

A study on antioxidant activity, phenol, and flavonoid content of seedpod of *Nelumbo nucifera* Gaertn.

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ABSTRACT

Objective: The objective of the present study was to evaluate the presence of phenol, flavonoid, tannin, antioxidant activity, and high-performance liquid chromatography (HPLC) analysis of quercetin (active component of *Nelumbo nucifera* [NN]) in ethanolic extract of the seedpod of *Nelumbo nucifera* (NNE). **Materials and Methods:** Preliminary screening of NNE was done for the presence of phenol, flavonoid, tannin, qualitatively, and quantitatively. Antioxidant activities of the NNE were estimated using extracts of aqueous, acetone, ethanol, and chloroform by *in vitro* antioxidant assays such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. **Results:** Total phenolic content in NNE was 93.45 mg gallic acid equivalents, the total flavonoid content was 295.312 mg/g quercetin equivalents in NNE, and tannin content is 508.7 mg/g tannin equivalents in NNE. The NNE was evaluated for antioxidant activities by DPPH and FRAP assays. Among the different solvent, the ethanolic extract recorded the most effective DPPH radical scavenging activity (69.2%) and FRAP value was 21.52 μ m. The results of HPLC analysis revealed the presence of active principle, namely quercetin. **Conclusion:** It can be concluded that NNE can be used as a potent natural antioxidant to overcome oxidative stress-induced diseases.

KEY WORDS: 1,1-Diphenyl-2-picrylhydrazyl, Antioxidant activity, Ferric-reducing antioxidant power, *Nelumbo nucifera*, Phenol and flavonoid

INTRODUCTION

Nelumbo nucifera (NN) Gaertn. (Family: Nelumbonaceae) commonly known as Indian lotus, a perennial aquatic plant been extensively used for nutritional and traditional medicinal purpose by people in many parts of the world.^[1] *Nelumbo* is represented by only two species, NN and *Nelumbo lutea*. NN is widely distributed in Southeast Asia and in India, it occurs from Kashmir in north to Kanyakumari in south.^[2] NN commonly known as American lotus is distributed in North and South America.^[3]

Numerous studies have shown that the whole plant possesses therapeutic potential such as anticancerous, antidiabetic, antipyretic, antimicrobial, antiviral, and antiobesity properties.^[4] Phytochemicals are the natural bioactive compounds found in plants, which forms the

basis for their medicinal activity.^[5-7] The phytochemicals are grouped into two main categories,^[8] namely primary constituents which include amino acids, common sugars, proteins, and chlorophyll and secondary constituents consisting of alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, and phenolic compounds.^[9,10] Phenolic compounds are known for their antioxidant activity as free radical scavengers which have been focused by a number of studies.^[11-13]

Free radicals and other reactive species produced during aerobic metabolism in the body can cause oxidative damage of various cellular components.^[14,15] Oxidative stress is the major factor in inducing many chronic and degenerative diseases such as immunosuppression, atherosclerosis, ischemic heart disease, diabetes mellitus, cancer, neurodegenerative diseases, and aging.^[16,17] The free radicals which cause the oxidative stress can be eliminated by antioxidants and those from natural sources can be effective in preventing free radical formation by scavenging them and help in suppressing such disorders.^[18,19] In recent

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years, the search of natural antioxidants has become the major priority in overcoming oxidative stress-induced diseases such as cancer, atherosclerosis, diabetes, and cardiovascular diseases, as well as aging.^[20]

The phenolic content and antioxidant activity of the seedpod of white lotus have rarely been compared in previous literature. In the present study, phenolic content and antioxidant activity of seedpod of white lotus were estimated and compared to provide valuable therapeutic aid for alleviating the ailments induced by oxidative stress.

MATERIALS AND METHODS

Collection of Seedpod of NN

Seedpod of NN was collected from a pond near Avadi in Chennai, Tamil Nadu, India. The voucher specimen was authenticated and deposited in the herbarium in National Institute of Siddha, Tambaram, Chennai (Authentication No: NISMB1442014).

Preparation of the Plant Extract

Preparation of the extracts was done according to a combination of the methods used by Pizzale *et al.*^[21] and Lu and Foo.^[22] About 15 g of dried fine powder seedpod of NN were extracted with 150 mL ethanol (75%), chloroform, acetone, and aqueous extract for 1 min using an Ultra Turrax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evaporator at 40°C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in airtight container in refrigerator <4°C.

