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LIPID FRACTIONS AND FATTY ACID COMPOSITION OF *CYPRESS SEMPERVIRENS* AND *THUJA ORIENTALIS* SEED OILS

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Cypress sempervirens seed oil (4.4%) and *Thuja orientalis* seed oil (17.2%) have been analysed for its physico-chemical characteristics and fatty acid composition. The fatty acids ranged from C₁₆ to C₂₄ in varying amounts.

Key words: Fatty acids, *Cypress sempervirens*, *Thuja orientalis*, Composition.

Introduction

Cypress sempervirens and *Thuja orientalis* belonging to N.O. Coniferae [1,2] are locally known as "Saru and Morpukh" respectively. Both are widely grown in Pakistan as an ornamental plant. *C. sempervirens* and *T. orientalis* both bear fruits in Feb. which ripen by the end of Nov. The seeds of both the species are enclosed in cones which contains essential oil. Although a lot of work has been carried out on the different aspects i.e. fatty acid and essential oil composition of *T. orientalis*[3,4] and *C. sempervirens*[5,6] yet the fixed oil of both the varieties especially from this part of the world has not been studied in details, except for the preliminary studies made by Zahid *et al.* [7] on the fatty acid composition of the seed oil of *T. orientalis*, using old methods. The results are inadequate as far as C₁₈ fatty acid composition is concerned. The present study was undertaken to provide detailed information regarding physico-chemical characteristics [Table-1] and fatty acid composition of different lipid fractions (Table- 3).

Material and Method

Seeds of both varieties were collected from locations around PCSIR Complex Lahore in the month of Dec. 1991, dried in the shade and separated from the coverings. The seeds were freed from dust and other plant materials. The seeds of each variety were dried, powdered and extracted with hexane in a Soxhlet apparatus on water bath for 6 hrs. The extracts were washed with water to remove the non-lipid contaminants, dried over anhydrous sodium sulphate and filtered. The solvent was removed in a rotatory evaporator at 40° under reduced pressure.

Various physico-chemical characteristics i.e. specific gravity, refractive index (Abbe's), acid value, saponification value, iodine and peroxide values were determined according to the standard procedures [8] and are given in Table 1.

Fractionation of the oil into lipid classes [9,10]: The oil (0.4g) was charged on ten 20x20 cm glass plates coated with silica gel of 0.5 mm. thickness (Kieselgel 60 G Art. 7731). Chromatograms were developed in hexane/diethylether/ace-

tic acid (80: 20: 2 v/v/v) and the resulting bands were visualised under UV light by spraying with 0.2% solution of 2',7'- dichlorofluorescein in methanol. Typical R_f's of the lipid classes were hydrocarbons (0.91), wax esters (0.83), triglycerides (0.52), free fatty acids (0.41), diglycerides (0.34), monoglycerides (0.17) and polar lipids (0.00). Lipid classes were identified by comparison of R_f's with those of standards under identical conditions. The bands were made visible under UV light by spraying the T.L.C. plates with 2',7'- dichlorofluorescein, marked, scrapped and extracted with chloroform/methanol (2:1 v/v) and filtered. The content of each lipid class is given in Table 2.

Methylesters of the oil were prepared by the standard method [11] of saponification, with alcoholic potash, removal of unsaponifiable matter by extraction with ether and esterification of liberated fatty acids with methanol and sulphuric

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF *CYPRESS SEMPERVIRENS* AND *THUJA ORIENTALIS* SEED OIL.

	<i>T. orientalis</i>	<i>C. sempervirens</i>
Moisture content of the seed (w/w)	7.6%	11%
Fixed oil (w/w)	17.2%	4.4%
Specific gravity at 25°	0.9765	0.9724
Refractive index at 25°	1.4735	1.4726
Acid value	2.5	5.5
Saponification value	143.8	144.6
Iodine value	154.7	168.3
Unsaponifiable matter	0.96%	0.68%

TABLE 2. *T. ORIENTALIS*.

Fractionation of the lipids	
Hydrocarbons	1.9%
Wax esters	1.5%
Triglycerides	87.3%
F.F.A.	2.8%
Diglycerides	2.7%
Monoglycerides	3.8%

acid. The methyl esters were purified on silica gel by column chromatography. The purity of the methyl esters was confirmed by a single spot on T.L.C. plate.

The methyl esters of triglycerides were prepared by using a mixture of boron trifluoride in methanol [12].

Analysis. Methyl esters were analysed on Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector under the following conditions.

Column. Packed glass 1.6 m L x 0.3 mm (i.d) 15% diethylene glycol succinate coated on Shimalite (AW 201) 60-80 mesh.

Column temperature 200° isothermal

Detector temperature 250°

Injector temperature 300°

Carrier gas nitrogen 45 ml/min.

Hydrogen flow rate 45-50 ml/min.

Air flow rate 500 ml/min.

Sample size 0.2 µl

Various components were identified by their retention time and co-injection of standard esters. Percentage composition of individual components was calculated on the basis of peak area using Shimadzu C-R-4A (chromatopac) data processor. Response factors were not determined.

Results and Discussion

The oil content of *T. orientalis* and *C. sempervirens* has been determined to be 17.2 and 4.4% respectively. The physico-chemical characteristic of oils are given in Table 1. The high linolenic content and high iodine value suggest the oils to be drying one, which can exclusively be used in the manufacture of paints, varnishes and printing inks. The fatty acid com-

position of triglycerides of both seed oils being reported for the first time is given in Table 3. Both the oils mainly consist of unsaturated fatty acids 80.8% and 84.5% respectively. It is quite interesting to note that the percentage of the total C₁₈ unsaturated fatty acids in *T. orientalis* is almost the same as reported by Zahid *et al.* [7], but there is a noticeable difference in the percentage of individual fatty acids. The results are inadequate because they have reported only one C₁₈ unsaturated fatty acids (C_{18:3}) without mentioning the composition of (C_{18:1} and C_{18:2}) where as we have not only reported the percentage composition of individual C₁₈ unsaturated but also the percentage composition of C₂₂ and C₂₄ fatty acids not reported earlier.

Studies on the proteins and carbohydrates contents and also the unsaponifiable matter of the oil are in hand and will be reported elsewhere.

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TABLE 3. FATTY ACID COMPOSITION OF TOTAL LIPIDS IN CYPRESS SEMPERVIRENS AND THUJA ORIENTALIS SEED OIL.

Fatty acid	Total lipids of <i>Thuja orientalis</i>	Triglyceride of <i>T. orientalis</i>	Total lipids of <i>Cypress sempervirens</i>	Triglyceride of <i>C. sempervirens</i>
C _{16:0}	5.9	4.0	9.3	10.8
C _{18:0}	3.5	2.0	3.0	3.0
C _{18:1}	18.1	12.3	13.0	12.5
C _{18:2}	17.2	16.7	28.8	23.4
C _{18:3}	45.5	55.5	41.7	33.0
C _{20:0}	0.2	-	-	-
C _{22:0}	1.6	1.3	4.2	3.0
C _{22:1}	-	0.8	-	10.8
C _{22:2}	-	7.4	-	2.6
C _{24:0}	7.4	-	-	-