Karaelmas Science and Engineering Journal
Journal home page: http://fbd.beun.edu.tr
DOI: 10.7212%2Fzkufbd.v8i1.682

Research Article
Received / Geliş tarihi : 07.11.2016
Accepted / Kabul tarihi : 18.05.2017

Chemotype Variation and Antimicrobial Properties of *Achillea millefolium* L. subsp. *millefolium* var. *millefolium*

Achillea millefolium L. subsp. millefolium var. millefolium'un Kemotip Varyasyonu ve Antimikrobiyal Özellikleri

Ibrahim Halil Gecibesler*

Bingol University, Faculty of Health Science, Department of Occupational Health and Safety, Laboratory of Natural Product Research, Bingol, Turkey

Abstract

Achillea millefolium L. subsp. *millefolium* var. *millefolium* were collected from six different localities, growing in wild natural habitat in the Eastern Anatolian province, Bingol/Turkey. Essential oils of the samples were isolated using an apparatus with Clevenger-type and analyzed by GC-MS/FID instrument. In total 56 components were identified from six samples collected from different localities and important qualitative and quantitative differences were observed. The most abundant components from all samples were found as α-pinene, β-pinene, sabinene, 1,8-cineol, endo-bornyl acetate and α-terpineol. The antimicrobial activities of essential oils of six different samples were tested against bacteria and yeast strains. The essential oils of sample collected from town of Sancak were exhibited higher activities against the microorganisms of *C. albicans, S. aureus* and *S. enterica* and it may be a valuable candidate for drug formulation

Keywords: *Achillea*, Antimicrobial activity, Aromatic plant, 1,8-cineol, α-terpineol, Yarrow

Öz

Türkiye'de Doğu Anadolu Bingöl ilinde yabani doğal habitatta yetişmekte olan *Achillea millefolium* L. subsp. *millefolium* var. *millefolium* altı farklı lokaliteden toplandı. Örneklerin uçucu yağı Clavenger-tipi bir aparat ile izole edildi ve GC-MS/FID spektroskopisi ile analiz edildi. Farklı lokalitelerden toplanan altı örnekten toplamada 56 tane bileşen tanımlandı ve önemli kalitatif ve kantitatif farklılıklar gözlendi. Bütün örneklerde en bol bulunan bileşenlerin α-pinen, β-pinen, sabinen, 1,8-sineol, endo-bornil asetat ve α-terpineol olarak bulundu. Altı farklı örneğin uçucu yağ bileşenlerinin antimikrobiyal aktiviteleri bakteri ve maya zincirlerine karşı test edildi. Antimikrobiyal aktivite test sonuçlarına göre, Sancak'tan toplanmış numuneden elde edilen uçucu yağlara karşı en hassas mikroorganizmaların *C. albicans, S. aureus* and *S. enterica* olarak tanımlandı ve bu uçucu yağ ilaç formülasyonları için değerli bir aday olabilir.

Anahtar Kelimeler: *Achillea,* Antimikrobiyal aktivite, Aromatik bitki, 1,8-sineol, α-terpineol, Civan perçemi

1. Introduction

Medicinal and aromatic plants possess pharmalogical and therapeutic properties due to their biologically active seconder metabolites such as essential oils and so have wide variety of usage in different class of disease treatments (Carlini 2003, Faustino et al. 2010). Essential oils are naturally occurring volatile components and responsible for plants and spices characteristic essence. There is an increasing demand for natural plant derived essential oil because they exhibit significant antimicrobial and antioxidant properties (Jallali et al. 2014, Erdogan et al. 2015, Moghaddam et al. 2015, Oke-Altuntas et al. 2016) and so they have wide variety of applications areas including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies. Natural essential oils are more preferable compared to the synthetic chemicals which are used for protection of food products against deterioration because despite having broad spectrum against both Gram-negative and Gram-positive bacteria they do not create any bacterial resistance and so safe for human health and do not cause environmental pollution as synthetic ones. Moreover essential oils can act as an antioxidant and neutralize

*Corresponding Author: igecibesler@bingol.edu.tr

Ibrahim Halil Gecibesler orcid.org/0000-0002-4473-2671

free radicals during the primary oxidative processes of phenolic compounds, monoterpene alcohols, sesquiterpene hydrocarbons and benzene derivatives which lead to decrease in food quality (Hussain et al. 2011, Pirbalouti et al. 2013, Kasrati et al. 2015).

A. millefolium L. subsp. *millefolium* var. *millefolium* taxon is a member of the Asteraceae family and in Turkey it is called as "Civanperçemi". Genus *Achillea* is represented by 42 species in Turkish flora and 23 of these species are endemic. The investigated plant *A. millefolium* is found widespread in Anatolia and distributed in Southwest and Central Asia (Davis 1975, Güner et al. 2000). Different *Achillea* species have been used in folk medicine for hundreds of years in various countries to cure several diseases as bleeding in North America and England, stomach and intestinal diseases in Germany and wound, pain, inflammation and digestive system complaints in Brazil (Baretta et al. 2012).

