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Composition and Antimicrobial Activity of the Essential Oil and Extract of *Hypericum elongatum*

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Abstract: HOFARIGHUN, RAEE flower, thousand eyes wort are popular names for *Hypericum* sp. in Persian language mostly called *H. perforatum*. It has been used as antispasmodic, diuretic, antimigraine, antiepileptic and cholagouge. Tisane of these plants in red wine was used as snake bite and burning remedy. 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. Pinene <α> (80.43%), Terpinene <γ> (4.23%) and Pinene <β> (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. Screening of the antimicrobials was investigated on Gram positive bacteria (*Staphylococcus aureus* PTCC 1112, *Staphylococcus epidermidis* PTCC 1114, *Bacillus subtilis* PTCC 1023, *Enterococcus faecalis* ATCC 8043), Gram negative bacteria (*Escherichia coli* PTCC 1338, *Pseudomonas aeruginosa* PTCC 1047, *Salmonella typhi* PTCC 1609), yeasts (*Candida albicans* ATCC 14053, *Candida kefyr* ATCC 3826) and fungi (*Aspergillus niger* PLM 1140, *Aspergillus fumigatus* PLM 712). The MIC of essential oil also was identified. Antimicrobial activity of essential oil against all of the microorganisms was observed, except *Aspergillus niger* and *Aspergillus fumigatus*. In spite of antimicrobial activity of hydroalcoholic extract against bacteria, there was no antimicrobial activity against fungi and yeasts. A survey of the literature revealed no reports dealing with chemical composition of essential oil and antimicrobial activity of *Hypericum elongatum*.

Key words: *Hypericum elongatum*, Guttiferae, Pinene <α>, antimicrobial, GC/MS

INTRODUCTION

Plants and their derivatives such as essential oils and oleoresins have long been used as food flavoring, beverages and antimicrobial agents. The genus *Hypericum* L. is the type genus of Hypericaceae, now usually included as a subfamily (Hypericoideae) in Clusiaceae (Guttiferae) and comprises more than 450 species in 36 sections, with worldwide distribution in warm temperate, subtropical and mountainous tropical regions (Petrakis *et al*., 2005) and well represented in the Mediterranean and the Near East area. Hyperici herb is widely used in folk medicine, being an important raw material in pharmaceutical industry (Gudzic *et al*., 1997).

HOFARIGHUN, RAEE flower, Thousand eyes wort are popular names for *Hypericum* sp., in Persian language mostly called *H. perforatum* (Lebaschi and Sharifi Ashourabadi, 2002). It has been used as antispasmodic, diuretic, antimigraine, antiepileptic and cholagouge. Tisane of these plants in red wine was used as snake bite and burning remedy (Babakhanlu *et al*., 1999) and also for diarrhea, dyspepsia, parasites, neuralgia, sciatica and rheumatism (Rotblatt and Ziment, 2002). There are 17 species of *Hypericum* in the flora of Iran (Lebaschi and Sharifi Ashourabadi, 2002). In recent years, *Hypericum* has attracted much attention for their antidepressant, antianxiety, antiviral, antimicrobial and wound healing activities. Thus, phytochemical investigations have led to the isolation of antimicrobial, anticancer, antidepressant, antiviral, antioxidant, cytotoxic and antifungal compounds (Cakir *et al*., 2005). It seems that better effects have been detected by researches who have studied on St. John's wort and the other species of *Hypericum* are partly forgotten. Low amount of essential oil in the species *Hypericum* could explain why there are only a few studies on volatile chemistry of this genus. The composition of the essential oil of *H. perforatum* was initially investigated in France in 1964, by Mathis and Ourisson (Radusiene *et al*., 2005) and then, some investigations have been done on this plant, collected

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from different regions such as southeastern France (Schowb *et al*., 2002a), Serbia (Saroglou *et al*., 2007), Lithuania (Radusiene *et al*., 2005), Greece (Petrakis *et al*., 2005; Smelcerovic *et al*., 2007) and other reports. Of late, some reports have appeared on the composition of the essential oils of other species of *Hypericum*. A survey of the literature revealed no reports dealing with chemical composition of essential oil and antimicrobial activity of *Hypericum elongatum*.

