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Invitro Antioxidant and Free Radical Scavenging Activity of Aqueous and Ethanolic Flower Extract of *Nymphaea Alba*

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Abstract

Nymphaea alba also known as the European White Waterlily, White Lotus or Nenuphar, is an aquatic flowering plant of the family Nymphaeaceae. The flowers are white and they have many small stamens inside. It contains the active alkaloids nupharine and nymphaeine, and is a sedative and an aphrodisiac/an aphrodisiac.In this study, the antioxidant activity of aqueous and ethanolic extracts from flower of Nymphaea alba was evaluated by various antioxidant assays including total antioxidant, hydrogen peroxide scavenging and nitric oxide scavenging activities. Both extracts have exhibited significant antioxidant activity in DPPH, Nitric oxide and Hydroxyl radical induced in-vitro assay methods. The results indicate that both the extracts firmly possess strong antioxidant effects .Comparatively the ethanolic flower extract showed more antioxidant activity than the aqueous extracts. The results obtained from the present study indicate that the Nymphaea alba flower extract can be a potential source of natural antioxidant.

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Nymphaea alba, Antioxidant activity; DPPH; Nitric oxide; Hydroxyl radical.

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Introduction

Free radicals or reactive oxygen species (ROS) exert oxidative stress towards the cells of the body by various mechanisms and cause damage to cellular proteins, nucleic acids, membrane lipids, and eventually cell death. All cells in the body has a

enzymatic antioxidant defense system such as superoxide desmutase (SOD), catalase, glutathione-s-transferase (GST) etc. and

Nonenzymatic antioxidant defense such as vitamin E and vitamin C to combat oxidative stress[1]. However, as per need occasionally synthetic antioxidant like butylated hydroxy anisole (BHA) or butylated hydroxy toluene (BHT) or propyl gallate (PG) are supplemented to reduce the oxidative damage but due to side effect these are not well accepted antioxidant[2]. So, attention directed to find out the alternative remedy from natural resources with strong antioxidant

properties having low toxicities. Plant and its products are rich sources of a phytochemicals

and have been found to possess a variety of biological activities including antioxidant potential [3]. Natural antioxidants are in high demand for application as nutraceuticals, bio-pharmaceuticals, as well as food additive because of consumer preference.

Nymphaea alba, also known as the **European White Waterlily**, **White Lotus**, or **Nenuphar**, is an aquatic flowering plant of the family Nymphaeaceae. It grows in water from 30-150 centimeters deep and likes large ponds and lakes. The leaves may be up to thirty centimeters in diameter and they take up a spread of 150 centimeters per plant. The flowers are white and they have many small stamens inside. It contains the active alkaloids nupharine and nymphaeine, and is a sedative and an aphrodisiac/anaphrodisiac depending on sources. The root of the plant was used by monks and nuns for hundreds of years as an anaphrodisiac, being crushed and mixed with wine. It is rich in tannic acid, gallic acid, alkaloids, sterols, flavonoids, glycosides, hydrolyzable tannins and high-molecular-weight polyphenolic compounds. [4] All the parts of the plant have medicinal uses in traditional system of medicine. It is used as an aphrodisiac, anodyne, antiscrophulatic, astringent, cardiotonic, demulcent, sedative and anti-inflammatory. Further, it also produces calming and sedative effects upon the nervous system, and is useful in the treatment of insomnia, anxiety and similar disorders. [5-7]Its anticarcinogenic action and inhibition of renal oxidative stress and hyperproliferative response were reported [8-10]

It also possess good anxiolytic activity.[11]In the past few years, interest in the study of

antioxidant activity of plant extract has been increased due to the fact that reactive oxygen

species is responsible for various diseases. So now a days antioxidants are added to a

variety of foods to prevent free radicals and the inhibition of free radical generation can serve

as facile model for evaluating the activity of anticancerous agents[12]. Gallic acid and ellagic acid are two widely occurring phenolic compounds present in *Nymphaea alba* flower, to which many biological activities including anticancer and antiviral activity have been attributed[13].

Understanding that, there has been increasing demand to evaluate the antioxidant and anticancer properties of bioactive compounds from plant origin rather than to look at synthetic options,

the objective of our study is to evaluate the antioxidant properties of Aqueous and Ethanolic flower extract of *Nymphaea alba* as a part of exploration of new and

novel bio-active compounds.

Materials and Methods:

Preparation of plant extract:

A fine dried powder (25 mesh) of sample (*Nymphaea alba* flower) was extracted using 50 ml of 70% ethanol at 75 ^{0}C for 2.5 h by reflx. The extracts were filtered through Whatman No.4 filter paper under reduced pressure, frozen and then

lyophilized (Ly-8-FMULE, Snijders). All the samples were redissolved in 70% ethanol at a concentration of 5.0 mg/ml and analysed for their

contents of polyphenols and flavonoids and DPPH radical-scavenging activity, nitric acid scavenging and hydroxyl radical scavenging activities.