Previous phytochemical investigations on *Achillea* genus reported essential oils that contain 1,8-cineole, camphor, borneol, α-thujone, β- pinene, santolina alcohol and in some cases sesquiterpenes germacrene D, caryophyllene and spathulenol as major compounds. In the literature there are many reports indicating the chemotype variation of the essential oils from *Achillea* species. Especially chemotype variation of *A. millefolium* is documented extensively in the literature (Kundakovic et al. 2007, Kordali et al. 2009, Rahimmalek et al. 2009) Comprehensively researches were revealed that the earlier investigations of *A. millefolium* L. presents several seconder metabolites, including essential oil (cineol, borneol, pinenes, camphor, menthol, eugenol, azulen, and chamazulene) sesquiterpenes (paulitin, isopaulitin, psilostachyin C, desacetylmatricarin, and sistenin), the alkaloid achilleine steroids (β-sitosterol, stigmasterol, cholesterol, campesterol), triterpenes (α-amyrin, β-amyrin, taraxasterol, pseudotaraxasterol) and flavonoids (such as centaureidin, casticin, apigenin, luteolin, rutin, quercetin, acacetin, isorhamnetin, and artemetin) (Miller and Chow 1954, Chandler et al. 1982, Guedon et al. 1993, Csupor-Loffler et al. 2009). It was proven by reports in Turkey that a number of these substances have some beneficial effects in several pathological conditions and have various ethnobotanical uses which includes treatment of abdominal pain, stomach ache, wounds and as insecticidal (Agelet and Valles 2001, Ezer and Arısan 2006). Many reports have shown that some selected Turkish medicinal plants exhibit a set of different biological activities and they are widely used in folk medicine in Turkey (Demirtas et al. 2013, Gecibesler et al. 2015). However, there isn't any research about chemical composition and their antimicrobial effects depending on localities of *A. millefolium* L. subsp. *millefolium* var. *millefolium*. For these purpose, we analyzed essential oil composition of *A. millefolium* L. subsp. *millefolium* var. *millefolium* species where it is grown in its wild natural habitat in six different localities in the province of Bingol in eastern Anatolia region of Turkey. We also revealed the qualitative and quantitative differences of chemical composition of this taxa which grows naturally in different localities of the same region. Furthermore, this study demonstrates the diversity of different chemical composition effects on the antimicrobial activity. To the best of our knowledge, this is the first study that compares the chemical composition and antimicrobial activity of *A. millefolium* L. subsp. *millefolium* var. *millefolium* variety.

2. Materials and Method

2.1. Plant Materials

As indicated in Table 1, aerial parts of *A. millefolium* L. subsp. *millefolium* var. *millefolium* varieties were harvested from natural habitats in province of Bingol, located in Turkey's Eastern Anatolia, specified in the locality and date. Plant samples were defined and confirmed by Dr. Alpaslan Koçak, Faculty member in Botany Department of Bingol University and they were stored with the herbarium numbers listed in Table 1.

2.2. Test Microorganisms

To determine the antimicrobial activities, a total of eight microorganisms including six bacteria species (*Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* EMCS, *Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes* NCTC5384, *S. enterica* ATCC13311) two yeast species (*Candida albicans* 96268, *Saccharomyces cerevisiae* RSKK04017) have been used.

2.3. Reagents and Standards

Dichloromethane and n-hexane were purchased from Merck (Darmstadt, Germany). Diethyl ether, gentamicin, fluconazole, Mueller Hinton Agar and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich. All of the chemicals and solvents used in this study were analytical grade.

2.4. Isolation of the Volatile Oils

Essential oils of *Achillea* samples were isolated by Clevenger type apparatus and subjected to hydrodistillation for 5

Table 1. Codes, localities, altitudes, dates and herbarium numbers of *A. millefolium* L. subsp. *millefolium* var. *millefolium* varieties.

Variety code	Collection locality	Altitude	Date	Herbarium No
Karlıova	Karlıova district-Kaynarpınar village	2000 m	10.07.2012	BIN-238
Elmalı	Elmalı village -Yedisu district	1520 m	11.07.2012	BIN-239
Ilıcalar	Ilıcalar and Alatepe village	1200 m	13.06.2012	BIN-241
Solhan	Solhan district	1150 m	12.06.2012	BIN-240
Sancak	Sancak district and Su Düğünü village	1650 m	11.07.2012	BIN-242
Genç	Genç district and Çayırtepe village	1200 m	13.06.2012	BIN-243

hours. Water in essential oils was removed using anhydrous sodium sulfate and the essential oils were kept at -20°C in amber vial with mouth cap for GC-MS/FID analysis and biological activity test. GC-MS/FID analyses were repeated three times for each volatile oil example and average of three measurements was computed. The essential oil yield was calculated based on dry weight.

