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Essential oil and fatty acid composition leaves of some lamiaceae taxa from Turkey

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Abstract
In this research leaf essential oil and fatty acid compositions of *Micromeria fruticosa* subsp. *serpyllifolia*, *Ballota nigra* subsp. *anatolica* and *Nepeta crinita* were analyzed by GC/GC-MS. Pulegone (25.62%) and Piperitone (10.26%) were detected the main compounds of *Micromeria fruticosa* subsp. *serpyllifolia*; β-caryophyllene (20.45%), germacrene D (16.20%) and caryophyllene oxide (10.55%) were detected the major constituents of *Ballota nigra* subsp. *anatolica*; β-caryophyllene (16.90%), germacrene D (10.89%) and nepetalactone (10.56%) were determined the main compounds of *Nepeta crinita*. The average major fatty acid composition of studied samples were found to be Palmitic (29.42%), Steraic (11.70%), Petroselinic (23.10%), Linoleic (10.04%) and Linolenic acid (23.50%); while other fatty acids were found in small proportions.

Keywords: Essential oil, fatty acid, *Micromeria, Ballota, Nepeta*

Introduction
Medicinal and aromatic plants have been used since ancient times to improve the sensory characteristics of food, to act as preservatives and for their nutritional and healthy properties in taritional medicine and ethnobotany. Medicinal and aromatic plants are generally recognized as safe and are excellent substitutes for chemical additives in human and animal life. In chemicals, essential oils obtained mainly by steam distillation, from medicinal and aromatic plants; in addition essential oils have many useful effects in human life. Lamiaceae is one of the most important families in the production of essential oils with antioxidants, antimicrobial and other biological activities. Medicinal and aromatic plants are rich in essential oils and are mainly found in the Mediterranean region, where the production of such oils is a profitable source of ecological and economic development. Lamiaceae taxa have been extensively studied with respect to their use as food preservatives and in ethnobotany. Plant extracts have been used since old times in traditional medicine; most of Lamiaceae taxa contains medicinal and aromatic plants used in traditional, in ethnobotany, in modern medicine and in the food and pharmaceutical industries. In Turkish traditional remedies most of Lamiaceae taxa are often used for the treatment of gastritis, infections, dermatitis, bronchitis, inflammation and other diseases. Essential oils can be synthesized by all plant organs (flowers, leaves, stems, twigs, seeds, fruits, roots, wood, or bark) and are complex mixtures of sesquiterpene and monoterpene hydrocarbons and alcohols, ketones, and aldehydes, fatty acids, oxides, and sulphur derivatives [1]. Essential oils shows antioxidant, antibacterial, antidiabetic, antimutagenic, non-toxigenic, and antimycotic properties which are promising for their use as bioactive compounds in different foods [2]. Plants protects itself by essential oils against fungi, bacteria, viruses, herbivores, allelopathic activity, defence against insects and attraction of pollinators and dispersal agents to favour the dispersion of seeds and pollens. In addition, the major activities of essential oils are antimicrobial, sedative, anti-inflammatory, bactericidal, antiviral, antifungal and preservative for foods [3]. In recent decades, scientists have carried out an intensive biological and chemical examination of the plant secondary metabolites particularly essential oils [4]. Recently, because of strong promotion on the reduction in use of synthetic products, many scientists began to understand the importance of traditional and alternative medicine; data regarding application of medicinal plants can be searched in historical manuscripts but have to be verified by a modern science in order to develop an effective drug. Medicinal and aromatic plants present a rich source of new biologically active compounds and they have been used for their medicinal properties for centuries. Lamiaceae consists of about 252 genera and more than 6700 species and in Turkey this family is the third largest family in Turkey with 46 genera, 782 taxa comprising of which 346 taxa are endemic and endemism ratio is ca. 44%.

The largest five genera of Lamiaceae as follows: *Stachys*, *Salvia*, *Sideritis*, *Phlomis* and *Teucrium* [5]. Essential oils are considered to be one of the potential sources for the screening of anticancer, antimicrobial, antioxidant and free radical scavenging agents [6]. Most of Lamiaceae taxa are frequently used in cooked dishes and are recognized as important preventive factor of many diseases and aromatic medicinal plants from this family have long been used in Turkey traditional medicine [7].

