WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 4, 2178-2189. Research Article ISSN 2277 – 7105

IN VITRO ANTIOXIDANT ACTIVITY OF *NYMPHAEA RUBRA* L. RHIZOME

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Article Received on
05 May 2014,
Revised on 01 June 2014,
Accepted on 25 Jun 2014

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ABSTRACT

The i*n vitro* antioxidant capabilities of methanol extract of *Nymphaea rubra* rhizome was evaluated by studying 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical scavenging activity, ABTS radical cation scavenging activity and reducing power using standard procedure. Total phenolic content was estimated by folin-ciocalteau method. Flavonoid was determined by Aluminium chloride method. The total phenolics and flavonoids in methanol extract were found to be 0.36 g $100g^{-1}$ and 0.67 g $100g^{-1}$ respectively. The methanol extract of rhizome showed potent *in vitro* antioxidant activities. Among different concentration tested, methanol extract of *N. rubra* rhizome at 1000 µg/mL showed potent scavenging activity in all models viz, DPPH, hydroxyl radical, superoxide and ABTS. The result clearly Indicates that, methanol extract of *N. rubra* rhizome is effective in scavenging free radicals and has the potential to be a powerful antioxidant.

KEY WORD: *Nymphaea rubra*, flavonoid, ABTS, reducing power.

INTRODUCTION

Nymphaea rubra L. (Nymphaceae) is a perennial aquatic rhizomatous herb. The plant is common throughout Asia and is cultivated throughout India. The whole plant serves as astringent, emmolient, diuretic and sudrofic and possesses antifungal, antipyretic and cardiotonic activity. The rhizome extract has antidiabetic, antimicrobial and anti-inflammatory properties.

The rhizomes are used for pharyngopathy, pectoralgia, spermatorrhoea, leucoderma, small pox, diarrohoea, dysentery, anthelmintic and cough. Leaves paste can be applied to the body during fever and inflammation of skin. Flowers are useful to treat diarrhoea, cholera, fever, hepatopathy and hyperdipsia. Seeds are used in the treatment of tissue inflammation, cancer, skin disease, leprosy, and poison antidote are generally prescribed to children as diuretic. [1-2] Keeping this in view, the present communication deals with the evaluation of *in vitro* antioxidant activity of rhizome of *N. rubra.*

MATERIALS AND METHODS

The rhizome of *Nymphaea rubra* L. were freshly collected from Injikuzhi, Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu.The plant specimen was identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology unit, Research department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of Plant extract

Rhizomes of the plant were dried in shade for one week, powdered and extracted with methanol using cold extraction in shaker for 48h at room temperature. The methanol extracts were concentrated in a rotary evaporator to obtain concentrated methanol extract which was then used for the estimation of total phenolic, flavonoid and assessment of antioxidant activities.

Estimation of Total Phenolics

Total phenolic content was estimated using Folin-Ciocalteau reagent based assay as previously described [3] with little modification. To 1mL of each extract (100µg/mL) in methanol, 5mL of Folin-Ciocalteau reagent (diluted ten-fold) and 4mL (75g/L) of Na_2CO_3 were added. The mixture was allowed to stand at 20ºC for 30min and the absorbance of the developed colour was recorded at 765nm using UV-VIS spectrophotometer. 1mL aliquots of 20, 40, 60, 80, 100 µg/mL methanolic gallic acid solutions were used as standard for calibration curve. The absorbance of solution was compared with gallic acid calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE g/100g dry weight of extract).

Estimation of Flavonoids

The total flavonoid content was determined according to Eom *et al.*[4] An aliquot of 0.5 mL of sample (1mg/mL) was mixed with 0.1 mL of 10% aluminium chloride and 0.1 mL of potassium acetate (1M). In this mixture, 4.3 mL of 80% methanol was added to make 5 mL volume. The mixture was vortexted and the absorbance was measured spectrophotometrically at 415 nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H. [5] The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method.[5] Briefly, an 0.1mM solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 mL of the solution of all extracts in methanol at different concentration (125, 250, 500 & 1000 µg/mL). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated by using the following formula. % inhibition= $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al.*[6] Stock solutions of EDTA (1mM), $FeCl_3$ (10 mM), Ascorbic Acid (1 mM), H_2O_2 (10 mM) and Deoxyribose (10 mM), were prepared in distilled deionized water. The assay was performed by adding 0.1mL EDTA , 0.01mL of $FeCl_3$**,** 0.1 mL H_2O_2, 0.36mL of deoxyribose, 1.0mL of the extract of different concentration (125, 250, 500 & 1000 µg/mL) dissolved in distilled water, 0.33mL of phosphate buffer (50 mM, pH 7.9), 0.1 mL of ascorbic acid in sequence . The mixture was then incubated at 37^0C for 1 hour. 1.0 mL

