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Research Article

Evaluation of Antioxidant Activity of Ethanolic and Chloroformic Extracts of *Nymphaeu nouchali* Leaves

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Abstract

The flower of *Nymphaea nouchali* is the national flower of Bangladesh and the plant parts are being used by the rural people of Bangladesh due their food and medicinal values. In this study the ethanol and chloroform extracts of *Nymphaea nouchali* leaves were investigated to evaluate their antioxidant property. Antioxidant property was evaluated by using total antioxidant capacity, DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging capacity, total phenol and flavonoid contents of the plant. In DPPH assay free radical scavenging activity of the extracts were evaluated comparing with ascorbic acid at 517 nm. The IC_{50} of ethanol extract of *Nymphaea nouchalli* is 10.102±0.23µg/ml and chloroform extract of *Nymphaea nouchalli* is 13.11±0.11µg/ml where as the IC_{50} of ascorbic acid was 19.89± 0.22µg/ml. Phenolic content was measured by Folin-Ciocalteu assay and is expressed as Gallic acid equivalents (GAE). The content of phenolic compound in the extracts correlates with the antioxidant activity, being higher in Ethanol extract of *Nymphaea nouchalli* (6.53±0.26 mg/g GAE) and lower in chloroform extract of *Nymphaea nouchali* (5.55±0.06mg/g GAE). And flavonoid contents were found 4.58±0.19 mg/g quercetin equivalent for chloroform extract and 5.99±0.33mg/g quercetin equivalent for ethanol extract respectively. But both of the extracts contain almost similar amount of total antioxidant capacity having 2.75±0.12 mg/g ascorbic acid equivalent (AAE) for ethanolic extract and 2.69±0.09 mg/g AAE for chloroformic extract. Data of this study reveals that the medicinal plant *Nymphaea nouchalli* is rich source of antioxidants. Further investigations are required to isolate the antioxidant molecules to treat free radical associated diseases like cancer, neurological disorder, inflammation and cardiac disorder etc.

1.0 Introduction

Herbs and plants make up a large portion of the ingredients used by people throughout the ages to help combat disease and illness. Many of these medicinal plants are still used today all around the world. In most of the traditional systems of treatment, the use of medicinal plant include the fresh or dried part, whole, chopped, powdered or an advanced form of the herb usually made via extraction with water, ethanol or an organic solvent play a major role and constitute the backbone of traditional medicine[1]. In this study ethanolic and Chloroform extract of leaves of *Nymphaea nouchali* have been testified to find out medicinal potentiality. *Nymphaea nouchali* flower is the national flower of Bangladesh and its local name is shapla. *It* is a day blooming nonviviparous plant with submerged roots and stems. *Nymphaea nouchali* is considered a medicinal plant in Indian Ayurvedic medicine and it was mainly used to treat indigestion[2]. Recent experiments have confirmed that it has medicinal qualities as an antihepatotoxic and antidiabetic[3, 4]. Powdered rhizomes are demulcent and diuretic; used in piles, dysentery and dyspepsia. Flowers are astringent, cardiotonic and refrigerant; alleviative of cough, bile, vomiting, giddiness, worms and burning of the skin. Filaments are astringent and cooling; useful in burning of the body, bleeding piles and menorrhagia. Seeds are used as a cooling medicine in cutaneous diseases[5]. It has also food values - its tubers and rhizomes are used as food items; they are eaten usually boiled or roasted. In the case of the red and blue water-lily, its tender leaves and flower peduncles are also valued as food [6].

Different solvent extracts of the entire plant haves shown the presence of sterols, alkaloids, saponins, tannins, gallic acid and quercetin which indicate the probability of the presence of antioxidant potentiality of this plant.[7] Since ancient times, the medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities. As antioxidants have been reported to prevent oxidative damage caused by free radical, it can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers[8, 9]. The potentially reactive derivatives of oxygen, attributed as reactive oxygen species (ROS), are continuously generated inside the human body. The generated ROS are detoxified by the antioxidants present in the body. However, overproduction of ROS and/or inadequate antioxidant defense can easily affect and persuade oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA[10]. ROS including superoxide radical, hydroxyl radical, singlet oxygen and H_2O_2 have been found to play an important role in the initiation and / or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular disease[11]. Although human body has its natural antioxidant defense but this natural antioxidant mechanism can be inefficient for severe and or continued oxidative stress. Being inspired by these fact scientists has been investigating medicinal plants for antioxidant potentialities for long time. Information of antioxidant activity of *Nymphaea nouchali* flower has been recognized but investigation of antioxidant activity of leaves is not done yet in extensively. In this study ethanolic and chloroformic extracts of leave of *Nymphaea nouchali* have been testified for antioxidant potentialities and a comparison has been made between them.

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2.0 Materials and Methods

2.1 Plant Material

The whole plants were collected from Jahangirnagar University, Savar, Dhaka, Bangladesh and identified by the taxonomist of Department of Botany, Jahangirnagar University.

