ORIGINAL RESEARCH PAPER

Biotechnology

# PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL EVALUATION OF *NYMPHAEA PUBESCENS* WILLD.: AN AQUATIC PLANT RESOURCE FOR DRUG DEVELOPMENT

**KEY WORDS:** N. Pubescens, Phytochemical Screening, Antioxidant Activity, Antibacterial Activity

**Dr. Sija. S.L*** Assistant Professor, on contract, Department of Biotechnology, Sree Narayana College, Kollam, Kerala, India *Corresponding Author

**Dr. Reshmi. S** Assistant Professor, PG Department of Biotechnology, GEMS Arts & Science College, Malappuram, Kerala, India

**Athira. T.S** Research Scholar, Microbiology Division, JN-TBGRI, Palode, Thiruvanantha puram, Kerala, India

**ABSTRACT**

The present study evaluates the phytochemical constituents, antioxidant potential and antibacterial properties of the methanol extracts of the water plant *N.pubescens*, using spectrophotometric techniques. The phytochemical screening revealed the presence of carbohydrates, alkaloids, saponins, resins, phenols, steroids, glycosides, flavonoids etc. in methanol extracts of various plant parts such as (root, stem, flower, leaves and seed). Spectrophotometric technique was used for the detection of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of various extracts. All extracts exhibited antioxidant activity and among the extracts, *N.pubescens* leaves demonstrated greater antioxidant potential in comparison with other plant parts. Then the antibacterial activity of methanol extract of various plant parts was also evaluated by using the agar well diffusion method against human pathogenic bacteria such as *B.subtilis, E.coli* and *S.aureus*. All extracts studied in this work showed antimicrobial activity against the test microorganisms. The present study revealed that *N.pubescens* is very rich in phytochemicals and is a good source of natural antioxidants confirms its use for the treatment of various diseases in human.

## INTRODUCTION

Secondary metabolites found in plants are also unusual sources of therapeutics. It has been discovered that domestic remedies and folk medicines served as the foundation for all traditional Indian medical systems [12]. Even though India's aquatic environments are rich reservoirs of diverse plant species, not much research has been done to list all of their potential medical applications. The most speciose, phenotypically varied, and geographically extensive genus within the Nymphaeales is Nymphaea.

### Taxonomical Hierarchy

Kingdom - Plantae
Subkingdom - Tracheobionta
Super division - Spermatophyta
Division - Magnoliophyta
Class - Magnoliopsida
Family - Nymphaeaceae
Genus - Nymphaea
Species - *Nymphaea pubescens* (Willdenow)

The global herbal medicine market found to be increasing day by day. With their proven antibacterial qualities, plant extracts and phytochemicals may play a major role in treatment and/or prevention strategies. The most important antimicrobial compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [9]. In view of the above, the present study has been made to investigate the phytochemical screening, antioxidant potential as well as the antimicrobial activities of a traditionally used medicinal water plant *N.pubescens* Willd.

## MATERIALS AND METHODS

### Source of plant materials

The whole aquatic plants of *N.pubescesns* willd used for the present study were obtained from Mylode pond, Pooyappally village, Kollam district of Kerala, India. The collected plant materials were brought into the laboratory. The root, stem, flower, leaves and seeds were sorted out from the plant and then washed thoroughly with running tap water to remove dust. Those plant materials were chopped into small pieces, shade dried until all the water particles evaporated and plants become well dried.

### Preparation of plant extracts

The air-dried plant materials were ground and used for preparing extracts. Powdered samples were extracted with methanol by maceration method and kept it for a period of 24 hrs at room temperature at a ratio of 5:100 (g:ml). Homogenized samples were centrifuged at 10,000 rpm for 15 minutes and supernatants were pooled. The extracts were filtered using Whatman No.1 filter papers and each extract was concentrated in a rotary evaporator to remove methanol. The residue thus obtained was dissolved in methanol and stored at 4-8ºC in a refrigerator for further analysis[11].

### Preliminary phytochemical studies

The preliminary phytochemical analysis of the plant extracts was carried out for the presence of alkaloids, tannins, saponins, terpenoids, steroids, phenolics, quinine, starch, carbohydrates and flavonoids using standard methods with some minor modifications[13].

