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Research Article

Essential Oil of Two Iranian Horehound Species: *Marrubium propinquum* and *Marrubium parviflorum*

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ABSTRACT

Background: Two species of genus *Marrubium* belonging to the family Lamiaceae, were studied for their volatile components.
Methods: The essential oils were extracted from aerial parts of the plants through hydrodistillation using a Clevenger apparatus. Later, CG and CG-MS analysis were applied to assess the chemical components of the essential oils.
Results: Analysis of the *M. propinquum* essential oil resulted in the identification of 22 components, representing 79.6% of the total essential oil that principally contained oleic acid (19%), β-caryophyllene (7.4%) and m-tolualdehyde (5.2%). In the case of *M. parviflorum*, 20 components were identified, representing 83% of the *M. parviflorum* essential oil, among them oleic acid (11.8%), α-pinene (10.2%) and germacrene D (9.8%) were the main compounds.
Conclusion: Regarding the results of this study in both essential oils after the non terpenoids, sesquiterpene hydrocarbons possessed the uppermost portion of the oils. We found some similarities and differences between *M. propinquum* and *M. parviflorum* essential oils and also in comparison with other species of genus *Marrubium* which might be due to different parameters such as agrotechnical factors.

Introduction

Herbal medicines have been limelight in the field of drug therapy due to their fundamental beneficial applications in humans' health care. One of the effective metabolites of herbal medicines has been known as essential oils. Various parts of plants could contain essential oils, accordingly they might have been used in medicine, pharmacy and food industry.[1] In this regard we aimed to study essential oils of two species of genus *Marrubium* from the family Lamiaceae.

Lamiaceae with about 220 genera and nearly 4000 species mainly contains flowering plants that are frequently aromatic in all parts.[2,3] Genus *Marrubium* from the family lamiaceae included 49 accepted species worldwide.[4] In Iran, *Marrubium* (horehound) is represented with 10 species, including *Marrubium propinquum* Fisch. & C.A.Mey and *Marrubium parviflorum* Fisch. & C.A.Mey.[5,6] Since ancient times, *M. vulgare* (white horehound) has been accepted for remedy of several disorders such as dyspeptic complaints, loss of appetite, cough, wound healing and as a choleretic in digestive and biliary complaints.[7]

Phytochemical analyses of *Marrubium* spp. demonstrated that they are rich in diterpenes,[8-15] polyphenols, flavonoids,[16] steroids, saponines, phenylpropanoid esters and glycosides.[1,16-22]

According to previous studies, different species of genus *Marrubium* possess various therapeutic effects such as a hypoglycemic effect,[23] antihypertensive,[24] antispasmodic,[25] antiproliferative,[26-30] antioxidant,[31-32] hepatoprotective,[33] gastroprotective,[34] antibacterial,[35-38] vasorelaxant,[13] hypolipidemic[39] and antioedematogenic.[40]

Several studies on essential oil composition of *Marrubium* species are found in literature.[41-57]

Evaluating the naturally occurring volatile constituents in the essential oil of a plant would be of value in characterizing species within a genus. According to the valuable results from previous studies on *Marrubium* species, *M. propinquum* and *M. parviflorum* were selected for assessing chemical components in their essential oils.

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Materials and Methods

Plant Material

Aerial parts of *Maruubium propinquum* were collected from Lighvan in East Azarbaijan province, Iran, during July 2014 at the flowering stage and authenticated by Amir-Hossein Talebpour from East Azerbaijan Education and Research Center for Agriculture and Natural Resources. Likewise, aerial parts of *M. parviflorum* were collected around *Misho-Dagh* mountainous near Marand in East Azarbaijan province, Iran, during July 2015 and authenticated by Atefeh Ebrahimi from Faculty of Pharmacy, Tabriz University of Medical Sciences. The voucher specimens were kept at the Herbarium of the Faculty of Pharmacy, Tabriz University of Medical science, Iran.

Essential oil Extraction

Air-dried plant materials of the *M. propinquum* and *M. parviflorum* were submitted to hydrodistillation using a Clevenger type apparatus to extract the essential oils for about 2 hrs. As the essential oils content was low in amount, xylene was used as an absorbing medium. The resultant essential oils were kept in dark glass bottles at 4°C until analysis by GC/MS.

