



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

Ahmad Reza Golparvar^{1*}, Amin Hadipanah², Ali Mehras Mehrabi³, Arezoo Armin¹

¹Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran;

*Email: dragolparvar@gmail.com

²Department of Horticultural, Science and Research Branch, Islamic Azad University, Tehran, Iran;

³Department of Plant Breeding, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran;

ARTICLE INFO

Type: Short Communication

Topic: Phytochemistry

Received November 6th 2014

Accepted January 5th 2015

Key words:

- ✓ *Marrubium vulgare* L.
- ✓ Chemical constituents
- ✓ GC/MS

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 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 Bardaiet 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 \pm 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 μ m 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 (C₅-C₂₄) 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 C₁₀ isoprenoids that are the major constituents of many plant essential oils and resins. These natural products play important chem-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 | <i>trans</i> -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 | Benzaldehyde | 965 | 0.19 |
| 7 | <i>p</i> -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 | β -Guaiene | 1491 | 0.92 |
| 26 | α -Farnesene | 1499 | 0.31 |
| 27 | α -Muulolene | 1503 | 0.23 |
| 28 | β -Bisabolene | 1507 | 0.81 |
| 29 | <i>trans</i> -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- <i>epi</i> -cubenol | 1616 | 0.17 |
| 36 | <i>Epi</i> - α -cadinol | 1643 | 0.25 |
| 37 | β -Eudesmol | 1652 | 0.19 |
| 38 | α -Cadinol | 1657 | 0.27 |
| 39 | β -Cubebene | 1674 | 0.18 |
| 40 | Citronellybutanoate | 1682 | 0.39 |
| 41 | α -Bisabolol | 1691 | 0.19 |
| 42 | Geranylthiglate | 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%).

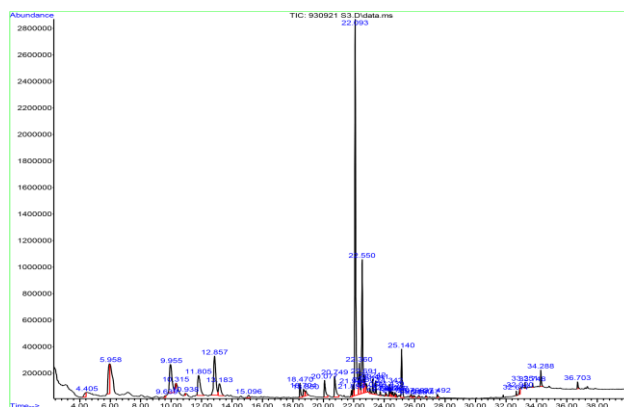


Fig 1. TIC of the essential oil from *Marrubium vulgare* L.

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* were carvacrol, E- β -farnesene and thymol. In Algeria, Abadi and Hassani (2013) reported the main components of the oil of *M. vulgare* were 4,8,12,16-tetramethyl heptadecan-4-ol (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 -cadinene. Nagy and Svajdenka (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 province were 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.

5. Acknowledgement

This research project has been supported by Islamic Azad University, Isfahan (Khorasgan) branch, Isfahan, Iran.

6. References

- Abadi, A. and Hassani, A. 2013. Essential oil composition and antioxidant activity of *Marrubium vulgare* L. growing wild in Eastern Algeria. *International Letters of Chemistry, Physics and Astronomy.*, 9(1): 17-24.
- Abedi, R., Golparvar, A.R. and Hadipanah, A. 2015. Identification of the essential oils composition from four ecotypes of *Mentha longifolia* (L.) Huds. growing wild in Isfahan province, Iran. *Journal of BioScience and Biotechnology.*, 4(2): 117-121.
- Adams, R.P. 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th edition (Allured Publishing Corporation, Carol Stream, IL) 456.
- Asadipour, A., Mehrabani, M., Nazeri, V. and Tabarraii, M. 2005. Composition of the essential oil of *Marrubium vulgare* L. *Ulum-i-Daroei.*, 2: 77-82.
- De Jesus, R.A.P., Cechinel-Filho, V., Oliveira, A.E. and Schlemper, V. 2000. Analysis of the antinociceptive properties of marrubiin isolated from *Marrubium vulgare*. *Phytomedicine.*, 7: 111-115.
- El Bardai, S., Morel, N., Wibo, M., Fabre, N., Llabres, G., Lyoussi, B. and Quetin-Leclercq, J. 2003. The vasorelaxant activity of marrubenol and marrubiin from *Marrubium vulgare*. *Planta Med.*, 69: 75-77.
- EL-Hawary, S., EL-Shabrawy, A., Ezzat, S. and ELShibany, F. 2013. Gas chromatography-mass spectrometry analysis, hepatoprotective and antioxidant activities of the essential oils of four Libyan herbs. *Journal of Medicinal Plants Research.*, 7(24): 1746-1753.
- Golparvar, A.R., Hadipanah, A. and Mehrabi, A.M. 2015. Diversity in chemical composition from two ecotypes of (*Mentha longifolia* L.) and (*Mentha spicata* L.) in Iran climatic conditions. *Journal of Biodiversity and Environmental Sciences.*, 6(4): 26-33.
- Hallahan, D.L. 2000. Monoterpenoid biosynthesis in glandular trichomes of labiate plants. *Advance Botanical Research.*, 31: 77-120.
- Hamdaoui, B., AidiWannes, W., Marrakchi, M., Ben Brahim, N. and Marzouk, B. 2013. Essential Oil Composition of Two Tunisian Horehound Species: *Marrubium vulgare* L. and *Marrubium aschersonii* Magnus. *Journal of Essential Oil Bearing Plants.*, 16(5): 608-612.
- Hamedeyazdan, S., Fathiazad, F. and Asnaashari, S. 2013. Chemical Composition of the Essential Oil from *Marrubium persicum* C. A. Mey. (Lamiaceae). *Pharmaceutical sciences.*, 19(2): 35-38.
- Hellen, K., Stulzer, H.K., Tagliari, M.P., Zampirolo, J.A., Cechinel-Filho, V. and Schlemper, V. 2006. Antioedematogenic effect of marrubiin obtained from *Marrubium vulgare*. *J. Ethnopharmacol.*, 108: 379-384.
- Kadri, A., Zarai, Z., Bekir, A., Gharsallah, N., Damak, M. and Gdoura, R. 2011. Chemical composition and antioxidant activity of *Marrubium vulgare* L.

