CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *Heliotropium europaeum*

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The genus *Heliotropium* (Boraginaceae) comprises 47 species distributed in Iran [1]. *Heliotropium europaeum* L. (Syn: *Heliotropium lasiocarpum* Fisch.& Mey., *Heliotropium tenuiflorum* Bunge) has been found in Azerbaijan, Fars, Golestan, Hamadan, Khorasan, Khuzestan, Kurdestann, Lurestan, Mazandaran, Qazvin, and Tehran Provinces of Iran [2, 3]. In Iranian traditional medicine, *H. europaeum* is used as antipyretic, cholagogue, emmenagogue, cardiotonic, and anthelmintic, in the treatment of headache and gout, and in external uses for the healing of wounds and treatment of warts [3]. This plant has also carcinogenic and hepatotoxic effects [4]. Some species of *Heliotropium* have been investigated for pyrrolizidine alkaloids and their *N*-oxides, sterols, flavonoids, and triterpenoids [5–11].

We found *H. europaeum* L. in Mazandaran province of Iran. A literature survey has shown that there is no report on the volatile constituents and antimicrobial activity of *H. europaeum*; thus we decided to investigate the chemical constituents and antimicrobial activity of the oil of *H. europaeum* for the first time.

The aerial parts of *H. europaeum* were collected in June 2006 from the suburb of Behshahr, Mazandaran province, North of Iran, and identified by the Department of Botany, Research Center of Natural Resources of Mazandaran. A voucher specimen (herbarium No. 305) was deposited at the Herbarium of the Department of Botany, Research Center of Natural Resources of Mazandaran.

The dried aerial parts (100 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 4 h. The essential oil with a light yellowish color and yield of 0.1% (v/w) was dried over anhydrous sodium sulfate and kept at 4°C in a sealed brown vial until required. The oil was submitted to GC and GC/MS analysis (Table 1).

TABLE 1. The Chemical Constituents of the Essential Oil of *Heliotropium europaeum*, %

Components	RI[a]	GC area, %	Components	RI[a]	GC area, %
trans-Linalool oxide	1074	1.6	*n*-Pentadecanol	1775	2.6
cis-Linalool oxide	1088	1.8	β-Eudesmol acetate	1794	1.2
Eugenol	1360	1.6	*n*-Hexadecanol	1877	1.8
β-Longipinene	1403	1.0	Phytol	1944	28.7
Geranyl acetone	1457	6.3	*n*-Octadecanol	2079	3.2
(*E*)-β-Ionone	1491	4.8	*cis*-Linoleic acid methyl ester	2093	7.3
n-Tridecanol	1574	1.9	*n*-Heneicosane	2100	3.2
Silphiperfol-6-en-5-one	1628	7.1	Phytol acetate	2220	4.3
Alloaromadendrene epoxide	1643	3.8	*n*-Pentacosane	2500	0.9
β-Eudesmol	1653	0.7	*n*-Hexacosane	2600	0.8
n-Tetradecanol	1675	2.4	*n*-Heptacosane	2700	1.3
Eudesma-7(11)-en-4-ol	1701	0.6	*n*-Octacosane	2800	1.1
E-Coniferyl alcohol	1737	1.1	Total identified		91.4
α-Oxobisabolene	1750	0.3			

[a]RI: retention indices on DB-5.

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TABLE 2. Antimicrobial Activity of the Essential Oil of *Heliotropium europaeum*[a]

Sample	Conc., µg/disk	Diameter of zone of inhibition (mm) (mean±C.D.)					
		Bacteria				Fungi	
		Bacillus subtilis (G+)	*Staphylococcus aureus* (G+)	*Escherichia coli* (G-)	*Salmonella typhi* (G-)	*Aspergillus niger*	*Candida albicans*
H. europaeum oil	250	-	-	-	-	-	-
	500	8.25±0.96	-	-	7.95±0.58	-	-
	1000	10.0±2	-	-	9.25±0.96	-	-
	2000	12.0±1.41	-	-	11.75±1.26	-	-
	4000	12.5±0.58	-	7.5±0.0	12.5±0.58	-	-
	8000	15.75±0.96	-	8.5±0.71	12.0±0.82	7.75±0.5	-
Gentamycin	50	29.8±1.9	37.3±2.5	31.6±3.2	29.0±2.5	-	-
Amikacin	3	21.9±1.55	24.9±3.1	23.8±2.5	16.8±3.1	-	-
Amphotericin B	100	-	-	-	-	22.7±2.1	223.3±2

[a]Values are inhibition zone (mm) and an average of triplicates; -, no inhibition.

Bacillus subtilis PTCC 1023, *Staphylococcus aureus* PTCC 1112, *Escherichia coli* PTCC 1330, *Salmonella typhi* PTCC 1639, *Aspergillus niger* PTCC 5011, and *Candida albicans* PTCC 5027 were used for testing the antimicrobial activity.

The diffusion method using filter paper disk (6 mm) was used for the screening of oil antibacterial and antifungal activities [12]. Bacterial and fungal strains were tested on Muller-Hinton agar and Sabouraud dextrose agar, respectively. Sterilized paper disks were loaded with different amounts of *H. europaeum* oil (250, 500, 1000, 2000, 4000 and 8000 µg /disk) and applied on the surface of agar plates. All plates were incubated at 37°C for 24 h for bacteria; at 25°C for 24 h for *C. albicans*; and at 25°C for 3 days for *A. niger*. Inhibition zone diameters were measured after the conventional incubation period. Gentamycin (50 µg/disk), Amikacin (3 µg/disk), and Amphotericin B (100 µg/disk) (obtained from Sigma) were used as positive controls (Table 2).

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