SHORT REPORT

A C G publications

Rec. Nat. Prod. 11:4 (2017) 389-394

records of natural products

Essential Oil Composition and Antimicrobial Activity of *Aster subulatus* Michx. from Turkey

Fatma Ayaz[1], Nurgün Küçükboyacı[1*] and Betül Demirci[2]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, Türkiye*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Türkiye*

(Received September 19, 2016; Revised March 08, 2017; Accepted March 14, 2017)

Abstract: Chemical composition and antimicrobial activity of the hydrodistilled essential oils from aerial parts and roots of *Aster subulatus* Michx. (Asteraceae) from Turkey were investigated. The essential oils of *A. subulatus* were analyzed by combination of GC-FID and GC-MS. Twenty-nine components comprising 79.0% of the essential oil of the roots and forty-nine components comprising 89.3% of the essential oil of the aerial parts were identified. The major constituents of the essential oil from the aerial parts were found to be elemol (21.5%), *β*-eudesmol (6.3%) and caryophyllene oxide (5.2%), while the main components of the root oil were hexadecanoic (33.0%), tetradecanoic (5.3%) and octanoic (4.6%) acids. The antimicrobial activities of the oils were tested against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* using a TLC-bioautography method. Both of the oils showed inhibitory activity against *S. aureus* and *C. albicans*, while the oils were found to be inactive against tested *E. coli* strain at 1 mg/mL concentration. This is the first report on the chemical composition and preliminary antimicrobial activity of the essential oils of *A. subulatus* from Turkey.

Keywords: Asteraceae; *Aster subulatus*; essential oil composition; antimicrobial activity.

1. Plant Source

The aerial parts and roots of *A. subulatus* were collected from Selçuk, İzmir, Turkey at the flowering-fruit stage in November 2013. The plant materials were dried at room temperature and identified by Prof. Dr. Hayri Duman from the Department of Botany, Faculty of Science, Gazi University. A voucher specimen (F. Ayaz 27) was retained in the Herbarium of the Gazi University (GAZI), Ankara, Turkey.

2. Previous Studies

The genus *Aster* L. (Asteraceae) consists of about two hundred fifty species native to North and South America, Europe, Africa and Asia [1]. In the flora of Turkey, the genus *Aster* is represented by ten species. *Aster subulatus* Michx. is an annual plant erecting with widely branched glabrous stems reaching 150 cm high [2-4].

The genus has long been used for the treatment of cold, fever, tonsillitis, bronchitis, snake bites and bee stings in traditional Chinese medicine [5]. Several biological activities such as allelopathic, antifungal, anti-inflammatory, insecticidal and expectorant activity have been reported on the essential oils from *Aster* species [6-10].

* Corresponding author: E-Mail: nurgunkucukboyaci@gmail.com; Phone +90-312-2023177.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 05/17/2017 EISSN: 1307-6167

There is a little study on the chemical composition of *A. subulatus* to date. It was reported that *A. subulatus* contained flavone and flavonol aglycones, monoglycosides and a diglycoside of kaempferol as well as chlorogenic acid [11]. Currently, a new compound, namely 1-[(butanoyl)phloroglucinyl]-*β*-D-glucopyranoside, as well as two known compounds, 3,5-dicaffeoylquinic acid and 3,5-dicaffeoyl-epiquinic acid, were isolated from this plant [12]. There is only one report concerning the chemical composition of the essential oil of *A. subulatus* [13]. Otherwise, only a few studies have been reported on the essential oil composition from different species of the genus *Aster* [5-10, 14-17].

3. Present Study

Crushed aerial parts (620 g) and roots (160 g) of the plant material were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce a small amount of essential oil, which was trapped in *n*-hexane. The samples were dried over anhydrous sodium sulphate and stored at +4°C in the dark until analyzed.

Gas Chromatography-Mass Spectrometry (GC-MS): The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

Gas Chromatography (GC): The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of the Essential Oil Components: Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 3 Library) [18,19] and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data, [20,21] was used for the identification.

