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Note

Chemical investigation of the seed oil of *Strychnos potatorum* and spectroscopic estimation of linoleic and linolenic acids

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To determine the suitability for use in paints, soaps and detergents etc., the fatty oil from *Strychnos potatorum* seed is separated. Seed oil obtained by extraction through petroleum ether has a sufficiently high (20.8%) unsaponifiable matter. Total fatty acids are found to be 60.4% with almost equal quantities of saturated and unsaturated fatty acids. Spectroscopic studies indicate linoleic acid (31.94%) and linolenic acid (8.18%) among the total of fatty acids. Physico-chemical characteristics of the oil and presence of specific natural organic substances are also investigated.

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Strychnos potatorum (*Nirmali*) belongs to the family Loganiaceae grown in Asia and Africa. In India its forests are in West Bengal, Central and South India. It is commonly known as clearing nuts tree. Berries are black when ripe, about 1.7cm diameter. Seeds, 1 or 2, are yellow, circular about 8 mm diameter, bluntly lenticular, not largely compressed, bitter bad taste, shinning with short oppressed silky hair. In Ayurveda and Yunani system the plant is widely used to cure different diseases[1,2]. Some chemical and biological aspects of *S. potatorum* have been studied[3-7]. Sajid *et al.*[8] in a very brief study have reported the presence of some fatty acids in the seed oil. In present study besides the study of physico-chemical properties, we have separated the fatty acids in the seeds oil and have been able to identify them and determine them quantitatively.

In fats and oils the conjugated unsaturation in a normal long chain exhibits selective absorption at specific wavelength in UV-region[9], thus it is possible to determine elaeostearic or any other conjugated acid present in a fat. Quantitative separation of the natural esters is difficult and often impossible even with the most refined technique. It is, therefore, common practice to convert natural fats and waxes to a single type of simple monoester, usually the methyl ester or to free acid, prior to any attempt at separation. This technique is used in the present work.

Results and Discussion

Oil obtained by extraction through petroleum ether is a dark brown viscous liquid with characteristic sharp odour (yield 2.51%). It consists of fatty oils and also the free fatty acids as indicated by a moderate acid value **(Table I)**. A low saponification value indicates the presence of comparatively the higher fatty esters. A good iodine value indicates the presence of sufficient amount of unsaturated fatty esters. The percentages of linoleic and linolenic acids confirm this observation. A small amount of aromatic amino acids seems to be present as indicated by a positive xanthoproteic test. Presence of some alkaloids, steroids and sterols is also indicated **(Table II)**. In fact, all natural fats contain some unsaponifiable matter that may consist of hydrocarbons, long-chain aliphatic alcohols, sterols

Table I — Physico-chemical properties of oil obtained by extraction through petroleum ether

Colour	dark brown
Physical state	viscous liquid
Odour	characteristic sharp
Yield	2.51%
Solubility	insoluble in water, soluble in *n*-hexane, petroleum ether, benzene and diethyl ether
Sp. gravity 30°C	0.957
Refractive index 30°C	1.432
Specific rotation 30°C	+2° 3'
λ_{max}(nm)	667, 319
Acid value	27.69
Saponification value	79.71
Iodine value	35.40
Acetyl value	15.39
R.M. value	0.185
Polenske value	5.75

Table II — Presence of specific natural products in the oil obtained by extraction through petroleum ether

Proteins	
Biuret test	−
Xanthoproteic test	+
Alkaloids	
Mayers' test	+
Dragendorff test	+
Steroids & Sterols	
Liebermann-Storch test	+
Salkowski reaction	+
Liebermann-Burchard reaction	+

Tests for anthocyanins, flavones, flavanols, flavanones, iso-flavones and leucoanthocyanins were negative.

etc. Presence of alkaloids in the seeds of *Strychnos potatorum* is supported by the reports of Singh *et al*[10], while the presence of steroids is supported by another report of Singh *et al*[11]. Though most of the vegetable oils of commerce contain 0.8-1.5 % of unsaponifiable material, in the case of *Strychnos potatorum* seeds the present study has indicated it to be higher (20.8%) in quantity. Separation of such a material is necessary; if unsaponifiable matter is not removed it will appear subsequently at some step in the separation of the fatty acids or esters[12]. The present study indicates the total fatty acid matter to be high (60.4%) with almost equal quantities of saturated and unsaturated fatty acids **(Table III)**. Linoleic and linolenic acids constitute almost eighty percent of the unsaturated fatty acid material, linoleic being 31.94% and linolenic acid 8.18% among the total fatty acid matter.

