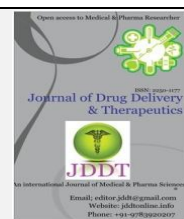


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Research Article

Phytochemical investigation and determination of phytoconstituents in flower extract of *Nelumbo nucifera*

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ABSTRACT

The aim of this study seeks to investigate the presence of qualitative and quantitative analysis of phytoconstituents of the flower of the plant *Nelumbo nucifera*. The present study provides evidence that successive solvent extract of *Nelumbo nucifera* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases. Maximum phenolic and flavanoid content was observed in ethanolic extract of *Nelumbo nucifera*. The DPPH scavenging potential of the ethanolic extracts of the herbs ranged from 33%-55%. In the present study analysis of free radical scavenging activity and total phenolic and flavonoid content showed that mainly the ethanolic extract of flower extract can be the potent source of natural antioxidants.

Keywords: Phytochemical, Ethanolic extract, Antioxidant activity, *Nelumbo nucifera*

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INTRODUCTION

Antioxidants are molecules that inhibit the initiation of oxidation chain reactions thereby preventing damage to human body cells¹. At present, synthetic antioxidants are available such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) but they were proven to be toxic for human beings². Medicinal plants containing polyphenols have been reported for antioxidant and other pharmacological activities³. Plant based natural antioxidants are at most interest worldwide because of non toxic nature. One such medicinal plant is *Nelumbo nucifera* (synonym: *Nelumbium speciosum Willd*), an aquatic plant belonging to the family Nelumbonaceae. This is a well-known medicinal plant widely used in the Ayurveda and Siddha system of medicine for the treatment of diabetes, inflammation, liver disorders, rinary disorders, menorrhagia, blenorrhagia, menstruation problem, as naphrodisiac, and as a bitter tonic with the plant having much beneficiary properties and having been traditionally claimed as an antidiabetic. Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules which protect the human body against various pathogens. Nature has been a source of medicinal agents for thousands of years and a large number of modern drugs have been isolated from natural sources. Herbal medicine is the oldest known healthcare system known to mankind. India has rich medicinal plants of nearly 7500 species. Out of these, 4635 species are commercially used to a fairly large scale⁴.

MATERIAL AND METHOD

Plant Material

Whole plant material of *Nelumbo nucifera* was collected from ruler area of Bhopal (M.P), India in the months of January 2016.

Extraction Procedure

Defatting of Plant Material

Powdered plant material of *Nelumbo nucifera* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place⁵.

Extraction by hot continuous percolation process

100 gm of *Nelumbo nucifera* dried plant material were exhaustively extracted with various solvent (Pet ether, chloroform, ethyl acetate, ethanol and water). The extracts were evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts

Qualitative phytochemical tests

Phytochemical examinations were carried out for all the extracts as per the standard methods⁷.

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids. **d) Hager's Test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test: Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of carbohydrates.

c) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Modified Borntrager's Test: Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammoniacal layer indicates the presence of anthranol glycosides.

b) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

7. Detection of proteins and amino acids

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

b) Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

11. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes

Estimation of total Phenolic and flavonoid Content

Total Phenolic content estimation

Principal: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method ⁷.

Preparation of Standard: 50 mg Gallic acid was dissolved in 50 ml methanol, various aliquots of 25- 125 μ g/ml was prepared in methanol.

Preparation of Extract: 10 mg of dried extracted dissolve in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenols.

Procedure: 2 ml of extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Principal: Determination of total flavonoids content was based on aluminum chloride method ⁷.

Preparation of standard: 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 25- 125 μ g/ml were prepared in methanol.

Preparation of extract: 10 mg of extract dissolved in 10 ml methanol and filter. Three (1mg/ml) of this extract was for the estimation of flavonoid. 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

In-Vitro free radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl - DPPH)

It is a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color⁷.

Procedure:

DPPH scavenging activity was measured by the spectrophotometer. Stock solution (1.5 mg/ml in methanol) was prepared such that 75 μ l of it in 3 ml of methanol gave an initial absorbance of 2.076. Decrease in the absorbance in presence of sample extract at different concentration (10-100 μ g/ml) was noted after 15 minutes.

Protocol for DPPH free radical scavenging activity:

- Preparation of stock solution of test sample: 100 mg of the extract was dissolved in 100 ml of methanol to get 1000 µg/ml solution.
- Dilution of test solution: 10, 20, 40, 60, 80 and 100µg/ml solution of the test samples were prepared from stock solution.
- Preparation of DPPH solution: 15 mg of DPPH was dissolved in 10 ml of methanol. The final solution was covered with aluminum foil to protect from light.

Estimation of DPPH radical scavenging activity:

- 75 µl of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading.
- 75 µl of DPPH and 50 µl of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken.
- Absorbance at zero time was taken for each concentration.
- Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

Calculation of % Reduction =

$$\frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \times 100$$

RESULTS AND DISCUSSION

The crude extracts so obtained after the soxhlet extraction process, each extracts were further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from different samples using different solvents are depicted in the table. 1.

Table 1: Result of Percentage Yield of Different Extract

S. No.	Solvents	Percentage Yield
1.	Pet. Ether	1.2%
2.	Chloroform	1.4%
3.	Ethyl Acetate	1.3%
4.	Ethanol	4.1%
5.	Water	3.1%

A small portion of the dried extracts were subjected to the phytochemical test using methods to test for alkaloids, glycosides, tannins, saponins, flavonoids and steroids separately for extracts of all samples. The outcomes of the results are discussed separately in the table 2.

