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*Full Length Research Paper*

# Antimicrobial potential of *Nymphaea lotus* (Nymphaeaceae) against wound pathogens

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**Ethanolic extracts of *Nymphaea lotus* leaves were tested for antimicrobial activity against gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*), and gram-negative bacteria (*Escherichia coli*, *Klebsiella Pneumoniae* and *Pseudomonas aeruginosa*) isolated from wounds by the disc diffusion method (DDM). The result of the phytochemical analysis of the extract showed the presence of bio-active compounds such as tannins, flavonoids, alkaloids, anthraquinones, saponins, cardiac glycosides and phenolics. The results also showed that *S. aureus*, *S. pyogenes* and *E. coli* isolated were highly susceptible to *N. lotus* with the zone of inhibition ranging from 8 to 25 mm while *K. pneumoniae* and *P. aureginosa* were moderately susceptible to this antimicrobial substance with the zone of inhibition ranging from 8 to 15 mm. The antibacterial activity of the extracts against these bacteria suggests that there is a scientific basis for its utilization for the treatment of bacterial wound infections.**

**Key words:** *Nymphaea lotus*, susceptibility, wound, phytochemical.

## INTRODUCTION

The use of plants by man for the treatment of diseases has been in practice for a very long time because of the high rate of mortality caused by bacterial infections and diseases in human population .For example, *Vibrio cholerae* and *E. coli* cause diarrhoea, *Mycobacterium spp* causes tuberculosis, *Clostridium perfringens* produces toxins that cause putrefactive decay of the infected tissue with gas production, while *Salmonella spp.* causes typhoid fever (Leven, 1987; Cheesbrough, 2004; Jawetz et al., 2004). Decreased efficiency and steadily increasing bacterial resistance of pathogens to existing antibiotics is a serious problem which has necessitated the development of new alternatives and continuing research into new classes of antimicrobial agents that can destroy these resistant microorganisms without any side effect and at a lower cost. (Essawi and Srour, 2000; Woodford, 2003; Iroha et al., 2007). Resistance to antibiotics by some bacteria that invade wound causing wound sepsis, injury to the tissue and interference with the normal functioning of the host and leading to chronic wounds had been reported and plants have been identified as the alternative in treating septic wounds with little or no microbial resistance. A special feature of higher plants is their capacity to produce a large number of organic chemicals of high structural diversity called secondary metabolites (Castello et al., 2002). Screening of compounds obtained from plants for their pharmacological assay has indeed been the vast source of innumerable therapeutic agents representing molecular diversity engineered by nature. One of the ways to prevent antibiotic resistance of pathogenic species is to use new compounds that are not based on existing synthetic antimicrobial agents (Shah, 2005). *Nymphaea lotus* belongs to Nymphaeaceae family. It is a perennial plant that grows up to 45 cm in height; it is a herbaceous aquatic plant, whose leaves float or submerge in water (Abu-Zaida et al., 2008). This plant prefers clear, warm, still and slightly acidic water and is localized to Central and Southern Europe, Asia, the Middle East, North Africa, tropical mountains in Africa and West Africa especially in Nigeria. Many bioactive and pharmacologically important compounds have been obtained from *Nymphaea spp* and used in medicine and

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pharmacy (Siddhanta et al., 1997). With the above in mind, the leaves of *N. lotus* were tested for antibacterial activity against some bacteria isolated from wounds, since it is being used in traditional medicine. However, neither the antimicrobial activity of *N. lotus* growing spontaneously in Nigeria nor the phytochemical screening has been published to the best of our knowledge.

## MATERIALS AND METHODS

### Plant materials

The leaves of *N. lotus* were collected from the stream at Itu village, near Uyo, Akwa Ibom State. The plants were cleaned of extraneous matter, and necrotic parts were removed and washed with fresh water. The plant was transported to the laboratory in polythene bags. In the laboratory, the leaves were washed thoroughly three times with running water and once with distilled water.

### Plant Identification

This plant was identified and confirmed as *N. lotus* by Dr. Ubom of the Botany Department, University of Uyo, Akwa Ibom State.

