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The Chemical Investigation of the Seed Fat of *Diospyros peregrina*

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The fixed oil from the seeds of *Diospyros peregrina* Gurke has been investigated for its physico-chemical properties and chemical composition. The gas chromatographic analysis has revealed the oil to consist of the glyceride of caprylic acid (0.95%), capric acid (1.26%), myristic acid (0.47%), palmitic acid (31.63%), stearic acid (8.24%), oleic acid (43.10%) and linoleic acid (14.26%). The unsaponifiable matter has been found to be a mixture of β-amyrin and β-sitosterol.

INTRODUCTION

Diospyros peregrina Gurke belongs to the natural order Ebenaceae and is locally known as **Tendu**. It is a moderate sized evergreen tree, found practically throughout India in shady wet places and near streams. It bears dark green foliage and large velvety fruits. It is cultivated for its ornamental value.[1] The present study describes the physico-chemical properties and chemical composition of the fatty acids in the seed oil of *Diospyros peregrina.*

EXPERIMENTAL

The seeds of *Diospyros peregrina* were collected locally. Five kgm. of dried powdered seeds were extracted with pet. ether (60–80°) in a soxhlet extractor for 10 hrs. The extract was concentrated by evaporating the solvent (yield *ca.* 2.87%).

Properties of the oil

The following physico-chemical characteristics of the oil were determined by the usual methods.[2] Yield 2.87%, specific gravity 0.9121 at 30°C, refractive index 1.2320 at 30°C, acid value 1.95, saponification value 151.22, iodine value 92.68, and unsaponified matter 1.00%.

50 gm of fixed oil was saponified by Hilditch's method.[3] The unsaponified matter was separated from the soap by usual procedure. The saponified matter was neutralized with $4N\text{–}H_2SO_4$ and extracted with ether. The ethereal solution was dried over anhydrous sodium sulphate. Removal of solvent from the extract gave the mixture of fatty acids.

Methylation of fatty acids

The methyl esters of mixed fatty acids were prepared by refluxing 15 gm of mixed fatty acids with 150 ml of methyl alcohol containing 1.5 ml

of conc. H_2SO_4 for 6 hrs. The esters formed were extracted with ether, washed with distilled water followed by 5% aqueous sodium carbonate and finally with distilled water. The extracts were dried over anhydrous sodium sulphate. Removal of solvent from ethereal extracts gave methyl esters of mixed fatty acids which were subjected to GLC.

Examination of methyl ester by GLC[4]

The chemical composition of the oil was determined by GLC of the methyl ester. Perkin Elmer model 881 GLC instrument equipped with FID was used having a column 2M × 3MM, packed with 15% carbowax 2M on chromosorb G; nitrogen was used as carrier gas with a flow rate of 4 ml/min. The column was operated isothermally at 210°C and the injection temperature was 250°C.

The identification was carried out by running a standard mixture of methyl esters under identical conditions and comparing their retention times. The percentage composition was recorded in Table 1.

TABLE 1

PERCENTAGE COMPOSITION OF THE ACIDS OF THE SEED OIL OF *DIOSPYROS PEREGRINA*

S. No.	Fatty acid	Percentage
1.	Caprylic acid	0.95
2.	Capric acid	1.26
3.	Myristic acid	0.47
4.	Palmitic acid	31.60
5.	Stearic acid	8.24
6.	Oleic acid	43.10
7.	Linoleic acid	14.26

Study of unsaponifiable matter

The unsaponifiable part of the oil was taken in ether, thoroughly washed with distilled water and dried over anhydrous sodium sulphate. Removal of the solvent gave brownish yellow coloured matter (yield 1.00%). It was chromatographed over a column of alumina (grade II) and eluted successively with pet. ether (60–80°) as such and with petroleum ether : benzene (80 : 20), petroleum ether : ethyl acetate (80 : 20), petroleum ether : ethyl acetate (70 : 30). About 5 ml of fractions were collected and the TLC of fractions was carried over silica gel G using benzene : ethyl acetate (90 : 10) as the solvent system and concentrated sulphuric acid as the detecting reagent. The fractions with the same R_f values were mixed together. The various chromatographic fractions collected are given in Table 2.

TABLE 2

VARIOUS CHROMATOGRAPHIC FRACTIONS OF THE OIL

Fraction	Eluant	Wt. of the fraction	No. of TLC spot	R_f value of the spot
1.	Petroleum ether (60–80°)	0.48	One	0.84
2.	Petroleum ether : Benzene (80 : 20)	0.50	One	0.56
3.	Petroleum ether : ethyl acetate (80 : 20)	0.01	Nil	0.00
4.	Petroleum ether : ethyl acetate (70 : 30)	0.01	Nil	0.00

Fraction 1 (Table 2)

Removal of the solvent gave a solid which was recrystallised with benzene and methanol when a crystalline compound was obtained (m.pt. 141°C); which on treatment with tetranitromethane gave yellow colour (triterpene). The compound was identified to be β-amyrin. The identity was confirmed by preparation of the benzoyl derivative (m.pt. and mixed m.pt. 236°C).

Fraction 2 (Table 2)

Removal of the solvent gave a pale yellow solid which was recrystallised with methanol when yellow crystals of the product were obtained (m.pt. 139°C). The compound was identified to be β-sitosterol. The identity was confirmed by its response to Libermann-Burchard reaction[5] (sterol) and by preparation of the acetate derivative m.pt. and mixed m.pt. 126°C.

Fraction 3 (Table 2)

This fraction did not give any spot on TLC and on removal of the solvent nothing could be separated.

Fraction 4 (Table 2)

The fraction 4 did not give any spot on TLC and on removal of the solvent nothing could be separated.

RESULT AND DISCUSSION

Table 1 indicated the seed oil of *Diospyros peregrina* to consist of the glycerides of caprylic acid (0.95), capric acid (1.26%), myristic acid (0.47%), palmitic acid (31.60%), stearic acid (8.24%), oleic acid (43.10%) and linoleic acid (14.26%). While Tandon *et al.*[6] have reported a low percentage of palmitic acid (8.4%), the present study has revealed the oil to contain a good proportion of palmitic acid (31.6%). Caprylic acid and capric acids have also been found in the oil for the first time. The unsaponifiable

matter has been found to be a mixture of β-amyrin and β-sitosterol while Tandon *et al.*[6] have reported the presence of β-amyrin alone.

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