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FATTY-ACID COMPOSITIONS OF *Silene vulgaris* AND *S. cserei* SUBSP. *aeoniopsis* SEEDS AND THEIR ANTIMICROBIAL ACTIVITIES

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The genus *Silene* L. (Caryophyllaceae), known as a large and polymorphic genus, is represented by 150 taxa in the Flora of Turkey, of which 67 are endemic [1–4].

To date, there has been little study of the chemical compositions of *Silene* species. The presence of triterpene saponins in *S. vulgaris* has been reported [5, 6]. In addition, a pectic polysaccharide named silenan was isolated from the aerial parts of *S. vulgaris*, which has been found to possess immunomodulatory activity [7]. On the other hand, several studies are devoted to the fatty acid composition of various *Silene* species [8–11]. In these studies, the seed oil of *S. vulgaris*, collected from two different origins, Greece and Spain, was investigated [8, 11]. To the best of our knowledge, there are no studies on the chemical composition of *S. cserei* subsp. *aeoniopsis*.

In this study, we investigated the constituents of the seed oil of *Silene vulgaris* (Moench) Garcke and *S. cserei* Baumg. subsp. *aeoniopsis* growing in Turkey by means of GC-MS. The oil yields were obtained 2.9% in *S. vulgaris* and 2.8% in *S. cserei* subsp. *aeoniopsis*. The composition of the fatty acids of the seed oils and their relative percentages are given in Table 1. Seventeen components representing 90.8% of *S. vulgaris* seed oil and 97.8% of *S. cserei* subsp. *aeoniopsis* seed oil were identified. The unsaturated fatty acid contents in these seed oils were found to be higher in *S. cserei* subsp. *aeoniopsis* (87%) than in *S. vulgaris* (68%). The ratio of unsaturated fatty acids to the saturated (U/S ratio) was found to be 0.3 in *S. vulgaris* and 0.1 in *S. cserei* subsp. *aeoniopsis*.

According to the GC/MS analysis results, seventeen fatty acids were detected in the seed oils of *S. vulgaris* and *S. cserei* subsp. *aeoniopsis*. The fatty acid compositions of the seeds of *S. vulgaris* and *S. cserei* subsp. *aeoniopsis* were very similar, but the percentages of main compounds were found to be different (Table 1). The main compounds were identified as unsaturated fatty acids such as 9,12-octadecadienoic acid (linoleic acid, 18:2ω6) (38.4 and 65.4%) and 9-octadecenoic acid (oleic acid, 18:1ω9) (28.6 and 17.8%), and a saturated fatty acid, hexadecanoic acid (palmitic acid, 16:0) (17.4 and 8.8%), in both seed oils, respectively. GC/MS analysis demonstrated that *S. cserei* subsp. *aeoniopsis* seed oil was remarkably rich in linoleic acid (65.4%). While percentage of the other essential fatty acid, 9,12,15-octadecatrienoic acid (α-linolenic acid), was found to be 0.8% in *S. vulgaris* seeds, it was determined as 3.6% in *S. cserei* subsp. *aeoniopsis*.

In addition, the hydrocarbons of both seed oils are given in Table 2. The results showed that total hydrocarbons in both oil samples was quite low, 0.8% in *S. vulgaris* and 0.3% in *S. cserei* subsp. *aeoniopsis*. In other words, it was lower in *S. cserei* subsp. *aeoniopsis* than in *S. vulgaris*.

Both of the oil samples were also screened against the standard strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii, Staphylococcus aureus,* and *Bacillus subtilis* for their antibacterial activity using the microdilution method, while their antifungal activity was tested against the yeast *Candida albicans* and the results compared to those obtained with reference agents (Table 3). Both of these oils displayed the same activity profile, having notable antibacterial activity against the Gram-negative bacterium *K. pneumoniae* at a concentration of 4 μg mL^{-1} and significant antifungal activity against *Candida albicans* (16 μg mL^{-1}).

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TABLE 1. Fatty Acid Composition of the Seed Oils of *S. vulgaris* and *S. cserei* subsp. *aeoniopsis**

Fatty acid	RT, min	*S. vulgaris*	*S. cserei* subsp. *aeoniopsis*	Fatty acid	RT, min	*S. vulgaris*	*S. cserei* subsp. *aeoniopsis*
8:0	9.02	0.8	Tr.	18:3	32.51	0.8	3.6
12:0	18.27	Tr.	Tr.	20:0	33.80	0.5	0.4
14:0	22.73	0.3	0.1	20:1	34.41	0.1	Tr.
15:0	24.81	0.2	0.1	22:0	36.88	1.5	0.1
16:0	26.93	17.4	8.8	24:0	38.25	Tr.	Tr.
16:1	27.60	0.1	0.1	25:0	39.60	Tr.	0.2
16:1	27.75	Tr.	0.1	Oil yield, %		2.9	2.8
18:0	30.62	2.1	1.1	$\sum_{Sat.}$		22.8	10.8
18:1	31.27	28.6	17.8	$\sum_{Unsat.}$		68	87
18:1	31.37	Tr.	Tr.	U/S		0.3	0.1
18:2	32.25	38.4	65.4	Total		90.8	97.8

*GC/MS analyses were replicated three times (mean RSD value is 0.1%); Tr.: traces.

