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Original Article

Antibacterial Properties of *Ajuga chamaecistus* Subsp. *Scoparia* and Chemical Composition of its Oils

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Abstract

In the present study, we reported the essential oils chemical composition and antibacterial activities of the aerial parts of *Ajuga chamaecistus* Ging. ex Benth. Subsp. *Scoparia* (Boiss.) Rech.f. that were collected during May 2013 and April 2014 and extracted by SDE (simultaneous distillation–extraction) and Clevenger apparatus. GC/MS analysis of the plant essential oils led to the identification of chemical composition of its oils. The main constituents of the essential oils in two SDE (simultaneous distillation–extraction) and Clevenger apparatus were *β*-Pinene (23.5%), *α*-Pinene (6.9%), Limonene (10.8%), Linalool (8.3) and Eugenol (7.7%). Essential oil was tested for their antibacterial activities using Gram-negative bacteria, Gram-positive bacteria. The plant was screened for its antibacterial activity and showed antibacterial activity against *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *P. vulgaris*, *S. paratyphi*, *B. subtilis*, *S. aureus, S. epidermidis* and *S. dysenteriae*.

Keywords: *Ajuga chamaecistus* Subsp. *Scoparia***,** Antibacterial activity, Essential oils, SDE and Clevenger

Introduction

The subspecies of *Ajuga* plants are irregularly given out in America, Australia, Korea, China, and Japan and also widespread in Europe [1]. There are five species of *Ajugu* L. in Iran including *Ajuga austro-iranica* Rech f., *Ajuga Chamaecistus* Ging, *Ajuga comate Stapf, Ajuga Orientalis* L. and *Ajuga reptans* L. Compounds isolated from plant of *Ajuga* have been demonstrated the biological activities including antibacterial activities against *Staphylococcus aureus* [2], Cancer chemopreventive [3], hypoglycemic activity [4], vasoconstrictor [5], antiarthritic effects in acute and chronic models [6], anti-inflammatory [7], cytotoxicity against Jurkat cells [8], anti-proliferation against tumor cells in vitro [9], neuroprotective effects against MPP^+ [10], antimalaria [11], activities. There have been many phytochemical screening on *Ajuga* species, focusing mainly on the isolation of phytoecdysteroids and diterpenes and on their antifeedant and insect-growth-inhibiting properties [12]. Consequently, the design of simple methods for the screening of chemical composition and biological properties this plant has commanded vast attention. The essential oils and plant crude extracts from the medicinal plants are one of the most useful bioactive groups of natural compounds for the accessibility and preparation of safer and easier antimicrobial agents. Antimicrobial have undergone a topic of growing attention in the past decade [13-18]. Microbial contamination is very important issue in the field of food, beverage, cosmetic, and pharmaceutical industries. According to these facts, the plant kingdom with a notable variety in producing natural compounds has achieved a special interest and, today, accessing to plant materials with dual antioxidant and

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antimicrobial capabilities is an ideal goal in the field of study on food additives.

The antimicrobial activities of essential oils have been known for a long time, and a number of researches have been conducted on their antimicrobial properties by means of bacteria and fungi. In the present research, we report the essential oils chemical composition and antimicrobial activities of the aerial parts of *Ajuga chamaecistus* Subsp. *Scoparia* by simple and standard methods.

Materials and Methods

Materials

Plant Material

Flowering samples of *Ajuga chamaecistus Subsp*. *Scoparia* were collected during May 2013 and April 2014 from Kashan area (Vadeqan, Iran) at an altitude of ca. 2100 m and tines of flowerer were separated, dried in the shade and ground (80 mesh). An authenticated specimen of the plant was also deposited in the herbarium of the Kashan Research Botanical Garden, Research Institute of Forests and Rangelands, Kashan, Iran.

Solvents and chemicals

Analytical grade methanol, ethanol, and dimethyl sulphoxide (DMSO), HPLC grade chloroform, standard Folin-Ciocalteu's, anhydrous sodium sulphate, sodium carbonate, and Tween 40 were obtained from Merck (Darmstadt, Germany). Ultra-pure water was used for the experiments.

Preparation of the Extracts

Isolation of the essential oils

Crushed flowers of the plant were hydrodistilled for 3.5 h using an all-glass Clevenger-type apparatus as recommended by European Pharmacopoeia [19]. The distilled essential oils were dried over anhydrous sodium sulphate, filtered and stored in amber vials at low temperature (4 °C) before use for analysis. Also, we used SDE (simultaneous distillation–extraction) apparatus.

