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Research Article

Phytochemicals and Antibacterial Activities of Leaf Extract of *Tridax procumbens* Linn. On *Staphylococcus aureus* and *Escherichia coli*

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Abstract: Medicinal plants have been used as remedies for human diseases because they contain components of therapeutic value. Plants serve as the greatest source to obtain new antimicrobials. An increasing interest in herbal remedies has been observed in several parts of the world. *Tridax procumbens* Linn. commonly known as 'coat button' is a weed and a pest plant. Previous researches have shown that the plant has antimicrobial activities and pharmacological effects. This study aimed at evaluating the phytochemical compounds and antibacterial activities of the leaf extract of *Tridax procumbens*. Leaf ethanol and water extracts were used in this study. The test organisms were *Staphylococcus aureus* and *Escherichia coli*. The antibacterial assay was performed by disc diffusion method. Treatment extract concentrations were 250mg/ml, 200mg/ml, 100mg/ml and 50mg/ml and distilled water (negative control) and an antibiotic Amphicloxa (positive control). Each treatment was replicated three times. The leaf extract was tested for the presence of tannins, saponins, flavonoids, terpenoids, alkaloids and glycosides. The plates were examined for zones of inhibitions. Data collected was subjected to analysis of variance. Treatments were separated and compared using LSD at $P < 0.05$. Both ethanol and water extracts were biologically active against the two microbes. Ethanol extract showed greater inhibition (9.29 mm) in comparison to water extract (7.19 mm). *Escherichia coli* showed greater susceptibility as compared to *Staphycoccus aureus*. The phytochemical screening of the leaf extract of *Tridax procumbens* revealed the presence of tannins, alkaloids, saponins, flavonoids, terpenoids and glycosides. It is possible that the growth inhibition observed in the study occurred due to the presence of these different chemical compounds. From these findings, ethanolic and water extracts of the leaf of *Tridax procumbens* Linn. showed a good potential as a source of new drug for treating infections caused by *Staphylococcus aureus* and *Escherichia coli*.

Keywords: antibacterial assay, Ethanol extract, new drug, phytochemicals, water extracts.

INTRODUCTION

The practice of traditional medicine is as old as the human race itself (Kigen *et al*., 2013; Mir *et al*., 2013). Traditional medicine is an important source of potentially new useful compounds for the development of chemotherapeutic agents (Orakwelu, 2011; Jain and Amita, 2012; Sharma and Kumar, 2008). About 80% of the population in the developing world is still dependent upon the traditional medicine available in their surrounding (Reema and Adel, 2011; Kiringe, 2006; Musyimi *et al., 2008;* Kigen *et al*., 2013; Kaigongi, 2014), vegetation, to meet their demands. Plants are considered the greatest source to obtain new antimicrobials. They rely on medicinal plants because of their effectiveness and lack of modern health care alternatives (Wadankar *et al*., 2011; Kiringe, 2006). Most rural communities depend on traditional medicine for the cure of diseases and ailments because most of the modern equipment's are expensive and service delivery too expensive to afford (Osazee *et al*., 2013; Kigen *et al*., 2013). Traditional medicine is widely used in Kenya and about 400 plant species have been recorded to be used in traditional remedies (Kaigongi, 2014). In order to find novel antimicrobial agents with new modes of action, plants have been explored as sources for the identification of new and effective antimicrobials. Plant materials continue to play an important role in the maintenance of human health as over 50% of all modern chemical drugs originates from natural plant source (Osazee *et al*., 2013; Kaigongi, 2014). Several plants are now being used in part or as whole to treat many diseases. Active components of

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these plants are now being investigated, extracted and developed into drugs with little or no negative effects or contra-indications (Osazee *et al*., 2013). Biological activities of plants are attributed to the class and concentration of phytochemical constituents which make some plant extracts exhibit a variety of activities (Kaigongi, 2014).

