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Antibacterial evaluation of aqueous and methanol extracts of *Tridax procumbens* flower on selected gram negative and gram-positive bacteria

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Abstract

It is obvious from available literatures that not much has been done with respect to *Tridax recumbence* on its effects bacterial in South - south Nigeria, hence the need to investigate the antibacterial activities of the aqueous and methanol flower extract of *Tridaxprocumbens*against the following selected clinical isolates: *Staphylococcus aureus, Lactobacillus and Shigella flexnerii.* Theflower extract of *Tridax recumbence*was obtainedusing cold maceration technique by weighing the powdered plant and macerated in distilled water and 70% methanol separately and allowed to stand at room temperature for a period of 4 days and 7 days respectively with frequent agitation. The antimicrobial assay done using agar well diffusion method and zones of inhibition for both methanol and aqueous extractand zones of inhibition recorded. The study shows that at a concentration of 300mg/ml of the methanol extract of *Tridax procumbence* on the test organisms; *S. aureus, Lactobacillus* and *S. flexnerii,* the zones of inhibition obtained were 8.0mm, 8.5mm and 6mm respectively. *Lactobacillus*and *S. aureus* showed an increase antibacterial effect when extract concentration was increased but *S. flexneriis*howed irregular zones inhibition with its value observed at 37.5mg/ml and 150mg/ml. The Minimum Inhibitory Concentration on the test organisms was 150mg/ml for *S. aureus* and 37.5mg/ml for *Lactobacillus* while *S. flexnerii* showed resistance to the extract at all the concentrations used. *Tridaxprocumbence* flower extract can be a good candidate for antibacterial agents and it is suggested that the flower extract could be a promising lead compound for the synthesis of effective chemotherapeutics.

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Introduction

In recent time, the use of traditional medicine has expanded and is fast becoming popular. Having formed the basis of sophisticated traditional medicine, plants have been used for thousands of years by people in Nigeria and many countries. It has been estimated that herbal medicines serve about 80% of the world population health need for millions of people in the rural areas of developing countries and more than 65% of the global population rely more on the use traditional medicine for basic health care (Kale and Shake, 2013).Gradually there have been a growing interest in the use of medicinal plants because herbal medicines have been proven to be safe and without any adverse effects especially when compared to Orthodox medicine. Plantsextracts and their chemical derivatives has been used to treat diseases which may be topical, subcutaneous or systemic and they have been reported to be effective (Mohale *et al*, 2014).

Plants with antimicrobial tendencies has become the need of today's research because mankind is currently faced with the problem of emerging resistance in virtually all pathogens (Peterson and Dalhoff, 2004).

The development and spread of multi drug resistant organisms especially in the hospital environment continues to be a burning global issue due to the indiscriminate and irrational use of antibiotics (Chitra *et al,* 2011). The high cost of existing synthetic antibiotics, their side effects and toxicities justify the need to search newer antimicrobial agents from plant origin.This study is therefore aimed at evaluating the antimicrobial activity of the aqueous and methanolic extract of *Tridax procumbence* Linn flowers using Gentamycin as a control.

Materials and methods

Materials and apparatus

Test tubes, beakers, petri dishes, glass rods, measuring cylinders, Durham's tubes, droppers, porcelain dishes, cotton wool, sterile 5ml and 2ml syringes, masking tape, disposable gloves, face masks, surgical blades, aluminium foil, spatula, sterile swab sticks, muslin cloth, filter paper (Whatman Limited England), spirit lamp, Autoclave, Incubator (Uniscope SM9052 laboratory incubator ®), Digital weighing balance (KERO BLG 300).

Culture media

Nutrient Agar (Titan Biotech Ltd, India), Nutrient Broth **(**Titan Biotech Ltd, India), Mueller Hinton agar.

Reagents

Methanol, savlon, distilled water

Collection of plant materials

The flowers of *Tridaxprocumbence* Linn were collected by handpicking from different areas of Abraka, Delta State and they were identified in the department of Pharmacognosy, Faculty of Pharmacy, Delta State University, Abraka. The freshly collected plant flowers were air dried under shade and reduced to coarse powders using a blender. This was then properly packaged and stored until use.

Plant Yield

About 200g of the dried powdered flowers of *Tridaxprocumbence* Linn yielded 27.6g of the aqueous extract which corresponds to 13.8% of the powdered plant and 51.5g of the methanol extract which corresponds to 10.3% of the powdered plant.

