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Essential oil composition and mineral element content *of Salvia Aethiopis* L. from Turkey

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Mediterranean sage (*Salvia aethiopis* L.) is a common species in Turkey belonging to the family Lamiaceae. It could be used for various medicinal purposes as an ointment, for treating multiple diseases, *etc*. Mediterranean sage was collected from the campus area of Yozgat Bozok University Erdoğan Akdağ in June 2017. The essential oil was isolated from leaves by hydrodistillation and analyzed utilizing GC and GC/MS. Among the 32 components identified in this essential oil (yield 0.15%), caryophyllene oxide (30.11%), aromadendrene (18.03%), α-humulene epoxide (5.78%), (E)-*α*-bisabolene (5.72%), and isoaromadendrene epoxide (4.30%) were found to be the major constituents. Concentrations of potentially harmful heavy metals (Al, Cd, Co, Cr, Ni) were below the respective toxic levels.

Keywords: *Salvia aethiopis*, essential oil, heavy metal

INTRODUCTION

Turkey showes a large distribution of various species (9753 species), 3035 of which are endemic. It is stated that there are 3649 (31.82%) endemic and 11707 taxon. Turkey is an important center of the Lamiaceae family [1]. Since ancient times, this family has had many medicinal plants, mainly in the Mediterranean basin [2]. It is a vast family with 200 genera and 3200 species. In Turkey, 45 genera, 546 species, and 731 taxa are spreading [3]. *Thymbra, Thymus, Origanum, Satureja, Mentha, Teucrium, Ballota, Stachys, Salvia, Ajuga, Prunella, Melissa, Lamium, Sideritis,* and *Marrubium* are among the known important genera of this family. Most of the members of the Lamiaceae family are rich in essential oils and other secondary metabolites. The family species are of great importance in areas such as medicine, pharmacy, food, cosmetics, and perfumery.

The leaves, flowers, and stems of *Salvia* species, which have been critical medicinal plants since ancient times, are used differently. Sage leaves are evaluated in various ways for the treatment of many ailments (soothing, pain relieving, antiperspirant, expectorant, cold and anti-cough, relieving muscle pain, lowering high blood pressure and disinfectant, *etc*.) in folk medicine [4, 5]. The consumption of some *Salvia* species as herbal tea and spices is widespread. *Salvia* species appeal to various consumer groups (food industry, pharmaceutical, chemical industry, *etc*.). Some of them are rich in macro- and microminerals. Herbal products can make an important contribution in meeting the daily mineral needs of the human body. Therefore, knowing the nutritional content and pharmacological functions of herbal teas it is crucial in determining the dosage of use [6].

Mediterranean sage (*S. aethiopis* L.) is a biennial or perennial herb that is 25-60 cm high. This species grows on steppes, igneous and limestone slopes, fallow fields, and roadside banks. Its leaves are simple, mostly basal, and ovate-elliptic to oblong [7].

The essential oil yield and composition have been studied in several countries such as Turkey [8-10], Serbia [11-13], Romania [14], Bulgaria [15], Spain [16], and Iran [17-20]. The eessential oil yield was determined between 0.2 and 0.5%, and the main components were sesquiterpene hydrocarbons β-caryophyllene (7.3-27.5%), α-copaene (9.15-22.4%), germacrene D (5.0-29.4%), bicyclogermacrene (9.3-41.5%), γ-muurolene (10.3%), β-cubebene (7.0-9.7%), δ-cadinene (5.0-8.7%), β-elemene (9.9%), and oxygen-containing sesquiterpene - spathulenole (8.3%). The variation in the yield and composition of the oil was probably due to the soil and geographical features of the plant's regions, even within a same country. The essential oil has been shown to have antimicrobial activity [14].

