Journal of Essential Oil Bearing Plants

ISSN: 0972-060X (Print) 0976-5026 (Online) Journal homepage: http://www.tandfonline.com/loi/teop20

Composition and Antimicrobial Activities of Marrubium astracanicum Jacq. subsp. astracanicum Essential Oil

Ömer Kiliç & Fethi Ahmet Özdemir

To cite this article: Ömer Kiliç & Fethi Ahmet Özdemir (2017) Composition and Antimicrobial Activities of Marrubium astracanicum Jacq. subsp. astracanicum Essential Oil, Journal of Essential Oil Bearing Plants, 20:5, 1400-1406, DOI: 10.1080/0972060X.2017.1392898

To link to this article: https://doi.org/10.1080/0972060X.2017.1392898

Published online: 20 Dec 2017.

Taylor & Francis
Taylor & Francis Group

ISSN Print: 0972-060X
ISSN Online: 0976-5026

Composition and Antimicrobial Activities of *Marrubium astracanicum* Jacq. subsp. *astracanicum* Essential Oil

Ömer Kiliç [1]*, Fethi Ahmet Özdemir [2]

[1] Department of Park and Garden Plants, Technical Science Vocational College, Bingol University, Turkey
[2] Department of Molecular Biology and Genetics, Faculty of Science and Art, Bingol University, Turkey

Received 27 June 2017; accepted in revised form 01 October 2017

Abstract: The aerial parts of essential oils were obtained by hydrodistillation in a modified clevenger-type apparatus and their analysis were performed by HS-SPME. The essential oil aerial parts of *Marrubium astracanicum* subsp. *astracanicum* was investigated. Thirty compounds were identified representing 85.5 % of the total components in the oils, respectively. β-caryophyllene (23.5 %), germacrene D (10.6 %), spathulenol (9.4 %) and bicyclogermacrene (9.3 %) were identified as major components of *M. astracanicum* subsp. *astracanicum*. The disc diffusion method was used to study the antibacterial activity of *M. astracanicum* subsp. *astracanicum* essential oil against sixteen bacteria strains. Zone diameter of inhibition from 6 to 13 mm for Gram (+) bacteria. Whereas, for Gram (-) bacteria strains have not shown the response to the essential oil action. In conclusion, this study results might be helpful chemotaxonomy, antimicrobial activity and potential usefulness of *Marrubium* taxa. Further research is required to evaluate the practical values of therapeutic applications.

Keywords: *Marrubium astracanicum;* HS-SPME, essential oil; antimicrobial activity.

Introduction

The genus *Marrubium* L. includes about 40 species, indigenous in the Mediterranean area, Europe and Asia [1]. Some *Marrubium* species are used in folk medicine, especially the aerial parts of the flowering plant of *M. vulgare* which are approved for dyspeptic complaints, loss of appetite, treating cough, healing wounds and as a choleretic in digestive and biliary complaints [2]. Secondary metabolites, especially essential oils have been in high demand from the manufacturers of foods flavoring, fragrance, cosmetics, and pharmaceutical industries due to the growing interest of consumers in ingredients from natural sources. Many plants, especially Lamiaceae taxa have been used for different purposes, such as food, drugs, perfumery and they have potential uses as alternative remedies for the treatment of many infections and preservation of foods from the toxic effects of oxidants [3]. Phytochemical evalution of the *Marrubium* has shown that it is rich in flavonoids, phenylpropanoids, diterpens, amino acids and saponoids [4-7]. The chemical composition of essential oils depends on climatic, seasonal and geographic conditions, harvest period. Many research report on the essential oil composition of *Marrubium* species; *M. parviflorum* [8], *M. vulgare* [9], *M. cuneatum* [10], *M. velutinum* and *M. peregrinum* [11]. *Marrubium astracanicum* subsp. *astracanicum* is not cultivated nowadays;

*Corresponding authors (Ömer Kiliç)
E-mail: < omerkilic77@gmail.com >

because of rich essential oil content and antimicrobial activity it can be cultivated in Turkey. Some species of *Marrubium*, especially *M. vulgare* is grows wild in dry sandy soils and wastelands; this species can be cultivated successfully in different part of world. *M. vulgare* was cultivated in Lithuania and harvested twice a year as a medicinal raw material.