Therapeutic plants can battle resistant pathogens and have rendered numerous convectional medications out of date in the treatment of diseases (Boadi *et al*., 2015). Numerous medication utilized in medicine are acquired from plants. The medicinal values of plants lie on their phytochemical constituents. The most active of these bioactive constituents of plants are alkaloids, tannins, steroids, terpenoids, phenolics and other compounds. These have been found to produce a definite physiological action on human body (Boadi *et al*., 2015; Taiye and Iyamah, 2014). The emergence of pathogenic microorganisms that are resistant or multi-resistant to major class of antibiotics has increased in recent years due to the indiscriminate use of synthetic antimicrobial drugs. This has led to the use and search for drugs and dietary supplements derived from plants for prevention or treatment of diseases (Orakwelu, 2011; Kaigongi, 2014).

A number of secondary metabolites isolated from plants have been demonstrated in animal models (*in vivo*) as active principles responsible for facilitating healing of wounds. Some of the most important ones include tannins and glycoprotein fractions (Wadankar *et al*., 2011).

Tridax procumbens, commonly known as coat button, is a widespread weed and a pest plant. It is native to the tropical America but it has been introduced to tropical, subtropical and mild temperate regions worldwide (Sujit and Amol, 2014). Traditionally, *Tridax procumbens* has been used for wound healing in the coastal parts of Kenya and in India (Sidi Michael personal communication, Sujit and Amol, 2014). A systematic search for useful bioactivities from the plant is now considered to be a rational approach in drug research. Several studies carried out on the plant have revealed the presence of alkaloids, carotenoids, flavonoids, saponins, tannins (Ikewuchi *et al*., 2009), phenolic compounds, steroids, anthraquinone, catechol, and terpenoids (Jain an Amita, 2012), anthracine glycosides and cyanogenic glycosides (Orakwelu, 2011). According to Orakwelu (2011), aqueous and methanol extract of *Tridax procumbens* showed zones of inhibition on *Staphylococcus aureus and Escherichia coli* using agar disc diffusion method while agar well diffusion method showed zone of inhibition on *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Salmonella and Staphylococcus aureus* using the same extracts.

Due to high cost of manufactured synthetic drugs in the market it's hard for local communities to access these drugs due to poverty. Besides, chemosynthetic drugs are associated with resistance, so more natural alternative drugs are required. There is need to investigate the potential antimicrobial activity and phytochemical constituents of plants. The essential values and uses of some medicinal plants has been worked out and published, but many of them remain unexplored in Kenya. (Sujit and Amol, 2014; Taiye and Iyamah, 2014). Although *Tridax procumbens* has been greatly tested on anti-hemorrhagic and anti-diabetic effects, very few studies have been conducted in Kenya. Information is thus required to clearly understand the antimicrobial activity of this plant. Plants are widely used in traditional medicine in Kenya and even in other developing countries (Kaigongi, 2014). Most people inhabiting these areas cannot afford the high cost for modern conventional drugs because they are expensive (Osazee *et al.,* 2013). *Tridax procumbens* has long been used by the Mijikenda people along the Kenyan coastal region. The plant is only regarded as a weed and its utilization around Maseno area is poor hence its availability and utilization is more considered for the study. The use of medicinal plants is wide spread in Africa while toxicity profile is not yet known, the dosage is often not well determined and prescribed in conditions where standardization has not been undertaken. This study was designed to investigate the phytochemical constituents and antimicrobial activity of *Tridax procumbens* against *Staphylococcus aureus* and *Escherichia coli. It was hypothesized that* The leaf extract of *Tridax procumbens* contain different phytochemical constituents and *has* inhibitory effect on the growth of *Staphylococcus aureus* and *Escherichia coli.*

MATERIALS AND METHODS

Sample Collection and Preparation

The leaves of *Tridax procumbens* were obtained from the environment of Maseno University and were identified by taxonomist in the department of botany at Maseno University. The leaf and flower parts of *Tridax procumbens* were used for identification of the plant. The leaves of *Tridax procumbens* were collected and dried at room temperature. The dried leaves were ground using an electrical grinding machine (ramtons blender with mill) to a fine powder (plate 1). The powder was the stored in a clean container. Fifty grams (50g) of the powdered sample was weighed and dissolved in100mlof distilled water in a sterile beaker. It was mixed thoroughly and was left for 24 hour. After 24 hours the mixture was filtered using whatmanNo.1 filter paper (plate 2). The filtrate was stored in a conical flask for analysis.

