Alongside with the effect on cell membranes, the antimicrobial action of PSMs can be directed toward intracellular processes such as DNA/RNA/protein synthesis and cell communication. This is the case with allicin, the main compound of crushed garlic (*Allium*

sativum), (Table **1**). It is well known that its thiosulfinate -S(O)S- moiety readily reacts with free SH groups of intracellular enzymes. As indicated by many studies, this reaction is non-specific [91, 133, 134]. Feldberg *et al*. [135] showed a very significant inhibition of RNA synthesis, while DNA and protein syntheses were less affected by the action of allicin. It has been shown that allicin's inhibitory action on enzymes can be reversible, since thiol containing compounds like glutathione or 2-mercaptoethanol can reactivate inhibited enzymes - papain, $NADP^+$ dependent alcohol dehydrogenase and NAD^+ dependent alcohol dehydrogenase from horse liver [78]. Garlic aqueous extract induces changes inside the cells, probably similar to the changes caused by allicin alone, showed by atomic force microscopy [134]. As one of the extensively studied secondary metabolites, recently reviewed for its mode of action [91], allyl isothiocyanate was found to generally inhibit enzymes and cause alterations of proteins by oxidative cleavage of disulfide bonds [136, 137]. Although Lin *et al.* [71] showed that allyl isothiocyanate induces membrane damage to *E. coli* and *Salmonella* sp. that leads to leakage of cellular metabolites, but not to cell lysis.

One of the major groups of active plant compounds, the flavonoids act through inhibiting both cytoplasmic membrane function and DNA synthesis. Protein and RNA syntheses are also affected but in a lesser extent. Apigenin and quercetin, together with several other flavonoids were found to inhibit DNA gyrase and β-hydroxyacyl-acyl carrier protein dehydratase activities [68, 138]. A further study [69] reported that quercetin binds to the GyrB subunit of *E. coli* DNA gyrase and inhibits the enzyme's ATPase activity. Together with this activity, quercetin was found to be a membrane active compound as well-it caused an increase in permeability of the inner membrane and a dissipation of the membrane potential [139]. Studies on the membrane action of flavonoids showed that sophoraflavanone G induces the reduction of membrane fluidity [140]. The same mode of action (membrane effect) was confirmed for several others flavonoids: (−)-epigallocatechin gallate [141], (−)-epicatechin gallate and 3-*O*-octanoyl-(+)-catechin [142], as well as 2,4,2'-trihidroxy-5'-methylchalcone [143, 144]. Finally, flavonoids can inhibit the energy metabolism, as showed in the study of Haraguchi *et al.* [145], where the tested licochalcones strongly inhibited oxygen consumption, probably as a consequence of bonding to the inhibition site on the respiratory electron transport chain. Recently, the mode of action of flavonoids was investigated in the study of Ulanowska *et al.* [146], which showed that genisteine (isoflavone) significantly affected the morphology of *Vibrio harvey*. The same compound inhibited DNA, RNA and protein synthesis in the mentioned bacteria. The mode of action of the highly aromatic quaternary alkaloids, such as berberine and harmane, is also intercalation with DNA [147]. Targets of activity of phenylpropanoids, biosynthetically related to flavonoids, have also been found to be varying. For example, *Cinnamomum verum* ethanol extract, with cinnamaldehyde and eugenol as the active compounds, inhibits the activity of the enzyme histidine decarboxylase [148]. Verbascoside isolated from *Buddleja cordata* Kunth. inhibits protein synthesis [75]. Coumarins cause a reduction in cell respiration and condensed pheylpropanoids - tannins act on microorganism membranes as well as bind to polysaccharides or enzymes promoting inactivation [149-151].

The mode of action of PSM can be without a terminal outcome, but the production of substances important for pathogenicity, such as bacterial toxins, might be affected. Essential oils of several spice plants, such as clove, thyme and cinnamon, reduced the production of listeriolizin O by *Listeria monocytogenes* [152], whereas Filgueiras and Vanetti [153] demonstrated the same effect of eugenol on these bacteria. Also, it was confirmed that carvacrol inhibits the production of toxins in *Bacillus cereus* and *Clostridium botulinum* [154]. Enterotoxin production by *S. aureus* was reduced after the treatment with oregano essential oil [114]. Aflatoxin production in the cells of *Aspergillus flavus* was significantly influenced by the treatment with both lime (*Citrus aurantifolia* (Christm.) Swingle) and kaffir lime (*Cytrus histrix* DC.) essential oils [155].

It is now well recognized that populations of bacteria from many bacterial species cooperate and communicate to perform diverse social behaviors including swarming, toxin production and biofilm formation [81, 156-159]. Biofilms are the default mode-of-life for many bacterial species and biofilm-based infections cause serious health problems worldwide. Recent publications demonstrated that the use of XTT (2,3-bis(2-methoxy-4-nitro-5-sulfo phenyl)-2*H*-tetrazolium-5-carboxanilide) reduction and/or crystal violet staining is useful in the determination of biomass (biofilm) formation and adherence under the influence of different medicinal plant extracts [160-164]. Among microorganisms that produce biofilms the most famous one is *C. albicans*. This fungus is a part of the normal oropharynx microflora, but in immunocompromised patients (e.g. with an HIV infection) it can cause oropharyngeal candidiasis [165]. Also almost all women in their life time experience vulvovaginal infections caused by *C. albicans* [166]. Biofilm production by *Candida* strains is considered an important virulence attribute for establishing and maintaining candidiasis. During the past decade there has been an increase in the number of resistant *C. albicans* biofilm producing strains to standard antimycotic agents such as amphotericin B, fluconazole, itraconazole and ketoconazole [166]. Essential oils have been used effectively against such infections since ancient times and without (much documented) side effects. Volatile oils of *Cymbopogon citratus* and *Syzygium aromaticum* have been shown to possess antifungal effects against oral and vaginal candidiasis in *in vitro* and *in vivo* studies and their specific anticandidal activity against planktonic forms is well established [100, 163, 166, 167]. This anti-candidal (and anti-biofilm) activity makes the use of these oils highly recommendable in the cases of illnesses that include biofilm formation [163].

Communication between bacterial cells involves the production and detection of diffusible signal molecules and has become commonly known as quorum sensing (QS) [81, 158, 168]. QS represents density-dependent communication system that regulates the bacterial expression of specific genes, whose products modify the local host environment favoring the invasion and persistence of the pathogen [168]. The discovery that many pathogenic bacteria employ QS to regulate their virulence makes this system interesting as a target for antimicrobial therapy, and certainly opens new therapeutic prospects. In theory, the ideal QS inhibitor would have to fulfill several criteria: to be a low molecular-mass molecule able to highly specifically reduce the expression of QS-controlled genes, but also chemically sufficiently stable and resistant to the metabolic and disposal processes of the host organism. According to the up to now obtained results on this subject, (plant) secondary metabolites seem to be very promising in this sense [81, 158, 168], at least as a starting point for future research. It has been already demonstrated that extracts of more than a few common plant species (crops), such are bean sprout, chamomile, carrot, garlic, habanero (*Capsicum chinensis* Jacq.), propolis, water lily and yellow pepper, inhibit *P. aeruginosa* QS [169-175]. Garlic extract, which contains at least three different QS inhibitors, is able to inhibit QS in a concentration-dependent manner and with a structure–activity relationship hypothesizing a competitive binding. It significantly reduces *P. aeruginosa* biofilm tolerance to tobramycin, and lowers the pathogenicity of *P. aeruginosa* in a *Caenorhabditis elegans* nematode model. Resveratrol and solenopsin A also interfere with *P. aeruginosa* QS, whereas hamamelitannin (2',5-di-*O*-galloyl-D-hamame lose) was found to inhibit QS in *S. aureus* and *S. epidermidis* [175].

3.2. Synergistic Approaches to Enhance Activity

If a single compound or several compounds cannot be found that fully explain the results of an antimicrobial assay on the entire plant extract, a great number of papers on antimicrobial PSMs put

forward an idea that it is highly likely that the mixture of these is in fact responsible itself for the onset of such activity i.e. the interactions occurring within the extract. Mixtures of bioactive compounds in botanical drugs are widely claimed to be superior over monosubstances, and many believe that a synergistic therapeutic effect is mainly responsible for this [121, 176, 177]. As demonstrated by many studies, a relatively small number of constituents of a given mixture are biologically active. However, in the last decades, the discovery of highly potent lead compounds, such as morphine, cocaine, digitoxin, lysergic acid, aconitine etc., has declined despite the great interest of the scientific community in this topic [178]. Perhaps this means that authors arguing that in phytotherapy, the mixture makes the medicine [7], and that the synergism is one of the key factors leading to the high potency of botanical drugs, are right [9]. A recent example corroborating this is the Chinese antimalarial plant *Artemisia annua* L. Although the responsible pharmacological lead compound, the sesquiterpene peroxide artemisinin, is known and even developed clinically, the use of the drug as a monotherapy is explicitly discouraged by the WHO [179]. Instead, artemisinin combination therapy is recommended not only because it has a cure rate of 95% against the malaria parasite (*Plasmodium* sp.) but since it may also contribute to curb resistance [179]. Multidrug therapy has become of paramount importance in the fight against multidrug resistant microbial strains [180]. Without the current multidrug approach used to treat tuberculosis (isoniazid, rifampicin, pyrazinamide, and ethambutol), the mortality of infected patients could reach global epidemic proportions. The application of only one of the abovementioned drugs in a monotherapy leads to an increase in the number of multiple-drug resistant strains [181].

A well-known combined commercial antibiotic is the mixture of amoxicillin (a β-lactam antibiotic) and clavulanic acid. Clavulanic acid binds to β-lactamase producing microorganisms and in that way protects amoxicillin from β-lactamase attack that in turn results in an extended spectrum of activity for amoxicillin. The concept of antimicrobial synergy is based on the principle that, in combination, the formulation may enhance efficacy, reduce toxicity, decrease adverse side effects, increase bioavailability, lower the dose and reduce the advance of antimicrobial resistance [182-184]. It could be interesting to mention that in Asia the number of different patents based on an apparent synergistic botanical formulation is increasing almost exponentially [7].

As nicely worded by Jürg Gertsch [7], plants clearly do not produce secondary metabolites to benefit mammals but to potentially cope with the diverse ecological pressures, such as microbial attack. Plant species often respond to stress by increasing the biosynthesis of different classes of molecules, rather than just an individual PSM. Although according to some authors, secondary metabolites are unimportant for the fitness of a plant organism [185, 186], the standpoint of others, however, is that every PSM is made because it possesses (or it possessed at some stage of evolution) a biological function that endows the producer-organism with increased fitness. According to Firn and Jones, these opposing views could be reconciled by recognizing that, because of the principles governing molecular interactions, potent biological activity is a rare property for any molecule to possess. In fact, there are relatively few pharmacologically highly potent secondary metabolites known, and they may represent less than 1% of the total natural products. Of more than 5000 distinct natural product scaffolds (chemical skeleta), less than 100 have so far inspired the development of biomedical monotherapies [67]. However, if we assume that phytochemical mixtures exert a synergistic biological effect; more effects per scaffold would be possible [7].

A high degree of pharmacological synergism should be expected in essential oils, which are prototypical mixtures of PSMs. Synergistic interactions between PSM compounds were confirmed by many studies. Pattnaik *et al.* [187] noted that minimal inhibitory concentrations (MIC) of essential oils were in many cases lower than that obtained when the major constituents of these oils were applied independently. For example, the major compound of *Filipendula vulgaris* Moench. oil (68.6% of salicylaldehyde) was less active than the entire essential oil [188]. When combined in a 60:40 molar ratio with linalool (1.8% of the *F. vulgaris* oil), a strong synergistic activity was noted, with the mixture of the two having a higher activity than the oil itself. In another study, mixtures of linalool and methyl chavicol (different *v/v* ratios) were tested and it was observed that when these two compounds were combined, a higher efficacy was achieved, compared to when they were assayed independently. The same is true for many other combinations of volatile PSMs and against different common human pathogens (carvacrol/thymol, terpinene-4-ol/myrcene, carvacrol/*p*-cymene, eugenol/thymol, eugenol/carvacrol, cinnamaldehyde/eugenol, citro nellol/geraniol etc.) [105, 189-193]. Ultee and co-workers [102, 103, 105] proposed an explanation for the mode of synergistic activity of *p*-cymene and carvacrol. According to them, *p*-cymene has a high affinity towards the cytoplasmic membrane, and its bonding to the membrane causes its expansion altering its potential and resulting in its higher sensitivity to the action of carvacrol. Recently, it was demonstrated that the biological effects observed for the major compounds of *Ocimum gratissimum* L. oil do not sum up to the overall effect of the essential oil, suggesting a possible synergy between the constituting PSMs [194]. Similarly linoleic and oleic acids were found to have a higher antimicrobial activity in combination than those they showed independently [195].

There are numerous studies showing that the pharmacokinetics of certain bioactive natural products can be improved by applying them as mixtures rather than as single compounds. Mixtures may simply affect the solubility and distribution of the potentially active PSM(s). For example, ichthytoxic lignans justicidin B and piscatorin, PSMs of the poisonous *Phyllanthus piscatorum* L. are readily soluble in water when administrated in the form of a plant extract, but almost water insoluble in pure state [8]. Piperine, the major alkaloid found in black pepper (*Piper nigrum* L.) has been shown to improve the oral bioavailability of otherwise poorly absorbable compounds [196]. One such example is the combination of piperine and curcumin (an anti-inflammatory and anticancer PSM from turmeric) [197]. It has been well documented that extracts of aromatic plants have superior activity over the essential oils prepared from the same plant material [198-200]. These studies showed that the enhancement of antimicrobial efficacy has its origin in the coexistence of volatile and nonvolatile constituents in the tested extracts. It is widely accepted that the administration of infused oil may act as a penetrative enhancer [201], and possibly the synergistic interactions noted may be a result of improved solubility and bioactivity of the active principles. These types of studies reinforce the concept of a multi-targeted approach in therapeutic strategies and prove the hypothesis formulated by Tyler [63], that searching for potent antimicrobial compounds is becoming more and more improbable and that research should be moving towards the investigation of combination of substances to achieve efficacy.

