Medicinal and Aromatic Plant Science and Biotechnology ©2011 Global Science Books

Hypericum Species as Sources of Valuable Essential Oils

Alessandra Bertoli[1*] • Cüneyt Çırak[2] • Jaime A. Teixeira da Silva[3]

[1] Department of Pharmaceutical Sciences, University of Pisa, via Bonanno, 33 56126 Pisa, Italy
[2] Department of Medicinal and Aromatic Plants Technology and Cultivation, University of Ondokuz Mayis,Vocational High School of Bafra, Bafra, Samsun, Turkey
[3] Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Ikenobe, 2393, Kagawa-ken, 761-0795, Japan

Corresponding author: * **bertoli@farm.unipi.it**

ABSTRACT

Since ancient times, the essential oils (EOs) of many aromatic plants have been used as bioactive ingredients in drug, food and cosmetic formulations all over the world. Significant biological properties have also been attributed to *Hypericum* EOs. *Hypericum* is a genus of about 450 species in the *Guttiferae* family, formerly often treated separately in their own *Hypericaceae* family. Despite the large number of species, only *Hypericum perforatum* has been studied in depth by the pharmaceutical industry to control the content of its well known bioactive constituents hypericins, hyperforins and flavonoids in the flowering aerial parts. As a consequence, efficient commercial products based on the hydroalcoholic extracts or oil of *H. perforatum* are already commercially available as antidepressive agents or to treat skin burns. However, only a few studies have been performed on the EO constituents of *H. perforatum* and other members of this species. In the last few years some papers have been published on *Hypericum* EOs, but the number of these studies is still limited and the results are not homogenous enough to justify the use of *Hypericum* EOs as phytomedicines or dietary supplements. The present study is an overview of the production of EOs from *Hypericum* species. A summary of the typical EO constituents found in wild or cultivated plants, as well as their biological activities, is provided to point out the most significant *Hypericum* species, valuable as potential sources of EOs and bioactive ingredients.

Keywords: antibacterial, antifungal, chemotaxonomy, essential oil, *Hypericum*, terpenes, terpenoids
Abbreviations: EO, essential oil; **GC-MS**, gas chromatography-mass spectrometry; **IPP**, isopenthenyl pyrophosphate; **ISO**, International Standard Organization; **SFE**, supercritical fluid extraction; **WHO**, World Health Organization

CONTENTS

INTRODUCTION

The essential oils (EOs) contain a large variety of volatile secondary metabolites such as terpenes, terpenoids, phenolic and aliphatic derivatives, generally characterized by a strong odour. In general, EOs were previously well known as important medicinal remedies (Burt 2004) and they were also used for their fragrance and in the preservation of food. Nowadays, these characteristics have been confirmed and much more is known about their biological mechanisms, e.g. as antimicrobial or potential anticancer agents.

A significant amount of information is currently available on the role of EOs in plant-plant, plant-animal or plant-insect interactions (Bakkali *et al.* 2008). In fact, the volatile constituents of aromatic plants are at present regarded not only as important bioactive ingredients, but also as target metabolites emitted by plants to balance their status in their natural habitat or in the agronomic conditions of fixed protocols (Guedes *et al.* 2004; Schwob *et al.* 2004; Bakkali *et al.* 2008).

Herbal medicines and dietary supplements have been increasing on world commercial market over the past few years. In the EU, Germany and France are indisputably in the lead in over-the-counter sales, and they have also had noteworthy markets for prescription of herbal preparations (Harrison 2004; Barnes 2007; EMA/MB/203131/2009). The success of the genus *Hypericum* is related especially to *Hypericum perforatum* L. (Hypericaceae, St. John's wort),

Received: 22 February, 2010. Accepted: 28 October, 2010.

Invited Review

used for the treatment of various depressive disorders (Butterweck 1998; Barnes *et al.* 2001; Butterweck *et al.* 2003; Roz *et al.* 2004; Shelton *et al.* 2009). The hydro-alcoholic extracts of *H. perforatum* and other species of this genus have been investigated as antiviral, antioxidant, antimicrobial, antifungal, anxiolytic and anticonvulsant agents (Wood *et al.* 1990; Vandenbogaerde *et al.* 2000; Couladis *et al.* 2002; Cakir *et al.* 2004, 2005; Skalkos *et al.* 2005; Ravindran *et al.* 2009). All these actions were attributed to a mixture of flavonoids, xanthones, tannins, phloroglucinols (hyperforin and adhyperforin) and naphtodianthrones (hypericin, protopseudohypericin, pseudohypericin and protohypericin) (Kitanov 2001; Avato *et al.* 2005). It has been reported that the volatile constituents take part in these types of activities. To better understand the biological activities of their EOs, several *Hypericum* species have been investigated in the past few years (Gudzic *et al.* 2002; Couladis *et al.* 2003; Cakir *et al.* 2005; Saraglou *et al.* 2007; Williams *et al.* 2007; Maggi *et al.* 2010).

The genus *Hypericum* belongs to the Hypericaceae (Clucicaceae) family and encompasses approximately 460 species accommodated in 36 sections (Robson 1968, 1977). Inter- and intraspecific variations in the EO composition of many species of this genus were previously reported, and depending on genetic and environmental factors, seasonal variation, plant organs and analytical methods used (Couladis *et al.* 2001; Bertoli *et al.* 2003; Schwob *et al.* 2004; Petrakis *et al.* 2005; Smelcerovic *et al.* 2007; Nogueira *et al.* 2008; Maggi *et al.* 2010).

This present work is a review of the studies undertaken on the EOs of *Hypericum* species from all over the world in order to evaluate the importance of this genus as a source of bioactive EOs. Furthermore, recent attempts to establish *in vitro Hypericum* cultures to produce volatile secondary metabolites has also been taken into consideration as some *Hypericum* species are very rare or are near extinction (Çirak 2007; Yee and Dirnbock 2009).

ESSENTIAL OILS: GENERAL

General definition, extraction and analytical methods

At present, approximately 3000 EOs are known, 300 of which are commercially important not only for pharmaceutical purposes, but also for agronomic, food, sanitary, cosmetic and perfume industries. EOs are volatile complex mixtures characterized by a strong odour and they contain volatile secondary metabolites biosynthesized by aromatic plants. They are liquid, transparent and rarely coloured, soluble in lipid or organic solvents with generally lower density than water. For their liquid nature at room temperature, EOs are called oils but they should not be confused with fixed oils which are composed of a naturally occurring mixture of lipids and are not volatile. Therefore, EOs differ entirely in both chemical and physical properties from fixed oils (Bruneton 2000). The EOs can be synthesized by all plant organs and are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes. EOs in plant materials can be categorized as superficial or subcutaneous oils (Denny *et al.* 1991; Hallahan *et al.* 2000). The superficial oils are contained in glandular hairs on the surface, which are generally broken by slight pressure to release the oil. Glandular hairs might regenerate and re-accumulate oils, or they may degenerate after a single act of excretion (Bruneton 2000). On the other hand, the subcutaneous oils are contained in some internal structures such as oil cells, ducts or secretory cavities. There are several methods to extract EOs, mainly low or high-pressure distillation employing boiling water or hot steam, but also by carbon dioxide (supercritical fluid extraction, SFE) or microwaves. Steam distillation, cold expression (especially for *Citrus*), together with SFE, are used for the industrial production of EOs.

Hydrodistillation by a Clevenger apparatus is recommended by several Pharmacopeias for the official quality control of raw plant material in order to evaluate EO yield and the typical volatile constituents. For perfumery formulations, extraction with lipophilic solvents and sometimes with supercritical carbon dioxide is favoured. It is important to point out that the chemical profile of the commercial EO can vary in quality, quantity and in composition according to the extraction method as well as climate, soil composition, plant organ, age and vegetative cycle stage (Figueiredo *et al.* 1997; Perry *et al.* 1999; Couladis *et al.* 2001; Masotti *et al.* 2003; Yee *et al.* 2009; Zhang *et al.* 2009). Thus, in order to obtain standardized EOs, they have to be extracted under the same conditions from the same organ of the plant, which has been growing on the same soil, under the same climate and has been picked in the same season. Nowadays, most EOs are studied by gas chromatography and mass spectrometry (GC-MS) techniques and various standardized procedures exist (Pharmacopoeia, ISO, WHO) to perform the quality control of commercial EOs (Massada 1976; Jennings 1980; Adams 2001).

Essential oils and composition

EOs are very complex plant products, which can contain about 20-100 components or more at quite different concentrations. A few major components generally characterize them by fairly high concentrations (20-70%) compared to other components present only in trace amounts. The EO components include two main groups with distinct biosynthetic origins.The main group is composed of terpenes, terpenoids and other aromatic and aliphatic constituents, all characterized by a low molecular weight. The biosynthetic pathways of terpenes and phenylpropanoid derivatives are generally separated in plants. Structurally and functionally different classes of terpenes are made from the combination of several 5-carbon-base units (C_5, isoprenes).

The biosynthesis of the terpenes includes different types of processes:

- synthesis of the isopentenyl diphosphate (IPP) precursor;
- repetitive addition of IPPs to form the prenyldiphosphate precursors of terpenes;
- modification of the allylic prenyldiphosphate by terpene specific synthetases to form the terpene skeleton;
- secondary enzymatic modification (redox reaction) of the skeleton to attribute functional properties to the different terpenes.

The most commonly found terpenes in the EOs are the monoterpenes (C_{10}) and sesquiterpenes (C_{15}), but hemiterpenes (C_5) and diterpenes (C_{20}) may also exist.

Furthermore, particular terpenes containing oxygen are called terpenoids.

Monoterpenes are formed from the coupling of two isoprene units (C_{10}) and they show a great variety of structures with different functions: hydrocarbons (acyclic, monocyclic, bicyclic), alcohols (acyclic, monocyclic, bicyclic), aldehydes (acyclic), ketones (acyclic, monocyclic, bicyclic), esters (acyclic, monocyclic, bicyclic), ethers, and phenols. Monoterpenes normally have a low boiling point and are water-insoluble.

Furthermore, these molecules can be optically active and the two enantiomers are often useful to distinguish among different species or habitats. The predominance of monoterpene (-)-enantiomers in the emission of some European *Pinus* and *Abies* species was explained by Persson (1990, 1993), who claimed their specific action as insect aggregation agents for breeding purposes. It is believed that the enantiomeric production of monoterpenes could also be used by plants to prevent the attack of herbivores on leaves, trunks and twigs. In general, aromatic plants can exploit either different toxicities of the (+)- and (-)-enantiomers towards ants, bugs and beetles or might use specific enantiomers to reveal the presence of a herbivore to its natural enemies who can then assist the plant indirectly (Wink 2003).

The sesquiterpenes (C_{15}) are formed from the assembly

of three isoprene units and the extension of the chain increases the possibility of cyclisations with a great variety of more or less derivatized chemical structures: hydrocarbons, alcohols, ketones, epoxide.

Terpenoids are synthesized from acetate units and they share their origins with fatty acids. They differ from fatty acids in that they contain extensive branching and are cyclized.

The other chemical class of typical constituents in the EOs are some aromatic compounds which are derived from phenylpropane and occur less frequently than all the terpenes mentioned above (Yazaki *et al.* 2009). The most common aromatic compounds are aldehydes such as cinnamaldehyde, alcohols (e.g. cinnamic alcohol), various phenols, methoxy and methylene dioxy derivatives. Nitrogenous or sulphur components such as glucosinolates or isothiocyanate derivatives can occur in some specific EOs. However, these last classes of compounds are really less frequent in comparison with mono- and sesquiterpenes and their derivatives.

The importance of EOs as bioactive phytocomplexes

The World Health Organization (WHO) noted that the majority of the world's population depends on traditional medicine for primary healthcare. Since ancient times, EOs have been widely used in popular medicines around the world not only as frangrances or food preservatives, but also for very important biological activities (e.g., antibacterial, antifungal, antiviral, antinflammatory) (Deans and Ritchie 1987). More recently, they have been screened as antioxidant ingredients in dietary supplements as well as potential complementary remedies in cancer treatment. However, the EOs have still been deeply investigated especially for their action against bacteria (Kim *et al.* 1995; Helander *et al.* 1998; Smith-Palmer *et al.* 2001; Seenivasan *et al.* 2006; Bakkali *et al.* 2008; Lo Cantore *et al.* 2009; Tyagi and Malik 2010) and fungi (Vijaya *et al.* 2001; Pawar *et al.* 2006; Cheng *et al.* 2008; Singh *et al.* 2008; Tatsadjieu *et al.* 2009; Prakash *et al.* 2010).

1. Antibacterial activity

It is well known that the antimicrobial activity of aromatic plant extracts used as flavouring agents in foods is due to their EO fraction (Conner 1993). In the modern food industry mild processes are applied in order to obtain safe products which have a natural or "green" image (Burt 2004). Under these considerations, the antimicrobial effects of plant EOs can be regarded as natural agents to reduce the proliferation of food-borne pathogens. Plant EOs and their components have broad-spectrum activity against both Gram-negative and Gram-positive food-borne pathogens (Burt 2004; Toker *et al.* 2006; Seenivasan *et al.* 2006; Bakkali *et al.* 2008; Ladeira *et al.* 2009; Nederostova *et al*. 2009; Lo Cantore *et al.* 2009; Tyagi and Malik 2010).

However, most studies on antimicrobial action of plant extracts have been conducted *in vitro* and little information exists regarding the antimicrobial activity of commercially available plant EOs used as flavouring agents in confectionery products (Gould 1996). The most interesting area of EO application is the inhibition and reduction of the most serious food-borne pathogens such as *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* (Burt 2004). For example, Salmonellosis is a growing concern to the chocolate industry as *Salmonella typhimurium* infections caused by contaminated chocolate products. The detection of the above-mentioned food-borne pathogens in chocolate and cocoa products as well as the increasing consumer demand for effective, safe, and natural products underlines the importance of plant EOs useful in chocolate confectionery (Kapperud *et al.* 1990; Pearson *et al.* 1990; Kotzekidou *et al.* 2008). Unfortunately, results obtained by different biological procedures using the same EOs are not always comparable. In the use and standardization of natural preservative such as EOs, it is also important to create reproducible and comparable antimicrobial data between *in vitro* studies and a real food system (Rios *et al.* 1988; Smith-Palmer *et al.* 1998; Burt 2004; Lo Cantore *et al.* 2009; Tyagi and Malik 2010). In fact, comparisons of microbiological studies that have used different methodologies are difficult, especially regarding minimal inhibitory concentrations (MICs).

The need for uniform and reliable procedures when testing biological activity has already been emphasized (Zaika 1988; Smith and Navilliat 1997; Sharm and Bhat 2009; Cabreira and Prieto 2010). Another important aspect in the increased interest in antimicrobial properties of plant EOs is the spread of conventional drug-resistant pathogens which is one of the most serious problems in the successful treatment with conventional antibacterial agents (Inouye 1983; Deans 1987; Brul and Coote 1999; Burt 2004; Apajalahti and Kettunen 2006; Isabel and Santos 2009). Nowdays, the alarming rate at which the human pathogens like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Candida albicans*, *Cryptococcus neoformans* evolve themselves as multidrug resistant "superbugs" towards the newly generated classes of new antibiotics and anti-fungal drugs, requests continuously the exploration of new chemical sources or biodiversity to combat this problem of infections caused by these superbugs. EOs could be regarded as a cheap and effec-tive alternative to antibiotics and potentially used to combat drug-resistant hospital due to superbugs (Carson *et al.* 1998; Bowler *et al.* 2001; Caplin *et al.* 2009; Hass 2010; Quave *et al.* 2010).

Therefore, it is reasonable to study the EO profiles of known and unknown species in order to investigate the large variety of volatile compounds produced by aromatic plants with specific or general antimicrobial activity.

2. Antifungal activity

Knobloch (1989) reported that 60% of EO derivatives examined to date were inhibitory for a large variety of fungi with important applications in the food and medicine industries. Eugenol, thymol, and carvacrol are well known phenolic volatile constituents in clove, thyme, and oregano EOs which have been demonstrated to have an inhibitory activity not only against bacteria but also fungi (Mahmoud 1994; Manohar *et al.* 2001; Fujisawa *et al.* 2002; Hwan, *et al.* 2005; Cheng *et al.* 2008; Chen *et al.* 2009; Devi *et al.* 2010). The mechanism of phenol toxicity towards fungi is based on the inhibition of fungal enzymes, which contain SH groups in their active sites (Cowan 1999).

Regarding the structure-activity relationship of unsaturated aldehydes, another typical EO constituent, the CHO group, when conjugated with a carbon to carbon double bond (C=C), was found to be responsible for their antifungal activity (Moleyar and Narasimham 1986).

There has been growing interest on research of the possible use of EOs which can be relatively less damaging for pest and disease control in agriculture.

