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Research Article

In vitro antioxidant properties of different parts of *Nelumbo nucifera* Gaertn.

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ABSTRACT

Medicinal plants constitute the major constituents of most indigenous medicines. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials. The mounting interest in appraisal of antioxidant power of herbal plant as medicine, the present study was carried out to explore the antioxidant potential of aqueous extracts of *Nelumbo nucifera* Gaertn *(Nelumbonaceae)* leaves, flower, seeds and rhizomes in-vitro. Antioxidant activity of plant parts aqueous extracts were determined by total phenolic, flavonoids and DPPH free radical scavenging assay, content using reference standard ascorbic acid, gallic acid and quercetin respectively. All the analysis was made with the use of UV-Visible Spectrophotometer. The highest total phenolic content (mg GAE/100g) was found to be 270.90±0.33 in seeds and 252.34±0.33 in leave and total flavonoids content (mg QE/100g) was 245.22±1.51 and 193.54±1.94 of aqueous extracts of leaves and flower. The DPPH radical scavenging activity of the extract was increased with the increasing concentration. The highest radical scavenging effect was observed in seeds and leaves extract with IC50, 20 and 25 µg /ml respectively. Among the various extracts in the study, the seeds and leave was found to improve the antioxidant property followed flowers and rhizomes. In the present study, results has capability to scavenge the free radicals and protect against oxidative stress causing diseases and *nelumbo nucifera* parts could be used as ingredients in food formulation. In future it may serve as a good pharmacotherapeutic agent which could be explored to provide affordable medicines to masses.

KEY WORDS: Antioxidant, Free radical scavenging, Flavonoids, Phenols, 1, 1, diphenly-2-picrylhydrazyl, *Nelumbo nucifera.*

INTRODUCTION

Free radicals are more with one or more unpaired electrons which makes them highly reactive[1]. Subsequently, the targeted molecule becomes a free radical itself and initiates a cascade of events that can ultimately lead to cellular damage[2]. However, at physiological levels, free radicals also help preserve hemostatic by acting as signal transducers[3]. Free radicals are the new "buzz word" in pathophysiology today. They have a special affinity for lipids, proteins and nucleic acids (DNA). Most molecules have all their electrons in pairs and are therefore not free radicals. Molecules are held together by pairs of electrons forming stable bonds, but breaking a bond forms highly reactive free radicals[4].

Therefore, medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects and more than 80% of population of developing countries is dependent on traditional folk medicine therapies for treating their ailments. Most of the plants have protective biochemical functions of naturally occurring antioxidants in the cells[5]. Several pharmaceutically active constituents of plants have been assessed to defend against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species. Naturally occurring antioxidants in plant cells include peptide defence mechanisms which are catalases, peroxidases, superoxide dismutases, glutathione and proteins, phenolic defence compounds which include

vitamin E, flavonoids, phenolic acids and other phenols, nitrogen compounds (alkaloids, amino acids and amines) and cartenoids and chlorophyll derivatives[6] .

Lotus (*Nelumbo nucifera*) is a perennial aquatic plant with yellow flowers. It is utilized as a dietary staple and also for a variety of medicinal purposes in Eastern Asia, particularly in China. This plant is belonging to the Family *Nelumbonaceae*. All parts of *N. nucifera* are used for various medicinal purposes in oriental medicine[7]. This plant is very effective to potential antioxidant activity[8], antipyretic[9], Antiplatelet activity[10] and Hypoglycemic activity[11]. Therefore, an attempt has been made investigate the antioxidant properties in different parts of *N. nucifera.*

MATERIALS AND METHODS

Sample collection and extract preparation:

Fresh leaves, flower, seed rhizomes of *Nelumbo nucifera* plant used in the present study were collected from village Ichoi, Milkipur, Faizabad, Uttar Pradesh. The plant parts were separated from weeds, washed and rinsed with distilled water. They were sun-dried for 5 days, ground in a mortar with a pestle into coarse powder and packed in an air-tight plastic container for further analysis. Methanol extracts were prepared by soaking 100g each of the dry powdered plant parts in 1 litre of methanol at room temperature for 48 h. The extracts were filtered first through a Whatmann filter paper No. 42 (125 mm) and then through cotton wool. The extracts were concentrated using a rotary evaporator with the hot water bath set at 40°C. The percentage yield of extracts ranged from 5 - 20% (w/w).

