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## Phytochemical screening and nutritional analysis of *Nelumbo nucifera* (Pink lotus) rhizomes to validate its edible value

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**Abstract**

*Nelumbo nucifera* or lotus rhizomes are edible and have been used as an indigenous medicine in India. The healing properties of the plant is due to the presence of active principles such as phenolics, tannins, alkaloids, and flavanoids which constitute of many pharmacologically active compounds. The aim of the present study to evaluate the phytochemical and nutritional analysis of lotus rhizome. The different solvents used for the extraction viz., ethanol, ethylacetate, hexane, chloroform, acetone and aqueous for phytochemical screening. Lotus rhizome extract shows that the presence of primary metabolites viz., Carbohydrates, proteins and lipids and the secondary metabolites such as phenols, flavanoids, tannins, alkaloids, sterols, terpenoids, cardiac glycosides, coumarin, quionone on phytochemical screening. The quantification of primary metabolites of the extract indicates its nutritive value and the secondary metabolites were responsible for its medicinal properties of the lotus rhizome. Results showed that phytochemical screening of rhizome extract has various phytochemicals such as carbohydrates, proteins, lipids, phenols, flavanoids, tannins, alkaloids, sterols, terpenoids, cardiac glycosides, coumarin, quionone. Quantification of phytochemicals revealed that the ethanolic extract has the maximum phenols, flavanoids and tannin content compared to other solvents used for the extraction. The lotus rhizome has good nutritional properties being a good source of carbohydrate and proteins with potential usage in flavouring agents, food production and bakery products.

**Keywords:** Lotus, phytochemicals, nutritional analysis

**Introduction**

*Nelumbo nucifera* is a genus that belongs to the family nelumbonaceae. The genus nelumbo has two species around the world, *Nelumbo nucifera* Gaertn and *Nelumbo lutea* wild. Lotus is a perennial aquatic edible plant commonly known as sacred lotus, which has been used as a traditional medicinal plant in China and India (Wang and Zhang, 2004) [22]. *Nelumbo nucifera* is widely distributed in South-East Asia. In India, it occurs from Kashmir in north to Kanyakumari in south, showing high phenotypic diversity with different shapes, sizes and shades of pink and white flowers ranging from 16-160 petals and is the national flower of the country. In certain areas the natural habitat for lotus has been destroyed and the plant populations have dramatically reduced (Tilt, 2010) [21]. Lotus is considered as endangered and threatened species in many parts of America (Sayre 2004) [16]. Lotus is considered to be sacred in many religions. It is defined as the symbol of purity, divine beauty, resurrection and enlightenment.

The lotus plant grows by extending a creeping rhizome through anaerobic sediments at the bottom of the water body. The color of the rhizome varies from yellowish white to yellowish brown in colour, smooth longitudinally striated with brown patches, bearing nodes and internodes. Each of the nodes produces a leaf. The rhizome exudates mucilaginous juice and shows a few large cavities surrounded by several larger ones, fracture is tough and fibrous. Lotus produces a number of important secondary metabolites, like alkaloids, flavonoids, steroids, triterpenoids, glycosides and polyphenols (Mukherjee *et al.*, 2009) [12] and different parts of the plant viz., leaves, seeds, flower, and rhizome can be used in traditional system of medicine.

In Asian culture, whole parts of lotus consisting of seeds, rhizomes, nodes, leaf, roots, young shoots, stamens, petals, stalks, and pericarps are eaten as food or used for medicinal purposes. In traditional system of medicine, different parts of the plant is reported to possess beneficial effects for the treatment of pharyngopathy, pectoralgia, spermatorrhoea, leucoderma, smallpox, dysentery, cough, haematemesis, epistaxis, haemoptysis, haematuria, metrorrhagia, hyperlipidaemia, fever, cholera hepatopathy and hyperdipsia (Baghel and Dubey, 2017) [17].

