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RESEARCH ARTICLE

Antioxidant and antimicrobial activity of *Nelumbo nucifera* Gaertn. leaf extracts

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Abstract

Nelumbo nucifera (Lotus plant) leaf extracts were prepared using different solvents (hexane, acetone and methanol) and evaluated for antioxidant and antimicrobial activity. DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical scavenging activity was high in methanolic leaf extract of *N. nucifera* compared to other extracts. Methanolic leaf extract showed maximum antibacterial activity against *Bacillus subtilis* whereas hexane and acetone showed maximum antifungal activity against *Candida albicans*. All solvent extracts exhibited high antifungal activity compared to standard.

Keywords: *Nelumbo nucifera,* hexane, methanol, *Bacillus subtilis, Candida albicans.*

Introduction

Nelumbo nucifera Gaertn. is a monogeneric plant belongs to family Nelumbonaceae, commonly known as sacred Indian lotus, rose of India, sacred water lily or East Indian lotus. *Nelumbo nucifera* is a perennial ornamental water plant grown in Asian countries for its edible rhizomes and seeds. *Nelumbo nucifera* is considered as an important traditional Chinese herb and all parts of the plant are used in medicine. The rhizome extract showed antidiabetic and antiobesity attributes (Ono *et al*., 2006). The leaves are known for their refrigerant, astringent and diuretic actions hence used for diverse applications for diarrhoea, high fever, hemorrhoids and leprosy. Koreans prepare traditional liquor (lotus liquor) from the blossoms and leaves which they have found to have antioxidant activities which reduced oxidative stress and risk of chronic diseases. The leaves of *Nelumbo nucifera* are considered best for overcoming body heat and stopping bleeding.

Various plant extracts are exploited in recent years for their antimicrobial activities. There are numerous literatures pertaining to antimicrobial activity of *N. nucifera.* Antifungal activity of *N. nucifera* extract was reported by Agnihotri (2008). Brindha Venkatesh and Arthi Dorai (2011) reported antibacterial activity of white and pink flower of *N. nucifera* hydro-ethanolic extracts against *Bacillus Subtilis, Staphylococcus aureus* and *Escherichia coli*. Antifungal activity of lotus rhizomes was reported by Matthews and Haas (1993) against *Aspergillus niger*, *Trichoderma viride* and *Penicillium* spp. In view of the above facts, this study was aimed to evaluate the antioxidant antimicrobial potential of *N. nucifera* leaf extracts.

Materials and methods

Chemicals: Analytical grade chemicals from Hi-Media, Loba, Merck and Sigma were used throughout the study. Methanol, acetone, hexane, chloroform, dichloromethane were of analytical grade. Analytical grade 1, 1-diphenyl-2, picrylhydrazyl (DPPH) was purchased from Merck. Ascorbic acid (vitamin C) purchased from Sigma Chemicals was used as the standard for antioxidant activity.

Collection of the plant material

Leaves of *N. nucifera* were collected from ponds in different localities of Tirunelveli and Nagercoil, Tamil Nadu between Dec 2011 to Jan 2012 (Fig. 1). Leaves were shade dried and dried parts were powdered using mechanical pulverizer and subjected for extraction.

Fig. 1. *Nelumbo nucifera* in its natural habitat.

Solvent and aqueous extracts

Air dried and powdered leaves were cold macerated with different solvents (Methanol, acetone and hexane) for 3 days with occasional stirring. The extract was then filtered through Whatmann filter paper (No. 1) and the solvent was removed at low temperature (40ºC-50°C) under reduced pressure in a rotary evaporator. Aqueous extract was prepared with hot water at 60°C.

DPPH radical scavenging activity

The capacity to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was determined according to Hatano *et al*. (1988). Various concentrations (200, 400, 600, 800 and 1000 µg/mL) of hexane, acetone, methanol and aqueous leaf extracts were mixed with of 0.1 mM DPPH solution in methanol. The mixture was shaken vigorously and incubated in dark for 30 min at room temperature. Absorbance was read at 517 nm using spectrophotometer. The control was prepared as above without the compound and methanol. Low absorbance indicated high free radical scavenging activity. DPPH scavenging effect was calculated as percentage of DPPH discoloration using the following equation:

$$\%\ \text{Scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

The assays were carried out in triplicate and the results were expressed as mean values ± standard deviations. The extracts concentration providing 50% inhibition (IC_{50}) was calculated from the values and graphs of percentage scavenging activity against concentration of samples were plotted. Ascorbic acid was used as standard.

