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Evaluation of the Phytochemicals and the Antibacterial Properties of *Sida Acuta* Leaf Extract and their Effects on Wound Bacterial Isolates

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ABSTRACT

This study was performed to evaluate the phyto-chemical and antibacterial properties of *Sida acuta* leaf extract against wound bacterial isolates. Standard methods were employed in the phytochemistry, the bacterial identification and the antibiotic susceptibility assay. Ethanol and acetone were used as the extraction solvents. Results obtained from the phytochemical analyses of the ethanol and acetone extracts revealed the presence of alkaloids, saponins, tannins, steroids, glycosides, polyphenols, oxalate and flavonoids. The sensitivity of the isolates to the extracts was represented by zones of inhibition at different concentrations. The highest zones of inhibition was observed at 50 mg/ml while the lowest was observed at 3.125 mg/ml. Gram-positive bacteria were found to be more sensitive to the extracts at different concentrations than the Gram-negative bacteria ($p > 0.05$). The minimum inhibitory concentration of both extracts against the Gram-positive and Gram-negative bacterial isolates were: 3.125 mg/ml and 6.25 mg/ml respectively. The minimum bacteriocidal concentration of both extracts against Gram-positive and Gram-negative bacterial isolates were: 12.5 mg/ml and 25 mg/ml respectively. The antibiogram of the isolates against standard antibiotics used as positive control revealed the resistant pattern of the isolates to conventional antibiotics used in medicine. The potential antibacterial effect of *Sida acuta* leaf extract has been revealed in this study, therefore its controlled use should be encouraged in the treatment of wounds and other infections caused by the bacterial isolates.

Keywords: Antimicrobial properties, Bacteria, Phytochemical analysis, *Sida acuta*, Wounds

INTRODUCTION

The problem of microbial resistance to drugs is continuously growing, hence the use of existing antimicrobial drugs in the future is still uncertain. Immediate action is therefore required to combat the problem, by encouraging research to develop new drugs; more so of herbal origin as synthetic drugs are known to cause side effects (Jindal *et al.*, 2012). The use of plants as medicines predates written human history (Omonkhelin *et al.*, 2007). Medicinal plants are distributed worldwide, but they are most abundant in the tropical countries (Calixto, 2000). The use of medicinal plants or herbs to treat diseases is almost universal among non-industrialized societies of the world and is often more affordable than purchasing expensive modern pharmaceuticals. World Health Organization (WHO) estimated that 80 percent of the population of some Asian and African countries presently use herbal medicines for some aspect of primary health care (Pooja *et al.*, 2015). A relative small percentage of medicinal plants are used as food by both humans and animals and it is possible that even more are used for medicinal purposes (Mbajiuka *et al.* 2014).

Sida acuta is a marvelous weed that frequently dominates improved pastures, waste and

disturbed roadside places (Okwu and Ekeke, 2003). The described pharmacological properties of the plants involve the antimicrobial, antioxidant and many other properties. It is however, worthy of note that the active ingredients contributing to the medicinal properties of plants are the phytochemicals, vitamins and minerals present in the plants (Mbajiuka *et al.* 2014). *Sida acuta* belongs to Malvaceae family. It is a taproot and perennial shrub, that is native to Mexico and Central America. However, today the plant is distributed in several parts of the tropics and the subtropics (Jindal *et al.*, 2012). The plant is frequently found in pastures, cultivated lands, roadsides and lawns. *Sida acuta* has wide application in Nigerian folk medicine. Some herbalist have claimed the traditional use of this plant to cure infections such as malaria, ulcer, fever, gonorrhoea, abortion, breast cancer following inflammation and wound infections (Edeoga *et al.* 2005). The leaf part of the plant is most frequently used against various (Iroha *et al.* 2009).

