An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

INVESTIGATING THE ANTIBACTERIAL EFFECTS OF LEAF EXTRACTS OF *NELUMBO NUCIFERA* ON FIVE TESTED PATHOGENS

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RTICLE INFO

Key Words:

Nelumbo nucifera
Anti-bacterial activity.
Gram negative bacteria,
human bacterial pathogens

ABSTRACT

Objective: To evaluate the antimicrobial potential of three different extracts namely ethanol, methanol and ethyl acetate of leaf extracts of *Nelumbo nucifera* (*N. nucifera*) against the human bacterial pathogens. **Methods:** Plant crude extracts were obtained by maceration method using three different solvents separately: ethanol, methanol and ethyl acetate was used to evaluate the antimicrobial property. The *E.coli*, *Pseudomonas sp*., *Staphylococcus sp*., *Bacillus sp*., and *Klebsiella sp*., were tested against the three crude extracts by the well diffusion method. Ampicillin acts as a standard drug. Different concentrations such as 100µl, 90 µl, and 80 µl were taken for analysis. Zone of inhibition was measured in millimeter. **Results:** The present study results showed the presence of wide range of antibacterial activities of the leaf extracts against all the above bacterial pathogens studied. The highest zone of inhibition was showed by methanol extract of *nelumbo nucifera* against the tested organisms followed by ethanol and ethyl acetate extract of *nelumbo nucifera*. **Conclusion:** This present study clearly evidenced that the *nelumbo nucifera* crude leaf extracts showed moderate to high antibacterial activity against the Gram –negative bacteria tested.

INTRODUCTION:

The prevalence and complications due to microbial infectious diseases are continuously increasing throughout the world. This might be due to increase microbial drug resistance towards commonly used antibiotic [1]. There was a tremendous increase in the need for a new antimicrobial drug which could effectively fight against resistant microbes. Consequently, this leads to the discovery of new approaches with most promising antimicrobial compounds. Plant –derived compounds are considered to be safer and target specific when compared with synthetic compounds and subsequently have different mechanisms of action against microbes [2-5]. Natural products from plants often act as a source for antimicrobial agents. From the previous reports, antimicrobial activities of some plant extracts have been reported to possess antimicrobial activity. *In vitro* antibacterial activity of different extracts such as water, hexane and ethyl acetate of *Coccinia grandis* leaf and stem against four gram positive and gram negative bacteria was investigated by Umbreen *et al*., 2008[6]. Subhan and Tariq 2005[7] reported antimicrobial activities of *Momordica charantia*, *Mentha*

piperata and *Pisum sativum*. Maria *et al*., 2006[8] screened the antimicrobial activity of ethanol extracts of *Cayaponia podantha* (Cucurbitaceae) and four Brazilian plants. In support of this antimicrobial activity of *Momordica charantia* seed essential oil was studied and proved by Alessandra *et al.,* 2008[9]. Similarly, Belsem *et al.,* 2009[10] also described the antimicrobial activities of *Citrulllus colocynthis*. The goal of the present research was to evaluate *in vitro* antibacterial efficiency of leaf extract of *Nelumbo nicifera* against five human pathogens causing several human diseases including *E.coli*, *Pseudomonas sp*., *Staphylococcus sp*., *Bacillus sp*., and *Klebsiella sp*., by the well diffusion method.

METERIALS AND METHODS

Collection of plant materials

Matured plant of *N. nucifera* used for this study was collected from local ponds at Eripudur, Vellore District, and Tamil Nadu in November 2016 and identified by Department of Botany, D.K.M College, Vellore, Tamil Nadu and India.

Preparation of plant extracts:

The leaves collected from plant source were washed with tap-water and distilled water for 2-3 times and the allowed to dry in shade, finally ground to a fine powder. 10gm of powdered material was soaked with 100ml of ethyl acetate, ethanol and methanol for 24 h and filtered. The filtrates of all the solvents were evaporated and stored under room temperature until screened.