### Determination of antioxidant activity

The ability of the extract to scavenge (DPPH) free radicals was assessed by the modified method[10]. DPPH (20 mg) was dissolved in methanol (250 ml) to obtain the concentration of 80 µg/ml. The stock solution of the plant extract was prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentration of 200 µg/ml. Ascorbic acid was used as standard in 1-125 µg/ml concentration. 1 ml of the diluted plant extract was mixed with 1 ml of DPPH. Spectrophotometric reading was taken at 517 nm after 30 minutes dark incubation at room temperature. Freshly prepared DPPH solution in methanol was used for absorbance measurements. Percentage of inhibition was calculated using the following equation.

$$\% \text{ of inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

### Determination of antibacterial activity

The antibacterial activity of the methanol extracts was screened against some human pathogenic bacteria; *B.subtilis* (MTCC 2340); *E.coli* (MTCC 56) and *S.aureus* (MTCC 9760) obtained from the Microbiology Laboratory of the Department of Biotechnology, Sree Narayana College, Kollam, Kerala. The bacteria were subcultured on nutrient agar slants incubated at 4ºC in the refrigerator to maintain stock culture.

### Antibacterial assay

Antibacterial activities of the different plant extracts were investigated by the agar well diffusion method [3] using Mueller-Hinton agar plates. The sterile liquid of Mueller-Hinton Agar media (pH 7.4 ± 2) was poured into sterile petridish. After solidification, the bacteria were swabbed with a sterile cotton swab under aseptic conditions. A known concentration of the plant extract (250mg/ml) was transferred to the well of 6 mm diameter. Bacterial plates were incubated at 37ºC for 24 hours. The zone of inhibitions produced by the inhibitory action of different plant extracts and control were taken as the antibacterial activity.

### Data Analysis

A statistical software programme (SPSS for Windows, ver. 17, 2012) was used to analyse the data. One-way analysis (ANOVA) was used to compare treatment means, and the Duncan's New Multiple Range test was used to analyse the data at the 0.05 level of significance ($p < 0.05$).

## RESULTS AND DISCUSSION

### Qualitative phytochemical analysis

Methanol extracts of various plant parts (root, stem, flower, leaves and seed) of *N.pubescens* were prepared by using maceration method (Fig.1 & Fig.2). The preliminary phytochemical screening carried out on the five different extracts revealed the presence of various bioactive secondary metabolites that were confirmed from their chemical colour reaction tests as shown in Fig.3 & Table 1. The presence of phytochemicals in various parts of *N.pubescens* was also reported [1][6][8].

**Figure 1:** Various parts of *N.pubescens* N1-Root, N2-Stem, N3-Flower, N4- Leaves, N5- Seed

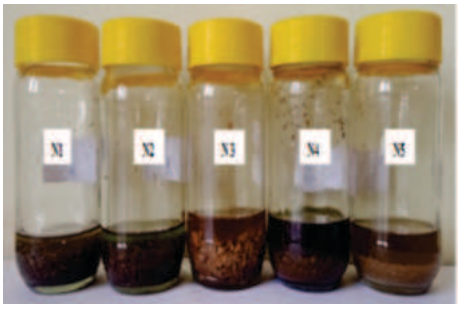


**Figure 2:** Plant extraction using Maceration method N1-Root, N2-Stem, N3- Flower, N4- Leaves, N5- Seed

**Table -1 Qualitative Phytochemical Analysis Of Various Extracts Of *N.pubescens***

| | Phytochemical test | Methanol extract of various plant parts of *N.pubescens* | | | | |
|---|---|---|---|---|---|---|
| | | Root | Stem | flower | Leaves | Seed |
| 1 | Alkaloids | + | - | + | +++ | ++ |
| 2 | Flavanoids | + | - | + | +++ | ++ |
| 3 | Glycosides | + | - | ++ | +++ | ++ |
| 4 | Phenolics | ++ | + | ++ | +++ | ++ |
| 5 | Quinones | +++ | +++ | +++ | +++ | +++ |
| 6 | Saponins | + | - | + | +++ | + |
| 7 | Starch | + | +++ | + | + | + |
| 8 | Steroids | + | ++ | - | - | - |
| 9 | Tannins | ++ | + | +++ | +++ | +++ |
| 10 | Terpenoids | + | - | ++ | +++ | ++ |

(+++) = present in high levels; (++) = present in medium level; (+) = present in low levels; (-) =Absence

