Antimicrobial Activity of *Bidens pilosa* Leaves Extracts Against *Staphylococcus aureus* and *Escherichia coli*

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SUMMARY

Resistance against synthetic antimicrobial agents is one of the major global public health challenges that compel scientists to search for alternatives including those of plants origin. *Staphylococcus aureus* and *Escherichia coli* are bacteria responsible for a variety of infections and diseases that causes significant morbidity and mortality in humans and animals. *E. coli* is widely distributed in nature and commonly found in lower gastro intestinal tracts of most warm-blooded animals associated with urinary tract infections and enterocolitis in humans and colibacillosis in poultry. This study was carried out to investigate antimicrobial activity of methanolic leaf extracts of *Bidens pilosa* against *S. aureus* and *E. coli*. Agar well diffusion method was used to assess antimicrobial activity of the leaf extracts at 20%, 50% and 70% concentrations respectively based on measured zone of inhibition. The leaf extracts of *Bidens pilosa* produced significant zone of inhibition indicating its antimicrobial activity against *E. coli and S. aureus*. The antimicrobial activity was demonstrated in all concentrations however, the highest zone of inhibition (18.5mm and 32mm) for *E. coli* and *S. aureus* respectively was at 70% concentration. The results shows that *Bidens pilosa* leaf extracts have antimicrobial activity against the tested bacteria and have the potential for further development including identification of active components that can be tested for treatment of *E. coli* and *S. aureus* associated conditions.

Key words: antimicrobial resistance, medicinal-plants, natural-products, blackjack, *E. coli, S. aureus*

INTRODUCTION

Antimicrobial resistance and spread of drug resistance are a major challenge in the treatment of clinical infections throughout the world due to the tendency of these organisms to rapidly develop resistance against antimicrobials in use (Aruljothi *et al*., 2014). The increasing ability of microorganism to resist of antibiotics has not only limited treatment options, but also has increased treatment costs and mortality rates in humans and animals (Dadgostar, 2019). Therefore, searching for alternative and more effective antimicrobials agents is unavoidable to ensure availability of effective treatment against different infectious diseases. One such possible source is the natural compounds present in plants. Human and animals greatly depend on plants which serve as source of food and medicine (Ajanaku *et al.,* 2018). The plant *Bidens pilosa* Linn. var. Radiata from family Asteraceae is widely distributed in the tropical and subtropical regions of the world (Chiang *et al.,* 2004). It is 30–100 cm in height with yellow flowers and commonly known as blackjack. *Bidens pilosa* is one of the plants widely used for variety of medicinal purposes such as anti-malarial, antibacterial, antiviral, wound healing for many years (Tajehmiri *et al.,* 2004).

However, it has been shown that, plants grown in different geographical location have different chemical composition and

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hence affects its pharmacological properties (Kumar *et al.*, 2017, Muhammad *et al.*, 2019).

This study aimed at evaluating the antibacterial activity of *Bidens pilosa* leaves extract that are locally sourced against one *E. coli* and *S. aureus*. The findings from this study will provide information on the effectiveness of this plant against *E. coli* and *S. aureus* and potentially pave way for further studies to identify and study the active phytochemicals in the plant.

MATERIALS AND METHODS

Preparation of plant extracts

Fresh plant leaves were collected in Morogoro around Sokoine University of Agriculture (SUA) premises. Leaves from fresh matured plant were collected from the field, washed using water, and dried under the shade for two weeks. Dried plant leaves were grinded using mortar and pestle to obtain a fine powder and stored in dry clean bottle before methanolic extraction. 95g of powdered leaves were soaked into 70% methanol in 1000 ml conical flask and shaken thoroughly to allow plant component to dissolve. The solution was incubated for 48 hours at room temperature (average of 29°C) to allow the alkaloids and other constituents found in in the *Bidens pilosa* leaves to dissolve into methanol. To prevent direct sun light to the mixture the conical flask was wrapped with aluminium foil. After incubation the solution was then filtered first using muslin cloth and then Whatmann No.1 filter paper. The rotary evaporator was used to separate methanol and extracted compounds. The extract was stored in a sealed bottle that does not allow light to pass (opaque) and stored in a dry clean and cool environment until antimicrobial testing. *Bidens pilosa* extracts was dissolved in distilled water to prepare 20, 50 and 70% concentration (w/v).

Preparation of growth media (Mueller Hinton Agar)

The Mueller Hinton Agar (OXOID^{TM}, UK) media was prepared strictly according to manufacturer's instruction (38g per 1 liter). The media were sterilized by autoclaving at 121°C for 15 minutes. After sterilization the agar was poured into appropriate petri dishes and allowed to cool down to a temperature of 40°C before use.

Preparation of McFarland turbidity standard

The 0.5 McFarland turbidity standard was prepared by mixing 1% solution of anhydrous Barium chloride ($BaCl_2$) and 1% solution of Sulphuric acid (H_2SO_4) to form a turbid suspension. The resulting mixture were put in a foil covered screw cap tube and then stored at room temperature ready for use.