### Microbial cultures

Fresh clinical isolates of *E. coli*, *S. aureus*, *S. pyogenes*, *K. pneumoniae* and *P. aeruginosa* isolated from wounds between June 2008 to September 2008 were obtained from the culture collection of the Microbiology Department, University of Uyo, Akwa Ibom State. Stock cultures were maintained on a nutrient agar slant at 4°C until needed.

### Preparation of plant extracts

A sample (50 g) of the shade-dried powdered leaves of *N. lotus* was soaked in 95% ethanol (200 ml) for 72 h. At the end of the extraction, the extract was filtered using Whatman No.1 filter paper. The filtrate was concentrated in vacuum at 30°C. After complete evaporation, the extract was weighed and preserved aseptically at 5°C .The graded concentrations (10, 20, 30 and 40 mg/ml ) of the extract were prepared and then subjected to antibacterial activity assays.

### Bioassay

The ethanol extract was tested for antibacterial activity by the disc diffusion method (NCCLS, 2004; Nair et al., 2005). Mueller-Hinton agar (MHA) was sterilized in flasks cooled to 45-50°C and then poured into sterilized Petri dishes. Sterile filter paper discs of 6 mm diameter were impregnated with extract solution of graded concentrations (10, 20, 30, and 40 mg/ml) and then placed on to agar plates which had previously been inoculated with the tested microorganisms (*S. aureus, S. pyogenes, E. coli, K. pneumoniae* and *P. aeruginosa*). Control experiments comprising streptomycin were set up. The plates were then incubated at 37°C for 24 h .The diameters of the inhibition zones were measured in millimeters.

### Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) of the extracts was determined for each of the test organisms in test tubes. To 0.5 ml of varying concentrations of the extracts (10, 20, 30 and 40, mg/ml) in test tubes, nutrient broth (2 ml) was added and then a loopful of the test organism. A tube containing nutrient broth and streptomycin was only inoculated with the test organism to serve as control. The culture tubes were then incubated at 37°C for 24 h. After incubation the tubes were then examined for microbial growth by observing for turbidity. To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated onto sterile nutrient agar by streaking. Nutrient agar plates were also streaked with the respective test organisms to serve as controls. All the plates were then incubated at 37°C for 24 h. After incubation the concentration at which no visible growth was considered as the minimum bactericidal concentration (MBC).

### Phytochemical screening

The preliminary phytochemical analysis of the plant extracts was performed to screen for the presence of bio-active components present in the leaves of *N. lotus* using the methods described by Evans (1989) and Sofowora (1993).

## RESULTS AND DISCUSSION

The *in vitro* antimicrobial activity of ethanolic extract of *N. lotus* leaves against the microorganisms employed was assessed qualitatively and quantitatively by the presence or absence of inhibition zones and zone diameters. The ethanolic extract of *N. lotus* showed *in vitro* antimicrobial activities against tested microorganisms (*S. aureus*, *S. pyogenes*, *E. coli*, *K. pneumoniae* and *P. aeruginosa*). In this study, the antimicrobial activities of ethanol extract were compared with standard streptomycin and this was used as positive control. Results from the antimicrobial disc diffusion assays were shown in Tables 1 and 2. The data indicated that *S. aureus, Streptococcus spp* and *E. coli* were the most sensitive bacteria tested to the ethanolic extracts of *N. lotus* with the highest inhibition zone of 25, 20 and 25 mm respectively. The *P. aureginosa* and *K. pneumoniae* were also found to be sensitive with the highest inhibition zones of 15 mm respectively. The ethanolic extracts of *N. lotus* also showed excellent activity against tested gram-positive bacteria (Table 3). *E. coli* was the most sensitive organism among gram-negative bacteria with the inhibition zone of 25 mm which was even more than that of standard streptomycin which was 20 mm. From the results obtained it was apparent that the ethanolic extract of *N. lotus* at 40 mg/ml concentration was the most effective as widest inhibitory zone were observed compared to other concentrations used. Phytochemical screening of ethanolic extract of *N. lotus* shows the presence of anthraquinones, cardiac glycosides, saponins, tannins, alkaloids, flavonoids and phenolics.

