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## Phytochemical Analysis and *in vitro* Antioxidant Activity of *Nymphaea lotus* L.

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**Abstract:** *Nymphaea lotus* L. is a perennial aquatic plant used in traditional medicine system as an aphrodisiac, anodyne, astringent, cardiotoxic, sedative, analgesic and as anti-inflammatory agent. The present study evaluates the phytochemical constituents and *in vitro* antioxidant potential of the aqueous and acetone extracts of the plant, using spectrophotometric techniques. The quantities of phenols, tannins, saponins and steroids were significantly higher ( $p < 0.05$ ) in the aqueous compared to the acetone extract while the proanthocyanidins and flavanols were significantly higher ( $p < 0.05$ ) in the acetone than the aqueous extract. The acetone extract of the plants displayed better DPPH and NO radical scavenging activity than the aqueous extract and this reflected in its low  $IC_{50}$  ( $0.016 \text{ mg mL}^{-1}$ ) which compares favourably with the standards. Conversely, aqueous extract of the plant showed better ABTS radical scavenging ability ( $IC_{50} 0.04 \text{ mg mL}^{-1}$ ) than the acetone extract ( $IC_{50} 0.15 \text{ mg mL}^{-1}$ ) while they displayed the same  $H_2O_2$  scavenging ability ( $IC_{50} 0.1 \text{ mg mL}^{-1}$ ). The present study revealed that *N. lotus* is very rich in phytochemicals and is a good source of natural antioxidants. This confirms and validates its use for the treatment of several diseases in human.

**Key words:** Phytochemicals, antioxidant, *Nymphaea lotus*, oxidative stress, medicinal plants

### INTRODUCTION

Reactive oxygen species (ROS), otherwise known as free radicals, are highly reactive chemical substances that move around in the body and have deleterious effect on body cells. They include different types of radicals such as superoxide, hydroxyl radical, or singlet oxygen (Alia *et al.*, 2003). They have been implicated in the aetiology of many diseases like cancer, diabetes, cataract, cardiovascular and neurodegenerative diseases (Okochi and Okpuzor, 2005). The natural defence mechanism of animals detoxifies these free radicals with the aid of its antioxidant molecules and enzymes. These antioxidants play the role of free radical scavengers by preventing and mitigating damage that may result from ROS (James *et al.*, 2011). However, oxidative stress results when the balance of free radicals and natural cellular antioxidants shifts in favour of the free radicals. This leads to the requirement for supplementary exogenous antioxidants compounds. There are several reports on the antioxidant potentials of medicinal plants such as *Diospyros abyssinica*, *Pistacia lentiscus* (Krishnaiah *et al.*, 2011), *Allium sativum* and *Origanum syriacum* (Al-Jaber *et al.*, 2011).

*Nymphaea lotus* L., commonly known as white water lily, belongs to the family Nymphaeaceae. The flowers are white, sometimes with a pink tinge. The leaves vary from green to red-brown, with a number of purple spots.

The plant is native to the Nile and is grown in various parts of East Africa and Southeast Asia (Wee, 1992). It is used in traditional medicine system as an aphrodisiac, anodyne, astringent, cardiotoxic, sedative, demulcent, analgesic and as anti-inflammatory agent (Madhusudhanan *et al.*, 2011). The plant produces calming and sedative effects on the nervous system, therefore, used for the treatment of insomnia, anxiety and other related disorders (Robin, 2001; Adnaik *et al.*, 2009). Many biological activities, including anticancer and antiviral, have been attributed to gallic acid and ellagic acid which are widely present in *N. lotus* (Thippeswamy *et al.*, 2011).

Despite these medicinal uses of *N. lotus*, there is little information on its quantitative phytochemical composition and antioxidant potential. The present study therefore, was aimed at evaluating the chemical components of the plant as well as the antioxidant properties of its aqueous and acetone extracts.

### MATERIALS AND METHODS

**Plant collection and extraction:** Fresh leaves of *Nymphaea lotus* were collected from southwest Nigeria in June, 2012 and were authenticated by Mr. Sanni of the Lagos State University herbarium. A voucher specimen (LSH. 2012/6) was prepared and deposited for future reference. The leaves were

carefully rinsed under running water, air dried to constant weight in the laboratory and later pulverized before extraction.

**Aqueous extraction:** About 100 g of powdered plant material was soaked in 1000 mL distilled water for 48 h at 30°C on an orbital shaker (Stuart Scientific Orbital Shaker, UK). The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was freeze dried using Savant Refrigerated Vapor Trap (RTV 4104, USA).

**Acetone extraction:** A known mass (200 g) of powdered leaves of *Nymphaea lotus* was soaked in 1000 mL acetone for 48 h at 30°C on an orbital shaker (Stuart Scientific Orbital Shaker, UK). The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was concentrated to dryness under reduced pressure at 50°C using rotary evaporator (Laborota 4000-efficient, Heidolph, Germany).