Fusarium oxysporum (vascular wilt), *Sclerotinia sclerotiorum* (water soaked spot), *Fusarium solani* (fruit rot) and *Phytophthora capsici* (fruit rot) cause severe damage to agriculture at pre- and post-harvest stages (Thompson *et al.* 1989; Mishra and Dubey 1994; Arras and Usai 2001; Mathew *et al.* 2010). Many other studies have also been performed on the evaluation of EO toxicity against fungi which generally cause deterioration of stored food packaging (Gould 1996; Costa 2000; Nielsen and Rios 2000; Ozcan and Boyraz 2000; Rodriguez *et al.* 2008; Reddy *et al.* 2010). Despite the wide use and familiarity of EOs, a better understanding of their biological action and side effects for new applications in human health, agriculture and environmental preservation is necessary. Some EOs may constitute effective alternatives or complements to synthetic compounds of conventional medicines as they do not show the same secondary effects (Carson and Riley 2003). EOs are

generally characterized by two or three major components, but the contituents present in traces may greatly influence the whole biological EO activity (Franzios *et al.* 1997; Cox *et al.* 2000; Santana-Rios *et al.* 2001; Cal 2006). The mechanism of action by terpene is not fully understood, but it is speculated to involve membrane disruption by lipophilic compounds. Considering all the important biological activities and applications of EOs, increased studies on their safety are urgently required. Depending on type and concentration, they exhibit cytotoxic effects on living cells, but they are usually not genotoxic (Hartman and Shankel 1990). EOs, due to their capacity to interfere with mitochondrial functions, may add prooxidant effects and thus become genuine antitumor agents (Atsumi *et al.* 2005; Zu *et al.* 2010). Many radical-producing agents are in fact used in antitumor treatments. In the case of EOs, radical production could be very well controlled and targeted without presenting, by itself, any toxic or mutagenic side-effects to healthy tissues (Yoo *et al.* 2005; Sharafi *et al.* 2010). Furthermore, recent studies showed that EOs may be included in vectorized liposomes (Fujisawa *et al*. 2002; Sinico *et al.* 2005) which would allow bettering definition of effective drug formulations. Thus, EOs can emerge from traditional to modern phytomedicine.

HYPERICUM SPECIES AND VOLATILE CONSTITUENTS

Typical chemical classes and structures

Even though commercial hydroalcoholic extracts or oil of *H. perforatum* have already been investigated in depth for naphthodianthrones (e.g., hypericin), phloroglucinols (e.g., hyperforin), xanthones, flavonoids, and biflavonoids (Tatsis *et al.* 2007; Wang *et al.* 2010), its EO is not so well studied. The composition of EOs of other *Hypericum* species native of different countries have only been investigated in the last few years, but a limited number of studies have been carried out on the different phenological stages or variations in the agronomic production of *Hypericum* plants. The investigated *Hypericum* EOs were generally obtained by hydrodistillation of air-dried aerial parts, collecting during the flowering stage which is considered balsamic period (Bruneton 2000).

In addition, the establishment of *in vitro* plant cultures specifically for production of hypericins and hyperforins or flavonoids, but few attempts were carried out to enhance EO production (**Tables 1, 2**) by *in vitro* biotechnologies. Perhaps, the low amount of EOs reported for *Hypericum* species could explain why there are only a few studies on volatile chemistry of this genus in comparison with the investigations on hypericins, hyperforins, and flavonoids. However, owing to the presence of significant volatile constituents in *Hypericum* EOs, an increasing interest towards EO production from several *Hypericum* species has arisen in the last few years (**Tables 1, 2**). In the present paper, the GC-MS profiles of the EOs extracted from *H. perforatum* (**Table 1**) and other species of the genus *Hypericum* (**Table 2**) are considered to give a state-of-the-art perspective on the research of volatile secondary metabolite production in this genus. The standardization of aromatic profiles of wild or cultivated plants of *Hypericum* species (parental plants) is of primary importance for the standardization of *in vitro* raw material riched in EOs or specific volatile constituents.

The different chemical classes of volatile constituents, which have been detected in various *Hypericum* species, are summarized in **Table 3**.

Hypericum perforatum

The most commercially important member of this genus, *H. perforatum* L., is already used as a valuable medicinal plant for treating nervous exhaustion, depression, and seasonal affective disorders (Bombardelli and Morazzoni 1995; Linde and Ramirez 1996; Obach 2000; Fegert *et al.* 2006; Canning *et al.* 2010; Linde 2010).

A wide spectrum of secondary active metabolites have been identified in its flowering aerial parts: naphthodianthrones (Kitanov *et al*. 2001; Radusiene and Bagdonaite 2002) acylphloroglucinols (Verotta *et al.* 2000), xanthones and flavonoids (Brolis *et al.* 1998; Radusiene *et al.* 2004) and tannins (Barnes *et al.* 2001). *H. perforatum* has been used in treating mild to moderate depression, as well as anxiety and insomnia (Bombardelli and Morazzoni 1995; Schulz *et al.* 1998). Taking all of these important pharmacological activities into consideration, phytochemical investigations were carried on hypericin and hyperforin contents, in particular, while a few studies have been published on the EO composition of *H. perforatum*.

As a very large number of volatile constituents have generally been detected, it seems that numerous metabolic pathways are elicited in *H. perforatum* secondary metabolism, generating the high complexity of its EO composition. The aerial parts of wild *H. perforatum* were collected during the flowering period, especially in different regions of Western Europe (France, Italy, Portugal, Spain, Greek, Serbia), but also in Turkey, Uzbekistan, Lithuania as well as in China and India. The GC-MS results of *H. perforatum* EOs reported in the literature are summarized in **Table 1**.

The first important studies were carried out in France where Mathis and Ourissons (1963, 1964a, 1964b, 1964c, 1964d) started with an investigation of some fresh samples collected in South-East France. This was the first attempt to consider *Hypericum* volatile constituents for chemotaxonomic purposes by distinguishing several *Hypericum* species in different sections. More recently, other studies focused more deeply on the EO composition of different populations of *H. perforatum* from other countries such as Serbia (**Table 1**). Comparing the EO production in *H. perforatum* collected in French and Serbian regions, hydrocarbons and sesquiterpenes characterized both types of EOs. However, *H. perforatum* collected from the Barelic region in Serbia contains an important quantity of α-pinene (8.6%), while the same species from the Rujan mountains did not contain α-pinene (Gudzic *et al.* 2001). Monoterpenes such as α- and β-pinene are the main constituents in the *H. perforatum* EO native of Greece (Petrakis *et al.* 2005).

On the other hand, the presence of hydrocarbon derivatives seem to be reduced in favour of mono- and sesquiterpenes, especially for Asian *H. perforatum* plants (Demirci *et al*. 2005; Çirak *et al.* 2010). Despite many recent papers on the EOs of *H. perforatum* native of different countries by the most modern techniques, the description of the extracted plant material and plant sampling are sometimes not detailed enough (**Table 1**).

Furthermore, the variety is rarely specified in the literature even if it is well known that significant variations in the EO composition may also be caused by varietal differences.

The major compounds in the EO of *H. perforatum* var. *angustifolium* collected in Italy (Sardinia) were 2-methyl octane (21.1%), germacrene-D (17.6%) and α-pinene (15.8%) (Pintore *et al*. 2005). French *H. perforatum* var. *angustifolium* samples were characterized by spathulenol (21.1%) and branched tetradecanol (9.1%) (Mathis and Ourissons 1964c; Schwob *et al.* 2002). The French *H. perforatum* plant samples initially investigated by Mathis and Ourisson (1964a, 1964b, 1064c) were especially characterized by 2-methyl octane (45%) and α-pinene (24%), but successive studies on the same species collected in Turkey, Serbia and India reported the monoterpene α-pinene as the main component (50-67%, 3-9% and 67%, respectively; **Table 1**).

However, recent studies have pointed out β-caryophyllene and caryophyllene oxide to be the principal constituents of *H. perforatum* EO collected in South-East France (16-19% and 16-17%, respectively; Schwob *et al.* 2004) and Serbia (β-caryophyllene, 14%; Gudzic *et al.* 2001) Therefore, a large variability in the EO composition of *H. perforatum* due to the origin of plant material has to be considered. (**Table 1**) However, it is difficult to compare all

Table 1 Studies on *Hypericum perforatum* essential oils.

Reference	Variety	Main compounds (%, relative percentage composition)	Plant origin	Plant organ	*EO yields
Baser *et al.* 2002		β–caryophyllene (11.7), caryophyllene oxide (6.3), spathulenol (6), α–pinene (5)	Uzbekistan	a.p., dried	0.1% w/w_{dw}
Cakir *et al.* 1997		α–pinene (61.7), 3-carene (7.5), β–caryophyllene (5.5), myrcene (3.6), cadalene (3.2), β–pinene (3)	Turkey	flowering a.p., dried	0.19% w/w_{dw}
Erken *et al.* 2001		α–pinene (50), carvacrol (22)	Turkey	flowering a.p., dried	
Chialva *et al.* 1981		2-methyloctane (16.4), α–pinene (11)	Italy	flowering a.p., dried	0.02% w/w_{dw}
Karim *et al.* 2007		α–pinene (10.3), β-caryophyllene (2.0)	Tunisia		
Mimica–Dukic *et al.* 1998	var. *perforatum*	β–caryophyllene (0.64-19.23), 10-methyl-1-undecene (0-14.66), 1-tetradecanol (5.08-23.75), palmitic acid (0-10.27), *n*-eicosane (0-30.86)	Serbia	a.p., dried	0.03-1.93%$_{dw}$
Mockute *et al.* 2003	var. *angustifolium*	dimethylheptane (0.6-6.6), α–pinene (1.1-6.9), β–caryophyllene (5.1-19.1), β–farnesene (tr-8.2), germacrene D (4.5-31.5), spathulenol (3.9-8.5), caryophyllene oxide (6.1-35.8), α–cadinol (2.2-6.2)	Lithuania	a.p., dried	0.1-0.4% w/w_{dw}
Nogueira *et al.* 1999		α–pinene (23.6-2.1), β–caryophyllene (3.7-10.0), germacrene D (5.1-13.4)	Portugal	a.p., dried	0.1-0.5% v/w_{dw}
Nogueira *et al.* 2008		α–pinene (39-64), β–pinene (2-3), *n*-nonane (12-24), *n*-undecane (3-9), germacrene D (0.3-4)	Portugal	flowering/fruit ication period	0.15% v/w_{dw}
Pavlovic *et al.* 2006		α–copaene (11.3), α–longipinene (9.7)			
Petrakis *et al.* 2005		2-methyl-octane (20.9), α–pinene (11.2), β–pinene (4.7), γ–muurolene (6.9), β–caryophyllene (5.8)	Greece		
Pintore *et al.* 2005		2-methyloctane (21.1), α–pinene (15.8), germacrene D (17.6)	Sardinia	a.p., dried	0.15% w/w_{dw}
Radusiene *et al.* 2005		β–caryophyllene (f: 4.2-14.2: l: 9.3-25.9), spathulenol (f: 4.5-11: l: 6.4-15.7), caryophyllene oxide (f: 7.7-34: l: 9.3-25.9), viridiflorol (f: 1.3-11.1: l: 0-9.5), *n*-tetradecanol (f: 0.l9-11.2: l: 0.5-24.5), manool (f: tr-27.6: l: 0-13.8)	Lithuania	a.p., dried	
Rancic *et al.* 2005		nonane (63.8), 2-methyloctane (2), 3-methylnonane (4.5), *p*-cymene (4.8), α–patchoulene (1.4), allo-aromadendrene (1.7), β–selinene (2.1)	Serbia	a.p., dried	0.15% w/w_{dw}
Saraglou *et al.* 2007		α–pinene (8.6), β–pinene (2.7), *trans*-ocimene (3.1), β–caryophyllene (3.9), β–farnesene (6.6), germacrene D (6.8), spathulenol (5.4), tetradecanol (3.4)	Serbia	flowering a.p., dried	0.02% v/w_{dw}
Schwob *et al.* 2002	var. *perforatum*	2-methyloctane, β–caryophyllene, caryophyllene oxide, β–farnesene, γ–cadinene, δ–cadinene, ar-curcumene, *cis*-calamenene, spathulenol, nerolidol, α–cadinol, 2-methyldodecane, dodecanol	South-East France	a.p., dried, different varieties	0.03-0.12% w/w_{dw}
Schwob *et al.* 2004	var. *angustifolium*	caryophyllene oxide, β–caryophyllene, spathulenol, β–funebrene, γ–muurolene, β–farnesene, caryophylladienol	South-East France	a.p., dried, phenological cycle	0.06-0.09% w/w_{dw}
Tognolini *et al.* 2006		2-methyloctane (36), α–pinene (26), 2-methylnonane (7), 2-methyldecane (4.8), caryophyllene oxide (4.2)	France		
Weyerstahl *et al.* 1995		α–pinene (67.3), nonane (4.6), geranyl acetate (4.8), β–caryophyllene (5.2), α–cuprenene (3.2)	North India	leaves	0.5%
Zeng H. *et al.* 2009		Sesquiterpenes = main constituents	China	leaves	
Mathis and Ourissons 1964b, 164c	*x quadrangulum*	2-methyloctane (18), nonane (32), 2-methyldecane (4), undecane(20), α–pinene (12), β–pinene (8), myrcene (2), limonene (4), *n*-octanal (+), decanal (+), caryophyllene (+++), humulene (+++)	France (June, August) after fructification flowering/fructification period	fresh a.p.	0.6-1.2*% w/w_{fw}
Mathis and Ourissons 1964b, 1964c		2-methyloctane (50-35, a.p.; 48, lea; 30, fr.), nonane (33-8, a.p.; 7, lea; 3, fr.), 2-methyldecane (tr-5, a.p.; 48, lea; 30, fr.), α–pinene (14-35), β–pinene (1-10), myrcene (-), limonene (tr),monoterpene alcohols (tr), *n*-octanal (+), decanal (+), caryophyllene (+++), humulene (+++)	France (June, August) after fructification	fresh a.p., lea., fr.	1-2.2*% w/w_{fw}
Çirak *et al.* 2010		β-caryophyllene (4.1-5.9), γ-muurolene (5.0-9.6), β-selinene (5.1-19.6), α-selinene (4.1-10.4), δ–cadinene (3.0-4.9), spathulenol (2.3-5.1), caryophyllene oxide (6.0-12.2)	Northern Turkey full flowering	a.p., dried, 10 populations	0.04-0.5% v/w_{dw}
Maggi *et al.* 2010	subsp. *perforatum*	(*E*)-caryophyllene (21.6–23.0), germacrene D (19.5–20.8)	Central Italy (Appennino Umbro-Marchigiano Mountains) flowering	dry a.p	0.07%$_{dw}$
	subsp. *veronense*	germacrene D (7.8–9.7), (*E*)- β-caryophyllene (6.0 –9.2)	Central Italy (Appennino Umbro-Marchigiano Mountains) flowering	dry a.p	0.04-0.06%$_{dw}$
Gudes *et al.* 2009	var. Topaz	1-octene (6.9), *n*-nonane (24.2), α-pinene (9.2), *n*-undecane (3.8), (*E*)-β–caryophyllene (7.7), germacrene D (16.5), γ-cadinene (3.8)		*in vitro* shoots	2.8 mg/g_{dw}

extraction methods: # isolation method by Stahl; * Clevenger apparatus; tr = traces; (-) = absence; (+) = less than 10%; (++) = 10-40%; (++) = more than 40%; le = leaves; fr = fruits; a.p. = aerial parts; fl. = flowers; dw = dry weight, fw = fresh weight.

this data because sometimes no information is reported on the environmental variables of the collection sites, the status of plant material (fresh/dry), the plant organs used for the hydrodistillation as well as the plant development cycle. Morphological data on *H. perforatum* have showed the presence of different types of secretory structures including translucent glands, black nodules and secretory canals. The EO of *H. perforatum* is synthesized either in translucent glands or in secretory canals that may be localized in leaves, petals, sepals and pistil (Ciccarelli *et al.* 2001) which are not present at every stage of the developmental cycle. Schwob *et al.* (2004) considered one population of *H. perforatum* var. *perforatum* in one French location. Therefore, in this study, the chemical profiles as well as the EOs yields could be compared only on the basis of the phenological cycle.

In fact, hydrodistillation of the aerial parts of *H. perforatum* gave yellowish oils with the lowest value at the fruiting stage and increased from 0.07 to 0.092% (**Table 1**) during anthesis, as observed also in other plant species (Juteau *et al.* 2002).

In this study, the common and main components were caryophyllene oxide, β-caryophyllene, spathulenol, 1-tetradecanol, 1-dodecanol. β-caryophyllene varied from 7.3 to 18.3% and the authors suggested that its variation during the phenological cycle should rather be analysed by also considering caryophyllene oxide and caryophylladienol levels, as these molecules share close metabolic pathways (Schwob *et al.* 2004). However, monoterpenoid composition and the levels of aliphatic alcohols seemed to be more related to the phenological cycle than sesquiterpenes. Considering the different groups of compounds, monoterpenoids were actually the group of terpenoid components less represented in *H. perforatum* EOs. However, numerous monoterpene hydrocarbons were identified in the EO of flowering shoots and were not present in other samples. Thus, the level of monoterpenoids may be linked, both quanti- and qualitatively, with the phenological stages (Schwob *et al.* 2004). Unlike aliphatic hydrocarbons, the hydrocarbon alcohols decreased from the vegetative to the fruiting stage and may be considered another important indicator to follow phenological processes. Based on these modifications during the phenological cycle in the EOs of *H. perforatum* var. *perforatum*, it was supposed that during this physiological process of ontogenesis, the morphological modifications occurring are concomittent with modifications in secondary metabolism (Schwob *et al.* 2004).

