Antioxidant and Antidiabetic Activities of Fresh Aqueous Pink Petals of Indian Lotus- *Nelumbo nucifera* Gaertn.

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**ABSTRACT**

The genus *Nelumbo* is endowed with a number of medicinally important activities antidiabetic, antipyretic, anti-inflammatory, anti-cancerous, antimicrobial, antiviral and anti-obesity properties. Furthermore, *Nelumbo nucifera* flowers are served as healthy beverages to treat hypertension, cancer, diarrhea, fever, weakness, infection and body heat imbalance. It has been widely used in folk medicine for the treatment of various inflammatory and infectious diseases. Current research studies were carried out for evaluating the antioxidant, antidiabetic and antibacterial activities of fresh aqueous pink petals of *Nelumbo nucifera*. Antioxidant activities such as DPPH radical scavenging, ABTS radical cation, phosphomolybdenum reduction and Fe³⁺ reduction were carried out for fresh aqueous pink petals of *Nelumbo nucifera*. The maximum DPPH radical scavenging activity was 88.33±0.47% at 300 µg/mL concentration and the IC₅₀ value was 131.68 µg/mL concentration. The maximum superoxide (O₂⁻) radical scavenging activity was 86.76±0.31% at 120 µg/mL concentration and the IC₅₀ value was 21.31 µg/mL concentration. The maximum ABTS radical cation scavenging activity was 69.55±0.26% at 30 µg/mL concentration and the IC₅₀ value was 10.82 µg/mL concentration respectively. The maximum Mo⁶⁺ reduction was 84.54±0.21% at 45 µg/mL concentration and the IC₅₀ value of Mo⁶⁺ reduction was 25.79 µg/mL concentration. The maximum Fe³⁺ reduction was 56.60±0.38% at 300 µg/mL concentration and the IC₅₀ value of Fe³⁺ reduction was 177.30 µg/mL concentration respectively. The maximum alpha amylase enzyme inhibition was 66.37±0.10% at 300 µg/mL concentration and the IC₅₀ value was 63.84 µg/mL concentration respectively. The antibacterial activity of fresh aqueous pink petals of *Nelumbo nucifera* showed maximum zone of inhibition of 14 mm for *B. subtilis* and minimum zone of inhibition of 10 mm for *E. coli* at 375 µg/mL concentration.

**Keywords:** Antioxidant, Superoxide (O₂⁻) radical, ABTS⁺ radical cation, Fe³⁺ reduction and alpha-amylase.

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**INTRODUCTION**

Traditional knowledge on medicinal uses of lotus plant is demonstrated by many scientific investigations. The whole plant is used as antifungal, antipyretic, emollient, sudorific, diuretic, and cardiotoxic. Uses of various parts of the lotus plant are common in tissue inflammation diarrea treatment and haemostasis. The flowers and leaves are of importance in treating bleeding disorders (Figure 1). Flowers consumption can promote conception and also are important to treat fever, diarrhea, hyperdipsia, cholera and hepatopathy. In traditional folk medicines, seeds are used for the treatment of poison antidote and disease of skin and usually prescribed as refrigerant and as diuretic to children. The sacred lotus is a perennial aquatic plant with rhizomes (often mistakenly called ‘roots’) that grow in the mud at the bottom of shallow ponds, lakes, lagoons, marshes and flooded fields. It's large, peltate (with the leaf-stalk attaching to the centre, rather than the edge) leaves rise above the water surface on 1 to 2 m long petioles. Lotus grows to a height of about 150 cm, with a 3-meter horizontal spread. In India, *N. nucifera*, commonly known as lotus, kamala or padma, is an aquatic species, requiring plenty of space and full sun in order to thrive. There are two varieties of *'kamala'*; one has white flowers and is commonly called *'pundarika'* or *'sveta kamala'*, the other has pink or reddish-pink flowers and is called *'rakta kamala'*. Sun-dried flower powder of *N. nucifera*, as well as the aqueous and alcoholic extract of the flower, produced significant hypoglycaemia in fasting normal albino rabbits.
Dietary and endogenous antioxidants prevent cellular damage by reacting with and eliminating oxidizing free radicals. However, in cancer treatment, a mode of action of certain chemotherapeutic agents involves the generation of free radicals to cause cellular damage and necrosis of malignant cells. So a concern has logically developed as to whether exogenous antioxidant compounds taken concurrently during chemotherapy could reduce the beneficial effect of chemotherapy on malignant cells. The importance of this concern is underlined by a recent study which estimates 23 percent of cancer patients take antioxidants. The study of antioxidant use in cancer treatment is a rapidly evolving area. Antioxidants have been extensively studied for their ability to prevent cancer in humans.

