ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 7:3 (2013) 177-183

records of natural products

Chemical Composition and Antimicrobial Activity of the Essential Oils from the Aerial Parts of *Astragalus hamzaoglui* Extracted by Hydrodistillation and Microwave Distillation

Nagihan Yilmaz İskender[1], Nuran Kahriman[1], Gonca Tosun[1], Salih Terzioğlu[2], Şengül Alpay Karaoğlu[3] and Nurettin Yayli [1*]

[1]*Department of Chemistry, Faculty of Sciences, Karadeniz Technical University, 61080, Trabzon, Türkiye*
[2]*Department of Forest Botany, Faculty of Forestry, Karadeniz Technical University, 61080, Trabzon, Türkiye*
[3]*Department of Biology, Faculty of Arts and Sciences, Rize University, 53100, Rize, Türkiye*
(Received October 19, 2010; Revised November 20, 2010, Accepted December 12, 2010)

Abstract: The essential oils from the areal parts (flower, leaf and stem) of *Astragalus hamzaoglui* were isolated by hydrodistillation (HD) and a microwave distillation (MD) process. The composition of the volatiles was analysed by GC-FID and GC-MS and screened for their in vitro antimicrobial activity. Totals of 68/64, 65/64, and 68/67 compounds were identified in the flower, leaf, and stem oils of *A. hamzaoglui* accounting for 92.9-95.0% in HD and 87.4-90.7% in MD, respectively. The essential oils consisted mainly of oxygenated monoterpenes (flower, HD: 33.3% vs. MD: 2.3%, leaf, HD: 8.0% vs. MD: 2.7%, stem, HD: 10.8% vs. MD: 4.7%), alcohols (flower, HD: 13.6% vs. MD: 10.4%, leaf, HD: 51.6% vs. MD: 3.6%, stem, HD: 35.4% vs. MD: 7.6%) and aldehydes (flower, HD: 15.5% vs. MD: 27.9%, leaf, HD: 10.0% vs. MD: 14.8%, stem, HD: 17.2% vs. MD: 24.5%). The major component of the oils of *A. hamzaoglui* was 1-octene-3-ol (flower, HD: 11.3% vs. MD: 8.8%, leaf, HD: 45.50% vs. MD: 3.3%, stem, HD: 32.6% vs. MD: 6.9%). Oxygenated sesquiterpenes were the minor constituents in all parts (flower, HD: 0.8% vs. MD: 0.4%, leaf, HD: 0.2% vs. MD: 0.4%, stem, HD: 0.4% vs. MD: 0.4%). of the *A. hamzaoglui*. Comparative study showed that the amount of total volatiles (flower, 95.0%, leaf, 92.9%, and stem, 95.0%) and the major constituent for the low molecular weight of essential oils were found to be better in HD of *A. hamzaoglui*. The oils were screened for antimicrobial activity against 12 microorganisms and showed antibacterial and antifungal activities against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* 702 Roma, *Mycobacterium smegmatis*, and *Saccharomyces cerevisiae*. The antifungal activity was observed for the all tested oils against *S. cerevisiae* except stem oil of HD.

Keywords: *Astragalus hamzaoglui*; Fabaceae; essential oil composition; antimicrobial activity.

1. Introduction

The legume genus *Astragalus* L. (Fabaceae) includes more than 2000 species in the world and is notable for its high endemism in Turkey [1,2]. It is estimated that ca. 478 species belonging to 64 sections are native in Turkey [3]. Among these the studied species is belong to section *Hypoglottis* Bunge which currently includes 21 species in Turkish flora [2]. *Astragalus hamzaoglui* O. Ketenoglu and Y. Menemen [4] is endemic to Turkey and newly recorded species from the East Black Sea region [4]. Materials of the study collected from a new distribution point of the species which is not far from

* Corresponding author: E-Mail: yayli@ktu.edu.tr ; Phone: +90 462 377422 ; Fax: +90 462 325 0570

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 5/28/2013 EISSN:1307-6167

its type place. Gum obtained from leaf and stem of some astragali species has been used as dye and to cure some throat diseases [5]. As well, this gum is an exported good of Turkey and whole plant and seed pods are commonly used for animal feed in Anatolia.

