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# *In vitro* antibacterial evaluation of aqueous and methanol extracts of *Tridax procumbens* flower on selected pathogenic organisms

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## Abstract

This study aimed at evaluating the antibacterial activity of the aqueous and methanol extracts of *Tridax procumbens* flowers against selected organisms. The powdered flowers of *Tridax procumbens* were extracted by cold maceration with water and methanol (70% v/v). The aqueous and methanolic extracts were subjected to antibacterial sensitivity test against selected organisms; *Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa* by determining the zone of inhibition using the agar well diffusion method. Results yielded varying MIC for each organism. The methanolic extract had an MIC value between 18.75mg/ml -150mg/ml and the aqueous extract had MIC values between 18.75mg/ml – 37.5mg/ml. In conclusion, both aqueous and methanolic extracts of *Tridax procumbens* flowers showed antibacterial activity against the selected isolates implying that the extracts of *Tridax procumbens* can serve as a good candidate for the antibacterial agent.

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## Introduction

Plants are an important source of drugs. They can serve as a direct or indirect source of drugs. The increasing resistance of many infectious agents to synthetic drugs is on the rise causing a need for the search for newer sources of antibiotics by research institutions, pharmaceutical companies and academia (Latha and Kannabarian, 2006). Natural products have been documented to play critical roles in modern drug development especially for antibacterial and antitumor agents. In the developing world, 80% of the total population is dependent on natural products because of their efficacy and safety tested over time (Veerasham, 2012). Each plant in nature possesses certain medicinal value, this makes them unique (Patel, 2015). These constituents may be housed by either the leaves, stem, bark, root, corm, bud, rhizome, flowers or seed. These constituents are termed active principles, they can work to inhibit greatly the life process of microbes especially the disease-causing ones, either singly or as a combination. Many works have been done which aimed at knowing the different phytochemicals and antimicrobial constituents of medicinal plants (Adamu *et al.,* 2005) and their use in the treatment of microbial infections (both topical and systematic applications) as an alternative to chemically synthetic drugs to which many infections have become resistant (Anyamene and Ezeadila, 2010). Gram-negative bacteria, particularly the Enterobacteriaceae family and non-fermenters are developing resistance to antibiotics. This antimicrobial resistance is a leading cause of morbidity and mortality as well as increased cost of health care and issues in the health concern of individuals. Their outer membrane protects them from many antibiotics (including penicillin), detergents and lysosomes, making them a medical challenge. Misuse of antibiotics is also a cause of antibiotic resistance.

The WHO defines antimicrobial resistance as a microorganism's resistance to an antimicrobial drug that was once able to treat an infection by that microorganism (WHO, 2014). Due to the emergence of increasing resistance to available synthetic antibiotics by microorganisms, there is a need for an alternative means of eradication of infectious agents by natural means. Plants provide a natural source of lead compounds that can be used in the control of the spread of infections globally. Hence,this study seeks to evaluate the antibacterial potential of the methanolic and aqueous extracts of the flowers of Tridax procumbens against the selected isolates of three gram-negative bacteria.This study aims to evaluate the antibacterial activity of methanol and aqueous extracts of the flowers of *Tridax procumbens* against selected isolates of three gram-negative bacteria.

## Materials and methods

*Identification of test microorganisms*

Biochemical tests were carried out to confirm the identity of the test organisms obtained for the study. The following biochemical tests were carried out on the isolates as described by Cowan, (1985): Oxidase, Indole, Citrate utilization, Catalase.

*Collection and identification of Tridax procumbens*

Taxonomically identified *Tridax procumbens* flowers were handpicked in October 2017. Identification and authentication of the flowers were done at the Department of Pharmacognosy and Traditional Medicine of the Faculty of Pharmacy, Delta State University, Abraka, Nigeria. The sample obtained was air-dried and reduced to a coarse powder using a sterile manual hand engine then stored at room temperature.

*Extraction of Tridax procumbens* Linn *flowers*

Coarsely powdered flowers of *Tridax procumbems* were extracted using a sufficient amount of 70% methanol and distilled water. Two hundred grams (200g) of the powdered *T.procumbens* sample was soaked into a mixture of 2900ml distilled water and 300ml of 10% methanol for 3 days. Two hundred grams (200g) of the powdered *T.procumbens* flowers was also soaked into 2000ml of 70% methanol for 7days. The extract was filtered and concentrated and stored in a refrigerator at 4$^{o}$C until when it was required for use.

*Qualitative phytochemical analysis*

Phytochemical analysis was carried out using the method of Trease *et al*, 1983; Harbourne, 1973. The extracts were screened for alkaloids, tannins, terpenes, saponins, and flavonoids.

*Preparation of media*

Each medium used was prepared according to the manufacturer's instruction.

*Determination of zone of inhibition*

Aqueous and methanolic extracts of *Tridax procumbens* flowers were screened for their antibacterial activity against E. *coli, P. aeruginosa,* and *P. vulgaris*. The medium was sterilized in an autoclave for 15 minutes at 121°C and poured into the plates then allowed to solidify. Mueller Hinton agar plates were prepared and stirred well.