Antioxidants supplements were once thought to be harmless but increasingly we are becoming aware of interactions and potential toxicity. Normal concentrations found in the body, vitamin C and beta-carotene are antioxidants; but at higher concentrations they are pro-oxidants and, thus, harmful and should be consumed accordingly needed by humans.

Free radicals attack three main cellular components.

**Lipids** - Peroxidation of lipids in cell membranes can damage cell membranes by disrupting fluidity and permeability. Lipid peroxidation can also adversely affect the function of membrane bound proteins such as enzymes and receptors.

**Proteins** - Direct damage to proteins can be caused by free radicals. This can affect many kinds of protein, interfering with enzyme activity and the function of structural proteins.

**DNA** - Fragmentation of DNA caused by free radical attack causes activation of the poly (ADP-ribose) synthetase enzyme. This splits NAD+ to aid the repair of DNA. However, if the damage is extensive, NAD+ levels may become depleted to the extent that the cell may no longer be able to function and dies. The site of tissue damage by free radicals is dependent on the tissue and the reactive species involved. Extensive damage can lead to death of the cell; this may be by necrosis or apoptosis depending on the type of cellular damage. When a cell membrane or an organelle membrane is damaged by free radicals, it loses its protective properties. This puts the health of the entire cell at risk.

**Taxonomic Classification of Nelumbo nucifera**

*Kingdom:* Plantae  
*Sub Kingdom:* Tracheobionta  
*Super Division:* Spermatophyta  
*Division:* Magnoliophyta  
*Class:* Magnoliopsida  
*Subclass:* Magnoliidae  
*Super order:* Proteanae  
*Order:* Proteales  
*Family:* Nymphaeaceae  
*Genus:* Nelumbo  
*Species:* *Nelumbo nucifera* Gaertn. – Sacred lotus.

**Materials and Methods**

**Collection of pink petals of Nelumbo nucifera and aqueous extraction**

The pink petals of *Nelumbo nucifera* were collected from the market at Maduvinkarai, Chennai, Tamil Nadu, India. The fresh pink petals of *Nelumbo nucifera* were subjected to soaking process in sterile distilled water and boiled in cookware for 15 min. The pink coloured supernatant was filtered, condensed in a hot plate at 50°C, which yields pale pink viscous extract.

**In vitro antioxidant activities**

**DPPH** radical scavenging activity

The radical scavenging activity of fresh aqueous pink petals of *Nelumbo nucifera* was carried out by the reduction DPPH-free radical method. One mL of fresh aqueous pink petals of *Nelumbo nucifera* extract with various concentrations (50-300 μg/mL) was mixed with 1 mL of 0.1 mM DPPH solution in methanol. The mixture was then allowed to stand for 30 min incubation in dark. One mL of methanol mixed with 1 mL of DPPH solution was used as the control. The decrease in absorbance was measured at 517 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

\[
\text{% of DPPH radical inhibition} = \left( \frac{\text{Control} - \text{Sample}}{\text{Control}} \right) \times 100
\]

**Superoxide (O$_2$-) radical scavenging activity**

Superoxide (O$_2$-) radical scavenging activity was carried out by the method and the reaction mixture contains different concentrations (20-120 μg/mL) of fresh aqueous pink petals of *Nelumbo nucifera* extract with 50 mM of phosphate buffer (pH 7.4), 200 μL of 1.5 mM of riboflavin, 200 μL 12 mM of EDTA and 100 μL 50 mM of NBT, added in that sequence. The reaction was started by illuminating the reaction mixture for 15 min in UV lamp. After illumination, the absorbance was measured at 560 nm. The percentage of inhibition was calculated as:

\[
\text{% of O$_2$- radical inhibition} = \left( \frac{\text{Control} - \text{Sample}}{\text{Control}} \right) \times 100
\]
measured at 590 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

\[
\% \text{ of Superoxide (O}_2^-\text{) radical inhibition} = \left( \frac{\text{Control} - \text{Sample}}{\text{Control}} \right) \times 100
\]

