



Antibacterial Activity of *Tridax procumbens* with Special Reference to Nosocomial Pathogens

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Research Article

Received 15th August 2011
Accepted 14th December 2011
Online Ready 25th December 2011

ABSTRACT

Aims: To evaluate the aqueous as well as ethanolic extracts of *Tridax procumbens* L., (Asteraceae) against various bacterial pathogens including strains obtained from community acquired and nosocomial infections.

Study design: Experimental study.

Place and Duration of Study: Department of Microbiology and Department of Pharmacology, M.G.M. Medical College and M.G.M. New Bombay Hospital, Navi Mumbai, India, between July 2010 and December 2010.

Methodology: After authentication of the plant, extracts were prepared from the leaves of *T. procumbens* using Soxhlet apparatus. Aqueous and ethanolic extracts were tested against some standard strains as well as clinical isolates of different bacteria by agar well diffusion technique. Nosocomial strains of *Pseudomonas aeruginosa* from different clinical samples were also tested.

Results: While the aqueous extract had no antibacterial activity, the alcoholic extract showed significant antibacterial activity against *Pseudomonas aeruginosa*. The antibacterial activity of the ethanolic extract against the nosocomial strains of *Pseudomonas* was significantly more compared to that of antibiotics such as augmentin, cefotaxime, and ciprofloxacin.

Conclusion: Narrow spectrum preparations like extracts of *Tridax* leaves may be useful for successful therapy against multidrug resistant pathogens like *Pseudomonas aeruginosa*.

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Keywords: *Tridax procumbens*; antibacterial activity; *Pseudomonas aeruginosa*; nosocomial infections.

1. INTRODUCTION

With 'Herbal Renaissance' happening all over the globe, medicinal herbs are staging a phenomenal comeback. Ethno botanical information from India estimates that more than 6000 higher plant species forming about 40% of the higher plant diversity, are used in its codified and folk healthcare traditions (Ved and Goraya, 2007). As sources of biologically active molecules and blue prints for the development of modified derivatives with enhanced activity and /or reduced toxicity, plant-derived drugs form an important segment of the modern pharmacopoeia. With ethno- pharmacologists, microbiologists and botanists combing the earth for such natural treasures, much of the research is being spotlighted on plants and their phytochemicals. This could translate into effective drugs as well as nutritional supplements (nutraceuticals) (Cowan, 1999).

Many ancient traditions including the Ayurveda, Siddha and the Unani systems of medicine have advocated the use of several herbal preparations like plant juices and extracts for diseases including infectious ones. 74% of the plant- derived medicines have a modern indication that correlates with their traditional, cultural and sometimes ancient uses (Wynn, 2001). Hence, traditional medicine is an important source for the development of novel chemotherapeutic agents which are less toxic and more economic (Racio *et al.*, 1989).

A major stumbling block to the successful management of infectious diseases has been the propensity with which microorganisms are able to develop resistance to routinely used antibiotics. The development and spread of multidrug resistant superbugs especially in the hospital environment, continues to be a burning global issue due to the indiscriminate and irrational use of antibiotics (Pitout *et al.*, 2008). In this context, researching into alternative sources of antimicrobial agents such as plant extracts is the need of the hour. Endowed with a wide variety of antimicrobial properties due to secondary metabolites such as alkaloids, tannins, terpenoids, saponins and flavonoids, herbs need to be evaluated for their scientific and successful application for the treatment of infectious diseases (Cowan, 1999). One such plant known to be associated with various pharmacological properties including antimicrobial ones is *Tridax procumbens*.

Tridax procumbens, a plant belonging to the daisy family, is found perennially in various tropical and subtropical regions as well as mildly temperate regions worldwide (Mundada and Shivhare, 2010). Listed as a weed and a pest plant, it has been known by several names including *Tridax* daisy in English, Jayanti veda in Sanskrit, Ghamra in Hindi, Dagadi pala in Marathi, Herbe caille in French and Thata poodu in Tamil. It habitats waste places, road sides and hedges throughout India. Some reports from tribal areas in India state that the leaf juice can be used to cure fresh wounds, stop bleeding and also as a hair tonic (Mundada and Shivhare, 2010; Perumal Samy *et al.*, 1997; Zambare *et al.*, 2010). A few reports have focused on the immense potential of this plant which has antimicrobial, wound healing, anti-inflammatory and immunomodulatory properties (Suseela *et al.*, 2002; Taddei and Rosas Romero, 2000). However, there is a paucity of reports on its effect on nosocomial and multidrug resistant pathogens. Hence this study was carried out to compare the antibacterial activity of aqueous and ethanolic extracts of the leaves of *T. procumbens* and to study the efficacy of the extracts against community acquired and nosocomial human

pathogens. To the best of our knowledge, the effect of *T. procumbens* against multidrug resistant nosocomial pathogens has not been studied so far.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