2.5. GC–MS/FID Analyses

The GC-MS/FID analysis of essential oil components were performed by using Agilent Technologies 7890A GC and Agilent Technologies 5975C inert MSD with Triple-Axis Detector System. HP5-MS column (30m x 0.25 mm i.d. 0.25 µm) was used for the chromatographic procedures and helium was used as carrier gas. The oven temperature program was as follows: 2 min at 50°C, 5°C/min up to 150°C, held for 15 min, then 4°C/min up to 240°C, for a total run time of 74.5 min; injector port and detector temperatures, 250°C; carrier gas, helium flow rate 1.3ml/min; injection volume of 1 µl, split ratio, 1:50 acquisition mass range: 29–400 m/z. The chemical structure characterization of volatile components were performed by comparing the mass spectra of the components found in electronic library NIST and WILEY and RI values with existing in literature (Adams 2001). This characterization process were also used in the laboratory and obtained from standard commercial volatile components. Percentage distribution of the chemical composition was calculated from the peak area that obtained from the FID received signals and percentage data was based on the average of three measurements.

2.6. Determination of Antimicrobial Activity by Disc Diffusion Method

The antimicrobial activities of the essential oils and positive controls were determined by disc diffusion method with slight modifications (Vlietinck and Vanden Berghe 1991, Eloff 1998). The essential oils were prepared in dimethylsulfoxide (DMSO) at a concentration of 25 mg/ml. Previous to use the essential oils were filtered using 0.22 µm nylon membrane filter. 200 µl aliquots were used from suspension cultures of microorganisms that contain approximately 106 colonies according to the equation Mc Farland 0.5, and then were transferred to petri dishes containing Mueller Hinton Agar allowed to spread to the surface homogenously. After that 40 µl of samples were injected into 6 mm diameter discs and placed with the help of a sterile forceps to petri dishes containing medium and microorganisms. Petri dishes incubated for 1 hour at 4°C then for 24 hours at 37°C. Negative control was used by injecting 40 µL of DMSO on the same size discs. Gentamicin and fluconazole were used as reference antimicrobial agent. Anti-microbial activity was determined by measuring the diameter of the inhibition zone in millimeters, including disc diameter (6 mm) with digital calipers.

2.7. Statistical Analysis

Hierarchical Cluster Analysis (HCA) was performed using SPSS software (SPSS Inc., Chicago, USA). Antimicrobial tests were performed in triplicate and the mean values were calculated. The data were subjected to analysis of variance and Duncan's multiple range tests were employed to gauge differences between means. A significant difference was judged to exist at a level of $p < 0.05$.

3. Results and Discussion

3.1. Chemotype Analysis

The essential oil yield of samples collected from Karlıova, Elmalı, Ilıcalar, Solhan, Sancak and Genç was found 0.38, 0.51, 0.17, 0.32, 0.29 and 0.18% respectively. The essential oils of *A. millefolium* L. subsp. *millefolium* var. *millefolium* varieties collected from six different localities are given in Table 2. The total essential oil for each sample was yielded as 92.61, 95.74, 90.61, 91.71, 92.93 and 95.26% from Karlıova, Elmalı, Ilıcalar, Solhan, Sancak and Genç, respectively using GC-FID and GC-MS analysis. As shown in Figure 1A-E,