In the present study reports the essential oil composition and antimicrobial activities of essential oil of *Marrubium astracanicum* subsp. *astracanicum* which was collected in the Eastern Anatolian region of Turkey.

Materials and methods

Plant material

Marrubium astracanicum subsp. *astracanicum* was collected from between Agaçeli-Çavuslar villages, steppe, slopes, 1350-1400 m, June 2016, during field work of project (BAP - TBMYO.2016.00.001). Plant materials collected and than identified with Flora of Turkey and East Aegean Islands [12], by taxonomist O. Kiliç. Voucher specimens were deposited in Department of Park and Garden Plants of Bingol University Turkey. All plant samples were air-dried at room temperature (20-24°C) in a shady place and kept away from direct light.

HS-SPME method

Aerial parts of dry samples powdered with a blender. 5 g powder plant sample were analyzed head space solid phase microextraction method using polydimethyl siloxane fiber. Before analysis fiber was preconditioned in the injection port of the gas chromatography. 5 g plant samples were weighed in a 40 ml vial. The vial was kept at 35°C with continuous internal stirring and the sample was left to equilibrate for 30 min; then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35°C. Then fiber was introduced into the gas chromatography injec-tor, and was left for 3 min to allow the analyses thermal desorption. In order to optimize method, sample volume, headspace volume, heating temperature and extraction time were studied on the extraction efficiency as previously reported [13].

GC-MS Analysis

Gas-chromatography and mass spectrometry is an analytical method that combines the features of different compounds within a test sample. A Varian 3800 gas chromatograph directly inter faced with a Varian 2000 ion trap mass spectrometer was used with 260°C injector temperature. Injection mode, splitless; column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 µm thickness. The oven temperature was adjusted as: 45°C held for 5 min, then increased to 80°C at the rate of 10°C/min, and to 240°C at 2°C/min. Helium was the carrier gas and used at a constant pressure of 10 psi; the transfer line temperature 250°C; acquisit ion range, 40 to 200 m/z; scan rate, 1 us^{-1}. Essential oil constituents were detected using the NIST and mass spectral library as described by Van den Dool and Kratz [14]. The relative amounts were computed on the basis of peak-area ratios.

Isolation of essential oil

A dried sample of the aerial parts was subjected to hydrodistillation in a Clevenger type apparatus for 4 h. The obtained essential oil was dried over anhydrous sodium sulfate and after filtration, stored at + 4°C until antimicrobial activities tested.

Antimicrobial activity

Microbial strains

Sixteen bacteria strains were used for antimicrobial activity studies. Seven Gram-positive bacteria; *Bacillus subtilis* ATCC 6337, *Brevibacillus brevis*, *Bacillus megaterium* DSM 32, *Bacillus subtilis* IM 622, *Bacillus cereus* EMC 19, *Staphylococcus aureus* 6538 P, *Listeria monocytogenes* NCTC 5348 and the nine Gram negative bacteria *Salmonella typhimurium* NRRLE 4413, *Pseudomonas fluorescens*, *Enterobacter aerogenes* CCM 2531, *Klebsiella pneumoniae* EMCS, *Escherichia coli* ATCC 25922, *Proteus vulgaris* FMC II, *Pseudomonas aeruginosa* DSM 50070, *Proteus vulgaris*, *Salmonella enterica* ATCC 13311.

Determination of the antimicrobial activity

The disc diffusion method was used to evaluate the zone of microbial growth inhibition at various dilutions of the essential oil. Essential oil dilu-

tions (10 µL) dimethyl sulfoxide (DMSO) were injected into sterilized discs which had a diameter of 6 mm (Bioanalyse). DMSO (10 µL) was injected as the negative control. Medium in petri plates surface were spread using a sterile swab containing the microbial suspension, petri plates were placed at 4°C for 2 h. they were incubated at 37°C for 24 h. The diameter of the clear zone around the disc was measured in mm as its antimicrobial activity. All the tests were repeated triplicate.

