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Hypericum sp.: Essential oil composition and biological activities

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Abstract:	Phytochemical characterization of several species of Hypericum genus has been studied for a long time. Several reviews, most of them on H. perforatum, have already been published concerning the characterization of alcoholic and water extracts as well as their biological activities. Studies on the essential oils of H. perforatum and other species of this genus have already been published, some of them reporting the positive biological activities of these essential oils. Additionally, variations on the essential oils of Hypericum species induced by seasonal variation, geographic distribution, phenological cycle and type of the organ in which essential oils are produced and/or accumulated have also been reported. However, so far, no review paper has been published gathering all the reported data on Hypericum essential oils and respective biological activities. Thus in this chapter we collect and summarize as many information as possible concerning composition and biological activities of essential oils and essential oil containing crude extracts of Hypericum species, as well the biotechnology approaches envisaging their improvement.

Hypericum sp.: Essential oil composition and biological activities

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Abstract

Phytochemical characterization of several species of *Hypericum* genus has been studied for a long time. Several reviews, most of them on *H. perforatum*, have already been published concerning the characterization of alcoholic and water extracts as well as their biological activities. Studies on the essential oils of *H. perforatum* and other species of this genus have already been published, some of them reporting the positive biological activities of these essential oils. Additionally, variations on the essential oils of *Hypericum* species induced by seasonal variation, geographic distribution, phenological cycle and type of the organ in which essential oils are produced and/or accumulated have also been reported. However, so far, no review paper has been published gathering all the reported data on *Hypericum* essential oils and respective biological activities. Thus in this chapter we collect and summarize as many information as possible concerning composition and biological activities of essential oils and essential oil containing crude extracts of *Hypericum* species, as well the biotechnology approaches envisaging their improvement.

Key Words

Antibacterial, antifungal, antiviral, antiangiogenic, antitumor activities; biotechnology

Introduction

Hypericum is an important genus of the family Clusiaceae (Guttiferae), which includes about 450 species of trees, shrubs and herbs. Some of the general morphological characteristics of this genus are: (i) opposite simple entire exstipulated leaves; (ii) (4-)5-merous flowers consisting of green sepals and free yellow petals (occasionally, the sepals as well as the petals can be red-tinged); (iii) free of more or less united ovary and; (iv) stamens organized in 5 fascicles. The main feature of some of the important species is the presence of translucent and often black or red schizogenous hypericin glands. Although, these glands are present in the plant parts like stem, flowers and leaves, their distribution differ between species. Hypericin was first identified in *Hypericum* species and serves as a chemotaxonomic marker of the genera. For more details about the taxonomy and distribution of this genus, refer Robson (2006) (Robson, 2006).

Many species of the *Hypericum* genus have a long traditional value as medicinal plants. Ancient Greeks knew about the medicinal properties of this genus. Greek herbalist Dioscorides recommends four species of *Hypericum* (Upericon, Askuron, Androsaimon, and Koris) for sciatica and burns. He referred that *H. crispum* and *H. barbatum* have antidiuretic and antimalarial properties. Theophrastus recommends *H. lanuginosum* for external application, while Pliny says it should be taken in wine against poisonous reptiles. *H. coris*, another Greek species, was mentioned by Hippocrates and Pliny. Yet another species, *H. perforatum* (St. John's wort) has been appreciated for its medicinal value since at least as early as the 1st century A.D. and was known by Hippocrates, Pliny, and Dioscorides who included it in the *De materia medica* (Bombardelli *et al.*, 1995; Upton, 1997)

Currently, several species of this genus has been used in ailments as knowledge-based medicine in many countries. In Portuguese folk medicine, some *Hypericum* species are used the major of them being *H. androsaemum*, *H. perforatum*, and *H. undulatum*. *H. androsaemum* (Hipericão do Gerês) is known for its diuretic effect and infusions are used for kidney and bladder ailments, whereas *H. undulatum* extract is used for liver problems. Flowers decoction of *H. undulatum* is also traditionally used for migraine, bladder ailments; and as renal antispasmodic and hepatic protector (Ferreira *et al.*, 2006). Moreover, decoctions of the flowering aerial parts of *H.*

perforatum have been used to treat liver troubles (Camejo-Rodrigues *et al.*, 2003), depression, liver disorders and rheumatism (Nogueira *et al.*, 1998). In the Canary Islands (Spain), infusions prepared from the flowers, leaves and fruits of various species of the genus *Hypericum* have been used as a vermifuge, diuretic, as well as a wound healing, sedative, antihysterical and antidepressive agent (Prado *et al.*, 2002; Rabanal *et al.*, 2002). In Italy, leaves and stems of *H. hircinum* have been widely used against cough (Pieroni *et al.*, 2004). *H. perforatum*, a well-known species of this genus, has been included in the traditional pharmacopeia of many countries. For instance, in the 1997 German List of Prescription Drugs described more than 50 preparations from *H. perforatum*. In the German Drug Prescription Report 1996, about 131 million of daily doses of products prepared from this species were prescribed. In Serbia, aerial parts of *H. perforatum* were cited to be used internally and externally for all ailments (Jaric *et al.*, 2007). Different parts of the plant and different preparations are externally applied or orally administrated for several ailments, including digestive, urinary, respiratory and cardiac diseases in Turkey (Kültür, 2007). In Bulgarian phytotherapy, aerial parts of *H. perforatum* have been recommended for treatment of gastric and duodenal ulcer; as regenerative and anti-inflammatory agent in digestive tract diseases and as epithelotonic agent (Ivanova *et al.*, 2005). In Italy, leaves and stems of *H. hircinum* have been widely used against cough (Pieroni *et al.*, 2004).

In the last decade, several pharmacological studies have been performed using crude extracts to evaluate the traditional knowledge. Results of those studies have revealed that extract of *Hypericum* exert several pharmacological properties including antidepressant, antimicrobial and wound healing effects (Ishiguro *et al.*, 1990; Decosterd *et al.*, 1991; Jayasuriyab *et al.*, 1991; Rocha *et al.*, 1995; Öztürk *et al.*, 1996; Sokmen *et al.*, 1999; Daudt *et al.*, 2000; Mukherjee *et al.*, 2000; Pistelli *et al.*, 2000; Avato *et al.*, 2004).

Plants produce and accumulate different types of compounds. Molecules with an important role in basic life functions such as cell division, growth, respiration, storage, and reproduction were described as primary metabolites (Bougard *et al.*, 2001). Several other compounds which are not essential for the above functions are known as secondary metabolites. For a long time, secondary metabolites have been considered as waste by-products of plant metabolism. Recent improvement of biochemical techniques and the rise of molecular biology have shown that these molecules play a major role in the adaptation of plants to their environment including biotic and abiotic challenges. They also act on animals (anti-feeding properties), pathogens (phytoalexins) and other plants (allelopathy) (Bougard *et al.*, 2001). Plant secondary metabolites include a vast array of compounds that to date sum up to more than 200,000 defined structures.

