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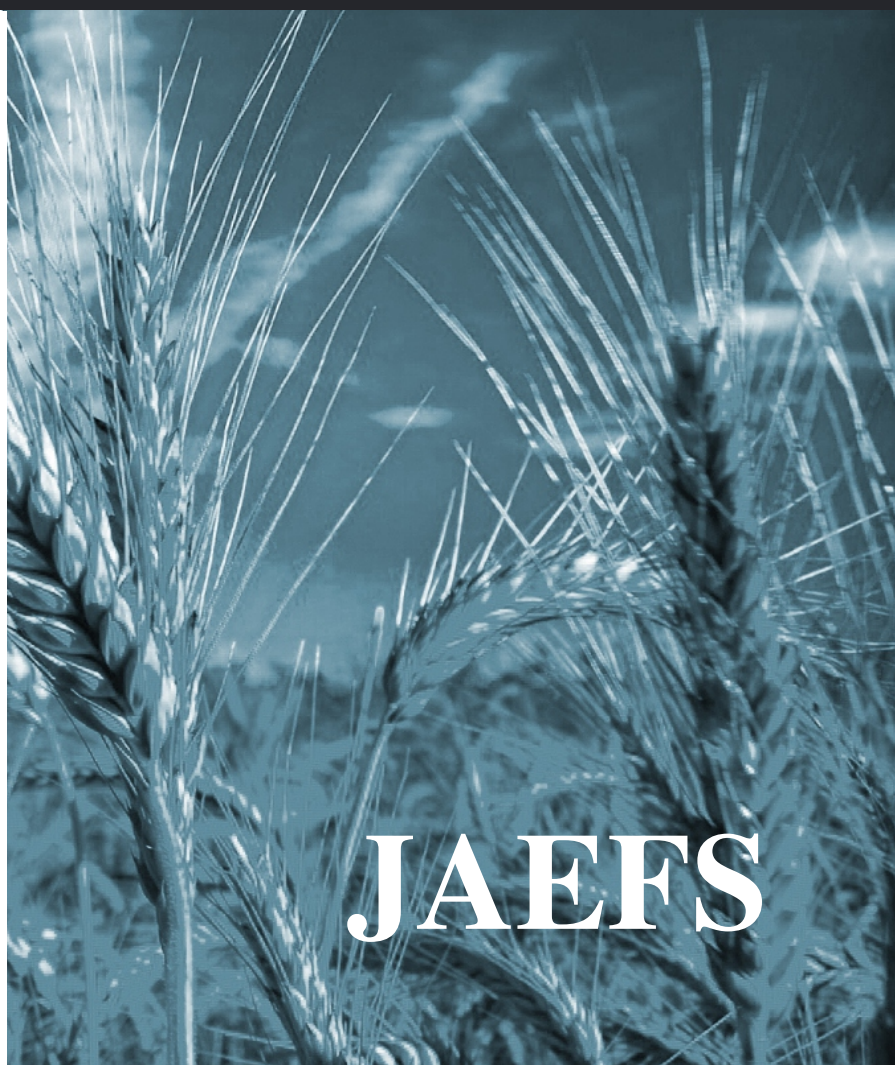

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Chemical compositions and antimicrobial activity of *Prunella vulgaris* L.

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Abstract

The popular medicinal plant *Prunella vulgaris* L., (Lamiaceae) is a perennial and an edible herbaceous plant which is widely distributed in the temperate zone and tropical mountains of Europe and Asia. Due to its medicinal and industrial importance, the demand for *P. vulgaris* has increased steadily in recent years. In the present study, the volatile compounds of the *P. vulgaris* were accumulated by Headspace-Solid Phase Microextraction (HS-SPME) technique, and, analysed by gas chromatography/mass spectrometry (GC/MS). The chemical composition of the methanolic extract (ME) and infusion (INF) of *P. vulgaris* were determined. Aerial parts of *P. vulgaris* INF of the major compounds were found hexanal (23.1%), ionol (10.7%), (Z)-3-hexenal (3.2%) and 3,5-octadien-2-one. The ME of *P. vulgaris* were characterized with α-fenchone (11.1%), hexanal (8.2%), 3,5-octadien-2-one (4.7%), methyl benzoate (4.5%) and selina-4,11-diene (3.1%). It was evaluated the antimicrobial activity of *P. vulgaris* extracts (ME, INF) in *in vitro* conditions against different kinds of microorganisms. The INF showed weak antimicrobial activity Minimum Inhibitor Concentration (MIC) against all tested microorganisms whereas ME showed weak antimicrobial effects *E. coli, S. aureus and Pseudomonas aeruginosa*; *S. pyogenes* (20 mg / mL) and *C. albicans* (15 mg / mL).

Keywords: *Prunella vulgaris*, HS-SPME, volatile compound, antimicrobial activity

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Introduction

Prunella is a genus of perennial herbaceous plants in the Lamiaceae family. There are approximately 15 species worldwide, distributed widely in the temperate regions and tropical mountains of Europe and Asia (Bai et al., 2016). In the genus *Prunella*, *P. vulgaris* L. also known as "self-heal;" contains several active components, including oleanolic acid, betulinic acid, ursolic acid, flavonoids and rosmarinic acid (Lamaison et al., 1991). Some pharmacological activities such as the immunmodulatory effect (Han et al., 2009), anti-viral activity against HSV-1, HSV-2 (Zhang et al., 2007), HIV (Yao et al., 1992), antioxidant activity (Psotova et al., 2003; Osakabe et al., 2002) and anti hyperglycemic action (Zhang et al., 2007) were confirmed. It has been used as a traditional medicine in the clinical treatment of herpetic keratitis and for its antioxidative and antimicrobial activities. In spite of its traditional uses as an antiseptic agent for treatment of wounds and sore throat, there are a few literatures on its antimicrobial activity.

Various bioactive constituents, such as terpenoids (Qi et al., 2009), polyphenols (Feng et al., 2010), flavonoids (Lee et al., 2008), and polysaccharides (Chiu et al., 2004) have been identified in the extracts of *P. vulgaris*. Various methods, such as steam distillation, Soxhlet extraction, and solvent extraction (Guan et al., 2007; Wang et al., 2008) can be used for the determination of volatile components. With the advantages of better selectivity and higher efficiency, headspace solid-phase microextraction (HS-SPME) has been introduced as a modern alternative to the traditional sample preparation technology (Adam et al., 2005).

The aim of this research was to determined volatile composition of the methanolic extract (ME) and infusion (INF) of *P. vulgaris* by headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) and examined antimicrobial activitis.

Materials and Methods

The aerial parts of *P. vulgaris* was collected in July 2017 from Beşikderesi, Eskişehir (Turkey). The ME and 5% INF of *P. vulgaris* were prepared. Their volatile compounds were trapped with Headspace Solid Phase Micro Extraction (HS-SPME) and analyzed by Gas Chromatography-Mass Spectrometry (GC/MS). ME and INF were examined for antimicrobial activity by the microdilution broth susceptibility assay against *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* NRRL B-3008, *Streptococcus pyogenes* ATCC 13615 and *Candida albicans* ATCC 90028.

Headspace-SPME

The manual SPME device (Supelco, Bellafonte, PA, USA) with a fiber-precoated 65 µm thick layer of polydimethylsiloxane/divinylbenzene (PDMS/DVB-blue) was used for extraction of the methanolic extract and

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infusion of *P. vulgaris* volatiles. The vial containing the plant extract was sealed with parafilm. The fiber was pushed through the film layer for exposure to the headspace of the extract for 15 min at 40 °C. The fiber was then inserted immediately into the injection port of the GC-MS for desorption of the adsorbed volatile compounds for analysis.

Analysis of volatile compounds

The ME and INF volatiles were analyzed by GC/MS using an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 m 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. The injector temperature was set to 250 C. Mass spectra were recorded at 70 eV. Mass range was *m/z* 35 to 450.

Identification of Components

Identification of the volatile components were 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 Software 4.0) (McLafferty and Stauffer,1989; Hochmuth, 2008) and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils. Relative percentage amounts of the separated compounds were calculated from TIC chromatograms. The volatile compounds identified are listed in Table 1.

Antimicrobial Activity

ME and INF were examined for antimicrobial activity by the microdilution broth susceptibility assay against *Staphyloccoccus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* NRRL B-3008, *Streptococcus pyogenes* ATCC 13615 and *Candida albicans* ATCC 90028.

Results and Discussion

The current study aimed the determination of the HS-SPME volatile profile of the methanolic extract (ME) and infusion (INF) of *P. vulgaris*. In addition, antimicrobial activities were also examined**.** HS-SPME was used to collect the volatile components of *P. vulgaris*. Aerial parts of *P. vulgaris* INF of the major compounds were found hexanal (23.1%), ionol (10.7%), (Z)-3-hexenal (3.2%) and 3,5-octadien-2-one (3.1%). The ME of *P. vulgaris* were characterized with α-fenchone (11.1%), hexanal (8.2%), 3,5-octadien-2-one (4.7%), methyl benzoate (4.5%) and selina-4,11-diene (3.1%) (Table 1). It was evaluated the antimicrobial activity of *P. vulgaris* extracts (ME, INF) in *in vitro* conditions against different kinds of microorganisms. The INF showed weak antimicrobial activity Minimum Inhibitor Concentration (MIC) against all tested microorganisms whereas ME showed weak antimicrobial effects *E. coli, S. aureus* and *Pseudomonas aeruginosa*; *S. pyogenes* (20 mg / mL) and *C. albicans* (15 mg / mL).

