IDDT

Available online on 15.09.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

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, Superoxide (O_2 -) radical, 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 (O_2 -) radical scavenging activity was 67.6±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 Fe³⁺ reduction was 84.54±0.21% at 45 µg/mL concentration and the RC₅₀ value of Mo⁶⁺ reduction was 25.79 µg/mL concentration. The maximum Fe³⁺ reduction was 56.00±0.38% at 300 µg/mL concentration and the RC₅₀ 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 (0_2) radical, ABTS $^{\bullet}$ radical cation, Fe $^{3+}$ reduction and alpha-amylase.

Article Info: Received 10 June 2019; Review Completed 18 Aug 2019; Accepted 29 Aug 2019; Available online 15 Sep 2019



Cite this article as:

Saraswathi K, Sivaraj C, Fransila B, Arumugam P, Antioxidant and Antidiabetic Activities of Fresh Aqueous Pink Petals of Indian Lotus-*Nelumbo nucifera* Gaertn., Journal of Drug Delivery and Therapeutics. 2019; 9(5):82-88 http://dx.doi.org/10.22270/jddt.v9i5.3565

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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 cardiotonic. Uses of various parts of the lotus plant are common in tissue inflammation diarrhea treatment and haemostasis¹. 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 children².

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^{3,4,5}. Lotus grows to a height of about 150 cm, with a 3-meter horizontal spread⁶. 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 reddishpink flowers and is called 'rakta kamala'⁷. 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 rabbits⁸.

ISSN: 2250-1177 [82] CODEN (USA): JDDTAO

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^{11,12}.

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 risk¹³.

Taxonomic Classification of Nelumbo nucifera

Kingdom: Plantae

Sub Kingdom: Tracheobionta
Super Division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Magnoliidae
Super order: Protaenae
Order: Protaeles

Family: Nelumbonaceae **Genus:** Nelumbo

Species: *Nelumbo nucifera* Gaertn. – Sacred lotus.





Figure.1: Habitat of Nelumbo nucifera

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^{14,15}.

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 16 . One mL of fresh aqueous pink petals of *Nelumbo nucifera* extract with various concentrations (50-300 $\mu g/mL$) 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 $1\,\text{mL}$ of DPPH solution was used as the control. The decrease in absorbance was measured at $517\,\text{nm}$ using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

% of DPPH' radical inhibition =
$$\frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Superoxide (02-) radical scavenging activity

Superoxide (O₂--) radical scavenging activity was carried out by the method 17 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

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measured at 590 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

% of Superoxide (O₂-) radical inhibition =
$$\frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

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 18 . 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 $\mu g/mL)$ 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:

% of ABTS⁺⁺ radical cation inhibition =
$$\frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Phosphomolybdenum reduction activity

The antioxidant capacity of the fresh aqueous pink petals of *Nelumbo nucifera* was assessed as described 19 . 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:

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

Ferric (Fe³⁺) 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 [K₃Fe (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:

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

Antidiabetic activity by Alpha-amylase inhibition method

The antidiabetic activity of fresh aqueous pink petals of *Nelumbo nucifera* was determined by slightly modified method²¹. 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:

% of alpha amylase inhibition =
$$\boxed{ \frac{ Sample - Control}{Sample} } *100$$

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 $\mu g/mL$, 200 $\mu g/mL$, 250 $\mu g/mL$, 375 $\mu g/mL$) was loaded in the clean lawns made using sterile cork borer. Tetracycline (30 μg) was used as standard. The plates were then incubated at $37^{\circ}C$ for 24 hours and after incubation, the inhibition diameter was measured and recorded.

