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PRELIMINARY PHYTOCHEMICAL AND ANTIBACTERIAL INVESTIGATIONS OF THE METHANOL EXTRACT OF *ALTERNANTHERA PHILOXEROIDES* (MART.) GRISEB

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ABSTRACT

The present work is aimed at exploring the phytochemical analysis and antibacterial activity of methanol leaf extract of *Alternanthera philoxeroides* (Mart.) Griseb. Many plants have been used medicinally because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. The plant material was extracted with methanol by cold maceration process. The preliminary phytochemical screening was carried out and the presence of carbohydrates, aminoacids, proteins, cardiac glycosides, alkaloids, flavonoids, tannins and phenolic compounds were observed. The methanol extract of *A.philoxeroides* possess appreciable levels of tannin and flavonoid contents, 5.6 mg of GAE/gm and 4.5 mg of rutin/gm of extract respectively. The antimicrobial activity was carried by agar well diffusion method against bacteria *S.aureus, B.subtilis, B.megaterium, E.faecalis, S.mutans, E.coli, K.pneumoniae, P.aeruginosa* and *P.vulgaris.* Methanol extract exhibited maximum activity (16±0.57mm) against *E.coli*. The results of MIC indicated that *S.aureus* and *E.coli* were the most sensitive bacteria to *A.philoxeroides* leaf methanol extract, inhibited at lowest concentration of 12.5µg/ml.

KEYWORDS: *Alternanthera philoxeroides,* tannin, flavonoid, antibacterial activity.

INTRODUCTION

Plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased world-wide. The

antimicrobial properties of plants have been investigated by a number of researchers worldwide. Antimicrobials of plant origin have enormous therapeutic potential. Plants accumulate various secondary metabolites including alkaloids, glycosides, polyphenols etc. They are effective in the treatment of infectious diseases with minimal side effects that are often associated with synthetic antimicrobials.[1] The tannin containing remedies are used as anthelmintics, antioxidants and antimicrobial agents. To promote the proper use and to determine the potential of plants as sources for new drugs, it is essential to understand their properties, safety and efficacy.

Alternanthera philoxeroides (Mart.) Griseb (Amaranthaceae) was reported for preventive and therapeutic effects against influenza,[2] antinociceptive and antihyperglycemic activities,[3] inhibitory action against human immunodeficiency virus[4] and dengue virus.[5]

The plant is used as leafy vegetable. It contains an immense variety of bioactive non-nutritive health enhancing factors such as antimicrobials, antioxidants, phytochemicals, essential fatty acids and dietary fiber.[6] Due to their dietary importance, many scientific studies have been carried out on the nutritive values of green leaves.[7] Therefore the present work is aimed to explore the phytochemical analysis and antibacterial activity of methanol leaf extract of *Alternanthera philoxeroides* (Mart.) Griseb.

MATERIALS AND METHODS

Plant Material

The fresh leaves of *Alternanthera philoxeroides* were collected from local market of Guntur, Andhra Pradesh, India. The plant specimen was authenticated from Botanical Survey of India, Ministry of Environment & Forests, Government of India, Southern Regional Centre, Coimbatore. The plant specimen was identified as *Alternanthera philoxeroides* (Mart) Griseb. The healthy leaves were shade dried and powdered to get a coarse powder.

Extraction of phytoconstituents

The phytoconstituents present in the leaves of *Alternanthera philoxeroides* were extracted by cold maceration process. 100g powder of dried leaves of *Alternanthera philoxeroides* was taken into conical flask. The phytoconstituents were extracted by adding 1000ml of methanol to the powder. The powder was extracted by keeping the flask on orbital shaker for 48 hrs. The extract was filtered through Whatmann filter paper and collected. The process was

repeated twice. The collected extract was pooled and concentrated by evaporation. The extract was preserved in desiccator for further study.

Bacterial cultures

In the study five Gram positive bacteria *Staphylococcus aureus, Bacillus subtilis, Bacillus megaterium, Enterococcus faecalis, Streptococcus mutans* and four Gram negative bacteria *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus vulgaris* were used.

Phytochemical screening

The reported phytoconstituents of the plant include phaeophytin a, phaeophytin a', oleanoic acid, β-sitosterol, 3β-hydroxystigmast-5-en-7-one, α-spinasterol, 24-methylene cycloartanol, cycloeucalenol and phytol.[8] The antitumour compounds alternanthin B and N-trans-feruloyl-3,5-dimethoxytyramine has been isolated from aerial parts of *A.philoxeroides.*[9] The plant is also reported for the presence of triterpenoid saponins. [10] The phytochemical screening for all the obtained extracts was carried using standard methods.[11, 12]