**Determination of total phenols:** The amount of phenols in the acetone and aqueous extracts of *N. lotus* was determined spectrophotometrically using the modified method of Oyedemi *et al.* (2012) with Folin-Ciocalteu reagent. Five milliliter of the aliquot extract (1 mg mL<sup>-1</sup>) was mixed with 5 mL Folin-Ciocalteu reagent (previously diluted with water 1:9 v/v) and 4 mL (75 g L<sup>-1</sup>) of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40°C for colour development. Absorbance was then measured at 765 nm using AII-C03 UV-VIS spectrophotometer. Results were expressed as mg g<sup>-1</sup> of tannic acid equivalent.

**Determination of flavonoids:** The amount of flavonoids in *N. lotus* was determined using the aluminium colorimetric assay method (Oyedemi *et al.*, 2010). A volume of 0.5 mL of 2% AlCl<sub>3</sub> ethanol solution was added to 0.5 mL of the sample solution. After 1 h at room temperature, the absorbance was measured at 420 nm. A yellow colour indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg mL<sup>-1</sup>. Total flavonoid contents were calculated as mg g<sup>-1</sup> of quercetin.

**Determination of total flavanols:** The reacting mixture of 2 mL of the plant extract with 2 mL of AlCl<sub>3</sub> in ethanol solution and 3 mL of 50 g L<sup>-1</sup> sodium acetate solution was allowed to stay for 2.5 h at 20°C in a water bath. A yellow colour indicated the presence of flavanols. The absorbance was measured at 440 nm. The flavanols content was calculated thus:  $Y = 0.0255x$ ,  $R^2 = 0.9812$ , where x is the absorbance and Y is the quercetin equivalent in mg g<sup>-1</sup>.

**Determination of proanthocyanidins:** A volume of 0.5 mL of 1 mg mL<sup>-1</sup> of the plant extract was added to 3 mL of vanillin-methanol (4%v/v) and 1.5 mL of hydrochloric acid was added to the reacting mixture and vortexed. The mixture was allowed to stand for 15 min at room temperature. Absorbance was measured at 500 nm. Proanthocyanin content was expressed as:  $Y = 0.5825x$ ,  $R^2 = 0.9277$ , where x is the absorbance and Y is the catechin equivalent in mg g<sup>-1</sup>.

**Determination of tannin contents:** The tannin content was determined according to the method of Oyedemi *et al.* (2012) with some modifications. A known mass (0.20 g) of the plant extract was added to 20 mL of 50% methanol, vortexed vigorously and later incubated at 80°C in a water bath for 1 h. The filtrate was mixed with 20 mL of distilled water, 2.5 mL of Folin-Dennis reagent and 10 mL of 17% aqueous Na<sub>2</sub>CO<sub>3</sub>. The mixture was made up to 100 mL with distilled water, mixed and allowed to stand for 20 min. A bluish-green colour developed at the end of the reaction mixture indicated the presence of tannins. The absorbance of the tannic acid standard solutions and the sample were measured after colour development at 706 nm. Results were expressed as mg g<sup>-1</sup> of tannic acid equivalent using the calibration curve:  $Y = 0.0593x - 0.0485$ ,  $R^2 = 0.9826$ , where x is the absorbance and Y is the tannic acid equivalent.

**Determination of alkaloids contents:** Total alkaloids contents were quantitatively determined according to the method of Harborne, 2005 with some modifications. A volume of 20 mL of 10% acetic acid prepared in ethanol was added to 5 g of the plant extract, covered and allowed to stand for 4 h. The mixture was filtered and the filtrate was concentrated to one-fourth of the original volume in a water bath. Concentrated ammonium hydroxide was added drop-wisely to the extract, till the completion of the precipitation. The whole solution was allowed to settle and re-filtered after washing with dilute ammonium hydroxide. The residue obtained was dried, weighed and the percentage composition was determined using the equation:

$$\text{Alkaloids (\%)} = \frac{\text{Final weight of the residue}}{\text{Initial weight of the sample}} \times 100$$

**Determination of saponins content:** Saponins content of the leaves of *N. lotus* was determined using the method of Obadoni and Ochuko (2002) with some modifications. A known mass (20 g) of the plant extract was added to 100 mL of 20% aqueous ethanol and kept in a shaker for 30 min. The mixture was heated over water bath for 4 h at

55°C and then filtered to collect the residue which was later re-extracted with 200 mL of 20% aqueous ethanol. The filtrate was concentrated over a water bath at 90°C to approximately 40 mL. The concentrate was transferred into a 250 mL separatory funnel and extracted with 20 mL diethyl ether. The ether layer was discarded while the aqueous layer was retained and to which 60 mL n- butanol was added. The mixture was washed twice with 10 mL of 5% aqueous sodium chloride. After evaporation, the samples were dried in the oven at 40°C to a constant weight. The saponins content was calculated using the equation:

$$\text{Saponin (\%)} = \frac{\text{Final weight of residue}}{\text{Initial weight of the sample}} \times 100$$

**DPPH radical scavenging assay:** The 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay was performed by using the method of Oyedemi *et al.* (2010). About 1.0 mL of the 0.004% methanol solution of DPPH was added to 1 mL of various concentrations (0.025-0.5 mg mL<sup>-1</sup>) of the extracts, rutin and vitamin C. The mixture was vortexed thoroughly and left at room temperature for 30 min in the dark. The absorbance was measured at 517 nm. Radical scavenging activity was calculated as percent inhibition using the equation:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

where, Abs control was the absorbance of DPPH<sup>+</sup>+methanol and Abs sample was the absorbance of DPPH radical+sample extract or standard.

