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Original Article

First Report on Screening of the Profiles of the Essential Oils and Volatiles from the Aerial Parts of *Marrubium persicum* Using Classical and Advanced Methods Prior to Gas Chromatographic Mass Spectrometric Determination

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Abstract

The Lamiaceae family consists of a broad spectrum of medicinal plants involving *Marrubium* L. genus. Regarding the diverse pharmaceutical uses of the plants belonging to this genus, they can be considered as proper alternatives for chemical drugs having harmful effects. The present work aims to identify and characterize chemical compositions of the essential oils and volatiles from the aerial parts of *Marrubium persicum* C. A. Mey. as an herbal plant in Iran using classical hydrodistillation. To establish a comprehensive comparison between the traditional techniques and advanced ones, microwave-based extraction techniques namely MAHD as well as SFME have also been utilized. In another part of this project, the profiles related to the volatile fractions from the aerial parts of *Marrubium persicum* C. A. Mey.have been assessed and compared with the other categories. The main components in the hydrodistillation (HD) method were α-pinene (21.5%), spathulenol (19.5%), α-thujene (17.4%), while the headspace solid-phase microextraction (HS-SPME) profile mainly consisted of β-caryophyllene (14%), eugenol (11.2%) and methyl eugenol (10.2%). On the other hand, using the SFME approach spathulenol (25.4%), α-pinene (17.4%) and germacrene D (9.5%) were found as the most abundant constituents. Moreover, in the MAHD profile caryophyllene oxide (13.1%), δ-elemene (12.4%), camphene (8.5%) were respectively the predominant natural compounds. According to gas chromatographic-mass spectrometric determinations, a total of 40 compounds were recognized in the corresponding profiles totally covering 94.6-99.7% of the whole chemical compositions. Sesquiterpene hydrocarbons were recognized as the most frequent groups of natural compounds in the profiles of the advanced approaches, whereas in the traditional one monoterpene hydrocarbons were found to be the dominant constituting group.

Keywords: *Marrubium persicum*, Gas chromatography-mass spectrometry (GC-MS), Hydrodistillation (HD), Headspace solid-phase microextraction method (HS-SPME), Microwave assisted hydrodistillation (MAHD), Solvent-free microwave extraction (SFME)

Introduction

Lamiaceae family is a broad family of the plants including a large number of genera which are aromatic in all growing areas. As given in the reference botanical books in Iran, the genus *Marrubium* L. from this family consists of about ten species growing wild in different climatic regions of the country of which the native Iranian species involve *M. anisodon* K. Koch, *M. propinquum* Fisch. & C. A. Mey., *M. persicum* C. A. Mey., *M. astracanicum* Jacq., *M. cordatum* Nábelek, *M. crassidens* Boiss., *M. cuneatum* Banks & Sol., *M. duabense* Murata, *M. parviflorum* Fisch. & C. A. Mey. and *M. procerum* Bunge [1,2].

In the scientific databases, a variety of useful natural compounds like ladanein, flavones,

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phenylethanoid glycosides [3,4], sterols [5], phenylpropanoids [6] and some types of flavonoids have been extracted from different species of the *Marrubium* L. genus up to present.

In addition, many reports could be found relating to promising biological activities along with phytochemical and medicinal usages of these herbal plants of which antiviral [7-9], anti-proliferative [10], gastro protective [11], antispasmodic [7,12,13], analgesic [14], antibacterial [8,15-18], antioxidant [19-28], antiseptic [18], anticancer [18,29], anti-hepatotoxic [30], inflammatory [20,31-33], anti-protozoal [34,35], anti-malarial [34], anti-fungal [18], immunomodulatory [36,37], anti-microbial activities [19,25,38-42] as well as cytotoxic properties are among the most significant characteristics [32,43,44] .

In the Iranian herbal and folk prescriptions, *M. persicum* which is called as "Ferasione Irani" has been frequently used and this medicinal plant can considerably lower the vaginal discharges in women and can highly regulate menstruation period of the women and performance of their hormones. More specifically, the extracts of this medicinal plant are capable of removing benign breast lumps and tumors and treating the ovarian cysts in the uterine. Furthermore, these extracts have found application in the treatment of men prostates, pain relief and as a powerful remedy against the progression of fatty liver [2,25].