Previous research carried out on this species demonstrated a wide range of ecological adaptation and morphological variation (Radusiene and Bagdonaite 2002). The most important feature distinguishing morphological types of *H. perforatum* may be the dimension of leaves. Robson (1968) classified *H. perforatum* into three varieties: var. *perforatum* with broad leaves, var. *angustifolium* with narrow leaves, and var. *microphyllum* with small leaves. The broad-leaved populations were confirmed as being predominant in Lithuania (Radusiene 2004). Radusiene *et al.* (2005) completed this research with quanti- and qualitative analyses of the EOs of 11 accessions of *H. perforatum* collected from various localities in Lithuania and grown in uniform field conditions in the second year. Thirty components were identified in the flowers and leaves and all accessions contained large proportions of oxygenated components derived from hydrocarbons, mono-, di- and sesquiterpenes. The oxygenated sesquiterpenes were the main group of compounds in all accessions (39.2-63.3% in flowers and 35.7-70.4% in leaves). However, there were differences in the amount of the main components: caryophyllene oxide (7.7-30 and 9.3-25.9% in flowers and leaves, respectively), spathulenol (4.5-11.0% flowers; 6.4-15.7% leaves), and viridiflorol (1.3-11.1% flowers; 0.5-9.5% leaves).

Oxygenated aliphatics, which account for 4.8-18.6% of total EO in flowers and 1.2-39.4% in leaves, were represented mainly by dodecanol (0.2-9.8% flowers; 0.3-19.2% leaves), tetradecanal (trace-8.9% flowers; 0-9.8% leaves) and tetradecanol (0.9-11.2% flowers; 1-24.5% leaves). It is important to point out that these aliphatic compounds varied greatly among the accessions and parts of the plant. The differences observed in volatile constituents of *H. perforatum* accessions gathered in the wilderness and presently cultivated in uniform conditions are very likely to be genetically determined. Although compounds with a caryophyllane skeleton were prevalent as volatiles from flowers and leaves of the majority of *H. perforatum* accessions investigated, the presence of some other components (spathulenol, dodecanol, tetradecanol, tetradecanal, carotol, manool) in considerably large and variable amounts testified the presence of EO chemotypes. On the other hand, no considerable differences were found in the composition of EOs between wild accessions and cultivars. Chemical variability of EOs of the analysed accessions seemed likely to result from genetic variability. However, more accessions are necessary to establish genetically determined chemo-polymorphism of this species (Shwob *et al.* 2002).

Other *Hypericum* species

The EO composition of 51 *Hypericum* species not including *H. perforatum* are summarised in **Table 2**. As a very large number of volatile constituents are generally detected, it seems that the numerous metabolic pathways that are elicited in the *H. perforatum* secondary metabolism, generating the high complexity of EO composition, are common also to the other *Hypericum* species. The aliphatic hydrocarbons, mono- and sesquiterpenes and their derivatives represent the typical constituents in the EOs of several *Hypericum* spp. even if significant quali- and quantitative differences were found depending on various endogenous and exogenous factors (**Table 3**). However, the same species may also show several variations in the constituents of EOs depending on the collection period, growth conditions, developmental stage, climate, interactions between plants and pathogens as well as the status of the plant material (fresh or dry) used for the distillation.

The variability of volatile constituents in different *Hypericum* species took into account different populations, especially in France, Portugal, Serbia and Greece (**Table 2**). Considering a non-European collection, the wild *H. brasiliense* EO obtained from fresh aerial parts collected in Brazil contained 30% caryophyllene (hydrocarbon sesquiterpene), while another Brazilian species *H. connatum* (dry whole plants) showed caryophyllene oxide (40%) and humulene epoxide II as main volatile constituents. In this case, the presence of higher amounts of oxygenated sequiterpenes in *H. connatum* than in *H. brasiliense*, despite their common geographic origin, may be due, in particular, to the status of the distilled plant material (dry instead of fresh) (Abreu *et al.* 2004; Ferraz *et al.* 2005).

In most papers reported in this review, the EOs were generally derived from wild plants and hydrodistilled after drying in air. In the case of some wild *Hypericum* species collected in Brazil (Ferraz *et al.* 2005), the fresh flowering aerial parts were compared and significant differences were found among the chemical classes of typical volatiles.

In fact, *H. caprifoliatum* and *H. myrianthum* are characterized by the production of *n*-nonane and *n*-undecane (60 and 38%, respectively), while *H. carinatum* and *H. ternum* by hydrocarbon sesquiterpenes (40 and 38%, respectively) such as β-caryophyllene, biciclogermacrene and α-humulene. The *H. connatum* EO was characterized by the oxygenated sequiterpenes caryophyllene oxide (40%) and humulene epoxide II (11%) even if significant amounts of hydrocarbon sesquiterpenes were also present (26%). In the case of *H. polyanthemum* and *H. ternum* collected in Brazil, the EOs were characterized by high amounts of peculiar volatile compounds such as benzopyranes (43 and 11%, respectively) (Ferraz *et al.* 2005).

Samples of French *H. scabrum* plants were rich in sesquiterpenes (Mathis and Ourissons 1964c), while the oil of the same species collected in Turkey consisted of 13 mono-

Table 2 Studies on the composition of essential oils extracted from various *Hypericum* spp.

Species	Main constituents (%, relative percentage composition)	Origin (period)	Plant organ	EO color	EO yields	References
H. acmosepalum	undecane (1.7), limonene (2.2), α–copaene (4.6), α–curcumene (13.0), γ–muurolene (8.7), β–selinene (16.0), caryophyllene oxide (9.0)	Asia	dry flowering a.p.			Demirci *et al.* 2005
H. alpinum	β–pinene (13.3), *cis*-ocimene (2.7), γ–terpinene (7.7), dodecanal (3.0), β–farnesene (1.0), germacrene D (3.4), δ–cadinene (4.3), γ–cadinene (1.4), caryophyllene oxide (4.8)	Serbia wild plants	dry flowering a.p.		0.01% w/w_{dw}	Saroglou *et al*. 2007
H. androsaemum	*n*-nonane (tr-8), α–pinene (2-20), β–pinene (15-25), myrcene (7-40), limonene (30-52), *n*-undecane (tr-5)	France (June-August)	fresh a.p., fl.,fr., le.	yellowish	0.6-1.4‰ w/w_{dw}	Mathis and Ourissons 1964b
	caryophyllene oxide (28 fl.;36 le.), ishwarane (30.5, le.), humulene epoxide II (5.6, le.), α–guaiene (40, fl.)	Iran (march) wild plants	dry le., fl.		0.97-1.30% w/w_{dw}	Morteza-Semnani *et al*. 2005
	n-nonane (1-9), β–pinene (2-4), limonene (2-4), β–caryophyllene (9-17), germacrene D (4-9), caryophyllene oxide (9-17)	Portugal wild plants	fresh le.		0.7-3.4 mg/g_{dw}	Guedes *et al.* 2004
	n-hexenal (2-8), *n*-nonane (1-4), α–pinene (0.3-3), β–pinene (6-2), limonene (15-2), β–caryophyllene (9-15), β–gurjunenene (6-15), germacrene D (5-7), γ–elemene (8-18), γ–muurolene (2-4)	Portugal cultivated plants seasonal variations	fresh le		0.9-4.1 mg/g_{dw}	Guedes *et al.* 2003
	β–pinene (2.1), limonene (1.3), *n*-undecane (3.8), β–caryophyllene (5.0), β–gurjunenene (8.6), γ–muurolene (15.3), germacrene D (4.3), γ–bisabolene (10.8), germacrene B (3.9), γ–elemene (9.8)		*in vitro* shoots		0.74 mg/g_{dw}	Guedes *et al.* 2003, 2009
H. barbatum	α–pinene (17.1), β–pinene (17.0), limonene (6.0), myrcene (2.5), β–caryophyllene (8.0), β–farnesene (1.8), β–selinene (3.7), α–selinene (1.9), δ–cadinene (3.0), spathulenol (2.7), caryophyllene oxide (12.2)	Serbia wild plants	dry flowering a.p.		0.02% w/w_{dw}	Saroglou *et al*. 2007
H. beanii	γ–muurolene (11), β–selinene (16), caryophyllene oxide (19)	Asia	dry flowering a.p.			Demirci *et al.* 2005
H. brasiliense	β–caryophyllene (29.5), α–humulene (12.7), ledene (6.4), γ–cadinene (4.4), ledol (5.7), caryophyllene oxide (9.9), cubenol (7.5)	Brazil wild plants	dry whole plant	yellowish	0.1% w/w_{dw}	Abreu *et al.* 2004
H. calycinum	*n*-nonane (3-6), *n*-undecane (tr-1), α–pinene (45-50), β–pinene (38-45), limonene (10-24), myrcene (3-5)	France (July, Sept) wild plants	fresh a.p., fl.,fr.		0.75-12‰ w/w_{dw}	Mathis and Ourisoons 1964b
	α–terpineol (11), β–pinene (29)	Asia wild plants	dry flowering a.p.			Demirci *et al.* 2005
	α–humulene (13.3 –15.1), germacrene D (10.5–14.5)	Central Italy (Appennino Umbro-Marchigiano Mountains)	dry flowering a.p.		0.11-0.15%$_{dw}$	Maggi *et al.* 2010
H. caprifoliatum	*n*-nonane (55.8), α–pinene (1.5), *n*-undecane (5.0),), β–caryophyllene (5.9), γ–muurolene (3.3), germacrene D (3.7), bicyclogermacrene (2.2)	Brazil (Nov., Jan.) Wild plants	fresh flowering a.p	yellowish	0.1% v/w_{fw}	Ferraz *et al.* 2005
H. carinatum	*n*-nonane (9.0), *n*-undecane (3.6),), α–copaene (2.6), β–caryophyllene (21.0), α–*trans*-bergamotene (10), α–humulene (5.7), β–farnesene (3.1), caryophyllene oxide (9.5), spathulenol (4.4), humulene epoxide II (2.1)	Brazil (Nov., Jan.) Wild plants	fresh flowering a.p	yellowish	0.2% v/w_{fw}	Ferraz *et al.* 2005
H. choisyanum	allo-aromadendrene (8.1), γ–muurolene (7.8), *cis*-eudesma-6,11-diene (11), γ–cadinene (3.9), spathulenol (2.7), caryophyllene oxide (3.3)	Asia	dry flowering a.p.			Demirci *et al.* 2005
H. connatum	β–caryophyllene (13.1), α–humulene (7.3), β–selinene (4.0), bicyclogermacrene (2.2), caryophyllene oxide (40.1), humulene epoxide II (10.5), α–cadinol (3.5)	Brazil (Nov., Jan.) wild plants	fresh flowering a.p	yellowish	0.2% v/w_{fw}	Ferraz *et al.* 2005
H. coris	α–curcumene (40), γ–muurolene (4.4), β–himachalene (4.6), β–selinene (4.0)	France (June)	dry a.p.		0.06% $_{dw}$	Schwob *et al.* 2002
H. dogonbadanicum	α–pinene (34.7), β–pinene (32.1), limonene (12.1)	Iran (June) wild plants	dry le. and fl.	Colorless	0.2% w/w_{dw}	Sajjadi *et al.* 2001
	a-pinene (12.8), β–pinene (4.7), limonene (8.2), camphene (3.9)	Iran wild plants		yellow	0.1% w/w_{dw}	Javidnia *et al.* 2008
H. empetrifolium	α–pinene (35.6), β–pinene (4.8), α–terpineol (4.9), β–caryophyllene (3.1), γ–gurjunene (10.5), germacrene D (1.5)	Greece (May-June)	dry flowering a.p			Petrakis *et al.* 2005
H. ericoides	*n*-nonane, decane, *n*-undecane (1-5), α–pinene (5-10), β–pinene, limonene (1-5), β–caryphyllene, α–copaene (1-5), γ–muurolene (5-10), α–curcumene (10-20), calamenene (1-5), δ–cadinene (5-10)	Spain	dry flowering a.p.		12% (by steam-distillation of *n*-hexane extract))	Cardona *et al.* 1983

Table 2 (Cont.)

Species	Main constituents (%, relative percentage composition)	Origin (period)	Plant organ	EO color	EO yields	References
H. foliosum	*n*-nonane (29-73), β–pinene (0.3-6), terpinolene (1-19), limonene (7-46), β–caryphyllene (1-7)	Azorean Islands (July) wild plants different collection sites	terminal cymose inflorescences		0.10-0.25% v/w_{fw}	Santos *et al.* 1999
H. forrestii	α–pinene (10), caryophyllene oxide (13)	Asia	dry flowering a.p.			Demirci *et al.* 2005
Hypericum helianthemoides	β-caryophyllene (23.3), spathulenol (17.4)	Iran		yellow	0.06% w/w_{dw}	Javidnia *et al.* 2008
H. heterophyllum	α–pinene (11.6), β–pinene (2.0), *n*-decane (5.8), isocaryophyllene (17.1), β–caryophyllene (4.5), α–humulene (2.4), γ–muurolene (8.2), germacrene D (3.1), β–selinene (3.0), valencene (3.8), γ–cadinene (5.5), δ–cadinene (9.5)	Turkey (August) wild plants	dry flowering a.p.	yellowish	0.09% w/w_{dw}	Çakir *et al.* 2004
H. hircinum	*n*-nonane (45-60), *n*-undecane (2-30), α–pinene (5-8), β–pinene (10-12), limonene (6-15), myrcene (6-18)	France (August-Sept) wild plants	fresh le. and fr.		2.2-3.5‰ w/w_{dw}	Mathis and Ourisoons 1964b
	n-nonane (35, fr.; 19.3, le.), β–pinene (17.3, fr.; 2.6, le.), limonene (12.7, fr.; 2.5, le.), undecane (4.8, fr.; 2.0, le.), *trans*-pinocarveol (3.2 fr), α–gurjunene (0.3, fr.; 10.7, le.), β–caryophyllene (0.1, fr.; 4.5, le.), β–gurjunene (0.2, fr.; 5.5, le.)	Italy (June-July) wild plants	dry le. and fr.	yellowish	0.1-0.25% v/w_{dw}	Bertoli *et al.* 2000
	subsp. *majus*: δ–selinene (18.5)	Central Italy (Appennino Umbro-Marchigiano Mountains)	dry fl.		0.04%$_{dw}$	Maggi *et al.* 2010
H. hirsutum	2-methyloctane (3), *n*-nonane (52),*n*-undecane (30), α–pinene (4), β–pinene (5), myrcene (3), sesquiterpenes (10-40)	France (July) wild plants	fresh flowering a.p.		1.4‰ w/w_{dw}	Mathis and Ourisoons 1964
	α–pinene (24.8), undecane (13.3), decanal (2.0), undecanone (4.1), β–caryphyllene (5.4), β–farnesene (2.2), germacrene D (1.3), γ–cadinene (1.4), δ–cadinene (2.6), caryophyllene oxide (5.6)	Serbia wild plants	dry flowering a.p.		0.02% w/w_{dw}	Saroglou *et al.* 2007
	(*E*,*E*)-α–farnesene (7.0–13.8) and *E*-β–farnesene (7.2–9.4)	Central Italy (Appennino Umbro-Marchigiano Mountains)	dry flowering a.p.		0.06-0.05%$_{dw}$	Maggi *et al.* 2010
H. hirtellum	β-caryophyllene (14.1), spathulenol (12.3)	Iran		yellow	0.07% w/w_{dw}	Javidnia *et al.* 2008
H. humifusum	α–pinene (44-77), β–pinene (4-7), *n*-undecane (0.2-7), β–caryophyllene (1-9), germacrene D (2-6)	Portugal flowering/fructification wild plants	dry flowering a.p.		0.23% w/w_{dw}	Nogueira *et al.* 2008
H. hyssopifolium	germacrene D (18.2), *E*-β–farnesene (6.5)	Central Italy (Appennino Umbro-Marchigiano Mountains)	dry flowering a.p.		0.1%$_{dw}$	Maggi *et al.* 2010
H. hyssopifolium ssp.elongatum	α–pinene (17.3), α–pinene (11.6), δ–cadinene (9.5), γ–muurolene (8.2), γ–cadinene (5.5), β–caryophyllene (4.5)	Turkey (August) wild plants	dry flowering a.p.	yellowish	0.1% w/w_{dw}	Çakir *et al.* 2004
H. hyssopifolium ssp. *hyssopifolium*	β–caryophyllene (8.4), dodecanol (9.3), γ–muurolene (8.0), spathulenol (19.5), tetradecanol (10.2)	South-East France (June) wild plants	dry flowering a.p.	yellowish	0.05% w/w_{dw}	Schwob *et al.* 2006
H. hyssopifolium var. microcalycinum	α–terpineol (2.1), α–humulene (1.3), α–amorphene (5.9), valencene (1.8), spathulenol (13.4), caryophyllene oxide (20.4), caryophyllene alcohol (9.0)	Suδ–East Turkey (June) wild plants			0.08% w/w	Toker *et al.* 2006
H. kouytchense	β–caryophyllene (2.3), *cis*-β–guaiene (10.7), γ–muurolene (12.4), γ–cadinene (8.4), caryophyllene oxide (9.0), humulene epoxide II (2.5)	Asia	dry flowering a.p.			Demirci *et al.* 2005
H. lancasteri	γ–muurolene (8.9), β–selinene (11.4), caryophyllene oxide (3.2), isospathunelol (6.8), β–eudesmol (4.1), eudesmadenione (10.8)	Asia	dry flowering a.p.			Demirci *et al.* 2005
H. leshenaultii	ar-curcumene (10.0), cuparene (24.8), γ–muurolene (16.8), caryophyllene oxide (2.8)	Asia	dry flowering a.p.			Demirci *et al.* 2005
H. linarifolium	α–pinene (19-31), β–pinene (5-11), germacrene D (4-7), *n*-undecane (1-7)	Portugal (flowering/fructification) wild plants	dry flowering a.p.		0.11% w/w_{dw}	Nogueira *et al.* 2008

Table 2 (Cont.)