Thin-Layer Chromatography (TLC): Chromatography was performed on 0.2 mm silica gel 60 F254 aluminium sheet TLC plates. Essential oils (10 μL; 1 mg/mL) were applied onto the TLC plate using minicaps capillary pipettes. Thereafter, the TLC plates were developed with toluene:ethyl acetate (93:7) as a mobile phase. A duplicate TLC plate for bioautography was prepared in parallel. After the development, TLC plates were evaluated at UV 254 nm and 366 nm for determination of fluorescent compounds. Alcoholic vanillin-sulphuric acid reagent was used to visualize the separated compounds and heated for 3 min at 110°C.

Preparation of Microorganisms and TLC Bioautography Method: After TLC separation of the 1 mg/mL samples, the antimicrobial activity of the essential oils was detected by direct bioautography. *Escherichia coli* NRRL B-3008, *Staphylococcus aureus* ATCC 6558 and *Candida albicans* ATCC 90028 were used for bioautography. Microbial suspensions were grown overnight in double strength Mueller-Hinton broth (MHB) were standardized to 10^8 $CFUmL^{-1}$ (corresponding to McFarland no: 0.5). TLC plates were layed on nutrient agar plates. And molten agar medium containing inocula was spread on TLC plates and incubated at 37 °C for 24 h after solidification of the agar layer. After 24 hours of incubation 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) 10% solution was sprayed on TLC plates. The treated plates were incubated at 37°C for another 2 hours. After incubation, the inhibition zones were visible as pale spots against a red background indicating the active inhibited zones.

The essential oils obtained from *A. subulatus* were simultaneously analyzed by GC and GC-MS analysis. The relative percentages and retention indices of the compounds in the essential oils are reported in Table 1. Twenty-nine components comprising 79.0% of the oil from the roots and forty-six components comprising 89.3% of the oil from the aerial parts were identified. The essential oil of the aerial parts consisted mainly of oxygenated sesquiterpenes (45.0 %), followed by sesquiterpene

hydrocarbons (21.6 %). The root essential oil contained majorly fatty acids (46.9 %). The main components of the oil from aerial parts were found elemol (21.5%), *β*-eudesmol (6.3%), caryophyllene oxide (5.2%), *α*-eudesmol (5.0%), *α*-muurolene (4.1 %), spathulenol (3.7%) and humulene epoxide II (3.0%). The root oil mainly contained hexadecanoic (33.0%), tetradecanoic (5.3%) and octanoic (4.6%) acids.

Up to date, only a few studies on the essential oil of *Aster* species have been investigated [5-10, 14-17]. In a recent study, taxonomical implications on the essential oil of the genus *Aster* was reported. This study revealed that the essential oils of *Aster* species consisted mainly of sesquiterpenes or monoterpenes without genus-related pattern [17]. According to our results, the aerial parts oil from *A. subulatus* was dominated by sesquiterpenes. In contrast, in a previous study conducted by Miyazawa & Hiromu [13], monoterpenes were predominantly found in the essential oil of *A. subulatus*. In that previous study, the essential oil was analyzed using gas chromatography and its constituents were identified through their infrared, mass and nuclear magnetic resonance spectral methods. According to its results, the essential oil was found to be rich in *β*-pinene (40.9%), limonene (11.3%), *α*-muurolene (4.7%), *α*-terpineol (3.8%), *β*-caryophyllene (3.8%), *α*-pinene (2.2%) and *α*-humulene (1.7%). In addition, the essential oil contained camphene, *β*-myrcene, *p*-cymene, *n*-$C_{13}H_{28}$, hexanol, 3-octanol, *trans*-linalool oxide, *cis*-linalool oxide, linalool, *α*-copaene, fenchyl alcohol, 4-terpineol, pinocarveol, butyric, valeric, isovaleric, caproic, caprylic, capric, lauric, myristic, palmitic and stearic acids as well as *o*-cresol, eugenol, isoeugenol and carvacrol [13]. The chemical composition of the essential oils of *A. subulatus* collected from Turkey has been found more different than reported in the previous study. According to the previously reported study by Miyazawa & Hiromu [13], while *β*-pinene (40.9%) was found to be major compound in the essential oil of *A. subulatus*, we principally determined elemol (21.5%) and hexadecanoic acid (33.0%) in the essential oils from aerial parts and roots of the plant, respectively. Otherwise, in our study, we did not identify limonene, *α*-pinene and linalool in both of the oils, while these compounds were found in the essential oil from *A. subulatus* in the previous study with 11.3, 2.2 and 2.1%, respectively. We also determined *β*-pinene (0.5%), *β*-caryophyllene (0.5%) and *α*-terpineol (0.3%) in much less quantity in the aerial parts oil in the present study. Moreover, *α*-muurolene (4.7%) and *α*-humulene (1.7%) were detected in the essential oil of *A. subulatus* in the previous study [13]. Similarly, *α*-muurolene and *α*-humulene were determined in the aerial parts oil in our study with 4.1 and 1.3%, respectively.