After obtaining approval from the Institutional Ethics Committee, the study was carried out at M.G.M. Medical College and M.G.M. New Bombay Hospital in Navi Mumbai, India. The leaves of *Tridax procumbens* were collected from the college campus of M.G.M. Medical College during the monsoon season (months of July and August). Herbarium specimen was also deposited at the Department of Botany, Agarkar Institute, Pune, India (Voucher No.WP-076). The taxonomic identity was confirmed as *Tridax procumbens* belonging to the family Asteraceae.

2.2 Preparation of Extract

Plant material was washed thoroughly with tap water and shade dried for 7 days. Coarse powder of leaves was obtained by crushing the leaves in an electronic blender. The extraction procedure was carried out with 100 grams of coarse powder of the leaves of *Tridax procumbens* using 500ml of 80% ethanol in a Soxhlet extractor for 48 hours. An aqueous extract was prepared similarly by using only the powder and 500 ml of sterile water. The extracts were stored in desiccators for preliminary phytochemical analysis and further testing of antimicrobial activity.

2.3 Antimicrobial Testing

The bacterial strains tested included standard American Type Culture Collection (ATCC) strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The bacterial isolates from various clinical samples of community acquired as well as nosocomial infections included gram positive organisms (*Staphylococcus aureus* and *Enterococcus faecalis*), gram negative bacilli (*Escherichia coli*, *Klebsiella pneumoniae*, *Shigella flexneri* and *Salmonella typhi*) and gram negative non fermenting bacilli (*Pseudomonas aeruginosa* and *Acinetobacter baumannii*).

The ethanolic and aqueous extracts were tested by agar well diffusion techniques as described by Perez *et al.* (1990). Different concentrations of the extract were used for testing (1g%, 2.5 g% and 5 g %). For the agar well diffusion method, Mueller Hinton agar plates were seeded with standard inoculum (1×10^6 CFU/ml) of the microorganism being tested. Wells of 10mm diameter were prepared in the plates with a sterile borer and 100 microlitres of the extract was pipetted directly into the wells to give final concentrations of 1mg, 2.5mg and 5mg respectively. The plates were incubated overnight at 37°C. Antibacterial activity was determined by measuring the diameter of the zones of inhibition of bacterial growth surrounding the wells with the extract. The test was carried out in triplicate and the mean zones of inhibition calculated. For each bacterial strain, controls were included that comprised of only sterile water (in the case of the aqueous extract) or pure ethyl alcohol (in the case of the ethanolic extract), instead of the *T. procumbens* extract. To calculate the effective zone size of the ethanolic extract, the zone size of the alcohol control was subtracted from the total zone of inhibition size.

The strains of *Pseudomonas aeruginosa* used in the study were also tested against five routinely used antibiotics, viz. Augmentin, Cefotaxime, Ciprofloxacin, Ticarcillin and Imipenem and the zone sizes were compared with that against the *Tridax* extract. The mean of three readings were taken. Data was analyzed statistically using Student's paired 't' test to determine the significance at $p < 0.05$.

3. RESULTS

3.1 Effect of Aqueous Extracts of *T. procumbens* on Various Bacterial Isolates

Zones of inhibition were not seen in the Mueller Hinton agar plates where different concentrations of *Tridax* were tested against the standard strains and isolates of community acquired as well as nosocomial pathogens.

Hence in this study, aqueous extract of the leaves showed no antibacterial activity.

The standard ATCC strains as well as clinical isolates of most of the Gram positive and Gram negative organisms did not show any sensitivity to the different concentrations of ethanolic extracts of *Tridax procumbens*.

