International Journal of Natural Sciences Research
2014 Vol. 2, No. 8, pp. 133-146
ISSN(e): 2311-4746
ISSN(p): 2311-7435

FREE RADICAL SCAVENGING POTENTIAL OF *NELUMBO NUCIFERA* GAERTN FLOWERS (WHITE AND PINK)

Brindha Durairaj[1] --- Arthi Dorai[2]
[1,2]Department of Biochemistry, PSG College of Arts and Science, Tamilnadu, India

ABSTRACT

Considering the growing interest in assessing the antioxidant capacity of herbal medicine, the present research was aimed to explore antioxidant potentials of NelumbonuciferaGaertn (Nelumbonaceae)flower in-vitro. Hydroethanolic extract of white Nelumbo nucifera (HEWNN) flower and pink Nelumbonucifera (HEPNN) flower were investigated for Total antioxidant capacity. Antioxidant activity by ferric thiocyanate (FTC), Thiobarbituric acid, Ferric reducing antioxidant power, Phosphomolybdenum, Hemoglobin glycosylation, and reducing power methods were estimated in both flowers and compared with standard ascorbic acid in dose dependent manner. In-vitro assays to inhibit free radicals such as 1,1-diphenyl 2-picryl radical (DPPH), superoxide, nitric oxide, hydroxyl radical and hydrogen peroxide (H_2O_2) were also carried out. Antioxidant capacity measured by FTC and compared with TBA method showed low absorbance values than control which indicated a high level of antioxidant potential. Both HEWNN and HEPNN flower extracts exhibited maximum activity 16.53 mg and 14.21 mg at 1000 µg/ml extract concentration in FRAP method and 62.5 mg and 56.3 mg ascorbic acid equivalents at 500 µg/ml extract concentration in phosphomolybdenum method. There was also significantly high antioxidant activity (55.5% & 41.6%) of haemoglobin followed reducing power (0.351 & 0.248 Abs) at same 500 µg/ml extract concentration. The results obtained suggest that alkaloids, phenols and flavonoids in flowers yield considerable antioxidant activity. The maximum scavenging activity of HEWNN and HEPNN against DPPH (67.52% & 55.51%), superoxide radical (81.2% & 64.5%), nitric oxide (70.2% & 57.7%), and hydroxyl radical (60.53% & 46.72%) and H_2O_2 (54.29% & 48.13%) were evaluated. The IC_{50} values were compared to the standard ascorbate in a dose dependent manner. The results obtained suggest that on comparison with HEPNN flower, HEWNN flower extract may act as a better chemo preventive agent providing promising antioxidant property and offering effective protection from free radicals. Our results clearly indicate that both HEWNN and HEPNN flower extracts have potent antioxidant and free radical scavenging capacity in all assays. Nelumbonucifera can be used as a lead compound for drug development in future.

Keywords: *Nelumbo nucifera*, Free radicals, Antioxidants, Superoxide, Hydroxyl radical, Ascorbic acid.

Contribution/ Originality

This study is one of very few studies which have investigated to compare the antioxidant status of two different varieties of *Nelumbonucifera* flower extracts. Our results clearly indicate that both HEWNN and HEPNN flower extracts have potent antioxidant and free radical scavenging capacity in all assays.

1. INTRODUCTION

In an aerobic environment all the animals and plants require oxygen and hence reactive oxygen species (ROS) are ubiquitous [1]. ROS formed *in-vivo*, such as superoxide anion; hydroxyl radical and hydrogen peroxide are highly reactive potentially damaging transient chemical species [2]. It was regulated by endogenous superoxide dismutase, glutathione peroxidase and catalase but due to over- production of reactive species, induced by exposure to external oxidant substances or a failure in the defense mechanisms, damage to cell structures, DNA, lipids and proteins [3]. In addition ROS has been implicated in more than 100 diseases [4]. The most notorious among them being neurodegenerative conditions like Alzheimer's disease [5], mild cognitive impairment [6] and Parkinson's disease [7].

Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals [8]. The natural antioxidant mechanism is inefficient and hence dietary intake of antioxidant compounds is important [9]. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants [10]. Thus the present investigation was aimed in development of new safe and effective antioxidant agents in *Nelumbonucifera* flowers.

The antioxidative phytochemicals in grains, vegetables and fruits have received increasing attention recently for their potential role in prevention of human diseases as well as in food quality improvement [11]. Lotus has been used both as food and medicine in Asia, particularly in India [12]. The antioxidant activity of plant extracts vary with methods [13, 14].

The Indian herb *NelumbonuciferaGaertn (*Nelumbonaceae), has been widely used to treat several diseases such as hypertension, cancer, diarrhea, fever, weakness, infection and body heat imbalance [15]. The flavonoids such as (+) -1(R)-coclaurine, (-)-1(S)-norcoclaurine and querctin 3-O-b-D-glucuronide, which are isolated from leaf showed the potency of anti HIV activity [16]. Several previous reports suggested that seed could suppress cell cycle progression, cytokine genes expression and cell proliferation in human peripheral blood mononuclear cells [17]. Recently the leaf of *Nelumbonucifera*showed that the hypotensive effects that mediated by vasodilation via nitric oxide [18] and betulinic acid isolated from rhizomes used as antitumor and melanoma specific cytotoxic agent [19]. However, scientific evidence on antioxidant potential in hydroethanolic extract of white and pink *Nelumbonucifera*is still unknown. The investigation was undertaken to explore the antioxidant potential of both white and pink *Nelumbonucifera*flowers.

