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A Comparative Study on The Composition of The Essential Oil of *Nepeta Menthoides* Growing Wild in Northwest of Iran (Sabalan Mountains in Ardabil Province)

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ABSTRACT
Hydrodistillation of fresh aerial parts of *Nepeta menthoides* growing wild in northwest of Iran (Sabalan mountains in Ardabil province), yielded 0.12% (w/w) essential oil. GC/MS analysis allowed identification of 33 components, which made up 93.61% of the total oil. The main components of the sample were 1,8-cineol (38.68%), geranyl acetate (12.05%), β-pinene (7.8%), cinerolon (3.57%) and α-terpineol (2.94%). The oil was rich in oxygenated monoterpenes (61.14) predominated over monoterpene hydrocarbons (15.4%). Comparison of these results with those from previous studies shows large differences, suggesting significant environmental influences on oil composition.

Keywords: *Nepeta menthoides*, essential oil, 1,8-cineol, geranyl acetate, β-pinene

INTRODUCTION

The genus *Nepeta* which belongs to lamiaceae family, is distributed in Europe, Asia and has also naturalized in north America. It is one of the largest genera within the family with about 300 species. Iran is one of the centers of diversity of species of *Nepeta* and about 75 species present in iran, 38 and 31 of which are endemic and native, respectively [1]. The composition of the oils of some Nepeta species such as *Nepeta balouchestanica, N. mahanesis, N. hormozganica* and *N. crispa* have been reported [2-4]. *Nepeta* plants have been of great interest to Iranian traditional medicine [5].

Composition of essential oil of *Nepeta menthoides* has been studied in many parts of Iran in different times of year. Compared to each other, different values have been reported depending on the conditions [6-8].

In Ardabil province, beverages obtaining from *Nepeta menthoides* are used traditionally for respiratory disorders, and their effect has nearly been proven.

In this work, we reported the antimicrobial behavior and composition of the oil from *Nepeta menthoides* collected from the south of Sabalan Mountains (in Ardabil province) at the height of 1500-2000m above sea level by means of GC and GC/MS in combination with retention indices.

As mentioned above, the plant has previously investigated in most parts of Iran, including Ardabil province.

A comparison of our data with that of other reports [6-8] will show that there are significant differences between the compositions of oils collected from different areas and or same area at different times of a year.

Plant Material

The plant material was collected on July 15, 2013 from the south of Sabalan Mountains in Ardabil area in northwest Iran at an altitude of 1500-2000m. A voucher specimen has been deposited in the herbarium of the department of Botany, Islamic Azad University, Ardabil, Iran.

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Table 1. Classification of identified terpenes in the essential oil

No.	Classification	Number of compounds	Percent %
1	monoterpene	9	15.4
2	oxygenated monoterpene	11	61.14
3	sesquiterpene	3	4.58
4	oxygenated diterpene	1	0.78
5	tritrpene	1	0.7

Isolation of Volatiles

The aerial parts of the fresh plant material (150g) were subjected to hydrodistillation for 4h using a Clevenger-type apparatus. The yield of the oil from the aerial parts, after drying over anhydrous sodium sulfate (Na_2SO_4) and recovery with n-hexane was 0.12% (w/w). The oil was then stored in sealed vials at low temperature (4^{o}C) before analysis.

GC Analysis

GC analysis was carried out on a Shimadzu 15A gas chromatograph equipped with split/splitless injector (operated at a split ratio of 1:50 and at 250^{o}C) and a flame ionization detector (250^{o}C). The carrier gas was nitrogen, at a flow rate of 1ml/min. The capillary column used was DB-5 (50m × 0.2mm, film thickness 0.32µm). The column temperature was kept at 60^{o}C for 3min and then heated to 220^{o}C at a rate of 5^{o}C /min, and kept at 220^{o}C for 5 min. The injection volume was 0.2 µl.