The plates were labelled according to the test organism being used in each plate and Gentamicin serving as a positive control. Using a 6mm cork borer, wells were punched in the agar plate. 1ml of each concentration of the extract was placed into the corresponding well using a Pasteur pipette. Gentamicin which served as a positive control for the experiment was placed in the well at the center of the plate.

This was done for the other test organisms using the methanol extract. The aqueous extract was also inoculated into agar plates streaked with the test organism using the same procedure as above. The plates were incubated for 24hour at 37°C. Antibacterial assay was carried out in duplicate and the average reading was taken.

*Determination of minimum inhibitory concentration (MIC)*

The MIC was determined using the serial dilution in solid medium. Mueller Hinton agar was prepared according to manufacturer instruction. 19ml of molten agar mixed with 1ml of the plant extract was poured onto the Petri dish and allowed to solidify. Each Petri dish contained different concentrations of the aqueous and methanolic plant extract.

The agar plates were divided into three parts and labelled for each test organism. Using a sterile swab stick, an overnight broth culture of the organism was spotted on the surface of the agar plate on the part of the plate labelled. The plates were then incubated for 24 hours at 37°C.

**Results and discussion**

*Tridax procumbens* has been documented to have many biological activities (Mir *et al* 2017). Research has also been carried out to determine its antiviral, antibiotic, wound healing properties, antioxidant, insecticidal, and anti-inflammatory activities *in-vitro* studies as well as in animal models (Suseela *et al*, 2002).

In this study, the antibacterial and phytochemical screening of flowers of *Tridax procumbens* was done.

**Table 1.** Biochemical test results on selsted organisms.

| Cultural characteristics | Gram stain | Citrate | Indole | Oxidase | Catalase | Tentative identification |
|---|---|---|---|---|---|---|
| Colonies were green and mucoid on agar plate | GNR | + | _ | + | + | *Pseudomonas aeruginosa* |
| Pink convex colonies with smooth edges | GNR | + | + | _ | + | *Eschericia coli* |
| Large, circular, gray, smooth colonies | GNR | + | + | _ | + | *Proteus vulgaris* |

GNR: gram negative rod; + : positive; _: negative.

Table 1 above is a presentation of some confirmatory test carried on the organisms in identifying the seleted organisms.

From Table 2 above, it shows that Terpene, Tannin, flavonoids, saponins and alkaloid were present in both the aqueous and methanol extract of *Tridax*

*Procumbens* with the work done previously by Oghenemaro *et al* 2019. The result obtained from this study showed that the methanolic and aqueous extracts of *Tridax procumbens* flowers possess antibacterial activity against the test organisms; *E.coli, P.vulgaris,* and *P.aeruginosa*. The antibacterial activity of the flowers of *Tridax procumbens* methanolic and aqueous extract was evaluated by comparing the zone of inhibition (mm) with that of the standard antibiotic Gentamicin using the agar well diffusion method as can be seen in Table 3 and 4 above.

**Table 2.** Qualitative analysis results revealing phytochemicals present in the extracts.

| S/N | Name of test | Aqueous extract | Methanolic extract |
|---|---|---|---|
| 1 | Terpene | + | + |
| 2 | Tannins | + | + |
| 3 | Flavonoids | + | + |
| 4 | Saponins | + | + |
| 5 | Alkaloids | + | + |

+ : present.

This indicated that the organisms varied in their sensitivities to various concentrations of the extracts. The test organisms showed better sensitivity to the methanolic extract of *Tridax procumbens* flowers compared to their corresponding sensitivity to the aqueous extracts.

**Table 3.** Zones of inhibition of aqueous extracts of *Tridax procumbens*.

| | Zones of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| Organism | 300 mg/ml | 150 mg/ml | 75 mg/ml | 37.5 mg/ml | 18.75 mg/ml | 9.375 mg/ml | Gentamicin |
| *E.coli* | 14.5 | 12.5 | 11.5 | 9.5 | 8 | 6 | 28.25 |
| *P.aeruginosa* | 13 | 12 | 10 | 9 | 9 | 6.5 | 25.25 |
| *P.vulgaris* | 15 | 13.5 | 11.5 | 8.5 | 6 | 4.5 | 29.5 |

The extracts showed reasonable efficacy in their activity when compared to the standard antibiotic Gentamicin. Table 3 and 4 and shows an interpretation of the sizes of inhibition zones obtained from the standard antibiotic and the plant extracts against the test organisms used. Clear zones that appeared around a well indicated the degree of inhibition provided by the concentration of the extract in a particular well against the test organism while those with cloudy appearance indicated that not all the bacteria in that region was eliminated by the extract.

