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FATTY ACID COMPOSITIONS OF SEED OILS OF THREE TURKISH *SALVIA* SPECIES AND BIOLOGICAL ACTIVITIES

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The fatty acid composition of seed extracts of Salvia bracteata, S. aethiopis, and S. candidissima *ssp*. candidissima *were analyzed by GC/MS. The main compound of* S. bracteata, S. aethiopis, *and* S. candidissima *ssp*. candissima *was found to be 9,12-octadecenoic acid at 64.3%, 73.4%, and 20.8%, respectively. The seed extracts of* S. bracteata *showed activity against* S. aureus E. coli, M. smegmatis, *and* C. albicans *with MIC values of 1.1, 0.5, 1.1, and 1.1 mg/mL, respectively, while the seeds extract of* S. aethiopis *showed activity against the same microorganisms with MIC values of 2.2, 2.2, 1.1, and 1.1 mg/mL, respectively. However, the seed extract of* S. candidissima *ssp.* candidissima *showed activity only against* M. smegmatis *with a MIC value of 0.25 mg/mL.*

Key words: *Salvia bracteata*; *S. aethiopis*; *S. candidissima*, fatty acids, antimicrobial activity, GC/MS.

Salvia species have many uses which include the treatment of stomach ailments and the common cold. The volatile oil of the species functions as an antiseptic and the tannin as a local antiinflammatory agent, and the bitter principles produce a pleasant sensory feeling in the mouth and throat. They also posses, antibacterial, carminative, diuretic, hemostatic, and spasmolitic activities and are used as calming agents in the form of herbal teas all around the world and Turkey [1–3]. Over 40 Turkish *Salvia* species have been investigated by Ulubelen et al. They have isolated and elucidated many new compounds such as abietane, clorodane, and pimarane diterpenoids, sesterterpenoids, triterpenoids and flavonoids together with known compounds. They also investigated the antibacterial, antifungal, antituberculosis, and cytotoxic activity of the isolated compounds from *Salvia* species [4–10]. Also recently, a review of the cardioactive and antibacterial activity of some Turkish *Salvia* species has been published [11]. The fatty acid compositions of the seeds of *Salvia hispanica* L. were reported in the literature [12–17]. The GC analysis of fatty acids of *S. scleara* was reported [18]. But to the best of our knowledge there are no studies on the fatty acid composition of seeds of *Salvia* species except these two species, and the biological activities of the seeds oil were not reported in the literature.

Many studies on the antibacterial acitivity of the fatty acids or crude extracts have been published and McGaw et al. reviewed them in 2002 [19]. An antibacterial activity study of linoleic and oleic acids isolated from *Helichrysum pedenculatum* was also published [20]. The fatty acid compositions of some Turkish *Salvia* species were reported by two different Turkish research group and they showed that the main constituents of the *Salvia* seed oils are unsaturated fatty acids [21, 22]. In this study, we report the fatty acid composition of seed oils of *S. bracteata*, *S. aethiopis*, and *S. candidissima* ssp. *candidissima* and their antibacterial activity.

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TABLE 1. Fatty Acid Composition of *S. bracteata*, *S. aethiopis*, *S. candidissima* ssp. *candidissima*

Compound	*S. bracteata*	*S. aethiopis*	*S. candidissima* ssp. *candidissima*
Tridecanoic acid[a] (13:0)	0.1	Tr.	-
Tetradecanoic acid[a] (14:0)	0.4	Tr.	0.4
Pentadecanoic acid[a] (15:0)	-	-	0.2
3-Hydroxytetradecanoic acid (15:0)	0.7	-	-
Hexadecanoic acid[a] (16:0)	8.2	10.5	20.8
7-Hexadecenoic acid (Z)[a] (16:1)	Tr.	Tr.	Tr.
9-Hexadecenoic acid (Z)[a] (16:1)	0.1	0.1	0.1
Octadecanoic acid[a] (18:0)	2.9	3.0	5.7
9-Octadecenoic acid (Z)[a] (18:1)	15.4	8.4	20.4
9,12-Octadecadienoic acid (Z,Z)[a] (18:2)	64.3	73.4	20.8
9,12,15-Octadecatrienoic acid[a] (18:3)	3.8	2.9	18.5
Eicosanoic acid[a] (20:0)	-	-	1.5
11-Eicosenoic acid[a] (20:1)	1.1	1.2	Tr.
Docosanoic acid[a] (22:0)	Tr.	Tr.	Tr.
Tetracosanoic acid[a] (24:0)	0.1	-	3.1
Total	97.1	99.5	91.5

[a]The compounds were indetified by co-injection and mass data.

TABLE 2. Antimicrobial Activity Results of Seeds of Three *Salvia* Species and the Main Compounds*

Tested plants (seeds)	*S. aureus*	*E. coli*	*M. smegmatis*	*C. albicans*
S. bracteata	1.1	0.5	1.1	1.1
S. aethiopis	2.2	2.2	1.1	1.1
S. candidissima ssp. *candidissima*	NA	NA	0.25	NA
Linoleic acid	2.2	NA	NT	NA
Linolenic acid**	1.6	3.1	NT	NT
Oleic acid	2.2	NA	NT	NA
Stearic acid	NA	NA	NT	NA
Gentamycin	$5\cdot10^{-4}$	$4\cdot10^{-3}$	NT	NT
Streptomycin	$1.1\cdot10^{-4}$	$2.2\cdot10^{-4}$	$5\cdot10^{-3}$	NT
Fluconazole	NT	NT	NT	$1.5\cdot10^{-2}$

*MIC values are given as mg/mL.