Studies on medicinal plants are rapidly increasing because of the search for new active molecules, and for the improvement in the production of plants or molecules for the herbal pharmaceutical industries (Poutaraud *et al.*, 2005). As a genus with several medicinal plant species, *Hypericum* has recently drawn the attention of phytochemists and pharmacologists. However, it is clear from the literature that *H. perforatum* is the only most characterized species of this genus in terms of phytochemistry and pharmacology. Under the stimulus of great scientific interest and economic value acquired by *H. perforatum*, studies with other plants of *Hypericum* genus have also been carried out revealing their antidepressant, analgesic, anti-inflammatory, antioxidant, antimicrobial and wound healing properties (Öztürk *et al.*, 1996; Apaydin *et al.*, 1999; Daudt *et al.*, 2000; Mukherjee *et al.*, 2001; Trovato *et al.*, 2001; Öztürk *et al.*, 2002; Prado *et al.*, 2002; Rabanal *et al.*, 2002; Sánchez-Mateo *et al.*, 2002; Cakir *et al.*, 2003; Couladis *et al.*, 2003; Heilmann *et al.*, 2003; Rabanal *et al.*, 2005).

Phytochemical characterisation of several species of this genus have revealed the presence of a variety of compounds, but not limited to phenylpropanes, flavonol derivatives, biflavones, proanthocyanidines, xanthones, phloroglucinols and naphtodianthrones (for review see (Bombardelli *et al.*, 1995; Nahrstedt *et al.*, 1997; Hölzl *et al.*, 2003)) and essential oils. In spite of the large size of *Hypericum* genus, the composition of volatile compounds is known in only a small number of species with the exception of *H. perforatum* (Saroglou *et al.*, 2007). The present review summarizes information available on the chemical composition of essential oils isolated from *Hypericum* genus. Additionally, the biological activities of this genus with special emphasis on essential oil composition are also discussed.

Chemical Composition of *Hypericum* Essential Oils

Interest in essential oils has revived in recent decades, with the popularity of aromatherapy, a branch of alternative medicine which claims that the specific aromas carried by essential oils have curative effects. Oils are volatilized or diluted in a carrier oil and used in massage or burned as incense. About 300 essential oils out of 3000 known are commercially important mainly for their flavours and fragrances (Burt, 2004).

Essential oils are aromatic oily extracts obtained from the plants. Composition of essential oils can differ between different parts of the plants. Steam distillation is the most commonly used method of extraction for commercial production of essential oils (Burt, 2004), although other extraction methods such as expression, fermentation, enfleurage or extraction are also in practice. In

the steam distillation, the water is heated and the steam passes through the plant material, thus vaporizing the volatile compounds. The vapors flow through a coil where they condense back to liquid, which is then collected in the receiving vessel. Because of the mode of extraction, mostly by distillation from aromatic plants, the extract contains a variety of volatile molecules such as terpenes and terpenoids, phenol-derived aromatic components and aliphatic components (Bakkali *et al.*, 2008).

Essential oils are very complex natural mixtures which can contain about 20–60 components at quite different concentrations. Hence, they are generally characterized by two or three major components at fairly high concentrations compared to other components that are present in trace amount (Bakkali *et al.*, 2008).

The main essential oil constituents identified in *Hypericum* species are summarised in Table 1. Characterization of essential oils from species of *Hypericum* genus revealed the presence of monoterpenoid and sesquiterpenoid compounds, as well as alkanes and aldehydes as the main compounds in the most of them (Mathis *et al.*, 1964a; Mathis *et al.*, 1964b; Mathis *et al.*, 1964c). In the essential oils of *H. ericoides*, α-curcumene, α-pinene, γ-muurolene and δ-cadinene were the most represented compounds (Cardona *et al.*, 1983).

In general, species of this genus have higher amount of sesquiterpenoids in their essential oils (Table 1). Indeed, *E*-caryophyllene was one of the most represented compounds in most of the studied *Hypericum* essential oils. Germacrene D was another major sesquiterpene hydrocarbon common to several species. Caryophyllene oxide, spathulenol and globulol, are the oxygenated-sesquiterpenes present in the top three most represented compounds of some *Hypericum* species. Likewise, α-pinene and β-pinene were the two mostly represented monoterpene hydrocarbons in essential oils from *Hypericum*. Interestingly, in *H. barbatum* the three most represented compounds are monoterpene hydrocarbons (α-pinene, β-pinene and limonene) (Saroglou *et al.*, 2007). *n*-Alkanes were found as the major compounds in few species of the genus. In *H. foliosium*, *H. hirsutum*, *H. myrianthum*, *H. richeri* and *H. triquetrifolium*, *n*-nonane is among the three most represented compounds. Some of these species also had high proportions of *n*-undecane (Santos *et al.*, 1999; Bertoli *et al.*, 2003; Ferraz *et al.*, 2005; Ferretti *et al.*, 2005; Saroglou *et al.*, 2007).

Table 1 – Main essential oil constituents of *Hypericum* species

Species	Origin	Main constituents	Reference
H. alpinum	Serbia	β-Pinene (13.3%)	(Saroglou *et al.*, 2007)

Species	Origin	Main constituents	Reference
		γ-Terpinene (7.7%) (-)-(*E*)-Caryophyllene (6.5%)	
H. acmosepalum	China	β-Selinene (16.3%) *ar*-Curcumene (12.6%)	(Demirci *et al.*, 2005)
H. aegypticum* ssp. *aegypticum	Libya	Ishwarane (14.4%) Eudesm-11-en-4-ol stereoisomer (9.6%) Eudesm-11-en-4-ol stereoisomer (10.7%)	(Crockett *et al.*, 2007)
H. aegypticum* ssp. *marrocanum	Northwestern Africa	Caryophyllene oxide (29.2%) β-Caryophyllene (15.1%) Caryophylladienol-II (9.7%)	(Crockett *et al.*, 2007)
H. androsaemum	Portugal	$C_{15}H_{24}$ (27.6%) Germacrene D (12.3%) β-Caryophyllene (14.0%)	(Nogueira *et al.*, 1998)
	Portugal	(*E*)-Caryophyllene (9.4-15.1%) γ-Elemene (8.0-17.9%) β-Gurjunene (6.1-15.5%)	(Guedes *et al.*, 2003)
	Portugal	(*E*)-Caryophyllene (9.0-17.0%) γ-Elemene (9.3-17.3%) β-Gurjunene (7.9-14.8%)	(Guedes *et al.*, 2004)
H. balearicum	Balearic Islands	α-Pinene (28.5%) β-Pinene (20.4%) β-Eudesmol (11.2%)	(Crockett *et al.*, 2007)
H. barbatum	Serbia	(-)-α-Pinene (17.1%) (-)-β-Pinene (17.0%) (-)-Limonene (6.0%)	(Saroglou *et al.*, 2007)
H. beanii	China	Caryophyllene oxide (18.7%) β-Selinene (16.3%) γ-Muurolene (11.3%)	(Demirci *et al.*, 2005)
H. brasiliense	Brazil	β-Caryophyllene (29.5%)	(Abreu *et al.*, 2004)

Species	Origin	Main constituents	Reference
		α-Humulene (12.7%) Caryophyllene oxide (9.9%)	
H. bupleuroides	Turkey	β-Sesquiphellandrene (33.2%) β-Caryophyllene (20.2%) Selina-3,7(11)-diene (7.0%)	(Demirci *et al.*, 2006)
H. calcynum	China	β-Pinene (29.2%) α-Terpineol (11.5%)	(Demirci *et al.*, 2005)
H. carinatum	Brazil	β-Caryophyllene (21.0%) α-*trans*-Bergamotene (10.0%) Caryophyllene oxide (9.5%)	(Ferraz *et al.*, 2005)
H. choisyanum	China	Cis-eudesma-6,11-diene (11.4%)	(Demirci *et al.*, 2005)
H. connatum	Brazil	Caryophyllene oxide (40.1%) β-Caryophyllene (13.1%) Humulene oxide II (10.5%)	(Ferraz *et al.*, 2005)
H. coris	France	α-Curcumene (40.1%) γ-Cadinene (14.7%) δ-Cadinene (6.6%)	(Schwob *et al.*, 2002)
H. delphicum	Arabian penninsula	Caryophyllene oxide (31.5%) β-Caryophyllene (18.2%) *n*-Undecane (17.5%)	(Crockett *et al.*, 2007)
H. foliosum	Portugal	*n*-Nonane (28.7-72.6%) Limonene (6.9-45.8%) Terpinolene (0.5-18.8%) β-Caryophyllene (1.1-6.9%) β-Pinene (0.3-6.3%)	(Santos *et al.*, 1999)
H. forrestii	China	Caryophyllene oxide (12.7%) α-Pinene (10.4%)	(Demirci *et al.*, 2005)
H. heterophyllum	Turkey	Isocaryophyllene (17.1%) α-Pinene (11.6%)	(Cakir *et al.*, 2004)