MATERIALS AND METHODS

Collection of plant materials: The aerial parts of *Hypericum elongatum* were collected while fruiting period from Sharestanak village, Karaj countryside, Tehran Province, Iran in September 2005. Voucher specimens were kept at the Herbarium of the Faculty of Pharmacy (260), Shiraz University of Medical Sciences.

Extraction of the essential oil: The essential oil was obtained by hydro distillation using a Clevenger type apparatus for 4 h from air dried aerial parts of *Hypericum elongatum* (Table 1). The oil was calculated on dry weight bases as 450 μL/100 g dried material, respectively and dried by anhydrous sodium sulfate. Then it was kept in -20°C until tests.

Extraction of the hydroalcoholic extract: The air dried and finely ground sample weighting about 25 g was extracted in 100 mL of 80° ethanol according to maceration method for 24 h. Then the extract was filtered and the waste washed by 100 mL of 80°C ethanol and then the result was added to the former extract. This extract was dried in room temperature and weighted for calculating the yield of dry extract. The stock solution in 80°C ethanol was prepared and kept in 20°C until tested.

Gas chromatography: The GC/MS analyses were carried out using a Hewlett-Packard 6890. The gas chromatograph was equipped with a HP-5MS capillary column (phenylmethylsiloxane, 25 m×0.25 mm i.d.). The oven temperature was programmed from 60°C (4 min) to 250°C at a rate of 3°C min^{-1} and finally 10 min at 250°C. The carrier gas was Helium with a flow rate of 1.2 mL min^{-1} and splitless. The mass spectrometer was operating in the EI mode at 70 eV. The interface temperature was 250°C and

Table 1: Composition of *Hypericum elongatum* aerial parts essential oil

Compounds	Ki[a]	Area (%[b])	Compounds	KI	Area (%)
Heptane	-[c]	tr[d]	Cermacrene D	1,481	1.11
Nonane	-	0.94	Unknown	1,485	0.46
Pinene <α>	-	80.43	Unknown	1,494	1.07
Camphene	-	0.05	Unknown	1,509	tr
Unknown	-	0.03	Cadinen <γ>	1,513	0.35
Pinene <β>	-	2.59	Cadinene <Δ>	1,524	0.83
Myrcene	-	1.23	Unknown	1,531	tr
Phellandrene <α >	1,005	0.09	Cadinene <α>	1,536	0.07
Terpinene <α>	1,020	1.18	Unknown	1,541	0.04
Cymene <ortho->	1,028	0.61	Unknown	1,572	tr
Carene <Δ-3>	1,033	0.93	Unknown	1,577	0.11
Ocimene <(Z)-β >	1,040	tr	Unknown	1,580	tr
Ocimene <(E)-β>	1,051	0.10	Unknown	1,641	tr
Terpinene <γ>	1,065	4.23	Eudesmol <β>	1,650	0.44
Terpinolene	1,090	0.58			
Undecane	1,101	0.28			
Nonanal	1,105	tr			
Campholenal <α >	1,128	0.07			
Terpinen-4-Ol	1,180	0.10			
Unknown	1,193	tr			
Cubebene <α>	1,350	tr			
Ylangene <α>	1,370	tr			
Copaene <α>	1,375	0.23			
Identification %		96.50			
β-Bourbonene	1,388	tr	Group components		
Unknown	1,391	tr	Monoterpene hydrocarbons		90.40
α-Gurjunene	1,406	tr	Oxygen-containing monoterpenes		0.10
Caryophyllene <E->	1,416	0.19	Sesquiterpenes		1.14
Copaene <β>	1,427	0.10	Oxygen-containing Sesquiterpenes		0.44
Aromadendrene	1,437	0.17	Aliphatic hydrocarbons		1.27
α-Humulene	1,451	tr	Others		0.15
Allo-aromadendrene	1,458	0.10			
Muurolene <γ>	1,479	0.82			

[a]: The retention index of compounds on the HP-5MS was determined; [b]: Percentage were calculated based on the concentration obtained in the same column; [c]: KI is not calculated, injected normal alkanes are more than c_9 so these constituents are identified by comparison between reported constituents in the literature and the present spectrum and the mass spectrum of Adam's library: [d]: Trace, less than 0.1%

then mass range was 30-600 m/z. Identification of components was based on a comparison of their RI and mass spectra with Wiley (275) and Adams libraries spectra (Adams, 2004).

