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Full Length Research Paper

Antimicrobial activity and chemical composition of essential oils of four *Hypericum* from Khorasan, Iran

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The essential oils obtained by hydrodistillation from the flowers, leaves, stems and roots or from four species *Hypericum* include *Hypericum perforatum* L.*, Hypericum hyssopifolium* Vill., *Hypericum helianthemoides* (Spach) Boiss. and *Hypericum scabrum* L. from plants growing wild in Khorasan, Northeast of Iran, were analyzed by gas chromatography (GC) and gas chromatography/mass spectral (GC/MS). In the oil of flowers, leaves, stems and roots from *H. perforatum,* 47, 43, 30 and 34 (0.06 to 0.1%w/w); from *H. hyssopifolium* 42, 48, 45 and 44 (0.05 to 0.09%w/w); from *H. helianthemoides* 50, 49, 39 and 46 (0.05 to 0.09%w/w); and from *H. Scabrum* 58, 43, 41 and 40 (0.04 to 0.1%w/w) components were identified, respectively. In the light conclusion and despite the morphological characters, *H. perforatum* L., *H. hyssopifolium* and *H. scabrum* could be placed in the pinene group, and *H. helianthemoides* in the ß-caryophyllene group. The *in vitro* antimicrobial activity of essential oils were also examined against seven microbial strains (gram positive and gram negative) by disc agar diffusion method and in general, the oils showed moderate activity against all tested microorganisms.

Key words: Antimicrobial activity, Essential oils composition, Iran, Khorasan, *Hypericum SP*, β-caryophyllene, α-pinene.

INTRODUCTION

The genus *Hypericum* L. (Family Hypericaceae) consists of over 460 species, which occur in all temperate parts of the world (Robson NKB 2003; Hosni K et al. 2008). Seventeen species of them are found in Iran, three of which are endemic: *Hypericum fursei* N. Robson, *Hypericum dogonbadanicum* Assad, and *Hypericum asperulum* Jaub.f Spach (Rechinger, 1980; Mozaffarian, 1996). Among them most commercially important member of this genus, *Hypericum perforatum* L., St. John's wort, demonstrated antidepressant, antimicrobial, antiseptic, antihelmintic antiviral, and anticancer activities (Mills et al., 2000; Barnes, 2001; Fnimh, 2001). *H. perforatum* also demonstrated anti dermatophyte activity (Larypooret al., 2009). This genus contains different natural product classes, including naphthodianthrones, prenylated phloroglucinols, xanthones, flavonoids, biflavonoids, tannins, proanthocyanidins, and phenolic acids (Javidnia et al., 2008). *H. perforatum* L., *Hypericum hyssopifolium* Vill., *Hypericum helianthemoides* (Spach) Boiss. and *Hypericum scabrum* L. are four *Hypericum* species from Khorasan provinces, Iran, whose essential oils from flowers, leaves, stems and roots have been subjected to analysis in this study. Many studies of the essential oil content of *Hypericum* have been performed. Essential oils and volatile constituents that have been most frequently reported from *Hypericum* include the aliphatic hydrocarbons *n*-nonane and *n*-undecane; the monoterpenes α- and β-pinene; and the sesquiterpenes β-caryophyllene and caryophyllene oxide (Crockett, 2010).

An examination of *H. perforatum* plants growing in 10 defined habitat types in Lithuania allowed the identification of three distinct chemotypes, dominated respectively by β-caryophyllene, caryophyllene oxide and germacrene D (Mockute et al., 2003, 2007). A similar study performed in southeastern Poland identified significant differences among both the content and composition of essential oils from plants growing in 16 habitats, although two major constituents (2-methyloctane and α-terpineol) were produced by representatives of most populations (Crockett, 2010). An examination of 11 accessions of *H. perforatum* leaves

and flowers growing in a single population in Lithuania indicated that β-caryophyllene and caryophyllene oxide dominated in leaves, while spathulenol, tetradecanol and viridiflorol were dominant constituents of the flowers (Radusiene et al., 2005). In June 2008 we studied *H. perforatum* plants growing in northeastern of Iran (northwestern of Neyshabur) revealed α- and β-pinene and α- and β-selinene as the primary volatile constituents of the leaves and flowers, while germacrene D was predominant in the oil extracted from the stems and roots (Motavalizadehkakhky et al., 2008). 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 (Bertoli et al., 2011).

H. perforatum collected from Serbia (Saraglou et al., 2007) 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). α- and β-pinene are major components in the oil of *H. perforatum* from Greece (Petrakis et al., 2005).

The amount of mono- and sesquiterpenes, seem reduced in *H. perforatum* from Turkey (Demirci et al. , 2005; Cirak et al., 2010). The main components in the oil of *H. prforatum* from Italy were 2-methyl octane (21.1%), germacrene-D (17.6%) and α -pinene (15.8%) (Pintore et al., 2005). Samples of French *H. scabrum* plants were rich in sesquiterpenes (Mathis et al., 1964), while the oil of the same species collected in Turkey consisted of 13 monoterpene hydrocarbon (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* collected from Iran (Morteza-Semnani et al., 2005). Hyperican content in flower and leaves of eight *Hypericum* (*helianthemoides, hyssopifolium, scabrum, perforatum,...*) species from Iran determined by HPLC (Jaymand et al., 2008). Chemical composition of leaves and flowers and fruits of *H. perforatum* from Kashan in Iran were determined by gas chromatography-mass spectrometry (Akhbari et al., 2009). Analysis of oil resulted in identification of 55 compounds (91.4%), for leaves, which α-pinene (29.33%) was the main components.

The analysis for flower and fruit part resulted in the identification of 26 compounds (95.96 %), which α-Amorphene (15.86%), α-pinene (11.34%), Thymol (7.27%) and α-Campholene aldehyde (6.63%) were the main components. Chemical composition of aerial parts of *H. perforatum* and *H. scabrum* from Tajikistan were analyzed by GC-MS. Sixty-six compound were identified in the oil of *H. prforatum* with Germacrene D (13.7%), α-pinene (5.1%), (E)-Caryophyllene (4.7%), n-dodecanol (4.5%), Caryophyllene oxide (4.2%), Bicyclogermacrene (3.8%), Spathulenol (3.4%) as the main constituents. Twenty-six compounds were identified in the oil of *H. scabrum* L. with α-pinene (44.8%), Spathulenol (7.1%), Verbenol (6.0%), trans-Verbenol (3.9%), and γ-Muurolene (3.5%) as the abundant compounds (Sharopov et al., 2010).