Mining, urban or industrial solid, gas and liquid wastes, pesticide and artificial fertilizer use, paint

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industry, and car exhaust gases cause excessive amounts of heavy metals to be released into nature. This heavy metal stress caused by environmental pollutants limits plant growth and reduces product yield and quality [21]. Some micronutrients such as copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and cobalt (Co) are necessary for plant growth and development. However, some heavy metals such as arsenic (As), mercury (Hg), cadmium (Cd), lead (Pb), and chromium (Cr) are elements that are not necessary for plant development [22]. The presence of heavy metals, with or without micronutrients, in the atmosphere, water, and soil, in a concentration above a certain level, causes severe problems for all living organisms [23]. This situation causes the accumulation of toxic elements in various vegetative organs of plants. The vast majority of medicinal, aromatic plants are collected from the natural area and exposed to multiple pollution factors.

The present study aimed to determine the yield and chemical composition of *Salvia aethiopis* L. essential oil obtained from Eastern Turkey characterized with continental climate, as well as the content of mineral elements, including heavy metals, to find opportunities for plant application as an additive in various food or cosmetic products.

MATERIAL AND METHODS

Yozgat Bozok University Erdogan Akdag Campus is located within the B5 square considering the grid system. In previous studies, it has been reported that there are 22 taxa, including 14 genera of Lamiaceae family, in the campus area of Yozgat Bozok University [27]. The aerial parts of the plant were collected in the complete flowering stage on 12 June 2017 from Yozgat Bozok University Campus (39º46´41.72´´; N 34º47´56.01´´; E, altitude 1346 m). Then dried and stored in a cool (20 °C), dark cabinet until further processed and analyzed. Species identification of the collected samples was made in the Department of Botany in Yozgat Bozok University. The flowers of the plants have white color (Picture 1), unlike species growing in other countries, registered that were colored pink and lilac.

Determination of essential oil content

After harvesting, the leaves were separated by hand, then dried to constant humidity in the dark, without direct sunlight, at a temperature of about 20 °C. The dried leaves were stored in double paper bags in a dark and dry place. Before analysis, they were crushed to a size of 1 cm, after which the moisture was determined [28].

Essential oil contents of dried leaves samples were determined by water distillation with a Clevenger type apparatus. An average of 50 g of dried plant sample was distilled in 700 mL of water for 3 h. Essential oil contents (%, v/w) of the samples were calculated on dry matter. The essential oils obtained were placed in dark colored bottles and stored in a refrigerator at +4 ºC until analysis.

Picture 1. *S. aethiopis* L. plant (The image is copyrighted).

Gas chromatography-mass spectroscopy analysis (GC / MS). The chemical composition of the essential oil was determined using standard methods [28].

Determination of mineral mater and heavy metal content. The mineral composition was determined using the iCAP-Qc ICP-MS spectrometer (Thermo Scientific) [28].

Each analysis in the study was carried out in triplicate and the results were presented as the mean value (± SD) from the three measurements. Significant differences ($p < 0.05$) were assessed by applying statistical tools such as ANOVA and Tukey's multiple comparison test.

RESULTS AND DISCUSSION

Essential oil rate (%) and essential oil components (%)

The essential oil rate obtained from the samples collected from the natural environment during the full flowering period was recorded as 0.15%. The essential oil yield was lower than that reported in the literature for plants' aerial parts, for example 0.27% [8], 0.5% [12], 1.6% [18], 0.23% [20], which could be explained by the growing conditions of the raw material.

The essential oil is a light yellow liquid with a characteristic -odor.

GC-MS results of essential oil obtained from the aerial parts of *S. aethiopis* species are given in Table 1.

Thirty-two components representing 91.18% of the essential oil obtained from the floral aerial parts of the *S. aethiopis* species were identified and caryophyllene oxide (30.11%) and aromadendrene (18.03%) were found as the main components. The major components were determined as follows: *α*-humulene epoxide (5.78%), (E)-*α*-bisabolene (5.72%), and isoaromadendrene epoxide (4.30%). The results indicated that the essential oil obtained from the aerial plants of *S. aethiopis* from Turkey was also a caryophyllene oxide and aromadendrene chemotype.

The classification of the identified compounds, based on functional groups, is summarized in Fig. 1.

According to the data analysis, oxygenated sesquiterpenes predominated in the oil. The comparative analysis of the main components in the essential oils of species growing in other countries of the Balkan Peninsula showed that the essential oil with a high content of sesquiterpene hydrocarbons was close to the data for Bulgaria [15], Serbia [12, 13], Romania [14], as in previous studies for Turkey [8-10].