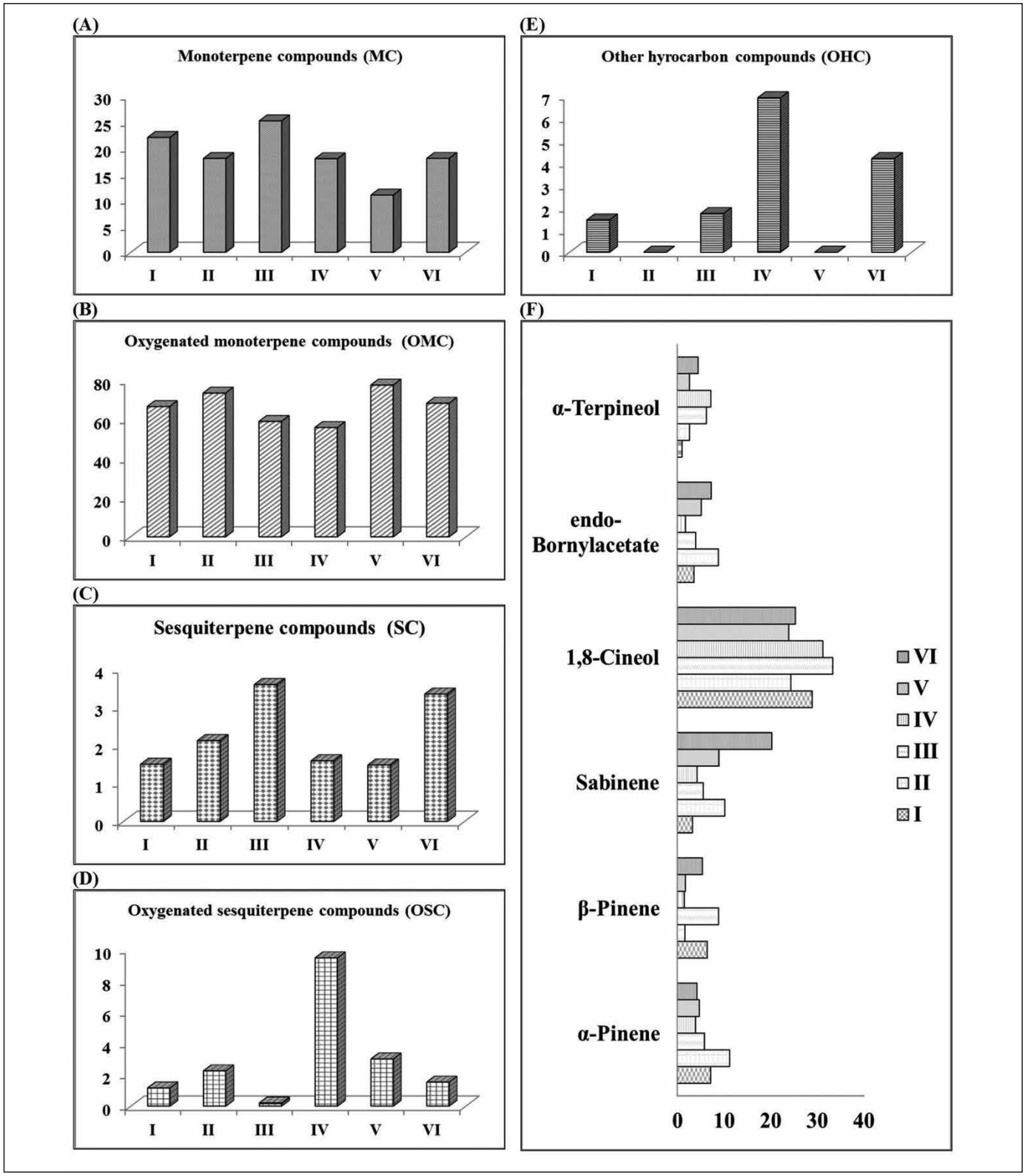

Figure 1. Chemotype variation in the essential oil composition of *A. millefolium* L. subsp. *millefolium* var. *millefolium* varieties from Turkey origin harvested from different localities Karlıova **(I)**, Elmalı **(II)**, Ilıcalar **(III)**, Solhan **(IV)**, Sancak **(V)** and Genç **(VI)**.

the essential components were divided into five groups for the convenience in comparison: monoterpene compounds (MC), oxygenated monoterpene compounds (OMC), sesquiterpene compounds (SC), oxygenated sesquiterpene compounds (OSC), and other hydrocarbon compounds (OHC).

The monoterpene compounds and oxygenated monoterpene compounds were the major fractions in the each sample according to results of GC-MS/FID. The oxygenated monoterpene compounds were detected as percentage of 66.52, 73.04 58.94, 55.83, 77.49 and 68.22% analyzed in sample of Karlıova, Elmalı, Ilıcalar, Solhan, Sancak and Genç, respectively and 1,8-cineole, which is oxygenated monoterpene, was constituted about half of these rates (Figure 1F). In the samples were found to be important variations in terms of monoterpene hydrocarbons. While sample of Ilıcalar had the highest monoterpene hydrocarbon content with a ratio of 25.16%, the example of Solhan had the lowest monoterpene hydrocarbon content with value of 10.95% (Figure 1A). On the other hand sample of Solhan is richest in terms of oxygenated sesquiterpene analyzed compounds (9.46%), but sample of Ilıcalar is poorest in terms of these compounds (0.2%) (Figure 1D). The other hydrocarbon compounds were constituted a very small fraction of the total (0.16-7.31%) (Figure 1E). When the table 2 is examined it was observed that volatile oil composition of all samples showed moderate qualitative similarity, but in the some of their main components showed significant differences in quantitative. The sabinene, one of the main components of the primary, was analyzed in sample of Karlıova, Elmalı, Ilıcalar, Solhan, Sancak and Genç with ratio of 3.27, 10.18, 5.56, 4.25, 8.92 and 20.25%. The major constituents of the isolated essential oils from the *A. millefolium* L. subsp. *millefolium* var. *millefolium* species were significantly different to previous reports on the chemistry of these oils isolated from *A. millefolium* L. or *A. millefolium* L. subsp. *millefolium* plants growing in Turkey or other countries. The differences in the chemical composition of oils might arise from several environmental (climatic, seasonal and geographical) and genetic factors as well as different nutritional status of the plants (Perry et al. 1999). Sources of compositional variability can also include the plant part extracted, phenological state of the plant, and time of year, as well as climatic and soil variations (Gholamreza et al. 2008, Figueiredo et al. 2008). As can be seen in Table 2, the *A. millefolium* L. subsp. *millefolium* var. *millefolium* examined contained oil without chamazulene. The main component of the distilled oil was 1,8-cineol, ranging from 24.33-33.28% with a maximum in the sample Ilıcalar. The Sabinene, α-pinene, β-pinene, endo-bornylacetate and α-terpineol were the predominant minor compounds in all samples. Also, the composition may show great differences in accordance with the provenance of the plant material. Forty *A. millefolium* samples that were collected in the wild in Lithuania could be classified into four groups according to a cluster analysis evaluating the essential oil composition (Gudaityte and Venskutonis 2007). Another study from the same country that considered 19 *A. millefolium* samples defined 6 chemotypes according to the main essential oil components. In these samples, 1,8-cineol ranged from 2.3 to 21.6% (Mockute 2003). From Iran, Afsharypuor et al. (1996) described an essential oil of *A. millefolium* subsp. *millefolium* rich in α-bisabolol (23%), spathulenol (12%) and *cis*-nerolidol (6%). The main constituents of *A. millefolium* essential oil from the Balkans were β-pinene (33%), β-caryophyllene (17%), sabinene (11%), and chamazulene (6%) (Boskovic et al. 2005). In *A. millefolium* subsp. *millefolium* from Turkey, 1,8-cineol (25%), camphor (17%) and α-terpineol (10%) were the main essential oil components (Candan et al. 2003). Two different diploid ecotypes of *A. millefolium* from the Indian Himalaya had β-caryophyllene (16%), 1,8-cineol (15%) and β-pinene (11% or 14%), β-caryophyllene (13%) and borneol (12%) as major compounds in the essential oil from the flowering parts. These plants were low in proazulenes (Agnihotri et al. 2005).