Estimation of Total Phenol Content in NN Seedpod

Total phenolic content in the NNE was determined by the Folin–Ciocalteu colorimetric method.^[23] For analysis, 0.5 ml of dry powdered NNE was added to 0.1 ml of Folin–Ciocalteu reagent (0.5 N) and the contents of the flask were mixed thoroughly. Then, about 2.5 ml of sodium carbonate (Na_2CO_3) was added, the mixture was allowed to stand for 30 min after mixing. The changes were measured at an absorbance of 760 nm in a UV-visible spectrophotometer. The total phenolic contents were expressed as mg gallic acid equivalents (GAE)/g extract.

Estimation of Total Flavonoid Content in NN Seedpod

Total flavonoids content in the NNE was determined by the aluminum chloride colorimetric method.^[24] 0.5 ml of NNE at a concentration of 1 mg/ml was taken and the volume was made up to 3 ml with methanol. Then, adding of 0.1 ml AlCl_3 (10%), 0.1 ml of potassium acetate, and 2.8 ml distilled water sequentially, test

solution was vigorously shaken. The changes were measured at an absorbance recorded at 415 nm after 30 min of incubation and a standard calibration plot was obtained at 415 nm using known concentrations of quercetin. The concentration of flavonoid in the test sample was calculated using the calibration plot and expressed as mg quercetin equivalent/g of sample.

Estimation of Tannins Content in NN Seedpod

Tannins content in NNE was estimated by standard method.^[25] 1 ml of extract was mixed with 0.5 ml of Folin–Ciocalteu's reagent followed by 1 ml of saturated sodium carbonate solution and 8 ml of distilled water. The reaction mixture was allowed to stand for 30 min at room temperature, supernatant was obtained by centrifugation and absorbance was recorded at 725 nm using UV-visible spectrophotometer. Different concentrations of standard tannic acid were prepared and a standard graph was plotted for the absorbance of various tannic acid concentrations.

The tannin content of the sample was expressed as mg tannic acid equivalent (TAE)/gram of the sample.

EVALUATION OF ANTIOXIDANT ACTIVITY

Qualitative Analysis of Antioxidant Activity of NN Seedpod

The antioxidant activity of NNE was determined by the method as described by George *et al.*^[26] 50 μL of NNE was taken in a microtiter plate, 100 μL of 0.1% methanolic 1,1-diphenyl-2-picrylhydrazyl (DPPH) was added over the samples and incubated for 30 min in dark condition. The discoloration of the samples from purple to yellow and pale pink was observed and recorded as strong and weak positive, respectively. A further quantitative analysis of the antioxidant positive samples was done.

Quantitative Analysis of Free Radical Scavenging Activity of NN Seedpod

The antioxidant activities were determined using DPPH (Sigma-Aldrich) as a free radical. 100 μL of NNE was mixed with 2.7 ml of methanol and then 200 μL of 0.1% methanolic DPPH was added. Incubation of the suspension was done for 30 min in a dark condition. Blank sample measured as control which contains same amount of methanol and DPPH solution was prepared.^[27] Following which, at every 5 min interval, the absorption maxima of the solution was measured using a UV double-beam spectra scan (Chemito, India) at 517 nm. Butylated hydroxytoluene (BHT) known synthetic standard of 0.16% was used for comparing the antioxidant activity of the sample. The experiment was carried out in triplicate.

The following formula was used to calculate the free radical scavenging activity of the sample.

$$\% \text{ DPPH radical scavenging} = \frac{[(\text{Absorbance of control} - \text{Absorbance of test sample}) / (\text{Absorbance of control})] \times 100.}$$

Ferric Reducing Antioxidant Power (FRAP) Assay in NN Seedpod

FRAP solution (3.6 ml) was added to distilled water (0.4 ml) and incubated at 37°C for 5 min. Then, this solution was mixed with 80 ml of NN seedpod, incubated at 37°C for 10 min and the reaction mixture was noted for absorbance at 593 nm. For the construction of the calibration curve, five concentrations of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1, 0.4, 0.8, 1, 1.12, and 1.5 mM) were used and the absorbance values were measured as for sample solutions.^[28]

