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Physicochemical and Phytochemical Investigation of Isolated seed oil of *Nelumbo Nucifera* (L.) and its Pharmacognostic parameters.

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ABSTRACT

Nelumbo nucifera (L.) belonging to Nelumbonaceae commonly known as lotus, aquatic herb grown and consumed throughout Asia. It is known in Sanskrit-Svetakamala, English-Sacred Lotus, Hindi- Kanwal and French-Nelumbo. All parts of *N. nucifera* used for various medicinal purposes in various systems of medicine including folk medicines, Ayurveda and Chinese traditional medicine. Many chemical constituents have been isolated from this plant. However, the bioactive constituents of lotus are mainly alkaloids and flavonoids. Traditionally the whole plant of lotus was used as astringent, emollient, diuretic, diarrhea and in the treatment of tissue inflammation and seeds are used in the cancer treatment. In this research work the fixed oil was isolated from lotus seeds with a solvent extraction method and its physicochemical and phytochemical parameters were evaluated along with TLC parameter. Seeds organoleptic, microscopical & physicochemical parameters were also evaluated.

Keywords: *Nelumbo nucifera*, Lotus seed, Padma, Sacred lotus, Svetakamala, Nelumbonaceae

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INTRODUCTION

Lotus is a perennial aquatic herb, belongs to Nelumbonaceae, comprising of only one genus *Nelumbo* with two species: *Nelumbo nucifera* Gaertn. and *Nelumbo lutea* Pear. It is symbol of purity in Buddhism.^{1,2} Lotus fruit famous for its long-life period. Herb with elegant sweet scented flowers, found in tanks and ponds throughout India, cultivated in Japan, China, Asia, Oceania, and America. It is widely cultivated for attractiveness and color, there are three main colors; white, red and yellow, yellow and red color is determined by the presences of carotenoids and anthocyanin respectively. The flowers used as beautifier and perfume. Seeds and the by-products of their processing are broadly consumed.¹⁻⁴

Leaves are two types aerial and floating, orbicular in shapes 20-90 cm in size and the fresh leaves are hard in texture. The petiole is very long, smooth with small prickles, greenish or greenish brown in color. There are two types of flowers, white variety is called as 'Pundarika' and pink or radish variety called as 'Rakta kamal'. Lotus herb bearing flowers, stem and root is called as 'Padmini' in Sanskrit. This aquatic herb develops with strong creeping rhizome, descending up to 1,800 m, yellowish white in color, size varies from 60-140 cm long and 0.5 to 2.5 cm in diameter. Fresh rhizome contain starch, water, fiber, sucrose and reducing sugar.^{3-7,12}

Seeds are hard, ovoid up to 1.0 cm long and 1.5 cm broad with brownish, grayish black hard pericarp. The Seed composes of three parts namely epicarp, embryo and cotyledons.

Seeds are rich in alkaloids: dauricine, lotusine, liensinine, roemerine, neferine and armepavine; flavonoids: kaempferol, quercetin, leucocyanidin, leucodelphinidin, catechin, isoquercetin and astragalin; proteins, amino acids: glutathione large amount contained in the plumule and cotyledons; tannin, unsaturated fatty acids and minerals: sodium, potassium, chromium, calcium, magnesium, zinc and copper. The seed polysaccharides are isolated are mainly composed of four types of monosaccharide such as D-galactose, L- arabinose, D-mannose and D-glucose. Lotus seeds contained 2.11% oil and they determined by gas chromatography and UV absorption.^{1,3,4,6,7}

This plant is great source of herbal medicine having strong astringent and cooling properties. The young leaves with sugar are used to treat rectal prolapse and paste of leaves applied to body in fever. Flowers are useful to treat diarrhea, fever and hepatopathy. The seeds are demulcent, nutritive and embryo for treating the high fevers, insomnia, cardiovascular disorders and powder mixed with honey to treat cough.¹⁷ Lotus alkaloids dilate blood vessels and reduce blood pressure. The sweet taste and nourishing properties of seeds are beneficial to spleen⁵⁻⁹. Seed oil is alternative source of energy and use as biofuel. Rhizome extract have anti-diabetic and anti-

inflammatory properties because of presence of steroidal triterpenoids. Powder of rhizomes used in dysentery, demulcent and chronic dyspepsia.¹²⁻¹⁵