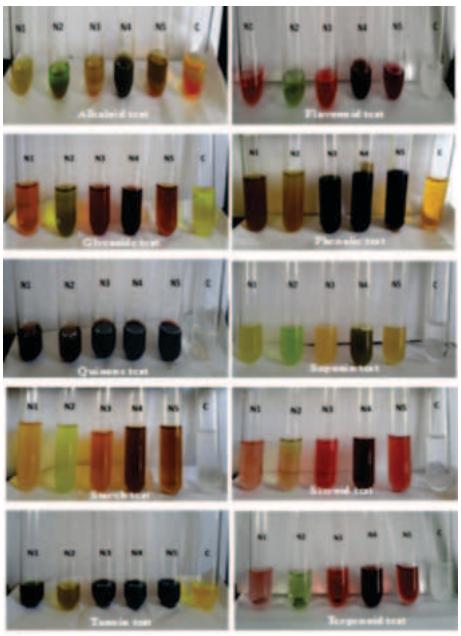

**Figure 3:** Phytochemical tests in methanol extract of *N.pubescens* N1-Root, N2-Stem, N3- Flower, N4- Leaves, N5- Seed and C- control

### Antioxidant activity

The antioxidant activity of the various plant extracts of *N.pubescens* were represented in Figure 4. The results of the present study showed that all the plant extracts showed antioxidant activity. The antioxidant activity of various plant parts can be ranked as: leaves (55.26±0.93 µg/ml) > flower (48.79±0.36 µg/ml) > stem (38.74±0.97 µg/ml) > seed (26.17±0.87 µg/ml) > root (19.30±0.18 µg/ml) (Fig.4). Many secondary metabolites (flavonoids, phenolic compounds etc.) serve as source of antioxidants and do scavenging activities in various plant parts of *N.pubescens* were also reported [5][7].

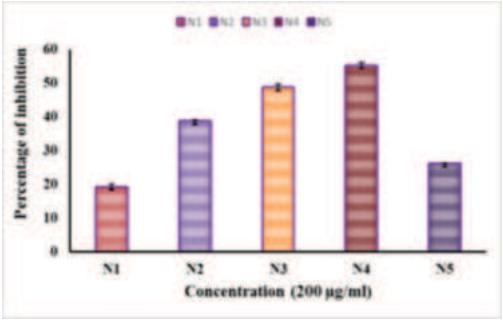


**Figure 4:** Antioxidant activity of investigated plant extracts of *N.pubescens* at the concentration of 200µg/ml. N1-Root, N2-Stem, N3- Flower, N4-Leaves, N5- Seed

### Antimicrobial activity

In the present study, methanol extracts of different plant parts of *N.pubescens* were tested for its antibacterial activity against three typical bacterial strains of *B.subtilis* (MTCC 2340); *E.coli* (MTCC 56) and *S.aureus* (MTCC 9760). All the plant extracts showed inhibitory action against *B.subtilis*, *E.coli* and *S.aureus* as given in the Table 2 and Fig.5. The control (methanol) showed no zone of inhibition against three typical bacterial strains.

**Table - 2 Zones Of Inhibition Produced By Methanol Extracts Of *N.pubescens***

| Test compound (Methanol extract) | | Zone of inhibition (mm) | | |
|---|---|---|---|---|
| | | Bacteria | | |
| | | B.subtilis | E.coli | S.aureus |
| N.pubescens | Root | 23 | 12 | 24 |
| | Stem | 11 | 16 | 16 |
| | Flower | 26 | 15 | 23 |

| | | | | |
|---|---|---|---|---|
| | Leaves | 30 | 15 | 22 |
| | Seed | 23 | 15 | 15 |

It was observed that among the five extract of *N.pubescens,* methanol extract of root showed comparatively high antimicrobial activity against *S.aureus* with a zone of inhibition 24mm. It also showed 12mm zone of inhibition against *E.coli*, 23mm zone of inhibition against *B.subtilis*. The seed showed same range of inhibitory action (15mm) against *E.coli* and *S.aureus*. Whereas stem showed least range of zone of inhibition (11mm) against *B.subtilis* compared to other plant extracts(Table 2, Fig.5). These findings were also in agreement with other researchers[2][4].