High-performance Liquid Chromatography (HPLC) Analysis of Quercetin Compound

The fine powder of NN was extracted with 75% of ethanol, and then, the extract was evaporated. The residue of extract was mixed with n-butanol and water (2:1) and both the upper layer of n-butanol and lower layer of water were separated and evaporated under vacuum. The residues were washed with petroleum ether to remove fatty components and then extracted with ethanol. Concentrated NN seedpod (10 mg/ml) was carefully transferred onto the upper surface of silica gel. The mobile phase used was methanol:chloroform in 2:1 ratio. The mobile phase was slowly passed through the column and a total of nine fractions were collected at an interval of 5 min at a flow rate of 1 ml/min. The collected fractions were subjected to quantitative antioxidant activity with BHT as standard.

The NNE seedpod (fraction-VI) was filtered through Sartorius RC membrane syringe filter (0.2 µm) and 30 µl of the sample used for the HPLC analysis. Chromatographic technique was performed using Shimadzu HPLC (Model SPD-10A UV-visible Detector) and Supelcosil LC-18 column (25 cm × 4.6 mm, 5 µm) with mobile phase, linear gradient elution profile started with methanol: water (75:25) and ended with methanol: water (50:50). Flow rate was maintained at 1.0 ml/min with a back pressure of 250 psi, and the compounds were read at 254 nm using a UV detector. The total run time was 20 min, but preferably, it was extended up to 40 min^[29] and the results were compared with the standard.

Table 1: Contents of total phenols, flavonoids, and tannins in NN seedpod

Parts of lotus	Phenol content (mg GAE/g)	Flavonoid content (mg QE/g)	Tannin content (mg TAE/g)
Seedpod	93.45 mg/g	295.312 mg/g	508.7 mg/g

GAE: Gallic acid equivalent, QE: Quercetin equivalent, TAE: Tannin acid equivalent, NN: *Nelumbo nucifera*

RESULTS

The quantitative analysis of NNE in the previous study showed that the total phenolic content measured by Folin–Ciocalteu method was 93.45 mg GAE/g in NNE, the total flavonoid contents measured by aluminum chloride method were 295.312 mg QE/g and tannin content was 508.7 mg TAE/g [Table 1].

The antioxidant activity of NNE was performed using DPPH and FRAP activity.

The percentage of DPPH radical scavenging activity of NNE is shown in Figure 1. That among the different solvents, ethanol showed the highest percentage of antioxidant activity (69.2%), followed by chloroform (61.41%), acetone (52.7%), and aqueous extract (52.7%).

Different Extractions of Seedpod of NN

According to Hodzic *et al.*,^[30] FRAP assay is simple and quick procedure which had been used to determine antioxidant activity. This assay forms one of the important indicators to assess body's antioxidant status to counteract the oxidative damage due to reactive oxygen species. The maximum antioxidant capacity of NNE was found to be 21.52 µM Fe(II)/g as obtained by FRAP assay [Table 2].

The NNE used for HPLC analysis recorded a R_t of 4.61 min [Figure 2] and standard quercetin compound recorded a R_t of 4.63 min, thus confirming the presence of quercetin compound in NNE [Figure 3]. The HPLC analysis of NNE along with the BHT as standard has been represented in Figure 4. It was observed that fraction VI of NNE showed the highest activity when compared with the other fractions at a time period of 25–30 min.