Lotus also has religious importance in South East Asia, where the seeds and its leaves are also eaten. These are sometimes also sold as a raw snack. The roasted seeds are good coffee replacer and popped like popcorn, ground into powder and eaten dry and used in bread making. Rhizomes widely used in traditional food preparation, used as pickle and fried chips.¹³⁻¹⁵

N. nucifera plant has antioxidant, anti-inflammatory, antidiarrheal, immunomodulatory, antibacterial, anticancer and antifertility activities¹. Different parts of *N. nucifera* are used as food and traditional medicine purpose and lotus seeds are edible^{2,3}. Different parts including leaves, flowers, rhizomes and seeds showed various pharmacological activities². Lotus flower extract enhance melanin synthesis in human melanocytes^{4,5}. *N. nucifera* and its other species have very significant advantages against the free radicals and ROS species damage⁶. *N. nucifera* had the highest antioxidant activity measured by DPPH radical scavenging assay^{6,7}. It is best Nutraceuticals protein rich food, seeds used as raw material in folk and Ayurvedic medicine⁸.

MATERIALS AND METHOD

Seeds of *Nelumbo nucifera*, Conc. HCl, Petroleum ether(60-80°C), Methanol, Chloroform, Sodium hydroxide, Alcohol, Glycerin, Phloroglucinol, Ethanol (AR grade),

TLC Silica gel 60 F254 (Merck)

Trinocular microscope-Olympus CX21i-TR-LED,

Brookfield viscometer LVD VII+ Pro.

Authentication, collection, drying and sizing

The dried lotus seeds were collected from local market, Nashik, Maharashtra. The authentication of seeds was done by Department of Botany, L.V.H's, Art, Science and Commerce College, Panchvati, Nashik.

Dried seed's blackish hard testa and greenish embryo were removed manually and remaining seeds kernel were powdered and passed through sieve No- 60 and packed in polyethylene bags.

Macroscopic characters

Macroscopic studies were carried out for organoleptic characteristics i.e shape, size, color, weight, texture of seeds.

Microscopic characters

Photomicrographs were obtained by observing free hand sections of seed kernel and examined under Trinocular microscope-Olympus CX21i-TR-LED using 10x and 45 x objective. Transverse

section of seed kernel were treated with different reagents like picric acid, Sudan red III, Iodine solution and photos were recorded.

Physicochemical study of *N.nucifera* seed^{16,18}

Physicochemical parameters of seed kernel powder were determined such as Total ash value, Water soluble ash value, Acid insoluble ash value, Foreign organic matter, Moisture content, Alcohol soluble extractive value and Water-soluble extractive value as per WHO guidelines.

EXTRACTION

Dried powder of seed kernel subjected for extraction using Petroleum ether (60-80°C) using Soxhlet assembly for 4-6 hrs. Petroleum extract concentrated by distillation to obtain crude fixed oil and was dehydrated by passing through anhydrous sodium sulphate. The isolated fixed oil was designated as NN-PT. The procedure is shown as flow chart wise in Figure 1.