Preparation of stock solution

The different solvent extract [Ethyl acetate, methanol and ethanol] were re suspended in DMSO [Dimethyl sulfoxide] to a final concentration of 1g/10ml of DMSO of the extracts and stored in 4°C for further analysis.

Bacterial strain, media and growth condition.

Gram negative bacteria *E. coli*, *Salmonella*, *Vibrio*, *Pseudomonas* and *Proteus* were used in the present studies. The five tested microorganisms (*E. coli*, *Salmonella*, *Vibrio*, *Proteus* and *Pseudomonas*) were maintained on nutrient broth medium was used to grow the bacterial stain at 37°C aerobically. Stock cultures of the above mentioned organisms were maintained at 4°C on slopes of nutrient agar. From the stock cultures a loop full of cells were transferred to test tube of 50 ml nutrient broth for the growth of bacterial strains. The broth was incubated at 37°C on a shaking incubator at 110rpm for 24h.

Antibacterial Activity

The well diffusion method was used to determine the growth inhibition of bacteria by the *N. nucifera* extracts. 25ml Muller Hinton agar (MHA) prepared was poured into sterile petri discs [14cm diameter] to act as base plate and agar was allowed to set at ambient temperature. Molten MH agar was swabbed with 15-18 hour broth culture of the test organism [*E. coil*, *Salmonella*, *Vibrio*, *Proteus* and *Pseudomonas*] and wells were made in the MHA media using sterile cork (8mm diameter) borer. Different concentration [100, 90, 80µl] of the extracts dissolved in DMSO was loaded into the wells using a sterile micropipette and allows the plates for diffusion at room temperature. Plates were evaluated for bacterial growth and incubated at 37°C after a minimum incubation period of 16 hours and occasionally till 24 hours. These concentrations were tested for exhibiting diameter of inhibition zones, zones were measured in transparent ruler in millimeter on the seeded plates. Commercial disc of ampicillin used as a reference drug [positive control] in same cultivation plate. All the experiment was done thrice for each extract. At the end of incubation period, diameter of inhibition zones, (growth free zone) was measured in milli meter (mm).