The present study is an endeavor to characterize the essential oils and volatiles obtained from the aerial parts of *M. persicum* as an herbal and medicinal plant growing wild in Iran. To the best of my knowledge, this is the first report focusing on chemical compositions of the essential oils and volatile fractions from the aerial parts of *M. persicum* obtained using the traditional and advanced methods.

Material and Methods

Plant Material and Botanical Identification

The aerial parts of *M. persicum* (Fig. 1) as the plant material were collected during the flowering stage on 23 March, 2015, in the Shamirzad regions, North of Iran, and on the Southern Slopes of the Alborz Mountains in Semnan Province at the geographical coordinates of 35° 46′ 22″ N 53° 19′ 43″ E at an altitude of 1935 m from the sea level. A voucher specimen was deposited at Herbarium of the Faculty of Agriculture, Shahrood University of Technology, Shahrood, Iran, for further authentication.

Fig. 1 Representation of the aerial parts of *Marrubium persicum* C. A. Mey.

Hydrodistillation Method

150-g portions of the air-dried aerial parts of *M. persicum* were subjected to hydrodistillation in a Clevenger-type apparatus for 4 h. The essential oils had pale yellowish colors and were subsequently dried with anhydrous sodium sulphate and stored at 4 °C in the absence of daylight until being analyzed**.** The percentage yields of essential oils, in terms of the weight of the collected oil per gram of dried plant, were 0.21%, 0.19%, 0.23 and 0.3% (w/w) for four replicate distillations.

Headspace Solid-Phase Microextraction (HS-SPME) Method

A commercially available manual SPME apparatus equipped with 75-μm diameter PDMS-CAR fibers obtained from Supelco (Bellefonte, USA) was used for the SPME procedure. Before use, the fiber was conditioned at 250 ˚C for 0.5 h in the GC injector. Then, one gram of powdered sample (*M. persicum*) was placed in a 20-mL sample vial sealed with septum-type caps provided from Supelco, which was heated for 15 min at 70 ˚C. After this time, the SPME needle pierced the septum, and the PDMS fiber was extended through the needle and exposed to the headspace above the sample for fifteen minutes to trap the volatile compounds in the upper space of the vials [45-51]. After the optimized extraction time (15 min), the fiber was drawn into the needle, and then the needle was removed from the septum and directly inserted onto the injection port of the GC. Desorption of the analytes from the fiber coating was performed by heating the fiber in the splitless (250 ˚C) mode of the injection port for

5 min. The sequential steps for the isolation of the volatiles from the aerial parts of *M. persicum* have been represented in Fig. 2.

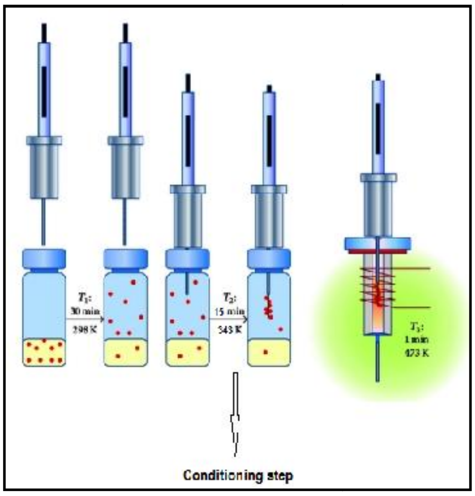

Fig. 2 Schematic representation of the respective steps using head space solid phase micro-extraction (HS-SPME).

Performance of Microwave Assisted Hydro-distillation (MAHD) Technique

A comprehensive detail concerning the application of the MAHD technique has been given in our previous papers in the literature [46,49,52-54]. Briefly, a microwave oven available from Samsung, South Korea regulated at a frequency of 2450 MHz having a proper internal capacity to house the glass Clevenger set up was employed which was connected to the condenser placed outside the system. It was found that 50-g portions of the dried aerial parts of *M. persicum* were adequate to finalize the extraction process (see Fig. 3). The yields of four experiments done using this method were 0.29%, 0.25%, 0.35% and 0.30% (w/w).