TABLE 2. Hydrocarbons of the Seed Oil of *S. vulgaris* and *S. cserei* subsp. *aeoniopsis*

Compound	RT, min	*S. vulgaris*	*S. cserei* subsp. *aeoniopsis*	Compound	RT, min	*S. vulgaris*	*S. cserei* subsp. *aeoniopsis*
Hexanal	5.62	0.6	Tr.	2-Dodecenal	19.68	0.1	0.1
Octanal	8.29	–	Tr.	2,4-Dodecadienal	21.31	0.1	0.1
2-Heptenal	9.68	Tr.	Tr.	Heptacosane	32.35	Tr.	Tr.
Nonanal	10.33	Tr.	Tr.	Nonacosane	36.65	Tr.	0.1
2-Octenal	12.02	Tr.	–	Squalane	37.20	Tr.	Tr.
2-Decenal	17.15	Tr.	–	Total hydrocarbons		0.8	0.3

Tr.: traces.

In addition, both of the samples were found to be active against Gram-negative bacteria, namely *E. coli*, *P. mirabilis*, and *A. baumannii* at 32 μg mL^{-1}, whereas the seed oils were less active against *P. aeruginosa* at 128 μg mL^{-1} of MIC values. As can be clearly seen from Table 3, against Gram-positive bacteria, namely *S. aureus* and *B. subtilis*, the antimicrobial activities (MICs) were 64 μg mL^{-1}. According to our results, obtained from antimicrobial tests, the seed oils of *S. vulgaris* and *S. cserei* subsp. *aeoniopsis* can be used to develop therapies for infections caused by *K. pneumoniae* in the future. To the best of our knowledge, the present work is the first report on the seed oil, fatty acid composition, and antimicrobial activities of *S. vulgaris* and *S. cserei* subsp. *aeoniopsis* growing in Turkey.

Plant Materials. The mature seeds of *Silene vulgaris* (Moench) Garcke were collected from the valley of Kecideresi, Kalecik, Ankara, Turkey in July 2005, and the mature seeds of *Silene cserei* Baumg. subsp. *aeoniopsis* (Bornm.) Chowdh. were collected from sidewalks in Bahcesaray, Van, Turkey in July 2001, at an altitude of 1750 m, in the natural habitats of the plants. The plants were identified by N. Adiguzel, Ph.D. Authenticated voucher specimens are preserved in the Herbarium of Gazi University (GAZI) as follows; N. Adiguzel 6330 and 3972, respectively.

Oil Extraction and Transesterification. The seeds of *S. vulgaris* and *S. cserei* subsp. *aeoniopsis* were separated from plant materials and dried under shade. The weighed seeds (2.5 g) were ground with anhydrous sodium sulfate and extracted with petroleum ether (bp. 40–60°C, Merck, USA) for 6 h in a Soxhlet apparatus. The lipophilic extracts were evaporated under vacuum at 40°C to dryness, and the obtained seed oils were weighed accurately and the percentage yields (w/w) calculated.

Fatty acid methyl esters (FAMEs) were obtained using boron trifluoride (BF_3) according to the AOAC method [12].

Gas Chromatography-Mass Spectrometry. The FAMEs were analyzed using a Trace 2000 GC series gas chromatograph and a Thermomass spectrometer. An SGE BP×70 column (60 m × 0.25 mm, 0.25 μm film thickness) was used.

TABLE 3. Antibacterial and Antifungal Activity of the Seed Oils of *S. vulgaris* and *S. cserei* subsp. *aeoniopsis* as MICs (μg mL^{-1})

Microorganism	MIC (μg mL^{-1})					
	Sample		Standard			
	S. vulgaris	*S. cserei* subsp. *aeoniopsis*	AMP	OFX	KET	FLU
Escherichia coli (ATCC 35218)	32	32	2	0.12	–	–
Pseudomonas aeruginosa (ATCC 10145)	128	128	–	1	–	–
Proteus mirabilis (ATCC 7002)	32	32	2	<0.12	–	–
Klebsiella pneumoniae (RSKK 574)	4	4	2	0.12	–	–
Acinetobacter baumannii (RSKK 02026)	32	32	2	0.12	–	–
Staphylococcus aureus (ATCC 25923)	64	64	0.12	0.5	–	–
Bacillus subtilis (ATCC 6633)	64	64	0.12	0.5	–	–
Candida albicans (ATCC 10231)	16	16	–	–	2	4

MIC: Minimum Inhibitory Concentration; AMP: ampicillin; OFX: oflaxocin; KET: ketokonazole; FLU: fluconazole.