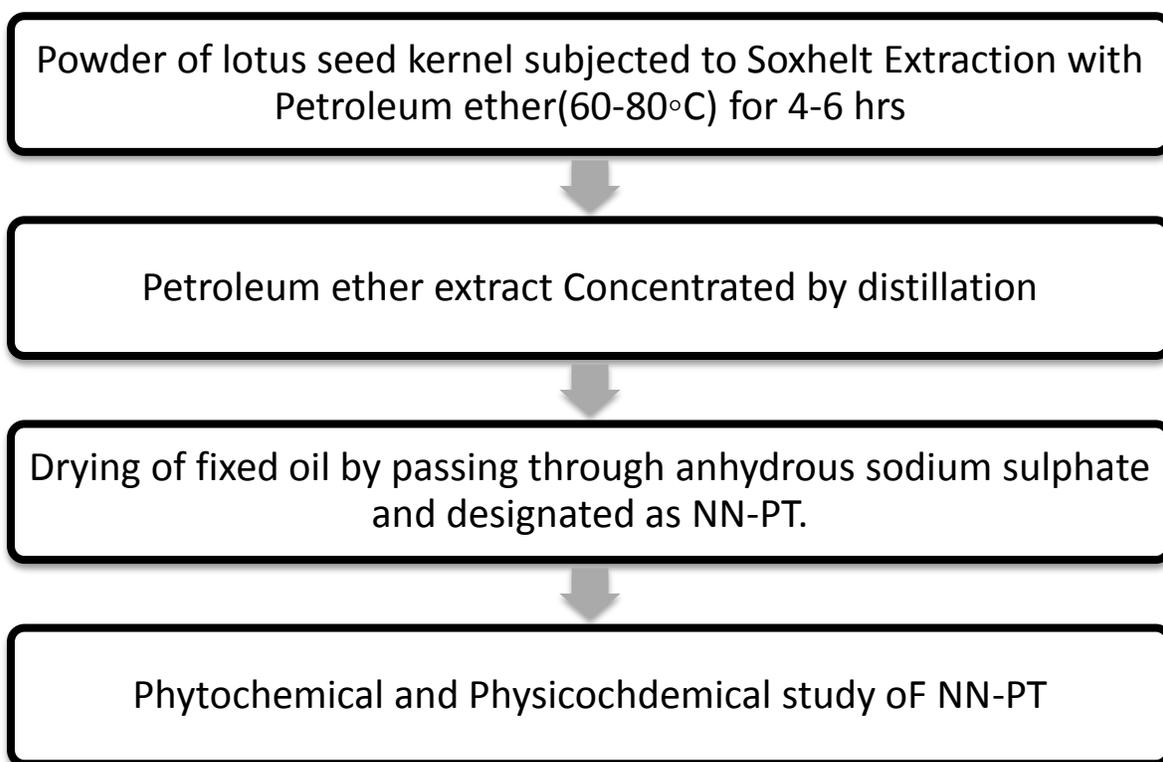


Figure 1: Flow Chart of extraction of fixed oil from Seed kernel of *N. nucifera*

Dried powder of seed kernel extracted with alcohol by reflux for 20 min. alcoholic extract filtered and concentrated by distillation. Alcoholic extract designated as NN-AL.

Phytochemical screening of NN-AL

NN-AL diluted with alcohol, various phytochemical screening tests were performed for identification of various primary and secondary metabolites ie test for alkaloids- Mayer's test, Hager's test, Wagner's test, Dragendorff's test; test for flavonoid- Shinoda test; test for tannin- Ferric acid test; test for carbohydrate- Molisch test, Benedict's test, Fehling test were performed.

Physical parameters evaluation of NN-PT

Physical parameters of isolated NN-PT determined such as color, odor, viscosity, density and specific gravity.

Physicochemical values of NN-PT

Physicochemical analysis of NN-PT oil was performed such as Acid value, Saponification value, unsaponifiable matter, ester value and iodine value determined as per IP (2014) and stain test performed on filter paper for physical properties as fixed oil. Phytochemical screening of NN-PT by TLC¹⁴

TLC parameter of NN-PT

Stationary phase: TLC Silica gel 60 F254

Mobile phase : Chloroform : Methanol (9:1)

Spraying reagent: Anisaldehyde-Sulphuric acid reagent.

Treatment after spray: Heat the plate at 105° C for 5-10 min.

Visualization: @254 nm, @365 nm and day light before spray and in day light after spray.

RESULTS AND DISCUSSION

The Morphology of seed were evaluated and parameters recorded. Lotus seeds are nut like round oval in shape, blackish grey in color and hard in texture. Each seed being surrounded by a testa shell and this shell turns quite hard when the seed dries. Kernel of seed is brownish and shows longitudinal wrinkles that gives rough texture as shown in Figure 2. Sizes of seeds are different according to varieties as 1.4-1.9 cm long and 0.8- 1.6 cm in diameter and seed kernel as 1.3 to 1.5 cm long and 0.6 to 1.4 cm in diameter. The average weight of 100 seeds were found as 1.2-1.4 kg. Transverse sections of seed kernel were examined under trinocular microscope using at 10X, 45X objective and photographs were obtained as shown in Figure 3.