Many recent example of antibacterial or antifungal activity of essential oils can be found in the *Hypericum* genus, not only for *H. prforatum*. In fact, several *Hypericum* species native to different region have been investigated on several types of bacteria and fungi (Warnke et al., 2009; Buchbauer et al., 2010, 2004; Pauli et al., 2010).

Essential oil from *H. maculatum* Crantz. in Serbia showed a large spectrum and a strong activity as antimicrobial agent especially against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Sarcina lutea* (Gudzic et al., 2002).

The antimicrobial activities of α- and β-pinene, as well as β-caryophyllene, have been well-documented and, as these compounds represent dominant components in the essential oils of many *Hypericum* species, such effects are not unexpected. Further investigations with essential oils, volatile fractions and infused oils from *Hypericum* species would be of interest due to the *ex vivo* anti-inflammatory activity and *in vivo* gastroprotective effects that have been demonstrated with *H. perforatum* infused oils (Zdunic et al., 2009; Lavagna et al., 2001).

The essential oil of fresh aerial parts of *Hypericum richeri* Vill. subsq. grisebachii obtained by hydrodistilation was analyzed by GC and GC-MS. One hundred and five constituents identified and tested against a panel of microbial strains by broth microdilution assay and it was found to was also moderate effect against all tested microorganisms (Dordevic et al., 2011).

The essential oils of *H. scabrum*, *H. scabroides* and *H. triquetrifolium* were studied for the first time for their antimicrobial activity against nine organisms. All the essential oils exhibited some broad spectrum antibacterial activity, at a concentration of 80 µg/ml. The essential oils of *Hypericum* species showed antibacterial activity against the tested organisms and a yeast (Kizil et al., 2004). The composition of the hydrodistilled oils obtained from aerial parts of *H. hyssopifolium* subsp. and *H. heterophyllum* Vent. were analyzed by means of GC and GC-MS, and 66 compounds were determined in total. The oils of *H. hyssopifolium*, is rich in monoterpenes consists α-pinene (57.3%), β-pinene (9.0%), limonene (6.2%) and α-phellandrene (4.4%). The oils were tested for antifungal activity using microbial growth inhibition assays *in vitro* against 10 agricultural pathogenic fungi. In general, the oils showed moderate activity against several fungal species (Cakir et al., 2004). The chemical composition of the essential oils of nine taxa from seven sections of *Hypericum* L. (Guttiferae, *H. perforatum* subsp*. perforatum, H. perforatum* subsp*. veronense, H. calycinum, H. montanum, H. richeri* subsp*. richeri, H. hyssopifolium, Hypericum hirsutum, Hypericum*

Table 1. Weight of plants, time and yield percentage of hydrodistillation.

Parts of plant	*H. perforatum*			*H. hyssopifolium*			*H. helianthemoides*			*H. scabrum*		
	Weight (g)	Time (h)	Yield (%)	Weight (g)	Time (h)	Yield (%)	Weight (g)	Time (h)	Yield (%)	Weight (g)	Time (h)	Yield (%)
Flower	128	3	0.1	120	2.8	0.09	132	3.2	0.09	120	3.5	0.1
Leaves	150	3	0.09	165	3	0.08	180	3	0.08	220	3	0.09
Stems	110	2.5	0.08	100	3	0.06	120	3.5	0.06	110	3	0.06
Roots	100	3.5	0.06	95	3.5	0.05	90	3.5	0.05	110	3.5	0.04

hircinum subsp. majus, and *Hypericum tetrapterum*) occurring in central Italy was analyzed by gas chromatography (GC) / flame ionization detector (FID) and GC/MS. A total of 186 compounds were identified, accounting for 86.9 to 92.8% of the total oils. The major fraction of the oil was always represented by sesquiterpene hydrocarbons (30.3 to 77.2%), while quantitative differences occurred between the other classes of volatiles depending on the taxa considered. Chemical composition of the nine *Hypericum* entities with respect to the taxonomical classification was discussed. Essential oils obtained from six taxa, were also tested for their antimicrobial properties against five different microbial strains by the broth-microdilution method, and they were found to have significant activity (expressed as MIC) (Maggi et al., 2010).

The volatile constituents, obtained from air-dried aerial parts of fruiting *Hypericum elongatum* were analyzed by GC/MS method. Thirty four components of about 96.50% of total oil were identified. α-Pinene (80.43%), γ-Terpinene (4.23%) and β-Pinene (2.59%) were the principal components (87.16%). The essential oil and hydroalcoholic extract were evaluated for antibacterial, antifungal and anti-yeast activities by using disc diffusion method (Ghasemi et al., 2007).

MATERIALS AND METHODS

Plant material

Four *Hypericum* were collected from Khorasan-Razavi and Khorasan-Shomali Provinces, Iran, in June 2010. *H. perforatum* L., *H. hyssopifolium* Vill., *H. helianthemoides* (Spach) Boiss. and *H. scabrum* L. were collected from Kharve in Este of Neyshabur [in last study we investigated *H. perforatum* L. from northeastern of Neyshabur (Motavalizadehkakhky et al., 2008)]; Bojnord; Mashhad (Akhlamad) and Chenaran, respectively. The plants were air dried and dried samples were crushed, then essential oils were obtained by hydrodistillation of their flowers, leaves, stems and roots, separately. Voucher specimens of the plant have been deposited in the herbarium.

Isolation of the Essential oils

90 to 220 g out of any parts (flowers, leaves, stems and roots) of plants were subjected to hydrodistilation for 2.5 to 3.5 h using an original Clevenger-type apparatus and yielded from 0.04 to 0.1% (w/w) of essential oils. After decanting, the obtained essential oils were dried over anhydrous magnesium sulfate and, after filtration, stored in refrigerator at - 4℃ until tested and analy zed (Table 1).

Analysis of the essential oils

Gas chromatography

Samples of the oils were diluted in acetone (1:9) and 1 µl was used for analysis. GC-MS analyses of the essential oil was analyzed on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer with an injector 7683B series device. An Agilent (9091)-413:325℃ HP-5 column (30 m x 320 x 0.25 µm) was used with helium as carrier gas at a flow rate of 3.35 ml/min. The GC oven temperature was initially programmed at 50℃ (hold for 1 min) and finally at 300℃ (hold for 5 min) at a ra te of 80℃/min while the trial temperature was 37.25℃.

The column heater was set at 250℃ in a split less mode while the pressure was 10.2 psi with an average velocity of 66.5 cm/s and a hold-up time of 0.75 min. Mass spectrometry was run in the electron impact mode (EI) at 70eV. The percentage compositions were obtained from electronic integration measurements using flame ionization detector (FID), set at 250℃.

