Fatty Acid Composition of Lipid Classes of *Nelumbo nucifera* Seed Oil

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Summary: The *Nelumbium Nucifera* seed oil (3.62%) was examined for their fatty acid composition. The oil was extracted from the ground seeds with a solvent mixture of chloroform and methonal (2:1v/v) and its different physicochemical values were evaluated. The oil was resolved by thin layer chromatography into hydrocarbon (0.58), wax ester (7.68%), triglycerides (60.54%), free fatty acids(19.17%), 1,3 diglycerides (1.04%), monoglycerides (3.05%) by using hexane,diethyl ether and acetic acid(80:20:2 v/v/v) solvent system.The %age of polar lipids in the oil was(6.68%). The oil and its lipid fractions except hydrocarbon were converted into methyl esters and analyzed for their fatty acid composition by application of gas chromatography.

Introduction

The *Nelumbo nucifera* commonly known as``Lotus" is an aquatic herb found every where in India and in Kashmir. It belongs to the family *Nymphaeacea* [1]. The plant also called padmini. The aquatic herb with its elegant sweat scanted flowers is generally met within tanks and ponds. The juice of flower stalks is useful in diarrhoea, cholera and in liver complaints and also in fevers; it is recommended also as cardiac tonic. Compounds decoction is useful in bilious fevers. Root, flowers, stalk and leaves in the form of infusion are used in fever as refrigerant and diuretic [2]. Roots also posses the ability to dechlorinate PCE to TCE or DCE in anaerobic culture [3]. The honey formed in flowers by the bees feeding upon the padma is useful in eye diseases. Syrup of flowers is used in coughs, to check hemorrhage from bleeding piles and in menorrhagia and dysentery .The Lotus plant freshness increase by means of method using food preservatives dicoloration inhibitors and antiseptic agents [4]. Seeds are used as an application in Leprosy and other skin infections. The seeds are eaten by Australian [5] because of its caloric value, protein, fat and also for contents of Na, K, Mg, Ca, Fe, Zn and Cu.

As part of continuous interest in the evaluation of seed oils from local resources, the present studies describe the chemical investigations on the seed oil of *N.nucifera*. Specific chromatographic techniques have been used for separation, purification, identification and characterization of lipid classes. The fatty acid compositions of the whole oil as well as of its lipid classes were determined by gas chromatography.

Results and Discussion

The lipid extracted from the seeds of *Nelumbium nucifera* in 3.62% yield were made free from unwanted materials by special washing solvent-mixture of chloroform, methanol and aqueous sodium chloride. The yield seemed not good but it is comparatively high than another species of Lotus which contained 2.11% oil [6]. The seeds of N. *nucifera* contain resins, tennin and fat so plant used extensively in medicine. It also used as cardiac tonic and So in the light of these advantages it was planned to investigate the quality of oil for its proper utilization, No doubt very little information is available on the seeds oil of *Nelumbium nucifera* but some work on the leaves and roots of the plant has been carried previously [2,3].

Chemical constituents are valuable to evaluate oil with respect to its utilization. Some of the important physicochemical properties of *Nelumbium nucifera* seeds oil were determined in Table1.The iodine value (90.0) of the oil confirmed that the oil is semi drying. Some earlier studies of the seeds of *Nelumbium nucifera* also indicate such character of the oil. [7]. The saponification value (175.80) of the extracted oil shows the presence of fatty acids having

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high molecular weight which was confirmed by GLC that higher molecular weight fatty acids present in large concentration. Less amount of free fatty acids (1.86) present in the oil so it can be used for edible purpose.

Table-1:Chemical Evaluation of *N.nucifera* Seed

Moisture%	7.26
Oil%	3.62
Physico chemical values of the oil of *N.nucifera*	
Saponification value	175.8
Iodine value	90
Free fatty acid (as oleic acid)	1.86
Unsaponifiable matter	0.52
Acid value	3.70
Ester value	172.1

The lipids were fractionated into neutral (95.24%) and polar (6.68%) lipid classes by thin layer chromatography. Neutral lipids were further fractionated into various classes and the composition of these classes are shown in Table-2. According to this table triglycerides (60.54%) is the predominant fraction while 1,2 diglycerides (1%) is lowest among the six classes of lipids found out in the neutral fraction. Polar lipids were also fractionated further and identified as phosphatidyl-ethanolamine, phosphatidyl-cholines, lysophatidyl-ethanolamine.