Species	Main constituents (%, relative percentage composition)	Origin (period)	Plant organ	EO color	EO yields	References
H. linarioides	δ–cadinene (7), *Z*-β–farnesene (5), γ–muurolene (6), spathulenol (5), α–selinene (4)	Turkey (July)	dry flowering a.p.	yellowish oil	0.1% w/w_{dw}	Cakir *et al.* 2005
H. lysimachioides var. lysimachioides	α–terpineol (4.9), α–longifolene (6.4), α–amorphene (4.9), β–selinene (6.7), α–selinene (4.3), spathulenol (4.9), caryophyllene oxide (30.8)	Suδ–East Turkey (June) wild plants			0.08% w/w	Toker *et al.* 2006
H. maculatum	*n*-undecane (8.2), β–caryphyllene (7.6), β–farnesene (10.0), γ–muurolene (5.2), δ–cadinene (4.2)	South-East Serbia (July) wild plants	dry flowering a.p.	yellow-green	0.35% w/w_{dw}	Gudzic *et al.* 2002
	nonane (5.5), α–pinene (4.4), β–pinene (1.5), undecane (3.5), δ–cadinene (2.9), β–farnesene (2.8), γ–cadinene (2.1), spathulenol (6.8), globulol (10)	Serbia wild plants	dry flowering a.p		0.04% w/w_{dw}	Saroglou *et al*. 2007
H. monogynum	tricosane (13), myrcene (10)	Asia	dry flowering a.p.			Demirci *et al.* 2005
H. montanum	germacrene D (11.4–26.1), *E*-β-caryophyllene (12.8 –13.1)	Central Italy (Appennino Umbro-Marchigiano Mountains)	dry flowering a.p.		0.07-0.05%$_{dw}$	Maggi *et al.* 2010
H. myrianthum	*n*-nonane (17.5), α–pinene (6.5), β–pinene (3.7), undecane (20.7), β–caryophyllene (5.8), α–humulene (2.4), dehydroaromadendrene (8.6), α–copaene (1.9)	Brazil (Nov., Jan.) wild plants	fresh flowering a.p	yellowish	0.5% v/w_{dw}	Ferraz *et al.,* 2005
H. olympicum	*E*-anethole (30.7), β–farnesene (12.4), γ–muurolene (7.5), germacrene D (4.3), δ–cadinene (8.7)	South-East Serbia wild plants	dry a.p.	yellow	0.45% w/w_{dw}	Gudzic *et al.,* 2001
	Linalool (2.8), α–copaene (2.1), β–caryophyllene (7.4), germacrene D (16), bicyclogermacrene (3.6), δ–cadinene (6.0), spathulenol (6.7), α–cadinol (4.0)	Greece wild plants	dry flowering a.p.		0.18% w/w_{dw}	Pavlovic *et al.,* 2006
H. patulum	Limonene (1.2), linalool (4.0), γ–muurolene (5.7), β–selinene (15), γ–cadinene (2.8), ar-curcumene (8.0), caryophyllene oxide (5.9), α–pinene (18),	Asia	dry flowering a.p.			Demirci *et al.* 2005
	benzocycloheptene (14), β–caryophyllene (9), longifolene (6)	China	dry a.p.			Zhang *et al.* 2009
H. perfoliatum	α–pinene (34-48), β–pinene (9.2), *n*-nonane (8.5), δ–cadinene (8.1), *n*-undecane (3.8), γ–muurolene (6.0), δ–cadinene (8.1), β–caryophyllene (3.8)	Greece (May-June) wild plants	dry flowering a.p.	yellowish	0.2-0.3% w/w_{dw}	Couladis *et al*. 2001
	α–pinene (41.3), β–pinene (6.5), *n*-nonane (6.1), δ–cadinene (6.2), γ–muurolene (4.1), *n*-undecane (3.2)	Greece (May-June) wild plants	dry flowering a.p.			Petrakis *et al.* 2005
	thymol (22.1), τ–cadinol (18.5), 4,5-dimethyl-2-ethylphenol (13.1), pentadecanone (4.8), spathulenol (4.5)	Algeria wild plants	dry a.p.			Touafek *et al.* 2005
	β–caryophyllene (13), *n*-undecane (8), α–humulene (5), linalool (5), δ–cadinene (5)	Portugal	dry flowering a.p.			Nogueira *et al*. 2002
	α–pinene (39-64), nonane (12-24), β–pinene (2.3), germacrene D (0.3-4), *n*-undecane (3-9), spathulenol (3.6)	Portugal (flowering/fructification)	dry flowering a.p.	pale yellowish	0.10% w/w_{dw}	Nogueira *et al*. 2008
	2-methyloctane (3.7), *n*-nonane (2.3), α–pinene (13.2), β–pinene (2.3), germacrene D (10.6), α–selinene (6.6), *n*-undecane (5.1), spathulenol (3.6)	Tunisia (June) wild plants			0.15% w/w_{dw}	Hosni *et al.* 2008
H. polyanthemum	Undecane (7.9), β–caryophyllene (4.0), benzopyranes (43.3), α–humulene (6.8), γ–muurolene (4.1), germacrene D (2.8), bicyclogermacrene (3.8)	Brazil (Nov., Jan.) wild plants	fresh flowering a.p	yellowish	0.5% v/w_{fw}	Ferraz *et al.* 2005
H. pseudohenryi	β–selinene (19)	Asia	dry flowering a.p.			Demirci *et al.* 2005
H. pulcrum	α–pinene (36-50), β–pinene (9-12), germacrene D (2-5), *n*-undecane (3)	Portugal (flowering/fructification)	dry flowering a.p.	pale yellowish	0.20% w/w_{dw}	Nogueira *et al.,* 2008
H. richeri	*n*-nonane (13.8), *Z*-β–ocimene (19.5), *E*-β–ocimene (8.0), β–bisabolene (8.7), *E*-nerolidol (5.1), α–cadinol (5.2)	Italy (June) wild plants	fresh flowering a.p.	yellowish	0.08% w/w_{dw}	Ferretti *et al.* 2005
	subsp. *richeri*: germacrene D (26.9)	Central Italy (Appennino Umbro-Marchigiano Mountains)	dry flowering a.p.		0.07%$_{dw}$	Maggi *et al.* 2010

Table 2 (Cont.)

Species	Main constituents (%, relative percentage composition)	Origin (period)	Plant organ	EO color	EO yields	References
H. rumeliacum var. *apollinis*	α–pinene (43.3), β–pinene (9.7), limonene (4.0), α–copaene (5.4), germacrene D (3.8), δ–cadinene (2.7)	Greece (May-June) wild plants	dry flowering a.p.			Petrakis *et al.* 2005
	α–pinene (18.5), β–pinene (21.5), limonene (7.1), myrcene (4.7), γ–terpinene (2.7), dodecanal (5.8), β–caryophyllene (1.6), germacrene D (2.9), α–selinene (1.9), δ–cadinene (1.2), caryophyllene oxide (1.5)	Serbia wild plants Greece (May)	dry flowering a.p flowering a.p.		0.02% w/w_{dw}	Saroglou *et al.* 2007
	α–pinene (43.8), β–pinene (9.8), myrcene (2.2), limonene (4.0), *n*-undecane (3.5), α–copaene (5.4), dehydro-aromadendrene (6.8), germacrene D (3.8), caryophyllene oxide (2.0), δ–cadinene (2.7)	Greece (May-June) wild plants	dry flowering a.p.	light yellow	0.22% w/w_{dw}	Couladis *et al.* 2003
H. scabrum	α–pinene (71.6), β–pinene (2.9), β–caryophyllene (4.8), myrcene (3.8)	Turkey wild plants	dry flowering a.p.	light yellow	0.19% w/w_{dw}	Çakir *et al.* 1997
	α–pinene (11.2), spathulenol (7.2), acetophenone (4.8), p-cymene (6.1), carvacrol (4.7)	Uzbekistan wild plants	dry a.p.		0.2% w/w_{dw}	Baser *et al.* 2002
		Suδ–East Turkey	dry flowering a.p.	yellowish	42 mg/196g_{dw}	Kizil *et al.* 2004
	n-nonane (5.6), α–pinene (45.3), β–pinene (2.5), limonene (2.6), thymol (5.3), carvacrol (3.3), germacrene D (2.8), bicyclogermacrene (1.7), δ-cadinene (1.3)	Iran (June) wild plants	dry flowering a.p.	yellowish	0.96% w/w_{dw}	Morteza-Semnani *et al.* 2006
	α–pinene (59.3), β–pinene (4.1), limonene (2.1)	Iran (wild plants)		light yellow	0.05% w/w_{dw}	Javidnia *et al.* 2008
H. ternum	*n*-undecane (4.8), β–caryophyllene (12.0), α–humulene (4.0), dehydro-aromadendrene (2.9), bicyclogermacrene (10.0), germacrene D (1.8), γ–cadinene (1.7), β–cadinene (5.0), caryophyllene oxide (1.0), benzopyranes (10.6)	Brazil (Nov., Jan.) wild plants	fresh flowering a.p	yellowish	0.2% v/w_{fw}	Ferraz *et al.* 2005
H. tetrapterum	*n*-undecane (7.4), α–longipinene (9.7), α–copaene (11.3), β–caryophyllene (6.1), β–gurjunene (4.4), δ–cadinene (6.1), caryophyllene oxide (8.9)	Greece wild plants	dry flowering a.p.		0.2% w/w_{dw}	Pavlovic *et al.* 2006
	α–copaene (12.7), α–longipinene (8.1)	Central Italy (Appennino Umbro-Marchigiano Mountains)	dry flowering a.p.		0.1%$_{dw}$	Maggi *et al.* 2010
H. tomentosum	*n*-octane (9.9), α–pinene (5.2), β–pinene (3.7), menthone (17.0), β–caryophyllene (5.3), germacrene D (2.2), δ–cadinene (1.1), caryophyllene oxide (2.3), spathulenol (2.2)	Tunisia (June) wild plants	dry flowering a.p.	pale yellowish	0.13% w/w_{dw}	Hosni *et al.* 2008
	undecane (8), α–humulene (5), β–caryophyllene (13), linalool (5), δ–cadinene (5)	Portugal	dry flowering a.p.			Nogueira *et al.* 2002
H. triquetrifolium		Sud-East Turkey	dry flowering a.p.	light yellow	22 mg/155g_{dw}	Kizil *et al.* 2004
	α–pinene (10.3), caryophyllene oxide (1.4)	Tunisia	a.p.			Hosni *et al.* 2007
	2-methyloctane (17), *n*-nonane (9.6), α–pinene (14.7), 2-methylnonane (5.5), β–caryophyllene (8.8), γ–muurolene (3.9), germacrene D (4.2), caryophyllene oxide (9.5), δ–cadinene (3.0)	Greece (May-June) wild plants	dry flowering a.p.			Petrakis *et al.* 2005
	n-nonane (8,le.; 15, fl), α-pinene (8 le.; 4, fl.), β-pinene (13, le.; 10, fl.), myrcene (16, le.; 5, fl.), β-caryophyllene (5 le.; 11, fl.), germacrene-D (10, le.; 13 fl.), sabinene (13, le.; 3, fl.), caryophyllene oxide (5, le.; 12 fl.)	Italy (June-July) wild plants	dry le. and fl.	yellowish		Bertoli *et al.* 2003
Hypericum undulatum Schousboe ex Willd.	*n*-nonane (48.4-37.1), α-pinene (2.3-5.4), β-pinene (8.1), *E*-β-ocimene (1.3-4.0), β-elemene (7.0-4.4), germacrene D (2.5-11.69)	Portugal Cultivated plants seasonal variation	Fresh plants		3.2-5.6 mg/g_{fw}	Guedes *et al.* 2009
	n-nonane (4930.3 mg/g_{dw}), β-pinene (415.5), *n*-undecane (256.2), β-elemene (240.7), α-cubebene (236.6)		*in vitro* shoots		4.9 to 10.4 mg/g_{dw}	Guedes *et al.* 2009
	n-nonane (58.9), β-pinene (11), β-elemene (4.3)		Micropropa-gated plants (8 months after transfer to plastic vessels)		10.5 mg/g_{dw}	Guedes *et al.* 2009

tr = traces; le = leaves; fl = flowers; a.p. = aerial parts; fr = fruits; empty cells = data not reported; dw = dry weight; fw = fresh weight.

Table 3 Characteristic volatile compounds detected in the EOs of different *Hypericum* spp.

HYDROCARBONS	
aliphatic (saturated/ unsaturated)	2-methyloctane, 3-methylnonane, *n*-decane, 2-methyl decane, *n*-undecane, 2,2,6-trimethyl-hepta-3,5-diene, 2-methyldodecane, *n*-tridecane, nonadecane, *n*-eicosane, *n*-heneicosane, *n*-docosane, *n*-tricosane, *n*-tetracosane, *n*-pentacosane, *n*-hexacosane, *n*-heptacosane, *n*-octacosane, *n*-nonacosane;
alcohols	*cis*-3-hex*en*-1-ol, 2-nonanol, undecanol, dodecanol, tetradecanol, pentadecanol;
aldehydes	benzene acetaldehyde, *trans*-2-hexenal, *n*-heptanal, *n*-octanal, *n*-nonanal, *E,E*-2,4-decadienal, *n*-octadecanal, *n*-dodecanal, 3,6-pentadecadienal;
ketone	acetophenone, 6-methyl-5-hepten-2-one, 2,6-dimethyl-3,5-heptanedione, 2-nonanone, 3-undecanone, 2-undecanone, 4-undecanone, pentadecan-2-one;
esters	methylbenzoate, 3-hexenylbenzoate;
eters	2-penthylfuran;
acids	myristic, hexanoic, *n*-octanoic, *n*-nonanoic, *n*-decanoic, *n*-dodecanoic, *n*-tetradecanoic, *n*-pentadecanoic, *n*-hexadecanoic.
MONOTERPENES They are formed from the coupling of two isoprene units (C_{10}) and the most common structures with different functions are:	
hydrocarbons	*acyclic:* myrcene, camphene, *cis*-β-ocimene, *trans*-β-ocimene, limonene; *monocyclic:* α-thujene, verbenene, γ-terpinene, *p*-cimene, α/β-phellandrenes, *cis* sabinene hydrate, α-terpinolene; *bicyclic:* α-/β-pinenes, 3-carene, camphene, sabinene;
alcohols	*acyclic*: geraniol, linalool, nerol; *monocyclic:* α-terpineol, δ-terpineol, 4-terpineol, *trans*-pinocarveol, *E*-anethole, *trans*-verbenol, myrtenol; *bicyclic:* borneol, fenchol;
aldehydes	*acyclic:* geranial, α-campholenal, myrtenal, cuminyl aldehyde;
ketones	*monocyclic*: *trans*-thujone, pinocarvone, verbenone, pulegone, menthones, carvone, piperitone, piperitenone; *bicyclic:* camphor, fenchone, thuyone, ombellulone, pinocamphone, pinocarvone;
esters	*acyclic:* linalyl acetate/propionate, citronellyl acetate; *monocyclic:* menthyl or α-terpinyl acetate, camphor; *bicyclic:* isobornyl acetate;
ethers	1,8-cineole;
phenols	thymol, carvacrol, menthol, *E*-carveol, nerol, methyleugenol;
acids	myristic.
SESQUITERPENES They are formed from the assembly of three isoprene units (C_{15}) and the extension of the chain increases the possibility of cyclisations with a great variety of structures and chemical functions.	
hydrocarbons	β-bourbonene, β-bisabolene, δ-cadinene, γ-cadinene, β-caryophyllene, α-/g-gurjunene, α-/γ-muurolene, germacrene D, bicyclogermacrene, α-copaene, α-humulene, longifolene, α-curcumene, valencene, *trans*-β-farnesene, alloaromadendrene, γ-bisabolene, α-/β-/δ-selinene, α-gurjunene, germacrene B, γ-elemene, α-cubebene, aromadendrene, alloaromadendrene, viridiflorene;
alcohols	α-/δ-/τ-cadinol, bisabol, β-nerolidol, farnesol, cubenol, β-santalol, viridiflorol, ledol, spathulenol, germacrenol;
ketones	pentadecanone, germacrone, *cis*-longipina*n*-2,7-dione, β-vetinone, turmerones;
epoxides	caryophyllene oxide, humulene epoxide II.

terpene hydrocarbons (85%) and α-pinene was the major component (72%). The predominance (45.3%) of α-pinene was also confirmed in dried flowering aerial parts of *H. scabrum* samples collected in Iran (Morteza-Semnani *et al.* 2005). *H. dogonbadanicum* Assadi is another herbal shrub endemic to Iran (Dogonbadan Mountains). Its EO is rich in monoterpenes (91.8%; α-pinene 34.7%; β-pinene 32.1%), and poor in sesquiterpenes (2.1%) (Sajjadi *et al.* 2001).

Among the reported data for *Hypericum* spp. EOs, the linear hydrocarbons and hydrocarbon monoterpenes are generally the most significant compounds even if a large variability has to be taken into consideration even for the same species depending on the phenological state.

In general, pinenes, limonene and 2-methyl-octane had already been used several decades ago as target compounds to classify *Hypericum* spp. in different sections by Mathis and Ourissons (1964b).