Gas chromatography-mass spectrometry

The essential oils were analyzed by GC-MS on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer with an injector 7683B series device. An Agilent (9091) 413:325℃ HP-5 column (30 m x 320 x 0.25 µm) was used with helium as carrier gas at a flow rate of 3.35 ml/min. GC oven temperature and conditions were as described previously. The injector temperature was at 250℃. Mass spectra were rec orded at 70 eV. Mass range was from *m/z* 30 to 500.

Identification of components

Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkenes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, 2007), and stored on the MS library (NIST 08.L database/ chemstation data system) with data previously reported in literature (McLafferty and Stauffer, 1989; Joulain and König, 1998).

The percentages of each component are reported as raw percentages base on total ion current without standardization. The chemical compositions of any parts of four *Hypericum* are summarized in Tables 2 and 3.

Table 2. Percentage of chemical composition of *Hypericum perforatum* L. and *Hypericum hyssopifolium* Vill. oils.

Compound	RI	*H. perforatum* L.				*H. hyssopifolium* Vill.			
		Flower	Leaves	Stems	Roots	Flower	Leaves	Stems	Roots
α-Pinene	939	27.5	16.5	2.0	3.5	17.3	13.6	14.0	12.5
Sabinene	975	0.1	5.5	-	-	0.4	0.2	0.5	0.9
β-Pinene	979	12.7	2.1	Tr	5.0	4.6	5.0	10.5	7.0
Myrcene	990	0.8	0.2	-	-	1.0	0.4	0.9	0.5
α-Phellandrene	1002	0.1	-	0.4		2.4	1.0	1.5	-
p-Cymene	1024	0.2	0.4	-	0.5	0.6	2.1	0.4	-
Limonene	1027	1.0	0.2	-	-	2.2	0.6	0.9	2.9
1,8-Cineole	1029	0.3	-	0.5	-	0.4	0.3	0.7	0.2
β-Phellandrene	1031	-	0.2	-	2.7	-	-	3.5	0.4
(E)-β-Ocimene	1044	0.7	0.3	-	1.0	0.3	0.9	-	0.3
Terpinolene	1088	-	Tr	7.2	-	0.1	-	1.2	0.3
Undecane	1098	-	-	1.8	Tr	-	0.8	0.8	-
Linalool	1102	0.4	0.3	-	-	0.2	1.2	-	0.5
α-Thujone	1107	0.7	0.5	-	0.2	0.1	-	0.6	-
β-Thujone	1112	1.6	-	0.3	-	-	-	0.4	0.5
Camphor	1141	-	-	1.6	-	-	0.3	0.3	0.2
Menthone	1152	0.2	0.3	-	0.1	-	0.5	-	0.1
Borneol	1165	0.6	0.4	-	-	0.4	1.1	2.0	1.2
Terpinen-4-ol	1174	0.4	0.5	-	Tr	0.3	0.5	-	0.2
Carvone	1243	0.5	0.3	0.1	-	0.1	0.3	-	-
Linalool acetae	1257	0.2	-	0.1	0.1	0.1	-	0.1	-
Thymol	1290	5.0	3.0	Tr	-	0.4	3.1	2.5	5.0
Carvacrol	1299	1.4	0.3	-	-	0.5	0.4	0.6	-
α-Cubebene	1348	0.7	0.4	-	0.5	-	0.6	-	0.3
α-Copaene	1376	0.4	0.3	2.0	1.9	-	-	-	-
β-Elemene	1390	0.7	0.3	1.0	2.8	0.4	0.4	1.7	3.1
α-Gurjunene	1409	0.9	0.7	-	-	-	-	0.3	1.6
β-Caryophyllene	1419	4.1	4.5	8.5	4.0	5.5	3.1	6.0	5.5
β-Copaene	1432	0.3	-	0.1	-	0.2	0.6	-	0.7
β-Humulene	1438	-	0.5	-	1.7	-	0.1	0.2	-
(Z)-β-Farnesene	1443	Tr	-	0.2	3.6	-	0.6	0.7	1.2
α-Humulene	1454	-	0.4	-	2.2	Tr	2.1	Tr	-
(E)-β-Farnesene	1456	5.1	2.7	3.5	-	5.5	6.3	4.2	3.7
β-Acoradiene	1466	0.4	-	-	0.3	0.3	-	-	2.2
Dodecanol	1470	-	-	-	-	5.3	0.2	4.0	3.2
γ-Gurjunene	1477	0.3	0.8	-	Tr	-	-	-	-
γ-Muurolene	1479	1.5	-	1.2	4.0	5.2	2.2	3.1	3.7
Germacrene D	1485	0.2	3.5	35.0	19.5	10.2	8.1	6.7	4.2
β-Selinene	1490	7.1	20.2	-	8.5	3.3	4.3	2.8	1.9
α-Selinene	1498	8.0	11.4	-	6.0	2.4	7.1	4.5	2.1
Bicyclogermacrene	1500	2.5	0.4	0.3	0.1	0.2	0.2	-	1.1
(Z)-α-Bisabolene	1507	-	-	-	1.9	0.1	0.1	0.2	0.1
Germacrene A	1509	0.6	0.3	0.4	0.1	0.7	0.5	0.2	-
γ-Cadinene	1513	0.3	0.2	-	3.0	4.5	3.5	1.7	1.9
Myristicin	1518	0.2	-	7.2	-	-	-	-	-
δ-Cadinene	1523	2.4	1.1	7.3	4.6	4.5	3.0	2.5	1.8
α-Calacorene	1545	0.3	0.4	1.4	-	0.1	1.5	0.3	1.2
Elemicin	1557	-	0.5	1.2	-	-	0.5	-	-
(3Z)-Hexenyl benzoate	1569	0.3	0.4	-	-	-	0.4	0.4	0.3
Spathulenol	1578	0.4	0.5	2.0	1.7	11.5	7.3	2.5	4.6

Table 2. Contd.

