PHYSICO-CHEMICAL AND PHYTOCHEMICAL STUDY OF HYDROETHANOLIC PETAL EXTRACT OF PINK NELUMBO NUCIFERA GAERTN

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ABSTRACT

Nature has given rise to medicinal agents for thousands of years. Various medicinal plants have been used for years in daily life to treat the disease all over the world. The therapeutic use of herbs is as old as human civilization and has evolved along with it. Medicinal plant drug discovery continues to provide new and important leads against various effects like cancer, malaria, cardiovascular diseases and neurological disorders. Interest in herbal drugs and natural medicine is undergoing a renaissance now. The curative properties of plants are due to the presence of active principles such as alkaloids, phenolics, tannins and flavonoids that constitute of many pharmacologically active compounds. The aim of the present study was to know about the physico-chemical evaluation of the extract, phytochemical screening and quantification of primary and secondary metabolites in the Nelumbo nucifera petal extract. The results obtained in the present study reveal the following: physico-chemical parameters show a good result in Nelumbo nucifera petal extract and can be used for the formulation of the drug. Phytochemical screening and quantification of primary and secondary metabolites in the Nelumbo nucifera petal extract shows the presence of primary metabolites viz., carbohydrates, proteins and lipids and the secondary metabolites such as tannins, phenols flavonoids and alkaloids on phytochemical screening. Quantification of primary metabolites of the extract shows its nutritive value and the secondary metabolites were responsible for its medicinal properties of the petals of the Nelumbo nucifera Gaertn that was used by the traditional herbalists. Finally, the present finding of the study concludes, that the hydro ethanolic Nelumbo nucifera petal extract possess abundant phytochemicals, which act as a source of natural antioxidants having a great importance in preventing various diseases and further study was needed to find out the active principle present in the Nelumbo nucifera petals.

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INTRODUCTION

The majority of people rely on this planet still on traditional Materia medica (medicinal plants and other materials) for everyday healthcare needs [1]. Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine and it was invoked as food supplements, nutraceuticals and in pharmaceutical industries and chemical entities for synthetic drugs [2]. The practice of traditional medicine is prevalent in various countries such as China, India, Japan, Pakistan, Sri Lanka and Thailand [3]. Herbal medicine was based on the assumption that plants contain natural substances that can promote health and alleviate illness [4, 5]. There is a popular belief that these medicines are safer, easily available and with fewer side effects. Plant products have been all part of phytomedicines since time immemorial derived from barks, leaves, flowers, roots, fruits, seeds [6]. Phytochemicals are the natural bioactive compounds found in plants, which are solely responsible for their medicinal activity. Knowledge on the Phytochemicals of plants is desirable because such information will be value for synthesis of complex chemical substances [7-9]. The Phytochemicals are grouped into two main categories [10] namely primary constituents which includes amino acids, common sugars, proteins and chlorophyll etc., and secondary constituents consisting of alkaloids, essential oils, tannins, terpenoids, saponins, phenolic compounds etc. [11,12]. Majority of phytochemicals have been known to bear valuable therapeutic activities such as insecticides [13], antibacterial, antifungal [14], anti-constipative [15], spasmolytic, anti-plasmodial and antioxidant [16] 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.

_Nelumbo nucifera_ Gaertn. A perennial aquatic plant, native to Tropical Asia and Queensland, Australia [17, 18]. It belongs to the family Nelumbonaceae with numerous common names (e.g. Indian lotus, Chinese water lily and sacred lotus) and synonyms _Nelumbium nucifero, N. speciosa, N. speciosum And Nymphaea nucifera_ [19]. The scientific classification of _N. nucifera_ was given in Table-1 and the pictorial view of the whole plant with flowers and the dried petals of the flower were given in Figure-1. It was equally the national flower of India and Vietnam. _N. nucifera_ was invoked as a medicinal herb in China, India and as popular traditional folk herbs in Thailand [20].In Siddha System of Medicine, _N. nucifera_ was reported to cure cardiac diseases, liver disorders and dysentery. Folks of Maharashtra used this _N. nucifera_ plant to cure kidney disorders [21]. So far, the studies have reported generally in the _N. nucifera_ flowers possessing Hepatoprotective, Antiplatelet, Antimicrobial, Hypoglycemic and Hypolipidemic, Whitening & anti-wrinkle, Antioxidant activities [22] and the Floral parts of _N. nucifera_ have also been used against many diseases like hypertension, cancer, weakness, and body heat imbalance, male sexual disorders, syphilis, stopping bleeding and to eliminate the stagnated blood [23].

