

## Antibacterial activities of extracts and their fractions of leaves of *Tridax procumbens* Linn

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### Abstract

#### Aims

Attempts were made to evaluate *in vitro* the antibacterial potential of crude extracts made with five solvents (n-hexane, butanol, ethanol, chloroform and water) from leaves of *Tridax procumbens* Linn and the of fractions from the ethanol extract aiming to locate active fractions.

#### Main Methods

Filter paper disc diffusion technique in agar was employed for determining antimicrobial activity. Minimum inhibition concentration (MIC) of the extracts was determined from the culture plate that had the lowest concentration and prevented and growth of bacterial strains.

#### Key Finding

Their antibacterial activities were evaluated *in vitro* against 9 clinical bacterial isolates. The extracts exhibited a pronounced activity against *E. coli* (38 mm); *Pseudomonas aeruginosa* (40 mm), *Bacillus subtilis* (47 mm), *Proteus vulgaris* (42 mm), *Klebsiella pneumoniae* (41 mm), *Micrococcus* sp (39 mm), *Staphylococcus aureus* (42 mm), *Citrobacter* sp (44 mm) and *Serratia marcescens* (41 mm). The minimum inhibitory concentration and minimum bactericidal concentration were found to be <80mg/ml for the ethanol extracts of *Tridax procumbens* against test organisms.

#### Significance

*Tridax procumbens* leaf extracts possess antibacterial activity against a wide range of microbes justify which could be its use in traditional medicine as a remedy for the treatment of wound.

**Keywords:** *Tridax procumbens*, antibacterial activity, minimum inhibitory concentration and minimum bactericidal concentration.

### Introduction

*Tridax procumbens* Linn. (Asteraceae) is one of the medicinally important plants commonly found in subtropical countries. The leaves of the plant are known to be used for the treatment of wound in traditional medicine. The young leaves are squeezed and rubbed on the affected parts two or three times per day. It is used as an antidote to arrow poison by applying the powdered leaves to the wound [2, 3]. But no report could be found in the literature on the evaluation of antimicrobial activity of crude extracts from the leaves of *Tridax procumbens*, which could provide scientific evidence for its use in traditional medicine.

Wound development is influenced by the following factors like age, obesity, malnutrition, endocrine and metabolic disorders. Local factors are like necrotic tissue, foreign bodies, tissue ischemia, hematoma formation and poor surgical management also cause wound infection. Microbiological contamination of wound depends on the type and virulence of organism, size of bacteriological dose and antibiotic resistance [1]. When these microbial factors are conducive, impaired host defenses set the stage for enacting the chain of events that produce wound infection. The usual pathogens on skin and mucosal surfaces are gram-positive cocci (notably *Staphylococci*); however gram-negative aerobes and anaerobic bacteria contaminate skin in the groin/perineal areas [1].

It is important to have a clear understanding of the terms used for wound infections. Since 1985 the most commonly used terms have included wound contamination, wound colonisation, wound infection and more recently critical colonisation. These terms can be defined as; wound contamination is the presence of bacteria within a wound without any host reaction; wound colonisation is the presence of bacteria within the wound which do multiply or initiate a host reaction, critical colonisation is the multiplication of bacteria causing a delay in wound healing usually associated with an exacerbation of pain not previously reported but still with no over host reaction and wound infection is the deposition and multiplication of bacteria in tissue with an associated host reaction.

Therefore, the present study was designed to study the medicinal use of *Tridax procumbens* to explain the rationale of its use in traditional medicine by *in vitro* assessment. The antibacterial activities of five crude extracts made from solvents (n-hexane, butanol, ethanol, chloroform and water) and that of some fractions from the ethanol extracts against clinical bacterial isolates of human pathogenic microorganisms were studied.

