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IN-VITRO ANTIBACTERIAL POTENTIAL OF *ALTERNANTHERA PHILOXEROIDES* (MART) GRISEB AGAINST MULTI-DRUG RESISTANT UROPATHOGENS

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Keywords:

Alternanthera philoxeroides, Urinary tract infection (UTI), Clinical isolates, Antibacterial activity

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ABSTRACT: Antimicrobial drug resistance is one of the major threats due to the wide usage of broad-spectrum antimicrobial drugs in the general population. The present study was undertaken to investigate the antibacterial potential of *Alternanthera philoxeroides* methanolic extract (APME) against multidrug-resistant UTI pathogens. The preliminary phytochemical screening of the extract showed the presence of carbohydrates, amino acids, proteins, alkaloids, glycosides, steroids, flavonoids, tannins, and phenolic compounds. The quantitative estimation of total phenolic, tannin, and flavonoid contents was determined for the crude extract of APME through standard curves of gallic acid and rutin. The phenolic content of APME was found to be 12.4 mg of gallic acid equivalent (GAE)/g of extract. The tannin content was found to be 5.6 mg of GAE/g of extract, whereas the flavonoid content was found to be 3.2 mg of rutin/g of extract. The antibacterial potential of the extract was observed as maximum against *Staphylococcus saprophyticus*, moderate against *Escherichia coli* and *Proteus vulgaris*, and minimum against *Proteus mirabilis*. The minimum inhibitory concentration was ranged from 12.5 to 25 mg/ml. The *Alternanthera philoxeroides* were found to contain some bioactive compounds with pronounced antibacterial activity, therefore further phytochemical studies and their characterization should be needed to isolate the active constituents and evaluate the antimicrobial activities against a wide range of microbial pathogens.

INTRODUCTION: Infectious diseases caused by pathogenic microorganisms are a major threat to public health worldwide [1]. Urinary tract infection is one of the most common bacterial infections in community practice. In the present scenario, the antimicrobial resistance of major uropathogens is on rise.

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Hence, the treatment of UTI infections became difficult to even several potent antibiotics are available. The uncontrolled and improper use of multiple antibiotics results in the development of antimicrobial resistance. Several herbal extracts were reported for their antimicrobial activity [2]. Many plants have been found to be effective against respiratory, gastrointestinal, cutaneous, and urinary tract infections. Plants are a rich source of secondary metabolites such as alkaloids, tannins, and flavonoids, which have been found to possess antimicrobial properties [3].

The phenomenon of antibiotic resistance exhibited by the pathogenic microorganisms has led to

screening several medicinal plants for their potential antimicrobial activity. In view of a growing problem with drug resistance of UTIs, the present study was undertaken with an objective to find more efficient antibacterial agents of plant origin and selected a green leafy vegetable *Alternanthera philoxeroides* (Mart.) Griseb.

FIG. 1: *ALTERNANTHERA PHILOXEROIDES* (MART.) GRISEB

Scientific Classification:

Kingdom	:	Plantae
Phylum	:	Spermatophyta
Subphylum	:	Angiospermae
Class	:	Dicotyledonae
Order	:	Caryophyllales
Family	:	Amaranthaceae
Genus	:	Alternanthera
Species	:	Philoxeroides
Binomial name	:	*Alternanthera philoxeroides* (Mart.) Griseb

Alternanthera philoxeroides (Mart.) Griseb is commonly known as alligator weed, pigweed **Fig. 1**. The synonyms of the plant include *Achyranthes philoxeroides* (Mart.) Standl., *Telanthera philoxeroides* (Mart.) Moq. In Telugu, it is known as "ponnaganti'. It can often be identified by its fleshy stems and white flowers.

Traditionally, the plant possesses various curative properties. Young shoot paste with black pepper is prescribed to cure acute cough. Leaves with a pinch of salt are orally administered to cure intestinal worms. It is also used as an aquarium plant.

The reported phytoconstituents of the plant include phaeophytin a, phaeophytin a', oleanolic acid, β-sitosterol, 3β-hydroxystigmast-5-en-7-one, α-spinasterol, 24-methylene cycloartanol, cycloeucalenol and phytol [4]. The antitumor compounds alternanthin B and N-trans-feruloyl-3, 5-dimethoxytyramine has been isolated from aerial parts of *A. philoxeroides* [5].

