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Biological activity of volatile diterpenoids

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Dubravka Stanetic

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Abstract

The aim of this literature review is to investigate if volatile diterpenoids, a small group of compounds, which can be found in essential oils, have any of the various biological activities which are generally attributed to essential oils. What is important to emphasize is the fact, that in the nature, the number of volatile and bio-active diteprenoids is very small. Different plants families such as Asteraceae and Lamiaceae as the richest ones and a lot of other families have been investigated and their essential oils have been extracted, compounds isolated and then analyzed. Almost all isolated diteprenoids shown to have cytotoxic, antioxidant or antimicrobial activities alone or more often in synergism with other essential oil compounds. For example labdane-type and abietane-type diterpenes have shown cytotoxicity against tumor cells and abietane-like compounds have an important role as antioxidants. For each isolated class of diterpenoids can be one or more biological activities credited.

Abstract

Das Ziel dieser Literaturübersicht war es zu untersuchen, ob die flüchtigen Diterpenoide, eine kleine Gruppe von Verbindungen, die in ätherischen Ölen zu finden sind, eine Vielzahl der biologischer Aktivitäten zeigen, die allgemein den ätherischen Ölen zugeschrieben werden. Was zu betonen ist, ist die Tatsache, dass in der Natur die Anzahl von flüchtigen und bioaktiven Diterpenoiden sehr klein ist. Verschiedene Familien wie Asteraceae und Lamiaceae als die reichsten und viele andere Familien wurden untersucht und deren ätherische Öle extrahiert und weiters deren Inhaltsstoffe isoliert und analysiert. Fast alle isolierte Diterpenoide zeigen zytotoxische, antioxidantive oder antimikrobielle Aktivitäten, allein oder häufiger in Synergismus mit anderen Komponenten, die in ätherischen Ölen enthalten sind. Zum Beispiel Labdan-Typ und Abietan-Typ Diterpenoide sind zytotoxisch gegen Tumorzellen und Abietan-ähnliche Verbindungen spielen eine wichtige Rolle als Antioxidantien. Der jeweils isolated Klasse von Diterpenoiden können je eine oder mehrere biologische Aktivitäten gutgeschrieben werden.

CONTENTS:

Introduction

There are about 215,000 to 500,000 different types of plants all over the world, but of this huge number, just about 6% have been noticed for their specific biological activity and about 15% have been studied phytochemically. Higher plants are very important, because these plants contain a wide range of natural compounds, which can be isolated from different parts of plants such as leaves, flowers, fruits, stems, seeds and roots, then analyzed and later used for the developing of new therapeutic agents. Good examples for that are essential oils, which possess a major spectrum of fundamental biological activities (Tirapelli et al., 2008; Tongnuanchan and Benjakul, 2014).

Volatile organic compounds (VOCs) can mostly be found in essential oils from different type of plants. Today essential oils, as secondary metabolites from the plants, are because of their wide range of biological activities such as antimicrobial, antiinflammatory, anticancer, antiviral, neuroprotective and antithrombotic activity, increasingly gained in importance (Lu et al., 2012; Katiyar et al., 2010; Wang et al., 2005). Essential oils are a mixture of many volatile and non-volatile compounds and they contain a lot of low molecular compounds such as hydrocarbons, alcohols, acids, aldehydes, acyclic lactones and acyclic esters (Nazzaro et al., 2013). Terpenes otherwise called terpenoids (terpenoids are actually terpene like compounds, they have the same biosynthesis pathway, same rearranged structure which may include an oxygen atom; therefore these two terms are used interchangeably (Bonito et al., 2006-2009)) or specially monoterpenes and sesquiterpenes, and aromatic polypropanoid compounds are the largest amounts in essential oils (Charles and Simon, 1990). Between these two types of terpenes, essential oils are also important source of volatile diterpenes. The topic of this paper gives importance to minor compounds of essential oils, namely to the diterpenes. In effect there are not so many volatile diterpenes (they

can be found in essential oils but in much lower percentage in comparison with terpenes with less carbon atoms), but the presence in small amounts of these compounds in volatile oils plays an important role and contributes to a variety of biological activities of these oils (Tisserand and Young, 2014; Bohlmann and Keeling, 2008). Because of a wide variety of compounds in essential oils, these can have diverse chemical properties and based on different chemical compounds, essential oils have potential in many therapeutic and medicinal procedures. These secondary plant metabolites find their usage in food industry, fragrances in perfumery, aromatherapy as also in pharmaceutical industry. So the safety, which is nowadays on a high level, of the essential oils is very important (Katiyar et al., 2010; Tognolini et al., 2006).

Diterpenoids as a part of terpenoid family

The terpenoid family is a very large family with a lot of different types of members. These lipophilic structures are very common in the nature and can be practically found everywhere, in bacteria, fungi, plants and insects and are vital for the growth of the plant and their development as also for the production of special substances for the interaction of plant with the environment. Terpenoids found in plants are specific because of their aromatic qualities and various spectrums of biological and pharmaceutical activities (Gershenzon and Dudareva, 2007; Gonzáles-Burgos and Gómez-Serranillos, 2012). They are used in traditional and also nowadays in herbal medicine and are verifying for antibacterial, antineoplastic, antioxidant, antiinflammatory and a lot of more other pharmaceutical properties (Bohlmann and Keeling, 2008). They are composed of five-carbon units called isoprene units deduced from either mevalonate or non-mevalonate pathway and converted and changed in many different ways (Cao et al., 2010; Dudareva et al., 2013; Bohlmann and Keeling, 2008). In the majority of

classes are multicyclic structures and difference between these can be based on their main carbon skeletons or more often in their functional groups. The number of isoprene units is a base for the classification of terpenoids. Every next member (class) of terpenoid family has one isoprene unit more than the previous member so that, hemiterpenes have C_5 (one isoprene unit), monoterpenes C_{10} (two isoprene units), sesquiterpenes C_{15} (three isoprene units), diterpene C_{20} (four isoprene units), sesterterpenoids C_{25} (five isoprene units) and etc. (Dubey et al., 2003; Bohlmann and Keeling, 2008; Tongnuanchan and Benjakul, 2014). The greater the number of isoprene units is, the lower are volatilities of these compounds. For example, monoterpenoids are the simplest model of all terpenoids, made only of two isoprene units. Low molecular weight of these compounds is responsible for the characteristic, very easy, volatility. The same group LMWT (low molecular weight terpenoids) includes also the sesquiterpenes with three isoprene units, so the sesquiterpenes volatilize as fast as monoterpenes and this explains why the low weight mono- and sesquiterpenes are the main compounds of essential oils. Third most common compounds in essential oils are phenylpropanoids, which are synthesized by the shikimic acid pathway. Phenylpropanoids have nine-carbon skeleton and although they do not belong to the LMWT, all characteristics of LMWT can equally be applied to phenylpropanoids (Bohlmann and Keeling, 2008; Greenhagen and Chappell, 2001; Wynn and Fougère, 2007; Tognolini et al., 2006; Wang et al., 2005).

Diterpenoids can be defined as C_{20} natural compounds, composed of four isoprene units, and because of the higher number of carbon atoms they appertain not to the group of LMWT and have much lower volatile characteristics in comparison with mono- and sesquiterpenes. Diterpenoids are high lipophilic structures and can be found in resins of the plants. Although they are very common and have diverse spectrum of biological activities, they were not so much investigated (Wynn and Fougère, 2007). The discovery of Taxol (Paclitaxel) from *Taxus brevifolia* and its amazing cytotoxic activities has increased the interest for this group of compounds (Heing and Jennewein, 2009).