2. MATERIALS AND METHODS

2.1. Plant Collection and Extract Preparation

The flowers of *Nelumbonucifera*were collected from different localities of Coimbatore District, Tamil Nadu and authenticated by Botanical Survey of India (BSI) in Tamil Nadu Agriculture University Coimbatore, Tamil Nadu, India. A voucher specimen (No.BSI/SC/5/23/09-10/Tech279) was deposited at the herbarium for future reference. The air dried and powdered flowers (100g of each) were cold macerated with 50% ethanol for three days, with occasional stirring. After 3 days, the suspension was filtered through a fine muslin cloth and was evaporated to dryness at low temperature (<40°C) under reduced pressure in a rotary evaporator. Dark brown colored crystals of approximately 8g was stored in airtight desiccators and used for further analysis.

2.2. Ferric Thiocyanate (FTC) Method

Antioxidant capacity was measured by Ferric thiocyanate (FTC) method according to the method of Kikuzaki and Nakatani [20]. A mixture consisting of 4mg of the sample in 4ml of absolute ethanol, 4.1ml of 2.52% linoleic acid in absolute ethanol, 8 ml of 0.05M phosphate buffer (pH 7.0) and 3.9 ml of distilled water was added to a vial with screw cap and then kept in an incubator at 40°C in dark place. To 0.1ml of this mixture 9.7ml of 75% ethanol and 0.1ml of 30% ammonium thiocyanate were added. After 3 minutes, 0.1ml of 0.02M ferrous chloride in 5% hydrochloric acid was added to reaction mixture (the absorbance of red color indicated the antioxidant activity). Absorbance was observed at 500nm for every 24 hours until the absorbance of the control reached maximum level. Both control and standard were subjected to the same procedures. The solvent was used for control and for the standard 4mg of the sample was replaced by 4mg of vitamin C.

2.3. TBA Method

TBA method of Ottolenghi [21] is used for evaluating the extent of lipid peroxidation. At low pH, and high temperature (100° C), melonaldehyde binds TBA to form a red complex that can be measured at 532nm. 2ml of 20% trichloroacetic acid and 2ml of 0.67% TBA solutions were added to 2ml of the mixtures containing the sample prepared in FTC method. This mixture was kept in water bath (100°C) for 10 minutes and after cooling to room temperature, was centrifuged at 3000rpm for 20 minutes. Antioxidant property was calculated based on the absorbance of the supernatant at 532nm on the final day of assay.

2.4. FRAP Assay

In FRAP assay, when a ferric tripyridyltriazine (Fe^{III}–TPTZ) complex is reduced to the ferrous (Fe^{II}) form, an intense blue colour with an absorption maximum at 593 nm develops and measured at low pH by the method of Benzie and Strain [22].Ferric tripyridyltriazine FRAP) reagent contained 2.5 mM ferric chloride and 2.5ml of 40mM TPTZ in dilute hydrochloric acid, 20ml of 2.5mM ferric chloride and 25ml acetate buffer was prepared freshly and warmed at 35°C.

FRAP reagent 1.5ml and sample solution 50µl at different concentration was incubated at 37°C for 10 minutes and absorbance was recorded at 593 nm [22].

2.5. Phosphomolybdenum Method

The phosphomolybdenum method was described by Prieto, et al. [23] and principle of this method is the reduction of Mo (IV) to Mo (V) by the plant extract. The tubes containing extract (at different concentrations) and reagent (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) were incubated at 95°C for 90min. Soon after the mixture is cooled at room temperature, the absorbance of each solution was measured at 695nm against blank. Methanol (0.3ml) in the place of extract served as the blank. The antioxidant activity was expressed as the number of equivalents of ascorbic acid.

2.6. Haemoglobin Glycosylation

Degree of non-enzymatic haemoglobin glycosylation was measured by Pal and Dutta [24]. Haemoglobin, 0.6mg/ml in 0.01M phosphate buffer (pH 7.4) was incubated in presence of 20 mg/ml concentration of glucose for 72 h, in order to find out the best condition for haemoglobin glycosylation. The assay was performed by adding 1ml of glucose solution, 1 ml of haemoglobin solution, and 1ml of gentamicin (0.2mg/ml), in 0.01 M phosphate buffer (pH 7.4). The mixture was incubated in dark at room temperature for 72h. The degree of glycosylation of hemoglobin in the presence of different concentration of extracts and their absence was colorimetrically measured at 520nm.

2.7. Reducing Power Assay

The reducing power was measured according to the method of Oyaizu [25]. Different concentrations of extract in 1ml of distilled water was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and Potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20min. Trichloroacetic acid (10%) was added to the mixture and centrifuged at 3000rpm for 10 minutes. The upper layer of the 2.5ml solution was mixed with distilled water (2.5ml) and ferric chloride (0.5ml 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated the increase in reducing power. Ascorbic acid served as a reference standard and potassium phosphate buffer (pH 6.6) was used as blank solution.

2.8. DPPH Radical Scavenging Assay

The free radical scavenging activity was measured *in-vitro* by DPPH assay. About 0.3mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 1ml of both the extract at different concentrations (100, 200, 300, 400 and 500µg/ml). This mixture was shaken and allowed to stand at room temperature for 30 minutes and the absorbance was measured at 517nm using a spectrophotometer. Decreased absorbance of the reaction mixture indicates higher free radical scavenging property. The percent DPPH scavenging effect was calculated using the following equation [11].

DPPH scavenging effect (%) = [$(A_0 - A_1)/ A_O$] X 100

Whereas A_0 is the absorbance of the control reaction mixture and A_1 is the absorbance of the plant extract or standard at different concentrations.