The essential oil composition of *A. millefolium* L. subsp. *millefolium* var. *millefolium* varied significantly according to the harvest location, where each major compound or chemical family presented a specific behavior. With a dissimilarity of 30, Hierarchical Cluster Analysis (HCA) allowed to distinguish the essential oil plant from Sancak (GA) of those obtained from the other locations (GB) (Figure 2). With a dissimilarity of 8, the group B is subdivided at two sub-groups: S-GC: contained essential oils plant from Ilıcalar, Genç and Solhan; S-GC: contained essential oils plant from Elmalı and Karlıova. Prior to HCA data (Table 3), the essential oil plant from Sancak location (GA), characterized by β-thujone (23.46%) as a chemotype, had a great difference (41.56%) in content between the monoterpene hydrocarbon (15.40%) and the oxygenated monoterpene (56.96%). The sub-group C (Ilıcalar, Genç and Solhan), was characterized by trans-chrysanthenon (14.34%) as a chemotype. The sub-group D (Elmalı and

Table 2. Chemical composition of *A. millefolium* subsp. *millefolium* var. *millefolium* essential oils.

		Percentage (%)[b]						IM[c]
RI[a]	Compounds	Karlıova	Elmalı	Ilıcalar	Solhan	Sancak	Genç	
798	Butyric acid	-	-	-	2.31	-	-	MS; RI
871	2-butenal	-	-	-	2.94	-	-	MS; RI
900	Benzenol	-	-	1.04	-	-	-	
923	Artemisia triene	-	0.57	0.46	1.67	-	-	MS; RI
935	β-citronellene		-	-	-		3.65	MS; RI
941	α-pinene	7.16	11.24	5.83	3.91	4.73	4.21	MS; RI; std
954	Camphene	3.3	0.95	0.53	-	0.57	0.64	MS; RI; std
955	Benzaldehyde	0.32	-	0.63	0.35	-	0.26	MS; RI
968	Sabinene	3.27	10.18	5.56	4.25	8.92	20.25	MS; RI; std
977	1-octene-3-ol	0.73	-	0.4	0.8	-	0.54	MS; RI
984	β-pinene	6.43	1.66	8.83	1.52	1.75	5.39	MS; RI; std
1011	δ-3-Carene	-	-	-	1.08	-	1.26	MS; RI; std
1014	α-terpinene	0.57	-	1.05	-	-	0.93	MS; RI; std
1024	β-cymene	1.97	3.28	3.47	1.95	1.97	3.39	MS; RI
1035	1,8-cineol	28.88	24.33	33.28	31.18	23.86	25.31	MS; RI; std
1037	*cis*-Ocimene	-	-	2.2	7.44	1.02	-	MS; RI; std
1055	γ-terpinene	2.24	-	2.16	-	0.91	1.66	MS; RI
1068	Artemisia ketone	0.92	0.27	-	-	-	0.24	MS; RI
1071	*cis*-sabinene hydrate	2.93	1.63	-	1.35	-	1.74	MS; RI
1074	Trans-4-thujanol	2.26	1.77	3.25	1.56	1.58	2.33	MS; RI
1087	Artemisia alcohol	-	-	0.33	0.75	-	-	MS; RI
1095	Fenchone	-	0.66	0.99	-	-	-	MS; RI
1098	Linalool	-	0.73	-	-	1.09	-	MS; RI
1103	Nonanal	0.19	-	0.13	-	-	-	MS; RI
1115	β-Thujone	-	-	-	-	23.46	-	MS; RI
1117	*trans*-chrysanthenone	13.31	15.36	-	0.96	-	-	MS; RI
1142	*cis*-verbenol	-	1.51	-	-	-	-	MS; RI
1147	Camphor	-	1.48	3.92	1.9	1.47	2.8	MS; RI; std
1165	Borneol	5.44	0.63	0.74	-	1.87	2.97	MS; RI
1163	α-phellandrene-8-ol	-	0.74	-	-	-	-	MS; RI
1165	*trans*-Chrysanthenol	0.63	-	-	-	3.6	-	MS; RI
1189	α-Terpineol	1.05	2.62	6.25	7.19	2.64	4.46	MS; RI; std
1194	Myrtenal	0.87	-	0.51	-	-	-	MS; RI
1197	Myrtenol	0.78	0.51	-	0.36	-	-	MS; RI
1203	Verbenone	-	0.23	-	-	-	-	MS; RI
1228	*cis*-carveol	-	0.34	0.32	0.21	-	-	MS; RI
1256	Piperitone	0.91	-	-	-	0.11	0.86	MS; RI
1260	Chrysanthenyl acetate	0.73	1.87	0.82	4.34	1.25	-	MS; RI
1263	*endo*-Bornyl acetate	3.57	8.81	3.96	1.78	5.13	7.29	MS; RI
1286	4-Thujen-2-α-yl-acetate	-	-	-	-	1.33	-	MS; RI
1293	Lavandulyl acetate	0.97	-	-	-	1.18	-	MS; RI
1346	α-Cubebene	1.23	-	-	-	-	-	MS; RI

Table 2. Cont.

		Percentage (%)[b]						IM[c]
RI[a]	Compounds	Karlıova	Elmalı	Ilıcalar	Solhan	Sancak	Genç	
1379	α-Copaene	-	0.25	-	-	1.03	-	MS; RI
1389	Germacrene-D	-	-	0.53	-	-	1.43	MS; RI
1411	Dehydro-aromadendren	0.27	0.24	0.51	0.74	-	0.26	MS; RI
1420	Caryophyllene	-	1.45	2.33	0.85	-	1.53	MS; RI
1481	γ-Himachalene	-	-	0.22	-	-	0.12	MS; RI
1507	β-Bisabolene	-	0.18	-	-	0.45	-	MS; RI
1557	Bisabolene oxide	-	-	-	-	0.35	-	MS; RI
1575	Spathulenol	-	0.34	-	-	0.3	0.55	MS; RI
1578	Azulene	0.24	-	-	-	-	-	MS; RI
1583	Caryophyllene oxide	0.92	1.51	0.2	0.95	0.64	0.98	MS; RI
1643	*cis*-(*Z*)-α-Bisabolene epoxide	-	-	-	8.51	-	-	MS; RI
1650	E-ocimenol	0.52	-	-	-	-	0.21	MS; RI
1657	β-Eudesmol	-	-	-	0.86	1.72	-	MS; RI
1724	Farnesol	-	0.4	0.16	-	-	-	MS; RI
	Total identified (%)	**92.61**	**95.74**	**90.61**	**91.71**	**92.93**	**95.26**	

*[a]**RI:** retention indices as determined on HP5-MS column using homologous series of C8–C26 alkanes. [b]Values are means of triplicate analyses.*
*[c]**IM:** Identification methods: **MS;** by comparison of the mass spectrum with those of the computer mass libraries and Adams (2001). **std;** by injection of an authentic sample. **–;** not detected.*

Karlıova), characterized by α-terpineol (5.97%) as a chemotype, had approximatively a ratio of 2/1 between the oxygenated monoterpene (41.54%) and the monoterpene hydrocarbon (19.92%).

3.2. Antimicrobial Activity

Essential oils and their individual components have been previously explored for their antimicrobial properties. The antimicrobial activity of these hydrophobic compounds has been attributed for their ability to disrupt the bacterial cell membrane by causing excessive cell permeability (Taweechaisupapong et al. 2012). Antimicrobial activity of essential oils is one of the most examined features, important for both food preservation and control of microbial human and animal diseases Numerous reports suggest strong antibacterial and antifungal activities of a wide range of essential oils, especially those belonging to the Asteraceae family (Rios 2015, Kazemi et al. 2015, Casiglia et al. 2016). However, in order to get more relevant data about the influence of some essential oil compounds on the activity cited, further examinations are necessary. Essential oils of the six *Achillea* samples antimicrobial activity were tested against eight different microorganisms and the results