ABTS\(^{+}\) (2,2-azinobis (3-ethylbenzo thiazoline-6-sulfonic acid) radical cation scavenging activity

The fresh aqueous pink petals of *Nelumbo nucifera* from the stock solution was taken in various concentrations and this assay was performed according to the method\(^{20}\). The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 hours at room temperature in the dark. Fresh ABTS solution was prepared for each experiment. Fresh aqueous pink petals of *Nelumbo nucifera* extract in varying concentrations (5-30 µg/mL) were allowed to react with 500 µL of the ABTS solution for 15 minutes in dark condition and the absorbance was measured at 734 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

\[
\% \text{ of ABTS}^{+} \text{ radical cation inhibition} = \left( \frac{\text{Control} - \text{Sample}}{\text{Control}} \right) \times 100
\]

Phosphomolybdenum reduction activity

The antioxidant capacity of the fresh aqueous pink petals of *Nelumbo nucifera* was assessed as described\(^{20}\). The fresh aqueous pink petals of *Nelumbo nucifera* extract with varying concentrations ranging (20-45 µg/mL) was combined with reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in water bath at 95°C for 90 min. The absorbance of the coloured complex was measured at 695 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as:

\[
\% \text{ of Phosphomolybdenum reduction} = \left( \frac{\text{Sample} - \text{Control}}{\text{Sample}} \right) \times 100
\]

Ferric (Fe\(^{3+}\)) reducing power activity

The reducing power of fresh aqueous pink petals of *Nelumbo nucifera* was determined by slightly modified method\(^{20}\). One mL of fresh aqueous pink petals of *Nelumbo nucifera* extract of different concentrations (50-300 µg/mL) was mixed with phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K\(_3\)Fe(CN)\(_6\)] (1 mL, 1 % w/v). The mixtures were then incubated at 50°C for 20 min in water bath. 500 µL of trichloroacetic acid (10 % w/v) was added to each mixture, followed by 100 µL of Ferric chloride (0.01%, w/v) was added and the absorbance was measured at 700 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as:

\[
\% \text{ of Fe}^{3+} \text{ reduction} = \left( \frac{\text{Sample} - \text{Control}}{\text{Sample}} \right) \times 100
\]

Antidiabetic activity by Alpha-amylase inhibition method

The antidiabetic activity of fresh aqueous pink petals of *Nelumbo nucifera* was determined by slightly modified method\(^{20}\). Different concentrations of fresh aqueous pink petals of *Nelumbo nucifera* extract (50-300 µg/mL) was mixed with 1 mL of methanol, followed by 10 µL of alpha amylase solution (1 % w/v). The mixture was then incubated in room temperature at 37°C for 10 min. 500 µL of starch (1 % w/v) was added to each mixture, mixed well and incubated in room temperature at 37°C for 60 min. 100 µL of freshly prepared 1N Hydrochloric acid was added to terminate the enzymatic reaction and 200 µL of freshly prepared iodine solution (1 % w/v) was added and shaken well. The absorbance was measured at 565 nm using UV-Vis spectrophotometer. Acarbose was used as the standard reference. The percentage of inhibition was calculated as:

\[
\% \text{ of alpha amylase inhibition} = \left( \frac{\text{Sample} - \text{Control}}{\text{Sample}} \right) \times 100
\]

Antibacterial activity by Agar well diffusion method

Nutrient agar was prepared and poured in the sterile Petri dishes and allowed to solidify. 24 hours grown bacterial pathogens were swabbed on nutrient agar plates.\(^{22}\) Then, the fresh aqueous pink petals of *Nelumbo nucifera* extract in varying concentrations (125 µg/mL, 200 µg/mL, 250 µg/mL, 375 µg/mL) was loaded in the clean lawns made using sterile cork borer. Tetracycline (30 µg) was used as standard. The plates were then incubated at 37°C for 24 hours and after incubation, the inhibition diameter was measured and recorded.