Synergistic interactions between PSMs and some common antibiotics against some microorganisms (*in vivo*) are also known (carvacrol/ciprofloxacin, carvacrol/ amphotericin B against *Bacillus cereus* and *C. albicans*; eugenol/ciprofloxacin and eugenol/ amphotericin B against *E. coli* and *C. albicans*) [9]. The mechanism of synergy in these cases may be attributed to complex multi-target effects, pharmacokinetic or physiochemical properties, neutralization principles, or even therapeutic approaches [177]. In a study on the synergistic interaction of *Punica granatum* L. constituents from its methanol extract with a range of antibiotics, the authors allude to the mode of action whereby the extract plays a role in efflux inhibition enhancing the uptake of the conventional drugs [9]. In another study, the mechanism of action of a combination of an isoflavanone from *Erythrina variegate* with mupirocin is thought to involve bac-

terial cell membranes [144]. Stermitz and co-workers in their study [202] focused on the antimicrobial action of berberine and 5′-methoxyhydnocarpin, and found strong evidences that 5′-methoxy hydnocarpin acts as a NorA multidrug resistance pump blocker and in that way promotes the antibiotic action of berberine. Studies like these, that give valuable insight into the specific modes of action of substances, should be strongly encouraged, as information they provide could be further used in the search/design of new potential antimicrobials and leads. Nevertheless, exploring this area of research may be extremely complex.

According to a vast amount of knowledge accumulated, some of which is mentioned above, it seems that synergism between a number of different PSMs definitely exists; however, the fact that major constituent(s) is(are) less active than the mixture as a whole is not a sufficient proof for the existence of synergism since some minor contributors may possess a very strong activity as well. For example, antimicrobial testing revealed that the sesquiterpene germacrone, the major oil constituent (49.7%) of *Geranium macrorrhizum* L. essential oil, was not the sole agent responsible for the high activity of the oil. Column chromatography of the oil enabled the isolation of germacrone-4,5- and -1,10-epoxides (0.4% of the total oil), that turned to be highly active against *Bacillus cereus* (MIC values for the 1,10- and 4,5-epoxide were 1.0 and 10.0 μg/ml, respectively) and *Pseudomonas aeruginosa* (MIC values for the 1,10- and 4,5-epoxide were 10.0 and 0.20 μg/ml, respectively) [203]. Thus, more in depth studies should be undertaken in order to confirm the proposed synergy of different natural products, as the simple unconfirmed statement of synergistic interactions existing in a complex PSM mixture could not just lead to erroneous conclusions but prevent us from spotting some very interesting, and highly potent minor PSMs.

When speaking of PSM mixtures as potential pharmaceuticals, one should not forget that antagonistic interactions between different PSMs, as well as between PSMs and non-plant derived compounds, are also possible [9]. For example, a strong antagonism was observed when a mixture of salicylaldehyde and methyl salicylate (60:40, molar ratio), constituents of the mentioned *F. vulgaris* oil, was assayed, alongside with pure substances and the essential oil [188], for antimicrobial activity. Similarly, it was demonstrated that antagonistic interactions between several commercial essential oils, extracted from *M. alternifolia*, *T. vulgaris*, *M. piperita* and *R. officinalis*, and conventional antimicrobials (ciprofloxacin and amphotericin B) also exist [204]. When *M. alternifolia* (tea tree) oil, which is often recommended for the treatment of skin ailments, was combined with ciprofloxacin and tested against *Staphylococcus aureus*, an antagonism was noted [204]. Cuzzolin *et al.* [38] have warned that there is a need for more systematic interactive studies to be undertaken to identify unfavorable combinations.

3.3. PSMs As Potential Oral Antimicrobials

When speaking of the possible applicability of PSMs as oral therapeutics, one must think about their pharmacokinetic and pharmacodynamic properties. To be more precise, their possible adsorption, distribution, metabolism and exertion pathways (ADME) that influence disposition of a compound within the organism should be taken into account. These four criteria all influence the level of the drug and kinetics of the tissues exposure to the drug and hence influence the performance and pharmacological activity of the compound as a drug [56, 205]. Unfortunately, despite promising recent findings, relatively little research is dedicated to the molecular pharmacology of PSMs [7].

One of the major issues that concern PSMs, that were shown to possess strong *in vitro* activity, is their *in vivo* way/route of application. Here, we tried to address the possible problems connected with oral application that could be regarded as the more favorable mode of administration in comparison to injection or inhalation, Fig. (**6**). The first barrier, for oral consumption, is the oral cavity itself. The flavor of a compound is the first possible problem in oral application, but it is a small one. A large number of active substances may have irritant properties on the oral mucosa that may involve the cheeks, gums, tongue, lips, and roof or floor of the oral cavity and can manifest themselves in different forms of irritation. Those conditions can be mild, in a form of redness and swelling, but also serious inflammatory reactions. There are publications that deal with clinical (*in vivo*) trials of certain natural products and their effect on oral cavity infections. For example the extract of the leaves of *Streblus asper*, extract of *Azadirachta indica* and 2.5% garlic mouthwash solution caused the reduction in salivary *S. mutans* during the trial period with no side effects, except in the case of the garlic mouthwash where there were reports of unpleasant taste, halitosis and nausea among the volunteers [206-208].

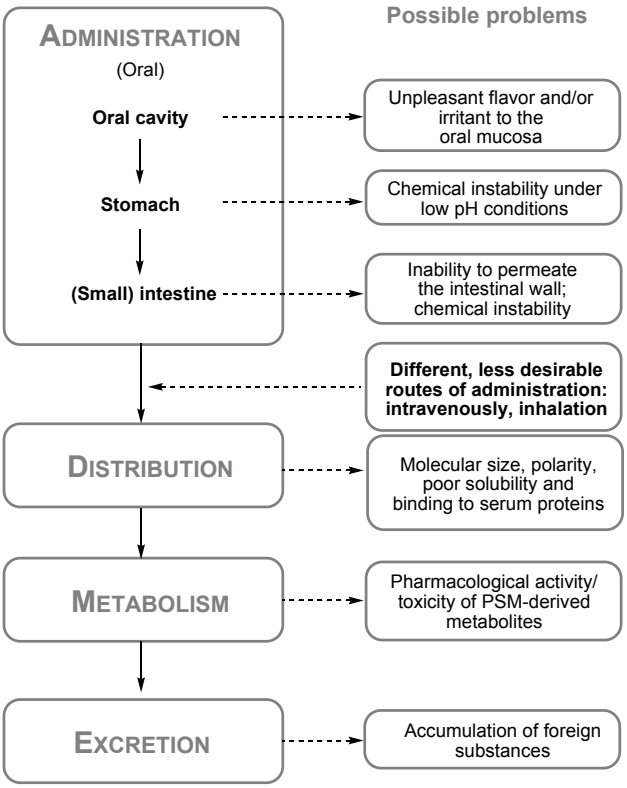


Fig. (6). Possible problems with PSMs, if used as (oral) human antibiotics.

The next large obstacle is the stomach. The low pH in stomach juices, from the present hydrochloric acid, and active gastric enzymes represent a strong line of "defense" of the organism. At this level of gastrointestinal tract, we could once again meet with the benefits of PSMs. The eradication of *Helicobacter pylori*, bacteria that play an important role in pathogenesis of different gastrointestinal tract disorders, can be an important cause and justification for the application of substance(s) that can not pass the stomach barrier or that mostly express their activity in the stomach. *Helicobacter pylori* infections are the main cause of peptic ulceration and gastric MALT (mucosa-associated lymphoid tissue) lymphoma and are a major risk factor for the development of gastric adenocarcinoma [209]. Frequently used as a chewing gum, mastic gum (*Pistacia lentiscus*), is marketed heavily in the UK and in other European countries and the USA as a natural treatment for *H. pylori* infection and peptic ulceration. The *in vitro* results showed that the mastic gum possesses strong bactericidal activity [210, 211], however, the *in vivo* assay (based on human volunteer subjects experiment) indicated that the mastic gum possess no effect on *H. pylori* infections

[212]. The volunteers that were submitted to a 14 day trial, withdrew after 5 days (one patient), because of side effects such as nausea and bloating, and two more subjects reported, at the end of the experiment, additional side effects in form of fatigue and constipation [212]. Bottom line, mastic gum does not have clinically significant effect against *H. pylori in vivo*, but it can still be used, in combination with anti-acid secretory drugs, in therapy of ulcers and prevention of relapse [212]. In another study, the interactive combination of cranberry and blueberry juices, and grape seed extract was tested against the same microorganism. This study was undertaken on the assumption that a diet rich in phytocompounds may act prophylactically to ward off infection. Of the five different combinations formulated, the synergistic mixture containing cranberry (75%) and blueberry juice extract (10%) and grape seed extract (15%) was active against *H. pylori* [213].

In the first part of the small intestine, duodenum, PSMs are exposed to different active substances (bile acids, pancreatic and gastric juices, as well as luminal enzymes) present in relatively high amount [214]. These substances could influence PSMs adsorption or could chemically modify them. Major absorption of all kinds of substances (carbohydrates, amino acids, fatty acid particles, vitamins, minerals, electrolytes and water), as well as ingested drugs, occurs through the specialized jejunal mucosa (SJM). If the ingested substance (individual PSM or PSM-mixture) succeed to reach SJM, it may be absorbed in the body's circulation and may live to our expectations and eradicate the cause of infection. Absorption critically determines the compound's bioavailability [214]. For example, less than 5% of dietary phytosterols, phytostanols, and their esters are absorbed by the gastrointestinal tract of rats and humans [215].

After entry into the systemic circulation PSMs will be subjected to the numerous distribution processes that tend to lower its plasma concentration. In the blood stream the solubility and affinity for protein binding are just two important examples of properties of the applied substance [214]. The concentration that can be reached in blood can be of vital importance and is in a close relationship to protein binding properties. If the lipophilic substance has a high affinity for transport proteins it will be hard for her to achieve any significant concentration in blood, and thus it will most probably have no beneficial effect. Also the penetration through the blood/brain barrier or joint capsule can be crucial due to the high amount of this kind of specific infections (meningitis, meningoencephalitis, arthritis, etc.) [214].

The metabolism of the first pass can easily change the activity of the applied molecule in both decreasing and increasing manner. Compounds begin to break down as soon as they enter the body. The majority of small-molecule drug metabolisms are carried out in the liver by cytochrome P450 redox enzymes. For example, the metabolism of thujone has been partially elucidated in mouse, rat, and human liver preparations *in vitro* and in mice, rats, and rabbits *in vivo*. Hydroxylations at various positions (7-hydroxylation as a major *in vitro* reaction), followed to varying extents by glucuronidation and reductions as minor reactions, are the principal metabolic pathways, although *in vitro* and *in vivo* metabolic profiles do not necessarily agree with each other [216, 217]. The degradation of some compounds could give pharmacologically inert products, which will reduce the effects of the parent drug on the body. However, metabolites may also be pharmacologically active, sometimes even toxic. For example, study of the relationship between the metabolism and toxicity of benzene (and other aromatic compounds, many of which are PSMs) indicates that several metabolites of benzene play significant roles in generating benzene toxicity [218]. Benzene is metabolized, primarily in the liver, to a variety of hydroxylated and ring-opened products that are transported to the bone marrow where subsequent secondary metabolism occurs. Two potential mechanisms by which benzene metabolites may damage cellular macromolecules to induce toxicity include the covalent binding of reactive metabolites of benzene and the capacity of benzene metabolites to induce oxidative damage [218].

In the end, compounds and their metabolites need to be removed from the body via excretion, usually through the kidneys (urine) or in the feces [214]. Unless excretion is complete, accumulation of foreign substances can adversely affect normal metabolism. Generally, nonpolar, nonelectrolyte substances of lower molecular weight (such is the great majority of PSMs) will readily dissolve in neutral body fats and would also readily accumulate in the body (fats constitute 15-20% of recommended body weight) [219].

Through the analysis of physicochemical properties of more than 2,000 drugs and candidate drugs in clinical trials, Lipinski and his colleagues [220] showed that a compound is more likely to be membrane permeable and easily absorbed by the body if it matches several simple criteria. Firstly, its molecular weight should be less than 500. The compound's lipophilicity, expressed as a quantity known as log*P* (the logarithm of the partition coefficient between water and 1-octanol), as well as the number of groups in the molecule that can donate hydrogen atoms to hydrogen bonds (usually the sum of hydroxyl and amine groups in a drug molecule), should be less than 5. The number of groups that can accept hydrogen atoms to form hydrogen bonds (estimated by the sum of oxygen and nitrogen atoms) should be less than 10. As all numbers mentioned in these rules are dividable by 5, the Lipinski rule is also known as a Rule of five. The rules, based on the 90-percentile values of the drugs' property distributions, apply only to absorption by passive diffusion of compounds through cell membranes; compounds that are actively transported through cell membranes by transporter proteins are exceptions to the rule. Due in no small part to their simplicity, the Lipinski criteria are widely used by medicinal chemists to predict not only the absorption of compounds, as Lipinski originally intended, but also the overall drug-likeness [221]. The data on the Lipinski properties of some characteristic PSMs are listed in (Table **2**). Obviously, some natural products nicely fit into the frame defined by Lipinski. Others however, do not fulfill even these basic requirements for oral pharmaceuticals. Chemical derivatization is one possibility how to improve inadequate molecular properties. Thus, if too many H-donors are present in the molecule, they could be alkylated. Too high lipophilicity could be lowered by introducing some polar groups (e.g. some monosaccharide unit may be attached to the PSM bearing an appropriate functional group). Nevertheless, fixing one problem may lead to new ones, even more serious. Chemical transformations could yield products with a completely different mode of action, adsorption/transport properties or serious side effects [56].

