

Full Length Research Paper

Antimicrobial activity of ethanolic and aqueous extracts of *Sida acuta* on microorganisms from skin infections

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The antimicrobial effect of the ethanolic and aqueous extracts of *Sida acuta* was investigated. Phytochemical analysis revealed the presence of saponins; tannins, cardiac glycosides, alkaloids and anthraquinones. The test isolates from human skin infections were *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Scopulariopsis candida*, *Aspergillus niger* and *Aspergillus fumigatus*. The zone of inhibition for the ethanolic extract varied from 10 mm for *P. aeruginosa* to 43 mm for *S. aureus* and from 4 mm for *P. aeruginosa* to 29 mm for *S. aureus* in the aqueous extract. Though the zone of inhibition increased with increase in concentration of the extract, the highest concentration of the ethanolic extract revealed a higher significant ($P > 0.05$) inhibition against *S. aureus* and *B. subtilis* compared to the inhibition effect on these organisms by gentamycin used as control. The aqueous extract had no significant effect on the test organisms. The extracts had no inhibitory effect on the fungi isolates. This study has shown that the extract of *S. acuta* if properly harnessed medically will enhance our health care delivery system.

Key words: Antimicrobial, inhibition, ethanolic extract, aqueous extract, Phytochemical and *Sida acuta*.

INTRODUCTION

Traditional medicine is the oldest method of curing diseases and infections and various plants have been used in different parts of the world to treat human diseases and infections (Caceres et al., 1991; Nweze et al., 2004; Vineela and Elizabeth, 2005). Different plant parts have also been used for various forms of diseases and infections. The following plants have been reported for the treatment of the following ailments: Garlic (*Allium sativum*) for dermatophytes (Davis et al., 2003, Wokoma et al., 2007), *Acalypha indica* and *A. torta* for superficial skin infection (Akinyanju et al., 1986), *Garcinia kola* for respiratory tract infection and diabetes (Kerharo and Adams, 1974; Adeleke et al., 2006).

Medicinal plants are known to owe their curative potentials to certain biological active substances, which exist in parts of the plants. The chemicals which are referred to as active principles or phytochemical substances include terpenes, flavonoid, bioflavonoid, benzophenones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthra-

quinones (Iwu, 1993; Asaolu, 2003).

Sida acuta is an erect, branched and perennial shrub with a woody tap root, hairy branched upto 1 m high and is reproduced from their seeds. The stem is woody, rounded and slender, and is fibrous and hairy especially when young. The leaves are simple and alternate while the inflorescence is solitary and axillary with stalks up to 1.3 cm long jointed about half of the length. The flowers are yellow with five petals and the fruit is capsuled with 5 - 6 carpels.

Sida acuta is a tropical weed of cultivated crops, pastures, roadsides and waste areas. Research carried out revealed that juice from leaf of *Sida acuta* is antihelminthic for intestinal worms (Sofowora, 1982), the root inhibit embryo implantation or growth in mice. Several medicinal plants have also been evaluated for their activities on different species of microorganisms. It has been reported that crude hot water extract of light edible leafy vegetable of *Lasianthera africana* and *Heinsia. Pulchella* exhibited bacteriostatic effects on six canned food borne pathogenic bacteria while *Ocimum gratissimum* and *Vernonia amygdalina* produced bacteriocidal effect on *Bacillus cereus* and *S. aureus* respectively (Itah and Opara, 1994; Itah, 1999).

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This study was designed to evaluate the efficacy of crude ethanolic and aqueous extracts of the leaf of *Sida acuta* on the microorganisms associated with skin infections.

MATERIALS AND METHODS

Collection of Sample

The leaves of the plant *Sida acuta* commonly called broom weed but locally called "Akanawan idipeke isoro" were obtained locally from gardens and road sides in Akwa Ibom State. This was taken for identification in the department of Pharmacology and Traditional Medicine, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

Preparation of ethanolic and aqueous extracts from the plant leaves

For the preparation of ethanolic extract, a modified method of Abdulrahman et al. (2004) was used. The fresh leaves were sun dried ground to fine particles with mechanical grinder. Thirty grains of the leaves was then macerated in 100 ml absolute ethanol (SDH Chemical Ltd) for 72 h properly covered and labelled in a conical flask. The extract was then filtered off with sterile filter paper (Whitman No 1). The filtrate was evaporated to dryness at 40°C in a vacuum using a rotary evaporator and store at 5°C in a refrigerator.