Experimental Section

Authentic seeds of *Strychnos potatorum* were procured from Yogi Pharmacy, Hardwar and authenticity was verified by F.R.I., Dehradun. A specimen has been deposited in the herbarium of Plant Medicine Section of the Chemistry Department of Gurukula Kangri University, Hardwar under the registry no. 08/15 and available for inspection. The seeds were washed with cold water and dried in shade. Extraction in petroleum ether was carried out by usual procedures to obtain the oily material.

Study of properties

Extracted oil was subjected to the study of specific gravity, refractive index, optical rotation, acid number, saponification value, iodine value, acetyl value, R.M. value and Polenske number using the methods described in the monographs of Indian Standards Institution[13]. The determination of various specific naturally occurring organic compounds was carried out following the methods given by Gilman[14] and Paech and Tracey[15]. The total fatty acids and the unsaponifiable matter were also determined[16]. Determination of percentages of total saturated and unsaturated fatty acids was followed by the spectrophotometric determination[17] of percentages of linoleic acid and linolenic acid.

Table III — Seed oil analysis

Unsaponifiable matter	20.8%
Total fatty acids	60.4%
Saturated fatty acids in total fatty acids	49.1%
Unsaturated fatty acids in total fatty acids	50.9%
Linolenic acid in total fatty acids	8.18%
Linoleic acid in total fatty acids	31.94%

For linolenic acid absorbance at 268 nm = 0.1306, $E_1 = 45.38$
For linoleic acid absorbance at 234 nm = 0.9612, $E_2 = 336.42$

Determination of unsaponifiable matter

The oil (5g) obtained by extraction through petroleum ether was dissolved in 20mL of 50% aqueous KOH solution slowly with constant stirring by magnetic stirrer and 5mL of ethanol added and kept till the complete saponification. Solution so obtained was diluted with water and extracted with diethyl ether thrice to bring all unsaponifiable matter in ether. It was separated using a separating funnel, 1.04g of unsaponifiable matter was collected after evaporation of diethyl ether. Soap solution was collected separately.

Determination of total fatty matter

The separated soap solution obtained above was hydrolysed by slow addition of conc. H_2SO_4 and kept overnight to obtain free fatty acids. The mixture of fatty acids was separated and purified by extraction with diethyl ether and evaporated to give 3.021g of fatty acids.

Determination of saturated and unsaturated fatty acids

From the obtained fatty acid mixture, the saturated and unsaturated fatty acids were separated by lead acetate complexes[18,19]. It gave 49.1% saturated fatty acids and 50.9% unsaturated fatty acids.

Spectrophotometric determination of linoleic and linolenic acids

Polyethanoid unsaturation in a long-chain carbon compound exhibits selective absorption at specific wavelengths in UV-region of spectrum[9]. By determining the extinction coefficients at the appropriate wavelengths, a measure of amount of the conjugated material present could be obtained, making it possible to determine conjugated acid present in a fat directly by spectrophotometric examination. The linoleic and linolenic acids in which the double bonds are separated by methylene groups, on heating in the presence of conc. alkali at 170°C or above (180°C for 60 min heating for linoleic acid and 170°C for 15 min for linolenic acid) partially rearrange to conjugated forms. At a fixed temperature the amount of rearrangement to the conjugated form is constant[20].

The mixture of fatty acids (0.2g) obtained was added into a loosely stoppered Pyrex test tube containing 10mL of KOH solution (0.75g KOH per 10 mL solution of pure redistilled ethylene glycol, which was heated to 190° and maintained at that temperature for 2 min and then cooled) and maintaining the requisite temperature for requisite time (180°/60 min and 170°/15 min) in an oil-bath. Contents were then rapidly cooled by immersion of the flask in a bath containing circulating cold water. Contents were transferred quantitatively to a 250 mL flask and made up to mark with spectroscopic alcohol. Keeping overnight at 0°C, the solution was filtered and spectrophotometric study was carried out. No further dilution was required as the strength was suitable for spectrophotometric determination. A blank on the prepared alkali solution, under identical conditions, was also made and used in compensator cell of the instrument. If the extinction coefficient $E^{1\%}_{1cm}$ at 268 nm after isomerisation at 170° for 15 min is found to be E_1, the percentage of linolenic acid is $E_1 \times 100/555$ and if the extinction coefficient at 234nm after isomerisation at 180°C for 60 min is found to be E_2, the percentage of linoleic acid is $\{E_2-(575E_1/555)\}\times 100/906$, the value $575E_1/555$ being the contribution of the isomerised linolenic acid to the total extinction coefficient value at this wavelength, i.e., E_2. 555 and 575 are spectrophotometric factors[9] for linolenic acid at respectively 268 and 234 nm and 906 for linoleic acid at 234 nm.

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