Over the last years, an increasing number of pharmacokinetic studies on the bioactive leads in botanical drugs have been carried out [7, 196, 197, 222-226], and valuable data were collected. However, these data are just a tip of the iceberg. If a standardized botanical drug is taken orally, in the best case, the pharmacokinetic properties (ADME) are known only for the major bioactive constituent [7]. But what about all the other PSMs present in the mixture, their mutual (synergism/antagonism) and interactions with the organism itself? Additionally, herbal medicine pharmacology requires a knowledge about the actual ligand concentration at a given receptor site. This is fundamental to the understanding of the pharmacodynamic behavior of any compound in a physiological context and ultimately the mechanism of action. If we have a mixture of several hundreds of potentially bioactive compounds, we should know what the plasma and tissue concentration of the individual compounds are and all of the respective receptor interactions at the given concentrations in order to understand how it works. To gather all the information required is not nearly an easy task.

Table 2. Lipinski Properties of Selected PSMs (Taken From SciFinder)

Class	Molecular Weight	log*P*	H-Donors	H-Acceptors
Monoterpenoids				
α-Pinene	136	4.3	0	0
Myrcene	136	4.3	0	0
α-Thujone	152	2.0	0	1
Piperitenone	150	1.8	0	1
Yomogi alcohol	154	2.7	1	1
Linalool	154	2.8	1	1
cis-Linalool oxide (furanoid)	170	1.8	1	2
α-Terpineol	154	2.7	1	1
Bornyl acetate	196	3.5	0	2
Geranyl pentanoate	238	**5.4**[a]	0	2
Sesquiterpenoids				
Germacrene D	204	**6.6**	0	0
Humulene	204	**6.6**	0	0
δ-Cadinene	204	**6.3**	0	0
Caryophyllene oxide	220	4.4	0	1
Mintsulfide	236	**5.5**	0	0
4(14)-Salvialene-1-one	220	3.9	0	1
(*E*,*E*)-2,6-Farnesal	220	5.0	0	1
β-Bisabolol	222	4.6	1	1
(*E*)-Nerolidol	222	4.7	1	1
Spathulenol	220	4.4	1	1
Diterpenoids				
m-Camphorene	272	**8.1**	0	0
Sandaracopimara-8(14),15-diene	272	**8.7**	0	0
Kaur-15-ene	272	**8.2**	0	0
Manoyl oxide	290	**6.9**	0	1
Icetexone	342	3.5	1	5
Labiatamide A	535	4.0	0	9
Euroabienol	462	3.0	2	8
(*E*)-Phytol	296	**8.2**	1	1
(*E*,*E*)-Geranylcitronellol	292	**7.0**	1	1
Marrubiin	332	3.8	1	4
Phenylpropanoids and related compounds				
Estragole	148	3.1	0	1
Cinnamaldehyde	132	1.9	0	1
Cinnamic acid	148	1.2	1	2
Eugenol	164	2.4	1	2
Apiole	222	1.9	0	4
Chlorogenic acid	354	≈0.4	**6**	9
Apigenin	270	2.1	3	5
Virolin	358	3.5	1	5
trans-Resveratrol	228	3.0	3	3
Umbelliferone	162	1.6	1	3
Alkaloids				
Nicotine	162	≈0.6	0	2
Taxol	**853**	4.0	4	**15**
Cocaine	303	2.3	0	5

(Table 2) contd….

Class	Molecular Weight	log*P*	H-Donors	H-Acceptors
Crotanecine	171	≈0.8	3	4
Coniine	127	2.2	1	1
Theobromine	180	≈1	1	6
Bufotenin	204	≈1.6	2	3
Gentianine	175	≈1.5	0	3
Codeine	299	≈1.4	1	4
Ibotenic acid	158	≈-0.5	4	6

[a]The values that deviate from Lipinski's criteria are given in bold type.

A chemist knows that even a "simple" reaction such as the nucleophilic substitution, conducted on a single substrate and with a single nucleophile, could give more than one product, and that the chemical analysis of the resulting reaction mixture could be quite challenging, from both experimental (analytical) and theoretical point of views. What to expect then in such complex systems, when we want a mixture of compounds to interact with a "mixture" of biomolecules?

3.4. Limitation of Phytocompounds As Antibiotics

Just a quick survey of the titles of publications appearing in *Journal of Ethnopharmacology*, *Planta Medica*, *Phytochemistry*, *Phytotherapy Research* etc, is sufficient to find a number of papers claiming extraordinary antimicrobial potency of plant secondary metabolites, most often in the form of crude extracts (e.g. essential oils). How then to explain the fact that not a single plant-derived antibacterial has been commercialized [227]? Maybe PSM compounds are not as perfect as we would like them to be? Moreover, some of these positive results may well be useless when speaking of a practical application (as was discussed previously) or are just overestimated. Some shortcomings of PSMs as antibiotics are given below:

a. Active Concentration

A common mistake found in many papers is to claim a significant degree of activity for slight dilutions (i.e. excessively high concentrations). The activity of extracts having MIC values higher than 1 mg/ml or pure compounds with MIC higher than 0.1 mg/ml is hardly worth mentioning, whereas those extracts and compounds that inhibit the growth of microorganisms in concentrations below 100 μg/ml and 10 μg/ml, respectively, deserve our full attention [16]. Nevertheless, screening the *in vitro* activity of crude and semi-purified extracts is of great importance, and the results of such studies, although sometimes modest, could potentially pinpoint novel antibiotics or new leads. Advances in different fields of biology, chemistry and medicine have now made it possible to obtain a high number of protein targets that are also known as high-throughput (automated testing of large collections (libraries) of compounds for activity as inhibitors or activators of specific biological targets) [56, 228]. Plant secondary metabolites, both in pure state or as crude or semi-purified extracts/mixtures, could be used as input libraries for such tests. However, the fact that the mechanism of PSM action are usually not one-target oriented (see previous subsections) could present a problem here [7].

b. Antimicrobial Assays and Usefulness of In Vitro Data

There are several *in vitro* methods commonly utilized for the evaluation of an antimicrobial potential of substances/mixtures, and the application of a specific assay most often depends on the available instrumentation and the training of the investigators (e.g. disc diffusion, dilution methods giving the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), "time kill", etc). The disc diffusion method is quick and easy to perform but usually burdened with several serious shortcomings, such as false positive and negative results due to poor test substance solubility and diffusion through the semi-solid nutritive medium. The dilution assay is a quantitative method that provides data on the lowest concentration of an antimicrobial that will inhibit visible growth of a microorganism after an overnight incubation. Assays involving MIC methodology are widely used and the value of MIC is an accepted criterion for measuring the susceptibility of microorganisms (e.g. bacteria in their planktonic phase) to inhibitors [156, 229, 99]. However, the results of *in vitro* MIC and MBC assays need not correlate with *in vivo* activities of tested compounds. The active concentrations in *in vitro* conditions frequently cannot be reached *in vivo* and the infecting microorganisms are never exposed to a static (constant) concentration of an antimicrobial during a 24 h period as is the case in a microtiter plate. Unless the therapeutic index (therapeutic ratio) of the *in vitro* tested antimicrobial is very high, it will not be likely that the substance with a high MIC (MBC) value (for example measured in mg/ml) is going to be used as a potential therapeutic agent. Additionally, the microorganisms in a microtiter plate are in a form of suspension, whereas the bacteria associated with different illnesses such as: urinary tract infections, middle-ear infections, formation of dental plaque, endocarditis and infections in cystic fibrosis, joint prostheses, heart valves etc. form biofilms [156, 157]. Biofilms are notoriously difficult to eradicate and are a source of many recalcitrant infections, thus this represents an extra challenge for antimicrobial agents. Thus, when assessing the antimicrobial potential of a sample (e.g. PSM or mixture of PSMs) in the case of bacteria in their biofilm phase, the results of a MIC method would not have unambiguous meaning and the minimum biofilm eliminating concentration (MBEC) assay would most certainly give a more realistic evaluation of the sample's activity. However, future trials are necessary to address the clinical efficacy and utility of the MBEC assay [156].

c. Therapeutic Window

Every effective drug has a given therapeutic window in which the effective dose is clearly distinguished from the adverse effects that are expected to occur at higher doses [7]. Rather intriguingly, botanical drugs or certain phytochemicals are often claimed to be nontoxic irrespective of the dose administrated, similar to vegetables, fruits and certain vitamins [37, 38]. However, there are a number of examples demonstrating quite the opposite. The ethnopharmacological usage of medicinal plants (decocts, macerates etc made from them), erroneously considered as being only benevolent, in the treatment of different kinds of infectious diseases can cause numerous side effects (fulminant hepatic failure, for example) [230]. Recently, a related compound to coniine, conmaculatin, a new piperidine alkaloid isolated from the highly poisonous *Conium maculatum* L., poison hemlock (Table **1**), was screened for antinociceptive activity in peripheral and central models of analgesia [50]. A dose dependent antinociceptive effect in a very narrow dose range (10–20 mg/kg) was observed for this volatile alkaloid, with no detectable activity below 10 mg/kg and high toxicity in doses

over 20 mg/kg. The essential oil of *Achillea umbellata* Sibth. et Sm. (a yarrow species) and its major constituents (fragranol and fragranyl acetate) and one more ester (fragranyl benzoate) were tested for acute toxicity in mice and antimicrobial activity against a panel of microorganisms, as well as assayed for anxiolytic and antinociceptive properties [231]. The value of the median lethal dose (LD_{50} = 853 mg/kg) determined put this oil in the group of toxic essential oils. Prior to death the treated mice showed signs of sedation and hypnosis that were very likely the consequence of intoxication and not a possibly beneficial effect of the plant's volatiles. A noted moderate antimicrobial activity against both bacteria and one yeast strain suggested that the mode of action of the oil and its constituents must be different for prokaryotic and eukaryotic cells. Thus, this *Achillea* sp. provides an excellent example that the effects of a number of medicinal plant species might have their status reinstated from being beneficial to health to the one that requires special caution in medicinal applications [231]. Since numerous natural products possess a non-specific toxicity towards (host) cells, this is a major problem in the fight against intracellular microorganisms, e.g. bacteria from the Mycobacteriaceae family (*Mycobacterium tuberculosis*, *Mycobacterium leprae*) that cause serious diseases in mammals, such as tuberculosis and leprosy [232]. Perhaps the only way to assess the safety of an antimicrobial substance is to ascertain whether is non-toxic for mammalian cells, i.e. to run both assays for the evaluation of MIC values for bacterial/fungal species and mammalian IC_{50} [233, 234].

d. Variable Composition of Botanical Drugs

European Chemical Agency (ECHA) defines plant extracts as UVCB substances (Substances of unknown or variable composition, complex reaction products or biological materials) [235]. UVCBs are substances with a relatively large number of constituents and/or with the composition, to a significant part, unknown and/or the variability of composition relatively large or poorly predictable. In the case of plant extracts, most often all of the stated above is true. Having this in mind, it is clear that the usage of botanical drugs has to be performed very cautiously, as it is quite difficult to assure standardized chemical composition. There are a great number of studies showing that different populations of the very same plant species differ significantly, even in respect of the dominant PSMs [236-239]. Thus, biological activity of the pharmaceutical mixtures prepared from different populations of the same taxa could also vary, leading to completely useless botanical drugs in some cases, or even toxic ones in others. Another scenario, both possible and dangerous, is the misidentification of two plant taxa that could be botanically (morphologically) very similar, but chemically very distinct [240]. If the wrongly identified taxon contains toxic constituents, this error could represent a serious health risk. It is not just in rural, underdeveloped areas, that people often collect medicinal plants and prepare botanical mixtures by themselves, but also it became an enjoyable practice among the more urban populations.

Thus, a positive biological activity is just one of the necessary prerequisites for substances to be applied as pharmaceutics. A lot of other data on its properties has to be accessible and beneficial as well. Among these, mechanisms of action and pharmacokinetic profiles are of special importance. For the majority of PSMs these are unknown, however, a significant number of natural products were studied in this sense. We will try to systematize the general current general knowledge available on the topic.