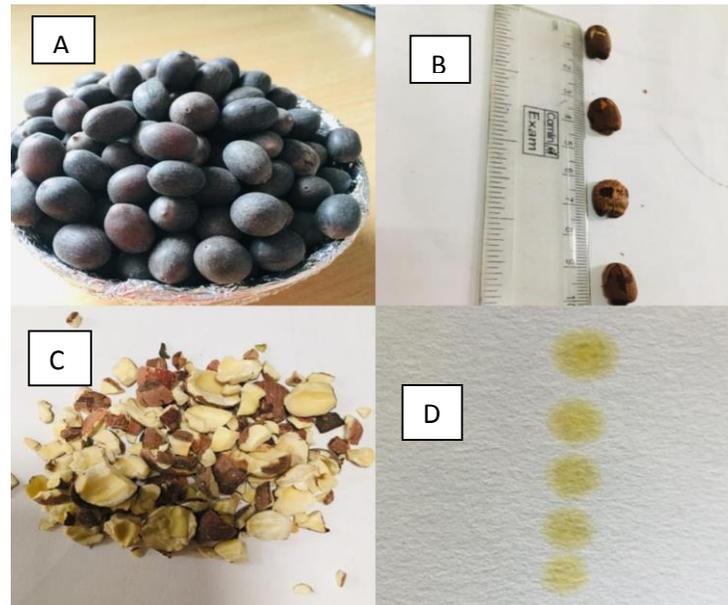


Figure 2: Morphology Evaluation of Seed & fixed oil stain test of *N. nucifera*

A- Seeds of *N. nucifera*, B-Size parameter of seed kernel of *N. nucifera*, C- seed kernel of *N. nucifera*, D- Staining test of NN-PT on filter paper

