AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Preliminary Phytochemical Screening and Free Radical Scavenging Activity on Aerial Plant of *Nelumbo Nucifera* Flowers

Kirithika.T[1*] Bhaigyabati Thoudam[1], Gomathi R[1], Usha K[1].

1.Department of Biochemistry, Biotechnology and Bio informatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore-43, Tamil Nadu, India.

ABSTRACT

Nelumbo nucifera Gaertn. (Family -Nymphaeaceae) commonly known as Indian lotus. Also known as the sacred lotus has been used in the indigenous system of medicine. Phytochemical constituents, free radical scavenging activity and total antioxidant activity of various extracts of *Nelumbo nucifera* flowers were carried out in the study. Phytochemicals were extracted from *Nelumbo nucifera flowers* using various solvents such as aqueous, benzene, chloroform, ethanol, ethyl acetate and methanol and petroleum ether. Screening of phytochemicals showed positive results for the presence of flavanoids, alkaloids, phenols, glycosides, carbohydrates and tannins. Phytochemicals were best extracted in methanol. The antioxidant activity of the extracts was measured in terms of reducing power and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity. The methanolic extract was found to possess comparatively higher antioxidant properties. So, the present data suggests that methanolic extract of lotus flower constitute significant amounts of phytochemical compounds and are good source of antioxidants.

Keywords: *Nelumbo nucifera,* Phytochemicals, methanolic extract, DPPH

*Corresponding Author Email: kirithimarx@gmail.com

Received 6 March 2013, Accepted 14 March 2013

Please cite this article in press as: Kirithika T *et al.*, Preliminary Phytochemical Screening and Free Radical Scavenging Activity on Aerial Plant of *Nelumbo Nucifera* Flowers. American Journal of PharmTech Research 2013.

INTRODUCTION

Nelumbo nucifera Garetn, namely known as Lotus, it is a perennial aquatic plant grown and consumed throughout Asia. All parts of *Nelumbo nucifera* have been used in oriental medicine for various medicinal purposes. Its leaves are known for diuretic and astringent properties and are used to treat fever, sweating, and strangury, and also used as a stypic. Moreover, the extract of *Nelumbo nucifera* leaves has been found to inhibit digestion, slower absorption of lipid metabolism, up-regulate energy expenditure, reduce the development of atherosclerosis, show hepatoprotective and antioxidant activity and inhibit human peripheral blood mononuclear cell proliferation in vitro[1].The ripe seed of plant are very effective in case of neurasthenia, spermatohoea and metrorrhoea. The decoction of leaves and seeds are very effect for insomnia, haemorrhage and haematemesis. Filaments in the form of decoction are used in treating body stool, haematourea, utrein haemorrhage and hematemesis[2].It also has antioxidant, physicopharmacological, antidiabetic, antiobisity, antimalarial and antifungal effect, anti diarrhoeal, hepatoprotective and free radical scavenging and antipyretic effect[1]. The most important macromolecules (lipids, proteins and nucleic acids) in human body as well as in food materials have been reported to undergo oxidative damage caused by free radicals such as superoxide and hydroxyl radicals generated in certain biochemical processes[3]. These free radicals may lead to protein denaturation, lipid peroxidation, DNA lesions and finally diseased conditions if not captured effectively. 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[4]. These antioxidant compounds prevent the oxidative damage to macromolecules by scavenging the free radicals produced in various biochemical processes occurring in human body[5]. 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[6].Usually all parts of this plant viz. rhizome, leaves and seeds, are utilized by humans as food products and herbal medicines. The rhizomes of lotus possess hypoglycaemic, diuretic, anti-inflammatory and antioxidant activities. These are recommended with other herbs as traditional medicines for the treatment of fever, sunstroke and various types of gastric problems[7]. The present work deals with the preliminary phytochemical investigation of various extracts (benzene, chloroform, ethanol, ethyl acetate, methanol and petroleum ether) of Nelumbo nucifera, to identify the major group of

phytochemicals which impart the medicinal property to the plant. Free radical scavenging activity using DPPH assay and total antioxidant of various extracts of *Nelumbo nucifera* were also analyzed.

MATERIALS AND METHODS

Collection of the Medicinal plant

The fresh white lotus flower was collected from Nagercoil, Kanyakumari district. Selected samples were authenticated (BSI/SRC/5/23/2012-13/Tech 1700) by Botanical Survey of India, Coimbatore.