2.9. Superoxide Radical Scavenging Assay

The scavenging activity of the extracts towards superoxide radicals was measured. Superoxide anions were generated from non-enzymatic phenazinemethosulfatenicotinamide adenine dinucleotide (PMS-NADH) system by the reaction of PMS, NADH, and oxygen. It was assayed by the reduction of nitro blue (16 mM, pH 80 containing 1ml of NBT (50µM) solution, 1ml of NADH (78µM) solution and different concentration of both the white and pink flower extracts. The reaction was initiated by the addition of 1ml of PMS (10µM) to the mixture indicated superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated [12].

2.10. Nitric Oxide Scavenging Activity Assay

Nitric oxide radical scavenging activity was determined [13]. Sodium nitroprusside in aqueous solution at physiological pH tends to generate nitric oxide, that interacts with oxygen to produce nitrite ions, which can be determined by using the GriessIllosvoy reaction. 2ml of 10mM sodium nitroprusside in 0.5ml phosphate buffer saline (pH 7.4) was added to 0.5 ml of extract at various concentrations and the mixture was incubated at 25◦C for 150 min. 0.5ml was taken out from the incubated mixture and added into 1.0ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. 1.0 ml naphthylenediaminedihydrochloride (0.1%W/V) was mixed and incubated at room temperature for 30min before measuring the absorbance at 540nm was measured with a spectrophotometer. The nitric oxide radical scavenging activity was finally calculated.

2.11. Hydroxyl Radical Scavenging Activity Assay

The scavenging activity for hydroxyl radicals was measured with Fenton reaction [14]. Reaction mixture consists of 60µl of 1.0 mM $FeCl_2$, 90 µl of 1mM 1,10-phenanthroline, 2.4ml of 0.2 M phosophate buffer (pH 7.8), 150µl of 0.17 M H_2O_2, and 1.5ml of extract at varying concentrations. The reaction starts only when H_2O_2 is added. The absorbance of the mixture at 560nm was measured spectrophotometrically after 5 min incubation at room temperature. The hydroxyl radicals scavenging activity was calculated from the absorbance.

2.12. Scavenging of Hydrogen Peroxide

A solution of hydrogen peroxide (40mM) was prepared buffer (pH7.4). Different concentration (100-500µg ml^{-1}) of plant extracts was added to hydrogen peroxide solution (0.6ml, 40mM). Absorbance of hydrogen peroxide at 230nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. Percentage activity of the extract and standard compounds was then calculated [26].

2.13. Statistical Analysis

The results are expressed as mean ± SD. Linear regression analysis was used to calculate the IC_{50} values.

3. RESULTS AND DISCUSSION

3.1. FTC and TBA Assay

There was significantly decreased absorbance value in FTC and TBA methods when compared to the control (Table 1) suggesting high level of antioxidant potential. The maximum antioxidant capacity of both HEWNN and HEPNN flower extract was found to be 16.53mg and 14.21mg at 1000µg/ml extract concentration in FRAP assay (Table 2) and 62.5mg and 56.3mg ascorbic acid equivalents at 500µg/ml concentration in phosphomolybednum method (Figure 1). In addition there was high degree of haemoglobin glycosylation (Figure 2) in HEWNN (55.5%) and HEPNN (41.6%) at 500µg/ml concentration. This is comparable to that of ascorbic acid (62.6%) at 300µg/ml concentration. In figure 3 the reducing power increased with increasing concentration of plant extract and maximum reducing power of HEWNN, HEPNN and ascorbic acid were 0.52, 0.45 and 0.64 respectively. These results clearly suggest that HEWNN flower extract have effective antioxidant activity than HEPNN flower extract, which may be due to phytochemical constituents.

In FTC method amount of peroxide formed at the primary stage of linoleic acid peroxidation is measured. The peroxide reacts with ferrous chloride pigment. The concentration of peroxide decreases as the antioxidant activity increases [26]. Antioxidant activity was based on the absorbance of the final day in TBA method which is used to measure the secondary product of oxidation such as aldehyde and ketone [27]. The higher absorbance value indicates the lower level of antioxidant capacity. The plant extracts tested showed low absorbance values than the control which indicated a high level of antioxidant activity when compared to Vitamin C. THE HEWNN flower showed lower absorbance in both FTC and TBA methods, which indicates that HE WNN flower extract, has strong antioxidant activity as compared to HEPNN flower extract.

3.2. FRAP Assay

FRAP assay is well known method to assess total antioxidant capacity and considered as a useful indicator of the body's antioxidant status to counteract the oxidative damage due to ROS. The advantage of FRAP assay is in being fast, easy to handle, with highly reproducible results [28]. Antioxidant activity by this method was evaluated on the basis of alkaloid and element content where ferric to ferrous ion reduction at low pH causes a ferrous-tripyridyl-triazine complex which has absorption at 593 nm [29]. Both HEWNN and HEPNN flower extracts shown concentration dependent increase in ferric reducing capacities. However, HEWNN flower extract was more potent than HEPNN flower which may be due to the presence of different levels of active constituents in both flowers.

3.3. Phosphomolybednum Assay

Phosphomolybednum assay used to determine the antioxidant capacity based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and the green phosphate/Mo (V) complex at acidic pH. This assay has been successful in the quantification of vitamin E antioxidant capacity and being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extracts [30]. Since the antioxidant activity is expressed as the number of equivalents of ascorbic acid, it is quantitative method. The good antioxidant activity of HEWNN and HEPNN might be attributed due to the presence of phytochemicals such as flavanoids and biflavones (Figure 1).

