

ANTIMICROBIAL ACTIVITY OF WHITE AND PINK NELUMBO NUCIFERA GAERTN FLOWERS

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ABSTRACT

Nelumbo nucifera Gaertn (Family: Nelumbonaceae), medicinally versatile and used as an important raw material of age-old traditional medical practices like Ayurveda and folk medicine. Bioassays for antimicrobial activities were carried out using hydroethanolic extract of both white and pink flowers of *Nelumbo nucifera Gaertn* plant. Both the flower extracts were tested against five important bacterial strains and two fungal strains. Further, Minimum Inhibitory Concentration (MIC) was evaluated against *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive) organisms. Both the flower extracts showed considerable activity against all tested bacteria and fungi strains. The white and pink flower extracts more or less showed similar antimicrobial activities. MIC for white flower extract

against *Escherichia coli* and *Staphylococcus aureus* was found to be 430 μ g and 450 μ g respectively and pink flower showed 480 μ g and 490 μ g respectively. The antibacterial and antifungal activities of both flower extracts were comparable to those of selected chemical antibiotics suggesting their potential as alternatives to orthodox antibiotics in the treatment of infectious caused by these microorganisms.

Keywords: *Nelumbo nucifera Gaertn*, antibacterial activity, Minimum Inhibitory Concentration (MIC), antifungal activity, agar disc diffusion.

INTRODUCTION

Medicinal plants are gifts of nature used to cure number of human diseases. To promote the proper use and to determine their potential as sources for new drugs, it is essential to study the medicinal plants [1]. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of phytomedicine to act against microbes [2]. Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drug. Therefore, researchers are increasingly turning their attention to folk medicine to develop better drugs against microbial infections [3].

Nelumbo nucifera Gaertn (Family: Nelumbonaceae) commonly known as Indian lotus, one of the oldest perennial aquatic herb consumed throughout Asia [4]. Pharmacological studies of the plant revealed that the whole plant possess antidiabetic, antipyretic, anti-inflammatory, anticancerous, antimicrobial, antiviral and anti-obesity properties [5]. Furthermore, *Nelumbo nucifera* flower has considerable reputation as a potent adjunct in the treatment of various ailments such as cancer, hypertension, diarrhea, fever, weakness, infection and body heat imbalance [6].

The major constituents isolated from the lotus plant are alkaloids (liensinine, neferine, nuciferine, remrefidine and isoliensinine) and flavonoids ((+)-1(R)-

coclaurine, (-)-1(S)-norcoclaurine and quercetin 3-O-b-D-glucuronide) [7]. Several previous reports suggested that seed could suppress cell cycle progression, cytokine genes expression and cell proliferation in human peripheral blood mononuclear cells [8]. Recently, the leaf of *Nelumbo nucifera* showed the hypotensive effects that mediated by vasodilatation *via* nitric oxide [9] and betulinic acid isolated from rhizomes used as anti-tumoral and melanoma specific cytotoxic agent [10].

A perusal of literature revealed that the flower parts of *Nelumbo nucifera* had not been subjected to screening for antibacterial and antifungal properties so far. From this viewpoint the present study was carried out to evaluate the antimicrobial activity of hydroethanolic extract of both white and pink *Nelumbo nucifera* flowers against five bacterial and two fungal isolates.

MATERIALS AND METHODS

Plant material

The flowers of *Nelumbo nucifera* were collected from different localities of Coimbatore District in September 2008 and authenticated by Botanical Survey of India (BSI) in “Tamil Nadu Agriculture University” Coimbatore, Tamil Nadu, India. A voucher specimen (No.BSI/SC/5/23/09-10/Tech.279) has been deposited at the Herbarium of the Botany department of “Tamil Nadu Agriculture University” for future reference.

Plant Extraction

The air-dried and powdered flowers (100g of each) were cold macerated with 50% ethanol for 3 days, with occasional stirring. After 3 days, the suspension was

filtered through a fine muslin cloth and was evaporated to dryness at low temperature (< 40°C) under reduced pressure in a rotary evaporator. Dark brown colored crystals of approximately 8g was stored in air-tight desiccator and used for further analysis.

Antibacterial Assay

Antibacterial activity was determined using the agar disc diffusion method described by Parekh [11]. The strains were maintained in agar at room temperature. Each bacterial inoculum was incubated in 2.5ml Mueller-Hinton broth at 37°C for 18 hours. Every inoculum was spread over plates containing Mueller-Hinton agar. Five millimeter discs containing 500µg/ml and 1000µg/ml of extract were placed on cultured pathogenic bacteria on agar plates and incubated at 37°C. The plates were checked for bacterial growth after a minimum of 16 hours and occasionally till 24 hours. The diameter of the zone of inhibition was then measured. Commercial disc of Chloramphenicol (30µg) was used as positive control and experiment was done thrice for each extract.