For the aqueous extract, the fresh leaves were sun dried and ground to fine particles with a mechanical grinder. It was then macerated in 500 ml of sterile distilled water for 72 h using a 500 ml conical flask. The conical flask was properly labelled and covered with aluminum foil to prevent contamination. The extracts were then filtered off with sterile filter paper (Whitman No 1). The prepared extract was also evaporated to dryness and stored in the refrigerator at 5°C for used.

The leaf extracts (both ethanolic and aqueous extracts) were screened for their phytochemical bases using the standard method of Harborne, 1973; Trease and Evans, 1989. The Phytochemical component analysed were alkaloids, saponins, flavonoids, tannins, anthraquinones and cardiac glycosides.

For alkaloids, 0.5 g of each extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. 1ml of the filtrate was treated with a few drops of the Dragendorff's reagent. The formation of orange colour indicated the presence of alkaloids.

For saponins, 0.5 g of the extract was added and mixed with Fehling's solution and then 5% of sodium trioxocarbonate solution was later added. The mixture was then boiled. The pink precipitate indicated the presence of saponins.

For flavonoids, 0.5 g of the extract and few pieces of magnesium strips was mixed with concentrated HCl. An orange faint colour of effervescence solution indicated the presence of flavonoids.

For tannins, 0.5 g of the plant extract was stirred with 1 ml of distilled water, filtered and ferric chloride solution or reagent was added to the filtrate. A blue black or blue green precipitate was taken as evidence for the presence of tannins.

For anthraquinones, 0.5 g of the plant extract was boiled with 1 ml of 10% sulphuric acid and filtered. 2.5 ml of benzene was added to the filtrate and shaken. The benzene layer was separated and half its own volume, 10% ammonia solution was added. The presence of a pink or red-violet colour in the lower ammonia phase indicated the presence of anthraquinones.

Isolation of test microorganisms

The bacteria species used in this study were isolated from infected and uninfected skin. The clinical samples were taken from wound

swab, whitlow, boils, the armpit of patients from the University of Uyo Teaching Hospital. On the other hand, the fungi samples were taken from ringworm and eczema infected skin. The samples were plated out on Blood Agar (Difco) for bacteria and Sabouraud Dextrose Agar (Difco) for fungi prepared according to manufacturers specifications. The isolates were purified after isolation through repeated subculturing and characterized using the methods of Cruickshank et al. (1975) and Cowan (1985). They were then stored in agar slants in the refrigerator at 4°C.

Antimicrobial susceptibility test

The ability of the various extracts to inhibit growth of clinical bacteria and fungi isolates was determined using the Agar Disc diffusion method (Cruickshank et al., 1975; Alade and Irobi, 1993). Sterile filter paper discs, 11 mm in diameter were impregnated with each extract concentration and dried at 30°C in the static incubator. They were then carefully placed aseptically with a forceps on the surface of the Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) plates that were preinoculated with the 24 h culture of bacteria and 0.1 ml spore suspension (1×10^5 spores/ml) of the fungi isolates respectively. The control antibiotics disc containing gentamycin (40 µg/ml) and griseofulvin solution were placed on each of the inoculated plates of Nutrient Agar and Sabouraud Dextrose Agar (SDA) respectively. The plates were left on the bench undisturbed for few minutes, after which the bacterial plates were incubated at 37°C for 24 h. Plates inoculated with fungi isolates were incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 5 days. The external diameters of visible zones of growth inhibition were measured after incubation.

RESULT

The analysis of the leaves extract of *Sida acuta* revealed that it contains the following phytochemical active agents in different proportions. They include alkaloids, saponins, anthraquinones, cardiac glycosides, tannins and flavonoids. It was observed that while tannins were present in high amount, flavonoids in trace amount, the others were present in moderate amount.

Microbial isolates

The test organisms isolated from infected and uninfected skin are presented in Table 1. The bacteria which were selected among others for this test were *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. The fungi species were *Scopulariopsis candida*, *Aspergillus niger* and *A. fumigatus*.