3.4. Haemoglobin Glycosylation

Haemoglobin glycosylation is an *in-vitro* non-enzymatic method [31]. HbA1c, or glycosylated haemoglobin, is formed through the non-enzymatic binding of circulating glucose to terminal of beta chain of hemoglobin molecule (glycation) [32]. Glycosylation comprises a series of reactions, such as the binding of acyclic form of sugar to specific amino acids in protein to form a Schiff base, rearrangement of the Schiff base to form an Amadori product (so called end products) and cross linking and subsequent degradation of protein to form advanced glycosylation end-products [33]. Degree of haemoglycosylation of HEWNN is much effective than HEPNN flower it may be due to its phytoconstituents. The study reveals that the antioxidant activity of the extract exhibits increasing trend with the increasing concentration of plant extract (Figure 2).

3.5. Reducing Power

The reducing ability of a compound generally depends on the presence of reductants (antioxidants) [34]. Which have been exhibited antioxidant potential by breaking the free radical chain by donating a hydrogen atom [35]. Reductants are also reported to react with certain precursors of peroxide thus preventing peroxide formation [36]. The presence of reductants in *Nelumbonucifera* extract causes the reduction of the Fe^{3+} /Ferricyanide complex to the ferrous form. The reducing power of HEWNN flower extract was very potent and the power of extract was increased with quality of sample (Figure 3).

3.6. DPPH Scavenging Activity

DPPH is a stable free radical which accepts an electron on hydrogen radical to become a stable diamagnetic molecule. Table indicates noticeable effect of HEWNN and HEPNN on DPPH scavenging activity. This activity was increased by increasing concentration of sample extract. DPPH antioxidant assay is based on the ability of 1,1-diphenyl 2-picryl radical to decolorize from purple to yellow in the presence of antioxidants. Both HEWNN and HEPNN showed maximum scavenging activity of 67.52% and 55.51% respectively at 500 µg/ml concentration which is comparable to scavenging activity of ascorbate (68.13%) at 400µg/ml. IC_{50} value of HEWNN was

350 µg/ml and HEPNN was 400 µg/ml as opposed to that of ascorbate (IC_{50} 250 µg/ml) which is a well-known antioxidant (Figure 4).

3.7. Superoxide Scavenging Activity

Superoxide anions damage biomolecules directly or indirectly by forming H_2O_2 peroxynitrile or single oxygen during aging and pathological events such as ischemic reperfusion injury. Lipid peroxidation is thus initiated. The superoxide anion radical scavenging activity of *Nelumbonucifera* flower extract assayed by the PMS-NADH system as shown in Table 2. Superoxide scavenging activity was increased markedly with increase in concentrations. Thus higher inhibitory effects of flower extracts on superoxide anion formation noted herein possibly renders them as promising antioxidants. The maximum scavenging activity of HEWNN, HEPNN and ascorbate at 500µl/ml was found to be 81.2%, 64.5% and 84.26% respectively. The half inhibition concentration (IC_{50}) of HEWNN was 310µg/ml. The results suggested that HEWNN flower has a potent superoxide radical scavenging activity when compared to HEPNN flower (Figure 5).

3.8. Nitric Oxide Scavenging Activity

Nitric oxide (NO) is a potent pleiotropic mediator of physiological process such as smooth muscle relaxant, neuronal signaling, and inhibition of platelet aggregation of cell mediated toxicity. Although nitric oxide and superoxide radicals are involved in host defense over production of these radicals contributes to the pathogenesis of some inflammatory diseases. Nitric oxide inhibitors possess beneficial effects on some aspects of inflammation and tissue damage seen in inflammatory conditions. In table 2 HEWNN and ascorbate showed maximum scavenging activity of 70.2% and 78.5% at 500µg/ml concentration respectively. However, maximum scavenging activity of HEPNN is 57.7% at 500µg/ml concentration, which is comparable to the scavenging activity of ascorbate (58.68%) at 300µg/ml concentration. The HEWNN and HEPNN significantly inhibited nitric oxide in dose dependent manner with IC_{50} value 325µg/ml and 410µg/ml respectively, whereas for ascorbate the IC_{50} value was 220 µg/ml (Figure 6).

3.9. Hydroxyl Radical Scavenging Activity

Hydroxyl radical is highly reactive oxygen centered radical formed from the reaction of various hydroperoxides with transition metal ions [37]. It attacks DNA polyunsaturated fatty acid in membranes, and most capable of abstracting hydrogen atoms from membrane lipids [38]. Both white and pink *Nelumbonucifera*flower extracts exhibited concentration dependent scavenging potential against hydroxyl radical with maximum scavenging activity 60.53% and 46.72% at 400µg/ml respectively. Standard ascorbate showed maximum scavenging effect of 70.51% at 500µg/ml. IC50 value of HEWNN, HEPNN and Ascorbate was found to be 360µg/ml, 450 µg/ml and 220 µg/ml respectively. The results suggest that the pink flower extract is less effective in scavenging hydroxyl radical than white flower extracts (Figure 7).

3.10. Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide is a weak oxidizing agent and tends to inactivate a free enzymes directly, usually by oxidation of essential thiol (-SH) groups. H_2O_2 can cross cell membrane rapidly once inside the cell it can react with Fe2+ and Cu2+ ions to form hydroxyl radical and this might be due to the origin of many of its toxic effects [39]. HEWNN, HEPNN excreted maximum scavenging activity of 54.29%, 48.13% at 400 µg/ml respectively. The IC_{50} value is 330µg/ml for white flower extract, 440µg/ml for pink flower extract and 360µg/ml for ascorbate. The decomposition of H_2O_2 by plant extract might result from its antioxidant and free radical scavenging activity.