A literature survey revealed few reports regarding the phytochemical work done on *Astragalus* L., which mainly concern the study of phenolic compounds, flavonoids, diterpenoids, lanostene type triterpenes, and flavonol glycoside [6-10]. To date, no previous reports dealing with any investigation of the volatiles of this species can be found in the literature. The aim of this work was set to perform a detailed areal compositional analysis of the volatiles isolated by HD and MD from the mentioned taxa originating from Turkey. Furthermore, we also tested the antimicrobial activity of the essential oils against a panel of microorganisms.

2. Materials and Methods

2.1. Plant Material

A. hamzaoglui was collected in Akçaabat,Trabzon-Turkey (at heights of ~800 m) in the northeastern part of Turkey in April, 2009. The plant was authenticated by Prof. S. Terzioğlu [1-4]. Voucher specimen was deposited in the Herbarium of the Faculty of Forestry, KATO (KATO: 8733), Karadeniz Technical University, Turkey.

2.2. Hydrodistillation apparatus and procedure

The fresh plant materials were separated into flower, leaf, and stem parts and then grounded into small pieces. The essential oils from fresh aerial parts (flower, leaf, and stem parts ~150 g, each) of *A. hamzaoglui* were isolated by hydrodistillation (HD) in a modified Clevenger-type apparatus [11] with cooling bath (-15 ^{o}C) system (4h) (yields: 0.09%, 0.06%, and 0.04% (v/w), respectively). The obtained oils were extracted with HPLC grade n-hexane (0.5 ml) and dried over anhydrous sodium sulphate and stored at 4-6 ºC in a sealed brown vial.

2.3. Microwave distillation apparatus and procedure

Microwave distillation (MD) [11-13] was performed at atmospheric pressure with a Milestone DryDIST microwave apparatus using a fixed power of 650 W for 30 min. Temperature was monitored by an external Infrared (IR) sensor. The fresh areal plant materials (flower, leaf, and stem parts ~150 g, each) were grounded into small pieces, then placed in a round bottom flask (2l) with 50 ml water and submitted to microwave distillation (MD) using a modified Clevenger-type apparatus with cooling bath (-15 ^{o}C) system (30 min) (yield (v/w): 0.08%, 0.06%, and 0.05%, respectively). The obtained oils were extracted with HPLC grade n-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at -5 ºC in a sealed brown vial.

2.4. Gas chromatography-mass spectrometry (GC/MS)

GC-MS analysis was performed using an Agilent-5973 Network System. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV) was used. The chromatographic column used for the analysis was HP-5 capillary column (30 m x 0.32 mm i.d., film thickness 0.25 μm). Helium was used as carrier gas, at a flow rate of 1 ml/min. The injections were performed in splitless mode at 230 °C. Two μL essential oil solution in hexane (HPLC grade) was injected and analyzed with the column held initially at 60 °C for 2 min and then increased to 240 °C with a 3 °C/min heating ramp.

2.5. Gas chromatography

The capillary GC-FID analyses were carried out under the same experimental conditions using the same column and same gas chromatograph type as described for the GC-MS. The percentage composition was computed from the GC peak areas without the use of correction factors.

2.6. Identification of constituents

Retention indices of all the components were determined by Kovats method using *n*-alkanes (C_6-C_{32}) as standards. The constituents of the oils were identified by comparison of their mass spectra with those of authentic standards (the n-alkanes and some of the terpenoids as indicated in table 1), literature [14-20] as well as those from Wiley, NIST, and MassFinder.

2.7. Antimicrobial screening

All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas auroginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Listeria monositogenes* ATCC 43251, *Bacillus cereus* 709 ROMA, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC 60193 and *Saccharomyces cerevisiae* RSKK 251. Essential oils were dissolved in diethyl ether to prepare extracts stock solution of (10000 µg/mL).

2.8. Agar well diffusion method

Simple susceptibility screening test using agar-well diffusion method [21] as adapted earlier [22] was used. Each bacterium was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth. The yeast like fungi was suspended in Yeast extracts broth. Then the microorganisms were diluted approximately 10^6 colony forming unit (cfu) perm L. For yeast like fungi, Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) were used. They were “flood-inoculated” onto the surface of MH and SD agars and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 µl of the extract substances were delivered into the wells. The plates were incubated for 18 h at 35°C. The *Mycobacterium smegmatis* was grown for 3 to 5 days on MHA plates at 35 °C [23]. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 µg), streptomycin (10 µg) and fluconazole (5 µg) were standard drugs. Diethylether was used as solved control.

