Phytochemical screening and nutritional analysis of *Nelumbo nucifera* (Pink lotus) flower petals and seeds

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**Abstract**

*Nelumbo nucifera* flower petals and seed extracts were evaluated for phytochemical constituents (flower petals and seeds) such as primary (carbohydrate, protein, fat) and secondary metabolites, flavonoids, phenols, tannins, steroids and glycosides. In addition, the nutritive value of the seeds was evaluated. The healing properties of the lotus plant is due to the presence of active principles such as phenolics, tannins, alkaloids, and flavanoids that are constituents of many pharmacologically active compounds. The Flower petals and seeds were extracted in solvents used for the extraction viz., ethanol, ethylacetate, hexane, chloroform, Acetone and aqueous for phytochemical screening. Lotus flower petals and seeds extract recorded the presence of primary metabolites viz., carbohydrates, proteins and lipids and secondary metabolites such as phenols, flavanoids, tannins, alkaloids, sterols, terpenoids, cardiac glycosides, coumarin (Petals) and quinone (Petals). The quantification of primary metabolites of the extract indicates its nutritive value while the secondary metabolites are responsible for the medicinal properties of the lotus seeds and flower petals. Quantification of phytochemicals revealed that ethanolic extract of flower and seed has the maximum phenols, flavanoids and tannin content compared to other solvents. This study indicates that lotus seeds can serve as an alternate protein source as it possess potential nutritional and medicinal properties.

**Keywords:** Lotus, flowers, seeds, phytochemicals, nutritional value

**Introduction**

Phytochemicals are bioactive and non-nutrient plant compounds protecting against oxidative damage which prevents cardiovascular disease, multiple cancers and also inhibiting cancer cell proliferation as reported by Gullett et al., 2010 [19], Heber, 2004 [20], Johnson, 2007 [21], Liu, 2003 [22], 2004 [23] and Surh, 2003 [24]. Phytochemicals are broadly classified into primary and secondary metabolites. The primary metabolites include carbohydrates, protein, lipids etc., and secondary metabolites consisting of phenols, flavanoids, tannins, alkaloids, sterols, terpenoids, saponins etc. Most of the phytochemicals rich in therapeutic activities like flavanoids having anti-inflammatory (Lee et al., 2003) [25], alkaloids have the activities like antimalarial (Dua et al., 2013) [26], antispasmodic, cytotoxicity and pharmacological effects (Thite et al., 2013) [27], antimicrobial (Benbott et al., 2012) [28] and anti-inflammatory (Augusto et al., 2011) [29]. Tannins having antibacterial (Hisanori et al., 2001) [30], antitumor and antiviral activities (Kumari and Jain, 2012) [31], Steroids have the activities of Cardiotonic effect, antibacterial and insecticidal properties (Alexei et al., 2009) [32]. Cardiac glycosides having activities against congestive heart failure and cardiac arrhythmia (Valadimir and Ludmila, 2001) [33]. Chopra et al., 1956 [14] reported that the flowers and leaves of lotus plants are used to treat many bleeding disorders, diarrhea, cholera, fever, hepatoapthy and hyperdipsia and seeds are also used to treat in tissue inflammation, cancer, skin diseases, leprosy, poison antidote. Arjun et al., 2012 [34], studied that seed powder mixed with honey is useful in treating cough, while roots with ghee (melted fresh butter), milk and promote strength, virility and intellect. Mukherjee et al., 1995 [16], Mukherjee, 2002 [15], reported that lotus seeds possess rich antimicrobial properties. Chen et al., 2007 [17], observed that lotus seed embryo are used in traditional Chinese drug called ‘Lian Zi Xin’, which primarily helps to overcome nervous disorders, insomnia, high fevers (with restlessness) and cardiovascular diseases (e.g. hypertension, arrhythmia). This study was aimed to analyse the phytochemical constituents and nutritional value of *N. nucifera* flowers and seeds.
Materials and Methods
Collection of planting material and preparation of the extract

The flowers were collected from ponds at Manickaputheri near Nagercoil area in Kanyakumari district. Fresh flower petals alone were removed, washed with water to eliminate mud and other dust particles. The seeds are also collected from seed pod. The seeds are cut into two halves. Then the petals and seeds were 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 aqueous solvents separately. The extracts were then filtered by Whatman filter paper No.1. Dried solvent extract was kept at 4°C for further analysis. Fresh seeds were 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 α-naphthol solution and 2 ml of Conc. H₂SO₄ 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-kiliani 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 saponins