Antimicrobial activity

In vitro antimicrobial activity of leaf extracts were determined by well diffusion method, described by Perez *et al.* (1990) and Nagarajan *et al.* (2010). Muller Hinton Agar (MHA) (Beef infusion, 300 g/L; Casein acid hydrolysate, 17.5 g/L; Starch, 1.5 g/L; Agar, 1.7 g/L; pH 7.3±0.1) medium was poured onto sterile petri dishes of 90 mm diameter. The agar was allowed to set at ambient temperature. Fresh human pathogenic bacterial cultures of two gram-positive bacteria, *Bacillus subtilis* (MTCC-441) and *Staphylococcus aureus* (MTCC-98), and two gram-negative bacteria, *Escherichia coli* (MTCC-1687) and *Proteus mirabilis* (MTCC-3310) were spread on the surface of the MHA plate with swabs. Wells were cut from the MHA in the petri dishes using a sterile cork (8 mm dia) borer. Different concentrations (50 µL, 75 µL and 100 µL) of the leaf extracts were loaded into the wells using a sterile micropipette. The inoculated plates were initially incubated for 15 min at room temperature and then they were incubated at 37ºC for 24 h. Turbidity was adjusted with sterile broth so as to correspond to 0.5 McFarland standards. Inhibition zones were recorded as the diameter of growth free zones including the diameter of the well in mm at the end of incubation period.

Antifungal activity

Antifungal activity of hexane, acetone, methanol and aqueous extracts were evaluated according to Marston and Hostettmann (1999). Sabouraud Dextrose Agar (SDA) (Meat peptone, 5 g; casein peptone, 5 g; dextrose, 40 g; agar, 15 g, distilled water, 1000 mL) was poured onto sterile petri dishes of 90 mm diameter. The agar was allowed to set at ambient temperature. The antifungal activity of the extracts was tested against two human pathogenic fungus *Candida albicans* and *C. krusei*. Fresh fungal culture was spread on surface of the SDA plate with the swab. Wells were cut from the SDA in the petri dishes using a sterile cork (8 mm dia) borer. Different concentrations namely 50 µL, 75 µL and 100 µL of the leaf extracts were loaded into the wells using a sterile micropipette. The inoculated plates were initially incubated for 15 min at room temperature and then they were incubated at 37°C for 24 h. Then, the plates were examined for any zone of growth inhibition. Inhibition zones were recorded as the diameter of growth free zones including the diameter of the well in mm at the end of incubation period. Percentage of inhibition was calculated by the formula:

$$\text{percentage of inhibition} = \frac{\text{I(Dia. of the inhibition zone in mm)}}{\text{90 (Dia. of the petri plate in mm)}} \times 100$$

Statistical analysis

The data were subjected to One-way Analysis of Variance (ANOVA) to evaluate the significant of difference of means of various treatment groups using SPSS statistical software package (Version: 10). The values are presented as mean ± S.D and $P<0.05$.

Results and discussion

DPPH radical scavenging activity

Excessive generation of ROS, induced by various stimuli and which exceed the antioxidant capacity of the organism, leads to a variety of patho-physiological processes such as inflammation, diabetes, genotoxicity and cancer (Gulcin *et al.,* 2002; Gulcin *et al.,* 2003). The free radical scavenging activity of leaf extracts are shown in Table 1. Different concentrations namely 200, 400, 600, 800, 1000 µg/mL of leaf extracts were used with ascorbic acid as standard (Table 1). In DPPH assay, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. The methanolic leaf extract showed strong antioxidant (94.97%) activity, followed by acetone (93.47%) and hexane (93.89%) at 1000 µg/mL. IC_{50} (Inhibitory Concentration) values of methanol is 230.62 µg/mL followed by hexane (231.91 µg/mL) and acetone (390.54 µg/mL) respectively. It was noticed that

Table 1. DPPH free radical scavenging activity of *N. nucifera* leaf extracts.

Concentration (µg/mL)	Concentration (µg/mL) (Aa)	% of radical scavenging activity			
		Hexane extract	Acetone extract	Methanol extract	Ascorbic acid
200	2	43.12±1.00	39.32±0.91	43.36±1.01	48.99±1.14
400	4	54.26±1.26	51.21±1.19	65.86±1.53	55.98±1.30
600	6	69.13±1.61	65.35±1.52	90.7±2.11	71.56±1.66
800	8	84.29±1.96	83.24±1.94	91.44±2.13	85.99±2.00
1000	10	93.89±2.19	93.47±2.18	94.97±2.21	91.65±2.13
IC_{50} Value (µg/mL)		231.91	390.54	230.62	3.57

Values are means of three replicates and the values in the columns with same letter are not significantly different at p<0.05 level. Aa = Ascorbic acid.

methanolic leaf extract of *N. nucifera* showed strong hydrogen donating abilities to act as an effective antioxidant. The scavenging effect increased with increasing concentration of the extract. The results fall in line with the findings of Olajire and Azeez (2011) who found a least IC_{50} value in *C. sativus* indicating its free radical scavenging activity.

Antimicrobial activity

Antibacterial activity of leaf extracts of *N. nucifera* were evaluated at three different concentrations (50, 75 and 100 µg/mL) against four bacterial strains by well diffusion method and was compared with the activity of standard (Tetracycline; 30 mcg) (Table 3). Hexane, acetone and methanol extracts showed various levels of inhibitory effects against human pathogenic bacteria and fungi except aqueous extract. The antimicrobial activities of these compounds were dose dependent and were found to be significant at 5 µg/mL concentration. Brindha Venkatesh and Arthi Dorai (2011) have reported antibacterial activity of the hydro-ethanolic extract of white and pink flower extracts of *N. nucifera.* The maximum zone of inhibition against *E. coli* (16 mm and 14mm), *B. Subtilis* (15 mm and 13 mm) and *S. aureus* (13 mm and 11 mm) was exhibited by the white and pink flower extracts in their study. In a similar earlier study by Li and Xu (2008) indicated that the leaf extracts of *N. nucifera* exhibited antibacterial activity.