3. Results and Discussion

The chemical composition of the essential oils from flower, leaf, and stem of *A. hamzaoglui* isolated by HD and MD is presented in table 1. A total of sixty-nine different components (flower, HD: 68 vs. MD: 64, leaf, HD: 65 vs. MD: 64, stem, HD: 68 vs. MD: 67) were identified by GC-FID and GC-MS with HP-5 column (Table 1) [15-20]. The amounts of compounds extracted by HD was greater in low molecular weight volatiles such as 1-octene-3-ol (flower, HD: 11.3% vs. MD: 8.8%, leaf, HD: 45.5% vs. MD: 3.3%, stem, HD: 32.6% vs. MD: 6.9%), is probably related to the possible degradation of products by oxidation or hydrolysis, because a longer extraction time (3 h for HD vs. 30 min for MD) and a greater quantity of water (2 l for HD vs. 50 ml for MD). But, the quantities of volatiles isolated by MD were higher for high molecular weight components like *cis*-phytol (flower, HD: 1.9% vs. MD: 3.0%, leaf, HD: 4.6% vs. MD: 21.7%, stem, HD: 5.4% vs. MD: 14.5%), hexahydrofarnesyl acetone (flower, HD: 4.0% vs. MD: 12.2%, leaf, HD: 0.6% vs. MD: 19.1%, stem, HD: 1.7% vs. MD: 12.3%), and pentadecanal (flower, HD: 5.9% vs. MD: 11.7%, leaf, HD: 2.1% vs. MD: 6.8%, stem, HD: 5.1% vs. MD: 8.8%).

The major component of the oils of *A. hamzaoglui* was 1-octene-3-ol (flower, HD: 11.3% vs. MD: 8.8%, leaf, HD: 45.50% vs. MD: 3.3%, stem, HD: 32.6% vs. MD: 6.9%). Oxygenated sesquiterpenes were the minor constituents in all parts (flower, HD: 0.8% vs. MD: 0.4%, leaf, HD: 0.2% vs. MD: 0.4%, stem, HD: 0.4% vs. MD: 0.4%). of the *A. hamzaoglui* (table 2). The MD oils

could be distinguished from the HD oils by their richness in oxygenated sesquiterpenes (flower, HD: 2.0% vs. MD: 3.1%, leaf, HD: 4.7% vs. MD: 22.0%, stem, HD: 5.5% vs. MD: 14.8%) and terpene related compounds (flower, HD: 6.0% vs. MD: 15.6%, leaf, HD: 1.7% vs. MD: 22.3%, stem, HD: 3.1% vs. MD: 16.4%). The HD oils could be differentiated from the MD oils by the greater richness in oxygenated monoterpenes (flower, HD: 33.3% vs. MD: 2.3%, leaf, HD: 8.0% vs. MD: 2.7%, stem, HD: 10.8% vs. MD: 4.7%). Comparative study showed that the amount of total volatiles (flower, 95.0%, leaf, 92.9%, and stem, 95.0%) and the major constituent for the most volatiles were found to be better in HD of *A. hamzaoglui*.

It is interesting to mention that some identified oil constituents could be formed by auto-oxidation during the hydrodistillation procedure. It is reported that hexahydrofarnesyl acetone might be formed by photodegradation of the chlorophyll phytyl side-chain [24]. In our case, eugenol acetate was observed only in the essential oil of flower which was isolated by HD. The numbers of the identified terpenoids from the areal parts of *A. hamzaoglui* using HD and MD were similar (flower, HD: 30 vs. MD: 27, leaf, HD: 30 vs. MD: 29, stem, HD: 31 vs. MD: 30), respectively. Comparison of volatiles with those mentioned in the literature [15-20] indicates that identified compounds are in most cases similar in the essential oil.

As noted previously, the reduced cost of extraction is clearly advantageous for the MD method in terms of time and energy [12,13]. In comparison of HD with MD offers important advantages for cleanliness of the process, shorter extraction times, and substantial savings of energy. In our case, we observed the similar results for the identified volatiles of *A. hamzaoglui* using two different isolation techniques (HD and MD) with different ratios.