There are different types of diterpenoids. They can be graded as linear or can have two, three, four, five or more circles, contingent on their skeletal core. In nature, they are commonly found in a polyoxygenated form with keto- and hydroxyl-groups, these last often esterified by small-sized aliphatic or aromatic acids. Diterpenoids are nowadays in the spotlight of researches because of their very useful and characteristic biological and pharmacological activities (Tirapelli et al., 2008). Several hundreds of diterpenes have been specified from overland and sea organisms, but just a few of them have passed through test and have been taken to the further procedure and clinical researches. Therapeutic-used diteprenoids will be specified together with other kindly bioactive diterpenes with point attention to those, which are extracted from plants. Higher plants are very rich on diterpenoids. Families like Asteraceae and Lamiacieae as the richest, also Flacourtiaceae, Araucariaceae, Euphorbiaceae and Celastraceae, have been particularly used for the isolation of important diterpenoids (Tirapelli et al., 2008; Lanzotti, 2013; Bohlmann and Keeling, 2008).

Biosynthesis of Terpenoids

There are three different types of pathways for the syntheses of VOCs (volatile organic compounds): MVA (mevalonic acid pathway), non-mevalonate MEP (methylerythroyl phosphate pathway) and Shikimic acid pathway, but only the MVA- and MEP-pathway allow the biosynthesis of terpenoids (Dudareva et al., 2013). Terpene synthesis is very unique and this process can also be called a chemical magic because the production of these compounds in nature runs perfectly. Which type of synthesis will happen depends on the type of terpenes and also on the biological organism. Volatile sesquiterpenes are made through the MVA pathway, while MEP pathway is responsible for the syntheses of volatile

hemiterpenes, mono- and diterpenes (Lichtenthaler, 2000; Dudareva et al., 2013; Zhang and Demain, 2005).

First pathway, mevalonate pathway is one cytoplasmatic six step enzymatic reaction and starts with producing of acetoacetyl CoA. This one is made through the condensation of two molecules of acetyl CoA via Claisen by acetyl-CoA C-acetyltransferase. Then upon an aldol-like reaction, which is catalyzed by hydroxymethylglutaryl-CoA-syntase, acetyl-CoA is bonded with a third molecule of acetyl-CoA and hydrolyzed to the metabolite 3-hydroxy-3-methylglutaryl-CoA. NADP-controlled reduction of the thioester group catalyzed by 3-hydroxy-3-methylglutaryl-CoA redutase leads to mevalonate production. Mevalonates primary hydroxyl group is phosporylated by mevalonate kinase and afterwards followed with important phosporhyrilisation at the tertiary hydroxyl group, which is catalyzed by mevalonate-5-diphosphate decarboxylase. At the same time there is loss of carbon dioxide and phosphate and this all results with building of 3-isopentenyl pyrophosphate (IPP) (Bohlmann, Keeling, 2008; Hunger et al., 2003; Zhang and Demain, 2005; Dudareva et al., 2013; Aharoni et al., 2005).

The second MEP pathway is a seven step enzymatic reaction and it is exclusively plastidic pathway. It was discovered much later then the mevalonate pathway and was rarely used (only by chloroplasts, algae and bacteria). Reaction starts with the bounding of pyruvate with D-glyceraldehyde-3-phosphate catalyzed by 1-deoxy-D-xylulose-5-phosphate-synthase (DXS) to 1-deoxy-D-xylulose-5-phosphate (DXP) with synchronal loss of carbon dioxide. Second step is an NADPH-dependent remodeling and reduction and as a catalyst deoxyxylulose-5-phosphate reductoisomerase (DXR) is used with the purpose to produce methyl-D-erythritol-4-phosphate (MEP). The cytidine-triphosphate (CTP) dependent conversion of MEP to 4-(diphosphocytidyl)-2-C-methyl-D-erythritol (CDP-ME) is the next step of the MEP pathway. Afterwards the enzyme CDP-ME kinase allows the phosphorylation of two hydroxyl groups on the CDP-ME resulting with building of the new molecule of CDP-MEP, which will be via 2-C-methyl-D-erythritol 2,4-cyclodiphosphate (MECP syntheses) transformed to

the MECDP. Last step is the conversion of MECDP to intermediate HMBPP (1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate) via HDS (4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase) and then via IDS (isopentenyl diphosphate synthase) to IPP and DMAPP (dimethylallyl pyrophosphate) (Bohlmann, Keeling, 2008; Hunger et al., 2003; Lichtenthaler, 2000; Wenke 2001; Dudareva et al., 2013; Dubey et al., 2003; Zhang and Demain, 2005; Aharoni et al., 2005).

Isopentenyl pyrophosphate isomerase (IPI) allows as a catalyst the reversible isomerisation of IPP (isopentenyl diphosphate) to DMAPP (dimethylallyl diphosphate, allylic isomer of IPP) and keeps the balance between them. The base for the terpenoid synthesis are these two C_5-precursors, IPP and DMAPP, which are via prenyltransferases (GPP-, FPP- and GGPP-synthases) bound to GPP (geranyl pyrophosphate), FPP (farnesyl pyrophosphate) and GGPP (geranyl geranyl pyrophosphate) tapped by phosphate groups (Greenhagen, Chappell, 2001). In cytosol TPS (terpene synthase) converts the precursor FPP to the terpene sesquiterpene, while the same enzyme in plasmid converts GPP to monoterpenes and GGPP to diterpenes (Dudareva et al., 2013; Dubey et al., 2003; Zhang and Demain, 2005; Bohlmann, Keeling, 2008; Hunger et al., 2003; Aharoni et al., 2005).

Three most common biological activities of essential oils which contain volatile diterpenoids

Antioxidant activity: During normal physiological processes, but also in stress conditions reactive oxygen species (ROS eng. Reactive Oxygen Species) and enzymatic antioxidants were produced in the human body. The term “antioxidants” is a general term for all components that can prevent or at least

reduce the oxidation of the substrate. The imbalance between the generated free radicals and antioxidants leads to oxidative damage of macromolecules, cells, such as peroxidation of membrane lipids, oxidative damage of nucleic acids, oxidation and other sulfuric groups in proteins (Aruoma, 1998; Zhu et al., 2007). All changes of cell-macromolecules are leading to the emergence of many health disorders in humans. Free radicals are molecules which have one or more unpaired electrons in its structure, and these unpaired electrons are responsible for their pronounced reactivity. Radicals possess an unpaired electron in an oxygen atom and belong to the group of reactive oxygen species. This group include: Superoxide radicals ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$), perhydroxy radicals ($HO_2^{\bullet}$) and alkoxy radicals ($RO^{\bullet}$). There are also non-radical species containing oxygen such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and ozone (O_3) (Ray et al., 2013; Di Matteo V. and E. Esposito, 2003). The absence of antioxidants, which have a role to neutralize reactive free radicals, leads to many diseases, such as cardiovascular disease and cancer, neurodegenerative diseases, Alzheimer's disease and inflammatory diseases (Gonzáles-Burgos and Gómez-Serranillos, 2012; Zhu et al., 2007) . As a health-protecting factor the antioxidants have big importance. Fruits, vegetables and grains are the elementary source of natural antioxidants. There are also many synthetic antioxidants which are used in the food industry such as butylated hydroxytoluene (BHT) and butylated hydroxyanisolen (BHA) (Brewer, 2011; Velioglu et al., 1998). Based on the claims that they can throw out free radicals the assessment of antioxidant activities as natural food additives is very substantial nowadays. Concentration of used compounds plays also an important role. Low-dosed retinol antioxidants show an antioxidant activity but high-dosed they can become genotoxic. The explanation for that is that the key for a normal function of the biological organism is in balance in the human body between oxidation and antioxidation (Bouayed and Bohn, 2010). There exists few different methods which can be used for the estimation of antioxidant activity: DPPH (stable free radical 2,2-diphenyl-1-picrylhydrazyl), ABTS (chemical compound 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and FRAP (fluorescence recovery after

photobleaching) (Molyneux, 2004; Rohman et al., 2010; Re et al., 1999; Antolovich et al., 2002).