**RESULTS AND DISCUSSION**

**DPPH\(^{+}\) radical scavenging activity**

Evaluation of antioxidant activity by DPPH method is the best screening option for herbal based drugs. DPPH\(^{+}\) (1,1-Diphenyl-2-picrylhydrazyl) is a stable nitrogen centered free radical which has an unpaired valence electron at one atom of nitrogen bridge\(^{23}\). The ability of fresh aqueous pink petals of *Nelumbo nucifera* to scavenge free radicals formed was assessed using 1,1-diphenyl-2-picryl hydrazyl radical (DPPH). The fresh aqueous pink petals of *Nelumbo nucifera* demonstrated high capacity for scavenging free radicals by reducing the stable DPPH (1,1-diphenyl-2-picryl hydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picryl hydrazine and the reducing capacity increased with increasing concentration of the extracts. The maximum DPPH\(^{+}\) radical scavenging activity of fresh aqueous pink petals of *Nelumbo nucifera* extract was 88.33±0.47% at 300 µg/mL concentration (Table 1). The IC\(_{50}\) value for the fresh aqueous pink petals of *Nelumbo nucifera* extract was found to be 131.68 µg/mL concentration respectively (Graph 1) and was compared with standard (Ascorbic acid, IC\(_{50}\) = 14.07 µg/mL concentration).

**Table 1: DPPH\(^{+}\) radical scavenging activity of fresh aqueous pink petals of *Nelumbo nucifera***

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/mL)</th>
<th>% of inhibition*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>19.14±0.28</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>37.97±0.14</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>61.00±0.35</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>73.79±0.10</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>83.62±0.22</td>
</tr>
<tr>
<td>6</td>
<td>300</td>
<td>88.33±0.47</td>
</tr>
</tbody>
</table>

(*Average value of 3 replicates*)
Superoxide (O$_2^-$) radical scavenging activity

Superoxide anion is also very harmful to cellular components and their effects can be magnified because it produces other kinds of free radicals and oxidizing agents. Flavonoids are effective antioxidants, mainly because they scavenge superoxide anions. Superoxide anions derived from dissolved oxygen by the riboflavin-light-NBT system will reduce NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT$^-$) to blue formazan, which is measured at 590 nm using UV-Vis spectrophotometer. Antioxidants are able to inhibit the blue NBT formation and the decrease of absorbance with antioxidants indicates the consumption of superoxide anion in the reaction mixture$^{24}$. The maximum superoxide (O$_2^-$) radical scavenging activity of fresh aqueous pink petals of *Nelumbo nucifera* extract was 86.76±0.31% at 120 µg/mL concentration (Table 2 and Graph 2) and the IC$_{50}$ value for the fresh aqueous pink petals of *Nelumbo nucifera* extract was found to be 21.31 µg/mL concentration respectively. It was compared with the standard ascorbic acid (IC$_{50}$ = 12.58 µg/mL concentration).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/mL)</th>
<th>% of inhibition$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>46.91±0.37</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>67.64±0.24</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>74.63±0.14</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>80.14±0.29</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>84.55±0.46</td>
</tr>
<tr>
<td>6</td>
<td>120</td>
<td>86.76±0.31</td>
</tr>
</tbody>
</table>

(*Average value of 3 replicates)

Table 2: Superoxide (O$_2^-$) radical scavenging activity of fresh aqueous pink petals of *Nelumbo nucifera*

Phosphomolybdenum reduction activity

The total antioxidant activity of fresh aqueous pink petals of *Nelumbo nucifera* extract was measured spectrophotometrically by phosphomolybdenum reduction method, which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm$^{26}$. The maximum phosphomolybdenum reduction of fresh aqueous pink petals of *Nelumbo nucifera* extract was 84.54±0.21% at 45 µg/mL concentration with the RC$_{50}$ value of 25.79 µg/mL concentration respectively (Table 4 and Graph 4). It was compared with the standard ascorbic acid (RC$_{50}$ = 7.86 µg/mL).