Determination of total phenolic content:

Total phenols were determined by Folin Ciocalteu reagent [12]. A dilute extract of each seed (0.5 ml of 1:10 g ml-1) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and 4 ml of aqueous sodium carbonate (1 M). The mixtures were kept at dark ambient condition for 15 min and the total phenols were determined by spectrophotometry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/ L solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

Determination of total flavonoids content:

Aluminum chloride colorimetric method was used for flavonoids determination[13]. Each seed extracts (0.5 ml of 1:10 g/ ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a UV-Visible Spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 g ml-1 in methanol.

DPPH radical scavenging activity:

The ability of the methanolic extracts to scavenge free radicals was determined against a very stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) determined spectrometrically [14]. Aliquots of the sample extract at different concentrations 0.02, 0.05, 0.1, 0.15, 0.25 mg/ml were added to 1 mM methanolic solutions of DPPH. Each mixture was vortexed vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage DPPH scavenging relative to control using the following equation:

DPPH scavenging activity (%) = (Absorbance of control − Absorbance of sample)/ Absorbance of control × 100

IC 50 Value was also calculated.

Statistical Analysis:

The results obtained were expressed as mean ±SD and analysis of variance of three determinations and also statistically analyzed to ascertain its significance. The significance was estimated at ($p \leq 0.05$level).

RESULT AND DISCUSSION

The level of total phenol and total flavonoid contents are shown in table .1 and Figure. 1 & 2 Total phenolic content in aqueous extracts were determined according to the Folin-Ciocalteu method and expressed as mg of gallic acid equivalent (GAE) per hundred gram of extract (mg GAE/100g) on dry weight basis. Total phenol content of plant parts ranged from 220.57±0.26 - 270.90±0.33. Seeds aqueous extract showed highest total phenolic content (270.90±0.33) followed by leave (252.34±0.33), rhizomes (241.01±0.36) and flowers (220.57±0.26). There were significant differences in the total phenol content between all the parts (leave, flowers, rhizomes and seeds) of *N. nucifera* at $p \leq 0.05$ level. Phenolic compounds are a class of antioxidant agents which act as free radical terminator[15]. It is also an important plant constituent due to their scavenging property and their hydroxyl groups which contributes directly to antioxidative action[16]. Results showed that whole plant contain good amount of phenolic content

in which seeds aqueous extract contain highest value while flowers had the lowest when compared to other plant parts extract which was comparable with the result observed by *Sagwan et al* [17] in *Pongamia pinnata L.* Likewise, *Sadiq et al*[18] have also reiterated that seed was found to be highest phenolic content as compared to other plant parts of *Eruca sativa*. Phenolic compounds are important constituents of plants, vegetables, and fruits because of their scavenging ability due to their redox properties and hydroxyl groups which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers[19]. The phenolic compounds may contribute directly to the antioxidative action. They are easily degraded (e.g., by oxidation or hydrolysis) and may also form covalent products and non-covalent complexes with various types of molecules. In addition, it was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation [20]. According to present study the appropriate amount of total phenol content in seeds, leave, rhizomes and flowers of *N. nucifera* can explain its high free radical scavenging activity. However its phenol rich extract was reported to be effective for the control of insulin resistance.

Total flavonoids content in aqueous extracts of *Nelumbo nucifera* plant parts were determined by using Aluminium Chloride method and the amount of flavonoids was expressed as mg quercetin equivalent (QE) per hundred gram of extract (QE/100g) dry weight. The total flavonoid content in the plant parts ranged from 140.75±0.58-245.22±1.51. Leave aqueous extract had the highest total flavonoid content (245.22±1.51) followed by flowers (193.54±1.94), seeds (161.84±0.32) and rhizomes (140.75±0.58). Data showed that there were significant differences among all the parts of *Nelumbo nucifera* at $p \leq 0.05$. Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants, characterized by a benzo-γ-pyrone structure[21]. They are one class of secondary plant metabolites that are also known as Vitamin P which are mostly used in plants to produce yellow and other pigments which play a vital role in the colors of plants. Moreover, Flavonoids contents are readily ingested by humans and they seem to demonstrate important anti-inflammatory, anti-allergic and anti-cancer properties [22]. The mechanisms of action of flavonoids are through scavenging or chelating process [23]. It also plays an important role in stabilizing lipid oxidation and is associated with antioxidant activity [24] . The study conducted by *Rebaya et al*[25] that the total flavonoids content in leave extract was higher than flower aqueous extract of *Halimium halimifolium* . This result was also in line with the study of *Bhardwaj et al* [26] and *Sharma et al*[27] that *Boerhaavia diffusa* L. and *Plumbago zeylanica* leave contain highest amount of total flavonoids content when compared to other parts.