Species	Origin	Main constituents	Reference
		δ-Cadinene (9.5%)	
H. hirsutum	Serbia	Nonane (24.8%) Undecane (13.3%) (-)-(*E*)-Caryophyllene (5.4%)	(Saroglou *et al.*, 2007)
	Serbia	*n*-Undecane (32.2%) Patchoulene (11.8%) Caryophyllene oxide (9.3%)	(Gudžic *et al.*, 2007)
H. humifusum	Portugal	α-Pinene (80.6%) β-Pinene (4.7%) Germacrene D (2.1%)	(Nogueira *et al.*, 1998)
	Portugal	α-Pinene (44.7-77.2%) β-Pinene (4.7-7.7%) β-Caryophyllene (1.2-9.3%) Germacrene D (1.9-6.1%)	(Nogueira *et al.*, 2008)
H. kouytchense	China	*cis*-β-Guaiene (10.7%) γ-Muurolene (12.4%)	(Demirci *et al.*, 2005)
H. lancasteri	China	β-Selinene (11.4%) Eudesmadienone (10.8%)	(Demirci *et al.*, 2005)
H. leschenaultii	China	Cuparene (24.8%) γ-Muurolene (16.8%)	(Demirci *et al.*, 2005)
H. linarifolium	Portugal	α-Pinene (31.1%) β-Caryophyllene (11.6%) *n*-Undecane (7.0%)	(Nogueira *et al.*, 1998)
	Portugal	α-Pinene (19.9-31.2%) β-Pinene (5.0-11.0%) β-Caryophyllene (6.6-11.6%)	(Nogueira *et al.*, 2008)
H. linarioides	Turkey	δ-Cadinene (6.9%) γ-Muurolene (5.5%) (Z)-β-Farnesene (5.2%)	(Cakir *et al.*, 2005)
H. lysimachioides	South eastern Anatolia	Caryophyllene oxide (30.8%) α-Longifolene (6.4%)	(Toker *et al.*, 2006)

Species	Origin	Main constituents	Reference
		β-Selinene (6.7%)	
H. maculatum	Serbia	Spathulenol (6.8%) Globulol (10.2%) Nonane (5.5%)	(Saroglou *et al.*, 2007)
H. monogynum	China	Tricosane (13.3%) Myrcene (10.4%)	(Demirci *et al.*, 2005)
H. myrianthum	Brazil	Undecane (20.7%) Nonane (17.5%) Dehydro-aromadendrene (8.6%)	(Ferraz *et al.*, 2005)
H. olympicum	Serbia	(*E*)-Anethole (30.7%) *β*- Farnesene (12.4%) *δ*-Cadinene (8.7%)	(Gudžić *et al.*, 2001)
	Greece	Germacrene D (16.0%) (*E*)-Caryophyllene (7.4%) Spathulenol (6.7%)	(Pavlović *et al.*, 2006)
H. patulum	China	*β*-selinene (14.7%)	(Demirci *et al.*, 2005)
H. polyanthemum	Brazil	HP1 Benzopyrans (26.7%) HP2 Benzopyrans (13.2%) Undecane (7.9%)	(Ferraz *et al.*, 2005)
H. pseudohenryi	China	*β*-Selinene (18.5%)	(Demirci *et al.*, 2005)
H. pulchrum	Portugal	*α*-Pinene (35.7-49.8%) *β*-Pinene (9.0-12.5%) Germacrene D (2.4-5.4%)	(Nogueira *et al.*, 1998)
	Portugal	*α*-Pinene (49.8%) *β*-Pinene (12.5%) Germacrene D (5.4%)	(Nogueira *et al.*, 2008)
H. richeri	Italy	(*Z*)-β-Ocimene (19.5%) *n*-Nonane (13.8%) *β*-Bisabolene (8.7%)	(Ferretti *et al.*, 2005)
H. roeperanum	East Africa	*γ*-Curcumene (15.6%)	(Crockett *et al.*,

Species	Origin	Main constituents	Reference
		(2*E*,6*E*)-Farnesol (7.8%) *ar*-Curcumene (7.7%)	2007)
H. scabrum	Uzbekistan	α-Pinene (11.2%) Spathulenol (7.2%) *p*-Cymene (6.1%)	(Baser *et al.*, 2002)
H. ternum	Brazil	β-Caryophyllene (12.0%) Bicyclogermacrene (10.0%) β-Cadinene (5.0%)	(Ferraz *et al.*, 2005)
H. tetrapterum	Greece	α-Copaene (11.3%) α-Longipinene (9.7%) Caryophyllene oxide (8.9%)	(Pavlović *et al.*, 2006)
H. tomentosum	Portugal	β-Caryophyllene (12.6%) *n*-Undecane (7.5%) α-Humulene (5.2%)	(Nogueira *et al.*, 1998)
H. xmoserianum	China	γ-Muurolene (10.7%) δ-Cadinene (10.2%)	(Demirci *et al.*, 2005)
H. tomentosum	Tunisia	Menthone (17.0%) *n*-Octane (9.9%) β-Caryophyllene (5.3%) α-Pinene (5.2%)	(Hosni *et al.*, 2008)
H. triquetrifolium	Italy	α-Humulene *cis*-Calamene δ-Cadinene α-Pinene (10.33%) Caryophyllene oxide (1.38%)	(Karim *et al.*, 2007)

Essential oils content and composition can be greatly affected by several parameters including seasonal variation (Guedes *et al.*, 2004), phenological cycle (Schwob *et al.*, 2004) and geographic distribution. We have summarised the most represented compounds of the essential oils of *H. perforatum*, *H. perfoliatum* and *H hyssopifolium* based on their geographical distribution in Table 2.

