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ANTIBACTERIAL ACTIVITY OF TRIBULUS TERRESTRIS L. AGAINST CLINICAL PATHOGENIC BACTERIA

Umadevi V[1], Arjun N. Shetty[1, 2] and Srinath Rao*[1]

[1]Department of P. G. Studies and Research in Botany, Gulbarga University, Kalaburagi. Karnataka, India.
[2]Department of P. G. Studies in Botany, Sharnbasva University, Kalaburagi, Karnataka, India.

***Corresponding Author Email: srinathraomm@gmail.com**

ABSTRACT

The microbial infections are growing at an alarming rate. The discovery and exploration of antibiotic is a continuous process. There is an urgent need to curb the growing bacterial infections. Now a days the emerging antibiotic resistant strains are threat to mankind. The phytochemicals and secondary metabolites explored from plants have immense therapeutical and pharmaceutical properties. Tibullus terrestris is a member of family Zygophyllaceae. It is a low trailing prostrate annual herb. Each part of the plant is enriched with diverse phytochemicals and is a rich repository for phytochemicals such as Saponins and Flavonoids. These secondary molecules act against different gram positive and gram-negative bacteria. In the present study different solvent (Methanol, Chloroform and Hexane) extract of the plant was evaluated against different clinical isolates such as Pseudomonas aerogenes, Klebsiella sp. and Escherichia coli. Antibacterial activity of the plant was carried out by agar diffusion method and the same was determined by measuring the zone of inhibition (mm) according to the standard clinical parameters. In our study the methanolic extract (500 µg) of plant found to be effective against all the tested organisms.

KEY WORDS

Tibullus terrestris, Methanol extract, Antibacterial activity, Phytochemicals

INTRODUCTION

People have been using plants as a traditional medicine from ancient days. Plants are the rich sources of different phytochemicals. A significant number of developing countries depend on plants to treat themselves against a variety of diseases. Throughout the world, human population has been using the knowledge of medicinal plants to fight diseases. Africans have been using natural therapies based on herbal medicine from long time [5]. Medicinal plants play a very prominent role in curing number of diseases and are also used as a supplement in case of deficiencies. The knowledge about the medicinal plant was known from ancient period. Importance of the medicinal herbs was cited in number of religious books. In Ayurveda Indian saints have mentioned about the different medicinal plants and their pharmacological properties in detail. Charka Samhita and Sushruta Samhita described the importance of 700 plants as herbal medicines. Plants have several pharmacological roles such as antioxidant, antiviral, anticancer, antimicrobial, antifungal and anti-parasitic [6]. In India, plants of therapeutic potential are widely used by all sections of people both as folk medicines in different indigenous systems of medicine like Siddha, Ayurveda, and Unani and also as processed product of pharmaceutical industry [15].

T. terrestris is a procumbent herb, distributed throughout India up to an elevation of 3600-meter altitudes very common in the upper Gangetic plain in wastelands, fields and sport grounds. The habitat includes disturbed places, road sides, railway, cultivated

field, waste places and walk ways [9]. The plant is a low trailing prostrate annual herb reaches the length of 4 to 5 feet. The shrub is annual or perennial and thrives in moist soils. It contains biologically active substances as steroids, saponins, flavonoids, alkaloids, unsaturated fatty acids, vitamins, tannins [2], glycoconjugates, oil and calcium [14]. The plant also has small amounts of alkaloid, tannin, potassium salts, cinnamicamide, resins and sugars [3] [4]. Production of the antibiotic from different plant extracts gaining much importance now a day due to emergent plant and animal diseases caused by different bacteria and viruses. Nosocomial and resistant pathogens are great threat to mankind. The researchers are in continuous effort to curb the resistant pathogens. The phytochemicals such as saponins and flavonoids produced by plant found to possess antibiotic activity. These compounds act against different gram positive and gram-negative bacteria. Keeping in mind the pharmacological property of the herb *T.terrestris* the antibacterial activity of the plant was assessed.

MATERIAL AND METHODS:

Plant material

T. terrestris leaf sample was collected from garden of Gulbarga University, Kalburgi. The leaves were washed with alcohol and then washed with tap water and later with deionised water to remove solid adherent dirt particles, then they were dried under shade and were ground to powder using house hold electric blender. Precisely 20 g of dried leaf powder was extracted in soxhlet apparatus using different solvents viz., Hexane, Chloroform and Methanol. Successive soxhlet extraction method was followed by knowing the polarity index of each solvents i.e., extraction of leaf powder initially done with the solvent hexane and the powder which is remained after hexane extraction is used for chloroform and then methanol extraction.