Micromeria Benth. taxa are numerous in the Mediterranean region, some of which are endemic. A number of Lamiaceae taxa are aromatic plants which are used for traditional medicine and as culinary herbs worldwide. Some *Micromeria* taxa are used in folk medicine and in ethnobotany for different purposes. In Spain *Micromeria graeca* is used for stomach pains; *Micromeria biflora* is used for treating disorders of the digestive tract; in Turkey *Micromeria fruticosa* is used to relieve headache, *Micromeria herpyllomorpha* and *Mivromeria varia* are used in the Canary Islands as a capillary tonic; in Israel a decoction of this species is used for bathing inflamed and sore eyes and an infusion is used in the treatment of abdominal pains and hypertension [8]. Most of *Micromeria* species have antimicrobial, antifungal and other biological activity [9, 10]. *Ballota* L. (Lamiaceae) genus comprised of about 90 species and widespread over the World [11]. In Turkey, the genus *Ballota* is represented by 12 species and 8 subspecies [12]. *Ballota nigra* is a perennial herb and bearing simple hairs, it is represented by five subspecies in Flora of Turkey. *Ballota nigra* is known as “yalancı ısırgan” in Turkey and aerial parts of some subspecies of *Ballota nigra* are used to treat inflammation, as an antiseptic for wounds, and against gastrointestinal disorders [13]. *Ballota nigra* subsp. *anatolica* is known as “gripotu” and has been used in folk medicine as an antiseptic, anti-inflammatory, anti-rheumatic, antioxidant, and antimicrobial agent, and also for nausea, vomiting, and nervous dyspepsia [14]. Some of *Nepeta* L. species are well known for their medicinal properties and biological activities; they are used in the folk medicine for their diuretic, diaphoretic, antitussive, antispasmodic, anti-asthmatic, febrifuge, emmenagogue, sedative and stomachic properties; pharmacological and biological effects are usually attributed to nepetalactones, especially found in *Nepeta* oils.

However, there is very lack study regarding *Ballota nigra* subsp. *anatolica*, *Micromeria cilicica*, *Nepeta crinita* are reported in the literature. Mostprobably, this could be the first report on the fatty acid compositions of studied samples from Turkey. In this research, essential oil and fatty acid composition leaves of *Micromeria fruticosa* subsp. *serpyllifolia*, *Ballota nigra* subsp. *anatolica* and *Nepeta crinita* were detected to contribute data about studied samples in the literature.

Material and Method

Plant materials

Micromeria fruticosa subsp. *serpyllifolia* was collected from Sancak (Bingol) Hasanova avenue surroundings, steppe and rocky areas, in June 2018, 1550-1600 m., by O. Kilic with 6887 collected number. *Ballota nigra* subsp. *anatolica* was collected from Sancak (Bingol) vicinity of Karacubuk village, roadside, moisty areas, in July 2018, 1750-1800 m., by O. Kilic with 7002 collected number. *Nepeta crinita* was collected from Levent valley (Malatya), steppe and rocky slopes, in July 2018, 1550-1650 m. by O. Kilic with 7102 collected number. Plant samples were identified with Flora of Turkey and East Aegean Islands [15]. Voucher specimens were deposited in the Pharmacy Faculty of Adıyaman University.

Gas chromatography/mass spectrometry (GC-MS) analysis

5 g of the each plant samples were homogenized in 10 mL of hexane/isopropanol at 10.000 rpm for 30 second and centrifuged at 5000 rpmfor 10 min [16]. The upper part was taken and put into the test tubes by filtration. Fatty acids need to be derivatized in order to be able to look at GC. Derivatization with methyl esters is often preferred. Forthispurpose Christiemethod was used [17]. 5 mL of 2% methanolic sulfuric acid was added and vortexed. This mixture was kept at 50 ºC for 15 hours of methylation. After 15 hours, the tubes were removed, cooled to room temperature, and vortexed with the addition of 5 mL of 5% NaCl. The fatty acid methyl esters formed in the tubes were extracted with 5 mL of hexane and the hexane phase was removed from the top by a pastry pipette and treated with 5 mL of 2% $KHCO_3$ and waited for 1-2 hours to separate the phases. The solvent of the mixture containing the methyl esters was then evaporated at 45 °C under nitrogen and the fatty acids below the test tubes were dissolved in 1 mL of hexane and analyzed by GC-MS using amber GC vials. The essential oils were analyzed using HP 6890 GC equipped with and FID detector was used. The column and analysis conditions were the same as in GC-MS. The percentage composition of the essential oils was computed from GC-FID peak areas without correction factors. The essential oil compounds were identified using the Wiley and Nist mass spectral library and the identified compounds of the essential oils are listed in Table 1. An Agilent brand 7890A/5970 C GC-MS instrument and a SGE Analytical BPx 90 100m x 0.25 mm x 0.25 um column were used for fatty acid analysis. The temperature program was gradually heated from 120 ^{0}C to 250 ºC and the total time was set to 45 min. The temperature program is like this; 120 ºC is heated up to 250 ºC at 5 ºC / min and is expected at this temperature for 19 min and the total time is 45 min. The autosamplers washed themselves in hexane 5 times before shrinking and after giving the collar. Injection volume was 1 uL and split ratio was 10: 1, solvent delay time was 12 minutes, carrier gas was He, and H_2 flow was 35 mL/min, flow rate was 350 mL/min, N_2 was 20.227 mL/min is automatically set by the program. The identified fatty acid compounds of studied taxa are listed in Table 2.

