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Antibacterial Activity of Ethanol Leaf Extract of *Sida acuta* Against Some Clinical Bacterial Isolates

Maimuna Hassan[1], Fatima M. Musa[1], Firdausi Aliyu[2], Aliyu Adamu[1]*

ABSTRACT

Persistent evolution of multidrug resistance bacteria due to inappropriate use of conventional antibiotics is undermining treatment intervention for infectious diseases, thus constituting substantial proportion of the global public health problem. This necessitated the search and development of new drugs particularly from plant origin that are effective against such superbugs. Therefore, the present study is designed to determine the phytochemical constituents in *Sida acuta* and their antibacterial effects on pathogenic bacterial species of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis*. The phytochemical components of the extract were identified using standard methods. Furthermore, the antibacterial activity of the leaf extract against the bacterial pathogens were assessed using agar well diffusion and broth dilution methods, at varying concentrations of the extract (37.50, 75, 150 and 300 mg/ml), and using Commercially obtained ciprofloxacin as control. Preliminary screening revealed that ethanolic leaf extract possesses many secondary metabolites such as alkaloids, flavonoids, phenol, tannins, terpenoids, glycosides and cardiac glycosides. The extract exhibited significant inhibitory effects at ($p<0.05$) against the reference isolates of bacteria. The highest antibacterial activity was exhibited by the highest concentration of the extract (300mg/ml). The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of ethanol crude extract of *Sida acuta* was found to be 37.5 and 75mg/ml, respectively against all the reference isolates of tested bacteria. The observed antibacterial activity suggests that *Sida acuta* could be used for the treatment of tested bacterial infections.

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Introduction

Medicinal plants represent the source of medical practice and are globally distributed, but mostly abundant in the tropics [1]. Historically, most native doctors were botanists, using medicinal plants to treat various kinds of diseases, if not all diseases. Currently, there are estimated 422,000 flowering plants distributed globally, out of which 50,000 were are used

[1] Department of Microbiology, Kaduna State University Kaduna, Tafawa Balewa Way Nigeria
[2] Department of Biotechnology, Nigerian Defence Academy, Kaduna Nigeria
* Corresponding Author**:** Aliyu Adamu, E-mail: aliyuadamu@kasu.edu.ng

as primary source of medicine as chewing sticks for dental care, anti-inflammatory, antimicrobials and anticancer [2].

The use of conventional drugs in chemotherapy for the treatment diseases is being undermined by the evolution of antimicrobial resistance. Microorganisms particularly bacteria have the ability to evolved into a resistance strains by either genetic adjustments such as mutations as a result of selective pressure incited by inappropriate antimicrobial use [3]; or acquisition of resistance genes [4][5]. Therefore, there is need to identify more therapeutic options especially from plant origin in order to ensure effective treatment.

Sida acuta, otherwise called in Hausa "Kalkashin kwado'' and "Stubborn grass'' in English is an important medicinal plant in Africa, for treatment some ailments [6]. This plant is a member *malvaceae* family, genus *Sida* species and species *acuta*. It can grow in almost all soil types except that originated from limestone and flooded soil. All the plant parts, including root, stem bark, leaf and flower were reported to be used for medicinal purposes [7]. It was gathered from various studies that the plant is used for management of sperm cell related fertility problems, cold, cough, abdominal pain, headache, tuberculosis, malaria, asthma, blood disorders, respiratory diseases, dysentery and diarrhea, cancer and inflammation, among others[8]. These biological activities were associated with phytochemical compounds contained within the plant including essential oil, tannins, flavonoids and alkaloids [9]. In India, hot water extract of the dried plant is administered orally as febrifuge and diuretic; and the leaf juice is taken to manage vomiting [10]. The plant fresh root is used for dysentery treatment in Guinea. It is therefore based on this, this study assessed the antibacterial efficacy of the plant leaf extract against some pathogenic bacterial species

Materials and Methods

Collection, authentication and preparation of plant material

Fresh leaves of *Sida acuta* plant was collected within kawo, Kaduna North Local Government area of Kaduna State. The plant was identified and authenticated by a taxonomist at the Department of Biological Science, Kaduna State University. The leaves were washed thoroughly under running water and dry under room temperature for 14 days.

It was grinded into coarse powder using mortar and pestle and stored in separate airtight bottles.

