Fatty acids and tocopherols of Turkish *Salvia fruticose, Salvia tomentosa, Stachys aleurites and Stachys cretica* subsp. *Anatolica* seed oils

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Salvia fruticosa, *Salvia tomentosa*, *Stachys aleurites*, and *Stachys cretica* subsp. *anatolica* species are members of the Lamiaceae family. Oil content, fatty acids, and the tocopherol composition of seed oils from these species were studied. The seed oil yield varied from 12.2% (*Salvia fruticosa*) to 17.2% (*Stachys aleurites*). Linoleic acid was found in high levels in the range from 44.13% (*Stachys aleurites*) to 61.7% (*Salvia tomentosa*), followed by oleic acid in the range from 23.1% (*Salvia tomentosa*) to 34.2% (*Stachys aleurites*). γ-Tocopherol was the most abundant tocopherol in the oils. *Salvia tomentosa* seed oil had the highest amount of γ-tocopherol (834.9 mg/kg), wherein the lowest amount was found in *Stachys cretica* seed oil (327.1 mg/kg). The work showed that the seed oils under study are rich sources of unsaturated fatty acids and γ-tocopherol that may contribute to human health.

Keywords: Lamiaceae, edible oils, antioxidants, bioactive lipids, tocols.

1. INTRODUCTION

Interest in new sources of oils and fats has increased. Seed oils are an important source of oils and fats that have been used for centuries by rural communities as food, medicine, cosmetic and fuel [1] Some non-conventional seed oils have a unique biochemical profile in terms of fatty acids, sterols, tocols, phenolics, and could increase the supply of novel products [2]. Minor components of seed oil such as unsaturated fatty acids, tocols, sterols and phenolic compounds play an important role in human health promotion, wherein the bioactive compounds must be obtained from the diet [3]. In addition, the fatty acid and tocol composition of plant seed oils can provide characteristic information that confirm phylogenetic and taxonomical relations in the plant kingdom [4].

There is a growing consumer interest in natural and/or organic products including food, beverages, cosmetics, herbal medicines, and pharmaceuticals with the major markets being Europe and North America. A multibillion dollar natural products industry has grown enormously with an annual growth rate of about 15-20% [1]. Thus, there has been a remarkable growth in the sales of natural products in Turkey and worldwide. *Salvia* L. is one of the largest genera in the family of Lamiaceae with over 900 species throughout the world. This genus is represented by 99 species in Turkey, wherein 50 of which are endemic [5]. *Salvia* species have been used as a folk medicine for colds, stomach aches, and sore throats since ancient times. Although there is a great number of *Salvia* species, only three *Salvia* species (*S. officinalis* L., *S. fruticosa* Mill., and *S. tomentosa* Mill.) are commercially important [6]. *Salvia fruticosa* is native to the

Eastern and Western Mediterranean including Israel, Palestine, Turkey, Italy, the Canary Islands, and North Africa. This plant has been widely used as herbal tea due to some medicinal properties [7]. Likewise, *S. tomentosa* is one of the most commonly used herbal teas. Besides, this plant has a wound-healing effect like that of iodine tincture [8].
The other member widely distributed of Lamiaceae family is *Stachys* L. that includes 300 species. This genus is represented by 89 species in Turkey [9]. *Stachys* species are known as "mountain tea" that is used as a herbal tea in Turkey. They are also used in the region of natural medicine to treat some health problems such as skin and stomach disorders [10]. *S. aleurites* Boiss. & Heldr. is an endemic plant to Turkey, where it prefers calcareous rocks near the coast in the Antalya province [11]. *S. cretica* L. subsp. *anatolica* Rech. f. is an endemic member of Lamiaceae family [12].
Even though these species are of the largest genera of the Lamiaceae, there are limited reports in the literature on their seed oil phytochemical profile especially fatty acids, and tocopherols content. In this study, we report on the fatty acids and tocopherols composition of *Salvia fruticosa*, *Salvia tomentosa*, *Stachys aleurites* and *Stachys cretica* subsp. *anatolica* seed oil.