Collection and confirmation of bacterial isolates

Bacterial colonies from pure cultures kept in the laboratory were assessed for macro morphology and micromorphology. Gram staining technique was used for microscopic examination of the isolates. After microscopic identification of the isolates, biochemical tests were performed to further confirm the isolates. For *E. coli* the tests include Triple sugar fermentation test and Indole, Methyl red, Voges-Proskauer and Citrate utilization test (IMViC test) which were Indole and Methyl red positive, Voges-Proskauer and citrate negative. For *Staphylococcus* species, the biochemical tests were catalase and coagulase tests which showed positive reaction for both.

Antimicrobial susceptibility test

Agar well diffusion method was used to determine antibacterial activity of plant leaves extracts. Mueller Hinton Agar (MHA) was used for conducting antibacterial tests. *E. coli* and *S. aureus* that were obtained from microbiology laboratory at SUA, were inoculated into the normal saline separately and form turbidity that was compared with that of 0.5 McFarland turbidity standard. Then were spread uniformly all over four MHA plates by using a sterile swab, where by two plates were for *E. coli* and the other two for *S. aureus* and left for few minutes to dry. For *E. coli* inoculated agar plates, the agar plate was divided into four parts. Three parts were

used for testing of leaf extract where 10µg from each of the three concentrations of 20%, 50%, and 70% was loaded in separate wells. In the fourth part of the agar plate, a control (15µg Erythromycin disc) was placed. The agar plates were then incubated at 37°C for 24 hours. Similar procedure was used for *S. aureus* inoculated agar plates except for the positive control used which was 2µg Clindamycin disc.

Data analysis

Inhibition zone that was seen around wells and disc were measured by using a ruler and recorded in millimeters. Mean zones of inhibition of plant extracts were calculated using Microsoft Excel (2010) and compared to those of positive control and between the two bacteria.

RESULTS

Bidens pilosa leaves extract showed antimicrobial activity against *S. aureus* and *E. coli*. The antimicrobial susceptibility for the two bacteria was variable but was concentration dependent as indicated by size of inhibition zone (Table 1). The highest inhibition was observed at the concentration of 70%. On average the zone of inhibition on *E. coli* and *S. aureus* 18.5mm and 32mm respectively and greater than those of positive controls (Table 1).

Table1: Antimicrobial activity of *Biden pilosa* leaf crude extracts against *E. coli* and *S. aureus*

Bacteria	*E. coli*				*S. aureus*			
Conc. (mg/ml)	200	500	700	PC1	200	500	700	PC2
Bacterial growth plate 1 (IZ)	11	15	19	13	28	30	33	25
Bacterial growth plate 2 (IZ)	10	14	18	9	25	29	31	20
Average	10.5	14.5	18.5	11	26.5	29.5	32	22.5

Conc: Concentration of leaf extracts of *Biden pilosa* (mg/ml), PC1: Positive control 1(Erythromycin) (15µg), PC2: Positive control 2 (Clindamycin) (2µg), IZ: Inhibition zone diameter in millimeter (mm)

DISCUSSION

Results from the present study has shown that *Bidens pilosa leaves* crude extracts possess antibacterial activity against both *E. coli* and *S. aureus* bacteria. The findings complement previous studies on the use of this plant against different pathogenic bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus sabtilis, proteus mirabilias* (Ajanaku *et al.,* 2018). This implies that *Biden pilosa* possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using methanol as the solvent. Indeed, roots and leaves of this plant have been reported to possess effective pharmacological properties like antibacterial activity (Arlene *et al.,* 2013). Preliminary phytochemical screening of *Bidens pilosa* aqueous extracts of leaves have revealed the presence of flavanols (Lawal *et al.,* 2015) and variety of other chemical constituents with variable pharmacological properties (Bairwa *et al.,* 2010; Tran and Tran, 2016). The actual active compound against bacteria is not clearly understood, it was earlier suggested in other studies that the anti-microbial activity of *Bidens pilosa* is likely to be caused by the presence of significant amounts of some monoterpenes and sesquiterpenes compounds which are known to posses a wide range of anti-microbial properties (Pattnaik *et al.,* 1997). In this study, the antimicrobial activities for *S. aureus and E. coli* was observed to increase with the increase in extracts concentrations from 200mg/ml to 700mg/ml, the highest concentration used in the study. This finding is not surprising as a similar finding has

been reported elsewhere using different plant based antibacterial agent (Singh *et al.*, 2017). Comparison of the effects of the leaf extracts between *S. aureus* than *E. coli* has indicated that the plant is more effective on *S. aureus* than *E. coli* based on the size of zone of inhibition shown by the extracts on the two bacteria. This could indicate probably the leaves are more effective against Gram positive bacteria than the Gram-negative bacteria. However, it is important to note that only one Gram positive bacteria was used in the present study and therefore it cannot be practical to generalize to all bacteria in the group. Testing against multiple Gram-positive and Gram negative bacteria could provide more conclusive results. In addition, the bacteria used in the present study were isolates obtained from the laboratory and not from the clinical cases. It is possible that the clinical case isolates could react differently. Still, the ability of the plant extract to suppress *E. coli* and *S. aureus* is promising due to the importance of the two bacteria in animal and human health. Therefore, *Bidens pilosa* leaves might be a good candidate in the search for a natural antimicrobial agent.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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