### Results

Five crude extracts (solvents) and four fractions from the ethanolic extract of *Tridax procumbens* leaves were investigated for their antibacterial potential *in vitro*. Results

**Table 1.** The antibacterial effect of *Tridax procumbens* hexane extracts against wound pathogenic bacteria analysed by disc diffusion method

S.No	Bacterial culture	Mortar & Pestle extract (µg/ml)					Soxhlet extract (µg/ml)					Streptomycin(µg/ml)	
		Diameter of inhibition (cm)										50	100
		20	40	60	80	100	20	40	60	80	100		
1	<i>Escherichia coli</i>	0.6	0.7	1	1.2	2.4	0.8	0.9	1.1	1.4	2.5	2.6	3.5
2	<i>P. aeruginosa</i>	0.3	1.2	1.7	1.9	2.2	0.5	1.3	1.9	2.1	2.3	3.2	4.1
3	<i>Proteus vulgaris</i>	0.5	0.8	1.1	1.6	2.4	0.6	0.9	1.4	1.9	2.5	2.0	3.3
4	<i>K. pneumoniae</i>	0.2	0.5	1.5	1.6	2.8	0.3	0.7	1.6	1.9	2.9	1.9	3.0
5	<i>Citrobacter sp</i>	0.1	0.6	1.5	1.6	2.7	0.4	0.8	1.7	1.8	2.7	2.1	3.1
6	<i>S.marcescens</i>	0.1	0.1	0.2	0.1	0.2	0.2	0.3	0.4	0.5	0.7	2.5	3.5
7	<i>Micrococcus sp</i>	0.1	0.8	1.2	1.5	2.1	0.4	0.9	1.7	1.6	2.3	2.4	3.3
8	<i>Staphylococcus aureus</i>	0.0.4	0.7	1	1.3	1.8	0.6	0.9	1.6	1.5	1.9	2.5	3.1
9	<i>Bacillus subtilis</i>	0.5	0.9	1.2	1.5	2.3	0.7	1.1	1.7	1.7	2.5	2.5	3.4

**Table 2.** The antibacterial effect of *Tridax procumbens* butanol extracts against wound pathogenic bacteria analysed by disc diffusion method

S.No	Bacterial culture	Mortar & Pestle extract (µg/ml)					Soxhlet extract (µg/ml)					Streptomycin(µg/ml)	
		Diameter of inhibition (cm)										50	100
		20	40	60	80	100	20	40	60	80	100		
1	<i>Escherichia coli</i>	0.6	0.8	1.8	2.5	3.6	0.9	1.1	1.4	3.0	4.1	2.6	3.5
2	<i>P. aeruginosa</i>	0.5	0.8	1.2	1.8	2.1	0.4	1.9	2.8	3.4	4.2	3.2	4.1
3	<i>Proteus vulgaris</i>	0.7	0.9	1.8	2.3	3.1	0.8	1.8	2.1	2.5	4.2	2.0	3.3
4	<i>K. pneumoniae</i>	0.5	0.9	1.8	2.3	2.5	0.9	1.9	2.4	2.7	3.9	1.9	3.0
5	<i>Citrobacter sp</i>	0.8	1.2	1.5	2.6	3.6	0.9	1.6	2.2	2.9	4.4	2.1	3.1
6	<i>S.marcescens</i>	0.8	1.4	1.8	2.5	2.8	0.7	2.1	2.5	3.1	4.1	2.5	3.5
7	<i>Micrococcus sp.</i>	0.8	1.2	1.5	2.1	2.5	0.6	1.5	2.2	2.6	3.7	2.4	3.3
8	<i>Staphylococcus aureus</i>	0.5	0.9	1.3	1.8	2.2	0.9	1.8	2.5	2.7	3.5	2.5	3.1
9	<i>Bacillus subtilis</i>	0.3	0.7	0.9	1.7	2.5	1.1	1.4	1.6	2.7	3.3	2.5	3.4

from the antibacterial study using disc method are presented in table 1. It is observed extract of ethanol and butanol of *Tridax procumbens* and standard antibiotic has similar antibacterial spectra. The other solvent extracts showed minimum level antibacterial effects only when compared to butanol extract. So butanol extracts were used for further analysis.

The extracts analysed anti microbial property with pathogenic microorganisms increment of extract concentration (100µg/ml) leads to high inhibition activity compared to lower concentration (80, 60, 40 and 20µg/ml) respectively. In the present investigation butanol extracts (100µg/ml) showed predominant inhibition 38, 40, 47, 42, 41, 39, 42, 44 and 41(mms) against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Micrococcus Sp*, *Staphylococcus aureus*, *Citrobacter Sp* and *Serratia marcescens* respectively. *In vitro* antibacterial assay is used to assess the efficacy of *Tridax procumbens* and to inhibit the growth of pathogenic microbes showed that the extract of various solvents like as hexane, butanol and chloroform, ethanol and water had broad spectrum of antibacterial potential (Tables – 1-5).