Plate 1. Powdered plant sample

Plate 2. Filtrate of aqueous leaf extract

Ethanol Extraction

Fifty grams (50g) of the powdered sample was weighed and dissolved in 100mlof 100% ethanol of in a sterile conical flask. It was the mixed thoroughly and was left to stand for 24 hours. After 24 hours the mixture was filtered using Whatman No. 1filter paper. The filtrate was then rotor vapored to concentrate the extracts. The concentrate was then collected in a sterile flask and used for analysis.

Qualitative Phytochemical Analysis

Chemical tests for the screening and identification of bioactive chemical constituents in *Tridax procumbens* were carried out in extracts using the standard procedure as described by Sofowara (1993) and Harbone (1973).

Test FOR Tannins

Half grams of powdered sample were boiled in 20ml of distilled water in a test tube and filtered. 0.1% $FeCl_3$ was added to the filtered samples and observed for brownish green or a blue black coloration which indicated the presence of tannins.

Test for Saponins

Two grams of powdered sample were boiled together with 20ml of distilled water in a water bath and filtered. Ten milliliters of the filtered sample was mixed with 5 ml of distilled water in the test tube and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and the formation of emulsion which indicates the presence of saponins.

Test for Flavonoids

A few chop of 1% NH_3 solution was added to the aqueous extract of each plant sample in a test tube. A yellow coloration indicates the presence of flavonoids.

Test for Terpenoids

Five milliliters of aqueous extract sample was mixed with 2 ml of $CHCl_3$ in a test tube, 3 ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration formed indicated the presence of terpenoids.

Test for Glycosides

One milliliter of concentrated H_2SO_4 was prepared in test tube. 5 ml of aqueous extract from each plant part sample was mixed with 2ml of glacial CH_3CO_2H containing 1 drop of $FeCl_3$. The above mixture was carefully added to 1ml of concentrated H_2SO_4 so that the concentrated H_2SO_4 was underneath the mixture. The presence of cardiac glycoside in the sample was indicated by a brown ring.

Test for Alkaloids

One milliliter of the extract was poured into a sterile test tube. Five milliliters of 2% hydrogen chloride was added into the same test tube. The solution in the test tube was heated in a water bath for10minutes. The solution was filtered after 10 minutes of heating using whatman No.1 filter paper. One milliliter of the filtrate was poured into a test tube. One milliliter of the wagner's reagent was added into the same test tube. The solution was mixed properly for observation of reddish brown color.

Determination of Antimicrobial Activity

Whatman filter paper (No. 1) discs of 6 mm diameter were made by punching the paper using a paper punch. The blank discs were then collected in a beaker and the beaker was covered with a foil paper and autoclaved to sterilize the blank discs. The sterile extracts of the plant (water and ethanol extracts) were serially diluted in two folds, using distilled water as a diluent. The disks were then dipped into the dilutions and placed on a glass plate to allow the solvents to evaporate and the test chemicals to be adsorbed in the discs. Disc impregnated with amphicloxa were used as a positive control and a disc impregnated with distilled water only were used as negative controls.

Test Organisms

The bacterial strains chosen based on their availability and pathogenicity were *Escherichia coli* and *Staphylococcus aureus*. There organisms were obtained from the medical biotechnology laboratory of Maseno university.

Disc Diffusion Method

The disc diffusion method followed by the clinical and laboratory standards institute protocol was used to evaluate antimicrobial activities (Kaigongi, 2014). To determine susceptibility, four concentrations (0, 50, 100, 200 and 250 mg/ml of extracts) , were prepared in ethanol and water respectively by dissolving 2.5 g of each extracts in 10 ml and serially diluting it to make 100 µl of the reconstituted extract and were dried completely under sterile conditions in a laminar flow. Each disc was gently pressed down to ensure complete contact with the agar inoculated with 1ml of *Escherichia coli* and *Staphylococcus aureus*. Extracts were tested in triplicates. Water saturated assay discs were used as negative control while antibiotic amphicloxa was used as positive control. The plates were incubated at 37^{o}C for 24 hours. Inhibition zones were recorded as the diameter of growth free zones. The experiment was arranged in a completely randomized design.

