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ORIGINAL ARTICLE

In-vitro Evaluation of Antioxidant Activity of *Nymphaea Stellata* Willd.

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NIKHIL K SACHAN, DOLI RANI DAS, ANUPAM K SACHAN, MOHD. SHUAIB: In-vitro Evaluation of Antioxidant Activity of Nymphaea Stellata Willd.

ABSTRACT

The present study was designed to investigate the radical scavenging activity of methanolic extract of Nymphaea stellata was evaluated in vitro with the spectrophotometric method based on reduction of stable 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical and compared with the positive control of Butylated hydroxytoluene. The result was expressed as IC50. The IC50 was calculated by plotting the graph between % inhibition vs concentration. The IC50 value of extract and Butylated hydroxytoluene was found to be 1.68291 mgm and 46.25 mgm for 2ml of 500 mM concentration of DPPH. Overall study reveals that the methanolic extract of Nymphaea stellata will show the moderate comparison to Butylated hydroxytoluene.

Key words: Nymphaea stellata, Antioxidant, Butylated hydroxytoluene, DPPH, IC50.

Introduction

The plant *Nymphaea stellata* Willd. (Nymphaceae), a medicinal herb has reported for its use in treatment of liver disorders in Ayurvedic literature. The leaves root and flowers have a wide range of pharmacological activities and are used for diabetes eruptive fevers and as cardiotonic, emollient, diuretic, narcotic, and as aphrodisiac [1]. The flowers of plant contain 6 flavonoid, gallic acid, astragalin, quercetin and kaempferol. And the seeds also contain 7 protiens, pentason, mucilage etc. But yet the plant has not been subjected to systematic scientific investigation to assets its antioxidant activity of this plant. There are many evidences indicates that free radical are responsible for birth of many disorders like inflammation, atherosclerosis, aging and hepatic toxicity [2].

Antioxidants or inhibitors of oxidation are the compounds which retard or prevent the oxidation in general and prolong the life of oxidizable matter [3]. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals and also by acting as reactive species scavenger. The antioxidants activities of the individual compounds may depends on structural factors, such as number of phenolic, hydroxyl or methoxyl groups and other structural features [4]. Among the antioxidative compounds vitamin C, E, A, Selenium, Carotenoids, Ascorbic acid shows very strong intensity of antioxidative activities [5]. Antioxidant determinations by the use of stable free radical were earliest being done in 1958 by Blois [6].

A free radical is a compound with one or more unpaired electrons in its outer orbital [7]. Such unpaired electrons make these species very unstable and therefore quite reactive with other molecules due to the presence of unpaired electrons and try to pair their electrons and generate a more stable compound [8].

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The molecule of DPPH is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm.

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 color (although there would be expected to be a residual pale yellow color from the picryl group still present). Representing the DPPH radical by Z and the donor molecule by AH, the primary reaction is;

$$Z^* + AH = ZH + A^* \quad (1)$$

Where, ZH is the reduced form and A* is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorized) by one molecule of the reductant. The reaction [1] is therefore intended to provide the link with the reactions taking place in an oxidising system, such as the autoxidation of a lipid or other unsaturated substance; the DPPH molecule Z* is thus intended to represent the free radicals formed in the system whose activity is to suppressed by the substance AH [9].

Materials and methods

Collection of Plant Material:

The flower of *Nymphaea stellata* plant was purchased from Khari-Bawoli market, Old Delhi.The plant was identified as *Nymphaea stellata*. F-Nymphaceae by the Dr. E Roshini Nayar (Principal Scientist), National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi. A voucher specimen (Specimen No: NHCP/NBPGR/2010-7/6652) is preserved in herbarium section of taxonomic department of NBPGR, New Delhi

Preparation of Extract:

The flower of plant after washing with distilled water was air dried at room temperature. The dried flowers were coarsely powdered and successfully extracted with methanol using Soxhlet apparatus at a temperature of 60-70^{0}C for a period of 6-8 hrs. The extract was then concentrated over water bath and finally dried to a constant weight.

Preparation of Reagents:

The 500µM solution of$DPPH was made by 23 mg of DPPH (Assay 85%) of Hi Media Laboratories Pvt. Ltd. CAS No. 1898-66-4. TRIS [2-amino-2 (hydroxy methyl) propane 1-3di-ol] Buffer pH 7.4 was prepared by adding 0.605g of TRIS Buffer 7.4 of Qualigens Fine Chemicals in 30 ml of water and adding 0.33 ml of concentrated hydrochloric acid, diluted to 100 ml with distilled water. TRIS buffer prevents the sudden pH change during the preparation of test dilutions [10].

Preparation of Reference Standard Solution:

Various dilutions of butylated hydroxytoluene were made with concentration of 5, 10, 15, 20, 25, 30, 35, 40, 45 & 50 µg per 0.5 ml of methanolic solution of butylated hydroxytoluene.

Preparation of Sample Solution and Dilutions:

Prepared the stock solution by dissolving 250 mg methanolic extract of *Nymphaea stellata* and made up the volume to 25 ml with methanol. Prepared the initial dilutions from stock solution using volume 0.25 ml , 0.5 ml , 0.75 ml , 1.0 ml , 1.25 ml ,1.50 ml 1.75 ml 2.0 ml, 2.25 ml ,and 2.5 ml and dilute upto 10 ml with methanol. The final concentrations used for taking the absorbance are 0.25 mg, 0.50 mg, 0.75 mg, 1.00 mg, 1.25 g, 1.5 mg, 1.75 mg, 2.00 mg, 2.25 mg, and 2.5mg per ml.