Scientifically, the plant was investigated for *in-vitro* inhibitory effect against dengue virus, [6] the whole plant methanolic extract for anti-nociceptive and anti-hyperglycemic effects, [7] methanolic extracts of leaf for phytochemical analysis and antibacterial efficacy,[8] ethanolic extracts of leaf for antimicrobial efficacy against various gram-positive, gram-negative bacteria and fungi [9].

Herein, we report the *in-vitro* antibacterial potential of *Alternanthera philoxeroides* (Mart.) Griseb against multidrug-resistant Uropathogens.

MATERIALS:

Plant Material: The plant *Alternanthera philoxeroides* were collected from fields in and around Guntur, Andhra Pradesh, India. The taxonomic identity was confirmed at the Botanical Survey of India, Ministry of Environment & Forests, Government of India, Southern Regional Centre, Coimbatore (Ref No. BSI/SRC/5/23/2013-14/Tech./1990).

Microbial Strains: Clinical strains of *Staphylococcus aureus, Staphylococcus saprophyticus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris* and *Proteus mirabilis* were procured from microbiology laboratory.

These microorganisms were isolated from urine samples of UTI infected patients. The number of isolates for each microorganism was mentioned in **Table 1**.

TABLE 1: LIST OF CLINICAL ISOLATES OF MICRO-ORGANISMS USED IN THE STUDY

S. no.	Name of the microorganism	No. of isolates
1	*Staphylococcus aureus*	13
2	*Staphylococcus saprophyticus*	14
3	*Enterococcus faecalis*	13
4	*Escherichia coli*	19
5	*Klebsiella pneumoniae*	18
6	*Pseudomonas aeruginosa*	19
7	*Proteus vulgaris*	11
8	*Proteus mirabilis*	12
	Total	119

EXPERIMENTAL METHODS:

Preparation of Plant Extract: The healthy leaves were shade dried and powdered to a coarse powder. The phytoconstituents present in the leaves were extracted by cold maceration process [10]. The powder of 1 kg was suspended in 1 L methanol and incubated in an orbital shaker for 3 days. The extract was filtered through 5 layers of muslin cloth, and the process was repeated twice. The collected extract was pooled, concentrated by evaporation, and labeled as APME.

Preliminary Phytochemical Screening: The preliminary phytochemical screening for APME 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 Optical Density against a concentration of tannins at 725 nm was estimated spectrophotometrically. Gallic acid was used as a standard and the total phenolic content was expressed as µg/ml GAE. Concentration 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 0.5 ml of each sample were introduced into test tubes and mixed with 0.5ml of a 1N dilute Folin-Ciocalteau reagent and 2.5 ml of 20% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 40 min at room temperature, and OD was read at 725 nm spectrophotometrically [13].

Determination of Tannin Content: Tannin content was determined using insoluble polyvinyl-polypyrrolidone (PVPP), which binds tannins [14]. Briefly 1 ml of extract (1 mg/ml) in which the total phenolics was determined was mixed with 100 mg of PVPP, vortexed, kept for 15 min at 4 °C and then centrifuged. 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: 0.5 mL of diluted extracts were added to test tubes and mixed with 0.1 ml of 10% ammonium nitrite, 0.1 ml of 1 M aqueous potassium acetate and 4.3 ml of methanol. After incubating for 40 min at room temperature, the absorbance was measured at 415 nm. Rutin was used as a standard compound in the range of 2-12 mg/ml concentration to construct a standard curve.

Determination of Sensitivity of Strains to Antibiotics: [15] The susceptibilities of the microbial strains to different antibiotics were determined by resazurin microtitre assay plate method (REMA). Antibacterial agents from different classes of antibiotics were used, which included ampicillin, amoxyclav, cefalexin, cephadroxil, ciprofloxacin, levofloxacin, tetracycline, doxycycline, clotrima-xazole, amikacin, gentamycin, erythromycin, azithromycin, nitrofurantoin.