portion of the incubated mixture was mixed with 1.0 mL of 10% TCA and 1.0 mL of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Srinivasan *et al*.[7] The superoxide anion radicals were generated in 3.0 ml of Tris – HCL buffer (16 mM, pH 8.0), containing 0.5 mL of NBT (0.3 mM), 0.5 mL NADH (0.936 mM) solution, 1.0 mL extract of different concentration (125, 250, 500 & 1000 µg/mL), and 0.5 mL Tris – HCl buffer (16 mM, pH 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12 mM) to the mixture, incubated at 25^{o}C for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula

Antioxidant activity by radical cation ($ABTS^{\cdot+}$)

ABTS assay was based on the slightly modified method of Huang *et al*.[8] ABTS radical cation ($ABTS^{+}$) was produced by reacting 7mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The $ABTS^{+}$ solution were diluted with ethanol to an absorbance of 0.70±0.02 at 734 nm. After addition of 100µL of sample or trolox standard to 3.9 mL of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC). The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Reducing power

The reducing power of the extract was determined by the method of Kumar and Hemalatha.[9] 1.0 mL of solution containing 50,100,200,400 &800µg/mL of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%). The mixture was incubated at 50^{o}C for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5^{o}C) in a refrigerator centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

Statistical analysis

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA). Estimates of mean, standard error for aforesaid parameters were calculated.

RESULT AND DISCUSSION

The total phenolics and flavonoid contents of methanol extract of *N. rubra* rhizome were found to be 0.36 g/100 g and 0.67 g/100 g respectively. Phenolics are diverse secondary metabolites abundant in plant tissues. Polyphenols possess ideal structural chemistry for free radical scavenging activity, and they have been shown to be more effective *in vitro* antioxidants than phenols and ascorbate. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors, from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain breaking function) and their ability to chelate transition metal ions (termination of the Fenton reaction). [10] Another mechanism underlying the antioxidative properties of phenolics is the ability of flavonoids to alter peroxidation kinetics by modification of the lipid packing order and to decrease fluidity of membranes.[11] These changes could sterically hinder diffusion of free radicals and restrict peroxidative reaction. Moreover, it has been shown recently that phenolic compounds can be involved in the hydrogen peroxide scavenging cascade in plant cells. [12]

Flavonoids, the major group of phenolic compounds reported for their antimicrobial, antiviral and spasmolytic activity. Flavonoids ability of scavenging hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals, which highlights many of the flavonoid health-promoting functions in organisms, which are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA. Flavonoids in human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms. Flavonoids, on the other hand, are potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anticancer activity.[13-14]

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The generation of free radicals can bring about thousands of reactions and thus cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radical.[15] Antioxidants may offer resistance against oxidative stress by scavenging the free

radicals or protecting the antioxidants defense metabolisms. Antioxidant effectiveness is measured by monitoring the inhibition of oxidation of a suitable substrate. Various methods are available for determining free radical scavenging effects. The rhizome of *N. rubra* extracted in methanol was tested for its *in vitro* antioxidant potential using various models viz DPPH, hydroxyl, superoxide and ABTS radical cation.

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical is widely used as a model system to investigate the free radical scavenging activities of several plant extracts. DPPH is stable, nitrogen centered free radical which produces violet colour in methanol solution. It was reduced to a yellow coloured product with, diphenyl picryl hydrazine, with the addition of the extracts. The reduction in the number of DPPH molecules can be calculated with the number of available hydroxyl groups.[16] The DPPH scavenging activity of methanol extract of *N. rubra* rhizome was found to increase in a concentration dependent manner. The results of DPPH radical scavenging activity of the extract and the standard ascorbic acid were presented in Figure 1. The extract exhibited potent DPPH radical scavenging activity. The IC_{50} value of the methanol extract of rhizome (18.26 µg/mL) was lower than standard ascorbic acid. (33.26 µg/mL) (Fig.2). IC_{50} is often used to express the amount of concentration of extracts needed to scavenge 50% of the free radicals. The IC_{50} value is inversely proportional to the scavenging activity of the extract.[17] The result of the present study showed that, the methanol extract of *N. rubra* contained high amount of radical scavenging compounds with proton donating ability.

