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Research Article

ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *IPOMOEA AQUATICA* FORSK.

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ABSTRACT

This study was carried out with an objective to investigate the antibacterial activity and phytochemical screening of the aqueous and methanol extracts of aerial part of Ipomoea aquatica Forsk. The antibacterial activity of the different extracts of Ipomoea aquatica were evaluated by disc diffusion method against three ATCC bacterial species. Zone of inhibition of the plant extracts were compared with ciprofloxacin and ampicillin standard discs. The presence of tannins, flavonoids, saponins, amino acids, steroid, etc in the different extracts were established. The methanol extract was effective against ATCC strains of Staphylococcus aureus whereas aqueous extract wasn't effective against ATCC strains of Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. The results suggested that aerial part of Ipomoea aquatica is effective against gram positive bacteria.

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INTRODUCTION

Globally, infectious diseases are the leading cause of death. Many infectious diseases were treated with herbal remedies throughout the history of mankind. Due to indiscriminate uses of synthetic antibacterial drugs, there are increase in number of drug resistant pathogens and clinical efficacy of many existing antibiotics is being threatened.[1,2] Therefore, it becomes an emergency for scientist to search for new antibacterial substances from different sources like the medicinal plants. Recent work on screening of antimicrobial agents revealed the potential of several herbs as sources of drugs. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes.[3]

The plant *Ipomoea aquatica* belongs to the family *Convolvulaceae*. This perennial plant is commonly known as Water spinach (English), Kolamani (Manipuri), Kalmisak (Hindi and Bengali) etc. This plant has a wide distribution and grows in moist soils and side-lines of fresh water usually all-round the year. This herb is commonly consumed as vegetable item in different regions of the world.[4] In Unani, *I. aquatica* is used for fever, bronchitis, biliousness and liver complaints. In Ayurveda, *I. aquatica* leaves are useful in jaundice, nervous debility, etc. Juice of plant with salt is traditionally used for ring worm infestation. The non-toxic nature of *Ipomoea aquatica* and its multiple beneficial effects has made it one of the most attractive plant to explore its protective role.[5-7] Therefore, the present attempt has been made to investigate its role for antibacterial property in *in-vitro* model.

Experimental Section

Set up: Department of Pharmacology and Department of Microbiology, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur, India.

Plant material: The fresh plants of *Ipomoea aquatica* Forsk. were collected from Lamphel area, Imphal, Manipur. The plant was identified and authenticated from Department of Life Sciences, Manipur University, Imphal, India. A voucher specimen was kept in the University herbarium for reference (Voucher no. 001351).

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Preparation of aqueous extract: The leaves and stems were separated, cleansed with water, cut into small pieces and dried in shade. It was then coarsely powdered by mixer grinder and stored in airtight container for future use. Aqueous extract of *Ipomoea aquatica* (AEIA) was prepared by soxhlet extraction method described by Verma SCL and Agrawal SL.[8] A brownish crude dry extract was obtained.

Preparation of methanol extract: Methanol extract of *Ipomoea aquatica* (MEIA) was prepared by soxhlet extraction method described by Dhiman A *et al*.[9] Coarse powder of stems and leaves of the plant was packed into a soxhlet apparatus and extracted upto 4 h with petroleum ether (60–80°C) for defatting. Defatted powder of the plant was removed from soxhlet apparatus and dried. Then dried defatted coarse powder of the plant was extracted with 99% of methanol. The solvent was evaporated and a greenish dried extract was obtained.

Phytochemical screening: Chemical tests were carried out by using aqueous and methanol extracts to identify various phytochemicals using standard methods.[10-13]