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) [26] with certain modifications. 200 µl (1 mg ml⁻¹) 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 about5 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) [27]. 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) [25]. 1 ml of extract was mixed with 0.5ml of Folin-
Ciocalteau’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 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) [30]. 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) [28].
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 H₂SO₄ 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 up to 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.

\[
\text{Nitrogen/kg = } \frac{\text{ml (HCl) } - \text{mlblank}) \times \text{normality x 14.01 \times final volume}}{\text{weight (g) x aliquot volume}}
\]

\[
\% \text{ Crude protein } = \frac{\text{Nitrogen \% \times 6.25}}{}
\]

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) [31]. 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 100º 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 70ºC). Moisture content was expressed in fresh weight basis in percentage and mean of replications was calculated from the following formula,

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

Estimation of crude fiber (Sadasivam & Manickam, 1997) [28].
A weighed portion of the finely ground sample was treated with ether for removal of fat. The residue was boiled with dilute H₂SO₄ (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% H₂SO₄, water and alcohol successively. The residue was transferred toashing dish (W1). The residue was dried at 130ºC for 2 hrs and weighed with ashing dish (W2). After ignition for 30 min at 600ºC, cool in a desiccator and weight of the ash with ashing dish was measured (W3).

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

Energy value
Energy value was calculated for each sample following Indrayan et al., (2005) [12].

\[
\text{Energy value } = 4x\%\text{protein } + 9x\%\text{fats } + 4x\%\text{carbohydrates}
\]

Results and Discussion
The phytochemical analysis was carried out for six different extracts (Ethanol, ethylacetate, hexane, chloroform, acetone and aqueous) of lotus flower petals and seeds and shown in table 1. The majority of phytochemicals present in ethanol extract of flower petals. the extract contains both primary (Carbohydrate, protein, fat) and secondary metabolites (Phenols, tannins, flavanoids, alkaloids, saponin, steroids, terpenoids, cardiac glycosides, coumarin, quinone) followed by hexane (phenols, tannins, flavanoids, alkaloids, saponin, steroids, terpenoids, cardiac glycosides, coumarin, quinone except saponin) and aqueous extract found to contain phenols, tannins, flavanoids, alkaloids, steroids, terpenoids, cardiac glycosides, coumarin, except alkaloids, saponin and quinone. The extract of other solvents viz., ethyl acetate (Carbohydrate, protein, phenols, flavanoids, tannins, cardiac glycosides quinone and coumarin), acetone (Carbohydrate, protein, phenols, flavanoids, alkaloids, cardiac glycosides, coumarin and terpenoids) and chloroform (carbohydrate, protein, phenols, tannins, flavanoids, alkaloids and coumarin) showed the presence of lesser number of components. Similar results were reported by Saraswathi and Gricilda Shoba 2015 [13], that the Preliminary phytochemical screening of N. nucifera petals using hydroethanolic extract show positive results for the presence of phytochemical constituents such as; carbohydrates, reducing sugars, proteins, steroids, flavonoids, tannins, alkaloids, phenols, glycosides and saponins. Baghel, and Dubey 2016 [10], states that lotus flowers, floral parts or their extracts have also been used against many diseases like consolidation of kidney function hypertension, cancer, weakness, body heat imbalance, syphilis, stopping bleeding
and to eliminate the stagnated blood. Venkatesh and Dorai 2011 [10], Durairaj and Dorai 2014 [11], reported that flowers, with their parts or extracts have shown to possess antimicrobial activities vasodilating effects, antihypertensive, antiarrhythmic abilities, aphrodisiac activity antioxidant and free radical scavenging capacity

The majority of phytochemicals were present in ethanol extract of lotus seed showed the presence of both primary (Carbohydrate, protein) and secondary metabolites (Phenols, tannins, flavanoids, alkaloids, saponin, steroids, terpenoids and cardiac glycosides) followed by hexane (carbohydrate, protein, phenols, tannins, flavanoids, steroids, terpenoids and cardiac glycosides), chloroform (Carbohydrate, protein, phenols, tannins, alkaloids, steroids, terpenoids and cardiac glycosides), acetone extract which exhibited carbohydrate, protein, phenols, tannins, flavanoids, alkaloids, steroids and cardiac glycosides. The extract of other solvent ethyl acetate (carbohydrate, protein, phenols, flavanoids, alkaloids and cardiac glycosides) showed lesser number of components.