The carrier gas was helium at a rate of 1 mL/min. GC oven temperature was kept at 100°C for 5 min and programmed to 240°C at a rate of 4°C/min, then kept constant at 240°C for 5 min. The injection temperature and source temperature were 250°C and 220°C, respectively. The 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 to 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 the computerized integrator [13].

Microbiological Studies. Test Materials. The oils of *Silene vulgaris* and *Silene cserei* subsp. *aeoniopsis* were dissolved in ethanol–*n*-hexane (1:1) using a 1% Tween 80 solution at a final concentration of 256 μg mL^{-1} and sterilized by filtration using 0.22 μm Millipore (MA 01730, USA) and used as the stock solutions.

Standard antibacterial powders of ampicillin (AMP, Fako) and oflaxocin (OFX, Hoechst Marion Roussel) and standard antifungal powders of ketoconazole (KET, Bilim) and fluconazole (FLU, Pfizer) were obtained from their respective manufacturers and dissolved in phosphate buffer solution (AMP, pH 8.0, 0.1 mol/L), dimethylsulfoxide (KET), and water (FLU, OFX).

Microorganisms. Standard strains of the bacteria *Escherichia coli* (American type culture collection; ATCC 35218), *Pseudomonas aeruginosa* (ATCC 10145), *Proteus mirabilis* (ATCC 7002), *Klebsiella pneumoniae* (Culture collection of Refik Saydam Central Hygiene Institute; RSKK 574), *Acinetobacter baumannii* (RSKK 02026), *Staphylococcus aureus* (ATCC 25923), and *Bacillus subtilis* (ATCC 6633) for the determination of antibacterial activity and standard strains of *Candida albicans* (ATCC 10231) for the determination of antifungal activity were used.

Inoculum Preparation. Mueller-Hinton Broth (MHB; Difco) and Mueller-Hinton Agar (MHA; Oxoid) for bacteria, and Sabouraud liquid medium (SLM; Oxoid) and Sabouraud dextrose agar (SDA; Oxoid) for fungi and culture suspensions were prepared as described in earlier publications using the microdilution method [14].

Antibacterial and Antifungal Tests. The microdilution method was employed for antibacterial and antifungal activity tests. The media were placed into each of the 96 wells of the microplates. Seed oil solutions at 256 μg mL^{-1} were added into the first rows of microplates, and twofold dilutions of the compounds (256–0.125 μg mL^{-1}) were made by dispensing the solutions to the remaining wells. 10 μL culture suspensions were inoculated into all the wells. The sealed microplates were incubated at 35°C for 24 h and 48 h in a humid chamber. The lowest concentration of the extracts that completely inhibits macroscopic growth was determined, together with the minimum inhibitory concentrations (MICs).

REFERENCES

1. M. J. E. Coode and J. Cullen, *Silene* L. in: *Flora of Turkey and the East Aegean Islands*, Vol. **2**, Edinburgh University Press, Edinburgh, 1967, p. 179–242.
2. P. H. Davis, R. R. Mill, and K. Tan, *Silene* L. in: *Flora of Turkey and the East Aegean Islands*, Vol. **10**, Edinburgh University Press, Edinburgh, 1988, p. 76–81.
3. M. Vural and N. Adiguzel, *The Herb. J. Syst. Bot*., **3**, 93 (1996).
4. A. Guner, N. Ozhatay, T. Ekim, and K. H. C. Baser, *Silene L.* in: *Flora of Turkey and the East Aegean Islands*, supl. **2**, Vol. **11**, Edinburgh University Press, Edinburgh, 2000, p. 50–53.
5. M. Glensk, V. Wray, M. Nimtz, and T. Schoepke, *J. Nat. Prod.*, **62**, 717 (1999).
6. S. Bouguet-Bonnet, M. Rochd, P. Mutzenhardt, and M. Henry, *Magn. Reson. Chem.*, **40**, 618 (2002).
7. O. A. Bushneva, R. G. Ovodova, A. S. Shashkov, A. O. Chizhov, and Y. S. Ovodov, *Biochem. (Moscow)*, **68**, 1360 (2003).
8. R. Alarcon, L. T. Ortiz, and P. Garcia, *Int. J. Food Sci. Tech.*, **41**, 1239 (2006).
9. I. Tolibaev, K. S. Mukhamedova, and A. I. Glushenkova, *Khim. Prir. Soedin.,* 512 (1993).
10. C. I. Vardavas, D. Majchrzak, K. H. Wagner, I. Elmadfa, and A. Kafatos, *Food Chem.*, **99**, 822 (2006).
11. A. G. Hanna and H. S. M. Soliman, *Egyptian J. Food Sci.*, **20**, 417 (1992).
12. Association of Official Analytical Chemists, *Official Methods of Analysis*, 14th Ed., Washington, DC: Association of Official Analytical Chemists, 1980.
13. T. Kilic, T. Dirmenci, and A. C. Goren, *Rec. Nat. Prod.,* **1**, 17 (2007).
14. B. Ozcelik, M. Aslan, I. Orhan, and T. Karaoglu, *Microbiol. Res.*, **160**, 159 (2005).