In addition, the antimicrobial activities of the essential oils were evaluated at 1 mg/mL concentration using an overlay TLC-bioautography method on two strains of bacteria, *Escherichia coli* and *Staphylococcus aureus*, and a strain of fungus, *Candida albicans*. Both of the oils showed inhibitory zones suggesting antimicrobial activity against the strains of *S. aureus* and *C. albicans*, while there was no inhibition zone against *E. coli* strain.

In the literature survey, it was concluded that sesquiterpenol components, such as α- and β-eudesmol and bicyclogermacrene, had often been associated with high antibacterial and antifungal activities [22]. Moreover, it has been reported that sesquiterpene alcohols such as elemol and eudesmol isomers rich essential oils from *Phebalium squamulosum* (Rutaceae) subspecies displayed moderate to high antimicrobial activities in a recent research. By contrast, very low activity was reported in the essential oils dominated by monoterpenes from that samples [23]. Elemol is also known as an important sesquiterpene alcohol with insecticidal and antitermite properties [24-26]. In addition, recently, ameliorative effects of the essential oil from *Chamaecyparis obtusa* were shown on atopic dermatitis by *in vitro* and *in vivo* models due to presence of eudesmol and particularly elemol as active components [27].

As a conclusion, elemol (21.5%) and hexadecanoic acid (33.0%) were found to be major components in the essential oils from the aerial parts and roots of *A. subulatus*, respectively. According to our results, *A. subulatus* could be considered a good source of elemol and eudesmol isomers having some important biological activities. Moreover, antimicrobial activity was shown on both of the essential oils against the strains of *S. aureus* and *C. albicans*. Our results demonstrated that sesquiterpenols dominated essential oil from the aerial parts of the plant, principally elemol and eudesmol isomers, could be responsible for its antimicrobial ability. To the best of our knowledge, this is the first report on the chemical composition and the preliminary antimicrobial evaluation of the essential oils from *A. subulatus* growing in Turkey.

Table 1. The chemical composition of the essential oils from aerial parts and roots of *Aster subulatus*