Table 2 - Variation in the essential oil composition of a few *Hypericum* species based on their geographical distribution

Species	Origin	Main constituents of essential oil	Reference
H. perforatum	Serbia	*cis*-Caryophyllene (48.5%) *β*- Farnesene (12.1%) 2-Methyl-dodecane (5.7%)	(Gudžić *et al.*, 1997)
	Serbia	*β*-Caryophyllene (14.2%) 2-Methyl-octane (13.1%) 2-Methyl-decane (7.9%)	(Gudžić *et al.*, 2001)
	Serbia	*α*-Pinene (8.6%) (*Z*)-*β*- Farnesene (6.6%) Germacrene D (6.8%)	(Saroglou *et al.*, 2007)
	Portugal	Germacrene D (20.0%) *β*-Caryophyllene (10.9%) 2-Methyl-octane (9.7%)	(Nogueira *et al.*, 1998)
	Uzbekistan	*β*-Caryophyllene (11.7%) Caryophyllene oxide (6.3%) Spathulenol (6.0%)	(Baser *et al.*, 2002)
	Lithuania	Caryophyllene oxide (6.1-35.8%) Germacrene D (4.5- 31.5%) *β*-Caryophyllene (5.1-19.1%)	(Mockutė *et al.*, 2003)
	Italy	2-Methyl-octane (21.1%) Germacrene D (17.6%) *α*-Pinene (15.8%)	(Pintore *et al.*, 2005)
	Greece	*α*-Pinene (21.0%) 2-Methyl-octane (12.6%) *γ*-Muurolene (6.9%)	(Pavlović *et al.*, 2006)
	India	Ishwarane *α*-cuprenene	(Weyerstahl *et al.*, 1995)
H. perfoliatum	Portugal	*α*-Pinene (50.0%) *n*-Nonane (16.8%) *n*-Undecane (8.8%)	(Nogueira *et al.*, 1998)

Species	Origin	Main constituents of essential oil	Reference
	Portugal	α-Pinene (39.4-64.3%) *n*-Nonane (11.9-23.8%) β-Pinene(1.9-3.2%)	(Nogueira *et al.*, 2008)
	Algeria	Thymol (22.1%) τ-Cadinol (18.5%) 4,5-Dimethyl-2-ethylphenol (13.0%)	(Touafek *et al.*, 2005)
	Greece	α-Pinene (48.6%) *n*-Nonane (8.5%) δ-Cadinene (4.6%)	(Couladis *et al.*, 2001)
	Greece	α-Pinene (34.2%) β-Pinene (9.2%) δ-Cadinene (8.1%)	(Couladis *et al.*, 2001)
	Greece	α-Pinene (41.3%) β-Pinene (6.5%) δ-Cadinene (6.2%) *n*-Nonane (6.1%)	(Petrakis *et al.*, 2005)
	Tunisia	α-Pinene (13.1%) *allo*-Aromadendrene (11.4%) Germacrene-D (10.6%)	(Hosni *et al.*, 2008)
H. hyssopifolium	South eastern Anatolia	Caryophyllene oxide (20.4%) Spathulenol (13.4%) Caryophyllene alcool (9.0%)	(Toker *et al.*, 2006)
	Turkey	α-Pinene (57.3%) β-Pinene (9.0%) Limonene (6.2%)	(Cakir *et al.*, 2004)
	France	Spathulenol (19.5 %) Tetradecanol (10.2%) Dodecanol (9.3%)	(Schwob *et al.*, 2006)

β-Caryophyllene and Germacrene D were abundant in essential oils of *H. perforatum* from Portugal and Lithuania, but not in the one from Greece. Gudžić and co-workers (1997; 2001) reported β-Caryophyllene as the most represented compound in essential oils of *H. perforatum* from Rujan mountain and Vlasina, both in Serbia (Gudžić *et al.*, 1997; Gudžić *et al.*, 2001). However this sesquiterpene was not one of the three major compounds in the essential oils of this species in Barelic, another region of Serbia (Saroglou *et al.*, 2007). α-Pinene predominance was registered in the essential oils from plants cultivated in Italy, Greece and Serbia (Barelic). Essential oils from Lithuania and Uzbekistan were the only ones in which oxygenated-sesquiterpenes were the major compounds, caryophyllene oxide and spathulenol for Uzbekistan and caryophyllene oxide for Lithuania. In the essential oils of *Hypericum perforatum* L. from North Indian, ishwarane and α-cuprenene were identified (Weyerstahl *et al.*, 1995). In essential oils of *H. perfoliatum* from Portugal, Greece and Tunisia some homogeneity was found in the major compounds. In all of them α-pinene was one of the major compounds, with *n*-nonane and β-pinene common to some of them. However, data on essential oils from plants cultivated in Algeria show a different composition regarding the three most represented compounds, without any mono-or sesquiterpene hydrocarbon between them. Essential oils of *H. hyssopifolium* from south eastern Anatolia and France shared the predominance of the oxygenated-sesquiterpene spathulenol. However, in Turkey the three most represented compounds in this species were monoterpene hydrocarbons, with α-pinene as the main constituent. Differences in the essential oils profile of plants cultivated in different locations can be attributed to different climatic and pedological conditions.

As shown in Table 3, *Hypericum* essential oil profile could also be dependent on the plant organs from which they are extracted. For example, essential oils extracted from flowers and leaves of *H. triquetrifolium* vary greatly in their composition. Flowers have a high representation of monoterpene hydrocarbons, whereas they are absent in the essential oils of leaves. On the other hand, although the essential oils composition of leaves and flowers were similar, the main constituent, caryophyllene oxide, varied in the concentration in both *H. androsaemum* and *H. perforatum*. Similarly, leaves and flowers of *H. caprifoliatum* varied in the nonane and β-caryophyllene concentrations.

Table 3 - Variation in the essential oil composition of some *Hypericum* species based, on the plant part from which they were extracted

Species	Main constituents of essential oil	Reference

	Leaves/ vegetative part	Flowers/ flowering top	
H. androsaemum	Caryophyllene oxide (35.8%) Ishwarane (30.5%) Humulene epoxide II (5.6%)	α-Guaiene (40.2%) Caryophyllene oxide (28.0%) Khusinol (4.2%)	(Morteza-Semnani *et al.*, 2005))
H. caprifoliatum	Nonane (44.6%) β-Caryophyllene (11.2%) Bicyclogermacrene (5.6%)	Nonane (55.8%) β-Caryophyllene (5.9%) Undecane (5.0%)	(Ferraz *et al.*, 2005)
H. perforatum	Caryophyllene oxide (9.3-25.9%) Spathulenol (6.4-15.7%) Tetradecanol (1.1-24.5%)	Caryophyllene oxide (7.7-34%) β-caryophyllene (4.2-14.2%) Viridiflorol (4.5-11%)	(Radusiene *et al.*, 2005)
H. triquetrifolium	Nonane (14.7%) Germacrene D (12.7%) β-caryophyllene (10.9%)	Myrcene (16.4%) α-Pinene (13.3%) Sabinene (13.1%)	(Bertoli *et al.*, 2003)

Biological Activities of *Hypericum* Essential Oils

Since the middle ages, essential oils have been widely used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal and cosmetic applications (Bakkali *et al.*, 2008). Nowadays, essential oils are used in pharmaceutical, sanitary, cosmetic, agricultural and food industries (Burt, 2004).

Although *Hypericum* extracts have been studied in terms of their biological activities, very few studies have so far been performed with essential oils. Antimicrobial activities have been the most reported biological activities for the essential oils of *Hypericum* species (Tables 4a. 4b. 4c).