4. CONCLUSION

In conclusion this study showed a potent antioxidant activity in both HEWNN and HEPNN flower extracts. Moreover in comparison with HEPNN flower, HEWNN flower extract showed effective antioxidant activity which may be due to structural variation of antioxidant compounds such as number of phenolic hydroxyl, keto-groups free carboxylic groups and other structural features [40]. Therefore if a systematic investigation in initiated the traditional medicinal systems practiced in India can offer promising leads for the discovery of potent antioxidants that can have therapeutic and dietary use globally. Further studies are required to establish its *in-vitro* antioxidant activity using different animal models.

REFERENCES

[1] T. Yosshikawa, S. Toyokuni, Y. Yamamoto, and Y. Naito, *Free radicals in chemistry biology and medicine* vol. 249. London: OICA International, pp.87-97, 2000.

[2] B. Halliwell and J. M. Gutteridge, *Free radicals in biology and medicine* vol. 3: Oxoford University Press, pp.124-143, 1999.

[3] M. Valko, C. J. Rhodes, J. Moncol, M. Izakovic, and M. Mazur, "Free radical metals and antioxidants in oxidative stress induced cancer," *Chemico Biological Interaction*, vol. 160, pp. 1-40, 2006.

[4] O. I. Aroma, "Free radicals, oxidative stress and antioxidants in human health and disease," *Journal of the American Oil Chemists Society*, vol. 75, pp. 199-212, 1998.

[5] M. A. Smith, P. L. Perry, L. M. Sayre, V. E. Anderson, and M. F. Beal, "Oxidative damage in Alzheimer's," *Nature*, vol. 382, pp. 120-126, 1996.

[6] I. Guidi, D. Galimberti, S. Lonati, and C. Novembrino, "Oxidative imbalance in patients with mild cognitive impairment and alzheimers disease," *Neurobiology of Aging*., vol. 27, pp. 262-269, 2006.

[7] J. L. Bolton, M. A. Trush, T. M. Penning, G. Dryhurst, and T. J. Monks, "Roe of quinones in toxicology," *Chemical Research in Toxicology*, vol. 13, p. 135, 2000.

[8] T. Finkel and N. J. Holbrook, "Oxidants, oxidative stress and biology of aging," *Nature*, vol. 408, pp. 239-247, 2000.

[9] K. Granelli, L. Bjorock, and L. A. Appleqvisit, "The variation of SOD and XO activities in milk using an improved method to quantitate SOD activity," *J Sci Food Agric*., vol. 67, pp. 85-95, 1995.

[10] G. Schwartsman, M. J. Ratain, G. M. Cragg, J. E. Wong, and N. Saijo, "Anticancer drug discovery and development throughout the world," *J. Clin. Oncol*, vol. 20, pp. 47-59, 2002.

[11] V. G. Kamatha, A. Chandrashekar, and P. S. Rajini, "Antiradical properties of sorghum (Sorghum bicolor L.Moench) flour extracts," *J Cereal Sci*., vol. 40, pp. 283-288, 2004.

[12] C. Kaur and H. C. Kapoor, "Antioxidant activity and total phenolic content of some Asian vegetables," *Inter J Food Sci Tech*., vol. 37, pp. 153-161, 2002.

[13] T. Sun and C. T. Ho, "Antioxidant activities of buckwheat extracts," *Food Chem*., vol. 90, pp. 743-749, 2005.

[14] G. C. Yen, P. D. Duh, and H. J. Su, "Antioxidant properties of lotus seed and its effect on DNA damage in human lymphocytes," *Food Chem*., vol. 89, pp. 379-385, 2005.

[15] C. Saengkhae , W. Arunnoppart, and P. Sungkhajorn, "Antioxidant avtivity of nelumbo nucifera Gaertn on oxidative stress induced erythrocyte haemolysis in hypertensive and normotensive rats," *Journal of Physiological sciences*, vol. 20, pp. 70-78, 2008.

[16] Y. Kashiwada, A. Asoshima, and Y. Ikeshiro, "Anti-HIV benzlisoquinoline alkaloids from leaves of nelumbo nucifera, and structural activity correlations with related alkaloids," *Bioorg Med Chem*., vol. 13, pp. 443-8, 2005.

[17] D. Bensky, S. Clavey, and E. Stoger, *Materia medica* vol. 3. USA: Eastland Press Inc, pp.151, 2004.

[18] P. Tongtorsak, N. Chaichit, and J. Panyajirawut, "Acute and chronic hypotensive effects of crude leaf extract of nelumbo nucifera gaertn," *TJPS*, vol. 17, pp. 68-9, 2004.

[19] P. K. Mukherjee, K. Saha, and B. P. Saha "Effect of nelumbo nucifera rhizome extract on blood sugar level in rats," *Journal of Entopharmacology*, vol. 58, pp. 207-213, 1997.

[20] H. Kikuzaki and N. Nakatani, "Antioxidants effect of water extract of aegle marmelos fruits in treptozotocin diabetic rats," *Journal of Ethnopharmacology*, vol. 87, pp. 207-210, 1993.

[21] H. Ottolenghi, "Interaction of ascorbic acid and mitochondria lipids," *Archives of Biochemistry and Biophysics*, vol. 79, pp. 355-357, 1959.