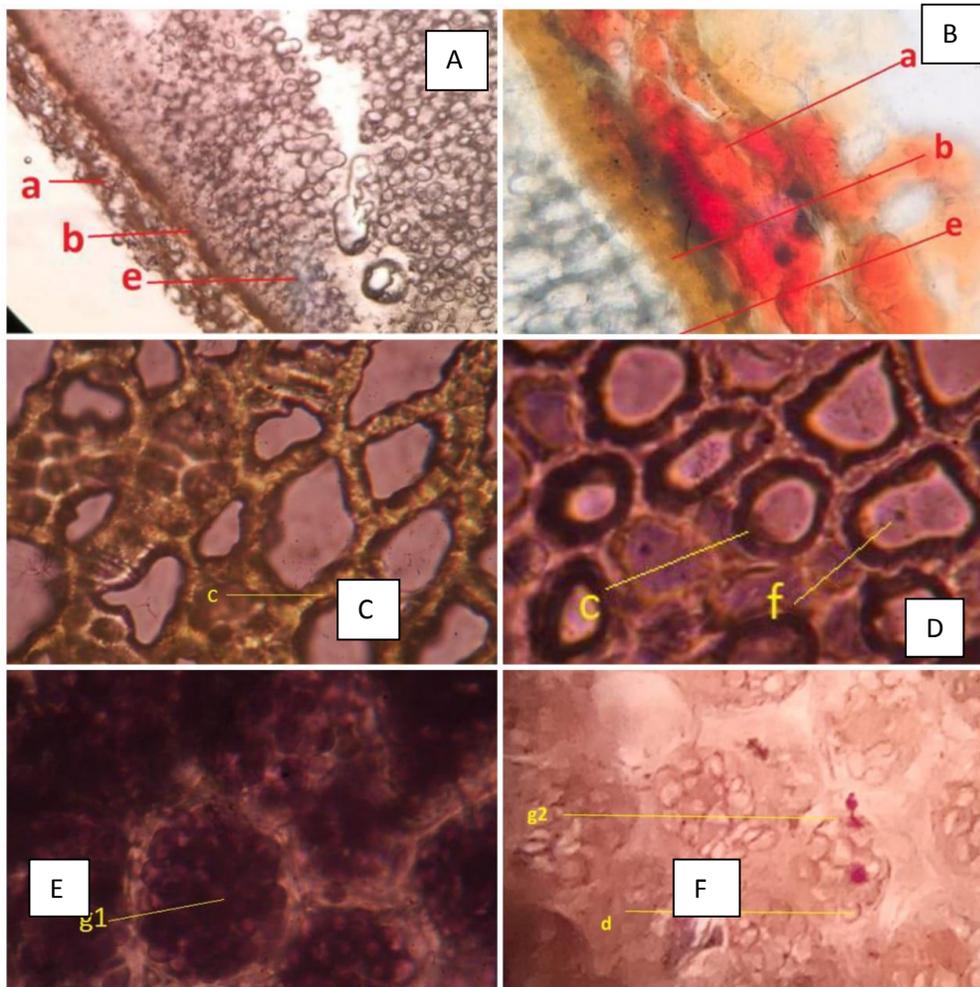


Figure 3: A: 10x T.S. of seed kernel of *N.nucifera* , B: 45x T.S of seed kernel of *N. nucifera*, C: 45x Endosperm, D: 45x Endosperm, E: 45x Endosperm stain with Iodine solution, F: 45 x Endosperm stain with Sudan III.

a- outermost layer of seed kernel, b- innermost layer of seed kernel, c-cell wall of endosperm, d- oil globules, e- endosperm, f- aleurone grains, g1- starch grain stained blue with iodine, g2- starch grain unstained

Seed kernel layer shows two zones: outermost zone and innermost zone. Outermost zone of seed kernel made up of 4-5 layers of tangential elongated parenchymatous cells filled with red pigment. Innermost zone of seed kernel made up of 2-3 layers of collapsed parenchymatous cells filled with brown pigment. This Seed kernel layer encloses whole endosperm. The endosperm cells are polygonal in shapes, thick walled, colorless and filled with starch grains, aleurone grains and fixed oil globules.

Microchemical test shows yellow color staining of aleurone grains with picric acid stain, bluish violet color stain for starch grains and reddish color to fixed oil droplets with Sudan III in glycerin in endospermic cell as ergastic inclusion as shown in Figure 3.

Physicochemical study of *N. nucifera* seed were evaluated and shown in Table 1. Phytochemical screening of NN-AL was performed for various primary and secondary metabolites and result shown in Table 2. Physical parameters of NN-PT were performed and result shown in Table 3. Physicochemical parameter NN-PT were performed such as Saponification value, Acid value, Iodine value, Ester value and unsaponifiable matter as per IP and result shown in Table 4

Phytochemical screening of NN-PT was performed by TLC as shown in Figure 4 and result shown in Table 5 under various light condition Under mentioned condition 10 spots were retained over TLC plate and gave characteristic value for NN-PT.

