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Fatty Acid Composition of *Heliotropium* Species (Boraginaceae): A First Chemical Report on the New Species *H. thermophilum*

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Fatty acid compositions of the seed oils of *H. thermophilum* Kit Tan, A. Celik & Y. Gemici, *sp. nova*, *H. europaeum* L., and *H. hirsutissimum* L. (Boraginaceae) were analyzed by GC/MS. The main fatty acid methyl esters were determined to be of palmitic (39.8-40.6%), linoleic (32.4-33.2%), oleic (10.6-12.1%) and stearic acids (7.9-8.5%). γ-Linolenic acid was found to be a minor component of the seed oils of the reported species. This is the first chemical report on the fatty acid composition of *H. thermophilum*, along with chemotaxonomic evaluation of the species.

Keywords: Boraginaceae, *Heliotropium*, fatty acid, linoleic acid, palmitic acid, chemotaxonomy.

Heliotropium, represented by around 250 species growing in warm regions, is a genus of plants belonging to the Boraginaceae family [1]. In Greek, Helios and tropein mean "the sun" and "turn", respectively. In the Turkish flora, there are 15 species of *Heliotropium*, two taxa of which are endemic. Some species, particularly *H. arborescens* L., are used as garden plants [2-5].

As a traditional medicine, *H. europaeum* L. is used in the treatment of gastrointestinal disorders as a coloretic, and the herb is believed to be a medicine for the treatment of wounds. Moreover, it is applied to warts, and to snake and scorpion bites in Anatolia.

The fatty acid compositions of seeds of *H. arborescens* and *H. europaeum* from Germany [6], and *H. crispum*, *H. curassavicum* L. and *H. pterecarpum* from Cape Verde Islands [7] are available in the literature, but only two studies have been published on *H. lasicarpum* and *H. dolosum* from Turkey [3,4]. The main fatty acid component of the species of *Heliotropium* from Germany was reported as linoleic acid (30.0 and 54.4%). Similar results have been reported for the species of *Heliotropium* collected in the Cape Verde Islands, with a linoleic acid content of over 50%. This difference might be the result of different climatic conditions, geographical and soil conditions, harvest periods and extraction procedures. Species of Anatolian *Heliotropium* appeared to have linoleic acid as a major fatty acid, together with palmitic and stearic acids. In this study, we report the fatty acid composition of the seed oils and the chemotaxonomic evaluation of *H. thermophilum*, *H. europaeum* and *H. hirsutissimum*, the first species of which is new and endemic. The habitat of *H. thermophilum* is a geothermal area with a ground temperature between 55-65ºC [1].

Eleven fatty acid methyl esters were determined in the seed oil of *H. thermophilum*, *H. europaeum* and *H. hirsutissimum*, which represented 95.6, 97.3 and 98.3% of the total oil, respectively. The total saturated fatty acid composition of the reported species was found to be around 50%, while the total unsaturated seed oil content was approximately 45% of the total composition. This is the characteristic

Table 1: Fatty acid composition of *Heliotropium* species (%).

	Compounds	*H. thermophilum*	*H. europaeum*	*H. hirsutissimum*
11:0	Undecanoic acid	0.2	0.2	0.2
14:0	Myristic acid	0.2	0.1	0.1
16:0	Palmitic acid	39.8	39.5	40.6
16:1	Palmiteloic acid	0.2	0.1	0.2
18:0	Stearic acid	8.1	7.9	8.5
18:1	Oleic acid	10.6	11.7	12.1
18:1	Vaccenic acid	0.5	0.7	0.5
18:2	Linoleic acid	32.4	33.2	32.2
18:3	γ-Linolenic acid	0.7	0.7	0.8
18:4	Stearidonic acid	t	t	t
20:0	Arachidic acid methyl ester	1.1	1.1	1.0
22:0	Heneicosanoic acid	1.8	1.6	1.9
	18:2/16:0	**0.81**	**0.84**	**0.79**
	18:3/18:2	**0.02**	**0.02**	**0.02**
	Total	95.6	97.3	98.3
	∑Unsaturated	44.4	46.4	45.8
	∑Saturated	51.2	50.9	52.5

t:<0.1

seed oil composition of *Heliotropium* species [3,4,6,7]. The main compounds of the total oil of the species examined were palmitic acid (39.8-40.6%), linoleic acid (32.2-33.2%), oleic acid (10.6-12.1%) and stearic acid (7.9-8.5%) (Table 1). γ-Linolenic acid (18:3) was found to be a minor component of the oils extracted from all the species of *Heliotropium*. This is similar to the previously reported γ-linolenic acid contents of *H. hirsutissimum, H. europaeum.* and *H.dolosum* [3-5].