Phytochemicals are the natural bioactive compounds found in plants, which are solely responsible for its medicinal activity. Knowing the importance of phytochemicals is desirable because such information will be of value for synthesis of complex chemical substances (Parekh *et al.*, 2008) [13]. The phytochemicals are grouped into two main categories namely primary constituents which includes amino acids, common sugars, proteins and chlorophyll etc., and secondary constituents consisting of alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds etc., (Krishnaiah *et al.*, 2007) [8]. Majority of (phytochemicals have been known to have valuable therapeutic activities such as insecticides Kambu *et al.*, 1982) [7], antibacterial, antifungal (Lemos *et al.* 1990) [11], anti-constipative (Ferdous *et al.*, 1992) [3], spasmolytic, anti-plasmodial and antioxidant (Vardar-Unlü *et al.*, 2003) [23] activities etc. Phytochemical screening of medicinal plants and quantification of primary and secondary metabolite is very important in identifying new sources of therapeutically and industrially important compounds.

(Mukherjee *et al.*, 2009) [12], Lotus plant yields a number of important medicinal secondary metabolites. Nutrition plays an important role in health, by not only providing vital nutrients, but also promoting health and inhibiting ailments. Pharmacological investigations carried on whole, different parts and rhizomes of *N. nucifera* have indicated important activities. Therefore the present study has been carried out to evaluate the phytochemical and nutritional analysis of lotus rhizome.

## Materials and methods

### Collection and preparation of the extract

The flowers were collected from ponds at Manickaputheri near Nagercoil area in Kanyakumari district. Fresh rhizomes were washed with distilled water to eliminate mud and other dust particles. The rhizomes were divided into very small pieces with a sharp knife. Then the rhizome was dried under shade, separated, crushed by a mechanical grinder and passed through a mesh sieve. A total of 10 g of the crushed plant material was taken and soaked for 3 days in 100 ml of ethanol, ethylacetate, hexane chloroform, acetone and water separately. The extracts were then filtered through Whatman filter paper No.1. Dried solvent extract was kept at 4°C until further analysis. Fresh rhizome was used for the determination of the moisture content.

### Qualitative phytochemical analysis

Phytochemical screening was carried out in the extracts using different solvents to identify the major natural chemical groups such as Carbohydrate, Protein, phenols, tannins, flavonoids, terpenoids, alkaloids, cardiac glycosides, coumarins and steroids.

### Test for carbohydrate

**Molisch's test:** Filtrate was treated with 2–3 drops of 1% alcoholic  $\alpha$ -naphthol solution and 2 ml of Conc.  $H_2SO_4$  was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids indicates the presence of carbohydrates.

### Test for protein

**Biuret test:** 2 ml of the extract, 5 drops of copper sulphate (1%) and 2 ml of sodium hydroxide (10%) were mixed. The development of violet colour indicated the presence of proteins.

### Test for Phenols

To 1ml of plant extract, 2ml of distilled water followed by few drops of 10 % ferric chloride was added. Formation of blue/ green colour indicated the presence of phenols.

### Test for tannins

**Ferric chloride test:** To 1ml of plant extract, 1ml of 5% ferric chloride was added. Formation of dark blue or greenish black colour indicated the presence of tannins.

### Test for flavonoids

**Shibita's test:** To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Formation of yellow colour indicated the presence of flavonoids.

### Test for alkaloids

**Mayer's test:** To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops Mayer's reagent was added. Presence of green color or white precipitate indicated the presence of alkaloids.

### Test for quinones

To 1ml plant extract, 1ml of concentrated sulphuric acid was added. Formation of red colour indicated the presence of quinones.

### Test for cardiac glycosides

**Keller-kilani test:** To 0.5 ml of plant extract, 2 ml of glacial acetic acid and few drops of 5 % ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardiac glycosides

### Test for terpenoids

To 0.5 ml of the plant extract, 2 ml of chloroform along with concentrated sulphuric acid was added. Formation of red brown colour at the interface indicated the presence of terpenoids.

### Test for steroids

To 0.5 ml of plant extract, 2 ml of chloroform and 1 ml of sulphuric acid was added. Formation of reddish brown ring at interface indicated the presence of steroids.

### Test for coumarin

To 1 ml of plant extract, 1 ml of 10 % sodium hydroxide was added. Formation of yellow colour indicated the presence of coumarins.

### Test for saponin

To 2ml of plant extract, 2ml of distilled water was added and shaken lengthwise in graduated cylinder for 15 min. Formation of 1cm layer of foam indicated the presence of saponins.

