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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF LOTUS (*NELUMBO NUCIFERA*) STEM

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ABSTRACT

Nelumbo nucifera commonly known as Indian lotus has been used as an indigenous medicine in India. The present study aims to evaluate the phytochemical activity and antioxidant activity of lotus stem. The lotus stem was extracted with solvents like ethanol, acetone, and water. Lotus stem was found to be an excellent source of protein and vitamin C, while it is a good source of fiber and Iron. Carbohydrate, flavanoids, quinones, cardiac glycosides, terpenoids, phenol, coumarines, sterols, phyto sterols are present. The Total Polyphenol Content (TPC) was found to be high in acetone extracts (130.88 mg GAE/100 ml). The Total Flavonoid Content (TFC) was found to be high containing in ethanolic extracts (77.8 mg Rutin equivalence/100 ml). Lotus stem can be used effectively as a therapeutic agent in nutritional therapy and phyto-therapy for the alleviation of various diseases. Plant derived nutraceuticals should be produced using Nelumbo nucifera stem.

KEYWORDS: **Nelumbo nucifera, Phytochemicals, Antioxidant**

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INTRODUCTION

Nelumbo nucifera Gaertn (Family Nymphaeaceae) commonly known as Indian Lotus is an aquatic herb with stout creeping yellowish white coloured rhizome. It has reported that rhizome extract showed anti-diabetic and anti-inflammatory effects, stalks extract showed anti-pyretic effect leaves and stamens extracts showed anti-oxidant effect and seeds extract showed hepatoprotective and free radical scavenging effects. The leaf of *Nelumbo nucifera* is bitter, sweet and neutral. It is aromatic and blue-green in colour [1].The leaves, root and the embryonic stage of the plant have reported to contain alkaloids such as roemerine, nuciferine, nornuciferine, nelumboside, anonaine and asimilobine[2]. It has been reported to have anti-strees, anti-obesity, anti-oxidant, hepatoprotective, anti-malaria, anti-fungal activity, anti-diabetic activity, anti-inflammatory and antipyretic activity [3].The stem is used in indigenous Ayurvedic medicine as a diuretic, anthelmintic, to treat strangury, vomiting, leprosy, skin disease and nervous exhaustion. The leaves are used for the treatment of haematemesis, epistaxis, haemoptysis, haematuria, metrorrhagia and hyperlipidaemia[4]. The flowers are useful in the treatment of diarrhoea, cholera, fever and gastric ulcers. The seeds and fruits are used as a health food in Asia and to treat many ailments, including poor digestion, enteritis, chronic diarrhoea, insomnia, palpitations, spermatorrhoea, leucorrhoea, dermatopathy, halitosis, menorrhagia, leprosy, tissue inflammation, cancer, fever and heart complaints, and as an antiemetic, poisoning antidote, diuretic and refrigerant[5]. Lotus seedpods are sometimes used as a traditional medicine for haemostatic function. The seed powder mixed with honey is useful in treating cough[6].Since the prevalence of human disease is on rise the antibiotic resistance exhibited by microbes have paved way for alternative therapy and one such is phytotherapy[7]. Lotus stem encompasses rich amount of polyphenolic compounds, which exhibit rich antioxidant properties[8]. However, industrial production of food products from lotus is not popular. Therefore, the present study aims to evaluate the phytochemical activity and antioxidant activity of lotus (*Nelumbo nucifera*) stem.

MATERIALS AND METHODS

The present study was carried out to evaluate the phytochemical and antioxidant composition assay of lotus (*Nelumbo nucifera*) stem.

Design of the study

The experimental design was carried out in the following phases.

Phase I: Collection and authentication of plant

The stem of *Nelumbo nucifera*, were collected from the ponds of Chengalpattu, kanchipuram District of Tamil Nadu, India and authenticated by Dr. Jeya Jothi, Taxonomist, Department of Plant Biology and Biotechnology, Loyola College, Chennai.

Phase II: Extraction of Nelumbo nucifera stem

Fresh stem were washed with distilled water to remove mud. The stems were crushed in the form of slices with a sharp knife .The stem was dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Five grams of the lotus stem powder were weighed. The lotus stem powder (5 g) was taken in Soxhlet apparatus and subjected to consecutive hot percolation extraction in solvents like ethanol, acetone and water. The extraction with each solvent was carried for 4 hours followed by filtration and evaporation of the solvent to dryness.