Table 1: Essential oil compounds of studied samples (%)

Compounds	*Micromeria fruticosa* subsp. *serpyllifolia*	*Ballota nigra* subsp. *anatolica*	*Nepeta crinita*
2-Hexanal	-	1.53	-
α-thujene	-	0.20	0.56
α-pinene	2.51	-	1.42
β-pinene	-	-	1.20
Camphene	-	0.23	-

Sabinene	0.76	-	2.81
3-octanol	1.95	-	-
1-octen-3-ol	-	5.42	-
α-terpinene	0.65	-	4.50
β-pinene	1.56	1.35	-
p-cymene	5.55	-	3.20
β-phellandrene	-	0.54	1.72
γ-terpinene	1.66	1.56	2.51
Limonene	3.45	0.53	3.65
1,8-cineole	4.55	1.80	6.82
Cis-*p*-menthone	4.66	-	-
δ-3-carene	-	0.52	1.20
α-terpinolene	-	1.14	-
β-thujone	1.25		2.30
Linalool	-	0.89	1.02
Carveol	0.47	-	0.12
Nerol	6.15	-	-
Camphor	-	1.45	1.23
Pulegone	25.62	-	3.52
Piperitone	10.26	-	-
Nepetalactone	-	-	10.56
Borneol	-	0.45	3.12
Bornyl acetate	0.85	-	-
α-terpineol	-	-	3.65
Trans-carveol	2.10	-	1.0
Carvacrol	1.63	1.26	-
α-terpinene	-	2.10	0.12
α-copaene	0.89	4.62	-
β-bourbenene	2.47	3.80	0.52
β-elemene	-	-	-
β-caryophyllene	4.56	20.45	16.90
Germacrene D	1.54	16.20	10.89
β-bisabolene	-	8.78	-
δ-cadinene	3.60	-	0.32
Spathulenol	0.65	5.6	-
Caryophyllene oxide	2.05	10.55	0.12
β-eudesmol	-	3.20	3.52
α-cadinol	1.54	-	-
β-bisabolene	-	-	0.16
Hexadecanoic acid	-	3.56	-
	90.74	93.19	89.66

Table 2: Leaf fatty acid composition of studied samples (%)

Studied Samples	Palmitic acid	Palmitoleic acid	Stearic acid	Petroselinic acid	Linoleic acid	Linolenic acid	Arasidic acid	Eicosatrienoic acid	Behenic acid
Micromeria fruticosa subsp. *serpyllifolia*	17.79	-	8.11	21.51	16.38	32.26	1.15	0.98	1.82
Ballota nigra subsp. *anatolica*	30.04	2.57	12.37	32.40	8.82	13.80	-	-	-
Nepeta crinita	40.43	-	14.79	15.39	4.94	24.45	-	-	-

Results and Discussion

In this study leaf essential oil and fatty acid compositions of *Micromeria fruticosa* subsp. *serpyllifolia*, *Ballota nigra* subsp. *anatolica* and *Nepeta crinita* were analyzed; pulegone (25.62%) and Piperitone (10.26%) were detected the main compounds of *Micromeria fruticosa* subsp. *serpyllifolia*; β-caryophyllene (20.45%), germacrene D (16.20%) and caryophyllene oxide (10.55%) were detected the major constituents of *Ballota nigra* subsp. *anatolica*; β-caryophyllene (16.90%), germacrene D (10.89%) and nepetalactone (10.56%) were found to be the main compounds of *Nepeta crinita*. The average major fatty acid composition of studied plant samples were determined palmitic (29.42%), steraic (11.70%), petroselinic (23.10%), linoleic (10.04%) and linolenic acid (23.50%), while other fatty acids were found in small proportions. In a research essential oil composition of *Micromeria thymifolia*, *Micromeria dalmatica*, and *Micromeria albanica* were investigated; as a result pulegone (32.81%, 15.94%, 13.43%) and piperitone (11.71%, 3.39%, 5.62%) were detected as main compounds of studied samples, respectively [9]. In another study essential oil composition of *Micromeria cilicica* were analysed (has been used in folk medicine); as a result 34 components in hydrodistillation, 30 components in steam distillation were detected and the major component characterized in the essential oils was pulegone (66.55, 64.10%) and other main components were determined as *cis-p*-menthone (21.71, 25.31%), *trans-p*-menthone (9.59, 5.59%), nerol (0.35, 2.49%) and 3-octonol (0.81, 0.25%), respectively [10]. Similarly in this research pulegone (25.62%) was detected the main compounds of *Micromeria fruticosa* subsp. *serpyllifolia;* whereas *cis-p*-menthone (4.66%) was determined small proportion (Table 1). A work has also clearly demonstrated the high anticandidal ffect of pulegone

