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Essential Oil Chemical Composition of *Mentha mozaffarianii* Jamzad Seeds

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Abstract. The seeds essential oil of the endemic species *Mentha mozaffarianii* growing wild in the south of Iran was analyzed by gas chromatography (GC) and GC-mass spectrometry (GC-MS). Characterization of individual components was performed using a commercial mass spectrometry library, and 25 components were identified. This analysis showed the presence of 3 volatile components, including piperitenone (35.6%), piperitone (27.1%) and 1,8-cineol (10.7%) as the main components.
Key words: *Mentha mozaffarianii*; essential oil; piperitenone; piperitone.

Resumen. Se obtuvo una muestra del aceite esencial de semillas de la planta endémica *Mentha mozaffarianii* en el sur de Irán y se analizó por GC y GC-MS. La caracterización de los compuestos individuales se realizó utilizando una biblioteca comercial para espectrometría de masa y se identificaron 25 compuestos. Este análisis mostró la presencia de tres compuestos volátiles, piperitenone (35.6%), piperitone (27.1%), y 1,8-cineol (10.7%) como principales componentes.
Palabras clave: *Mentha mozaffarianii*; aceite esencial; piperitenone; piperitone.

Introduction

The Iranian endemic plant *Mentha mozaffarianii* Jamzad belongs to the Lamiaceae family and is known locally as "Pooneh-Koohi" [1]. Six species and several subspecies of the genus *Mentha* are found in Iran, among which just *M. mozaffarianii* is endemic. It has a limited geographical range in the south of Iran and is just found in Siyahoo, Qotb-Abad, Damtang and Sikhoran in Hormozgan Province [2]. The leaves and seeds have been commonly used in Iranian traditional medicine as antiseptic, analgesic, and to treat painful menstruation, dyspepsia, arthralgia, fever, headache, common cold, and healing wound [1-3]. Literature survey revealed reports just on the essential oil composition of the leaves and the aerial parts of *M. mozaffarianii* and there was no attempt to study the essential components of *M. mozaffarianii* seeds up to now. Regarding the significant pleasant odor of the seeds, we were prompted to investigate the essential oil composition of this part of *M. mozaffarianii* for the first time.

Results and Discussion

The hydrodistillation of *M. mozaffarianii* seeds gave pale yellow oil with pleasant odor and yield of 2.4% (v/w) based on the fresh weight. Fig. 1 shows the gas chromatogram of *M. mozaffarianii* seed essential oil.

Table 1 shows the list of compounds whose GC-MS concentration is not less than 0.1% of total peak concentration. According to Table 1, 25 components were identified in the seeds essential oil which presented about 96.8% of the total composition. The major constituents of *M. mozaffarianii* seed oil were characterized as piperitenone (35.6%), piperitone (27.1%) and 1,8-cineol (10.7%). The studied essential oil comprised one hydrocarbon (0.2%), 17 monoterpenoids (94.4%), six sesquiterpenoids (3.2%) and one phenylpropanoid (0.3%).

Piperitenone as the major component of the studied oil is a monoterpene ketone. According to Fujita et al. [4] piperitenone appears first in young leaves of *Mentha* species and then converts to pulegone or piperitone via two separate pathways in older leaves. The current results showed that most of the first appeared piperitenone was converted to piperitone. High and low amounts of piperitone (27.1%) and pulegone (4.6%) respectively in the seeds oil demonstrate a characteristic metabolic pathway in *M. moaffarianii* in which piperitenone could highly be metabolized to piperitone rather than pulegone. As literature survey there were just two reports on the essential oil composition of the leaves and aerial parts of *M. mozaffarianii* [3,5]. A comparison of the results with the literature showed that the seeds oil composition was so similar to the leaves oil