Graph 1: DPPH$^-$ radical scavenging activity of fresh aqueous pink petals of *Nelumbo nucifera*

Graph 2: Superoxide (O$_2^-$) radical scavenging activity of fresh aqueous pink petals of *Nelumbo nucifera*

Graph 3: ABTS$^{**}$ radical cation scavenging activity of fresh aqueous pink petals of *Nelumbo nucifera*

Table 3: ABTS$^{**}$ radical cation scavenging activity of fresh aqueous pink petals of *Nelumbo nucifera*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/mL)</th>
<th>% of inhibition$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>39.84±0.26</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>46.21±0.11</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>52.76±0.18</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>55.70±0.41</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>62.10±0.46</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>69.55±0.26</td>
</tr>
</tbody>
</table>

(*Average value of 3 replicates)

ABTS$^{**}$ is a blue chromophore produced by the reaction between ABTS and potassium persulfate and ABTS$^{**}$ radical cation gets reduced in the presence of fresh aqueous pink petals of *Nelumbo nucifera* and the remaining radical cation concentration was then quantified at 734 nm. It can be prepared using K$_2$SO$_3$ as an oxidant. The blue-green colour of ABTS solution is formed by the loss of an electron by the nitrogen atom of ABTS (2, 2-azinobis (3-ethylbenzothiazolin-6-sulphonic acid)). The decolourization of the solution takes place in the presence of hydrogen donating antioxidant (nitrogen atom quenches the hydrogen atom$^{25}$). The maximum ABTS$^{**}$ radical cation scavenging activity of fresh aqueous pink petals of *Nelumbo nucifera* extract was 69.5±0.26% at 30 µg/mL concentration (Table 3 and Graph 3) and the IC$_{50}$ value for the fresh aqueous pink petals of *Nelumbo nucifera* extract was found to be as 10.82 µg/mL concentration respectively, which was compared with standard ascorbic acid (IC$_{50}$ = 4.87 µg/mL concentration).
Table 4: Phosphomolybdenum reduction activity of fresh aqueous pink petals of *Nelumbo nucifera*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/mL)</th>
<th>% of reduction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>38.1±0.10</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>48.4±0.39</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>59.8±0.16</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>77.7±0.33</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>82.9±0.24</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>84.5±0.21</td>
</tr>
</tbody>
</table>

(*Average value of 3 replicates)

Graph 4: Phosphomolybdenum reduction activity of fresh aqueous pink petals of *Nelumbo nucifera*

**Ferric (Fe³⁺) reducing power activity**

The reducing power of Fe³⁺ to Fe²⁺ by fresh aqueous pink petals of *Nelumbo nucifera* was studied and showed reduction ability in a dose dependent manner. The maximum reduction of fresh aqueous pink petals of *Nelumbo nucifera* extract was 56.00±0.38% at 300 µg/mL concentration (Table 5 and Graph 5). Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action27. The RCo value for the fresh aqueous pink petals of *Nelumbo nucifera* extract as found to be 177.30 µg/mL concentration respectively and was compared with the standard (20.93 µg/mL concentration) Ascorbic acid.

Table 5: Ferric (Fe³⁺) reducing power activity of fresh aqueous pink petals of *Nelumbo nucifera*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/mL)</th>
<th>% of reduction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>13.4±0.37</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>28.2±0.30</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>43.4±0.12</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>50.75±0.18</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>53.16±0.22</td>
</tr>
<tr>
<td>6</td>
<td>300</td>
<td>56.00±0.38</td>
</tr>
</tbody>
</table>

(*Average value of 3 replicates)

Graph 5: Ferric (Fe³⁺) reducing power activity of fresh aqueous pink petals of *Nelumbo nucifera*