Table 4a - Antimicrobial activities of *Hypericum* essential oils on standard micro organisms in different *in vitro* assays evaluated by the Minimum Inhibitory Concentration (MIC)

***Hypericum* sps**	**Test organism**	**Antimicrobial activity (MIC)**	**Reference**
H. alpinum	*Escherichia coli*	50.0 µg/ml	(Saroglou *et al.*, 2007)
	Proteus mirabilis	no activity	
	Agrobacterium tumefaciens	25.0 µg/ml	
	Pseudomonas aeruginosa	no activity	
	Pseudomonas tolaasii	50.0 µg/ml	
	Salmonella enteritidis	50.0 µg/ml	
	Staphylococcus aureus	12.5 µg/ml	
	Micrococcus luteus	12.5 µg/ml	
	Sarcina lutea	12.5 µg/ml	
	Bacillus cereus	12.5 µg/ml	
	Candida albicans	no activity	
H. barbatum	*Escherichia coli*	25.0 µg/ml	(Saroglou *et al.*, 2007)
	Proteus mirabilis	50.0 µg/ml	
	Agrobacterium tumefaciens	25.0 µg/ml	
	Pseudomonas aeruginosa	50.0 µg/ml	
	Pseudomonas tolaasii	25.0 µg/ml	
	Salmonella enteritidis	25.0 µg/ml	
	Staphylococcus aureus	6.25 µg/ml	
	Micrococcus luteus	6.25 µg/ml	
	Sarcina lutea	6.25 µg/ml	
	Bacillus cereus	6.25 µg/ml	
	Candida albicans	25.0 µg/ml	
H. canariense	*Bacillus cereus* var. *mycoides*	0.05 mg/ml	(Rabanal *et al.*, 2005)
	Micrococcus luteus	0.05 mg/ml	
	Staphylococcus aureus	0.05 mg/ml	

Hypericum sps	Test organism	Antimicrobial activity (MIC)	Reference
	Staphylococcus epidermis	0.11 mg/ml	
	Bordetella bronchiseptica	0.11mg/ml	
H. coris	*Escherichia coli*	no activity	(Schwob *et al.*, 2002)
	Enterococcus hirae	no activity	
	Staphylococcus aureus	CI 100µg/ml	
	Candida albicans	no activity	
	Saccharomyces cerevisiae	CI 100µg/ml	
H. glandulosum	*Bacillus cereus* var. *mycoides*	0.22 mg/ml	(Rabanal *et al.*, 2005)
	Micrococcus luteus	0.15 mg/ml	
	Staphylococcus aureus	0.09 mg/ml	
	Staphylococcus epidermis	0.22 mg/ml	
	Bordetella bronchiseptica	0.11mg/ml	
H. grandifolium	*Bacillus cereus* var. *mycoides*	0.05 mg/ml	(Rabanal *et al.*, 2005)
	Micrococcus luteus	0.11 mg/ml	
	Staphylococcus aureus	0.09 mg/ml	
	Staphylococcus epidermis	0.15 mg/ml	
	Bordetella bronchiseptica	0.18 mg/ml	
H. hirsutum	*Escherichia coli*	50.0 µg/ml	(Saroglou *et al.*, 2007)
	Proteus mirabilis	no activity	
	Agrobacterium tumefaciens	50.0 µg/ml	
	Pseudomonas aeruginosa	no activity	
	Pseudomonas tolaasii	50.0 µg/ml	
	Salmonella enteritidis	50.0 µg/ml	
	Staphylococcus aureus	25.0 µg/ml	
	Micrococcus luteus	25.0 µg/ml	
	Sarcina lutea	12.5 µg/ml	

Hypericum sps	Test organism	Antimicrobial activity (MIC)	Reference
	Bacillus cereus	12.5 µg/ml	
	Candida albicans	no activity	
H. maculatum	*Escherichia coli*	25.0 µg/ml	(Saroglou *et al.*, 2007)
	Proteus mirabilis	50.0 µg/ml	
	Agrobacterium tumefaciens	25.0 µg/ml	
	Pseudomonas aeruginosa	25.0 µg/ml	
	Pseudomonas tolaasii	25.0 µg/ml	
	Salmonella enteritidis	25.0 µg/ml	
	Staphylococcus aureus	12.5 µg/ml	
	Micrococcus luteus	12.5 µg/ml	
	Sarcina lutea	12.5 µg/ml	
	Bacillus cereus	12.5 µg/ml	
	Candida albicans	50.0 µg/ml	
	Escherichia coli	25.0 µg/ml	(Saroglou *et al.*, 2007)
	Proteus mirabilis	50.0 µg/ml	
	Agrobacterium tumefaciens	25.0 µg/ml	
	Pseudomonas aeruginosa	50.0 µg/ml	
	Pseudomonas tolaasii	25.0 µg/ml	
	Salmonella enteritidis	25.0 µg/ml	
H. perforatum	*Staphylococcus aureus*	12.5 µg/ml	
	Micrococcus luteus	12.5 µg/ml	
	Sarcina lutea	12.5 µg/ml	
	Bacillus cereus	12.5 µg/ml	
	Candida albicans	50.0 µg/ml	
	Aspergillus niger	15.0 µg/ml	(Rančcić *et al.*, 2005)
	Aspergillus flavus	15.0 µg/ml	

Hypericum sps	Test organism	Antimicrobial activity (MIC)	Reference
	Cladosporium cladosporioides	15.0 µg/ml	
	Penicillium funiculosum	15.0 µg/ml	
	Trichoderma viride	15.0 µg/ml	
H. rumeliacum	*Staphylococcus aureus*	7.83 mg/ml	(Couladis *et al.*, 2003)
	Staphylococcus epidermidis	11.2 mg/ml	
	Escherichia coli	3.8 mg/ml	
	Enterobacter cloacae	17.2 mg/ml	
	Klebsiella pneumoniae	9.3 mg/ml	
	Pseudomonas aeruginosa	7.35 mg/ml	
	Candida albicans	6.34 mg/ml	
	C. tropicalis	5.25 mg/ml	
	C. glabrata	4.75 mg/ml	
	Escherichia coli	25.0 µg/ml	(Saroglou *et al.*, 2007)
	Proteus mirabilis	50.0 µg/ml	
	Agrobacterium tumefaciens	25.0 µg/ml	
	Pseudomonas aeruginosa	25.0 µg/ml	
	Pseudomonas tolaasii	25.0 µg/ml	
	Salmonella enteritidis	25.0 µg/ml	
	Staphylococcus aureus	6.25 µg/ml	
	Micrococcus luteus	12.5 µg/ml	
	Sarcina lutea	6.25 µg/ml	
	Bacillus cereus	12.5 µg/ml	
	Candida albicans	25.0 µg/ml	

Table 4b - Antimicrobial activities of *Hypericum* essential oils on standard micro organisms in different *in vitro* assays evaluated by the zone of inhibition (mm).

***Hypericum* sps**	**Test organism**	**Antimicrobial activity (mm)**	**Reference**
H. perforatum	*Candida albicans*	5 mm (2.5µl)	(Rančcić *et al.*, 2005)
	Escherichia coli	7 mm (1µl)	
	Micrococcus luteus	6 mm (1µl)	
	Pseudomonas tolaasii	3 mm (1µl)	
	Salmonella enteritidis	4 mm (1µl)	
	Salmonella typhimurium	4 mm (1µl)	
	Staphylococcus aureus	5 mm (1µl)	
	Staphylococcus epidermidis	4 mm (1µl)	
H. scabrum	*Escherichia coli K12*	18 mm (40µg/disc)	(Kızıl *et al.*, 2004)
	Escherichia coli PBR322	10 mm (40µg/disc)	
	Escherichia coli PUC9	14 mm (60µg/disc)	
	Bacillus brevis ATCC	10 mm (40µg/disc)	
	Bacillus cereus DM65	14 mm (60µg/disc)	
	Streptococcus pyogenes DM41	10 mm (60µg/disc)	
	Pseudomonas aeruginosa DMC66	16 mm (40µg/disc)	
	Staphylococcus aureus DMC70	16 mm (40µg/disc)	
	Candida albicans DM31	18 mm (60µg/disc)	
H. scabroides	*Escherichia coli K12*	16 mm (40µg/disc)	(Kızıl *et al.*, 2004)
	Escherichia coli PBR322	16 mm (40µg/disc)	
	Escherichia coli PUC9	20 mm (40µg/disc)	
	Bacillus brevis ATCC	12 mm (40µg/disc)	
	Bacillus cereus DM65	10 mm (60µg/disc)	
	Streptococcus pyogenes DM41	10 mm (40µg/disc)	
	Pseudomonas aeruginosa DMC66	14 mm (40µg/disc)	