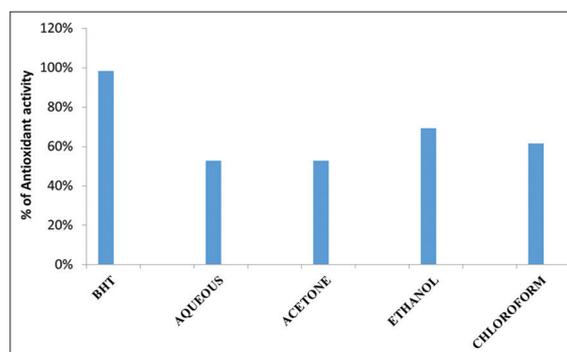


Figure 1: Quantitative antioxidant activity of *Nelumbo nucifera* seedpod

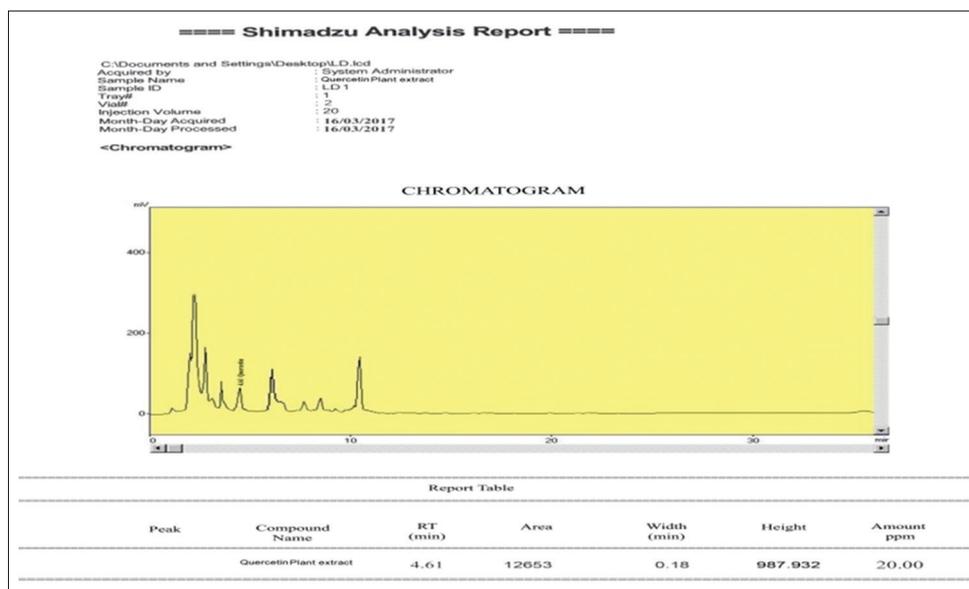


Figure 2: High-performance liquid chromatography analysis of quercetin compound from *Nelumbo nucifera* seedpod

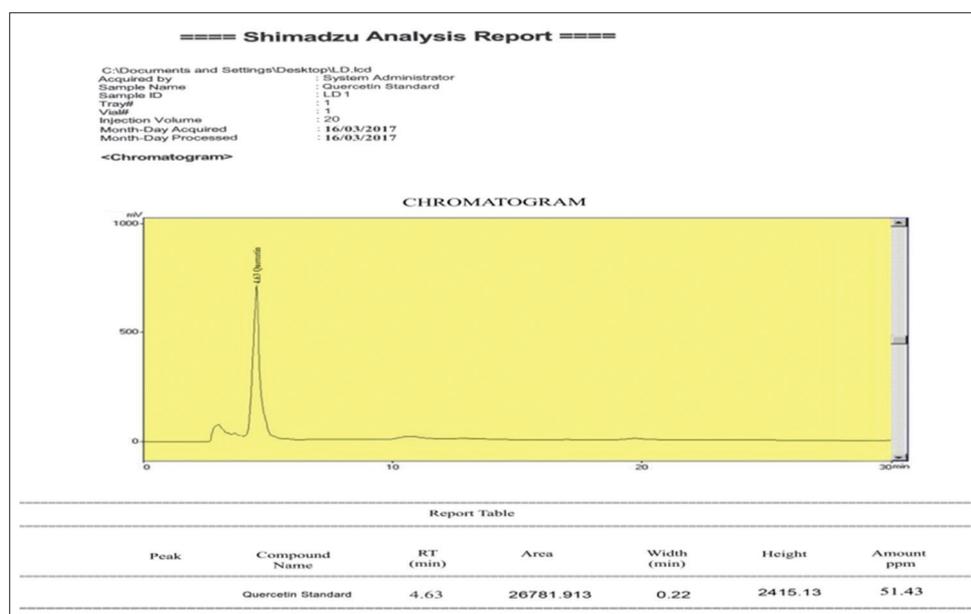


Figure 3: High-performance liquid chromatography analysis of quercetin compound from *Nelumbo nucifera* seedpod

Table 2: FRAP value in NN seedpod ($\mu\text{M Fe (II)/g}$)