Table 1. Chemical composition of *S. aethiopis* essential oil obtained from aerial parts.

Component	RI_{cal}*	Content, %
Copaene	1375	1.17 ± 0.01
β-Bourbonene	1382	1.19 ± 0.01
Caryophyllene	1384	0.16 ± 0.0
trans-β-Caryophyllene	1419	0.38 ± 0.0
Aromadendrene	1439	18.03 ± 0.16
Dihydro-β-ionol	1475	0.21 ± 0.0
Germacrene D	1477	1.87 ± 0.01
β-Selinene	1483	0.64 ± 0.0
Cubedol	1484	0.47 ± 0.0
Bicyclogermacrene	1491	1.14 ± 0.01
α-Cadinene	1509	2.22 ± 0.02
endo-1-Bourbonanol	1514	0.84 ± 0.0
(E)-*α*-Bisabolene	1536	5.72 ± 0.04
Germacrene B	1542	1.32 ± 0.01
Endo-1,5-Epoxysalvial-4(14)ene	1561	0.36 ± 0.0
Germacrene D-4-ol	1571	0.57 ± 0.0
Caryophyllene oxide	1575	30.11 ± 0.28
Viridiflorol	1584	0.79 ± 0.0
α-Humulene epoxide	1600	5.78 ± 0.05
Hexadecane	1601	0.44 ± 0.0
delta-Cadinol	1644	0.28 ± 0.0
α-Cadinol	1651	2.20 ± 0.02
Valerenol	1668	0.19 ± 0.0
Shyobunol	1682	1.92 ± 0.01
Isoaromadendrene epoxide	1739	4.30 ± 0.04
2-Pentadecanone, 6,10,14-trimethyl ester	1838	2.84 ± 0.02
Sclareoloxide	1991	2.51 ± 0.02
Phytol	2074	1.13 ± 0.01
7-Hexyldocosane	2192	0.16 ± 0.0
Tetracosane	2381	0.41 ± 0.0
Pentacosane	2494	1.27 ±0.01
Nonacosane	3119	0.56 ± 0.0

* RI_{calc} - Kovats Retention Index, calculated by authors; TIC - Total Ion Current.

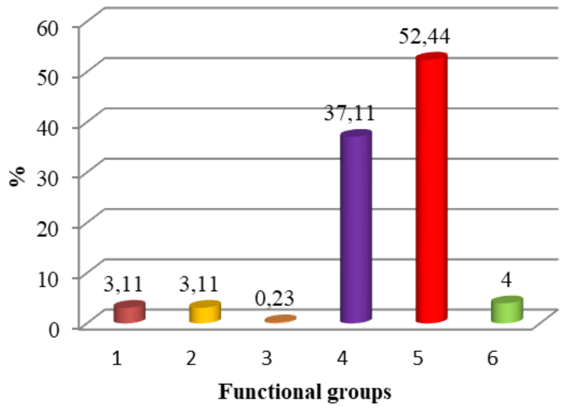

Fig. 1. Classification of the identified compounds, %. 1 - aliphatic hydrocarbons, 2 – oxygenated aliphatics, 3 - oxygenated monoterpenes, 4 - sesquiterpene hydrocarbons, 5 - oxygenated sesquiterpenes, 6 - diterpenes

According to [8] the main components of the *S. aethiopis* oil were germacrene D (29.0%), α-copaene (19.8%), β-cubebene+β-elemene (9.9%), bicyclogermacrene (9.3%), δ-cadinene (8.7%), and β-caryophyllene (7.3%). The variations observed in this study and those reported by other authors could be related to climatic alterations. Furthermore, differences in the amounts of some of the components obtained in this study could be explained by the variations of the soil and rainfed conditions between the countries.