Extraction of plant material

The leaves phytochemicals were extracted by percolation method as we described previously [11]. 150 g of the leaf powder was mixed with 2000 ml of 70% Ethanol in a flask and vigorously mixed. The mixture was kept in tightly sealed vessels for 72 hours at room temperature and constantly shaking. Whatman no 1 filter paper was used to filter the mixture and the solvent was removed from the filtrate at 28˚C by evaporation in rotary vacumm evaporator and the recovered extract finally tried in air.

Phytochemical screening of *Sida acuta*

Phytochemical components of the leaf extract were screened using the appropriate chemical test as we described previously [11]. The components analysed were Alkaloids, Flavonoids, Saponins, Phenol, Tannins, Steroids, glycosides, Terpenoids, Phlobatannins and Cardiac glycosides.

Collection of test bacteria

The organism used in this study were reference bacterial isolates consisting of *Escherichia coli* (ATCC 43888), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027) and *Bacillus subtilis* (ATCC 6633). They were obtained from National Veterinary Research Institute (NVRI), VOM, Jos, Plateau State.

Preparation of extract concentrations

In a test tube, 3 g of leaf crude extract was dissolved in 10 ml of 10% Dimethyl Sulfoxide (DMSO) to obtain 300 mg/ml as the stock concentration. From the stock solution and using 10 % DMSO as a diluent, two-fold dilutions was used to obtained 150 mg/ml, 75 mg/ml and 37.5 mg/ml concentrations [12].

Standardisation of bacterial inoculums

The number of bacterial cells in the inoculums were standardised using the McFarland Scale No.1. The bacterial cells from overnight growth culture suspended separately, in 2 mL of sterile physiological saline. The turbidity of the bacterial suspensions were then adjusted to match that of 0.5 McFarland standards.

Antibacterial activity of crude extracts

The antibacterial activity of the ethanolic crude extract of leaves of *Sida acuta* against the reference, *S. aureus, E. coli, B. subtilis and P. aeruginosa* was determined using agar well diffusion method [13]. 100 µl of standardised bacterial suspension was inoculated (in triplicates) into MH agar. Using swab stick, the inoculum was spread evenly over the entire surface of the plates and allow to stay for 10 minutes, after which a sterile cork borer was used to dig wells of 9 mm. 100 µl 300 mg/ml, 150 mg/ml, 75 mg/ml and 37.5 mg/ml extract concentrations were each filled into the wells. DMSO was used as negative control and was used to fill in seprated well in the plate. To ensure appropriate diffusion of the well content (extract) in to the agar, the plates were allowed to stay at room temperature for 10 minutes. The plates were then incubated for 24 hours at 37˚C. For each bacteria tested and concentration, the zone of inhibition of growth was measured using a meter rule and the means were computed.

Determination of minimum inhibitory concentration (MIC)

Using broth dilution method as described in (Slue and Agbabiaka 2018), the MICs of crude extract the test bacteria. 1ml of the different concentrations () were added to separate tubes containing 9 ml of Mueller Hinton broth and 100 µl of the standardized bacterial inoculum. The test tube was incubated for 24 hours at 37˚C, after which were observe for turbidity as indication of bacterial growth. The lowest concentration of extract which inhibited the growth of a test bacteria was recorded as the MIC. Negative and positive controls constituting of the broth and extract; and broth, inoculum, and ciprofloxacin, respectively were used.

Determination of minimum bactericidal concentration (MBC)

The MBC was determined by plate method as described by [14]. The tubes that yielded no growth from MIC were sub-cultured on nutrient agar plates for 24 hours at 37°C, after which were examined for growth. The tube with minimum concentration of extract, in which the growth was completely stopped was considered as the MBC.

Results and Discussion

The phytochemical screening carried out on the crude extract of *Sida acuta* leaves using qualitative methods, revealed the presence of many compounds as shown in Table 1, which include alkaloids, flavonoids, phenol, tannins, terpenoids, glycosides, cardiac glycosides, alkaloids, flavonoids, phenol, tannins, terpenoids, glycosides, cardiac glycosides steroids and phlobatannins. However, saponins were found to be absent. The phytocompounds detected in *S. acuta* leaf extract are similar to the compounds detected in many medicinal plants [15][16]. These phytocompounds are known to be biologically active and could confer antibacterial activities via various mechanisms. Alkaloids for example, being one of the compounds present in *Sida acuta* is one of the largest groups of phytochemicals in plants, with amazing effects in humans, which is used for development of a powerful pain killer medication [15].