Table 2: Result of Phytochemical Screening of Extracts

S. No.	Constituents	A	B	C	D	E
1.	Alkaloids	-	-	-	+	-
2.	Glycosides	-	-	-	-	-
3.	Flavonoids	-	-	+	+	+
4.	Diterpenes	+	-	+	+	-
5.	Phenolics	-	-	+	+	-
6.	Amino Acids	-	-	-	-	-
7.	Carbohydrate	-	-	-	-	-
8.	Proteins	-	-	-	-	-
9.	Saponins	-	-	+	+	+

A- Pet. Ether, B- Chloroform, C- Ethyl acetate, D- Ethanol, E- Water

From the results obtained it is clear that the *Nelumbo nucifera* plant shows the presence of alkaloids, saponins, flavonoids, phenol, diterpenes were found present in flower parts when extracted with different solvents using soxhlet extraction procedure. The phytochemical analysis of *Nelumbo nucifera* plant indicates the presence of phenols and flavonoids present in sufficiently enough quantity according to preliminary phytochemical analysis. Phenolic and flavonoids are the phytochemical that are present in ethyl acetate and ethanol.

The content of total phenolic content (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y =$

$0.008x + 0.004$, $R^2 = 0.999$, where x is the Gallic acid equivalent (GAE) and Y is the absorbance.

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $Y = 0.008 X + 0.007$, $R^2 = 0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance. The results are given in Table 3.

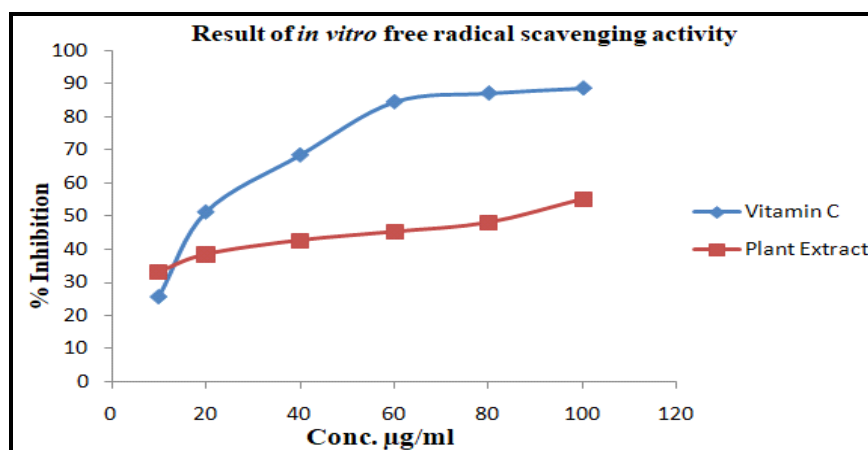
The total phenolic content in ethanolic extract was 10.68mg of gallic acid equivalent/100mg of dry extract and total flavonoid content was 15.98 mg of quercetin equivalent/100mg of dry extract. The results are given in Table 3.

Table 3: Results of Total Phenolic and flavonoid content

S. No.	Extract	Total Phenol (mg/100mg)	Total Flavonoid (mg/100mg)
1.	Ethanolic	10.68	15.98

Table 4: Result of *in vitro* free radical scavenging activity

S. No	Vitamin C			Plant Extract		
	Conc. $\mu\text{g/ml}$	Test	% Inhibition	Conc. $\mu\text{g/ml}$	Test	% Inhibition
1	10	1.524	25.72	10	1.447	33.29
2	20	1.012	51.25	20	1.421	38.55
3	40	0.655	68.44	40	1.375	42.76
4	60	0.322	84.48	60	1.322	45.31
5	80	0.268	87.09	80	1.285	48.1
6	100	0.236	88.61	100	1.242	55.17
IC₅₀ ($\mu\text{g/ml}$)			24.65	IC₅₀		

Absorbance of 0.1mM DPPH (A_o) = 2.076Figure 1: Graph of *in vitro* free radical scavenging activity

DPPH scavenging activity has been used by various researchers as a rapid, easy and reliable parameter for screening the *in vitro* antioxidant activity of plant extracts. DPPH is a stable free radical and accepts an electron to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was at 517nm. It was observed that with the increase of concentration, there is decrease of absorbance value. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidants molecules and radical, progresses, which results in the scavenging of the radical by electron donation. IC₅₀ for standard ascorbic acid was found to be 24.65 $\mu\text{g/ml}$ and for ethanolic extract of *Nelumbo nucifera* was found to be 200.58 $\mu\text{g/ml}$. Thus the anti-oxidant activity of sample was less than the standard table 4 and fig. 1.

Free radicals are the cause for several major disorders. So, evaluation of antioxidant activity in plants could result in the discovery of natural antioxidants with pharmacological and food value. The importance of phenol compounds in plants as natural antioxidants and their use as substitutes to synthetic antioxidants in food additives is well known. Therefore there observation can be used in pharmaceutical to explore new drugs. Thus the present aim is to assess the antioxidant activity of *Nelumbo nucifera* by DPPH method and also compared the % antioxidant activity with standard ascorbic acid.

CONCLUSION

Thus, from the present study the plant flower extract of *Nelumbo nucifera* showed presence of number of phytochemical as secondary metabolites and they can be used in the pharmaceutical industries for producing a potent drug against various disease. The ethanolic flower extract was found to constitute alkaloids, saponins, phenol, diterpenes flavonoids etc. In the present study analysis of free radical scavenging activity and total phenolic and flavonoid content showed that mainly the ethanolic extract of flower extract can be the potent source of natural antioxidants.

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