Considering the above pharmacological benefits that were reported in the _N. nucifera_ flower, as well as in the floral parts, the present study was aimed to analyze physicochemical and phytochemical screening as well as quantification of respective phytochemicals that were present more particularly in the _N. nucifera_ petal extract, which was not reported yet among the floral parts of _N. nucifera_.

### Table 1: Scientific Classification.

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order</td>
<td>Proteales</td>
</tr>
<tr>
<td>Family</td>
<td>Nelumbonaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Nelumbo</td>
</tr>
<tr>
<td>Species</td>
<td>nucifera</td>
</tr>
<tr>
<td>Binomial name</td>
<td>Nelumbo nucifera Gaertn.</td>
</tr>
</tbody>
</table>

**Figure 1:** (A) _Nelumbo nucifera_ Gaertn. (Plant with flower). (B) Dried petals of _N. nucifera_.

**MATERIALS AND METHODS**

**Collection and Authentication of plant**

_Nelumbo nucifera_ Gaertn. Flowers were purchased from the Koyambedu flower bazaar of Chennai in Tamil Nadu, India. Prof. P. Jayaraman, Plant Anatomy Research Centre (PARC), and Chennai did the taxonomic identification of the flower by comparing with the voucher specimen (Voucher no.1236).
Preparation of plant powder and extract

The petals alone removed from the flower of the collected *N. nucifera* Gaertn. Flower and washed thoroughly with water to eliminate the earthy matters, freed from the debris, shade dried under room temperature for a few weeks, and coarsely powdered using a food processor.

Extraction was performed by hot continuous percolation method using soxhlet’s apparatus. About 500g of coarsely powdered *Nelumbo nucifera* petals were extracted in 70% ethanol by the continuous hot extraction method at 50 °C was decanted from the Soxhlet apparatus and the filtrate was evaporated for the total elimination of alcohol using a Rota flash Vacuum evaporator. The concentrated liquid extract obtained was then transferred to a China dish and kept in a water bath for 50 °C for dryness. The residual extract was transferred to an airtight container free from contamination until it was used.

Chemical reagents

The standards such as Quercetin and Gallic acid were procured from HiMedia Laboratories, Mumbai and Piperine from sigma Aldrich, USA. The other chemicals like aluminium chloride, ferric chloride, phosphate buffer, sodium carbonate, sodium acetate, trichloro acetic acid and 2-thiobarbituric acid were procured from Sisco research lab, Mumbai and Dragendorff’s reagent from Loba Chemie, Mumbai. The chemicals, which used for this study, are in analytical grade.

Physico-Chemical Analysis

According to Indian Pharmacopoeia, the evaluation of Physico-chemical parameters such as, ash values viz., total ash, acid insoluble ash and water-soluble ash, sulphated ash, and loss on drying were determined [24] using petal powder. Evaluation of extractive values of the extract such as alcohol soluble extractive value, and water-soluble extractive values were determined by following the method given in Practical Pharmacognosy by Kokate CK [25].

Preliminary phytochemical screening

The preliminary phytochemical screening was used to determine the presence/absence of phytochemicals in the petal extract. The phytochemical screening of *N. nucifera* petal extract (NNPE) was carried out according to the method given by Harbone [26].

Quantification of primary metabolites

Primary metabolites were directly involved in growth and development of the plants. The quantification of primary metabolites viz. Carbohydrate, protein and lipid were determined as given below.

Total carbohydrate content

According to the method described by Hedge and Hofreider [27], Total soluble Carbohydrate content was determined. The procedure was described briefly as follows; the standard was taken in the range of 0.2-1.0 ml in the test tubes and the NNPE was taken in 1ml duplicates. With all the test tubes, the volume was made to 1 ml by adding distilled water and 1 ml of distilled water alone serve as blank. To this 4 ml of Anthrone reagent was added and were heated for eight minutes in a water bath and cooled. The green colour developed was read at 630nm using UV/visible spectrophotometer (Perkin Elmer, Lambda 25. USA). 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.

Total protein content

The total protein content was estimated depending on Lowry et al [28]. To 0.1 ml of the NNPE in test tubes, the volume was made to 1ml with distilled water and 1 ml of distilled water alone serves as blank. 5 ml of alkaline copper sulphate reagent was added, mixed well and allowed to stand for 10 min and then 0.5 ml of Folins-Ciocalteau’s reagent was added and mixed well. The mixture was allowed to stand under dark for 30 min. The blue colour developed was read at 660nm using UV/visible spectrophotometer (Perkin Elmer, Lambda 25. USA). The protein content of the extract was calculated from the standard graph of Bovine Serum Albumin and the results were expressed as mg/g.