2. MATERIALS AND METHODS

2.1 MATERIALS

The flowering aerial parts of *Salvia fruticosa* were collected in Turkey, C3 Antalya, Kemer district (36º25'30"N, 30°27'13"E), in a *Pinus brutia* forest from sea level up to 15 meters of altitude, at the end of June 2007. A voucher specimen is deposited at AKDU (Herbarium of the Biology Department, Akdeniz University) as Göktürk 6062.
The flowering aerial parts of *Salvia tomentosa* were collected in Turkey, C3 Antalya, Göynük (36°40'50"N, 30°31'33"E), in a macchie from sea level up to 70 meters of altitude, at the end of June 2007. A voucher specimen is deposited at AKDU (Herbarium of the Biology Department, Akdeniz University) as Göktürk 6063.
The flowering aerial parts of *Stachys aleurites* were collected in Turkey, C3 Antalya, Konyaalti, Varyant (36° 53' 68"N, 30° 40' 69"E), on calcareous rocks near the coast, about 10 meters above the sea level during mid July 2007. A voucher specimen is deposited at AKDU (Herbarium of the Biology Department, Akdeniz University) as Göktürk 6067.
The flowering aerial parts of *Stachys cretica* subsp. *anatolica* were collected in Turkey, C3 Antalya, Geyikbayiri village, Feslikan plateau (36°52'42"N, 30º26'37"E), clearing *Pinus brutia* forest, from 1100-1200 meters above sea level during mid July 2007. A voucher specimen is deposited at AKDU (Herbarium of the Biology Department, Akdeniz University) as Göktürk 6069.
The flowering aerial parts of *S. fruticosa, S. tomentosa, S. aleurites*, and *S. cretica* were collected in nature during the flowering stage in Antalya, Turkey. The herb samples were dried in the shade at room temperature before the seeds were separated. Seed samples separated after drying were ground immediately using a laboratory-scale mill (Retsch, Germany) before analysis.

2.2 METHODS

2.2.1 Seed oil extraction and fatty acid analysis

The ground seeds were extracted with *n*-hexane by Soxhlet apparatus. The oil content for each sample was calculated and expressed as % (w/w) of the seeds. The fatty acid composition of the oils was determined by gas chromatography (GC) as fatty acid methyl esters (FAME). FAME were prepared according to the official IUPAC method [13]. The chromatographic analysis was performed in a Shimadzu GC-2010 chromatograph using a DB-23 fused-silica capillary column (30 m, 0.25 mm i.d., 0.25 m film thickness, Agilent J. & W., USA). Helium was used as a carrier gas at a flow rate of 1.00 mL/min. The column temperature was isothermal at 190°C, wherein the injector and detector temperatures were 230°C and 240°C, respectively. FAME were identified by comparison of their retention times with those of the reference standards.

2.2.2 Tocopherol analysis

According to AOCS [14], the tocopherol composition of oils was determined using HPLC with a SCL-10Avp System controller, SIL–10ADvp Autosampler, LC-10ADvp pump, CTO-10 Avp column heater and fluorescence detector with wavelengths set at 295 nm for excitation and 330 nm for emission. A solution of oil in heptane was analyzed on a silica gel Supelcosil Luna column (particle size 5 µm, 15 cm, 34.6 mm i.d.; Supelco, Inc. Bellefonte, PA). The mobile phase was consisted of heptane: tetrahydrofuran (95:5, v/v) at a flow rate of 1.2 mL/min and the injection volume was 10 µL. The data were integrated and analyzed using Shimadzu Class-VP chromatography Laboratory Automated Software. Standard of tocopherols isomers were dissolved in heptane and used for identification and quantification of peaks. The amounts of tocopherols in the oils were calculated as mg per kg oil using external calibration curves (r^2 0.999) which were obtained for each tocopherols standard.