Caryophyllene oxide	1583	1.7	0.4	1.9	2.2	2.0	2.2	1.9	1.9
Junenol	1619	0.2	-	Tr	0.1	0.1	0.2	0.6	0.4
5-Cedranone	1630	-	-	-	3.2	-	-	-	-
β-Eudesmol	1650	0.2	0.2	0.6	-	0.3	4.0	2.5	4.5
α-Cadinol	1654	2.5	0.3	-	Tr	1.2	1.0	2.1	0.9
Germacra-4(15),5, 10 (14)-triene-1-α-ol	1686	0.4	1.2	-	-	-	0.5	0.8	0.7
Junicedranol	1692	-	-	-	-	3.2	1.4	2.5	2.2
6,10,14-trimetyl-2-pentadecanone	1849	-	3.5	-	-	-	-	-	-
Unidentified	1956	0.5	-	-	-	-	-	-	-
(E)-Phytol	1943	0.6	-	0.3	-	-	0.2	0.5	0.1
Heneicosane	2100	-	6.0	-	1.0	0.6	1.0	1.0	0.9
Number of identified compounds		47	43	30	34	42	48	45	44
Yield of the oil (%)		0.1	0.09	0.08	0.06	0.09	0.08	0.06	0.05
Monoterpenes		43.1	25.4	9.6	12.7	28.9	23.8	33.4	24.8
Oxygenated monoterpenes		11.1	5.6	2.5	0.3	2.4	7.7	7.1	7.9
Sesquiterpenes		35.8	48.1	60.9	64.7	43.1	44.3	35.1	36.3
Oxygenated sesquiterpenes		5.4	2.6	4.5	4.0	17.3	16.6	12.9	15.2
Diterpenes		-	-	-	-	-	-	-	-
Oxigenated diterpenes		0.6	-	0.3	-	-	0.2	0.5	0.1
Others		1.2	10.4	10.3	4.3	6.0	2.9	6.3	4.4
Total		97.2	92.1	88.1	86.0	97.7	95.5	95.3	88.7

Tr : trace(< 0.05%).

Antimicrobial assay of the Oils

In vitro antibacterial assay of the oils carried out according to disc agar diffusion method (Jirovets, 1999; Kumar, 2004). Antibacterial activity of the oils were tested against gram positive bacterial strains such as *Bacillus cereus* (MTCC430), *B. subtilis* (MTCC441), *S. aureus* subsp. Aureus (MTCC2940); and Gram-negative bacterial strains such as *K. pneumonia* (MTCC109), *E. coli* (MTCC443), *Proteus vulgaris* (MTCC426) and *Salmonella typhi* (MTCC733), were grown in nutrient broth for 24 h (pH 7.2 to 7.4) and were used as inoculums. The Mueller-Hinton agar medium were poured into the plates to uniform depth of mm and allowed to solidify. Then the microbial suspensions were streaked over the surface of media using a sterile cotton swab to ensure the confluent growth of the organism. Aliquots of 10 µl of the oil at 1:2 dilutions in dimethyl sulfoxide (DMSO) were impregnated on Whatman No. 1 filter paper discs of 6 mm diameter. These discs were aseptically applied to the surface of the agar plates at well-spaced intervals. The plates were incubated at 37℃ for 24 h and observed inhibition z ones including the diameter of the discs were measured. Control discs impregnated with 10 µl of the solvent DMSO and streptomycin (10 µl /disc), reference for bacteria were used alongside the test discs in each experiment. The results are presented in Table 3.

RESULTS AND DISCUSSION

Chemical composition of the essential oils

1. *H. perforatum* L.

The compounds identified in flowers, leaves, stems and roots of *H. perforatum* L. essential oils are listed in Table 2.

(a) Flowers: Forty seven constituents accounted for 97.2% of the total flowers oil. α-Pinene (27.5%), β-Pinene (12.7%), β-Caryophyllene (4.1%), (E)-β-Farnesene (5.1%), β-Selinene (7.1%), and α-Selinene (8.0%) were major components. Monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes were 43.1, 11.1, 35.8 and 5.4%, respectively.

(b) Leaves: Forty three constituents accounted for 92.1% of the total leaves oil. α-Pinene (16.5%), Sabinene (5.5%), β-Pinene (2.1%), β-Caryophyllene (4.5%), β-Selinene (20.2%), Germacrene D (3.5%), Heneicosane (6.0%) and α-Selinene (11.4%) were major components. Monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes were 25.4, 5.6, 48.1 and 2.6%, respectively.

(c) Stems: Thirty compounds identified in stems oil (88.1%). Terpinolene (7.2%), β-Caryophyllene (8.5%), Germacrene D (35.0%), Myristicin (7.2%) (E)-β-Farnesene (3.5%), and δ-Cadinene (7.3%) were main components. Monoterpenes (9.6%) decreased, but sesquiterpenes (60.9%) increased while oxygenated mono- and sesquiterpenes decreased (2.5 and 4.5%, respectively).

(d) Roots: Thirty four constituents representing 86.0% of

Table 3. Percentage of chemical composition of *Hepericum helianthemoides*(Spach) Boiss. and *Hypericum scabrum* L. oils.