Total lipid content

The total lipid content was estimated according to the method Zak and his co-authors [29]. To 0.1 ml of the NNPE was made up to 5 ml with working ferric chloride acetic acid reagent and the tubes were kept at room temperature for 10 min, to this 3ml of Con Sulphuric acid was added and the tubes were kept in ice cold condition for 20 minutes and the developed pink color was read at 540nm using UV/visible spectrophotometer (Perkin Elmer, Lambda 25. USA). The lipid content of the extract was calculated on the basis of the standard graph of cholesterol and the results were expressed as mg/g.

Quantification of secondary metabolites

Drug discovery from medicinal plants has played a significant role in the treatment of various diseases and indeed, most new clinical applications of plants secondary metabolites and their derivatives over the last century. Secondary metabolites are important mediators of ecological interactions between plants and their environment.
Total phenol determination

Total phenolic content was identified using the method given by McDonald et al [30]. 1 ml of NNPE was added with 5 ml Folin's phenol reagent followed by 4 ml of sodium carbonate and the mixture was permitted to stand for 15 min at room temperature. The developed blue colour was read at 765 nm using UV/visible spectrophotometer (Perkin Elmer, Lambda 25, USA). The total phenol content was calculated on the basis of the standard graph of Gallic acid and the results were expressed as Gallic acid equivalent (mg/g).

Total flavonoid determination

The total flavonoid content was determined from Chang et al [31] methods. 0.5 ml of prepared extract / standard was separately mixed with 4.5 ml of methanol and to this 0.1 ml of 10% aluminum chloride and 0.1 ml of 1M sodium acetate were added and the mixture was allowed to stand for 15 min and the absorbance of the reaction mixture was read at 415 nm using UV/visible spectrophotometer (Perkin Elmer, Lambda 25, USA). The content of flavonoids was calculated using standard graph of quercetin and the results were reported in quercetin equivalent (mg/g).

Total tannin determination

According to Schanderl [32], the total tannin content of the extract was identified. The protocol was briefly described as below. To the 1 ml of the extract followed by adding 0.5 ml of Folin's phenol reagent (1:2) followed by 5 ml of 35% sodium carbonate was added & allowed to stand at room temperature for 5 min. The developed blue color was read at 640 nm using UV/visible spectrophotometer (Perkin Elmer, Lambda 25, USA). The content of tannins was calculated using standard graph of Gallic acid and the results were reported in Gallic acid equivalent (mg/g).

Alkaloid determination

The alkaloids estimation was performed by spectrophotometric method of Dragendorf’s reagent as Sreevidya and Mehrotra [33] described it. Briefly, 10 ml NNPE extract was centrifuged over 10 min (3000 rpm) to remove residual suspended particles and then 5 ml of the supernatant were mixed with 1 ml of HCl 0.1 N. Then, 2.5 ml of Dragendorff’s reagent was added to the previous mixture for precipitation and the precipitate was centrifuged over 5 min (3000 rpm). This precipitate was further washed with 2.5 ml of ethanol. The filtrate was discarded and the residue was then treated with 2.5 ml of disodium sulfide solution. The brownish black precipitate formed was then centrifuged (5 min, 3000 rpm). This residue was dissolved in 2 ml of concentrated nitric acid, with warming if necessary; this solution was diluted to 10 ml in a standard flask with distilled water and 1 ml was then pipetted out and mixed with 5 ml of thio urea solution. The absorbance of this solution was measured at 435 nm against a blank containing 1 ml of concentrated nitric acid and 2.5 ml of thiourea solution. The content of alkaloids was calculated using standard graph of piperine and the results are expressed as piperine equivalent (mg/g).

RESULT

Physico-chemical analysis

On using the NNPE powder, the physiochemical parameters were done according to procedures given in materials and method section. The results were expressed in mg/g and the data were given in Table-2.

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Parameters</th>
<th>Nelumbo nucifera Gaertn. ( % w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total ash</td>
<td>24.31</td>
</tr>
<tr>
<td>2.</td>
<td>Water soluble ash</td>
<td>5.94</td>
</tr>
<tr>
<td>3.</td>
<td>Acid insoluble ash</td>
<td>15.9</td>
</tr>
<tr>
<td>4.</td>
<td>Sulphated ash</td>
<td>31.31</td>
</tr>
<tr>
<td>5.</td>
<td>Alcohol soluble extractive</td>
<td>8.15</td>
</tr>
<tr>
<td>6.</td>
<td>Water soluble extractive</td>
<td>11.21</td>
</tr>
<tr>
<td>7.</td>
<td>Loss on drying</td>
<td>05.13</td>
</tr>
</tbody>
</table>

Percentage yield

The percentage yield for hydroethanolic NNPE extract was found to be 7.6% w/w.