Compared with the previous study results, the essential oil rates and composition of taxa were evaluated in the research scope; some results appeared to be similar or compatible but with very different results. These differences may be caused by genetic and environmental factors (temperature, precipitation, exposure time and intensity, altitude, orientation, drought, salinity, structure of soil and condition of plant nutrients, *etc.*) [9]. Plant parts used in the analysis, plant life cycle, and collecting time can also be effective [29]. Plants utilize secondary metabolites in order to counteract biotic and abiotic stressors. However, larger amounts of secondary metabolites are often synthesized when plants get stressed. For example, essential oil rates for medicinal and aromatic plants grown in hot and dry regions are higher than those for plants growing in cool and rainy areas [29]. In this context, it could be said that environmental factors significantly impact the differentiation of the findings obtained from our research from other studies.

Mineral matter and heavy metal content (ppm)

Some macro- (Ca, K, P and S) and microelement (Fe, Mn, Zn, Cu, B and Na) and heavy metal (Al, Cd, Co, Cr and Ni) contents of *S. aethiopis* species were determined and the results are presented in Figure 2.

In our study, Ca content was 270.15 ppm, K content was 468.80 ppm, and S content was 12.29 ppm. The proximity of agricultural areas to the cities, deterioration of urbanization, domestic and industrial wastes, heavy metals, or flue fumes emitted by motor vehicles cause undesirable results in all species. On the other hand, heavy metals from high doses of the food chain have a negative effect on human health. Medicine and aromatic plants collected from the natural environment or under cultural conditions may be exposed to various pollution factors. This situation causes the accumulation of toxic elements in the vegetative organs of plants, especially in their leaves. Toxic elements such as lead, cadmium, aluminum, and mercury can cause serious health problems. Therefore, the mineral content of medicinal and aromatic plants is an essential indicator of overall human health. Al, Cd, Co, Cr, and Ni contents of plants were evaluated as heavy metals. Among these, the amount of Al was determined as highest, which could be due to the level of precipitation, climatic factors and soil conditions (its pH) [21, 22]. The analytical determination of heavy metals in medicinal and aromatic plants is among the most important quality parameters in determining the plants' purity, safety, and effectiveness [30]. The limit values for Cd, Cr, and Ni, determined by the WHO/FDA (World Health Organization / American Food and Drug Administration), are 0.3, 0.02, and 1.63 ppm, respectively [31]. It was found that the detected quantities of the three metals were below the maximum allowed by the WHO/FDA.

CONCLUSION

Based on the results for the analysis of the chemical content of *S. aethiopis* essential oil, it was revealed that its main components were caryophyllene oxide, aromadendrene, *α*-humulene epoxide, *trans-α*-bisabolene, and isoaromadendrene epoxide. No heavy metals did occur in any significant concentrations in the plant samples. Therefore, *S. aethiopis* essential oil could be considered a prime potential additive or ingredient for application in the food and cosmetic industries, and will be a subject to further research.

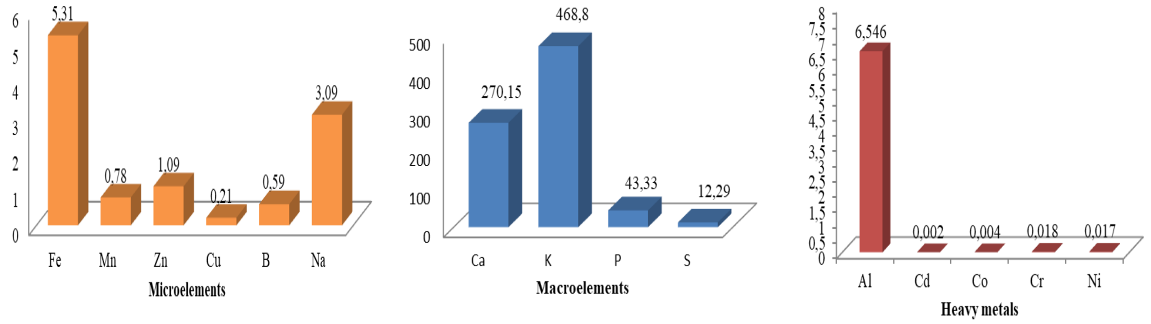

Fig. 2. Mineral matter and heavy metal content (ppm) of *S. aethiopis* L. taxa

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