The test antibiotic (1% w/v) of 100 µL was added in the first row wells and other wells filled with 50 µL of sterile nutrient broth medium. The antibiotic solution was serially diluted by transferring 50 µL to the next well and so on so that all wells contained 50 µL. Then 10 µL of respective bacterial suspension and finally 30 µL of resazurin (0.02%) were added. The experiment was set with positive control a column with all the solutions except antibiotic and negative control a column with sterile nutrient broth except antibiotic solution and bacterial suspension. The plates were covered and incubated at 37 °C for 24 h. After incubation observes the color change as from blue to pink indicates a positive response. Resazurin, which is blue in the oxidized state, turns pink when reduced by viable cells.

The least concentration where the colour change was observed was noted as the inhibitory concentration of antibiotics with respect to the bacterial strain and is represented as drug resistance concentration exhibited by microbial strain.

Agar Well Diffusion Assay: The antibacterial screening of APME was tested using agar well diffusion on solid media [16, 17]. The inoculums (1 × 10^8 CFU/ml) were spread on Mueller Hinton agar medium using sterile swabs. The inoculated agar medium was punched with 6 mm diameter wells. The wells were filled with different concentrations of the extract (500, 750, 1000 µg/ml). The activity was compared with positive (Nitrofurantoin, 300 µg/disc) and negative (DMSO) controls. The plates were incubated in an upright position at 37 °C for 24 h. After incubation, the zone of growth inhibition was measured.

Determination of Minimum Inhibitory Concentration (MIC) by Micro-Broth Dilution: The minimum inhibitory concentrations (MIC) of extracts were determined by the micro-broth dilution method using resazurin indicator [18, 19]. The extract of 100 µL (10% w/v) was added in the first row wells and other wells filled with 50 µL of sterile nutrient broth. Transfer 50 µL of test material to the next well so that all wells contain 50 µL and for serial dilution. Then 10 µL of respective bacterial suspension (1 × 10^8 CFU/mL) and 30 µL of resazurin (0.02%) were added. The plates were covered and incubated at 37 °C for 24 h.

RESULTS:

TABLE 2: RELIMINARY PHYTOCHEMICAL SCREENING OF APME

Name of the test	APME
Alkaloids	+
Glycosides	+
Aminoacids	+
Proteins	+
Carbohydrates	+
Steroids	+
Flavonoids	+
Tannins & Phenolic compounds	+

"+" indicates positive

TABLE 3: TOTAL PHENOLIC, NON-TANNIN, TANNIN AND FLAVONOID CONTENTS PRESENT IN APME

Parameter	Unit	APME
Total phenolic content	mg of GAE/g of extract	12.4
Non-tannin content	mg of GAE/g of extract	6.8
Tannin content	mg of GAE/g of extract	5.6
Flavonoid	mg of rutin eqvt./g of extract	3.2