Extraction, Preparation and Isolation of the selected medicinal plants:

The flower of *Nelumbo nucifera* was dried initially under shade. It was preserved in air tight containers and it was powdered with the use of mechanical grinder. For the preliminary study, Fresh samples of *Nelumbo nucifera* were screened for the presence of phytochemicals. Five grams of *Nelumbo nucifera* were weighed, mashed and homogenized with 50ml of alcohol, acid (1% HCl) and water separately. These were boiled for one hour, cooled, filtered and used for the analysis of phytochemicals. The extract of *Nelumbo nucifera* flowers were subjected to preliminary phytochemical screening such as flavanoids, phenols, anthocyanins, tannins, saponins, steroids, alkaloids and terpenoids using standard procedure[8].

Preparation of the Organic Extract for Phytochemical Analysis

Dried and powdered flowers were defatted to remove fatty material. 20 gm of weighed powdered flowers of *Nelumbo nucifera* were packed in Soxhlet extractor separately and extracted with petroleum ether at 60-80°C for 36 hrs and completion of extraction was confirmed by pouring a drop of extract from the thimble on a filter paper, which does not show the presence of any oil spot. The column pack of leaves and flowers were removed and dried, then they were subjected separately to continuous hot extraction with absolute methanol in soxhlet apparatus for 24 hrs and completion of extraction was confirmed by pouring a few drop of extract from the thimble, left no residue on evaporation. After complete extraction the solvent was evaporated and concentrated to dry residue for further analysis. The qualitative phytochemical tests of various extracts of *Nelumbo nucifera* were carried out using standard procedure[9].

Determination of Free Radical Scavenging Activity (DPPH Radical Scavenging Activity)

The free radical scavenging activity of the methanolic extract was determined according to the method of Braca[10]. Briefly, 0.1 ml of extract and standard compound BHA (butylated hydroxyanisole) of variable concentration (100-500 µg/ml) was added to 3 ml of 0.004% DPPH (in ethanol). The samples were incubated for 30 min in the dark and the absorbance of the

reaction mixture was measured at 517 nm spectrophotometrically[11]. The standard compound BHA was used for comparison. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percent of DPPH radical scavenging activity was calculated as [(Ac-Ae)/Ac] 100, where Ac is the absorbance of the control and Ae is the absorbance of the extract/standard [11.]

Determination of Antioxidant Capacity by Phosphomolybdenum Method:

The antioxidant activity of the methanolic extract was determined by the phosphomolybdenum method as described by Prieto[12]. Briefly, 0.3 ml of extract was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95 °C for 90 min and cooled to room temperature. Finally, absorbance was measured at 695 nm using a spectrophotometer12 against blank. Methanol (0.3 ml) in place of extract was used as the blank. The total antioxidant capacity was expressed as the number of equivalents of ascorbic acid (AAE) in micrograms per milligram of extract [13.]

$$\text{Total antioxidant activity} = 100\ [1- (A_o-A_t) / (Ao0–A_t0)]$$

Where A_o is the OD of the sample at time to minutes and A_t is the time of the sample at time t = 90minutes. A_o0 and A_t0 represent the OD of the control at time t = 0 minutes and t = 90 minutes respectively.

RESULT & DISSCUSION

Phytochemical analysis

The preliminary phytochemical screening showed the presence of Phytoconstituents such as alkaloids, carbohydrates, proteins, phenolic compounds, glycosides, flavanoids and tannins.

Table -1 Phytochemical constituent of Various Extracts of *Nelumbo nucifera*

Phytochemicals	Inference						
	A	B	C	E	EA	M	PE
Amino acids	+	-	-	+	-	+	-
Anthraquinones	-	+	+	+	+	+	-
Alkaloids	+	-	-	+	_	+	-
Carbohydrates	+	-	+	+	+	+	-
Flavanoids	+	+	+	+	+	+	-
Glycosides	-	+	+	+	+	+	-
Saponins	-	-	-	-	-	_	-
Steroids	+	+	-	+	+	+	-
Tannins	+	+	+	+	+	+	-
Terpenoids	+	+	+	+	+	+	-
Phenols	+	+	+	+	+	+	-
Fixed oils and fats	-	-	-	-	-	-	+

+ Presence; - Absence

A -Aqueous; B-Benzene; C-Chloroform; E-Ethanol; EA -Ethyl acetate; M -Methanol; PE-Petroleum ether

The phytochemical analysis of the aqueous, benzene, chloroform, ethanol, ethyl acetate, methanol and petroleum ether extracts of Nelumbo nucifera showed positive results for the presence of flavonoids, alkaloids, phenols, steroids, glycosides, carbohydrates, aminoacids, terpenoids and tannins. Methanolic extract of *Nelumbo nucifera* flower gave the maximum extraction of phytochemicals than any other extracts. Methanolic extract was followed by the presence of phytochemical constituents.

