

## FATTY ACID CONTENT OF THREE *Cistus* SPECIES GROWING IN TURKEY

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*The seed oils of Cistus laurifolius, C. salviifolius, and C. creticus were investigated for their fatty acids by employing capillary GC and capillary GC-MS. The results of this study indicated that palmitic, linoleic, linolenic, oleic, stearic, and behenic acids were found in all of these three seed oils of Turkish origin. In addition, an important polyunsaturated fatty acid, linoleic acid, was the major fatty acid in all of these oil samples.*

**Key words:** *Cistus laurifolius, Cistus salviifolius, Cistus creticus*, fatty acid, GC-MS, GC, *Cistaceae*, Rockrose.

The *Cistaceae* family comprises about 8 genus and 175 species [1], which are mainly found in the Mediterranean area, north-west Africa, east Asia, and south and east America [2]. *Cistus* genus has 17 species, which are widely grown north of the Mediterranean, in the west and middle parts of Europe [1, 3]. There are 5 *Cistus* species; *C. laurifolius* L., *C. salviifolius* L., *C. creticus* L., *C. parviflorus* L., *C. monspeliensis* L. grown in Turkey [4].

Hippocrates called this genus "Cistos" [5]. The *Cistus* genus has widespread utilization in Turkish folk medicine such as against rheumatism, for hemorrhoids, to cure sterility, kidney and urinary inflammations, as a hemostatic, antipyretic, expectorant, sedative, and for peptic ulcer, as well as diabetes mellitus [6–9]. The potent anti-ulcerogenic activity of the flowers and buds of *C. laurifolius* was reported in our previous papers by using different *in vivo* peptic ulcer models in rats and mice [10–11]. This utilization was also shown in *Materia Medica*. In addition, the chloroform fraction of methanol extracts from flowers of *C. laurifolius* showed potent inhibitory activity against *Helicobacter pylori* [12]. Moreover, anti-inflammatory, anti-hypertensive, anti-microbial, and cytotoxic effects of *Cistus* species were also reported [13–16]. "Labdanum" or "Ladanum", which is the oleoresin of *Cistus* species, has been used in cosmetology for many years [6].

In this study, the fatty acids of seed oils of three *Cistus* species (*C. laurifolius, C. salviifolius, C. creticus*) were investigated by capillary gas chromatography (GC) and capillary gas chromatography-mass spectrometry (GC-MS). The amount of oils is shown in the Table 1. According to the table, seeds of *C. laurifolius, C. salviifolius, and C. creticus* gave 7.64%, 3.33%, and 6.00% oil yields respectively, and the highest oil content was in the seeds of *C. laurifolius*.

The composition of fatty acids of the seed oils and percentages of average yields of fatty acids detected are given in Table 2. Linoleic, palmitic, and oleic acids were found to be major contents of each seed oil obtained from the three *Cistus* species of Turkish origin. Since we could not separate oleic acid and linoleic acid by using GC/MS, we used capillary GC. Stearic acid was presented in all of the samples and their percentages in all these samples were found to be similar (3.33%, 4.52%, and 4.56%). The percentages of oleic acid in *C. salviifolius* (14.06%) and *C. creticus* (14.41) were very close. Palmitic acid was found as the main saturated fatty acid in the oils. Fatty acids, especially essential fatty acids, are of vital significance for human beings. Linoleic acid, which is an important fatty acid for human health, was the dominant (41.53–48.48%) in all of the three seed oils. The amount of linoleic acid was the same in oil from the seeds of *C. salviifolius* and *C. creticus*, while *C. laurifolius* had a higher amount (48.48%). The major constituents of the oils were unsaturated fatty acids. 71.05% of the oils of *C. laurifolius*, 67.92% of the oils of *C. creticus*, and 65.35% of the oils of *C. salviifolius* consist of linolenic, linoleic, and oleic acids, which are known to be valuable commercial materials in the food industry and cosmetology. The total saturated fatty acid content of *C. laurifolius, C. salviifolius, and C. creticus* was 21.22%, 29.54%, and 30.06%, respectively.