Table-2:R_f Values and the % Composition of Neutral Lipids of *Nelumbium nucifera* Seeds Oil

Lipid fractions	%age	R_f value
Hydrocarbon& wax ester	7.68	0.93
Triglycerides	60.54	0.90
Free fatty acid	19.17	0.65
1,3 diglycerides	1.04	0.55
1,2 diglycerides	1.0	0.50
Monoglycerides	3.05	0.25

The total lipid as well as its fractions including triglycerides, free fatty acids, 1,3:diglycerides, monoglycerides showed high percentage of saturated fatty acids except that of 1,2:diglycerides and wax esters which showed high percentage of unsaturated fatty acids

The conversion of various classes of lipids into methyl esters by reacting with boron tri fluoride-methanol reagent was preferred, as it allows one to work with small quantities of material. The fatty acid moiety which plays a vital role for the formation of various classes of lipids was characterized by use of gas chromatography is shown in Table-4 which

Table-3:Percentage of Saturated and Unsaturated Fatty Acids of *Nelumbo nucifera* Seed Oil and its Fractions

Lipid fractions	Saturated Fattyacid %age	Unsaturated Fattyacid %age	Unidentified
Whole oil	48.3	41.3	10
Wax ester	48.2	51.7	0.6
Triglycerides	62.4	37.3	0.4
Free fatty acid	53.8	41.2	6.0
1,3 Diglycerides	50.7	47.3	2.0
1,2 Diglycerides	35.8	60.1	5.0
Monoglycerides	65.5	34.9	--
Phosphatidylethanolamine	50.0	48.6	1.4
Phosphatidylcholines	63.6	34.1	2.3
Lysophalidylethanolamine	47.6	46.7	6.5

shows that fatty acid composition ranged from C_{10} to C_{20} in the lipids of *N.nucifera*. Palmitic acid $C_{16:0}$ was found to be maximum in saturated fatty acids and linoleic acid was maximum among unsaturated fatty acids i.e 33.27% and17.9% respectively. Saturated fatty acids were present in comparatively greater amount than unsaturated fatty acid.This finding is not according to an earlier study of another [6] specie of Nelumbium which contained unsaturated acid in larger amount than saturated fatty acid .This difference in composition may be due to the difference of species.

Gaffendes (1969) studied the seed lipids of 20 species of water plants and [7] found low content of stearic acid in them.The results of the present investigation in this aspect are according to the earlier studies because the GLC report of *Nelumbium nucifera* oil also shows 3% stearic acid in it [8]. Table-4 a and4b shows the fatty acid composition of neutral and polar lipids which indicated that $C_{18:0}$ was next to palmitic acid among saturated acids and oleic acid was the next to linoleic acid among unsaturated fatty acids.

The results of physico-chemical values conclude that the *N.nucifera* oil does not show any value which can have any adverse effect if it is used for human consumption. Furthermore the oil also contains a higher amount of essential fatty acids like linoleic and linolenic acid which are considered to be useful for human body [9]. The appreciated amount of oleic acid in the oil improves the shelf life of the oil [10] and the high concentration of $C_{18:2}$ is also useful because it lower HDL(High density lipoprotein) which decrease CVD(Cardio vascular diseases) risk[11].

Table4.Fatty Acid Composition of Various Lipid Classes and Oil of *Nelumbium nucifera* Seeds

4a) Neutral

Acids	Whole oil	Wax	T.G	FFA	1,3 D.G	1,2 D.G	M.G
Capric acid $C_{10:lower}$	2.09	0.1	0.9	0.5	--	1.9	1.9
Lauric acid $C_{12:0}$	2.04	1.7	2.0	1.3	0.4	1.2	4.6
Myristic acid $C_{14:0}$	3.21	1.9	3.0	3.3	0.2	0.7	10.8
Palmitic acid $C_{16,0}$	33.27	41.0	48.1	40.6	24.1	20.7	33.5
Palmitoleic acid $C_{16:1}$	5.7	--	0.3	--	--	--	--
Mygaric acid $C_{17:0}$	0.2	0.3	1.1	3.8	10.8	2.3	4.5
Stearic acid $C_{18:0}$	3.0	2.5	7.0	4.2	6.9	4.0	5.0
Oleic acid $C_{18:1}$	11.7	10.7	9.9	6.4	6.5	4.9	3.0
Linoleic acid $C_{18:2}$	19.9	35.3	22.8	32.76	32.5	40.2	--
Linolenic acid $C_{18:3}$	3.4	5.7	2.3	1.1	8.3	5.0	5.7
Arachidic acid $C_{20\&higher}$	5.5	1.7	2.3	1.1	8.3	5.0	2.7
Unknown	10	0.6	0.4	6.0	2.0	5.0	--