[22] I. F. F. Benzie and J. J. Strain, "Ferric reducing antioxidant power and ascorbic acid concentration," *Methods in Enzymology*, vol. 299, pp. 15-27, 1999.

[23] P. Prieto, M. Pineda, and M. Aguilar, "Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybednum complex: Specific application to the determination of vitamin E," *Anal Biochem*, vol. 269, pp. 337-41, 1999.

[24] D. K. Pal and S. Dutta, "Evaluation of antioxidant activity of the roots and rhizomes of cyperus rotundus L," *Indian Journal of Pharmaceutical Sciences*, vol. 68, pp. 256-258, 2006.

[25] M. Oyaizu, "Studies on product of browning reaction prepared from glucose amine," *Jap J Nutr*., vol. 44, pp. 307-15, 1986.

[26] F. Aquil, I. Ahmad, and Z. Mehmood, "Antioidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants," *Turkish Journal of Biology*, vol. 30, pp. 177-183, 2006.

[27] J. W. Anderson, A. E. Jones, and S. Ridell Manson, "Ten different dietary fibers have significantly different effects on serum and liver lipids of cholesterol fed rats," *Journal of Nutrition*, vol. 124, pp. 78-83, 1994.

[28] N. Faujan, A. Noriham, A. S. Norrakiah, and A. S. Babji, "Antioxidant activity of plants methanolic extracts containing phenolic compounds," *African Journal of Biochemistry*, vol. 8, pp. 484-489, 2009.

[29] R. S. Farag, A. Z. Badei, F. M. Hawed, and G. Baroty, "Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media," *Journal of American Oil Chemists Society*, vol. 66, pp. 7932-799, 1989.

[30] G. Cao and R. L. Prior, "Comparison of different analytical methods for assessing total antioxidant capacity of human serum," *Clin Chem.*, vol. 44, pp. 1309-1315, 1998.

[31] Y. Lu and L. Y. Foo, "Antioxidant activities of polyphenols from sage (Salvia offincialis)," *Food Chem.*, vol. 75, pp. 197-202, 2001.

[32] R. J. Koeing, C. M. Peterson, R. L. Jones, and C. Saddek, "Correlation of glucose regulation and HBA1C in diabetes," *N. Eng .J. Med.*, vol. 295, pp. 417-419, 1976.

[33] K. M. Reiser, "Nonenzymatic glycation of collagen in aging and diabetes," *Proc Soc Exp Biol Med.*, vol. 196, pp. 17-29, 1991.

[34] S. Meir, J. Kannner, B. Akiri, and P. Hadas, "Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves," *J. Agric Food Chem.*, vol. 43, pp. 1813-1817, 1995.

[35] P. D. Duh, Y. Y. Tu, and G. C. Yen, "Antioxidant activity of water extract of harng jyur (Chrysanthemum Molifolium Ramat)," *Lebesnm- Wiss Technol*, vol. 32, pp. 269-77, 1999.

[36] M. H. Gordon, *The mechanism of the in-vitro antioxidant action of food antioxidants*. London: Elseiver, 1990.

[37] O. I. Aruoma, "Free radical scavenging potential of saussarea costus," *Asia Pacific J Clin Nut.*, vol. 8, p. 53, 1999.

[38] M. J. Miller, S. Chotinaruemol, J. L. Kakkis, and D. A. Clark, "Determination of in-vitro antioxidant and radical scavenging activities of protocol," *J Pharamcol Exp Therap*, vol. 264, pp. 11-15, 1993.

[39] J. M. C. Gutteridge, "Age pigments and free radicals: Fluorescent lipid complexes formed by iron and copper containing proteins," *Biochem Biophys Acta*, vol. 834, pp. 144-8, 1993.

[40] D. E. Patt and B. J. Hudson, "Natural antioxidants not exploited commercially. In: Food antioxidants," *Elsevier Applied Science*, vol. 41, pp. 171-191, 1990.

Figure-1. Phosphomolybednum radical scavenging potential of white and pink *Nelumbonucifera Gaertn* flowers

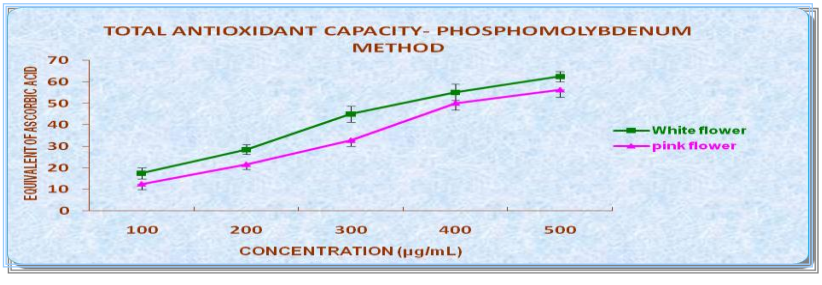

Figure-2. Total antioxidant potential of white and pink *NelumbonuciferaGaertn*flower

