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Essential oil composition of *Marrubium vulgare* L. from Iran

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ABSTRACT

Background & Aim: White horehound (*Marrubium vulgare* L.) is a perennial medicinal plant of the family Lamiaceae. The aim of this study was to identify of the chemical components of white horehound collected from Isfahan.

Experimental: The aerial parts of *M. vulgare* were collected from (Kamu Mountain) Isfahan province central of Iran, during 2014. The essential oils of samples were obtained by hydro-distillation, and analyzed using gas chromatography–mass spectrometry (GC–MS).

Results & Discussion: Results of GC/MS indicated that 44 compounds were identified in the essential oil from the aerial parts of *M. vulgare*. The major constituents of the essential oil were β-caryophyllene (32.19%), (E)-β-farnesene (11.39%), 1,8-cineole (8.17%), and α-pinene (6.64%). A comparison of our results with different reports, differences in the volatile composition of the plants could be attributed to genetic (genus, species, and ecotype), chemotype, distinct environmental and climatic conditions, seasonal sampling periods, geographic origins, plant populations, vegetative plant phases, and extraction and quantification methods.

Industrial and practical recommendations: The biosynthesis of secondary metabolites, although controlled genetically, is strongly affected by the environmental influences of a particular growing region.

1. Introduction

It is a major constituent of many species of the genus *Marrubium* (Lamiaceae) and includes about 97 species found along the Mediterranean, Asia, America, and Australia and also in temperate regions. In Iran, nine of them are endemic. The plant is a perennial, C3, herbaceous plant, stems usually branched to form a rounded bushy plant (100 cm) tall. The leaves arranged opposite along stem, leaf blades broadly ovate, oval, in axils of upper leaves; flowers sessile and crowded in dense whorls. The flowers are white. Chromosome number of *M. vulgare* is polyploid (2n=34) (Mozaffarian, 2008). Extensive pharmacological studies have demonstrated that marrubiin displays a suite of activities including antinociceptive (De Jesus *et al.*, 2000), antioxidant, antigen toxic, cardioprotective (Mnonopi *et al.*, 2011), vasorelaxant (El Bardai*et al.*, 2003), gastro protective, antispasmodic (Paula de Olivera *et al.*, 2011), immunomodulating (Karioti *et*

al., 2003), antioedematogenic (Hellen *et al.,* 2006), analgesic (Meyre-Silva *et al.,* 2005), and antidiabetic properties (Mnonopi *et al.,* 2012). The chemical composition of plants is known to be influenced by several external factors including climate, as some compounds may be accumulated at a particular period to respond to environmental changes (Abedi *et al.,* 2015; Golparvar *et al.,* 2015).

Khanavi *et al.* (2006) showed that the major component of *M.* vulgare from other region of Iran were β-bisabolene (25.4%), β-caryophyllene (11.6%), germacrene D (9.7%) and *E*-β-farnesene (8.3%). Asadipour *et al.* (2005) found that caryophyllene oxide (18.7%), β-caryophyllene (12.8%) and germacrene D (10.0%) were the major compounds of *M. vulgare* collected from another region of Iran. Hamedeyazdan *et al.* (2013) reported that the major constituents of the *Marrubium persicum* essential oil were m-tolualdehyde (19.2%) followed by acetophenone (14.6%), germacrene D (10.5%), β-caryophyllene (7.4%), β-farnesene (6.2%), and α-pinene (4.6%). In studies (Kadri *et al.,* 2011) indicated the major constituents obtained of *M.* vulgare the essential oil were γ-eudesmol (11.93%), β-citronellol (9.90%), citronellyl formate (9.50%) and germacrene D (9.37%). The aim of this study was to identify of the chemical components of (*Marrubium vulgare* L.) from Iran.

2. Materials and Methods

2.1. Plant material

The aerial parts of the plant samples of (*Marrubium vulgare* L.) were collected from (Kamu Mountain) Isfahan province. Kamu is a city in Qamsar district, Kashan County, Isfahan province, in center Iran (33°, 36´ N and 51°, 14´ E), during 2014. The samples of the plants were identified by regional floras and authors with floristic and taxonomic references, and voucher specimens were deposited at the Herbarium of Agriculture Researches Islamic Azad University, Isfahan (Khorasgan), Iran.