Hypericum sps	Test organism	Antimicrobial activity (mm)	Reference
	Staphylococcus aureus DMC70	10 mm (40µg/disc)	
	Candida albicans DM31	16 mm (40µg/disc)	
H. triquetrifolium	*Escherichia coli K12*	12 mm (60µg/disc)	(Kızıl *et al.*, 2004)
	Escherichia coli PBR322	10 mm (40µg/disc)	
	Escherichia coli PUC9	10 mm (40µg/disc)	
	Bacillus brevis ATCC	10 mm (40µg/disc)	
	Bacillus cereus DM65	12 mm (40µg/disc)	
	Streptococcus pyogenes DM41	12 mm (60µg/disc)	
	Pseudomonas aeruginosa DMC66	12 mm (40µg/disc)	
	Staphylococcus aureus DMC70	16 mm (40µg/disc)	
	Candida albicans DM31	12 mm (40µg/disc)	
H. hyssopifolium* var. *microcalcynum	*Escherichia coli K12*	14 mm (40µg/disc)	(Toker *et al.*, 2006)
	Escherichia coli PBR322	10 mm (40µg/disc)	
	Escherichia coli PUC9	8 mm (40µg/disc)	
	Bacillus brevis ATCC	16 mm (60µg/disc)	
	Bacillus cereus DM65	10 mm (40µg/disc)	
	Streptococcus pyogenes DM41	14 mm (60µg/disc)	
	Pseudomonas aeruginosa DMC66	12 mm (40µg/disc)	
	Staphylococcus aureus DMC70	12 mm (40µg/disc)	
	Candida albicans DM31	12 mm (40µg/disc)	

Table 4c - Antimicrobial activities of *Hypericum* essential oils on standard micro organisms in different *in vitro* assays evaluated by % of inhibition

***Hypericum* sps**	**Test organism**	**Antimicrobial activity (% Inhibition)**	**Reference**
H. linarioides	*Alternaria solani*	0% (5 mg/ml)	(Cakir *et al.*, 2005)
	Fusarium acuminatum	0% (5 mg/ml)	
	Fusarium culmorum	13.6% (5 mg/ml)	
	Fusarium equiseti	0% (5 mg/ml)	
	Fusarium oxysporum	0% (5 mg/ml)	
	Fusarium sambucinum	9.6% (5 mg/ml)	
	Fusarium solani	0% (5 mg/ml)	
	Verticillium albo-atrum	36.0% (5 mg/ml)	
	Rhizoctonia solani AG-5	0% (5 mg/ml)	
	Rhizoctonia solani AG-9	87.5% (5 mg/ml)	
	Rhizoctonia solani AG-11	2.8% (5 mg/ml)	
H. heterophyllum	*Rhizoctonia solani* AG-3	0% (5 mg/ml)	(Cakir *et al.*, 2004)
	Rhizoctonia solani AG-4	0% (5 mg/ml)	
	Rhizoctonia solani AG-5	6% (0.5 mg/ml)	
	Rhizoctonia solani AG-9	12% (0.5 mg/ml)	
	Rhizoctonia solani AG-11	54% (0.5 mg/ml)	
	Fusarium oxysporum	0% (5 mg/ml)	
	Fusarium culmorum	0% (5 mg/ml)	
	Fusarium sambucinum	0% (5 mg/ml)	
	Fusarium solani	0% (5 mg/ml)	
	Fusarium acuminatum	0% (5 mg/ml)	
H. hyssopifolium	*Rhizoctonia solani* AG-3	0% (5 mg/ml)	(Cakir *et al.*, 2004)
	Rhizoctonia solani AG-4	11% (0.5 mg/ml)	

Hypericum sps	Test organism	Antimicrobial activity (% Inhibition)	Reference
	Rhizoctonia solani AG-5	28% (0.5 mg/ml)	
	Rhizoctonia solani AG-9	6% (0.5 mg/ml)	
	Rhizoctonia solani AG-11	46% (0.5 mg/ml)	
	Fusarium oxysporum	6% (0.5 mg/ml)	
	Fusarium culmorum	6% (0.5 mg/ml)	
	Fusarium sambucinum	0% (5 mg/ml)	
	Fusarium solani	0% (5 mg/ml)	
	Fusarium acuminatum	0% (5 mg/ml)	

It is likely that the antimicrobial properties of the *Hypericum* essential oils are based in a great part due to the presence of α-pinene, β-pinene and (*E)*-caryophyllene as these compounds are known for their antimicrobial effects (Aligiannis *et al.*, 2001; Mourey *et al.*, 2002; Cakir *et al.*, 2004; Costa *et al.*, 2008). As these compounds are widely found in the essential oils of *Hypericum*, it is not surprising to see many reports on antimicrobial activities of species of this genus (Gudžić *et al.*, 1997; Schwob *et al.*, 2002b; Couladis *et al.*, 2003; Cakir *et al.*, 2004; Cakir *et al.*, 2005; Schwob *et al.*, 2006; Toker *et al.*, 2006; Saroglou *et al.*, 2007). Anti-inflammatory and anticarcinogenic activities have been attributed to β-caryophyllene (Zheng *et al.*, 1992; Martin *et al.*, 1993; Kubo *et al.*, 1996; Tambe *et al.*, 1996). The monoterpene hydrocarbon, α-pinene have also shown to exert anti-inflammmatory activity in rats (Martin *et al.*, 1993).

Antibacterial activity

Essential oils extracted from the flowers of *H. perforatum* grown in the Vlasina region, Serbia had antibacterial activity against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Sarcina lutea, Bacillus subtilis 841, Salmonella enteritidis* and *Klebsiella pneumoniae* (Gudžić *et al.*, 1997). However, no activity was found against *Pseudomonas aeruginosa*. (Schwob *et al.*, 2002b) The volatile fraction of *H. coris* aerial parts, consisting mainly of α-curcumene showed moderate antibacterial activity against *Staphylococcus aureus* whereas, no activity was found against *Escherichia coli* and *Enterococcus hirae* (Schwob *et al.*, 2002b). On the other hand,

moderate growth inhibitory activity was found in the essential oils of *H. hyssopifolium* against *E. hirae* and *S. aureus*, while no activity was detected against *E. coli* (Schwob *et al.*, 2006). Essential oils of *H. lysimachioides* and *H. hyssopifolium* were highly effective in growth inhibition of nine microorganisms (*Escherichia coli K12, E. coli PBR 322, E. coli PUC 9, Bacillus brevis ATCC, B. cereus DMC65, Streptococcus pyogenes DMC41, Pseudomonas aeruginosa DMC66,* and *Staphylococcus aureus DMC70*) at a concentration of 60 to 80 μg (Toker *et al.*, 2006). Essential oil isolated from the aerial parts of *H. rumeliacum* exhibited moderate activities against all the tested bacteria (*S. aureus, S. epidermidis, E. coli, Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas aeruginosa*), with a minimal inhibitory concentration (MIC) of 3.80–17.20 mg/ml (Couladis *et al.*, 2003). The *H. rumeliacum* essential oil showed its highest activity against a Gram-negative strain of *E. coli,* while *E. cloaceae* appeared to be the most resistant. According to the authors, the antibacterial properties of *H. rumeliacum* oil could be associated with the high percentage of α-pinene and β-pinene.

