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# Chemical Examination of the Fruits of *Diospyros peregrina* Gurke

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The brownish-yellow oil obtained from the petroleum ether extract of the fruits of *Diospyros peregrina* was found to be a glyceride of myristic, palmitic, stearic, oleic, linoleic and palmitoleic acids. The glycoside from the alcoholic extract was characterized as β-sitosterol-D-glucoside. The free sugars isolated were found to be fructose, galactose, glucose, lactose, mannose and sucrose. The pectin precipitated from the water extract on hydrolysis revealed the presence of arabinose, galactose and galacturonic acid in it. The protein from the defatted fruits on hydrolysis gave histidine, lysine, glycine, aspartic acid, glutamic acid, alanine, proline, methionine, leucine and tryptophan.

**Key Words: *Diospyros peregrina* Gurke; Fruits, Glyceride; Glycoside; Free sugars; Pectin; Protein; Amino acids.**

*Diospyros peregrina* Gurke known as Riber Ebony in English has been reviewed by many authors[1–4]. As the fruit was reported to cure ulcers and used in diseases of the blood, urinary losses and to remove stone in the urinary tract, it was thought worthwhile to undertake the analysis of the fruits.

100 g of crushed fruits were extracted in a Soxhlet successively with petroleum ether, benzene, solvent ether, chloroform, acetone, alcohol and water. The extracts after the removal of the solvent under reduced pressure were examined qualitatively by established methods[5, 6] for common plant constituents. For detailed examination, the extractions were made several times successively with petroleum ether, ethanol and water.

**Petroleum ether extract:** Brownish-yellow oil obtained in an yield of 1.04% and having the physico-chemical constants: specific gravity at 30°C, 0.9418; $n_D^{30}$ 1.4760; saponification value 154.45; iodine value 93.28, was found to be a glyceride of myristic (4.32%), palmitic (19.79%), stearic (29.68%), oleic (28.36%), linoleic (11.57%) and palmitoleic (6.28%) acids by paper, thin layer chromatographic techniques. The identification was further confirmed by gas liquid chromatography (2 m column with Reoplex 400; 250°C; 2.17 $h^{-1}$ $N_2$; FID).

**Alcoholic extract:** The syrupy gummy mass obtained after concentrating the ethanolic extract under reduced pressure was dissolved in minimum amounts of

methanol and was precipitated by the addition of large amounts of ether. The precipitation was repeated to get an amorphous pale brownish mass. The mass showed two distinct spots on silica gel-G plates developed with a solvent system containing chloroform; methanol: water (65 : 35 : 10) and sprayed with conc. $H_2SO_4$. The components were separated by column chromatography and the first fraction 'X' was found to be a glycoside[5] while the other fraction 'Y' contained free sugars.

**Fraction 'X':** The fraction on removal of the solvent gave colourless plates (m.p. 292°C) whose IR spectrum (KBr) shows bands at 3450–3400, 2945, 2840, 1648, 1480, 1382, 1362, 1065, 1100–800 $cm^{-1}$ (sugar moiety). The fraction on hydrolysis with 2N-$H_2SO_4$ for about 4 h followed by pouring the hydrolysed material into a large amount of water gave a precipitate which responded to Lieberman-Burchard reaction for sterol. IR spectrum of the aglycone shows peaks at 3330 due to hydroxy; 2910, 1645 due to C-$CH_3$; 1460, 1385, 1130, 1065, 1020, 960 due to cyclohexane; 918, 845, 802, 760 and 718 $cm^{-1}$. The aglycone identified as β-sitosterol by Co-TLC was further confirmed by its acetyl derivative (m.p. 127°C) whose IR (Nujol) shows bands at 2945, 1758, 1480, 1380, 1285, 1260, 1145, 1045, 992, 975, 910, 890, 850 and 750 $cm^{-1}$. The benzoate derivative (m.p. 147°C) of the fraction further confirms the aglycone as β-sitosterol. The sugar portion of the fraction 'X' revealed the presence of only D-glucose by ascending and descending chromatographic techniques along with the authentic specimen. The studies revealed it to β-sitosterol-D-glucoside whose identity was further established by the preparation of its tetraacetate derivative (m.p. 170°C).

**Fraction Y:** The free sugars isolated[5] were identified by paper chromatography[7] with aniline hydrogen phthalate reagent[8, 9] as fructose, galactose, glucose, lactose, mannose and sucrose.

**Preliminary studies of pectin (Water extract):** The fruits left after extraction with ethanol were heated with water on a water bath for about 40 h and filtered while hot. The filtrate concentrated was subjected to precipitation by the addition of alcohol. The fibrous material thus obtained was washed several times with alcohol and acetone respectively and was dried in a vacuum desiccator. The precipitate was subjected to acid hydrolysis (2N-$H_2SO_4$) by heating on a water bath for about 40 h. The hydrolysate neutralized and filtered was concentrated and separated into methanol soluble portion 'A' (in hot condition) and an amorphous powder as methanol insoluble portion 'B'. The fraction 'A' showed the presence of arabinose and galactose while fraction 'B' was identified as D-galacturonic acid by paper chromatography [descending with n-butanol : acetic acid : water (4 : 1 : 5 v/v) and ascending with acetic acid: isopropanol : pyridine : water (1 : 8 : 8 : 4 v/v) solvent systems] with authentic specimen.

**Examination of protein:** Protein extracted from the defatted fruits with 10% NaCl solution and precipitated at pH between 3.5 to 4.5 from the saline extract on hydrolysis with 6N-HCl gave histidine, lysine, glycine, aspartic acid, glutamic acid, alanine, proline, methionine, leucine and tryptophan (tryptophan was identified from the alkali hydrolysate of the protein) which were identified by

paper chromatography (ascending and descending) and two dimensional thin layer chromatography[10].

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