ISSN: 1304-7981 Number: 6, Year: 2014, Pages: 44-50 http://jnrs.gop.edu.tr

Gaziosmanpasa University
Graduate School of Natural and Applied Sciences
Journal of New Results in Science

Received: 06.06.2014
Accepted: 29.06.2014
Editors-in-Chief: Naim Çağman
Area Editor: Yakup Budak

Chemical Composition of Essential Oil from *Marrubium Vulgare* L. Leaves

Burcu Bayir[a] (burcu_bilge_garip@hotmail.com)
Hatice Gündüz[a] (haticegunduzq@gmail.com)
Tuba Usta[a] (t.usta1041@gop.edu.tr)
Esma Şahin[a] (esma_sahinkimyager@hotmail.com)
Zeynep Özdemir[a] (z.ozdemir1034@gop.edu.tr)
Ömer Kayır[a] (mkkimyager89@gmail.com)
Özkan Şen[a] (ozkan.sen@gop.edu.tr)
Hüseyin Akşit[a] (huseyin.aksit@gop.edu.tr)
Mahfuz Elmastaş[a] (mahfuz.elmastas@gop.edu.tr)
Ramazan Erenler[*a] (ramazan.erenler@gop.edu.tr)

[a]*Department of Chemistry, Faculty of Art and Science, Gaziosmanpasa University, 60240 Tokat, Turkey*

Abstract – The essential oils are significant for pharmaceutical, food and cosmetic industries. *Marrubium vulgare* L. has been used as a traditional medicine to treat the various illnesses. The chemical composition of the essential oil from leaves of *Marrubium vulgare* L. was obtained by steam distillation using the Clevenger apparatus. The oil was analyzed by gas chromatography and mass spectrometry (GC-MS). The main constituent of the oil was α-pinene (28.85%).

***Keywords* –** α-Pinene, *Marrubium vulgare* L., *essential oil*

1. Introduction

Many compounds, having significant variety of chemical structures and displaying biological activities have been provided by plants. About 80% of drugs either natural products or inspired by a natural compounds [1].

Marrubium genus, belonging to the Lamiaceae family, known as horehound or hoarhound, has 30 species distributed Europe, North Africa and Asia [2]. Photochemical investigation of the *Marrubium* species revealed the isolation and identification of labdane diterpene [2-5], monoterpene [6], flavonoids [7-10]. *Marrubium.* genus displays a large variety of

[*] Corresponding Author

biological activities including antimicrobial [11, 12], cardioprotective [13], antitumor [14, 15], immunomodulatory [16], antioxidant [17-19], anti-diabetic [20], antiprotozoal [21], gastroprotective [22], antiprotozoal [23], antiviral [24], antibacterial [25], antihepatotoxic [6], vasorelaxant [26], antioedematogenic [27], insecticidal [28], antihypertensive activities [29]. Essential oils of *Marrubium* species have also antibacterial, antifungal and cytotoxic properties [30], as well as exhibiting antioxidant and antimicrobial activities [31-33].

Marrubium vulgare L. (White horehound), a perennial herb is used as a traditional medicine to treat bronchitis, coughs and colds. The leaves and flowering stems are used as diuretic, antispasmodic, antiseptic, antidiabetic [20].

Essential oils (EOs) are volatile, aromatic oily liquids; natural products with terpene structure described by an intense smell and are constituted by aromatic plants as secondary metabolites. In nature, EOs play a significant function in the protection of the plants as antibacterials, antivirals, antifungals, insecticides and also against herbivores by reducing their desire for such plants [34]. Diversity in the chemical contents of essential oils have been attributed to many factors, such as environment, abiotic stress, genetic heritance and the phenological stages of the plants [35]. The main components of essential oil achieved from the Tunisian *Marrubium vulgare* L. were β-caryophyllene (7.8 %), (E)-β-farnesene (7.4 %) and 1,8-cineole (4.8 %) [36]. Another work carried out on the plant material grown in Poland revealed that the main compound of the essential oil was caryophyllene (44.54%), bicyclogermacrene (20.6%) and alpha-humulene (5.79%) [37].

The aim of this research is to present the chemical profile of essential oil of *Marrubium vulgare* L. leaves.