Phytochemical studies indicate that *P. vulgaris* contains oleanolic, betulinic, ursolic, 2α,3α- dihydroxyurs-12-en-28-oic and 2α,3α-ursolic acids, triterpenoids, flavonoids, tannins and anionic polysaccharide prunelline (Ruy et al., 2000). More recently, the organic fraction of *P. vulgaris* was found to exhibit antioxidative and antimicrobial activities (Psotova et al., 2003). *P. vulgaris* is rich in phenolic acids and its main component is rosmarinic acid (Lamaison et al., 1991; Psotova et al., 1998; Han et al., 2009).

Rosmarinic acid or α-O-caffeoyl-3-4-dihydroxyphenyllactic acid is the mulifunctional caffeic acid ester with antimicrobial activity against *Bacillus cereus*, *B. subtilis* and *B. polymyxa*. Gram-negative bacteria were previously reported to be highly susceptible to rosmarinic acid (Askun et al., 2009). The volatile compounds of *P. vulgaris* from different parts of the herb (cultivated in Jiangsu) and from different geographical regions were comparatively analyzed by HS-SPME combined with GC-MS. And the following 12 were found in all origins: 1-nonanol, dodecane, tridecane, *a*-bourbonene, tetradecane, geranyl acetone, pentadecane, caryophyllene oxide, hexadecane, tetradecanal, isobutyl phthalate, and n-butyl hexadecanoate (Yang et al., 2013).

In another study performed by Golembiovska et al. in 2014 (16) on *P. vulgaris* from 104 components were identified in flowers, leaves, stems and roots and the main constituents were observed squalene, myristic acid, spathulenol, viridiflorol, germacrone. In recent years, studies have found that *P. vulgaris* also exhibits certain anti-pathogen effects on plant pathogens. Antibacterial activity of the methanolic extract of *P. vulgaris* extracts was reported against *E. coli*, *S.aureus*, *S. typhimurium* and *K. pneumonae* (Rasool et al., 2010). Studies by Yoon et al. found that the methanol extract of *P. vulgaris* had a strong anti-fungal and antioomycete activity on *Phytophthora infestans*, rice blast fungus, red pepper anthracnose and wheat leaf rust fungus (Yoon et al., 2010). An Iranian study by Mahboubi et al. (2015) showed that the methanol extract of *P. vulgaris* exhibited the best activity against *St. mutans* (MIC 3.2 mg.7ml), *S. aureus*, *S. epidermidis*, *S. sobrinus*, S. *sanguis, S. salivarius, S. dysenteriae, S. flexeneri, P. aeruginosa* (MIC 3.2, 6.4 mg/ml). *S. saprophyticus, S. pneumoniae, S. pyogenes, E. faecalis, E. faecium, S. agalactiae, K. pneumoniae, E. aerogenes, A. flavus, A. niger* and *S. marcescenes* with MIC and MLC 6.4 and 12.8 mg/g had lower sensitivity to methanol extract. Methanol extract had cidal activity against *E. coli*, *B. subtilis*, *B. cereus*, *C. albicans*, and *C. glabrata*.

Several researchers have evaluated the composition of the essential oil of *P. vulgaris* growing in different geographic areas. These studies revealed some chemical differences in the oil compositions, probably related to the different subspecies and/or the geographical origin of the plants. In conclusion, using HP-SPME-GC-MS, it was possible to quantify different volatile compounds like as hexanal, α-phenchone in *P. vulgaris* which belong to different chemical classes. Results shows that the extracts of *P. vulgaris* tested exhibited significant antimicrobial activity. Our results indicated that the methanol extracts of *P. vulgaris* showed higher antimicrobial activity than INF of *P. vulgaris* against tested microorganisms. In addition, the extract of *P. vulgaris* were observed more efficiency against skin patogenes.

Conclusion

By using headspace technology coupled with GC/MS, volatile profile of the methanolic extract (ME) and infusion (INF) of *P. vulgaris* were determined. The GC/MS analysis results of the samples led to identification of 74 compounds. Our results showed that the number of components were different in the other studies.

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The observed differences and variability of the essential oil of *P. vulgaris* of are likely due to different environmental and genetic factors. In addition, it was evaluated the antimicrobial activity of *P. vulgaris* extracts (ME, INF) in in vitro conditions against different kinds of microorganisms. Among the tested microorganisims, *S. pyogenes* and *C. albicans* were found to be more sensitive to the ME.

Table 1. The Volatile Composition of the methanolic extract (ME) and infusion (INF) of *P. vulgaris*

RRI	Compound	ME %	INF %	IM
1093	Hexanal	**8.2**	**23.1**	RRI, MS
1194	Heptanal	-	2.5	RRI, MS
1225	*(Z)*-3-Hexenal	-	**3.2**	MS
1260	1-Pentanol	-	1.0	MS
1197	Methyl hexanoate	1.5	-	RRI, MS
1213	1,8-Cineole	2.9	-	RRI, MS
1244	2-Pentyl furan	0.9	-	MS
1296	Octanal	-	0.8	RRI, MS
1348	6-Methyl-5-hepten-2-one	1.7	0.9	MS
1360	1-Hexanol	0.5	-	MS
1391	*(Z)*-3-Hexenol	-	0.3	MS
1398	2-Nonanone	0.4	-	MS
1400	Nonanal	2.4	2.3	MS
1406	*α*-Fenchone	**11.1**	-	RRI, MS
1416	3-Octen-2-one	1.1	-	MS
1441	*(E)*-2-Octenal	-	1.5	MS
1452	1-Octen-3-ol	1.1	-	MS
1463	1-Heptanol	-	0.5	MS
1479	*(E,Z)*-2,4-Heptadienal	-	2.3	MS
1496	2-Ethyl hexanol	2.1	2.2	MS
1497	*α*-Copaene	0.4	-	MS
1500	Methyl nonanoate	0.3	-	RRI, MS
1500	Pentadecane	0.6	-	RRI, MS
1506	Decanal	0.6	-	MS
1507	*(E,E)*-2,4-Heptadienal	0.6	2.5	MS
1520	3,5-Octadien-2-one	**4.7**	**3.1**	MS
1532	Camphor	1.2	0.7	RRI, MS
1535	*β*-Bourbonone	1.8	-	MS
1541	Benzaldehyde	0.7	1.1	RRI, MS
1548	*(E)*-2-Nonenal	-	0.8	MS
1553	Linalool	1.4	1.4	RRI, MS
1562	Octanol	0.4	0.9	RRI, MS
1573	*(E,E)*-3,5-Octadien-2-one	1.8	1.1	MS
1582	*cis*-Chrysanthenyl acetate	0.5	-	MS
1597	*β*-Copaene	0.3	-	MS
1600	Hexadecane	1.0	-	RRI, MS
1604	2-Undecanone	0.6	-	MS
1611	Terpinen-4-ol	2.6	1.2	RRI, MS
1638	*β*-Cyclocitral	0.7	0.9	MS
1641	Methyl benzoate	**4.5**	-	RRI, MS
1655	*(E)*-2-Decenal	-	0.6	MS
1664	1-Nonanol	-	0.6	MS
1704	*γ*-Muurolene	2.3	-	MS
1706	*α*-Terpineol	0.5	0.4	RRI, MS
1715	*(E,E)*-2,4-Nonadienal	-	0.3	MS
1719	Borneol	0.3	-	RRI, MS
1740	*α*-Muurolene	0.8	-	MS
1744	Selina-4,11-diene	**3.1**	-	MS
1747	3,4-Dimethyl-2,5-furandione	0.3	-	MS
1751	Carvone	0.8	-	RRI, MS
1773	*δ*-Cadinene	1.7	-	MS
1776	*γ*- Cadinene	1.1	-	MS
1798	Methyl salicylate	0.2	-	RRI, MS
1802	Cumin aldehyde	-	0.6	RRI, MS

1849	Calamenene	1.3	-	MS
1868	*(E)*-Geranyl acetone	0.6	0.5	MS
1870	Hexanoic acid	1.3	-	RRI, MS
1878	2,5-Dimethoxy-*p*-cymene	0.3	0.1	MS
1896	Benzyl alcohol	0.5	-	RRI, MS
1935	Phenyl ethyl alcohol	0.4	-	RRI, MS
1958	*(E)*-*β*-Ionone	0.6	0.8	MS
2009	*trans*-*β*-Ionon-5,6-epoxide	0.4	-	MS
2019	2,3,6-Trimethyl benzaldehyde	0.3	0.4	MS
2045	Carotol	1.2	-	MS
2179	3,4-Dimethyl-5-pentyliden-2(5H)-furanone	tr	-	MS
2228	Acorenone B	0.2	-	MS
2300	Tricosane	0.3	0.9	RRI, MS
2380	Dihydroactinidiolide	0.4	-	MS
2500	Pentacosane	0.5	1.5	RRI, MS
2600	Hexacosane	0.6	1.7	RRI, MS
2700	Heptacosane	0.8	1.6	RRI, MS
2800	Octacosane	0.9	1.7	RRI, MS
2900	Nonacosane	0.7	1.3	RRI, MS
2931	Hexadecanoic acid	0.6	-	RRI, MS
	Total	**81.6**	**63.3**	

RRI: Relative retention indices calculated against *n*-alkanes
*%: calculated from TIC data
tr: Trace (< 0.1 %)
IM: Identification method based on the relative retention indices (RRI) of authentic compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data

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Research Article

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Elemental characterization of buckwheat (*Fagopyrum esculentum* Moench) cultivated in Turkey

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Abstract

This study, mineral and heavy metal contents of buckwheat (*Fagopyrum esculentum* Moench) newly imported foreign origin in our country were investigated. Buckwheat seeds cultivated at Konya ecological conditions at five different sowing dates and at different doses of fertilizer (0, 10 and 20 kg / da DAP-18-46) were obtained macro elements (Na, K, Ca, Mg and P), micro elements (Fe, Zn, Cu and Mn) and heavy metal (Al), were determined by ICP-OES. The analyses of mineral compositions in buckwheat seeds were determined using NMKL 161 method. It was determined that the amount of phosphorus (P) from macro nutrients in buckwheat seeds ranged from 1197-3778 ppm according to different sowing dates, and the amount of iron (Fe) from micro nutrients varied between 20.5-393.10 ppm. The amount of aluminum (Al) in heavy metals varied between 47.03-328.30 ppm. In this research, it was found that the seeds of the buckwheat (*Fagopyrum esculentum* Moench) grown at different planting dates and fertilizer doses show significant differences in mineral content.