4. CONCLUSIONS

During the extensive survey of the literature [1-240] we undertook, which focused on the studies dealing with PSMs as potential antimicrobial agents, several important points emerged. First of all, if we are to use plant secondary products as antimicrobials, the most effective way to do that appears to be to do it in the very same manner plant species do it, and that means that we should use PSMs mixtures rather than individual compounds. Plants do not rely on single compounds in their biochemical warfare with pathogens. Instead of trying to use a "single golden bullet", they use a whole arsenal of different PSMs, each aimed at more different targets [7, 9]. If we want to develop an antibiotic based on a single PSM, in a way, we would be ignoring the evolutionary advantage that plants may have developed to overcome emerging resistance via the various metabolic mechanisms for the production of structurally and functionally diverse compounds. There is also a great chance that we will fail in our quest. And again, one should bare in mind that only a limited number of cases are known of microorganism resistance developed to compounds of plant origin (PSM) (e.g. reserpine, a plant alkaloid produced by *Rauwolfia serpentina*, Apocynaceae) [39]. However, the only reason for this may lie in the fact that they are not nearly as exploited in the treatment of microbial borne infections in humans as commercial antibiotics. Thus, human pathogens are not extensively exposed to these agents, and perhaps still had not had the need or chance to develop an adaptive mechanism in their combat with PSMs. Common antibiotics are extensively utilized and frequently even misused. If some PSM (individually or in a combination) is to be repeatedly utilized as, for example, penicillins are/were, there is a great chance that bacteria or fungi will quickly "learn" how to fight these new, at the moment, still promising, medicinal weapons. For example, there is proof that bacteria can adapt in such a way as to maintain their membrane functionality in the presence of subinhibitory concentrations of thymol, a PSM that possesses verified significant antimicrobial properties [77, 241]. This perhaps means that in the future, bacteria might develop some type of resistance to thymol (or other PSMs). Yet again, it is much harder for microorganisms to simultaneously shield themselves against all these different PSM-bullets and one must not forget the fact, corroborated by numerous studies on synergistic interactions of PSMs, that these "bullets" tend to cooperate [9].

But then, if a mixture (botanical drug) is better than a monosubstance, how to acquire such a mixture? Isolation from plant material provides a relatively easy access to these highly diverse and specific structures, and this is certainly a big bonus. Synthesis could be costly, difficult and sometimes even impossible on a large scale, and simple combining of different pure PSMs would never result in mixtures as complex as nature has to offer. However, the uniformity of a PSM profile could be, and usually is, highly susceptible to external factors (geographical, climatic, and ecological) that (in) directly influence plant metabolism. Perhaps it is possible to solve this by a combination of controlled breeding and hybridization experiments performed with the selected, most promising species.

Some other problems with PSMs as potential antibiotics, that have to be addressed, are those mainly related to the non-equivalence of their *in vitro* and *in vivo* activity, their active window, and the possible toxicity and specificity of the routes of application. Irrespective of the importance of traditional medicine in developing countries and their growing popularity in the industrialized West, the therapeutic potential of botanical drugs has to be scientifically addressed. More rigorous studies on the bioavailability, pharmacodynamics and mechanism of action of PSMs, as well as understanding of pharmacological synergies and potential network pharmacology of complex mixtures, are needed to make real progress in this area [7]. Probably the best way of corroborating the pharmacological efficiency of botanical drugs would be to reengineer the mixtures, Fig. (**7**). By taking apart (separating, isolating) and reassembling the mixtures of all bioactive constituents, one would be able to determine which natural products contribute to the pharmacodynamics of a given pharmacological effect, either indirectly (i.e. by modulating solubility and bioavailability) or directly (by interacting with particular targets) [7]. New techniques, such as metabolomics and the dual application of chemometric data analy-

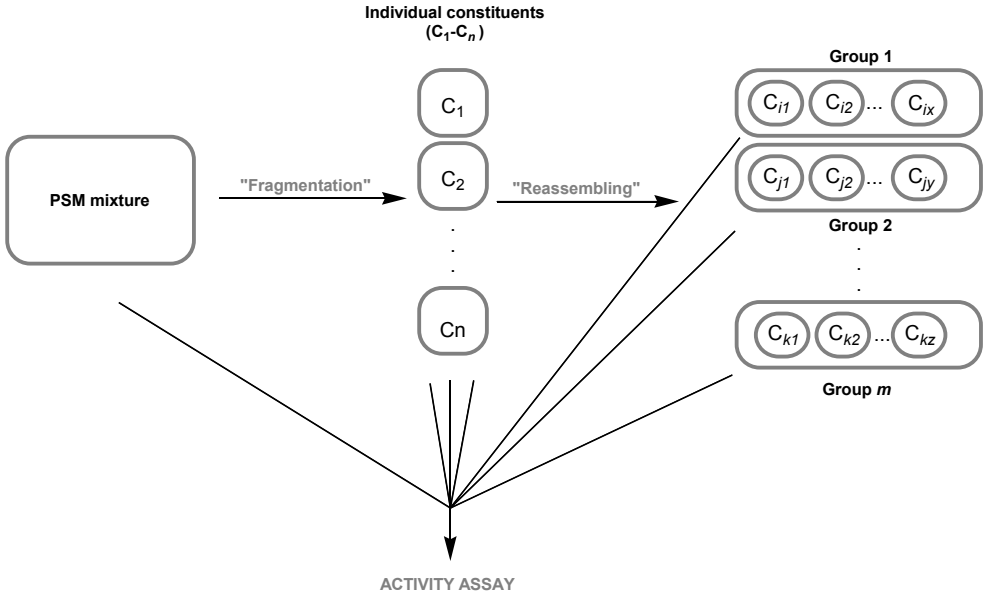


Fig. (7). Ways of assessing the mechanism of action of a botanical drug. Fragmentation of PSM mixture (comprised of *n* different individual constituents (ICs)) and subsequent reassembling of all bioactive constituents would result in a high number (*m*) of groups of compounds (PSM submixures comprised of (different number of) ICs). Assay of biological/pharmacological activity of PSM (sub)mixtures and ICs could provide insight into the pharmacodynamics and the role of different ICs.

sis methods, are providing the researcher with new tools with which to explore such fascinating phenomena which will undoubtedly become increasingly important in our continued quest to understand the mechanism of action of complex herbal preparations. Elucidating mechanisms of action of bacteriostatic and bactericidal compounds, as well as those behind the resistance to antibacterial agents is certainly going to be a boost for basic microbiological research, and provide especially useful information on the regulation of fundamental cellular processes.

In the end, we must keep constantly on our minds the well known evolutionary adaptive capabilities of microorganisms. Thus, the only conclusion possible is that antibiotics themselves have to evolve. Although the era of synthetic and microorganism-derived antibiotics is not nearly over, we desperately need a new approach. Despite a number of shortcomings, plant secondary metabolites seem to be an excellent starting point in the search for/design/ development of new antimicrobial agents. It is now up to medicinal chemists, pharmacologists, phytochemists and researchers from many other related fields to find the way how to use this enormous potential hidden in and provided by *Plantae*. There is a lot of work to be done, but although the stakes are high, so are the rewards.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This work was funded by the Ministry of Education and Science of Serbia (Project 172061). PB is supported by UNESCO-L'OREAL National Fellowships Programme for “Women in Science” (Serbia).

REFERENCES

[1] Fraser, C.M.; Eisen, J.A.; Salzberg, S.L. Microbial genome sequencing. *Nature*, **2000**, *406*, 799-803.

[2] Global Health Observatory Data Repository [online database]. Cause-specific mortality **2008**. Geneva, World Health Organization. http://apps.who.int/ghodata/ (accessed July 18, 2012).

[3] World health statistics 2012. World Health Organization. http://www.who.int/gho/publications/world_health_statistics/EN_WHS2012_Full.pdf (accessed July 18, 2012)

[4] Global Report for Research on Infectious Diseases of Poverty. World Health Organization on behalf of the Special Programme for Research and Training in Tropical Diseases 2012, WHO Document Production Services, Geneva, Switzerland. http://www.who.int/tdr/stewardship/global_report/Summary_advocacy_web.pdf (accessed July 18, 2012)

[5] Wiggins, P. Efflux pumps: an answer to Gram-negative bacterial resistance? *Expert Opin. Investg. Drugs.*, **2004**, *13*(8), 899-902.

[6] Hoban, D.J.; Biedenbach, D.J.; Mutnick, A.H.; Jones, R.N. Pathogen of occurrence and susceptibility patterns associated with pneumonia in hospitalized patients in North America: results of the SENTRY Antimicrobial Surveillance Study. *Diagn. Microbiol. Infect. Dis.*, **2003**, *45*(4), 279-285.

[7] Gertsch, J.; Botanical drugs, synergy, and network pharmacology: forth and back to intelligent mixtures. *Planta Med.*, **2011**, *77*(11), 1086-1098.

[8] Gertsch, J.; Tobler, R.T.; Brun, R.; Sticher, O.; Heilmann, J. Antifungal, antiprotozoal cytotoxic and piscicidal properties of justicidin B and a new arylnaphthalide lignan from *Phyllanthus piscatorum*. *Planta Med.*, **2003**, *69*(5), 420-424.

[9] Van Vuuren, S.; Viljoen, A. Plant-based antimicrobial studies–methods and approaches to study the interaction between natural products. *Planta Med.*, **2011**, *77*(11), 1168-1182.

[10] Radulović, N.S.; Dekić, M.S.; Stojanović-Radić, Z.Z., Zoranić, S.K. *Geranium macrorrhizum* L. (Geraniaceae) essential oil: A potent agent against *Bacillus subtilis*. *Chem. Biodivers.*, **2010**, *7*(11), 2783-2800.

[11] Radulović, N.S.; Djordjević, N.D.; Stojanović-Radić, Z.Z. Volatiles of the Balkan endemic *Daucus guttatus* ssp. *zahariadii* and cultivated and wild-growing *D. carota* - A comparison study. *Food Chem.*, **2011**, *125*(1), 35-43.

[12] Blagojević, P.D.; Radulović, N.S.; Palić, R.M.; Stojanović, G.S. Chemical composition of the essential oils of Serbian wild-growing *Artemisia absinthium* and *Artemisia vulgaris*. *J. Agr. Food Chem.*, **2006**, *54*(13), 4780-4789.

[13] Radulović, N.S.; Dekić, M.S.; Stojanović-Radić, Z.Z.; Palić, R.M. Chemical composition and antimicrobial activity of the essential oils of *Geranium columbinum* L. and *G. lucidum* L. (Geraniaceae). *Turk. J. Chem.*, **2011**, *35*(3), 499-512.

[14] Radulović, N.S.; Dekić, M.S.; Stojanović-Radić, Z.Z. Chemical composition and antimicrobial activity of the volatile oils of *Geranium sanguineum* L. and *G. robertianum* L. *Med. Chem. Res.*, **2012**, *21*(5), 601-615.

[15] Stojanović-Radić, Z.Z.; Comić, Lj.R.; Radulović, N.S.; Dekić, M.S.; Randjelović, V.N.; Stefanović, O.D. Chemical composition and antimicrobial activity of *Erodium* species: *E. ciconium* L., *E. cicutarium* L., and *E. absinthoides* Willd. (Geraniaceae). *Chem. Pap.*, **2010**, *64*(3), 368-377.

[16] Rios, J.L.; Recio, M.C. Medicinal plants and antimicrobial activity. *J. Ethnopharmacol.*, **2005**, *100*(1-2), 80-84.

[17] Potterat, O.; Hamburger, M. Drug discovery and development with plant-derived compounds. *Prog. Drug. Res.* **2008**, *65*, 45-118.

[18] Radulović, N.S.; Blagojević, P.D.; Palić, R.M. Comparative study of the leaf volatiles of *Arctostaphylos uva-ursi* (L.) Spreng. and *Vaccinium vitis-idaea* L. (Ericaceae). *Molecules*, **2010**, *15*(9), 6168-6185.

[19] Carson, C.F.; Hammer, K.A.; Riley T.V. *Melaleuca alternifolia* (Tea Tree) oil: a review of antimicrobial and other medicinal properties. *Clin. Microbiol. Rev.*, **2006**, *19*(1), 150-162.

[20] Carson, F.C.; Mee, B.J.; Riley, T.V. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time kill, lysis, leakage and salt tolerance assays and electron microscopy. *Antimicrob. Agents Chemother.*, **2002,** *46*(6), 1914-1920.

[21] Cox, S.D.; Mann, C.M.; Markham, J.L. Interactions between components of the essential oil of *Melaleuca alternifolia*. *J. Appl. Microbiol.*, **2001**, *91*(3), 492-497.

[22] Cox, S.D.; Mann, C.M.; Markham, J.L.; Bell, H.C.; Gustafson, J.E.; Warmington, J.R.; Wyllie, S.G. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *J. Appl. Microbiol.*, **2000**, *88*(1), 170-175.

[23] Cox, S.D.; Mann, C.M.; Markham, J.L.; Gustafson, J.E.; Warmington, J.R.; Wyllie, S.G. Determining the antimicrobial actions of tea tree oil. *Molecules*, **2001**, *6*(2), 87-91.

[24] Leonti, M.; Casu, L.; Sanna, F; Bonsignore, L. A comparison of medicinal plant use in Sardinia and Sicily-De Materia Medica revisited? *J. Ethnopharmacol.*, **2009**, *121*(2), 255-267.

[25] Thomas, E.; Vanderbroek, I.; Goetghebeur, P. The relationship between plant use and plant diversity in Bolivian Andes, with special reference to medicinal plant use. *Hum. Ecol.*, **2008**, *36*(6), 861-879.

[26] Moerman, D.E. Symbols and selectivity: a statistical analysis of native American medical ethnobotany. *J. Ethnopharmacol.*, **1979**, *1*(2), 111-119.

[27] Moerman, D.E. An analysis of the food plants and drug plants of native North America. *J. Ethnopharmacol.*, **1996**, *52*(1), 1-22.

[28] Qui, J. Traditional medicine: a culture in the balance. *Nature*, **2007**, *448*, 126-128.

[29] Kong D.X.; Li, X.J.; Zhang, H.Y. Where is the hope for drug discovery? Let history tell the future. *Drug Discov. Today*, **2009**, *24*(3-4), 115-119.

[30] Touwaide, A.; Pollio, A.; Aliotta, G.; Piomelli, D.; De Santo, N.G. Medicinal plants for the treatment of urogenital tract pathologies according to Dioscorides' De Materia Medica. *Am. J. Nephrol.*, **1997**, *17*(3-4), 241-247.