Antimicrobial screening: Disc diffusion method was employed for determination of antimicrobial activity of the essential oil and hydroalcoholic extract. Briefly, a suspension of the tested microorganism that contained 1.5×10^8 cfu mL^{-1} was prepared and then spread on a solid media (nutrient agar) by a swab. Paper discs were impregnated with different amounts of the oil and ethanol extract and placed on inoculated plates. After remaining for 15 min at room temperature, the plates were incubated at 37°C for 24 h for bacteria and 27°C for 48 h for the yeasts and fungi. The diameters of inhibition zones were measured in millimeters. Amphotericin-B, Ampicillin and Gentamicin were used as positive controls for microorganisms (Baron and Finegold, 1990).

Determination of Minimum Inhibitory Concentration (MIC): A microdilution broth susceptibility assay was used to evaluate antimicrobial activity of the essential oil. Two milliliter of a microbial suspension containing 5×10^5 cfu mL^{-1} of nutrient broth was prepared. Then according to the serial dilution different amounts of the essential oil was added to each tube. One of the tubes contained no essential oil and it was kept as positive control and the other one as a negative one which contained no microorganism. After incubation for 24 h for bacteria and 27°C for 48 h for the yeast and fungus, the first tube without turbidity was determined as the Minimal Inhibitory Concentration (MIC) (Baron and Finegold, 1990).

RESULTS AND DISCUSSION

The *in vitro* antimicrobial tests of the essential oil and ethanol extract of *H. elongatum* resulted in a range of growth inhibition pattern against pathogenic microorganisms. The results were obtained using disc diffusion method, followed by the measurement of MIC for essential oil. According to the disc diffusion method results, some of the microorganisms were inhibited by the amount of 8 μL $disc^{-1}$ of pure essential oil. Actually, only fungi could resist against the essential oil. In the other side, hydroalcoholic extract could inhibit microbial growth except fungi and yeasts (Table 2 and 3). The

Table 2: Antimicrobial activity of *H. elongatum* essential oil

	Inhibition zone[a], (μL)[b]					
Microorganisms	2	4	8	Gentamicin (μg $disc^{-1}$)	Ampicillin (10 μg $disc^{-1}$)	Amphotericin-B (20 μg $disc^{-1}$)
Staphylococcus aureus	++	++	+++		++++	
Staphylococcus epidermidis	+	++	+++		++++	
Bacillus subtilis	++	+++	+++		++++	
Enterococcus faecalis	na	+	++		++++	
Escherichia coli	na	na	+	++++		
Pseudomonas aeruginosa	++	++	+++	++++		
Salmonella typhi	na	++	+++	++++		
Candida albicans	+	++	++			++++
Candida kefyr	+	++	+++			++++
Aspergillus niger	na	na	na			++++
Aspergillus fumigatus	na	na	na			++++

a: + = 6.4-8 mm, ++ = 8-10 mm, +++ = 10-12 mm, ++++ = > 12 mm, na: not active (6.4 is the diameter of disc.); b: μL of the *H. elongatum* pure essential oil

Table 3: Antimicrobial activity of *H. elongatum* hydroalcoholic extract

	Inhibition zone[a] (μL)[b]					
Microorganisms	20	40	60	Gentamicin (μg $disc^{-1}$)	Ampicillin (10 μg $disc^{-1}$)	Amphotericin-B (20 μg $disc^{-1}$)
Staphylococcus aureus	++	++	+++		++++	
Staphylococcus epidermidis	++++	++++	++++		++++	
Bacillus subtilis	++	+++	+++		++++	
Enterococcus faecalis	+	++	++		++++	
Escherichia coli	++	++	++	++++		
Pseudomonas aeruginosa	++++	++++	++++	++++		
Salmonella typhi	+	+	+	++++		
Candida albicans	na	na	na			++++
Candida kefyr	na	na	na			++++
Aspergillus niger	na	na	na			++++
Aspergillus fumigatus	na	na	na			++++

a: + = 6.4-8 mm, ++ = 8-10 mm, +++ = 10-12 mm, ++++ = > 12 mm, na: not active (6.4 is the diameter of disc.); b: μL of the *H. elongatum* hydro alcoholic extract were applied to the discs (Each μL contains 1.5 μg of dried extract)