Results and discussion

The chemical components of essential oils was tabulated in Table 1. β-caryophyllene, germacrene D, bicyclogermacrene and spathulenol were identified as the major components of studied sample. β-eudesmol (11.93 %), α-citronellol (9.90 %), ledene (5.35 %), δ-cadinene (3.30%) were present in fairly good amounts; 1,8 cineole (3.72 %) and geranial (2.74 %) were also detected in appreciable amounts *Marrubium vulgare* L. essential oil from Tunisia [15]. About the chemical composition of *M. vulgare* from different parts of the world, Saleh and Glombitza [16] reported tricyclene, α-pinene and bisabolol as the main compounds of *M. vulgare* [16]; Morteza and Saeedi [17] reported that the major constituents of the essential oil of *M. vulgare* from Iran were β-bisabolene (20.4 %), γ-cadinene (19.1 %) and isocaryophyllene (14.1 %) [17]; Khanavi *et al.,* [8] showed that the major component of *M. vulgare* from other region of Iran were β-bisabolene (25.4 %), β-caryophyllene (11.6 %) and germacrene D (9.7 %) [8]; Asadipour *et al.,* [18] 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 [18]. In our research, β-caryophyllene and germacrene D were detected the major components of *M. astracanicum* subsp. *astracanicum* (Table 1).

The composition of essential oil samples aerial parts of *M. parviflorum* Fisch. & C.A.Mey. and *M. vulgare* L. were investigated, both essential oils were characterized by a high amount of bicyclogermacrene (26.3 %), germacrene D (21.5 %) and β-caryophyllene (15.6 %) as the major constituents of *M. parviflorum*, and β-bisabolene (25.4 %), β-caryophyllene (11.6 %), germacrene D (9.7 %) and β-farnesene (8.3 %) as the major component of *M. vulgare* [8]. Bicyclogermacrene, germacrene D and β-caryophyllene were also detected as the main compounds in this study (Table 1). The steam-distilled essential oil obtained from aerial parts of *M. astracanicum* was examined and twenty-five compounds were characterized; the major components were caryophyllene oxide (35.8 %), citronellal (16.9 %) and β-caryophyllene (13.1 %) [19].

In another study, essential oils of aerial parts of *M. globosum* subsp. *libanoticum* and *M. cuneatum* growing wild in Lebanon were analysed and the main components of both oils were β-caryophyllene (12.4 %-5.2 %), hexadecanoic acid (7.4 %-6.5 %) and spathulenol (5.2 %-6.5 %) [20]. Similarly, β-caryophyllene, germacrene D, bicyclogermacrene and spathulenol were the major components of this sudy (Table 1). Interestingly, there were some differences between the main components of *Marrubium* taxa and the essential oil composition published in other studies. According to our results there were some similarities and differences in essential oil composition between studied samples and the other oils of *Marrubium* spp. The variations of oil components of studied *Marrubium* taxa may be because of the collection time, chemotypes, drying conditions, mode of distillation, geographic and climatic factors.

The antimicrobial activities of *M. astracanicum* subsp. *astracanicum* essential oil against microorganisms examined in the present study and their potency were assessed by the presence or absence of inhibition zones and zone diameter. This essential oil displayed varied antibacterial activities across the studied gram positive bacteria (Table 2). Zone diameter of inhibition from 6 to 13 mm for Gram (+) bacteria. Whereas, for Gram (-) bacteria strains have not shown the response to the essential oil action (Table 2). Antimicrobial activity of *Marrubium astracanicum* essential oil was observed against *Staphylococcus aureus* 6538 P (13 mm), *Brevibacillus brevis* (10 mm), *Listeria monocytogenes* NCTC 5348 (9 mm), *Bacillus megaterium* DSM 32 (8 mm), *Bacillus*

Table 1. Essential oil composition of *Marrubium astracanicum* subsp. *astracanicum* (%)