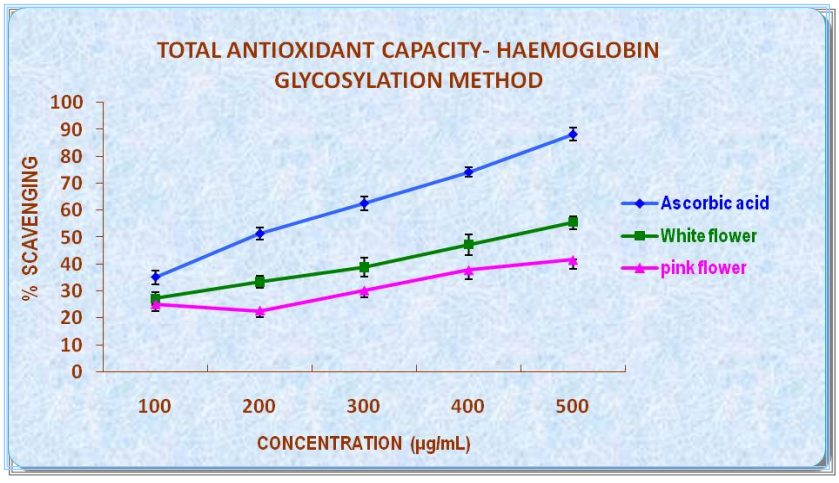

Figure-3. Reducing potential of white and pink *NelumbonuciferaGaertn*flowers

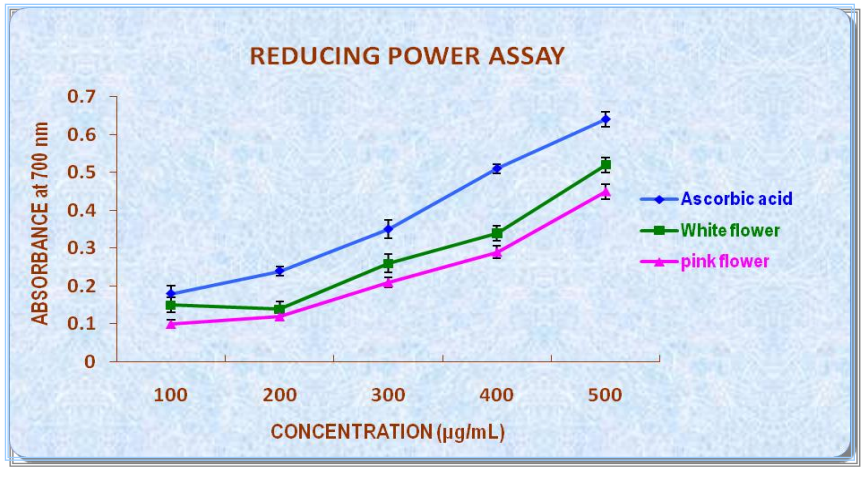

Figure-4. DPPH radical scavenging effect of white and pink *NelumbonuciferaGaertn*flowers

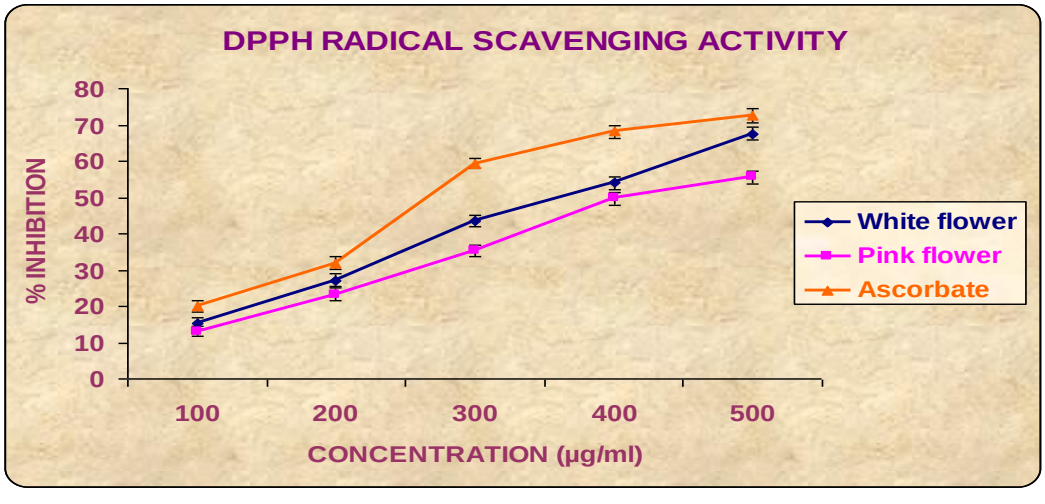

Figure-5. SO radical scavenging potential of white and pink *NelumbonuciferaGaertn*flowers

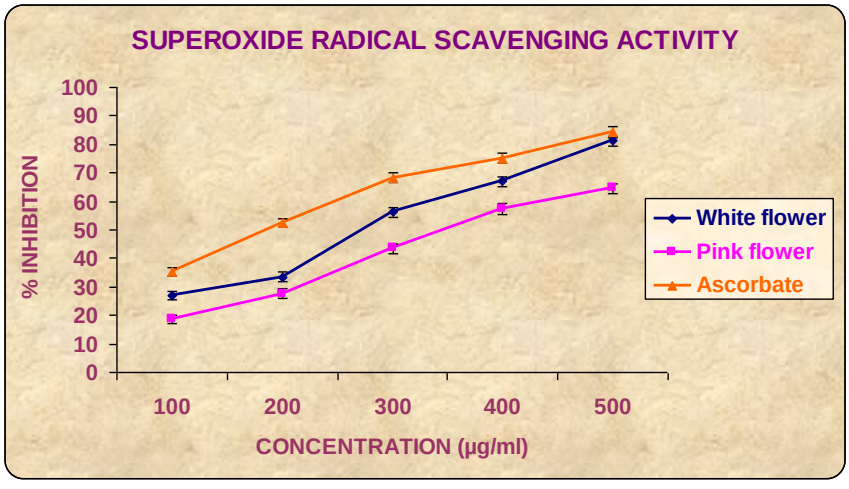

Figure-6. NO radical scavenging potential of white and pink *NelumbonuciferaGaertn*flowers