Determination of Minimum Inhibitory Concentration (MIC)

MICs are considered as the “gold standard” for determining the susceptibility of the organisms to

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antimicrobials. MICs are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. MIC of antibiotics was evaluated using standard microbroth dilution method [12] against *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive) organisms.

Antifungal Assay

For Antifungal activity extract was serially diluted with Dimethyl Sulfoxide (DMSO) to get final concentration of 500µg/ml and 1000µg/ml. A volume of 0.5ml of microorganism suspensions containing approximately 4×10^6 cells were used to inoculate the surface of the solidified media prepared using Sabouraud Dextrose agar (SDA) medium and allowed to set and then incubated at 37°C for 1- 4 weeks. The results were read by noting the presence or absence of growth of the organisms and compared with standard Clotrimazole (30µg) [13].

Statistical Analysis

The experiments were set up in triplicate. The mean zones of inhibition and MICs were calculated from the values of the three experiments for each isolates and reported as final results.

RESULTS

The antibacterial activity of the hydroethanolic extract of both white and pink *Nelumbo nucifera* flower extracts were evaluated at two different concentrations (500

& 1000µg) against five bacterial strains by the disk diffusion method and the results were summarized in Table 1.

Table 1 Antibacterial activity of both white and pink *Nelumbo nucifera* flowers by Disc diffusion method

Microorganism	Inhibition Zone (mm)*				
	White <i>Nelumbo nucifera</i> flower		Pink <i>Nelumbo nucifera</i> flower		Chloramphenicol (Control) 30µg/ml
	1000µg/ml	500µg/ml	1000µg/ml	500µg/ml	
<i>Escherichia coli</i>	16	11	14	9	30
<i>Klebsiella pneumonia</i>	12	6	10	8	22
<i>Pseudomonas aeruginosa</i>	9	7	8	5	29
<i>Bacillus Subtilis</i>	15	9	13	5	26
<i>Staphylococcus aureus</i>	13	10	11	7	27

* : mean of three replicates

The antibacterial activity of both *Nelumbo nucifera* flower extracts was found to be increased in dose dependent manner. The maximum zone of inhibition was exhibited by both white and pink *Nelumbo nucifera* flowers against *Escherichia coli* (16mm & 14mm), *Bacillus Subtilis* (15mm & 13mm) and *Staphylococcus aureus* (13mm & 11mm). The moderate zone of inhibition was found in both white and pink flower extracts against *Klebsiella pneumonia* (12mm & 10mm) and *Pseudomonas aeruginosa* (9mm & 8mm). Gram-negative bacteria were more susceptible to the *Nelumbo nucifera* flower extracts than gram-positive

bacteria which contradict the previous reports that plant extracts are more active against gram-positive bacteria than gram-negative bacteria.

However, the results revealed that the hydroethanolic extract of white *Nelumbo nucifera* flower showed effective antibacterial activity when compared to pink *Nelumbo nucifera* flower which may be due to its variation in phytochemical constituents like flavonoids, alkaloids and tannins which was also reported by Bose *et al.*, [14] and these results were compared with the standard

antibiotic chloramphenicol (30µg/ml). Similar work by *Tithonia diversifolia* against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* showed that the plant can be used in the treatment of gastrointestinal infection and diarrhea in human. The minimum inhibitory concentration for white *Nelumbo nucifera* flower extract against *Escherichia coli* and *Staphylococcus aureus* was found to be 430µg and 450µg respectively and pink flower

Rogger *et al.*, [15] showed that antibacterial effect of showed 480µg and 490µg respectively (Figure 1 &2). The lowest MIC was exhibited by white *Nelumbo nucifera* flower extract against both the microorganisms when compared to pink *Nelumbo nucifera* flower.

Figure 1 Minimum inhibitory concentration of both white and pink *Nelumbo nucifera* Flowers against *Escherichia coli*

