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Analysis of the Essential Oil of *Marrubium crassidens* Bioos. and *M. astracanicum* Jacq.

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Abstract: The volatile composition of two *Marrubium* species has been studied. The investigated taxa are *Marrubium crassidens* Bioss. and *Marrubium astracanicum* Jacp. (Labiatae), which are native in Iran. The essential oils were obtained by hydrodistillation in a modified Clevenger-type apparatus and their analysis were performed by GC and GC-MS. Twenty-two components in the oil of *M. crassidens* representing 90.5% of the total oil and 24 components in the oil of *M. astracanicum*, representing 91.9% of the total oil, were identified. Both essential oils were characterizied by a high amount of sesqiterpens with germacrene D (14.2%), bicyclogermacrene (14.2%), β-caryophyllene (29.0%) and spathulenol (5.6%) as the major constituents of *M. crassidens* and germacrene D (23.4%), α-humulene (33.7%), bicyclogermacrene (11.9%) and spathulenol (6.8%) as the major components of *M. astracanicum*. Some differences in the essential oil of *Marrubium crassidens* and *Marrubium astracanicum*, growing under different environmental conditions, have been found.

Key words: *Marrubium*, Labiatae, essential oil, bicyclogermacrene, germacrene D, α-humulene

INTRODUCTION

The family Labiatae is composed of more than 240 genera, including *Marrubium* genus that comprises approximately 30 species considered oil-poor species. Ten species of the genus *Marrubium* (Labiatae) are found in Iran (Mozaffarian, 1996; Rechinger, 1982) including *M. crassidens* and *M. astracanicum*. Phytochemical evaluation of the *Marrubium* has shown that it is rich in Flavonoids, phenylpropanoids, diterpens, amino acids and saponoids (Rigano *et al*., 2007; Hayet *et al*., 2007; Nakano and Kanal, 1995; Kurbatova *et al*., 2003). Many studies have been shown various properties of this genus, such as hypoglycemic effect, anti- Schistosoma, antioxidant, calcium channel blocker, vasorelaxant, tonic, abortifacient, hypoglycemic, hypertensive, antimicrobial and cytotoxic activity (Khanavi *et al*., 2005; Madari and Jacobs, 2004; Hajhashemi *et al*., 2000; Rigano *et al*., 2007; Hayet *et al*., 2007; Sahpaz *et al*., 2002; El Bardai *et al*., 2003, 2001). The chemical composition of essential oils depends on climatic, seasonal and geographic conditions, harvest period. Many research report on the essential oil composition of *M. parviflorum* (Khanavi *et al*., 2005), *M. vulgare* (Khanavi *et al*., 2005; Nagy and Svajdlenka, 1998), *M. cuneatum* (Baher *et al*., 2004), *M. velutinum* and *M. peregrinum* (Lazari *et al*., 1999; Nagy and Svajdlenka, 1998) and *M. astracanicum* (Morteza-Semnani and Saeedi, 2004; Baher and Mirza, 2003), whereas the oil of *M. crassidens* has never been studied before. Especially *M. crassidens* is endemic in Iran and so it importance for the study. In the present study, we have investigated the oil composition of two *Marrubium* species which grow in different regions of Iran province.

MATERIALS AND METHODS

Plant material: The aerial part of *M. crassidens* was collected from Kohpaye, in Kerman Province, Iran, at an altitude of 2700 m, in May 2007 during the flowering stage and the flowering aerial part of *M. astracanicum* was collected from Alboorz mountain (between Firouzkouh and Veresk, in Tehran Province, Iran, at an altitude 1800 m in June 2007. Voucher specimens have been deposited at the Herbarium of the Faculty of Sciences, Islamic Azad University of Research and Sciences Unit, Tehran, Iran.

Isolation of the essential oil: Five hundred gram of air-dried flowering aerial parts of both species were subjected to hydrodistillation using a Clevenger-type apparatus for 4 h. The oils were dried over anhydrous Sodium Sulfate and submitted to GC and GC-MS analysis.

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Gas Chromatography (GC): GC analysis were performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 40°C min^{-1}. Injector and detector (FID) temperature were 260°C; helium was used gas with a linear velocity 32 cm sec^{-1}.

Gas chromatography-mass spectrometry: GC-MS analyses were carried out on a Varian 3400 system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d.); Oven temperature was 40 to 240°C at a rate of 4°C min^{-1}, transfer line temperature 260°C, injector temperature 250°C, carrier gas helium with a linear velocity of 31.5 cm sec^{-1}, split ratio 1/60, flow rate 1.1 mL min^{-1}, Ionization energy 70 eV; scan time 1 sec; mass range 40-350 amu.

Identification of components: The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature (Adams, 2001; Lawrence *et al*., 1988). Identification of some compounds by co-injection. The retention indices were calculated for all volatile constituents using a homologous series of n-alkenes.