The *Nelumbo nucifera* plant has antioxidant, anti-diabetic, anti-obesity, anti-fungal activity that are the due to the presence of alkaloids, nuciferin, nor-nuciferine, flavonoids, glycosides and polyphenolic constituents[14].

Free radical scavenging activity of *Nelumbo nucifera*

The stable free radical scavenging activity by DPPH method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific plant extract[15].Figure 1 indicates the percentage of free radicals scavenging activity in various extractions with different concentrations (10-100µg) of Nelumbo nucifera.

The result showed that methanolic extract of Nelumbo nucifera gave the highest DPPH scavenging activity among the various extracts. Among the extracts, chloroform extract showed the second highest scavenging activity and the least activity was noted in ethyl acetate fraction. The IC_{50} of methanol extract of Nelumbo nucifera was found to be 69µg/ml. This indicates the Nelumbo nucifera is a rich source of antioxidant.

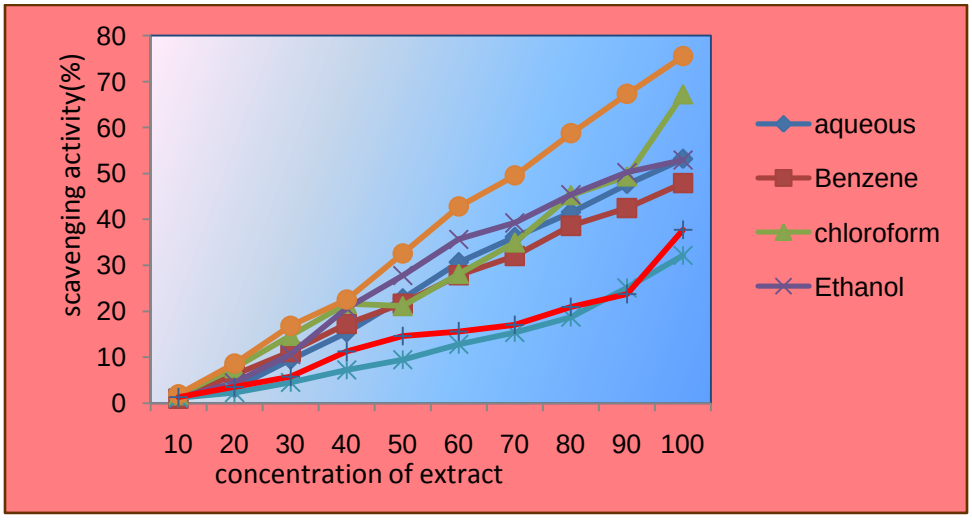

Figure1 (DPPH free radical scavenging activity)

Total Antioxidant Capacity of *Nelumbo Nucifera*

The total antioxidant potential of various extracts of *Nelumbo nucifera* was analyzed. Figure 2 was shown the total antioxidant activity of various extracts of *Nelumbo nucifera*. Methanolic

extract of Nelumbo nucifera exhibited strongest total antioxidant activity of 89.75 µg of ascorbic acid/mg of extract among the various extracts while ethyl acetate yielded the lowest value of 32.56 µg of ascorbic acid/mg of extract. The antioxidant activity of methanolic extract of *Nelumbo nucifera* may be due the presence of flavanoids and phenols in the extract. The results indicate that *Nelumbo nucifera* rich in phytochemical, which may be responsible for its medicinal property. The antioxidant activity of *Nelumbo nucifera* may be due the presence of flavanoids and tannins. Among the extracts methanolic extract showed the maximum activities for DPPH and total antioxidant activity. Further studies are under progress in our laboratory for the isolation of the active compounds