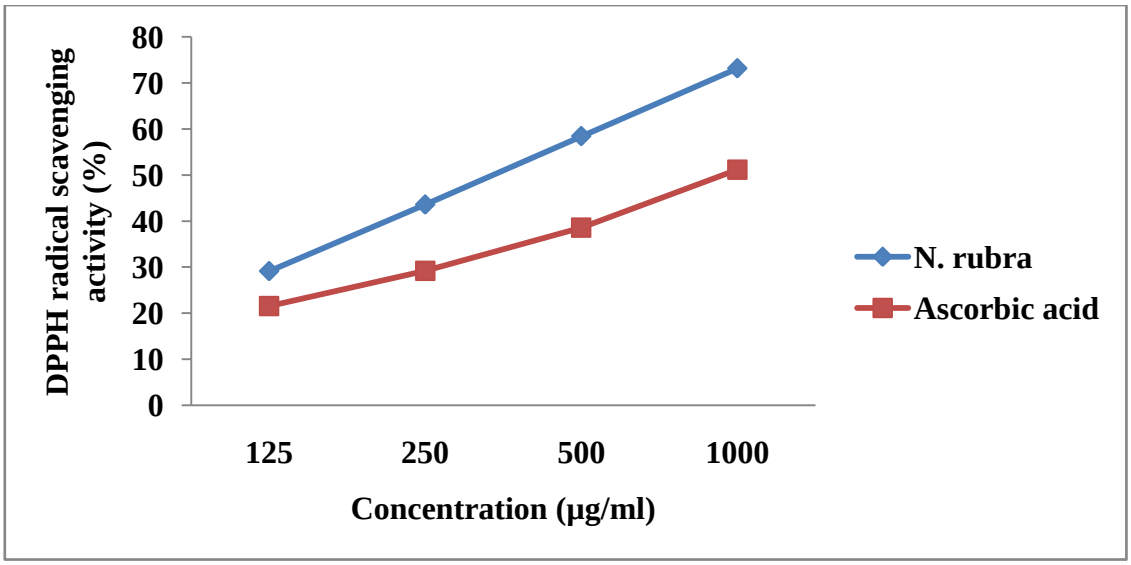

Fig. 1: DPPH radical scavenging activity of methanol extract of *Nymphaea rubra* rhizome.