Compounds	RRI*	*M. astracanicum* subsp. *astracanicum*
α-Pinene	1025	0.5
Sabinene	1052	-
Mrycene	1068	0.4
Limonene	1090	0.8
γ-Terpinene	1115	-
α-Terpinolene	1140	0.1
Linalool	1145	2.4
Borneol	1200	0.5
α-Terpineol	1215	-
Carvacrol	1231	4.1
Camphene	1252	1.1
Decanal	1263	-
Bornyl acetate	1282	0.6
α-Cubebene	1286	2.5
Thymol	1297	0.5
β-Bourbenene	1365	-
β-Cubebene	1369	1.1
β-Caryophyllene	1393	23.5
β-Farnesene	1415	0.3
α-Humulene	1418	2.8
Aromadendrene	1421	1.6
Dodecanal	1425	0.1
Germacrene D	1432	10.6
β-Selinene	1441	0.5
Bicyclogermacrene	1443	9.3
Naphtalene	1450	2.3
α-Zingiberene	1455	0.7
β-Bisabolene	1458	3.5
δ-Cadinene	1485	0.3
Spathulenol	1495	9.4
Caryophyllene oxide	1498	1.7
Muurolene	1523	1.0
Copaene	1534	0.7
α-Cadinol	1539	-
β-Bisabolol	1660	0.4
Hexadecanoic acid	1665	2.1
Total		85.5

RRI*: Relative Retention Index

subtilis ATCC 6337 (7 mm), *Bacillus subtilis* IM 622 (7 mm) and *Bacillus cereus* EMC 19 (6 mm). Control disks with DMSO showed no inhibition zone (Table 2). A large number of *in vitro* studies have shown a high antimicrobial avtivity of essential oils [21-27]. Substantial number of previous studies has shown that resistance of gram negative bacteria is common and caused by a combi-

Table 2. Antimicrobial activity of the essential oil of *Marrubium astracanicum* subsp. *astracanicum* using disc diffusion method

Bacteria	Gram	Inhibition zone diameter (mm)
Bacillus subtilis ATCC 6337	Positive	7
Brevibacillus brevis	Positive	10
Bacillus megaterium DSM 32	Positive	8
Bacillus subtilis IM 622	Positive	7
Bacillus cereus EMC 19	Positive	6
Staphylococcus aureus 6538 P	Positive	13
Listeria monocytogenes NCTC 5348	Positive	9
Salmonella typhimurium NRRLE 4413	Negative	-
Pseudomonas fluorescens	Negative	-
Enterobacter aerogenes CCM 2531	Negative	-
Klebsiella pneumoniae EMCS	Negative	-
Escherichia coli ATCC 25922	Negative	-
Proteus vulgaris FMC II	Negative	-
Pseudomonas aeruginosa DSM 50070	Negative	-
Proteus vulgaris	Negative	-
Salmonella enterica ATCC 13311	Negative	-
DMSO	Control	-

nation of factors, such as different cellular organization and poor permeability of the cell membrane, which acts as a barrier for antibacterial agents [28-30]. Several researchers also report mono- and sesquiterpenoids as the major components of essential oils which are phenolic in nature [31,32]. It is therefore reasonable to assume that their antimicrobial activity might be related to the abundance of phenolic compounds. Germacrene-D is known has significant antibacterial activities [33]. Therefore, essential oils always represent a complex mixture of different chemical components, thus it is very difficult to reduce the antibacterial effect of the total oil to a few active principles. In conclusion, studied *Marrubium* species could be a source of β-caryophyllene, germarene D, bicyclogermacrene and spathulenol. The chemical results from this study might be helpful chemotaxonomy, potential usefulness and cultivation of *Marrubium* taxa. Microbiological tests of studied sample may be helpful after further testing in practice. Besides, due to their various bioactivities, further researchs should be carried out on the drug development of *Marrubium* extracts and their constituents.

Acknowledgements

The authors acknowledge the Scientific and Research Council of Bingol University (BAP-TBMYO.2016.00.001) for support this study.