RESULTS AND DISCUSSION

DPPH' radical scavenging activity

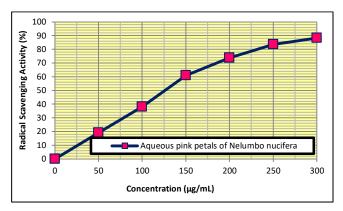
Evaluation of antioxidant activity by DPPH method is the best screening option for herbal based drugs. DPPH (1,1-Diphenyl-2-picrylhydrazyl) is a stable nitrogen centered free radical which has an unpaired valence electron at one atom of nitrogen bridge²³. The ability of fresh aqueous pink petals of Nelumbo 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 IC₅₀ 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, IC50 = 14.07 μg/mL concentration).

Table 1: DPPH radical scavenging activity of fresh aqueous pink petals of *Nelumbo nucifera*

S.No	Concentration (μg/mL)	% of inhibition*
		DPPH' radical
1	50	19.14±0.28
2	100	37.97±0.14
3	150	61.00±0.35
4	200	73.79±0.10
5	250	83.62±0.22
6	300	88.33±0.47

(*Average value of 3 replicates)

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

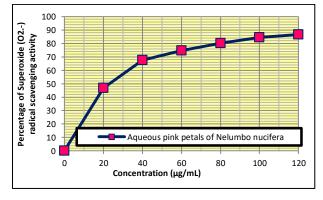
Superoxide (02-) radical scavenging activity

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 (NBT2+) 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 mixture²⁴. The maximum superoxide $(O_2$ -) radical scavenging activity of fresh aqueous pink petals of Nelumbo nucifera extract was $86.76\pm0.31\%$ at 120 µg/mL concentration (Table 2 and Graph 2) and the IC₅₀ 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 of ascorbic acid (IC₅₀ = $12.58 \mu g/mL$ concentration).

Table 2: Superoxide (O₂--) radical scavenging activity of fresh aqueous pink petals of *Nelumbo nucifera*

S.No	S.No Concentration (μg/mL)	% of inhibition*
		Superoxide (O ₂) radical
1	20	46.91±0.37
2	40	67.64±0.24
3	60	74.63±0.14
4	80	80.14±0.29
5	100	84.55±0.46
6	120	86.76±0.31

(*Average value of 3 replicates)



Graph 2: Superoxide (O₂--) radical scavenging activity of fresh aqueous pink petals of *Nelumbo nucifera*

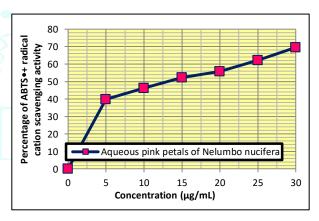
ABTS ** radical cation scavenging activity

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 K₂S₂O₈ 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 (3ethylbenzothiazolin-6-sulfonic acid)). The decolourization of the solution takes place in the presence of hydrogen donating antioxidant (nitrogen atom quenches the hydrogen atom²⁵. The maximum ABTS++ radical cation scavenging activity of fresh aqueous pink petals of Nelumbo nucifera extract was 69.55±0.26% at 30 μg/mL concentration (Table 3 and Graph 3) and the IC50 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 (IC₅₀ = $4.87 \mu g/mL$ concentration).

Table 3: ABTS*+ radical cation scavenging activity of fresh aqueous pink petals of *Nelumbo nucifera*

S.No	Concentration	% of inhibition*
	(μg/mL)	ABTS**radical cation
1 1	5	39.84±0.26
2		46.21±0.11
3	15	52.36±0.18
4	20	55.70±0.41
5	25	62.10±0.46
6	30	69.55±0.26

(*Average value of 3 replicates)



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

Phosphomolybdenum reduction activity

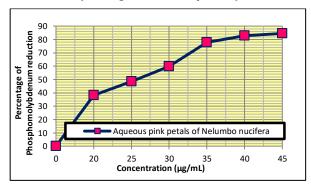
The total antioxidant activity of fresh aqueous pink petals of Nelumbo nucifera extract measured was spectrophotometrically by phosphomolybdenum reduction method, which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm²⁶. 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 RC₅₀ value of 25.79 μg/mL concentration respectively (Table 4 and Graph 4). It was compared with the standard ascorbic acid ($RC_{50} = 7.86$ $\mu g/mL$).