Gas Chromatography-Mass Spectrometry (GC-MS)

The essential oils were analyzed by GC-MS using a Shimadzu GC-MS-QP 5050A gas chromatograph fitted with a DB1 (methyl phenyl syloxane, 60 m x 0.25 mm i.d., 0.25 μm film thickness) capillary column. The GC was set at the following conditions with helium as the carrier gas; flow rate of 1.3 mL/min; linear velocity: 29.6 cm/s; Split ratio, 1:24; column temperature, 2 min in 50°C, 50-270 °C at 3 °C/min; injector temperature, 250 °C, and 1 μL of volume injection of the essential oil. The MS operating parameters were as follows: ionization potential, 70 eV; ion source temperature; 270 °C; quadrupole 100 °C, Solvent delay 2 min, scan speed 2000 amu/s and scan range 30-600 amu, EV voltage 3000 volts.

Identification of the compounds

Retention indices for all compounds were determined according to the kovats retention indices using n-alkanes series as standards. The components of the essential oils were identified by comparison of the retention indices and mass spectral data with those for the standards and by computer matching with the Wiley 229, Nist 107, Nist 21 Library, as well as by comparing the fragmentation patterns of the mass spectra with those reported in the literature. For quantification purpose, relative area percentages were obtained by FID without the use of correction factors, where the FID detector condition was set on a duplicate of the same column applying the same operational conditions.

Results

All the identified components in both essential oils are shown in Table 1, based on the order of their elution on DB1-MS column; retention indices and percentages. The GC–MS analysis of the essential oil from *M. propinquum* resulted in the identification of 22 components, representing 79.6% of the total essential oil. As shown in Figure 1, the components of *M. propinquum* essential oil were classified to non-terpenoids (38.8%), sesquiterpene hydrocarbons (20.9%), oxygenated sesquiterpenes (7.3%) monoterpene hydrocarbons (3.4 %), and oxygenated monoterpenes (2.9%).

Among the non-terpenoid compounds, m-tolualdehyde, p-tolualdehyde, and oleic acid were the dominant constituents with 5.2%, 2.9% and 19%, correspondingly. The major sesquiterpenes of *M. propinquum* essential oil were identified as β-caryophyllene (7.4%), bicyclogermacrene (4.6%), germacrene D (4.1%) and β-farnesene (3.7%). Besides, limonene and α-pinene were considered as the most frequent monoterpenes of the *M. propinquum* essential oil with the relative percentages of 1.9% and 1.4%, respectively. Considering the oxygenated monoterpenes and oxygenated sesquiterpenes, anethole (2.9%) and caryophyllene oxide (5.4%) were the most dominant components of the groupings. To sum up, essential oil of *M. propinquum* principally contained oleic acid (19%), β-caryophyllene (7.4%), m-tolualdehyde (5.2%) bicyclogermacrene (4.6%), and germacrene D (4.1%). In the case of *M. parviflorum* essential oil, 20 components were identified, representing 83% of the total essential oil that are classified to non-terpenoids (28.2%), sesquiterpene hydrocarbons (24.9%), monoterpene hydrocarbons (13.9%), oxygenated sesquiterpenes (8.4%) and oxygenated monoterpenes (2.0%). The most abundant sesquiterpenes were shown to be germacrene D (9.8%), β-caryophyllene (5.3%), β-farnesene (3%), and bicyclogermacrene (5.8%). Moreover, results showed that oleic acid (11.8%) (a monounsaturated omega-9 fatty acid) was the foremost constituent. Additionally, monoterpenes of *M. parviflorum* essential oil were rich in α-pinene, and limonene with the relative percentages of 10.2%, and 2.2%. In view of the oxygenated monoterpenes and oxygenated sesquiterpenes, verbenol (1.3%) and caryophyllene oxide (5.2%) were the leading components of the groupings. Taken as a whole, the major constituents of the *M. parviflorum* essential oil were detected to be oleic acid (11.8%), followed by α-pinene (10.2%), germacrene D (9.8%), bicyclogermacrene(5.8%) and β-caryophyllene (5.3%).