Nutrient analysis
The nutrient content of lotus flower petals and seed were analyzed. The current study shows that the moisture content was higher (9.10%) than those reported by Indrayan et al., (2005) [12]. According to the present study the carbohydrate content of lotus rhizome was 33.13%. The crude protein content of lotus rhizome was 13.32% which was higher when compared to that (10.60%) reported by Indrayan et al., 2005 [12]. The crude fat content of lotus seed was 2.82% which was higher when compared to that (1.93%) reported by Indrayan et al., 2005 [12]. The fiber content of lotus rhizome was 20.27%. The carbohydrates, crude protein, crude fat and crude fibre estimates of the Nelumbo nucifera seeds of the present study were higher than the earlier reports (Indrayan et al., 2005) [12]. This is due to difference in the geographical area, cultivation methods, soil type, fertilizers, climatic conditions, temperature, humidity and rainfall in Tamil Nadu, India. Therefore the nutritive value of Nelumbo nucifera seeds were 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. Phenols are also having antibacterial and antifungal activities and are usually responsible for vital roles in biological system. The other role of phenolic compounds include antiaging, anti-inflammatory, antioxidant and antiproliferative agents. The total phenolic content of lotus flower petals in the present study was higher in ethanolic extract (20.9±0.91) followed by ethylacetate (18.93±0.91) and the lowest phenolic content was observed in hexane extract (15.78±0.89). Saraswathi and Shoba 2015 [13], found that the hydroethanolic extract was 18.56±2.33 which was found to be lower than ethanolic extract but higher when compared to other extracts.

Table 1: Qualitative phytochemical analysis in lotus flower petals and seeds (Nelumbo nucifera)

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Flower extract</th>
<th>Seed extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coumarin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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

Table 2: Nutritional analysis in seeds of Nelumbo nucifera

<table>
<thead>
<tr>
<th>Contents g/100g</th>
<th>Lotus seed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>9.10</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>33.13</td>
</tr>
<tr>
<td>Crude protein</td>
<td>13.32</td>
</tr>
<tr>
<td>Crude fat</td>
<td>2.82</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>20.27</td>
</tr>
<tr>
<td>Calorific value (kcal)</td>
<td>211.18</td>
</tr>
</tbody>
</table>
In the present study, the tannin content was higher in the ethanolic extract (23.85±0.46) followed by ethyl acetate (23.14±1.76) of T. marcelo, reported that tannin content of lotus flower petals showed the highest content of tannins, flavonoids and phenols. In future, lotus seeds can serve as an alternate protein source in addition to its potential nutritional and medicinal properties.

**References**


**Table 3:** Total phenol, total flavonoid and tannin content of different extract of *Nelumbo nucifera* (pink flower petals)

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Total phenol (mg/g Gallic acid equivalent)</th>
<th>Total flavonoid (mg/g Quercetin equivalent)</th>
<th>Total tannin mg/g Gallic acid equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>20.9±0.91</td>
<td>7.16±0.24</td>
<td>23.85±0.46</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>18.93±0.91</td>
<td>6.56±0.45</td>
<td>18.09±0.35</td>
</tr>
<tr>
<td>Hexane</td>
<td>15.78±0.89</td>
<td>5.25±0.37</td>
<td>14.41±0.27</td>
</tr>
<tr>
<td>Chloroform</td>
<td>17.29±0.57</td>
<td>6.26±0.38</td>
<td>16.74±0.13</td>
</tr>
<tr>
<td>Acetone</td>
<td>16.93±1.11</td>
<td>5.70±0.53</td>
<td>15.71±0.12</td>
</tr>
<tr>
<td>Aqueous</td>
<td>16.64±1.12</td>
<td>5.49±0.45</td>
<td>15.59±0.15</td>
</tr>
</tbody>
</table>

(18.09±0.35). The lowest tannin content was observed in the hexane extract (14.41±0.27).

**Conclusion**

In the present study, the phytochemical screening, quantification and nutritional analysis on lotus flower petals and seeds revealed that it has important properties in traditional medicine, phytochemical composition and pharmacological activities. The results obtained in the present study indicated that the lotus flowers and seeds of *N. nucifera* have the potential to act as a source of useful medicinal and nutritive values because of the presence of various phytochemical components such as carbohydrate, protein, tannin, phenols, flavonoids and alkaloids. The ethanolic extract of *N. nucifera* flower petals showed the highest content of tannins, flavonoids and phenols. In future, lotus seeds can serve as an alternate protein source in addition to its potential nutritional and medicinal properties.