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

S.No	Concentration	% of reduction*
	(µg/mL)	Mo ⁶⁺ reduction
1	20	38.16±0.10
2	25	48.46±0.39
3	30	59.86±0.16
4	35	77.75±0.33
5	40	82.90±0.24
6	45	84.54±0.21

(*Average value of 3 replicates)



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

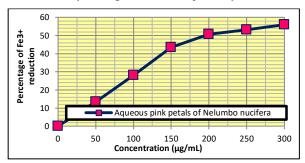
Ferric (Fe3+) reducing power activity

The reducing power of Fe $^{3+}$ to Fe $^{2+}$ 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 action 27 . The RC $_{50}$ 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*

S.No	Concentration	% of reduction*
	(μg/mL)	Fe ³⁺ reduction
1	50	13.46±0.37
2	100	28.20±0.30
3	150	43.43±0.12
4	200	50.75±0.18
5	250	53.16±0.22
6	300	56.00±0.38

(*Average value of 3 replicates)



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

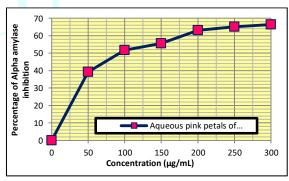
Antidiabetic activity by Starch-iodine colour method

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 αglucosidase 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 levels^{30,31}. 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 ($IC_{50} = 12.19 \,\mu g/mL$ concentration).

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

S.No	Concentration (μg/mL)	% of inhibition*
		Starch-Iodine method
1	50	39.16±0.15
2	100	51.67±0.24
3	150	55.52±0.19
4	200	63.08±0.36
5	250	65.08±0.41
6	300	66.37±0.10

(*Average value of 3 replicates)



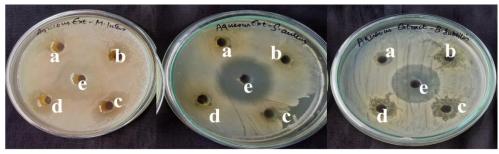
Graph 6: Antidiabetic activity of fresh aqueous pink petals of *Nelumbo nucifera*

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).

Zone of inhibition (mm) S.No **Bacterial pathogens** Standard 125 200 250 375 Tetracycline - 30 μg μg μg μg Micrococcus luteus 10 11 13 10 2 Staphylococcus aureus 31 10 10 10 11 3 Bacillus subtilis 29 11 12 14 14 4 Escherichia coli 28 10 10 10 10 Proteus vulgaris 29 10 11 11 5 11 13 12 Shigella flexneri 29 12 13 6

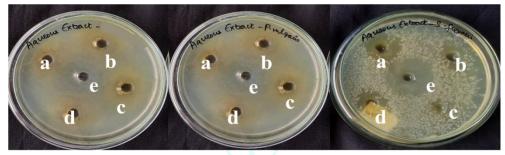
Table 7: Antibacterial activity of fresh aqueous pink petals of Nelumbo nucifera



Micrococcus luteus

Staphylococcus aureus

Bacillus subtilis



Escherichia coli

Proteus vulgaris

Shigella flexneri

Concentration range of fresh aqueous pink petals of *Nelumbo nucifera*: a-125 µg/mL, b-200 µg/mL, c-250 µg/mL, d-375 µg/mL e-30 µg/mL-Standard (Tetracycline)

Figure.2: Antibacterial activity of fresh aqueous pink petals of Nelumbo nucifera

The maximum inhibitory effect for fresh aqueous pink petals of Nelumbo nucifera extract against Bacillus subtilis was 14 mm at 375 $\mu g/mL$ 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*.

ACKNOWLEDGEMENT

The authors are thankful to Armats Biotek Training and Research Institute for providing facilities to carry out research work.

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ISSN: 2250-1177 [88] CODEN (USA): JDDTA0