Table 1: Mean zones of inhibition of gram positive and gram negative organisms by aqueous extract of *T. procumbens*

Sl. No.	Bacterial strains	Source	Zone sizes for different concentrations of <i>T. procumbens</i> (aqueous extract)		
			1 mg	2.5 mg	5 mg
1	<i>S. aureus</i> (standard strain)	ATCC (25923)	No zone (R)	No zone (R)	No zone (R)
2	<i>S. aureus</i>	Pus	No zone (R)	No zone (R)	No zone (R)
3	<i>Enterococcus</i>	Sputum	No zone (R)	No zone (R)	No zone (R)
4	<i>E. coli</i> (standard strain)	ATCC (25922)	No zone (R)	No zone (R)	No zone (R)
5	<i>E. coli</i>	urine	No zone (R)	No zone (R)	No zone (R)
6	<i>Klebsiella pneumoniae</i>	urine	No zone (R)	No zone (R)	No zone (R)
7	<i>Shigella flexneri</i>	stool	No zone (R)	No zone (R)	No zone (R)
8	<i>S. typhi</i>	blood	No zone (R)	No zone (R)	No zone (R)
9	<i>P. aeruginosa</i> (std. strain)	ATCC (27853)	No zone (R)	No zone (R)	No zone (R)
10	<i>Pseudomonas aeruginosa</i>	Pus	No zone (R)	No zone (R)	No zone (R)
11	<i>Acinetobacter</i>	Tracheal secretions	No zone (R)	No zone (R)	No zone (R)

R- Resistance.

3.2 Effect of Ethanolic Extracts of *T. procumbens* on Various Bacterial Isolates

Table 2: Mean zones of inhibition of gram positive and gram negative organisms by ethanolic extract of *T. procumbens*

Sl. No.	Bacterial strain	Source	Zone sizes for different concentrations of <i>T. procumbens</i> (Ethanolic extract)		
			1 mg	2.5 mg	5 mg
1	<i>S. aureus</i> (standard strain)	ATCC (25923)	No zone(R)	No zone (R)	No zone (R)
2	<i>S. aureus.</i>	Pus	No zone (R)	No zone (R)	No zone (R)
3	<i>Enterococcus</i>	Sputum	No zone (R)	No zone (R)	No zone (R)
4	<i>E. coli</i> (standard strain)	ATCC (25922)	No zone (R)	No zone (R)	No zone (R)
5	<i>E. coli</i>	urine	No zone (R)	No zone (R)	No zone (R)
6	<i>Klebsiella pneumoniae</i>	urine	No zone (R)	No zone (R)	No zone (R)
7	<i>Shigella flexneri</i>	stool	No zone (R)	No zone (R)	No zone (R)
8	<i>S. typhi</i>	blood	No zone (R)	No zone (R)	No zone (R)

R- Resistant.

Table 3: Mean zones of inhibition of Gram negative nonfermenters including nosocomial strains by alcoholic extract of *T. procumbens*.

Sl. No.	Bacterial Strain	Source	Zone sizes for different concentrations of <i>T. procumbens</i> extract		
			1 mg	2.5 mg	5 mg
1	<i>Pseudomonas aeruginosa</i> (P1)	ATCC (27853) std strain	No zone (R)	20 mm (S)	21 mm (S)
2	<i>Pseudomonas aeruginosa</i> (P2)	Nosocomial strain from tracheal secretion	No zone (R)	25 mm (S)	27 mm (S)
3	<i>Pseudomonas aeruginosa</i> (P3)	Nosocomial strain from BAL fluid	No zone (R)	24 mm (S)	25 mm (S)
4	<i>Pseudomonas aeruginosa</i> (P4)	Nosocomial strain from Urine	No zone (R)	21 mm (S)	21 mm (S)
5	<i>Pseudomonas aeruginosa</i> (P5)	Nosocomial strain from Blood	No zone (R)	22 mm (S)	24 mm (S)
6	<i>Pseudomonas aeruginosa</i> (P6)	Pus	No zone (R)	24 mm (S)	25 mm (S)
7	<i>Pseudomonas aeruginosa</i> (P7)	Pus	No zone (R)	27 mm (S)	28 mm (S)
8	<i>Pseudomonas aeruginosa</i> (P8)	Sputum	No zone (R)	30 mm (S)	32 mm (S)
9	<i>Pseudomonas aeruginosa</i> (P9)	Urine	No zone (R)	22 mm (S)	24 mm (S)
10	<i>Acinetobacter baumannii</i> (A)	nosocomial strain from tracheal secretion)	No zone (R)	No zone (R)	No zone (R)

BAL: Broncho alveolar lavage; R - resistant; S - sensitive.