Compound	RI	*H. helianthemoides*				*H. scabrum* L.			
		Flower	Leaves	Stems	Roots	Flower	Leaves	Stems	Roots
α-Pinene	939	13.5	12.0	10.0	7.0	31.5	33.0	32.5	25.7
Camphene	950	0.5	1.0	4.3	5.1	0.5	0.9	0.8	0.4
trans-Pinane	975	-	2.0	3.5	4.2	0.4	-	0.5	-
β-Pinene	979	1.5	0.6	1.7	3.1	2.9	3.5	4.0	3.7
Myrcene	990	0.4	0.5	0.7	2.0	3.1	4.0	3.2	4.0
α-Phellandrene	1002	0.2	0.5	0.1	-	0.1	-	-	0.8
p-Cymene	1024	0.4	0.5	-	0.2	3.6	2.5	-	1.8
Limonene	1029	2.3	1.9	0.4	-	2.8	0.9	0.8	1.0
1,8-Cineole	1030	-	1.0	-	-	0.2	0.6	0.6	-
Sylvestrene	1030	-	1.7	0.4	1.9	-	-	-	-
(Z)-β-Ocimene	1037	6.7	3.7	2.8	0.9	1.0	0.9	0.2	0.1
(E)-β-Ocimene	1044	0.6	0.8	-	0.6	0.5	-	0.7	-
p-Mentha-2,4(8)-diene	1088	Tr	-	0.2	0.1	0.2	0.2	-	0.1
trans-Sabinene hydrate	1098	-	-	-	-	0.3	-	-	-
Undecane	1100	0.2	-	-	-	0.5	0.8	0.5	-
Linalool	1100	0.2	0.6	0.5	0.9	0.2	1.0	2.2	2.1
1,3,8-*p*-Menthatriene	1110	-	-	-	-	0.9	0.8	1.2	0.6
Myrcenol	1122	-	-	-	-	0.6	-	0.7	-
Camphor	1141	1.2	2.3	4.0	0.5	-	-	-	-
Trans-Verbenol	1144	1.7	1.7	0.9	0.6	2.1	-	0.1	0.4
Menthone	1152	-	-	-	-	2.5	2.2	1.7	1.9
Pinene oxid	1159	0.1	-	0.7	0.3	1.4	0.5	1.5	0.5
Borneol	1165	0.2	0.6	-	0.8	0.9	-	-	-
Lavadulol	1169	-	-	-	-	0.5	0.8	0.6	-
p-Cymen-8-ol	1184	0.1	1.2	2.2	0.5	0.7	0.9	-	0.5
α-Terpineol	1189	0.4	0.3	0.6	1.0	1.9	2.1	1.9	0.8
Verbenone	1207	0.3	0.2	-	-	2.5	0.2	-	-
Citronellol	1225	-	-	-	-	0.9	0.8	1.0	1.2
cis-Pulegol	1229	-	-	-	-	0.8	0.9	0.2	0.1
Thymol	1290	0.4	2.5	2.9	3.1	2.9	-	0.9	0.8
Carvacrol	1299	0.9	1.9	1.6	0.5	2.5	2.1	3.1	1.2
Pulegone	1237	1.7	2.6	3.1	1.7	-	-	-	-
2-Ethyl menthone	1286	0.5	0.3	0.4	-	-	-	-	-
δ-Terpinyl acetate	1317	-	-	-	-	0.3	-	-	-
Eugenol	1359	-	-	-	-	0.6	0.5	0.3	-
iso-Longifolene	1390	0.3	0.8	0.7	1.5	-	-	-	-
β-Isocomene	1407	-	-	-	-	-	0.2	0.9	1.0
β-Caryophyllene	1419	19.0	10.2	15.9	9.0	2.1	2.5	1.0	2.1
β-Copaene	1432	1.5	7.2	2.5	9.1	0.5	-	0.7	1.1
γ-Elemene	1436	-	-	-	-	0.2	0.8	0.6	-
α-Guaiene	1439	0.1	0.2	-	0.3	1.5	-	1.0	-
(Z)-β-Farnesene	1443	0.2	1.7	0.6	1.5	-	-	-	-
α-Humulene	1454	1.2	2.5	0.9	0.8	1.0	1.6	3.1	0.9
dehydro-Aromadendrane	1462	0.7	0.6	0.3	0.1	0.2	-	-	-
Dauca-5,8-diene	1472	0.1	-	-	-	-	-	-	-
Dodecanol	1470	0.3	-	-	-	0.6	0.8	-	1.9
β-Chamigrene	1477	-	0.2	-	0.9	-	-	-	-
α-Amorphene	1481	0.6	-	0.9	1.2	1.3	0.8	0.2	0.1
Germacrene D	1485	2.5	4.2	3.5	3.9	2.5	2.4	3.1	3.0

Table 3. Contd.

β-Selinene	1490	0.8	2.5	3.1	1.2	1.8	3.1	2.6	0.9
α-Selinene	1496	-	1.7	-	1.8	-	-	-	-
Bicyclogermacrene	1500	0.8	-	0.3	-	0.4	0.3	-	0.5
(Z)-α-Bisabolene	1505	-	0.4	-	0.6	0.3	0.3	0.1	0.2
Germacrene A	1509	0.5	0.1	0.5	-	0.4	-	-	1.1
γ-Cadinene	1513	2.1	3.2	2.6	2.9	2.9	3.4	1.7	3.9
Myristicin	1518	-	-	-	-	0.4	-	-	-
δ-Cadinene	1523	1.2	1.7	1.0	1.9	0.8	1.6	-	1.1
Selina-3,7(11)-diene	1546	0.4	0.2	-	0.1	0.5	-	-	0.1
Elemicin	1557	-	-	-	-	0.4	0.3	0.2	0.5
(3Z)-Hexenyl benzoate	1569	0.2	0.1	-	0.3	-	-	-	-
Caryolane-8-ol	1572	3.7	2.7	3.2	2.6	0.4	3.1	3.0	2.0
Spathulenol	1578	8.0	6.5	7.0	4.0	4.5	7.0	6.2	5.9
Caryophyllene oxide	1583	2.1	1.1	2.2	1.4	-	-	-	-
Cubeban-11-ol	1595	0.3	0.2	-	-	-	-	-	-
Epi-Cedrol	1619	-	-	-	-	0.7	0.5	0.2	-
β-Eudesmol	1650	2.7	1.1	1.7	2.1	0.7	3.5	4.2	4.7
α-Cadinol	1654	0.5	0.6	-	0.7	1.8	0.7	-	-
14-Hydroxy-9-epi-(E)-Caryophyllene	1669	4.9	2.2	1.9	2.1	1.2	0.2	0.1	0.6
Tetradecanol	1672	1.4	-	-	0.9	-	-	-	-
α-Chenopodiol	1856	-	-	-	-	0.9	0.7	0.1	-
(E)-Phytol	1943	0.1	0.2	-	0.5	0.3	0.2	-	0.1
Heneicosane	2100	Tr	0.5	0.4	0.9	Tr	-	-	-
Number of identified compounds		50	49	39	46	58	43	41	40
Yield of the oil (%)		0.09	0.08	0.06	0.05	0.1	0.09	0.06	0.04
Monoterpenes		26.1	25.2	24.1	25.1	47.5	46.7	43.9	38.2
Oxygenated monoterpenes		7.2	15.2	16.5	9.9	21.5	12.6	14.8	9.5
Sesquiterpene		32.0	37.4	32.8	36.8	16.4	17.0	15.0	16.0
Oxygenated sesquiterpenes		22.2	14.4	16.0	12.9	10.2	15.7	13.8	13.2
Diterpenes		-	-	-	-	-	-	-	-
Oxigenated diterpenes		0.1	0.2	-	0.5	0.3	0.2	-	0.1
Others		2.6	0.9	0.4	2.1	2.2	1.9	0.7	2.4
Total		90.2	93.3	89.8	87.3	98.1	94.1	88.2	79.4

Tr : trace(< 0.05%).

essential oils were identified in the essential oils of *H. perforatum* L. roots .12.7, 0.3, 64.7 and 4.0 were percentages of mono- , oxygenated mono- , sesqui and oxygenated sesquiterpenes, respectively.

2. *H. hyssopifolium*

The compounds identified in flowers, leaves, stems and roots of *H. hyssopifolium* essential oils are listed in Table 2.

