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In vitro Evaluation of Antimicrobial Potential of Three Crude Extracts of *Tridax procumbens* Leaves

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JJO and AN designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors JJO and PE managed the literature searches, analyses of the study performed the spectroscopy analysis and author AN managed the experimental process. Author PE identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The decreasing sensitivity of microbial isolates to most available standard antimicrobial agents currently in use necessitates the search for novel and clinically effective ones especially from plant sources. In this research, effort was made to assay *in vitro* antimicrobial potential of three crude extracts of *Tridax procumbens* leaves.
Methodology: Disc diffusion method was used for the assay against six bacteria isolates: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Proteus vulgaris* and *Klebsiella pneumoniae*. The study was carried out in three replicates for a period of 72 hrs.
Results: The results showed that highest sensitivity was observed with chloroform extract against *Streptococcus pyogenes* (34.00±0.58 mm), *Escherichia coli* (28.00±1.15 mm) and *Klebsiella pneumoniae* (28.00±0.58 mm). Ethanol extract exhibited moderate activity, while little antibacterial activity was seen with the aqueous extract. There was no inhibition of bacterial growth at all in the negative control (Distilled water). The sensitivity of chloroform extract of *Tridax procumbens* at 80µg/ml was higher but not significantly different (P>0.05) from ciprofloxacin (50 µg/ml) against

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Escherichia coli, *Proteus vulgaris*, *Streptococcus pyogenes* and *Klebsiela pneumoniae*.
Conclusion: *Tridax procumbens* appear to be a source of novel broad spectrum antimicrobial agent.

Keywords: Extract; leaves; Tridax procumbens; antibacterial activity.

1. INTRODUCTION

History reveals that plants have always been considered as an important source of medicine. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties [1].

Plant extracts represent a continuous effort to find new compounds with the potential to act against multi-resistant bacteria [2]. Africa's traditional healers use hundreds of indigenous plants for remedies [3] in traditional medicine which is still the first point of healthcare for many people in sub-Saharan Africa where there has been a long and rich tradition of obtaining treatments from herbs and trees [4].

Tridax procumbens Linn. (Asteraceae) has been used for treatment of boils, blisters and cuts by local healers [5] among many other ailments in humans. The leaves of the plant are squeezed and the extract applied to the affected part [6]. But comparative pharmacologic potential of *Tridax procumbens* with standard antimicrobial agents have only partially been investigated.

Tridax procumbens is a plant of open waste places, native of central tropical America, rich in flavonoids, now widely occurring in the tropics [7]. Flavonoids have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [1] Moreso, lipophilic flavonoids may also disrupt microbial membranes [8].

Plants extracts like *T. procumbens* and *Cybopogon citratus* were observed to show antimicrobial activity against multidrug resistant pathogens [9,10]. Therefore this research was designed to explore the *in vitro* therapeutic potential of *Tridax procumbens* using water, ethanol and chloroform as solvents. It is anticipated that the study will probably lead to the discovery of a potentially active drug to be added to available antimicrobial arsenal and perhaps serve as therapeutic alternative to mitigate the growing antimicrobial resistance, given that the effective life span of any antibiotic is limited [1].

2. MATERIALS AND METHODS

2.1 Collection of Plant

2.1.1 Plant material, identification and extraction

Fresh leaves of *Tridax Procumbens* were collected within Makurdi metropololis and identified at the Botany unit of the Department of Biological Sciences, University of Agriculture, Makurdi. The identified leaves were later air dried in the laboratory and powdered.

Extraction was done using water, ethanol and chloroform as solvents. Ten grams of the powdered leaves was weighed separately and transferred into three 500 ml capacity conical flask. 200 ml each of distilled water, ethanol and chloroform were added separately to each flask and refrigerated for 72 hrs with the flask shaken intermittently on daily basis. After 72 hrs the contents of the flasks were filtered through Whatman® No.1 filter paper and centrifuged at 1500 rpm for 10 minutes. The filtrates were evaporated to dryness in pre-weighed evaporating petri dishes on hot water bath at 40°C. The crude extracts so obtained were labeled and stored in screw capped vials in the refrigerator at 4°C until used for antibacterial assay.