2. Material and Methods

Plant Materials

Marrubium vulgare L. was collected from Almus-Tokat and identified by Prof.Dr. İsa Telci, Süleyman Demirel University, Faculty of Agriculture, Isparta, Turkey.

Isolation of essential oil

The *Marrubium vulgare* L. leaves (70 g) and water (250 ml) were placed in a flask and steam distillation process was applied using the Clevenger apparatus for 3h. The essential oil sample was separated (90 mg), dried over sodium sulphate and stored at the fridge until usage.

GC analysis

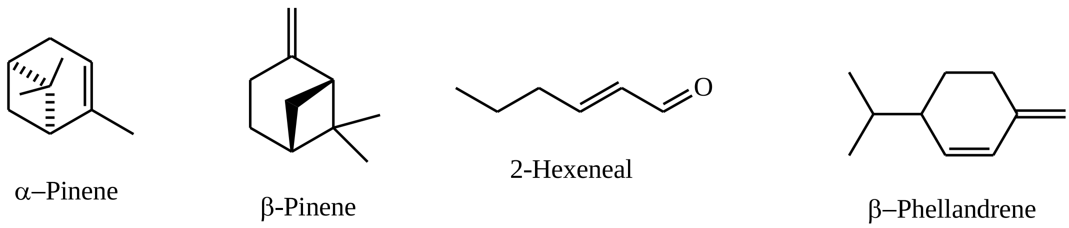
GC analyses of essential oil were performed on Perkin-Elmer Clarus 500 model Autosystem GC. Acetone was used for dilation of oil (1:10) then injected in the HT-5 column (25 m×0.22 mm×0.1μm film). The column temperature was programmed from 50 to 120 °C at 3 °C/min, 120 to 220 °C 5 °C/min with initial and final temperatures held for 0,64 min (totally 44 min). Helium was used as carrier gas at 5 psi inlet pressure. The temperature was 250 °C for both injector and detector (FID). Diluted samples (1.0 μL) were injected in the split/splitless (50:1 split) mode.

GC–MS analysis

GC/MS analyses were performed on Perkin-Elmer mass spectrometer using BPX5 column (30 m×0.25 mm×0.25µm film). An electron ionization system with ionization energy of 70 eV was used for GC–MS detection. The carrier gas was helium with a flow rate of 1.3 ml/min. Injector and MS transfer line temperatures were set at 230 °C and 250 °C, respectively. The oven temperature was the same as with GC analysis. Diluted samples (1/10 in acetone, v/v) of 1.0µL were injected in the split/splitless (5:1 split) mode.

3. Results and Discussion

The essential oil of *Marrubium vulgare* L. was produced by hydrodistillation using Clevenger apparatus and chemical constituents were determined by gas chromatography and mass spectrometry (GC-MS). The major constituents of *M. vulgare* leaves essential oil were identified as α-Pinene (28.85%), β-Pinene (18.31%), β-Phellandrene (17.40%), 2-hexenal (14.80%) (Table 1). The chemical structures of main constituents are given in Scheme 1.



Scheme 1. Chemical structures of main constituents of essential oil from *Marrubium vulgare* L. leaves

In comparison of these values with the same species (*M. vulgare* L.) grown in different countries revealed that the essential oil constituents are rather different, this has to do the environmental effects, stress conditions and heritance. For instance, *M. vulgare* L. essential oil grown in Tunisia has a potent antimicrobial, antifungal and anticancer activities. The main components of this oil were γ-eudesmol (11.93%), germacrene-D (9.37), citronellyl formate (9.50), β-citronellol (9.90%) [30]. α-Pinene and β-Pinene, main constituents of essential oil of *M. vulgare* L. have pharmaceutical and medicinal properties [38]. Total synthesis of α-Pinene exhibiting the antimicrobial activity was achieved [39]. A research revealed that the main constituents of α-Pinene and β-Pinene of essential oil from *Ferula microcolea* exhibited the antioxidant activity [40]. An essential oil exhibited the antibacterial activities against food spoilage pathogens included the α-Pinene and β-Pinene as major compounds. Xu and coworker presented the double effects of α-pinene on turpentine beetle, *Dendroctonus valens:* α-pinene inhibited the feeding activities of bark beetle and the bark beetle exploited it to produce pheromones [41].