[31] European Chemical Agency, ECHA. http://apps.echa.europa.eu/legacy/doc/webinars/monoconstituent_suvi_takala_echa.pdf (Accessed July 16, 2012)

[32] Neldner, K.H. Complementary and alternative medicine. *Dermatol. Clin.*, **2000**, *18*(1), 189-193.

[33] World Health Organization. Traditional, complementary and alternative medicines and therapies. Washington DC: WHO Regional Office for the Americas/Pan American Health Organization (Working Group OPS/OMS). 1999.

[34] World Health Organization. Traditional medicine. http://www.who.int/mediacentre/factsheets/fs134/en/ (Accessed July 23, 2012).

[35] Newman, D.J.; Cragg, G.M. Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.*, **2007**, *70*(3), 461-477.

[36] Croteau, R.; Kutchan, T.M.; Lewis, N.G. In: *Biochemistry & Molecular Biology of Plants*, Buchanan, B.; Gruissem, W.; Jones, R., Eds.; American Society of Plant Physiologists, **2000**; pp. 1250-1318.

[37] Zeng, Z.P.; Jiang, J.G. Analysis of the adverse reactions induced by natural product-2derived drugs. *Br. J. Pharmacol.*, **2010**, *159*(7), 1374-1391.

[38] Cuzzolin, L.; Zaffani, S.; Benoni, G. Safety implications regarding use of phytomedicines. *Eur. J. Clin. Pharmacol.*, **2006**, *62*(1), 37-42.

[39] Ahmed, M.; Borsch, C.M.; Nayfakh, A.A.; Schuldiner, S. Mutants of the *Bacillus subtilis* multidrug transporter Bmr with altered sensitivity to the antihypertensive alkaloid reserpine. *J. Biol. Chem.*, **1993**, *268*(15), 11086-11089.

[40] Daglia, M. Polyphenols as antimicrobial agents. *Curr. Opin. Biotechnol.*, **2012**, *23*(2), 174-181.

[41] Arif, T.; Bhosale, J.D.; Kumar, N.; Mandal, T.K.; Bendre, R.S.; Lavekar, G.S.; Dabur, R.J. Natural products antifungal agents derived from plants. *J. Asian Nat. Prod. Res.*, **2009**, *11*(7), 621-638.

[42] Yu, H.; Zhang, L.; Li, L.; Zheng, C.; Guo, L.; Li, W.; Sun, P.; Qin, L. Recent developments and future prospects of antimicrobial metabolites produced by endophytes. *Microbiol. Res.*, **2010**, *165*(6), 437-449.

[43] Bero, J.; Frederich, M.; Quetin-Leclercq, J. Antimalarial compounds isolated from plants used in traditional medicine. *J. Pharm. Pharmacol.*, **2009**, *61*(11), 1401-1433.

[44] Negi, A.S.; Kumar, J.K.; Luqman, S.; Saikia, D.; Khanuja, S.P. Antitubercular potential of plants: a brief account of some important molecules. *Med. Res. Rev.*, **2010**, *30*(4), 603-645.

[45] Buzzini, P.; Arapitsas, P.; Goretti, M.; Branda, E.; Turchetti, B.; Pinelli, P.; Ieri, F.; Romani, A. Antimicrobial and antiviral activity of hydrolysable tannins. *Mini Rev. Med. Chem., 2008, 8*(12), 1179-1187.

[46] Radulović, N.S.; Dekić, M.S.; Stojanović-Radić, Z.Z. Antimicrobial volatile glucosinolate autolysis products from *Hornungia petraea* (L.) Rchb. (Brassicaceae). *Phytochem. Lett.*, **2012**, *5*(2), 351-357.

[47] Radulović, N.S.; Dekić, M.S.; Stojanović-Radić, Z.Z. A new antimicrobial glucosinolate autolysis product, 4-isothiocyanatobutanoic acid, from the diffuse wallflower (*Erysimum diffusum*): Methyl 4-isothiocyanatobutanoate, a long unrecognized artifact of the isolation procedure? *Food Chem.*, **2011**, *129*(1), 125-130.

[48] Radulović, N.S.; Denić, M.; Stojanović-Radić, Z.Z. Antimicrobial phenolic abietane diterpene from *Lycopus europaeus* L. (Lamiaceae). *Bioorgan. Med. Chem.*, **2010**, *20*(17), 4988-4991.

[49] Radulović, N.S.; Blagojević, P.D.; Palić, R.M. Composition of diethyl ether flower extracts of *Lonicera fragrantissima* Lindl. & Paxton (Caprifoliaceae). *Nat. Prod. Commun.*, **2009**, *4*(11), 1581-1584.

[50] Radulović, N.S.; Djordjević, N.D.; Denić, M.; Gomes, P.M.; Fernandes, P.D.M.; Boylan, F. A novel toxic alkaloid from poison hemlock (*Conium maculatum* L., Apiaceae): Identification, synthesis and antinociceptive activity. *Food Chem. Toxicol.*, **2012**, *50*(2), 274-279.

[51] Radulović, N.S.; Miltojević, A.B.; McDermott, M.; Waldren, S.; Parnell, J.A.; Gomes P.M.; Fernandes P.D.; de Sousa Menezes F. Identification of a new antinociceptive alkaloid isopropyl *N*-methylanthranilate from the essential oil of *Choisya ternata* Kunth. *J. Ethnopharmacol.*, **2011**, *135*(3), 610-619.

[52] Dictionary of Natural Products on CD-Rom, version 14.1, 1982-2006; Chapman & Hall/CRC, New York, **2006**.

[53] Dewick, P.M. *Medicinal natural products: a biosynthetic approach*, 2nd ed.; Wiley & Sons, LTD: Chichester, England, **2001**.

[54] Stojanović-Radić, Z.Z.; Čomić, Lj.R.; Radulović, N.S.; Blagojević, P.D.; Denić, M.; Miltojević, A.B.; Rajković, J.; Mihajilov-Krstev, T.M. Antistaphylococcal activity of *Inula helenium* L. root essential oil: eudesmane sesquiterpene lactones induce cell membrane damage. *Eur. J. Clin. Microbiol.*, **2012**, *31*(6), 1015-1025.

[55] Avonto, C.; Taglialatela-Scafati, O.; Pollastro, F.; Minassi, A.; Di Marzo, V.; De Petrocellis, L.; Appendino, G. An NMR spectroscopic method to identify and classify thiol-trapping agents: revival of Michael acceptors for drug discovery. *Angew. Chem. Int. Ed.*, **2011**, *50*(2), 467-471.

[56] Patrick, G.L. *An introduction to medicinal chemistry*, 4th ed.; Oxford University Press, New York, **2009**; pp. 213-239.

[57] Schaumann, R.; Rodloff, A.C. Activities of quinolones against obligately anaerobic bacteria. *Anti. Infect. Agents. Med. Chem.*, **2007**, *6*(1), 49-56.

[58] Wermuth, C.G. Selective optimization of side activities: the SOSA approach. *Drug Discov. Today*, **2006**, *11*(3-4), 160-164.

[59] Jones, W.P.; Kinghorn, D.A. In *Natural products isolation (Methods in Biotechnology)*; Sarker, S.D.; Latif, Z.; Gray, A.I., Eds.; Humana Press: Totowa, New Jersey, **2005**; Vol. *20*, pp. 323-351.

[60] Recio, M.C.; Rios, J.L.; Villar, A. Antimicrobial activity of selected plants employed in the Spanish Mediterranean area. Part II. *Phythother. Res.*, **1989**, *3*(3), 77-80.

[61] Rios, J.L.; Recio, M.C.; Villar, A. Antimicrobial activity of selected plants employed in the Spanish Mediterranean area. *J. Ethnopharmacol.*, **1987**, *21*(2), 139-152.

[62] De Vos, P. European material medica in historical texts: longevity of a tradition and implications for future use. *J. Ethnopharmacol.*, **2010**, *132*(1), 28-47.

[63] Tyler, V.E. Phytomedicines: back to the future. *J. Nat. Prod.*, **1999**, *62*(11), 1589-1592.

[64] Feng, C.; Liu, M.; Shi, X.; Yang, W.; Kong, D.; Duan, K.; Wang, Q. Pharmacokinetic properties of paeoniflorin, albiflorin and oxypaeoniflorin after oral gavage of extracts of Radix Paeoniae Rubra and Radix Paeoniae Alba in rats. *J. Ethnopharmacol.*, **2010**, *130*(2), 407-413.

[65] Wegener, T.; Wagner, H. The active components and the pharmacological multi-target principle of STW 5 (Iberogast). *Phytomedicine*, **2006**, *13* (Suppl. 5), 20-35.

[66] Lv, H.; Sun, H.; Sun, W.; Liu, L.; Wang, P.; Wang, X.; Cao, H. Pharmacokinetic study of a Chinese triple herbal drug formula. *Phytomedicine*, **2008**, *15*(11), 993-1001.

[67] Cordell, G.A.; Colvard, M.D. Some thoughts on the future of ethnopharmacology. *J. Ethnopharmacol.*, **2005**, *100*(1-2), 5-14.

[68] Zhang, L.; Kong, Y.; Wu, D.; Zhang, H.; Wu, J.; Chen, J.; Ding, J.; Hu, L.; Jiang, H.; Shen, X. Three flavonoids targeting the β-hydroxyacyl-acyl carrier protein dehydratase from *Helicobacter pylori*: Crystal structure characterization with enzymatic inhibition assay. *Protein Sci.*, **2008**, *17*(11), 1971-1978.

[69] Plaper, A.; Golob, M.; Hafner, I.; Oblak, M.; Solmajer, T.; Jerala, R. Characterization of quercetin binding site on DNA gyrase. *Biochem. Biophys. Res. Commun.*, **2003,** *306*(2), 530-536.

[70] Parveen, M.; Hasan, M.K.; Takahashi, J.; Murata, Y.; Kitagawa, E.; Kodama, O.; Iwanashi, H. Response of *Saccharomyces cerevisiae* to a monoterpene: evaluation of antifungal potential by DNA microarray analysis. *J. Antimicrob. Chemother.*, **2004**, *54*(1), 46-55.

[71] Lin, C.M.; Preston, J.F.; Wei, C.I. Antibacterial mechanism of allyl isothiocyanate. *J. Food Prot.*, **2000**, *63*(6), 727-734.

[72] Di Pasqua, R.; Hoskins, N.; Betts, G.; Mauriello, G. Changes in membrane fatty acids composition of microbial cells induced by addition of thymol, carvacrol, limonene, cinnamaldehyde and eugenol in the growing media. *J. Agric. Food Chem.*, **2006**, *54*(7), 2745-2749.

[73] Cox, S.D.; Gustafson, J.E.; Mann, C.M.; Markham, J.L.; Lie, Y.C.; Hartland, R.P.; Bell, H.C.; Warmington, J.R.; Wyllie, S.G. Tea tree oil causes K^+ leakage and inhibits respiration in *Escherichia coli*. *Lett. Appl. Microbiol.*, **1998**, *26*(5), 355-358.

[74] Bennis, S.; Chami, F.; Chami, N.; Bouchikhi, T.; Remmal, A. Surface alteration of *Saccharomyces cerevisiae* induced by thymol and eugenol. *Lett. Appl.*

Microbiol., **2004**, *38*(6), 454-458.

[75] Avila, J.G.; de Liverant, J.G.; Martinez, A.; Martinez, G.; Munoz, J.L.; Arciniegas, A.; de Vivar, A.R. Mode of action of *Buddleja cordata* verbascoside against *Staphylococcus aureus*. *J. Ethnopharmacol*., **1999**, *66*(1), 75-78.

[76] Bouhdid, S.; Abrini, J.; Amensour, M.; Zhiri, A.; Espuny, M.J.; Manresa, A. Functional and ultrastructural changes in *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells induced by *Cinnamomum verum* essential oil. *J. Appl. Microbiol*., **2010**, *109*(4), 1139-1149.

[77] Di Pasqua, R.; Betts, G.; Hoskins, N.; Edwards, M.; Ercolini, D.; Mauriello, G. Membrane toxicity of antimicrobial compounds from essential oils. *J. Agric. Food Chem.*, **2007**, *55*(12), 4863-4870.

[78] Rabinkov, A.; Miron, T.; Konstantinovski, L.; Wilchek, M.; Mirelman, D.; Weiner, L. The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. *Biochim. Biophys. Acta*, **1998**, *1379*(2), 233-244.

[79] Osbourn, E. Saponins in cereals. *Phytochem.*, **2003**, *62*(1), 1-4.

[80] Rasmussen, T.B.; Skindersoe, M.E.; Bjarnsholt, T.; Phipps, R.K.; Christensen, K.B.; Jensen, P.O. Andersen, J.B.; Koch, B.; Larsen, T.O.; Hentzer, M. Eberl, L-; Hoiby, N-; Givskov, M. Identity and effects of quorumsensing inhibitors produced by *Penicillium* species. *Microbiol.*, **2005**, *151*(Pt 5), 1325-40.

[81] Williams, P. Quorum sensing, communication and cross-kingdom signaling in the bacterial world. *Microbiol*., **2007**, *153*(Pt 12), 3923-3938.

[82] Garvey, M.I.; Rahman, M.M.; Gibbons, S.; Piddock, L.J.V. Medicinal plant extracts with efflux inhibitory activity against Gram-negative bacteria. *Intern. J. Antimicrob. Agents*, **2010**, *37*(2), 145-151.

[83] Sikkema, J.; de Bont, J.A.M.; Poolman, B. Mechanisms of membrane toxicity of hydrocarbons. *Microbiol. Rev.,* **199.5**. *59*(2), 201-222.

