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Extraction and Antibacterial Evaluation of Some Extracts of *Tridax procumbens*

Asmita D. Jadhav*, T.B. Sawant Mahesh and S. Chavadar

U.G. and P.G. Department of Microbiology Yashwantrao Chavan College of Science, Karad *Vidyanagar,* Karad- 415 124, India.

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Adverse effects of popular antibiotics and multidrug resistant strains of pathogens have lead rapid search for new antimicrobials. Traditional medicine is an important source of potentially useful new drugs. They could be the unique templates that could serve as a starting point for synthetic analogues. The medicinal properties of *Tridax procumbens* such as wound healing, immunomodulatory properties are well documented. However it is necessary to determine the role of this plant as an antimicrobial agent that also may be helpful in fast wound healing by controlling the pathogens. Successive solvent extraction procedure was utilized to obtain various extracts flowers and leaves of *T. procumbens.* These extracts were used to study their capacity to control bacterial agents causing urinary tract infections. Agar diffusion method was used in the study. Among the five compounds obtained in the extraction process, quarternary alkaloids were found to be effective, while polar compounds extracts showed poor activity at tested conditions. The determination of MIC and TA dictates the effectiveness of the extracts. Results of the present study indicate that *T. procumbens* and can be exploited for future antibacterial drugs.

Key words: *Tridax procumbens,* MIC, TA, antibacterial drug.

Tridax procumbens L (Asteraceae) is common grass native of tropical America and naturalized in tropical Africa, Australia and Asia including India[1]. The plant has been used as a traditional medicine. The juice of the leaves of this plant is used by villagers to arrest bleeding from cuts and bruises in animals. *T. procumbens* is known for its wound healing activities. Whole plants is made into paste and applied on fresh cuts [2]. Antidiabetic properties of the plants are also documented recently[3].

* To whom all correspondence should be addressed. E-mail: jadhavasmita19@yahoo.co.in

Bacteria such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella* spp. etc. are known to cause various infections in human and animals[4]. The present investigation is carried out to evaluate antimicrobial properties of *T. procumbens* (leaves and flowers) against these pathogens. Five different compounds obtained from leaves and flowers of *T. procumbens*. The study was carried out using agar diffusion (agar well) method.

MATERIAL AND METHODS

Plant material and extraction process

Different parts of *Tridax procumbens* (leaves and flowers) were collected in the month of August from southern parts of India (Karad,

Maharashtra). The selected plant parts of *T. procumbens* were weighed property and then subjected to successive solvent extraction process extraction[5].

After completion of the extraction process, five different compounds (polysacchrides, polar compounds , alkaloids, quarternary alkaloids and lipids) were obtained which were accurately weighed and reconstituted in suitable solvent system (polysacchrides, polar compounds, alkaloids, quarternary alkaloids in the sterile distilled water and lipids in methanol : distilled water [3:1]). The reconstituted solvents were filtered through the bacteriological filters and the filtrates were collected in sterile test tubes and stored at 4°C until further use.

Screening of antibacterial activity

Test pathogens: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*. And *Salmonella* spp. were obtained from Microbiology Department, Yashwantrao Chavan College of science, Karad & maintained on the suitable agar medium. Agar diffusion method[ref6] (agar well method), using nutrient agar plates, was performed for antibacterial screening. 0.1 ml of test inoculums was spread on agar plate, and on each plate three wells (cups) were prepared using a metal cork borer (11mm). For each pathogen two plates were used; that allows one well for each extract and one for solvent control. The wells were filled aseptically with 0.2 ml of the extract (10mg/ml) after which the plates were kept at 4°C for 1 hr. for diffusion of extract in the medium. The plates were then incubated at 37°C for 24 hrs. The results were recorded in terms of inhibition zone (IZ).