**Nitric oxide scavenging activity:** The method of Vaijanathappa *et al.* (2008) was adopted to evaluate the scavenging activity of *N. lotus* against nitric oxide radical. A volume of 2 mL sodium nitroprusside (10 mM) prepared in 0.5 mM phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of plant extract or rutin or vitamin C at various concentrations (0.025-0.5 mg mL<sup>-1</sup>). The mixture was incubated at 25° for 2.5 h. After incubation, 0.5 mL of the reaction mixture was removed; 1 mL of sulfamic acid reagent (0.33 in 20% glacial acetic acid) was mixed and allowed to stand at room temperature for 5 min for complete diazotization reaction. 1 mL of naphthyl ethylene diamine dichloride (0.1%w/v) was added and the mixture was allowed to stand for 30 min at room temperature. Absorbance was measured at 540 nm:

$$\text{Nitric oxide percent inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

**ABTS scavenging assay:** The method described by Otang *et al.* (2012) was used to determine the ability of *N. lotus* extracts to scavenge ABTS radical. The ABTS diammonium salt (ABTS<sup>+</sup>) was generated by mixing two stock solutions of 7 mM ABTS and 2.4 mM potassium persulfate in the same ratio and allowed to interact in the dark for 12 h at room temperature. The solution was diluted with methanol (1 mL of ABTS<sup>+</sup> in 60 mL methanol) until the absorbance reached 0.706 at 743 nm. Different concentrations (0.025-0.5 mg mL<sup>-1</sup>) of the extracts and the standard drugs were allowed to react with the ABTS radical in the dark for 7 min. The absorbance was later measured at 743 nm. Percentage inhibition of ABTS<sup>+</sup> was calculated thus:

$$\text{Percentage inhibition of ABTS}^+ = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

**Ferric reducing power assay:** The reducing power of both aqueous and acetone extracts of *N. lotus* was evaluated according to the method of Gamie *et al.* (2011). Different concentrations (0.025-0.5 mg mL<sup>-1</sup>) of the extracts and standard drugs were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium hexacyanoferrate II. The mixture was incubated at 50° for 20 min, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant was mixed with 2.5 mL distilled water and 0.5 mL of 0.1% FeCl<sub>3</sub>. The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated stronger reducing power.

**Hydrogen peroxide scavenging activity:** The method of Talaz *et al.* (2009) was used to assess the ability of the extracts to scavenge hydrogen peroxide. A volume of 0.6 mL of 4 mM H<sub>2</sub>O<sub>2</sub> solution prepared in 0.1M phosphate buffer (pH 7.4) was mixed with different concentrations (0.025-0.5 mg mL<sup>-1</sup>) of the extracts and the standard drugs. The absorbance of the solution was measured at 230 nm after 15 min against a blank solution containing phosphate buffer without H<sub>2</sub>O<sub>2</sub>. The scavenging activity of the plant extract on H<sub>2</sub>O<sub>2</sub> was expressed as:

$$\text{Percentage scavenged [H}_2\text{O}_2\text{]} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{sample}}} \times 100$$

**Statistical analysis:** All experiments were done in triplicates and where applicable, the data were subjected to one way Analysis of Variance (ANOVA) and differences between samples were determined by Duncan's Multiple Range test using the Minitab program (version 12 for windows). p-values <0.05 were regarded as significant.

## RESULTS AND DISCUSSION

**Phytochemical composition:** The 100 g of dried plant material of *N. lotus* extracted in water yielded 1.856 g of dry extract while the 200 g extracted in acetone yielded 1.214 g of dry extract. Table 1 presented the result of quantitative phytochemical analysis on the aqueous and acetone extracts of *N. lotus*. This result revealed the presence of proanthocyanidins, phenols, tannins, flavanols, flavonoids, saponins, alkaloids and steroids. The quantities of phenols (189.94 mg g<sup>-1</sup>), tannins (74.51 mg g<sup>-1</sup>), saponins (17.90%) and steroids (32.54%) were significantly higher (p<0.05) in the aqueous compared to the acetone extract while the proanthocyanidins (299.02 mg g<sup>-1</sup>) and flavanols (42.94 mg g<sup>-1</sup>) were significantly higher (p<0.05) in the acetone than the aqueous extract. There were no significant differences (p>0.05) in the quantity of flavonoids and alkaloids in both extracts.

