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Essential Oil Composition and Antibacterial Activity of *Nepeta glomerulosa* Boiss from Iran

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Abstract: The chemical compositions of the essential oil of *Nepeta glomerulosa* Boiss aerial parts, grown in Iran were determined by GC-MS. Fifty-two compounds (97.2 %) were determined. The major compounds were geranyl acetate (17.0 %), limonene (12.0 %), eucalyptol (5.8 %), (bornyl acetate (5.3 %), citronellal (4.9 %), spathulanol (4.2 %), sabinene (3.9 %), β -ocimene (3.9), β -sesquiphellandrene (2.8 %), neryl acetate (2.5 %), α -humulene (2.4 %), α -pinene (2.3 %), humulene oxide (2.2 %), norsolanadione (2.1 %) and terpinen-4-ol (2.0 %). The yield of the oil was 1.1(v/w) %. The essential oil showed antibacterial activity for *Staphylococcus aureus*.

Key words: *Nepeta glomerulosa* Boiss, essential oil, hydrodistillation, geranyl acetate.

Introduction: The genus *Nepeta* (Lamiaceae) comprises 280 species that are distributed over a large part of central and southern Europe, West, central, and Southern Asia. About half of the existing species are recorded in Iran. The genus *Nepeta* is represented in Turkey by 33 species and altogether 38 taxa, 17 of these being endemic in Turkey ¹. *Nepeta* species are widely used in folk medicine because of their antispasmodic, diuretic, antiseptic, antitussive, antiasthmatic, ethnobotanical effect, diaphoretic, vulneary, antispasmodic, tonic, febrifuge ²⁻⁵. The feline attractant properties of several *Nepeta* species have been known for a long time. The compounds of essential oil of *Nepeta* are considered to be responsible for the feline attractant activity of *Nepeta* species ⁶⁻⁷.

As far as our literature survey, there are no reports on the chemical compositions of essential oil and antibacterial activity of the essential oil of *N. glomerulsa* Boiss. Thus, this study is the first report on this plant. The aims of this work are to identify of the chemical compositions and a brief study of antibacterial activity of essential oil of *N. glomerulsa* Boiss obtained by using a Clevenger distillation apparatus. The chemical compositions of the essential oil were evaluated by using gas chromatography-mass spectrometry (GC-MS).

Experimental

Plant material: The aerial parts of *Nepeta glomerulosa* Boiss was collected during August

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2008 from Zhoshke Mountain, Mashhad, Iran. The plant was identified at FUMH Herbarium, Ferdowsi University of Mashhad, Iran, and a Voucher specimen is kept at FUMH Herbarium (27628 FUMH).

Isolation procedure: The air dried leaf of specimens (70g) were extracted by hydrodistillation using Clevenger-type apparatus for 4 h. The oil was dried over anhydrous sodium sulfate. The corresponding oils were isolated in yield of 1.1% (v/w).

Identification of oil components: The essential oil was analyzed by gas chromatography mass spectrometry (GC-MS). The GC-MS analysis was carried out on a Shimadzu GC-MS model QP 5050. The capillary column was DB-5 (30 × 0.2 mm, film thickness 0.32 μm). The initial temperature of column was 60°C (held 1 min) and then heated to 200°C with a 3°C/min rate and then heated to 250°C and kept constant for 2 min. The flow rate of Helium as carrier gas with (1.7 mL/min). The analysis uses split ratio 1/28. The injector and detector temperatures were both at 280°C; volume injected 0.1 μl of the essential oil and ionization potential 70 eV. The same condition of temperature programming used for n-alkenes mixture to calculate the retention index (RI).

Identification of components in the oil was based on the retention index (RI), Wiley computer library and literature survey⁸. The relative percentage of the oil constituent was calculated.

Antibacterial activity: Antibacterial activity by disc diffusion method and determination of inhibition zones at different oil dilutions were done for *Staphylococcus aureus*.