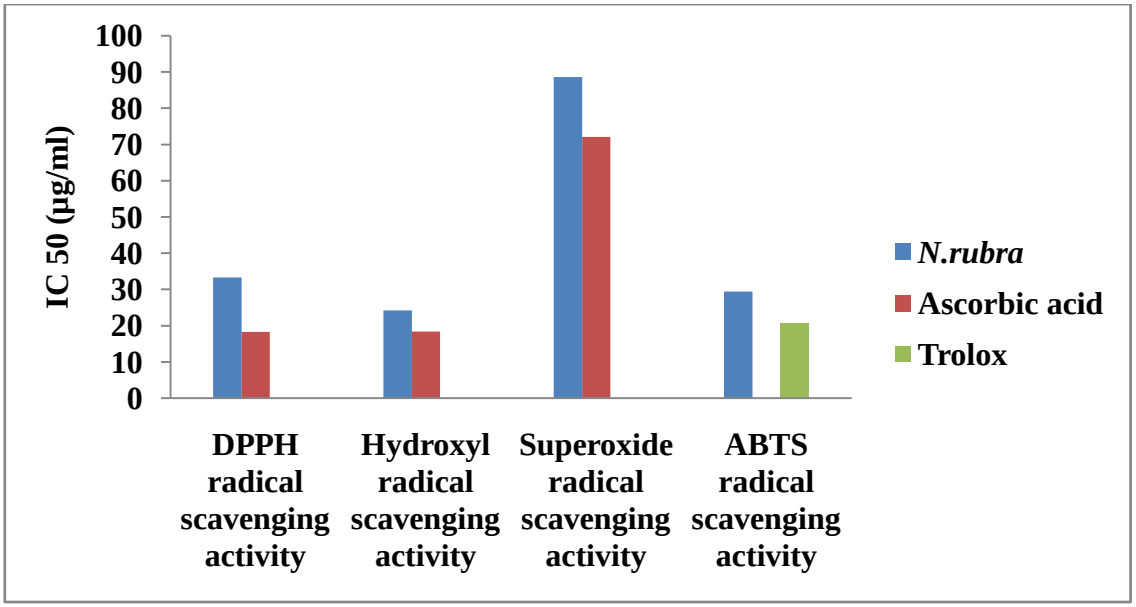

Fig. 2: IC $_{50}$ values of methanol extract of *Nymphaea rubra* rhizome.

The hydroxyl radical scavenging activity is measured as the percentage of inhibition of hydroxyl radicals generated in the Fenton reaction mixture by studying the competition between deoxyribose and the extract for hydrogen radicals generated from Fe^{3+}/EDTA/H_2O_2 systems. The hydroxyl radicals attack deoxy ribose which eventually results in TBARS formation.[18] The hydroxyl radical scavenging activity of methanol extract of *N. rubra* rhizome was found to increase in a concentration dependent manner. All the concentration of *N. rubra* showed higher activity than the standard ascorbic acid. Figure 3 showed hydroxyl radical scavenging activity of methanol extract of *N. rubra* rhizome compared with standard ascorbic acid. It was observed that methanol extract of rhizome exhibited more hydroxyl scavenging activity than standard ascorbic acid at all the concentrations. At a concentration of 1000 µg/mL, hydroxyl scavenging activity of methanol extract reached 76.90% with low IC_{50} value of 18.46 µg/mL (Fig. 2) while at the same concentration, ascorbic acid was found to be 69.73% (IC_{50} value=24.19 µg/mL)

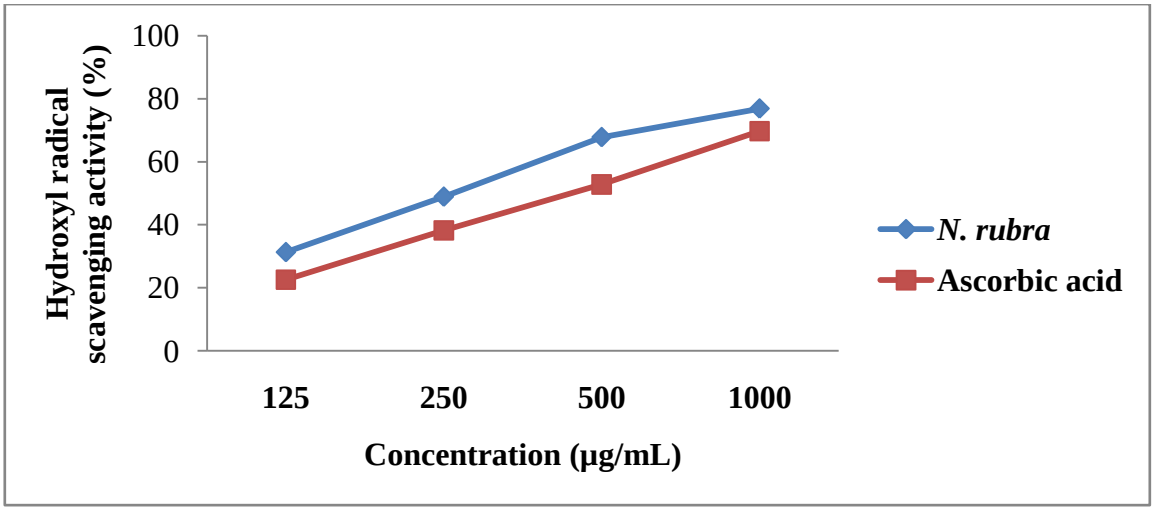

Fig. 3: Hydroxyl radical scavenging activity of methanol extract of *Nymphaea rubra* rhizome.