Almost all the phytochemicals have antioxidant activity that protects the cells from free radical damage (oxidative stress) and therefore, reduces the risk of developing certain degenerative diseases (Aruoma, 2003). The amounts of phenols, proanthocyanins, tannins, flavanols, flavonoids and saponins in *N. lotus* were considerable. Natural phenolic compounds play an important role in cancer prevention and treatment (Huang *et al.*, 2009). Oki *et al.* (2002) reported positive correlation between free radical scavenging activity and total phenolic compounds.

Flavonoids are involved in scavenging the oxygen derived free radicals (Nijveldt *et al.*, 2001). It has been discovered in a number of studies that flavonoids contain hypolipidemic potential (Harnafi and Amrani, 2007; Narender *et al.*, 2006). It has also been established that flavonoids from medicinal plants possess high antioxidant potential due to their hydroxyl groups and protect humans more efficiently against any free radical related diseases (Vaya *et al.*, 2003). It has been confirmed experimentally that flavonoids enhance vaso-relaxant process (Bernatova *et al.*, 2002) and prevent platelet activity-related thrombosis (Wang *et al.*, 2005) and thereby reducing risk of cardiovascular mortality. The presence of saponins in the extracts may also boost their antioxidant properties thereby conferring pharmacological potentials ranging from anti-inflammatory, antitumor and sedative properties (Hamburger and Hostettmann, 1991).

Steroids have been reported to have antibacterial, antiviral and aphrodisiac properties. The presence of steroids in the extracts could support the antibacterial, antifungal and aphrodisiac properties reported in the

Table 1: Phytochemical analysis of aqueous and acetone extracts of *Nymphaea lotus*

Phytochemicals	Aqueous	Acetone
Proanthocyanidins (mg g <sup>-1</sup> )	211.84±0.89	299.02±1.47*
Phenols (mg g <sup>-1</sup> )	189.94±0.12	96.31±0.56*
Flavanols (mg g <sup>-1</sup> )	22.45±0.050	42.94±0.12*
Flavonoids (mg g <sup>-1</sup> )	5.93±0.100	6.34±0.33
Tannins (mg g <sup>-1</sup> )	74.51±0.120	64.71±0.45*
Saponins (%)	17.90±0.120	12.31±0.21*
Alkaloids (%)	0.04±0.010	0.86±0.25
Steroids (mg g <sup>-1</sup> )	32.54±0.050	21.62±0.13*

Data are Means±SEM (n = 3), \*Significant difference (p<0.05)

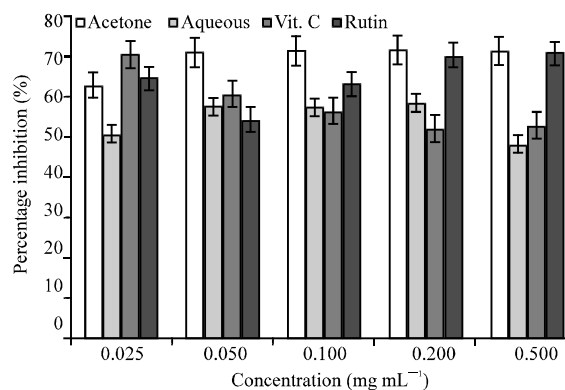


Fig. 1: DPPH scavenging activity of acetone and aqueous extracts of *N. lotus*, vitamin C and rutin by spectrophotometric detection of DPPH radicals. Results are means of three replicates

literatures (Ebana *et al.*, 1991; Cushnie and Lamb, 2005; Akinjogunla *et al.*, 2009). Steroids are very important compounds due to their relationship with sex hormones and are useful in the treatment of sexual dysfunction (Oyedemi and Afolayan, 2011). Several alkaloids were reported with pharmacological properties since time immemorial and this includes their cytotoxic and sedative potentials. Robin (2001) reported the presence of alkaloids nympheine and nupharine in *Nymphaea lotus* which are good sedative agents. This makes *Nymphaea lotus* to produce calming and sedative effects upon nervous system and thereby useful in the treatment of insomnia, anxiety and similar disorders (Robin, 2001).

**DPPH Radical Scavenging activity:** Figure 1 showed the DPPH radical scavenging activity of the acetone and aqueous extracts of *Nymphaea lotus*. Comparison of the *in vitro* antioxidant activity of the acetone and aqueous extracts of *N. lotus* and the standard antioxidant compounds showed that *Nymphaea lotus* is a good source of natural antioxidants. In DPPH scavenging assay, 0.5 mg mL<sup>-1</sup> acetone extract exhibited similar percentage inhibition as the standard antioxidant drug rutin. However, the percentage inhibition of this

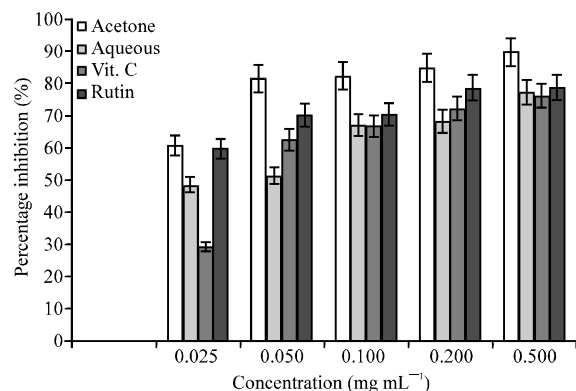


Fig. 2: Nitric oxide scavenging activity of *Nymphaea lotus* extracts, vitamin C and rutin. Results are means of three replicates

radical by acetone was significantly different ( $p < 0.05$ ) when compared with aqueous extract and vitamin C.