**Antidiabetic activity by Starch-iodine colour method**

Diabetes mellitus (DM) is a chronic disease characterized by a deficiency in insulin production and its action or both. That leads to prolonged hyperglycemia with disturbances in most metabolic processes inside the human body28. The most popular methods for diagnosis of diabetes includes the measuring of fasting plasma glucose level (FPG), which is done in the early morning. Patients with FPG below 100 mg/dl are considered normal; those between 100 and 125 mg/dl indicate pre-diabetic while those individuals with glucose levels above 125 mg/dl are considered diabetic29. The alpha-glucosidase inhibitors “starch blockers” inhibit certain enzymes responsible for the breakdown of carbohydrates in the small intestine. They act mainly by decreasing the rate of carbohydrate absorption in the body. Moreover, acarbose, an important example in this class, reversibly inhibits both pancreatic α-amylase and α-glucosidase enzymes by binding to the carbohydrate-binding region and interfering with their hydrolysis into monosaccharides. This results in a slower absorption together with a reduction in postprandial blood-sugar levels30,31. The ability of for α-amylase enzyme inhibition was assessed by starch-iodine method. The maximum α-amylase enzyme inhibition of fresh aqueous pink petals of *Nelumbo nucifera* extract was 66.37±0.10% at 300 µg/mL concentration (Table 6 and Graph 6) and the IC₅₀ value for the fresh aqueous pink petals of *Nelumbo nucifera* extract was 63.84 µg/mL concentration respectively. It was compared with the standard Acarbose (IC₅₀ = 12.19 µg/mL concentration).

Table 6: Antidiabetic activity of fresh aqueous pink petals of *Nelumbo nucifera*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/mL)</th>
<th>% of inhibition*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>39.16±0.15</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>51.67±0.24</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>55.52±0.19</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>63.08±0.36</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>65.08±0.41</td>
</tr>
<tr>
<td>6</td>
<td>300</td>
<td>66.37±0.10</td>
</tr>
</tbody>
</table>

(*Average value of 3 replicates)

Graph 6: Antidiabetic activity of fresh aqueous pink petals of *Nelumbo nucifera*

**Antibacterial activity by Agar well diffusion method**

The fresh aqueous pink petals of *Nelumbo nucifera* were investigated for *in vitro* antibacterial activity against microorganisms including Gram-positive bacteria (*Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli, Proteus vulgaris, Shigella flexneri*). The antibacterial sensitivity of the fresh aqueous pink petals of *Nelumbo nucifera* extract and their potency were assessed quantitatively by measuring the inhibitory zone around the wells in the petriplates (Table 7).
Table 7: Antibacterial activity of fresh aqueous pink petals of *Nelumbo nucifera*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Bacterial pathogens</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Standard Tetracycline - 30 µg</td>
</tr>
<tr>
<td>1</td>
<td><em>Micrococcus luteus</em></td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td><em>Staphylococcus aureus</em></td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td><em>Bacillus subtilis</em></td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td><em>Escherichia coli</em></td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td><em>Proteus vulgaris</em></td>
<td>29</td>
</tr>
<tr>
<td>6</td>
<td><em>Shigella flexneri</em></td>
<td>29</td>
</tr>
</tbody>
</table>

The maximum inhibitory effect for fresh aqueous pink petals of *Nelumbo nucifera* extract against *Bacillus subtilis* was 14 mm at 375 µg/mL concentration respectively (Figure 2). The antibacterial activity of the fresh aqueous pink petals of *Nelumbo nucifera* could be correlated as due to the presence of secondary metabolites such as flavonoids, phenolic compounds, terpenoids, tannins and alkaloids that adversely affect the growth and metabolism of microbes.

CONCLUSION

Ethno-medicinal knowledge has already helped humans to combat many diseases. *Nelumbo nucifera* has also been extensively used for nutritional and traditional medicinal purpose by people in many parts of the world. Based on the antioxidant, antidiabetic and antibacterial activities, petals of *Nelumbo nucifera* could be used as a source of therapeutic agents. Further, the individual active compounds can be isolated by chromatographic techniques and the fractions shall be evaluated separately for FTIR, NMR to identify the compound functional group, nature and structure for converting as a new active drug from *Nelumbo nucifera*.

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REFERENCES


22. Elff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants J. Ethnopharmacol, 1998; 60: 1-8.