Phytochemical analysis

Preliminary phytochemical screening of hydroethanolic NNPE revealed the presence of various components such as carbohydrates, proteins, phenols, flavones, tannins, saponins and alkaloids among which phenols, tannins and flavones were the most prominent ones and the results are summarized in Table 3.
Table-3: Phytochemical analysis of *Nelumbo nucifera* Gaertn Petals.

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Phytochemicals</th>
<th>Presence/Absence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carbohydrates</td>
<td>++</td>
</tr>
<tr>
<td>2.</td>
<td>Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Proteins</td>
<td>++</td>
</tr>
<tr>
<td>5.</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Phenols</td>
<td>+++</td>
</tr>
<tr>
<td>7.</td>
<td>Flavones</td>
<td>+++</td>
</tr>
<tr>
<td>8.</td>
<td>Tannins</td>
<td>+++</td>
</tr>
<tr>
<td>9.</td>
<td>Saponins</td>
<td>++</td>
</tr>
<tr>
<td>10.</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>Anthroquinones</td>
<td>-</td>
</tr>
<tr>
<td>12.</td>
<td>Quinones</td>
<td>-</td>
</tr>
</tbody>
</table>

*: Presence (+ mild, ++ moderate, +++ high), -: Absence.

Quantification of Primary metabolites

Quantitative estimation of Primary metabolites were analysed in NNPE and the results were expressed in mg/g. the data were given Table-4.

Table-4: Quantitative estimation of Primary metabolites of *Nelumbo nucifera* Petals.

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Primary metabolites</th>
<th>Quantity present in the NNPE (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carbohydrates</td>
<td>15.66</td>
</tr>
<tr>
<td>2.</td>
<td>Total Proteins</td>
<td>4.12</td>
</tr>
<tr>
<td>3.</td>
<td>Total Lipids</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Quantification of Secondary metabolites

Quantitative estimation of secondary metabolites were done depending upon the presence of phytochemicals during the phytochemical screening of NNPE, the experiment was repeated in triplicate, the results were expressed in mg/g, and the data were given Table-5.

Table-5: Quantitative estimation of secondary metabolites of *Nelumbo nucifera* Petals.

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Secondary metabolites</th>
<th>Quantity present in the NNPE (% w/w)</th>
<th>Equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total Phenols</td>
<td>18.56±2.33 GE/g</td>
<td>Gallic acid</td>
</tr>
<tr>
<td>2.</td>
<td>Total Flavanoids</td>
<td>6.77±0.05 QE/g</td>
<td>Quercetin</td>
</tr>
<tr>
<td>3.</td>
<td>Total Tannins</td>
<td>23.14±1.76 GE/g</td>
<td>Gallic acid</td>
</tr>
<tr>
<td>4.</td>
<td>Total Alkaloids</td>
<td>4.55±0.04 PIPE/g</td>
<td>Piperine</td>
</tr>
</tbody>
</table>

*Values are expressed as mean±SEM (n=3). GE: Gallic acid equivalent; QE: Quercetin equivalent; PIPE: Piperine equivalent.

DISCUSSION

Physico-chemical parameter can be used as standard to ensure the quality of crude drug [34], which is summarised in Table-2. Ash values, represent the inorganic salts occur in the drug. High ash value of NNPE shows the presence of very high inorganic content. The total ash method is used to measure the total amount of material remaining after ignition. This includes both ‘physiological ash’ that is derived from the plant tissue itself, and ‘Non-physiological ash’, which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Both total ash and acid insoluble ash contents are useful parameters to illustrate the quality as well as the purity of herbal medicine [35]. Water-soluble ash is used to measure the quantity of soluble portion of total ash in water [36, 37].

Extractive values give information about availability of soluble phytoconstituents in particular solvent. The water-soluble extractive values indicated the presence of sugar, acids and inorganic compounds[24,36] and the alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids [24,37]. In the present study suggests that the Alcohol soluble extractive value (8.15w/w) is more as compared to aqueous extractive value (11.21w/w) suggesting alcoholic extract would be more beneficial as compared to aqueous extract for therapeutic aspect. Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. If the water content is high, the crude drugs can be easily deteriorated due to fungus and the moisture content of the crude drugs [36] was found to be 05.13 w/w, which signify that the NNPE powder was properly dried and properly stored.