Extraction procedures

Aqueous extract: 200g of the coarse powder was placed in a glass bottle and three litres of distilled water was added. The sample was kept for 4 days with intermittent shaking. The percolates were filtered with muslin cloth and the filtrates was concentrated at 60^{O}C under reduced pressure. The final product was then stored prior to testing.

Methanol extract: 200g of the coarse powder was placed in a glass bottle and one litre of 70% Methanol was added. The sample was kept for 7 days with intermittent shaking. The percolates were filtered with muslin cloth and the filtrates was concentrated at 60^{O}C under reduced pressure. The final product was then stored prior to testing.

Source of organisms

The test organisms are human pathogenic bacteria from Pharmaceutical Microbiology laboratory, Department of Microbiology, Faculty of Pharmacy, Delta State University, Abraka. *Staphylococcus aureus, Lactobacillus, and Shigella flexnerri*were used. These bacteria were confirmed using standard biochemical tests after which they served as test bacteria.

Biochemical tests

Catalase test, Hydrogen Sulphide test, Fermentation test, Oxidase test, Citrate utilization test, Urease test and gram staining technique were all carried out as described by Monica 2004.

Preparation of media

Each of the media used was prepared according to manufacturer's instruction. Serial dilution: For the serial dilution of the aqueous and methanol extract, 2ml of sterile water was transferred aseptically into 6 different sterile test-tubes. A 2ml volume of the aqueous extract was transferred into the first tube and the tube was rocked slowly, and from this another 2ml was taken and transferred to the second tube and likewise rocked for proper mixing. This process was repeated for every other test tube until the final tube where 2ml volume was equally taken and discarded. The above dilution process resulted in six concentrations of the extract. (300mg/ml, 150mg/ml, 75mg/ml, 37.5mg/ml, 18.75mg/ml, 9.375mg/ml).

Standardization of bacteria inoculum

All the test organisms were subcultured on nutrient agar for 24 hours and few colonies were transferred into 5ml of sterile nutrient broth in test tubes and incubated for 30 minutes at 37^{O}C.

Determination of antimicrobial activity

The spread plate method was used to assay for the antimicrobial activity of the flower extracts. Using sterile cork borer (6mm in diameter), seven wells were made on an already set Mueller Hinton agar plateand were asceptically flooded with the standardized bacterial culture. Gentamycin (40mg/ml) was used as the control.

0.1ml of the various concentrations of the extract was introduced into the bored holes. Twelve plates of the agar were prepared, with two plates for each organism. This was done for the aqueous and methanolic flower extract. Gentamycin was also placed on each plate as control. The inoculated petri dishes were left for few minutes for the extract to diffuse into the agar. The plates were then incubated at 37^{O}C for 24 hours. The zone of Inhibition was then measured using a millimeter rule.

Determination of minimum inhibitory concentration (MIC)

Agar diffusion techniquewas employed in the determination of the MIC. 1ml of the extract was mixed with 19ml of molten Mueller Hinton agar gently rocked allowed to solidify. This procedure is repeated for the six concentrations of the aqueous extract. The test organisms were streaked on the agar and incubated for 24 hours at 37^{O}C. The MIC was taken as the least concentration that inhibited the growth of the test organisms.

Results

From Table 1b the zone of inhibition *S. aureus* was within the range of 2.5mm and 8mm while for *L. bacillus* and *S. flexnerii*, the inhibition zones were within 5mm -8.5mm and 6mm–7.5mm.

Table 1a. Inhibition zones of aqueous extracton the different isolates.

Organism	9.375 mg/ml	18.75 mg/ml	37.5 mg/ml	75 mg/ml	150 mg/ml	300 mg/ml
S. aureus	-	-	-	-	-	-
Lactobacillus	-	-	-	-	-	-
S. flexnerri	-	-	-	-	-	-

Key: - means no zone of inhibition

From Table 1a the aqueous extract showed no zone of inhibition against the organism at all concentration.

The control antibiotic showed high zone of inhibition on the organism compared to what was observed in Table 1a and b.

Discussion

The result obtained from this study shows that the aqueous extract of *Tridaxprocumbens* does not possess antimicrobial activity against *Staphylococcus aureus, Lactobacillus and Shigella flexnerii* (Table 1a.) The results also show that the methanol extract of *Tridaxprocumbens* possess antimicrobial activity against the following organisms;*Staphylococcus aureus, Lactobacillus and Shigella flexnerii* as shown in the Table 1b with their zone of inhibition observed at different concentrations and their minimum inhibitory concentration which is in line with the work done byBharathi *et al*. 2012.