Keywords: Buckwheat, Fagopyrum esculentum Moench, planting time, mineral element, seed

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Introduction

Buckwheat (*Fagopyrum esculentum* Moench), a kind of plant belonging to the family Polygonaceae, is most commonly cultivated and produced in China, Russia, Ukraine and Kazakhstan, but in recent years, it has been cultivated all over the world and in particular in Asia, Europe and America (Kara, 2014). Meanwhile, Buckwheat has been produced in Turkey recent years.. Buckwheat is one of the most important pseudo cereal and a valuable raw material for functional foods and drug production since it is rich in essential amino acids, fatty acids, routine and vitamins, and it is also a good source of minerals. Additionally, researches conducted proved that buckwheat could also behave as antioxidant.

New entrance to the field of agriculture in our country in recent years, the plant is one of the buckwheat plant. In recent years, the researches on buckwheat in Turkey have been carried out in both production and Research & Development activities by different institutions and universities.. Buckwheat is a valuable source of minerals for the people who consume it. Buckwheat contains a relatively high level of some minerals (Ikeda and Yamashita, 1994; Ikeda et al., 1995). Buckwheat grains are an important source of micro elements, such as: Zn, Cu, Mn, Se and macro elements: K, Na, Ca, Mg (Wei et al.2003). Buckwheat seed contains some kinds of minerals at relatively high levels. Buckwheat minerals have beneficial effects on health: magnesium may contribute to maintenance of normal muscle and nerve function, healthy immune function, and bone health; potassium may reduce the risk of high blood pressure and stroke, in combination with a low sodium diet; zinc is the component of many enzymes and its deficiencies lead to retarded development of children, skin affections, acne and weakening of taste; phosphorus is an essential component of bones and teeth (Anonymus, 2009).

In this research, it was found that the seeds of the buckwheat (*Fagopyrum esculentum* Moench) cultivated in different planting dates and fertilizer doses showed significant differences in mineral content.

Material and Method

Material: The seeds used in the study were obtained from Selcuk University, Faculty of Agriculture, Medicinal Plants Research and Application Farm, (Konya, Turkey).

The field experiments were carried out in Konya Seljuk University, Faculty of Agriculture, Medicinal Plants Research and Application Farm. Field experiments were conducted in 2012. Depending on the climatic conditions, buckwheat seeds were planted directly in the field, with the first sowing date in March 2012, the second sowing date in April 2012, the third sowing date in May 2012, the 4th sowing date in June 2012 and the 5th sowing date in June 2012. The fertilizer used in the experiments diammonium phosphate (DAP-18-46) was applied with three different doses (0, 10 and 20 kg / da).

The analyses of mineral compositions in Bucwheat Seeds: Mineral contents were determined in the seeds of buckwheat applied at different sowing times and fertilizer doses. The analyses of mineral compositions in buckwheat

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seeds (P, K, Ca, Mg, Mn, Zn, Al, Cu, Fe, Na) were determined using NMKL 161 method. A Perkin-Elmer Optima 2000 inductively coupled plasma–optical emission spectrophotometer (ICP–OES) was used to analyze the elements in buckwheat seeds.

Results and Discussion

The average results of the mineral contents determined in different fertilizer doses and buckwheat seeds cultivated at planting dates are given in Table 1. Macro elements (Na, K, Ca, Mg, P), micro elements (Fe, Zn, Mn, B) and Al were investigated.

The highest amount of phosphorus (P) one of the major macro elements (3778 ppm) was obtained from the first sowing date and the buckwheat seeds produced in the control plots, the lowest phosphorus content (1197 ppm) was obtained from the fifth sowing date and 20 kg / da fertilizer dose application. As can be seen, phosphorus content decreased as the sowing date was delayed. The amount of calcium (Ca) in the buckwheat seeds was the highest (13350 ppm) in the control plots at the first sowing date. On the other hand, the lowest amount of calcium (1051 ppm) was obtained from the third sowing date at 20 kg / da fertilizer dose application. Reduces were observed when the sowing date delayed in calcium content such like the phosphorus content. The highest amount of potassium (K) (5815 ppm) of buckwheat seeds was obtained from the first sowing date and produced from 10 kg / da fertilizer dose application, the lowest amount of potassium (2927 ppm) was determined in buckwheat seeds produced at the fifth sowing date and 20 kg / da fertilizer dose application. The highest amount of magnesium (Mg) was found at 2174 ppm and the second sowing date was determined at 10 kg / da fertilizer dose application, the lowest Mg was determined at 1636 ppm and the fifth sowing date at 20 kg / da fertilizer dose application. The highest sodium (Na) (179.1 ppm) was obtained from fertilizer dose application at 10 kg / da. The lowest Na was determined at 10 kg / da fertilizer dosing and first sowing date. There was no correlation between the amount of fertilizer applied and the macro elements accumulated in the seeds.

The amount of iron (Fe) from the micro nutrients was obtained from the highest second sowing date and 10 kg / da fertilizer dose application (393.10 ppm). The lowest amount of Fe was obtained from the fifth sowing date (20.5 ppm) and 20 kg/da fertilizer dose application. The highest amount of Zinc (Zn) was 55.59 ppm from the fifth sowing date and 20 kg / da fertilizer doses and the lowest amount of Zinc (Zn) was determined as 20.17 ppm from the first sowing date and 20 kg / da fertilizer dose.

Aluminum (Al) mineral is specified as heavy metal. Accordingly, the highest amount of Al was determined to be 375.5 ppm, the lowest amount of Al was found to be 50.44 ppm. The highest Al value was determined at 10 kg / da fertilizer dose and second sowing date, the lowest amount of Al was obtained of third sowing date and 20 kg / da fertilizer dose application.

In a study conducted under Konya ecological conditions, the average amount of phosphorus (P) was reported as 3666.70 ppm and the amount of iron (Fe) as 87.94 ppm (Kan, 2011). In addition, they determined that the buckwheat was rich in K, Zn, Ca, Mg, Mn and Na minerals (Wei et al., 2003). The content of Ca, Fe, Mg, Mn, Cu in the buckwheat flour was higher than that of wheat pasta (Tanaka, 1996). The lowest values of mineral nutrient content in buckwheat were determined in the plots without fertilizer (Kara and Telli, 2016). It should be noted that the high amounts of phosphorus and iron obtained in this study may be due to the ecological characteristics of the years of cultivation as well as the date of sowing applied.

Conclusion

The results indicated that mineral composition of buckwheat seeds significantly varied according to the fertilizer doses and sowing dates. High level of Mg, Fe, Mn and Cu obtained from the first and second sowing dates, despite that, high level of zinc (Zn) was determined from the 4th and the 5th sowing date. High level of Mn and Al were found in the seed of buckwheat. The compositions mineral of buckwheat seeds were effected by different fertilizer doses applied.

Table 1. Value of Buckwheat Seed Mineral Substances (ppm)

Sowing date	Fertilizer Dose (kg/da)	Minerals (ppm)									
		Ca	K	Mg	Na	P	Cu	Fe	Mn	Zn	Al
1st Sowing Date (21.03.2012)	Control	13350	5427	2042	115,2	3778	6,969	328,30	21,06	28,02	129,4
	10	1493	5815	1980	179,1	3634	6,24	108,60	13,84	30,2	69,82
	20	2317	5288	2037	60,01	3610	4,912	164,10	14,74	21,17	136
2nd Sowing Date (15.04.2012)	Control	1831	4972	1918	72,67	3618	5,347	165,20	18,82	22,91	111,2
	10	4268	5250	2174	96,73	3551	5,846	393,10	23,9	25,83	375,5
	20	2886	5541	2096	155,7	3467	6,021	215,40	17,66	31,96	177,5
3rd Sowing Date (07.05.2012)	Control	2598	4590	1971	118,8	2698	4,551	129,40	9,539	22,3	103
	10	2671	4654	1832	113,4	2384	4,715	163,70	11,92	23,73	130,3
	20	1051	4461	1701	71,64	2420	4,912	72,33	9,135	67,78	50,44
4th Sowing Date ı (22.05.2012)	Control	1678	4160	1808	105,62	2237	4,821	120,20	10,60	43,01	123,5
	10	2478	3913	1774	105,85	2029	4,729	105,57	9,63	45,53	118,9
	20	2986	3667	1739	106,07	1821	4,638	90,94	8,67	48,04	114,3
5th Sowing Date (11.06.2012)	Control	1896	3420	1705	106,30	1613	4,547	76,31	7,70	50,56	109,7
	10	2154	3174	1670	106,53	1405	4,455	61,68	6,74	53,07	105,1
	20	1896	3420	1705	106,30	1613	4,547	76,31	7,70	50,56	109,7