DPPH is characterized as stable free radical by virtue of the delocalization of the spare electron, where the molecule as a whole do not dimerise, as would be the case with other free radicals (Arokiyaraj *et al.*, 2008). The phenolic compounds present in the *N. lotus* extracts could be responsible for the observed DPPH radical scavenging activity, since phenols can readily donate hydrogen atom to the radical (Tung *et al.*, 2009). Oki *et al.* (2002) reported that the quantity of phenolic compounds in an extract is directly proportional to its free radical scavenging ability. Siriwardhana *et al.* (2003) also reported positive correlation between DPPH radical scavenging activity and total phenolic content. Huang *et al.* (2009) further reported that phenolic compounds have chemopreventive properties such as antioxidant, anticarcinogenic and anti-inflammatory effects.

**Nitric oxide scavenging activity:** Nitric oxide radical is a highly reactive compound capable of changing the structural and functional behavior of many cellular components (Ashokkumar *et al.*, 2008; Hazra *et al.*, 2009). Figure 2 showed that the extracts of *N. lotus* inhibited nitric oxide in a concentration dependent manner. Acetone extract exhibited highest percentage inhibition of 89.93% which was significantly different from the aqueous extract and the standard antioxidants (vitamin C and rutin). The inhibitory potentials of the extracts on NO radical can be attributed to the presence of flavonoids which are able to compete with oxygen and its derivatives because they can easily donate electrons to the radicals (Maccocci *et al.*, 1994).

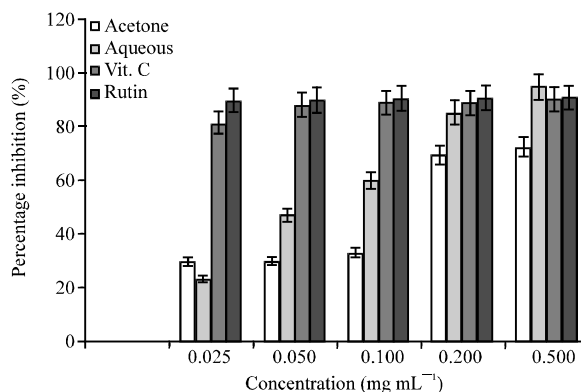


Fig. 3: ABTS scavenging activity of the extracts of *N. lotus*, vitamin C and rutin. Results are means of 3 replicates

Flavonoids are involved in scavenging the oxygen derived free radicals (Nijveldt *et al.*, 2001; Vaya *et al.*, 2003).

**ABTS radical scavenging activity:** Figure 3 depicted that aqueous extract exhibited the highest scavenging activity against ABTS radical. The extracts exhibited a dose response scavenging activity against ABTS radical. The order of inhibition was aqueous>rutin>vitamin C>acetone extract.

ABTS is a blue green radical that is reactive towards phenolics, thiols and other antioxidants (Walker and Everette, 2009). As a result, the blue-green radical becomes colourless and is measured spectrophotometrically at 734 nm. The extracts exhibited a dose response scavenging activity on ABTS radical with highest percentage inhibition of 72.15% for acetone extract and 94.30% for aqueous extract. The result of the inhibitory activities of the extracts on ABTS radical followed a similar trend as that obtained from inhibition of DPPH radical. This is contrary to the findings of Wang *et al.* (1998) who reported that compounds which possess ABTS radical scavenging activity might not be able to scavenge DPPH radical. The ABTS scavenging activity of the extracts can be added to the concentration of their phenolic contents.

**Hydrogen peroxide scavenging capacity:** Hydrogen peroxide is a pro-oxidant that is capable of crossing the membrane to oxidize a number of compounds. H<sub>2</sub>O<sub>2</sub> can give rise to hydroxyl radical thereby interacting with cellular components to cause tissue damage and eventually cell death (Reddy *et al.*, 2010). Figure 4 showed that both the aqueous and acetone extracts of *N. lotus* scavenged the hydrogen peroxide radical. The percentage

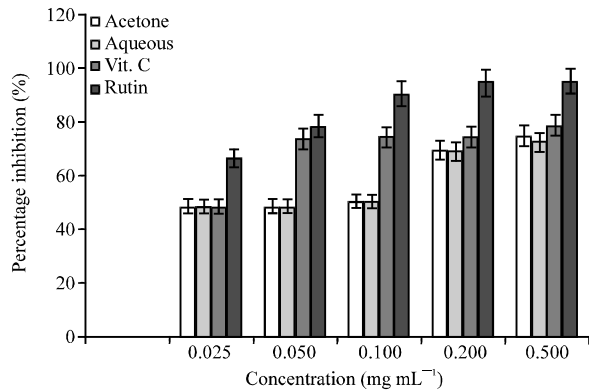


Fig. 4: Hydrogen peroxide scavenging activity of aqueous and acetone extracts *N. lotus*, vitamin C and gallic acid. Results are means of three replicates