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined for each extracted compound showing antimicrobial activity against test pathogen. Broth micro dilution method[6] was followed for the determination of MIC values. The extracts were diluted as 0.2 mg/ml to 2.4 mg/ml, and one ml of each diluted extract was added in sterile nutrient broth (5ml) tube containing respective test pathogen. The tubes were incubated at 37°C for 24 hrs. The final results were noted by visual detection of turbidity (growth) in broth tubes. The MIC value of extract for respective test pathogen was taken as the lowest concentration of extract in the tube that showed no turbidity after incubation.

Determination Total Activity (TA)

Total activity is the volume at which test extract can be diluted with the ability to kill micro-organisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract and is expressed in ml/g of extract[7].

RESULTS AND DISCUSSION

Various extracts obtained from the leaves and flower of *T. procumbens* were tested against selected pathogens and were recorded in terms of inhibition zone (Table 1) and minimum inhibitory concentration (Table 2). All the plant extracts used

Table 1. Antimicrobial activity of the extracts of *T. procumbens*

Plant part	Extracts	Inhibition zone diameter in cm. (including 11 mm dia of cork borer.)				
		E.coli	*S. aureus*	*K. pneumoniae*	*P. aeruginosa*	*Salmonella spp.*
Flowers	Polysaccharides	26	12	28	-	22
	Alkaloids	30	-	23	-	15
	Quarternary alkaloids	40	38	29	37	30
	Polar comp.	20	22	12	-	-
	Lipids.	32	36	36	28	30
Leaves	Polysaccharides	-	23	18	12	18
	Alkaloids	18	19	18	18	14
	Quarternary alkaloids	38	28	29	24	25
	Polar comp.	18	19	-	21	-
	Lipids.	23	16	22	24	20

in this study possessed considerable antibacterial activity. The lipid and quarternary alkaloid of flower inhibited the all five microorganisms. The same results were observed for alkaloids, quarternary alkaloids and lipids of the leaves. Extracted polysaccharide of flower inhibited the *E.coli, S. aureus, K. pneumoniae* and *Salmonella spp.* while the polysaccharides of the leaves inhibited the test pathogens except *E. coli.*

In flowers, quarternary alkaloids showed the best antibacterial activity against *E. coli* where IZ of 40 mm, 41.58 ml/g TA and MIC value of 0.6 mg/ml was observed. *P. auroginasa* was found to be most resistance to the three extracts, except lipids and quarternary alkaloids that showed some antibacterial effect against the pathogen. *E. coli* and *K. pneumoniae* were inhibited by all the five extracts. For *S. aureus* the best antibacterial activity was showed by lipids where IZ of 36 mm., 8.07 ml/g TA and MIC value of 1.2 mg/ml and also by quarternary alkaloids where IZ of 38 mm, 20.79 ml/g TA, 1.2 mg/ml of MIC. Comparatively low antibacterial activity was observed for polysaccharide against *S. aureus* (IZ of 12 mm, TA 1.88 ml/g and MIC 1.8 mg/ml) and for polar compounds against *K. pneumoniae* (IZ 12 mm, Ta 0.96 ml/g, MIC 1.8 mg/ml). The lowest MIC value of 0.6 mg/ml was recorded in case of *E. coli* by lipid, alkaloids and quarternary alkaloids indicate significant antimicrobial potential of the extracts against the test organisms.

In the extracts of the leaves showed variable results than those of extracts obtained from the flowers. Alkaloids, quarternary alkaloids, lipids of leaves were found to be most potent inhibiting all the test pathogens. *E. coli* showed resistance to the polysaccharides, while it was greatly inhibited by quarternary alkaloids (IZ - 38 mm, TA – 105.85 ml/g, MIC - 0.2 mg/ml). For *S. aureus* the best antibacterial activity was showed by quarternary alkaloids with IZ of 28 mm., 52.92 ml/g TA and MIC value of 0.4 mg/ml. Comparatively low antibacterial activity was observed for polysaccharide against *P. aeruginosa* (IZ of 12 mm, TA 2.16 ml/g and MIC 0.6 mg). The lowest MIC value of 0.2 mg/ml was recorded in case of *E. coli*, *K. pneumoniae* and *P. aeruginosa* for quarternary alkaloids, polysaccharides and lipids respectively points out the significance of these compounds as potential antimicrobial agents.