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Research Article

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Essential oil yield and compositions of sage (*Salvia officinalis* L.) cultivated in different province of Turkey

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Abstract

The essential oils from three Salvia species from Konya, Karaman and Elazığ locations were analyzed by gas chromatography – mass spectrometry (GC-MS). In this study, ıt was investigated essential oil yield and compositions of sage (*Salvia officinalis* L.) cultivated in different province Turkey (Elazıg, Karaman, Konya). The air-dried herb parts of sage (*Salvia officinalis* L.) were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce essential oil. The GC-MS analysis was carried out with Agilent 7890 GC-MS system. The relative percentages of the separated compounds were calculated from total ion chromatograms. The identification of the oil components was based on the Wiley and NIST mass spectral library. The sage essential oil was determined as 1.7% in Konya, 1.6% in Karaman and 1.1% in Elazığ, respectively. The essential oil of the sage cultivated in Konya is 1.7% and its major components are α-thujone (15,04%), 1,8 cineole (13,46%) and camphor (8,90%). The essential oil of sage cultivated in Karaman is 1.6 % and the major components are camphor (26.22%), α-thujone (20.02%) and 1.8 cineole (10.54). The essential oil of the sage cultivated in Elazığ is 1,1% and the major constituents are α-thujone (24,55%), 1,8 cineloe (14,42%) and camphor (11,15%). According to the results of this study, it was determined that significant differences between essential oil yield and components of sage (*Salvia officinalis* L.) produced in different provinces of our country.

Keywords: Sage, *Salvia officinals,* Essential oil composition, Location

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Introduction

Salvia officinalis is known as sage or garden sage. Salvia comes from the Latin salveo and salvaro, terms that mean “save” and “cure.” (Anonymus, 2018). Sage is a perennial plant rich in volatile oil belonging to Lamiaceae family. The genus Salvia, belonging to Lamiaceae,are represented by over 900 species worldwide. Anatolia is a major centre for Salvia in Asia, 47 of its 90 species endemic to Turkey (Kan et al, 2007). The sage is a herbaceous or bushy plant that can be grow up to 60-100 cm, and the flowers could differ from the blue to white and it has green leaves with a burning smell. It is fibrous rooted and drought resistant. The branching feature of the plant is quite large. Herbs of sage are used in the pre-flowering period (Felice Senatore et al.,2006). Salvia genus comprises herbaceous, suffructicous or shrubby perennials, rarely biennial or annual, often strongly aromatic plants. Approximately 900 species have been recorded widespread throughout the world. The plant grows mainly in mild and hot climates. Some members of this genus are of economic importance, since they have been used in folk medicine all around the world for their antibacterial, antitumor, antidiabetic antituberculosus, activities and as a flavoring agent in perfumery and cosmetics (Werker et al, 1985; Tzakau et al, 2001). There are some significant activities and properties of Salvia essential oils, including antimicrobial, antioxidant, anticholinesterase, improvement of cognitive performance and mood, reducing work-related stress, antimutagenic, anticancer, antiinflammatory, choleretic activities. The present review reported the main and significant pharmacological activities of salvia essential oils. (Fu et al, 2013).

This study was carried out to determine essential oil yields and volatile oil components in sage herba grown in Konya, Karaman and Elazığ provinces.

Material And Method

Plant Material: Sage seeds used in this study were obtained from Selcuk University Faculty of Agriculture, Department of Medicinal Plants. The Seedlings of *Salvia officinalis* have been cultivated in three different cultivated trials. Samples of *S. officinalis* were harvested from the cultivated trial from Konya, Karaman and Elazığ provinces in Turkey. Plant samples to be used in the analysis of essential oil were harvested in pre-flowering period.

Essential oil yield (%): "Water Distillation Method" was used to obtain the essential oil yield of sage. According to this method, 100 g dry herb samples were subjected to water distillation for 3 hours and a volatile oil was obtained. Volumetric (ml / 100 g) volatile oil yield was determined by Clevenger type essential oil equipment.

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Determination of essential oil components: After the essential oil was obtained, essential oil was identified to the GC-MS to determine its components. The chemical composition of the *S. officinalis* essential oil was performed by GC-MS. The composition of the essential oil was calculated as percentage. The identification of essential oil components was carried out by comparison of the obtained mass spectra with the NIST and Wiley library.

Results and Discussion

The essential oil of the three samples was obtained in yields of 1.1, 1.4 and 1.7% (w/w), based on the dry weight of the plant from cultivated trials in Elazığ, Karaman and Konya respectively. The qualitative and quantitative composition of the oils is presented in Table 1, Figure 1,2,3,4. 12 compounds were identified accounting for 84.88-92.50 %. The 12 components commonly identified in all three province are camphor, α-thujone, 1,8-cineole, viridiflorol, borneol, camphene, limonene, salvene, α-pinene, β-pinene, β-thujone and γ-terpinene It was determined a significant difference in composition between with variations in the amounts of the main compounds: the highest amount of essential oil of 1.8 cineole was obtained from Elazığ province (14.420 %) while the lowest amount was obtained from Karaman province (10.540). In these samples, thujones, characteristic compounds of sage oils, are present in very high amounts (α-thujone 15.040 – 24.552 % and β-thujone 4.716-8.483 %). The highest amount of α-thujone was 24.552 % from Elazığ province, the lowest amount of α-thujone was 20.023 % from Karaman. β-thujone was determined the highest amount with 8.483 % from Elazığ, the lowest amount with 4.716 % from Konya. The amount of β-thujone was determined to 5.192 % from Karaman province.

The other studies reported that *Salvia officinalis* contains 1-2.5% yield of essential oil. It is desired that the yield of essential oil to *Salvia officinalis* is at least 1.5% according to European Pharmacopoeia. The major active compositions of sage essential oil contain R-and β-tocopherol, camphor, cineole, borneol, as well as R-humulene from sesquiterpenes and β-chiophyllene in larger quantities (Mathe et al, 2007). In the other study conducted under the conditions of Çukurova Region Drog was investigated according to different planting times of medicinal sage (*Salvia officinalis* L) and the effects of this plant on the essential oil yield and essential oil components were investigated. Essential oil yield was determined as 1.73-4.80% ,the main components of essential oil are determined as follows: Camphor (16.69%), cineole (12.67%) and thujone (10.69%) (Kırıcı et al, 1996). In a Tunisian study, the main essential oil components were identified as: Camphor (25.14%), α-thujone (18.83%), 1,8-cineole (14.14%), viridiflorol (7.98%), β-thujone (4.46%) and β-caryophyllene (3.30%) (Khedler et al, 2017). The essential oil components were alpha-pinene, beta-pinene, 1,8 cineole, alpha-thujone, beta-thujone, camphor, borneol, alpha-humulene, viridiflorol, and manool account at least 81% of weight in essential oil samples. (Santos-Gomes et al, 2001). It is also seen in our work that the main components are the same as other studies and they are parallel to the quantities.

Conclusion

Results of this study showed monoterpenes 1,8-cineole, camphor and α-thujone are the major constituents of *Salvia officinalis* essential oil. The essential oil composition of *S. officinalis* is highly influenced by genetic and environmental factors, plant parts age, climate conditions, and seasonality (Farhat et al, 2009). The results showed that the sage cultivated from Konya province can be prefered with regard to 1.8 cineloe. It can be used as a high quality raw material for the production of phytopreparations.

Table 1. Common Essential Oil Compounds from Three Locations

RI*	Compounds	% Percentage according to locations		
		Konya	Karaman	Elazığ
1585	Camphor	8,901	26,216	11,151
1425	α-thujone	15,040	20,023	24,552
1250	1,8-cineole	13,460	10,543	14,420
1794	Viridiflorol	9,440	3,816	4,534
1030	α-pinene	4,208	3,786	5,717
1118	β-pinene	6,866	2,839	3,032
1442	β-thujone	4,716	5,192	8,483
1665	Borneol	8,440	1,429	4,291
1073	Camphene	2,708	4,818	3,014
1229	Limonene	1,715	2,076	1,678
1238	γ-terpinene	0,422	0,308	0,392
930	Salvene	0,155	0,130	0,255
1590	Caryophyllene	5,919	1,710	-
1695	β-selinene	5,547	-	3,360
2051	Manool	5,015	4,612	-
TOTAL		92,55	87,50	84,88

RI: Retention indices relative to C8 to C24 *n*-alkanes.