**Table 1.** Antibacterial activity of *N. lotus* against gram-positive bacteria isolates.

| Bacteria | Mean zone of inhibition (MM) ± SD | | | | Streptomycin 30ug/disc |
|---|---|---|---|---|---|
| | 10mg/ml | 20mg/ml | 30mg/ml | 40mg/ml | |
| *S. aureus* | 8.0 ± 1.0 | 10.0 ± 2.0 | 20.0 ± 2.5 | 25.0 ± 1.0 | 24.0 ± 3.0 |
| *S. aureus 2* | 15.0 ± 2.0 | 17.0 ± 1.5 | 20.0 ± 1.5 | 25.0 ± 1.5 | 32.0 ± 2.0 |
| *S. aureus 3* | 8.0 ± 0.5 | 10.0 ± 1.0 | 15.0 ± 1.0 | 20.0 ± 2.0 | 20.0 ± 3.0 |
| *S. aureus 4* | 8.0 ± 1.0 | 8.0 ± 2.0 | 12.0 ± 2.0 | 15.0 ± 3.0 | 23.0 ± 1.0 |
| *S. pyogenes 1* | 8.0 ± 1.0 | 10.0 ± 1.0 | 13.0 ± 1.0 | 15.0 ± 1.5 | 30.0 ± 2.0 |
| *S. pyogenes 2* | 10.0 ± 2.0 | 15.0 ± 1.0 | 18.0 ± 3.0 | 20.0 ± 2.0 | 24.0 ± 1.0 |
| *S. pyogenes 3* | 8.0 ± 1.0 | 8.0 ± 1.5 | 10.0 ± 1.5 | 15.0 ± 1.0 | 26.0 ± 3.0 |
| *S. pyogenes 4* | 9.0 ± 0.5 | 12.0 ± 1.0 | 15.0 ± 2.0 | 20.0 ± 1.0 | 30.0 ± 1.0 |

Zone of Inhibition, Z.I ±SD

**Table 2.** Antibacterial activity of *N. lotus* against gram-negative bacteria isolates.

| Bacteria | Mean Zones of inhibition (MM)±SD | | | | Streptomycin 30ug/disc |
|---|---|---|---|---|---|
| | 10mg/ml | 20mg/ml | 30mg/ml | 40mg/ml | |
| *Escherichia coli 1* | 8.0 ± 1.0 | 9.0 ± 1.0 | 14.0 ± 1.5 | 20.0 ± 2.5 | 22.0 ± 2.5 |
| *E. coli 2* | 10.0 ± 1.5 | 12.0 ± 2.0 | 15.0 ± 1.0 | 18.0 ± 1.5 | 18.0 ± 0.5 |
| *E. coli 3* | 15.0 ± 1.0 | 17.0 ± 1.0 | 20.0 ± 1.5 | 25.0 ± 1.5 | 20.0 ± 1.5 |
| *E. coli 4* | 8.0 ± 1.0 | 10.0 ± 1.5 | 15.0 ± 0.5 | 20.0 ± 1.0 | 20.0 ± 1.0 |
| *K. pneumoniae 1* | - | 8.0 ± 0.5 | 9.0 ± 1.0 | 12.0 ± 0.5 | 20.0 ± 0.5 |
| *K. pneumoniae 2* | 8.0 ± 1.0 | 8.0 ± 1.0 | 8.0 ± 1.5 | 10.0 ± 1.0 | 18.0 ± 1.5 |
| *K. pneumoniae 3* | - | 9.0 ± 1.0 | 12.0 ± 2.0 | 15.0 ± 1.5 | 22.0 ± 2.0 |
| *K. pneumoniae 4* | - | 8.0 ± 0.5 | 8.0 ± 1.5 | 12.0 ± 1.0 | 20.0 ± 2.0 |
| *P. aureginosa 1* | - | 8.0 ± 0.5 | 9.0 ± 1.5 | 12.0 ± 1.0 | 18.0 ± 1.5 |
| *P. aureginosa 2* | 8.0 ± 1.0 | 8.0 ± 1.5 | 10.0 ± 1.0 | 14.0 ± 1.5 | 18.0 ± 1.0 |
| *P. aureginosa 3* | - | 8.0 ± 0.5 | 8.0 ± 1.0 | 10.0 ± 1.5 | 14.0 ± 1.5 |
| *P. aureginosa 4* | 8.0 ± 1.0 | 8.0 ± 1.0 | 10.0 ± 1.5 | 15.0 ± 0.5 | 20.0 ± 1.0 |