In a recent study, essential oils of six *Hypericum* species (*H. alpinum, H. barbatum, H. rumeliacum, H. hirsutum, H. maculatum* and *H. perforatum*) were tested against several bacteria (*Escherichia coli* strain ATCC 35218, *Proteus mirabilis, Agrobacterium tumefaciens, Pseudomonas aeruginosa, Pseudomonas tolaasii, Salmonella enteritidis* strain ATCC 13076, *Staphylococcus aureus* strain ATCC 6538, *Micrococcus luteus, Sarcina lutea* strain ATCC 9341 and *Bacillus cereus*) (Saroglou *et al.*, 2007). They found that *H. barbatum* essential oil was proved to be the most active, while the essential oils of *H. alpinum* and *H. hirsutum* were inactive against the clinical species of *P. mirabilis* and *P. aeruginosa*.

Antifungal activity

Essential oil of *H. perforatum* flowers was found to be effective against the fungus *Aspergillus niger*, but not against *Candida albicans* (Gudžić *et al.*, 1997). Similarly, *H. coris* essential oil consisting mainly of α-curcumene did not show any antimicrobial activity against *Candida albicans* (Couladis *et al.*, 2003), whereas antimicrobial activity was found against *Saccharomyces cerevisiae*. Couladis and co-workers (Couladis *et al.*, 2003) tested the activity of essential oil isolated from the aerial parts of *H. rumeliacum* against three fungi (*Candida albicans, C. tropicalis,* and *C. glabrata*) and reported the MIC value of 4.75–6.34 mg/ml. Saroglou *et al* (Couladis *et al.*, 2003) tested essential oils of six *Hypericum* species against *Candida albicans*. Among them, *H. barbatum, H. rumeliacum, H. maculatum* and *H. perforatum* essential oils were effective. The MIC for *H. barbatum* and *H. rumeliacum* was 25 μg/ml whereas, *H. maculatum* and

H. perforatum showed an MIC of 50 µg/ml, whereas *H. alpinum* and *H. hirsutum* essential oils were inactive.

Cakir and co-workers tested the essential oils of three species of *Hypericum* against several phytopathogenic fungi using agar diffusion method for the possible use in agricultural pest and disease control. A moderate antifungal activity of *H. hyssopifolium* and *H. heterophyllum* was found against *Fusarium acuminatum*, and *Rhizoctonia solani* (strains AG-5, AG-9 and AG-11) (Couladis *et al.*, 2003). *H. linarioides* essential oils was active against *Rhizoctonia solani* strain AG-9 and *Verticillium alboatrum* (Couladis *et al.*, 2003).

Although the antimicrobial activity of essential oils from many plant species has been extensively surveyed, their accurate mechanism of action has not been reported in great details. However, it is thought that it might involve membrane disruption by lipophlic compounds, such as terpenoids (Cowan, 1999). It has been demonstrated that α-pinene and β-pinene are able to destroy cellular integrity, and thereby, inhibit respiration and ion transport processes, and they can also increase the membrane permeability in yeast cells and isolated mitochondria (Andrews *et al.*, 1980; Uribe *et al.*, 1985). Griffin *et al.* (1999) postulated a relative inactivity of hydrocarbons, which is correlated to their limited hydrogen capacity and water solubility. On the other hand, besides being active, ketones, aldehydes and alcohols have different specificity and activity levels. These facts seem to be associated to the functional group and with hydrogen-bonding parameters. Indeed, it seems that there is a relationship between the compounds' chemical structure and the antimicrobial activities they exert (Griffin *et al.*, 1999). Several data suggest that a great antimicrobial potential could be ascribed to the oxygenated terpenes (Panizi *et al.*, 1993; Adam *et al.*, 1998; Saroglou *et al.*, 2007).

Antiangiogenetic activity

Recently, antiangiogenetic activity of essential oil of *Hypericum perforatum* has been demonstrated using the chicken chorio allantoic membrane (CAM) assay by Demirci *et al* (2008) (Demirci *et al.*, 2008). The essential oils at various concentrations (5-50 microgram/pellet) remarkably prevented new blood vessel growth in the *in vivo* chicken embryo compared to standards. Antiangiogenic effect of *H. perforatum* has also been attributed to hyperforin, a phenolic type compound that do not takes part of the essential oil (Martínez-Poveda B *et al.*, 2005). This compound has shown to inhibit angiogenesis both *in vitro* and *in vivo*. Thus, hyperforin and essential oil may have potential use in cancer and metastasis inhibition, as well as in treatment of angiogenesis-related pathologies.

Antioxidant activity

Antioxidant and inhibitory activity of acetylcholinesterase were shown in assays performed with essential oils of *Hypericum undulatum* (Ferreira *et al.*, 2006). According to the authors, further investigations should be done with this essential oils to evaluate its potential use in preventing or alleviating patients suffering from Alzheimer's Disease (Ferreira *et al.*, 2006).

Biological Activities of *Hypericum* Extracts

The most of the pharmacological properties described in the previous section related with *Hypericum* essential oils coincides well with biological activities reported to *Hypericum* crude extracts. Hence, we speculate that at least some of the biological activities exhibited by the crude extract should also be present in the essential oils. Here we summarize some of the important biological activities of *Hypericum* crude extract, which have not so far been demonstrated with essential oils, but it would be worth to be done.

Antitumor activity

Hartwell (1970) reported 14 entries referring to *Hypericum* as a folk remedy for various neoplastic conditions in a survey on the use of plants for the treatment of cancer (Hartwell, 1970). Thus, in the last years, *Hypericum* extracts have been investigated for their potential use as antitumor agent. Antiproliferative activity of crude methanolic extracts of *H. caprifoliatum* Cham. & Schlecht., *H. carinatum* Griseb., *H. connatum* Lam., *H. myrianthum* Cham. & Schlecht., *H. polyanthemum* Klotzsch ex Reichardt and *H. ternum* A. St. Hil. were tested against two cell lines (HT-29 human colon carcinoma cells and H-460 non-small cell lung carcinoma). The most active crude methanolic extracts were those from *H. caprifoliatum, H. myrianthum* and, to a lesser extent, from *H. connatum* (Ferraz *et al.*, 2005a). *H. perforatum* extracts (commercially available St. John's wort preparations) showed potential anticarcinogenic effect, since they functioned as potent inhibitors of the major human procarcinogen-activating enzyme, the isoform CYP1A1 (Schwarz *et al.*, 2003). These extracts should be also evaluated for cancer chemopreventive potential. Different antiproliferative effects of *Hypericum* methanlic extracts on leukemia cell lines (K562 and U937), human colon carcinoma cells (HT-29) and non-small cell lung carcinoma (H-460) were also reported (Hostanska *et al.*, 2003a; Ferraz *et al.*, 2005a).