The chemotaxonomic importance of the fatty acid composition of seed oils in plant species is widely accepted [6-15]. From a chemotaxonomical approach, the linoleic and palmitic acid contents of the seed oil of *Heliotropium* species are expected to be in excess of the other components, while the γ-linolenic acid content of the oils is at a minor level [3,6,7]. Considering these points, further evaluation was performed of the 18:2/16:0 and 18:3/18:2 ratios of the fatty acids in the seed oils. The results showed that the ratio of 18:2/16:0 was in the range 0.79-0.84, while the ratio of 18:3/18:2 was 0.02 for all three species of *Heliotropium* (Table 1).

A new taxon, *H. thermophilum*, from south west Turkey was reported recently [1]. In parallel with this report, and in addition to its botanical taxonomic proof, the ratios of the fatty acid contents of the species given in this work also showed that the species is a member of the *Heliotropium* genus. In conclusion, the chemotaxonomical approach described above is strong evidence for the new species, *H. thermophilum.*

Experimental

Plant material: *H. thermophilum* Kit Tan, A. Celik & Y. Gemici, *sp. nova,* and *H. hirsutissimum* were collected in Denizli, west Aegean, and *H. europaeum* L. in Balıkesir, north-west Aegean, during the seed development period in 2005. The species were identified by one of the authors Dr Ali Celik. The voucher specimens were deposited in the Herbarium of the Faculty of Sciences, Aegean University, Turkey. The herbarium numbers of *H. thermophilum*, *H. hirsutissimum* and *H. europaeum* are EGE 40763, EGE 31626 and EGE 20816, respectively.

Sample extraction and derivatization: Seeds were separated from the aerial parts of the plants. The seeds of *H. thermophilum* (16.8 g), *H. hirsutissimum* (15.2 g) and *H. europaeum L* (20.1 g) were extracted with *n*-hexane using a Soxhlet extraction apparatus. The solvents were evaporated to yield crude viscose products [0.6 g (3.6%), 0.4 g (2.6%) and 1.1 g (5.4%), respectively].

The crude product (100 mg) was refluxed with 0.1 M KOH in ethanol (2 mL) for 1 h. The solution was cooled to room temperature and water (5 mL) was added. The aqueous mixture was first neutralized with HCl (0.5 mL) and then extracted with *n*-hexane: diethyl ether mixture (1:1; 3 x 5 mL). Lastly, the organic layer was washed with water (10 mL), dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. To the remaining residue dissolved in toluene (1 mL) in a test tube was added H_2SO_4 in methanol (2 mL, 1%). The mixture was left overnight at 50ºC before sodium chloride solution (5 mL, 5%) was added and the mixture extracted with *n*-hexane (2 x 5 mL). The organic layer was separated using a Pasteur pipette, washed with potassium bicarbonate solution (4 mL, 2%), dried over anhydrous Na_2SO_4 and filtered. The organic solvent was removed under reduced pressure to give fatty acid methyl esters [8,9]

GC/MS conditions: The fatty acid methyl esters were analyzed using a Trace 2000 GC series gas chromatography and Thermo mass spectrometer. A SGE BP x 70 column (60 m x 0.25 mm, 0.25 μm film thicknes) was used. The carrier gas was helium at a flow rate of 1 mL/min. GC oven temperature was kept at 100ºC for 5 min and programmed to 240ºC at a rate of 4ºC/min and kept constant at 240ºC for 5 min. The injection and source temperatures were 250ºC and 220ºC, respectively. The MS interface temperature was 240ºC. The injection volume was 0.5 μL with a split ratio of 1:30. EI/MS were recorded 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 delays. The library search was carried out using the NIST, Wiley GC-MS, and TUBITAK-UME libraries. Supelco™ 37 components FAME mixture (Catalog no: 47885-U) was used to compare the GC chromatograms. The relative percent of the separated compounds was calculated from Total Ion Chromatography by the computerized integrator.

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