DATA ANALYSIS

Data collected from this study was subjected to analysis of variance (ANOVA) using SAS statistical package. Treatment means were separated and compared at ($p \leq 0.05$).

RESULTS

Phytochemical screening of the leaf aqueous extract (Table 1 and plate 3) showed that *Tridax procumbens* contain tannins, saponins, terpenoids, flavonoids, glycosides and alkaloids.

Table 1: Phytochemical screening of secondary metabolites present in the leaf aqueous extract of *Tridax procumbens*

Tests	Observations	Deduction
Tannins	Colour change from green to blue black	+
Saponins	An emulsion formed	+
Flavonoids	Colour change from green to yellow	+
Terpenoids	An interface formed. A colour change from green to reddish brown	+
Glycosides	A brown ring was formed	+
Alkaloids	Colour change from green to reddish brown	+

KEY: - absent, + present.

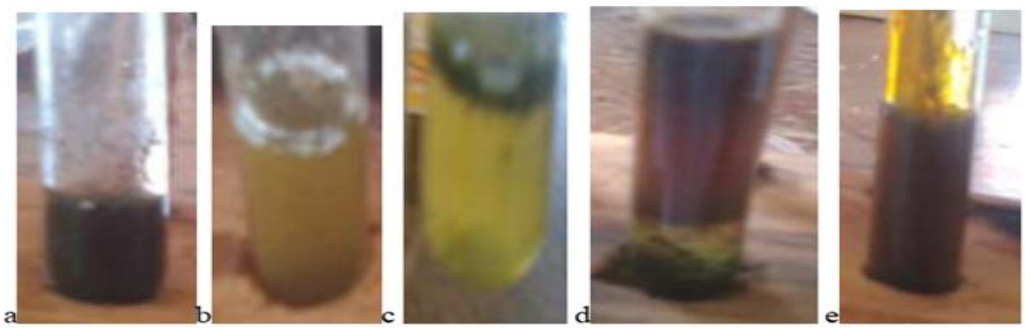

Plate 3: Showing the observations for phytochemical analysis in the order, tannins, saponins, flavonoids, terpenoids and alkaloids respectively.

The antimicrobial activity varied with the extract fraction tested; extract concentration and the test bacteria. Ethanol extract fraction of *T. procumbens* had greater antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli.* However, all bacterial tested were very sensitive to the amphicloxa positive control. Ethanolic extract had significantly ($p < 0.05$) greater effect on *Escherichia coli* than on *Staphylococcus aureus* (tables 2 and 3, plate 4).

Table 2: Analysis of mean data of antibacterial activity of *Tridax procumbens* leaf extracts, comparison of ethanol and water extract and the different concentration used.

	Zone of growth inhibition (mm)
Microbe	
Escherichia coli	8.7a
Staphylococcus aureaus	7.7b
lsd	0.37
Extracts	
Ethanol extracts	9.3a
Water extracts	7.2b
lsd	0.64
Extract Concentration (mg/ml)	
0	0d
50	7.5c

100	8.9b
200	9.2b
250	9.3b
Amphicloxa	14.5a
lsd	0.45

Means followed by different letter(s) are significantly different at p≤ 0.05. Data values are means of three replicates.

Table 3. Analysis of variance of antibacterial activity of *Tridax procumbens* leaf extracts, comparison of ethanol and water extract and different concentrations used.

Source	DF	Mean of squares	F value	Pr > F
Model	61	81.6	42.8	< 0.0001
Error	154	1.91		
Corrected total	215			
Pathogen	1	51.04	26.8	< 0.0001
Treatment	1	238.6	125.2	< 0.0001
Concentration	5	797.4	418.5	< 0.0001

Plate 4. Showing growth inhibition zones of ethanol extract on *S. aureus* and *E. coli* respectively at 250mg/ml.