Phase III: Evaluation of Selected Chemical Constituents

1. Proximate Principles: Proximated principles like carbohydrate, fat, protein and fiber in *Nelumbo nucifera* stem was evaluated using standard AOAC procedures.
2. Minor Nutrients: Quantative analysis of vitamin C and iron was carried out.
3. Determination of ash content: Ash content was determined by gravimetrically. About 2.5gms of sample was weighed into a porcelain crucible. The sample was burned with a Bunsen burner before

placing in a model Furnace at 580°C overnight to complete the ashing process.

4. Determination of moisture content: Moisture content was determined by thermo gravimetrically. About 3gms of sample was weighed into a moisture dish. The sample was dried at hot air oven for 4hrs at 105^{o}C. Empty weight of dish, before and after drying was measured to calculate moisture content in lotus stem (% Moisture = M2-M3/M2-M1 X 100).

Phase IV: Qualitative phytochemical analysis

The phytochemical tests was carried out using standard methods of analysis of carbohydrates, tannins, saponins, flavanoids, alkaloids, quinines, glycosides, cardiac-glycosides, terpenoids, triterpenoids, coumarins, steroids, phytosteroids, phlobatanins and anthroquinones[9].

a. Test for Carbohydrates

To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.

b. Test for Tannins

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

c. Test for Saponins

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

d. Test for Flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

e. Test for Alkaloids

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

f. Test for Quinones

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

g. Test for Glycosides

To 2ml of plant extract, 3ml of choloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

h. Test for Cardiac Glycosides

To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides.

i. Test for Terpenoids

To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates presence of terpenoids.

j. Test for Phenols

To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

k. Test for Coumarins

To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins.

l. Tests for Steroids and Phytosteroids

To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

m. Tests for Phlobatannins

To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of phlobatannins.

n. Tests for Anthraquinones:

To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones.

Phase V: Quantitative phytochemical analysis

Determination of total phenol content

The amount of total phenol content of different solvent extracts was determined by Folin-Ciocalteu's reagent method[10.,]. The various concentrations of the extract were made up to 3.5ml with distilled water and 0.125 ml of Folin-Ciocalteu's reagent was mixed and the

mixture was incubated at room temperature for 6 minutes. Then, 1.25 ml of saturated sodium carbonate solution (0.7 N) was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm using a digital spectrophotometer, against a blank sample. The calibration curve was made by preparing gallic acid (20 to 100 µg ml-1) solution in distilled water. Total phenol content is expressed in terms of Gallic acid equivalent (mg g-1 of extracted compounds)[11].

Determination of flavonoid content

The amount of flavonoid content of different solvent extracts was determined by aluminium chloride colorimetric method[12]. The various concentration of the extract were made upto 2.5 ml using distilled water. The reaction mixture consisted of 0.1 ml of sample , 75µg of 5% $NaNo_2$, 150 µg of 10% aluminium chloride and was incubated at room temperature for 6 min. Then 0.5ml of 1 M NaOH was added and the absorbance of all samples was measured at 510 nm using a digital spectrophotometer, against a blank sample. The calibration curve was made by preparing a standard rutin (50 to 250 µg ml-1) solution in ethanol. The flavonoid content is expressed in terms of standard rutin equivalent (mg g-1 of extracted compound).

Ferric reducing antioxidant power (FRAP)

The antioxidant activity of the different solvent extracts of *Nelumbo nucifera* stem was evaluated by FRAP assay. The reducing ability of different solvent extracts was determined by FRAP assay[13] .FRAP assay is based on the ability of antioxidants to reduce Fe3+ to Fe2+ in the presence of TPTZ, forming an intense blue Fe2+-TPTZ complex with an absorption maximum at 750 nm. This reaction is pH-dependent (optimum pH 3.6). 0.1 ml extract is added to 3.0 ml FRAP reagent [10 parts 300 mM sodium acetate buffer at pH 3.6, 1 part 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl and 1 part 20 mM FeCl3] and the reaction mixture is kept in a water bath at 50°C for 20 min and then the absorbance was measured at 750 nm. FeSO4 (100 to 1000 µM ml-1) was used as a positive control[14].

RESULTS AND DISCUSSION

Evaluation of Selected Chemical Constituents of Nelumbo nucifera stem

Proximated principles like carbohydrate, protein, fat and fiber in lotus stem was evaluated using standard AOAC procedures. Foods providing 10-19 percent of the RDA are considered to be a good source of nutrients and foods providing 20 percent or more of the RDA is considered to be high or excellent sources of a nutrient, but foods providing lower percentages of the RDA also contribute to a healthful diet. The chemical and Nutrient constituents of lotus stem as compared to the RDA is presented in Table 1.