Table 4: MIC[a] of *Hypericum elongatum* essential oil

	MIC, (μL mL^{-1})[b]					
Microorganisms	1	2	4	6	8	B[c]
Staphylococcus aureus	+[d]	+	+	+	+	+
Bacillus subtilis	+	+	+	+	-[e]	+
Escherichia coli	+	+	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+	+	+
Candida albicans	+	+	+	+	+	+
Aspergillus niger	+	+	+	+	+	+

a: Minimum inhibitory concentration; b: μL of pure essential oil per each milliliter of culture media; c: Blank; d: Microbial growth exist; e: microbial growth doesn't exist

above mentioned essential oil, possessed antimicrobial activity against *Bacillus subtilis* resulting MIC values of 4 μL/1 mL of nutrient broth (Table 4).

In general, susceptibility of Gram negative and Gram positive bacteria against the essential oil of *H. elongatum* has no significant difference. The most sensitive bacteria against hydroalcoholic extract were *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*.

Previous investigations on the antimicrobial activity of several *Hypericum* sp. essential oils have been carried out. The oil of *Hypericum linarioides* showed antifungal activity (Cakir *et al*., 2005), as well as the oils of *Hypericum hyssopifolium* and *Hypericum heterophyllum*. Similarly, the oils of *Hypericum scabrum*, *Hypericum scabroides* and *Hypericum triquetrifolium* showed antimicrobial activity against nine organisms. The essential oil of *Hypericum runteliacum* showed a moderate *in vitro* antimicrobial activity (Saroglou *et al*., 2007), while the volatile fraction of *Hypericum coris* was active against *Saccharomyces cerevisiae* (Schwob *et al*., 2002b). It seems that low amount of Sesquiterpene hydrocarbons and aromatics is the cause of low anti fungal activity of the essential oil of *H. elongatum*.

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. Pinene <α> (80.43%), Terpinene <γ> (4.23%) and Pinene <β> (2.59%) were the principal components (87.16%) (Table 1).

According to the former studies, it could be said that there are two major groups in the composition of essential oils, obtained from different species of genus of *Hypericum*, collected from different regions where these species are growing up. One of them is the group that is introduced by essential oils with a low content of non-terpene compounds and a high content of terpene compounds. The other group is contrariwise. On the other hand, it is observed that in essential oils with a high content of terpene compounds, mono and sesquiterpens, are individually taking place as major compounds. It means that in many of the essential oils of these species detected with low amounts of monoterpenes, high amounts of Sesquiterpenes could be predictable (Toker *et al*., 2006; Schwob *et al*., 2002a, b, 2004; Cakir *et al*., 2005; Gudsic *et al*., 1997; Pintore *et al*., 2005; Radusiene *et al*., 2005; Petrakis *et al*., 2005).

At the present study, the essential oil of *H. elongatum* contained a very high content of terpene hydrocarbons, something about 95% of the total known composition of the oil, whereas 96.5% of the whole content of composition was identified. The noticeable subject of this proportion was that most of this amount was due to the presence of monoterpene hydrocarbons with an amount of 90.04% and was exactly introduced by (α + β) Pinene, by 83.02% of total known oil.

CONCLUSION

In spite of antimicrobial activity of the essential oil of *H. elongatum* it is acceptable why its hydroalcoholic extract of *Hypericum* was used as topical opportunistic pathogens growth prevention in injury and burns. And about the composition of essential oil, its surprising content of (α+β) Pinene (83.02%) in fruiting period could introduce *H. elongatum* as a chemotype.

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