References

1. **Willis, J.C. (1966).** A Dictionary of the Flowering Plants and Ferns. 7th Edition, Cambridge Univ. Press.
2. **Gruenwald, J., Brendler, T. and Jaenicke, C. (2000).** PDR for Herbal Medicines, 2nd edn. Medical Economics Company: Montvale, NJ. 401.
3. **Barlow, S.M. (1990).** Toxicological Aspects of Antioxidants Used as Food Additives, in Food Antioxidants, Hudson BJF (Ed.). (pp. 23). Elsevier, Amsterdam.
4. **Rigano, D., Formisano, A., Basil, A., Lavitola, F., Senatore, F., Rosselli, S. and Brunu,**

M. (2007). Antibacterial activity of flavonoids and phenylpropanoids from *Marrubium globosum* ssp. *libonaticum.* Phyto. Res. 21: 395-397.

5. **Hayet, E., Samia, A., Patrick, G., Mahjoub, M.A., Mastouri, M., Gutmann, L., Zine, M. and Aouni, M. (2007).** Antimicrobial and cytotoxic activity of *Marrubium alysson* and *Retama raetam* grown in Tunisi. Pak. J. Biol Sci. 10: 1759-1762.
6. **Nakano, K. and Kanal, K. (1995).** New labdane diterpenoids from *Marrubium astracanicum.* Chem. Pharm. Bull. 43: 1454-1457.
7. **Kurbatova, N.V., Muzychkina, R.A., Mukhitdinov, N.M. and Parshina, G.N. (2003).** Comparative phytochemical investigation of the composition and content of biologically active substances in *Marrubium vulgare* and *Marrubium alternidens.* Chem. Nat. Comp. 39: 501-502.
8. **Khanavi, M., Ghasemian, L., Hosseiny, M.E., Hadjiakhoondi, A. and Shafiee A. (2005).** Chemical Composition of The Essential Oils of *M.parviflorum* and *M.vulgare* from Iran. Flav. Frag. J. 20: 324-326
9. **Nagy, M. and Svajdlenka, E. (1998).** Composition of the essential oils from *Marrubium vulgare* and *Marrubium peregrinum*. J. Essent. Oil Res. 10: 585-587.
10. **Bahar, N.Z., Mirza, M. and Shahmir F. (2004).** Essential oil of *Marrubium cuneatum* and its secretory elements. Flav. Frag. J. 19: 233-235.
11. **Lazari, D.M., Skaltsa, H.D. and Constantinidis, T. (1999).** Essential oils of *Marrubium velutinum* and *Marrubium peregrinum* two species growing wild in Greece. Flav. Frag. J. 14: 290-292.
12. **Davis, P.H. (1982).** Flora of Turkey and the East Aegean Islands, Un. Press, Edinburgh. 7: 321.
13. **Verzera, A., Zýno, M., Condurso, C., Romeo, V. and Zappala, M. (2004).** Solid-phase microextraction and GC-MS for the rapid characterisation of semi-hard cheeses. Anal. Bio. Chem. 80: 930-936.
14. **Van den Dool, H. and Kratz, P.D. (1963).** A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatograph. J. of Chromatog. 11: 463-471.
15. **Kadri, A., Zied, Z., Békir, A., Néji, G., Mohamed, D. And Radhouane G. (2011).** Chemical composition, antioxidant activity of *M. vulgare* essential oil from Tunisia. African J. Biotech. 10: 3908-3914.
16. **Saleh, M.M. and Glombitza, K.W. (1989).** Volatile Oil of *Marrubium vulgare* and its Anti-schistosomal Activity. Planta. Medica. 55: 105-108.
17. **Morteza-Semnani, K. and Saeedi, M. (2004).** The Essential Oil Composition of *Marrubium astracanicum* From Iran. J. Essent. Oil Bearing Plant. 7: 239-242.
18. **Asadipour, A., Mehrabani, M., Nazeri, V. and Tabarraii M. (2005).** Composition of the essential oil of *Marrubium vulgare*. Ulum-i-Daroei. 2: 77-82.
19. **Baher, Nik, Z. and Mirza, M. (2003).** Composition of the Essential Oil of *Marrubium astracanicum*. J. Essent. Oil Res. 15: 342-343.
20. **Armando, G., Felice, S., Nelly, A., Maurizio, B., Franco, P., Daniela, R., Carmen, F. (2006).** Chemical Composition and Antimicrobial Activity of the Essential Oils from Aerial Parts of Two *Marrubium* sp. Growing Wild in Lebanon. Polish. J. Chem. 80: 623-628.
21. **Miletic, P., Marjanovic-Balaban, Z. and Kalaba, V. (2009).** Antimicrobial activity of essential oil from Norway spruce (*Picea abies*). VIII Symposium "Advanced Technology and Economic Development. Proceed. Leskovac. 8: 1-9.
22. **Kalaba, V., Marjanovic-Balaban, Z., Stijepic, M., Glušac, J. and Kalaba, D. (2014).** Anti-microbial activity of selected essential oils against of Staphylococcus aureus compared with anti-microbial drugs, II International Congress Food Technology Quality and Safety, Novi Sad,