Antimicrobial activity: Although the pharmaceutical industry has lately itself very developed, the number of pathogenic microorganisms that are resistant to antibiotics increased. Pharmaceutical industry is trying through the changes of the molecular structure of existing antibiotics to improve their efficiency. However, bacteria possess the genetic ability quickly to reach and spread resistance. Because the synthesized molecules can also produce toxic reactions, it is essential to investigate new therapeutic drugs. Nowadays natural products are a major source of new preventive and therapeutic agents for various diseases including diseases caused by pathogenic microorganisms. The interest for natural drugs increased so much, that they are a first choice in primary health care (Silva and Fernandes Júnior, 2010; Bhalodia and Shukla, 2011; J. Davies and D. Davies, 2010). In recent years intensive studies have been directed towards examining the possibilities the use of plant extracts and essential oils in treatments against pathogenic bacteria and mushrooms. There are many chemical compounds isolated from plants such as phenolic acids, quinones, flavones, flavonoids, tannins, coumarins, terpenoids and alkaloids, which have been shown to have antimicrobial activity (Cowan, 1999). Of all the above compounds, it has been shown that constituents of essential oils, terpenoids possess the highest antimicrobial activity. Natural compounds, and between them also terpenoids and diterpenoids, act toxic on the cell membrane. Because of their lipophillic character, these compounds can move easily from aqueous phase and incorporate in membrane structures. The consequence of this action is that the membrane increases its permeability and fluidity, thus the membrane protein ratio can be disbalanced and the ion transport inhibited (Trombetta et al., 2005). At the beginning of the investigation of essential oils, their volatile components and their biological activity, namely their antimicrobial activity, were in spotlight. But nowadays, the interest also for the antifungal activity increased. It is assumed that natural antifungal compounds may have inhibitory effects on some of the

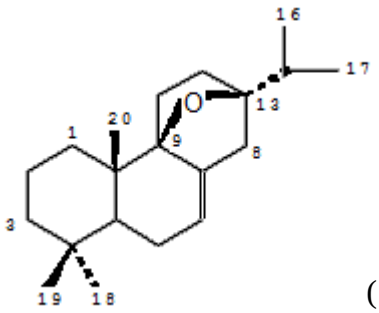
enzymes that are involved in the synthesis of mycotoxins and they can also react with sulfonic protein groups in the membrane. Although there are a non-specific protein bonds which lead to membrane damage that inevitably affects the balance of inorganic ions (Cowan, 1999; Lambert et al., 2001). There are three different types of methods for the measurement of the antimicrobial activities of essential oils and their isolated compounds, two qualitative techniques, the bioautographic and diffusion methods, which finnish only a statement about the presence or absence of the microorganisms, and one quantitative technique, the dilution method, which defines also the minimal inhibitory concentration (Valgas et al., 2007).

Cytotoxic activity: Plants and their extracts have the ability to induce an apoptosis of human cancer cells and that is why they have been therapeutically used since ancient times in cancer therapy (Bahnot et al., 2011). Nature is a very good source of anticancer drugs, so that today about 50% of anticancer drugs are derived from the nature. Cancer is nowadays the most common cause of death, so that today anticancer studies are worldwide guided in the hope of finding new better drugs (Grbovic et al., 2013). It is a degenerative disease and it is characterized as an uncontrolled multiplication of the cells and their rapid spread in the whole body, resulting with abnormal cell formations with constant progression till the human death. Stressful lifestyle, smoking and drinking habits, fast food or toxic drugs can be the cause for the cancer formation, in fact it is an ensemble acting of genetic and environment factors (Fatemeh and Khosro, 2013; Nataru et al., 2014; Umadevi et al., 2013). Usage of anticancer agents is complicated, because these drugs on the one hand have the desired effect on tumor cells, but on the other hand produce a huge number of toxic side effects and also can be life-threatening for the drug-user. Very often this heavy therapy requests a dosis-adjusment (reduction) of chemotherapeutic agents and sometimes also even the break off the therapy. Thereby nowadays these side effects of standard anticancer therapies and limited accomplishment of clinical therapies

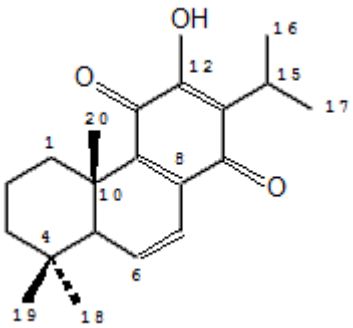
(surgeries, immunomodulation, chemo- and radiotherapy) are the motivation of the development of therapeutic effective drugs from the nature with reduced toxicity. What is also the second reason for the increased usage of plant products in therapies at all, is that these products are easily available, are much cheaper and show notably lower toxicity compared to the allopathic drugs (Umadevi et al., 2013; Nawab et al., 2011).

Volatile diterpenoids identified in essential oils from different plant types and their biological activities

Tetradenia riparia (Hochstetter) otherwise called *Iboza riparia* or *Moschosma riparium*. It is assigned to the Lamiaceae family and it comes from South Africa, where it is one of the most aromatic and popular medicinal plants. This exotic plant is popularly known as false myrrh, lemon verbena, lavandula, misty plume, or incense. The Lamiaceae family as one of the richest families based on the content of essential oils was studied to detect compounds and potential of these oils. Analyses of the oil of *T. riparia* showed that this oil contains a complex mixture of terpenoids: Mono-, sesqui- and diterpenes and the most representative composition are oxygenated sesquiterpenes, specifically 14-hydroxy-9-*epi*-caryophyllene. Biological activity of the essential oil of *T. riparia* and all components together which have been detected and analyzed are anti-spasmodic, -mycobacterial, -malarial, larvicidal and insectidal und etc. Based on the analysis of this essential oil two new interesting diterpene compounds have been isolated and screened for their biological activity. These compounds are 9β,13β-epoxy-7-abietene (**1**) and an 6,7-dehydroroyleanone (**2**) (Figure 1) and they have been screened for their cytotoxic and antioxidant activities (Gazim et al., 2014).



(1)



(2)

Figure 1: Structural formula of compounds 9β,13β-epoxy-7-abietene (**1**) and an 6,7-dehydroroyleanone (**2**) (Gazim et al., 2014).

MTT colorimetric technique has been used to estimate the cytotoxic activity of these two interesting diterpene compounds, as also to estimate the activity of whole essential oil. The biological activity was tested on three different cell lines: MDA-MB-435 (human melanoma cell), HCT-8 (human colon tumor cell line) and SF-295 (nervous system human tumor cell line). The outcome displayed high cytotoxic potential of the whole essential oil and of the isolated diterpene 9β,13β-epoxy-7-abietene with approximately 78% and 94% clear inhibition of SF-295, and 85% and 86% of HCT-8 too. The values approximately 59% for the essential oil and 45% for 9β,13β-epoxy-7-abietene on the MDA-MB-435 showed that the effect on this cell line was lower in compared with the effect on the two other cell lines. The diterpene 6,7-dehydroroyleanone did not show an effect on any of the considered lines (Table 1) (Gazim et al., 2014).