Wounds offer bacteria an attractive environment in which they can potentially flourish, and if left unchecked, cause significant damage. With potential for damaging infections and the surge in multi-drug resistant bacteria, practitioners

have become increasingly challenged to find solutions to wound infections. Infection of a wound is the successful invasion and proliferation by one or more species of microorganisms anywhere within the body's sterile tissues, sometimes resulting in pus formation (Calvin, 1998). Wound infections may occur following accidental trauma and injections, but post-operative wound infections in hospital are most common. Some infections are endogenous in which infection occurs from patient's own bacterial flora such as *Staphylococcus aureus* from skin, anterior nares or coliforms (Mohammed *et al.* 2013). Many infections are exogenous; skin and anterior nares are important sources of Staphylococci. Spread of organisms from hospital staff and visitors occur by direct and indirect airborne routes. Organisms commonly found in infected wounds include Gram-positive cocci such as *Staphylococcus aureus*, *Streptococcus* sp., Gram-negative bacilli mostly *Acinetobacter* sp, *Enterobacter* sp, *Escherichia coli*, *Proteus* sp., *Pseudomonas aeruginosa* and anaerobic bacteria such as *Propionibacterium* sp. and *Klebsiella* sp. (Taiwo *et al.*, 2002).

Development of wound infection depends on the interplay of many factors. The breaking of the host protective layer, the skin, and thus disturbing the protective functions of the layer, will induce many cell types into the wound to initiate host response (Collier, 2003). Despite optimal treatment, some wounds are slow to heal. The challenge clinically and microbiologically is to identify those wounds in which healing is impaired as a result of infection or heavy bacterial burden and in which systemic or topical antimicrobial treatment will be of benefit (Healy and Freedman, 2006).

The aim of this research work was to evaluate the phyto-chemical and antibacterial efficacy of *Sida acuta* leaf extract on wound bacterial isolates.

MATERIALS AND METHODS

Collection of Plant Material:

Fresh *Sida acuta* leaves were obtained from surroundings of homes and gardens in Benin City, Edo State, Nigeria. These were identified at the Department of Plant Biology and Biotechnology of the University of Benin, Benin City. Identification was confirmed using appropriate literature sources (Akobundu and Agyakwa, 1998).

Preparation of Extract:

The leaves of the test plants were well-dried and then grinded to powder. About 200 g of the powder were separately soaked in 400 ml of 95 % ethanol in a 500 ml reagent bottle for 24 hrs. These were then centrifuged at 3000 rpm to enable paper diffusion of the active ingredients into the

extraction medium. The fluids were then filtered using Whatman No1 filter paper, and the filtrate was evaporated to dryness using boiling water bath at 100 °C. This procedure was also carried out with acetone to obtain acetone extract. The extracts were then stored at 4 °C in a refrigerator.

Test Organisms

Bacterial cultures of the test organisms including, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Klebsiella pneumoniae* were obtained from the Department of Medical Microbiology, University of Benin teaching hospital, Benin City, Nigeria. Their identity was confirmed using cultural, morphological and biochemical test as previously described (Cheesebrough *et al.*, 2006) and (Prescott *et al.*, 2008; Akinnibosun *et al.*, 2008a, b). All of the bacterial cultures were maintained on nutrient agar slants at 4 °C prior to use.

Phytochemical Screening of the Extracts:

Phytochemical screening of the extracts was carried out according to methods described by Odebiyi and Soforowa, (1978) and Trease and Evans, (1989).

Antimicrobial Sensitivity Bioassay

The antimicrobial assay was performed by using the agar well diffusion method. Wells of 10 mm in diameter were made into previously seeded nutrient agar plates. Each well was filled with 1 ml of the extract. The same quantity of sterile distilled water without the plant extract served as negative control. The plates were pre-incubated for 2 hrs at room temperature to allow diffusion of extract before incubating overnight at 37°C. The diameter of clear zone was measured in mm. Triplicate plates were prepared for each extract and controls.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was determined by diluting the extracts double-fold (beginning with 40 mg/ml) with nutrient broth in a series of test tubes. To each of the tubes, equal volume of the test organism was added and incubated at 37°C for 24 hours. Controls were prepared by inoculating tubes without the extracts but with the cell suspensions. The tubes were then examined for the presence of turbidity after the incubation period. The least concentration with no observable bacterial growth when compared with the control was considered as the Minimum Inhibitory Concentration.