2.2.3 Statistical analysis

The results of this study were reported as mean values of three replicates and standard deviation. Statistical

analysis was performed using SPSS 10.0 statistical package program. Significant differences among the means of the samples were evaluated by analysis of variance using Duncan's multiple range tests at 95% confidence (p <0.05).

3. RESULTS AND DISCUSSION

Oil content of the seeds analyzed in the present study was ranged from 12.2% to 17.2% with *Stachys aleurites* seeds yielding the highest percentage of oil (Table I). Oil contents of *Salvia fruticosa*, and *Salvia tomentosa* seeds in Turkey were reported as 11% and 4.6%, respectively [15]. The other work demonstrated that *Salvia fruticosa* seeds contained 11%, and *Salvia tomentosa* seeds contained 8% oil [16]. In this work, the oil content of *Salvia fructicosa* (12.20%), and *Salvia tomentosa* (13.59%) are greater than those previously reported [15-16]. To the best of our knowledge, no investigations have been conducted on the oil content of these *Stachys* species seeds.

54.4%), and oleic acid (34.2% and 29.1%). The other identified unsaturated fatty acid was petrocelinic acid (Δ^6-*cis*-octadecenoic acid, 18:1 *n*-12), which accounted for 7.61% in *Stachys aleurites* oil. *in vitro* studies showed that petroselinoyl moieties in triacylglycerols are hydrolyzed by pancreatic lipase at lower rates than those containing other C-18 acyl moieties [17]. *Stachys cretica* subsp. *anatolica* oil had linolenic acid with 5.87%. Palmitic and stearic acids were determined as saturated fatty acids and their content comprises 9.00%, 7.71%, and 3.81, 2.84% of total fatty acids, respectively. There was no literature on the fatty acid composition of these *Stachys* species' seed oils. On the other hand, a research emphasized that linoleic (27.1%-64.3%) and oleic (20.2%-48.1%) acids were the major fatty acids of the total fatty acid in seed oils from 13 different *Stachys* species [18]. The fatty acid composition of *Stachys aleurites*, and *Stachys cretica* subsp. *anatolica* seed oils showed similarity with data previously published for 13 different *Stachys* species [18]. Some differences could

Table I - Oil content and fatty acid composition (%) of *Salvia fruticosa*, *Salvia tomentosa*, *Stachys aleurites*, and *Stachys cretica* seed oils*

	S. fruticosa	***S. tomentosa***	***S. aleurites***	***S. cretica* subsp. *anatolica***
Total lipids (%)*	12.20±1.00 b	13.59±1.00 b	17.22±1.00 a	15.83±0.76 a
Palmitic acid ($C_{16:0}$)	11.04±0.07 a	11.21±0.12 a	9.00±0.14 b	7.71±0.09 c
Stearic acid ($C_{18:0}$)	3.37±0.08 b	2.96±0.14 c	3.81±0.03 a	2.84±0.08 c
Oleic acid ($C_{18:1}$, n-9)	24.01±0.06 c	23.18±0.28 d	34.22±0.28 a	29.13±0.09 b
Linoleic acid ($C_{18:2}$)	60.73±0.18 b	61.72±0.28 a	44.13±0.48 d	54.46±0.06 c
Linolenic acid ($C_{18:3}$)	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	5.87±0.03 a
Petroselinic acid ($C_{18:1}$, n-12)	0.00±0.00 b	0.00±0.00 b	7.61±0.09 a	0.00±0.00 b

*Different tiny letters in the row (a-c) indicate significant differences among plant species ($p \leq 0.01$)

The fatty acid composition of oil samples is given in Table I. The oils of *Salvia fruticose*, and *Salvia tomentosa* found to be a rich source of linoleic acid with 60.73% and 61.72%, respectively. The other identified unsaturated fatty acid of these oils was oleic acid $C_{18:1}$ (24.01% and 23.18%). Palmitic acid $C_{16:0}$ was the major saturated fatty acid (11.04%, and 11.21%), followed by stearic acid $C_{18:0}$ (3.37%, and 2.96%). A research by Gören et al. [16] exhibited that linoleic acid (47.6%) was the major fatty acid, followed oleic acid (31.0%), and the other identified fatty acids were palmitic (12.1%), and stearic (5.1%) in *Salvia fruticosa* oil. In the same work, *Salvia tomentosa* oil was rich in linoleic acid (59.1%), as well as oleic (19.5%), palmitic (11.5%), and stearic (2.2%) acids. The results are close to those reported by Gören et al. [16].