Table 1. Chemical constituent of the essential oils obtained from aerial parts of *M. propinquum* and *M. parviflorum.*

No.	Compounds	RI[a]	*M. propinquum(%)*	*M. parviflorum(%)*	Identification Method
1	α-Pinene	917	1.4	**10.2**	GC/MS, RI
2	1-Octen-3-ol	-	1.7	5.1	GC/MS
3	Sabinene	933	-	1.5	GC/MS, RI
4	Limonene	960	1.9	2.2	GC/MS, RI
5	m-Tolualdehyde	-	5.2	2.7	GC/MS
6	p-Tolualdehyde	-	2.9	1.6	GC/MS
7	Linalool	992	-	0.7	GC/MS, RI
8	Verbenol	1019	-	1.3	GC/MS, RI
9	Anethole	1078	2.9	-	GC/MS, RI
10	β-Bourbenene	1392	-	1.0	GC/MS, RI
11	β-Caryophyllene	1410	**7.4**	5.3	GC/MS, RI
12	β-Farnesene	1424	3.7	3.0	GC/MS, RI
13	Germacrene D	1440	4.1	**9.8**	GC/MS, RI
14	Bicyclogermacrene	1448	4.6	5.8	GC/MS, RI
15	Beta-bisabolene	1452	1.0	-	GC/MS, RI
16	Spathulenol	1484	1.9	2.5	GC/MS, RI
17	Caryophyllene oxide	1488	**5.4**	5.2	GC/MS, RI
18	Eicosane	-	1.7	-	GC/MS
19	α- Bisabolol	1532	-	0.7	GC/MS, RI
20	Oleic acid	1821	**19**	**11.8**	GC/MS, RI
21	Phytol	-	4.6	5.6	GC/MS
22	Linoleic acid	1897	1.7	-	GC/MS, RI
23	Heneicosane	-	5.2	-	GC/MS
24	Tricosane	-	1.9	4.3	GC/MS
25	Tetracosane	-	1.2	2.7	GC/MS
Total identified			**79.6**	**83**	
Non-terpenoids			**38.8**	**28.2**	
Monoterpene hydrocarbons			**3.4**	**13.9**	
Oxygenated monoterpenes			**2.9**	**2.0**	
Sesquiterpene hydrocarbones			**20.9**	**24.9**	
Oxygenated sesquiterpenes			**7.3**	**8.4**	
Oxygenated diterpenes			**1.7**	**5.6**	
Unidentified			**20.4**	**17**	

RI[a] is the Retention Index relative to C8–C20 n-alkanes on the DB-1 column.

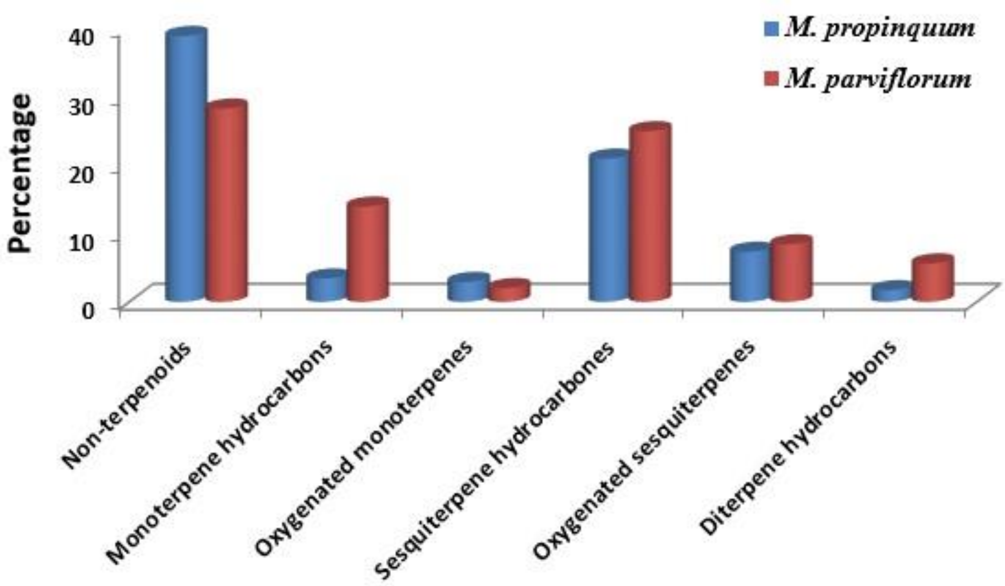

Figure 1. Comparison between the percentage of identified chemical groups of *M. propinquum* and *M. parviflorum* essential oils.