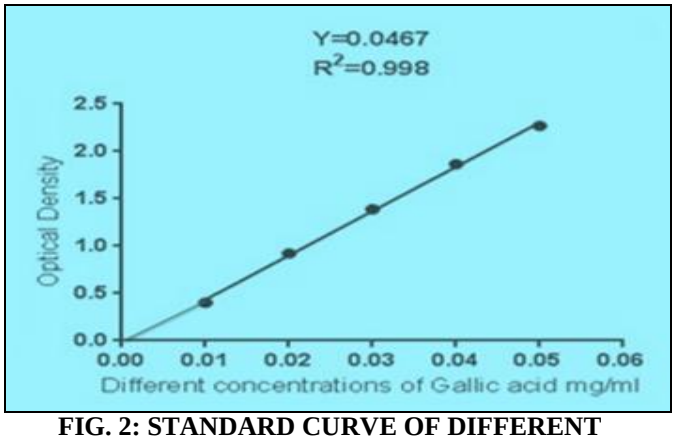

FIG. 2: STANDARD CURVE OF DIFFERENT CONCENTRATIONS (mg/mL) OF GALLIC ACID AND THEIR RESPECTIVE OPTICAL DENSITY AT 725 nm

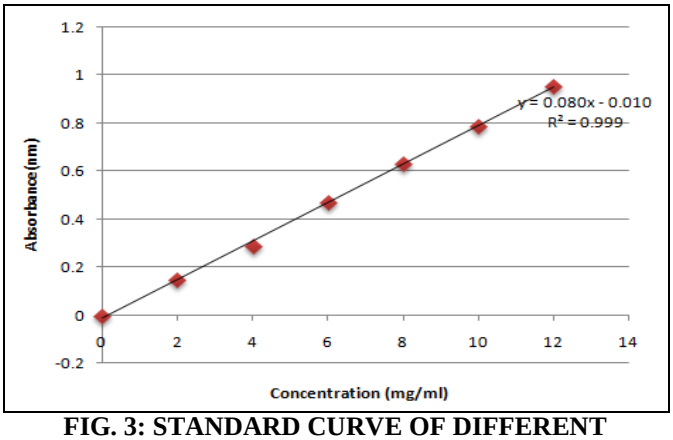

FIG. 3: STANDARD CURVE OF DIFFERENT CONCENTRATIONS (mg/mL) OF RUTIN AND THEIR RESPECTIVE OPTICAL DENSITY AT 506 nm

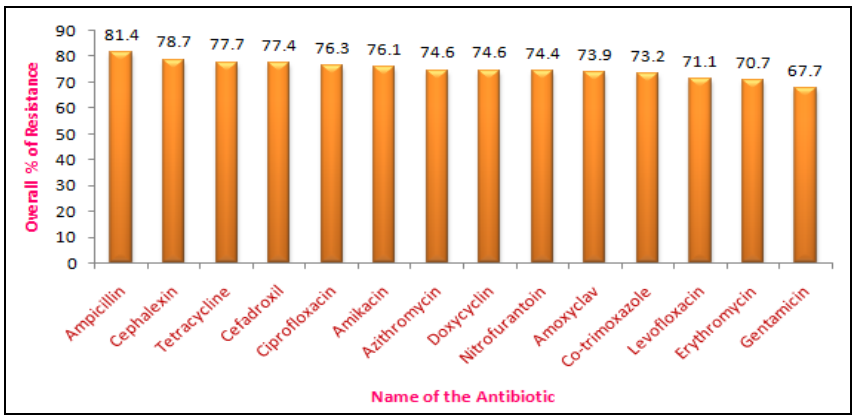

FIG. 4: OVERALL % OF ANTIBIOTIC RESISTANCE PATTERNS OF CLINICAL ISOLATES OF UTI PATHOGENS

TABLE 4: ANTIBACTERIAL SCREENING OF APME AGAINST CLINICAL ISOLATES OF UTI PATHOGENS

Name of the organism	Diameter of Zone of inhibition in mm				
	500 µg	750 µg	1000 µg	NF 300 µg	DMSO
S. aureus	-	14.33 ± 0.57	18.33 ± 0.57	14.66 ± 0.57	-
S. saprophyticus	-	15.33 ± 0.57	19.33 ± 0.57	13.66 ± 0.57	-
E. faecalis	-	10.66 ± 0.57	14.33 ± 0.57	13.33 ± 0.57	-
E. coli	8.33 ± 0.57	12.66 ± 0.57	15.33 ± 1.52	12.66 ± 0.57	-
K. pneumoniae	-	10.66 ± 0.57	16.33 ± 0.57	13.66 ± 0.57	-
P. aeruginosa	8.33 ± 0.57	12.66 ± 0.57	14.66 ± 0.57	12.33 ± 0.57	-
P. vulgaris	-	11.66 ± 0.57	15.33 ± 0.57	14.33 ± 0.57	-
P. mirabilis	-	8.33 ± 0.57	11.33 ± 0.57	13.33 ± 0.57	-