KI[a]	RRI[b]	Compound	A (%)[c]	B (%)[c]
1117[d]	1118	*β*-pinene	0.5	-
1122[e]	1132	sabinene	0.1	-
1483[e]	1492	cyclosativene	0.6	-
1488[f,g]	1497	α-copaene	0.6	-
1523[e]	1535	*β*-bourbonene	1.1	-
1541[e]	1549	*β*-cubebene	-	0.6
1575[e]	1586	pinocarvone	0.6	-
1590[e]	1600	*β*-elemene	0.2	-
1579[e]	1597	*β*-copaene	0.4	-
1601[e]	1611	terpinen-4-ol	0.6	-
1608[f,g]	1612	*β*-caryophyllene	0.5	-
1631[e]	1648	myrtenal	0.2	-
1661[e]	1670	*trans*-pinocarveol	0.7	-
1663[f,g]	1687	*α*-humulene	1.3	0.6
	1688	selina-4,11-diene *(=4,11-eudesmadiene*)	0.2	-
1689[e]	1704	*γ*-muurolene	1.8	0.6
1694[e]	1706	*α*-terpineol	0.3	-
1708[e,g]	1726	germacrene D	1.2	t
1723[e]	1740	*α*-muurolene	4.1	0.9
1734[e]	1755	bicyclogermacrene	1.2	-
1755[e]	1773	*δ*-cadinene	2.5	0.3
1763[e]	1776	*γ*-cadinene	t	-
1790[e]	1804	myrtenol	0.6	-
1808[e]	1827	(*E,E*)-2,4-decadienal	0.8	0.4
1854[e]	1868	(*E*)-geranyl acetone	-	0.1
1921[e]	1941	*α*-calacorene	0.7	0.6
1950[e]	1981	heptanoic acid	-	1.6
1999[f,g]	2008	caryophyllene oxide	5.2	2.4
2041[e]	2041	pentadecanal	1.7	0.9
2047[e]	2071	humulene epoxide-II	3.0	1.7
2057[e]	2084	octanoic acid	0.9	4.6
2078[e]	2096	elemol	21.5	-
2124[e]	2131	hexahydrofarnesyl acetone	1.9	1.7
2144[h]	2144	spathulenol	3.7	1.5
2176[e]	2185	*γ*-eudesmol	3.1	-
	2204	eremoligenol	1.2	-
2209[h]	2209	*T*-muurolol	1.2	0.4
2222[e]	2250	*α*-eudesmol	5.0	-
2238[e]	2257	*β*-eudesmol	6.3	-
2252[e]	2273	selin-11-en-4α-ol	-	2.3
	2312	9-geranyl-*p*-cymene	1.7	-
	2400	tetracosane	0.8	1.4
	2438	kaur-16-ene	1.5	-
	2500	pentacosane	0.7	2.1
2486[e]	2503	dodecanoic acid	-	1.5
	2600	hexacosane	1.7	2.7
2586[e]	2607	*1*-octadecanol	1.7	2.6
2613[e]	2622	phytol	0.8	2.7
2686[e]	2670	tetradecanoic acid	0.2	5.3
	2700	heptacosane	2.1	3.2
	2800	octacosane	-	2.4
2822[e]	2822	pentadecanoic acid	-	0.9
2913[e]	2931	hexadecanoic acid	2.6	33.0
		Monoterpene Hydrocarbons	0.6	-
		Oxygenated Monoterpenes	3.0	-
		Sesquiterpene Hydrocarbons	21.6	6.0
		Oxygenated Sesquiterpenes	45.0	5.9
		Fatty acids	3.7	46.9
		Diterpenes	4.0	2.7
		Others	11.4	17.5
		Total identified	**89.3**	**79.0**

[a] KI Kovats indices from literature d) [28], e) [29], f) [30], g) [31], h [32] [b] RRI: Relative retention indices calculated against *n*-alkanes for a polar column, % calculated from FID data, t Trace (< 0.1 %), - not detected, A aerial parts, B root.

Acknowledgements

The authors would like to thank to Prof. Dr. Hayri Duman from the Department of Botany, Faculty of Science, Gazi University, Ankara, Turkey for the identification of the plant.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] A.M. Armitage (2008). *Herbaceous Perennial Plants: A Treatise on Their Identification, Culture, and Garden Attributes.* 3rd ed. Champaign: Stipes Publishing.

[2] A.J.C. Grierson (1975). *Aster* L. In: *Flora of Turkey and the East Aegean Islands*, Ed. P.H. Davis, vol. 5, Edinburgh: University Press, 118-121.