Chromatographic Analysis

Gas chromatographic

Oils obtained from the aerial parts were analyzed using an Agilent HP-6890 gas chromatograph (Agilent Technolo-gies, Palo Alto, CA, USA) with HP-5MS 5% phenylmethylsiloxane capillary column (30 m×0.25 mm, 0.25µm film thickness; Restek, Bellefonte, PA) equipped with a flame ionization detector (FID). Oven temperaturewas kept at 60 ºC for 3 min initially, and then raised with a rate of 3 ºC/min to 250 ºC. Injector and detector temperatures were set at 220 and 290 ºC, respectively. Helium (1 ml/min) was used as carrier gas and diluted samples (1/1000 in *n*-pentane, v/v) of 1.0 µl were injected manually in the splitless mode. Peak area percent of each compound relative to the area percent of the entire spectrum (100%) were used for obtaining its quantitative data. The injection was repeated three times and the peak area percents were reported as means ± SD of triplicates. Co-injection of selected commercially available components of the essential oil were also carried out and led to the enrichment of the respected picks in the spectrum and further confirmation of their identities.

Gas Chromatography/Mass Spectrometry:

GC/MS analysis of the oil was carried out on an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5MS 5% phenylmethylsiloxane capillary column (30 m×0.25 µm, 0.25 µm film thickness; Restek, Bellefonte, PA) equipped with an Agilent HP-5973 mass selective detector in the electron impact mode (Ionization energy: 70 eV) operating under the same conditions as described above. Retention indices were calculated for all components using a homologous series of *n*-alkanes injected in conditions equal to the sample one. Identification of components of essential oil was based on retention indices (RI) relative to *n*-alkanes and computer matching with the Wiley275.L and Wiley7n.L libraries, as well as comparisons of the fragmentation pattern of the mass spectra with data published in the literature [20].

Antimicrobial Activity

Microbial Strains

The essential oils tested against a set of 9 microorganisms. Following microbial strains were provided by Iranian Research Organization for Science and Technology (IROST) and used in this research: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 10536), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29737), *Klebsiella pneumonia* (ATCC 10031), *Staphylococcus epidermidis* (ATCC 12228), *Shigella dysenteriae* (PTCC 1188), *Proteus vulgaris* (PTCC 1182), *Salmonella paratyphi-A*

serotype (ATCC 5702). Bacterial strains were cultured overnight at 37 ºC in nutrient agar (NA).

Disk diffusion assay

Determination of antimicrobial activities of essential oil was accomplished by agar disk diffusion method [21]. The plant extracts were dissolved in DMSO to a final concentration of 30 mg/ml and filtered by 0.45 µm Millipore filters for sterilization. Anti-microbial tests were carried out using the disk diffusion method reported by Murray [22], and employing 100 µl of suspension containing 10^8 CFU/ml of bacteria. The disks (6 mm in diameter) impregnated with 10 µl of the essential oil, its major components or the extracts solutions (300µg/disk) and DMSO (as negative control) were placed on the inoculated agar. The inoculated plates were incubated for 24 h at 37 ºC for bacterial strains. Gentamicin (10 µg/disk), and rifampin (5 µg/disk) were used as positive controls for bacteria. The diameters of inhibition zones were used as a measure of antimicrobial activity and each assay was repeated thrice.

Determination of minimal inhibition concentrations (MIC)

Bacterial strains sensitive to the essential oil of the plant in disk diffusion assay were studied for their minimal inhibition concentration (MIC) values using micro-well dilution assay method [23]. The inocula of the microbial strains were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oil dissolved in 10% DMSO solution were first diluted to the highest concentration (5 mg/ml) to be tested, and then serial twofold dilutions were made in a concentration range from 0.078 to 5 mg/ml in 10 ml sterile test tubes containing brain heart infusion (BHI) broth for bacterial strains. The 96-well plates were prepared by dispensing 95 µl of the cultures media and 5 µl of the inoculum into each well. A 100 µl aliquot from the stock solutions of the plant initially prepared at the concentration of 5 mg/ml was added into the first wells. Then, 100 µl volumes from their serial dilutions were transferred into six consecutive wells. The last well containing 195 µl of the cultures media without the test materials and 5 µl of the inoculum on each strip was used as the negative control. The final volume in each well was 200 µl. Gentamicin and rifampin for bacteria were used as standard drugs for positive control in conditions identical to tests materials. The plates were covered with sterile plate sealers. Contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. The MIC value was defined as the lowest concentration of the plant essential oil required for inhibiting the growth of microorganisms. All tests were repeated three times.