Practical Details of the Solvent-Free Microwave Extraction (SFME) Approach

The main principles of the solvent-free microwave extraction (SFME) approach have been extensively discussed in our previous reports which are very similar to that of the MAHD method (see Fig. 4) [46,49,54,55]. The main difference between this approach and the MAHD relates to soaking 60-g portions of the dried and ground aerial parts of the plant material (*M. persicum*) in the SFME method and immediately after draining off the excess water. This was mainly to hydrate the external layers of the plant material and to condition them for better extraction of the essential oils from the respective secretary glands. In addition, for four replicate extractions the yields were found to be 0.19%, 0.22%, 0.30% and 0.20% (w/w), respectively.

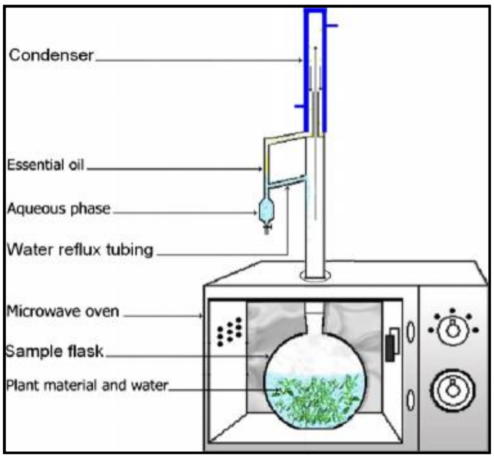

Fig. 3 Schematic representation of the microwave-assisted hydrodistillation (MAHD) apparatus used in this study.

Chromatographic Analyses

GC analyses were performed on a Shimadzu 15A gas chromatograph equipped with a split/splitless (ratio 1:30) injector and a flame ionization detector, both operating at 250 ˚C. High purity nitrogen was used as the carrier gas (1 mL/min) and the capillary column used was DB-5 (50 m×0.2 mm, film thickness 0.32 μm). The column temperature was kept at 60 ˚C for 3 min, then heated to 220 ˚C with a 5 ˚C /min rate and finally kept constant at 220 ˚C for 5 min.

Fig. 4 Schematic representation of the SFME apparatus used in this study to separate essence from aerial parts of *Marrubium persicum* C. A. Mey.

Table 1 Chemical composition of the volatile constituents from the aerial parts of *Marrubium persicum* C. A. Mey. obtained by using classical and advanced methods [a]