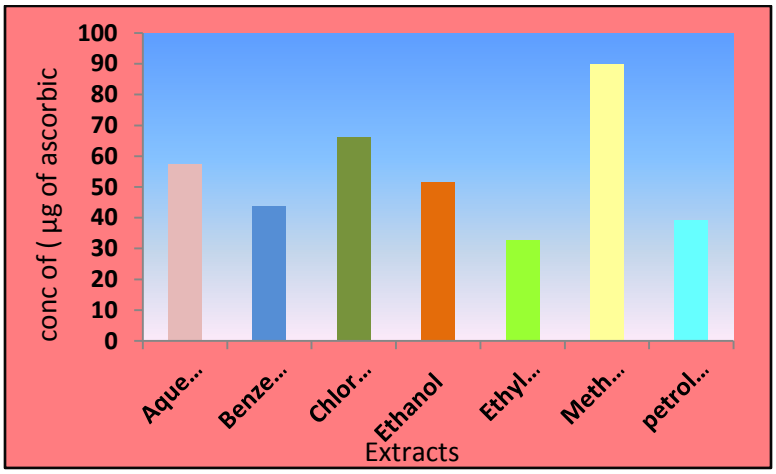

Figure 2 Total antioxidant activity of various extract of *Nelumbo nucifera* flower)

CONCLUSION

From the study, it may be concluded that *Nelumbo nucifera* is a rich source of phytochemical and has antioxidant property. Phytochemical were extracted best in methanol among the solvents and methanolic extract showed the maximum DPPH and total antioxidant activities.

ACKNOWLEDGEMENT

The Authors give grateful thanks to Dr. Nilesh, Scientist, Botanical survey of India, Coimbatore. For their help rendered for identification of Plants.

REFERENCE

1. K.R. Sridhar, B. Rajeev. Lotus- A potential nutraceutical source.J Agri Tech, vol. 3, 2007:143-155.
2. P.N. Tongtorsak, N. Chaichit and J. Panyajirawut. Acute and chronic hypotensive effects of crude leaf extract of Nelumbo nucifera Gaertn. TJPS 2004;.17:68-69.
3. C.W. Saengkhae, Arunnopparat and P. Sungkhajorn. Antioxidant activity of Nelumbo nucifera Gaertn on oxidative stress-induced Erythrocyte hemolysis in hypertensive and normotensive rats.

J. Physiol. Sci 2008;20:70-78.

4. Rai S, Wahile A, Mukherjee K, Padas aha B and Mukherjee PK. Antioxidant activity of Nelumbo nucifera (Sacred lotus) Seeds. J Ethanopharmacol 2006: 104,322-327.
5. Mukherjee PK, Saha K, Balasubramanian R, Pal M, Saha BP. studies on psychopharmacological effect of *Nelumbo nucifera* Gaertn, rhizome extract. J Ethanopharmacol 1996: 54, 63-67.
6. D.K. Pa, S. Dutta. Evaluation of the Antioxidant activity of the roots and rhizomes of Cyperusrotundus L. Indian J. Pharma Sci 2006;68: 256-258.
7. Mensor, LI, Menezes, FS, Leitao, GG, Reis, AS., dos Santos, T., Coube, C.S and Leitao, S.G. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical metho. Phytotherapy Res 2001:15, 127-130.
8. J. Parekh, S. Chanda. Antibacterial activity of the crude methanol extract of Flower (Lythraceae). Brazilian J Microbiology 2007;38: 204-207.
9. M.V.Kumaraswamy, H.U.Kavitha and S.Sathish. Antibacterial evaluation and phytochemical analysis of Betula utilis against some human pathogenic bacteria. Word journal of agricultural science 2008;.4 (5): 661-664.
10. M. Valko, C.J. Rhodes, J. Moncol, M. Izakovic and M. Mazur. Free radical metals and antioxidants in oxidative stress-induced cancer.Chemico. Biol. Interaction 2006; 160:1–40.
11. C.R. Suresh, V. Annam, K. Pratibha K, M. Prasad. Total antioxidant capacity-a novel early biochemical marker of Oxidative stress in HIV infected individuals. J. Biomed. Sci 2009;1: 61-65.
12. P. D. Duh, Y. Y. Tu, G. C. Yen .Antioxidant activity of water extract of HarngJyur (Chrysanthemum moifolium Ramat. Lebensm–WissTechnol 1999;32:269-277.
13. Y.Lu, L.Y.Foo. Antioxidant activities of polyphenols from sage (Salvia officinalis). Food chemistry. 2000;75: 197-202.
14. C.Nagarajan, D.Alam, G. Subhan and P.Nyeen. Antioxidant and hepatoprotectant action of crude alcoholic extract of Wrightiatomentosa. Oriental Pharmacy and Experimental medicine 2008;.81:241-248.
15. Y. Rogger, L. Stanier, L. Mark. Assessment of antimicrobial and antifungal activity of common medicinal plants. Int J Pharmacology 2000;3(6):499-504