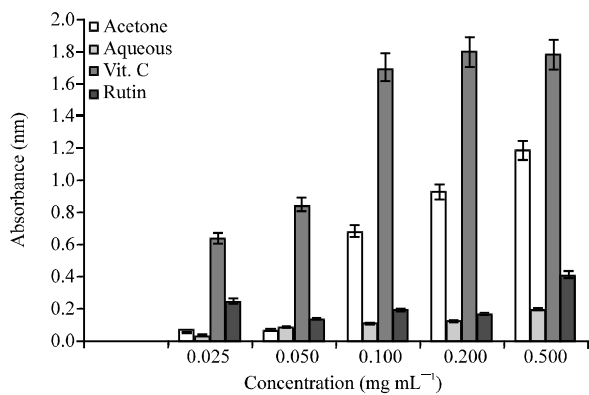


Fig. 5: Ferric reducing power of aqueous and acetone extracts *N. lotus*, gallic acid and rutin. Results are means of 3 replicates

inhibitions by acetone (74.73%) and aqueous extracts (72.35%) were significantly lower ( $p < 0.05$ ) than those of gallic acid (78.63%) and rutin (94.89%). Nevertheless, the inhibition of  $H_2O_2$  radical by the extracts can be attributed to the proton donating abilities of their phenolic contents.

**Ferric reducing power:** The ability of *N. lotus* extracts to reduce ferric cyanide to its ferrous form is presented in Fig. 5. At all the concentrations tested, acetone extract showed significantly higher ( $p < 0.05$ ) reducing power than aqueous extract and rutin while gallic acid had highest reducing power. The result of the reduction of Fe radical by the extracts and the standard drugs observed was in the order gallic acid > acetone > rutin > water. It was also observed that increase in concentrations of the extracts and the standard drugs increased the reducing power.

Table 2: Free radical scavenging activity of *Nymphaea lotus* ( $IC_{50}$  mg mL<sup>-1</sup>)

Sample	DPPH	ABTS	NO	$H_2O_2$
Acetone	0.016±0.001 <sup>a</sup>	0.150±0.003 <sup>a</sup>	0.022±0.001 <sup>a</sup>	0.100±0.011 <sup>a</sup>
Aqueous	0.025±0.001 <sup>a</sup>	0.040±0.002 <sup>b</sup>	0.061±0.010 <sup>b</sup>	0.100±0.009 <sup>a</sup>
Vitamin C	0.052±0.002 <sup>b</sup>	0.016±0.001 <sup>c</sup>	0.045±0.004 <sup>b</sup>	-
Rutin	0.020±0.001 <sup>a</sup>	0.010±0.000 <sup>c</sup>	0.030±0.002 <sup>a</sup>	0.013±0.001 <sup>b</sup>
Gallic acid	-	-	-	0.025±0.001 <sup>b</sup>

Values are the mean of three replicates. Values with different alphabetical superscripts along columns are significantly different ( $p < 0.05$ )

**Estimation of  $IC_{50}$ :** Table 2 presented the result of the  $IC_{50}$  generated for the inhibition of the various radicals used in this experiment. The concentration required to scavenge 50% free radical generated ( $IC_{50}$ ) was determined from the results of a series of concentrations tested. The lower the  $IC_{50}$  the higher the free radical scavenging activity of the extract. The  $IC_{50}$  values of the tested samples in DPPH scavenging assay were in the order vitamin C > acetone extract > aqueous extract. For ABTS assay, the  $IC_{50}$  values were in the order: acetone extract > vitamin C > aqueous extract. Nitric oxide scavenging assay  $IC_{50}$  values were in the order vitamin C > acetone extract > aqueous extract while Hydrogen peroxide  $IC_{50}$  values were in the order gallic acid > acetone extract > acetone extract > aqueous extract.

Acetone extract of *Nymphaea lotus* has a lower  $IC_{50}$  for DPPH (0.016 mg mL<sup>-1</sup>) and nitric oxide (0.022 mg mL<sup>-1</sup>) radical scavenging ability compared to aqueous extract while aqueous extract has lower  $IC_{50}$  for ABTS radical scavenging activity (0.040 mg L<sup>-1</sup>). However, both extracts have the same  $IC_{50}$  for hydrogen peroxide radical scavenging ability (0.1 mg mL<sup>-1</sup>). Therefore, it can be inferred that the acetone extract of *Nymphaea lotus* is a more potent antioxidant than the aqueous extract.