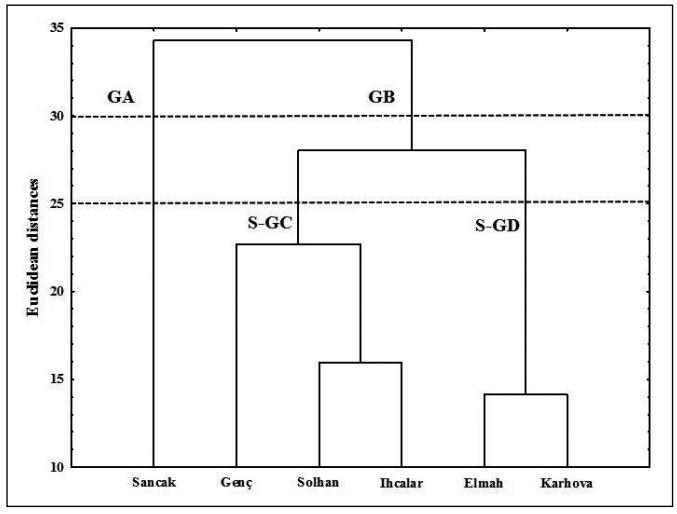

Figure 2. Dendrogram obtained by HCA, based on matrix linking percentages of the components to the essential oils extracted from A. millefolium L. subsp. millefolium var. millefolium harvested at different locations.

are shown in Table 4. It was found that the essential oils of all samples were showed inhibitory effects against the test microorganisms. The antimicrobial activities of the essential oils were examined against the three gram positive bacteria, three gram negative bacteria and two yeast strains.

In general, each essential oil exhibited notable bactericidal and fungicidal activity. The essential oil of Sancak sample showed higher antibacterial and antifungal activity especially against *S. enterica* (43.29±0.38 mm), *S. aureus* (46.44±0.71 mm) and *C. albicans* (47.43±1.32 mm) strains. On the other hand *E. coli* was the second most sensitive strains (36.23±0.43 mm) against the sample of Solhan followed by *B. subtilis* (29.67±4.36 mm), *K. pneumonia* (28.15±0.56 mm), *S. cerevisiae* (27.91±0.17 mm) and *L. monocytogenes* (17.02±0.49 mm) In general, yeast strains seem to be more sensitive to the essential oils of Genç sample. Strains of *K. pneumoniae* showed high resistant (12.10±0.72 mm) to the essential oils of Ilıcalar sample, which is of a particular interest. Furthermore, essential oils of Karlıova sample exhibited moderate activity against *S. enterica* (15.20±1.08 mm) and *B. subtilis* (14.28±1.47 mm). Essential oil of Elmalı and Genç samples were less effective than other samples of essential oil against both bacteria and fungi. Bacterial strains were more sensitive according to yeast strains. Essential oil extracted from Solhan sample was notable more effective against both yeast species compared with all other essential oil samples and it showed zone diameter changing in the range of 18.90±0.72 and 40.43±0.45 mm. These results suggest that Solhan and Sancak samples of *Achillea* taxa can be used as a natural food preservative againts tested bacteria and yeast pathogens which are known to cause food borne illness (Ultee et al. 2002, Gallucci et al. 2009, Saei-Dehkordi et al. 2012). It is known that many of different *Achillea* species exhibit antimicrobial activity (Kotan et al. 2010, M. Kazemi and Rostami 2015) and overall evaluation of tested six *Achillea* sample were also aroused a conviction that they could be suitable for use as an antimicrobial agent Sancak sample, quite rich in terms of oxygenated sesquiterpene, and this incident elucidate why it was more effective against the gram-negative bacteria strains. Bioactive molecules may provide antimicrobial activity for Karlıova, Elmalı and Genç samples which contain a high concentrations of α-pinene, 1,8-cineole, β-pinene, Sabinene and endo-bornyl acetate (Boulila et al. 2015). Also β-thujone component that give rise to chemotype variation among *Achillea* samples might be contribute to the antimicrobial activity for Sancak sample. According to the literature (Kotan et al. 2010, M. Kazemi and Rostami 2015, Polatoğlu et al. 2016). the other minor components such as camphene, linalool and camphor detected in *Achillea* samples during our study could also be good antimicrobial agents.

4. Conclusion

The screening of antimicrobial activities of the essential oils from *A. millefolium* L. subsp. *millefolium* var. *millefolium* varieties revealed that tested samples possesses significant antimicrobial activity, which is due to the presence of antimicrobial agents within a complex of analyzed lipophilic compounds. Further, this activity comparable to gentamicin and fluconazole. The development of natural antimicrobial formulations will help to reduce the quality deterioration in many food systems, the formation of toxic compounds and significant loss of a food's nutritional quality as well as the microbial based human and animal diseases. The essential oil composition of *A. millefolium* L. subsp. *millefolium* var. *millefolium* varieties from the different localities of Turkey was presented noticeable qualitative and quantitative differences. During comparison of the essential oils from

Table 3. HCA data for the essential oils extracted from *A. millefolium* L. subsp. *millefolium* var. *millefolium* harvested at different locations.