Table 1
Chemical and Nutrient constituents of lotus stem as compared to the RDA

Principle	Nutritive value per 100g	RDA (Adult man)	% RDA	Source of Nutrient
Carbohydrate (g)	13.40	-	-	-
Protein (g)	14.60	60	**24.3%**	**Excellent**
Fat (g)	0.30	30	1%	Low
Fibre (g)	5.04	30	**16.8%**	**Good**
Iron(mg)	1.65	17	**10%**	**Good**
Vitamin C (mg)	20.07	40	**50.1%**	**Excellent**
Moisture (%)	13.48	-	-	-
Ash (g)	3.88	-	-	-

Recommended Dietary Allowance for Indians, ICMR, 2009
10-19% DV = Good source of nutrient ≥ 20% DV = High/excellent source of nutrient

The nutrient content of lotus stem was analysed and was compared with the RDA given by ICMR. Lotus stem was found to be an excellent source of protein and vitamin C, while it is a good source of fiber and Iron. As compared to the available literature, the carbohydrate content of lotus stem (13.4 g) found to be low compared to root (17.2 g). Lotus stem is a rich source of protein (14.6 g) when compare to root (2.6 g). Scarcity of

protein rich food and food supplements are responsible for protein energy malnutrition particularly in India. These stem proteins would play a great role in the management of malnutrition[15].

Phytochemical analysis of lotus stem

The phytochemical tests was carried out using standard methods of analysis of carbohydrates, tannins, saponins, flavanoids, alkaloids, quinines, glycosides, cardiac-glycosides, terpenoids, triterpenoids, coumarins, steroids, phytosteroids, phlobatanins and anthroquinones. Qualitative analysis of phytochemical present in Lotus stem is presented in Table 2.

Table 2
Qualitative analysis of phytochemical present in Lotus stem

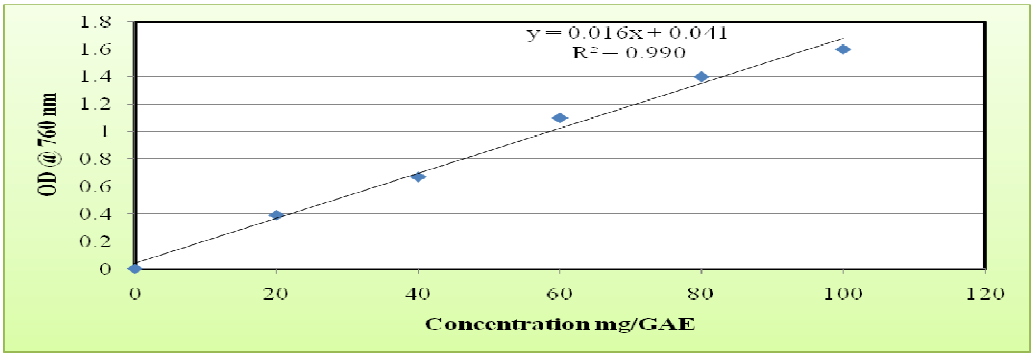
Phytoconstituents	Stem extracts		
	Ethanol	Aqueous	Acetone
Carbohydrate	+	+	+
Tannin	-	+	-
Saponins	-	-	-
Flavanoids	+	+	+
Alkaloids	-	+	-
Quinines	+	-	+
Glycosides	-	-	-
Cardiac glycosides	+	-	+
Terpenoides	+	+	+
Phenol	+	+	+
Coumarins	+	+	+
sterols and phytosterols	+	+	+
Phlobatannins	-	-	+
Anthoquinones	-	-	-

