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Original Research Paper

ANTIBACTERIAL ACTIVITY OF FLOWER EXTRACTS OF *NYMPHAEA NOUCHALI*

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ABSTRACT

The flowers of *N. nouchali* were collected from lakes in Thane district dried, powdered and extracted with Pet ether, toluene, chloroform, acetone, ethyl acetate and methanol. These crude extracts were tested for antibacterial activity by agar streak method, the extracts found to be active were subjected to Minimum inhibitory concentration (MIC) determination by TTC assay, the extracts were prepared according to the MIC and antibacterial susceptibility test was carried out using Agar well diffusion method. All the extracts showed antibacterial activity against the tested strains. But the polar extracts showed a greater antibacterial potential as compared to the non polar extracts. Methanolic extract was the most active. The highest activity was seen against *Salmonella paratyphi A* and the lowest activity was seen against *Salmonella paratyphi B*. The present study showed the effectiveness of the crude plant extract against the tested bacterial strains and indicates the potential use of the extract as antimicrobial agent for the control of infectious diseases.

Keywords: *Nymphaea nouchali*, Well diffusion assay, Agar streak method, antibacterial activity.

INTRODUCTION

Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules which protect the human body against various pathogens. Nature has been a source of medicinal agents for thousands of years and a large number of modern drugs have been isolated from natural sources. Herbal medicine is the oldest known healthcare system known to mankind. India has rich medicinal plants of nearly 7500 species. Out of these, 4635 species are commercially used to a fairly large scale.[1] Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% prescribed medicines in industrialized countries are derived directly or indirectly from plants.[2] Secondary metabolites are phytochemical compounds found in plants that are not required for normal functioning of the body but have a beneficial effect on health or play an active role in amelioration of the disease. It becomes pertinent to identify such phytochemicals due to increased awareness of the limited ability of synthetic pharmaceuticals. Many antibiotics have limited effective life and the public is becoming increasingly aware of the problems with the over prescription of these antibiotics. Many people mainly in the developed countries are interested in having autonomy over their medical care so self-medication has become a common phenomenon.[3]

The plant *Nymphaea nouchali* belongs to the genus *Nymphaea* belongs to the family *Nymphaeaceae* and consists of hardy and tender plants of aquatic habitat. The genus contains 50 species and commonly called the water lily. *Nymphaea nouchali* is a day blooming

nonviviparous plant with submerged roots and stems. Parts of the leaves are submerged, while others rise slightly above the surface. The leaves are round and green on top; they usually have a darker underside. The floating leaves have undulating edges that give them a crenellate appearance. Their size is about 20–23 cm and their spread is 0.9 to 1.8 m .This water lily has a beautiful flower which is usually violet blue in color with reddish edges. Some varieties have white, purple, mauve or fuchsia coloured flowers. The flower has 4-5 sepals and 13-15 petals that have an angular appearance making the flower look star-like from above. The cup-like calyx has a diameter of 11–14 cm. Ayurveda and Siddha systems of medicines for the treatment of diabetes, inflammation, liver disorders, urinary disorders, menrrhagia, blenorrhagia, menustration problems, as an aphrodisiac and as a bitter tonic. There seems to be an agreement between the traditional use and experimental observations, such as, hepatoprotective, anti-inflammatory, and particularly antidiabetic activity.[4] *N. stellata* are ingredients of many ayurvedic formulations like, Asokarista, Arvindasava, Usirasava, Candanasava, Kalyanaka Ghrta, Samangadi Curna, Kanaka Taila, Jatyadi Taila, Tungadrumadi Taila, Manjesthadi Taila, Candanadi Lauha, and TriphalaGhrta (The Ayurvedic Pharmacopeia of India). Numerous studies have identified compounds within herbal plants that are effective as antibiotics[6] and a number of such isolated compounds are good source of antibacterial prototypes. Thus such encouraging results indicate the need for research into the traditional health systems. It also facilitates pharmacological studies leading to the synthesis of a more potent drug with reduced toxicity.[7,8] The purpose of this study is to investigate the antibacterial properties of *Nymphaea nouchali*. In this paper we report of the above study in order to direct further investigations towards the finding of better and safe antibacterial phytocompounds.

MATERIALS AND METHODS

Collection of Plant Material

The fresh flowers of *Nymphaea nouchali* free from disease were collected in the month of June-July from various lakes in Thane District, Maharashtra. The flowers were washed with tap water and latter with demonized water and dried under shade. The plant material was regularly checked for fungal growth or rotting. After the plant material was dried it was powdered with the help of an electric blender and sieved through size 80 sieve to obtain a uniform fine particle size. This plant material was stored in airtight containers at 4ºC.

Preparation of Plant Extracts

20 gms of the sieved powder was accurately weighed and extracted with solvents of varying polarity individually; they were hexane, chloroform, pet ether, acetone, ethyl acetate, methanol, ethanol in a Soxhlet's extractor for 72 hours. The extracts thus obtained were concentrated under reduced pressure to yield crude plant extracts. These crude plant extracts were reconstituted in Dimethyl sulphoxide (DMSO) maintaining the concentration of 10000 µg/ml. These extracts were stored at -20°C in amber coloured glass stoppered vials.