No.	Compound	Class	R.I.		Isolation methods			
			Cal. [g]	Lit. [h]	HD	HS-SPME	SFME	MAHD
1	α-Thujene	MH [b]	922	924	17.4	4.1	3.4	5.2
2	α-Pinene	MH	930	932	21.5	1.8	17.4	0.8
3	Camphene	MH	944	946	-	-	0.4	8.5
4	Sabinene	MH	968	969	0.2	-	-	-
5	α-Phellandrene	MH	1015	1003	0.1	-	0.2	3.0
6	p-Cymene	MH	1036	1025	4.1	-	-	0.2
7	Limonene	MH	1040	1029	-	-	-	0.9
8	*Trans*-β-Ocimene	MH	1052	1050	0.3	4.8	0.5	1.6
9	*Cis*-Sabinene hydrate	OM	1095	1097	0.6	-	-	-
10	α-Terpinolene	MH	1100	1089	0.1	3.0	0.1	3.9
11	Linalool	OM [c]	1104	1097	-	-	2.1	0.5
12	Camphor	OM	1144	1146	0.1	4.7	0.4	0.2
13	Isopulegol	OM	1150	1150	0.1	-	-	-
14	Pinocarvone	OM	1161	1160	-	-	-	0.1
15	Artemisyl acetate	OM	1162	1162	-	0.4	0.1	0.2
16	Camphor	OM	1165	1146	0.2	7.7	4.1	0.8
17	Geranial	OM	1279	1267	0.8	-	0.1	1.7
18	*n*-Tridecene	NH [d]	1293	1292	0.1	-	-	-
19	Bornyl acetate	OM	1303	1289	0.4	-	-	-
20	δ-Elemene	SH [e]	1357	1338	7.5	16.9	6.8	12.4
21	Eugenol	NH	1361	1359	0.9	11.2	0.8	0.1
22	Geranyl acetate	OM	1383	1381	-	-	-	0.6
23	β-Elemene	SH	1390	1391	0.1	4.4	1.2	5.9
24	α-Copaene	SH	1399	1377	0.1	-	0.1	-
25	Methyl eugenol	NH	1400	1403	0.1	10.2	6.4	3.7
26	β-Bourbonene	SH	1412	1388	0.6	2.1	1.0	2.4
27	β-Caryophyllene	SH	1450	1419	7.4	14.0	6.1	8.0
28	γ-Himachalene	SH	1478	1482	0.1	-	-	-
29	Aromadendrene	SH	1483	1460	0.3	1.5	2.3	2.1
30	α-Amorphene	SH	1498	1485	-	1.4	-	0.1
31	Germacrene-D	SH	1509	1485	0.7	0.1	9.5	6.1
32	*allo*-Aromadendrene	SH	1518	1460	0.1	0.4	0.2	-
33	α-Selinene	SH	1525	1498	0.6	0.2	5.0	7.2
34	γ-Cadinene	SH	1540	1514	0.7	0.2	0.4	0.9
35	δ-Cadinene	SH	1544	1523	2.6	0.1	0.5	-
36	*Cis*-Calamenene	SH	1549	1540	0.1	-	0.1	-
37	Elemol	OS [f]	1573	1550	2.7	-	0.7	0.2
38	Spathulenol	OS	1574	1576	19.5	2.1	25.4	8.1
39	Caryophyllene oxide	OS	1580	1583	2.8	8.4	3.4	13.1
40	α-Bisabolol	OS	1683	1685	1.7	-	0.1	0.7
Total percentage					94.6	99.7	98.8	99.2
MH (%)					43.7	13.7	22.0	24.1
OM (%)					2.2	12.8	6.8	4.1
SH (%)					20.90	41.3	33.2	45.1
OS (%)					26.7	10.5	29.6	22.1
NH (%)					1.1	21.4	7.2	3.8
NIC [i]					33	22	29	30

[a] The compounds have been sorted according to their retention indices on an HP-5MS capillary column; [b] Monoterpene hydrocarbon; [c] Oxygenated monoterpene; [d] Non-terpene hydrocarbon; [e] Sesquiterpene hydrocarbon; [f] Oxygenated sesquiterpene; [g] Calculated Kovatz retention indices; [h] Kovatz retention indices given in the literature; [i] Non-identified compounds

Relative percentage amounts were calculated from peak areas using a CR5 Shimadzu CR pack without the use of correction factors. In addition, GC-MS analyses were performed using a Hewlett-Packard 5973 instrument equipped with an HP-5MS column (30 m×0.25 mm, film thickness 0.25 μm). The effluent of the GC column was introduced directly into the source of the MS. The column temperature programming was the same with the GC analysis. The flow-rate of helium carrier gas was 1 mL/min and final column temperature was adjusted at 230 ˚C. The detector temperature was set at 250 ˚C and MS spectra were taken at 70 eV (E1) using an electron multiplier voltage of 1800 eV over the mass range 30-350 amu and scan times of 2 scans/ sec.

Qualitative and Quantitative Analyses

Identification and determination of the constituents of each volatile profile were tentatively made by comparison of their mass spectral fragmentation patterns and retention indices (RI) relative to C_9-C_{25} *n*-alkanes both with those given in the literature [56] and those stored in the MS library (Wiley 275). For further reliability, we utilized some of the spectral patterns as well as Kovatz indices based on the previous findings of our research group [52,53,57-75]. Relative percentages of the components were calculated from peak areas using a Shimadzu C-R4A chromatopac on the DB-5 column, without the use of a correction factor.

Results and Discussion

Chemical Profiles Using the Aforementioned Methodologies

As for as I know, this is the first report dealing with the quantitative and qualitative characterization of the volatile fractions and the essential oils from the aerial parts of *M. persicum* in Iran. This study showed the presence of several monoterpene hydrocarbons (MH), oxygenated monoterpenes (OM), sesquiterpene hydrocarbons (SH), oxygenated sesquiterpenes (OS) and non-terpene hydrocarbons (NH), as well.