[3] P.H. Davis, R.R. Mill and T. Kit (1988). *Aster* L. In: *Flora of Turkey and the East Aegean Islands*, Eds. P. H. Davis, R.R. Mill, T. Kit, vol. 10, Edinburgh: University Press, 160-161.

[4] A. Güner, A. Özhatay, T. Ekim and K.H.C Başer (1988). *Aster* L. In: *Flora of Turkey and the East Aegean Islands*, Ed. T. Kit, vol. 11, Edinburgh: University Press, 160-11.

[5] T.Y. Chung, J.P. Eiserich and T. Shibamoto (1993). Volatile compounds isolated from edible Korean chamchwi (*Aster scaber* Thunb), *J. Agric. Food Chem.* **41**, 1693-1697.

[6] N. Tabanca, B. Demirci, S.L. Crockett, K.H. Başer and D.E. Wedge (2007). Chemical composition and antifungal activity of *Arnica longifolia*, *Aster hesperius*, and *Chrysothamnus nauseosus* essential oils, *J. Agric. Food Chem.* **55**, 8430-8435.

[7] B. Yang, Y.Q. Xiao, R.X. Liang, R.J. Wang, W. Li, C. Zhang, Y. Cao, Q.P. Wang, L. Wang and Y.Y. Wang (2008). Studies on expectorant compounds in volatile oil from root and rhizome of *Aster tataricus*, *China J. Chinese Mat. Med.* **33**, 281-283.

[8] J.F.G. Dias, O.G. Miguel and M.D. Miguel (2009). Composition of essential oil and allelopathic activity of aromatic water of *Aster lanceolatus* Willd:(Asteraceae), *Braz. J. Pharm. Sci.* **45**, 469-474.

[9] S.S. Chu, S.L. Liu, Q.Z. Liu, G.H. Jiang and Z.L. Liu (2013). Chemical composition and insecticidal activities of the essential oil of the flowering aerial parts of *Aster ageratoides*, *J. Serb. Chem. Soc.* **78**, 209-216.

[10] C. Kim, H.J. Bu, S.J. Lee, C-G. Hyun and N.H. Lee (2014). Chemical compositions and anti-inflammatory activities of essential oils from *Aster spathulifolius* and *Vitex rotundifolia* Maxim, *J. App. Pharm. Sci.* **4**, 12-15.

[11] N.H. El-Sayed, A. Lenherr, S. Sundberg and T.J. Mabry (1987). Flavonoids of *Aster subulatus*, *Biochem. Syst. Ecol.* **15**, 549-550.

[12] R.K. Ko, M.C. Kang, B.S. Kim, J.H. Han, G.O. Kim and N.H. Lee (2009). A new phloroglucinol glycoside from *Aster subulatus* Michx, *Bull. Korean Chem. Soc.* **30,** 1167-1169.

[13] M. Miyazawa and K. Hiromu (1977). The constituents of the essential oil from *Aster subulatus* Michx, *J. Agric. Chem. Soc. Jap. (Japan)* **51**, 23-26.

[14] Y.Q. Tu, X.P. Zong and X.P. Dong (2006). Chemical component analysis of essential oil from *Aster poliothamnus* Diels. by GC-MS, *West China J. Pharm. Sci.* **21**, 445-447.

[15] M. Miyazawa, J. Kawata, K. Kohno, M. Imai and T. Ono (2008). Essential oil and headspace constituents from the aerial parts of *Aster ageratoides* Turez. var. *ovatus* Nakai, *J. Essent. Oil Res.* **20**, 9-11.

[16] H.S. Choi (2012). Comparison of the essential oil composition between *Aster tataricus* and *A. koraiensis*, *Anal. Chem. Lett.* **2**, 139-151.

[17] N. Rajčević, P. D. Marin, L. Vujisić, Z. Krivošej Z., V. Vajs and P. Janaćković (2015). Chemical composition of *Aster albanicus* Deg. (Asteraceae) essential oil: Taxonomical implications, *Arch. Biol. Sci.* **67**, 1055-1061.