Chemicals

1-diphenyl-2-picrylhydrazyl (DPPH) and Naphthylethylenediamine dihydrochloride was purchased from Sigma Chemical Co. All other reagents were used of analytical grade.

DPPH Radical Scavenging assay:

It is one of the most extensively used antioxidant assay for plant samples.This assay is based on the measurement of the scavenging ability of antioxidant test substances towards the stable radical. The free radical scavenging activity of aqueous and ethanolic extracts was examined *in vitro* using DPPH radical [14,15]. The test extracts were treated with different concentrations from a maximum of 250 µg/ml to minimum of 4 µg/ml. The reaction mixture consisted of 1 ml of 0.1mM DPPH in ethanol, 0.95 ml of 0.05 M Tris-HCl buffer (pH 7.4), 1 ml of ethanol and 0.05 ml of the herbal extract. The absorbance of the mixture was measured at 517 nm exactly 30 sec after adding extract. The experiment was performed (in triplicate) .

DPPH radical scavenging that is calculated by the Formula:%DPPH radical scavenging

$$= \frac{\text{sample absorbance}}{\text{control absorbance}} \times 100$$

Ascorbic acid is used as a positive control.

Hydroxyl Radical Scavenging Activity[16]:

The Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe3+ / ascorbate/ EDTA/ H_2O_2 system. The reaction mixture contained deoxy ribose (2-8mM), Fecl (0.1mM), EDTA (0.1mM), H_2O_2 (lmM), ascorbate (0.1mM), KH PO - KOH buffer (20mM, 2 4 pH 7.4) and various concentrations (25-400 µm of extracts and std 10 to 80 µm /ml) of standard drug in a final volume of 1 ml. The reaction mixture was incubated for 1hr at 37°C, deoxyribose degradation was measured with spectrophotometer at 532 nm.

The percentage of hydroxyl radical scavenging activity was calculated by the formula:

$$\%\text{Hydroxyl radical scavenging} = 1 - \frac{\text{Difference in sample absorbance}}{\text{Difference in control absorbance}} \times 100$$

Nitric Oxide Scavenging assay[17]

Sodium nitroprusside (5 µM) in standard phosphate buffer solution was incubated with different concentration of the test extracts dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25°C for 5 hr. After 5 hr, 0.5 ml of incubation solution was removed and diluted with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water). The absorbance of chromophore formed was read at 546 nm. The control experiment was also carried out in similar manner, using distilled water in the place of extracts. The experiment was performed (in triplicate) and The activity was compared with ascorbic acid, which was used as a standard antioxidant.

Nitric oxide radical scavenging that is calculated by the Formula:

$$\%\text{Nitric oxide radical radical scavenging} = \left(\frac{\text{Control OD - Sample OD)}}{\text{Control OD}}\right) \times 100$$

Result: Statistical Analysis: The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnet,s t – test, P- values < 0.05 were considered as significant.

Table 1: *In vitro* free radical scavenging effect of *Nymphaea alba* extract by DPPH method

Drug	4 µg/ml	8 µg/ml	15 µg/ml	30 µg/ml	60µg/ml	125 µg/ml
AENA	20.33 ± 0.002*	21.45 ± 0.001*	22.82 ± 0.001*	29.70 ± 0.001*	35.50 ± 0.001*	46.35 ± 0.002*
EENA	27.02 ± 0.002*	29.31± 0.002*	31.85 ± 0.001*	33.31 ± 0.001*	45.44 ± 0.002*	47.03 ± 0.002*
Vit C	0.1 µl/ml	0.2 µl/ml	0.4 µl/ml	0.6µl/ml	0.8 µl/ml	1 µl/ml
	5.82 ± 0.002	14.12 ± 0.001*	28.21 ± 0.001*	45.18 ± 0.003*	61.25 ± 0.001*	77.12 ± 0.001*

AENA = Aqueous extract of *Nymphaea alba*, EENA = Ethanolic extract of *Nymphaea alba*.*
P < 0.001 compared to reagent blank

Table 2: *In vitro* free radical scavenging effect of *Nymphaea alba* extract by hydroxyl radical scavenging method.

Drug	25 µg/ml	50 µg/ml	100 µg/ml	200µg/ml	400µg/ml
AENA	16.21 ± 1.13	28.52 ± 1.11	33.62 ± 0.82	46.71 ± 1.21	52.20 ± 0.76
EENA	17.14 ± 1.11	29.47 ± 0.82	41.52 ± 0.45	57.17 ± 0.58	72.25 ± 0.92
Vit C	10 µg/ml	20 µg/ml	40 µg/ml	60µg/ml	80 µg/ml
	26.34 ± 0.95	40.51 ± 0.72	55.21 ± 0.84	67.32 ± 0.52	78.42 ± 0.45

AENA = Aqueous extract of *Nymphaea alba*, EENA = Ethanolic extract of *Nymphaea alba*.*
P < 0.001 compared to reagent blank.