Antimicrobial activity

The activities of the ethanolic and aqueous extracts of the plants *Sida acuta* on both the bacteria and the fungi isolates are presented in Figures 1 - 4. The ethanolic extract inhibited the bacteria with zone of inhibition ranging from 6 to 43 mm compared to the gentamicin with the zone of inhibition 18 to 35 mm (Figure 1).

Significant inhibition was however observed with

Table 1. Microorganisms isolated from human skin and used as test organisms.

Bacteria		Fungi	
<i>Staphylococcus aureus</i>	<i>Scpulariopsis candida</i>		
<i>Psuedomonas aeruginosa</i>	<i>Aspergillus niger</i>		
<i>Escherichia coli</i>	<i>Aspergillus fumigates</i>		
<i>Bacillus subtilis</i>			

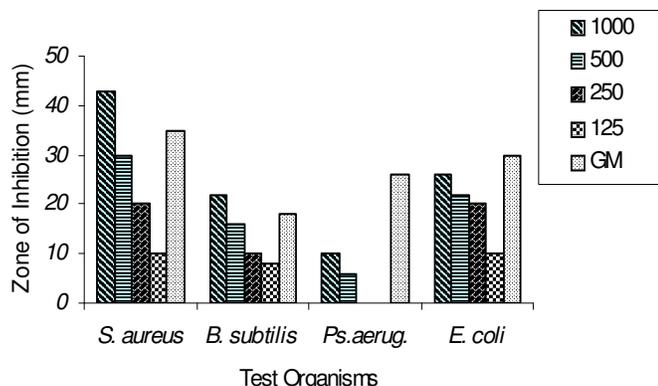


Figure 1. Sensitivity of different organisms to different concentrations of ethanolic extract of *Sida acuta*.

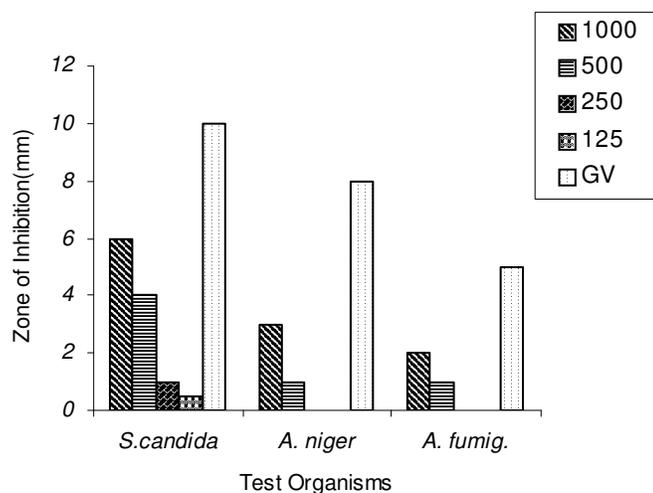


Figure 2. Sensitivity of different organisms to different concentrations of ethanolic extract of *Sida acuta*.

Staph.aureus at the highest concentration of the extract 1000 mg/ml compared to the gentamicin.

The aqueous extract inhibited the bacteria isolates but exhibited lower zones of inhibition (Figure 2). Both the ethanolic and aqueous extracts had no significant effect on the fungi isolates compared to the zone of inhibition exhibited by the antifungal agents (griseofulvin) (Figures

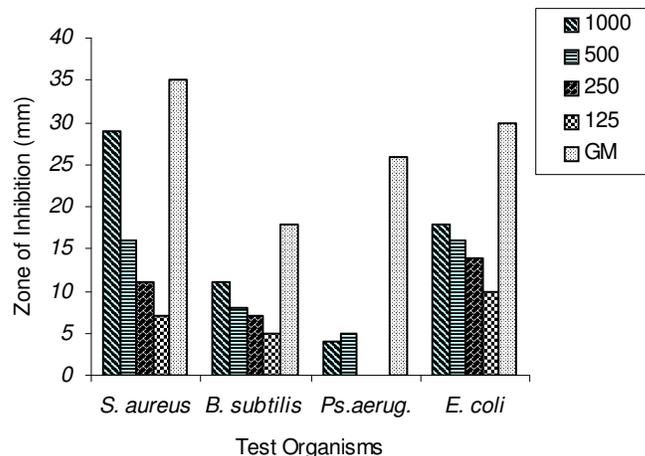


Figure 3. Sensitivity of different organisms to different concentrations of aqueous extract of *Sida acuta*.