Determination of Minimum Microbicidal Concentration

(MMC):

The minimum microbicidal concentration (MMC), which includes minimum bactericidal (MBC) and minimum fungicidal concentrations (MFC), of the essential oil was determined according to the MIC values for 24 h at 37 ºC for bacteria. The lowest concentration in the medium which had fewer than five colonies was taken as the minimum microbicidal concentration (MMC) [14].

Results and Discussion

Chemical Composition of the Essential Oils

The essential oils of the aerial parts of ***Ajuga chamaecistus Subsp. Scoparia*** were obtained by SDE and Clevenger apparatus in the yields of 0.018% and 0.094% (v/w) for April 2014 and 0.005% and 0.053% (v/w) for May 2013 respectively. (Table1). Essential oils were analyzed by GC/FID and GC/MS systems and the oils components were identified both quantitatively and qualitatively. Seventy components were identified in the essential oils obtained from the aerial parts of ***Ajuga chamaecistus* Subsp. *Scoparia*** that were collected during May 2013 and April 2014 and extracted by SDE and Clevenger apparatus (Table 2). The main constituents of the essential oils in two SDE and Clevenger apparatus were β-Pinene, α-Pinene, Limonene, Linalool and Eugenol (Fig. 1).

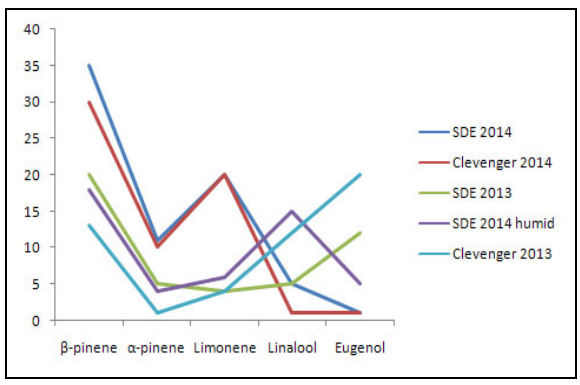

Fig. 1 A compare of components of essential oils

Table 1 Yields of The essential oils of the aerial parts *Ajuga chamaecistus* Subsp. *Scoparia*

Entry	Method	Yield% May 2013	Yield% April 2014
1	Clevenger	0.01	0.02
2	SDE	0.05	0.09
3	SDE for humid aerial parts	-	0.02

Table 2 Chemical composition of the essential oils of *Ajuga chamaecistus* Subsp. *Scoparia*