The DPPH radical scavenging activity of the aqueous extracts were evaluated by using the parameter IC_{50} which means the concentration of antioxidant required for 50% scavenging of DPPH radicals in the particular time period. *In vitro* activities of all the plant part extracts were measured with the standard antioxidant (Ascorbic acid). The *N. nucifera* aqueous extract had dose dependent action, *i.e.* the scavenging activity of DPPH increased proportionate to the increase in concentration of the extracts. Here, The DPPH free radical scavenging activities of different plant parts leaves, flower, rhizome and seed extracts are shown in (Table 2 and Figure 3). The antioxidant activity of *Nelumbo nucifera* plant parts with IC_{50} (Inhibitory Concentration) values ranged from (20-60 µg/ml). Seed extract exhibited highest antioxidant activity with IC_{50} Values (20µg/ml) followed by leave (25µg/ml), rhizomes (43µg/ml) and flowers (60µg/ml). Leave showed lowest antioxidant activity with IC_{50} value (60µg/ml). The DPPH is a stable nitrogen centered free radical with absorption maximum band around 515 to 528 nm. In this assay, the antioxidants reduce the DPPH radial (purple color) to a yellow coloured compound, diphenylpicrylhydrazine. The extent of color change depends on hydrogen donating ability of the antioxidants [28]. This method illustrates that there was a decrease in the concentration of DPPH radicals due to the scavenging ability of the soluble constituents in the aqueous extracts of *Nelumbo nucifera* leave, flowers, rhizomes, seeds with their standard ascorbic acid as a reference compound. DPPH assay is based on the ability of DPPH which is a stable free radical which decolorize in the presence of antioxidants. It is a direct and reliable technique for determining radical scavenging action. The aqueous *nucifera* seeds and leave extract showed strong antioxidant activity. It was noticed that seed and leave extract of *N. nucifera* showed strong hydrogen donating abilities to act as an effective antioxidant. Contrary to the present study, Artun, *et al* [29] that the young *Carica papaya* leaves aqueous methanol extract exhibited a higher DPPH scavenging effect than seed aqueous methanol extract. This result was also in line with the study of *Sadiq et al*[30] that maximum DPPH scavenging activity was found in methanolic *Eruca sativa* seeds extracts than leaf, stem and flower extracts of plants.

Table 1
The antioxidant activity of different plant parts of *Nelumbo nucifera* (G.) on dry weight basis.

Plant Part (Aqueous Extract)	Total Phenolic Content (mg GAE/100g)	Total Flavonoids (mg QE/100g)
Leave	252.34 ± 0.33[a]	245.22 ± 1.51[a]
Flowers	220.57 ± 0.26[b]	193.54 ± 1.94[b]
Rhizomes	241.01 ± 0.36[c]	140.75 ± 0.58 [c]
Seeds	270.90 ± 0.33[d]	161.84 ± 0.32[d]

Values are Mean ± Standard deviation of triplicate determinations. Values sharing same uppercase letter between rows are not significantly different at $p < 0.05$.

Table 2
The DPPH free radical scavenging activity of different plant parts of *Nelumbo nucifera* (Gaertn.) on dry weight basis.

Concentration (µg/ml)	Ascorbic Acid	Leave	Flowers	Rhizomes	seeds
20	87.44	45.01	36.80	43.60	48.40
40	88.35	66.80	42.20	47.20	68.50
60	89.72	67.20	48.90	57.50	71.50
80	90.60	69.00	49.10	58.10	72.88
100	91.09	70.40	49.30	62.01	72.90
120	92.46	71.01	50.10	62.70	74.66
140	93.80	72.0	51.20	63.10	75.33
160	94.20	72.70	57.40	63.40	78.66
180	95.20	74.20	58.10	64.01	79.45
200	97.03	77.11	62.40	64.70	79.50
IC_{50} Values(µg/ml)	-	25.00	60.00	43.00	20.00