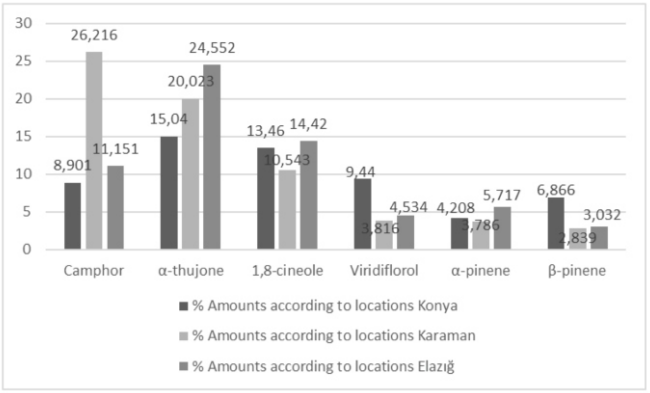

Figure 1. Common Essential Oil Components from Three Location

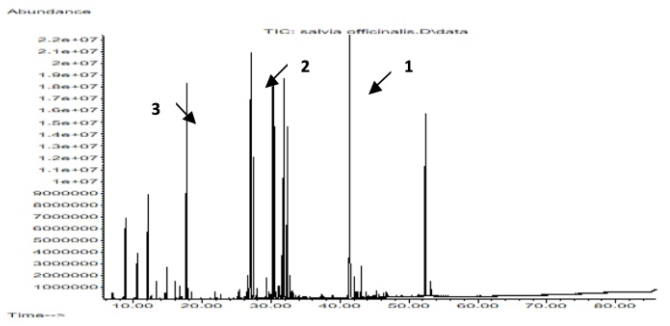

Figure 2. Essential oil composition choromatogram in Konya province 1. α-thujone, 2. 1.8-cineole, 3. camphor

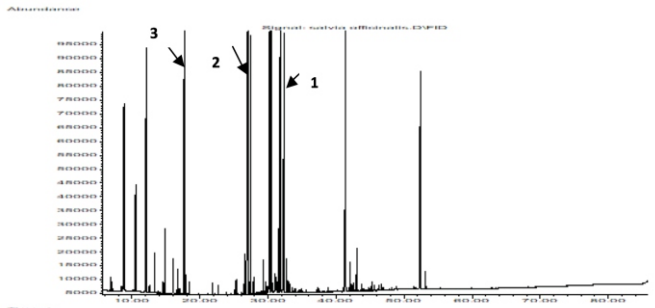

Figure 3. Essential oil composition choromatogram in Karaman province 1.Camphor, 2.α-thujone, 3.1.8-thujone

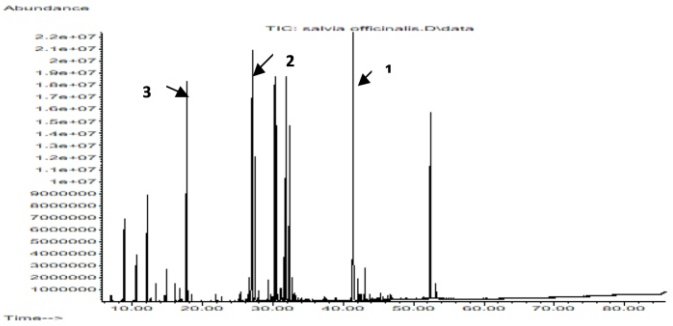

Figure 4. Essential oil composition choromatogram in Elazığ province. 1. α-thujone, 2. 1.8-cineole, 3. Camphor

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Investigation of routine contents of buckwheat (*Fagopyrum esculentum* Moench) cultivated in Turkey

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Abstract

It was determined by the routine contents of herbs and seeds of buckwheat plant grown at Konya ecological conditions at March, April, May, June 2012, 2013 and different fertilizer doses (0, 10 and 20 kg / da DAP-18-46) for two years. Routine analyses were performed using an Agilent Technologies1200 series high pressure liquid chromatography (HPLC), including a binary pump, vacuum degasser, auto sampler, diode array detector, and coupled to an Agilent Technologies 1200 series Model VL single quadrupole mass spectrometer equipped with an multimode ionization interface. At different planting times; the amount of routine in herbs of buckwheat plant ranged from 2.15 to 2.99%, ranged from 2.27 to 2.73% at the applied fertilizer doses. Routine contents of buckwheat seeds were relatively low compared to herb plants and ranged from 0.031 to 0.071%. In this study, it was determined that the buckwheat (*Fagopyrum esculentum* Moench) grown at different planting times investigated significant differences in the routine contents of herbs and seeds.

Keywords: Buckwheat, *Fagopyrum esculentum*, Planting Time, Fertilizer, Routine, Herb, Seed

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Introduction

Buckwheat (*Fagopyrum esculentum* Moench) is a plant of the Polyganeceae family, with a single year and a short vegetative period (80-90 days) (Kan, 2014). The buckwheat is a plant based on ancient times and is of Central Asian origin. First, the buckwheat plant grown in the Hun Empire, China and Japan is grown in many countries today. Buckwheat, which is produced in many countries of the world (Russia, Ukraine, Kazakhstan, France, Czech Republic, Slovakia etc.), has been started to produce in our country in the last two years and we have two kinds of buckwheat . The buckwheat plant has fast-growing, leafy and white-pink flowers. The flowers are fragrant and suitable for nectar accumulation of honey bees (Anonymus, 2012). The buckwheat plant is benefited both from the seed (grain) and from each plant. Buckwheat grains are rich in vitamins, especially those of B group (Fabjan et al.2003). The amino acid composition of buckwheat proteins is well balanced and of a high biological value (Kato et al.2001), although the protein digestibility is relatively low (Liu et al.2001). Buckwheat grains are a rich fibre, soluble dietary fibre (SDF), and are applied in the prevention of obesity and diabetes (Brennan,2005) source of TDF (total dietary). With 80% unsaturated fatty acids more than 40% are constituted by polyunsaturated fatty acid (PUFA) (Krkošková & Mrázová2005). Buckwheat grains are an important source of microelements, such as: Zn, Cu, Mn, Se (Stibilj et al.2004), and macroelements: K, Na, Ca, Mg (Wei et al.2003).

Buckwheat plant exhibits high biological activity because it is rich in flavonoids, phenolic acids, tannins, phytosterols and phagopyrins. Six flavonoids were isolated from the buckwheat seed and the most significant amount was determined routine in total flavonoids. Rutin is one of many well-known flavonoids with a high biological activity. The routine inhibits blood platelet aggregation, increases capillary strength, and reduces their 3 permeability; additionally, it reduces the cholesterol level in the blood, has anti-oxidative and antiinflammatory effects, and contributes favourably to hepatoprotection (Chen and Hsieh, 2010).Biological activities such as neuroprotective, acetylcholinesterase, butyrylcholinesterase and antioxidant were studied according to the total phenol, flavonoid and routine contents of the extracts prepared from seeds, stems and herbs of buckwheat cultivated in Konya ecological conditions. It has been concluded that these important compounds containing buckwheat which are main compounds responsible for activity.

In this study, it was determined by the routine contents of herbs and seeds of buckwheat cultivated at five different sowing dates and different fertilizer doses in Konya ecological conditions.

Material and Method

The seeds of buckwheat were cultivated Selcuk University, Faculty of Agriculture, Medicinal Plants Research and Application Farm, (Turkey). The herbs and seeds were powdered using pestle and mortar into fine

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powder. Then the powdered samples of the seeds and herbs were submitted to successive solvent extraction sequentially with ethanol (EtOH) at room temperature for 48 h (2×500 mL). After filtration,the organic phases were evaporated by using a rotary evaporator (Buchi, Switzerland) at 40 °C to dryness in vacuo.

Routine analyses were performed using an Agilent Technologies 1200 series high pressure liquid chromatography (HPLC), including a binary pump, vacuum degasser, auto sampler, diode array detector, and coupled to an Agilent Technologies 1200 series Model VL single quadrupole mass spectrometer equipped with an multimode ionization interface. Standard solutions containing rutin (5.1–1020 g mL−1) was prepared in ethanol (70%). 10 L of injections were achieved in triplicate for each standard solution to see the reproducibility of the detector response at each concentration level. Five concentrations of routine were subjected to regression analysis to calculate calibration equation and correlation. All the calculations concerning the quantitative analysis were performed with external standardization by measurement of peak areas (Gülpınar et al, 2012)

Results and Discussion

The routine of herbs and seeds content of buckwheat grain samples is given in Table 1 and Table 2 respectively. According to the variance analyzes performed, the effect of different sowing dates and fertilizer doses on the routine rate of buckwheat herb was statistically significant at 1% level.

In this study, it was determined that to different sowing dates, the highest amount of routine obtained from all herbs of buckwheat was obtained from the application of third sowing date in 2012 (2.99%), while the lowest amount of routine (2.15%) was obtained from first sowing date applications. According to different fertilizer applications; the highest amount of routine was obtained from application of fertilizer (2.90%) at 10 kg / da and the lowest amount of routine was obtained from control plots (2.27%).

In this study, it was determined that to the variance analyzes performed, the effect of different sowing dates on the routine rate of buckwheat seeds was statistically significant at 1% level. According to this study, the highest amount of routine obtained from the seeds of the buckwheat was obtained at the second sowing date (0.07%) and the lowest at the fifth sowing date (0.03%) in the seeds when both trial years were evaluated together. It has been concluded that the different fertilizer doses applied on the routine contents of buckwheat seeds are not effective. It was determined that the buckwheat plant is much more in herbs than the routine contents. Routine quantities found in other studies were found to be lower than this study. The content of routine of buckwheat seeds and herbs were affected by different sowing dates, different production techniques and ecological factors. At the same time, the content of routine is depend on genotype, sowing dates, cultivating conditions, development period, part of plant and harvesting time. Different cultivars of buckwheat may have different contents of routine with potential variation also in different part of plants (Ahmed et. al,2013). Most of routine is accumulated in the in florescence (up to 0.12 mg/g DW), in stalks (0.004–0.01 mg/g DW),upper leaves (0.08–0.10 mg/g DW) (Hagels 1999) and 0.12–0.36 mg/g DW in grains depending on the variety and growth conditions (Kitabayashi et al.1995;Brunori et al.2010; Park et al.2011). Routine contents of dry matter in buckwheat seed were reported to ranged from 12.6-35.9 mg / 100g (Tian et al., 2002) and from 0.05 g / 100 g to 1.35 / 100 g (Bai et al., 2015). It is concluded that different fertilizer doses applied on the routine contents of buckwheat seeds are not effective. The routine contents of buckwheat were found to be much higher in herbs than in seeds. The same time, ecological factors such as cultivating conditions may also have a great influence on routine content (Kreft et al., 2002).