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TABLE 1. Color and Yield of Oil from Seeds of Three *Cistus* Species

Plant	Seed weight, mg	Oil		
		weight, mg	yield, %	color
<i>C. laurifolius</i>	2000	153	7.64	Yellow
<i>C. salviifolius</i>	3000	100	3.33	Light yellow
<i>C. creticus</i>	4000	240	6.00	Yellow

TABLE 2. Fatty Acids Content in the Seeds of Three *Cistus* Species

Fatty acids	Relative percentage of fatty acids*		
	<i>C. laurifolius</i>	<i>C. salviifolius</i>	<i>C. creticus</i>
16:0	17.70±0.66	19.58±0.76	20.24±0.88
18:0	3.33±0.13	4.56±0.19	4.52±0.16
18:1	17.87±0.49	14.06±0.38	14.41±0.76
18:2	48.48±0.06	41.53±0.71	41.60±0.75
18:3	4.70±0.46	9.76±0.38	11.91±0.64
22:0	0.19±0.11	5.40±0.28	5.30±0.60

\*Data are expressed as mean ± S. E. M.

According to our findings, the seed oils of these *Cistus* species might be evaluated to be used in preparations of some cosmetic products and should be considered new alternative sources of linoleic acid. This is the first report on the fatty acid content of oil from the seeds from *C. laurifolius*, *C. salviifolius*, and *C. creticus*.

## EXPERIMENTAL

**Plant Materials.** The seeds of *C. laurifolius* were collected from Koroglu-Afyon (Turkey), in July 2002. Two *Cistus* species were collected from Mordogan, Cesme-Izmir (Turkey) in July 2002. The voucher specimens were identified by (Prof.) Dr. Songul Turkoz at the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University. The herbarium specimens are stored in the Herbarium of Gazi University, Faculty of Pharmacy (*C. salviifolius*: GUE 2298; *C. creticus*: GUE 2299; *C. laurifolius*: GUE 2300).

**Extraction.** The powdered seeds of *C. laurifolius*, *C. salviifolius*, and *C. creticus* were extracted with petroleum ether (30 ml × 10). The extracts were concentrated to dryness under reduced pressure at 35°C. Yield of extraction is presented in Table 1.

**Methylation of Fatty Acids.** Before the analysis by GC/MS, the oils were saponified with 0.5 N NaOH solution, and free acids were converted to their methyl esters according to Morrison and Smith's method using boron trifluoride (BF<sub>3</sub>)-methanol complex (20%; Sigma Co.) reagent [17]. BF<sub>3</sub>-MeOH reagent (2 ml) was added to each of the oil samples in petroleum ether (2 ml) and heated for 2 min in a boiling water bath. The methyl esters of fatty acids were dissolved in CHCl<sub>3</sub> and injected into the GC/MS.

**GC/MS Analysis.** As to fatty acid analysis, capillary gas chromatography-mass spectrometer (GC/MS) analysis was carried out on Hewlett Packard HP 6890 series equipment. Separation of 0.2 µl injection was achieved on a 25 m × 200 µm ID, 0.33 µm film thickness, HP-5 (cross linked 5% phenyl silicone gum phase, model number: HP 19091J-102) capillary column, split ratio 1:10, 130°C at rate 2°C/min with helium linear velocity 0.5 ml/min and injection temperature 250°C, detector 280°C, mass range *m/z* 20–440. The fatty acids were identified by comparison of their retention times with those of standards and by using Wiley library database search. Data obtained from the three analyses of the seed oils were expressed as means.

**GC Analysis.** Methylated oil samples of GC/MS analysis were used for GC analysis. Fatty acid methyl esters were analyzed by capillary gas chromatography (GC) in a Hewlett Packard Model 5890 Series II gas chromatograph with flame ionization detector. The capillary column was Ultra 1 (cross linked methyl silicone gum phase, 50 m × 0.2 mm × 0.3 mm). The temperature was programmed from 170 to 230°C at the rate of 2°C/min. The detector and injector temperature was set at 250°C, Nitrogen at the flow rate of 0.7 ml/min was used as carrier gas. The split ratio was 1/50 and the chart speed was 1 cm/min. The peaks were identified by comparing them with standart samples, and the relative amounts of fatty acids were calculated by Integrator Hewlett Packard HP 3396A.

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