It is important to point out that the *Hypericum* spp. EOs have been characterized in particular by their aliphatic hydrocarbons and derivatives (**Tables 1-3**), which are generally not present in such a large variety and yield in other aromatic plants (**Table 4**). Regarding the production of EOs from cultivated *Hypericum* spp., no comprehensive studies are reported in the literature apart from a study on *H. androsaemum* harvested in Portugal and to assess seasonal variation (Guedes *et al.* 2003). In this study, the EO yields obtained by the hydrodistillation of the aerial parts of these cultivated plant materials varied from 0.94 to 4.09 mg/g dw, depending on the time of harvest. Most of the volatile compounds were sesquiterpene hydrocarbons, corresponding to 43-78% of the total EO. The other compounds were hydrocarbon and oxygenated monoterpenes, hydrocarbon and oxygenated sesquiterpenes, *n*-alkanes and 1-alkenes. Even though the same agronomic protocol was performed, many differences in EO composition were found depending on harvest time. In fact, the EO sampled in November from the whole aerial parts was characterized by the highest levels of sesquiterpene hydrocarbons and a high number of *n*-alkanes and 1-alkenes, from C_{18} to C_{28}, whereas sampling in June showed the highest levels of *n*-nonane and 1-octene as well as monoterpene hydrocarbons. A significant percentage of the *H. androsaemum* plants harvested in November was mainly composed by three sesquiterpenes: β-caryophyllene (15.1%), α-gurjunene (15.5%), and γ-elemene (17.9%). These compounds were among the five major constituents of the EOs of plants harvested in July and June. Therefore, independently of the harvest time, the sesquiterpene hydrocarbons constituted the major compound group, accounting for >40-78% of the total EO. Despite the fact that the identity of the most of them was unknown, they were considered to be responsible for the specific essential oil olfactroscopic pattern of *H. androsaemum* L. (Nogueira *et al.* 1999) (**Tables 2, 4**).

In a following study on the leaves of *H. androsaemum* L. cultivated in the same region, Guedes *et al.* (2004) found for the EOs hydrodistilled from yields seasonally dependent ranges (0.7 to 3.4 mg/g dw) comparable with those obtained in the previous work. Furthermore, the trend in the sesquiterpene production was substantially confirmed: at the end of winter the EO was dominated by sesquiterpene hydrocarbons and accumulated a high number of intermediate to long chain *n*-alkanes and 1-alkenes.

However, few details about agronomic protocols were given and a comparison with the volatile composition of the corresponding wild plant material was not made in both those works.

Most of the studies on the EO production of *Hypericum* spp. during different phenological stages have been carried out on flowering plants as this is considered to be the balsamic period in the Pharmacopoeia Hyperici monograph. However, the collection period or the status of the analysed plant samples has not reported in some papers included in this review. In addition, few studies specified if the analysed flowering aerial parts also contained fruits or not. As *Hypericum* spp. are widespread all over the world and the flowering and fructification periods are very different in each country, an indication of the collection time is necessary to allow a real comparison among several results. Fur-

Table 4 The essential oil composition and the classification of *Hypericum* species proposed by Mathis and Ourissons (1964a, 1964b, 1964c, 1964d).

limonene (>10%)	no other predominant constituents	*H. canariense* L.	sect. *Webbia*
myrcene (+/-)*	pinenes	*H. androsaemum* L.	sect. *Androasaemum*
		H. elatum L.	
		H. chinense L.	sect. *Norysca*
		H. calycinum L.	sect. *Eremanthe*
	linear saturated hydrocarbons	*H. hircinum* L.	sect. *Androasaemum*
		H. inodorum Willd.	
		H. patulum Thunb.	sect. *Norysca*
		H. hookerianum Wight	
	other predominant constituents (mixture)	*H. prolificum* L.	sect. *Myriandra*
		H. kalmianum L.	
limonene (<5%) myrcene (+)	*n*-nonane (≥ 80%)	*H. ascyron* L.	sect. *Roscyna*
		H. gebleri L.	
	methyl-2-octane (≥ 30%)	*H. perforatum* L.	sect. *Euhypericum*
		H. olympicum L.	
		H. polyphyllum Boiss.	
	linear sature hydrocarbons (methyl-2-octane, +)	*H. tetrapterum* Fries	sect. *Euhypericum*
		H. undulatum Schousb.	
		H. quandrangulum L.	
		H. hirsutum L.	
		H. elegans Steph.	
		H. coris L.	
	pinenes (methyl-2-octane, -)	*H. montanum* L.	
		H. pulchrum L.	
		H. humifusum L.	
	other predominant constituents (mixture)	*H. orientale* L.	sect. *Euhypericum*
		H. tomentosum L.	
		H. atomarium Boiss.	
		H. degenii Bornm.	
		H. barbatum Jacq.	
		H. rumelicum Boiss.	
		H. rhodopeum Friv.	sect. *Campylopus*

* +/- = present or not

thermore, it is important to study the EOs composition related to other physiological or environmental aspects as very few studies were performed on the variability of EOs considering the same species in its phenological stages or seasonal variations or collected in different sites (Santos *et al.* 1999; Couladis *et al.* 2001; Petrakis *et al.* 2005; Nogueira *et al.* 2008; Maggi *et al.* 2010).

Morphological structures in *Hypericum* genus for the production of EOs

Morphologically, the genus *Hypericum* is characterized by the presence of different types of secretory structures including translucent glands, black nodules and secretory canals (Baroni Fornasiero *et al.* 1998; Bottega *et al.* 1999; Baroni Fornasiero *et al.* 2000; Ciccarelli *et al.* 2001; Łotocka and Osińska 2010). Not all of these structures are present in all *Hypericum* species and their presence and/or frequency vary among plant organs (Robson 1968). The secretory structures, which are sites of synthesis and/or accumulation of biologically active substances, are important for discrimination among taxa (Robson 1977, 1981; Pignatti 1982). EOs are synthesized either in translucent glands, or in secretory canals that may be localized in leaves, petals, sepals and pistil which have not be found at every stage of the developmental cycle (Ciccarelli *et al.* 2001). Recently, the anatomy and ultrastructure of internodes, leaves and petals were compared in *Hypericum elegans*, *H. inodorum*, *H. olympicum*, and *H. forrestii* as well as in two genotypes of *H. perforatum* (Łotocka and Osińska 2010). Significant differences were found in the content and composition of the EO in leaves, flowers and stems of these investigated species. The EO content ranged from traces to 0.35%. In most of the samples, the dominant constituents appeared to be 2-methyl-octane and αα-pinene. The content of 2-methyl-octane ranged from 12.33 to 39.43% and the content of α-pinene ranged from 1.07 to 16.42%. Other compounds present in appreciable concentrations were: α-terpineol (1.45-10.09%), β-pinene (0.61-8.90%), β-caryophyllene (0.92-9.73%) and α-humulene (1.05-3.67%). In addition, variations in the EO composition of *Hypericum* may be due to the different species and also within the same species of a population, chemotypes, geographic or climatic factors, collection time, plant organ, drying conditions, and extraction method. Schwob *et al.* (2004) found that the levels of monoterpenoids and aliphatic alcohols in *H. perforatum* EO varied with the phenological cycle and the number of compounds detected increased during ontogenesis. In the EOs obtained from the flowers and leaves in 11 accessions of *H. perforatum* EO, differences were not attributed to monoterpenoids, but to some sesquiterpenes (caryophyllene oxide, spathulenol, viridiflorol) and hydrocarbons (tetradecanal, tetradecanol). The concentrations of some sesquiterpenes such as β-caryophyllene and caryophyllene oxide varied greatly between leaves and flowers, higher in the former, whereas other oxygenated sesquiterpenes (spathulenol and viridiflorol) and oxygenated hydrocarbons (dodecanol and tetradecanol) were higher in the latter. Furthermore, the chemical variability of EOs can also result from genetic variability, since the influence of different environmental factors has been eliminated (Radusiene *et al.* 2005).

Volatile constituents and their chemotaxonomic significance in *Hypericum* genus

The genus *Hypericum* is the type genus of Hypericaceae, now usually included as a subfamily (Hypericoideae) in the Clusiaceae (= Guttiferae), and comprises more than 450 species divided in 36 sections (Robson 2001). The first important studies were carried out in France by Mathis and Ourissons (1964) with the investigation of several fresh *Hypericum* spp. samples collected in South-East France

(**Table 4**). This was the first attempt to classify the numerous *Hypericum* species into different sections by their volatile constituents.

Successive studies confirmed that *Hypericum* species are very rich in terpenes and terpenoids and they may play an important role in explaining their geographic distribution within the genus (Couladis *et al.* 2001, 2002, 2003). Recently, chemotaxonomic similarity and differences of nine *Hypericum* spp. collected in Serbia were showed by the EO composition (Smelcerovic *et al.* 2007). In this study, the contents of non-terpenes, mono- and sesquiterpenes of the species *H. barbatum*, *H. richeri* and *H. rumeliacum* (section Drosocaprium) were similar. The *H. hirsutum* and *H. linarioides* EOs (section Taeniocarpium) contained a high percentage of *n*-nonane, while *H. maculatum*, *H. perforatum* and *H. tetrapterum* (section *Hypericum*) were more homogeneous in non-terpene and sesquiterpene contents. The *H. olympicum* (section Olympia) EO differed from that of other EOs by its higher terpene content. The greatest similarity between the EO content and the sectional botanical classification (Robson 1977) was observed for the Drosocaprium section (*H. barbatum*, *H. richeri* and *H. rumeliacum*). In addition, the greatest similarity between the EO contents for different years was found for *H. maculatum H. olympicum* and *H. perforatum*. Furthermore, cluster analysis confirmed that both genetic and environmental factors play a role in determining the composition of EOs of nine *Hypericum* species collected in Serbia (Smelcerovic *et al.* 2007). Smelcerovic and Spiteller (2006) had already concluded that a stronger correlation (r=0.99) exists between Robson's sectional classification (Robson 1977) and flavonoid contents (hypericin, pseudohypericin, hyperforin, hyperoside and quercitrin) of some *Hypericum* spp. (*H. barbatum* Jacq., *H. hirsutum* L., *H. linarioides* Bosse, *H. maculatum* Crantz, *H. rumeliacum* Boiss. and *H. tetrapterum* Fries) in contrast to a rather low correlation with the corresponding EO constituents.

Nogueira *et al.* (2008) also contributed to an understanding of the chemotaxonomic significance of *Hypericum* spp. EOs by reporting the geographical distribution and analysis of EOs of wild Portuguese *Hypericum* spp. Belonging to three different sections: *H. perfoliatum* (section Drosocarpium), *H. humifusum* and *H. linarifolium* (section Oligostema), and *H. pulchrum* (section Taeniocarpium). Monoterpene hydrocarbons constituted the main fraction of all EOs (43-69, 53-85, 28-45 and 48-65% for *H. perfoliatum*, *H. humifusum*, *H. linarifolium* and *H. pulchrum*, respectively). On the other hand, sesquiterpene hydrocarbons (2-13, 6-18, 21-27 and 16-18%, respectively) and a third fraction of non-terpenic compounds (20-29, 3-16, 2-14 and 5-11%, respectively) were relatively high in the EOs. Furthermore, cluster and principal component analyses were very useful tools in the chemotaxonomical investigation of the four analysed species. They were based on a range of select specific EO constituents (α-pinene, β-pinene and *n*-nonane) despite the fact that all these species are rich in α-pinene: α-pinene (39-64%)/*n*-nonane (12-24%), β-pinene (2-3%) for *H. perfoliatum* (section Drosocarpium); α-pinene (45-77%), β-pinene (4-8%), *n*-nonane (n.d.-1%) for *H. humifusum* (section Oligostema); α-pinene (20-30%), β-pinene (5-11%), *n*-nonane (n.d.-1%) for *H. linarifolium* (section Oligostema); α-pinene (36-50%), β-pinene (9-13%), *n*-nonane (2-5%) for *H. pulchrum* (section Taeniocarpium). The results of this study also supported the taxonomical classification based on morphological characters by Robson (1977).

In addition, a previous study on two Greek populations of *H. perfoliatum* (Couladis *et al.* 2001; Petrakis *et al.* 2005) showed similar composition of EOs to that reported by Nogueira (2008) for the same species. In contrast, an Algerian sample (Touafek *et al.* 2005) of *H. perfoliatum* showed not only different percentages of the typical constituents such as α-pinene (0.5%) and γ-cadinol (19%), but also two components, thymol (22%) and 4,5-dimethyl-2-ethylphenol (13%), which are quite unusual for *Hypericum* spp. (Nogueira *et al.* 2002). Nogueira's investigations support the previous studies of Mathis and Ourisson (1964a, 1964b, 1964c) in which *H. humifusum* (section Oligostema) and *H. pulchrum* (section Taeniocarpium) were grouped in an α-pinene dominant group, but showed, in addition, that *H. linarifolium* (section Oligostema) and *H. perfoliatum* (section Drosocarpium) can also be included in this group (Nogueira *et al.* 2008).

The studies of Mathis and Ourissons continue to be an important step in the classification of *Hypericum* spp. Despite the fact that different locations, phenological phases, and extraction procedures were used, the EO composition of four species (*H. humifusum*, *H. pulchrum*, *H. linarifolium*, and *H. perfoliatum*) showed qualitative similarities that could be correlated with the taxonomical classification based on morphological characters. Three volatile constituents (α-pinene, β-pinene, and *n*-nonane) could be used to separate the four species into specific sections using PCA and cluster analysis (Nogueira *et al.* 2008).

Production of EOs by *Hypericum in vitro* cultures

The effectiveness of the St. John's wort phytocomplex has been focused, especially on extracts enriched in dianthrones, acylphloroglucinols, and flavonoids. Nowadays, hypericins and hyperforin are well known for their multitarget activities.

Although the biosynthesis of hypericins and hyperforin in St. John's wort have not yet been fully understood, the biotechnological aspects involved in regulating their production both in intact plants and *in vitro* cultures have been extensively elucidated over the past several years (Kirakosyan *et al.* 2008). Most of these studies were carried out to evaluate the effects of different concentrations of plant growth regulators, medium and *Agrobacterium* cultures on biomass and accumulation of hypericins, hyperforin, and flavonoids in several types of *H. perforatum in vitro* plant material (Gadzovska *et al*. 2005; Liu *et al.* 2007a, 2007b; Pavliäk *et al*. 2007; Danova *et al.* 2010). More recent investigations revealed that St. John's wort can be grown efficiently in large-scale sterile bioreactors for the production of hypericin, pseudohypericin and hyperforin (Zobayed *et al*. 2003, 2004; Cui *et al.* 2010).

On the other hand, very few studies have been reported in the literature on the production of volatile constituents by *in vitro* cultures of *Hypericum* species and consequentely on the potential of bioreactors.

To the best of our knowledge, Guedes *et al*. (2003, 2009) carried out the only research on the production of EOs by *in vivo* and *in vitro* plant material from *H. androsaemum*. In this study, the EO yield obtained by the hydrodistillation of *in vitro H. androsaemum* shoots (0.74 mg/g dw) was lower than the minimum value obtained from the cultivated plants. Either the different growth conditions or the immaturity of the *in vitro* shoots compared to those of *in vivo* plants may be responsible for the correspondingly low EO content. All the volatile constituents of *H. androsaemum* shoots were common to the EOs of *in vivo* plants. Sesquiterpene hydrocarbons were the major group, representing more than 80% of the total EO, a value higher than that of the same group from the *H. androsaemum* EOs of *in vivo* plants harvested in November 1999 when they were dominant. The *in vitro* typical sesquiterpenes, γ-muurolene (15.3%) and *E*-γ-bisabolene (10.8%) were not included among the five most represented constituents of the EOs extracted from *in vivo* plants (lower than 5%).

On the other hand, from a series of *n*-alkanes and 1-alkenes identified in *in vivo* plants, only *n*-hexacosane (0.2%) was found in Eos of *in vitro* plants. The quali- and quantitative differences between the EOs of *H. androsaemum in vitro* shoots and *in vivo* plants may be due to the immaturity of the shoots and/or the absence of elicitor factors (**Table 2**).

Guedes *et al.* (2003) considered the shoot stage as a practical point to compare *in vitro* and *in vivo* EO production for two important reasons: the development and envi-

ronment of this type of culture can be maintained under strict control; *in vitro* shoots or plantlets are the most suitable *in vitro* system models for studies on the metabolism of terpenes because they resemble the *in vivo* plants most closely.

The same authors carried out an investigation of the volatile constituents produced by *in vitro* plant material of *H. androsaemum* L., *H. perforatum* L. and *H. undulatum* Schousboe (Guedes *et al.* 2009; **Tables 1** and **2**). In this study, primary explants (apical buds and nodal segments) from *in vivo* plants were used to establish *in vitro* shoot cultures of *H. androsaemum* and *H. undulatum*, respectively. *H. perforatum* shoot cultures were established from nodal segments of axenic seedlings. EOs from *in vivo* plants and *in vitro* cultures of *H. androsaemum*, *H. perforatum* and *H. undulatum* were isolated by hydrodistillation in a Clevenger type apparatus and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

The most represented volatiles in the EO obtained from *in vivo* plants were sesquiterpene hydrocarbons (43-78%) and significant seasonal variations on their content were registered. Sesquiterpene hydrocarbons dominated the EO of leaves and stems of *H. androsaemum*, while monoterpene hydrocarbons dominated the EO of the ripened seed capsules.

(*E*)-β-caryophyllene, β-gurjunene and γ-elemene were the major compounds in the EO of *H. androsaemum*. Moreover, this species was characterized by a high number of intermediate to long chain *n*-alkanes and 1-alkenes. Almost 80% of the total EO hydrodistilled from *in vitro* shoots of *H. androsaemum* was represented by sesquiterpene hydrocarbons, with γ-elemene as the only major constituent common to the EO of cultivated plants. EOs from the aerial parts of *H. perforatum* plants, sampled over one year, revealed high levels of sesquiterpene hydrocarbons and low levels of oxygenated compounds. Germacrene D, (*E*)-β-caryophyllene and β-selinene were the major compounds. The highest EO content was found in flowers (12-17 $mg/g_{dry\ biomass}$), in which sesquiterpene hydrocarbons was the major compound group and 2-methyl-octane the most represented compound (22-29%).