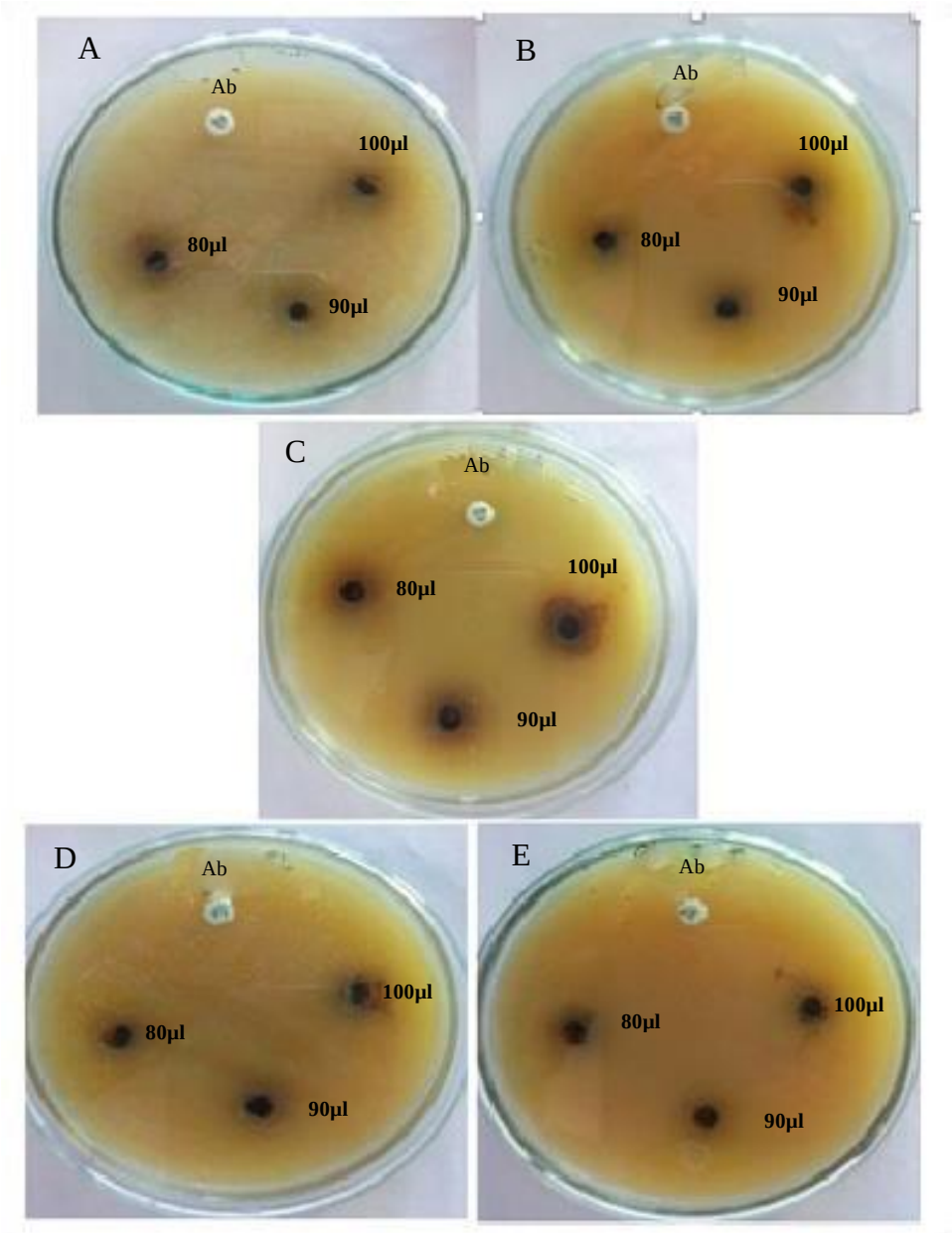
Statistical Analysis: The zones of inhibition were calculated for each experiment of three extracts and the diameter in millimeter as reported as final result.

RESULT

The antibacterial activity of ethyl acetate, ethanol and methanol extract of *N. nucifera* leaves were assessed by MHA well diffusion method at different concentration (100 µl, 90 µl, and 80 µl) by measuring the inhibition zone. The results are presented in figure 1, 2, 3. Overall, the results show a dose

- dependent activity. To ethyl acetate extract *E.coli* and *Vibrio* were found to be most sensitive followed by *Salmonella* and *Proteus* which were found to be more sensitive when compared to *Pseudomonas* at highest concentration of 100 µl.

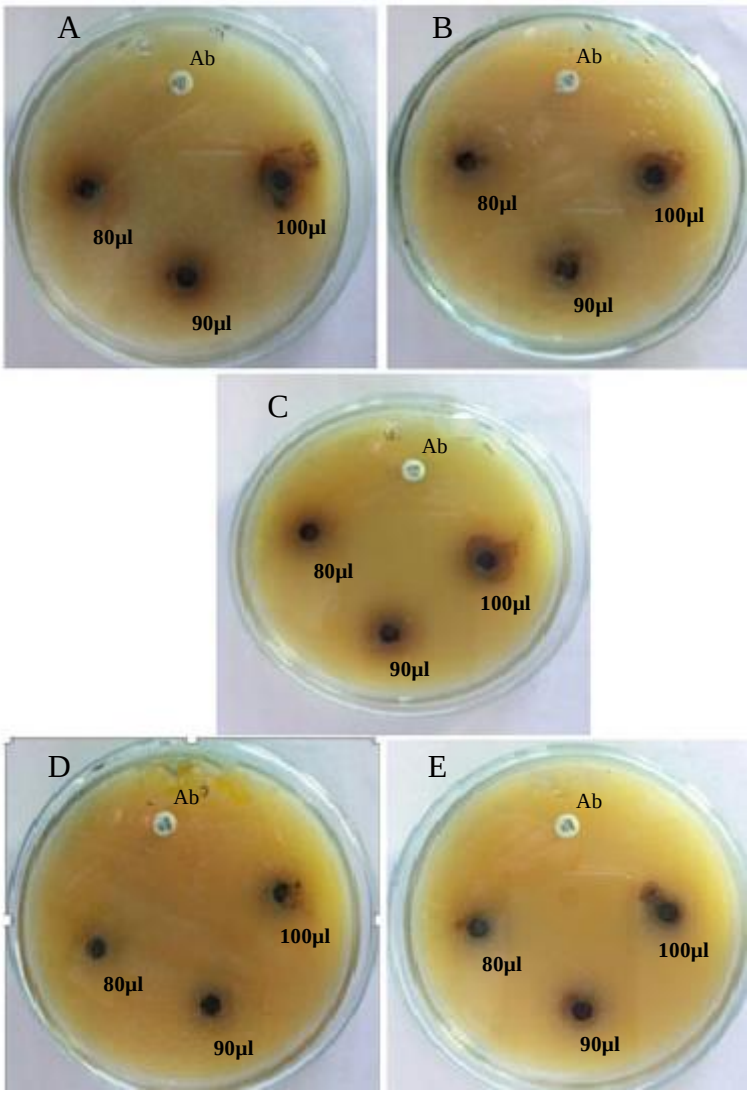
Figure 1: Susceptibility of ethyl acetate crude leaf extracts of *N.nucifera* against different microorganisms.