## Quantitative phytochemical screening

### Total phenol content

Total phenol content of the rhizome extracts was estimated by Folin–Ciocalteu colorimetric method as described by Singleton and Rossi, (1965) [18] with certain modifications. 200  $\mu$ l (1 mg ml<sup>-1</sup>) of extract was mixed with 8.5 ml of distilled water and to this 0.5ml of Folin–Ciocalteu reagent was added and mixed thoroughly for about 5 min. After adding 20% sodium carbonate (1 ml), the test tubes were incubated in dark for 60 min at room temperature. The

absorbance was measured at 760nm by using UV – visible spectrophotometer. The calibration curve was prepared using gallic acid equivalent and the results were expressed as mg per gram of gallic acid equivalent.

#### Total flavonoid content

Total flavonoid content of the rhizome extracts was estimated as per the aluminium chloride method described by Liu *et al.*, (2008) [9]. Extract of 0.5 ml was added to 3 ml of sodium nitrate (5%) followed by 2.5 ml of distilled water. It was mixed thoroughly and incubated at room temperature for 3 min. To this 0.3 ml of 10% aluminium chloride was added. The test tubes were allowed to stand for 5 minutes and then 2 ml of 1M sodium hydroxide was added. After 60 min, the final volume was made up to 10 ml and the absorbance was measured at 415 nm using UV- visible spectrophotometer. The standard curve was prepared using quercetin and the results are expressed in milligram per gram (mg/g) quercetin equivalent.

#### Estimation of Tannin content

Tannins content in extracts of *Nelumbo nucifera* rhizome was estimated using standard method (Bhardwaj and Modi, 2016) [1]. 1 ml of extract was mixed with 0.5ml of Folin-Ciocalteu's reagent followed by 1ml of saturated sodium carbonate solution and 8ml of distilled water. The reaction mixture was allowed to stand for 30 min at room temperature. The supernatant was obtained by centrifugation and absorbance was recorded at 725 nm using UV-Visible Spectrophotometer.

#### Nutritional analysis

##### Total carbohydrate

The total carbohydrate content in the rhizome was estimated by anthrone method Hodge and Hofreiter, 1962) [5]. The samples were hydrolysed with 2.5 N- HCl for three hours in a boiling water bath and neutralized with solid sodium carbonate. Make up the volume to 100ml and centrifuged. Collected the supernatant. To 1ml aliquot added 4 ml of Anthrone reagent and heated for eight minutes in a boiling water bath. The green to dark green colour developed by the anthrone reagent was measured at 630 nm. The carbohydrate content of the extract was calculated on the basis of the standard graph of glucose and the results were expressed as mg/g.

##### Crude protein determination: (Sadasivam & Manickam, 1997) [15]

A known weight of the sample was transferred to 250 ml Kjeldahl flask for determination of nitrogen by Micro-kjeldahl method. Into the flask, catalyst mixture (potassium sulphate + mercuric oxide) and concentrated H<sub>2</sub>SO<sub>4</sub> were added. The mixture was boiled and digestion was continued until the colour of the digest was colourless. The volume of the digest was made upto a known volume. Similarly, a blank without the sample was run. The reduced nitrogen extracted by steam distillation from a definite volume of the digest was collected in boric acid solution. The nitrogen present in the boric acid solution was estimated by titrating with 0.02 N HCl using mixed indicator (methyl red and methylene blue). The blank distillation and titration were carried out and calculation was done as below.

$$\text{Nitrogen/kg} = \frac{\text{ml (HCL)} - \text{ml blank} \times \text{normality} \times 14.01 \times \text{final volume}}{\text{weight (g)} \times \text{aliquot volume}}$$

$$\% \text{ Crude protein} = \text{Nitrogen \%} \times 6.25$$

#### Determination of crude fats

Fat was determined by the soxhlet extraction method using 200 ml n-hexane as the extracting solvent in soxhlet apparatus (Ranganna, 1986) [14]. A weighed portion of the finely ground sample was transferred to a thimble. The top of the thimble was plugged with a wad of fat free cotton. Then it was placed in the extraction tube of the Soxhlet refluxing apparatus. The extraction tube was then attached to a Soxhlet flask. Approximately 75 ml of anhydrous ether was poured into the flask. The top of the extraction tube was connected to a condenser. Extraction of fat from the sample was done for at least 16 hours on a heating mantel. Ether collected from the Soxhlet flask was evaporated out and crude fat thus obtained was dried at 100<sup>o</sup> C for 1 hr and weighed.