Results are mean ± SD of triplicate analysis

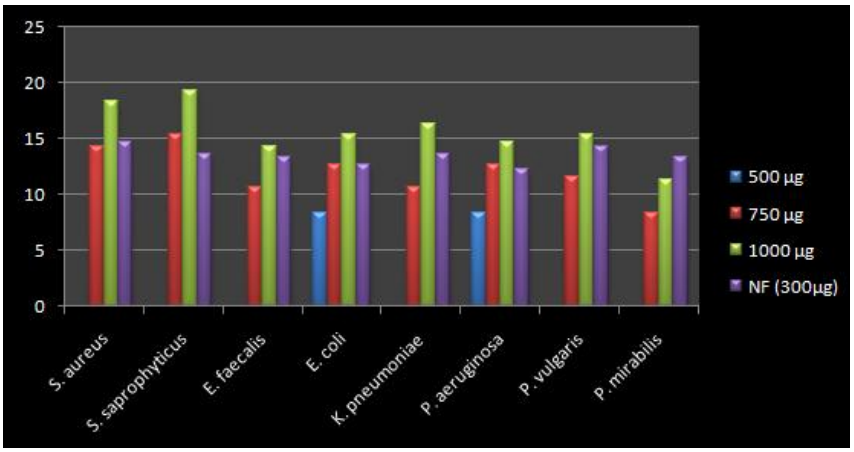

FIG. 5: ANTIBACTERIAL SCREENING OF APME AND NF AGAINST CLINICAL ISOLATES OF UTI PATHOGENS

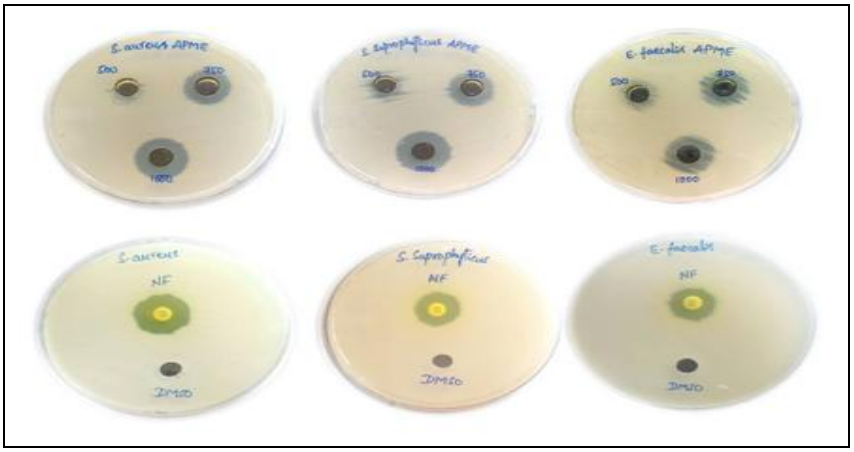

FIG. 6: ANTIBACTERIAL POTENCY OF ATME, NF & DMSO AGAINST *S. AUREUS, S. SAPROPHYTICUS* & *E. FAECALIS*

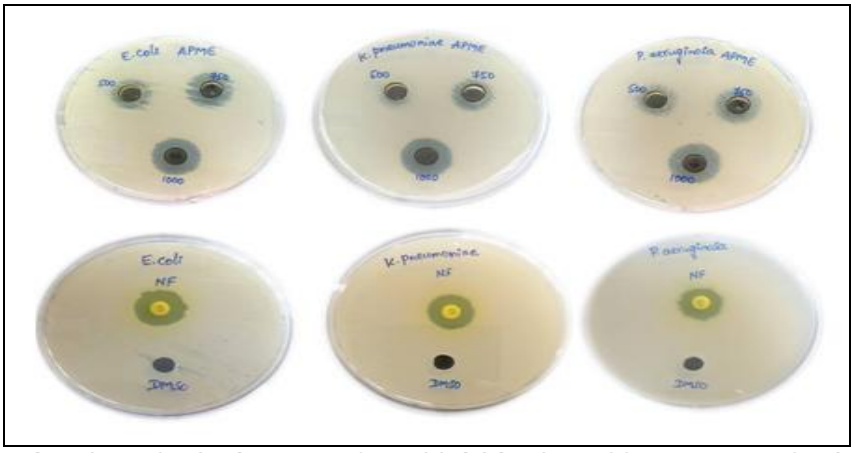

FIG. 7: ANTIBACTERIAL POTENCY OF ATME, NF & DMSO AGAINST *E. COLI, K. PNEUMONIAE* & *P. AERUGINOSA*

FIG. 8: ANTIBACTERIAL POTENCY OF ATME, NF & DMSO AGAINST *P. VULGARIS* & *P. MIRABILIS*

TABLE 5: MINIMUM INHIBITORY CONCENTRATION OF APME AGAINST CLINICAL ISOLATES OF UTI PATHOGENS (µg/mL)