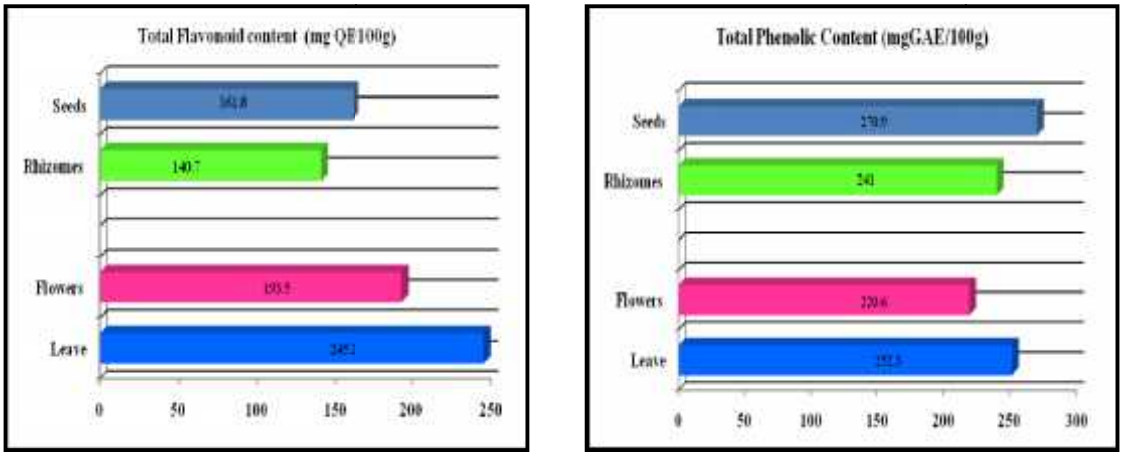

Figure 1 and 2
The Total phenol and Total flavonoid content of different plant parts of *Nelumbo nucifera* (G.) on dry weight basis.

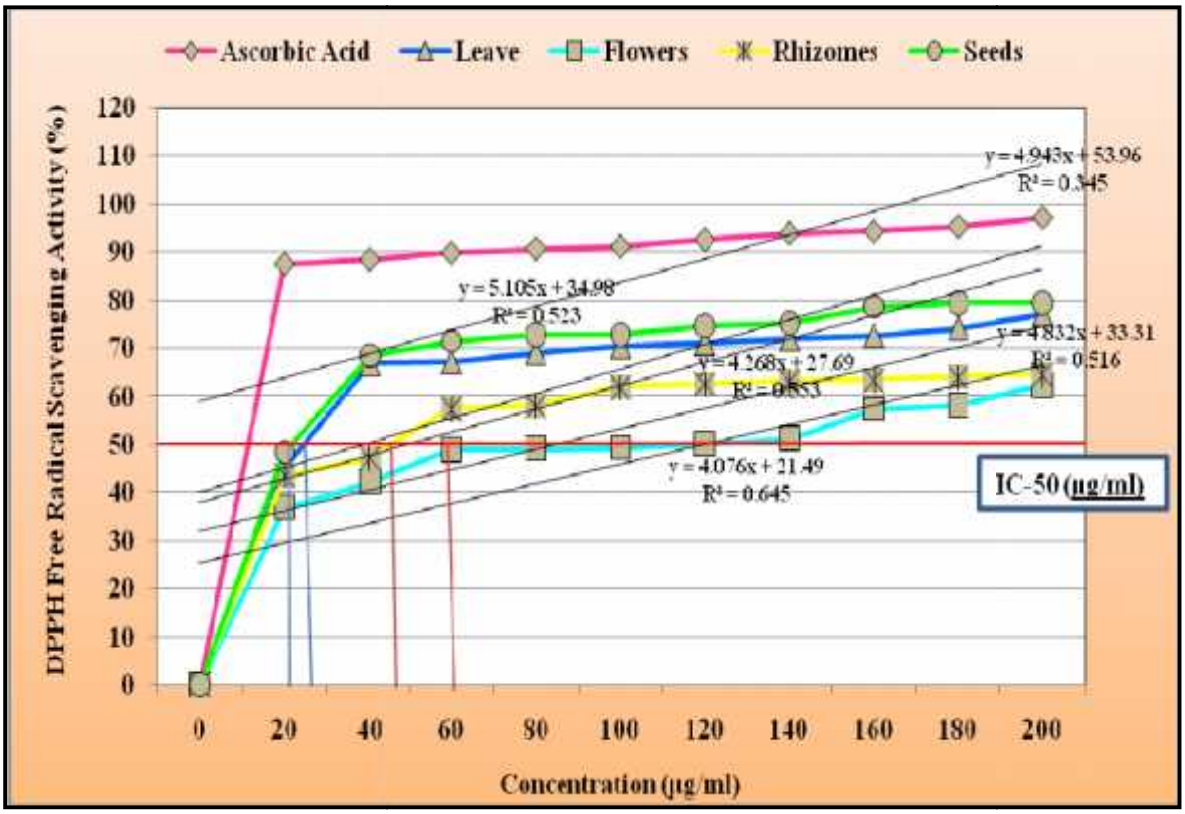

Figure 3
The DPPH activity of different plant parts of *Nelumbo nucifera* (G.) on dry weight basis.

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