Proceedings. 434-439.

23. **Nikolic, M., Glamodzlija, J., Ciric, A., Markovic, T., Markovi, D., Peric, T. and Sokovic, M. (2013).** Chemical composition and antimicrobial activity of essential oil from mint. Lek Sirov., 33: 63-72
24. **Mahmmod, Z.A. (2013).** The effect of chamomile plant as feed additives on productive performance, carcass characteristics and immunity response of broiler. Int. J. Poult Sci. 12: 111-116.
25. **Jakubcova, Z., Zeman, L., Horky, P., Mrkvicova, E., Mares, P., Mrazkova, E. and Stastnik, O. (2014a).** The influence of the addition of chamomile extract to the diet of chickens. Proceedings of the conference Mendel Net, Brno, Czech Republic. 147-150.
26. **Jakubcova, Z., Zeman, L., Mares, P., Mlcek, J., Jurikova, T., Dostalova, L., Mrazkova, E., Mrkvicova, E., Balla, S. and Sochor, J. (2014b).** Effect of chamomile supplements to feeding doses on antimicrobial parameters in poultry. Pot Ravinarstvo. 8: 228-232.
27. **Al-Mashhadani, E.H., Al-Mashhadani, H. and Al-Shamire, J.S. (2013).** Effect of supplementing different levels of chamomile oil on broiler performance, physiological traits. Int. J. Poult Sci. 12: 426-429.
28. **Stanojevic, L.P., Marjanovic-Balaban, Z.R., Kalaba, V.D., Stanojevic, J.S. and Cvetkovic, D.J. (2016).** Chemical composition, antioxidant and antimicrobial activity of chamomile flowers essential oil. J. Essent. Oil Bearing Plant. 19: 2017-2028
29. **McDonnell, G. and Russell, A.D. (1999).** Antiseptics and disinfectans: activity, action and resistance. Clin. Microbiol. Rev. 12: 147-179.
30. **Lambert, P.A. (2002).** Mechanisms of antibiotic resistance in *P. aeruginosa*. J.R. Soc. Med. 95: 22-26.
31. **Oyedeji, O. and Afolayan, A. (2005).** Comparative study of the essential oil composition and antimicrobial activity of *L. leonurus* and *L. ocymifolia* in the Eastern Cape. African J. Bot. 71: 114-116.
32. **Cakir, A., Kordali, S., Zengin, H. and Izumi, S. (2004).** Composition and antifungal activity of essential oils isolated from *Hypericum hyssopifolium* and *H. heterophyllum*. Flav. Frag. J. 19: 62-68.
33. **Sahin, F., Gulluc, M., Daferera, D., Sokmen, A., Sokmen, M., Polissiou, M., Agar, G. and Ozer, H. (2004).** Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the eastern Anatolia region of Turkey. Food Control. 15: 549-557.