Antioxidant activity

The actual knowledge that reactive oxygen species are involved in the genesis of several diseases, such as arteriosclerosis, rheumatism, some cancers and ageing has prompted the search for new antioxidants (Heilmann *et al.*, 2003). Thus, there is an increasing demand for phytochemicals with antioxidative activity, not only plant extracts but also isolated compounds. The high amount of phenolic compounds produced by species of the genus *Hypericum* makes its extract very interesting from the potential antioxidant activity point of view. *H. barbatum* Jacq., *H. hirsutum* L., *H. linarioides* Bosse, *H. maculatum* Crantz, *H. olympicum* L., *H. perforatum* L., *H. richeri* Vill., *H. rumeliacum* Boiss. and *H. tetrapterum* Fries crude methanolic extracts have been tested for their antioxidant activity (Radulovic *et al.*, 2007). Even though, all the extracts of the *Hypericum* species studied possess a significant antioxidant activity, it was higher in the crude methanolic extracts of the flowers of *H. perforatum*, followed closely by aerial parts of *H. barbatum*. The antioxidant activity of ethanolic extracts of *H. perforatum* has also been demonstrated (Silva *et al.*, 2005). Methanolic extracts of *Hypericum rumeliacum* Boiss. also revealed antioxidant activity (Galati *et al.*, 2008).

H. triquetrifolium, is another species of the genus, which water and methanol extracts had significant antioxidant activities (Conforti *et al.*, 2002; Tawaha *et al.*, 2007). Ethanol and water extracts of the flowers of *H. venustum* showed a strong reducing power, free radicals and hydrogen peroxide scavenging activity (Spiteller *et al.*, 2008). Amongst 21 species of medicinal plants widely used in Bulgaria, extracts of *H. perforatum* prepared as infusions with water, showed considerable antioxidant activity (Ivanova *et al.*, 2005). Various extracts from *H. undulatum*, such as essential oils, decoctions and ethanolic extracts showed inhibitory activity of acetylcholinesterase and antioxidant activity, demonstrating that it may help to prevent or alleviate patients suffering from Alzheimer's Disease (Ferreira *et al.*, 2006). Recently, water and methanol extracts of *H. triquetrifolium*, from Jordania, revealed high antioxidant activities, concomitant with high amounts of phenolic compounds. Significant antioxidant activity has also been attributed to methanolic extracts of *H. triquetrifolium* (Conforti *et al.*, 2002).

Antiviral activity

Schmitt and co-workers (2001) tested aqueous and methanolic extracts of *H. connatum*, *H. caprifoliatum* and *H. polyanthemum* for their antiviral activity against feline immunodeficiency

virus (FIV). Feline immunodeficiency virus (FIV) is a causative agent of acquired immunodeficiency syndrome (AIDS)-like disease in cats. The fact that FIV has similarity to HIV-1 in many molecular and biochemical properties, and the pathogenesis of FIV infection is thought to be similar to that of HIV infection makes it an attractive model for AIDS research. From the three plants studied only *H. connatum*, used in traditional medicine, showed activity against FIV (Schmitt *et al.*, 2001). Antiviral activity against herpes simplex viruses (HSV) was also shown in crude methanolic extracts of *H. connatum* (Fritz *et al.*, 2007).

Wound-healing and anti-inflammatory activities

Besides the above mentioned, several other biological activities have been attributed to the *Hypericum* extracts. Wound-healing activity of *H. perforatum* alcoholic extracts was recently shown, when extracts were incubated with chicken embryonic fibroblasts from fertilized eggs (Öztürk *et al.*, 2007). The authors found that the mechanism of action of these extracts was similar to that of titrated extract of *Centella asiatica*, which is used in Europe in wound healing drugs (Maquart *et al.*, 1999). Indeed, both extracts seemed to stimulate collagen synthesis in fibroblast cultures and increase the tensile strength of tissues.

Analgesic and topical anti-inflammatory activities in mice were also reported for methanol extracts of three other *Hypericum* species, *H. reflexum*, *H. canariense* L, and *H. glandulosum* Ait. (Rabanal *et al.*, 2005; Sánchez-Mateo *et al.*, 2006). Furthermore, the methanol extracts of *H. perforatum, H. empetrifolium, H. triquetrifolium* and *H. rumeliacum* have been shown to exhibit anti-inflammatory activity (Apaydin *et al.*, 1999; Öztürk *et al.*, 2002; Galati *et al.*, 2008). Analgesic activity was also attributed to the *H. empetrifolium* extracts (Trovato *et al.*, 2001).

Biotechnology in *Hypericum* Essential Oil Improvement

Recent data have shown a complex composition of the essential oils as well as the influence of different parameters in their quality. Indeed, as mentioned above, the composition of essential oils depends on climatic and pedological conditions, plant organ and vegetative cycle stage. Thus, it is of utmost importance to characterize the essential oils composition as well as the influence of the referred parameters on its quality, in order to obtain essential oils of constant composition. According to Bakkali (2008), this could only be possible if essential oils are 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.

However, one possible alternative to conventional agriculture practices for the production of essential oils could be the use of *in vitro* cultures (Rao *et al.*, 2002; Lila, 2005). This technology mostly offers the possibility of having a controlled system of production, ensuring a continuous supply of products with uniform quality and yield (Lila, 2005). To the best of our knowledge, essential oils composition upon *in vitro* cultures has been reported only in *H. androsaemum* (Guedes *et al.*, 2003). In the essential oils isolated from shoots of *H. androsaemum*, sesquiterpene hydrocarbons was the major group of compounds, representing more than 80% of the total essential oil. Its major constituent was γ-muurolene (15% of the total essential oil) (Guedes *et al.*, 2003).

Genetic transformation methods can also be used to improve the essential oils content. Transformation is currently used for genetic manipulation of major economic crops, vegetables, ornamental, medicinal, fruit, tree and pasture plants, using *Agrobacterium*-mediated or direct transformation methods (Riva *et al.*, 1998). Genetic transformation on species of *Hypericum* has only been performed in *H. perforatum*. A successful protocol of particle bombardment-mediated transformation for *H. perforatum* L. has been reported (Franklin *et al.*, 2007). According to the authors, this species remains highly recalcitrant towards *Agrobacterium*-mediated transformation. Further data have shown a reduction of *Agrobacterium*-viability when in contact with *H. perforatum* cells. Possibly, this can be due to the activation of defence mechanisms of the plant cells against the bacteria (Franklin *et al.*, 2008). The establishment of successful transformation procedures and the knowledge of biosynthetic pathways of useful secondary metabolites can make possible the modulation of its production. In the last decades, great progress has been made in the elucidation of plant terpenoids biosynthetic pathways at the gene and enzyme levels. Indeed, Metabolic Engineering is seen as a powerful tool to improve secondary metabolites production. For *Hypericum* essential oils, the knowledge of the enzymes involved in the production of its compounds as well as their regulatory mechanisms can possibly lead to the increase in the production of essential oils with higher amount of the desirable compounds. For instance, *β*-caryophyllene, one of the major constituents of essential oils of some *Hypericum* species, should be an interesting molecule to increase production due to its abovementioned pharmacological activities. Moreover, the enzyme responsible for its synthesis, β-caryophyllene synthase, has already been isolated and characterized in several species (*Artemisia annua* (Cai *et al.*, 2002), *Arabidopsis thaliana* (Chen *et al.*, 2003) and *Cucumis sativus* (Mercke *et al.*, 2004). Different approaches can be used in order to modulate its production, such as over-expression of genes

involved in the production of the desirable compounds, manipulation of transcription factors controlling the expression of cascades of genes, or even the identification of tissue/organ-specific promoters (Lange *et al.*, 1999; Mahmoud *et al.*, 2002).

Conclusion

Without doubt, *Hypericum* is an important genus containing a vast array of secondary metabolites distributed by water, alcoholic extracts and essential oils. Although essential oil composition of several *Hypericum* species has been reported, its pharmacological studies are scarce. Essential oils characterization of *H. perforatum, H. androsaemum* and *H. undulatum*, are under way in our laboratory.

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