Tested compounds	MDA-MB-425	SF-295	HCT-8
***T.riparia* essential oil**	60	78	85
9β,13β-epoxy-7-abietene	45	94	86
6,7-dehydroroyleanone	3	15	12

Table 1: Cytotoxic activity (growth inhibition in %) of tested compounds on three different cell lines MDA-MB-435 (human melanoma cell), HCT-8 (human colon tumor cell line) and SF-295 (nervous system human tumor cell line).

Three methods (DPPH radical scavenging, β-carotene-linoleic acid and ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) have been used for estimation of the antioxidant activity of the essential oil of *T. riparia* and two new isolated diterpene compounds from the same essential oil. 9β,13β-epoxy-7-abietene, which showed a high cytotoxic activity, did not exhibit any antioxidant activities, while the second compound 6,7-dehydroroyleanone with no cytotoxic activity showed a higher antioxidant activity then the whole essential oil. The IC_{50} values of all three methods were much lower for the diterpene 6,7-dehydroroyleanone compared with the essential oil (especially with the DPPH method, 1500 times lower ($p\pm<0.01$)) (Gazim et al., 2014). Suhaj (2006) claimed that the oxidation is one of the most important triggers for chemical and biological damages. In comparison with quercetin (one of the most common antioxidants) 6,7-dehydroroyleanone showed a much better antioxidant activity.

Inga laurina Willd. appertain to the *Inga genus* (Leguminosae), is otherwise called Angá or Ingá Branco and is mostly disseminated in Central and South America. The essential oils were extracted from the leaves and bark of *I.laurina* collected in two different seasons (rainy and dry season) by hydrodistillation using Clevenger-type apparatus and these oils were used to prove the antimicrobial activity against aerobic and anaerobic oral bacteria. In these different seasonal times different compounds were synthesized in the plant depending on the ecological requirement. The production of some compounds (especially terpenoids) is light dependent and thus the amount of these compounds in different seasons is variable. Diterpene phytol was isolated from the essential oils of *I. lauriana*. The phytol concentration was the largest in the essential oil from the leaves collected in rainy season (circa 33%), lower concentration was found in the essential oil from stem bark collected in dry season (circa 9%) and much lower concentrations in leaves collected in dry season (circa 2%) and in stem bark collected in rainy season (circa 1%). Except

phytol other compounds found in the essential oil collected during the rainy season are mostly fatty acids, then nonacosane, (*Z*)-hex-3-en1-ol, heptacosane and esters. In comparison to the rainy season, the essential oils from the dry season had almost the same compounds, but much higher concentration of ester-compounds. The growth inhibition potential against anaerobic microorganisms of tested oils from the rainy season is represented with MIC values (minimal inhibitory concentration) from 50 to 400 μg/mL and MIC values for aerobic microorganismus from 25 to 50 μg/mL. With regard to the dry season the MIC values for the anaerobic microorganisms are between 100 and 400 μg/mL and for the aerobic microorganisms between 100 and 200 μg/mL (Table 2). The results showed that the highest antimicrobial activity exerted the essential oil from the leaves collected during the rainy season (this oil contain also the highest amount on phytol), then the essential oil from the stem bark collected during the dry season, than the oil from the leaves collected during dry season and the lowest activity was found in the oil from stem bark collected during rainy season. The sequence of the activity of these four oils is based on the concentration of the isolated diterpene and is also proportional to the phytol concentration, so that the oil with highest percentage of phytol had the highest antimicrobial activity. It is also important to say that the other compounds found in the essential oils of *I.laurina* showed separately an antimicrobial activity and so the activities of the oils may be connected either to the majority compounds in oils or to the synergistic interaction between all compounds in the mixture (Furtado et al., 2014).

		Sample/Season			
		Dry		Rainy	
MO		Stem bark	Leaves	Stem bark	Leaves
Anaerobic	*P.gingivalis*	100	100	100	50
	P.nigrescens	200	100	400	100

	F.nucleatum	>400	>400	400	200
	A.naeslundii	>400	>400	>400	400
	B-fragilis	>400	>400	>400	>400
Aerobic	*S.mutans*	200	200	25	50
	S.sanguins	200	100	50	50
	S.salivarius	200	100	25	25
	S.sobrinus	200	200	25	25
	S.mitis	100	100	50	50

Table 2: MIC values (in μg/mL) from the essential oils isolated from the leaves and bark from *I.laurina* in different seasons against anaerobic (Porphyromonas gingivalis, Prevotella nigrescens, Fusobacterium nucleatum, Actinomyces naeslundii, Bacteroides fragilis) and anaerobic (Streptococcus mutans, Streptococcus sanguinis, Streptococcus salivarius, Streptococcus sobrinus, Streptococcus mitis) MO's (Furtado et al., 2014).

Cryptomeria japonica D.Don (Taxodiaceae), in Japanese called 'sugi', is broadly a widespread tree in Taiwan. Cheng et al. (2005) and Shyur et al. (2008) proved that the leaf essential oil from *C.japonica* has excellent antifungial, insecticidal, anti-inflammatory, mosquito larvicidal and repellent effects. The leaf essential oil from *C.japonica* has been extracted by two methods, hydrodestillation (HD) and steam distillation (SD) and analyzed by GC-FID and GC/MS analyses. Cheng et al. (2012) proved that there was no major difference between oils obtained by these two extraction methods. However, Marongiu et al. (2005) proved that the oil extraction from other plants such as *Commiphora myrrha*, *Eugenia caryophyllata* and *Acorus calamus* with HD method had a much higher income compared with the oil extracted through SD method. Using the HD extractiom method the major compounds extracted from the leaf essential oil of *C.japonica* were the diterpene kaur-16-ene (23,3%), then β-elemol (18,3%), α-pinene (8,5%), γ-eudesmol (8,2%), limonene (6,5%) and α-eudesmol (5,2%). The main constituents extracted from the same oil using SD method were kaur-16-ene (29,7%) and then β-elemol (17,0%), sabinene (10,7%), γ-eudesmol (5,6%), α-pinene (5,5%) and α-eudesmol (5,2%). Based on these outcomes the conclusion is

that kaur-16-ene and β-elemol are the major compounds in the essential oil of *C.japonica.* The antitermitic activity of these compounds extracted from the essential oil of *C.japonica* against *Coptoterms formosanus* has been tested. The outcome showed that the highest antitermic effect (100% after 7 days) against *C.formosanus* had α-terpineol and β-elemol. Extracted kaur-16-ene as well other isolated substituents exhibited very low termite-mortality (value was less than 46%). Kaur-16-ene, although is one of the major compounds in the essential oil of *C.japonica,* has not shown a significant antitermitic activity against *Coptoterms formosanus* (Cheng et al., 2012).