A- *E. coil*, B- Proteus, C- *Vibrio*, D- *Salmonella* and E- *Pseudomonas*.

Ab- Ampicillin Antibiotic, µl – micro litre, 100 µl, 90 µl, 80 µl – Different concentrations of ethyl acetate crude extract.

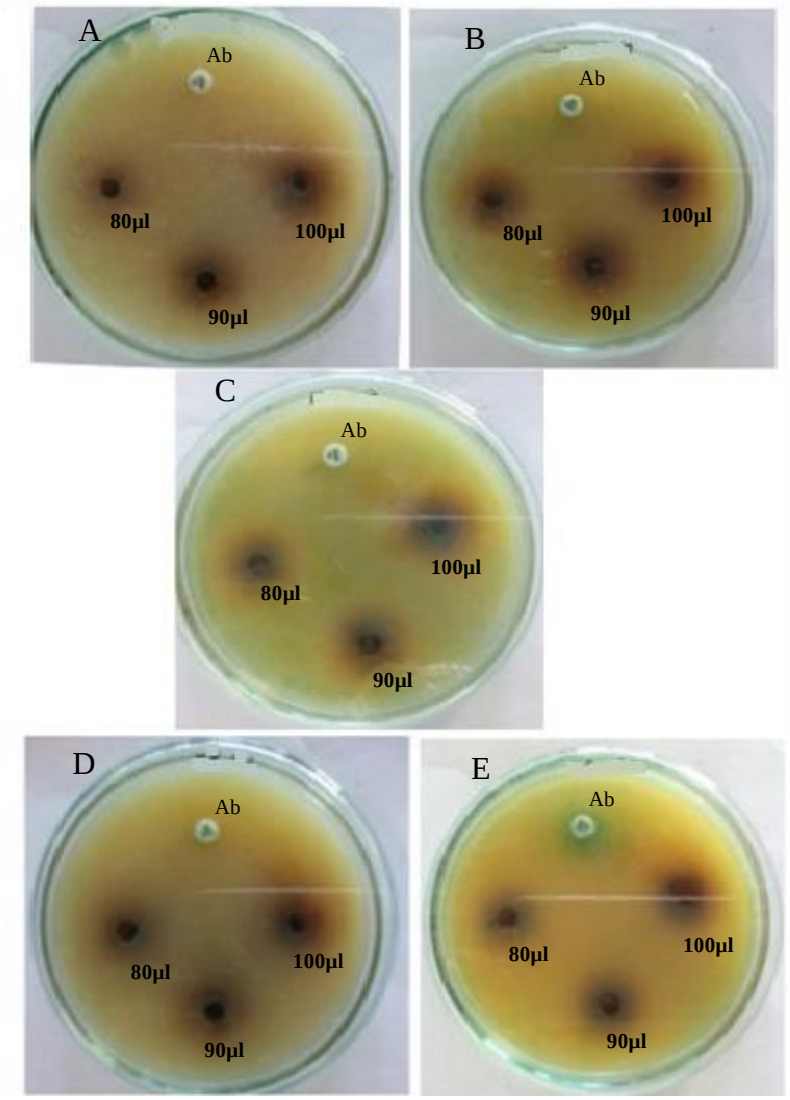
Figure 2: Susceptibility of ethanol crude leaf extracts of *N.nucifera* against different microorganisms.