In this study, it was determined that the buckwheat (*Fagopyrum esculentum* Moench) cultivated at different sowing date investigated significant differences in the routine contents of herbs and seeds. The routine content of buckwheat, in other pseudo-cereals no routine compound is found.

Table 1. Routine amounts (%) obtained from buckwheat herb according to different applications

Year	Sowing Date	Fertilizer Dose (kg/da)			Average
		Control	10	20	
2012	1st sowing date (21.03.2012)	2,15r	2,16qr	2,15r	2.15h
	2nd sowing date (15.04.2012)	2,17qr	2,51klm	2,52jkl	2.40f
	3rd sowing date (07.05.2012)	2,55ıj	3,21c	3,20c	2.99a
	4th sowing date (22.05.2012)	2,20q	2,99d	2,30o	2.50e
	5th sowing date (11.06.2012)	2,25p	3,65a	2,27op	2.72b
	Average	2.27e	2.90a	2.49d	2.55a
2013	1st sowing date (26.03.2013)	2,25p	2,28op	2,34n	2.29g
	2nd sowing date (29.04.2013)	2,72ef	2,74e	3,45b	2.97a
	3rd sowing date (14.05.2013)	2,47m	2,52jkl	2,54jk	2.51e
	4th sowing date (10.06.2013)	2,55ıjk	2,59hı	2,61h	2.58d
	5th sowing date (21.06.2013)	2,49lm	2,67g	2,69fg	2.61c
	Average	2.49d	2.56c	2.73b	2.59a

Table 2. Routine amounts (%) obtained from buckwheat seeds according to different applications

Year	Sowing Date	Fertilizer Doses (kg/da)			Average
		Control	10	20	
2012	1st sowing date (21.03.2012)	0,03g	0,08a	0,07b	0.06b
	2nd sowing date (15.04.2012)	0,07b	0,07b	0,07b	0.07a
	3rd sowing date (07.05.2012)	0,06bc	0,06bc	0,06bc	0.06b
	4th sowing date (22.05.2012)	0,06bc	0,06bc	0,05c	0.06b
	5th sowing date (11.06.2012)	0,05c	0,05c	0,06bc	0.05c
	Average	0.05	0.06	0.06	0.06
2013	1st sowing date (26.03.2013)	0,07b	0,06bc	0,06bc	0.06b
	2nd sowing date (29.04.2013)	0,05c	0,06bc	0,05c	0.05c
	3rd sowing date (14.05.2013)	0,04e	0.04e	0.04e	0.04e
	4th sowing date (10.06.2013)	0,04e	0.03f	0.04e	0.04f
	5th sowing date (21.06.2013)	0,03f	0,03f	0,02g	0.03g
	Average	0.05	0.04	0.04	0.04

Conclusion

Buckwheat is not a cereal, it is actually a gluten-free seed which is a suitable for people who are sensitive to wheat or other cereal grains that contain protein glutens. Buckwheat is very good source of manganese and good source of magnesium, copper and diatery fibre. Buckwheat has more protein, phenolic compounds (especially routine) than rice, wheat, millet, corn. Buckwheat, compared to rice, wheat and corn, has low on the glycemic index. The parts of the buckwheat plant that contains the highest routine is its herb.

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Research Article

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A study on essential oil yield and components of dryed and fresh foliage of peppermint (*Mentha piperita* L.) cultivated in Turkey

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Abstract

In the study, essential oil yield and its components dryed and fresh foliage obtained from peppermint [*Mentha piperita* L. (Lamiaceae)] cultivated in Konya ecological conditions were investigated. The yield of essential oil from dryed and fresh peppermint foliage was determined to be 3.2 % and 2.9 %, respectively. The major essential oil components of dryed and fresh foliage peppermint oil were determined as mentone (50.80 %), mentol (34.55 %) and mentone (48.18 %), mentol (21.77), respectively. The aim of this research attempts to contribute to knowledge of differences between essential oil yield and components of the dryed and fresh foliage peppermint (*Mentha piperita* L.) cultivated Konya ecological conditions, Turkey.

Keywords: *Peppermint, Mentha piperita L.,* Essential oil, Component, Menthone, Menthol

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Introduction

The increase in the world population, the diversity of human needs and the increase in demand for natural products and the importance of medicinal and aromatic plants have also increased (Polatcı et al.,2009). Turkey is one of the rare countries with ecological conditions suitable for the agriculture of cultivated medical and aromatic plants. Medicinal and aromatic plants that are exported from our country or consumed in the inner market have been usually collected from flora of Turkey (Kan, 2005).

Mentha species are commercially cultivated in many countries because of the essential oil and herb. In Turkey, peppermint cultivated in the gardens, in front of the houses and on the fields since ancient times have been used for medicinal purposes such as antispasm, carminative, refreshing, stimulant and diuretic effects and ıt widely has been used as spices, culinary herb and herbal teas. Mint is the richest natural source of menthol, caffeic acids, flavonoids such as apigenin (Gruenwald, 2004) which has a wide application area in medicine, food and cosmetics industry (Baytop, 1984).

Pepermint (*Mentha piperita* L.) is a perennial, herbaceous that is belong to Lamiaceae (Labiatae) family. The origin of pepermint is the Mediterranean Region, especially Anatolia and Egypt (Esetlili et all., 2015) and widespread in cultivation throughtout all over the world(Rita and Animesh, 2011). Pepermint is a species that emerges as a result of hybridization of *Mentha aquatica* L. and *Mentha spicata* L. species (*Mentha piperita* L. *M. aquatica* x *M. spicata*) (Büyükbayraktar, 2009). In addition ıt is known as the British mint, this plant is also known as "nane" in Turkey (Baytop, 1994). This plant, has an important place in terms of essential oil and essential oil content and among to mint species is the most benefited from essential oil in the world. The essential oil of pepermint obtained from herb's leaves and widely uses in traditional medicine applications for the purpose of analgesic, anesthetic, antiseptic, astringent, carminative, decongestant, expectorant, nervine, stimulant, stomachic, inflammatory diseases, ulcer and stomach problems(Shrivastava, 2009).

The aim objective of the work presented was to determine the effects differences between essential oil yield and components of the dryed and fresh foliage peppermint (*Mentha piperita* L.) cultivated Konya ecological conditions in Turkey.

Materials and Methods

Plant material: The plant material used in the trial, is Pepermint (*Mentha piperita* L.). This study was carried out to essential oil yield and components of the dryed and fresh foliage peppermint cultivated, Selçuk University, Faculty of Agriculture, Medical Plants Research and Application Farm in Konya ecological conditions. The harvested leaves of this plant were dryed at the shade conditions.

Essential Oil Distillation and Analysis: The foliage of the plants were subjected to hydrodistillation for 3 h using Clevenger type apparatus to produce essential oil. Essential oil is calculated as volume (ml / 100 g) GC-MS instrument was used to determine the essential oil components. The essential oils were stored at -20^{0}C until analyzed. GC analysis was performed on a Agilent 6890N Network GC system combined with Agilent 5975C VL MSD Network Mass Selective Detector. The GC conditions were; column, DB Waxe tr; 60.0m x 0.25mm x 0.25µm; oven temperature programme: The column held initially at 60^{o}C for 10 min

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after injection, then increased to 220 °C with 4°C/min heating ramp for 10 min and increased to 240°C with 10°C/min heating ramp without hold; inject or temperature 250°C; carrier gas; He; in let pressure, 9.60psi; linear gasvelocity, 7 cm/sec; initial flow 0.3 ml/min ;split ratio,65.0:1; injected volume 1.0µl (EP6).

Results and Discussion

Essential oil yield (%): In the study, the yield of essential oil from dryed and fresh peppermint follage was determined to be 3.2 % and 2.9 %, respectively. It can be said that dryed pepermint on essential oil yield is effective in this study. At the same time, it is known that effect of plant genetic structure, ecological and cultivated conditions are important in the yield of essential oil. In the other researchs, The oil yield of pepermint obtained the fresh or partly dryed plant, varied from 0.1 -3.75 % (Aflatuni, 2005). The differences between the study in Konya ecological conditions and the results obtained from other works, it could be said that from research conditions.

Essential oil components (%): It was identified commonly total 23 chemical components of essential oils from dryed and fresh pepermint that cultivated in Konya ecological conditions. A total of 23, accounting for 89.88 and 83.39 % of the total oil, were identified in the *M. piperita* L. essential oils.

In this study, ıt was determined which menthol and menthone as major components of peppermint volatile oil. The major essential oil components of dryed and fresh foliage peppermint oil were determined as mentone (50.80 %), menthol (34.55 %) and mentone (48.18 %), menthol (21.77), respectively. the amount of 1.8 cineole in the pepermint essential oil components varied from 3.21 to 3.83 % (Table 1). It has been determined that the effect of dryed on the volatile oil components of the peppermint obtained from this work is important.

The chemical composition of *M. piperita* is characterized by the presence of oxygenated monoterpenes such as menthol, menthone, menthyl acetate, sabinene hydrate menthofurone and 1,8 cineole.

Kızıl et al. (2010) reported that menthol content 35.64 %, menthone content 38.06 % and cineole content 3.62% obtained from *M. piperita* essential oil. Moreover, menthol content of different peppermint origin varied from 10 to 63% and menthone content from 12 to 76%.

The other studies were determined main component as menthol (% 26-30), menthone (% 14-21) (Zheljazkov ve ark.2009). According to monographs of European Pharmacopoeia are cineole (3.5-14.0%), menthone (14.0-32.0%) and menthol (30.0-55.0%) (Shrivastava, 2009). The results obtained from this study were found to be appropriate when compared to the pharmacopoeia of Europe.