Total activity (TA) of the respective extracts was calculated and recorded in Table No.3. In flower extracts as well as extracts of leaves quarternary alkaloids showed highest total activity against all five selected test microorganisms. High TA also showed by flower lipid against *E.coli* as 16.15 ml/g, while the lowest TA was showed by Polar compounds of flower against *S. aureus* (0.96 ml/g) and against *K. pneumoniae* (0.96 ml/g). The lipid extract from leaves showed the highest TA against *P. aeruginosa* (15.5 ml/g)

Table 2. MIC of extract *T. procumbens*

Plant part	Extracts	MIC of the extract against test pathogen (mg/ml)				
		E.coli	*S. aureus*	*K. pneumoniae*	*P. aeruginosa*	*Salmonella spp.*
Flowers	Polysaccharides	1.8	1.8	1.4	-	1.8
	Alkaloids	0.6	-	1.8	-	1.2
	Quarternary alkaloids	0.6	1.2	1.2	1.2	1.8
	Polar comp.	1.2	1.8	1.8	-	-
	Lipids.	0.6	1.2	1.2	1.8	1.2
Leaves	Polysaccharides	-	0.6	0.2	0.6	0.4
	Alkaloids	0.8	0.6	0.6	0.6	0.6
	Quarternary alkaloids	0.2	0.4	0.4	0.4	0.6
	Polar comp.	0.6	0.4	-	0.8	-
	Lipids.	0.8	0.4	0.4	0.2	0.8

Table 3. Quantity and total activity of extracts *T. procumbens*.

Plant part	Extracts	Amount of extract (mg/g of plant part)	Total activity (ml/g)				
			E. coli	*S. aureus*	*K. pneumoniae*	*P. aeruginosa*	*Salmonella spp.*
Flowers	Polysaccharides	3.39	1.88	1.88	1.4	—	1.88
	Alkaloids	0.74	1.23	—	0.41	—	1.61
	Quarternary alkaloids	24.95	41.58	20.79	20.79	20.79	13.86
	Polar comp.	1.73	1.44	0.96	0.96	—	—
	Lipids.	9.69	16.15	8.07	8.07	4.03	8.7
Leaves	Polysaccharides	1.3	—	2.16	2.16	2.16	3.25
	Alkaloids	0.94	1.17	1.56	1.56	1.56	1.56
	Quarternary alkaloids	21.17	105.85	52.92	52.92	52-92	35.28
	Polar comp.	0.45	0.75	1.12	—	0.56	—
	Lipids.	3.01	3.76	7.52	7.52	15.05	3.76

Total Activity (TA)= Extract per gram dried plant part / MIC of extract

CONCLUSION

Antibiotic resistance by microorganisms is becoming an increasing public health concern throughout the world. This results in treatment failure and thus in increased morbidity and mortality rate. The discovery of newer antibiotics is failing short due to the development of the multidrug resistant pathogens. The effectiveness and safety of drugs of herbal origin might be a solution over these problems.

Pharmacological evaluation of *T. procumbens* was done by some earlier workers[8], but the present study reports extraction and use of five different extracts. All the five plant extracts used showed considerable antibacterial activity. Quarternary alkaloids and lipids showed broad spectrum of activity by inhibiting the test microorganisms. In this study it was founds that all five extracts of *T. procumbens* were potent inhibitors of pathogens like *E. coli*, *K. pneumoniae*, *S. aureus* etc. Determination of MIC and TA in the study will be useful in establishing the extracts as an antibacterial agent. Some of the pathogens used in the study were known to cause urinary tract infections (UTI) also; thus the extracts of *T. procumbens* could control the UTI also.

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