Table 1. Chemical composition of the *Marrubium vulgare* L. essential oil analyzed by gas chromatography-mass spectrometry

Compounds	Rt (min)	Oil composition (%)
2-Hexenal	3,98	14,80
α-Phellandrene	4,27	1,55
α-Pinene	4,51	28,85
β-Phellandrene	5,56	17,40
β-Pinene	5,67	18,31
β- myrcene	5,82	5,07
α-limonen	7,24	3,88
Sabinen	8,33	0,49
caryophyllene	23,37	3,86
β-Farnesene	24,11	0,88
Germacrene	25,92	1,05

As a consequence, *Marrubium vulgare* L. grown in Tokat has the essential oil containing biologically important compounds.

4. References

[1] Newman, D.J.; Cragg, G.M., Natural products as sources of new drugs over the last 25 years. J Nat Prod, 2007, 70, (3), 461-477.

[2] Argyropoulou, C.; Karioti, A.; Skaltsa, H., Labdane diterpenes from Marrubium thessalum. Phytochemistry, 2009, 70, (5), 635-640.

[3] Argyropoulou, C.; Karioti, A.; Skaltsa, H., Minor Labdane Diterpenes from Marrubium thessalum. Chem Biodivers, 2011, 8, (10), 1880-1890.

[4] Dendougui, H.; Seghir, S.; Belloum, Z.; Benayache, F.; Leon, F.; Brouard, I.; Bermejo, J.; Benayache, S., A New Labdane Diterpene and Other Constituents from Marrubium deserti Noe ex coss. Records of Natural Products, 2011, 5, (4), 300-304.

[5] Piccoli, P.N.; Bottini, R., Accumulation of the labdane diterpene Marrubiin in glandular trichome cells along the ontogeny of Marrubium vulgare plants. Plant Growth Regulation, 2008, 56, (1), 71-76.

[6] Ahmed, B.; Masoodi, M.H.; Siddique, A.H.; Khan, S., A new monoterpene acid from Marrubium vulgare with potential antihepatotoxic activity. Natural Product Research, 2010, 24, (18), 1671-1680.

[7] Haid, S.; Novodomska, A.; Gentzsch, J.; Grethe, C.; Geuenich, S.; Bankwitz, D.; Chhatwal, P.; Jannack, B.; Hennebelle, T.; Bailleul, F.; Keppler, O.T.; Ponisch, M.; Bartenschlager, R.; Hernandez, C.; Lemasson, M.; Rosenberg, A.; Wong-Staal, F.; Davioud-Charvet, E.; Pietschmann, T., A Plant-Derived Flavonoid Inhibits Entry of All HCV Genotypes Into Human Hepatocytes. Gastroenterology, 2012, 143, (1), 213-U772.

[8] Pukalskas, A.; Venskutonis, P.R.; Salido, S.; de Waard, P.; van Beek, T.A., Isolation, identification and activity of natural antioxidants from horehound (Marrubium vulgare L.) cultivated in Lithuania. Food Chemistry, 2012, 130, (3), 695-701.