RESULTS AND DISCUSSION

Table 1 shows the constituent of essential oil of *Marrubium crassidens* and *Marrubium astracanicum*. Oils were light yellow with a distinct sharp odor, in a yield of 0.26 and 0.19% (w/w) based on dry weight, for *M. crassidens* and *M. astracanicum*, respectively. Twenty-two components were detected in the oil of *M. crassidens*, representing 90.5% of the total oil. The major constituents were germacrene D (14.2%), bicyclogermacrene (14.2%), β-caryophyllene (29.0%) and spathulenol (5.6%). In the essential oil of *M. astracanicum*, 24 components were identified; representing 91.9% of the total oil, that germacrene D (23.4%), α-humulene (33.7%), bicyclogermacrene (11.9%) and spathulenol (6.8%) were the major components. Both samples contained different amounts of similar components (77.2% for *M. crassidens* oil and 83.5% for *M. astracanicum* oil, respectively). Germacrene D and bicyclogermacrene were identified as the major components of the oils. The oil of *M. crassidens* contained sesquiterpens hydrocarbon (74.0%), oxygenated sesquiterpens (13.4%) and monoterpens hydrocarbon (2.8%). The oil of *M. astracanicum* contained sesquiterpens hydrocarbon (72.3%), oxygenated sesquiterpens (13.4%) and monoterpens

Table 1: Chemical composition (%) of the oil of *Marrubium crassidens* and *M. astracanicum*

No.	Compound	RI	*M. crassidens* (%)	*M. astracanicum* (%)	Method of Identification
1	α-pinene	942	1.2	1.0	MS, RI
2	1-octene-3-ol	982	-	0.4	MS, RI
3	Myrcene	995	-	1.7	MS, RI
4	Limonene	1027	1.6	0.3	MS, RI
5	Pregeijerene	1286	0.3	1.0	MS, RI, CoI
6	α-cubebene	1358	-	0.4	MS, RI
7	Bicycloelemene	1380	1.0	-	MS, RI
8	Trans-β-damascenone	1384	-	1.1	MS, RI, CoI
9	α-copaene	1386	1.3	0.2	MS, RI
10	β-bourbonene	1388	1.4	0.4	MS, RI
11	β-elemene	1390	1.6	0.4	MS, RI
12	Longifolene	1412	-	0.2	MS, RI
13	β-caryophyllene	1422	29.0	0.9	MS, RI
14	α-bergamoten	1439	1.3	-	MS, RI
15	Aromadendrene	1448	-	0.2	MS, RI
16	α-humulene	1457	3.1	33.7	MS, R I, CoI
17	Germacrene D	1484	14.2	23.4	MS, RI
18	Ledenes	1490	2.9	-	MS, RI
19	Bicyclogermacrene	1508	14.2	11.9	MS, RI
20	β-bisabolene	1510	2.1	-	MS, RI
21	δ-cadinene	1521	1.9	0.6	MS, RI
22	Spathulenol	1576	5.6	6.8	MS, RI
23	Caryophyllene oxide	1485	4.6	-	MS, RI
24	Globulol	1586	-	1.3	MS, RI
25	Viridiflorol	1590	0.6	1.1	MS, RI, CoI
26	Isospathulenol	1620	0.6	0.8	MS, RI
27	T-muurolol	1643	-	1.3	MS, RI, CoI
28	α-cadinol	1656	0.4	-	MS, RI
29	T-cadinol	1662	1.0	-	MS, RI
30	Hexa hydro farnesyl acetone	1727	0.6	1.0	MS, RI, CoI
31	1,2-benzendicarboxylic acid	1760	-	1.8	MS, RI
Total			90.5	91.9	

MS: Mass spectroscopy, RI: Retention indices, CoI: Co-injection

hydrocarbon (3.0%). The total amounts of sesquiterpens in the oil of *M. crassidens* and *M. astracanicum* (87.4 and 85.7%, respectively) were higher than monoterpens (2.8 and 3.0%, respectively). In the essential oil of *M. astracanicum* growing in Behshahr (Mazandaran Province, North of Iran) (Morteza-Semnani and Saeedi, 2004) the major components were methylcyclopentane (15.5%), thymol (10.6%), n-heptane (7.4%) and in the plant collected from Damavand (Tehran Province, Iran), (Baher and Mirza, 2003) caryophyllene oxide (35.8%), citronellal (16.9%) and β-caryophyllene (13.1%) were the major compounds. These compounds were not identified in our study. Instead, α-humulene (33.7%) found in this study, as one of the major compounds of *M. astracanicum* was not mentioned in previous study on this species (Morteza-Semnani and Saeedi, 2004; Baher and Mirza, 2003).

CONCLUSIONS

The variations of oil components of *Marrubium* species growing in different provinces of Iran may be due to the collection time, drying conditions, extraction methods, chemotypes, geographic and climatic factors. In addition, in most oils of this genus, sesquiterpens were present in higher content than monoterpens (Bal *et al*., 1999; Nagy and Svajdlenka, 1998; Khanavi *et al*., 2005).

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