Methanol, acetone and hexane extract showed antimicrobial activity against all microorganisms. Methanol extract recorded maximum (16.55%) antibacterial activity against Gram-positive *B. subtilis*. Acetone extracts showed maximum activity against gram-positive *B. subtilis* (15.55%) and gram-negative (15.55%) organisms. Hexane extract showed moderate activity against all the organisms tested. All the extracts showed antifungal activity against *Candida tropicalis* and *C. krusei*. Among these extracts, hexane and acetone extracts showed maximum (14.44%) activity against *C. albicans* (Fig. 3). All the extracts showed good and moderate antimicrobial inhibition activity.

Fig. 2. Antibacterial activity of methanolic leaf extract of *N. nucifera* against *B. subtilis*.

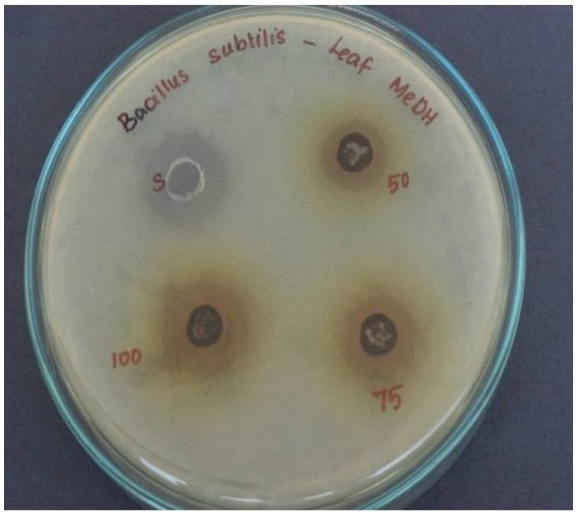

Fig. 3. Antifungal activity of acetone leaf extract of *N. nucifera* against C. *krusei*.

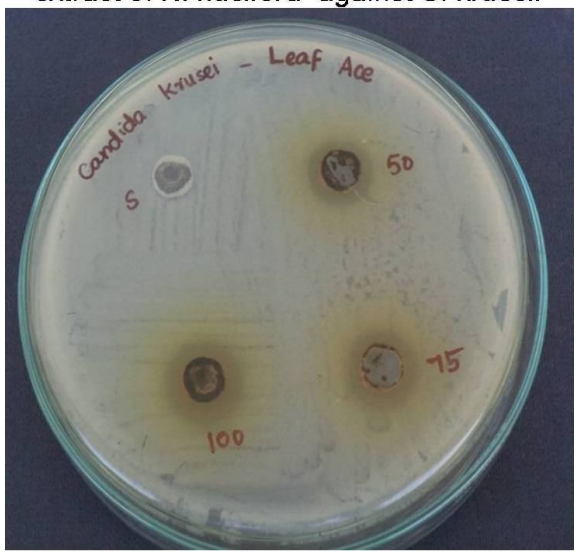

Table 2. Antimicrobial activity of *N. nucifera* leaf extracts.

Organisms	Zone of inhibition (mm)																			
	Hexane extract						Acetone extract						Methanol extract						Standard	
	50 µL		75 µL		100 µL		50 µL		75 µL		100 µL		50 µL		75 µL		100 µL		25 µL	
	I	I%	I	I%	I	I%	I	I%	I	I%	I	I%	I	I%	I	I%	I	I%	I	I%
B. subtilis	11	12	12	13	13	14	10	11	11	12	14	15	13	14	14	15	15	16	16	17
S. aureus	10	11	11	11	12	13	10	11	11	12	12	13	10	11	11	12	12	13	24	25
E. coli	9	10	10	11	11	12	9	10	10	11	12	13	9	10	10	11	11	12	10	11
P. mirabilis	9	10	11	12	13	14	-	-	10	11	14	15	9	10	11	12	13	14	14	15
**C. albicans*	11	12	12	13	13	14	11	12	12	13	13	14	9	10	10	11	11	12	-	-
**C. krusei*	-	-	10	11	11	12	-	-	11	12	12	13	-	-	8	9	10	11	-	-

*Values given are mean values with triplicate (n=3); I= Zone of inhibition; I % = Percentage of zone of inhibition, *Fungi.*

A plot of percentage of inhibition against various bacterial strains is summarized in Table 2. The results revealed the importance of plant extracts when associated with antibiotics, to control resistant microorganisms, which are becoming a threat to human health. Sittiwet (2009) reported that all parts of *N. nucifera* are edible. Antifungal activity of *N. nucifera* extract has also been reported by Agnihotri *et al.* (2008).

Conclusion

This study showed the antioxidant and antimicrobial properties of *N. nucifera* leaf extracts. To conclude, further investigations are necessary to find out the active ingredients responsible for its antioxidant and antibacterial activity.

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