NN	FRAP $\mu\text{M Fe (II)/g}$
Seedpod	21.52

FRAP: Ferric reducing antioxidant power, NN: *Nelumbo nucifera*

DISCUSSION

Phenolics are the most widespread secondary metabolite consist of diverse groups of compounds have received much interest as potential natural antioxidant in terms of their ability to act as radical scavengers.^[31] According to Rice-Evans *et al.*,^[32] the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors, and singlet oxygen quenchers which can suppress lipid peroxidation through different

chemical mechanisms. Thus, antioxidant capacity of a sample can be attributed mainly to its phenolic compounds.^[33] Similarly, Shahidi and Naczk, 1995,^[34] reported that naturally occurring phenolics exhibit antioxidant activity. Flavonoids are one of the most widespread groups of natural phenolic constituents found in plants which are free radicals scavengers showing antioxidant activity.^[35] It has been shown that the flavonoids prove two important actions in oxidative stress i.e., quenching of reactive oxygen species and enhancing the cellular antioxidant defense system. Antioxidative phytochemicals in food products such as grains, vegetables, and fruits have received importance in preventing diseases of humankind and hence need further analysis on the various natural plants as a

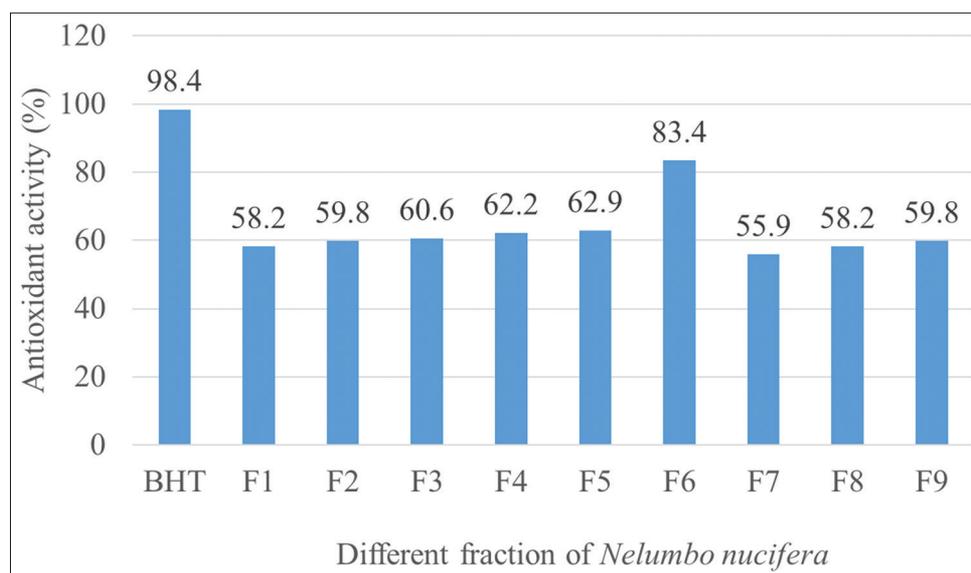


Figure 4: Antioxidant activity of different fractions of *Nelumbo nucifera* seedpod

food source.^[36] Herbal medicines have become one of the most important fields of alternative medicines all over the world. Hence, it is essential to study the importance of medicinal plants to promote their proper use and to determine their potential as a source for the preparation of new drugs.^[37] The antioxidant activity of plants varies with different extracts and different assays, a single assay may not be adequate to assess its activity. Hence, the DPPH and FRAP assays were done to confirm its antioxidant activity. This is the first study to show the antioxidant activity and isolation of active component quercetin from seedpod in the white lotus in Chennai, Tamil Nadu, India. According to Moure *et al.*,^[38] quality of natural extracts and antioxidant activities does not only depend on storage time, geographic origin, and harvesting date but also environment and technological factors as well. Since many neurodegenerative diseases and various other diseases are produced due to an imbalance in oxidants and antioxidants in our body, this study shows its impact of overcoming these by providing a natural source of antioxidants from Mother Nature.

CONCLUSION

The results of the study suggest that the NNE exerts potent antioxidant activity which might be due to the presence of rich polyphenolic constituents. HPLC analysis revealed the identification of active compound, namely quercetin present in the NNE of white lotus. The results emphasize the significance of plant materials as a major source of antioxidants.

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AUTHORS' CONTRIBUTIONS

P.N. Ruvanthika contributed to carry out experiment, preparation of manuscript, and computation of data. S. Manikandan contributed for data analysis and editions.

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