[84] Kohanski, M.A.; Dwyer, D.J.; Collins, J.J. How antibiotics kill bacteria: from targets to networks. *Nat. Rev. Microbiol*., **2010**, *8*(3), 423-435.

[85] Helander, I.M.; Alakomi, H.; Latva-Kala, K.; Mattila-Sandholm, T.; Pol, I.; Smid, E.J.; Gorris, L.G.M.; von Wright, A. Characterization of the action of selected essential oil components on Gram-negative bacteria. *J. Agric. Food Chem.*, **1998,** *46*(9), 3590-3595.

[86] Lambert, R.J.W.; Skandamis, P.N.; Coote, P.J.; Nychas, G.J.E. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol*., **2001**, *91*(3), 453-462.

[87] Walsh, S.E.; Maillard, J.Y.; Russell, A.D.; Catrenich, C.E.; Charbonneau, D.L.; Bartolo, R.G. Activity and mechanisms of action of selected biocidal agents on Gram-positive and negative bacteria. *J. Appl. Microbiol.,* **2003**, *94*(2), 240-247.

[88] Horváth, G.; Kovács, K.; Kocsis, B.; Kustos, I. Effect of thyme (*Thymus vulgaris* L.) essential oil and its main constituents on the outer membrane protein composition of *Erwinia* strains studied with microfluid chip technology. *Chromatographia*, **2009**, *70*(11-12), 1645-1650.

[89] Di Pasqua, R.; Mamone, G.; Ferranti, P.; Ercolini, D.; Mauriello, G. Changes in the proteome of *Salmonella enterica* serovar Thompson as stress adaptation to sublethal concentrations of thymol. *Proteomics*, **2010**, *10*(5), 1040-1049.

[90] Bang, K.H.; Lee, D.W.; Park, H.M.; Rhee, Y.H. Inhibition of fungal cell wall synthesizing enzymes by *trans*-cinnamaldehyde. *Biosci. Biotechnol. Biochem*., **2000**, *64*(5), 1061-1063.

[91] Hyldgaard, M.; Mygind, T.; Meyer, R.L. Essential oils in food preservation: mode of action, synergies and interactions with food matrix components. *Front. Microbiol*., **2012**, *3*, 1-24.

[92] Kwon, J.A.; Yu, C.B.; Park, H.D. Bacteriocidal effects and inhibition of cell separation of cinnamic aldehide on *Bacillus cereus. Lett. Appl. Microbiol.*, **2003**, *37*(1), 61-65.

[93] Gill, A.O.; Holley, R.A. Disruption of *Escherichia coli, Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. *Int. J. Food Microbiol*., **2006**, *108*(1), 1-9.

[94] Gill, A.O.; Holley, R.A. Inhibition of membrane bound ATPases of *Escherichia coli* and *Listeria monocytogenes* by plant oil aromatics. *Int. J. Food Microbiol*., **2006**, *111*(2), 170-174.

[95] Fitzgerald, D.J.; Stratford, M.; Gasson, M.J.; Narbad, A. Structure-function analysis of the vanillin molecule and its antifungal properties. *J. Agric. Food Chem.*, **2005**, *53*(5), 1769-1775.

[96] Fitzgerald, D.J.; Stratford, M.; Gasson, M.J.; Ueckert, J.; Bos, A.; Narbad, A. Mode of antimicrobial action of vanillin against *Escherichia coli, Lactobacillus plantarum* and *Listeria innocua*. *J. Appl.Microbiol.*, **2004**, *97*(1), 104-113.

[97] Patwardhan, B.; Vaidya, D.B.; Chorghade, M.; Joshi, S.P. Reverse pharmacology and systems approaches for drug discovery and development. *Curr. Bioact. Compd*., **2008**, *4*(4), 201-212.

[98] Denyer, S.P.; Stewart, G.S. Mechanisms of action of disinfectants. *Int. Biodeter. Biodegr.*, **1998**, *41*(3-4), 261-268.

[99] Kalemba, D.; Kunicka, A. Antibacterial and antifungal properties of essential oils. *Curr. Med. Chem.*, **2003**, *10*(10), 813-829.

[100] Ahmad, A.; Khan, A.; Akhtar, F.; Yousuf, S.; Xess, I.; Khan, L.; Manzoor, N. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against *Candida*. *Eur. J. Clin. Microbiol. Infect. Dis.*, **2011**, *30*(1), 41-50.

[101] Deva, R. Metabolism of arachidonic acid and formation of novel 3-hydroxyoxylipins by *Candida albicans* and interaction of HeLa cells *Candida albicans* as a model for vulvovaginal candidiasis: redundancy of signaling pathways for activation of COX-2. PhD Disertation, Faculty of Human Medicine. Freie Universität Berlin, 2010.

[102] Ultee, A.; Slump, R.A.; Steging, G.; Smid, E.J. Antimicrobial activity of carvacrol toward *Bacillus cereus* in rice. *J. Food Protect.*, **2000**, *63*(5), 620-624.

[103] Ultee, A.; Kets, E.P.W.; Smid, E.J. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. *Appl. Environ. Microbiol.*, **1999**, *65*(10), 4606-4610.

[104] La Storia, A.; Ercolini, D.; Marinello, F.; Di Pasqua, R.; Villani, F.; Mauriello, G. Atomic force microscopy analysis shows surface structure changes in carvacrol-treated bacterial cells. *Res. Microbiol.*, **2011**, *162*(2), 164-172.

[105] Ultee, A.; Bennik, M.H.J.; Moezelaar, R. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus. Appl. Environ. Microbiol*., **2002**, *68*(4), 1561-1568.

[106] Ben Arfa, A.; Combes, S.; Preziosi-Belloy, L.; Gontard, N.; Chalier, P. Antimicrobial activity of carvacrol related to its chemical structure. *Lett. Appl. Microbiol.*, **2006**, *43*(2), 149-154.

[107] Veldhuizen, E.J.A.; Tjeerdsma-Van Bokhoven, J.L.M.; Zweijtzer, C.; Burt, S.A.; Haagsman, H.P. Structural requirements for the antimicrobial activity of carvacrol. *J. Agric. Food Chem*., **2006,** *54*(5), 1874-1879.

[108] Rhayour, K.; Bouchikhi, T.; Tantaoui-Elaraki, A.; Sendide, K.; Remmane, A. The mechanism of bactericidal action of oregano and clove essential oils and their phenolic major constituents on *Escherichia coli* and *Bacillus subtilis. J. Essent. Oil Res*., **2003**, *15*(5), 356-362.

[109] Ait-Ouazzou, A.; Cherrat, L.; Espina, L.; Lorán, S.; Rota, C.; Pagán, R. The antimicrobial activity of hydrophobic essential oil constituents acting alone or in combined processes of food preservation. *Innov. Food Sci. Emerg. Technol.*, **2011**, *12*(3), 320-329.

[110] Trombetta, D.; Castelli, F.; Sarpietro, M.G.; Venuti, V.; Cristani, M.; Daniele, C.; Saija, A.; Mazzanti, G.; Bisignano, G. Mechanisms of antibacterial action of three monoterpenes. *Antimicrob. Agents Chemother.*, **2005** *49*(6), 2474-2478.

[111] Somolinos, M.; García, D.; Condón, S.; Mackey, B.; Pagán, R. Inactivation of *Escherichia coli* by citral. *J. Appl. Microbiol*., **2010**, *108*(6), 1928-1939.

[112] Espina, L.; Somolinos, M.; Lorán, S.; Conchello, P.; García, D.; Pagán, R. Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. *Food Control*, **2011**, *22*(6), 896-902.

[113] Nguefack, J.; Leth, V.; Amvamzollo, P.H.; Mathur, S.B. Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. *Int. J. Food Microbiol.,* **2004**, *94*(3), 329-334.

[114] De Souza, E.L.; De Barros, J.C.; De Oliveira, C.E.V.; Da Conceição, M.L. Influenceof *Origanum vulgare* L. essential oil on enterotoxin production, membrane permeability and surface characteristics of *Staphylococcus aureus. Int. J. Food Microbiol.*, **2010**, *137*(2-3), 308-311.

[115] Oussalah, M.; Caillet, S.; Lacroix, M. Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of *Escherichia coli* O157:H7 and *Listeria monocytogenes. J. Food Protect.*, **2006**, *69*(5), 1046-1055.

[116] Pérez-Fons, L.; Aranda, F.J.; Guillén, J.; Villalaín, J.; Micol, V. Rosemary (*Rosmarinus officinalis*) diterpenes affect lipidpolymorphism and fluidity in phospholipid membranes. *Arch. Biochem. Biophys.*, **2006**, *453*(2), 224-236.

[117] Rasooli, I.; Rezaei, M.B.; Allameh, A. Ultrastructural studies on antimicrobial efficacy of thyme essential oils on *Listeria monocytogenes. Int. J. Infect. Dis.*, **2006**, *10*(3), 236-241.

[118] Miksusanti, M.; Jenie, B.S.L.; Priosoeryanto, B.P.; Syarief, R.; Rekso, G.T. Mode of action Temu Kunci (*Kaempferia pandurata*) essential oil on *E. coli* K1.1 cell determined by leakage of material cell and salt tolerance assays. *Hayati J. Biosci.*, **2008**, *15*(2), 56-60.

[119] Bouhdid, S.; Abrini, J.; Zhiri, A.; Espuny, M.J.; Manresa, A. Investigation of functional and morphological changes in *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells induced by *Origanum compactum* essential oil. *J. Appl. Microbiol.*, **2009**, *106*(5), 1558-1568.

[120] Turgis, M.; Han, J.; Caillet, S.; Lacroix, M. Antimicrobial activity of mustard essential oil against *Escherichia coli* O157:H7 and *Salmonella typhi. Food Control*, **2009**, *20*(12), 1073-1079.

[121] Gill, A.O.; Delaquis, P.; Russo, P.; Holley, R.A. Evaluation of antilisterial action of cilantro oil on vacuum packed ham. *Int. J. Food Microbiol.*, **2002**, *73*(1), 83-92.

[122] Silva, F.; Ferreira, S.; Duarte, A.; Mendonça, D.I.; Domingues, F.C. Antifungal activity of *Coriandrum sativum* essential oil, its mode of action against *Candida* species and potential synergism with amphotericin B. *Phytomedicine*, **2011**, *19*(1), 42-47.

[123] Silva, F.; Ferreira, S.; Queiroz, J.A.; Domingues, F.C. Coriander (*Coriandrum sativum* L.) essential oil: its antibacterial activity and mode of action evaluated by flow cytometry. *J. Med. Microbiol*., **2011**, *60*(Pt 10), 1479-86.

[124] Hafedh, H.; Fethi, B.A.; Mejdi, S.; Emira, N.; Amina, B. Effect of *Mentha longifolia* L. ssp. *longifolia* essential oil on the morphology of four pathogenic bacteria visualized by atomic force microscopy. *Afr. J. Microbiol. Res.*, **2010**, *4*(11), 1122-1127.

[125] Stojanović-Radić, Z.; Comić, L.; Radulović, N.; Blagojević, P.; Mihajilov-Krstev, T.; Rajković, J. Commercial Carlinae radix herbal drug: Botanical identity, chemical composition and antimicrobial properties. *Pharm. Biol*., **2012**, *50*(8), 933-940.

[126] Pajohi, M.R.; Tajik, H.; Farshid, A.A; Hadian, M. Synergistic antibacterial activity of the essential oil of *Cuminum cyminum* seed and nisin in a food model. *J. Appl. Microbiol.* **2011**, *110*(4), 943-951.

[127] Paul, S.; Dubey, R.C.; Maheswari, D.K.; Chul Kang, Shttp://www.sciencedirect.com/science/article/pii/S0956713510003592 - affa#affa. *Trachyspermum ammi* (L.) fruit essential oil influencing on membrane permeability and surface characteristics in inhibiting food– borne pathogens. *Food Control*, **2011**, *22*(5), 725-731.

[128] Zeng, W.C.; Zhu, R.X.; Jia, L.R.; Gao, H.; Zheng, Y.; Sun, Q. Chemical composition, antimicrobial and antioxidant activities of essential oil from *Gnaphlium affine*. *Food Chem.Toxicol.* **2011**, *49*(6), 1322-1328.

[129] Takaisi-Kikuni, N.B.; Kriiger, D.; Gnann, W.; Wecke, J. Microcalorimetric and electron microscopic investigation on the effects of essential oil from *Cymbopogon densiflorus* on *Staphylococcus aureus*. *Microbios*, **1996**, *88*(354), 55-62.

[130] Gustafson, J.E.; Liew, Y.C.; Chew, S.; Markham, J.; Bell, H.C.; Wyllie, S.G.; Warmington, J.R. Effects of tea tree oil on *Escherichia coli*. *Lett. Appl. Microbiol*., **1998**, *26*(3), 194-198.

[131] Hammer, K.A.; Carson, C.F.; Riley, T.V. Antifungal effects of *Melaleuca alternifolia* (tea tree) oil and its components on *Candida albicans*, *Candida glabrata* and *Saccharomyces cerevisiae*. *J. Antimicrob. Chemother.*, **2004**, *53*(6), 1081-1085.

[132] Hsieh, P.C.; Siegel, S.A.; Rogers, B.; Lewis, K. Bacteria lacking a multidrug pump: a sensitive tool for drug discovery. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*(12), 6602-6606.

[133] Miron, T.; Rabinkov, A.; Mirelman, D.; Wilchek, M.; Weiner, L. The mode of action of allicin: its ready permeability through phospholipid membranes may contribute to its biological activity. *Biochim. Biophys. Acta*, **2000**, *1463*(1), 20-30.