Superoxide radical scavenging activity of methanol extract of *N. rubra* rhizome extract was presented in figure 4. Superoxide produced from molecular oxygen due to oxidative enzymes of body as well as via non-enzymatic reactions such as antioxidation by catecholamines. The decrease in absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture.[19] The superoxide radical scavenging activity of *N. rubra* rhizome was found to increase in a concentration dependent manner. Methanol extract of *N. rubra* rhizome showed maximum scavenging activity of 84.16% (IC_{50} value=88.61 µg/mL) at 1000 µg/mL while at the same concentration ascorbic acid was found to be 95.34% (IC_{50} value=72.08 µg/mL) (Fig. 2). The superoxide radical scavenging activity of rhizome extract was comparable to ascorbic acid.

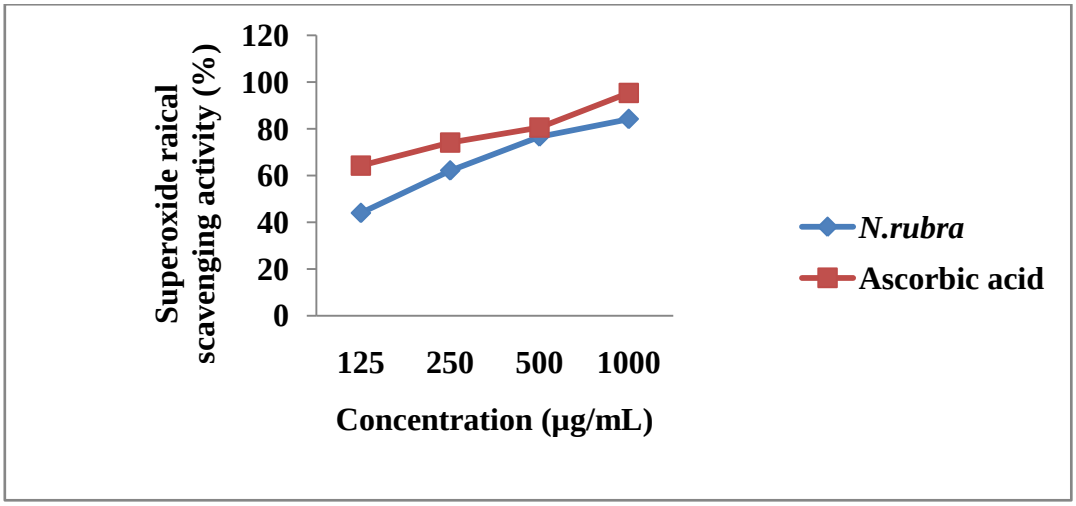

Fig. 4: Superoxide radical scavenging activity of methanol extract of *Nymphaea rubra* rhizome.

The ABTS activity is based on the inhibition of the absorbance of the radical cation $ABTS^{+}$, which has a characteristic long wavelength absorption spectrum.[20] The ABTS radical cation scavenging potential of the methanol extract of rhizome of *N. rubra* was presented in figure 5. The ABTS radical cation scavenging activity was found to increase in a concentration dependent manner. All the concentration of *N. rubra* showed higher activity than the standard trolox. The percentage scavenging activity and IC_{50} of the investigated extract at 1 min. of reaction time was calculated. Methanol extract of *N. rubra* rhizome showed a maximum scavenging activity of 58.27% at 1000 µg/mL concentration with low IC_{50} value of 20.67 µg/mL whereas trolox showed IC_{50} value of 29.46 µg/mL (Fig. 2).