The antimicrobial activity of essential oils were tested against *E. coli*, *E. aeroginosa*, *Y. pseudotuberculosis*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *L. monocytogenes*, *B. cereus*, 702 Roma, *M. smegmatis*, *C. albicans*, *C. tropicali*s, and *S. cerevisiae*. The obtained inhibition zone results along with the standard antibiotics are presented in Table 3. From these results, the essential oil of the flower and leaf isolated by MD showed a higher activity against *E. coli*, *S. aureus*, *B. cereus*, *M. smegmatis* and *S. cerevisiae*. The essential oil of the leaf and stem extracted by HD gave activity only *M. smegmatis*. All tested oils gave antifungal inhibition in the range of 6 to 12 mm for *S. cerevisiae* except the stem oil isolated by HD. The essential oil of the leaf form MD showed only activity against *C. albicans*. The rest of the all microorganisms turned out to be completely resistant to all tested oils.

Table 1. Identified volatile components in the essential oils from flower, leaf, and stem of *A. hamzaoglui*

Lit. RI	Exp. RI	Compound	Flower		Leaf		Stem	
			HD	MD	HD	MD	HD	MD
			% Area	% Area	% Area	% Area	% Area	% Area
902	900	Heptanal	0.2	0.1	0.1	0.1	0.1	0.1
930	932	*α*-Thujene	0.2	0.1	0.2	0.2	0.2	0.2
939	943	*α*-Pinene[c]	0.3	0.2	0.3	0.3	0.3	0.2
952	955	2*E*-Heptenal	0.2	0.1	0.1	0.1	0.2	0.4
960	960	Benzaldehyde	0.2	0.2	0.2	0.4	0.7	0.9
979	982	1-Octen-3-ol	11.3	8.8	45.5	3.3	32.6	6.9
984	987	3-Octanone	0.7	1.6	3.6	0.5	1.1	1.1
993	991	2-Pentylfuran	1.0	0.9	2.4	-	1.3	1.1
997	1001	3-Octanol	1.5	1.0	4.7	-	1.5	0.4
999	1004	Octanal	0.2	3.8	1.9	1.0	2.6	2.9
1014	1014	2*E*,4*E*-Heptadienal	0.3	0.3	0.5	0.1	0.5	0.3
1029	1030	Limonene[c]	2.7	1.8	3.5	0.4	5.8	1.2
1042	1046	Benzen acetaldehyde	0.6	0.8	0.6	0.6	1.5	0.8
1060	1062	*γ*-Terpinene[c]	0.2	0.2	0.3	0.2	0.2	0.5
1067	1070	2-Octen-1-ol	0.8	0.6	1.4	0.3	1.3	0.3
1089	1089	Terpinolene	0.1	0.1	0.1	0.2	0.2	0.1
1097	1103	Linalool[c]	0.9	0.5	3.2	0.5	3.4	0.7
1101	1109	Nonanal	3.3	4.4	3.0	1.7	2.5	3.9
1155	1158	2*E*,6*Z*-Nonadienal	0.1	0.1	0.1	0.1	0.3	0.2