[18] W.A. Koenig, D. Joulain and D.H. Hochmuth (2004). *Terpenoids and Related Constituents of Essential Oils*, Hamburg, Germany: MassFinder 3.

[19] F.W. McLafferty and D.B. Stauffer (1989). *The Wiley/NBS Registry of Mass Spectral Data*, New York: J Wiley and Sons.

[20] ESO 2000 (1999). *The Complete Database of Essential Oils*, The Netherlands: Boelens Aroma Chemical Information Service.

[21] D. Joulain and W.A. Koenig (1998). *The Atlas of Spectra Data of Sesquiterpene Hydrocarbons*, Hamburg: EB-Verlag.

[22] A.R. Koroch, H.R. Juliani and J.A. Zygadlo (2007). *Bioactivity of essential oils and their components* In: *Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability*, Ed. R.G. Berger, New York: Springer, 87-103.

[23] N.J. Sadgrove, I.R. Telford, B.W. Greatrex and G.L. Jones (2014). Composition and antimicrobial activity of the essential oils from the *Phebalium squamulosum* species complex (Rutaceae) in New South Wales, Australia, *Phytochemistry* **97**, 38-45.

[24] M. Krings, T.N. Taylor and D.W. Kellogg (2002). Touch-sensitive glandular trichomes: a mode of defence against herbivorous arthropods in the Carboniferous, *Evol. Ecol. Res.* **4**, 779-786.

[25] S.S. Cheng, C.Y. Lin, M.J. Chung and S.T. Chang (2012). Chemical composition and antitermitic activity against *Coptotermes formosanus* Shiraki of *Cryptomeria japonica* leaf essential oil, *Chem. Biodivers.* **9**, 352-358.

[26] J.F. Carroll, G. Paluch, J. Coats and M. Kramer (2010). Elemol and amyris oil repel the ticks *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) in laboratory bioassays, *Exp. Appl. Acarol.* **51**, 383-392.

[27] H. Yang, E.M. Jung, C. Ahn, G.S. Lee, S.Y. Lee, S.H. Kim, I.G. Choi, M.J. Park, S.S. Lee, D.H. Choi and E.B. Jeung (2015). Elemol from *Chamaecyparis obtusa* ameliorates 2,4-dinitrochlorobenzene-induced atopic dermatitis, *Int. J. Mol. Med.* **36**, 463-472.

[28] A. Abdelwahed, N. Hayder, S. Kilani, A. Mahmoud, J. Chibani, M. Hammami, L. Chekir-Ghedira and K. Ghedira (2006). Chemical composition and antimicrobial activity of essential oils from Tunisian *Pituranthos tortuosus* (Coss.) Maire, *Flavour Fragr. J.* **21**, 129-133.

[29] V.I. Babushok, P.J. Linstrom and I.G. Zenkevich (2011). *Retention* indices for frequently reported compounds of plant essential oils, *J. Phys. Chem. Ref. Data*. **40**, 1-47.

[30] H.E. Temel, B. Demirci, F. Demirci, F. Celep, A. Kahraman, M. Doğan and K.H.C. Başer (2016). Chemical characterization and anticholinesterase effects of essential oils derived from *Salvia* species, *J. Essent. Oil Res.* **28**, 322-331.

[31] A. Kaya, M. Dinç, S. Doğu and B. Demirci (2017),. Compositions of essential oils of *Salvia adenophylla*, *Salvia pilifera*, and *Salvia viscosa* in Turkey, *J. Essent. Oil Res.* **29,** (3), 233-239.

[32] A. Ali, N. Tabanca, B. Demirci, E. K. Blythe, K. H. C. Baser and I. A. Khan (2016). Chemical composition and biological activity of essential oils from four Nepeta species and hybrids against *Aedes aegypti* (L.) (Diptera: Culicidae), *Rec. Nat. Prod.* **10**, 137-147.