GC/MS Analysis

A Hewlett-Packard 5973 apparatus fitted with a HP-5MS column (30m × 0.25mm, film thickness 0.25µm) was used. The column temperature was kept at 60^{o}C for 3min and increased to 220^{o}C for 5min. The carrier gas was helium at a flow rate of 1ml/min. Electron impact (EI) ionization was used at an ionization voltage of 70eV. Mass spectra were taken in the full scan mode within the scan range of 40-450 amu at a scan velocity of 0.2 Sec/scan.

Identification of constituents in the oil were made by comparison of their mass spectral fragmentation patterns and retention indices (Kovats indices) relative to C_9 – C_{21} n-alkanes with those given in the literature [9] and stored in the mass spectral database (Wiley 275). Relative percentages of the components were calculated from peak areas using a Shimadzu C-R4A chromatopac, without the use of a correction factor.

RESULTS AND DISCUSSION

The composition of the oil of aerial parts of *Nepeta menthoides* is given in **Table 2**. In this oil, 33 components, representing 93.61% of the total composition, were identified. The identified components of the oil consisted of nine monoterpene hydrocarbons (15.4), eleven oxygenated monoterpenes (61.14%), three sesquiterpene hydrocarbons (4.58%), one oxygenated diterpene (0.78%) and one triterpene hydrocarbon (0.7%). Also, three compounds other than terpenes exist among the identified compounds. This classification is summarized in **Table 1**.

As it can be seen in **Table 2**, 1,8-cineol (38,68 %), geranyl acetate (12.05%), β-pinene (7.8%) and *tran*-α-bisabolene are the major components in the oil, and oxygenated monoterpenes predominate over monoterpene hydrocarbons.

Table 2. The composition of the oil of aerial parts of *Nepeta menthoides*

NO.	Compound	K.I.	Percent %
1	α-Thujene	940	0.6
2	α-pinene	948	1.51
3	Camphene	962	0.12
4	β -Pinene	990	7.8
5	β-myrcene	1008	0.77
6	δ-3-Carene	1023	1.25
7	α-Terpinene	1038	1.16
8	1,8-Cineol	1058	38.68
9	3-Methylbutanoic acid butyl ester	1071	0.16
10	γ-Terpinene	1086	1.73
11	*cis*-Sabinene hydrate	1096	1.35
12	α-Terpinolene	1120	0.46
13	2-Methylbutyric acid 2-Methylbutyl ester	1138	5.1
14	1-(2-Propenyl)cyclopentene	1164	0.13
15	*trans*-Pinocarveol	1177	0.28
16	Pinocarvone	1205	0.17
17	δ-Tterpineol	1213	1.36
18	4-Terpineol	1225	1.74
19	α-Terpineol	1245	2.94
20	2-Methoxy-*p*-cresol	1270	0.71
21	Geraniol	1322	0.44
22	Perilla alcohol	1377	0.2
23	Ethyl 3-Phenyl propanoate	1439	0.36
24	Cinerolon	1458	3.57
25	Geranyl acetate	1483	12.05
26	Nepetalactone	1497	1.93
27	*trans*-β-Farnesene	1574	0.29
28	3-Methy butanoic acid 2-Phenyl ethyl ester	1615	0.94
29	*trans*-α-Bisabolene	1637	4.24
30	δ-Cadinene	1662	0.05
31	Thunbergol	2411	0.78
32	Squalene	-	0.70
33	Bis(2-ethylhexyl)phthalate	-	0.2
Total			93.61%

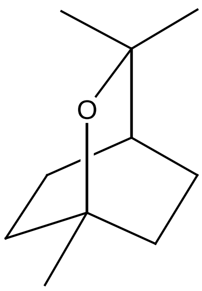

Figure 1. Structure of 1,8-cineol

Compared to the present work, a previous study on the essential oil of this plant showed nearly similar composition with different values of components, containing 1,8-cineol (57.3%), β-pinene (8.8%), geranyl acetate (8.1%) and other natural compounds (altogether 18 compounds) [7].

The difference may be because of environmental conditions such as height, rich soil, humidity, temperature, time of year and etc., influencing the plant. Therefore, careful analysis of the oils should be done before they put to use.

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