(a) Flowers: Forty two constituents accounted for 97.7% of the total flowers oil. α-Pinene (17.3%), β-Pinene (4.6%), β-Caryophyllene (5.5%), (E)-β-Farnesene (5.5%), γ-Muurolene (5.2%), Germacrene D (10.2%), Spathulenol (11.5%), δ-Cadinene (4.5%) and γ-Cadinene (4.5%) were major components. Monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes were 28.9, 2.4, 43.1 and 17.3%, respectively.

(b) Leaves: Forty eight constituents accounted for 95.5% of the total leaves oil. α-Pinene (13.6%), β-Pinene (5.0%), β-Caryophyllene (3.1%), β-Selinene (4.3%), (E)-β-Farnesene (6.3%), Germacrene D (8.1%), Spathulenol (7.3%) , β-Eudesmol (4.0%) and α-Selinene (7.1%) were major components. Monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes were 23.8, 7.7, 44.3 and 16.6%,

Table 4. Antibacterial activity (zoon inhibition) of the oil of flowers ,leaves, stems and roots of four *Hypericum* against four gram positive and gram negative bacteria (%) as compare Streptomycin (N.T=Not Tested).

	Oil in DMSO (1:2)	MTCC No.	Streptomycin 1 mg/ml	Gram-positive bacteria			Gram-negative bacteria			
				B. cereus	*B. subtillis*	Staphylococcus	Escherichia	Klebsiella	Proteus	Salmunella
			-	430	441	2940	443	109	426	733
Zone of inhibition (mm)	*H. perforatum*	Flower	11.5	12.5(108)	13(113)	12.5(108)	12.5(109)	11.5(100)	13(113)	10(87)
		Leaves	10.5	13(124)	11.5(109)	10(95)	12(114)	11(105)	10(95)	9(86)
		Stems	8	10(125)	8(100)	7(86)	7(87)	6.5(81)	7.5(94)	8(100)
		Roots	6.5	7(108)	6(86)	5(77)	6(92)	5(77)	5.5(100)	6.5(100)
	H. hyssopifolium	Flower	12	13(108)	12(100)	13.5(120)	13(108)	12.5(104)	11.5(96)	10(83)
		Leaves	11	11(100)	12(109)	11.5(104)	12(109)	12.5(114)	13(118)	10.5(95)
		Stems	10.5	9(86)	8.5(81)	9.5(90)	13(124)	12.5(119)	13(124)	11.5(109)
		Roots	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T
	H. helianthemoides	Flower	13	12.5(96)	12(92)	11.5(88)	13(100)	12.5(96)	11(85)	10.5(81)
		Leaves	11.5	10.5(91)	10(87)	9(78)	13(113)	12(104)	10(87)	12(104)
		Stems	9	7(78)	6(67)	6(67)	10(111)	8(89)	7.5(83)	7(78)
		Roots	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T
	H. scabrum	Flower	12	14(116)	13(108)	13.5(112)	11(92)	12(100)	12.5(104)	10(83)
		Leaves	10	11(110)	11.5(115)	12(120)	11(110)	12(120)	12(120)	11.5(115)
		Stems	8	10.5(131)	9(112)	9.5(119)	N.T	N.T	N.T	N.T
		Roots	6.5	6(92)	5(77)	5.5(85)	6(92)	7(108)	8(123)	5.5(85)

respectively.

(c) Stems: Forty five compounds identified in stems oil (95.3%). β-Caryophyllene (6.0%), Germacrene D (6.7%), β-Pinene (10.5%), (E)-β-Farnesene (4.2%), and α-Selinene (4.5%) were main components. Monoterpenes (33.4%), sesquiterpenes (35.1%), oxygenated mono- and sesquiterpenes (7.1 and 12.9%, respectively) were identified.

(d) Roots: forty four constituents representing88.7% of essential oils were identified in the essential oils of *H. hyssopifolium* roots, 24.8, 7.9, 36.3 and 15.2 were percentages of mono-, oxygenated mono-, sesqui and oxygenated sesquiterpenes respectively. α-Pinene (12.5%), β-Pinene (7.0%), Thymol (5.0%), β-Caryophyllene (5.5%), Germacrene D (4.2%) , β-Eudesmol (4.5%) and Spathulenol (4.6%) were main components.

3. *H. helianthemoides*

The compounds identified in flowers, leaves, stems and roots of *H. helianthemoides* essential oils are listed in Table 3.

(a) Flowers: Fifty constituents accounted for 90.2% of the total flowers oil. α-Pinene (13.5%), β-Caryophyllene (19.0%), (Z)-β-Ocimene (6.7%), Germacrene D (2.5%) and Spathulenol (8.0%) were major components. Monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes were 26.1, 7.2, 32.0 and 22.2%, respectively.

(b) Leaves: Forty nine constituents accounted for 93.3% of the total leaves oil. α-Pinene (12.0%),(Z)-β-Ocimene (3.7%), β-Copaene (7.2%), β- Caryophyllene (10.2%), Spathulenol (6.5%) and Germacrene D (4.2%) were major

components. Monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes were 25.2, 15.2, 37.4 and 14.4%, respectively.

(c) Stems: Thirty nine compounds identified in stems oil (89.8%). α-Pinene (10.0%), Camphene (4.3%), Camphor (4.0%), β-Caryophyllene (15.9%), Germacrene D (3.5%) and Spathulenol (7.0%) were main components. Monoterpenes (24.1%), sesquiterpenes (32.8%), oxygenated mono- and sesquiterpenes (16.5% and 16.0% respectively) were identified.

(d) Roots: Forty six constituents representing 87.3% of essential oils were identified in the essential oils of *H. helianthemoides* roots . 25.1, 9.9, 36.8 and 12.9 were percentages of mono-, oxygenated mono-, sesqui and oxygenated sesquiterpenes respectively. α-Pinene (7.0%), trans-Pinene (4.2%), Camphene (5.1%), β-Caryophyllene (9.0%), Germacrene D (3.9%), β-Copaene (9.1%) and Spathulenol (4.0%) were main components.

4. *H. scabrum*

The compounds identified in flowers, leaves, stems and roots of *H. scabrum* essential oils are listed in Table 3.

(a) Flowers: Fifty eight constituents accounted for 98.1% of the total flowers oil. α-Pinene (31.5%), Myrcene (3.1%), *p*-Cymene (3.6%), Germacrene D (2.5%) and Spathulenol (4.5%) were major components. Monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes were 47.5, 21.5, 16.4 and 10.2%, respectively.