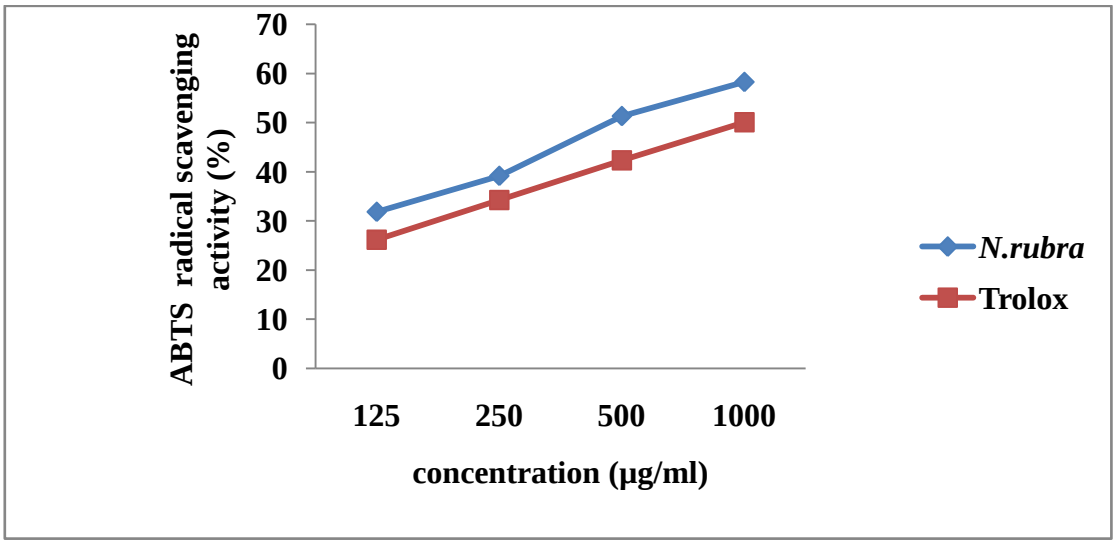

Fig. 5: ABTS radical cation scavenging activity of methanol extract of *Nymphaea rubra* rhizome.

Figure 6 showed the reducing capabilities of the plant extract compared to ascorbic acid. The reducing ability of a compound generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the free radical chains, donating the hydrogen atom. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.[21] The reducing capacity of *N. rubra* rhizome is a significant indicator of its potential antioxidant activity. The reducing power of methanol extract of rhizome of *N. rubra* was very potent and the reducing power of the extract was increased with quantity of extract. At a concentration of 1000µg/mL reducing power of methanol extract of rhizome was 0.694%

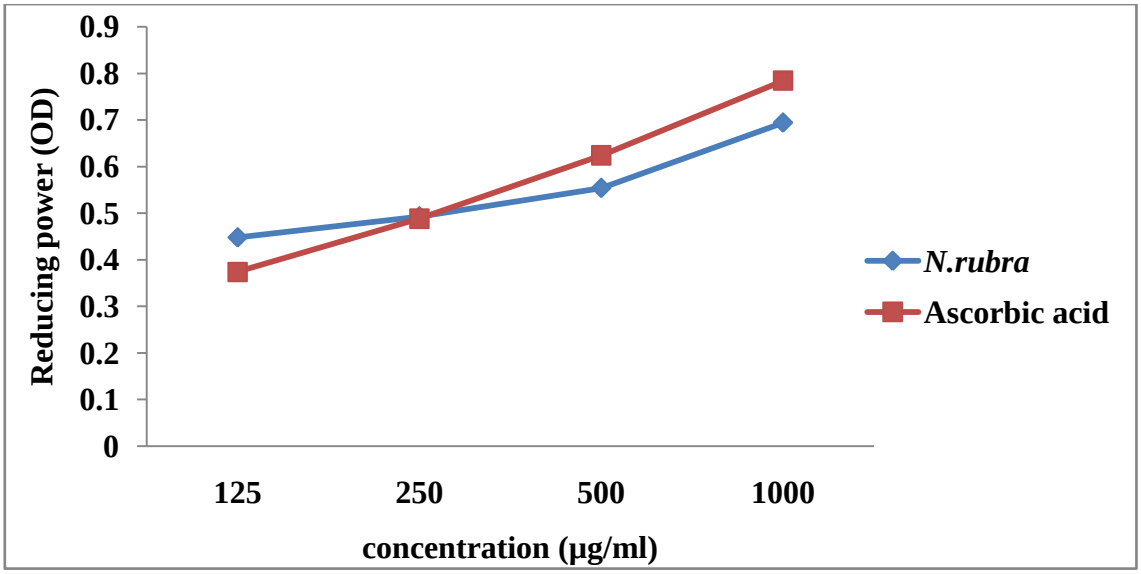

Fig. 6: Reducing power ability of methanol extract of *Nymphaea rubra* rhizome.

CONCLUSION

The present study clearly indicated that, the methanol extract of *N. rubra* rhizome showed strong antioxidant activity by inhibiting DPPH free radical scavenging, hydroxyl radical scavenging, superoxide radical scavenging and ABTS radical cation scavenging activities when compared with standard ascorbic acid and trolox. The rhizome of *N. rubra* as promising natural sources of antioxidants suitable for application in nutritional/pharmaceutical fields, in the prevention of free radical mediated diseases. Further studies are needed to explore the potential phenolic compound(s) from *N. rubra* and *in vivo* studies are needed for better understanding on their mechanism of action.