2.2 Collection of Bacteria Samples and Cultivation

2.2.1 Sample collection

Samples of test organisms (from wounds adjudged to be infected) were obtained from laboratories of Benue State University Teaching Hospital, Federal Medical Centre, Bishop Murray Medical Centre, Immaculate Conception Hospital, General Hospital North bank and Madonna Hospital, in Makurdi Benue State, Nigeria.

2.2.2 Bacteria cultivation

Various culture media were prepared according to manufacturer's instruction. Each specimen

collected was cultured by inoculating the appropriate culture media (Mac conkey agar and Mueller Hinton) plates by streaking. The inoculated plates were then incubated at 37°C for 24 hrs. After 24 hrs the various cultured plates were read and the bacteria were clinically isolated based on standard microbiological methods such as colonial morphology, microscopy and biochemical characteristics. They were maintained in nutrient broth culture for 48 hrs and sub-cultured into nutrient agar 18-24 hrs before been used for the study.

2.3 Antimicrobial Bioassay

The Kirby-Bauer disc diffusion method [11,12] was used to evaluated the antibacterial activity *Tridax procumbens (T. procumbens)* extracts *in vitro*. Six bacteria strains were employed in the bioassay: *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Streptococus pyogenes* and *Klebsiella pneumoniae* isolated from different patients diagnosed of wound infection.

Each isolated bacteria strain was transferred to a test tube containing 5ml nutrient broth and the turbidity of each broth culture adjusted with saline to obtain turbidity visually comparable to that of a 0.5 McFarland standard.

The nutrient agar plates were inoculated by using sterile inoculating wire loop two hours after suspension of the test organisms in the broth. Sterile filter paper discs (Glass Microfibre filters, Whatman®; 6 mm in diameter) impregnated with 20 µg/ml, 40 µg/ml, 80 µg/ml and 100 µg/ml of *T. procumbens* extract were placed over plates seeded with the respective test organisms. Standard ciprofloxacin antibiotic discs at concentrations of 50 µg/ml and 100 µg/ml served as positive control, while distilled water was used as negative control. There were three replicates in each plate and for each test organism. The effective zones of inhibition of microbial growth was determined as the difference between zone of test agents and zone of inhibition of negative control (distilled water).

2.4 Statistical Analysis

Analysis of variance (ANOVA) was used to analyse all data using MINITAB statistical software. Results are expressed as standard error of the means. $P<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

The crude extracts of *T. procumbens* in three different solvents were tested for potential *in vitro* antimicrobial activity against six microorganisms. Results from the study showed that inhibition of bacterial growth occurred in a concentration-dependent manner (Tables 1, 2, 3 and 4) and agrees with the findings of [6]. Sensitivity of the test organisms was observed to be highest in the chloroform extract and least in aqueous extract (Table 1).

The sensitivity of chloroform extract (Table 3) at 80µg/ml was 28.00±1.15 mm, 25.00±0.00 mm, 27.67±0.88 mm, 23.67±0.33 mm, 34.00±0.58 mm, and 28.00±0.58 mm for *E. coli, P. aeruginosa, P. vulgaris, S. aureus, S. pyogenes* and *K. pneumoniae* respectively. The highest activity of chloroform extract at 80 µg/ml was against *S. pyogenes* (34.00±0.58 mm) and least against *S. aureus* (23.67±0.33 mm) both of which are gram positive. This activity profile indicates that the chloroform extract of *T. procumbens* is pharmacologically active against both gram positive and gram negative bacteria isolates.

Antimicrobial activity of ethanol extract of *T. procumbens* (Table 2) increased with increase in concentration. At highest concentration of 100µg/ml, ethanol extract showed bacterial growth inhibition of 20.00±1.15 mm, 19.00±0.58 mm, 17.00±1.15 mm, 18.00±1.15 mm, 17.67±1.45 and 14.00±0.58mm against for *E. coli, P. aeruginosa, P. vulgaris, S. aureus, S. pyogenes* and *K. pneumoniae* respectively. This result is in contrast with the report of [9] who reported resistance of *E. coli, P. vulgaris, S. aureus* and *K. pneumoniae* to ethanolic extracts of *T. procumbens* at 500 mg.