2.2 Chemicals and Reagents

1, 1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid, quercetin and gallic acid were obtained from Sigma Chemical Co (MO, USA). Folin-ciocalteu reagent (FCR) and Griess reagent were purchased from Merck, Germany. Ethanol and Chloroform and all other chemicals and reagents were of analytical grade.

2.3 Preparation of Plant Extract

The plant parts were collected in fresh condition. They were sun-dried first for three days and then, dried in an oven at reduced temperature (<50 ºC) to make suitable for grinding purpose. The powdered plant materials were submerged in sufficient volume of ethanol and chloroform in an air-tight flat bottomed container for seven days, with occasional shaking and stirring. The extracts were then filtered and dried on electrical water bath.

2.4 Phytochemical Screening of Crude Plant Extract

In phytochemical screening the ethanolic and chloroformic extracts were quantitatively checked for various components using the following reagents and chemicals – Molishch's reagents - for carbohydrate test; aqueous sodium hydroxide solution- for glycoside test; dilute sulphuric acid and NaOH solution- for glycoside test; fehling's solution- for glycoside test; 10% Ammonia solution- for anthraquinone glycoside test; mayer's reagent, wagner's reagent Hager's reagent & Dragendroff's reagent- all for alkaloid tests; Conc. Hydroclric acid – for flavanoid test; conc. Sulphuric acid- for steroid test; $FeCl_3$ (5%) - for tannin test. Components were identified by observing the Characteristic color changes.[12]

2.5 Determination of Total Phenol

The content of total phenolic compounds in ethanolic and chloroformic extracts of *Nymphaea nouchali* were determined by Folin–Ciocalteu Reagent[13]. 1.0 ml of each plant extracts (10 µg/µl) or standard of different concentrations (250,200,150,100 and 50) µg/ml solution were taken in different test tubes. 5 ml of Folin – ciocalteu (Diluted 10 fold) and 4 ml of Sodium carbonate reagent solutions were added to the test tubes. The test tubes were incubated for 30 minutes at 20^0C to complete the reaction. The absorbance of the solutions was measured at 765 nm using a spectrophotometer against blank. Total content of phenolic compounds in plant ethanolic and chloroformic extracts are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

2.6 Determination of Flavonoid Content

Aluminum chloride colorimetric method was used for flavonoids determination[14]. 1 ml of sample was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2ml of 1 M potassium acetate and 5.6 ml of distilled water. It was kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing Quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol.

2.7 Determination of Total Antioxidant Capacity

The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. [15]The antioxidant capacity is expressed as ascorbic acid equivalent (AAE). The plant extract (0.3 ml) was combined with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated at 95^0C for 90 minutes. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm against blank. Total antioxidant capacity of extract was measured from the calibration curve constructed by using ascorbic acid standard solutions.

2.8 DPPH Scavenging Activity

DPPH scavenging activity of the extracts was measured by the method developed by Braca et *al.*, 2001[16]. The sample extract (0.2 ml) was diluted with methanol and 2 ml of DPPH solution (0.5 mM) was added. After 30 minutes, the absorbance was measured at 517 nm. IC_{50} values (concentration of samples required to scavenge 50% of free radicals) were calculated from the regression equation, developed by plotting concentration of samples versus percentage inhibition of free radicals. The percentage inhibition activity was calculated from $[(A_0-A_1) / A_0] \times 100$, where A_0 is the absorbance of control and A_1 is the absorbance of the extract/ standard. Ascorbic acid was used as positive control here.

2.9 Statistical Analysis

Experimental results are presented as the mean standard deviation (SD) of three parallel measurements. Probability values of less than 0.05 were regarded as significant. All statistical analysis is performed using Excel 2007 and STATISTICA computer software packages.

3.0 Results and Discussions

The ethanolic and chloroformic extracts of leaf of the plant *Nymphaea nouchali* were screened for possible chemical constituents of the plant as chemical constituents are responsible for their therapeutic and medicinal activities. The results of the phytochemical screening are summarized in Table 1 and Table 2.

The presence of flavonoid and tannin indicate the antioxidant potentiality of the plant extract of *N. nouchali*. That's why the present study was designed to evaluate the antioxidant property of two different solvent extracts of leaves of *Nymphaea nouchali*. In this investigation antioxidant activity was determined by applying four established method – total phenolic content, total flavonoid content, total antioxidant property and DPPH scavenging activity.

The Total phenolic content (TPC) assay is a common assay widely used to estimate relative amounts of phenolic compounds present in an extract[17]. Phenolic compounds are a class of antioxidant agents which act as free radical terminators[8]. These are commonly found in both edible and inedible plants and they have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[18]. The TPC results were expressed as mg gallic acid equivalent as this compound represents the most simple form of a phenolic compound. Phenol compounds present in the extract undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the TPC[19]. The total phenolic contents of the two extracts were calculated using the standard curve of gallic acid ($y = 0.008x + 0.222$, $R^2 = 0.914$) In comparison with standard gallic acid Ethanolic extract of leaf showed the highest amount of phenol contents among the two extracts (Table-3).