REFERENCE

1. Indian Herbal Pharmacopoeia. Indian Drug Manufacturing Association, Mumbai, 254-264, 2002.
2. Behera SK, Mohapatra TK, Dash V. Anthelmintic activity of rhizomes of *Nymphaea rubra* Linn. Anc Sci Life, 2010; 29:33-36.
3. McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem, 2001; 73:73-84.
4. Eom SH, Cheng WJ, Hyoung JP, Kim EH, Chung MI, Kim MJ, Yu CY, Cho DH. Far Infra red ray irradiation stimulates antioxidant activity in *Vitis flexuosa* Thunb. Berries. Korean J Med Crop Sci, 2007; 15: 31-32.

5. Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P. Antioxidant activity *in vitro* of selenium-contained protein from the se-enriched. *Bifodobacterium animalis* 01. *Anaerobe*, 2010; 16:380-386.
6. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: a simple test to be assay for determination of rate constants for reaction of hydroxyl radicals. Ana Biochem, 1987; 165: 215-219.
7. Srinivasan R, Chandrasekar MJN, Nanjan MJ, Suresh B. Antioxidant activity of *Caesalpinia digyna* root. J Ethnopharmacol, 2007; 113:284-291.
8. Huang MH, Huang SS, Wang BS, Sheu MJ, Hou WC, *et al.* Antioxidant and anti-inflammatory properties of *Cardiospermum halicacabum* and its reference compounds *ex vivo and in vivo.* J Ethnopharmacol, 2011; 133:743-750.
9. Kumar RS, Hemalatha S. *In vitro* antioxidant activity of alcoholic leaf extract and subfractions of *Alangium lamarckii* Thwaites. J Chem Pharm Res, 2011; 3:259-267.
10. Sanjay J, Bhumilke S, Rakesh B. Evaluation of antioxidant potential of *Tinospora cordifolia* and *Tinospora sinensis.* Int J Pharmaceu Sci Re, 2010; 1:122-128.
11. Abirami B, Gayathri P, Uma D. *In vivo* antioxidant potential of *Pterocarpus marsupium* bark. Int J Chem Pharmaceau Sci, 2012; 3:17-24.
12. Patel VR, Patel PR, Kajal SS. Antioxidant activity of some related medicinal plants in Western region of India. Adv Biol Res, 2010; 4:23-26.
13. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem, 2010; 48:909-930.
14. Mbaebie BO, Edeoja HO, Ajolayan AJ. Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq. Asian Pac JTrop Biomed, 2012; 2:118-124.
15. Sasikumar JM, Maheshu V, Aseervatham GSB, Darsini DTP. *In vitro* antioxidant activity of *Heydibis corymbosa* (L) Lam. aerial parts. Indian J Biochem Biophy, 2010; 47:49-52.
16. Merinal S, StellaBoi GV. *In vitro* antioxidant activity and total phenolic content of leaf extracts of *Limonia crenulata* (Roxb.). J Nat Prod Plant Resour, 2012; 2:209-214.
17. Hue SM, Boyce AN, Somasundram C. Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato (*Ipomea batatas*). Australian J Crop Sci, 2012; 6:375-380.
18. Battu CR, Ethadi SR, Vede PG, Swathi PK, Chandrika K, Rao VA *et al.* Evaluation of antioxidant and anti-inflammatory activity of *Euphorbia heyneana* Sprang. Asian Pac J Trop Biomed, 2011; 1:191-194.

19. Basniwal PK, Sutha M, Rathore GS, Gupta R, Kumar v, Pareek A, Jain D. *In vitro* antioxidant activity of hot aqueous extract of *Helicteris isora* Linn. fruits. Nat Prod Radi, 2009; 8:483-487.
20. Vijayabaskar P, Shiyamala V. Antioxidant properties of seaweed polyphenol from *Turbinaria ornata* (Turner) J.Agardh.1848. Asian Pac J Trop Biomed, 2012; 590-598.
21. Arulmozhi S, Mazumdar PM, Narayanan LS, Thakurdesai PA. *In vitro* antioxidant and free radical scavenging activity of fractions from *Alstonia scholaris* (L.)R.Br. Int J Pharm Tech Res, 2010; 2:18-25.