Results and discussion: The compositions of essential oil and antibacterial activity of aerial parts of *Nepeta glomerulosa* Boiss are shown in Table 1 and 2, respectively. Fifty-two constituents, representing 97.2% of the total components in the oil, have been identified in the essential oil extracted from the aerial parts of this plant.

The essential oil with major compositions of geranyl acetate (17.0%), limonene (12.0%), eucalyptol (5.8%), bornyl acetate (5.3%), showed moderate antibacterial activity and inhibited the growth of the tested bacteria. Due to the high amount of geranyl acetate (17.0%), limonene (12.0%), eucalyptol (5.8%), and other terpenoids in the oil of *Nepeta glomerulosa* Boiss, it can be concluded that the herb and essential oil of *Nepeta glomerulosa* Boiss can be used as flavoring agents in food and also in the medicinal and perfume industries. These main components have been reported in the literatures for *Nepeta* genus⁹⁻¹¹. The results indicating that *Nepeta glomerulsa* Boiss has potential use in phytotherapy.

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Table 1. Chemical composition of *Nepeta glomerulosa* Boiss essential oil

No.	Compound	RI	%
1	α -Thujene	927	0.2
2	α -Pinene	935	2.3
3	Camphene	950	1.1
4	β -Pinene	979	0.5
5	β -Myrcene	994	1.9
6	Octan-3-ol	1002	0.2
7	α -Terpinene	1021	0.3
8	Limonene	1038	12.0
9	Cyclooctyne	1045	0.2
10	β -Ocimene	1057	3.9
11	Sabinene	1069	4.0
12	Sabinene hydrate	1072	0.5
13	Terpinolene	1090	0.1
14	Linalool	1105	0.2
15	1-Pentylallyl acetate	1120	0.4
16	Thujone	1138	0.3
17	Camphor	1143	0.5
18	Citronellal	1163	4.9
19	Borneol	1170	1.4
20	Terpinen-4-ol	1182	2.0
21	α -Terpineol	1193	0.7
22	Chrysanthemal	1211	0.1
23	<i>iso</i> -Borneol	1225	0.1
24	Nerol	1235	0.2
25	D-Pulegone	1237	1.4
26	Citronellol	1244	0.4
27	Geraniol	1267	1.4
28	β -Farnesene	1272	0.2

table 1. (continued).

No.	Compound	RI	%
29	Bornyl acetate	1290	5.3
30	Limonene oxide	1314	2.0
31	Eucalyptol	1359	5.8
32	Citronellyl acetate	1361	0.7
33	Neryl acetate	1372	2.5
34	Geranyl acetate	1396	17.0
35	Caryophyllene	1407	0.7
36	Bergamotene	1432	1.3
37	α -Humulene	1441	2.4
38	Hotrienyl acetate	1466	0.1
39	Nerolidol	1476	1.4
40	β -Bisabolene	1501	0.5
41	β -Sesquiphellandrene	1518	2.8
42	Norsolanadione	1537	2.1
43	Spathulanol	1546	4.2
44	<i>iso</i> -Caucalol	1568	1.1
45	Caryophyllene oxide	1581	1.2
46	Nepetalactol	1586	1.1
47	Humulene oxide	1591	2.2
48	β -Elemene	1607	0.2
49	Lancifold	1613	0.1
50	Citral	1675	0.2
51	Terpinyl acetate	1692	0.1
52	Iso-isopulegol acetate	1732	0.8
	Total		97.2

Table 2. Antimicrobial activity of *Nepeta glomerulosa* Boiss essential oil

Microorganism	Inhibition zone [mm] ^a						Standard antibiotics	
	The ratio of oil dilutions (with methanol)							
	1	1/2	1/4	1/8	1/16	Ampicillin ^b	Tetracycline ^c	
<i>Staphylococcus aureus</i>	19.5*	16	12.5*	9	7	13	20	

^a includes diameter of the disc (6mm) and the ranges are: (7-13) moderately active; (>14) highly active. The results are average of two experiments

^b tested at 10 μ g/disc

^c tested at 30 μ g/disc

^a similar inhibitory type of activity of the oil to that of standard antibiotics