W.E Wax esters
T.G Triglycerides
FFA Free fatty acids
1,3 DG 1,3 Diglycerides
1,2 DG 1,2 Diglycerides
M.G Monoglycerides

4b) Polar

Acids	Phosphatidylethanolamine	Phosphatidylcholines	Lysophatidylethanolamine
Capric acid $C_{10:lower}$	0.3	0.9	0.5
Lauric acid $C_{12:0}$	1.9	0.4	0.2
Myristic acid $C_{14:0}$	0.5	2.3	1.5
Palmitic acid $C_{16,0}$	46.4	56.6	43.8
Palmitoleic acid $C_{16:1}$	--	--	--
Stearic acid $C_{18:0}$	1.0	1.2	0.6
Oleic acid $C_{18:1}$	18.1	9.2	15.6
Linoleic acid $C_{18:2}$	23.0	24.5	24.9
Linolenic acid $C_{18:3}$	7.5	0.4	6.2
Arachidic acid $C_{20\&higher}$	0.9	2.2	1.0
Unknown	1.4	2.3	6.5

Experimental

Extraction of Oil

The seeds of *N. nucifera* were dried in oven at 105°C crushed into fine powder. The lipids were extracted with 500ml chloroform: methanol (2:1v/v) [12] mixture at room temperature by shaking for 2 hours. After filtration the residual material was treated three times with 100ml of same solvent mixture. All the extracts were combined and three consecutive washings with Folch solution [13] were given to remove the non lipid impurities. After removal of solvent under reduced pressure the oil of *N.nucifera* was stored in an inert atmosphere.

Physicochemical Values of the Oil

The physicochemical values like saponification value, iodine value, peroxide value, free fatty acid were determined according to British standard specification and procedure [14] and Nicholls 1952 [15]. Refractive index was determined with Abbe's refrectometer.Ester value and acid valuewere derived from the above determined values.

Saponification of the Fatty Matter and Liberation of Fatty Acids

The oil of *N.nucifera* was refluxed on water bath with 0.5N alcoholic potassium hydroxide solution for 3 hours. The solvent was distilled out under reduced pressure and the residual soap was washed thrice with petroleum ether to remove the unsaponified matter. The soap was dissolved in water and acidified with 0.2N sulfuric acid and were refluxed on water bath for one hour [16,10]. The liberated fatty acids were extracted with diethyl ether and dried over anhydrous sodium sulfate. After the removal of solvent a mixture of fatty acids was obtained. The unsaponified matter was extracted by means of hexane [12].

Separation of Lipid Classes of N.nucifera Oil

Thin-layer glass plates (20x20 cm) of 0.5 mm thickness were prepared by using kieselgel 60G. These plates were activated at 105°C for two to three hours. Aliquots of the lipids (100mg) were streaked on five plates which were developed in the solvent

system of hexane - diethyl ether - aceticacid (80:20:2 v/v /v) for neutral lipids and 40mg lipids were streaked on two plates which were developed in the solvent system of chloroform: methanol: 30% ammoniumhydroxide:water (60:35:5:2.5v/v/v/v) for polar lipids [17]. The resulting bands were made visible under UV lamp by spraying with 2,7dichloro-fluoroscein in methanol.Neutral lipid classes were identified by comparison of their R_f values with those of standards.The bands made visible under UV light were marked and then scraped from the plates.

Preparation of Methyl Esters of Fatty Acids

The fatty acids mixture of *N.nucifera* seeds and its purified lipid fractions were treated with borontrifluoride- methanol reagent under standard conditions for the formation of methyl esters[18], which were purified by the application of thin – layer chromatography using hexane-ether (9:1v/v) solvent system.

Identification of Fatty Acids by GLC

The methyl ester of lipid fractions were analyzed on Shimadzu GC-4A gas chromatograph equipped with flame ionization detector and polar (PEG) capillary column (25mx 0.2mm i.d). The column temperature was programmed at 180°C for 0 min. with3°C/min rise. The final temperature of column was 220°C. Nitrogen was used as carrier gas with a flow rate of 30ml/min. The temperature of injector and detector was maintained at 230°C and 250°C respectively, 0.5µl of the methyl ester sample was injected into the injector and the resolution of the sample into individual fatty acids was recorded on Shimadzu CR-4A chromatopac. These unknown fatty acids were identified by comparing their retention times with those of the standard methyl esters injected under the same condition of temperature and pressure.

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