2.2. Essential oil extraction

The essential oils were extracted from 100 g of ground tissue in 1 L of water contained in a 2 L flask and heated by heating jacket at 100 °C for 3 h in a Clevenger-type apparatus, according to procedures outlined in the British Pharmacopoeia. The collected essential oil was dried over anhydrous sodium sulfate and stored at 4°C ± 1°C until analyzed.

2.3. GC/MS analysis

Compositions of the essential oils were determined by GC–MS. The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. HP-5MS column (30 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas with flow rate of 1.0 mL/min. The oven temperature was kept 20°C at 50°C for 4 min and programmed to 280°C at a rate of 5°C /min, and kept 20°C constant at 280°C for 5 min, at split mode. The injector temperature was at 20°C at 280°C. Transfer 20 line temperatures 280°C. MS were taken at 70 eV. Mass range was from *m/z* 35 to 450. Retention indices were calculated for all components using a homologous series of *n*-alkanes (C_5-C_{24}) injected under conditions used with the oil samples. Identification of the essential oil components was accomplished based on comparison of retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILLEY/ChemStation data system) (Adams, 2007).

3. Results and discussion

The chemical constituents identified by GC-MS, are presented in Table 1. GC–MS analysis resulted in identification of 44 constituents of the oil composition. Their sum constituted the bulk of the oils and ranged from 99.89% oil. The results indicated that the major components were β-caryophyllene (32.19%), (E)-β-farnesene (11.39%), 1,8-cineole (8.17%) and α-pinene (6.64%) (Fig 1).

The biosynthesis of secondary metabolites, although controlled genetically, is strongly affected by the environmental influences of a particular growing region, and also by the agronomic conditions, harvesting time and the type of processing. In addition, for maximum oil production, long days and high light intensities are required during the maturation period (Salehi *et al.,* 2014). Monoterpenes are a large and diverse class of volatile C_{10} isoprenoids that are the major constituents of many plant essential oils and resins. These natural products play important chemo-ecological roles in the interactions of plants with their environments (Hallahan, 2000). Previous works from different countries showed that yields of *M. vulgare*

dried aerial parts EO varied depending on many factors such as climatic, seasonal and geographic conditions; it's about 0.07% for Slovakia (Nagy and Svjdlenka, 1998), 0.08% for Iran (Khanavi *et al*., 2006).

Table 2. Chemical composition of essential oils of *Marrubium vulgare* L.

Row	Compound	RI	%
1	*trans*-2-Hexanal	823	0.77
2	Heptanal	901	4.26
3	α-Thujene	931	0.22
4	α-Pinene	937	6.64
5	Camphene	945	0.36
6	Benzaldeyde	965	0.19
7	p-Cymene	1025	4.76
8	1,8-Cineole	1035	8.17
9	γ-Terpinene	1063	2.62
10	linalool	1092	0.43
11	γ-Terpineol	1189	1.39
12	Decanal	1209	0.95
13	Carvone	1219	0.64
14	Piperitone	1225	2.11
15	Eugenol	1355	2.91
16	α-Copaene	1378	0.51
17	β-Cubebene	1382	1.08
18	β-Caryophyllene	1417	32.19
19	Geranyl linalool	1439	2.58
20	(E)-β-Farnesene	1443	11.39
21	α-Humulene	1454	1.59
22	Alloaromadendrene	1474	0.97
23	Germacrene D	1479	0.41
24	β-Ionone	1482	1.16
25	ß-Guaiene	1491	0.92
26	α-Farnesene	1499	0.31
27	α-Muurolene	1503	0.23
28	β-Bisabolene	1507	0.81
29	*trans*–calamenene	1510	0.64
30	δ–Cadinene	1526	0.36
31	α-Calacorene	1529	0.37
32	Spathulenol	1573	0.24
33	Caryophyllene oxide	1579	4.06
34	Viridiflorol	1592	0.28
35	1,10-di-*epi*-cubenol	1616	0.17
36	*Epi*-α-cadinol	1643	0.25
37	β-Eudesmol	1652	0.19
38	α-Cadinol	1657	0.27
39	β-Cubebene	1674	0.18
40	Citronellylbutanoate	1682	0.39
41	α-Bisabolol	1691	0.19
42	Geranyltiglate	1712	0.18
43	Benzyl benzoate	1762	1.08
44	Cyclononasiloxane	2195	0.47
	Total		99.89

RI: Retention indices determined on HP-5MS capillary column.