[9] Hussain, A.; Perveen, S.; Malik, A.; Afza, N.; Iqbal, L.; Tareen, R.B., Urease Inhibitiory Flavone Glucosides from Marrubium anisodon. Polish Journal of Chemistry, 2009, 83, (7), 1329-1335.
[10] Hennebelle, T.; Sahpaz, S.; Skaltsounis, A.L.; Bailleul, F., Phenolic compounds and diterpenoids from Marrubium peregrinum. Biochemical Systematics and Ecology, 2007, 35, (9), 624-626.
[11] Ortega-Ramirez, L.A.; Rodriguez-Garcia, I.; Leyva, J.M.; Cruz-Valenzuela, M.R.; Silva-Espinoza, B.A.; Gonzalez-Aguilar, G.A.; Siddiqui, M.W.; Ayala-Zavala, J.F., Potential of Medicinal Plants as Antimicrobial and Antioxidant Agents in Food Industry: A Hypothesis. Journal of Food Science, 2014, 79, (2), R129-R137.
[12] Robles-Zepeda, R.E.; Velazquez-Contreras, C.A.; Garibay-Escobar, A.; Galvez-Ruiz, J.C.; Ruiz-Bustos, E., Antimicrobial Activity of Northwestern Mexican Plants Against Helicobacter pylori. Journal of Medicinal Food, 2011, 14, (10), 1280-1283.
[13] Yousefi, K.; Soraya, H.; Fathiazad, F.; Khorrami, A.; Hamedeyazdan, S.; Maleki-Dizaji, N.; Garjani, A., Cardioprotective effect of methanolic extract of Marrubium vulgare L. on isoproterenol-induced acute myocardial infarction in rats. Indian Journal of Experimental Biology, 2013, 51, (8), 653-660.
[14] Yildirim, A.B.; Karakas, F.P.; Turker, A.U., In vitro antibacterial and antitumor activities of some medicinal plant extracts, growing in Turkey. Asian Pacific Journal of Tropical Medicine, 2013, 6, (8), 616-624.
[15] Hamedeyazdan, S.; Fathiazad, F.; Sharifi, S.; Nazemiyeh, H., Antiproliferative Activity of Marrubium persicum Extract in the MCF-7 Human Breast Cancer Cell Line. Asian Pacific Journal of Cancer Prevention, 2012, 13, (11), 5843-5848.
[16] Daoudi, A.; Aarab, L.; Abdel-Sattar, E., Screening of immunomodulatory activity of total and protein extracts of some Moroccan medicinal plants. Toxicology and Industrial Health, 2013, 29, (3), 245-253.
[17] Edziri, H.; Mastouri, M.; Aouni, M.; Verschaeve, L., Polyphenols content, antioxidant and antiviral activities of leaf extracts of Marrubium deserti growing in Tunisia. South African Journal of Botany, 2012, 80, 104-109.
[18] Yumrutas, O.; Saygideger, S.D., Determination of in vitro antioxidant activities of different extracts of Marrubium parviflorum Fish et Mey. and Lamium amplexicaule L. from South east of Turkey. Journal of Medicinal Plants Research, 2010, 4, (20), 2164-2172.
[19] Matkowski, A.; Piotrowska, M., Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae. Fitoterapia, 2006, 77, (5), 346-353.
[20] Boudjelal, A.; Henchiri, C.; Siracusa, L.; Sari, M.; Ruberto, G., Compositional analysis and in vivo anti-diabetic activity of wild Algerian Marrubium vulgare L. infusion. Fitoterapia, 2012, 83, (2), 286-292.
[21] Kirmizibekmez, H.; Atay, I.; Kaiser, M.; Yesilada, E.; Tasdemir, D., In vitro Antiprotozoal Activity of Extracts of five Turkish Lamiaceae Species. Natural Product Communications, 2011, 6, (11), 1697-1700.
[22] de Oliveira, A.P.; Santin, J.R.; Lemos, M.; Klein, L.C.; Couto, A.G.; Bittencourt, C.M.D.; Cechinel, V.; de Andrade, S.F., Gastroprotective activity of methanol extract and marrubiin obtained from leaves of Marrubium vulgare L. (Lamiaceae). Journal of Pharmacy and Pharmacology, 2011, 63, (9), 1230-1237.
[23] Kirmizibekmez, H.; Atay, I.; Kaiser, M.; Yesilada, E.; Tasdemir, D., In vitro antiprotozoal activity of organic and aqueous extracts of several Turkish Lamiaceae species. Planta Medica, 2011, 77, (12), 1426-1426.

[24] Edziri, H.; Mastouri, M.; Mahjoub, M.A.; Ammar, S.; Mighri, Z.; Gutmann, L.; Aouni, M., Antiviral activity of leaves extracts of Marrubium alysson L. Journal of Medicinal Plants Research, 2011, 5, (3), 360-363.

[25] Ulukanli, Z.; Akkaya, A., Antibacterial Activities of Marrubium catariifolium and Phlomis pungens Var. Hirta Grown Wild in Eastern Anatolia, Turkey. International Journal of Agriculture and Biology, 2011, 13, (1), 105-109.

[26] Baccelli, C.; Navarro, I.; Block, S.; Abad, A.; Morel, N.; Quetin-Leclercq, J., Vasorelaxant activity of diterpenes from Croton zambesicus and synthetic trachylobanes and their structure-activity relationships. Journal of Natural Products, 2007, 70, (6), 910-917.