The minimum inhibitory concentrations of extracts of *Tridax procumbens* is given in table 6. The results showed that the minimum inhibitory butanol extracts is 20µg/ml against test bacteria. Similarly MIC of other extracts was 40, 60, 40 and 80µg/ml for hexane, chloroform, ethanol and aqueous extracts respectively.

The minimal inhibitory concentration of *Tridax procumbens* against pathogens is less in butanol extracts compared to other solvent extracts. However the MIC for chloroform extracts of *Tridax procumbens* against pathogens was 40µg/ml. *Tridax procumbens* extracts against gram positive bacteria is less compared to gram negative bacteria

in extracts of all solvents. The minimum inhibitory concentration of *Tridax procumbens* for different gram negative was in the range of 20-60µg/ml. Outcome of present investigation clearly demonstrates that the butanol and chloroform extracts of *Tridax procumbens* leaves in the highest concentration (100µg/ml) effectively control the growth of bacteria.

The minimum bacterial concentration of the extracts in comparison to those for streptomycin showed appreciable activity by *Tridax procumbens*. Minimum bactericidal concentrations 20-80µg/ml for the gram negative bacteria ranged from 20-60 µg/ml against gram negative bacteria. MIC for standard antibiotic streptomycin was 10µg/ml for both gram positive and gram negative bacteria. The butanol extract of *Tridax procumbens* was found more effective in reducing the growth of bacteria tested, than the hexane, chloroform, ethanol and extracts (Table 6).

## Discussion

Gram positive bacteria and gram negative bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Micrococcus Sp*, *Staphylococcus aureus*, *Citrobacter Sp* and *Serratia marcescens* showed a reduction in their growth on treatment with the different solvent extracts of *Tridax procumbens*. The degree of inhibition as measured by the disc diffusion method, reported that the gram negative bacteria were more inhibited than the gram positive bacteria. Various workers also reported similar findings from different plants sources [9, 10, 11].

The minimum inhibitory concentrations *Tridax procumbens* for gram positive bacteria is (20-80µg/ml) and (20-60µg/ml) for gram negative bacteria. The average size of

**Table 3.** The antibacterial effect of *Tridax procumbens* chloroform extracts against wound pathogenic bacteria analysed by disc diffusion method

S.No	Bacterial culture	Mortar & Pestle extract (µg/ml)					Soxhlet extract (µg/ml)					Streptomycin(µg/ml)	
		Diameter of inhibition (cm)										50	100
		20	40	60	80	100	20	40	60	80	100		
1	<i>Escherichia coli</i>	0.9	1.7	1.9	2.3	2.9	0.9	2.1	2.1	2.4	3.3	2.6	3.5
2	<i>P. aeruginosa</i>	0.8	1.2	1.4	2.2	2.6	0.9	1.8	1.8	2.3	3.2	3.2	4.1
3	<i>Proteus vulgaris</i>	0.9	1.6	1.8	2.9	3.2	1.2	1.7	2.4	2.9	3.9	2.0	3.3
4	<i>K. pneumoniae</i>	0.4	0.6	1.2	1.8	2.5	1.2	1.8	2.4	3.2	4.0	1.9	3.0
5	<i>Citrobacter sp</i>	0.8	1.3	1.5	1.9	2.5	1.1	1.9	2.2	2.6	3.2	2.1	3.1
6	<i>S.marcescens</i>	0.8	1.4	1.8	2.1	2.8	0.7	1.5	2.2	2.6	3.0	2.5	3.5
7	<i>Micrococcus sp</i>	0.6	1.2	1.5	1.9	2.7	0.7	0.9	1.5	2.4	3.0	2.4	3.3
8	<i>Staphylococcus aureus</i>	0.2	1.7	1.8	2.1	2.6	1.1	1.4	1.7	2.5	2.9	2.5	3.1
9	<i>Bacillus subtilis</i>	0.2	0.3	0.2	0.2	0.1	1.1	1.6	1.4	2.2	2.9	2.5	3.4

**Table 4.** The antibacterial effect of *Tridax procumbens* ethanol extracts against wound pathogenic bacteria analysed by disc diffusion method