Table 1. Essential oil composition of dryed and fresh foliage peppermint (%)

RT*	Compounds	Percentage	
		Dryed peppermint	Fresh peppermint
1022	α-Pinene	0.24	0.54
1150	Camphene	0.05	0.02
1197	β-Pinene	0.46	0.83
1203	Sabinene	0.26	0.42
1218	Myrcene	0.18	0.27
1234	Menthol	34.55	21.77
1237	(Z)-β-Ocimene	0.23	0.24
1243	γ-Terpinolene	0.36	0.26
1577	L-Menthone	49.18	50.09
1584	Cis-3-Hexenyl Isovalerate	0.31	0.05
1687	Linalool	0.34	0.19
1695	Trans-SabineneHydrate	0.12	0.1
1724	Isopulegone	0.16	0.25
1751	Cis-Isopulegone	0.16	0.07
1760	Trans-Caryophyllene	1.11	1.81
1768	IsoMenthol	0.15	0.11
1828	1.8-Cineole	3.22	3.83
1943	Germacrene D	1.82	1.4
2007	Piperitone	0.75	0.99
2014	Delta Cadiene	0.03	0.03
2193	Limonen-10-yl Acetate	0.04	0.05
2236	Cis-Jasmone	0.05	0.06
2185	(E)-IsoEugenol	0.02	0.03
	Total	**89.88**	**83.39**

Conclusion

According to the results of our study, it was reported that differences between essential oil yield and components of the dryed and fresh foliage peppermint (*Mentha piperita* L.) cultivated in Konya ecological conditions, Turkey. Compared with these results, it was determined that dryed foliage had better yields of essential oil yield and essential oil contents than fresh foliage.

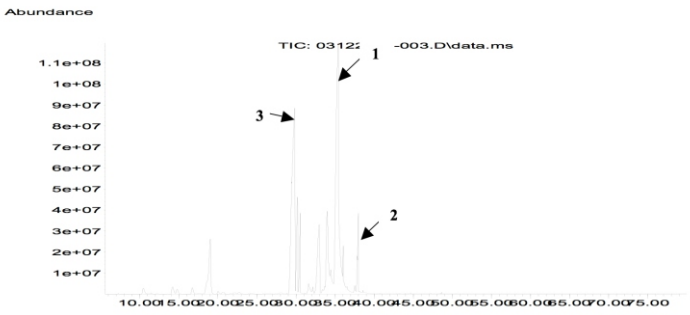

Figure 1. Essential oil composition of dryed foliage peppermint choromatogram 1. L-Menthone, 2. 1.8-cineole, 3. Menthole

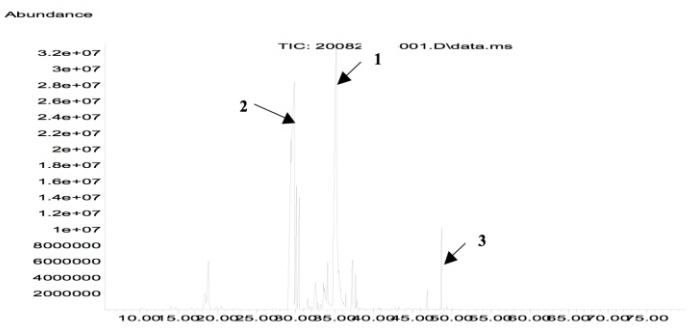

Figure 2. Essential oil composition of fresh foliage peppermint choromatogram. 1. L-Menthone, 2. Menthole, 3. 1.8-cineole

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Research Article

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The Fourth International Mediterranean Symposium on Medicinal and Aromatic Plants (MESMAP-4) 2018 Special Issue

Essential oil yield and compositions of chamomile (*Matricaria Chamomilla* L.) cultivated in different province Turkey

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Abstract

In this study, it was investigated essential oil yield and compositions of Chamomile (*Matricaria chamomilla* L.) cultivated in Konya and Karaman ecological conditions, Turkey. Essential oil yield of chamomile was determined as 0.73 % in Konya and 0.62 % in Karaman. The most important essential oil component of chamomile, chamazulene content is 1.13 % in Konya location and 1.36 % in Karaman location. Alpha-bisabolol content from the essential oil components in the Konya and Karaman province was determined as 38.60% and 27.36%, respectively. The aim of this research attempts to contribute to knowledge of differences between essential oil yield and components of Chamomile (*Matricaria chamomilla* L.) cultivated in Konya and Karaman ecological conditions, Turkey.

Keywords: Chamomile, *Matricaria chamomilla* L., Essential oil, Composition, Chamazulene

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Introduction

Chamomile (*Matricaria chamomilla* L.) is annual, herbaceous that is plant belongs to Asteraceae family. The origin of chamomile is in eastern Europe and Asia Minor and it has been almost every flora of Turkey and all over the word. Chamomile is one of the important medicinal herb that has been used in herbal remedies for thousands of years. The parts of the plant used for medical purposes are flowers and the blue-green essential oil obtained from the flowers.

The most important essential oil contents of chamomile are chamazulene, bisabolol (Kazemi, 2014; Singh et al., 2011), bisabololxyzd, bisabololonoxid and parneson (Ceylan, 1983). This plants contains seconder metabolite actived. The most important of them are sesquiterpenes, flavonoids, coumarins, polyacetylenes and essential oil. This plant use as food, cosmetics, pharmaceutical application, sanitary, ornamental plant and the treatment of many diseases in traditional medicine applications (Sharafzadeh et al., 2011). It has been determined that studies on Chamomile (*Matricaria chamomilla* L.) have antiseptic, antiallergic and anti-inflammatory, antimicrobial, fungusid, antispasmodic, gastroprotective, allergic reaction, carminative and sedative activities (D' Andrea, L., 2002; Gruenwald, 2004; Lopez et al., 2016;Kazemi, 2014; Zeybek et al., 2011).

The aim objective of the work presented was to determine the effects differences between essential oil yield and components of Chamomile (*Matricaria chamomilla* L.) cultivated in Konya and Karaman ecological conditions, Turkey.

Materials and Methods

Plant materials

The seed used in the study were obtained from Selçuk Universty, Faculty of Agriculture, Medicinal Plants Research and Application Farm, Turkey. The plant material used in the trial, is chamomile (*Matricaria chamomilla* L.). This study was carried out to essential oil yield and components of chamomile cultivated in Konya and Karaman ecological conditions. The harvested flower of this plant were dried at the shade.

Essential oil distillation and analysis

The air-dried flowers of chamomile were subjected to hydrodistillation for 3h using a Clevenger-type apparatus to produce essential oil. The essential oils were stored at -20^{0}C until analyzed. The GC-MS analysis was carried out with Agilent 7890 GC-MS system. The relative percentages of the separated compounds were calculated from total ion chromatograms. The identification of the oil components was based on the Wiley and NIST mass spectral library. The GC conditions were; column, DB Waxe tr; 60.0m x 0.25mm x 0.25µm; oven temperature programme: The column held initially at 60^{o}C for 10 min after injection, then increased to 220 ^{o}C with 4^{o}C/min heating ramp for 10 min and increased to 240^{o}C with 10^{o}C/min heating ramp without hold; inject or temperature 250^{o}C; carrier gas; He; in let pressure, 9.60psi; linear gasvelocity, 7 cm/sec; initial flow 0.3 ml/min; split ratio, 65.0:1; injected volume 1.0µl (EP6, 2007).

Results and Discussion

Essential oil components of Chamomile cultivated in Konya and Karaman were given Table 1. According to the results obtained this study; The highest essential oil yield was determined from in Konya location (0.73%), while the lowest essential oil yield was determined in Karaman location(0.62%). It can be said that the essential oil yield of Konya location is effective in this study. At the same time, it is known that effect of plant genetic structure, ecological and

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cultivated conditions are important in the yield of essential oil. Pirzad et al.(2008) reported that essential oil yield of Chamomile varied from 0.626- 0.754%. The other study conducted that the oil yield of Chamomile varied from 0.5 -1.5 % (Ceylan, 1983). The results obtained from this study were found to be appropriate when compared to this research.

It was identified commonly total 8 chemical components of major essential oils from *Matricaria chamomilla* L. that cultivated in Konya and in Karaman ecological conditions. (Figure 1; Figure 2) A total of 8 commonly compounds, accounting for 91.37 and 80.06 % of the total oil, were identified the essential oil of *Matricaria chamomilla* L. that cultivated in Konya and in Karaman ecological conditions, respectively. These components are β-ocimene, β-farnesene, Germancrene D, Bisabololoxide, Bisaboloneoxide, Alpha-bisabolol, Chamazulene, Alpha-bisabololoxide.

The most important essential oil component of chamomile, Chamazulene content was 1.13% in Konya location and 1.36 % in Karaman location. Alpha-bisabolol content from the essential oil components in the Konya and Karaman province was determined as 38.60% and 27.36%, respectively. The yields of β-farnesene content of essential oil was 30.15% in Konya and 25.05 % in Karaman. According to our results, Chamomile cultivated in Konya is higher than in Karaman on account of both the yield of essential oil and the amount of the important essential oil contents. Lopez et al.(2016) reported that chamomile flowers have large amount of the sesquiterpene hydrocarbon *trans*-beta-farnesene (38.22%) followed of alpha-bisabolol oxide A (16.74%) were found in the chamomile essential oil and chamazulene was found 1.5-4.44%. According to monographs of European Pharmacopoeia are chamazulene (≥1.0%), bisabolol oxides (29-81%).