A- *E. coil*, B- Proteus, C- *Vibrio*, D- *Salmonella* and E- *Pseudomonas*.

Ab- Ampicillin Antibiotic, µl – micro litre, 100 µl, 90 µl, 80 µl – Different concentrations of ethanol crude extract.

Figure 3: Susceptibility of methanol crude leaf extracts of *N.nucifera* against different microorganisms.



A- *E. coil*, B- Proteus, C- *Vibrio*, D- *Salmonella* and E- *Pseudomonas*.

Ab- Ampicillin Antibiotic, µl – micro litre, 100 µl, 90 µl, 80 µl – Different concentrations of methanol crude extract.

The *E-coli* showed the zone of inhibition of 18 mm (80 µl), 19 mm (90 µl) and 21 mm (100 µl) towards ethyl acetate extract. A similar trend is observed in the case of other test organisms with the inhibition zone of 21 mm (100 µl), 19 mm (90 µl), 18 mm (80 µl) for *Vibrio*, 20 mm (100 µl), 18 mm (90 µl), 17 mm (80 µl) for *Salmonella*, 20 mm (100 µl), 18 mm (90 µl), 16 mm (80 µl) for *Proteus* and 19 mm (100 µl), 18 mm (90 µl) and 16 mm (80 µl) for *Pseudomonas*, respectively. The antibacterial result for methanol extract indicates that the extract was found to be more effective towards *Proteus, E.coli* and *Pseudomonas* compared to *Vibrio* and *Salmonella*. Among the tested concentration 100 µl, 90 µl and 80 µl, 100 µl were more effective against the tested organisms when compared to 90 µl and 80 µl concentrations. 100 µl concentration on evaluation recorded

25 mm, 25 mm, 24 mm, 23 mm and 21 mm for *Proteus, E.coli, Pseudomonas, Vibrio* and *Salmonella*, respectively. The results obtained from 90 µl revealed a typical inhibition zone of 22 mm, 22 mm, 20 mm, 18 mm and 19 mm for *Proteus, E.coli, Pseudomonas, Vibrio* and *Salmonella*, respectively. The extract at 80 µl concentration measured zone of inhibition of 19 mm for *Proteus*, 18 mm for *E.coli*, *Pseudomonas, Salmonella* and 16 mm for *Vibrio*. In the present study, the antibacterial activity of Ethanol leaf extract prepared was determined against organisms *E.coli*, *Vibrio*, *Salmonella*, *Proteus* and *Pseudomonas*. The results of Ethanol extract displayed a potential antibacterial activity with highest inhibition zone of 25 mm, 22 mm, 20 mm against *Proteus*, 24 mm, 22 mm, 19 mm against *E.coli* at 100 µl, 90 µl and 80 µl respectively. Similarly Ethanol extract also exhibited moderate antibacterial activity towards *Vibrio* (23 mm, 21 mm and 19 mm) at 100 µl, 90 µl, 80 µl concentration respectively, while a least activity was showed against *Pseudomonas* and followed by *Salmonella* 100µl (22 mm each), 90 µl (21 mm, 20 mm) and (19 mm, 18 mm, respectively). Result of antibacterial activity of three extracts suggested that methanol extract was most effective extract against pathogens followed by ethanol and ethyl acetate extracts.