Thuja belongs to the Cupresaceae family, includes four species *T.occidentalis 'globosa'*, *T.occidentalis 'aurea'*, *T.plicata* and *T.plicata 'gracialis'* and are widespread grown in Europe and North America. The leaf essential oils from these four different species of *Thuja* have been isolated using hydrodistillation on a Clevenger-type apparatus and than analysed by GC-FID and GC-MS. The essential oils from *T.occidentalis 'globosa'* and *T.occidentalis 'aurea'* comprises the major constituent the monoterpen ketone α-thujone (circa 50%), then diterpene beyerene further the monoterpenes sabinene, camphor, β-thujone and fenchone and another diterpene, namely rimuene (β-thujone and fenchone can be found in *T.occidentalis 'globosa'* in much higher concentration while *T.occidentalis 'aurea'* was richest of the diterpene rimuene). The same constituents are also found in the essential oils from *T.plicata* and *T.plicata 'gracialis'* with difference that *T.plicata* had a higher content of fenchone and *T.plicata 'gracialis'* of beyerene. All four essential oils have been tested for their antimicrobial activity against six Gram-positive and –negative bacteria and three pathogenic fungi (Tsiri et al., 2009). Pavela (2005) reported that the essential oil from *T.occidentalis* possessed a high antimicrobial activity and was labelled as highly toxic. The highest antimicrobial activity showed the oil from *T.plicata* with MIC values 0.50-1.25 mg/mL as also the antifungal activity with MIC values 0.87-1.12 mg/mL. *T.plicata 'gracialis'* exhibited slightly lower antimicrobial and

antifungal activity with MIC values 0.75-1.24 mg/mL and 1.15-1.45 mg/mL. Actually these activities can be assigned to the essential oil constituent α-thujone and β-thujone, which showed the strongest antimicrobial activity with MIC values of 0.09-0.83 mg/mL. The diterpene beyerene was isolated and also characterized as one of the compounds with a relative good antimicrobial and antifungal activity with MIC values of 0.87-1.37 mg/mL and 1.15-1.50 mg/mL (Tsiri et al., 2009). The single diterpene has not shown excite a major antimicrobial activity, but the high antimicrobial activity of the essential oil can be attributed to this compound in and to synergistic effects of all compounds in oil.

Leonurus japonicas Houtt. is widespread mostly in East Asia and also belongs to the Lamiaceae family. From this plant two different essential oils have been obtained. The first one, called "Yimucao" has been obtained from the herb harvested in summer and the second one "Chongweizi" from the fruits in autumn. Both oils were extracted by hydrodistillation using Clevenger type apparatus. The usage of these olis is different. "Yimucao" is used for the treatment of acute nephritis, oligouria and dispel edema, while the "Chongweizi" is more used as a tea or a drug for the reduction of high blood pressure and ameliorates memory. The antimicrobial activity of these oils and their isolated constituents has been assessed using the micro-dilution assay. "Yimucao" essential oil is very interesting because of the high content of diterpenes (32.8%), represented with phytone (19.0%) and phytol (13.8%). The second most common group in this oil are sesquiterpenes (45.4%) represented by caryophyllene oxide (11.5%) and β-caryophyllene (9.9%). The antibacterial activity of the whole "Yimucao" oil and of the isolated constituents was tested against several Gram-positive and Gram-negative bacteria. "Yimucao" essential oil showed great antimicrobial activity against Gram-positive bacteria *M. caseolyticus*, *S. aureus*, *S. epidermidis*, *methicillin-resistent S- aureus*, *E. faecium* and *E. faecalis* with MIC values from 0.032 to 0.256 mg/mL and MBC value (Minimal Bacterial Concentration Test) from 0.064 to 0.256 mg/mL. The isolated diterpene compounds have also been

separated screened for their antimicrobial activity. It has been shown that these diterpenes had exhibited an activity only against *Staphylococcus auricularis* and *Macrococcus caseolyticus* with MIC values of 0.128 mg/mL and MBC values 0.512 mg/mL. The activity of these diterpenes was lower compared with the activity of β-caryophyllene, which are responsible for the antimicrobial activity of the whole essential "Yimicao" oil and which showed an activity against all tested Gram-positive bacteria. Although the isolated diterpenes have shown lower antibacterial activity against the tested bacteria, they are very important constituents of the essential "Yimicao" oil and very likely act synergistically with the most efficient compound β-caryophyllene resulting in the desired antimicrobial activity of this oil (Xiong et al., 2013).

Nepeta belongs to the Lamiaceae family and it includes 250 different species. It can be found in Asia, Europe, North Africa and America. Interesting biological activities can be connected with several *Nepeta* species. For example, in traditional medicine it is often used as a laxative for the treatment of kidney and liver diseases, it is also used as a diuretic, antispasmolitic, tonic, antiasthmatic und etc. Essential oils are not rare in *Nepeta* genus and these have interesting antimicrobial activities. Genus *N. clarkei* grows wild in India (Himalayas of Kashmir) and its essential oil has been extracted by hydrodistillation using the Clevenger-type apparatus and analyzed with GC-MS. The main part of the essential oil of *Nepeta clarkei* are diterpenes (74.2%) represented mostly by kaur-16-ene (36.6%) and several types of oxygenated diterpenes such as pimara-7,15-dien-3-one (19.7%), phytol (1.2%), methyl isopimarate (3.0%) and methyl abietate (5.3%). The second major group of constituents of the essential oil of N. clarkei is sesquiterpenoids with 20.3% of the total oil composition (Rather and Hassan, 2011). The essential oil of *Nepeta clarkei* showed *in vitro* antimicrobial activity against six pathogenic bacteria and two fungal strains, based on the zone of inhibition and MIC values. The essential oil of *N. clarkei* showed the stongest antimicrobial activity against *Plasmodium aeruginosa* with zone inhibition of 22

mm and MIC value 0.15 µL/mL and was also effective against *E. Coli*, *S. aureus*, *P. multocida*, *P. vulgaris*, *S. marcescens*, *C. albicans* and *T. rubrum* but in much lower zone inhibition and much higher MIC values (Bisht et al., 2010). Besides the essential oil of *N. clarkei*, there are also many different essential oils isolated from other *Nepeta* species. These oils contain very diverse compounds and thus have different antimicrobial activities, e.g. are effective against different bacterial and fungal strains. The general activity for all essential oils from *Nepeta* species has been displayed with zone inhibition values from 6 to 28 mm for bacterial and 9.3 to 20.0 mm for fungal strains and MIC values 0.15 to 30.34 µL/mL for bacterial and 0.19 to 12.5 µL/mL for fungal strains. The conclusion from this is that the diterpene rich essential oil from *N. clarkei* possesses a very good antimicrobial activity.

Porcelia macrocarpa R.E. Fries (Annonaceae) can be found in the heartland of Brasil as also on the Atlantic coast. The leaf essential oil and the ripe fruits essential oil from this plant have been studied, hydrodistillated using Clevenger type apparatus and analyzed by GC-MS. The leaf essential oil from *P. macrocarpa* has shown to exhibit an antimicrobial activity, while the ripe fruit oil showed very low to no activity. The main constituents of the leaf essential oil are sesquiterpenes represented by bicyclogermacrene and germacrene D, then monoterpenes and only one diterpene. The content of these compounds in the essential oil of *P. macrocarpa* is variable. Depending on seasons the percentage of diverse compounds is lower or higher. The only diterpene found in this oil is phytol. The phytol concentration in the oil was the largest in February with 18±2%, then in January with 7.3±0.9% and March with 3.2±0.2%. In the leaf essential oil collected in August no diterpene was found. The antimicrobial activity of the essential oil was tested against four *Cryptococcus* strands (*C. neoformans* serotype A, B, C and D) and determinated with Broth Microdilution Assay (MIC values). The oil showed the best antimicrobial activity against *C. neoformans* serotype D with a dosage of 0.06 mg/mL and an inhibition rate of

85%. Against *C. neoformans* serotype A the dosage of 0.5 mg/mL exhibited the inhibition rate of 80%. Higher doses of the essential oil (around 1.0 mg/mL) are needed against *C. gattii* serotype B und C to cause an inhibition rate of 98% and 61% (Biolcati et al., 2013). The represented results are the result of whole essential oil. The diterpene phytol as part of this oil together with existing monoterpenes and sesquiterpenes plays role in the antimicrobial activity.