Name of the Microorganism	APME
S. aureus	25
S. saprophyticus	12.5
E. faecalis	12.5
E. coli	25
K. pneumoniae	25
P. aeruginosa	25
P. vulgaris	25
P. mirabilis	25

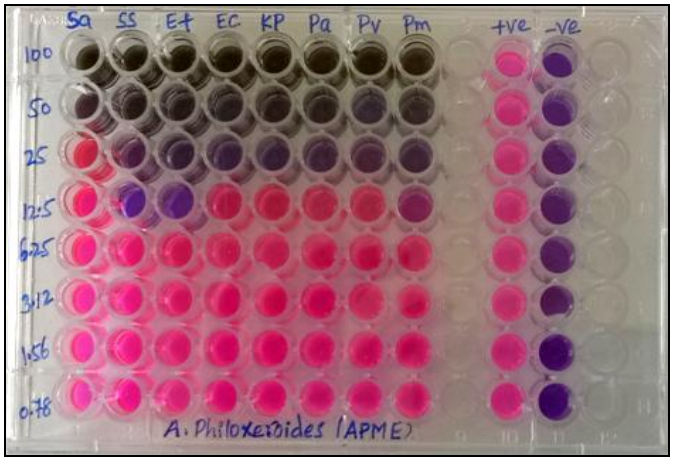

FIG. 9: MINIMUM INHIBITORY CONCENTRATION OF APME AGAINST CLINICAL ISOLATES OF UTI PATHOGENS (µg/mL)

DISCUSSION: The preliminary phytochemical screening of APME revealed the presence of various constituents. The extract showed the presence of carbohydrates, amino acids, proteins, alkaloids, glycosides, steroids, flavonoids, tannins, and phenolic compounds and they were shown in **Table 2**.

The quantitative estimation of total phenolic, tannin, and flavonoid contents was determined for APME through standard curves of gallic acid and rutin.

The phenolic content of APME was found 12.4 mg of gallic acid equivalent (GAE)/g of extract, the tannin content was found to be 5.6 mg of GAE/g of extract and the flavonoid content was found to be 3.2 mg of rutin/g of extract **Table 3, Fig. 2, 3**.

The significant findings of the study on the percentage of resistance pattern showed by all the isolates high resistance towards ampicillin (81.4%), moderate resistance to azithromycin, doxycycline (74.6%) & nitrofurantoin (74.4%) and low resistance to gentamicin (67.7%). The overall % of antibiotic resistance patterns of clinical isolates of UTI pathogens were represented in **Fig. 4**.

These findings demonstrate the importance of using more modern broad-spectrum antibiotics to treat urinary tract infections. Promoting the rational use of antibiotics to treat UTI's may be useful to reduce the emergence of multidrug-resistant strains.

The crude extract APME showed potent antibacterial efficacy against tested multidrug-resistant uropathogens. The inhibition rate of APME at the highest concentration (1000 µg/ml) was found to be *S. saprophyticus* > *S. aureus* > *K. pneumoniae* > *E. coli* & *P. vulgaris* > *E. faecalis* & *P. aeruginosa* > *P. mirabilis*.

The results obtained were comparable with zones of inhibition produced by standard drug, Nitrofurantoin (300 µg/disc), which is a drug of choice for urinary tract infections. It showed high inhibition against *S. aureus, P. vulgaris* > *S. saprophyticus, K. pneumoniae* > *E.f aecalis* & *P. mirabilis* > *P. aeruginosa* and *E. coli*. The results were reported in **Table 4, Fig. 5, 6, 7** and **8**.

The Minimum Inhibitory Concentration (MIC) values of APME were noted between 12.5 to 25 mg/ml. It was observed that *S. saprophyticus, E. faecalis* was inhibited 12.5 mg/ml whereas *S. aureus, E. coli, K. pneumoniae, P. aeruginosa, P. vulgaris* and *P. mirabilis* at 25 mg/ml. The results were reported in **Table 5** and **Fig. 9**.

CONCLUSION: The present study concludes that the APME showed potent antibacterial efficacy against one or more clinical isolates of UTI

pathogens. The plant requires extensive investigation to identify the antibacterial principles which may act as accessible alternatives to synthetic antibiotics.