Quantitative Determination of Phytoconstituents

Determination of Total Phenolic Content

The total phenolic content was determined using Folin Ciocalteau reagent. A standard calibration curve was prepared and the absorbance against concentration of tannins at 725nm was estimated spectrophotometrically. Gallic acid was used as a standard and the total phenolic content was expressed as µg/ml gallic acid equivalents (GAE). Concentrations of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentration of 1mg/ml of plant extract was prepared in methanol and 0.5ml of each sample were introduced into test tubes and mixed with 0.5ml of a 1N dilute Folin-Ciocalteau reagent and 2.5ml of 20% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 40 minutes at room temperature and absorbance was read at 725nm spectrophotometrically.[13]

Determination of Tannin Content

Tannin content was determined using insoluble polyvinyl-polypyrrolidone (PVPP), which binds tannins.[14] Briefly 1ml of extract (1mg/ml) in which the total phenolics was determined, was mixed with 100mg of PVPP, vortexed, kept for 15min at 4^0C and then centrifuged for 10 min at 3000 rpm. In the clear supernatant non-tannin phenolics were

determined the same way as that of total phenolics. Tannin content was calculated as a difference between total and non-tannin phenolic content.

Determination of Flavonoid Content

The diluted methanol extract (0.5 ml, 1% w/v) were taken in test tube and mixed with 0.1 ml of 10% aluminum nitrate, 0.1 ml of 1 M aqueous potassium acetate and 4.3 ml of methanol. After standing for 40 min at room temperature, the absorbance of the reaction mixture was measured at 506 nm. Rutin was used as a standard compound in the range of 2-12 mg/ml concentrations to construct a standard curve.

Determination of Antibacterial activity

The antibacterial efficacy of methanolic leaf extract of *A.philoxeroides* was evaluated by agar well diffusion method.[15] To determine the susceptibility patterns of bacteria against compounds of *A.philoxeroides* the overnight grown culture in nutrient broth (Himedia, Mumbai, India) served as inoculums. The sterile Muller Hinton agar (Himedia, Mumbai, India) medium was poured into sterile petri plates at 40-45^{0}C and allowed to solidify. The nutrient agar plates were inoculated with the overnight grown cultures. The wells (6mm) were prepared on the inoculated plates equidistantly. The extracts were dissolved in DMSO (Dimethyl Sulfoxide) and each well is filled with 500µg/ml of crude methanolic extract. The wells were also filled with positive (Amikacin 100µg/ml) and negative (DMSO) controls. After proper diffusion of extract into the media the plates were incubated for 24hrs at 37^{o}C in upright position and zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration of *A.philoxeroides* leaf methanol extract was determined by microtitre plate assay. 100µl of test material (10% w/v in DMSO) was added in the first row of 96 wells microtitre plate and other wells filled with 50µl of sterile Muller Hinton broth (Himedia, Mumbai, India). Transfer 50µl of test material to the next well so that all wells contain 50µl and for serial dilution. 10µl of respective bacterial suspension, MacFarland's standard (1x10^8 CFU/ml) and finally 30µl of resazurin (0.02% w/v) were added. The plates were covered and incubated at 37°C for 24 hours. The colour change from purple to pink indicated a positive response. The lowest concentration at which colour change was noted is denoted as minimum inhibitory concentration (MIC) values for the test material against bacterial strain. The results were compared with positive control (contains all

solutions except test compound) and negative control (contains all solutions except test compound and bacterial suspension)

RESULTS & DISCUSSION

Herbs have been a source of medicinal compounds since time immemorial. History of use of herbal medicine in the treatment of diseases can be identified with the history of medicine and with the history of civilization itself. All parts of plants were used in Ayurveda, Unani and Homeopathic systems of medicine for the treatment of number of human diseases such as wound infections, typhoid, dysentery, ulcers, cough, urinary tract infections and a number of skin diseases. They were also used to treat bacterial, fungal and viral diseases. However studies with reference to their specific antibacterial activity had been done to negligible extent.

The medicinal value of plants depends on the presence of phytoconstituents. The methanolic leaf extract of *Alternanthera philoxeroides* showed the presence of carbohydrates, aminoacids, proteins, cardiac glycosides, alkaloids, flavonoids, tannins and phenolics compounds. The results of preliminary phytochemical screening was reported in table:1.

The quantitative determination of total phenolics content, non-tannin, tannin and flavonoid contents. The presence of tannins and flavonoids in the plants exhibited various biological activities like antibacterial, antifungal, antioxidant and anthelmintic. The methanol extract of *A.philoxeroides* possess tannin content 5.6 mg of GAE/gm of extract. The results of total phenolics, tannin and flavonoid contents were represented in table:2. The total phenolic content and tannin content were estimated through the standard calibration curve of gallic acid (Fig:1). The flavonoid content was estimated through the standard calibration curve of rutin (Fig:2). The methanol extract of *A.philoxeroides* possess flavonoid content 4.5 mg of rutin/gm of extract.