Determination of Minimum Bacteriocidal Concentration (MBC)

The positive MIC tubes were sub-cultured on nutrient agar plates with proper labels followed by incubation at 37 °C for 24 hrs. They were then

examined for growth of bacteria. The tube with minimum concentration of extract in which the growth was completely stopped was clearly noted as the minimum bacteriocidal concentration.

Statistical Analysis

The Statistical Package for Social Scientists (SPSS, version 16.0) was used for the analysis of the data that was obtained (Ogbeibu, 2005).

RESULTS AND DISCUSSION

Results of the phytochemical screening of the acetone and the ethanol extracts of *Sida acuta* are shown in Table 1. Alkaloids, saponins, flavonoids and other phytochemicals were found to be present in the plant extracts. These results are in general agreement with the reports of Raimi *et al.* (2014), who also observed the presence of these phytochemicals in *Sida acuta*. The sensitivity of the bacterial isolates to ethanol and acetone extracts of *Sida acuta* is shown in Figs. 1 and 2. The highest zones of inhibition was observed at 50 mg/ml concentration while the lowest was at 3.125 mg/ml. The data obtained showed that the inhibitory effects of the sample on the various investigated microorganisms were dose-dependent. This observation is in agreement with the findings of Akinnibosun and Itedjere (2013). Gram-positive bacteria was found to be more sensitive to the extracts at different concentrations than the Gram-negative bacteria ($p > 0.05$). These results are consistent with those of Dicko *et al.* (2005). Iroha *et al.* (2009) reported the antimicrobial effect of aqueous and ethanolic leaf extract of *S. acuta* against 45 clinical isolates of *Staphylococcus aureus* isolated from nasal cavity of Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) infected patients from University of Nigeria Teaching Hospital. The results indicated that *S. acuta* extracts have appreciable antimicrobial activity against *S. aureus* which is in perfect agreement with the one obtained in this study where the ethanolic and the acetone extracts of the leaf were able to show significant activity at 6.25 mg/ml. The minimum inhibitory concentration and minimum bacteriocidal concentration of ethanol and acetone extracts of *Sida acuta* leaf against bacterial isolates

are shown in Figs. 3 and 4. The mean MIC of both extracts for Gram-positive organisms was 3.125 mg/ml and 6.25 mg/ml for Gram-negative bacteria. The minimum bacteriocidal concentration of both extracts was 12.5 mg/ml for Gram-positive and 25 mg/ml for Gram-negative. The minimum inhibitory concentration and minimum bacteriocidal concentration also revealed the potency of the extract against Gram-positive bacteria compared to Gram-negative species used in the study. Tables 2 and 3 show the antibiogram of the Gram-positive and the Gram-negative bacterial isolates respectively, against standard antibiotics used as positive control. From the results of this study, it was observed that *Sida acuta* extracts compared favourably with the standard antibiotics. Appreciable resistance was observed for all isolates to the different antibiotics which have found use in clinical medicine. The microorganisms were found to be resistant to many of the standard antibiotics used. The resistant nature of these microorganisms may have been acquired via plasmid transfer or chromosomally mediated (Walsh, 2000; Cohen, 2002; Coutinho and Siqueira-Junior, 2010). Drug abuse and indiscriminate misuse of antibiotics among the general population has favoured the emergence of resistant strains. Multidrug resistance was observed for most of the test bacteria as they were resistant to more than one drug (Wasfy *et al.*, 2000; Akomie and Akpan, 2013). The worldwide escalation in both community and acquired antimicrobial resistant bacteria has threatened the ability to effectively treat patients, emphasizing the need for continued surveillance, more appropriate antimicrobial prescription, prudent infection control and new treatment alternatives (Rhomberg *et al.*, 2006; Chikere *et al.*, 2008; Okonko *et al.*, 2009b). The susceptibility of antibiotic resistant bacterial strains to the plant extract is quite interesting and these plant extracts can be used as an alternative in the treatment of diseases caused by these microorganisms (Cohen, 2002). The broad spectrum of activity displayed by the samples in this study appears to justify and explain the scientific basis for their uses in traditional medicine. It is hoped that this study would lead to further investigations that would enhance the preparation of antibacterial drugs of natural origin for the treatment of wounds and infections caused by the test organisms.