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CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Shuman EK: Global climate change and infectious diseases. N Engl J Med 2010; 362: 1061-63.
2. Islam B, Khan SN, Haque I, Alam M, Mushfiq M and Khan AU: Novel anti-adherence activity of mulberry leaves: inhibition of *Streptococcus mutans* biofilm by 1-deoxynojirimycin isolated from *Morus alba*. J Antimicrob Chemother 2008; 62(4): 751-57.
3. Lewis K and Ausubel FM: Prospects of plant derived antibacterials Nat. Biotechnol 2006; 24: 1504-07.
4. Fang JB, Duan HQ, Zhang YW and Yoshihisa T: Chemical constituents from herb of *Alternanthera philoxeroides*. China Journal of Chinese Material Medica 2006; 31: 1072-75.
5. Fang JB, Jia W, Gao WY, Yao Z, Teng J and Zhao AH: Antitumour constituents from *Alternanthera philoxeroides*. J. Asian Nat. Prod. Res 2007; 9: 511-15.
6. Jiang WL, Luo XL and Kuang SJ: Effects of *Alternanthera philoxeroides* Griseb against dengue virus *in-vitro*. Academic Journal of First Medical College of PLA 2005; 25: 454-56.
7. Khatun F, Zaman F, Mosaiab F, Zaman M, Rehana F, Nasrin D, Jamal F, Nahar N and Rahmatullah M: Evaluation of antinociceptive and antihyperglycemic activities in methanol extracts of whole plants of *Alternanthera philoxeroides* (Mart.) Griseb. (Amarantheceae) in mice. Pak. J. Pharm. Sci 2012; 25(3): 583-87.
8. Pulipati S, Babu PS and Devi BS: Preliminary Phytochemical and antibacterial investigations of the methanol extract of *Alternanthera philoxeroides* (Mart.) Griseb. European Journal of Pharmaceutical and Medical Research 2015; 2(4): 347-56.
9. Pulipati S, Babu PS, Sree NB, Kumar UE, Shaheela S, Krishna JM and Chakradhar T: Phytochemical analysis and antimicrobial investigations of ethanolic leaf extract of *Alternanthera philoxeroides* (Mart.) Griseb. World Journal of Pharmacy and Pharma Sciences 2015; 5(2): 1122-29.
10. Handa SS, Khanuja SPS, Longo G and Rakesh DD: Extraction Technologies for Medicinal and Aromatic plants, Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology 2008; 1(66): 83-88.
11. Evans WC: Trease and Evans Pharmacognosy. Elsevier Pub., New Delhi, India 2006; 15: 538-47.
12. Kokate CK, Purohit AP and Gokhale SB: Pharmacognosy 1997; 7: 105-44.
13. Koleckar V, Kubikova K, Rehakova Z, Kuca K, Jun D and Jahodar L: Condensed and hydrolysable tannins as antioxidants influencing the health. Mini Reviews in Medicinal Chemistry 2008; 8: 436-47.
14. Makkar HPS, Bluemmel M, Borowy NK and Becker K: Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. Journal of Science Food Agriculture 1993; 61: 161-65.
15. Mezger A, Gullberg E, Goransson J, Zorzet A, Herthnek D, Tano E, Nilsson M and Andersson DI: A general method for rapid determination of antibiotic susceptibility and species in bacterial infections 2015; 53(2): 425-32.
16. Valgas C, DeSouza SM and Smania EFA: Screening methods to determine antibacterial activity of natural products, Braz J Microbiol 2007; 38: 369-80.
17. Mishra MP, Rath S, Swain SS, Ghosh G, Das D and Padhy RN: *In-vitro* antibacterial activity of crude extracts of 9 selected medicinal plants against UTI causing MDR bacteria. Journal of King Saud University-Science 2015; 29(1): 84-95.
18. Ngemenya MN, Djeukem GGR and Nyongbela KD: Microbial, phytochemical, toxicity analyses and antibacterial activity against multidrug resistant bacteria of some traditional remedies sold in Buea Southwest Cameroon. BMC Complement Altern Med 2019; 19: 150.
19. Jorgensen JH and Turnidge JD: Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, eds. Manual of clinical microbiology, Washington, DC: American Society for Microbiology 2007; 9: 1152-72.

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