The result from the present study is very encouraging and indicates that the *N. nucifera* leaf extract inhibited the growth of microorganisms more extensively compared with ampicillin, a commonly used antibiotic. The standard antibiotic ampicillin showed less inhibition towards tested gram - negative bacteria. Thus the herb could be potential in the treatment infectious disease.

DISCUSSION

The antimicrobial activity of *Camellia sinensis* was reported by Zakir *et al*., 2005[11] against human pathogenic bacteria. All the tested bacteria *E.coli, Pseudomonas aeuroginosa* and *S. aureus* exhibited a prominent sensitivity towards *Camellia sinensis* extracts. These results are similar to our antibacterial activity. Similarly the present results are in accordance to Radji *et al.,* 2013[12] who reported a significant antimicrobial activity of *Camellia sinensis* against human pathogenic bacteria *S. aureus MRSA, P. aeruginosa* and *MDR-p aeruginosa.* Majid *et al.,* 2013[13]; Jazani *et al.,* 2009[14] have also reported a similar antibacterial activity of *Camellia sinensis* towards *S. aureus* and *P. aeruginosa*. The present results of antimicrobial activity of *N. nucifera* leaf extracts are also in agreement to *Terminalia arjuna* against six different pathogenic bacteria [15]. It was observed that *Pseudomonas aruginosa, Enterobacter aerogenes, Raoultella planticola, Agrobacterium tumefacines, Bacillus subtilis* and *E.coli* showed better sensitivity towards *Terminalia arjuna* extracts. Likewise, Mandal *et al.,* 2013[16] also observed a significant antimicrobial potential as like *Terminalia arjuna* against test microbes [*S. aureus* and *E.coli*]. Aneja *et al.,* 2012 [17] also reported an antimicrobial activity against *S. aureus, S. aeruginosa* and *E.coli* on *Terminalia arjuna* treatment and these results are in accordance to our results. The antibacterial activity of red beet root extracts (*B. Vulgaris*) against *E.coli* as reported by Winkles *et al.,* 2005[18] was similar to our antibacterial activity. On the other hand, Mostafa *et al.,* 2007[19] antibacterial activity of *S. aromaticum* extract against *B. celeus, P. aeraginosa* and *E.coli* reports are similar to the present findings against *E.coli, Salmonella, Vibrio, Proteus* and *Pseudomonas*. In the present investigation, *N. nucifera* leaf extracts exhibited a variation in the pattern of inhibition among the test organisms towards the pathogens due to difference in polarity of the solvents and the ability of the solvents to extract specific phytochemicals. A number of authors Babu *et al.*, 2007, Caceres *et al.*, 1993, Kwo *et al.*, 1996, Desta 1993, Ekpendu *et al*., 1994 and lqbal *et al.,* 1998[20-25] showed different inhibition trends among the crude extract prepared with different polarity solvent against the test organisms. These difference in sensitivity may be due to different phytochemicals present in the organic solvent crude extracts. Borris, 1996[26] proved that the crude extract obtained by extracting plant material with methanol solvent contains a variety of phytochemicals compared to water. The antimicrobial activity of *N. nucifera* extract towards the tested pathogenic bacteria might be due to interaction of antimicrobial components with enzymes and proteins present on the microbial cell membrane and causing its disruption to disperse a proton flux

towards the cell exterior. Thus change in disturbance in structure and permeability induces cell death or inhibits the enzymes necessary for various biosynthesis as suggested by various researchers Burt *et al.*, 2004; Friedman *et al.*, 2004; Gill *et al.*, 2006a; Gill *et al.*, 2006b; Tiwari *et al.*, 2009 and Onah *et al.*, 1994[27-32].

CONCLUSION

In conclusion, of the present investigation, *Nelumbo nucifera* contain potential antibacterial components that may be of great use for the development of new antimicrobial drugs against various diseases. The result also indicates that all the three extracts possess inhibitory activity against the tested organisms at highest concentration and also provides a baseline information on the possible use of this plant in the treatment of bacterial infections.

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