#### Calculation

$$\% \text{ Crude fat} = \frac{\text{Weight of the ether soluble material} \times 100}{\text{weight of the sample}}$$

#### Moisture content

The moisture content was estimated after recording fresh weight and dry weight of rhizomes (kept in hot air oven at 70°C). Moisture content was expressed in fresh weight basis in percentage and mean of replications was calculated from the following formula,

$$\text{Moisture content} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Fresh weight}} \times 100$$

#### Estimation of crude fiber: (Sadasivam & Manickam, 1997) [15]

A weighed portion of the finely ground sample was treated with ether for removal of fat. The residue was boiled with dilute H<sub>2</sub>SO<sub>4</sub> (0.255 N) and filtered through muslin cloth. The residue was washed with boiling water until washings are no longer acidic and boiled again with 0.313N NaOH and filtered through muslin cloth again and washed with boiling 1.25% H<sub>2</sub>SO<sub>4</sub>, water and alcohol successively. The residue was transferred to ashing dish (W1). The residue was dried at 130°C for 2 hrs and weighed with ashing dish (W2). After ignition for 30 min at 600 °C, cool in a desiccator and weight of the ash with ashing dish was measured (W3).

$$\text{Crude fibre \%} = \frac{\text{Loss in weight on ignition (W2-W1)} - (\text{W3-W1}) \times 100}{\text{Weight of the sample taken}}$$

#### Energy value

Energy value was calculated for each sample following Indrayan *et al.*, (2005) [6]

$$\text{Energy value} = 4\% \text{ protein} + 9\% \text{ fats} + 4\% \text{ carbohydrates}$$

#### Results and Discussion

The phytochemical analysis was carried out for six different extracts (ethanol, ethylacetate, hexane, chloroform, acetone and aqueous) of lotus rhizome (Table 1). Majority of phytochemicals were present in ethanol extract recording the presence of both primary (carbohydrate, protein, fat) and secondary metabolites (phenols, tannins, flavanoids, alkaloids, steroids, terpenoids, cardiac glycosides, coumarin, quinone) followed by ethylacetate and chloroform extract. Alkaloids and coumarin were not detected in ethyl acetate and

chloroform extracts. The extract of *Nelumbo nucifera* rhizomes in other solvents showed lesser number of components. Similar results were reported by Ullah *et al.*, (2018) [24] where preliminary phytochemical screening of *N. nucifera* rhizome positive results for the presence of phytochemical constituents such as; flavonoids, tannins, alkaloids, phenols, coumarin, cardiac glycosides, phlobatanins and saponins.

### Nutrient analysis

The nutrient content of lotus rhizome was analyzed. The current study shows that the moisture content was higher (62.70±0.95) than those reported by Ullah *et al.* (2018) [24] (55.3±0.80 g/ 100g) and (6.86±0.17g/100g) reported by Shukla *et al.*, 2015 [20]. According to the present study the carbohydrate content of lotus rhizome was 38.40±0.98. The crude protein content of lotus rhizome was 11.22±1.05 which was higher when compared to that (9.9±1.45) reported by Ullah *et al.* (2018) [24], Ullah *et al.* (2018) [24] reported high fat content of 3.1±1.67. The fiber content of lotus rhizome was 5.62±0.63. The carbohydrates, crude protein and crude fibre estimates of the *nelumbo nucifera* rhizomes of the present study were higher than the earlier reports (Ullah *et al.*, 2018 [24], Gnana *et al.*, 2014) [4]. The geographical area, cultivation methods, soil type, fertilizers, climatic conditions, temperature, humidity and rainfall in village Majokey, Charsadda district, Pakistan is different from Tamil Nadu, India. Therefore the nutritive value of *Nelumbo nucifera* was collected from natural lotus pond at Nagercoil area in Tamil Nadu shows significant variation for the above nutrient content.

### Total phenolic content

Phenols are the largest group of plant secondary metabolites and they are related to defense activity in the plant. They also having antibacterial and antifungal activities and they are usually responsible for vital roles in biological system. The other role of phenolic compounds have antiaging, anti-inflammatory, antioxidant and antiproliferative properties. The total phenolic content of lotus rhizome was higher in ethanolic extract (18.93±0.91) followed by ethylacetate (17.04±0.22) and the least phenolic content was observed in hexane extract (14.78±0.89). Ullah *et al.* (2018) [24] reported phenol content in methanolic extract to be higher compared to other solvent extracts. However the phenolic content in ethanol extract of *Nelumbo nucifera* rhizome was higher than that reported by Ullah *et al.* (2018) [24].