Table 1. Phytochemical Screening of *Sida acuta*

Phyto-chemical constituents	Ethanol extract	Acetone extract
Alkaloids	+	+
Steroids	+	+
Saponins	+	-
Tannins	+	+
Flavonoids	+	+
Oxalate	+	+
Polyphenols	+	+
Glycosides	+	-

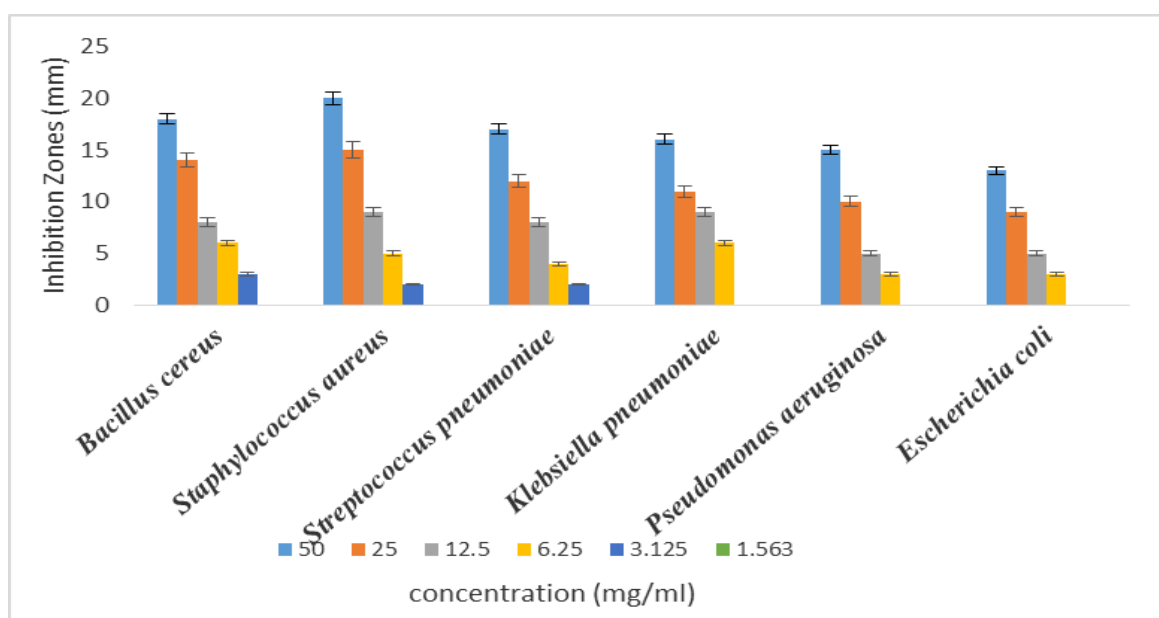


Fig. 1: Sensitivity of Ethanolic Extract of *Sida acuta* Against the Bacterial Isolates