Chemical class	GA (Sancak)	S-GC		S-GD	
		Range (%)	Average (%)	Range (%)	Average (%)
α-Pinene	4.73	3.91-5.83	4.65	7.16–11.24	9.20
β-Pinene	1.75	1.52-8.83	5.25	1.66-6.43	4.05
Sabinene	8.92	4.25–20.25	10.02	3.27–10.18	6.73
Monoterpene hydrocarbon	**15.40**	**9.68–29.85**	**19.92**	**16.86–23.08**	**19.97**
1,8-Cineol	23.86	25.31–33.28	29.92	24.33–28.88	26.61
β-thujone	**23.46**	0.00	0.00	0.00	0.00
Endo-bornyl acetate	5.13	1.78-7.29	4.34	3.57-8.81	6.19
Trans-chrysanthenon	0.00	0–0.96	0.32	13.31–15.36	**14.34**
α-Terpineol	2.64	4.46-7.19	**5.97**	1.05–2.62	1.84
Borneol	1.87	0–2.97	0.99	0.63-5.44	3.04
Oxygenated monoterpene	**56.96**	**40.03-43.49**	**41.54**	**51.75-52.25**	**52.00**

Table 4. Antimicrobial activity of the studied essential oils using disc diffusion assay.

	Inhibition zone diameter (mm)							Positive Controls	
Microorganisms	**Karlıova**	**Elmalı**	**Ilıcalar**	**Solhan**	**Sancak**	**Genç**	**NC**	**Gentamicin**	**Fluconazole**
Bacteria (Gram-negative)									
K. pneumoniae	17.12±1.56^{b}	10.11±0.61^{c}	12.10±0.72^{a}	30.01±0.59^{d}	28.15±0.56^{b}	9.04±0.41^{c}	-	43.51±2.01^{e}	nt
S. enterica	15.20±1.08^{a}	10.15±0.39^{c}	38.21±0.44^{e}	26.45±0.65^{b}	43.29±0.38^{e}	6.15±0.75^{a}	-	37.87±1.87^{d}	nt
E.coli	21.47±1.93^{c}	20.34±0.22^{c}	18.67±0.54^{b}	18.90±0.72^{a}	36.23±0.43^{d}	9.17±0.35^{c}	-	24.54±1.29^{a}	nt
Bacteria (Gram-positive)									
S. aureus	26.15±0.03^{d}	9.08±0.91^{b}	41.13±0.17^{f}	37.11±0.68^{e}	46.44±0.71^{f}	7.03±0.27^{a}	-	32.72±0.79^{e}	nt
B. subtilis	14.28±1.47^{a}	9.51±1.67^{b}	20.45±0.02^{c}	28.54±1.82^{c}	29.67±4.36^{c}	7.54±1.18^{b}	-	38.10±1.42^{d}	nt
L. monocytogenes	32.21±1.71^{e}	7.22±0.28^{a}	37.71±0.61^{e}	40.43±0.45^{f}	17.02±0.49^{a}	8.10±0.39^{b}	-	29.53±1.59^{b}	nt
Yeast									
C. albicans	26.45±0.04^{d}	10.19±0.62^{c}	20.63±0.73^{c}	29.21±0.02^{c}	47.43±1.32^{f}	19.10±0.24^{d}	-	nt	41.0±0.05^{b}
S. cerevisiae	37.02±1.87^{f}	19.25±0.86^{d}	31.27±3.38^{d}	19.09±0.34^{a}	27.91±0.17^{b}	39.61±0.37^{e}	-	nt	36.0±0.32^{a}

Results were expressed as mean ± standard error. Means with different letters within a row are significantly different ($p < 0.05$).
***NC**: negative control (used DMSO), **nt**: not tested, **-**: not susceptibility.*
Diameter of inhibition zone including disc diameter of 6 mm, by the agar disc diffusion method at a concentration of 40 µL of oil or positive and negative controls/disc.

different locations, numerous factors such as environmental conditions, soil characteristics, time of harvest, methods of drying, extraction and analytical conditions and used plant parts which may affect the composition of the essential oils should be considered.

5. Acknowledgements

The author thanks for financial support the Scientific Research Project Department of Bingol University (Project No: BAP-21-217-2014 and BAP-21-322-2015). The author thanks to Gülden Koçlar and İbrahim Demirtaş for grammatical revision of the manuscript. The author is grateful to Alpaslan Koçak and Vecdi Çakıcı for botanical studies. The author thanks also Dr. Abderrahmane Djouahri for his constructive advices and statistical analysis.

6. Disclosure statement

No potential conflict of interest was reported by the authors.

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