Carbohydrate, flavanoids, quinones, cardiac glycosides, terpenoids, phenol, coumarines, sterols, phyto sterols is present in the extracts of lotus stem. Tannins and alkaloids were present only in the aqueous extract. Flavanoids present in the lotus stem might help to reduce risk of asthma, certain types of cancer, and coronary heart disease. Generally, all the phytonutrients present in the plant help to prevent disease and keep our body working properly. Tannins help to reduce ulcerative colitis[16] .The major phytochemicals present in lotus seeds are alkaloids (e.g dauricine, lotusine, nuciferine, pronuciferine, liensinine, isoliensinine, roemerine, nelumbine, neferine)[17] . Dauricine and neferine block the Na+, K+ and Ca2+ transmembrane currents in cardiac cells . As anti-arrhythmic, neferine significantly inhibit the rabbit platelet aggregation[18] .Moreover, a large number of natural compounds such as ascorbic acid, tocopherols, phenolic acids, and other phytochemical compounds present in food materials have been reported to possess antioxidant properties due to the presence of hydroxyl groups in their chemical structures[19]. These antioxidant compounds prevent the oxidative damage to macromolecules by scavenging the free radicals produced in various biochemical processes occurring in the human body. The other possible mechanisms reported for the activity of antioxidant compounds include prevention of chain initiation, prevention of hydrogen abstraction, peroxide decomposition and reduction of metal ions[8] .

Determination of total phenol content

Total phenol compounds, as determined by Folin-Ciocalteu method, are reported as gallic acid equivalents by reference to standard curve ($y=0.016x + 0.041$, $R^2 = 0.990$). The Total Polyphenol Content (TPC) of lotus stem extracts was found to be high containing 52.50, 30.63 and 130.88 mg GAE/100 ml for ethanolic, aqueous and acetone extracts respectively. Acetone extract of lotus stem was found to have high polyphenol content. Considering the wide variation in the total polyphenol content, foods are divided into two groups, namely foods high in polyphenol content (>100 mg gallic acid equivalents (GAE) 100 ml) and low polyphenol content foods (<100 mg GAE/100 ml)[10] .

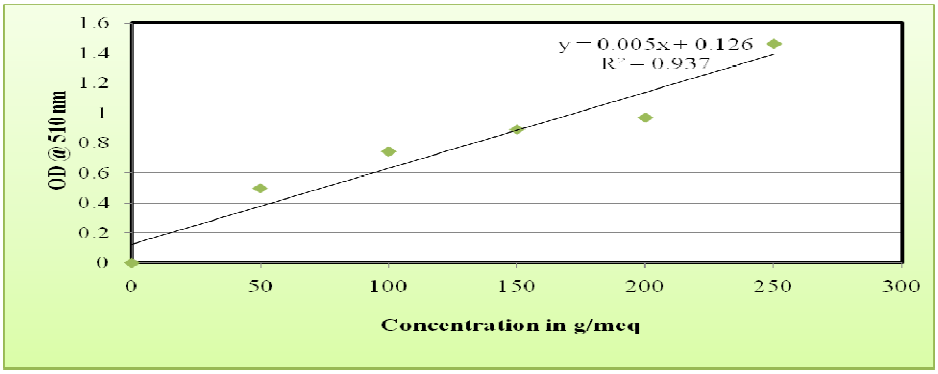
Graph 1
Standard Calibration Curve of Gallic Acid



Determination of flavonoid content

Total flavonoid content is reported as rutin equivalents by reference to standard curve (y=0.005x + 0.126, R^2 = 0.937). The Total Flavonoid Content (TFC) of lotus stem extracts was found to be high containing 77.8, 3.8 and 2.6 mg Rutin equivalence/100 ml for ethanolic, aqueous and acetone extracts respectively. Compared to the other extracts aqueous extract of lotus stem is found to have the highest flavonoid content. The flavonoids are a diverse group of polyphenolic compounds widely distributed in the plant kingdom and over 4000 structurally unique flavonoids have been identified in plant sources. These are primarily recognized as the pigments responsible for the many shades of yellow, orange, and red in of flowers, fruit, and leaves. The major actions of flavonoids are those against cardiovascular diseases, ulcers, viruses, inflammation, osteoporosis, diarrhea and arthritis[20].