Table 1. Essential oil components of chamomile in Konya and Karaman (%)

RI	Components	Percentage (%)	
		Konya location	Karaman location
20.536	β-ocimene	0.41	1.05
35.711	β-farnesene	30.15	25.05
37.260	Germancrene D	6.11	4.35
48.026	Bisabolol oxide	1.30	5.61
49.050	Bisabolone oxide	0.17	1.35
49.926	α-bisabolol	38.60	27.36
54.937	Chamazulene	13.50	13.93
56.156	α-bisabolol oxide	1.13	1.36
	Total	91.37	80.06

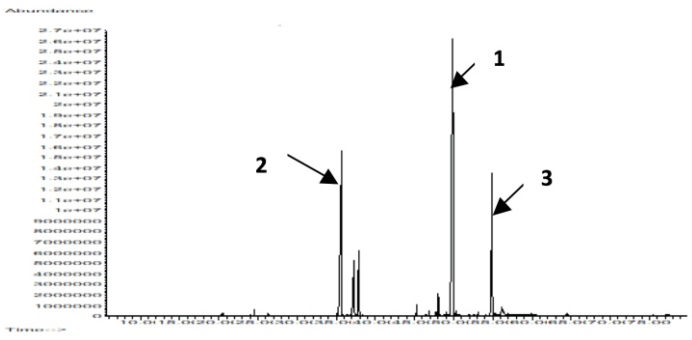

Figure 1. Essential oil composition choromatogram in Konya location 1. α-bisabolol, 2. β-farnesene 3. Chamazulene

Conclusion

According to the results of this study, it was determined that has differences between essential oil yield and components of chamomile (*Matricaria chamomilla* L.) produced in different province of our country were determined. Compared with these results, it was determined that yields of essential oil composition obtained from Chamomile cultivated in Konya and Karaman were appropriated to European Pharmacopedia.

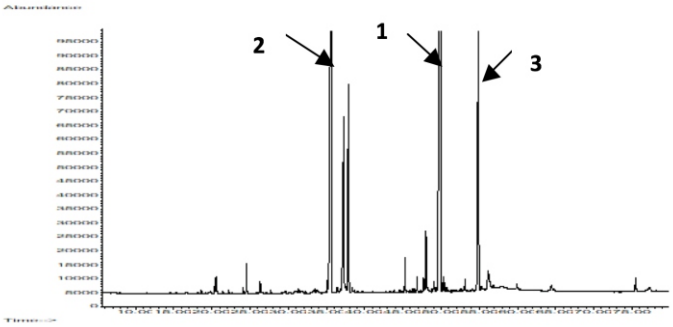

Figure 2. Essential oil composition choromatogram in Karaman location 1. α-bisabolol, 2. β-farnesene 3. Chamazulene

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Research Article

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The Fourth International Mediterranean Symposium on Medicinal and Aromatic Plants (MESMAP-4) 2018 Special Issue

Essential oil yield and compositions of endemic mountain tea (*Sideritis libanotica* Labill. ssp. linearis (Bentham) Borm. and *Sideritis bilgerana* P.H. Davis) cultivated in Konya ecological conditions of Turkey

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Abstract

In this study, it was investigated essential oil yield and compositions of endemic mountain tea (*Sideritis libanotica* Labill. and *Sideritis bilgerana* P.H. Davis) cultivated in Konya ecological conditions, Turkey. The essential oil yield of cultivated *Sideritis libonitica* Labill. flowers was 0.20 % while the essential oil yield of *Sideritis bilgerana* P.H. Davis flowers was 0.15%. The highest essential oil components were determined as 19.82 % β-pinene, 14.60 % α-pinene from *Sideritis bilgerana* P.H. Davis; 25.92 % hexadecanoic acid and 21.49% δ-cadinene from *Sideritis libonitica* Labill. According to the results of this study, it was determined that significant differences between essential oil yield and components of cultivated the endemic mountain tea species in Konya ecological conditions were determined.

Keywords: Mountain tea, *Sideritis*, Essential oil, Composition, Endemic

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Introduction

Sideritis L. belongs to the family of Lamiaceae (Labiatae) which is one of the most common and diverse plants of the world. Over 150 species of the genus Sideritis are mainly found in the Mediterranean area. The aerial parts of plants from the genus Sideritis, generally known as 'mountain tea', are widely used as a popular folk medicine in Spain, Greece and Turkey. The genus Sideritis is represented in the Turkish flora by 46 species, 31 of which are endemic (Demirtaş et al, 2011), including Sideritis libanotica Labill. ssp. linearis (Bentham) Borm. Sideritis species are used in the treatment of gastrointestinal ailments and common colds as well as a herbal tea in Turkish folk medicine (Baytop, 1999; Yesilada et al., 1995). Several studies have been conducted on various biological activities of Sideritis species such as antimicrobial, antioxidant, antiinflammatory, antispasmodic, antiulcerative, nervous system stimulant anticonvulsant, carminative, analgesic and sedative effects. Çarıkçı et al., 2012; Gümüşçü et al., 2011; İşcan et al., 2005; Tepe et al., 2006; Gonzales et al. 2011). Many chemical constituents have been identified in sideritis genus such as terpenes, flavonoids, essential oil, iridoids, coumarins, lignanes and sterols. Diterpenes, flavonoidts and essential oil ocur in almost every species. In fact, they are the main pesponsible for the pharmacological activity. Sideritis species grown in Turkey are has got a rich yield and compositions of essential oils.

This study was carried out to determine essential oil yields and compositions oil from two endemic mountain tea species cultivated in Konya, Turkey.

Materials and Methods

Plant material: Sideritis bilgerana and Sideritis libonitica were cultivated at the Medicinal Plant Farm, in Konya (Turkey). The species were identified by Dr. Hayri Duman, at Gazi University. Voucher specimens were deposited at the Herbarium of Faculty of Agriculture, Selçuk University, Konya, Turkey.

Essential oil yield (%): Plant samples to be used in the analysis of essential oil were harvested in flowering period. The essential oils were obtained by hydrodistillation using a Clevenger-type apparatus for 3 h, from aerial parts of the Sideritis libonotica and Sideritis bilgerana. The oil yields were calculated on a dry weight basis as 0.20 and 0.15%, respectively.

Determination of essential oil components: After the essential oil was obtained, essential oil was identified to the GC-MS to determine its components. The chemical composition of *Sideritis libanotica* and *Sideritis bilgerana* essential oil was performed by GC-MS. The composition of the essential oil was calculated as percentage. The identification of essential oil components was carried out by comparison of the obtained mass spectra with the NIST and Wiley library.

Results and Discussion

The essential oil yield of cultivated Sideritis libonitica Labill. flowers was 0.20 % while the essential oil yield of Sideritis bilgerana P.H. Davis flowers was 0.15%. The percentage constituents of essential oils from cultivated *Sideritis libanotica* and *Sideritis bilgerana* were given Table 1.

The essential oils of *S. bilgerana* and *Sideritis libonotica* were analyzed both by GC and GC/MS to determine their

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constituents (Table 1). As a result of GC and GC/MS analyses, 22 and 18 components were identified, representing 85.013 and 97.000 % of the total for both *S. bilgerana* and *S. libonotica* oils, respectively. GC/MS analyses of the oils have revealed the occurrence of β -pinene (19,82%) and α -pinene (14.40 %) as the main constituents of *S. bilgerana*. δ-cadinene (21.49%) and hexadecanoic acid (25,92%) was also characterized as a main component in the oil of *S.libonotica.* To the best of our knowledge, the literature contains no information on the essential oil from *S. libanotica* and *S. bilgerena* produced in the control of cultivation conditions. With it, Iscan et al. (2005) reported β -pinene (48.40 %) and α -pinene (31.90 %) as the main constituents of *S. bilgerana* collected from naturally growing in flora of Turkey. The essential oil yield and composition of is highly could be influenced by genetic and environmental factors, cultivation and ecological conditions.

Conclusion

According to the results of this study, it was determined that significant differences between essential oil yield and components of the endemic mountain tea species cultivated in Konya ecological conditions according to *Sideritis* spp. grown in natural flora.

Table 1. The composition of essential oils from *Sideritis bilgerana* and *S. libonotica* cultivated

RI	Components	Percentage	
		Sideritis bilgerana	***Sideritis libonotica***
1022	α-pinene	14.606	0.075
1111	β -pinene	19.028	0.284
1165	α -cubebene	1.841	-
1197	Limonene	1.126	0.108
1204	β-phellandrene	0.356	0.101
1206	Muurol-5-En-4-α-Ol (cıs)	1,348	-
1208	Muurola-4(14),5-Diene(Trans)	0,322	-
1210	2-hexenal	0.087	0.096
1289	Linalool	0,791	-
1549	Muurola-3,5-Diene(Cıs)	0,654	-
1599	Cadına-1(6),4-Diene(Trans)	0,61	-
1606	Caryophyllene	2.446	4.528
1665	β-farnesene	1.103	0.817
1669	γ-curcumene	2.022	0.872
1727	Zingiberene	3.075	2.207
1735	β -bisabolene	9.861	20.286
1748	Bicyclogermacrene	-	3.151
1781	Bisabolene	-	0.289
1783	α-curcumene	1.125	0.933
1869	δ-cadinene	7.932	21.49
1987	Caryophyllene oxide	4.326	7.409
2126	Pentadecanone	-	1.831
2142	3-hexen-1-ol	0.958	3.530
2224	α- bisabolol	5.437	3.073
2894	Hexadecanoic acid	5.959	25.92
	Total	85.013	97.000

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