Campanula portenschlagiana ROEM. et SCHULT (Campanulaceae) can be found in Croatian (mostly Dalmatian mountains) as also in Bosnian and Herzegovina and on the European and World Red List, but in significantly lower amount. The volatile oil of *C. portenschlagiana* has been isolated by hydrodestilation and then analyzed by GC-FID and GC/MS. The major compounds of the volatile oil of *C. portenschlagiana* are diterpene alcohols, represented with labda-13(16),14-dien-8-ol (29.6%). Other diterpene alcohols found in this oil are phytol with 5.1%, abienol with 2.6% and feruginol with 1.7%. Besides, the above-mentioned oil contains the nonoxygenated diterpene cembrene A with 6.2%, then sesquiterpenes represented by β-caryopyllene with 3.1%, further the alkane tricosane with 3.2%, pimarinal diteprene aldehyde with 2.7% as also often oxygenated constituents represented by farnesyl acetone (2.7%), methyl stearate (2.1%) and nonanal (2.4%). The same diterpene, namely labda-13(16),14-dien-8-ol can also be found in a large amount in the volatile oil of *Anthemis werneri* L. spp. Werneri (Asteraceae) (Saroglou et al., 2006). The antimicrobial activity of the volatile oil from the *C. portenschlagiana* against several Gram-positive and Gram-negative bacteria as also against several fungi species has been analyzed and assessed by inhibition zone diameters in the disc-diffusion (DD) assay. The volatile oil exhibited a very good antimicrobial activity against the tested bacteria and fungi (Table 3). The effectiveness of the oil against Gram-positive bacteria (*Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Clostridium perfringens* and *Listeria monocytogenes*) at a concentration of 250 µg/disc can be presented with inhibition-zone diameters between 19.6 and

24.8 mm and MIC (minimal inhibitory concentration) in a range between 62.5 and 125.0 µg/ml. *Bacillus cereus* is found to be the most sensitive Gram-positive bacterium against the volatile oil of *C. portenschlagiana*. The activity against Gram-negative bacteria, namely *Escherichia coli, Klebsiella pneumonieae* and *Pseudomonas aeruginosa* was assessed in the same way and DD values found in a range between 24.7 and 28.3 mm as also MIC-values between 7.8 and 62.5 µg/ml. The highest antimicrobial activity of this volatile oil has been noticed against *P.aeruginosa*. The essential oil of *C. portenschlagiana* was also tested against several fungi species such as *Candida albicans, Penicillium* sp. and *Rhizopus stolonifer* and it showed good efficiency (DD-values in the range between 24.7 and 29.5 mm and MIC-values between 3.9 and 7.85 µg/ml). This activity was compared with the activity of 15 µg/disc of gentamicin against bacteria species and amphotenicin B against fungi species, and it was confirmed that this essential oil showed higher DD-values against all tested species. As a conclusion, it can be confirmed that the volatile oil of *C. portenschlagiana* possess a very good antimicrobial activity against the tested species. In plants the phenolic diterpenes and their derivates were found to have an important role and to show significant antimicrobial activity. In this volatile oil the main constituents are labda-13(16),14-dien-8-ol, phytol, primarinal, cembrene A and abienol, which alone or in combination with each other can exert this very good antimicrobial effectiveness against Gram-positive and Gram-negative bacteria as also against a few fungi species (Politeo et al., 2013).

Microorganisms	**Volatile oil**		**Gentamicin/Amphoternicin B**	
	DD	MIC	DD	MIC
Bacteria				
Bacillus cereus	24.8	62.5	18.2	4.0
Enterococcus faecalis	19.6	125.0	14.6	4.0
Staphylococcus aureus	21.4	125.0	23.9	1.2

Clostridium perfringens	21.8	125.0	21.7	0.5
Listeria monocytogenes	22.8	125.0	19.4	2.0
Escherichia coli	24.7	62.5	11.5	32.0
Klebsiella pneumoniae	27.9	15.6	18.2	8.0
Pseudomonas aeruginosa	28.3	7.8	9.5	64.0
Fungi				
Candida albicans	29.5	3.9	21.6	1.0
Penicillium sp.	24.7	62.5	17.3	4.0
Phizopus stolonifer	27.3	7.8	19.2	2.0

Table 3: Antimictobial activity of the volatile oil of the *C. portenschlagiana* compared with standard antibiotic gentamicin and the antifungal drug amphoternicin B. DD- Inhibition-zone diameter in [mm] obtained by disc-diffusion method at the concentration of 250 µg/disc for the volatile oil, of 15µg/disc of gentamicin and of 10 µg/disc amphoternicin B; MIC. Minimum inhibitory concentration [µg/ml] (Politeo et al., 2013).

One of the largest genuses of the Lamiaceae family is the genus *Stachys L*. which can be found in warm regions of the Mediterranean Basin, southwestern Asia and less in North America. In Italy, in the Monti Sibillini National Park, one of five endemic taxa *Stachys tymphaea* HAUSSKN. is widespread. The essential oil from *S. tymphaea* has been hydrodistilated and *in vitro* analyzed for their antimicrobial, antioxidant and cytotoxic activities using the MTT test on human cell lines, radical scavenging assays DPPH, ABTS and FRAP. This essential oil comprises 62 constituents (91.5% of all compounds). The main constituents are sesquiterpene hydrocarbons with 54.6%, represented with 30% of germacrene D and with 12.4% of (*E*)-β-farnesene. The second major constituents are the diterpene (*E*)-phytol with 11.9% and two unknown diterpenes. These two unknown diterpene (with the molecular weight 272 and 288) have both been found in the essential oil of *S. tymphaea* in percentages of 2.7 and 4.7%.

Different types of human cells (MDA-MB 231-human breast carcinoma, A375-human malignant melanoma, HCT116-human colon carcinoma cell lines) have been used for the investigation of the cytotoxic activity of the essential oil of *S. tymphaea*. For the test the MTT assay has been used. The cells were treated with increasing concentration of the essential oil for 72h. An outcome confirmed that the essential oil of *S. tymphaea* had possesses excellent cytotoxic activity against all three cell lines. The activity is in effect dependent on the concentration (concentration range stands between 0.78 µg/ml at the beginning and 200 µg/ml at the end of the third day). So, depending on the concentration of this oil, the cytotoxic effect (IC_{50} values) varies between 20.4 and 28.1 µg/ml on the MDA-MB 231 cell line, 30.5 and 38.7 µg/ml on the HCT116 cell line and 20.8 and 31.0 µg/ml on the A375 cell line. The cytotoxic activity was compared with cisplatin which showed IC50 values around 3.0, 2.5 and 0.4 µg/ml on the cell lines MDA-MB 231, HCT116 and A375. Two often major constituents, namely germacrane D and (*E*)-phytol, in the essential oil of the *S. tymphaea* have been isolated and separately analyzed for their cytotoxic activities. Germacrane D showed an activity on human breast (MDA-MB 231 and MCF-7), ductal (Hs 578T) and hepatocellular (Hep G2) carcinoma and (*E*)-Phytol against KB, MCF-7, A549 and CasKi cell lines. Based on the presence of these two cytotoxic active compounds and their synergistic effect the activity of the volatile oil of *S. tymphaea* can be explained (Venditti et al., 2014).