1162	1164	2*E*-Nonenal	0.4	0.8	0.3	0.6	0.5	0.8
1177	1179	Terpinen-4-ol	0.1	0.2	0.1	0.2	0.8	0.2
1189	1193	*α*-Terpineol[c]	0.3	0.2	0.4	0.1	0.5	0.1
1197	1198	Safranal	0.1	0.1	0.1	0.1	0.1	0.2
1200	1200	Dodecane[c]	0.1	0.1	0.1	0.3	0.1	0.1
1202	1208	Decanal	0.6	0.8	0.4	0.6	0.7	1.1
1221	1220	*β*-Cyclocitral	0.3	0.3	0.6	0.8	0.4	0.7
1230	1235	Nerol	0.2	0.1	0.2	-	0.5	-
1254	1254	*cis*-Piperitone epoxide	0.1	0.1	0.2	0.2	0.8	1.1
1256	1258	*trans*-Piperitone epoxide	0.1	-	0.1	0.2	0.1	0.3
1253	1262	Geraniol	1.8	0.5	2.1	0.5	3.9	1.1
1293	1296	2*E*,4*Z*-Decadienal	0.4	0.1	-	0.2	0.2	0.4
1300	1299	Tridecane[c]	0.1	0.6	0.1	0.2	0.1	0.1
1307	1309	Undecanal	0.2	0.4	0.1	0.2	0.2	0.4
1317	1321	2*E*,4*E*-Decadienal	1.6	1.9	0.2	0.7	0.7	1.4
1359	1365	Eugenol	29.4	0.3	1.0	0.1	0.3	0.3
1385	1383	*E*-*β*-Damascenone	0.1	0.1	0.1	0.1	0.1	0.2
1400	1400	Tetradecane[c]	0.1	0.1	0.1	0.3	0.1	0.2
1414	1412	*E*-*β*-Damascone	0.1	0.2	0.1	0.2	0.1	0.1
1419	1416	*E*-Caryophyllene	1.1	-	0.1	0.2	0.1	0.3
1455	1451	*α*-Humulene	0.2	-	0.2	0.1	0.1	0.1
1455	1455	Geranyl acetone	0.1	0.1	0.1	0.2	0.1	0.1
1457	1459	*β*-Farnesene	0.1	0.1	0.2	0.1	0.1	0.1
1465	1465	Farnesane	2.6	3.3	0.6	1.5	0.2	0.7
1489	1485	*β*-Ionone	0.1	0.2	0.3	0.3	0.2	0.3
1500	1491	Bicyclogermacrene	-	-	0.1	-	0.1	0.1
1500	1499	Pentadecane[c]	0.1	0.1	0.1	0.2	0.1	0.2
1510	1512	Tridecanal	0.2	0.2	0.1	0.3	0.2	0.2
1523	1535	Eugenol acetate	3.3	-	-	-	-	-
1563	1566	Nerolidol E	0.3	0.3	0.2	0.3	0.3	0.2
1583	1579	Caryophyllene oxide	0.5	0.1	-	0.1	0.1	0.2
1600	1598	Hexadecane[c]	0.1	0.1	0.1	0.4	0.1	0.2
1613	1613	Tetradecanal	0.7	1.2	0.2	0.8	0.8	1.1
1700	1699	Heptadecane[c]	0.2	0.4	0.2	0.7	0.5	0.7
1713	1718	Pentadecanal	5.9	11.7	2.1	6.8	5.1	8.8
1814	1815	Hexadecanal	0.4	1.0	0.1	0.5	0.4	0.8
1847	1847	Hexahydrofarnesyl acetone	4.0	12.2	0.6	19.1	1.7	12.3
1891	1891	Ethyl linoleolate	1.5	5.0	2.5	7.8	4.8	9.1
1919	1917	Farnesyl acetone	1.4	2.7	0.2	1.9	0.6	2.3
1924	1924	Methyl hexadecanoate	0.1	0.3	0.1	0.3	0.1	0.4
1948	1946	Isophytol	0.1	0.1	0.1	0.3	0.1	0.3
1984	1997	Hexadecanoic acid	7.4	12.1	-	0.7	6.1	0.7
2100	2098	Heneicosane[c]	0.1	0.6	0.1	0.4	0.1	0.4
2098	2095	Methyl linolenate	0.2	0.2	0.1	0.5	0.3	0.5
2114	2114	*cis*-Phytol	1.9	3.0	4.6	21.7	5.4	14.5
2200	2199	Docosane[c]	0.1	0.2	0.1	0.2	0.1	0.2
2300	2213	Phytol acetate	0.2	0.1	0.3	0.5	0.3	1.1
2300	2298	Tricosane[c]	0.6	0.5	1.4	5.3	0.2	0.3
2400	2398	Tetracosane[c]	0.1	0.2	0.1	0.2	0.1	0.3
2500	2499	Pentacosane[c]	0.5	2.2	0.2	0.4	0.1	0.7
		Total isolate	95.0	90.7	92.9	87.4	95.0	88.2

[a] RI calculated from retention times relative to that of n-alkanes (C_6-C_{32}) on the non-polar HP-5 column.
[b] Percentages obtained by FID peak-area normalization.
[c] Identified by authentic samples.

Table 2. The chemical class distribution in the essential oils from areal parts of *A. hamzaoglui*.