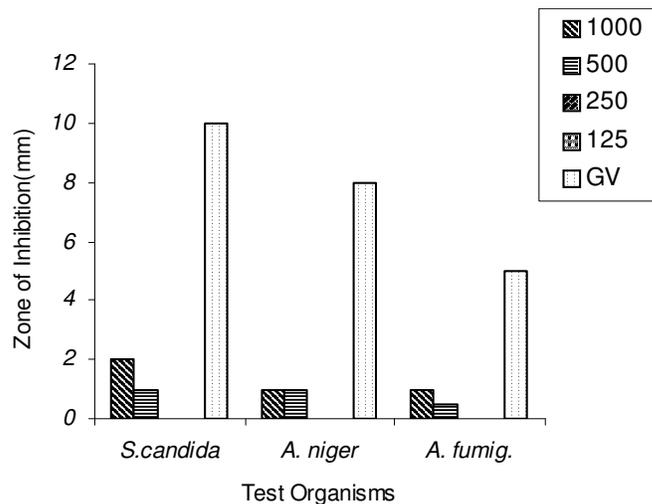


Figure 4. Sensitivity of different organisms to different concentrations of aqueous extract of *Sida acuta*.

3 - 4). Generally, the isolates showed variable sensitivity to the ethanolic extracts.

DISCUSSION

The leaves of the plant *Sida acuta* contains the active component tannins in high proportion while alkaloids, saponins anthraquinones, cardiac glycosides occurred in only moderate quantity. Flavonoids, however was observed to be in trace amount. This is in agreement with the work of Karou et al. (2007), who attributed the great potential of *Sida acuta* to the present of different components.

In this study, it was observed that the ethanolic extracts had a significantly higher antimicrobial activity than the

aqueous extract. This difference is attributed to the solubility of the active component in different solvents. Karou et al. (2007), observed that the IC₅₀ of chloroformic fraction of *Pterocarpus erinaceus* was higher than the IC₅₀ of the petroleum ether fraction while there was no significant difference between the chloroformic and aqueous fraction with *Sida acuta*. He noted that the differences in the IC₅₀ of the difference extracts were as a result of the differences in the solubility of the active agents in the extracting solvents.

It was observed that different isolates exhibited varying degree of resistance to the ethanolic extract of the *Sida acuta*. This result supports the findings of Anani et al. (2000), who noted that methanolic extract of *Sida acuta* had a significant activity on *S. aureus*, *E. coli*, *B. subtilis* and *Mycobacterium phlei* as against no inhibition effect recorded on *Streptococcus faecalis* and *Klebsiella pneumoniae*. Similar results were obtained by Rajakaruna et al. (2002), Saganuwan and Gulumbe (2006) with methanolic extract of *Sida acuta*. This difference in susceptibility can be attributed to two factors. The inherent resistant factor of the different species of the isolates and the previous exposure of the organism to other antimicrobial drugs or agents, as a result of drug abuse in the population.

Finally, it was observed that the highest concentration of the ethanolic and aqueous extract of the plant has no significant effect on the fungi isolates. They had lower zone of inhibition compared to the antifungal agents used as control. Similar result was reported by Adeleke et al. (2006), who worked on the methanolic extracts of *Garcinia kola* and noted that the MIC values obtained for most bacteria species were lower than those for fungi species. This difference in their susceptibility could be traced to the structural and chemical differences in their cells and cell-wall structures.

Conclusion

Sida acuta has been known to be medicinally important in many respects. However, this study has revealed that the leaves of *Sida acuta* have several active agents that are inhibitory to microorganisms. The significant activity of the ethanolic extract against microorganisms from infected skin, confirms their traditional usefulness in the cure of wound infections. This report has also revealed that the solvents used for extraction plays a very important role in its level of activity. Unlike the root of the plant which has been reported to exhibit antifungal activity, the extracts from the leaves has been shown to be less active against fungi.

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