Measurement of in Vitro Antioxidant Activity:

The antioxidant activity of the flower of *Nymphaea stellata* was determined by using a method based on the reduction of methanolic solution of colored-free radical 1, 1 di-phenyl-1-2 picryl hydrazyl (DPPH). The radical scavenging activity of tested sample was expressed as an inhibition percentage. Butylated hydroxyl toluene was used as reference standard. In 5 ml volumetric flasks added 1 ml of DPPH solution, 0.5 ml of TRIS Buffer and 0.5 ml of final dilutions of different concentrations range prepared from *N. stellata* stock solution and made up the volume to 5 ml with methanol. In same way, prepared the control dilutions of DPPH, replacing 0.5 ml of prepared dilutions (the drug solution under investigation) with methanol. The absorbances of all the dilutions were taken after 30 minutes at λ max 517nm using methanol as blank.

Statistical Analysis:

The percentage inhibition was calculated using:

$$\text{Percent Inhibition} = \frac{(A_C - A_S)}{A_C} \times 100$$

Where, Ac is absorbance of control, As is the absorbance of sample. IC_{50} value (a concentration at 50% inhibition) was determined from the curve between percentage inhibition and concentration. All determinations were done in triplicate and the IC_{50} value was calculated by using the equation of line [11].

Results and discussion

The methanolic extract of *Nymphaea stellata* tested for *in vitro* using DPPH showed moderate free radical scavenging activity, as evidenced by low IC_{50} values. Fig-1 depicted that the IC_{50} value of *Nymphaea stellata* was 1.68291.

The absorbance of sample (methanolic extract of *Nymphaea stellata* and standard Butylated hydroxytoluene) were taken in triplicate.

With the increase of concentration, the decrease of absorbance value and the calculated percentage inhibition has shown with the help of tables.

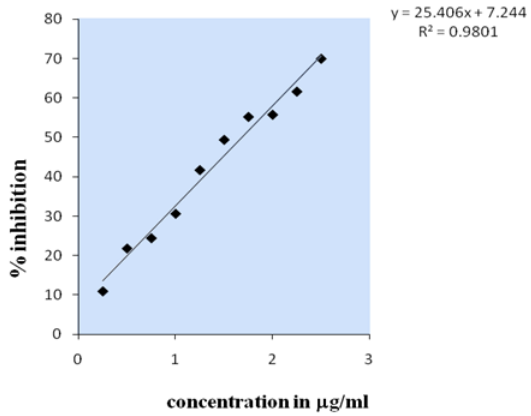

Fig. 1: Graph between concentration (mgm) vs % inhibition of methanoilc extract of flower of *Nymphaea stellata*.
X Axis = Concentration in µg,Y Axis = percentage Inhibition, From the equation, When Y=50, X= 1.68291

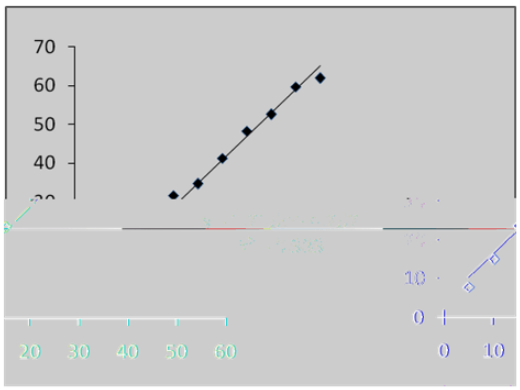

Fig. 2: Graph between concentration (mgm) vs % inhibition of methanoilc extract of flower of *Nymphaea stellata*.
X Axis = Concentration in µg, Axis = % Inhibition, From the equation, When Y=50, X= 46.25

Table 1: Values of absorbance and percentage inhibition with increase in concentration of methanolig extract of Nymphaea stellata.

Concentration(µgm)	Absorbance	%Inhibition
0.250	1.623	10.97
0.500	1.425	21.83
0.750	1.377	24.46
1.000	1.264	30.66
1.250	1.062	41.74
1.500	0.922	49.42
1.750	0.816	55.24
2.00	0.806	55.78
2.250	0.699	61.65
2.500	0.547	69.99

Table 2: Values of absorbance and percentage inhibition with increase in concentration of methanolic solution of butylated hydroxytoluene (standard antioxidant).

Concentration(µg)	Absorbance	Percentage Inhibition
5	2.163 + 0.01	7.78
10	1.996 + 0.04	14.91
15	1.792 + 0.06	23.58
20	1-601 + 0.02	31.75
25	1.531 + 0.09	34.72
30	1.375 + 0.00	41.36
35	1.211 + 0.01	48.36
40	1.111 + 0.00	52.62
45	0.945 + 0.01	59.69
50	0.892 + 0.01	61.94

Conclusion:

Polyphenolic compounds like flavonoids and phenolic acids, commonly found in plants, have been reported to have multiple biological effects, including antioxidant activity [12]. In vitro study shows that *Nymphaea stellata* has moderate free radical scavenging action. Antioxidant property of *Nymphaea stellata* can be attributed to the presence of flavonoids and polyphenols and which in turn may be responsible for its anti-stress effect.

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