(b) Leaves: Forty three constituents accounted for 94.1% of the total leaves oil. α-Pinene (33.0%), Myrcene (4.0%), β-Caryophyllene (2.5%), Spathulenol (7.0%), β-Eudesmol (3.5%) and Germacrene D (2.4%) were major components. Monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes were 46.7, 12.6, 17.0 and 15.7%, respectively.

(c) Stems: forty one compounds identified in stems oil (88.2%). α-Pinene (32.5%), Myrcene (3.2%), β-Pinene(4.0%), α-Humulene (3.1%), β-Eudesmol (4.2%), Germacrene D (3.1%) and Spathulenol (6.2%) were main components. Monoterpenes (43.9%), sesquiterpenes (15.0%), oxygenated mono- and sesquiterpenes (14.8% and 13.8%) were identified, respectively.

(d) Roots: forty constituents representing 79.4% of essential oils were identified in the essential oils of *H. scabrum* roots. 38.2, 9.5, 16.0 and 13.2 were percentages of mono-, oxygenated mono-, sesqui and oxygenated sesquiterpenes respectively. α-Pinene (25.7%), β-Pinene (3.7%), Myrcene (4.0%), Germacrene D (3.0%), γ-Cadinene (3.9%), β-Eudesmol (4.7%) and Spathulenol (5.9%) were main components.

As a light conclusion according to Cakir et al. (2005), *Hypericum* species can be divided in two groups, based on the monoterpene and sesquiterpene content with emphasis on ß-caryophyllene and α-pinene as the major and characteristic compounds. Hence, *H. perforatum* L., *H. hyssopifolium* and *H. scabrum* could be placed in the pinene group, because α-pinene is major components in the essential oils of them, and *H. helianthemoides* in the ß-caryophyllene group because it's essential oils is rich in ß-caryophyllene.

Antimicrobial activity

The results of antimicrobial activity of the four *Hypericum* genus essential oils against seven bacterial (gram positive and gram negative) are listed in Table 4. Zone of inhibition diameters (mm) determinations were obtained by disc agar diffusion method . In general, the oils showed moderate activity against all tested microorganisms.

The *H. perforatum* L., *H. hyssopifolium* , *H. helianthemoides* and *H. Scabrum* oils obtained from flowers, leaves, stems and roots in DMSO (1:2 dilutions) showed 77 to 125, 81 to 120, 67 to 96 and 77 to 131% inhibition against gram positive bacteria : *B. cereus*, *B. subtilis* and *S. aureus* subsp. aureus, respectively, as compared to the standard, streptomycin at 10 µl/disc (Table 4).

The *H. perforatum* L. *H. hyssopifolium* ,*H. helianthemoides* and *H. Scabrum* oils obtained from flowers, leaves, stems and roots in DMSO (1:2 dilutions) showed 77 to 114, 83 to 124, 78 to 113 and 83 to 123% inhibition against gram negative bacteria : *K. pneumonia*, *E. coli*, *P. vulgaris* and *S. typhi* respectively, as compared to the standard, streptomycin at 10 µl/disc (Table 4).

The antimicrobial potential of four *Hypericum* genus essential oils could be explained by its high content of terpenoids. This activity is suspected to be associated with the high percentage of sesquiterpenoid fraction, since it was previously reported that ß-caryophyllene, ß-pinene and caryophyllene oxide possessed moderate to strong activities against a number of microorganisms (Magiatis et al., 2002; Bougatsos et al., 2004).

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REFERENCES

Adams RP (2007). Identification of Essential oil Components by Gas Chromatography / Mass Spectrometry, 4th Ed. Allured Publishing Co. Carol Stream, Illinois.

Akhbari M, Batooli H (2009). Composition of *Hypericum perforatum* L. volatile oil from Kashan. Am-Eurasian J. Sustain. Agric., 3(1): 107-110.

Barnes J, Anderson LA, Phillipson JD (2001). St.John's Wort (Hypericum perforatum L.) : a review of its chemistry, pharmacology

