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# Antibacterial Activity and Composition of Essential Oil of *Nepeta pungens* Benth. from Iran

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# ABSTRACT

The oils obtained by hydrodistillation from fresh and dried aerial parts of *Nepeta pungens* Benth. at the flowering stage were analyzed by GC and GC-MS to investigate the variations of oil yields, oil components along with their percentages in fresh and dry stages. Forty-ninecompounds (97.2%) were determined. The major compounds were geranyl acetate (17.0%), limonene (12.0%), eucalyptol (5.8%), (bornylacetate (5.3%), citronellal (4.9%), spathulanol (4.2%), sabinene (3.9%),  $\beta$ -ocimene (3.9),  $\beta$ -sesquiphellandrene (2.8%), nerylacetate (2.5%),  $\alpha$ -humulene (2.4%),  $\alpha$ -pinene(2.3%), humuleneoxide (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*.

Keywords: Nepeta pungens Benth., 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 38taxa, 17 of these beingendemicin Turkey (Davis, 1982). Nepeta species are widely used in folk medicine because of the antispasmodic, diuretic, antiseptic, antitussive, antiasthmatic, ethnobotanicaleffect, diaphoretic, vulneary, antispasmodic, tonic, febrifuge (Ghannadi et al., 2003; Gkinis et al., 2003; Dorman et al., 2000; Zenasni at al., 2008). 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 (Dabiri et al., 2003; Hussain et al., 2009). As far as our literature survey, the reareno reports on the chemical compositions of essential oil and antibacterial activity of the essential oil of N.pungens Benth. 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. pungens Benth. Obtained by using a Clevenger distillation apparatus. The chemical compositions of the essential oil were evaluated by using gas chromatography- massspectrometry (GC-MS).



#### MATERIAL S AND METHODS

# Plant material

The aerial parts of *Nepeta pungens* Benth. was collected during August 2011 from Sepidan Mountain, Fars, Iran. The plant was identified at SUMSH Herbarium, Shiraz university of Medical Science, Iran, and a Voucher specimen is kept at SUMSH Herbarium (27628SUMSH).

# **Isolation procedure**

The air dried leaf of specimens (70g) were extracted by hydro-distillation using Clevenger-type apparatus for4 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 QP5050. The capillary column was DB-5 ( $30\times0.2$  mm, film thickness  $0.32\mu$ m). The initial temperature of column was  $60^{\circ}$ C (held 1 min) and then heated to  $200^{\circ}$ C with a  $3^{\circ}$ C/min rate and then heated to  $250^{\circ}$ C and kept constant for 2 min. The flow rate of Helium as carrier gas with (1.7mL/min). The analysis uses split ratio 1/28. The injector and detector emperatures were both at  $280^{\circ}$ C; volume injected 0.1 µl of the essential oil and ionization potential 70eV. The same condition of temperature programming used from-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 survey (Adams, 1995). 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 pungens* Benth. are shown in Table 1 and 2, respectively. Forty-nine constituents, representing 96.1 % 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 anti-bacterial 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 pungens* Benth. it can be concluded that the herband essential oil of *Nepeta pungens* Benth. can beused as flavoring agents in food and also in the medicinal and perfume industries. These main components have been reported in

the literatures for *Nepeta* genus (Rustaiyan *et al.*, 1999a; Sefidkonet *et al.*, 2002). There sults indicating that *Nepeta pungens* Benth. has potential use in phytotherapy.

Table.1: Chemical composition of Nepeta pungens Benth. 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	iso-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
30	Limonene oxide	1290	5.3
31	Eucalyptol	1314	2.0
32	Citronellyl acetate	1359	5.8
33	Neryl acetate	1361	0.7
34	Geranyl acetate	1372	2.5
35	Caryophylene	1396	17.0
36	Bergamotene	1407	0.7
37	α-Humulene	1432	1.3
38	Hotrienyl acetate	1441	2.4
39	Nerolidol	1466	0.1
40	β-Bisabolene	1400	1.4
41	β-Sesquiphellandrene	1501	0.5
42	Norsolanadione	1518	2.8
43	Spathulanol	1518	2.0
44	iso-Caucalol	1546	4.2
45	Caryophyllene oxide	1568	1.1
46	Nepetalactol	1581	1.1
40	Humulene oxide	1586	1.2
48	β-Elemene	1591	2.2
49	Lancifold	1607	0.2
.,	Total	1007	96.1

Table. 2: Antimicrobial activity of Nepeta pungens Benth. essential oil.

Microorganism	Inhibition zone [mm] <sup>a</sup> The ratio of oil dilutions (with methanol)				Standard antibiotics		
	1	1/2	1/4	1/8	1/16	Ampi- cillin <sup>b</sup>	Tetracyc- line <sup>c</sup>
Staphylococcus aureus	19.5*	16	12.5*	9	7	13	20

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

<sup>b</sup>testedat 10µg/disc

° testedat30µg/disc

\*similar inhibitory type of activity of the oil to that of standard antibiotics.

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