Alkanes which represented no more than 9% of the total EO obtained from the corrispondent cultivated plants, was the second major group in the EO of *in vitro* shoots. In particular, *n*-nonane was accounted for more than 24% of the total EO. EOs of plants and *in vitro* shoots of *H. undulatum* Schousboe ex Willd had *n*-nonane as the major constituent (more than 40%). This compound was that most contributed for *n*-alkanes group even if sesquiterpene hydrocarbons constituted the dominant one. The highest yield of *H. undulatum* EO was obtained from leaves, followed by ripened seed capsules, flowers and stems. The EO contents observed in *in vitro H. undulatum* shoots (4-10 $mg/g_{dry\ weight}$) were higher than those observed in the aerial parts of field growing plants. An important issue highlighted by this study was that, although variations in the composition of the EO from shoots grown on two different basal media (MS basal medium and Mg basal medium) were registered, the group of alkanes was the major one independently of the culture conditions. However, the highest contents of *n*-nonane were recorded in the EO from shoots grown on Mg basal medium. In order to get hairy root cultures of *H. androsaemum*, *H. perforatum and H. undulatum*, the influence of several factors (effect of explant pre-culture, bacterial density, explant wounding, addition of acetosyringone to the bacterial suspension and co-culture medium, as well as co-culture period) were evaluated, using the *A. rhizogenes*-mediated transformation as the main approach. Several assays were performed, but the hairy roots production was not achieved in any of the tested explants (leaves, internodal segments and roots) (Guedes *et al*. 2009).

Taking into account these few data available in the literature on the production of EOs by *in vitro Hypericum* spp. (**Table 1, 2**), further studies are requested to define the real potentiality of the different types of *in vitro* plant material dedicated to the production of EOs and specific volatiles.

BIOLOGICAL ACTIVITIES OF *HYPERICUM* ESSENTIAL OILS

Several compounds have been isolated and identified from the *Hypericum* genus and many species are widely used in folk medicine. Important pharmacological proprieties have been attributed to *H. perforatum* extract as antidepressive agents (Bombardelli *et al.* 1995; Linde 1996; Shelton 2009; Wang *et al.* 2010).

H. perforatum has been the most investigated, but also other related species in the genus have been shown to possess antiviral, wound-healing, antioxidant, cytotoxic, antimicrobial, antifungal, anxiolytic and anticonvulsant activities (Bombardelli and Morazzoni 1995; Vandenbogaerde *et al.* 2000; Butterweck 2003; Couladis *et al.* 2003; Cakir *et al.* 2005; Skalkos *et al.* 2005; Toker *et al.* 2006; Sevim *et al.* 2010). These actions were attributed especially to the different identified chemical classes of constituents: phloroglucinols (hyperforins), naphtodianthrones (hypericins), flavonoids, xanthones, tannins (Bombardelli and Morazzoni 1995; Kitanov 2001).

The research on the biological activities of *Hypericum* EOs, as for many other aromatic plants, have been dedicated especially to their antibacterial and antifungal properties which are considered useful as potential phytomedicines, or important natural food preservatives alternative to synthetic substances without resistant problems (Avato *et al.* 2005; Chorianopoulos *et al.* 2007; Saddiqe *et al.* 2010; Sevim *et al.* 2010).

Antibacterial and antifungal activity

Many recent examples of antibacterial or antifungal activities of EOs can be found in the *Hypericum* genus, not only for *H. perforatum*. In fact, several *Hypericum* species native to different regions have been investigated on several types of bacteria and fungi.

The EOs obtained from *Hypericum maculatum* Crantz (Serbia) showed a large spectrum and a strong activity as antimicrobiological agent especially against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Sarcina lutea*, as well as antifungal against *Aspergillus niger* and *Candida albicans* (Gudzic *et al.* 2002). The EO hydrodistilled from the aerial parts of *Hypericum linarioides* Bosse was characterized by high content of sesquiterpenes (64.2%). It contained mainly δ-cadinene (6.9%), (*Z*)-β-farnesene (5.2%), γ-muurolene (5.5%), spathulenol (4.8%), hexahydrofarnesyl acetone (4.5%) and α-selinene (4.0%). The oil was tested for antifungal activity using mycelial growth inhibition *in vitro* assays against 11 agricultural pathogenic fungi, which consisted of six *Fusarium* species (*Fusarium acuminatum*, *Fusarium culmorum*, *Fusarium equiseti*, *Fusarium oxysporum*, *Fusarium sambucinum* and *Fusarium solani*) and three anastomosis groups of *Rhizoctonia solani* (AG-5, AG-9 and AG-11), *Alternaria solani* and *Verticillium albo-atrum*. The *H. linarioides* EO showed significant antifungal activity especially against AG-9 and *V. albo-atrum* (Cakir *et al.* 2005).

The EO of the aerial parts of *Hypericum rumeliacum* was dominated by the monoterpenes α-pinene (43.8%) and β-pinene (9.8%) and exhibited moderate activities (MIC values 3.80–17.20 mg/ml) against both Gram-negative (*Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*) (Couladis *et al.* 2003). The oil showed the strongest activity against the Gram-negative strain of *E. coli*, while *E. cloaceae* appeared to be the most resistant. The antibacterial properties of the oil could be associated with the high percentage of α-pinene and β-pinene, which are known to possess strong antibacterial activities (Couladis *et al.* 2000). Furthermore, the *Hypericum rumeliacum* oil exhibited a

stronger activity against the pathogenic fungi *Candida albicans*, *C. tropicalis* and *C. glabrata* (MIC values 4.75–6.34 mg/ml) (Couladis *et al.* 2003). The volatile constituents of *H. cordatum* (fresh leaves) were isolated by hydrodistillation. The main components of the EO were myrcene (40.18%), α-pinene (16.40%), and limonene (12%). The antibacterial activities of the EO were tested against *Saccharomyces aureus*, *E. coli* and the anti-fungal activities against *Cladosporium cladosporioides* and *C. sphaerospermum*. The EO showed antibacterial activity especially against the bacterium *S. aureus* and anti-fungal activity against both tested fungi (Ladeira *et al.* 2009). The chemical composition of *H. elongatum* EO and its biological activities were investigated for the first time by Ghasemi (2007).

The EO showed a very high content of terpene hydrocarbons and the most of amount was due to the presence of monoterpene hydrocarbons (90% and in particular to (α + β) Pinene (83% of total known oil) It showed significant antimicrobial activity both on Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Enterococcus faecali*) and Gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*). No significant activity were registered against the fungi *Aspergillus niger* and *Aspergillus fumigatus* (Ghasemi *et al.* 2007). The EOs of six Serbian *Hypericum* species (*Hypericum alpinum*, *Hypericum barbatum*, *Hypericum rumeliacum*, *Hypericum maculatum*, *Hypericum perforatum*, *Hypericum hirsutum*) has been tested on several bacteria (Gram-negative, Gram-positive) and one fungus (*Candida albicans*) (Saroglou *et al.* 2007). Among the investigated species, *H. barbatum* EO was the most active, while the EOs of *H. alpinum* and *H. hirsutum* were inactive against the clinical species of *Pseudomonas mirabilis* and *Psudomonas aeruginosa*. The reduced or missing activity of *H. hirsutum* EO against the tested microorganisms could be attributed to its high content in aliphatics (52.1%). In fact, Griffin *et al.* (2000) showed that hydrocarbons tend to be relatively inactive because of their limited hydrogen capacity and water solubility.

Ketones, aldehydes and alcohols were found more active, but with differing specificity and levels of activity, depending on the functional group and also the hydrogen-bonding parameters (Griffin *et al.* 2000). Previous results on the EOs of some Mediterranean Lamiaceae (*Satureja montana* L., *Rosmarinus officinalis* L., *Thymus vulgaris* L., and *Calamintha nepeta* L. Savi, *Origanum vulgare*, *Mentha spicata*, *Lavandula angustifolia*, *Salvia fruticosa*) showed that greater antimicrobial potential could be ascribed to the EOs enriched in oxygenated terpenes (Panizzi *et al.* 1993; Adam *et al.* 1998).

The composition of the hydrodistilled EOs obtained from the aerial parts of *H. hyssopifolium* subsp. *elongatum* var. *elongatum* and *H. heterophyllum* were analyzed and tested for *in vitro* antifungal activity against 10 agricultural pathogenic fungi, which consisted of five *Fusarium* species (*F. oxysporum*, *F. culmorum*, *F. sambucinum*, *F. solani and F. acuminatum*) and five anastomosis groups of *Rhizoctonia solani* (AG-3, AG-4, AG-5, AG-9 and AG-11). The most significant results were obtained against AG-11 by *H. heterophyllum* EO. In addition, the antifungal activity of 13 pure major components in the EOs of *Hypericum* species was studied against these fungal species. Among these compounds, β-caryophyllene oxide exhibited a significant inhibition effect (range 33–85%) on the growth of all tested pathogenic fungi.

In particular, this compound also displayed a greater inhibition effect on the anastomosis groups of *R. solani* than on the *Fusarium* species. Likewise, α-terpineol showed an important activity on the growth of all anastomosis groups of *R. solani*. However, α-terpineol was not active against *Fusarium* species, except for *F. sambucinum* (48%).

The total oils of *H. hyssopifolium* and *H. heterophyllum* showed a moderate antifungal activity against the growth of some fungal species. No significant correlation between the activity and the percentage of some their major EOs components was pointed out. For example, while none of the pure compounds, except β-caryophyllene oxide, showed activity against *Fusarium* species, both of the oils had a moderate activity against *F. acuminatum.* Although the antifungal activity of an EO can be attributed mainly to its major compounds, the synergistic and antagonistic effect of one compound in minor percentage in the mixture should be taken into account. Among the pure compounds, β-caryophyllene oxide significantly inhibited the growth of all fungi. Although the inhibitory effects assayed for β-caryophyllene oxide were lower than that for commercial antifungal reagent (Benomyl), the fact that it showed activity against all fungi species was a significant finding. Therefore, β-caryophyllene oxide and/or the EOs containing a high proportion of this compound may be used as antifungal reagents to protect plants against fungal diseases (Cakir *et al.* 2004).

H. perforatum EO showed potent antifungal activity against *Trichophyton mentagrophytes* (dermatophyte), but no or slight activity was observed with the herbal teas (Inouye 2008). The EO of St. John's wort growing in Bulgaria was as effective as antibiotics currently applied in clinical practice against food Gram-positive and Gram-negative bacteria. *Staphylococcus aureus* was the most susceptible, but *Pseudomonas aeruginosa* was the most resistant to the EO (Gochev *et al.* 2008).

CONCLUDING REMARKS

Hypericum perforatum and the other species in this genus have been investigated in depth especially for their naphtodiantrones (hypericins), phloroglucinols (hyperforin) and flavonoids.

On the other hand, the EOs of many wild or cultivated *Hypericum* species have been studied only more recently. In these studies, variations in the typical EO constituents are related to plant organ, genetic, enviromental and seasonal factors.

However, improvement in the knowledge of EO constituents of *Hypericum* species is important to achieve two important goals:

- to define the chemotaxonomy of such a large number of species.
- to study the relationship between biological activities and EOs.

Furthermore, the establishment of *in vitro* cultures from *Hypericum* species represents another potential approach to standardize plant material not only with regards to flavonoids, hypericins, and hyperforin, but also other significant bioactive metabolites such as volatile constituents.

REFERENCES

Abreu IN, Porto ALM, Marsaioli AJ, Mazzafera P (2004) Distribution of bioactive substances from *Hypericum brasiliense* during plant growth. *Plant Science* **167**, 949-954

Adam K, Sivropoulou A, Kokkini S, Lanaras T, Arsenakis M (1998) Antifungal activities of *Origanum vulgare* subsp. hirtum, *Mentha spicata*, *Lavandula angustifolia* and *Salvia fruticosa* essential oils against human pathogenic fungi. *Journal of Agricultural and Food Chemistry* **46**, 1738-1745

Adams RP (2001) *Identification of Essential Oil Components by Gas Chromatography/Quadruple Mass Spectroscopy*, Allured Publishing, Carol Stream, IL, 455 pp

Adams RP (1989) *Identification of Essential Oils by Ion Trap Mass Spectroscopy*, Academic Press, San Diego, CA, 302 pp

Apajalahti J, Kettunen A (2006) Rational development of novel microbial modulators. In: Barug D, De Jong J, Kies AK, Verstegen MWA (Eds) *Antimicrobial Growth Promoters. Where do we go from here?* Wageningen Academic Publishers, Wageningen, pp 165-181

Arras G, Usai M (2001) Fungitoxic activity of 12 essential oils against four postharvest *Citrus* pathogens: chemical analysis of *Thymus capitatus* oil and its effect in subatmospheric pressure conditions. *Journal of Food Protection* **64**, 1025-1029

Atsumi T, Fujisawa S, Tonosaki K (2005) A comparative study of the antioxidant/prooxidant activities of eugenol and isoeugenol with various concentrations and oxidation conditions. *Toxicology in Vitro* **19**, 1025-1033