**Table 4.** Zones of inhibition of methanolic extracts of *Tridax procumbens*.

| | Zones of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| Organism | 300mg/ml | 150mg/ml | 75mg/ml | 37.5mg/ml | 18.75mg/ml | 9.375mg/ml | Gentamicin |
| *E.coli* | 11 | 8.5 | 7.5 | 4.5 | 3.5 | 2.5 | 25.5 |
| *P.aeruginosa* | 11.5 | 8 | 7 | 4.5 | 4 | 2.5 | 28.75 |
| *P.vulgaris* | 9.5 | 7 | 5.5 | 4 | 3.5 | 2.5 | 29 |

The minimum inhibitory concentration was also carried out as indicated in Table 5 and 6. The MIC of the methanolic extract varied between 18.75-150 mg/ml while that of the aqueous extract varied between 18.75-37.5 mg/ml. Hence, the aqueous extract showed higher activity compared to the methanolic extracts. The results of the MIC showed that *E.coli* and *P.aeruginosa* were more sensitive to

the aqueous extract and *P.vulgaris* was more sensitive to the methanolic extract with *E.coli* having a MIC of 37.5 mg/ml for the methanolic extract and 18.75 mg/ml for the aqueous extract, *P.vulgaris* had a MIC of 18.75 mg/ml for the methanolic extract and 37.5 mg/ml for the aqueous extract, while *P.aeruginosa* showed reasonable sensitivity to the aqueous extract with a MIC of 18.75 mg/ml whereas the methanolic extract gave a MIC of 150mg/ml.

The extracts showed considerable efficacy in their activity when compared to the standard antibiotic, gentamicin. Low MICs observed for the aqueous extract of *T.procumbens* flowers could be of significance in health care as it could be used as an alternative to Orthodox antibiotics in the treatment of diseases due to *E.coli* and *P.aeruginosa*, especially as microorganisms quickly develop resistance to some known synthetic antibiotics and this will, in turn, minimize the cost of obtaining better health care. Tables 4 and 5 show an interpretation of the sizes of the zones of inhibition obtained from the extracts and the standard antibiotic at different concentrations against the test organisms used. Phytochemical screening of both extracts revealed the presence of saponins, alkaloids, tannins, terpenes, and flavonoids.

**Table 5.** MIC for Methanolic extract.

| | Concentration of extract | | | | | |
|---|---|---|---|---|---|---|
| Organism | 300mg/ml | 150mg/ml | 75 mg/ml | 37.5mg/ml | 18.75mg/ml | 9.375mg/ml |
| *E. coli* | - | - | - | - | + | + |
| *p. vulgaris* | - | - | - | - | - | + |
| *p. aeruginosa* | - | - | + | + | + | + |

+ = Growth; - = No growth.

These compounds are known to be biologically active, they are termed secondary metabolites and are believed to be responsible for the observed antibacterial effects. Flavonoids are phenol-containing compounds present in plants known to be synthesized in response to microbial infection. Jamine *et al*, 2007 have reported flavonoids to be effective antibacterial substances *in-vitro* against a wide array of infectious agents. Tannins are water-soluble polyphenolic compounds also known to be potential antimicrobial agents. They have been reported to inhibit the growth of many fungi, bacteria, yeasts and viruses by precipitating microbial protein. Dhanabalan *et al*, 2008 reported antiparasitic, antiphlogistic, antisecretolytic and antimicrobial effects as physiological effects produced by tannins. The use of tannin-containing plants in the phytotherapy of inflammation of the mouth, non-specific diarrhea, throat and slightly injured skin has been reported by Naveen *et al*, 2008.

**Table 6.** MIC for aqueous Extract.

| | Concentration of extract | | | | | |
|---|---|---|---|---|---|---|
| Organism | 300mg/ml | 150mg/ml | 75 mg/ml | 37.5mg/ml | 18.75mg/ml | 9.375mg/ml |
| *E. coli* | + | - | - | - | - | + |
| *p. vulgaris* | + | - | - | - | + | + |
| *p. aeruginosa* | + | - | - | - | - | + |

+ = Growth; - = No growth.

The phytochemical investigation of *T.procumbens* by Aarti, 2010 revealed the presence of alkaloids, tannins, saponins, carbohydrates, steroids, proteins, cardiac glycosides and flavonoids. Different studies have provided evidence that some medicinal plants might be potential sources of new antibacterial agents (Kone *et al*, 2004; Rahman *et al*, 2011). Since time immemorial, early man has been said to use plants in the treatment of various ailments. Herbal medicine is still practiced in many parts of the world for the

treatment and prevention of diseases especially in local regions with a variety of vegetation.

It can be concluded that methanolic and aqueous extracts of *T. procumbens* flowers have antibacterial activity and supports their use in traditional medicine for the treatment of bacterial infections. This proves that herbal medicines can be as efficacious as Orthodox medicine in combating some pathogenic bacteria.

**Conclusion**

The antibacterial activity of aqueous and methanolic extracts of the flowers of *Tridax procumbens* was evaluated in this study. From the study, both the aqueous and methanolic extracts had significant antibacterial activity against the selected organisms which are mainly organisms implicated in wound infections, however, the effect of the aqueous extract was higher than that of the methanolic extract. Therefore, *Tridax procumbens* extracts can serve as a good candidate for antibacterial agent particularly against the organisms used in this study, especially the aqueous extract. The extracts of *T.procumbens* could serve as an alternative source of the antibacterial agent as against currently available synthetic antibiotics to which most organisms have developed resistance.

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