Three different tests have been used to test the antioxidant activity of the essential oil of the *S. tymphaea* $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and FRAP assay and all three were compared with *Trolox*®. The results showed that the volatile oil exhibited a very good antioxidant activity using all three test methods (IC_{50} values using first two methods, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ were about 71- or 28-fold higher than the values of *Trolox*®; the last method FRAP is the most important and proves that the oil has a big attraction for iron binding and therefore exhibit the major antioxidant activity). This essential was also tested for its antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* in comparison to the

antibiotic ciprofloxacin. No antimicrobial activity has been proved for this oil and no other investigations have been conducted (Venditti et al., 2014).

Polylepis besseri belongs to the Rosaceae family and is specific because of its ability to grow in unfavorable regions as also of its erosion-protective role of the soil. The essential oil from aerial parts of *P. besseri* has been isolated by hydrodistillation using Clevenger-apparatus and then analyzed by GC and GC/MS, which showed that the essential oil of *P. besseri* possesses a high percentage of diterpenes (54%) and the following oxygenated constituents: 3.8% of α-cadinol and 2.6% of abietol. Interestingly, this oil showed a low percentage of monoterpenes with 11.7% and sesquiterpenes with 14.1%. Antimicrobial and insecticidal activities of this essential oil have been studied. The antimicrobial activity was tested against *Shigella flexneri*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*, but the effectiveness of the oil against these four bacteria species was very low. Significantly better activity of the essential oil was found against *Aedes aegypti-larvae* and *Triatoma infestans* 4^{th}-instar nymphs. Normally, the insecticidal activity of the oil is attributed to the presence of mono- and sesquiterpenes. Because of the low content of these constituents and remarkably high content of diterpenes, the insecticidal activity of the essential oil of *P. besseri* can be assigned to the presence of the diterpenes (Loayza et al., 2002).

Pinus peuce Griseb. (Pinaceae), named a Macedonian pine, is widespread mostly throughout the southern and western regions of R. Macedonia, as well as in Serbia, Greece, Bulgaria and Albania. From ancient times the needle essential oil has been used in traditional medicine for the treatment of various respiratory infections, such as bronchitis, asthma and emphysema. The essential oils from the needles with or without branches have been isolated by hydrodistillation using Clevenger-apparatus and then analyzed by GC, GC-FID and GC/MS. Among the

107 different constituents, 40 monoterpenes and 37 sesquiterpenes have been found as the major compounds, as well as 9 diterpenes and 21 other constituents (Karapandzova et al., 2010). The antimicrobial activity of the essential oil of *P. peuce* growing on the Pelister Mountain was tested against 13 different bacteria strains (Gram-positive [*S. aureus, S. epidermidis, S. pneumoniae, S. agalactiae, S. pyogenes and Enterococcus*] and Gram-negative bacteria [*Acinetobacter spp, E. coli, S. enteritidis, K. pneumoniae, P. aeruginosa, H. influenzae and P. mirabilis*]) and one strain of *Candida albicans* A, using Hole-plate diffusion and broth dilution methods. The needle essential oil of *P. peuce* showed antimicrobial activity against *S. pneumoniae, S. aureus, Acinetobacter spp*. with MIC-values of 31.25 µl/ml and a MIC-value of 62.5 µl/ml for *S. epidermidis*. Not any of diterpene constituents have been screened for their antimicrobial activity, but the high antimicrobial activity of the essential oil can be attributed to synergistic effects of all compounds in the oil (Karapandzova et al., 2011).

Some other volatile diterpenoids identified in plant essential oils

Stemodia trifoliata (Link.) Reichb. belongs to the Scrophulariaceae family and includes around 40 species that are widespread in Australia, America, Africa and Asia. The leaf essential oil of *S. trifoliata* was hydrodistillated using Clevenger-apparatus and analyzed by GC-MS and GC-FID. The main constituents of this essential oil are sesquiterpenes with 33.0-48.0% (represented mainly by β-caryophyllene (9.4-15.4%) and caryophyllene oxide (6.2-9.0%)) as also diterpenes with 44.6-51.2% (represented mainly by 6α-hydroxymanoyl oxide (25.1-29.7) and 6α-acetoxymanoyl oxide (13.9-23-2%)). Although the essential oil of *S. trifoliata* possesses a high percentage on volatile diterpenoids, no biological activity of this oil was studied and proved so far (Da Silva et al.,2009).

Mikania plants are a part of Asteraceae family which comprises 450 different species and about 150 of them can be found throughout Brazil. The aerial part essential oils of *M. hagei* and *M. jeffreyi* and the stems and leaves essential oil of *M. hookeriana* were hydrodistillated using Clevenger-apparatus and analyzed by GC-MS. The main constituent of the essential oil of *M. hagei* is represented with β-selinene (45.8%). α-Pinene (23.4%), germacrene D (11.2%) and kaurenal (6.3%) are major constituents of *M. hookeriana* essential oil, and limonene, α- and β-pinene those of *M. jeffreyi* essential oil. The diterpene constituents are found only in traces in all three essential oils. Kaurane diterpenes with 9.5% are the only identified diterpenes from the essential oil of *M. hookeriana* of all three oils. These oils performed so far no biological activities (Reis et al., 2008).

The twig essential oil of *Juniperus cedrus* Webb. & Berth. (Cupressaceae) which is growing in ecological areas of Madeira has been studied, isolated by hydrodistillation using Clevenger-type apparatus and analyzed by GC and GC/MS. The result showed that the oil consists mostly of monoterpene hydrocarbons with 53.1-87.8% (α-pinene, limonene, Δ-3-carene and β-myrcene, and oxygenated constituents, namely α-terpinyl acetate). The second main group are sesquiterpenes (4.1-22.3%) represented with *E*-β-caryophyllene as the major constituent. Small amounts of diterpenoides are found in the twig essential oil of *J. cedrus*, namely sandracopimaradiene, isoabienol and trans-totarol with 0.1-6.1%, 1.5-1.3% and 0.4-2.2%. No biological activity of the essential oil of *J. cedrus* has been studied so far (Cavaleiro et al., 2002).

In Anaimalai hills of India, the rare type of plant can be found, called *Pogostemon hirsutus* Benth. (Lamiaceae). Its leaf essential oil has been extracted and analyzed by GC-FID and GC-MS. This oil showed a high percentage of monoterpenes and diterpenes, 42.6% of the constituents belong to abietane-type diterpenes, namely abietatriene, dehydroabietal and dehydroabietol with 16.3%,

3.5% and 21.0%. The biological function of the essential oil of *P. hirsutus* still has not been tested so far (Murugan et al., 2013).

The seeds essential oil of Korean fir, otherwise called *Abies koreana* (Pinaceae), has been hydrodistillated and analyzed by GC-FID, GC-MS and NMR methods. As the main constituents of this oil monoterpenes with a significantly high percentage of 70-95% and oxygenated monoterpenes with smaller amount of 1-20% are found. The remaining 2-8% belong to hydrocarbons and oxygenated forms of sesquiterpenes and diterpenes. The presence of diterpenes in the essential oil of *A. koreana* has been proved, but the biological activity neither of the essential oil or of the diterpenes has been tested (Wais-Bonikowska et al., 2013).

Conclusion

Nature is a big source of substances with potential biological activities. From ancient times plants have been used and studied in different treatments and they opened absolutely new therapeutically accesses. Plants, microorganisms and marine organisms and their secondary metabolites have been used as a basis in traditional treatment of human diseases and they have are also an important source as lead substances for the development of new therapeutic drugs. It is interesting that almost 50% of the drugs introduced into the market, observed in the last years, are actually directly or indirectly nature products. Also recently, many types of natural products isolated from the plants have been marked as profitable as therapeutic drugs or nutraceuticals, and they have been published on the markets all over the world.