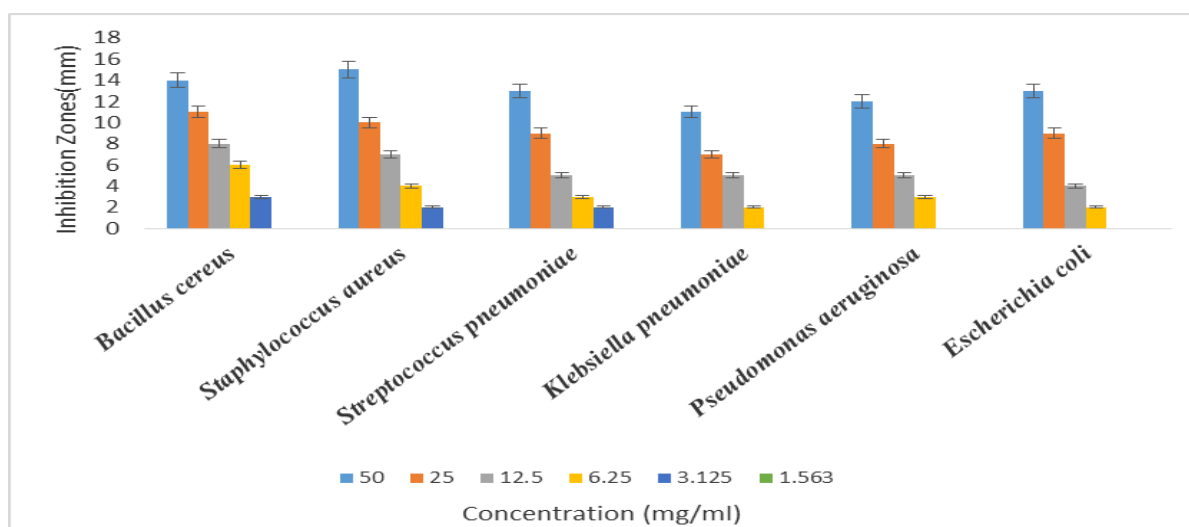


Fig. 2: Sensitivity of Acetone Extract of *Sida acuta* Against the Bacterial Isolates.