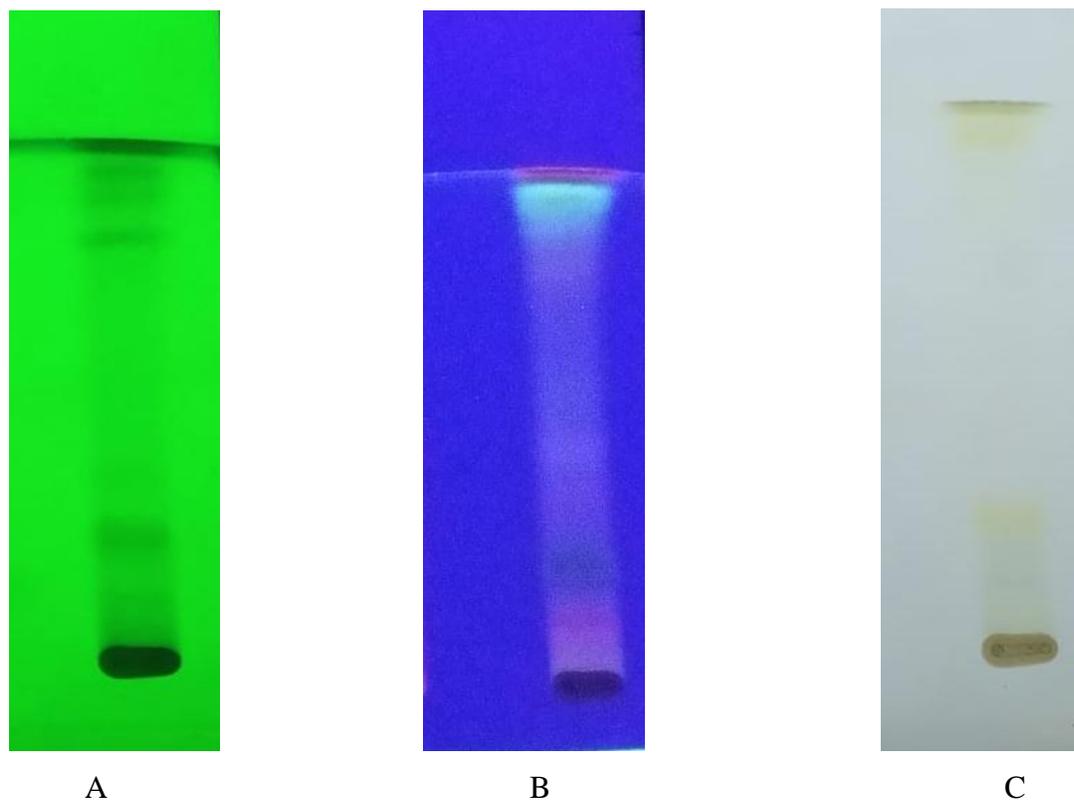


Figure 4 : TLC of NN-PT A: @254nm, B: @366nm, C: TLC Day light after spray

Table 1: Physicochemical study of *N. nucifera* seed¹⁶

Sr.no.	Parameter	Result % w/w
1.	Total ash	5.5
2.	Water-soluble ash	2.4
3.	Acid insoluble ash	3.2
4.	Crude fiber	12
5.	Moisture content	11

Table 2: Preliminary phytochemical screening of NN-AL^{14,16}

Chemical test	NN-AL
Alkaloids	++
Carbohydrate	+++
Saponins	+
Proteins	+++
Flavonoid	+
Tannins	+

+++ Strongly positive, ++ - moderately positive, + positive, - negative

Table 3: Physical parameters of NN-PT

Feature	Observation
Color	Pale yellow
Odor	Pleasant
Density	1.05 gm/ml
Specific gravity	1.09
Viscosity	98.5 CP
Yield	10.50 gm

Table 4: Physicochemical values of NN-PT^{11,16}

Physicochemical parameter	Values
Saponification Value	168.3
Unsaponifiable matter	0.45
Acid Value	4.12
Iodine Value	88.8
Ester Value	161.57

Table 5 : Evaluation of TLC OF NN-PT

Track No.	Spot No.	@ 254 nm	Before spray @366nm	Day light	After spray in day light	RF
1	1	Dark green	Dark violet	Yellow	Yellow	0.10
	2	Faint green	Violet	Faint yellow	Faint yellow	0.13
	3	Faint green	Violet	Faint yellow	Faint yellow	0.15
	4	Faint green	Violet	Faint yellow	Faint yellow	0.19
	5	Faint green	Blue	Yellow	-	0.22
	6	-	Blue	-	-	0.28
	7	-	Faint blue	-	Yellow	0.34
	8	Faint green	Faint blue	Yellow	Yellow	0.50
	9	Faint green	Greenish blue	Yellow	-	0.57
	10	Faint green	Greenish blue	Yellow	Yellow	0.59

CONCLUSION:

Seed shows presence of primary metabolites in microscopic evaluation and various secondary metabolites ie. Alkaloids, flavonoids, tannins in phytochemical test. Physical parameter of isolated fixed oil NN-PT confirmed as fixed oil with staining test on filter paper & its various physico chemical values can be use for its characteristic evaluation. NN-PT evaluated for TLC which is novel in research work. It's TLC pattern with mentioned TLC parameter can be use for its characteristic evaluation.

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