Compound class	Flower				Leaf				Stem			
	HD		MD		HD		MD		HD		MD	
	% Area	NC[a]	% Area	NC[a]	% Area	NC[a]	% Area	NC[a]	% Area	NC[a]	% Area	NC[a]
Terpenoids												
Monoterpene hydrocarbons	3.5	5	2.4	5	4.4	5	1.3	5	6.7	5	2.2	5
Oxygenated monoterpenes	33.3	10	2.3	9	8.0	10	2.7	9	10.8	10	4.7	9
Sesquiterpene hydrocarbons	4.0	4	3.4	2	1.2	5	1.9	4	0.6	5	1.3	5
Oxygenated sesquiterpenes	0.8	2	0.4	2	0.2	1	0.4	2	0.4	2	0.4	2
Oxygenated diterpenes	2.0	2	3.1	2	4.7	2	22.0	2	5.5	2	14.8	2
Terpene related compounds	6.0	7	15.6	7	1.7	7	22.3	7	3.1	7	16.4	7
Alcohols	13.6	3	10.4	3	51.6	3	3.6	2	35.4	3	7.6	3
Aldehydes	15.5	17	27.9	17	10	16	14.8	17	17.2	17	24.5	17
Hydrocarbons	2.1	11	5.1	11	2.5	11	8.6	11	1.6	11	3.4	11
Esters	5.1	4	5.5	3	2.7	3	8.6	3	5.2	3	10.0	3
Others	9.1	3	14.6	3	6.0	2	1.2	2	8.5	3	2.9	3

[a]NC: Number of compounds

Table 3. Screening for antimicrobial activity of the essential oils from areal parts of *A. hamzaoglui* (50µL).

Sample	Stoc. µg/mL	Microorganisms and inhibition zone (mm)									
		Ec	Yp	Pa	Sa	Ef	Li	Bc	Ms	Ca	Sc
Flower (HD)	10000	-	-	-	-	-	-	-	-	-	12
Leaf (HD)	10000	-	-	-	-	-	-	-	6	-	10
Stem (HD)	10000	-	-	-	-	-	-	-	7	-	-
Flower MD)	10000	7	-	-	6	-	-	6	-	-	8
Leaf (MD)	10000	7	-	-	7	-	-	7	10	6	6
Stem (MD)	10000	-	-	-	6	-	-	-	7	-	6
Amp.	10 µg	10	18	18	35	10	10	15		-	-
Srp.	10 µg								35		
Flu	5 µg									25	>25

Ec*: Escherichia coli*, Ea: *Enterobacter aeroginosa*, Yp: *Yersinia pseudotuberculosis*, Pa: *Pseudomonas aeruginosa*, Sa: *Staphylococcus aureus*, Ef: *Enterococcus faecalis*, Li: *Listeria monocytogenes*, Bc: *Bacillus cereus* 702 Roma, Ms: *Mycobacterium smegmatis*, Ca: *Candida albicans*, Ct: *Candida tropicalis*, *Saccharomyces cerevisiae*, Amp.: Ampicillin, Str.: Streptomycin, Flu.: Fluconazole, (-): no activity.

Acknowledgements

This work was supported by grants from Karadeniz Technical University Research Fund and State Planning Agency (DPT) of Turkey.

References

[1] G. Burnie, S. Forrester, D. Greig, S. Guest, M. Harmony, S. Hobley, G. Jackson, P. Lavarack, M. Ledgett, R. McDonald, S. Macoboy, B. Molyneux, D. Moodie, J. Moore, T. North, D. Newman, K.