S.No	Bacterial culture	Mortar & Pestle extract (µg/ml)					Soxhlet extract (µg/ml)					Streptomycin(µg/ml)	
		Diameter of inhibition (cm)										50	100
		20	40	60	80	100	20	40	60	80	100		
1	<i>Escherichia coli</i>	0.85	1.1	1.3	2.5	3.2	0.9	1.4	1.7	2.4	3.2	2.6	3.5
2	<i>P. aeruginosa</i>	0.6	0.8	1.2	2.9	3.6	0.9	1.2	2.6	3.2	3.8	3.2	4.1
3	<i>Proteus vulgaris</i>	0.4	1.3	2.5	2.6	3.8	0.5	0.9	1.4	1.9	2.4	2.0	3.3
4	<i>K. pneumoniae</i>	0.1	1.8	2.5	2.7	2.9	0.9	1.5	1.9	2.8	3.5	1.9	3.0
5	<i>Citrobacter sp</i>	0.2	1.6	1.8	2.8	2.9	0.9	1.4	1.7	2.9	3.8	2.1	3.1
6	<i>S.marcescens</i>	0.8	1.2	2.1	2.6	2.8	1.1	1.5	2.1	2.7	3.1	2.5	3.5
7	<i>Micrococcus sp</i>	0.7	1.5	2.1	2.3	3.1	0.6	1.1	1.5	1.9	2.4	2.4	3.3
8	<i>Staphylococcus aureus</i>	0.5	2.0	2.2	2.2	2.8	0.5	0.9	1.1	1.9	2.8	2.5	3.1
9	<i>Bacillus subtilis</i>	0.1	0.3	1.3	2.1	2.5	0.5	0.9	1.4	1.9	2.1	2.5	3.4

the inhibition zone for *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* at the concentration of 100µg/ml were 20 and 26mm respectively in all extracts except water extract. The extract which produces the inhibition zone against all pathogens were reduced 19mm to 47mm at 100µg/ml except aqueous extract. The minimum inhibition concentration for 20-80µg/ml and it was 60µg/ml for all the Gram negative bacteria. The minimum bactericidal concentrations calculated for different extracts against then pathogenic microbes also showed the results similar to disc inhibition assay and the optical density measured for plant extracts at a concentration of 100µg/ml was close to the bactericidal effect of standard antibiotic. The present investigation confirmed the previous work [4, 6] of *Tridax procumbens* antimicrobial activity against pathogenic organisms.

## Materials and methods

### Sample collection

Young leaves of *Tridax procumbens* were collected from the premises of Sri Kaliswari College, Sivakasi, Virudhunagar district, Tamilnadu, India. It was air dried and reduced to powder.

### Preparation of extracts and fractionation

Ten grams each of powdered leaves were separately macerated and exhaustively percolated with hexane, butanol, ethanol, chloroform and water. The resulting macerate and percolate were combined and evaporated *in vacuum* to yield corresponding semi dried extracts of 4.6g (46%), 2.4g (14%), 0.8g (8%) and 0.5g (5%) denoted as extracts A, B, C, D and E respectively.

Extract A (10) gm was dissolved in 100ml distilled water, filtered and exhaustively extracted with diethyl ether, ethyl acetate and n-butanol. Each fraction treated as described above was evaporated to give correspondingly dried extracts denoted as A1 (2.8g, 20%), A2 (1.2g, 12%) and A3 (0.5g, 5%) respectively. The residual aqueous phase was also evaporated to dryness and denoted as A4 (4.2g, 42%).

### Preparation of test samples

Ten milligrams each of dried extracts and fractions were dissolved in 10ml ethanol to obtain corresponding stock solution of 1mg/ml. These stock solutions were diluted with the same solvent to have a series of concentration of the test samples such as 20, 40, 60, 80 and 100 µg/ml.

### Anti bacterial testing

Selected microorganisms include bacteria such as *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Micrococcus sp*, *S. aureus*, *Citrobacter sp* and *Serratia marcescens* isolated from different pathologic medium from patients diagnosed to have various wound infection at the laboratory Joys, Nagercoil, Kanyakumari (district) Tamilnadu. The antibacterial activities of *Tridax procumbens* extracts were evaluated *in vitro* by a disc diffusion method using Muller Hinton medium.