Row	Compound	Composition(%)							
		family	SDE April 2014	SDE May 2013	SDE (humid) April 2014	Clevenger April 2014	Clevenger May 2013	RI[a]	RI[b]
1	2E-Hexenal	O[c]	0.8	2.5	-	-	-	859	855
2	3Z-Hexenol	O	-	-	13.1	-	-	866	859
3	2E-Hexenol	O	-	-	2.3	-	-	874	862
4	α-Thujene	M[d]	7.1	3.7	4.6	9.5	1.5	935	930
5	α-Pinene	M	11.1	6.0	4.6	10.5	2.6	944	939
6	Camphene	M	0.3	-	-	0.2	-	957	954
7	Sabinene	M	-	-	0.6	-	-	981	975
8	β-Pinene	M	35.9	19.3	17.7	31.3	13.4	993	979
9	β-Myrcene	M	5.5	1.8	2.3	3.6	1.1	1002	990
10	α-Phellandrene	M	0.6	-	-	0.6	-	1014	1002
11	2E,4E-Heptadienal	O	-	0.6	-	-	-	1022	1016
12	o-cymene	M	-	0.8	-	-	-	1034	1026
13	Limonene	M	19.5	5.5	6.5	18.8	4.1	1042	1029
14	Salicylaldehyde	O	-	0.8	-	-	-	1055	1044
15	β-Ocimene	M	0.2	-	-	0.2	-	1055	1050
16	γ-Terpinene	M	-	-	-	0.1	-	1069	1059
17	α–Terpinolene	M	-	-	-	0.3	-	1097	1088
18	Linalool	OM[e]	5.0	7.2	16.2	1.7	11.2	1113	1096
19	α-Campholenal	OM	-	-	-	-	0.7	1137	1126
20	E-Pinocarveol	OM	-	3.5	-	0.1	4.0	1154	1139
21	E-Verbenol	OM	-	1.9	0.8	0.1	1.9	1157	1144
22	Pinocarvone	OM	-	1.0	-	-	1.5	1175	1164
23	n-Nonanol	O	-	-	-	-	2.1	1183	1169
24	Terpinene-4-ol	OM	0.2	-	-	0.1	-	1188	1177
25	α–Terpineol	OM	0.5	1.2	2.4	0.4	1.6	1203	1188
26	Myrtenal	OM	-	1.0	-	-	3.9	1210	1195
27	Myrtenol	OM	-	1.0	-	-	-	1213	1195
28	Verbenone	OM	-	0.7	-	-	-	1224	1205
29	E-Carveol	OM	-	0.7	-	-	-	1233	1216
30	Geraniol	OM	-	0.6	-	-	-	1266	1252
31	n-Decanol	O	-	-	-	-	1.1	1182	1269
32	Thymol	OM	-	-	-	2.4	2.0	1304	1290
33	p-vinyl-guaiacol	O	0.3	3.9	-	-	-	1328	1309
34	δ-Elemene	S[f]	0.2	-	-	0.1	-	1346	1338
35	Eugenol	P[g]	1.3	12.5	4.7	1.1	18.7	1373	1359
36	α-Copaene	S	2.2	1.0	-	2.3	2.2	1387	1376
37	Geranyl acetate	OM	-	-	-	0.1	-	1393	1381
38	B-Damascenone	O	-	1.6	-	-	-	1396	1384
39	β-Bourbonene	S	-	-	-	0.1	1.3	1397	1388
40	β-Cubebene	S	-	-	-	0.2	-	1400	1388
41	Z-Jasmone	O	-	-	1.0	-	-	1411	1392
42	Methyl eugenol	P	-	-	-	0.6	-	1420	1403
43	α-Cedrene	S	-	0.6	-	-	-	1429	1411
44	E-Caryophyllene	S	-	-	-	0.1	-	1431	1419
45	α-Bergamotene	S	-	-	-	0.2	-	1445	1434
46	Coumarin	P	-	1.2	1.1	-	-	1450	1434
47	Z-Farnesene	S	0.8	-	2.8	0.7	0.8	1453	1442
48	Geranyl acetone	O	-	1.3	-	-	4.1	1464	1455
49	E- Farnesene	S	0.8	-	2.1	1.4	-	1465	1456
50	GermacreneD	S	0.7	0.4	-	2.3	1.6	1493	1485
51	β-Ionone	O	-	0.8	0.6	0.2	1.6	1500	1488

52	Bicyclogermacrene	S	2.7	0.8	2.2	3.9	3.4	1510	1500
53	β-Bisabolene	S	1.2	0.5	3.1	0.8	1.3	1519	1505
54	δ-Cadinene	S	1.9	0.8	3.1	1.8	1.5	1536	1523
55	Nerolidol	OS[h]	-	-	-	0.1	-	1577	1563
56	3Z-Hexenyl benzoate	O	-	-	0.7	-	-	1583	1566
57	GermacreneD-4-ol	OS	0.6	-	1.2	1.6	-	1592	1575
58	Spathulenol	OS	-	3.2	1.1	-	5.3	1595	1578
59	Globulol	OS	-	-	-	0.2	-	1600	1590
60	Junenol	OS	-	-	-	0.2	-	1620	1619
61	τ-Muurolol	OS	-	-	-	0.2	-	1657	1642
62	Z-Methyl benzoate	O	-	-	0.8	-	-	1661	1649
63	α-Cadinol	OS	0.2	-	-	0.3	-	1668	1654
64	Shyobunol	OS	-	-	-	-	1.5	1708	1656
65	Myristic acid	O	-	1.7	-	-	-	1792	
66	hexahydrofarnesyl acetone	O	-	1.4	-	-	2.7	1855	
67	5E,9E-Farnesyl acetone	O	-	0.5	-	-	-	1929	1913
68	E-Phytol	OD[i]	0.2	2.7	1.5	0.9	1.0	1943	1943
69	Palmitic acid	O	-	3.2	-	-	-	1976	1960
70	Geranyl linalool	OD	-	-	-	0.6	-	2040	2027
Σ			100	97.9	97.1	99.9	100		

a: Relative retention indices to C8–C24 n-alkanes on HP-5MS column.

b: Literature retention indices.