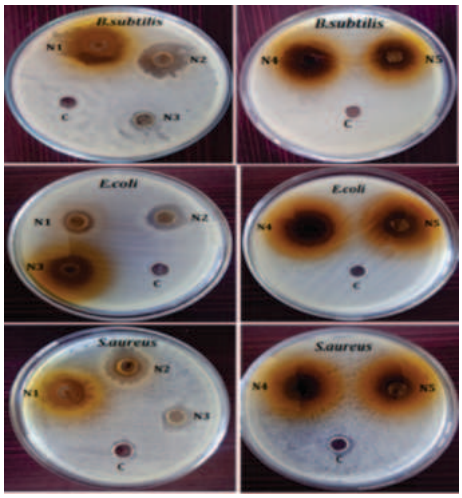


**Figure 5:** Zones of inhibitions revealed by various plant parts of *N.pubescens* against *B.subtilis, E.coli & S.aureus* N1-Root, N2-Stem, N3- Flower, N4- Leaves, N5- Seed, C-control

## CONCLUSIONS

This study provides information about the phytochemical constituents, antioxidant property and antibacterial activity of various plant parts of *N.pubescens*. Hence, it is identified that these parts can be used as a source for the manufacturing of drugs of scavenging property and antibacterial activity. Moreover those natural antioxidants can have potential advantages among various diseases with oxidative stress and for that *N.pubescens* may be one of the alternatives. With more resources and time, further investigation of chemical constituents of *N.pubescens* and other poorly studied plants can be revealed. There is a large untapped reservoir that is just waiting to be explored, thus additional research and investigations are necessary.

**Acknowledgement**

The authors are thankful to the Department of Biotechnology, Sree Narayana College, Kollam- 691 001, Kerala, India for providing the laboratory facilities to carry out the present work.

## REFERENCES:

[1] Ace, B., Shanti, D.L., and Winda, A. (2015), "Phytochemical screening and antioxidant activity of seeds extract of water plant (*N.stellata* and *N.nucifera*)",Journal of Chemical and Pharmaceutical Research, 7(11), 221-224.
[2] Akinjogunla, J.O., and Eghafona, N.O. (2010), "Antibacterial activity of *N.lotus* (Nymphaeaceae) leave extract against methicillin resistant *S.aureus* (MRSA) and vancomycin resistant *S.aureus* (VRSA) from clinical samples," Electron.J. Environ. agric.Food Chem, 9, 1064-1073.
[3] Alzoreky, N.S., and Nakahara, K. (2003), "Antibacterial activity of extracts from some edible plants commonly consumed in Asia", International Journal of Food Microbiology, 80, 223-230.
[4] Boontarika, T., Kittima, V.A., and Pannapa, P. (2023), "*In Vitro* Antimicrobial Activity of *N.pubescens* (Pink Water Lily) Leaf Extracts". Plants, 12(20), 3588.
[5] Edison, D.D., and Veerabahu, R.M. (2013), "Total phenolics, flavonoids and *in vitro* antioxidant activity of *N.pubescens* wild rhizome". World Journal of Pharmacy and Pharmaceutical Sciences, 22(55) 371-372.
[6] Elegami, A.A., Bates, C., Gray, A.I., and Mackay, S.P. (2003), "Two very unusual macro cyclic flavonoids from the water lily *N.lotus*." Phytochem 63(6), 727-731.
[7] Hazara, B., Biswas, S., and Mandal, N. (2008), "Antioxidant and free radical scavenging activity of *Spondias pinnata*". BMC Comple and AlterMed, 1-10.
[8] Kehinde, M.A., Mustapha, M., and Mohammed, M.M. (2016), "Phytochemical screening and antibacterial activities of crude extract of *N.lotus* (water lily) against fish pathogens", Journal of Biomedical Sciences, 2(4), 38.
[9] Kumarappan, C.T., Thilagam, E., and Vijakumar, C. (2012). "Modulatory effect of polyphenolic extracts of *Ichnocarpus frutescens* on oxidative stress in rats with experimentally induced diabetes". Indian J Med Res, 136, 815-821.
[10] Kumarasamy, Y., Byres, M., and Sarker, S.D. (2007), "Screening seeds of some Scottish plants for free radical scavenging activity", Phytother.Res, 21, 615-621.
[11] Muthusamy, R., Karthiga, V., and Bernala., W. (2013), "Phytochemical screening and antibacterial activity of methanol extract of *Tridax procumbens*." International Journal of Pharmacy and Biological Sciences, (3)1, 521-524.
[12] Pareek,A., and Kumar, A. (2014), "Nutraceutical Value of Aquatic Plants". J. Acad. (N.Y.), 4(1), 10-21.
[13] Shaikh, J.R., and Patil, M.K. (2020), "Qualitative tests for preliminary phytochemical screening": An overview. 8. 603-608.