Said-Al Ahl *et al*. (2015) reported that the major constituents of the *Marrubium vulgare* essential oil cultivated in Egypt were carvacrol (36.28%), β-phellandrene (15.49%), carvyl acetate (11.52%), transcaryophyllene (4.06%), linalool (3.86%), α-terpinene (3.83%), β-pinene (3.53%), trans-sabinene hydrate (3.29%), β-thujone (2.93%), 1-octen-3-ol (2.48%), 1,8-cineol (1.49%), α-Pinene (1.44%) and borneol (1.12%). Zawislak, (2012) reports that the main components of the oil of *Marrubiumvulgare* L. were E-caryophyllene (25.91–32.06%), germacrene D (20.23–31.14%) and δ-amorphene (8.38–10.22%), while in the oil of *Marrubium incanum* Desr. the following compounds were germacrene D (32.46–37.87%), E-caryophyllene (22.49–30.79%) and α-cadinol (14.36–17.87%). Morteza-Semnani and Saeedi (2004) reported that the major constituents of the essential oil of *Marrubium astracanicum* from Iran were β-bisabolene (20.4%), 8-cadinene (19.1%) and isocaryophyllene (14.1%).

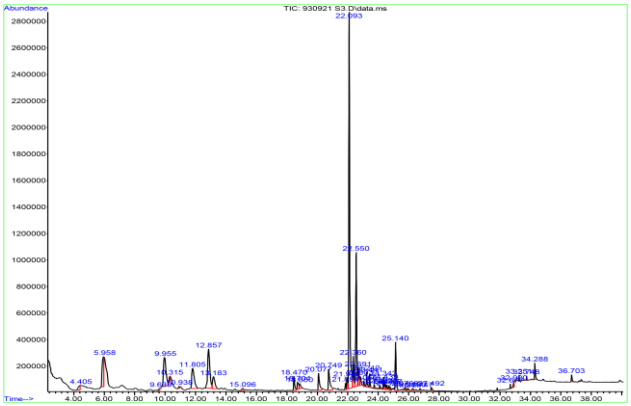

Fig 1. TIC of the essential oil from *Marrubium vulgare* L.

About the chemical composition of *M. vulgare*from different parts of the world, Saleh and Glombitza (1989) reported tricyclene, β-pinene, bisabolol, β-elemone and isomenthon-8-thiol as the main compounds of *M. vulgare*. From Libya, EL-Hawary *et al.* (2013) reported the main components of the oil of *M. vulgare*werecarvacrol, E-β-farnesene and thymol. In Algeria,Abadiand Hassani (2013) reported the main components of the oil of *M. vulgare*were 4,8,12,16-tetramethyl heptadecan-4-olid (16.97 %), germacrene D-4-ol (9.61%), α- pinene (9.37 %), phytol (4.87%), dehydro-sabina ketone (4.12 %), piperitone (3.27%), δ-cadinene (3.13%), 1-octen-3-ol (2.35%) and benzaldehyde (2.31%).

In Tunisian, Hamdaoui *et al.* (2013) reported the main components of the oil of *M. vulgare*were β-bisabolene (28.3%), (*E*)-β-farnesene (7.4%) and β-caryophyllene (7.8%). In Egypt, Salama *et al.* (2012) reported the main components of the oil of *M. vulgare* were thymol and *γ*-cadineneas. Nagy and Svajdlenka (1998) found that the main constituent of *M. vulgare* from Slovakia were β-caryophyllene (45.8%) and germacrene-D (14.4%). Weel *et al.* (1999) reported that (Z)-β-farnesene, β-caryophyllene, (E)-2-hexenal, α-humulene and germacrene-D were the main components of *M. vulgare* growing in Lithuania.

4. Conclusions

In conclusion, the results obtained in the study indicated that the major components of the oil of *Marrubium vulgare* L. collected from (Kamu Mountain) Isfahan provincewere β-caryophyllene, (E)-β-farnesene, 1,8-cineole and α-pinene. A comparison of our results with different reports, differences in the volatile composition of the plants could be attributed to genetic (genus, species, and ecotype), chemotype, distinct environmental and climatic conditions, seasonal sampling periods, geographic origins, plant populations, vegetative plant phases, and extraction and quantification methods.

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