Using the classical hydrodistillation approach (Table 1), 33 compounds were found in the profile accounting for 94.6% of the total structure of which seven were monoterpene hydrocarbons (43.7%), six oxygenated monoterpenes (2.2%), thirteen sesquiterpene hydrocarbons (20.90%), four oxygenated sesquiterpenes (26.7%) and three non-terpne hydrocarbon (1.1%). Thus, the respective order of these natural products classes is as MH>OS>SH>OM>NH.

Accordingly, the water-distilled essential oil from the aerial parts of *M. persicum* was dominated by the presence of high quantities of natural compounds involving α-pinene (21.5%), spathulenol (19.5%), α-thujene (17.4%), δ-elemene (7.5%) and β-caryophyllene (7.4%).

In the methods based upon the application of microwave beams (SFME and MAHD), twenty nine and thirty compounds were respectively identified representing 98.8% and 99.2% of the respective profiles (Table 1). It was realized that in the former approach (SFME), six monoterpene hydrocarbons, five oxygenated monoterpenes, twelve sesquiterpene hydrocarbons, four oxygenated sesquiterpenes and finally two non-terpene hydrocarbons contribute to the overall composition respectively relating to 22.0%, 6.8%, 33.2%, 29.6% and 7.2% of the category.

On the other hand, the profile of the oil obtained by the latter approach (MAHD), consisted of eight monoterpene hydrocarbons (24.1%), seven oxygenated monoterpenes (4.1%), nine sesquiterpene hydrocarbons (45.1%), four oxygenated sesquiterpenes (22.1%) and two non-terpene hydrocarbons (3.8%). Regarding these percentages, the final rank of the natural compounds groups for the SFME and MAHD are respectively as: SH>OS>MH>NH>OM and SH>MH>OS>OM>NH. It was found that the major fraction of the SFME oil involved spathulenol (25.4%), α-pinene (17.4%), germacrene-D (9.5%), δ-elemene (6.8%), methyl eugenol (6.4%) and β-caryophyllene (6.1%) while the oil of the MAHD method mainly consisted of caryophyllene oxide (13.1%), δ-elemene (12.4%), camphene (8.5%), spathulenol (8.1%), β-caryophyllene (8.0%), α-selinene (7.2%), germacrene-D (6.1%) and α-thujene (5.2%).

Moreover, using the HS-SPME approach (Table 1) led to recognition of twenty-two compounds totally comprising 99.7% of the volatile structure containing four monoterpene hydrocarbons (13.7%), three oxygenated monoterpenes (12.8%), eleven sesquiterpene hydrocarbons (41.3%), two oxygenated sesquiterpenes (10.5%) and two non-terpene hydrocarbons (21.4%). In this profile, the most frequently occurring compounds were found to be respectively as δ-elemene (16.9%), β-caryophyllene (14.0%), eugenol (11.2%), methyl eugenol (10.2%), caryophyllene oxide (8.4%), β-caryophyllene (7.7%) and *trans*-β-ocimene (4.8%).

Table 2 Main components of the essential oils from different species of *Marrubium* L. genus