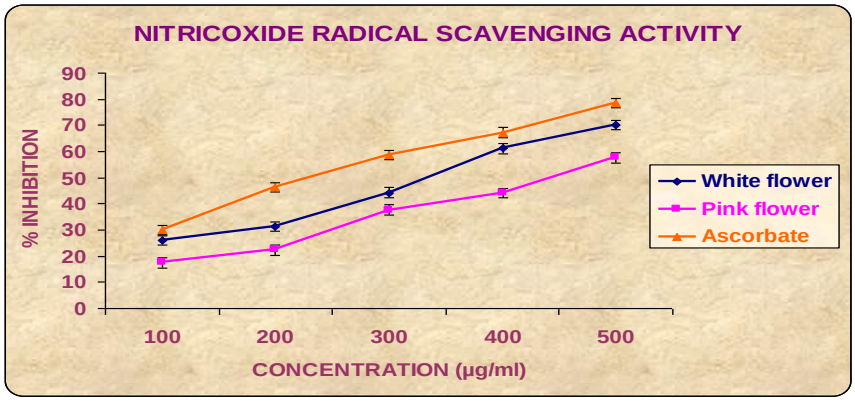

Figure-7. Hydroxyl radical scavenging potential of white and pink *NelumbonuciferaGaertn*flowers

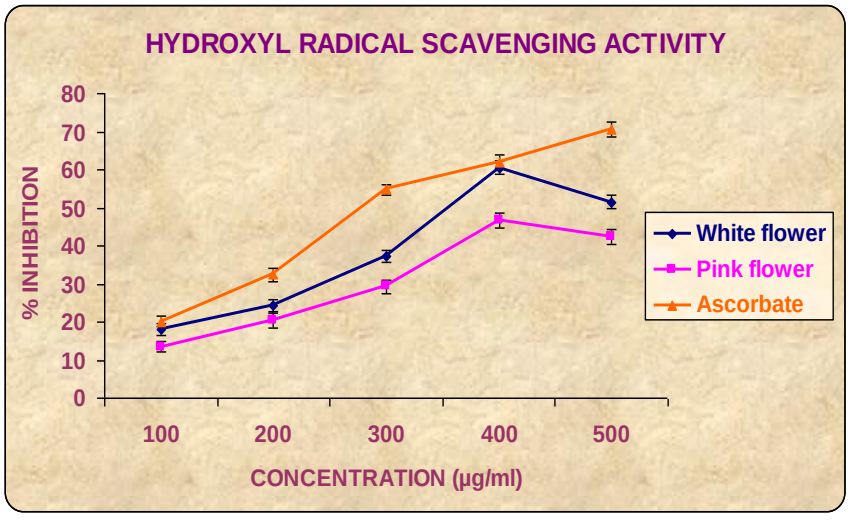

Figure-8. H_2O_2 radical scavenging potential of white and pink *NelumbonuciferaGaertn*flowers

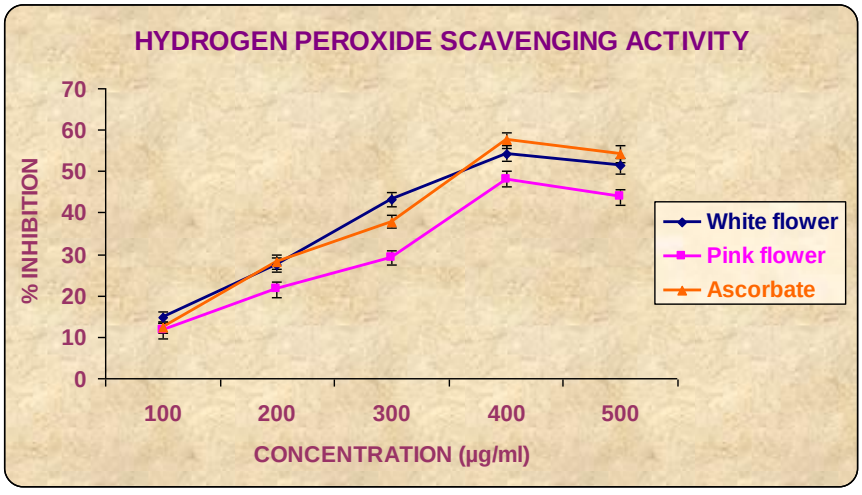

Table-1. Antioxidant activities of hydroethanolic extract of white and pink *Nelumbonucifera*Gaertn flower by FTC and TBA method [20, 21]

Extract	FTC Method								TBA method
	Absorbance								Absorbance
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7	Day-8	Day 9
Control	0	0.083	0.109	0.145	0.179	0.225	0.234	0.257	0.264
Vitamin C	0	0.040	0.043	0.051	0.063	0.070	0.074	0.083	0.085
HEWNN	0	0.019	0.021	0.033	0.035	0.038	0.043	0.045	0.049
HEPNN	0	0.025	0.031	0.047	0.051	0.054	0.056	0.061	0.068

Source: the data were processed and presented in table1

Table-2. Antioxidant activities of hydroethanolic extract of white and pink *NelumbonuciferaGaertn* flower by FRAP assay[22].

S.No	Concentration (µg/ml)	HEWNN (mg)	HEPNN (mg)
1.	200	3.165	2.861
2.	400	8.612	6.327
3.	600	10.18	7.562
4.	800	14.22	12.57
5.	1000	16.53	14.21

Source: the data were processed and presented in table2