**Values were taken from the literature [26].

NA: not active; NT: not tested.

GC/MS analysis of the seed extract of *S. bracteata* showed 13 fatty acids. The main compounds are 9,12-octadecadienoic acid (linoleic acid) (64.3 %), hexadecanoic acid (palmitic acid) (8.2 %), 9,12,15-octadecatrienoic acid (linolenic acid) (3.8%), and 9-octadecenoic acid (oleic acid) (15.4%), and the seed extracts of *S. aethiopis* of more or less the same components were found to contain 73.4, 10.5, 12.9 and 8.4%, respectively. The fatty acid composition of the third species, S. *candidissima* ssp. c*andidissima*, showed a difference in the percentage of linoleic acid. In contrast to the others, the linoleic acid content of the species was only 20.8%. The other components, similar to the previous species, were 20.8% for hexadecanoic acid methyl ester (palmitic acid), 20.8% for 9,12,15-octadecatrienoic acid methyl ester, 20.4% for 9-octadecenoic acid methyl ester, and 3.1% tetracosanoic acid methyl ester (Table 1).

The seed extracts of the three species were tested on gram positive (*S. aureus*) and a gram negative (*E. coli*) bacteria. The seed extract of *S. bracteata* showed activity on both gram positive and gram negative bacteria. MIC values are found to be 1.1 mg/mL and 0.5 mg/mL for the bacteria, respectively. The second plant also showed activity against *S. aureus* and *E. coli*

with 2.2 mg/mL MIC values. However, the third species, *S. candidissima* ssp. *candidissima*, did not show activity against both bacteria. The main components, linoleic acid, oleic acid, and stearic acid, were also tested against *S. aureus* and *E. coli*. Linoleic acid and oleic acid were found active against *S. aureus*. The antibacterial activity of another unsaturated compound, linolenic acid, was reported in the literature. The reported MIC values are 1.56 mg/mL for *S. aureus* and 4.4 mg/mL for *E. coli* [24]. However, linoleic acid and oleic acid did not show activity against the gram negative bacteria *E. coli*. Another main compound, stearic acid, did not show activity against the tested bacteria (Table 2).

Antifungal activity assays were carried out against *C. albicans.*, *S. bracteata* and *S. aethiopis*, showed antifungal acitivity while *S. candidissima* ssp. *candidissima* and all pure compounds did not show antifungal acitivity. Antituberculosis activity test was also carried out against *M. smegmatis*. All of the seed extracts showed antituberculosis activity with 1.1, 1.1, and 0.5 mg/mL MIC values, respectively.

EXPERIMENTAL

Plant Species and Sample Collection. *Salvia bracteata* and *S. aethiopis* were collected from Afyon province at 1000 m altitude while *S. candidissima* ssp. *candidissima* was collected from Icel, Demirozu at 1300 m altitude. *Salvia* species were collected during the seed period. The voucher specimens were deposited in the Herbarium of Department of Biology, Faculty of Arts and Science, Balikesir University. The herbarium numbers of plants are TD 1383, TD 1385, TD 1409, respectively.

Preparation of the Fatty Acids. Seeds of the species were separated from plant parts. Seeds weighing 7.1, 8.2, and 20 g were obtained from *S. bracteata*, *S. aethiopis*, and S. *candidissima* ssp. *candidissima*, respectively. Extractions of fatty acids were carried out by Soxhlet extraction in hexane [23]. After the Soxhlet extraction, 0.62 g extract was obtained from *S. breactata* (8.7%), 0.33 g from *S. aethiopis* (7.2%), and 0.120 g from S. *candidissima* ssp. *candidissima* (0.6%). For the GC/MS analysis 60 mg of extracts were esterified by acidic methanol [24]. The remaining extracts were used for the biological activity assessment.

Gas Chromatography Mass Spectrometry. The fatty acid methyl esters were analyzed using Trace 2000 GC series gas chromatography and Thermo mass spectrometer. An SGE BP × 70 column (60 m × 0.25 mm, 0.25 μm film thicknes) was used, with helium as a carrier gas at a rate of 1 mL/min. The GC oven temperature was kept at 100°C for 5 min and programmed to 240°C at a rate of 4°C/min and kept constant at 240°C for 5 min. The injection temperature and source temperature were 250 and 220°C, respectively. MS interface temperature was 240°C. The injection volume was 0.5 μL with a split ratio of 1:30. EI/MS were taken at 70 eV ionization energy. Mass range was from *m/z* 50–650 amu. Scan time was 0.5 sec with 0.1 interscan delay. The library search was carried out using NIST and Wiley GC-MS library and TUBITAK-UME library. Supelco^{TM} 37 component FAME mixture (Catalog No 47885-U) was used for the comparison of the GC chromatograms. The relative percentage of the separated compounds was calculated from Total Ion Chromatography by a computerized integrator.

Biological Assessments. The seed extracts, linoleic acid, oleic acid, and stearic acid, were tested against a gram positive bacteria *Staphylococus aureus* ATCC 6538, a gram negative bacteria *Escherichia coli* ATCC 8379, *Mycobacterium smegmatis*, and a fungi *Candida albicans* ATCC 10231 for antimicrobial activity. The tested materials were dissolved in hexane and hexane was used as a control. For the positive control experiment, gentamycin, streptomycin, and fluconazole were used. The test procedure is described in the literature [25]. The tested lineloic acid, oleic acid, and stearic acid were purchased from Aldrich Company.

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