Test Cultures

The following strains were used for the study and were maintained by the Microbiology department of K. J. Somaiya College of Science and Commerce.

- *Escherichia coli*
- *Klebsiella pneumonia*
- *Proteus vulgaris*
- *Pseudomonas aeruginosa*
- *Salmonella paratyphi A*
- *Salmonella paratyphi B*
- *Shigellaflexineri*
- *Staphylococcus aureus*

These cultures were maintained on sterile nutrient agar (Himedia, Mumbai) slants and stored at 4 ºC until further use.

Preparation of Inoculums

The bacteria were subcultured on nutrient agar slants for 24 hours. Loopful of these cultures were suspended in sterile saline (0.85% NaCl w/v) to obtain a transmittance of 25% at 560 nm

using an UV-Vis spectrophotometer. This concentration corresponded to 10^6 colony forming units (CFU) per mL. McFarland standards were used to adjust the turbidity of bacterial suspension. The McFarland standard was made by adding 0.05 mL of $BaCl_2$ (1.17% w/v of $BaCl_2.2H_20$) to 9.95 mL of 0.18 M H_2SO_4 with constant mixing. This standard was kept in a test tube and sealed to prevent loss by evaporation.[9]

Antimicrobial Assay

The antibacterial potential of the extracts was first assessed with the help of agar streak method. The extracts which were found to inhibit bacteria were further subjected to micro dilution method to find the minimum inhibitory concentration. The solutions of minimum inhibitory concentration were further subjected to well diffusion assay to determine the potency of the extracts.

Preliminary Analysis by Agar Streak Method

All the extracts were screened for their antibacterial activity by agar streak method. In this method, 15 mL of molten sterile agar butts were taken and 1 mL of extract (10000 µg/ml) was added to it. This mixture was poured into a sterile petri dish and allowed to solidify. Now, the bacterial suspensions were streak inoculated onto the above agar plates using a sterile nichrome loop. These plates were incubated at 37 °C for 24 hrs. The results were recorded as growth or no growth. The results are shown in table 1.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration was determined by micro-well dilution method in nutrient broth (Himedia, Mumbai). 0.01%w/v 2,3,5-triphenyltetrazolium chloride (TTC Sigma Aldrich, India) was used as a visual indicator of growth. In each well of the 96 well-plate 95 µL nutrient broth and 5 µL inoculums was added, to this 100 µL of extract of concentrations 500 µg/mL, 250 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 10 µg/mL &5 µg/mL and 100 µL of TTC were added. Negative control was solvent in place of extract and penicillin and streptomycin were used as positive control. The plate was incubated at 37 ºC for 24 hrs. In presence of bacteria the TTC gets converted to red formazan, this red formazan indicates the viability or activity of the cells.[10] The results were noted as growth or no growth based on the colour of TTC and are represented in table 2.

Well Diffusion Method

The extracts that were found to be active by the previous method were examined for their zone of inhibition by agar well diffusion method. The bacterial cultures were sub cultured a day before on agar slants and the inoculums were prepared as stated earlier. The inoculums were added to 20 mL of sterile molten nutrient agar. This was homogenized well and poured in petri dishes. The agar was allowed to solidify and harden. Later the required four wells were made using sterile metallic cork borer. The wells were filed with the extracts (0.1mL). Streptomycin and Penicillin were used as reference standards and the solvent (DMSO) was used as negative control. The plates were incubated at 37 °C for 24 hours. The zone of inhibition was measured using vernier calipers and reported in table 3.

RESULTS

The inhibition zone assay revealed primarily two types of observations which were wells without any surrounded clear or inhibition zones which could be attributed to the absence of any inhibitory activity and clear inhibition zone representing the bacteriostatic or bactericidal action of the tested plant extract. The minimum inhibitory concentration for the extracts was determined with the help of 2,3,5-triphenyltetrazolium chloride as the visual indicator of growth. The increase in concentration of the extracts resulted in lower cell count which proportionately reduced the intensity of the red colour due to lower formazan formation. This is the indicator of antibacterial activity of the extract and also the dose dependence of the activity. The results of the microbial assay indicate that the crude extracts of *N. nouchali* are active against the tested bacterial strains. The flower extracts showed variable activity with different organisms. The petroleum ether extract had no measurable

effect on the tested bacteria. The zone of inhibition ranged from 2 cm to 27 cm. The non polar extracts were less active as compared to their polar counterparts. Amongst all the extracts tested the methanolic extract proved to be the most effective. It showed maximum zone of inhibition (27.0) against *Salmonella paratyphi A* (table 3). Most of the extracts showed activity against both gram negative and gram positive bacteria thus indicating a wide spectrum of antibacterial activity.