c: other

d: monoterpene

e: oxygenated monoterpen

f: sesquiterpene

g: phenylpropene

h: oxygenated sesquiterpene

i: oxygenated diterpen

Table 3 Antimicrobial activities of the essential oil

	Essential oil			Rifampin		Gentamicin	
Test microorganisms	DD [a]	MIC[b]	MMC[c]	DD	MIC	DD	MIC
Gram-negative bacteria							
P. aeruginosa	9	1000	2000			23	500
E. coli	12	250	500	11	500	20	500
K. pneumoniae	16	1000	4000	7	250	22	500
P. vulgaris	14	1000	2000	10	125	23	500
S. paratyphi-A serotype	10	4000	>4000			21	500
Gram-positive bacteria							
B. subtilis	10	125	250	13	15.26	21	500
S. aureus	10	2000	>4000	10	250	21	500
S. epidermidis	15	250	500	40	250	35	500
S. dysenteriae	16	1000	4000	8	250	18	500

A dash (-) indicate no antimicrobial activity.

[a] Inhibition zone in diameter (mm) around the impregnated discs.

[b] Minimal inhibition concentrations (as µg/ml).

[c] Minimum microbicidal concentration

Antimicrobial Activity

The antimicrobial activity of *Ajuga chamaecistus* Subsp. *Scoparia* essential oil were evaluated against a panel of 9 microorganisms and their potency were assessed qualitatively and quantitatively by the presence or absence of inhibition zones, zone diameters and MIC values. The results are given in Table 3. The plant essential oil showed antimicrobial activities significantly. In most cases, antibacterial activities of the plant samples were evaluated from half to near to that of

the positive control drugs rifampin and gentamycin. The maximum inhibition zones and MIC values for microbial strains sensitive to the plant products were in the range of 9–16 mm and 125-4000 µg/ml, respectively. Maybe, the main constituents of the essential oils (β-Pinene, α-Pinene, Limonene, Linalool and Eugenol) are mainly responsible for their antimicrobial activity.

Conclusion

In conclusion, we have described the essential oils chemical composition and antibacterial activity of the aerial parts of *Ajuga chamaecistus* Subsp. *Scoparia.* The plant was screened for its antibacterial activity and showed antibacterial activity against *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *P. vulgaris*, *S. paratyphi*, *B. subtilis*, *S. aureus, S. epidermidis* and *S. dysenteriae*. Water-distilled essential oil from *Ajuga chamaecistus* Ging, an endemic growing wild in Iran, was investigated by hosseini *et al*. [24] and eight compounds were identified representing 93.3% of the oil whereas p-cymene (34.5%), β-pinene (18.0%), α-phellandrene (17.8%) and α-pinene (15.2%) were major constituents. The main constituents of the essential oils of *Ajuga chamaecistus* Subsp. *Scoparia* were *β*-Pinene (23.5%), *α*-Pinene (6.9%), Limonene (10.8%), Linalool (8.3) and Eugenol (7.7%). To the best of our knowledge, there is no report on the chemical composition of the essential oil and its antibacterial potential of *Ajuga chamaecistus* Subsp. *Scoparia* in the literatures.