No zone of inhibition.

**Table 3.** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts of *N. lotus* on gram-positive bacteria.

| Bacteria | MIC (mg/ml) | MBC (mg/ml) |
|---|---|---|
| *S. aureus 1* | 10 | 10 |
| *S. aureus 2* | 10 | 10 |
| *S. aureus 3* | 10 | 20 |
| *S. aureus 4* | 10 | 20 |
| *S. pyogenes 1* | 20 | 20 |
| *S. pyogenes 2* | 10 | 20 |
| *S. pyogenes 3* | 10 | 20 |
| *S. pyogenes 4* | 20 | 20 |

The lowest minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 10 - 20 mg/ml was demonstrated against *S .aureus* and *S. pyogenes* while the MIC and MBC values ranging between 10-30 mg/ml were demonstrated against the rest of the test bacteria(Tables 4 and 5). The presence of secondary metabolites in plants, produce some biological activity in man and animals and it is responsible for their use as herbs (Sofowora, 1986). Hence, the presence of the secondary metabolites such as anthraquinones, cardiac glycosides, saponins, tannins, alkaloids, flavonoids and phenolics in *N. lotus* may be responsible for its potential use as a drug against pathogenic bacteria. According to Ebana et al. (1991) and Cushnie and Lamb (2005) both alkaloids and flavonoids had antimicrobial activities. Tannins are important in herbal medicine in treating wounds and to arrests bleeding (Nguyi, 1988). The results obtained showed that ethanolic extracts of *N. lotus* exhibited inhibitory activities against the tested bacteria with different degrees as demonstrated by measuring the diameters of inhibition zones and these results are in conformity with the results obtained by Abu-Zaida et al. (2008).

In conclusion, the demonstration of antimicrobial activity of this plant against both gram-negative and gram-

**Table 4.** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts of *N. lotus* on gram-negative bacteria.

| Bacteria | MIC (mg/ml) | MBC (mg/ml) |
|---|---|---|
| *E. coli 1* | 10 | 10 |
| *E. coli 2* | 10 | 20 |
| *E. coli 3* | 20 | 20 |
| *E. coli 4* | 10 | 20 |
| *K. pneumoniae 1* | 30 | 30 |
| *K. pneumoniae 2* | 10 | 20 |
| *K. pneumoniae 3* | 10 | 20 |
| *K. pneumoniae 4* | 20 | 30 |
| *P. aeruginosa 1* | 20 | 30 |
| *P. aeruginosa 2* | 10 | 20 |
| *P. aeruginosa 3* | 30 | 30 |
| *P. aeruginosa 4* | 10 | 20 |

**Table 5.** Phytochemical analysis of the ethanol extracts of Nymphaea lotus leaves.

| Plant Constituents | Tests used | Occurrence |
|---|---|---|
| Alkaloids | Drangendorff's test | +++ |
| Flavonoids | General test | ++ |
| Phenolics | Frothing test | ++ |
| Saponins | General test | + |
| Tannins | General test | + |
| Anthraquinones | General test | +++ |
| Cardiac glycosides | Leberman' s Test | +++ |

Keys: + = Present in small concentrations
++ = Present in moderately high concentrations
+ + + = Present in high concentrations

positive bacteria was an indication that the plant is a potential source for production of drugs with a broad spectrum of activity and this also supports the traditional application of the plant and suggests that the plant extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel drugs for the treatment of wound infections.

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