The extracts show great promise as antibacterial agent against the above mentioned bacterial strains. The standards of penicillin and streptomycin were used as reference standards and at least the polar extract showed greater zone of inhibition than the standards used. The inhibitory action of the extracts on the bacterial strains was dose dependent as it is evident from the fact that decrease in concentration showed lower activity for all bacterial strains.

DISCUSSION

Plants are important source of pharmacophore which will function as new chemotherapeutic agents. The first step to develop a chemotherapeutic agent from plants would be the assay of in vitro antibacterial activity. The extracts thus found active will help to identify the active compounds responsible for the activities from the plant. In recent years multi drug resistance is seen in pathogenic bacteria which has revived interest in the search of new antibacterial agents from natural sources. In fact, gram negative bacteria *P. aeruginosa* are frequently reported to have developed multi drug resistance to many of the antibiotics.[11] But, the extracts especially the polar ones show a good activity against *P. aeruginosa*. The antibacterial agents from natural sources also eliminate the side effects of synthetic or semi synthetic antibacterial agents. The antibacterial activity of the plant extract was variable with various organisms. The zones of inhibitions ranged from 0 mm to 27 mm. The results obtained in the present study shows that N. nouchali possesses antibacterial activity against *Klebsiella pneumonia, Salmonella paratyphi A, Shigella flexineri, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Salmonella Paratyphi B and Pseudomonas aeruginosa*. These results were compared with standard antibiotics Penicillin and Streptomycin. The polar extracts showed greater activity than the standard antibiotics.

The antibacterial activity of plant extracts are attributed to certain bactericidal compounds that are synthesized by the plant. The antibacterial activity thus varies if solvents of varying polarity are used because the solubility of these compounds may be different in these solvents. Hence, as evident from the results, extracting solvents exerts a great influence on the antibacterial properties. The activity also depends upon the ability to resolve and diffuse in the media used in the assay.[12]

In spite of the fact that these extracts are not pure compounds, the results obtained clearly suggest the potency of the extracts. Thus, these extracts can further be fractionated to yield antibacterial compounds which can be used in the development of phytomedicine against these microbes. Inhibitory activities of the crude extracts are in general greater or comparable to that of the standards used. These extracts being of natural origin are safer and less prone to development of drug resistance in bacteria.

The above mentioned pathogens cause a number of life threatening diseases which can be managed by the use of synthetic antibiotics which have their own side effects. The increasing problems associated with drug resistance in bacteria and the increasing cost of synthetic antibacterial agents pharmaceutical companies are looking for other alternatives.[13] Our aim is to find plants which have antibacterial activity without many side effects. A detailed study needs to be carried out to isolate bioactive compounds that show antibacterial activity.

CONCLUSIONS

From the above study it is evident that the flowers of *N. Nouchali* have significant anti bacterial action and can be employed as an antibacterial agent. Also fractionation of the extract to yield antibacterial compounds should be carried out.

CONFLICT OF INTEREST

All the authors declare that there is no conflict of interest.

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Table 1: Preliminary antibacterial screening by agar streak method

Bacteria	EtOAc	MeOH	Chloroform	Acetone	Toluene	Pet Ether
Klebsiella pneumonia	+	+	+	-	+	-
Salmonella paratyphi A	+	+	-	+	+	-
Shigella flexineri	+	+	+	+	+	-
Staphylococcus aureus	+	+	+	+	+	-
Escherichia coli	+	+	+	+	-	-
Proteus vulgaris	+	+	+	-	-	-
Salmonella paratyphi B	+	+	-	+	-	-
Pseudomonas aeruginosa	+	+	-	+	-	-

Table 2: Minimum inhibitory concentration (MIC) of extracts of N. nouchali in µg/mL

Bacteria	Ethyl acetate	Methanol	Chloroform	Acetone	Toluene	Penicillin
Klebsiella pneumonia	100	5	50	500	100	250
Salmonella paratyphi A	50	25	500	50	100	25
Shigella flexineri	50	5	100	50	50	No activity
Staphylococcus aureus	100	5	100	50	50	10
Escherichia coli	25	25	50	100	250	250
Proteus vulgaris	100	100	100	250	250	No activity
Salmonella paratyphi B	100	100	No activity	100	Not detected	No activity
Pseudomonas aeruginosa	100	50	250	100	No activity	No activity

Table 3: Determination of zone of inhibition of extracts of N. nouchali in mm

Bacteria	Ethyl acetate	Methanol	Chloroform	Acetone	Toluene	Penicillin	Streptomycin
Klebsiella pneumonia	19	25	13	0	12	0	13
Salmonella paratyphi A	23	27	0	20	12	26	20
Shigella flexineri	12	26	14	14	17	0	0
Staphylococcus aureus	17	19	15	26	18	20	14
Escherichia coli	18	21	12	22	0	0	16
Proteus vulgaris	20	15	13	0	0	0	18
Salmonella paratyphi B	12	13	0	12	0	0	16
Pseudomonas aeruginosa	23	24	0	2	0	0	0

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