## CONCLUSION

This study revealed that *Nymphaea lotus* is very rich in phytochemicals that are of high medicinal importance, this confirms and validates its uses for the treatment of several diseases in humans. It was also discovered that the plant possesses high antioxidant power. It can be suggested that the presence of phenolic compounds may be responsible for the high antioxidant power of this plant. Since phenolic compounds can readily donate electrons to the free radicals and thus can be used as a source of natural antioxidants since the standard antioxidant compounds have been linked to different adverse effects.

## REFERENCES

Adnaik, R.S., P.T. Pai, V.D. Sapakal, N.S. Naikwade and C.S. Magdum, 2009. Anxiolytic activity of *Vitex negundo* Linn. in experimental models of anxiety in mice. Int. J. Green Pharm., 3: 243-247.

- Akinjogunla, O.J., A.A. Adegoke, I.P. Udokang and B.C. Adebayo-Tayo, 2009. Antimicrobial potential of *Nymphaea lotus* (Nymphaeaceae) against wound pathogens. *J. Med. Plants Res.*, 3: 138-141.
- Al-Jaber, N.A., A.S. Awaad and J.E. Moses, 2011. Review on some antioxidant plants growing in Arab world. *J. Saudi Chem. Soc.*, 15: 293-307.
- Alia, M., C. Horcajo, L. Bravo and L. Goya, 2003. Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. *Nutr. Res.*, 23: 1251-1267.
- Arokiyaraj, S., S. Martin, K. Perinbam, P.M. Arockianathan and V. Beatrice, 2008. Free radical scavenging activity and HPTLC finger print of *Pterocarpus santalinus* L. an *in vitro* study. *Indian J. Sci. Technol.*, 1: 1-3.
- Aruoma, O.I., 2003. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant food. *Mutation Res. Fundam. Mol. Mech. Mutagen.*, 523-524: 9-20.
- Ashokkumar, D., V. Thamilselvan, G.P. Senthilkumar, U.K. Mazumder and M. Gupta, 2008. Antioxidant and free radical scavenging effects of *Lippia nodiflora*. *Pharm. Biol.*, 46: 762-771.
- Bematova, I., O. Pechanova, P. Babal, S. Kysela, S. Stvrtnina and R. Andriantsitohaina, 2002. Wine polyphenols improve cardiovascular remodeling and vascular function in NO-deficient hypertension. *Am. J. Physiol. Heart Circ. Physiol.*, 282: H942-H948.
- Cushnie, T.P.T. and A.J. Lamb, 2005. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents*, 26: 343-356.
- Ebana, R.U.B., B.E. Madunagu, E.D. Ekpe and I.N. Otung, 1991. Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola*, *Borreria ocymoides*, *Kola nitida* and *Citrus aurantifolia*. *J. Applied Microbiol.*, 71: 398-401.
- Ganie, S.A., E. Haq, A. Masood, A. Hamid and M.A. Zargar, 2011. Antioxidant and protective effect of ethyl acetate extract of podophyllum hexandrum rhizome on carbon tetrachloride induced rat liver injury. *Evid. Based Complement Altern. Med.*, 2011: 238020-238020.
- Hamburger, M. and K. Hostettmann, 1991. Bioactivity in plants: The link between phytochemistry and medicine. *Phytochemistry*, 30: 3864-3874.
- Harborne, J.B., 2005. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd Edn., Springer Pvt. Ltd., New Delhi, India, ISBN-13: 9788181283108, pp: 145-156.
- Harnafi, H. and S. Amrani, 2007. Flavonoids as potent phytochemicals in cardiovascular diseases prevention. *Pharm. Rev.*, 1: 193-202.
- Hazra, B., R. Sarkar, S. Mandal, S. Biswas and N. Mandal, 2009. Studies on antioxidant and antiradical activities of *Dolichos biflorus* seed extract. *Afr. J. Biotechnol.*, 8: 3927-3933.
- Huang, W.Y., Y.Z. Cai and Y. Zhang, 2009. Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. *Nutr. Cancer*, 62: 1-20.
- James, O., E.G. Unekwajo and A.A. Ojochenemi, 2011. Assessment of biological activities: A comparison of *Pergularia daemia* and *Jatropha curcas* leaf extracts. *Br. Biotechnol. J.*, 1: 85-100.
- Krishnaiah, D., R. Sarbatly and R. Nithyanandam, 2011. A review of the antioxidant potential of medicinal plant species. *Food Bioprod. Process.*, 89: 217-233.
- Madhusudhanan, N., T. Lakshmi, S.G. Kumar, G. Venu and Ramakrishnan *et al.*, 2011. *In vitro* antioxidant and free radical scavenging activity of aqueous and ethanolic flower extract of *Nymphaea alba*. *Int. J. Drug Dev. Res.*, 3: 252-258.
- Marcocci, L., L. Packer, M.T. Droy-Lefaix, A. Sekaki and M. Gardes-Albert, 1994. Antioxidant action of *Ginkgo biloba* extract EGb 761. *Methods Enzymol.*, 234: 462-475.
- Narender, T., T. Khaliq, A. Puri and R. Chander, 2006. Antidyslipidemic activity of furano-flavonoids isolated from *Indigofera tinctoria*. *Bioorg. Med. Chem. Lett.*, 16: 3411-3414.
- Nijveldt, R.J., E. van Nood, D.E.C. van Hoorn, P.G. Boelens, K. van Norren and P.A.M. van Leeuwen, 2001. Flavonoids: A review of probable mechanisms of action and potential applications. *Am. J. Clin. Nutr.*, 74: 418-425.
- Obadoni, B.O. and P.O. Ochuko, 2002. Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global J. Pure Applied Sci.*, 8: 203-208.
- Oki, T., M. Masuda, S. Furuta, Y. Nishiba, N. Terahara and I. Suda, 2002. Involvement of anthocyanins and other phenolic compounds in radical-scavenging activity of purple-fleshed sweet potato cultivars. *J. Food Sci.*, 67: 1752-1756.
- Okochi, V.I. and J. Okpuzor, 2005. Micronutrients as therapeutic tools in the management of sickle cell disease, malaria and diabetes. *Afr. J. Biotechnol.*, 4: 1568-1579.
- Otang, W.M., D.S. Grierson and R.N. Ndip, 2012. Phytochemical studies and antioxidant activity of two South African medicinal plants traditionally used for the management of opportunistic fungal infections in HIV/AIDS patients. *BMC Comp. Alter. Med.*, Vol. 12. 10.1186/1472-6882-12-43