DISCUSSION

The medicinal value of plants lies in some chemical substances with definite physiological action on the human body (Musyimi *et al*., 2008). Bioactive compounds include alkaloids, flavonoids, tannins and phenolic compounds (Edeoga, 2005). Both the extracts inhibited the growth of both *Staphylococcus aureus* and *Escherichia coli*. This could be due to the presence of the active components acting by inhibiting the bacterial colonization, lowering surface tension of extracellular medium or by lysing bacterial membrane (Al-Bayati and Al-Mola, 2008), inactivating enzymes and disrupting bacterial membrane (Victor *et al.*, 2005; Ogunwenmo *et al.*, 2007). Results on antimicrobial activity of the leaf extract of *Tridax procumbens* against the test microorganisms at different concentrations revealed that inhibition zone diameters increased with increase in extract concentration. This means that the more concentrated the extract, the higher the activity against the organism. This could be due to the polarity of the solvent that conferred the ability to extract a variety of compounds and could be the justification for the reasons why it is used in traditional medicine.

The phytochemical screening of the aqueous leaf extract of *Tridax procumbens* revealed the presence of saponins, tannins, terpenoids, flavonoids and cardiac glycosides. The presence of some of these plant secondary metabolites in a significant amount in the investigated parts of *Tridax procumbens* may have conferred antimicrobial activity on leaf extracts of this plant. The results agree with those of Ikewuchi (2009) on the leaf of *Tridax procumbens* and Mir Amin *et al., (*2013). Plants water extract of different plants would contain various components (Kaigongi, 2014). The susceptibility of *Staphylococcus aureus* and *Escherichia coli* to both ethanol and water extracts of *Tridax procumbens* is an indication that the plant has a potential to serve as a source of drugs against the test microorganisms. The antibacterial activity demonstrated by the extracts of *Tridax procumbens* may therefore explain some of the previous claims that the plant is used traditionally for the treatment of fever, typhoid fever, cough, asthma, epilepsy and diarrhea (Tejaswini *et al.*, 2011). Results from the present study have given us the scientific basis for use of such plants in traditional medicine practices.

CONCLUSION

From this study it is clear that the leaves of *Tridax procumbens* are rich in phytochemicals especially the tannins, saponins, flavonoids, cardiac glycosides, terpenoids and alkaloids thus are of medicinal value. Both ethanol and water extract of leaves of *Tridax procumbens* have antimicromial activity since they inhibited the growth of both *E. coli* and *S. aureus.* The ethanol extract was found to be more active against *E. coli* and *S. aureus* than the water

extract. More detailed investigations at molecular level should be undertaken to unveil the exact mechanism of action of *Tridax procumbens* leaf extracts. Since only a few isolates were tested, further studies involving a large number of resistant pathogens are necessary to draw meaningful conclusions.