As shown in Table I, the main fatty acids in the studied *Stachys aleurites*, and *Stachys cretica* subsp. *anatolica* seed oils were linoleic acid (44.1% and be due to different species, locations, climatic conditions, and post-harvest treatments.

Tocopherol content of *Salvia fruticose*, and *Salvia tomentosa* seed oils was exhibited in Table II. γ-Tocopherol was found abundant in the oils of *Salvia fruticose*, and *Salvia tomentosa* seeds and its quantities in these oils were 620.5 and 834.9 mg/kg, respectively. α-tocopherol in *Salvia fruticosa* oil accounted for 23.05 mg/kg, and α-tocopherol amount was 22.85 mg/kg in *Salvia tomentosa* oil. The least found tocopherol was β-tocopherol in Salvia species' oils. Similar results were reported for some *Salvia* species which were rich in γ- and α-tocopherol for *S. cryptantha* Montbret & Aucher ex Bent., *S. syriaca* L., *S. limbata* C.A.Mey., and *S. virgata* Jacq. [19]. The main tocopherol in *Stachys aleurites*, and *Stachys cretica* subsp. *anatolica* oil samples was γ-tocopherol (327.1 and 308.7 mg/kg), wherein δ-tocopherol (18.50 mg/kg) was found to be

Table II - Tocopherol composition (mg/kg oil) of *Salvia fructicosa*, *Salvia tomentosa*, *Stachys aleurites* and *Stachys cretica* seed oils*

	S. fruticosa	***S. tomentosa***	***S. aleuretes***	***S. cretica* subsp. *anatolica***
α-tocopherol*	23.05±0.95 a	22.85±0.45 a	15.85±0.45 b	13.15±0.15 c
β-tocopherol	7.80±0.00 a	7.35±0.15 b	1.50±0.30 c	0.00±0.00 d
γ-tocopherol	620.5±0.20 b	834.9±21.95 a	327.1±4.85 c	308.7±0.95 c
δ-tocopherol	16.90±0.20 b	28.0±1.80 a	18.50±0.20 b	3.27±0.06 c

*Different tiny letters in the row (a-c) indicate significant differences among plant species ($p \leq 0.01$)

the second abundant tocopherol in *Stachys aleurites* seed oil. The second most detected tocopherol in *Stachys cretica* subsp. *anatolica* oil was α-tocopherol (13.15 mg/kg).

Tocols in vegetable oils are believed to protect unsaturated fatty acids from oxidation. Despite many studies reported that α-tocopherol is the most efficient antioxidant *in vivo*, however, when compared to γ-tocopherol *in vitro*, reports indicated a considerable discrepancy in its antioxidant effectiveness [20]. People with low antioxidant intake may have an increased risk for certain types of cancer and for atherosclerosis. Levels of tocopherols detected in seed oils under investigation may contribute to the stability of the oils. Oils under study could be considered as important sources for tocopherols especially γ-tocopherol which contribute significantly to antioxidant activity and health-promoting traits.

4. CONCLUSION

This study intended to determine the levels of fatty acids, and tocopherols in four seeds of the Lamiaceae family (*Salvia fruticosa*, *Salvia tomentosa*, *Stachys aleurites* and *Stachys cretica* subsp. *anatolica*) grown in Turkey. This work showed that these seed oils are a good natural source for polyunsaturated fatty acids (PUFA), and γ-tocopherol for industrial applications of functional products. The results are important for the economical utility of seeds as a non-traditional source of oils for food, beverages, cosmetics, herbal medicines, and pharmaceuticals.

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Compliance with ethical standards
Conflict of interest: none.
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