One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms, which have been widely studied for their potential use in the elimination and reduction of human cancer cells [17]. Table 2 summarises the antibacterial activities of the plant extract against the test bacteria. The ethanolic extract was observed to produce visible zone of inhibition against all the four test bacteria and across all concentrations tested. The effect of the extract against all the test bacteria was found to be bactericidal with MIC and MBC values of 37.5 mg/ml and 75 mg/ml, respectively (Table 3). Being *E. coli* and *P. aeruginosa* Gram negative, and *B. subtilis* and *S. aureus* Gram positive, indicates the extract has broad spectrum activity. This could probably be the explanation why *S. acuta* is effective traditional treatment of tested bacterial infections.

E. coli was highly susceptible to the extract with mean zones of inhibition of 29.25 ± 0.35, while *B. subtilis* was less susceptible to the extract with mean zones of inhibition of 21.50 ±0. The highest antibacterial activity was exhibited by the highest concentration of the extract (300 mg/ml). Also, it has been reported by [18] that the nature and composition of some biologically active components (saponins, alkaloids, phenol etc) are enhanced in the presence of ethanol due to the stronger extraction capacity as well as solubility of the compound in the solvent.

In addition, the differences in susceptibilities of bacterial isolates to varied concentrations of crude extracts of *S. acuta* could be attributed to mode of actions and structural properties of the bacteria. Ethanol leaf extract of *S. acuta* was active against both Gram positive and Gram negative bacteria as observed in this study. Gram positive bacteria (*Staphylococcus aureus* and *B. subtilis*) with no lipopolysaccharrides tend to allow more diffusion of the active components and Gram negative bacteria (*E. coli* and *P. aeruginosa*) possess lipopolysaccharrides that might have minimized penetration of active components of the extracts which shows the effectiveness of this plant. Similar antibacterial activities by ethanolic extract of various plant were also observed in many studies [16].

Table 1 Phytochemical Constituents of Ethanol leaf extract of *Sida acuta*

S/NO	Constituents	Test	Ethanolic Extract
1	Alkaloids	Mayer's	+
2	Flavonoids	Sodium hydroxide	+
3	Saponins	Frothing	−
4	Phenol	Ferric chloride	+
5	Tannins	Ferric chloride	+
6	Steroids	Sulphuric acid	+
7	Terpenoids	Salkowski	+
8	Cardiac glycosides	Keller Killani	+
9	Glycosides	Ferric chloride	+
10	Phlobatannins	HCL	+

Key: + = present, − = Absent

Table 2 Antibacterial Activity of *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa* to test concentration of Ethanol leaf extract of *Sida acuta*

Bacterial Isolates treated	Mean zone of Inhibition (mm)				
	37.5 mg/ml	75 mg/ml	150mg/ml	300mg/ml	Ciprofloxacin **50μg**
E. coli (ATCC 43888)	13.50±1.41	20.50±0.71	24.50±1.41	29.25±0.35	35.00±0.00
P. aeruginosa (ATCC 9027)	12.25±1.77	20.50±0.00	21.00±0.71	22.50±1.41	28.00±0.00
S. aureus (ATCC 6538)	11.75±1.06	17.25±0.35	20.25±1.00	26.50±0.71	27.00±0.00
B. subtilis (ATCC 6633)	13.00±1.41	15.50±0.00	19.50±0.00	21.50±0.71	30. 00±0.00

KEY: Values are means ± Standard deviation

Table 4 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ethanol leaf Extract of *Sida acuta* against Reference isolates of bacteria

Bacteria	MIC (mg/ml)	MBC (mg/ml)
E. coli	37.50	75.00
S. aureus	37.50	75.50
P. aeruginosa	37.50	75.50
B. subtilis	37.50	75.50

Conclusion

The current study found that leaf extract of *Sida acuta* contains different phytochemicals including alkaloids, flavonoids, phenol, tannins, terpenoids, glycosides and cardiac glycosides. *In vitro* antibacterial assay shown the ethanolic plant extract to be active against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis* with MIC and MBC of approximately 37.5 and 75mg/ml, respectively, suggesting it potential use for treatment of infectious diseases.

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