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References

1. Camps F, Coll J. Insect allelochemicals from ajuga plants, Phytochem. 1993; 32:1361-1370.
2. Chen H, Tan RX, Liu ZL, Zhang Y. Antibacterial Neoclerodane Diterpenoids fromAjuga lupulina, J. Nat. Prod. 1996; 59:668-670.
3. Konoshima T, Takasaki M, Tokuda H, Nishino H, Cancer chemopreventive activity of an iridoid glycoside, 8-acetylharpagide, fromAjuga decumbens. Cancer Lett. 2000;157:87-92.
4. Kutepova TA, Syrov VN, Khushbaktova ZA, Saatov Z. Hypoglycemic activity of the total ecdysteroid extract from Ajuga turkestanica. Pharm. Chem. J. 2001;35:608-609.
5. Breschi MC, Martmotti E, Catalano S, Flamini G, Morelli I, Pagni AM. Vasoconstrictor activity of 8-0-acetylharpagide from ajuga reptans. J. Nat. Prcd. 1992;55:1145-1148.
6. Kaithwas G, Gautam R, Jachak SM, Saklani A. Antiarthritic effects of Ajuga bracteosa Wall ex Benth. in acute and chronic models of arthritis in albino rats, Asian Pac J Trop Biomed. 2012;2:185-188.
7. Hsieh WT, Liu YT, Lin WC. Anti-inflammatory properties of Ajuga bracteosa in vivo and in vitro study and their effects on mouse model of liver fibrosis**.** J. Ethnopharmacol*,* 2011;135:116-125.
8. Akbay P, Gertsch J, Calis I, Heilmann J, Zerbe O, Sticher O. Novel Antileukemic Sterol Glycosides fromAjuga salicifolia, Helv. Chim. Acta. 2002; 85:1930-1942.
9. Chiou CT, Kuo YH, Chan YY, Juang SH, Chan HH, Wu TS. Ajugalide-B (ATMA) is an anoikis-inducing agent fromAjuga taiwanensis with antiproliferative activity against tumor cellsin vitro. Phytochem. 2012; 80: 64–69.
10. Guo P, Li Y, Xu J, Guo Y, Jin DQ, Gao J, Hou W, Zhang T. Neo-Clerodane diterpenes fromAjuga ciliataBunge and their neuroprotective activities. *Fitoterapia* 2011;82:1123-1127.
11. Cocquyt K, Cos P, Herdewijn P, Maes L. Van den Steen P E, Laekeman G, Ajuga remota Benth: From ethnopharmacology to phytomedical perspective in the treatment of malaria, Phytomed. 2011;18:1229-1237.
12. Ben Jannet H, H-Skhiri F, Mighri Z, Simmonds MSJ, Blaney WM. Antifeedant activity of plant extracts and of new natural diglyceride compounds isolated fromAjuga pseudoialeaves againstSpodoptera littoralislarvae, Ind. Crop. Prod. 2001; 14:213-222.
13. Maggi F, Cecchini C, Cresci A, Coman MM, Tirillini B, Sagratini G, Papa F, Vittori S. Chemical Composition and Antimicrobial Activity of the Essential Oils from Several Hypericum Taxa (Guttiferae) Growing in Central Italy (Appennino Umbro-Marchigiano). Chem. Biodivers. 2010;7:447-466.
14. Li WJ, Nie SP, Liu XZ, Zhang H, Yang Y, Yu Q, Xie MY. Antimicrobial properties, antioxidant activity and cytotoxicity of ethanol-soluble acidic components from Ganoderma atrum. Food. Chem. Toxicol. 2012; 50: 689–694.
15. Ye CL, Dai DH, Hu WL. Antimicrobial and antioxidant activities of the essential oil from onion (Allium cepaL.) Food Control. 2013;30:48-53.
16. Ebrahimabadi AH, Djafari-Bidgoli Z, Mazoochi A, Jookar Kashi F, Batooli H. Essential oils composition, antioxidant and antimicrobial activity of the leaves and flowers of Chaerophyllum macropodum Boiss. Food Control. 2010;21:1173-1178.
17. Sökmen A, Vardar-Ünlü G, Polissiou M, Daferera D, Sökmen M, Dönmez E. Antimicrobial Activity of

Essential Oil and Methanol Extracts of Achillea sintenisii Hub. Mor. (Asteraceae). Phytother. Res. 2003;17:1005-1010.
18. Casetti F, Bartelke S, Biehler K, Augustin M, Schempp CM, Frank U. Antimicrobial Activity Against Bacteria with Dermatological Relevance and Skin Tolerance of the Essential Oil from Coriandrum sativum L. Fruits Phytother. Res. 2012; 26: 420–424.
19. Anonymous. European pharmacopoeia (3rd ed.). Strasburg, France: Council of Europe, 1996. pp.121-122.
20. Adams RP, Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Co., Carol Stream, IL, USA, 2001
21. Wayne, PA, National Committee for Clinical Laboratory Standard. In Performance standards for antimicrobial disk susceptibility test. Sixth approved standard. M2-A6,1997.
22. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolke, R H. Manual of clinical microbiology (7th ed., pp. 1773). Washington, DC: ASM. 1995.
23. Gulluce M, Sokmen M., Sahin F, Sokmen A, Adiguzel A. Ozer H. Biological activities of the essential oil and methanolic extract ofMicromeria fruticosa (L) Druce ssp. Serpyllifolia (Bieb) PH Davis plants from the eastern Anatolia region of Turkey. J. Sci. Food Agric. 2004;84:735-741.
24. Hosseini MM. Pazoki A. Zamani HA. Akhlaghi H. Chemical Composition of the Essential Oil from Aerial Parts of *Ajuga chamaecistus* Ging. subsp. Scopria in Brackish Regions of Iran. Jeobp. 2011;14:101-105.