Total antioxidant capacity of the ethanolic and chloroformic extracts of leaf of the plant *Nymphaea nouchalli* were determined by using the phosphomolebdenum method expressed as ascorbic acid equivalents (AAE) per gram of plant extract. The natural antioxidant ascorbic acid is used as a standard. The total antioxidant capacities of ethanolic and chloroformic extracts of N. nouchalli were determined from the calibration curve ($y = 0.002x + 0.904$) extablished by ascorbic acid at 695nm. Ascorbic acid equivalents of ethanolic extract and chloroformic extract were 2.75±0.12mg/g and 2.69±0.09mg/g respectively (Table-3).

Table 1: Phytochemical screening of extracts of *Nymphaea nouchalli*

Plant Extract	Carbohydrate	Flavonoid	Glycoside	Sapanin	Glucoside	Steroid	Tanin
Ethanolic	+	+	-	-	-	+	+
Chloroformic	+	+	+	-	-	-	+

Table 2: Alkaloid tests of extracts of *Nymphaea nouchali*

Plant extracts	Meyers Reagent	Hagers Reagen	Wagners reagent	Dragendroff reagent
Ethanolic extract	-	+	+	-
Chloroformic extract	+	+	-	-

Flavonoid contents of the ethanolic and chloroformic extracts of leaf of the plant *Nymphaea nouchalli* were determined by Aluminium chloride colorimetric method. Flavonoids protect cells against the damageing effects of reactive oxygen species, such as singlet oygen, superoxide, peroxyl radicals, hydroxyl racidals and peroxynitrite. The total flavonoid content was calculated by using the standard curve of quercetin (y = 0.009x - 0.036,) and was expressed as quercetin equivalents (QE) per gram of the plant extract. The flavonoid contents of ethanolic extract and chloroformic extract were found 4.58mg/g and 5.99mg/g quercetin equivalents.

Table 3: Antioxidant property of leaves extracts of *N. nouchali.*

Sample	Total Phenol Content (mg/g of Galic Acid Equivalent)	Total Flavonoid Content(mg/g Quercetin Equivalent)	DPPH Free radical scavenging activity (IC_{50}µg/ml)	Total Antioxidant capacity(mg/g of Aacorbic Acid Equivalent)
Ascorbic Acid			19.89± 0.22	
Ethanolic extract	6.53±0.26	4.58±0.19	10.102±0.23	2.75±0.12
Chloroformic extract	5.55±0.06	5.99±0.33	13.11±0.11	2.69±0.09

A method based on the scavenging of the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) has been used extensively to predict the antioxidant activities of extracts of plants[20]. DPPH is nitrogen centered free radical that shows strong absorbance at 517 nm. DPPH radical accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Extent of DPPH radical scavenged was determined by the decrease in intensity of violet colour in the form of IC_{50} values. Lower IC_{50} value represents higher antioxidant activity. The antioxidant activity was compared with ascorbic acid (ASC) as standard. IC_{50} value of ethanolic extract was found 10.102±0.23 µg/ml and that of chloroformic extract was found 13.11±0.11 µg/ml whereas the IC_{50} value was found 19.89± 0.22µg/ml. Although here both of the extract showed IC_{50} values greater than standard; the cause may be that the quality of ascorbic acid was not so good as there are evidence that IC_{50} value of Ascorbic acid is also 10.10±0.02 µg/ml[7]. But both of the extract showed high antioxidant potentiality.

4.0 Conclusion

The experimental data of all the tests extracts reveal that although there are small differences among the values of total phenol content, total flavonoid content, total antioxidant capacity and DPPH scavenging activity; but both of the ethanolic and chloroformic extracts possess strong antioxidant activity.

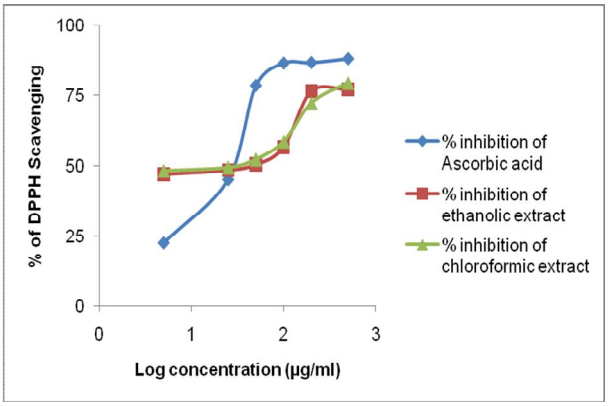

Figure 1: DPPH radical scavenging activity of ethanolic and chloroformic extract of leaf of *Nymphaea nouchali.*

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