### Total flavanoid content

Flavanoids are one of the most important secondary metabolites and they are having various biological activities including anti-cancer, anti-viral, anti-inflammatory properties. The flavanoids might be one of the safest non-immunogenic drugs because they are small organic compounds which are normally absorbed by the human body (Lee *et al.*, 2007) [10]. The total flavanoid content was maximum in the ethanol extract (7.12±0.45) of lotus rhizome followed by ethyl acetate (6.89±0.86). Ullah *et al.* (2018) [24] reported higher flavanoid content in methanolic extract of lotus rhizome compared to ethanolic extract. Among all extracts, hexane extracts recorded minimum flavanoid content.

### Tannin content

Tannins are water soluble polyphenols and are important secondary metabolites. Tannins have antimicrobial activity,

anticarcinogenic, antimutagenic potentials that might be related to antioxidative property, which is important in protecting cellular oxidative damage including lipid peroxidation, accelerate blood clotting, reduce blood pressure, decrease the serum lipid level (Chung *et al.*, 1998) [2]. The tannin content of *Nelumbo nucifera* rhizome was higher in the ethanolic extract (3.81±0.16) followed by ethyl acetate (2.94±0.18). The lowest tannin content was observed in the hexane extract (2.41±0.27).

**Table 1:** Qualitative phytochemical analysis in lotus rhizome (*Nelumbo nucifera*)

Chemical constituents	Rhizome extract					
	Eth.	EA	HE	Chl.	Ace.	Aqu.
CHO	+	+	+	+	+	+
Protein	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Flavanoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Alkaloids	+	-	-	-	-	+
Steroids	+	+	-	+	-	-
Terpenoids	+	+	-	+	+	-
Cardiac glycosides	+	+	-	+	+	+
Coumarin	+	-	-	-	-	-
Quinones	+	+	-	+	-	-

Present (+), absent (-), Eth. -Ethanol, EA -Ethyl acetate, HE - Hexane, Chl. - Chloroform, Ace. - Acetone and Aqu. - Aqueous extract

**Table 2:** Nutritional analysis of *Nelumbo nucifera* (rhizome)

Contents g/100g	Lotus rhizome ± SD
Moisture content	62.70±0.95
Carbohydrate	38.40 ±0.90
Crude protein	11.22±1.05
Crude fat	0.59±0.33
Crude fiber	5.62±0.63
Calorific value (kcal)	203.79

**Table 3:** Total phenol, total flavonoid and tannin content of the different extract of *Nelumbo nucifera* (rhizome)

Solvents	Total phenol (mg/g Gallic acid equivalent)	Total flavonoid (mg/g Quercetin equivalent)	Total tannin mg/g Gallic acid equivalent
Ethanol	18.93±0.91	7.12±0.45	3.81±0.16
Ethyl acetate	17.04±0.22	6.89±0.86	2.94±0.18
Hexane	14.78±0.89	4.25±0.37	2.41±0.27
Chloroform	16.29±0.57	5.82±0.25	2.74±0.13
Acetone	15.93±1.11	5.70±0.53	2.71±0.16
Aqueous	15.64±1.12	5.49±0.45	2.59±0.16



Lotus rhizome (dried)





Lotus rhizome (powdered)

### Conclusion

In the present study, the phytochemical screening, quantification and nutritional analysis carried on lotus rhizome revealed that it has important primary and secondary metabolites with properties in traditional medicine, phytochemical composition and pharmacological activities. The results obtained in the present study indicates that the rhizomes of *N. nucifera* have the potential to act as a source of useful medicinal and nutritive value because of the presence of various phytochemical components such as carbohydrate, protein, tannin, phenols, flavonoids and alkaloids. The quantitative screening indicated that the ethanolic extract of *N. nucifera* rhizome showed the highest contents of tannins, flavonoids and phenols. The lotus rhizome is a good source of carbohydrate and proteins and have good nutritional properties have the benefits of being used in flavouring agents, food production and bakery products. In other studies conducted by our lab on the compounds in *Nelumbo nucifera* we have identified compounds that would be used in flavaouring, food production and bakery products. The present study validates the presence of valuable secondary metabolites in *Nelumbo nucifera* rhizome, which could be an added value for the lotus flowers.

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