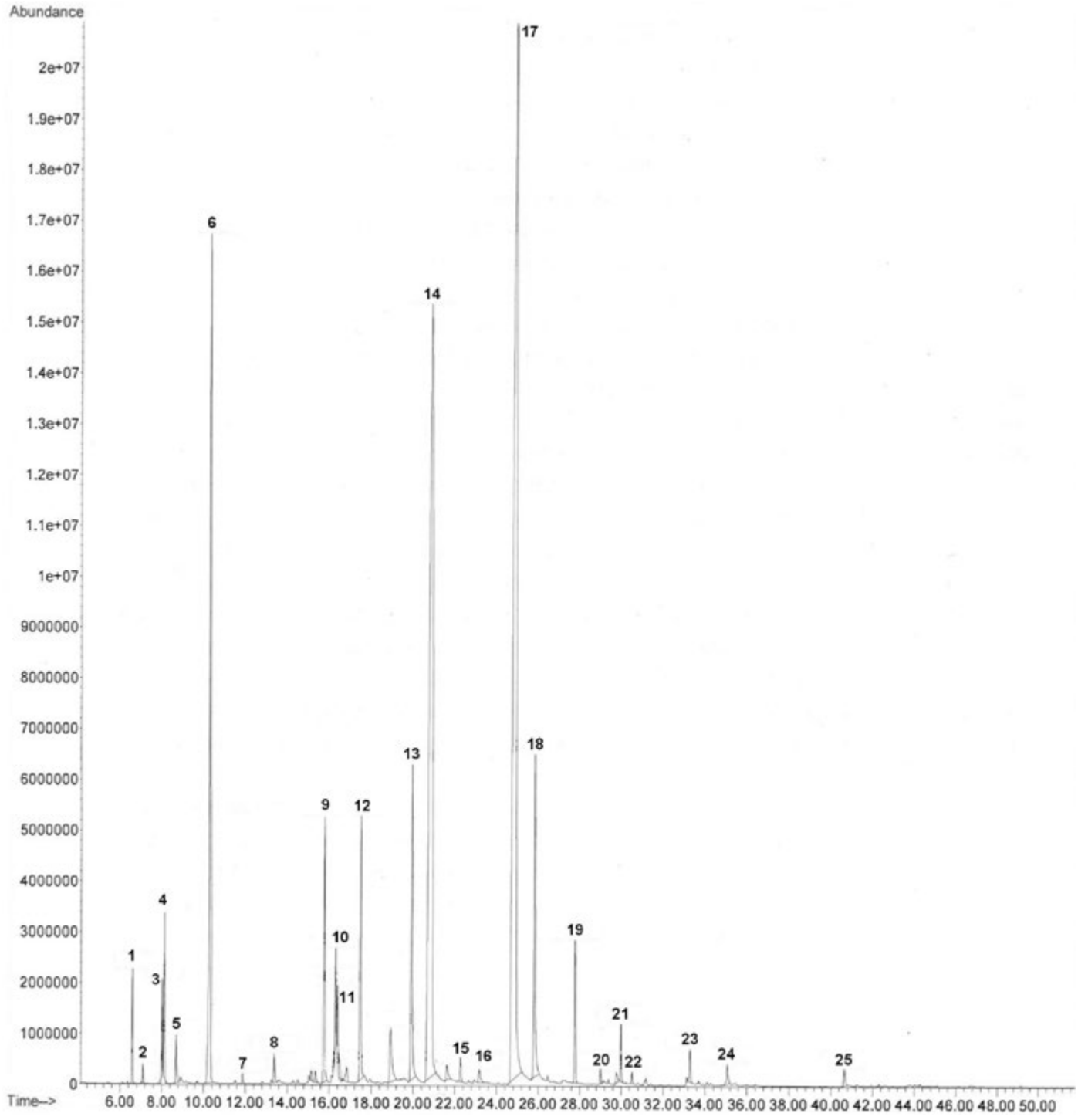

Fig. 1. Gas chromatogram of *M. mozaffarianii* seed essential oil.

composition reported by Arman et al. [3]. That is because both samples were collected from the same region in Hormozgan Province. Regarding the results reported by Arman et al [3], presence of piperitenone as the main component in the seeds and the leaves oils of *M. mozaffarianii* is characteristic. However, the piperitenone content in *M. mozaffarianii* leaves oil was reported as two times more than that in the seeds oil. Higher amounts of piperitenone (59.5%) in the essential oil of *M. mozaffarianii* leaves could be related to the stage of plant growth. According to Arman *et al*. *M. mozaffarianii* was collected at full flowering stage. Since the leaves were young at this stage, piperitenone content was expected in maximum and Arman *et al*. report also confirmed it. Mature seeds appear at the time the plant gets older. As the current results about half of

Table 1. GC-MS analysis of *M. mozaffarianii* seed essential oil.