and the water extract of *Micromeria cilicica* against the yeast *Candida albicans* [10], hence the extracts of *Micromeria cilicica* may be useful as an alternative antimicrobial agent in natural medicine for the treatment of many infectious diseases. Antifungal activities of essential oils from *Micromeria dalmatica*, *Micromeria albanica*, *Micromeria thymifolia* [9], *Micromeria cristata* were evaluated [18]. Biological assays showed strong toxicity against fungi of oil from these species. In another study, *Micromeria nervosa* was found to be active against *Candida albicans* [19]. Essential oil composition of *Ballota nigra* subsp. *foetida* was analyzed and 37 identified compounds of the oil, β-caryophyllene (20.0%), germacrene D (18.0%) and caryophyllene oxide (15.0%) were detected as major compounds [20]; similarly β-caryophyllene (20.45%), germacrene D (16.20%) and caryophyllene oxide (10.55%) were detected the major constituents of *Ballota nigra* subsp. *anatolica* from studied sample (Table 1).

In a study twenty-two compounds were identified from the oil of *Ballota nigra* subsp. *uncinata* representing 96.9% of the total oil and fourteen compounds were identified from the oil of *Ballota nigra* subsp. *anatolica* representing 88% of the total oil; the major components were characterized as caryophyllene oxide (21.2%), hexadecanoic acid (19.9%), β-caryophyllene (18.9%) for *Ballota nigra* subsp. *uncinata* and hexadecanoic acid (40.9%) and β-bisabolene (13.4%) for *Ballota nigra* subsp. *anatolica*, respectively [21]. In this study β-caryophyllene (20.45%), germacrene D (16.20%) and caryophyllene oxide (10.55%) were detected the major constituents of *Ballota nigra* subsp. *anatolica* (Table 1). The essential oil composition of some *Ballota* taxa have been previously studied; in 2003, Bader *et al*. Reported that β-caryophyllene (25.1%) and germacrene D (24.2%) as the main compounds of *Ballota nigra* subsp. *foetida* from Jordan [22]. In a study of caryophyllene oxide (7.9%), *epi*-α-muurolol (6.6%), o-cadinene (6.5%), and α-cadinol (6.3%) were found to be the main constituents of *B. nigra* [23]. A literature survey has shown that germacrene D (18.1%), nerolidol epoxyacetate (15.4%), sclareol oxide (12.1%), linalyl acetate (11.5%), and β-caryophyllene (10.5%) were found to be the main constituents of *B. nigra* subsp. *anatolica* growing in Iran [11]. *Ballota nigra* dominated by β-caryophyllene and germacrene D. In 2014, Fraternale and Ricci reported β-caryophyllene, caryophyllene oxide and germacrene-D as major compounds of *B. nigra* subsp. *foetida* [23]. The difference in the oil and fatty acid composition of the present study and previous research may be due to the collection time, chemotypes, drying conditions, mode of distillation, and geographic and climatic factors. The essential oil aerial parts of *Nepeta baytopii* Hedge and Lamond., *Nepeta cataria* L. and *Nepeta fissa* CA Mey. were investigated by GC and GC-MS. The yield of oils are *ca*. 0.40, 0.45 and 0.50 mL/100 g, respectively; forty six, fourty seven and forty nine compounds were identified representing 92.4, 91.2 and 92.5% of the oil, respectively; 1, 8-cineole (23.2%) and nepetalactone (12.8%) in *Nepeta baytopii*, nepetalactone (27.5%), 1, 8-cineole (10.8%) and germacrene D (9.2%) in *N. cataria*, 1, 8-cineole (24.3 %) and nepetalactone (17.6%) were identified as major components in *N. fissa* [24]. In this study β-caryophyllene (16.90%), germacrene D (10.89%) and nepetalactone (10.56%) were found to be the main compounds of *Nepeta crinita* (Table 1). In the literature there is lack study about fatty acid composition of *Micromeria fruticosa* subsp. *serpyllifolia*, *Ballota nigra* subsp. *anatolica* and *Nepeta crinita;* so this study also contribute to fatty acid composition of *Micromeria*, *Ballota* and *Nepeta* taxa. The average major fatty acid composition of studied samples were found to be Palmitic (29.42%), Steraic (11.70%), Petroselinic (23.10%), Linoleic (10.04%) and Linolenic acid (23.50%); while other fatty acids were found in small proportions.

Conclusion

In conclusion, some qualitative and quantitative differences were detected between studied species in view of main compounds of essential oil and fatty acid. These variations depending on genetic, environmental factors, ontogeny, season, plant part analyzed, analytical methods or defence and protection from insects, animals or pathogens. The findings showed that the studied plant taxa had some variations in fatty acid and essential oil composition. Detected main compounds can be chemotaxonomic marker of studied samples.

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