The low antimicrobial activity of ethanol extract of *T. procumbens* (20.00±1.15, 19.00±0.58, 17.00±1.15, 18.00±1.15, 17.67±1.45, 14.00±0.58) was statistically different ($P>0.05$) from that of ciprofloxacin at the same concentration with sensitivity of 45.33±0.88 mm, 34.00±0.58 mm, 35.67±0.88 mm, 41.33±0.88, 40.33±1.20 and 33.00±0.58 mm against *E. coli, P. aeruginosa, S. aureus, S. pyogenes* and *K. pneumoniae* respectively. In the contrary, [6] reported higher antibacterial activity for similar organisms using butanol fractionated extract of *T. procumbens*. Corroborating, [9] reported antipseudomonal activity of ethanolic extract of *T. procumbens* (20 mm) at concentration of 2.5 mg similar to 19.00±0.58 mm obtained at a concentration of 100 µg/ml.

Table 1. Antibacterial activity of *Tridax procumbens* extracts at 20 µg/ml concentration

Test drug	Mean diameter of inhibition of bacteria growth (mm) ± SEM					
	E. coli	***P. aeruginosa***	***P. vulgaris***	***S. aureus***	***S. pyogenes***	***K. pneumoniae***
Ciprofloxacin (50 µg/ml)	25.67±0.88	34.00±0.58	25.00±0.58	25.00±0.00	32.33±0.33	21.67±0.88
Chloroform extract	20.00±1.15	15.00±0.58	20.00±0.58	17.67±0.88	21.33±1.20	17.00±0.58
Ethanol extract	10.67±0.88	R	9.67±0.33	11.00±1.15	9.00±0.58	R
Aqueous extract	R	R	R	R	R	R
Distilled water	R	R	R	R	R	R

(R)= Resistant

Table 2. Antibacterial activity of *Tridax procumbens* extracts at 40 µg/ml concentration

Test drug	Mean diameter of inhibition of bacteria growth (mm) ± SEM					
	E. coli	***P. aeruginosa***	***P. vulgaris***	***S. aureus***	***S. pyogenes***	***K. pneumoniae***
Ciprofloxacin (50 µg/ml)	25.67±0.88	34.00±0.58	25.00±0.58	25.00±0.00	32.33±0.33	21.67±0.88
Chloroform extract	20.67±1.20	18.30±0.88	19.00±1.73	20.00±0.59	27.00±0.00	21.33±1.20
Ethanol extract	13.33±0.33	11.00±0.58	12.00±0.00	14.00±0.58	11.67±0.88	9.00±0.58
Aqueous extract	R	R	R	R	R	R
Distilled water	R	R	R	R	R	R

(R)= Resistant

Table 3. Antibacterial activity of *Tridax procumbens* extracts at 80 µg/ml concentration

Test drug	Mean diameter of inhibition of bacteria growth (mm) ± SEM					
	E. coli	***P. aeruginosa***	***P. vulgaris***	***S. aureus***	***S. pyogenes***	***K. pneumonae***
Ciprofloxacin (50 µg/ml)	25.67±0.88	34.00±0.58	25.00±0.58	25.00±0.00	32.33±0.33	21.67±0.88
Chloroform extract	28.00±1.15	25.00±0.00	27.67±0.88	23.67±0.33	34.00±0.58	28.00±0.58
Ethanol extract	18.67±0.88	15.00±0.57	15.00±0.00	17.00±0.58	15.00±0.58	13.67±0.88
Aqueous extract	9.00±0.58	8.00±0.57	R	9.00±0.00	8.33±1.20	8.33±0.67
Distilled water	R	R	R	R	R	R

(R)= Resistant

Table 4. Antibacterial activity of *Tridax procumbens* extracts at 100 µg/ml concentration

Test drug	Mean diameter of inhibition of bacteria growth (mm) ± SEM					
	E. coli	***P. aeruginosa***	***P. vulgaris***	***S. aureus***	***S. pyogenes***	***K. pneumonae***
Ciprofloxacin (100 µg/ml)	45.33±0.88	34.00±0.58	35.67±0.88	41.33±0.88	40.33±1.20	33.00±0.58
Chloroform extract	34.00±0.58	30.00±0.58	31.67±0.33	28.00±1.15	38.00±0.58	43.00±0.58
Ethanol extract	20.00±1.15	19.00±0.58	17.00±1.15	18.00±1.15	17.67±1.45	14.00±0.58
Aqueous extract	10.00±1.15	8.67±0.33	R	10.33±0.88	8.33±0.58	10.33±0.33
Distilled water	R	R	R	R	R	R