1. Test for tannins: Ferric chloride Test- In 2-3 ml of the extract (alcohol or aquoues) few drops of $FeCl_3$ solution were added→ blue colour (hydrolysable tannins) or green colour (condensed tannins) indicated presence of tannins.
2. Test for flavonoids: Lead acetate test- 1 ml of extract was added to 1ml of 10% lead acetate solution → yellow coloured precipitation indicated presence of flavonoids.
3. Test for starch (non-reducing sugar): Iodine test- In 2 ml of extract four drops of iodine solution was added → blue colour of starch which disappeared in heating and reappeared in cooling.
4. Test for protein: Biuret test- in 2 ml of test solution 2 ml 4% of sodium hydroxide and few drops of 1% copper sulphate solution were added → violet/ pink colour indicates the presence of proteins. Precipitation test- In the test solution trichloroacetic acid or 5% lead acetate was added → white precipitation was observed.
5. Test for amino acid: Ninhydrin test- Heat 3 ml of extract and 3 drops of ninhydrin solution (0.2 gm ninhydrin in 100 ml ethanol or in 94 ml distilled water and 6 ml acetone) were added and kept in boiling water bath for 10 minutes → purple or bluish colour indicated the presence of amino acid.
6. Test for carbohydrates: Benedict's test (reducing sugars)- 2 ml extract was added to 2ml benedict's reagent & heated in boiling water bath for 5 minutes → appearance of green (<1000 mg/dl), yellow (1000-1500 mg/dl), orange (1500-2000 mg/dl) or red to brick red (>2000 mg/dl) colour confirmed the presence of reducing sugars.
7. Test for saponins: Froth formation test- 50 mg extract was diluted with 20 ml of distilled water and suspension was shaken vigorously in a test tube for 15 minutes. Stable (1-2 minutes) 2 cm froth formation indicated presence of saponins.
8. Test for steroids: Sulfur powder test- small amount of sulfur powder was added to the extract solution→ sulfur powder sink at the bottom → indicated presence of steroids.

Anti-bacterial activity: Anti-bacterial study was done by disc diffusion method using Kirby-Bauer disk diffusion susceptibility test[14] protocol according to CLSI guidelines.[15] ATCC stains of *Staphylococcus aureus* 25923. *Escherichia coli* 25922 and *Pseudomonas aeruginosa* 27853 were collected from Department of Microbiology, RIMS, Imphal, Manipur. Zone of inhibition (ZOI) of the plant extracts were compared with ciprofloxacin and ampicillin standard discs.

Preparation of plant extracts discs: Whatman filter papers (No.1) were used to prepare discs approximately 6 mm in diameter. The disc was then sterilized by dry heat at 140℃ for 60 min. Filter paper discs containing 50, 100 and 200 µg extract prepared from 5, 10 and 20 mg/ml extract solutions, respectively by transfusing 10 µL solution by micropipette in each disc. Prepared extract discs were dried before placing over Muller Hinton (MH) agar. Methanol and aqueous extracts were used to prepare the methanol and aqueous extract discs, respectively.[16]

Antibiotic testing using Kirby-Bauer disc diffusion method: Few colonies of the organisms to be tested were picked up in a sterilized wire loop and transferred into a test tube containing normal saline to make bacterial suspension. The test tube was stirred to make the suspension turbid. Then it was compared with the standard 0.5 McFarland for the desired cloudiness and adjusted accordingly. A sterile cotton swab was then dipped into the bacterial suspension and surplus suspension was removed by pressing the swab against the side of the tube. To make lawn culture, this swab was then streaked in a MH agar plate in 3 planes. The antibiotic discs to be tested along with the standard were placed over this agar evenly using a flamed forceps. After overnight incubation at 37°C the zone of inhibition (ZOI) was measured using a scale.[14] Then ZOI was graded according to Kang SN *et al*.[17] shown in Table 1.

Table 1 Grading of zone of inhibition

Diameter of ZOI	Antibacterial activity
6 – 8 mm	No antimicrobial activity
8.1–9 mm	Slight antimicrobial activity
9.1–12 mm	Moderate antimicrobial activity
12.1–15 mm	Clear antimicrobial activity
>15 mm	Strong antimicrobial activity

RESULTS AND OBSERVATION

Phytochemical screening: Phytochemical analysis of aqueous and methanol extracts of *Ipomoea aquatica* shown in Table 2.