Avato P (2005) A survey on the *Hypericum* genus: Secondary metabolites and bioactivity. *Studies in Natural Products Chemistry* **30**, 603-634
Bakkali F, Averbecka S, Averbecka D, Idaomar M (2008) Biological effects of essential oils – A review. *Food and Chemical Toxicology* **46**, 446-475
Barnes J, Anderson LA, Phillipson JD (2001) St. John's wort (*Hypericum perforatum* L.): A review of its chemistry, pharmacology and clinical properties. *Journal of Pharmacy and Pharmacology* **53**, 583-600
Baroni Fornasiero R, Bianchi A, Pinetti A (1998) Anatomical and ultrastructural observations in *Hypericum perforatum* L. leaves. *Journal of Herbs, Spi-ces and Medicinal Plants* **5**, 21-33
Baser KHC, Ozek T, Nuriddinov HR, Demirci AB (2002) Essential oils of two *Hypericum* species from Uzbekistan. *Chemistry of Natural Compounds* **38**, 54-57
Bertoli A, Menichini F, Mazzetti M, Spinelli G, Morelli I (2003) Volatile constituents of the leaves and flowers of *Hypericum triquetrifolium* Turra. *Flavour and Fragrance Journal* **18**, 91-94
Bertoli A, Pistelli L, Morelli I, Spinelli G, Manichini F (2000) Constituens of *Hypericum hircinum* oils. *Journal of Essential Oil Research* **12**, 617-620
Bombardelli E, Morazzoni P (1995) *Hypericum perforatum*. *Fitoterapia* **66**, 43-68
Boonchird C, Flegel TW (1982) *In vitro* antifungal activity of eugenol and vanillin against *Candida albicans* and *Cryptococcus neoformans*. *Canadian Journal of Microbiology* **28**, 1235-1241
Bottega S, Garbari F, Pagni AM (1999) Secretory structures in *Hypericum elodes* L. (Hypericaceae). I. Preliminary observations. *Atti della Società Toscana di Scienze Naturali Memorie Serie B* **106**, 93-98
Bowler PG, Duerden BI, Armstrong DG (2001) Wound microbiology and associated approaches to wound management. *Clinical Microbiology Reviews* **14**, 244-269
Brolis M, Gabetta B, Fuzzati N, Pace R, Panzeri F, Peterlongo F (1998) Identification by high-performance liquid chromatography-diode array detection-mass spectrometry and quantification by high-performance liquid chromatography-UV absorbance detection of active constituents of *Hypericum perforatum*. *Journal* of *Chromatography A* **825**, 9-16
Brul S, Coote P (1999) Preservative agents in foods mode of action and microbial resistance mechanisms. *International Journal of Food Microbiology* **50**, 1-17
Bruneton J (2000) *Pharmacognosy, Phytochemistry and Medicinal Plants* (3rd Edn), Paris, Intercept-Lavoisier
Burt S (2004) Essential oils: Their antibacterial properties and potential applications in foods – a review. *International Journal of Food Microbiology* **94**, 223-253
Butterweck V (2003) Mechanism of action of St John's wort in depression: What is known? *CNS Drugs* **17**, 539-562
Butterweck V, Petereit F, Winthrhoff H, Nahrstedt A (1998) Solubilized hypericin and pseudohypericin from *Hypericum perforatum* exert antidepressant activity in the forced swimming test. *Planta Medica* **64**, 1121-1122
Cakir A, Duru ME, Harmandar M, Ciriminna R, Passannati S, Piozzi F (1997) Comparison of the volatile oils of *Hypericum scabrum* L. and *Hypericum perforatum* in Turkey. *Flavour Fragrance Journal* **12**, 285-287
Cakir A, Kordali S, Zengin H, Hirata T (2004) Composition and antifungal activity of essential oils isolated from *Hypericum hyssofilium* and *Hypericum heterophyllum*. *Flavour and Fragrance Journal* **19**, 62-68
Cakir A, Kordali S, Kilic H, Kaya E (2005) Antifungal properties of essential oil and crude extracts of *Hypericum linarioides* Bosse. *Biochemical Systematics and Ecology* **33**, 245-256
Cal K (2006) Skin penetration of terpenes from essential oils and topical vehicles. *Planta Medica* **72**, 311-316
Canning S, Waterman M, Orsi N, Ayres J, Simpson N, Dye L (2010) The efficacy of *Hypericum perforatum* (St John's Wort) for the treatment of Premenstrual Syndrome: A randomized, double-blind, placebo-controlled trial. *CNS Drugs* **24**, 207-225
Caplin JL, Allan I, Hanlon GW (2009) Enhancing the *in vitro* activity of *Thymus* essential oils against *Staphylococcus aureus* by blending oils from specific cultivars *International Journal of Essential Oil Therapeutics* **3**, 35-39
Cardona ML, Marco JA, Sendra JM, Seoane E, Torres Ibañez J (1983) Waxes and volatile oils in *Hypericum ericoides* (Guttiferae). *Lipids* **18**, 439-442
Carson CF, Riley TV, Cookson BD (1998) Efficacy and safety of tea tree oil as a topical antimicrobial agent. *Journal of Hospital Infection* **40**, 175-178
Carson CF, Riley TV (2003) Non-antibiotic therapies for infectious diseases. *Communicable Diseases Intelligence* **27**, S143-S146
Chen F, Shi Z, Neoh KG, Kang ET (2009) Antioxidant and antibacterial activities of eugenol and carvacrol-grafted chitosan nanoparticles. *Biotechnology and Bioengineering* **104**, 30-39
Cheng S-S, Liu J-Y, Chang E-H, Chang S-T (2008) Antifungal activity of cinnamaldehyde and eugenol congeners against wood-rot fungi *Bioresource Technology* **99**, 5145-5149
Chialva F, Gabri G, Liddle PAP, Ulian F (1981) Indagine sulla composizione dell' olio essenziale di *Hypericum perforatum* L. e di *Teucrium chamaedrys* L. *Rivista Italiana E.P.P.O.S.* **LXIII**, 286-288
Chorianopoulos NG, Evergetis ET, Aligiannis N, Mitakou S, Nychas GJE, Haroutounian SA (2007) Correlation between chemical composition of Greek essential oils and their antibacterial activity against food-borne pathogens. *Natural Product Communications* **2 (4)**, 419-426
Ciccarelli D, Andreucci AC, Pagni AM (2001) Translucent glands and secretory canals in *Hypericum perforatum* L. (Hypericaceae): Morphological, anatomical and histochemical studies during the course of ontogenesis. *Annals of Botany* **88**, 637-644
Çırak C (2007) Seeds for *ex situ* conservation of some *Hypericum* species from Turkey. *American Journal of Plant Physiology* **2**, 287-294
Çırak C, Bertoli A, Pistelli L, Seyis F (2010) Essential oil composition and variability of *Hypericum perforatum* from wild populations of northern Turkey. *Pharmaceutical Biology* **48**, 906-914
Conner DE (1993) Naturally occurring compounds. In: Davidson PM, Branen AL (Eds) *Antimicrobials in Foods*, New York: Marcel Dekker, pp 441-468
Costa TR, Fernandes FLF, Santos SC, Oliveria CMA, Liao LM, Ferri PH, Paulo JR, Ferreira HD, Sales BHN, Silva MRR (2000) Antifungal activity of volatile constituents of *Eugenia dysenterica* leaf oil. *Journal of Ethnopharmcology* **72 (2)**, 111-117
Couladis M, Tanimanidis A, Tzakou O (2000) Essential oil of *Pholomis lanata* growing in Greece: Chemical composition and antimicrobial activity. *Planta Medica* **66**, 670-672
Couladis M, Badisa RB, Baziou P (2002) Antioxidant and cytotoxic activities of *Hypericum* sp. on brine shrimps and human cancer cell lines. *Phytotherapy Research* **16 (8)**, 719-722
Couladis M, Baziou P, Petrakis PV, Harvala C (2001) Essential oil composition of *Hypericum perfoliatum* L. growing in different locations in Greece. *Flavour and Fragrance Journal* **16**, 204-206
Couladis M, Chinou IB, Tzakou O, Petrakis PV (2003) Composition and antimicrobial activity of the essential oil of *Hypericum rumeliacum* subsp. *apollinis* (Boiss. & Heldr.). *Phytotherapy Research* **17**, 152-154
Cowan MM (1999) Plant products as antimicrobial agents. *Clinical Microbiology Reviews* **12**, 564-582
Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, Wyllie SG (2000) The mode of antimicrobial action of essential oil of *Melaleuca alternifolia* (tea tree oil). *Journal of Applied Microbiology* **88**, 170-175
Cui XH, Chakrabarty D, Lee EJ, Paek KY (2010) Production of adventitious roots and secondary metabolites by *Hypericum perforatum* L. in a bioreactor. *Bioresource Technology* **101**, 4708-4716
Danova K, Čellárová E, Macková A, Daxnerová Z, Kapchina-Toteva V (2010) *In vitro* culture of *Hypericum rumeliacum* Boiss. and production of phenolics and flavonoids. *In Vitro Cellular and Devevelopmental Biology – Plant* **8**, 1-8
Deans SG, Ritchie GA (1987) Antimicrobial properties of plant essential oils. *International Journal of Food Microbiology* **5**, 165-180
Demirci B, Baser KHC, Crockett SL, Khan IA (2005) Analysis of the volatile constituents of Asian *Hypericum* L. (Clusiaceae, Hyperidoideae) species. *Journal of Essential Oil Research* **17**, 659-663
Denny EFK (1991) *Field Distillation for Herbaceous Oils* (2nd Edn), Denny McKenzie Associates, Lilydale (Tasmania), Australia, 266 pp
Devi KP, Nisha SA, Sakthivel R, Pandian SK (2010) Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. *Journal of Ethnopharmacology* **130**, 107-115
European Medicines Agency (2009) Work programme 2010 of the European Medicines Agency, EMA/MB/203131/2009. Available online: www.ema.europa.eu
Erken S, Malyer H, Demirci F, Demirci B, Baser KHC (2001) Chemical investigations on some *Hypericum* species growing in Turkey. *Chemistry of Natural Compounds* **37**, 5
Fegert JM, Kölch M, Magno Zito J, Glaeske G, Janhsen K (2006) Antidepressant Use in Children and Adolescents in Germany. *Journal of Child and Adolescent Psychopharmacology* **16**, 197-206
Ferraz ABF, Limberger RP, Bordignon SAL, von Poser GL, Henriques AT (2005) Essential oil composition of six *Hypericum* species from southern Brazil. *Flavour and Fragrance Journal* **20**, 335-339
Ferretti G, Maggi F, Tirillini B (2005) Essential oil composition of *Hypericum richeri* Vill. from Italy. *Flavour and Fragrance Journal* **20**, 295-298
Figueiredo, AC, Barroso JG, Pedro LG, Scheffer JJC (1997) Physiological aspects of essential oil production. In: *Basic and Applied Research*, Allured Publishing Corp., Carol Stream, IL, pp 95-107
Franzios G, Mirotsou M, Hatziapostolou E, Kral J, Scouras ZG, Mavragani-Tsipidou P (1997) Insecticidal and genotoxic activities of mint essential oils. *Journal of Agricultural and Food Chemistry* **45**, 2690-2694
Fujisawa S, Atsumi T, Kadoma Y, Sakagami H (2002) Antioxidant and prooxidant action of eugenol-related compounds and their cytotoxicity. *Toxicology* **177**, 39-54
Gadzovska S, Maury S, Ounnar S Righezza M, Kascakova S, Refregiers M, Spasenoski M, Joseph C, Hagège D (2005) Identification and quantification of hypericin and pseudohypericinin different *Hypericum perforatum* L. *in vitro* cultures. *Plant Physiology and Biochemistry* **43**, 591-601
Ghasemi Y, Khalaj A, Mohagheghzadeh A, Khosravi AR, Morowvat MH (2007) Composition and antimicrobial activity of the essential oil and extract of *Hypericum elongatum*. *Journal of Applied Sciences* **7 (18)**, 2671-2675
Gochev V, Stoyanova A, Atanasova T, Atanasova T (2008) Antimicrobial activity of essential oil of St. John's Wort (*Hypericum perforatum* L.) grow-

ing in Bulgaria. *Plovdiv* **55 (1)**, 239-244
Gould GW (1996) Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. *Journal of Food Protection* **13**, 82-86
Griffin GS, Markham LJ, Leach ND (2000) An agar dilution method for the determination of the minimum inhibitory concentration of essential oils. *Journal of Essential Oil Research* **12**, 149-255
Gudzic B, Djokovic D, Vajs V, Palic R, Stojanovic G (2002) Composition and antimicrobial activity of the essential oil of *Hypericum maculatum* Crantz. *Flavour and Fragrance Journal* **17**, 392-394
Gudzic B, Dordevic S, Nedeljkovic J, Smelcerovic A (2004) Essential oil composition of *Hypericum atomarium* Boiss. *Chemical Industry* **58**, 413-415
Gudzic B, Dordevic S, Palic R, Stojanovic G (2001) Essential oils *Hypericum olympicum* L. and *Hypericum perforatum* L. *Flavour and Fragrance Journal* **16**, 201-203
Guedes AP, Amorim LR, Vicente AMS, Ramos G, Fernandes-Ferriera M (2003) Essential oils from plants and *in vitro* shoots of *Hypericum androsaemum* L. *Journal of Agricultural Food Chemistry* **51**, 1399-1404
Guedes AP, Amorim LR, Vicente A, Fernandes-Ferreira M (2004) Variation of the essential oil content and composition in leaves from cultivated plants of *Hypericum androsaemum* L. *Phytochemical Analysis* **15**, 146-151
Guedes AP (2009) Essential oils from plants and *in vitro* shoot cultures of *Hypericum androsaemum* L., *H. perforatum* L. and *H. undulatum* Schousboe ex. Wild. PhD thesis, Universidade do Minho. Available online: http://hdl.handle.net/1822/9876
Hallahan DL (2000) Monoterpenoid biosynthesis in glandular trichomes of labiate plants. *Advances in Botanical Research* **31**, 77-120
Harrison RA, Holt D, Pattison DJ, Elton PJ (2004) Who and how many people are taking herbal supplements? *International Journal Vitamin and Nutrition Research* **74**, 183
Hass L (2010) Losing touch in the era of superbugs? *Annals of Family Medicine* **8**, 461-463
Hartman PE, Shankel DM (1990) Antimutagens and anticarcinogens: a survey of putative interceptor molecules. *Environmental Molecular Mutagenesis* **15**, 145-182
Helander IM, Alakomi HL, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ (1998) Characterization of the action of selected essential oil components on Gram-negative bacteria. *Journal of Agricultural and Food Chemistry* **46**, 3590-3595
Hosni K, Msaada K, Taarit MB, Chahed T, Kallel M, Marzouk B (2007) Essential oil composition of *Hypericum triquetrifolium* Turra aerial parts. *Italian Journal of Biochemestry* **56**, 40-46
Hosni K, Msaada K, Taarit MB, Ouchikha O, Kallel M, Marzouk B (2008) Essential oil composition of *Hypericum perfoliatum* L. and *Hypericum tomentosum* L. growing wild in Tunisia. *Industrial Crops and Products* **27**, 308-314
Inouye S, Goi H, Miyauchi K, Muraki S, Ogihara M, Iwanami Y (1983) Inhibitory effect of volatile constituents of plants on the proliferation of bacteria. *Journal of Antibacterial and Antifungal Agents* **11**, 609-615
Inouye S, Takahashi M, Abe S (2008) Anti-trichophyton activity of hydrosols, herbal teas and related essential oils. *International Journal of Essential Oil Therapeutics* **2 (4)**, 139-144
Isabel B, Santos Y (2009) Effects of dietary organic acids and essential oils on growth performance and carcass characteristics of broiler chickens. *Journal of Applied Poultry Research* **18**, 472-476
Javidnia K, Miri R, Soltani M, Gholami M, Khosravi AR (2008) Essential oil composition of four *Hypericum* species from Iran. *Chemistry of Natural Compounds* **44**, 374-377
Jennings W, Shibamoto T (1980) *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*, Academic Press, New York
Juteau F, Masotti V, Bessiere JM, Viano J (2002) Compositional characteristics of the essential oil of *Artemisia campestris* var. *glutinosa*. *Biochemistry, Systematics and Ecology* **11**, 1065-1070
Kapperud G, Gustavsen S, Hellesnes I, Hansen AH, Lassen J, Hirn J (1990) Outbreak of *Salmonella typhimurium* infection traced to contaminated chocolate and caused by a strain lacking the 60-megadalton virulence plasmid. *Journal of Clinical Microbiology* **28**, 2597-2601
Kotzekidou P, Giannakidis P, Boulamatsis A (2008) Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens *in vitro* and on the fate of inoculated pathogens in chocolate. *LWT - Food Science and Technology* **41**, 119-127
Kim J, Marshall MR, Wei C (1995) Antibacterial activity of some essential oil components against five foodborne pathogens. *Journal of Agricultural and Food Chemistry* **43**, 2839-2845
Kirakosyan A, Gibson DM, Kaufman PB (2008) The production of dianthrones and phloroglucinol derivatives in St. John's Wort. In: Ramawat KG, Merillon J (Eds) *Bioactive Molecules and Medicinal Plants*, XXIV Springer, Berlin, pp 149-162
Kitanov GM (2001) Hypericin and pseudohypericin in some *Hypericum* species. *Biochemistry and Systematic Ecology* **29**, 171-178
Kizil G, Toker Z, Ozen HC, Aytekin C (2004) The antimicrobial activity of essential oils of *Hypericum scabrum*, *Hypericum scabroides* and *Hypericum triquetrifolium*. *Phytotherapy Research* **18 (4)**, 339-341
Knobloch K, Pauli A, Iberl B, Weigand H, Weis N (1989) Antibacterial and antifungal properties of essential oil components. *Journal of Essential Oil Research* **1**, 119-128
Ladeira AM, da Silva GB, Raggi L, Young MCM, Agripino DG, Lima MEL, Moreno Paulo RH (2009) Chemical composition and antimicrobial activities of the essential oil of *Hypericum cordatum*. *Journal of Essential Oil Research* **21**, 558-560
Linde K, Ramirez G (1996) St. John's Wort for depression – an overview and meta-analysis of randomized clinical trials. *British Medical Journal* **313**, 253-258
Linde K (2010) *Hypericum* extract worse than placebo in a trial in irritable bowel syndrome patients. *Focus on Alternative and Complementary Therapies* **15**, 242-243
Liu X-N, Zhang X-Q, Zhang S-X, Sun J-S (2007a) Regulation of metabolite production by precursors and elicitors in liquid cultures of *Hypericum perforatum*. *Plant Cell, Tissue and Organ Culture* **91**, 1-7
Liu X-N, Zhang X-Q, Sun J-S (2007b) Effects of cytokinins and elicitors on the productionof hypericins and hyperforin metabolites in *Hypericum sampsonii* and *Hypericum perforatum*. *Plant Growth Regulation* **53**, 207-214
Lo Cantore P, Shanmugaiah V, Iacobellis NS (2009) Antibacterial activity of essential oil components and their potential use in seed disinfection. *Journal of Agriculture and Food Chemistry* **28**, 9454-9461
Łotocka B, Osińska E (2010) Shoot anatomy and secretory structures in *Hypericum* species (Hypericaceae). *Botanical Journal of the Linnean Society* **163**, 70-86
Maggi F, Cecchini C, Cresci A, Coman MM, Tirillini B, Sagratini G, Papa F, Vittori S (2010) Chemical composition and antimicrobial activity of the essential oils from several *Hypericum* taxa (Guttiferae) growing in central Italy (Appennino Umbro-Marchigiano). *Chemistry and Biodiversity* **7**, 447-466
Mahmoud LE (1994) Antifungal action and antia: Atoxigenic properties of some essential oil constituents. *Letters in Applied Microbiology* **19**, 110-113
Manohar V, Ingram C, Gray J, Talpur NA, Echard BW, Bagchi D, Preuss HG (2001) Antifungal activities of origanum oil against *Candida albicans*. *Molecular and Cellular Biochemistry* **228**, 111-117
Masotti V, Juteau F, Bessiere JM, Viano J (2003) Seasonal and phenological variations of the essential oil from the narrow endemic species *Artemisia molinieri* and its biological activities. *Journal of Agricultural Food Chemistry* **51**, 7115-7121
Massada Y (1976) *Analysis of Essential Oils by Gas Chromatography and Mass Spectrometrometry*, John Wiley & Sons, New York
Mathew S, Thomas G, Ahmad T (2010) An evaluation on the impact of fungi on the post-harvested stored wheat grains. *International Journal of Biotechnology and Biochemistry* **6**, 995-1002
Mathis C, Ourisson G (1963) Etude chimio-taxonomique du genre *Hypericum* I. Repartition de l'Hypericine. *Phytochemistry* **2**, 157-171
Mathis C, Ourisson G (1964a) Etude chimio-taxonomique du genre *Hypericum* II. Identification de constituants de diverses huiles essentielles d'*Hypericum*. *Phytochemistry* **3**, 115-131
Mathis C, Ourisson G (1964b) Etude chimio-taxonomique du genre *Hypericum* III. Repartition des carbures satures et des monoterpenes dans les huiles essentielles d'*Hypericum*. *Phytochemistry* **3**, 133-141
Mathis C, Ourisson G (1964c) Etude chimio-taxonomique du genre *Hypericum* IV. Repartition des sesquiterpenes, des alcools monoterpeniques et des aldehydes satures dans les huiles essentielles d' *Hypericum*. *Phytochemistry* **3**, 377-378
Mathis C, Ourisson G (1964d) Etude chimio-taxonomique du genre *Hypericum* V. Identification de quelques constituants non volatils d' *Hypericum perforatum*. *Phytochemistry* **3**, 379
Mimica-Dukic N, Ivanec-Tumbas I, Igic R, Popovic M, Gasic O (1997) The content and composition of essential oil of *Hypericum perforatum* from Serbia. *Pharmaceutical and Pharmacological Letters* **8**, 26-28
Mishra AS, Dubey NK (1994) Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Applied and Environmental Microbiology* **60**, 1101-1105
Mockutė D, Bernotienė G, Judžentienė A (2003) Volatile compounds of the aerial parts of wild St. John's wort (*Hypericum perforatum* L.) plants. *Chemija* **14**, 108-111
Moleyar V, Narasimham P (1986) Antifungal activity of some essential oil components, *Food Microbiology* **3**, 331-336
Nielsen PV, Rios R (2000) Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. *International Journal of Food Microbiology* **60**, 219-229
Nederostova L, Kloucek P, Kokoska L, Stolcova M, Pulkrabek J (2009) Antimicrobial properties of selected essential oils in vapour phase against foodborne bacteria. *Food Control* **20**, 157-160
Nogueira T, Curto MJ, Figueiredo AC, Barroso JG, Luis GP, Rubiolo P, Bicchi C (2008) Chemotaxonomy of *Hypericum* genus from Portugal: Geographical distribution and essential oils composition of *Hypericum perfoliatum*, *Hypericum humifusum*, *Hypericum linarifolium* and *Hypericum pulchrum*. *Biochemical Systematics and Ecology* **36**, 40-50
Nogueira T, Duarte F, Tavares R, Curto MJM, Capelo J, Freitas AC (1999) Comparative study of the aromas of *Hypericum* L. species from Portugal

using olfactroscopy. *Flavour and Fragrance Journal* ***14***, 195-199

Obach RS (2000) Inhibition of human cytochrome p450 enzymes by constituents of St. John's Wort, an herbal preparation used in the treatment of depression. The *Journal of Pharmacology and Experimental Therapeutics* **294**, 88-95