### Disc diffusion method

Filter paper disc diffusion technique in agar [5] was employed for determining antimicrobial activity. Whatman No.1 filter paper discs of 6mm diameter, placed in dry Petri plates, were autoclaved. The test extracts in measured quanti-

**Table 5.** The antibacterial effect of *Tridax procumbens* water extracts against wound pathogenic bacteria analysed by disc diffusion method

S.No	Bacterial culture	Mortar & Pestle extract (µg/ml)					Soxhlet extract (µg/ml)					Streptomycin(µg/ml)	
		Diameter of inhibition (cm)										50	100
		20	40	60	80	100	20	40	60	80	100		
1	<i>Escherichia coli</i>	0.2	0.3	0.5	0.6	0.6	0.4	0.5	0.8	1.0	1.2	2.6	3.5
2	<i>P. aeruginosa</i>	0.1	0.3	0.5	0.6	0.9	0.4	0.6	0.7	0.9	1.1	3.2	4.1
3	<i>Proteus vulgaris</i>	0.2	0.3	0.5	-	-	0.2	0.3	0.5	0.9	1.4	2.0	3.3
4	<i>K. pneumoniae</i>	-	-	-	-	-	0.2	0.3	0.5	0.7	0.8	1.9	3.0
5	<i>Citrobacter sp</i>	0.1	0.3	0.5	-	0.9	0.3	0.6	0.9	1.2	1.4	2.1	3.1
6	<i>S.marcescens</i>	0.2	0.5	-	-	-	0.1	0.2	0.4	0.6	0.7	2.5	3.5
7	<i>Micrococcus sp</i>	-	-	-	-	-	0.2	0.4	0.6	0.9	1.3	2.4	3.3
8	<i>Staphylococcus aureus</i>	0.2	0.3	0.5	-	-	0.1	0.2	0.4	0.6	0.8	2.5	3.1
9	<i>Bacillus subtilis</i>	-	-	-	-	-	0.4	0.5	0.7	0.8	0.8	2.5	3.4

**Table 6.** The minimum inhibitory effect different solvent extracts of *Tridax procumbens* against wound pathogenic bacteria

S.No	Extract	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Proteus vulgaris</i>	<i>Klebsiella pneumoniae</i>	<i>Citrobacter sp</i>	<i>Serratia marcescens</i>	<i>Micrococcus sp</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
1	Hexane	20	40	40	20	50	50	70	70	60
2	Butanol	10	20	10	10	10	20	20	20	20
3	Chloroform	20	30	50	40	60	50	80	70	60
4	Ethanol	40	50	30	60	40	50	60	70	80
5	Water	30	40	60	20	40	50	70	60	80

ties were dissolved in minimum amount of acetone. Sterile filter paper No.1 discs were loaded with the crude extracts of *Tridax procumbens* [7]. The amount of extracts loaded in each disc was in the concentrations 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml. Similarly discs were prepared with standard antibiotic streptomycin (w/v) in two different concentrations (20µg/ml and 40µg/ml).

The pathogenic strains were suspended in nutrient broth (Hi Media) by transferring a loop full of grown 24 h on agar slopes. The suspensions were vortexed and 0.1 ml aliquots were spread over respective agar medium plates. The extracts and streptomycin loaded discs were then placed over the plates seeded with respective microorganisms. The plates were incubated at 37°C for 36h. The antibacterial activity was determined by measuring the inhibition zone around the discs.

#### MIC and MBC test

Minimum inhibition concentration (MIC) of the extracts was determined from the culture plate that had the lowest concentration and prevented growth of bacterial strains. Minimum bacterial concentration (MBC) was determined by using the method of Samy and Ignacimuthu (2001) [8]. The test containing 3ml of Muller Hinton broth and 0.1ml of bacterial suspension and 0.1ml plant extract were incubated at 37°C for 24hrs. Bacterial turbidity was measured at 650 nm to determine the rate of inhibition of bacterial growth. Streptomycin at 20 and 40µg/ml was used as a reference for determination of minimum bactericidal concentrations. The tubes containing only the growth medium and each of the organisms were used as control. The minimum bactericidal concentration that showed reduction of the bacterial growth as measured from the turbidity of the culture assay optical density value.

Total bacterial count for each bacterial species was estimated by counting the number of bacteria in each test tube

incorporated with different concentration of plant extracts and control. The average of three counting was taken as the final count (CFU/ml) of colony forming bacterial suspensions.

#### Conclusion

The antibacterial drug preparations were alternative medicines for synthetic drugs. It is suggested that using the extracts are effective and economic herbal drugs may be prepared for bacterial infections. The preliminary phytochemical screening also supported antimicrobial activity of *Tridax procumbens*. The broad spectrum of antibacterial activity of *Tridax procumbens* is highly promising for further phytochemical evaluation and being continued in further studies.

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