Plant name	Main components (%)	Part (s)	Employed technique (s)	Country	Ref.
M. globosum	β-Caryophyliene (12.4%), hexadecanoic acid (7.4%) and spathulenol (5.2%)	Aerial parts	Hydrodistillation	Lebanon	[76]
M. cuneatum	Germacrene D (15.6%), hexadecanoic acid (6.5%), spathulenol (6.5%), caryophyllene oxide (6.2%), *p*-methoxyacetophenone (5.4%) and bicyclogermacrene (5.2%)	Aerial parts	Hydrodistillation	Lebanon	[76]
M. vulgare	Flower: Eugenol (50.1%)	Glandular trichomes	Distillation extraction [A]	Algeria	[88]
M. vulgare	Vegetative: β-Bisabolene (29%) and eugenol (16.2%)	Glandular trichomes	Hydrodistillation	Algeria	[88]
M. vulgare	β-Bisabolene (28.3%), β-caryophyllene (7.8%), (*E*)-β-farnesene (7.4%) and 1,8-cineole (4.8%)	Aerial parts	Hydrodistillation	Tunisia	[91]
M. aschersonii Magnus	β-Bisabolene (22.0%), α-thujene (10.3%), eugenol (10.1%), α-humulene (6.2%)	Aerial parts	Hydrodistillation	Tunisia	[91]
M. parviflorum Fisch & C. A. Mey	Bicyclogermacrene (26.3%), germacrene D (21.5%) and β-caryophyllene (15.6%)	Aerial parts	Hydrodistillation	Iran	[92]
M. vulgare	β-Bisabolene (25.4%), β-caryophyllene (11.6%), germacrene D (9.7%) and (*E*)-β-farnesene (8.3%)	Aerial parts	Hydrodistillation	Iran	[92]
M. velutinum Sm.	γ-Muurolene (27.28%), β-caryophyllene (24.25%) and β-caryophyllene oxide (6.03%)	Aerial parts	Steam distillation	Greece	[93]
M. peregrinum	(*Z*)-β-Farnesene (12.04%-16.47%), (*E*)-β-farnesene (21.49%-24.16%), *epi*-bicyclosesquiphellandrene (3.53%-12.31%) and bicyclogermacrene (4.81%-11.03%) [b]	Aerial parts	Steam distillation	Greece	[93]
M. crassidens Boiss	β-Caryophyllene (20.3%), germacrene D (12.9%), caryophyllene oxide (11.1%) and cubenol (11.0%)	Aerial parts	Hydrodistillation	Iran	[94]
M. vulgare	Thymol (34.6%)	Aerial parts	Steam distillation	Egypt	[89]
M. vulgare	(*E*)-Caryophyllene (25.91%-32.06%), germacrene D (20.23%-31.14%) and δ-amorphene (8.38%-10.22%)	Whole structure	Steam distillation	Poland	[95]
M. incanum Desr.	Germacrene D (32.46%-37.87%), (*E*)-caryophyllene (22.49%-30.79%) and α-cadinol (14.36%-17.87%)	Whole structure	Steam distillation	Poland	[95]
M. thessalum	Caryophyllene oxide (21.7%), β-caryophyllene (17.6%), germacrene D (15.3%), β-bisabolene (12.6%) and *trans*-β-farnesene (8.1%)	Aerial parts	Hydrodistillation	Greece	[96]
M. bourgaei	β-Caryophyllene (23.2%), (*Z*)-β-farnesene (13.5%) and germacrene D (10.3%)	Aerial parts	Hydrodistillation	Turkey	[81]
M. astracanicum	β-Caryophyllene (21.2%) and valeranone (5.4%)	Aerial parts	Hydrodistillation	Iran	[82]
M. vulgare	γ-Eudesmol (11.93%), β-citronellol (9.90%), citronellyl formate (9.50%) and germacrene D (9.37%).	Aerial parts	Hydrodistillation	Tunisia	[23]