Discussion

According to the data in table 1, both *M. propinquum* and *M. parviflorum* were assigned as higher content of non-terpenoid compounds plants. Previous reports on the essential oils indicated that a higher numbers of sesquiterpenes were present in different species of *Marrubium* genus.

A review on prior studies on *M. parviflorum* essential oil from different localities represented various compounds as the foremost constituents of the essential oils. For instance, bicyclogermacrene, germacrene D and β-caryophyllene with virtual percentages of 26.3%, 21.5% and 15.6% were specified in *M. parviflorum* essential oil collected from Khalkhal, Iran. In another report on the essential oil of *M. parviflorum* from Nevshehir Turkey, hexadecanoic acid (15.4%), germacrene D (11.1%) and β-caryophyllene (10.0%) were determined as the major chemical compounds.[54,55] Whereas in the present study, oleic acid (11.8%), α-pinene (10.2%) and germacrene D (9.8%) were specified as the main compounds of the *M. parviflorum* essential oil which was collected from Marand region, Iran.

Based on the findings of our study, the major constituents for *M. propinquum* was revealed to be β-caryophyllene (7.4%), bicyclogermacrene (4.6%) and germacrene D (4.1%) which was different from the previous report in the literature. In the study conducted by Tajbakhsh et al on *M. propinquum* essential oil, it was reported that the plant collected from the suburb of Gadouk Mazandaran province in northern Iran contained mainly β-farnesene (43.8%), β-caryophyllene (20.1%) and germacrene D (4.115.8%).[56]

Having compared the essential oils of *M. propinquum* and *M. parviflorum*, we could bring about the prime difference between *M. propinquum* and *M. parviflorum* essential oil due to the monoterpenoid groupings, as regards in the Figure 1. *M. parviflorum* essential oil was rich in monoterpene hydrocarbons (13.9%) and α-pinene with the relative percentages of 10.2% was determined as a chief monoterpene. On the contrary, in *M. propinquum*, monoterpene hydrocarbons were relatively in a lower amount of 3.4% of the essential oil. In comparison, in essential oil of *M. propinquum* not only allocated α-pinene and limonene in higher percentages but also contained a monoterpene hydrocarbons, sabinene, which was not identified in *M. propinquum*. In addition, the constituents of the oxygenated monoterpenes were completely different in the relative essential oils. So that, linalool and verbenol were present as oxygenated monoterpenes in *M. parviflorum* whereas, anethole was the main component in *M. propinquum* essential oil. Nonetheless, in both essential oils after the non terpenoids, sesquiterpene hydrocarbons possessed the uppermost portion of the oils. On the whole, we could find much similar compounds in different quantities accounting for 79.6% of *M. propinquum* essential oil and 83% of *M. parviflorum* essential oil.

Upon earlier reports germacrene D was ascertained as the major sesquiterpene hydrocarbone in *M. anisodon* (44.2%) and *M. incanum* Desr. (28.75-32.14%).[52,56] Likewise, bicyclogermacrene, one of the major compounds of the sesquiterpene hydrocarbons in *M. propinquum* and *M. parviflorum* essential oils, was usually absent in the essential oil of *M. vulgare*, the most famous species of *Marrubium* genus. As we know, geographical, seasonal and climatic issues would commonly influence the chemical composition of the essential oils within various populations of these species.

Conclusion

Concerning the results of this study we could find some similarities and differences between *M. propinquum* and *M. parviflorum* essential oils and also in comparison with other species of *Marrubium* genus. Generally, mentioned similarities and differences might be attributed to both intrinsic parameters of the plants (genetic, growth stage, etc.) and extrinsic factors such as climatic conditions, seasonal variations, environmental issues, distillation processes and etc.

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Conflict of interests

The authors claim that there is no conflict of interest.

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