Biological screening of plant extracts are most frequently carried out as determination of antibacterial activity. These evaluations are done by means of standard *in vitro* assays (agar well diffusion) utilizing a broad spectrum of pathogenic bacteria. In the present study gram positive (*S.aureus, B.subtilis, B.megaterium, S.mutans, E.faecalis*), gram negative (*E.coli, K.pneumoniae, P.aeruginosa and P.vulgaris*) bacterial strains were used. These organisms are responsible for various minor or major infections in humans.

The results of inhibitory effect of methanolic leaf extract of *A.philoxeroides* were shown in table:3. The results showed that different bacterial species exhibited different sensitivities towards the extract. The antibacterial activity of tested methanol extract was compared with standard drug amikacin. The highest antibacterial activity was exhibited against *E.coli* (16±0.57mm), moderate activity against *S.aureus* and *P.vulgaris* (15±1.15mm) least activity was exhibited by *P.aeruginosa* (11±0.57mm). The MIC of methanolic leaf extract ranged from 50 to 12.5µg/ml. The results from MIC indicated that *S.aureus* and *E.coli* were the most sensitive bacteria to *A.philoxeroides* leaf extract, inhibited at lowest concentration of 12.5µg/ml.

Table-1: Preliminary phytochemical screening of leaves of *Alternanthera philoxeroides*

Name of the Test	Methanolic Extract
Carbohydrates	+
Protiens	+
Aminoacids	+
Steroids	+
Cardiac glycosides	+
Flavonoids	+
Alkaloids	+
Tannins & phenolic compounds	+

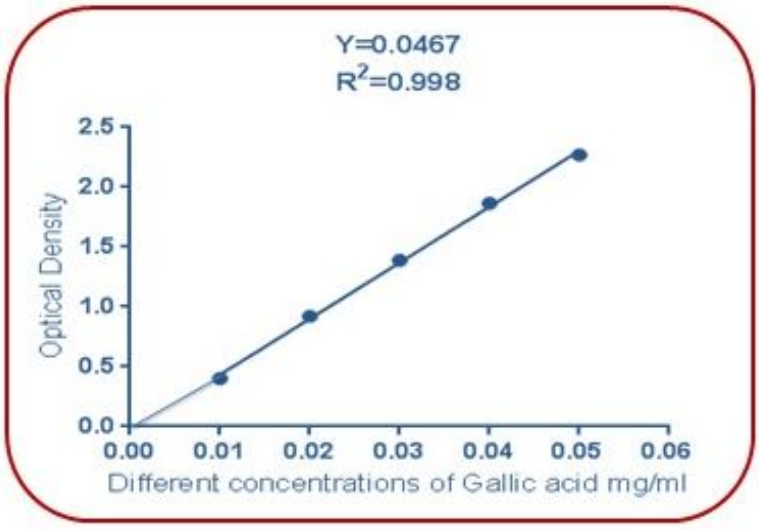


Figure-1: Standard curve of different concentrations (mg/ml) of Gallic acid and their respective optical density at 725nm

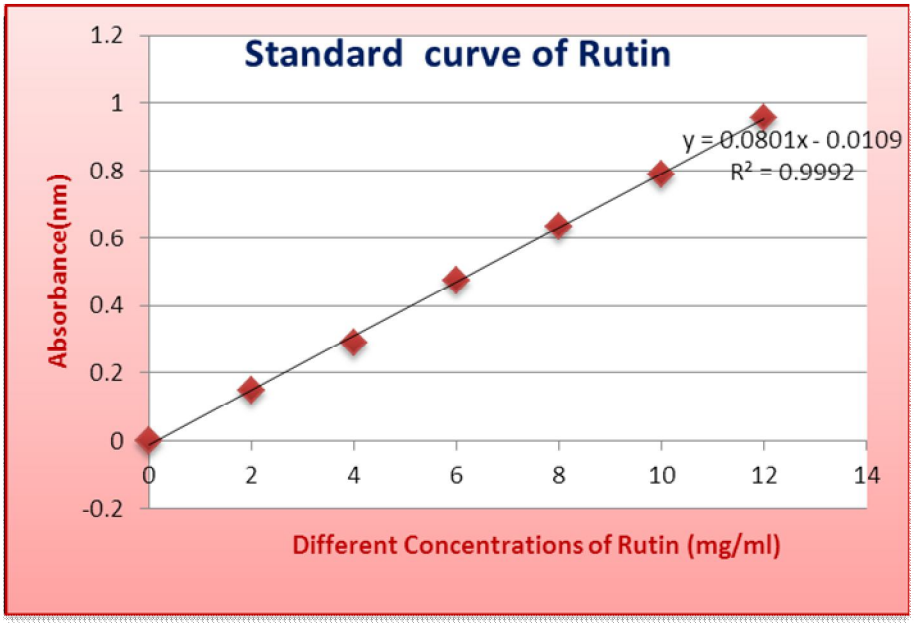


Figure-2: Standard curve of different concentrations (mg/ml) of Rutin and their respective optical density at 506nm