Table 3: *In vitro* free radical scavenging effect of *Nymphaea alba* extract by nitric oxide scavenging method

Drug	4 µg/ml	8 µg/ml	15 µg/ml	30 µg/ml	60µg/ml	125 µg/ml
AENA	1.25 ± 0.002	6.35 ± 0.001*	6.71 ± 0.003*	7.73 ± 0.003*	9.42 ± 0.004*	15.72 ± 0.001*
EENA	43.21 ± 0.002*	43.72± 0.002*	43.35 ± 0.001*	44.51 ± 0.001*	44.62 ± 0.002*	47.25 ± 0.002*
Vit C	0.1 µl/ml	0.2 µl/ml	0.4 µl/ml	0.6µl/ml	0.8 µl/ml	1 µl/ml
	3.21 ± 0.002	13.46 ± 0.001*	32.13 ± 0.001*	41.24 ± 0.003*	63.31 ± 0.001*	76.40 ± 0.001*

AENA = Aqueous extract of *Nymphaea alba*, EENA = Ethanolic extract of *Nymphaea alba*.*
P < 0.001 compared to reagent blank.

Results and Discussion:

Non Enzymatic Assay :

1.DPPH radical Scavenging assay:

In the present study several free radical scavenging activities of Aqueous and Ethanolic Flower extract of *Nymphaea alba* were evaluated by DPPH scavenging assay . Aqueous and Ethanolic Flower extracts of *Nymphoea alba* have got profound antioxidant activity. DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, which gets decolorized in the presence of antioxidants.[18,19] The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, it gets decolorized which can be quantitatively measured from the changes in absorbance at 517nm.The Aqueous and Ethanolic Flower extracts of *Nymphoea alba* exhibited a significant dose dependent inhibition of DPPH activity. In this study, at 125 µg /ml, the ethanolic extract showed highest inhibition of DPPH activity when compared to the Aqueous Flower extract of *Nymphaea alba* shown in (Table 1).The results of DPPH-free radical scavenging assay suggest that the EENA flower extract is more capable of scavenging free radicals than AENA.

2. Hydroxyl radical Scavenging assay:

The hydroxyl radical is an extremely reactive free radical formed in biological system. It has been implicated as a major active oxygen centered radical formed from the reaction of various hydroperoxides with transition metal ions, which is capable of damaging almost every molecule found in living system causing lipid peroxidation and biological damage [20,21]. The maximum *Hydoxyl* radical scavenging effect was found at 400µg /ml concentration. The EENA flower extract showed higher scavenging activity than AENA shown in (Table 2).This ability of the extracts shows the quenching ability of hydroxyl radicals, which seems to be a good scavenger, of active oxygen species thus reducing the rate of chain reaction.

3. Nitric oxide Scavenging assay:

Nitric oxide (NO) is a potent pleiotropic mediator of physiological process such as smooth muscle relaxant, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical, which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities[22]. Although nitric oxide and superoxide radicals are involved in host defense, over production of these two radicals contributes to the pathogenesis of some inflammatory diseases[23]. Moreover in the pathological conditions, nitric oxide reacts with superoxide anion and form potentially cytotoxic molecules, peroxynitrite. Nitric oxide inhibitors have been shown to have beneficial effects on some aspects of inflammation and tissue damage seen in inflammatory diseases[24]. EENA significantly inhibited nitric oxide in a dose dependent manner (Table 3) than the AENA at a concentration of 125µg/ml. The results indicates that, the extract might contain compounds capable of inhibiting nitric oxide and offers scientific evidence for the use of the *Nymphaea alba* flower extract in the indigenous system in treatment of various diseases.

Conclusion :

The use of traditional medicine is widespread and plants still represent a large source of natural antioxidants that might serve as leads for the development of novel drugs.

Several anti-inflammatory, digestive, anti necrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have an antioxidant and/or radical scavenging mechanism as part of their activity [25-27] . The data presented in our study reveals that the ethanolic extract of *Nymphaea alba* (EENA) flower showed strong antioxidant activity than Aqueous Extract (AENA) by inhibiting DPPH, hydroxyl radical, nitric oxide scavenging activities when compared with standard drug Ascorbic acid. *Nymphaea alba* flower extract possesses free radical scavenging activity which could exert a beneficial action against liver damage induced by different exogenous and endogenous sources. The present study also suggests that phenolic compounds of the *Nymphaea alba* flower like Gallic acid and ellagic acid is found to provide a good source of antioxidants that could offer potential protective effects against lipid oxidation. These results remain important as the first step in screening antioxidant activity of *Nymphaea alba* flower. It can be concluded that, the free radical scavenging effects of the aqueous and ethanolic flower extract of *Nymphaea alba* is highly promising to be an effective antioxidant.and can be used as an accessible source of natural antioxidants with consequent health benefits.

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