Diterpenoids are high lipophilic structures which can be found in resins of the plants. This group of compounds remained in the shadow of investigation of mono- and sesquiterpene compounds, because the latter are much more common in nature and have broader spectrum of biological activities (Wynn and Fougère, 2007). Only after discovery of the anticancer drug Taxol® (Paclitaxel), diterpenoids have fallen into the focus of nowadays researches (Heing and Jennewein, 2009). The point of this work was the review on the biological activities of a special group of diterpenoids, namely the volatile ones, isolated from the essential oils from different plant families, such as Asteraceae and Lamiaceae as the richest two (Tirapelli et al., 2008; Lanzotti, 2013; Bohlmann and Keeling, 2008). What is important to emphasize is the fact, that in the nature, the number of volatile and at the same time bio-active diterpenoids is relatively small. These volatile diterpenoids can be found in essential oils, but the amount of these constituents of essential oils is much lower in comparison with other terpene compounds (mono- and sesquiterpenes). There are many diterpenoids which show

excellent biological activities, the best example for that is Taxol® with its cytotoxic activity (Heing and Jennewein, 2009), but Taxol® is not volatile and therefore cannot be included as relevant in this paper. From the essential oils of *T. riparia*, *I. laurina*, *C. japonica*, different *Thuja* and *Napeta* species, *P. macrocarpa*, *L. japonicas* and *C. portenschlagiana* many types of volatile diterpenoids were isolated, such as phytol and phyton, kaurane-type diterpenes, abietane-type diterpenes, rimuene and beyerene, primarane, labdane and etc. All these compounds were tested as well in essential oils and as separately isolated and tested for their biological activities. In comparison to the variety of biological activities which are generally attributed to the essential oils, the single volatile diterpenoids showed to exhibit prevailing antimicrobial, antioxidant and cytotoxic activities. To each of these isolated diterpenes one or more activities can be credited. Thus, 13β-epoxy-7-abietene from *T. riparia*, 6,7-dehydroroyleanone and (*E*)-phytol and two unknown diterpenes from *S. tymphea* showed to have cytotoxic and antioxidant activities. Other isolated diterpenes like kaur-16-ene, beyerene, rimuene, pimar-7,15-dien-3-ene of different essential oils, such as from *C. japonica, Thuja* and *Nepeta* species and *P. macrocarpa* have shown to possess antimicrobial activity against different Gram-positive and Gram-negative bacteria as also against some fungi species (Gazim et al., 2014; Furtado et al., 2014; Cheng et al., 2012; Tsiri et al., 2009; Xiong et al., 2013; Bisht et al., 2010; Biolcati et al., 2013; Politeo et al., 2013; Venditti et al., 2014). Not all of the isolated diterpenoids have separately shown to have a strong or any biological activity at all, some of them exert the biological activity only in synergism with other in essential oil constituents. Although they are rare in nature and are there in essential oils in small amounts, they presence plays an important role and contributes to a variety of biological activities of these oils (Tisserand and Young, 2014; Bohlmann and Keeling, 2008).

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Curriculum vitae

Name:	Dubravka Stanetic
Birth Place:	Banja Luka, Bosnian and Herzegovina
Date of Birth:	29.09.1989
Citizenship:	Bosnian and Herzegovina
Family status:	Single

Education:

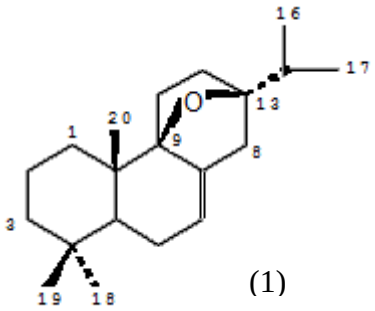
1997-2004 Primary school „Vuk Stefanovic Karadzic", Banja Luka, Bosnia and Herzegovina

2004-2008 High school „Gymnasium Banja Luka", Banja Luka, Bosnia and Herzegovina

2008-2014 Pharmacy studies, University of Vienna, Austria

Tabellen und Formeln:

Tetradenia riparia (Hochstetter) Lamiaceae



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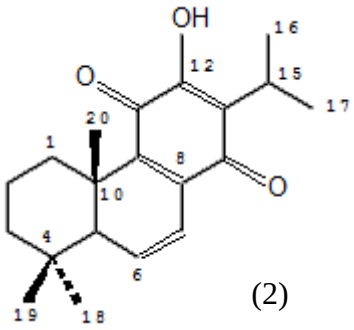

(2)

Figure 1: Structural formula of compounds 9β,13β-epoxy-7-abietene (**1**) and an 6,7-dehydroroyleanone (**2**) (Gazim et al., 2014).

Tested compounds	MDA-MB-425	SF-295	HCT-8
***T.riparia* essential oil**	60	78	85
9β,13β-epoxy-7-abietene	45	94	86
6,7-dehydroroyleanone	3	15	12

Table 1: Cytotoxic activity (growth inhibition in %) of tested compounds on three different cell lines MDA-MB-435 (human melanoma cell), HCT-8 (human colon tumor cell line) and SF-295 (nervous system human tumor cell line).

Inga laurina Willd. Leguminosae

		Sample/Season			
		Dry		Rainy	
MO		Stem bark	Leaves	Stem bark	Leaves

Anaerobic	*P.gingivalis*	100	100	100	50
	P.nigrescens	200	100	400	100
	F.nucleatum	>400	>400	400	200
	A.naeslundii	>400	>400	>400	400
	B-fragilis	>400	>400	>400	>400
Aerobic	*S.mutans*	200	200	25	50
	S.sanguins	200	100	50	50
	S.salivarius	200	100	25	25
	S.sobrinus	200	200	25	25
	S.mitis	100	100	50	50

Table 2: MIC values (in µg/mL) from the essential oils isolated from the leaves and bark from *I.laurina* in different seasons against anaerobic (Porphyromonas gingivalis, Prevotella nigrescens, Fusobacterium nucleatum, Actinomyces naeslundii, Bacteroides fragilis) and anaerobic (Streptococcus mutans, Streptococcus sanguinis, Streptococcus salivarius, Streptococcus sobrinus, Streptococcus mitis) MO's (Furtado et al., 2014).

Campanula portenschlagiana ROEM. et SCHULT (Campanulaceae)

Microorganisms	**Volatile oil**		**Gentamicin/Amphoternicin B**	
	DD	MIC	DD	MIC
Bacteria				
Bacillus cereus	24.8	62.5	18.2	4.0
Enterococcus faecalis	19.6	125.0	14.6	4.0
Staphylococcus aureus	21.4	125.0	23.9	1.2
Clostridium perfringens	21.8	125.0	21.7	0.5
Listeria monocytogenes	22.8	125.0	19.4	2.0

Escherichia coli	24.7	62.5	11.5	32.0
Klebsiella pneumoniae	27.9	15.6	18.2	8.0
Pseudomonas aeruginosa	28.3	7.8	9.5	64.0
Fungi				
Candida albicans	29.5	3.9	21.6	1.0
Penicillium sp.	24.7	62.5	17.3	4.0
Phizopus stolonifer	27.3	7.8	19.2	2.0

Table 3: Antimictobial activity of the volatile oil of the *C. portenschlagiana* compared with standard antibiotic gentamicin and the antifungal drug amphoternicin B. DD- Inhibition-zone diameter in [mm] obtained by disc-diffusion method at the concentration of 250 µg/disc for the volatile oil, of 15µg/disc of gentamicin and of 10 µg/disc amphoternicin B; MIC. Minimum inhibitory concentration [µg/ml] (Politeo et al., 2013).