Table 4: Comparison of Zones of inhibition sizes of *Pseudomonas aeruginosa* strains against antibiotics and *Tridax* extract

Strain of <i>Pseudomonas</i> tested	Zone sizes (in mm) against different Antibiotic discs and <i>Tridax</i> extract						
	Augmentin	Cefotaxime	Ciprofloxacin	Ticarcillin	Imipenem	<i>Tridax</i> extract (2.5 mg)	<i>Tridax</i> extract (5mg)
P1	22 (S)	23 (S)	22 (S)	24 (S)	28 (S)	20 (S)	21 (S)
P2	12 (R)	8 (R)	14 (R)	22 (S)	26 (S)	25 (S)	27 (S)
P3	14 (R)	8 (R)	12 (R)	12 (R)	22 (S)	24 (S)	25 (S)
P4	6 (R)	7 (R)	6 (R)	10 (R)	20 (S)	21 (S)	21 (S)
P5	11 (R)	13 (R)	08 (R)	12 (R)	21 (S)	22 (S)	24 (S)
P6	10 (R)	15 (S)	12 (R)	20 (S)	25 (S)	24 (S)	25 (S)
P7	11 (R)	15 (S)	19 (S)	22 (S)	24 (S)	27 (S)	28 (S)
P8	17 (S)	18 (S)	20 (S)	24 (S)	29 (S)	30 (S)	32 (S)
P 9	16 (S)	18 (S)	21 (S)	22 (S)	24 (S)	22 (S)	24 (S)

(S)- Sensitive; (R)-Resistant.

All the strains of *Pseudomonas* were sensitive to the *Tridax* extract.

Only the ethanolic extract of *T. procumbens* in the 2.5mg and 5mg concentration showed very good antibacterial activity against different strains of *Pseudomonas* including the standard strain and isolates from nosocomial and community acquired infections.

Best sensitivity was seen against *Pseudomonas* isolated from Sputum followed by Pus, Tracheal secretions and BAL fluid. Sensitivity to a lesser extent was seen in isolates from Blood and Urine.

The difference between activity of 1mg extract (no activity) and 2.5mg extract (good antibacterial activity) in terms of differences in zone sizes was statistically significant, ($p < 0.05$) while there was no significant difference in the activity of 2.5 mg and 5mg extracts.

The isolates of *Pseudomonas* (P2-P5) obtained from nosocomial infections were resistant to several routinely used antibiotics such as Augmentin, Cefotaxime and Ciprofloxacin, while they showed variable sensitivity to Ticarcillin and good sensitivity to Imipenem. All the strains showed 100% sensitivity to *Tridax* extract. The sensitivity was comparable to a higher antibiotic i.e., Imipenem. In the case of the isolates from community acquired infections (P6-P9), most strains showed good sensitivity to the other antibiotics tested as well as to the *Tridax* extract. Statistically significant difference was noted in the zone sizes produced by *Tridax* extracts as compared to those produced against Augmentin, Cefotaxime and Ciprofloxacin when *Pseudomonas* strains from nosocomial infections were tested.

3.3 Phytochemical and Biochemical Analysis

Table 5: Phytochemical analysis of leaves of *T. procumbens*

Sl. No.	Parameters	(% W/W) (Mean)
1	Water soluble extractive value	28.8 %
2	Alcohol soluble extractive value	6.9%
3	Acid insoluble ash	5.2%
4	Water soluble ash	3.3%
5	Total ash value	22.83%
6	Sulphated ash value	22.5%
7	Moisture content	13%

Table 6: Preliminary Biochemical evaluation of extracts of dried *T. procumbens* leaves.

Sl. No	Chemical Tests	Aqueous extract	Ethanolic extract
1.	Carbohydrates	+	+
2.	Tannins	+	++
3.	Flavonoids	+	+++
4.	Glycosides	-	+
5.	Saponins	+	+
6.	Alkaloids	+	+
7.	Steroids	+	++

'+' : Phyto constituent present. '-' : Phyto constituent absent.

The ethanolic extract of *T. procumbens* had greater amounts of phytoconstituents such as Flavonoids and Tannins as compared to the aqueous extracts.

4. DISCUSSION

Tridax procumbens has been used as a phytomedicine by traditional healers and practitioners of Unani and Ayurvedic systems of medicine. This traditional usage stems from the fact that *Tridax* is associated with antibacterial activity (Mundada and Shivhare, 2010). We studied the efficacy of aqueous and ethanolic extracts of *Tridax* as antibacterial agents against human pathogens including nosocomial strains. While the ethanolic extract was associated with antibacterial activity the aqueous extract was not. Similar findings have been reported in another study (Aniel and Naidu, 2010). The difference in activity between the aqueous and alcoholic extracts can be explained by the fact that different solvents have varying capacities to extract phytoconstituents based on their solubility and polarity. The antibacterial activity of *Tridax* is known to be due to alkaloids, flavonoids, tannins and saponins (Mundada and Shivhare, 2010). Ethanol could have led to better extraction of these active principles with enhanced antibacterial activity of *Tridax* leaves in the present study.