[27] Stulzer, H.K.; Tagliari, M.P.; Zampirolo, J.A.; Cechinel, V.; Schlemper, V., Antioedematogenic effect of marrubiin obtained from Marrubium vulgare. Journal of Ethnopharmacology, 2006, 108, (3), 379-384.

[28] Pavela, R., Insecticidal activity of certain medicinal plants. Fitoterapia, 2004, 75, (7-8), 745-749.

[29] El Bardai, S.; Lyoussi, B.; Wibo, M.; Morel, N., Comparative study of the antihypertensive activity of Marrubium vulgare and of the dihydropyridine calcium antagonist amlodipine in spontaneously hypertensive rat. Clinical and Experimental Hypertension, 2004, 26, (6), 465-474.

[30] Zarai, Z.; Kadri, A.; Ben Chobba, I.; Ben Mansour, R.; Bekir, A.; Mejdoub, H.; Gharsallah, N., The in-vitro evaluation of antibacterial, antifungal and cytotoxic properties of Marrubium vulgare L. essential oil grown in Tunisia. Lipids Health Dis, 2011, 10.

[31] Laouer, H.; Yabrir, B.; Djeridane, A.; Yousfi, M.; Beldovini, N.; Lamamra, M., Composition, Antioxidant and Antimicrobial Activities of the Essential Oil of Marrubium deserti. Natural Product Communications, 2009, 4, (8), 1133-1138.

[32] Sarikurkcu, C.; Tepe, B.; Daferera, D.; Polissiou, M.; Harmandar, M., Studies on the antioxidant activity of the essential oil and methanol extract of Marrubium globosum subsp globosum (lamiaceae) by three different chemical assays. Bioresource Technology, 2008, 99, (10), 4239-4246.

[33] Grassia, A.; Senatore, F.; Arnold, N.A.; Bruno, M.; Piozzi, F.; Rigano, D.; Formisano, C., Chemical composition and antimicrobial activity of the essential oils from aerial parts of two Marrubium sp (Lamiaceae) growing wild in Lebanon. Polish Journal of Chemistry, 2006, 80, (4), 623-628.

[34] Bakkali, F.; Averbeck, S.; Averbeck, D.; Waomar, M., Biological effects of essential oils - A review. Food and Chemical Toxicology, 2008, 46, (2), 446-475.

[35] Türkmen, N.; Öz, A.; Sönmez, A.; Erol, T.; Gülümser, D.; Yurdakul, B.; Kayır, Ö.; Elmastas, M.; Erenler, R., Chemical Composition of Essential Oil from Rosmarinus Officinalis L. Leaves. Journal of New Results in Science, 2014, (6), 27-31.

[36] Hamdaoui, B.; Wannes, W.A.; Marrakchi, M.; Ben Brahim, N.; Marzouk, B., Essential Oil Composition of Two Tunisian Horehound Species: Marrubium vulgare L. and Marrubium aschersonii Magnus. Journal of Essential Oil Bearing Plants, 2013, 16, (5), 608-612.

[37] Zawislak, G., The Chemical Composition of the Essential Oil of Marrubium Vulgare L. From Poland. Farmacia, 2012, 60, (2), 287-292.

[38] Rufino, A.T.; Ribeiro, M.; Judas, F.; Salgueiro, L.; Lopes, M.C.; Cavaleiro, C.; Mendes, A.F., Anti-inflammatory and Chondroprotective Activity of (+)-alpha-Pinene: Structural and Enantiomeric Selectivity. Journal of Natural Products, 2014, 77, (2), 264-269.

[39] Dhar, P.; Chan, P.; Cohen, D.T.; Khawam, F.; Gibbons, S.; Snyder-Leiby, T.; Dickstein, E.; Rai, P.K.; Watal, G., Synthesis, Antimicrobial Evaluation, and Structure-Activity Relationship of alpha-Pinene Derivatives. J Agr Food Chem, 2014, 62, (16), 3548-3552.

[40] Amiri, H., Chemical Composition and Antioxidant Activity of Essential Oil and Methanolic Extracts of Ferula Microcolea (Boiss.) Boiss (Apiaceae). Int J Food Prop, 2014, 17, (4), 722-730.

[41] Xu, B.B.; Liu, Z.D.; Sun, J.H., The effects of alpha-pinene on the feeding performance and pheromone production of Dendroctonus valens. Entomol Exp Appl, 2014, 150, (3), 269-278.