- Oyedemi, S.O., G. Bradley and A.J. Afolayan, 2010. *In vitro* and *in vivo* antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg. Afr. J. Pharm. Pharmacol., 4: 70-78.
- Oyedemi, S.O. and A.J. Afolayan, 2011. Antibacterial and antioxidant activities of hydroalcoholic stem bark extract of *Schotia latifolia* Jacq. Asian Pac. J. Trop. Med., 4: 952-958.
- Oyedemi, S.O., B.O. Oyedemi, S. Arowosegbe and A.J. Afolayan, 2012. Phytochemicals analysis and medicinal potentials of hydroalcoholic extract from *Curtisia dentata* (Burm. f) C.A. Sm stem bark. Int. J. Mol. Sci., 13: 6189-6203.
- Reddy, K.H., P.V.G.K. Sharma and O.V.S. Reddy, 2010. A comparative *in vitro* study on antifungal and antioxidant activities of *Nervilia aragoana* and *Atlantia monophylla*. Pharm. Biol., 48: 595-602.
- Robin, D., 2001. *Nymphaea odorata* (White pond lily) in Medical Herbalism. Mater. Med. Pharm., 11: 231-233.
- Siriwardhana, N., K.W. Lee, Y.J. Jeon, S.H. Kim and J.W. Haw, 2003. Antioxidant activity of hizikia fusiformis on reactive oxygen species scavenging and lipid peroxidation inhibition. Food Sci. Technol. Int., 9: 339-346.
- Talaz, O., I. Gulcin, S. Goksu and N. Saracoglu, 2009. Antioxidant activity of 5,10-dihydroindeno[1,2-b]indoles containing substituents on dihydroindeno part. Bioorganic Med. Chem., 17: 6583-6589.
- Thippeswamy, B.S., B. Mishra, V.P. Veerapur and G. Gupta, 2011. Anxiolytic activity of *Nymphaea alba* Linn. in mice as experimental models of anxiety. Indian J. Pharmacol., 43: 50-55.
- Tung, Y.T., J.H. Wu, C.Y. Huang, Y.H. Ku and S.T. Chang, 2009. Antioxidant activities and phytochemical characteristics of extracts from *Acacia confusa* bark. Bioresour. Technol., 100: 509-514.
- Vaijanathappa, J., S. Badami and S. Bhojraj, 2008. *In vitro* antioxidant activity of *Enicostemma axillare*. J. Health Sci., 54: 524-528.
- Vaya, J., S. Mohmod, A. Goldblum, M. Aviram and N. Volkavor *et al.*, 2003. Inhibition of LDL oxidation by flavonoids in relation to their structure and calculated enthalpy. Phytochemistry, 62: 89-99.
- Walker, R.B. and J.D. Everette, 2009. Comparative reaction rates of various antioxidants with ABTS radical cation. J. Agric. Food Chem., 57: 1156-1161.
- Wang, M., J. Li, M. Rangarajan, Y. Shao, E.J. LaVoie, T.C. Huang and C.T. Ho, 1998. Antioxidative phenolic compounds from sage (*Salvia officinalis*). J. Agric. Food Chem., 46: 4869-4873.
- Wang, W.N., X.B. Yang, H.Z. Liu, Z.M. Huang and G.X. Wu, 2005. Effect of *Oenanthe javanica* flavone on human and duck hepatitis B virus infection. Acta Pharmacol. Sin., 26: 587-592.
- Wee, Y.C., 1992. A Guide to Medicinal Plants. 3rd Edn., Singapore Science Centre, Singapore, Pages: 160.