Table 2 Continued

Plant name	Main components (%)	Part(s)	Employed technique(s)	Country	Ref.
M. peregrinum	No.1: β-Caryophyllene (13.20%), γ-muurolene (5.59%), bicyclogermacrene (7.63%), germacrene-D (6.79%), spathulenol (5.18%) and caryophyllene oxide (4.23%) [B]	Whole structure	Hydrodistillation	Serbia	[83]
	No.2: β-Caryophyllene (14.34%), γ-muurolene (5.56%), bicyclogermacrene(6.42%), germacrene-D(8.56%), spathulenol(5.68%) and caryophyllene oxide (3.73%) [B]				
	No.3: β-Caryophyllene (17.99%), γ-muurolene (6.29%), bicyclogermacrene (9.80%), germacrene-D (9.05%), spathulenol (3.76%) and caryophyllene oxide (4.98%) [B]				
M. anisodon	(Z)-β-Farnesene (20.2%), nonacosane (18.5%) and β-caryophyllene (13.3 %)	Aerial parts	Hydrodistillation	Turkey	[84]
M. bourgaei	Hexadecanoic acid (33.3%) and hexahydrofarnesyl acetone (6.4%)	Aerial parts	Hydrodistillation	Turkey	[87]
M. deserti	Germacrene D (45.7%)	Aerial parts	Hydrodistillation	Algeria	[85]
M. vulgare	β-Bisabolene (20.4%), δ-cadinene (19.1%) and isocaryophyllene (14.1%)	Aerial parts	Hydrodistillation	Iran	[78]
M. astracanicum	Caryophyllene oxide (35.8%), citronellal (16.9%) and β-caryophyllene (13.1%)	Aerial parts	Steam distillation	Iran	[90]
M. cuneatum	Bicyclogermacrene (37.9%) and germacrene D (24.1%)	Aerial parts	Steam distillation	Iran	[97]
M. incanum	(*E*)-Caryophyllene (27.0%), germacrene D (26.2%) and bicyclogermacrene (11.5%)	Aerial parts	Hydrodistillation	Serbia	[41]
M. globosum	Spathulenol (15.8%), β-caryophyllene (9.0%), caryophyllene oxide (7.9%), germacrene D (6.5%) and bicyclogermacrene (3.1%)	Whole structure	Hydrodistillation	Turkey	[98]
M. anisodon	Germacrene D (44.2%) and β-caryophyllene (10.4%)	Aerial parts	Hydrodistillation	Iran	[77]
M. propinquum	(*E*)-β-Farnesene (43.8%), β-caryophyllene (20.1%) and germacrene D (15.8%)				
M. vulgare	(*Z*)-β-Farnesene (9.61%), β-caryophyllene (8.50%), germacrene D (4.71%), γ-cadinene (4.96%), α-copaene (3.08) and (*E*)-hex-2-enal (4.67%)	Leaves	Hydrodistillation	Lithuania	[79]
M. vulgare	γ-Eudesmol (11.93%), β-citronellol (9.90%), citronellyl formate (9.50%) and germacrene D (9.37%)	Aerial parts	Hydrodistillation	Tunisia	[18]
M. vulgare	V.P. [C]: (*E*)-Caryophyllene (44.54%), germacrene D (23.85%), bicyclogermacrene (20.06%), α-humulene (5.79%) and caryophyllene oxide (5.56%)	Whole structure	Steam distillation[E]	Poland	[86]
	F.P. [D]: Germacrene D (43.36%), (*E*)-Caryophyllene (24.79%), bicyclogermacrene (9.86%), carvacrol (9.48%) and caryophyllene oxide (1.63%)				

[A] Using a Likens-Nickerson-type; [B] First population (No. 1) was collected in June 1998, in an area north-west of Domokos (Fthiotida, central Greek mainland), where it was growing on serpentine. The second population (No. 2) was found at the foothills of Mt. Parnassos, where it was growing on limestone;[C] Vegetative phase; [D] Flowering phase; [E] Using a Deryng apparatus, according to the Polish Pharmacopoeia VII

Chemical Profiles of Different Species of *Marrubium* L. in Previous Reports

There have been several reports in the literature dealing with the constituents of the essential oils from a broad spectrum of *Marrubium* L. species in different parts of the world (see Table 2). In the majority of these studies, sesquiterpene hydrocarbons constitute most of the chemical profile [41,76-86]. On the other hand, eugenol and hexadecanoic acid have been reported as the most abundant constituent components in some chemical profiles accounting for dominancy of non-terpene hydrocarbons [76,87,88].

In addition, only one chemical profile was characterized as a rich source of oxygenated monoterpenes particularly having thymol as the major constituting natural compound [89]. Oxygenated sesquiterpenes were detected as the major class of the natural compounds occurred in another profile, as well [90].

More specifically, all of the identified profiles of the different species of *Marrubium* were characterized by either low frequency or absence of the monoterpene hydrocarbons.

However, in contrast to these findings and specifically in the hydrodistilled essential oils from the aerial parts of *M. persicum* considerable amounts of monoterpene hydrocarbons were detected.

Conclusion

In this attempt, the essential oils and volatile fractions from the aerial parts of *M. persicum* were successfully separated and subsequently analyzed using the GC-MS and GC instrumentations. In this regard, 33, 22, 29 and 30 components were identified representing 94.6%, 99.7%, 98.8% and 99.2% of the total chemical compositions, using the HD, HS-SPME, SFME and MAHD methods, respectively. The most fractions of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and non-terpene hydrocarbons were respectively found in the HD, HS-SPME, MAHD, SFME and HS-SPME profiles of the used approaches.

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