Chemicals:

All the chemicals and reagents used in the present study are of analytical grade Methanol, Chloroform, Peptone, Agar, NaCl and Beef Extract procured from Himedia Pvt. Ltd., India.

Microbial isolates

Clinical isolate used in the present study are *Pseudomonas aerogenes, Klebsiella* sp. and *Escherichia coli.* The bacterial isolates were cultured on nutrient agar a night before the experiment and they were incubated at 37°C. 1-2 colonies of bacteria were suspended in normal saline and their turbidities were adjusted to 0.5 McFarland (1×108 CFU/ml). All the bacterial cultures were obtained from Department of Microbiology, Gulbarga University, Kalburgi, Karnataka, India. 1.0 ml of bacterial suspension was aseptically inoculated in autoclaved bottle containing 100 ml nutrient broth (NB) medium and incubated for overnight on orbital shaker. Next day each microorganism's cultures were dispensed into 20 mL of nutrient broth and incubated at 37 ºC for 4-8 h or till culture reaches optical density 0.6-0.8. A loop full of the culture was used for the antimicrobial assay. Nutrient Agar inoculated with the isolate was incubated at 37 °C overnight. The colonies with characteristic growth were subjected to routine biochemical test according to the Bergey's manual of systematic bacteriology [8].

Antibacterial Testing by Agar Well Diffusion Method

Preliminary evaluation of the antibacterial activity of the extracts was determined by agar well diffusion method. Inoculums of each bacterial strains were plated using sterile swabs into Petri dishes containing approximately 25 ml of Nutrient agar, where 6 mm wells were made and filled with different concentration (30 and 50 µl) of Methanol, Chloroform and Hexane extracts. The Petri dishes were pre-incubated for 3 h at room temperature, allowing the complete diffusion of the samples and then, incubated at 37±1^{0}C for 24 h. Further the antibacterial activity of the above samples was determined by measuring the zone of inhibition (mm) according to the standard clinical parameters. Each test was carried out in triplicate.

RESULTS AND DISCUSSION

The antibacterial activity of leaf extracts of plant with all the three solvents was evaluated against clinical bacterial pathogens. Among three solvents used for antibacterial assay, hexane extract did not show zone inhibition in all the concentrations tested. Maximum zone of inhibition is observed from methanolic extract of 50 µl (500 µg) against *E. coli, Pseudomonas* and *Klebsiella* sp with a zone of inhibition of 16, 22 and 17 mm respectively, which is almost near to the values of standard drug cefotaxin (CTX)**.** Chloroform extract had also showed zone of inhibition, however maximum activity is recorded from 50 µl (500 µg) against *E. coli* and *Pseudomonas* i.e., 09 and 18 mm respectively but

which was very less when compared to the activity shown by methanolic extract (Table: 1; Fig: 1).

Table 1: Antibacterial properties of Methanolic and Chloroform extract of *T. terrestris*

Sl. No.	Test organisms	Zone of Inhibition in mm					
		Std. (CTX 30 µg)	ME		Std. (CTX 20 µg)	CE	
			M_{50} (50 µl)	M_{30} (30 µl)		C_{50} (50 µl)	C_{30} (30 µl)
1	***E. coli***	24	16	14	11	09	07
2	***Pseudomonas***	25	22	17	10	18	13
3	***Klebsiella***	27	17	12	19	-	-