Graph 2
Standard Calibration Curve of Rutin

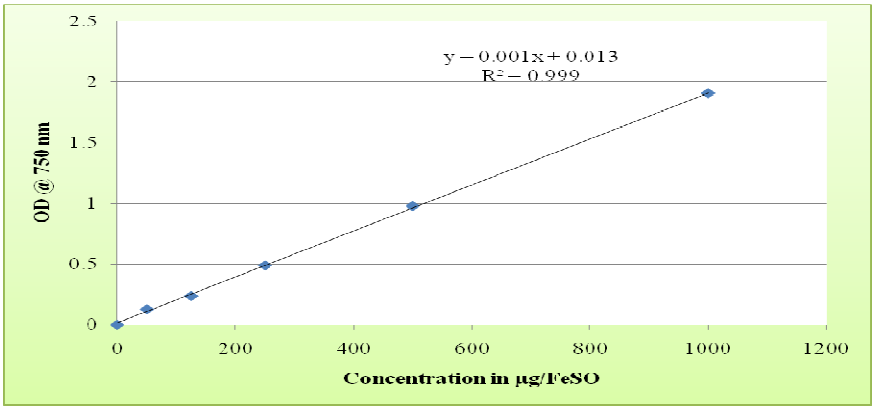


Antioxidant activity of lotus stem by FRAP method

Antioxidant activity of ethanolic, acetone and aqueous extract of lotus stem was detected using the FRAP Method. In FRAP assay, when a ferric tripyridyltriazine (FeIII -TPTZ) complex is reduced to the ferrous (FeII) form, an intense blue colour with an absorption maximum at 750nm develops and measured at low pH. The antioxidants present in lotus stem as determined by FRAP assay, are reported as $FeCl_2$ equivalents by reference to standard curve (y=0.001x + 0.013, R^2 = 0.999). The antioxidant of lotus stem extracts was found to be high containing 227, 451, 1168 mg $FeCl_2$/100 ml for ethanolic, aqueous and acetone extracts respectively.The free radical scavenging and protective effects of lotus seed extracts (LSE) against reactive nitrogen, sodium nitroprusside (SNP), peroxynitriteinduced cytotoxicity and DNA damage in macrophage RAW 264.7 cell lines.Inhibitory effects of the seeds extracted with water (LSWE), ethyl acetate (LSEAE) and hexane (LSHE) were evaluated. Results showed that all the extracts inhibit nitric oxide

accumulation in LPS-activated RAW 264.7 cells. The extracts (0.01-0.2 mg/ml) showed dose-dependent inhibitory effect on the accumulation of nitric oxide upon decomposition of SNP. The potency of inhibitory activity was high in LSEAE followed by LSWE and LSHE. Results on the effect of three seed extracts on macrophage DNA damage[21].

Graph 3
Standard Calibration curve of ferric chloride



CONCLUSION

The result of this study clearly indicates that nutritive value of lotus stem is high compared to nutritive value of lotus root as seen in the literature. Phytochemicals present in the lotus stem were carbohydrate, flavanoids, quinones, cardiac glycosides, terpenoids, phenol, coumarines, sterols, phyto sterols. These phytochemical are used to reduce risk of asthma, diabetes, certain types of cancer, and coronary heart disease, ulcerative colitis, and other innumerable diseases and Similar results were also obtained by various researchers[22,23,24].The genus n*elumbo* is endowed with a number of medicinally important activities antidiabetic, antipyretic, anti-inflammatory, anticancerous, antimicrobial, antiviral and anti-obesity properties.Furthermore, *N.nucifera* flowers are served as healthy beverages to treat hypertension, cancer,diarrhea, fever, weakness, infection and body heat imbalance. It has been widely used in folk medicine for the treatment of various inflammatory and infectious diseases. Different parts of the lotus plant are useful in treatment of diarrhea, tissue inflammation and haemostasis. The rhizome extract has anti-diabetic and anti-inflammatory properties due to presence of asteroidal triterpenoid. Rhizomes are used for pharyngopathy, pectoralgia,spermatorrhoea, leucoderma, small pox, diarrehoea, dysentery and cough. The stem is used in indigenous Ayurvedic medicines as diuretic, anthelmintic and to treat strangury, vomiting, leprosy, skin disease and nervous exhaustion.In India lotus is used in many ways like pickle preparation and cooking. The lotus root can be processed into flour and used in preparation of number of products. Together with the lotus seeds, the edible root contains an abundant amount of starch, sugars, proteins, lipids, vitamins and minerals. They are easily digested and are a good and nutritious food for all ages. Composition of the lotus reveals starch as the main component. The fresh root contains 15% of starch. Starch as a raw material has various applications in the manufacturing of food products like imparting texture and consistency and to acts as functional ingredients like thickeners, stabilizers and gelling agent[25].Pharmaceutical and food industries have exploited terpenoids for their potentials and effectiveness as medicines and flavor enhancers. Lotus stem contains several phytochemicals, polyphenols and flavonoids. These compounds are powerful antioxidants. In conclusion, the present study on the phytochemical and antioxidant activity

of Nelumbo nucifera stem signify that Lotus stem can be used effectively as a therapeutic agent for nutritional therapy and phyto-therapy for the alleviation of various diseases. Plant derived nutraceuticals should be produced using Nelumbo nucifera stem.

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