REFERENCES

1. Al-Bayati, F. A., & Al-Mola, H. F. (2008). Antibacterial and antifungal activities of different parts of Tribulus terrestris L. growing in Iraq. *Journal of Zhejiang University Science B*, *9*(2), 154-159.
2. Armstrong, W. P. (1998). Plants that make you loco; Mind altering plant alkaloids. Available from: http://waynesword.palomar.edu/www0703.htm. – accessed on 1/4/2016.
3. Boadi, N. O., Saah, S. A., Mensah, J. K., Badu, M., Addai-Arhin, S., & Mensah, M. B. (2015). Phytoconstituents, antimicrobial and antioxidant properties of the leaves of Persea americana Mill cultivated in Ghana.
4. Cowan, M. M. (1999). Plant products as anti-microbial agents. *Clinical microbiology review*, 12:564-582.
5. Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African journal of biotechnology*, *4*(7), 685-688.
6. Lowy, F. D. (2003). Antimicrobial resistance: the example of Staphylococcus aureus. *The Journal of clinical investigation*, *111*(9), 1265-1273.
7. Harbone, J.B. (1973). Phytochemical methods, Chapman and Hall, Ltd., London, pp49-188.
8. Ikewuchi, C., & Ngozi, M. I. (2009). Chemical profile of Tridax procumbens Linn. *Pakistan Journal of Nutrition*, *8*(5), 548-550.
9. Jain, A., & Amita, J. (2012). *Tridax procumbens* Linn: A Weed with Immense Medical Importance: A Review; *International Journal of Pharma and Bio Sciences* 3(1),544-552
10. Kaigongi, M. M. (2014). Antimicrobial activity, toxicity and phytochemical analysis of four medicinal plants traditionally used in Msambweni District, Kenya (Doctoral Dissertation, School of Biological Sciences, University of Nairobi).
11. Kigen, G. K., Ronoh, H. K., Kipkore, W. K., & Rotich, J. K. (2013). Current trends of traditional herbal medicine practice in Kenya: a review. *African Journal of Pharmacology and Therapeutics*, *2*(1).
12. Kiringe, J. W. (2006). A survey of traditional health remedies used by the Maasai of Southern Kaijiado District, Kenya. *Ethnobotany Research and Applications*, *4*, 061-074.
13. Mir, M. A., Sawhney, S. S., & Jassal, M. M. S. (2013). Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale. *Wudpecker Journal of Pharmacy and Pharmocology*, *2*(1), 1-5.
14. Musyimi, D. M., Ogur, J. A., & Muema, P. M. (2008). Phytochemical compounds and antimicrobial activity of extracts of aspilia plant (Aspilia mossambicensis)(Oliv) Wild. *International Journal of Botany*, *4*(1), 56-61.
15. Ogunwenmo, K. O., Idowu, O. A., Innocent, C., Esan, E. B., & Oyelana, O. A. (2007). Cultivars of Codiaeum variegatum (L.) Blume (Euphorbiaceae) show variability in phytochemical and cytological characteristics. *African Journal of Biotechnology*, *6*(20).
16. Orakwelu, N.U. (2011). Studies on the medicinal potential of *Tridax procumbens* Linn. Available on http://www.naulibrary.org/dglibrary/admin/book_directory/thesis/10316.pdf. Accessed on 5/10/2015.
17. Osazee, J.O., Daniel, O.E., Ogunsan, F.E., & Elimian, O.K. (2013). In-vitro Screening of Antibacterial Potential of Aspilia Africana leaves; *The Bio Scientist Journal.* 1(2), 140-146 *aureus: Future of Microbiology Journal. 2(3), 323-334.*
18. Sawant, R. S., & Godghate, A. G. (2013). Preliminary phytochemical analysis of leaves of Tridax procumbens Linn. *International Journal of Science, Environment and Technology*, *2*(3), 388-394.
19. Al-Hussaini, R., & Mahasneh, A. M. (2011). Antibacterial and antifungal activity of ethanol extract of different parts of medicinal plants in Jordan. *Jordan Journal of Pharmaceutical Sciences*, *4*(1), 57-69.
20. Sofowara, A. (1993). Screening Plants for Bioactive Agents. Medicinal Plants and Traditional Medicinal in Africa. 2nd ED. Spectrum Books Ltd,
21. Sujit, S.K., & Amol, S.D. (2014).*Tridax procumbens*: A Medicinal Gift of Nature; *Asian Journal of Research in Biological and Pharmaceutical Sciences 2(4),159-162.*
22. Fasola, T. R., & Iyamah, P. C. (2014). Comparing the phytochemical composition of some plant parts commonly used in the treatment of malaria. *International Journal of Pure and Applied Sciences and Technology*, *21*(1), 1.
23. Tejaswini, K., Vishwanath Pradeep, B., Rudrama Devi, K., Shylaja, S., & Jyothsna, K. (2011). Phytochemical screening and antimicrobial activities of plant extract of Tridax procumbens. *The Bioscan*, *6*(2), 321-323.
24. Victor, J., Siebert, S., Hoare, D., & Wyk, B. V. (2005). Sekhukhuneland grasslands: A treasure house of biodiversity. *FAO, Rome*.
25. Wadankar, G. D., Malode, S. N., & Sarambekar, S. L. (2011). Traditionally used medicinal plants for wound healing in the Washim District, Maharashtra (India). *International Journal of PharmTech Research*, *3*(4), 2080-2084.