Table 1b. Inhibition zones of methanol extracton the different isolates.

Organism	9.375 mg/ml	18.75 mg/ml	37.5 mg/ml	75 mg/ml	150 mg/ml	300 mg/ml
S. aureus	2.5 mm	5 mm	5.5 mm	6.5mm	7 mm	8 mm
Lactobacillus	5 mm	5 mm	5.5 mm	7.5mm	7 mm	8.5 mm
S. flexnerri	6 mm	6 mm	7.5 mm	7mm	7.5 mm	6 mm

The antimicrobial effectiveness of the flowers extracts of *Tridaxprocumben s*is seen to be dependent on the concentration. The presence of the zones of inhibition showed that the extract possesses antibacterial activity which was more effective against gram positive organisms than the gram-negative organisms. Although the zones of inhibition were lower than that exhibited by the control drug (Gentamycin) as seen in Table 1c. This could be due to the fact that the plant extract was in the crudestate and contains other constituents that do not possess antibacterial property.

Table 1c. Zone of inhibition of the control antibiotic.

Organism	Gentamycin (40mg/ml)
S. aureus	30 mm
Lactobacillus	30 mm
S. flexnerri	24.5 mm

The highest zone of inhibition was seen in *Staphylococcus aureus*and *Lactobacillus* at the highest concentrations of the extract (300mg/ml) where inhibition were 8mm and 8.5mm respectively except for *Shigella flexnerii*which was seen at 37.5mg/ml and 150mg/ml having a zone of inhibition of 7.5mm. *Shigella flexnerii*showed irregular growth inhibition by the extract which suggests that the growth of the organisms is only being inhibited or depleted at a fixed concentration.

However, in the case of *S. aureus* and *Lactobacillus* as the concentration increased the antibacterial effect also increased as shown from the zone of inhibition. This study shows that the increase or decrease in concentration in proportion to the growth rate of the organism is sometimes directly or inversely based on each organism.

It can however be deduced that the crude flower extract of the plant *Tridaxprocumbens*is active against the different test microbes at varying degrees.

This result confirms its use ethnomedically for the treatment and management of different bacteria and fungi infectionswhich could also be seento be similar to the work done bySuffredini *et al* 2004.The MIC showed that the flower extract had antimicrobial properties against all the test organisms in varying degree except *Shigella flexnerii Staphylococcus*

aureus and *Lactobacillus* were susceptible to the flower extract but not at all concentrations used. *Staphylococcus aureus* showed resistance at 9.375mg/ml, 18.75mg/ml, 37.5mg/ml, 75mg/ml and was susceptible at 150mg/ml and 300mg/ml. *Lactobacillus* showed resistance at 9.375mg/ml, 18.75mg/ml, and was susceptible at 37.5mg/ml, 75mg/ml, 150mg/ml and 300mg/ml.

Table 2. Minimum inhibitory concentration (Methanol extract).

Organism	9.375 mg/ml	18.75 mg/ml	37.5 mg/ml	75 mg/ml	150 mg/ml	300 mg/ml
S. aureus	G	G	G	G	NG	NG
Lactobacillus	G	G	NG	NG	NG	NG
S. flexnerri	G	G	G	G	G	G

Key: NG = No Growth.

G = Growth.

These suggests that the higher the concentration of the flower extract the higher the antimicrobial activity are shown in Table 2. However, *Shigella flexneri*is howed resistance to the extract at all the concentrations used. The results from the study show that the flower extract of *T. procumbens* has good therapeutic activities against the gram-positive organisms although at different level of effectiveness and minimal activities against gram negative organisms.

Conclusion

The aqueous extract of *Tridaxprocumbens* flowers was found not to possess any antibacterial activity against the selected isolates while the methanol extract does which therefore supports the traditional use of the plant for treatment of ailments associated with these bacteria.

Based on this research it is therefore recommended that medicinal plants like *Tridax procumbens* be used as cheap and readily available remedies for bacterial infections in developing countries especially for infections caused by the tested isolates. However, there is need for further investigation to isolate and characterize the bioactive compounds to develop new antibacterial drugs.

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