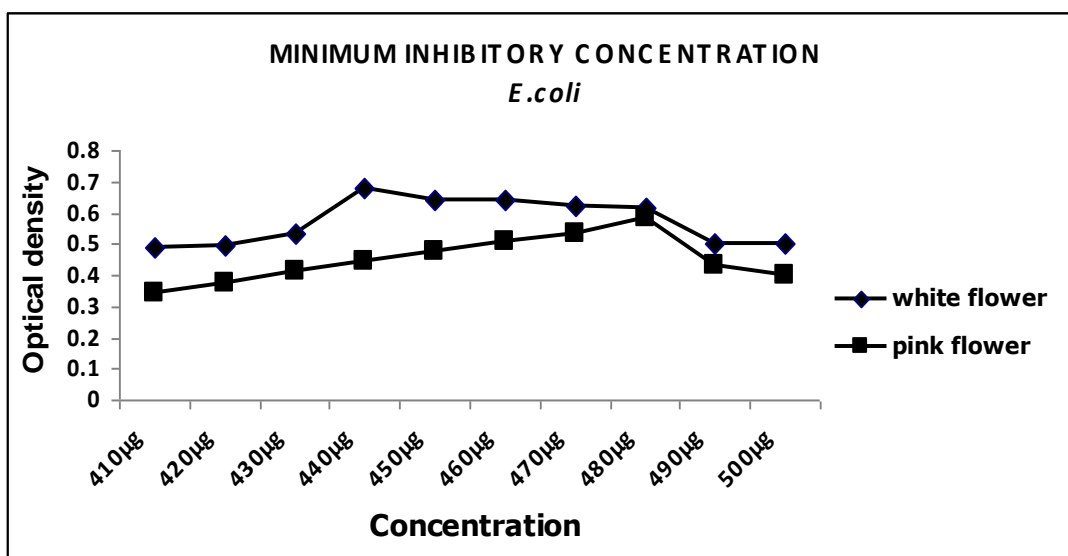
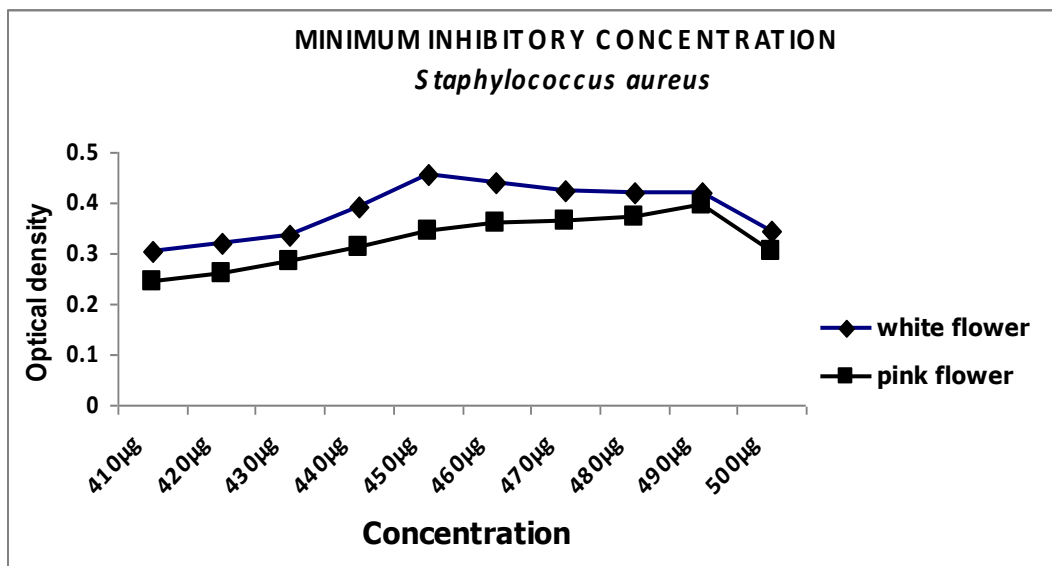


Figure 2 Minimum inhibitory concentration of both white and pink *Nelumbo nucifera* Flowers against *Staphylococcus aureus*



Comparison with pertinent data from literature indicated that the results obtained are most converge with the results of Ushimaru and Silva, [16] who have reported that medicinal plants like *Allium sativum*, *Zingiber officinale* have antibacterial activity against *Salmonella typhimurium*, *Staphylococcus aureus* at minimum inhibitory concentration of 450µg/ml.

The antifungal activity of the hydroethanolic extract of both white and pink *Nelumbo nucifera* flower extracts were determined against two fungal strains and recorded in Table 2.

Table 2 Antifungal screening of both white and pink *Nelumbo nucifera* flowers by Disc plate method

Microorganism	Inhibition Zone (mm)*				
	White <i>Nelumbo nucifera</i> flower		Pink <i>Nelumbo nucifera</i> flower		Clotrimazole (Control) 30µg/ml
	1000µg/ml	500µg/ml	1000µg/ml	500µg/ml	
<i>Aspergillus niger</i>	15	10	11	9	23
<i>Monascus purpureus</i>	13	9	12	8	21

* : mean of three replicates

The antifungal activity was also observed in dose dependent manner and highest activity was observed with hydroethanolic extract of white flower against *Aspergillus niger* (15mm) and moderate activity was seen in pink

flower (11mm). Both the flower extracts showed more or less same activity against *Monascus purpureus* (13mm & 12mm). These results were compared with the standard Clotrimazole (30µg/ml). These results clearly indicated that

both the flower extracts possess substantial antifungal properties and support folkloric use in the treatment of fungal infections. Our findings are in accordance with the observations of Ravindra *et al.*, [17] who proved that highest antifungal activity was observed with methanolic extract of *Capparis pepiaria* against the tested fungal strains.

The findings of this study confirmed the therapeutic potency of both white and pink *Nelumbo*

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nucifera flowers used in traditional medicine. These results offer promising lead for the discovery of potent antimicrobial compounds in therapeutic and dietary use globally.

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