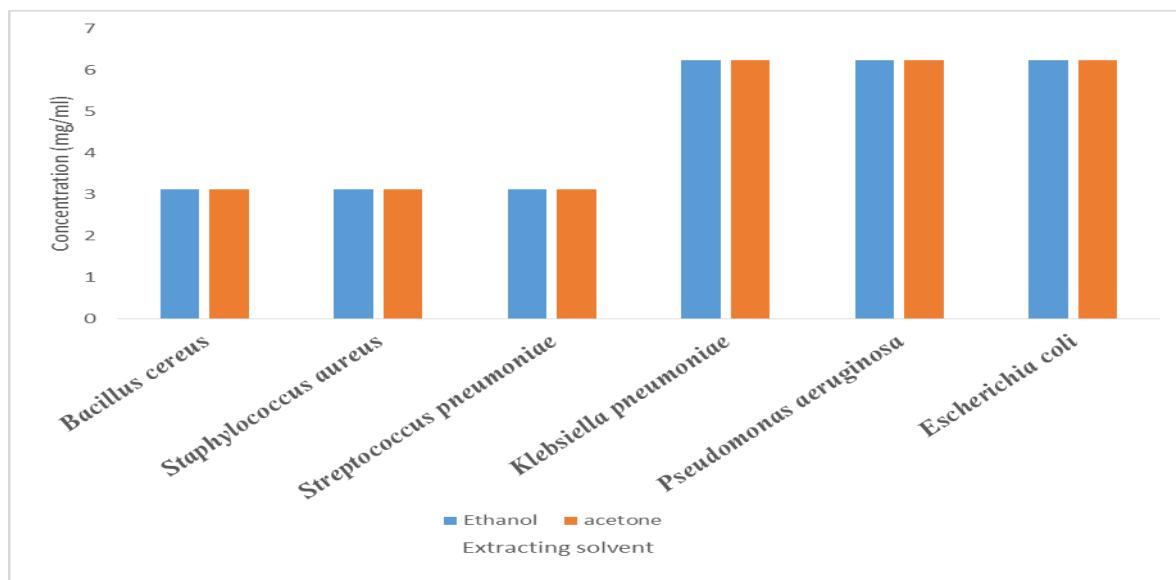


Fig. 3: Minimum Inhibitory Concentration of *Sida acuta* Against the Isolates

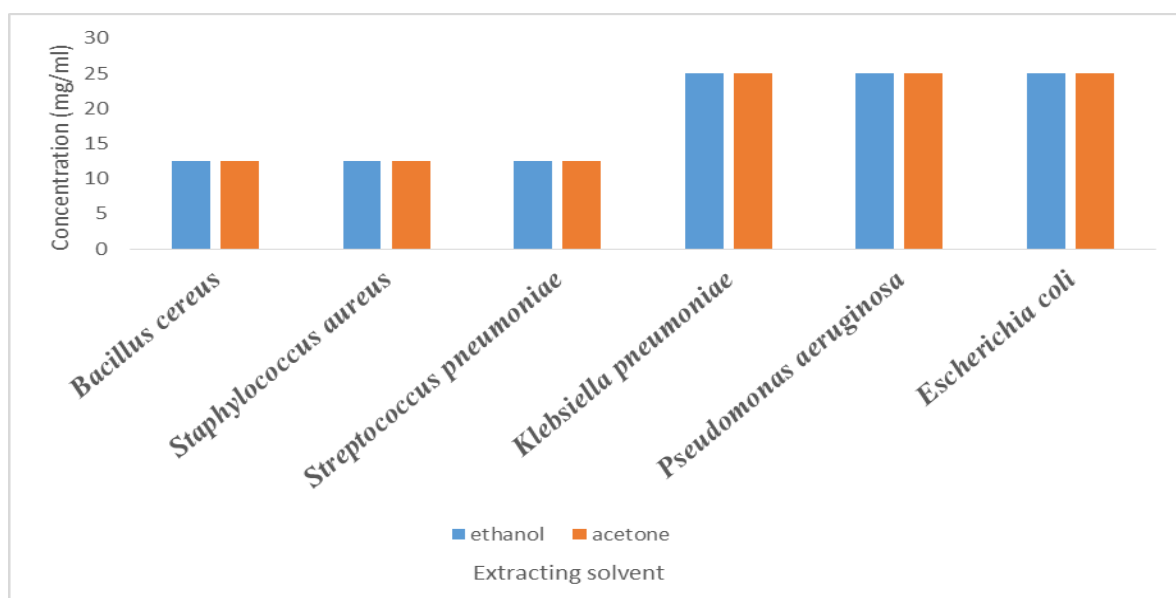


Fig. 4: Minimum Bacteriocidal Concentration Of *Sida acuta* Against the Isolates

Table 2: Antibiogram of Gram-negative (-ve) Bacterial Isolates Against Standard Antibiotics

Gram -ve	AM	AU	GN	PEF	OFX	STR	SXT	CH	SP	CN
<i>K. pneumoniae</i>	R	R	I	R	I	I	R	I	R	R
<i>P. aeruginosa</i>	R	R	R	S	S	R	R	R	R	S
<i>E. coli</i>	R	R	S	S	I	R	R	R	R	I

Legend: AM-Amoxicillin (30µg); AU-Augmentin (30µg); GN-Gentamycin (30µg); PEF-Pefloxacin (30µg); OFX-Ofloxacin (50µg); STR-Streptomycin (30µg); SXT-Septin (30µg); CH-Chloramphenicol (30µg); SP-Sparfloxacin (30µg); Cikatin (30µg).

Table 3: Antibiogram of Gram-positive (+ve) Bacterial Isolates Against Standard Antibiotics

Gram+ve	AM	R	CPX	S	SXT	E	PEF	GN	APX	Z
<i>B. cereus</i>	R	R	S	I	R	R	S	S	I	I
<i>S. aureus</i>	R	I	S	R	R	R	I	S	R	I
<i>S. pneumoniae</i>	R	R	S	S	R	R	S	S	I	I

Legend: AM-Amoxicillin (30µg); GN-Gentamycin (30µg); PEF-Pefloxacin (30µg); SXT-Septtrin (30µg); CPX-Ciprofloxacin (50µg); R-Rifampin (30µg); E-Erythromycin (30µg); APX-Ampiclox (30µg); Z-Zinnacef (30µg); S - Streptomycin (30µg).

CONCLUSION

The increasing nature of antibacterial resistance has necessitated the search for alternative and more effective therapy to alleviate disease conditions. In this study, *Sida acuta* leaf extract has shown potential antibacterial effect against bacterial pathogens and their resistance to conventional antibiotics. The leaf extract has shown greater spectral activity against Gram-positive bacteria than it has shown to Gram-negative bacteria. This effect makes the *Sida acuta* leaf extract to possess the potential for use against Gram-positive bacterial infections in the future. Further research to test the efficacy of the plant against fungi and possibly viruses would be very ideal. In addition, toxicity and lethality studies on the plant and the extracts would be needed in order to ascertain the relevant safety margins.

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