and clinical properties. J. Pharm. Pharmacol., 53: 583-600.
Bertoli A, Cirak C, Silva JAT (2011). *Hypericum* as Sources of Valuable Essential Oils. Med. Aromat. Biotechnol., 1: 29-47.
Bougatsos C, Ngassapa O, Runyoro DKB, Chinoua IB (2004). Chemical composition and in vitro antimicrobial activity of the essential oils of two Helichrysum species from Tanzania. Z. Naturforsch, 59c: 368-372.
Buchbauer G (2004). Biological effects of fragrances and essential oils. Vienna Medzinische wkly. 154: 539-547.
Buchbauer G (2010). Biological activities of essential oils. In: Baser KHC, Buchbauer G ; editors. Handbook of essential oils: science, technology and applications. Taylor and Francis; New York: editors. Handbook of essential oils: Sci., Technol. Appl., pp. 235-280.
Cakir A, Kordali S, Zengin H, Izumi S, Hirata T (2004). Composition and antifungal activity of essential oils isolated from *H. hyssopifolium* and *H. heterophyllum*. Flavour Fragr. J., 19(1): 62-68.
Cakir A, Kordali S, Kilic H, Kaya E (2005). Antifungal properties of essential oils and crude extracts of Hypericum linarioides. Biochem. Syst. Ecol., 33: 245 .
Cirak C, Bertoli A, Pistelli L, Seyis F (2010). Essential oil composition and variability of *Hypericum perforatum* from wild populations of northern Turkey. Pharm. Biol., 48: 906-914.
Crockett SL (2010). Essential Oil and Volatile Components of the Genus *Hypericum* (Hypericaceae) Nat. Prod. Commun., 5(9): 1493-1506.
Demirci B, Baser KHC, Crockett SL, Khan IA (2005). Analysis of the volatile constituents of Asian *Hypericum* L. species. J. Essent. Oil Res., 17: 659-663.
Dordevic A, Zlatkovic B, Lazarevic J, Palic R (2011). A detailed chemical composition and antimicrobial activity of *H. richeri* Vill. subsq. essentiall oil from Serbia. J. Med. Plants Res., 5(23): 5486-5492.
Fnimh AC (2001). *Encyclopedia of Medicinal Plants*, Dorling Kindersley, London, P. 108.
Ghasemi Y, Khalaj A, Mohagheghzadeh A, Khosravi AR, Morowvat MH (2007). Composition and Antimicrobial Activity of the Essential Oil and Extract of *Hypericum elongatum*. J. Appl. Sci., 7: 2671-2675.
Gudzic B, Djokovic D, Vajs V, Palic R, Stojanovic G (2001). Essential oils *Hypericum olympicum L*. and *Hypericum perforatum* L. Flavour Fragr. J., 16: 201-203.
Gudzic B, Djokovic D, Vajs V, Palic R, Stojanovic G (2002). Composition and anntimicrobial activity of the essential oil of *Hypericum maculatum* Crantz. Flavour Fragr. J., 17: 392-394.
Hosni K, Msaada K, Taarit MB, Ouchikh O, Kallel M, Marzouk B (2008). Essential oil composition of *Hypericum perfoliatum* L. and *Hypericum tomentosum* L. growing wild in Tunisia. Ind. Crops Prod*.,*
27(3): 308-314.
Jaymand K, Rezaei MB, Mozafarian VA, Azadi R, Naderi HB, Meshkizadeh S, Golipour M (2008). Determination of hyperican content in flower and leaves of eight *Hypericum* species. J. Med. Plants, 7(25): 26-55.
Javidnia K, Miri R, Soltani M, Gholami M, Khosravi AR (2008). Essential oil composition of four *Hypericum* species from iran. Chem. Nat. Compds. 44(3): 374-377.
Jirovets L, Buchbauer G, Puschmann CH, Fleischhacker W, Shafi PM, Rosamma MK (1999). Analysis of the essential oil of the fresh leaves of *Syzygium cumini* and *Syzygium* travancoricoricum from South India. J. Essent. Oil Bear. Plant, 2: 68-77.
Joulain D, König WA (1998). The atlas of spectral data of sesquiterpene hydrocarbons. WieneHamburg: E.B Press.
Kizil G, Toker Z, Ozen HC (2004). The antimicrobial activity of essential oils of *Hypericum scabrum, Hypericum scabroides* and *Hypericum triquetrifolium*. Phytother. Res., 18(4): 339-341.
Kumar A, Naqvi AA, Kahol AP, Tanden S (2004). Composition of leaf oil of *Syzygium cumini L*. from north India. Perfume, 48: 439-441.
Larypoor M, Akhavansepahy A, Rahimifard N, Rashedi H(2009).Antidermatophyte activity of the essential oil of *Hypericum perforatum* of north of Iran. J. Med. Plants, 8(31): 110-117.
Lavangna SM, Secci D, Chimenti P, Bonsignore L, Ottaviani A, Bizzarri B (2010). Efficacy of *Hypericum* and *Calendula* oils in the epithelial reconstruction of surgical wounds in childbirth with cesarean section. Farmacology, 56: 451-453.
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). Chem. Biodivers., 7(2): 447-466.
Magiatis P, Skaltsounis AL, Chinou I, Haroutounian SA (2002). Chemical composition and in-vitro antimicrobial activity of the essential oils of three Greek Achillea species. Z. Naturforsch, 57c: 287-290.
Mathis C, Ourisson G (1964). Chemico-taxonomic study of the genus Hypericum II. Identification ofvarious essential oils, constituents of Hypericum. Phytochemistry, 3: 115-131.
McLafferty FW, Stauffer DB (1989). The Wiley/NBS registry of mass spectral data. New York: Wiley & Sons .
Mills S, Bone K (2000). Principles and practice of phytotherapy. Churchill Livingstone, New York, Pp. 542-552.
Mockute D, Bernotiene G, Judzentiene A (2003) .Volatile compounds of the aerial parts of wild St. John's wort (*Hypericum perforatum* L.) plants. chemistry, 14: 108–111.
Mockute D, Bernotiene G, Judzentiene A (2007). The essential oils with dominant germacrene D of *Hypericum perforatum* L. growing wild in Lithuania. J. Essent. Oil Res., 20: 128–131.
Morteza-Semnani K, Saeedi M (2005). The essential oil composition of *Hypericum androsaemum* L. leaves and flowers from Iran. Flavour Fragr. J., 20: 332-334.
Motavalizadehkakhky A, Rustaiyan A, Masoudi S, Tabatabaei-Anaraki M, Aboly J (2009) . Chemical composition of the essential oils from flowers, leaves, stems and roots of *Hypericum perforatum* L. from Iran. J. Essent. Oil-Bearing Plants, 11: 548–552.
Mozaffarian VA (1996). Dictionary of Iranian Plant Names. Farhang Moaser, Tehran.
Pauli A, Schilcher H (2010). *In vitro* antimicrobial activities of essential oils monographed in the European Pharmacopoeia. In: Baser KHC; Buchbauer G., editors. Handbook of essential oils. science, technology and applications. 6th Edition. CRC Press; Boca Raton, Florida: 6th Edition. CRC Press; Boca Raton, Florida:Pp. 353-548.
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. Biochem. Syst. Ecol., 33: 873-898.
Pintore G, Chessa M, Boatto G, Cerri R, Usai M, Tirillini B (2005). Essential oil composition of *Hypericum* Growing Wild in Sardinia(Italy). J. Essent. Oil Res., 17: 533-535.
Radusienė J, Judzentiene A, Bernotiene G (2005). Essential oil composition and variability of *Hypericum perforatum* L. growing in Lithuania. Biochem. Syst. Ecol., 33: 113–124.
Rechinger KH (1980). Hypericum In: Flora Iranica, Hypericaceae, No., Rechinger, K.H., Hedge I.C. (eds). Akademische Druck and verlagsanstalt, Graz, Austria.
Robson NKB (2003). *Hypericum Botany*, in: E. Ernst (ed), *Hypericum, the Genus Hypericum*, Taylor Francis, London, P. 1.
Saroglou V, Martin PD, Rancic A, Veljic M, Skaltsa H (2007). Composition and antimicrobial activity of the essential oil of six *Hypericum* species from Serbia. Biochem. Syst. Ecol., 35: 146-152.
Sharopov SS, Gulmurodov IS, Setzer WN (2010). Essential oil composition of *Hypericum perforatum* L. and *Hypericum scabrum* L. Growing in Tajikistan. J. Chem. Pharm. Res., 2(6): 284-290.
Warnke PH, Becker ST,Podschun R, Sivanathan S, Springer IN, Russo PAJ,Wiltfang J, Fickenscher H, Sherry E (2009). The battle against multi-resistant strains:Renaissance of antimicrobial essential oils as a promising force to fight hospital-acquired infections. J. Cranio-Maxillofacial Surg., 37: 392-397.
Zdunic G, Godevac D, Milenkovic M, Vuclcevcc D, Savikin K, Menkovic N, Petrovic S (2009). Evalution of *Hypericum perforatum* oil extracts for an anti-inflammatory and gastroprotective activity in rats. Phytother. Res., 23: 1559-1564.