(R)= Resistant

All the tested organisms were resistant the aqueous crude extract of *T. procumbens* at concentrations below 80 µg/ml of crude extracts which agrees with the findings of [9]. Only marginal antibacterial activities of 10.00±1.15 mm, 8.67±0.33 mm, 10.33±0.88 mm, 8.33±0.58 mm and 10.33±0.33 mm against *E. coli, P. aeruginosa, S. aureus, S. pyogenes* and *K. pneumoniae* respectively was observed. It appears that the antibacterial activity of the crude extract of *T. procumbens* is negligible with increasing polarity and decrease in molecular weight of the solvent.

Comparatively, the antibacterial activity of crude extract of *T. procumbens* assayed is the order: chloroform extract> Ethanol extract>Aqueous extract.

4. CONCLUSION

Extracts of *T. procumbens* appears to possess good but varying sensitivity for the test organisms *in vitro.* The chloroform extract of *T. procumbens* showed better profile of bacteria growth inhibition than aqueous and ethanol extracts. The activity of chloroform and ethanol extracts of *T. procumbens* against tested organisms is suggestive of antimicrobial activity comparable to the broad spectrum (flouroquinolone) ciprofloxacin and may be a potential novel source of broad spectrum antimicrobial agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Marjorie MC. Plant products as antimicrobial agents. Clinical Microbiology Review. 1999;12(4):564-582.
2. Aliyu MS, Hanwa UA, Tijjani MB, Aliyu AB, Ya'u B Phytochemical and antibacterial properties of leaf extract of *Stereospermum kunthianum* (Bignoniaceae). Nigerian Journal of Basic and Applied Science. 2009;17(2):235-239.
3. Peter K, Susnne D, Sabine S, Rudeka M, Stefaine H, Tanja P, Paul K, Achim H, Annette K. *In vitro* and *in vivo* Antimalarial Activity Assays of seeds from *Balanites aegyptiaca*: Compounds of the Extract show Growth Inhibition and Activity against Plasmodium Aminopeptidase. Journal of Parasitological Research. 2011;2011:9. Article ID 368692.
   DOI: 10.1158/2011/368692. (Accessed on 4/1/2015)
4. Arzu B, Fatma PK, Arzu UT. *In vitro* antibacterial and antitumor activities of some medicinal plant extracts, growing in Turkey. Asian Pacific Journal of Tropical Medicine. 2013;2012:616-624.
5. Sreermulu N, Sateesh S, Rangan A, Vatsavaya SR. Ethnobotanical-medicne for common human ailments in Nalgonda and Warangal district of Telanagana, Andhra Pradesh, India. Annals of Plant Science. 2013;2(070):222-229.
6. Dharsarathan P, Hemalatha N, Theriappan P, Ranjitsingh AJA. Antibacterial activities of extracts and the fractions of leaves of *Tridax procumbens* Linn. Australian Journal of Biomedical Science. 2011;1(1):13-17.
7. Gamboa-Leon R, Vera-Ku M, Peraza-Sanchez SR, Ku-Chulim C, Horta-Baas A., Rosada-Vallado, M. Antileishmanial activity of a mixture of *Tridax procumbens* and *Allium Sativum* in mice. Parasite. 2014;21:15-27.
   DOI:10.1051/parasite/2014016. PMC 3980668. PMID 24717526.
8. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma M. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. Journal of Ethnopharmacology. 1996;50:27–34.
9. Chitra P, Ujjwala K, Manjusha B, Sowmya M, Mrudula P, Yashwant D. Antibacterial Activity of *Tridax procumbens* with Special Reference to Nosocomial pathogens. British Journal of Pharmaceutical Research. 2011;1(4):164-173.
10. Oloche JJ, Mailafia S. Antimicrobial assay of methanolic extract of fresh lemon gass (*Cymbopogon citratus*) in Zaria, Nigeria. Journal of Advancement in Medical and Pharmaceutical *Sciences.* 2011;5(1):33-39.

11. Ellof JN. A sensitivity and quick microplate method to determine the minimum inhibitory concentration of plant extracts for bacteria. Plant Medica. 1998;64:711-713.

12. Andrews JM. BSAC standardized disc susceptibility testing method. Journal of Antimicrobial Chemotherapy. 2004;53:713-728.

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