Pienaar, G. Purdy, J. Silk, S. Ryan and G. Schien (1999). Botanica. Australia: Random House Australia Pty Ltd.
[2] D.F. Chamberline and V.A. Matthews, L. *Astragalus* in P.H. Davis (1970). Flora of Turkey and the East Aegean Islands, Edinburgh University Press, Vol. 3, p. 49
[3] A. Uzun, S. Terzioğlu, S. Palabaş Uzun and K. Coşkunçelebi (2009). *Astragalus ansinii* sp. nov. (Fabaceae) from Turkey, and a contribution to the sectional taxonomy, *Nordic J. Botn.* **27,** 397-401.
[4] O. Ketenoğlu and Y. Menemen (2003). *Astragalus hamzaoglui* (Fabaceae), a new species from the east Black Sea region, Turkey, *Ann. Bot. Fennici*. **40**, 59-62.
[5] T. Baytop (1999). Therapy with Medicinal Plants in Turkey, University of Istanbul, Istanbul, p. 273.
[6] V. Niknam and H. Ebrahimzadeh (2002). Phenolics content in *Astragalus* species, *Pak. J. Bot*. **34**, 283-289.
[7] Y. Jayaprakash Rao, Ch. Ramakrishna Reddy, N. Gangadhar and G.L.D Krupadanam (2009). Phytochemical Investigation of the Whole Plant of *Astragalus leucocephalus*, *Ind. J. Chem*. **48**, 1329-1332.
[8] I. Krasteva, I. Nikolova, N. Danchev and S. Nikolov (2004). Phytochemical analysis of ethyl acetate extract from *Astragalus corniculatus* Bieb. and brain antihypoxic activity, *Acta Pharm.* **54**, 151-156.
[9] A.R. Jassbi, S. Zamanizadehnajari, P.A Azar and S. Tahara (2002). Antibacterial diterpenoids from Astragalus brachystachys, *Z. Naturforsch.* **57**, 1016-1021.
[10] E. Bedir, I. Çalis, S. Piacente, C. Pizza and I.A. Khan (2000). A New Flavonol Glycoside from the Aerial Parts of *Astragalus* vulneraria, *Chem. Pharm. Bull.* **48,** 1994-1995.
[11] N. Kahriman, G. Tosun, H. Genç and N. Yaylı (2010). A Comparative Essential Oil Analysis of *Geranium sylvaticum* Extracted by Hydrodistillation and Microwave distillation, *Turk. J. Chem.* **34**, 969-976.
[12] M.E. Lucchesi, F. Chemat and J. Smadja (2004). Solvent free microwave extraction of essential oils from aromatic herbs: comparison with conventional hydro-distillation, *J. Chrom. A*. **1043**, 323-327.
[13] M.A. Ferhat, B.Y. Meklati, F. Visinoni, M.A. Vian and F. Chemat (2008). Solvent free microwave extraction of essential oils Green chemistry in the teaching laboratory, *Chem. Today.* **26**, 47-50.
[14] R.P. Adams (2004). Identification of essential oil components by Gas Chromatography / Quadrupole Mass Spectroscopy. Allured, Carol Stream, IL, USA.
[15] F. Sefidkon and M.S. Khajavi (1999). Chemical composition of the essential oils of two Salvia species from Iran: Salvia verticillata L. and Salvia santolinifolia Boiss., *Flavour Fragr. J.* **14**, 77-78.
[16] N. Yaylı, A. Yaşar, C. Güleç, A. Usta, S. Kolaylı, K. Coşkunçelebi and Ş. Karaoğlu (2005). Composition and Antimicrobial Activity of Essential Oil from Centaurea sessilis and Centaurea armena, *Phytochemistry.* **66**, 1741-1745.
[17] K. Javidnia, R. Miri, M. Kamalinejad, H. Sarkarzadeh and A. Jamalianm (2004). Chemical composition of the essential oils of *Anthemis altissima* L. grown in Iran, *Flav. Fragr. J*. **19**, 213-216.
[18] O. Üçüncü, N. Yaylı, A. Yaşar, S. Terzioğlu and N. Yaylı (2008). Chemical composition of the essential oils from flower, leaf, and stem of *Senecio trapezuntinus* Boiss., *Nat. Prod. Com.* **3**, 925-928.
[19] A. Usta, O. Üçüncü, T.B. Cansu, S. Terzioğlu and N. Yaylı (2009). Chemical Composition of the Essential Oils from the Flower, Leaf and Stem of Aquilegia olympica Grown in Turkey, *Asian J. Chem.* **21**, 6569-6574.
[20] N.Y. İskender, N. Yaylı, N. Yıldırım, T.B. Cansu and S. Terzioğlu (2009). The Volatile Constituents of the Flower, Leaf, and Stem of Verbascum wiedemannianum Grown in Turkey, *J. Oleo Sci*. **58**, 117-121.
[21] C. Perez, M. Pauli and P. Bazerque (1990). An antibiotic assay by the agar well diffusion method. *Acta Biol. Med. Exper.* **15**, 113-115.
[22] I. Ahmad, Z. Mehmood and F. Mohammed (1998). Screening of some Indian medicinal plants for their antimicrobial properties, *J. Ethnopharma.* **62**, 183-193.
[23] G.L. Woods, B.A. Brown Elliott, E.P. Desmond, G.S. Hall, L. Heifets, G.E. Pfyffer, J.C. Ridderhof, R.J. Jr. Wallace, N.C. Warren and F.G. Witebsky (2003). *Approved Standard. NCCLS document* M24-A.
[24] J.F. Rontani, P. Cuny and V. Grossi (1996). Photodegradation of chlorophyll phytyl chain in senescent leaves of higher plants, *Phytochemistry*. **42**, 347-351.