- essential oil from Tunisia. *African Journal of Biotechnology*, 10(19): 3908-3914.
- Karioti, A., Skopeliti, M., Tsitsilonis, O., Heilmann, J. and Skaltsa, H. 2007. Cytotoxicity and immunomodulating characteristics of labdanediterpenes from *Marrubium cylleneum* and *Marrubiumvelutinum*. *Phytochemistry*, 68, 1587–1594.
- Khanavi, M., Ghasemian, L., Motlagh, E.H., Hadjiakhoondi, A. and Shafiee, A. 2006. Chemical composition of the essential oils of *Marrubium parvi florum* Fisch. & C. A. Mey. And *Marrubium vulgare*L. from Iran. *Flav. Fragr. J.*, 20: 324-326.
- Meyre-Silva, C., Yunes, R.A., Schlemper, V., Campos-Buzzi, F. and Cechinel-Filho, V. 2005. Analgesic potential of marrubiin derivatives, a bioactive diterpene present in *Marrubium vulgare* (Lamiaceae). *Farmacol.*, 60: 321–326.
- Mnonopi, N., Levendal, R.A., Davies-Coleman, R.T. and Frost, C.L. 2011. The cardioprotective effects of marrubiin, a diterpenoid found in *Leonotis leonurus* extracts. *J. Ethnopharmacol.*, 138: 67–75.
- Mnonopi, N., Levendal, R.A., Mzilikezi, N. and Frost, C.L. 2012. Marrubiin, a constituent of *Leonotis leonurus*, alleviates diabetic symptoms. *Phytomedicine*, 19: 488–493.
- Morteza-Semnani, K. and Saeedi, M. 2004. The Essential Oil Composition of *Marrubium astracanicum* Jacq. From Iran. *J. Essent. Oil Bearing Plants*, 7: 239-242.
- Mozaffarian, V. 2008. A pictorial dictionary of botany botanical taxonomy Latin-English-French-Germany-Persian. Germany: Koeltz Scientific Books. 522.
- Nagy, M. and Svajdlenka, E. 1998. Comparison of Essential Oils from *Marrubium vulgare* L. and *M. peregrinum* L. *J. Essent. Oil Res.*, 10:585-587.
- Paula de Olivera, A., Santin, J.R., Lemos, M., Klein, L.C.J., Couto, A.G., Bittencourt, C.M.S., Cechinel, F. and Valdir, F.A. 2011. Gastroprotective activity of methanol extract and marrubiin obtained from leaves of *Marrubium vulgare* L. (Lamiaceae). *J. Pharm. Pharmacol.*, 63: 1230–1237.
- Said-Al Ahl, H.A.H., Gendy, A.S.H., Mahmoud, A.A. and Mohamed, H.F.Y. 2015. Essential Oil Composition of *Marrubiumvulgare* L. Cultivated in Egypt. *International Journal of Plant Research*, 1 (4): 138-141.
- Salama, M.M., Taher, E. E. and El-Bahy, M.M. 2012. Molluscicidal and mosquitocidal activities of the essential oils of *Thymus capitatus* HOFF. ET LINK. And *Marrubium vulgare* L. *Rev. Inst. Med. Trop. Sao Paulo*, 54(5): 281-286.
- Saleh, M.M. and Glombitza, K.W. 1989. Volatile Oil of *Marrubium vulgare* and its Anti-schistosomal Activity. *Planta Med.*, 55:105-108.
- Salehi, S., Golparvar, A.R. and Hadipanah, A. 2014. Effect of harvest time on yield and quality of *Thymus vulgaris* L. essential oil in Isfahan province, Iran. *Agriculturae Conspectus Scientificus*, 79(2): 115-118.
- Weel, K.C.G., Venskutonis, P.R., Pukalskas, A., Gruzdiene, D. and Linssen, J.P.H. 1999. Antioxidant activity of horehound (*Marrubium vulgare*) grown in Lithuania. *Fett/Lipid.*, 101(10): 395-400.
- Zawislak, G. 2012. Chemical composition of essential oils of *Marrubium vulgare* L. and *Marrubium incanum* Desr. grown in Poland. *Chemija*, 23(2): 136–140.