[134] Perry, C.C.; Weatherly, M.; Beale, T.; Randriamahefa, A. Atomic force microscopy study of the antimicrobial activity of aqueous garlic versus ampicillin against *Escherichia coli* and *Staphylococcus aureus*. *J. Sci. Food Agric.*, **2009**, *89*(6), 958-964.

[135] Feldberg, S.R.; Chang, S.C.; Kotik, A.N.; Nadler, M.; Neuwirth, Z.; Sundstrom, D.C.; Thompson, N.H. *In vitro* mechanism of inhibition of bacterial cell growth by allicin. *Antimicrob. Agents Chemother.*, **1988**, *32*(12), 1763-1768.

[136] Luciano, F.B.; Holley, R.A. Enzymatic inhibition by allyl isothiocyanate and factors affecting its antimicrobial action against *Escherichia coli* O157:H7. *Int. J. Food Microbiol*., **2009**, *131*(2-3), 240-245

[137] Ahn, E.-S.; Kim, Y.-S.; Shin, D.-H. Observation of bactericidal effect of allyl isothiocyanate on *Listeria monocytogenes*. *Food Sci. Biotechnol*., **2001**, *10*(1), 31-35.

[138] Cushnie, T.P.; Lamb, A.J. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents*, **2005**, *26*(5), 343-356.

[139] Mirzoeva, O.K.; Grishanin, R.N.; Calder, P.C. Antimicrobial action of propolis and some of its components: the effects on growth, membrane potential and motility of bacteria. *Microbiol. Res*., **1997**, *152*(3), 239-246.

[140] Tsuchiya, H.; Iinuma, M. Reduction of membrane fluidity by antibacterial sophoraflavanone G isolated from *Sophora exigua*. *Phytomedicine*, **2000**, *7*(2), 161-165.

[141] Ikigai, H.; Nakae, T.; Hara, Y.; Shimamura, T. Bactericidal catechins damage the lipid bilayer. *Biochim. Biophys. Acta*, **1993**, *1147*(1), 132-136.

[142] Stapleton, P.D.; Shah, S.; Hamilton-Miller, J.M.T. et al. Anti-*Staphylococcus aureus* activity and oxacillin resistance modulating capacity of 3-*O*-acyl-catechins. *Int. J. Antimicrob. Agents*, **2004**, *24*(4), 374-380.

[143] Sato, M.; Tsuchiya, H.; Akagiri, M.; Takagi, N.; Iinuma, M. Growth inhibition of oral bacteria related to denture stomatitis by anticandidal chalcones. *Aust. Dent. J*., **1997**, *42*(5), 343-346.

[144] Sato, M.; Tanaka, H.; Yamaguchi, R.; Kato, K.; Etoh, H. Synergistic effects of mupirocin and an isoflavanone isolated from *Erythrina variegata* on growth and recovery of meticillin-resistant *Staphylococcus aureus*. *Int. J. Antimicrob. Agents*, **2004**, *24*(3), 241-246.

[145] Haraguchi, H.; Tanimoto, K.; Tamura, Y.; Mizutani, K.; Kinoshita, T. Mode of antibacterial action of retrochalcones from *Glycyrrhiza inflata*. *Phytochem*., **1998**, *48*(1),125-129.

[146] Ulanowska, K.; Tkaczyk, A.; Konopa, G.; Węgrzyn, G. Differential antibacterial activity of genistein arising from global inhibition of DNA, RNA and protein synthesis in some bacterial strains. *Arch. Microbiol.*, **2006**, *184*(5), 271-278.

[147] Phillipson, J.D.; O'Neill, M.J. New leads to the treatment of protozoal infections based on natural product molecules. *Acta Pharm. Nord*., **1987**, *1*, 131-144.

[148] Wendakoon, C.N.; Morihiko, S. Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. *J. Food Prot*., **1995**, *58*(3), 280-283.

[149] Chung, K.T.; Lu, Z.; Chou M.W. Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. *Food Chem. Toxicol.*, **1998**, *36*(12), 1053-1060.

[150] Cowan, M.M. Plant products as antimicrobial agents. *Clin. Microbiol. Rev*., **1999**, *12*(4), 564-582.

[151] Ya, C.; Gaffney, S.H.; Lilley, T.H.; Haslam, E. Carbohydrate-polyphenol complexation. In: Hemingway RW, Karchesy JJ, eds. Chemistry and significance of condensed tannins. Plenum Press; New York **1988**: 553.

[152] Smith-Palmer, A.; Stewart, J.; Fyfe, L. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Lett. Appl. Microbiol.*, **1998**, *26*(2), 118-122.

[153] Filgueiras, C.T.; Vanetti, M.C.D. Effect of eugenol on growth and listeriolysin O production by *Listeria monocytogenes*. *Braz. Arch. Biol. Techn*., **2006**, *49*(3), 405-409.

[154] Daifas, D.P.; Smith, J.P.; Blanchfield, B.; Sanders, G.; Austin, J.W.; Koukoutisis, J. Effects of mastic resin and its essential oils on the growth of proteolytic *Clostridium botulinum*. *Int. J. Food Microbiol*., **2004**, *94*(3), 313-322.

[155] Rammanee, K.; Hongpattarakere, T. Effects of tropical citrus essential oils on growth, aflatoxin production, and ultrastructure alterations of *Aspergillus flavus* and *Aspergillus parasiticus*. *Food Bioprocess Technol*., **2011,** *4*(6), 1050-1059.

[156] Sepandj, F.; Ceri, H.; Gibb, A.; Read, R.; Olson, M. Minimum inhibitory concentration (MIC) versus minimum biofilm eliminating concentration (MBEC) in evaluation of antibiotic sensitivity of gram-negative bacilli causing peritonitis. *Per. Dialysis Int*., **2004**, *24*(1), 65-67.

[157] Lewis, K. Riddle of biofilm resistance. *Antimicrob. Agents. Ch*., **2001**, *45*(4), 999-1007.

[158] Zucca, M.; Crivellaro, S.; Savoia, D. In: *Cystic fibrosis: etiology, diagnosis and treatments*; Leatte P.N. Ed.; Nova Publ., **2009**; pp. 1-9.

[159] Kociolek, M.G. Quorum-sensing inhibitors and biofilms. *Anti-infective Agents Med. Chem*., **2009**, *8*(4), 315-326.

[160] Quave, C.L.; Plano, L.R.W.; Pantuso, T.; Bennett B.C. Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resisant *Staphylococcus aureus*. *J. Ethnopharmacol*., **2008**, *118*(3), 418-428.

[161] da Silva, T.D.; Giordani, R.B.; Zimmer, K.R.; da Silva, A.G.; da Silva, M.V.; Correia M.T.S.; Baumvold, I.J.R.; Macedo, A.J. Potential of medicinal plants from the Brazilian semi-arid region (Caatinga) against *Staphylococcus epidermidis* planktonic and biofilm lifestyles. *J. Ethnopharmacol,*. **2011**, *137*(1), 327-335.

[162] Wang, Y.; Wang, T.; Hu, J.; Ren, C.Y.; Lei, H.; Hou, Y.; Brantner, A.H. Anti-biofilm activity of TanReQing, a Traditional Chinese Medicine used for the treatment of acute pneumonia. *J. Ethnopharmacol*., **2011**, *134*(1), 165-170.

[163] Khan, M.S.A.; Ahmad, I. Biofilm inhibition by *Cymbopogon citratus* and *Syzygium aromaticum* essential oils in the strains of *Candida albicans*. *J. Ethnopharmacol*., **2012**, *140*(2), 416-423.

[164] Furletti, V.F.; Teixeira, I.P.; Obando-Pereda, G.; Mardegan, R.C.; Sartoratto, A.; Figueira, G.M.; Duarte, R.M.T.; Rehder, V.L.G.; Duarte, M.C.T.; Höfling, J.F. Action of *Coriandrum sativum* L. essential oil upon oral *Candida albicans* biofilm formation. *Evid-Based. Compl. Alt.*, **2011**, doi:10.1155/2011/985832.

[165] Davies, A.N.; Brailsford, S.R.; Beighton, D. Oral candidosis in patients with advanced cancer. *Oral Oncol*., **2006**, *42*(7), 698-702.

[166] Seneviratne, C.J.; Jin, L.; Samaranayake, L.P. Biofilm lifestyle of *Candida:* a mini review. *Oral Dis*., **2008**, *14*(7), 582-590.

[167] Ahmad, N.; Alam, M.K.; Shehbaz, A.; Khan, A.; Mannan, A.; Rashid, H.S.; Bisht, B.; Owais, M. Antimicrobial activity of clove oil and its potential in the treatment of vaginal candidiasis. *J. Drug Target*., **2005**, *13*(10), 555-561.

[168] Bjarnsholt, T.; Givskov, M. Quorum-sensing blockade as a strategy for enhancing host defenses against bacterial pathogens. *Philos. Trans. R. Soc .Lond. B Biol. Sci*., **2007**, *362*(1483), 1213-1222.

[169] Rasmussen, T.B.; Bjarnsholt, T.; Skindersoe, M.E.; Hentzer, M.; Kristoffersen, P.; Kote, M.; Nielsen, J.; Eberl, L.; Givskov, M. Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. *J. Bacteriol*., **2005**, *187*(5), 1799-1814.

[170] Docherty, J.J.; Fu, M.M.; Tsai, M. Resveratrol selectively inhibits *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *J. Antimicrob. Chemother*., **2001**, *47*(2), 243-244.

[171] Fulghesu, L.; Giallorenzo, C.; Savoia, D. Evaluation of different compounds as quorum sensing inhibitors in *Pseudomonas aeruginosa*. *J. Chemother*., **2007**, *19*(4), 388-391.

[172] Park, J.; Kaufmann, G.F.; Bowen, J.P.; Arbiser, J.L.; Janda, K.D. Solenopsin A, a venom alkaloid from the fire ant *Solenopsis invicta*, inhibits quorum-sensing signaling in *Pseudomonas aeruginosa*. *J. Infect. Dis*., **2008**, *198*(8), 1198-1201.

[173] Adonizio, A.; Kong, K.F.; Mathee, K. Inhibition of quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa* by South Florida plant extracts. *Antimicrob. Agents Chemother*., **2008**, *52*(1), *98*-203.

[174] Koh, K.H.; Tham, F.Y. Screening of traditional Chinese medicinal plants for quorum-sensing inhibitors activity. *J. Microbiol. Immunol. Infect*., **2011**, *44*(2), 144-148.

[175] Pan, J.; Ren, D. Quorum sensing inhibitors: a patent overview. *Expert Opin. Ther. Pat*., **2009**, *19*(11), 1581-1601.

[176] Kan, Y.; Ucan, U.S.; Kartal, M.; Altun, M.L.; Aslen, S.; Sayar, E.; Cayhan, T. GC-MS analysis and antibacterial activity of cultivated *Satureja cuneifolia* Ten. essential oil. *Turk. J. Chem*., **2006**, *30*(2), 253-259.

[177] Wagner, H.; Ulrich-Merzenich, G. Synergy research: approaching a new generation of phytopharmaceuticals. *Phytomedicine*, **2009**, *16*(2-3), 97-110.

[178] Thornburg, C.C.; Zabriskie, T.M.; McPhail, K.L. Deep-sea hydrothermal vents: potential hot spots for natural products discovery? *J. Nat. Prod*., **2010**, *73*(3), 489-499.

[179] Hastings, I. How artemisinin-containing combination therapies slow the spread of antimalarial drug resistance. *Trends Parasitol*., **2011**, *27*(2), 67-72.

[180] Efferth, T.; Koch, E. Complex interactions between phytochemicals. The multi-drug target therapeutic concept of phytotherapy. *Curr. Drug Targets*, **2011**, *12*(1), 122-132.

[181] Cox, H.; Hargreaves, S.; Ismailov, G. Effect of multidrug resistance on global tuberculosis control. *Lancet*, **2003**, *362*(9398), 1858-1859.

[182] Li, R.C.; Schentag, J.J.; Nix, D.E. The fractional maximal effect method: a new way to characterize the effect of antibiotic combinations and other non-linear pharmacodynamic interactions. *Antimicrob. Agents Chemother.*, **1993**, *37*(3), 523-531.

[183] Inui, T.; Wang, Y.; Deng, S.; Smith, D.C.; Franzblau, S.G.; Pauli, G.F. Counter-current chromatography based analysis of synergy in an anti-tuberculosis ethnobotanical. *J. Chromatogr. A*, **2007**, *1151*(1-2), 211-215.

[184] Cottarel, G.; Wierzbowski, J. Combination drugs, an emerging option for antibacterial therapy. *Trends Biotechnol.*, **2007**, *25*(12), 547-555.

[185] Firn, R.D.; Jones, C.G. A Darwinian view of metabolism: molecular properties determine fitness. *J. Exp. Bot.*, **2009**, *60*(3), 719-726.

[186] Firn, R.D.; Jones, C.G. The evolution of secondary metabolism – a unifying model. *Mol. Microbiol.*, **2000**, *37*(5), 989-994.

[187] Pattnaik, S.; Subramanyam, V.R.; Bapaji, M.; Kole, C.R. Antibacterial and antifungal activity of aromatic constituents of essential oils. *Microbiology*, **1997**, *89*(358), 39-46.

[188] Radulović, N.S.; Mišić, M.; Aleksić, J.; Djoković, D.; Palić, R.M.; Stojanović, G.S. Antimicrobial synergism and antagonism of salicylaldehyde in *Filipendula vulgaris* essential oil. *Fitoterapia*, **2007**, *78*(7-8), 565-570.