Table-2: Total phenolic, Non-tannin, Tannin & Flavonoid content present in methanolic leaf extract of *Alternanthera philoxeroides*

Parameter	Unit	Methanolic extract
Total phenolic content	mg of GAE/gm of extract	12.4
Non tannin content	mg of GAE/gm of extract	6.8
Tannin content	mg of GAE/gm of extract	5.6
Flavonoid content	mg of rutin/gm of extract	4.5

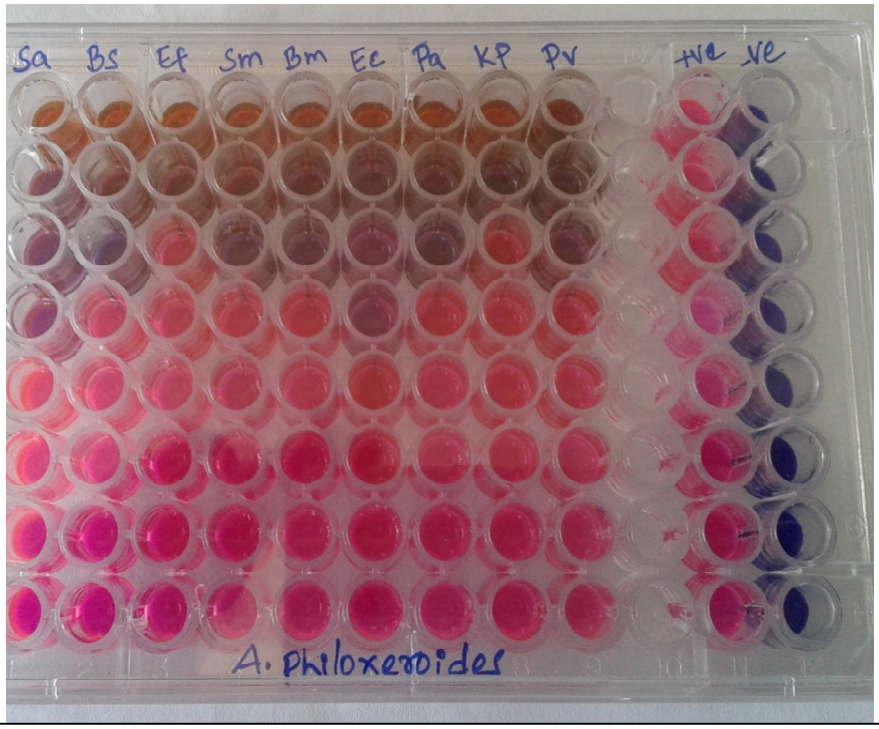
Table-3: Antibacterial activity of methanolic leaf extract of *Alternanthera philoxeroides*

Name of the organism	Diameter of zone of inhibition in mm	
	Methanolic Extract 500µg/ml	Amoxicillin 100µg/ml
S.aureus	15±1.15	23±0.57
B.subtilis	13±0.57	17±0.57
E.faecalis	14±0.57	19±0.57
B. megaterium	12±0.57	16±1.15
S.mutans	12±0.57	17±0.57
E.coli	16±0.57	24±0.57
P. vulgaris	15±1.15	21±0.57
P.aeruginosa	11±0.57	17±1.15
K.pneumoniae	14±1.15	20±0.57

Values are mean ± SD of triplicates

Table-4: Minimum inhibitory concentration of methanolic leaf extract of *Alternanthera philoxeroides*

Name of the Organism	Minimum Inhibitory Concentration (MIC) (µg/ml)
S.aureus	12.5
B.subtilis	25
E.faecalis	50
B. megaterium	25
S.mutans	25
E.coli	12.5
P. vulgaris	25
P.aeruginosa	25
K.pneumoniae	50



Sa - *S.aureus,* Bs - *B.subtilis,* Ef - *E.faecalis,* Sm - *S.mutans,* Bm- *B.megaterium,* Ec - *E.coli,* Pa - *P.aeruginosa,* Kp - *K.pneumoniae,* Pv - *P.vulgaris*

Figure-3: Minimum inhibitory concentration of methanolic leaf extract of *Alternanthera philoxeroides*

CONCLUSION

The present study suggested that, the crude methanolic leaf extract of *A.philoxeroides* have a great potential as antimicrobial agent against tested pathogenic bacteria and it can be used as an alternative medicine in the treatment of infectious diseases. The results of this study have shown that the plant is potentially a good source of antibacterial agent.

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