Compound[a]	KI[b]	KI[c]	Percentage
1. α-Pinene	942	939	0.9
2. Camphene	950	954	0.2
3. Sabinene	972	975	0.7
4. β-Pinene	976	979	1.4
5. β-Myrcene	996	991	0.5
6. 1,8-Cineol	1028	1031	10.7
7. *cis*-Sabinene hydrate	1074	1070	0.1
8. Linalool	1101	1097	0.3
9. Menthone	1149	1153	3.0
10. Borneol	1166	1169	1.0
11. Terpinene-4-ol	1179	1177	0.3
12. α-Terpineol	1190	1189	3.3
13.Pulegone	1241	1237	4.6
14. Piperitone	1255	1253	27.1
15. Bornyl acetate	1287	1289	0.3
16. Thymol	1293	1290	0.3
17. Piperitenone	1339	1343	35.6
18. Piperitenone oxide	1372	1369	4.4
19. *trans*-Caryophyllene	1418	1419	1.6
20. α-Humulene	1457	1455	0.2
21. Germacrene D	1489	1485	0.6
22. Bicyclogermacrene	1496	1500	0.1
23. Caryophyllene oxide	1579	1583	0.4
25. 6,10,14-Trimethyl-2-pentadecanone	1843	1840	0.2
Total			96.8

[a] Compounds listed in order of elution.
[b] KI (Kovats index) measured relative to *n*-alkanes (C_9-C_{28}) on the non-polar DB-5 column under condition listed in the Materials and Methods section.
[c] KI, (Kovats index) from literature.

the piperitenone content has converted to piperitone. Absence of piperitenone epoxide in the seeds oil is considerable. It was reported by Arman *et al*. [3] as the second major component in the leaves oil of *M. mozaffarianii*. Rustayian *et al*. [5] reported 1,8-cineol with a high amount of 53.5% in the essential oil of aerial parts of *M. mozaffariani* while it contained 10.7% of the seed oil. Differences between *M. mozaffarianii* seeds oil chemical profile and that of the leaves reported by Rustayian *et al*. could be mainly related to the factors such as geographic origin and the season where the collection took place.

As piperitenone is found to be one of the main metabolites of the potent hepatotoxin, pulegone in the body [6], *M. mozaffarianii* could be regarded as a toxic plant and due to its traditional use as a folklore medicine future toxicology investigations are suggested.

This paper presents the essential oil composition of seeds of the endemic species *M. mozaffarianii* for the first time. Regarding the essential oil major components, further biological studies are suggested to investigate the pharmacological and therapeutic properties of the seeds.

Experimental

Plant material

Fresh seeds of *M. mozaffarianii* were collected in June 2015 from Bekhan village, Fareghan, Hadji-Abad County (Tangezaq Mountains), Hormozgan Province, Iran: (28°18′33″N, 55°54′06″E, 1700 m). Specimens were identified by R. Asadpour and voucher was deposited in the Herbarium of Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran under code number 419-PMP/A. Seeds were powdered and submitted to hydrodistillation in a Clevenger-type apparatus for 3 hours. At the end of distillation the oil was collected, dried with anhydrous Na_2SO_4, measured, and transferred to a

clean glass vial and kept at a temperature of −18 °C for further analyses.

Analysis of the essential oil

Oil sample analyses were performed on a HP-6890 gas chromatograph (GC) equipped with a FID and a DB-5 capillary column, 30 m × 0.25 mm, 0.25 μm film thickness, temperature programmed as follows: 60-240 °C at 4 °C/min. The carrier gas was N_2 at a flow of 2.0 ml/min; injector port and detector temperature were 250 °C and 300 °C, respectively. Sample was injected by splitting and the split ratio was 1:10. GC-MS analysis was performed on a Hewlett-Packard 6890 /5972 system with a DB-5 capillary column (30 m × 0.25 mm; 0.25 μm film thickness. The operating conditions were the same conditions as described above but the carrier gas was He. Mass spectra were taken at 70 eV. Scan mass range was from 40-400 *m/z* at a sampling rate of 1.0 scan/s. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the oil were identified by their retention time, retention indices, relative to C_5-C_{28} n-alkanes, computer matching with the Wiley 275.L library and as well as by comparison of their mass spectra with data already available in the literature [7,8]. The percentage of composition of the identified compounds was computed from the GC peaks areas without any correction factors and was calculated relatively. The analyses of the essential oil are the average of three replicates.

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