The ethanolic extract showed very good antibacterial activity only against gram negative non fermenters like *Pseudomonas aeruginosa*. There was no activity against gram positive as well as gram negative bacteria. These findings corroborate with some other studies (Mahato and Chaudhary, 2005). The *in vivo* antibacterial activity of *Tridax* against *Pseudomonas aeruginosa* in experimental animals has also been well established (Oladunmoye, 2006).

In contrast to these findings, some other studies have demonstrated the efficacy of *Tridax* extracts even against other bacteria such as *S. aureus*, *E. coli*, *Klebsiella pneumoniae* and *Proteus* (Sharma and Kumar, 2009; Das *et al.*, 2009; Yoga Latha *et al.*, 2010). These differences could have arisen due to a number of factors. One reason is the use of standard strains of bacteria and bovine pathogens in these studies instead of human pathogens. Our study included clinical isolates in addition to the standard ATCC strains. Many of the strains were isolated from nosocomial infections and were multi drug resistant to routinely used antibiotics. To the best of our knowledge there have been no studies on the effect of *Tridax* on nosocomial pathogens. Sharma *et al* found no activity of extract of leaves against *E. coli* and *S. aureus* while the flower extract showed antibacterial activity. In this study, only the leaves of *Tridax* were used. The effect of extracts of flowers and other parts of the plant needs to be elucidated. Differences in the extraction procedures such as cold percolation versus hot extraction methods and in the nature of solvents used in extraction are other factors which can influence the nature of the active principles present in the extract. Hence, a comparative study of extracts with different types of solvents and different parts of the plants would optimize the procedures for the best possible antibacterial activity. Another reason for a difference in activity as compared to other studies could be due to differences in the time of plant collection.

The highlight of this study was that the ethanolic extract showed very good activity against *Pseudomonas aeruginosa* at a concentration of 5mg/ml. There was a statistically significant increase in the zones of inhibition as compared to some control antibiotics. The nosocomial strains of *Pseudomonas* isolated from tracheal secretions and bronchoalveolar lavage (from cases of ventilator associated pneumonia), urinary tract infection and bloodstream infections were mostly resistant to routinely used antibiotics such as augmentin, ciprofloxacin, cephalexin and even ticarcillin with sensitivity only to imipenem. It is a well-established fact

that *Pseudomonas aeruginosa* is one of the leading gram-negative organisms associated with nosocomial infections (Paramythiotu *et al.*, 2004). The increasing frequency of multi-drug-resistant *Pseudomonas aeruginosa* (MDRPA) strains is a matter of concern as efficacious antimicrobial options are severely limited (Obritsch, 2005). In this context, the effect of *Tridax* extract on multidrug resistant *Pseudomonas* isolated from nosocomial infections is an encouraging finding. In traditional medicine crude extracts made by crushing leaves have been used on cuts and wounds. The antibacterial activity of ethanolic extract of *Tridax procumbens* can be attributed to the presence of flavonoids and tannins which are substances known to have several mechanisms of action such as inhibition of DNA gyrase, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, etc. (Cushnie and Lamb, 2005)

Flavonoids obtained from tea are known to be inhibitory to a number of microbes including phytopathogens such as *Pseudomonas* (Friedman, 2007). The finding of phytoconstituents such as flavonoids and tannins in the extract of *T. procumbens* along with the ability to inhibit the growth of nosocomial pathogenic strains of *Pseudomonas* in our study, reiterates this fact.

In order to make more scientific formulations and for optimizing the clinical usage, further research will be needed in this direction. In the wake of development of multidrug resistant strains, this can have long term applications in the future for the treatment of nosocomial infections by *Pseudomonas aeruginosa*.

5. CONCLUSION

The herb *Tridax procumbens*, found growing commonly in tropical countries, is endowed with antibacterial properties. Our study demonstrated that this activity was associated only with the ethanolic extract and was prominently seen only against *Pseudomonas aeruginosa* strains. Multi drug resistant nosocomial strains of *Pseudomonas* isolated from ventilator associated pneumonia, urinary tract infection as well as blood stream infection showed significant sensitivity to *Tridax* extracts. Our study corroborates the efficacy of *Tridax* as an anti pseudomonal agent and its value as a source of formulations for treatment of nosocomial infections caused by *Pseudomonas aeruginosa*.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Indian Council of Medical Research (ICMR), New Delhi for providing student's grant towards this project.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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