Table 2 Phytochemical analysis of aqueous and methanol extracts of leaves and stems of *Ipomoea aquatica*

Sl. No.	Phytochemicals	Aqueous extract	Methanol extract
1.	Tannins	+	+
2.	Flavonoids	+	+
3.	Starch	+	+
4.	Protein	+	+
5.	Amino acid	+	+
6.	Carbohydrates	+	+
7.	Saponins	+	+
8.	Steroid	+	+

Antibacterial study: The ZOI of discs of aqueous and methanol extract of *Ipomoea aquatica* and standard antibiotic discs shown in Figure 1 and Figure 2, respectively. Discs of AEIA did not show any antibacterial activity against *S. aureus*, *E. coli*

and *P. aeruginosa*. Discs of MEIA did not show any antibacterial activity against *E. coli* and *P. aeruginosa*. However, MEIA disc at 100 µg/disc showed slight and at 200 µg/disc showed moderate antibacterial activity against ATCC strain of *S. aureus*.

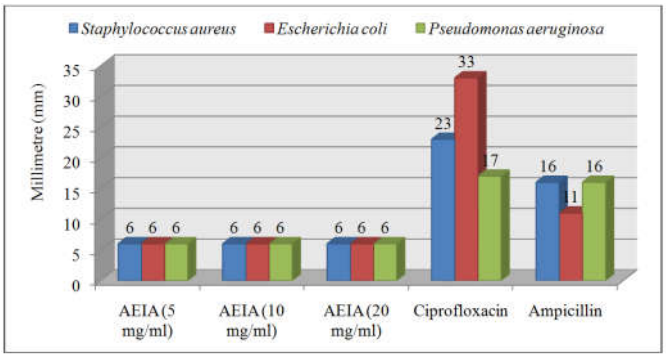

Figure 1 Antibacterial activity (ZOI) of aqueous extract of *Ipomoea aquatica* (AEIA) and standard discs.

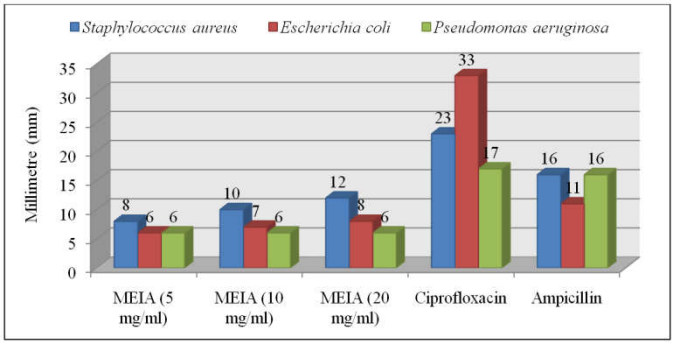

Figure 2 Antibacterial activity (ZOI) of methanol extract of *Ipomoea aquatica* (MEIA) and standard discs.

DISCUSSION

Kirby Bauer method of antimicrobial susceptibility testing by disc diffusion is the most standardized method used routinely in labs for anti-bacterial susceptibility testing. Antibiotic within the disc gradually diffuses into the surrounding area. If the organism is sensitive to that particular antibiotic then there will be a zone of inhibition (ZOI). The size of ZOI is not only determined by the sensitivity of the particular organism to the antibiotic, but also other factors like concentration of the antibiotic in the disc and diffusibility of an antibiotic in a particular media.

There are several hypothesises explaining the usage of plant extracts as antimicrobials in traditional medicine. It was found that *Ipomoea aquatica* contains many active principles like flavonoids, phenols, saponins, tannins, etc. Aqueous extract of the plant showed no antibacterial activity whereas methanol extract showed mild to moderate antibacterial activity against *S. aureus* but not to *E. coli* and *P. aeruginosa*. *S. aureus* is a gram positive organism whereas *E. coli* and *P. aeruginosa* are gram negative organism, this result shows MEIA might have antibacterial activity against only the gram positive bacteria. Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent. Methanol extract might have had higher solubility for more phytoconstituents, consequently showed highest antibacterial activity.[18]

CONCLUSION

The study suggested that aqueous extract of leaves and stems of *Ipomoea aquatica* did not show any antibacterial activity. But methanol extract of the plant showed moderate antibacterial activity against *Staphylococcus aureus*. However, further studies are needed to elucidate the exact mechanism of action of antibacterial activity offered by its phytoconstituents and its clinical application to prevent or to cure infectious diseases.

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Declarations

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Ethical approval: Taken from Institutional Animal Ethics Committee.

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