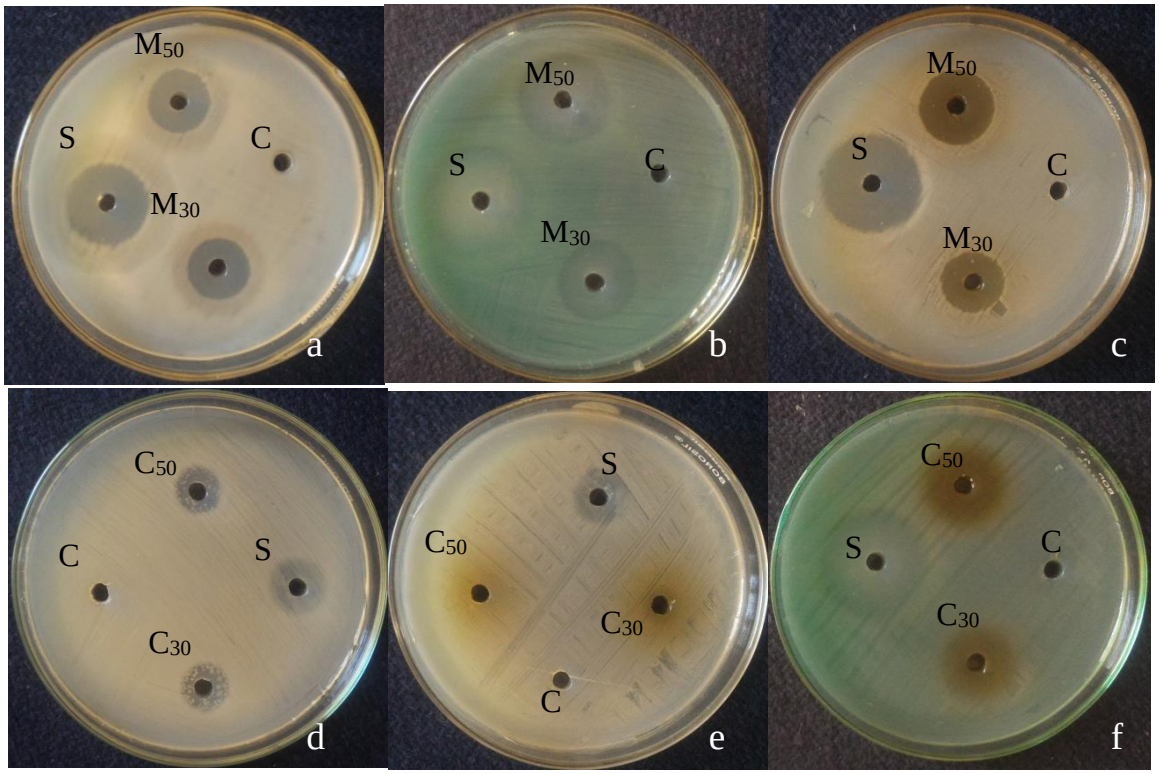

Figure 1: Antibacterial activity of Methanolic extract of.*T. terrestris* showing antibacterial activity against **a**. *E. coli*, **b**. *Pseudomonas aerogenosa* and **c**. *Klebsiella* sp. And Chloroform extract of.*T. terrestris* showing antibacterial activity against **d**. *E. coli*, **e**. *Klebsiella* sp and. **f**. *Pseudomonas aerogenosa*.

Where, chloroform extract at both the concentrations (30 and 50 µl) tested against *Klebsiella* had not shown beneficial effect. Similarly, many attempts were made other research groups on antimicrobial activity using different extracts of *T. terrestris*. Fazly Bazzaz reported that *T. terrestris* extract exhibited good antibacterial activity against *S. sanguis, A. viscosus,* and *E. faecalis* in agar dilution method. Studies conducted in Iraq Turkey and India showed that the *T.terrestris* extract has a good antibacterial activity against gram positive bacteria such as *S. aureus* and *E. faecalis* and gram negative bacteria such as *E. coli* [1 and 11]. Our results are in agreement with the results of Ranade et al., [13] and Kianbakht and Jahaniani, [10] and they have reported antibacterial activity of *T. terrestris* against human pathogenic bacteria i.e., ESβl producing *E. coli* strain and *Staphylococcus aureus*, *Enterococcus faecalis*, *E. coli, Pesudomonas aeroginosa* respectively. And also reported the beneficial effect of methanolic extract against all the tested bacterium than other solvent extract. Mohammed et al., [12] reported that the saponins extracted from *T. terrestris* had elevated

antimicrobial activity and it has shown inhibiting effect on both Gram positive and negative bacteria, indicating presence of broad-spectrum antibiotic compounds or simply general metabolic toxins in the plant. Antibacterial activity of the *T. terrestris* was attributed by the presence of saponins. Saponins have capacity to lyse the bacterial membranes. From our study it can be concluded that the plant extract can be used as a potent drug to bacterial infections. Phytochemicals present in the plant are responsible for its therapeutic activity. It also helps to discover novel class of antibiotics against bacterial infections.

CONCLUSION

The wonder medicinal plant *T. terrestris* is having a potent Antibacterial activity. The phytochemicals produced from the plants were used extensively in different pharmaceutical industries. In our study the antibacterial activity of the plant was carried out on different clinical pathogens such as *E. coli, Pseudomonas* and *Klebsiella* sp. From our study it can be concluded that the methanolic and Chloroform extract of 50 µl showed the maximum zone of 22 and 18 mm against *P. aerogenosa* respectively. Further studies are required to evaluate the specific compound which is responsible for the present activity and their effect on other pathogenic bacteria.

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***Corresponding Author:**
Srinath Rao*
Email: srinathraomm@gmail.com