Low value of moisture content does not promote microbial contamination as the general requirement of moisture content in crude drug is not more than 14% (W/W) [34].
Phytochemical, natural compound occurs in plants such as medicinal plants, vegetables, fruits and flowers that work with nutrients and fibers to act against diseases or more specifically to protect against diseases. The information obtained from the preliminary phytochemical screening reveals the chemical nature of the drug and the preliminary phytochemical screening of hydroethanolic NNPE shows the presence of tannins, phenolics, flavonoids and alkaloids were the prominent phytochemicals present in the extract (Table-3).

Plants are rich sources of high value metabolites like proteins, phenols, sugars, starch and lipids are useful in flavoring, fragrances, insecticides, sweeteners and organic dyes [38]. The primary metabolites present in the NNPE were summarized in the Table-4. Carbohydrates are one such group of carbon compounds that are essential to life. Almost all organisms use carbohydrates as building blocks of cells and actually, exploit their precious supply of potential energy to maintain life [39]. Quantification of carbohydrate, in the hydroethanolic NNPE shows that it serves as a rich source of energy (15.66 w/w). Very moderate amount of protein (4.12 w/w) was quantified in the present extract. Very low lipid content (0.38w/w) was found in the hydroethanolic NNPE.

Secondary metabolites have evolved in nature in response to the needs and challenges of the plant environment. Secondary metabolites analysis is necessary for extraction, purification, separation, crystallization, identification of various phytocompounds. The quantities of phytochemicals present in the hydroethanolic NNPE were given in the order as Tannins > Phenolics > Flavonoids > Alkaloids.

Our present study shows a high concentration of tannin (23.14±1.76 mg/g) comparing to the other phytocompounds present in the NNPE. Tannins were said to be water-soluble polyphenolic compounds. Tannins act as an antimicrobial agent against the growth of fungi and yeasts [40], bacteria and viruses [40 - 42]. The presence of large amount of tannins confirms its astringent property. This compound can also be effective in protecting the kidneys [43].

Hydroethanolic NNPE showed the phenols concentration at 18.56±2.33 mg/g than the other phytoconstituents. Phenolic compounds were ubiquitously distributed in the plant kingdom that exhibit a wide range of pharmacological and medicinal properties, including UV protective agents, defensive compounds against herbivores and pathogens, contributors to plant colors, contributors to the taste of food, drink, and pharmaceuticals [44, 45]. Polyphenols could inhibit the activity of digestive enzymes and/or precipitate nutritional proteins [46]. Phenolic substances and flavonoids were associated with antioxidant activity and play an important role in stabilizing lipid-per oxidation [47,48] by adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [49,50].

In the present study, Flavonoids have been reported to be the third highest concentration (18.56±2.33 mg/g) after phenol, exert wide range of biological activities includes, Anti-inflammatory, antibacterial, antiviral, anti-allergic [51-53], cytotoxic antitumour, treatment of neurodegenerative diseases, vasodilator action [54-56]. In addition, flavonoids were well known for inhibit lipid-per oxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities. They exert these effects as antioxidants, free radical scavengers, chelators of divalent cation [53, 56 and57]. These are also reported inhibiting a variety of enzymes like hydrolases, hyaluronidase, alkaline phosphatase, arylsulphatase, cAMP phosphodiesterase, lipase, α-glycosidase, kinase [58].

In the present study, the levels of alkaloids were found to be (4.55±0.04 mg/g) in the hydroethanolic NNPE. Alkaloids have dramatic physiological activities; therefore, they are commonly used in the development of medicines [26, 59]. Some, alkaloids may be beneficial against HIV infection [60, 61] as well as intestinal infections associated with AIDS [62]. Combinations of alkaloids with Nitrogenous compounds are widely used as therapeutic agents in the management of cancer [63].
CONCLUSION

Thus, in the present study, the important phytochemicals were identified and quantified in the hydroethanolic Petal extract of *Nelumbo nucifera* Gaertn. The results obtained in the present study indicates that the petals of *N. nucifera* have the potential to act as a source of useful drugs because of the presence of various phytochemical components such as carbohydrate, protein, tannin, phenols, flavonoids and alkaloids. The results are very much encouraging but scientific validation is necessary before being implemented into practice.

Authors’ Statements

**Competing Interests**

The authors declare no conflict of interest.

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NNPE</td>
<td><em>Nelumbo nucifera</em> petal extract</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
</tbody>
</table>

**REFERENCE**


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