[189] Lachowicz, K.J.; Jones, G.P.; Briggs, D.R.; Bienvenu, F.E.; Wan, J.; Wilcock, A.; Coventry M. J. The synergistic preservative effects of the essential oils of sweet basil (*Ocimum basilicum* L.) against acid-tolerant food microflora. *Lett. Appl. Microbiol.*, **1998**, *26*(3), 209-214.

[190] Moleyar, V.; Narasimham, P. Antibacterial activity of essential oil components. *Int. J. Food Microbiol.*, **1992**, *16*(4), 337-342.

[191] Didry, N.; Dubreuil, L.; Pinkas, M. Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. *Pharm. Acta Helv.*, **1994**, *69*(1), 25-28.

[192] Inoue, Y.; Shiraishi, A.; Hada, T.; Hamashima, H.; Shimada, J. The antibacterial effects of myrcene on *Staphylococcus aureus* and its role in the essential oil of the tea tree (*Melaleuca alternifolia*). *Nat. Med.*, **2004**, *58*(1), 10-14.

[193] Lis-Balchin, M.; Hart, S.; Deans, S.G.; Eaglesham, E. Comparison of the pharmacological and antimicrobial action of commercial plant essential oils. *J. Herbs Spices Med. Plants*, **1996**, *4*(2), 69-86.

[194] Galindo, L.A. Pultrini, A.D.M.; Costa, M. Biological effects of *Ocimum gratissimum* L. are due to synergic action among multiple compounds present in essential oil. *J. Nat. Med.*, **2010**, *64*(4), 436-441.

[195] Dilika, F.; Bremner, P.D.; Meyer, J.J.M. Antibacterial activity of linoleic- and oleic acid isolated from *Helichrysum pedunculatum*, a plant used during circumcision rites. *Fitoterapia*, **2000**, *71*(4), 450-452.

[196] Atal, C.K.; Dubey, R.K.; Singh, J. Biochemical basis of enhanced drug bioavailability by piperine: evidence that piperine is a potent inhibitor of drug metabolism. *J. Pharmacol. Exp. Ther.*, **1985**, *232*(1), 258-262.

[197] Anand, P.; Thomas, S.G.; Kunnumakkara, A.B.; Sundaram, C.; Harikumar, K.B.; Sung, B.; Tharakan, S.T.; Misra, K.; Priyadarsini, I.K.; Rajasekharan, K.N., Aggarwal, B.B. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. *Biochem. Pharmacol.*, **2008**, *76*(11), 1590-1611.

[198] Van Vuuren, S.F.; Viljoen, A.M. In vitro evidence of phyto-synergy for plant part combinations of *Croton gratissimus* (Euphorbiaceae) used in African traditional healing. *J. Ethnopharmacol.*, **2008**, *119*(3), 700-704.

[199] Van Vuuren, S.F.; Viljoen, A.M.; van Zyl, R.L.; van Heerden, F.R.; Başer, K.H.C. The antimicrobial, antimalarial and toxicity profiles of helihumulone, leaf essential oil and extracts of *Helichrysum cymosum* (L.) D. Don subsp. *cymosum*. *S. Afr. J. Bot.*, **2006**, *72*(2), 287-290.

[200] Van Vuuren, S.F. Antimicrobial activity of South African medicinal plants. *J. Ethnopharmacol.*, **2008**, *119*(3), 462-472.

[201] Shealy, C.N. *The illustrated encyclopaedia of healing remedies.* Australia: Element Books, **1998**.

[202] Stermitz, F.R.; Lorenz, P.; Tawara, J.N.; Zenewicz, L.A.; Lewis, K. Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin, a multidrug pump inhibitor. *P. Natl. Acad. Sci. USA*, **2000**, *97*(4), 1433-1437.

[203] Radulović, N.S.; Zlatković, D.B.; Dekić, M.S.; Stojanović-Radić, Z.Z. In *Proceedings of the 50th Meeting of the Serbian Chemical Society*, Belgrade, Serbia, June 14-15, 2012; *Serbian Chemical Society*, Belgrade, Serbia, **2012**; pp. 108.

[204] Van Vuuren, S.F.; Suliman, S.; Viljoen, A.M. The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials. *Lett. Appl. Microbiol.*, **2009**, *48*(4), 440-446.

[205] Balani, S.K.; Miwa, G.T.; Gan, L.S.; Wu, J.T.; Lee, F.W. Strategy of utilizing *in vitro* and *in vivo* ADME tools for lead optimization and drug candidate selection. *Curr. Top. Med. Chem.* **2005**, *5*(11), 1033-1038.

[206] Taweechaisupapong, S.; Wongkham, S.; Chareonsuk, S.; Suparee, S.; Srilalai, P.; Chaiyarak, S. Selective activity of *Streblus asper* on Mutans streptococci. *J. Ethnopharmacol.*, **2000**, *70*(1), 73-79.

[207] Pai, M.R.; Acharya, L.D.; Udupa, N. Evaluation of antiplaque activity of *Azadirachta indica* leaf extract gel-a 6-week clinical study. *J. Ethnopharmacol.*, **2004**, *90*(1), 99-103.

[208] Groppo, F.C.; Ramacciato, J.C.; Motta, R.H.; Ferraresi, P.M.; Sartoratto, A. Antimicrobial activity of garlic against oral streptococci. *Int. J. Dent. Hyg.*, **2007**, *5*(2), 109-115.

[209] Blaser, M.J.; Berg, D.E. *Helicobacter* genetic diversity and risk of human disease. *J. Clin. Invest.*, **2001**, *107*(7), 767-773.

[210] Marone, P.; Bono, L. ; Leone, E.; Bona, S. ; Carretto, E.; Perversi, L. Bactericidal activity of *Pistacia lentiscus* mastic gum against *Helicobacter pylori*. *J. Chemother.*, **2001**, *13*(6), 611-614.

[211] Huwez, F.U.; Thirlwell, D.; Cockayne, A.; Ala'Aldeen, D.A. Mastic gum kills *Helicobacter pylori*. *N. Engl. J. Med.*, **1998**, *339*(26), 1946.

[212] Bebb, J.R.; Bailey-Flitter, N.; Ala'Aldeen, D.; Atherton. J.C Mastic gum has no effect on *Helicobacter pylori* load *in vivo*. *J. Antimicrob. Chemoth.*, **2003**, *52*(3), 522.

[213] Vattem, D.A.; Lin, Y.T.; Ghaedian, R.; Shetty, K. Cranberry synergies for dietary management of *Helicobacter pylori* infections. *Process Biochem.*, **2005**, *40*(5), 1583-1592.

[214] Rang, H.; Dale, P.; Maureen M.; Ritter, J.M. *Rang and Dale's Pharmacology*, 6th ed.; Elsevier Science Health Science, **2007**.

[215] European Food Safety Authority (EFSA). Scientific opinion on the safety of stigmasterol-rich plant sterols as food additive. *EFSA Journal*, **2012**, *10*, 2659 [39 pp.].

[216] Ishida, T.; Toyota, M.; Asakawa, Y. Terpenoid biotransformation in mammals. V. Metabolism of (+)-citronellal, (±)-7-hydroxycitronellal, citral, (−)-perillaldehyde, (−)-myrtenal, cuminaldehyde, thujone and (±)-carvone in rabbits. *Xenobiotica*, **1989**, *19*(8), 843-855.

[217] Höld, K.M.; Sirisoma, N.S.; Casida, J.E. Detoxification of α- and β-thujones (the active ingredients of absinthe): Site specificity and species differences in cytochrome P450 oxidation *in vitro* and *in vivo*. *Chem. Res. Toxicol.*, **2001**, *14*(5), 589-595.

[218] Snyder, R.; Hedli, C.C. An overview of benzene metabolism. *Environ. Health Perspect.*, **1996**, *104*(Suppl 6), 1165-1171.

[219] Rose, V.E.; Cohrssen, B. *Patty's industrial Hygiene*, 4th ed.; Wiley & Sons: New Jersey, **2011**, pp. 73.

[220] Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. *Drug Deliver Rev.*, **1997**, *23*(1-3), 3-25.

[221] Leeson, P. Drug discovery: Chemical beauty contest. *Nature*, **2012**, *481*, 455-456.

[222] Atal, C.K.; Zutshi, U.; Rao, P.G. Scientific evidence on the role of Ayurvedic herbals on bioavailability of drugs. *J. Ethnopharmacol.*, **1981**, *4*(2), 229-232.

[223] Wang, S.P.; Liu, L.; Wang, L.L.; Jiang, P.; Zhang, J.Q.; Zhang, W.D,; Liu, R.H. Screening and analysis of the multiple absorbed bioactive components and metabolites in rat plasma after oral administration of Jitai tablets by high-performance liquid chromatography/diode-array detection coupled with electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.*, **2010**, *24*(11), 1641-1652.

[224] Woelkart, K.; Feizlmayr, E.; Dittrich, P.; Beubler, E.; Pinl, F.; Suter, A.; Bauer, R. Pharmacokinetics of bilobalide, ginkgolide A and B after administration of three different *Ginko biloba* L. preparations in humans. *Phytother. Res.*, **2010**, *24*(3), 445-450.

[225] He, S.M.; Li., C.G.; Liu, J.P.; Chan, E.; Duan, W.; Zhou, S.F. Disposition pathways and pharmacokinetics of herbal medicines in humans. *Curr. Med. Chem.*, **2010**, *17*(33), 4072-4113.

[226] Schmid, B.; Kotter, I.; Heide, I. Pharmacokinetics of salicin after oral administration of a standardized willow bark extract. *Eur. J. Clin. Pharmacol.*, **2001**, *57*(5), 387-391.

[227] Gibbons, S. Plants as a source of bacterial resistance modulators. *Phytochem. Rev.*, **2005**, *4*(1), 63-78.

[228] Koehn, F.E.; Carter, G.T. The evolving role of natural products in drug discovery. Nat. Rev. Drug Discov., **2005**, 4(3), 206-220.

[229] Lambert, R.J.W.; Pearson, J. Susceptibility testing: accurate and reproducible minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) values. *J. Appl. Microbiol.*, **2000**, *88*(5), 784-790.

[230] Estes, J.D.; Stolpman, D; Olyaei, A.; Corless, C.L.; Ham, J.M.; Schwartz, J.M.; Orloff, S.N. High prevalence of potentially hepatotoxic herbal supplement use in patients with fulminant hepatic failure. *Arch. Surg.*, **2003**, *138*(8), 852-858.

[231] Radulović, N.S.; Dekić, M.S.; Randjelović, P.J.; Stojanović, N.; Zarubica, A.R.; Stojanović-Radić, Z.Z. Toxic essential oils: Anxiolytic, antinociceptive and antimicrobial properties of the yarrow *Achillea umbellata* Sibth. et Sm. (Asteraceae) volatiles. *Food Chem. Toxicol.*, **2012**, *50*(6), 2016-2026.

[232] Ryan, K.J.; Ray, C.G. *Sherris Medical Microbiology*, 4th ed.; McGraw Hill, **2004**.

[233] Orme, I. Search for new drugs for treatment of tuberculosis. *Antimicrob. Agents Ch.*, **2001**, *45*(7), 1943-1946.

[234] Falzari, K.; Zhu, Z.; Pan, D.; Liu, H.; Hongmanee, P.; Franzblau, S.G. *In vitro* and *in vivo* activities of macrolide derivatives against *Mycobacterium tuberculosis*. *Antimicrob. Agents Ch.*, **2005**, *49*(4), 1447-1454.

[235] European Chemical Agency, ECHA. http://echa.europa.eu/documents/10162/13587/10_sb_siduvcb_d1_lrws_20120203_en.pdf http://apps.echa.europa.eu/legacy/doc/webinars/monoconstituent_suvi_takala_echa.pdf (Accessed July 16, 2012)

[236] Radulović, N.S.; Blagojević, P.D. Plant volatiles providing additional evidences to the occurrence of a wild-growing population of *Calamintha*

vardarensis (GREUTER et BURDET) SILIC outside of its natural habitat. *Chem. Biodivers*., **2010**, *7*(12), 2856-2868.

[237] Radulović, N.S.; Blagojević, P.D. Volatile secondary metabolites of *Micromeria dalmatica* BENTH. (Lamiaceae): Biosynthetical and chemotaxonomical aspects. *Chem. Biodivers.*, **2012**, *9*(7), 1303-1319.

[238] Radulović, N.S.; Blagojević, P.D. Volatile Profiles of *Artemisia alba* from contrasting serpentine and calcareous habitats. *Nat. Prod. Commun*., **2010**, *5*(7), 1117-1122.

[239] Radulović, N.S.; Blagojević, P.D.; Rabbitt, K.; Menezes F.S. Essential oil of *Nepeta* x *faassenii* Bergmans ex Stearn (*N. mussinii* Spreng. x *N. nepetella* L.): A comparison study. *Nat. Prod. Commun*., **2011**, *6*(7), 1015-1022.

[240] Radulović, N.S.; Blagojević, P.D.; Skropeta, D.; Zarubica, A.R.; Zlatković, B.K.; Palić, R.M. Misidentification of tansy, *Tanacetum macrophyllum*, as yarrow, *Achillea grandifolia*: a health risk or benefit? *Nat. Prod. Commun*., **2010**, *5*(1), 121-127.

[241] Turina, A.D.V.; Nolan, M.V.; Zygadlo, J.A.; Perillo, M.A. Natural terpenes: self-assembly and membrane partitioning. *Biophys. Chem.*, **2006**, *12*(2), 101-113.

Received: August 11, 2012 **Revised: October 18, 2012** **Accepted: October 20, 2012**