Ozcan M, Boyraz N (2000) Antifungal properties of some herb 6 decoctions. *European Food Research and Technology* **212**, 86-88

Panizzi L, Flamini G, Cioni PL, Morelli I (1993) Composition and antimicrobial properties of essential oils of four Mediterranean Lamiaceae. *Journal of Ethnopharmacology* **39**, 167-170

Pavliäk M, Vacek J, Klejdus B, Kubaäný (2007) Hypericin and hyperforin production in St. John's wort *in vitro* culture: Influence of saccharose, polyethylene glycol, methyljasmonate, and *Agrobacterium tumefaciens*. *Journal of Agricultural and Food Chemistry* **55**, 6147-6153

Pawar VC, Thaker VS (2006) *In vitro* efficacy of 75 essential oils against *Aspergillus niger*. *Mycoses* **49**, 316-323

Perry NB, Anderson RE, Brennan NJ, Douglas MH, Heane AJ, McGimpsey JA, Smallfield BM (1999) Essential oils from Dalmatian sage (*Salvia officinalis* L.): Variations among individuals, plant parts, seasons, and sites. *Journal of Agricultural and Food Chemistry* **47**, 2048-2054

Persson M, Sjodin K, Borg-Karlson AK, Norin T, Schafer I, Wolf A, Ciccioli P, Brancaleoni E, Cecinato A, Ekberg P (1990) Relative amounts and enantiomeric compositions of monoterpene hydrocarbons in xylem and needles of *Picea abies*. *Phytochemistry* **42**, 1289-1297

Persson M, Borg-Karlson AK, Norin T (1993) Enantiomeric composition of the six main monoterpene hydrocarbons in different tissues of *Picea abies* (L.) Karst. *Phytochemistry* **33**, 303-307

Petrakis PV, Couladis M, Roussis V (2005) A method for detecting the biosystematic significance of the essential oil composition: The case of five Hellenic *Hypericum* L. species. *Biochemistry and Systematic Ecology* **33**, 873-898

Pignatti S (1982) *Flora d'Italia* (Vol 1), Edagricole Eds., Bologna, pp 349-350

Pintore G, Chessa M, Boatto G, Cerri R, Usai M, Tirillini B (2005) Essential oil composition of *Hypericum perforatum* L. var. *angustifolium* DC Growing Wild in Sardinia (Italy). *Journal of Essential Oil Research* **17**, 533-535

Prakash B, Shukla R, Singh P, Kumar A, Mishra PK, Dubey NK (2010) Efficacy of chemically characterized *Piper betle* L. essential oil against fungal and aflatoxin contamination of some edible commodities and its antioxidant activity. *International Journal of Food Microbiology* **142**, 114-119

Quave CL, Plano LWR, Bennett BC (2008) Quorum sensing inhibitors for methicillin-resistant *Staphylococcus aureus* from Italian medicinal plants. In: *49th Annual Meeting of the Society for Economic Botany*, Durham, NC. June 1-5, Duke University, p 27

Radušienė J, Bagdonaitė E (2002) Phenotypic variation in *Hypericum perforatum* L. and *H. maculatum* Cranz wild populations in Lithuania. In: Johnson CB, Franz Ch (Eds) *Breeding Research on Aromatic and Medicinal Plants*, Harworth Herbal Press, New York, pp 345-351

Radušienė J, Bagdonaitė E, Kazlauskas S (2004) Morphological and chemical evaluation on *Hypericum perforatum* and *H. maculatum* in Lithuania. *Acta Horticulture* **629**, 55-62

Radušienė A, Judžentienė G, Bernotienė A (2005) Essential oil composition and variability of *Hypericum perforatum* L. growing in Lithuania. *Biochemical Systematics and Ecology* **33**, 113-124

Rančić A, Soković M, Vukojević J, Simić A, Marin P, Duletić-Laušević S, Djoković D (2005) Chemical composition and antimicrobial activities of essential oils of *Myrrhis odorata* (L.) Scop, *Hypericum perforatum* L. and *Helichrysum arenarium* (L.) Moench. *Journal of Essential Oil Research* **17 (3)**, 341-345

Ravindran AV, Lam WR, Filteau MJ, Lespérance F, Kennedy HS, Parikh SV, Patten SB (2009) Canadian Network for Mood and Anxiety Treatments (CANMAT). Clinical guidelines for the management of major depressive disorder in adults. V. Complementary and alternative medicine treatments. *Journal of Affective Disorders* **117**, S54-S64

Reddy KRN, Nurdijati SB, Salleh B (2010) An overiew of plant-derived products on control of mycotoxigenic fungi and mycotoxins. *Asian Journal of Plant Sciences* **9**, 126-133

Rios JL, Recio MC, Villar A (1988) Screening methods for natural products with antibacterial activity: A review of the literature. *Journal of Ethnopharmacology* **23**, 127-149

Robson NKB (1968) Guttiferae (Clusiaceae). In: Tutin TG (Ed) *Flora Europaea* (Vol 2), Cambridge, pp 261-269

Robson NKB (1977) Studies in the genus *Hypericum* L. (Guttiferae) I. Infrageneric classification. *Bulletin of the British Museum Natural History (Botany)* **5**, 293-355

Robson NKB (2001) Studies in the genus *Hypericum* L. (Guttiferae). 4(1). Sections 7. Roscyna to 9. *Hypericum sensu lato* (part 1). *Bulletin of the British Museum Natural History (Botany)* **31**, 37-88

Rodriguez A, Nerin C, Batlle R (2008) New cinnamon-based active paper packaging against rhizopusstolonifer food spoilage. *Journal of Agriculture and Food Chemistry* **56**, 6364-6369

Roz N, Rehavi M (2004) Hyperforin depletes synaptic vesicles content and induces compartemental redistribution of nerve ending monoamines. *Life Science* **75**, 2641-2850

Saddiqe Z, Naeem I, Maimoona A (2010) A review of the antibacterial activity of *Hypericum perforatum* L. *Journal of Ethnopharmacology* **131**, 511

Sajjadi SE, Rahiminezhad MR, Mehregan I, Poorassar A (2001) Constituents of essential oil of *Hypericum dogonbadanicum* Assadi. *Journal of Essential Oil Research* **13**, 43-44

Santana-Rios G, Orner GA, Amantana A, Provost C, Wu SY, Dashwood RH (2001) Potent antimutagenic activity of white tea in comparison with green tea in the *Salmonella* assay. *Mutation. Research* **495**, 61-74

Santos PAG, Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJC (1999) Composition of the essential oil of *Hypericum foliosum* Aiton from five Azorean islands. *Flavour and Fragrance Journal* **14**, 283-289

Saroglou V, Marin PD, Rancic A, Veljic M, Skaltsa H (2007) Composition and antimicrobial activity of the essential oil of six *Hypericum* species from Serbia. *Biochemical Systematics and Ecology* **35**, 146-152

Schulz H, Jobert M, Huebner WD (1998) The quantitative EEG as a screening instrument to identify sedative effects of single doses of plant extracts in comparison with diazepam. *Phytomedecine* **5**, 449-458

Schwob I, Bessiere J, Masotti V, Viano J (2004) Changes in essential oil composition in Saint John's wort (*Hypericum perforatum* L.) aerial parts during its phenological cycle. *Biochemical Systematics and Ecology* **32**, 735-745

Schwob I, Bessiere JM, Dherbomez M, Viano J (2002) Composition and antimicrobial activity of the essential oil of *Hypericum coris*. *Fitoterapia* **73**, 511-513

Schwob I, Bessiere JM, Viano J (2002) Composition of the essential oils of *Hypericum perforatum* L. from southeastern France. *Comptes Rendus Biologies* **325**, 781-785

Schwob I, Viano J, Jann-Para G, Bessiere JM, Dherbomez M (2006) Composition and antimicrobial activity of the essential oil of *Hypericum hyssopifolium* ssp. *hyssopifolium* from southeast France. *Journal of Essential Oil Research* **18**, 469-471

Seenivasan P, Manickkam J, Savarimuthu I (2006) *In vitro* antibacterial activity of some plant essential oils. *BMC Complementary and Alternative Medicine* **6**, 39

Senatore F (1996) Influence of harvesting time on yield and composition of the essential oil of a thyme (*Thymus pulegioides* L.) growing wild in Campania (Southern Italy). *Journal of Agricultural and Food Chemistry* **44**, 1327-1332

Sevim A, Betul D, Gokalp I, Yavuz K, Baser B, Can KH (2010) Composition and anticandidal activity of the essential oil of *Hypericum perforatum* L. *Asian Journal of Chemistry* **22**, 1315-1320

Sharafi SM, Rasooli I, Owlia P, Taghizadeh M, Astaneh SDA (2010) Protective effects of bioactive phytochemicals from *Mentha piperita* with multiple health potentials. *Pharmacognosy Magazine* **6**, 147-153

Sharma OP, Bhat TK (2009) DPPH antioxidant asy revisited. *Food Chemistry* **113**, 1202-1205

Shelton RC (2009) St John's Wort (*Hypericum perforatum*) in major depression. *Journal of Clinical Psychiatry* **70**, 23-27

Singh P, Srivastava B, Kumar A, Dubey NK (2008) Fungal contamination of raw materials of some herbal drugs and recommendation of *Cinnamomum camphora* oil as herbal fungitoxicant. *Microbial Ecology* **56**, 555-556

Sinico C, De Logu A, Lai F, Valenti D, Manconi M, Loy G, Bonsignore L, Fadda AM (2005) Liposomal incorporation of *Artemisia arborescens* L. essential oil and *in vitro* antiviral activity. *European Journal of Phamaceutics and Biopharmaeutics* **59**, 161-168

Skalkos D, Stavropoulos NE, Tsimaris I, Gioto E, Stalikas CD (2005) The lipophilic extract of *Hypericum perforatum* exerts significant cytotoxic activity against T24 and NBT-II urinary bladder tumor cells. *Planta Medica* **71**, 1030-1035

Smelcerovic A, Mimica-Dukic N, Djordjevic S (2004) Essential oil composition of *Hypericum perforatum* L. ssp *angustifolium* from South Serbia. *Journal Essential Oil-Bearing Plants* **7**, 275-278

Smelcerovic A, Spiteller M, Ligon AP, Smelcerovic Z, Nils R (2007) Essential oil composition of *Hypericum* L. species from Southeastern Serbia and their chemotaxonomy. *Biochemical Systematics and Ecology* **35**, 99-113

Smelcerovic A, Spiteller M (2006) Phytochemical analysis of nine *Hypericum* L. species from Serbia and the F.Y.R. Macedonia. *Pharmazie* **61**, 251-252

Smelcerovic A, Verma V, Spiteller M, Ahmad SM, Puri SC, Qazi GN (2006) Phytochemical analysis and genetic characterisation of six *Hypericum* species from Serbia. *Phytochemistry* **67**, 171-177

Smith MD, Navilliat PL (1997) A new protocol for antimicrobial testing of oils. *Journal of Microbiological Methods* **28**, 21-24

Smith-Palmer A, Stewart J, Fyfe L (1998) Antimicrobial proprieties of plant essential oils and essences against five important food-borne pathogens. *Letters in Applied Microbiology* **26**, 118-122

Smith-Palmer A, Stewart J, Fyfe L (2001) The potential application of plant essential oils as natural food preservatives in soft cheese. *Food Microbiology* **18**, 463-470

Swigar AA, Silverstein RM (1981) *Monoterpenes*, Milwaukee, Aldrich Chem. Comp. Eds

Tatsadjieu NL, Jazet Dongmo PM, Ngassoum MB, Etoa FX, Mbofung CMF (2009) Investigations on the essential oil of *Lippia rugosa* from Cameroon for its potential use as antifungal agent against *Aspergillus flavus* Link ex. Fries. *Food Control* **20**, 161-166

Tatsis EC, Boeren S, Exarchou V, Troganis AN, Vervoort J, Gerothanassis

IP (2007) Identification of the major constituents of *Hypericum perforatum* by LC/SPE/NMR and/or LC/MS. *Phytochemistry* **68**, 383-393

Thompson DP (1989) Fungitoxic activity of essential oil components on food storage fungi. *Mycologia* **81**, 151-153

Toker Z, Kizil G, Oezen HC, Kizil M, Ertekin S (2006) Compositions and antimicrobial activities of the essential oils of two *Hypericum* species from Turkey. *Fitoterapia* **77**, 57-60

Tognolini M, Barocelli V, Ballabeni E, Bruni R, Bianchi A, Chiavarini M, Impicciatore M (2006) Comparative screening of plant essential oils: Phenylpropanoid moiety as basic core for antiplatelet activity. *Life Sciences* **78**, 1419-1432

Touafek O, Nacer A, Kabouche A, Kabouche Z (2005) Analysis of essential oil of Algerian *Hypericum perfoliatum* (L). *Flavour and Fragrance Journal* **20**, 669-670

Tyagi AK, Malik A (2010) Antimicrobial action of essential oil vapours and negative air ions against *Pseudomonas fluorescens International Journal of Food Microbiology* **143**, 205-210

Vandenbogaerde A, Zanoli P, Puia G (2000) Evidence that total extract of *Hypericum perforatum* affects exploratory behavior and exerts anxiolytic effects in rats. *Pharmacology*, *Biochemistry and Behavior* **65**, 627-633

Verotta L, Appendino G, Jakupovic J, Bombardelli E (2000) Hyperforin analogues from St. John's wort (*Hypericum perforatum* L.). *Journal of Natural Products* **63**, 412-415

Vijaya M, Ingram C, Gray J, Talpur NA, Echard BW, Bagchi D, Preuss HG (2001) Antifungal activities of origanum oil against *Candida albicans*. *Molecular and Cellular Biochemistry* **228**, 111-117

Wang D, Bai J, Sun F, Yang D (2010) Chemical constituents and antidepressant activity of the new species *Hypericum enshiense* occurring in China. *Phytomedicine* **17**, 410-413

Weyerstahl P, Splittgerber U, Marschall H, Kaul VK (1995) Constituents of the leaf essential oil of *Hypericum perforatum* L. from India. *Flavour and Fragrance Journal* **10**, 365-370

Williams LAD, Porter RB, Junor GO (2007) Biological activities of selected essential oils. *Natural Product Communications* **2**, 1295-1296

Wink M (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry* **64**, 3-19

Wood SG, Huffman J, Weber N, Andersen D, North J (1990) Antiviral activity of naturally occurring anthraquinones and anthraquinone derivatives. *Planta Medica* **56**, 651-652

Yee W, Dirnbock T (2009) Models for analyzing species'presence/absence data at two time points. *Journal of Theoretical Biology* **259**, 684-694

Yazaki K, Sasaki K, Tsurumaru Y (2009) Prenylation of aromatic compounds, a key diversification of plant secondary metabolites. *Phytochemistry* **70**, 1739-1745

Yoo C-B, Han K-T, Cho K-S, Ha J-H, Park H-J, Nam J-H, Kil U-H, Lee K-T (2005) Eugenol isolated from the essential oil of *Eugenia caryophyllata* induces a reactive oxygen species-mediated apoptosis in HL 60 human promyelocytic leukemia cells. *Cancer Letters* **225**, 41-52

Zaika LL (1988) Spices and herbs: Their antimicrobial activity and its determination. *Journal of Food Safety* **9**, 97-118

Zhang LS, Dong GP, Liu GM (2009) Study on chemical constituents from essential oil of *Hypericum patulum*. *Journal of Chinese Medicinal Material* **32**, 224-226

Zu Y-G, Yu H-M, Liang L, Fu Y-J, Efferth T, Liu X, Wu N (2010) Activities of ten essential oils towards *Propionibacterium acnes* and PC-3, A-549 and MCF-7. *Cancer Cells Molecules* **15**, 3200-3210