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Antimicrobial and Phytochemical Analyses of *Sida acuta* Leaf Extracts on Selected Wound Isolates

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Abstract

Medicinal plants have been intensively screened for their bioactivity in order to treat various diseases in human. This study was performed to evaluate the antimicrobial and phytochemical properties of *Sida acuta* leaf extracts against selected wound microbes. The plant extracts were obtained sequentially using n-hexane, aqueous and acetone respectively in order of increasing polarity. The extracts were tested for their antimicrobial activities against *Staphylococcus aureus, Candida albicans, Klebsiella pneumoniae, Enterococcus faecium, Acinetobacter baumannii, Salmonella typhi* and *Pseudomonas aeruginosa* using agar-well diffusion method. The sensitivity of the test organisms to the extracts was represented by zones of inhibitions (mm) at different concentrations. There was corresponding increase in the zones of inhibitions (mm) on the test organisms as the concentration of the extracts increased from 64 mg/ml – 512 mg/ml. Among the plant extracts, the aqueous extract of *Sida acuta* leaf revealed significantly higher zones of inhibitions (mm) from 7.50±2.12 - 25.00±1.40 on all isolates. This was closely followed by the acetone extract while the n-hexane extract produced the lowest zones of inhibitions (mm) on all the test organisms except for *Acinetobacter baumannii* which has 9.80±0.42 at the concentration of 512 mg/ml. Results obtained on the phytochemical analyses of the aqueous and acetone extracts revealed the presence of tannins, saponins, alkaloids and flavonoids. Hence, the antimicrobial effects of *Sida acuta* leaf extracts has been revealed in this study, therefore its controlled use should be encouraged in the treatment of wounds and other infections caused by these microorganisms.

Keywords: Antimicrobial properties, Isolates, Phytochemical analyses, *Sida acuta*, Wound

Introduction

The problem of microbial resistance to drugs is growing continuously hence, the use of existing antimicrobial drugs in the future is not certain. Immediate action is therefore required to fight the problem, by supporting and encouraging research to develop new drugs; especially those of herbal origin as synthetic drugs are known to have side-effects (Akinnibosun & Pela, 2015). The use of plants as treatments predates written human history. Medicinal plants are distributed throughout the world, but they are most bountiful in the tropical countries. The use of medicinal plants to handle

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diseases is almost common among non-industrialized societies of the world and is often more affordable and available than purchasing the expensive modern pharmaceuticals (Jindal *et al.,* 2012).

World Health Organization (WHO) arrived at an estimation that about 80% population of some Asian and African countries currently use herbal medicines for some aspect of primary health care service. A relatively lower percentage of medicinal plants are used as food or supplements by both humans and animals and there is a possibility that even more are used for medicinal purposes (Pooja *et al.,* 2015).

Sida acuta is a wonderful and exciting weed which belongs to the Malvaceae family. It is a taproot and perennial shrub that is native to Mexico and Central America. It is a small and erect plant of about 30-150 m in height. It is hairy branded up to 1 m high and the plant is reproduced from their seeds. The stems are fibrous with a thread-like bark; the leaves are simple and alternate with stalks of 1.3 cm long jointed at half the length. The flowers are yellow with five petals and the fruit has capsules with 5-6 carpel (Chinelo & Miracle, 2018). The plant is found in cultivated and non-cultivated lands, pastures and lawns but mostly found in abundance in tropical areas (Shittu & Alagbe, 2020).

Sida acuta has wide applications, in Nigeria in folk medicine. Some herbalists have claimed the use of this plant traditionally to cure infections and ailments such as fever, ulcer, gonorrhea, malaria, and breast cancer following inflammations and wound infections. The described pharmacological attribute of the plant includes antioxidant, antimicrobial, anti-inflammatory and several others (Mbajiuka *et al.,* 2014). The leaf part of the plant is the most commonly and frequently used against various diseases (Shittu & Alagbe, 2020).

Wounds give microorganisms an attractive abode in which they can powerfully flourish, and if left unobserved, can result into a significant damage. With the ability to damage infections and the sudden increase in the development of multi-drug resistant microorganisms, medical practitioners have become more challenged to look for solutions to wound infections. Infection of a wound is the successful invasion by one or more species of microorganisms anywhere within the body's sterile tissues which sometimes results in pus formation. Wound infections may occur due to an accidental trauma and injections, but post-operative wound infections in hospital and medical centers are most common (Jindal *et al.,* 2012).

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.

Development of wound infection depends on the interactions of many factors. The breakdown of the protective layer of the host's skin and thus**,** the alteration of the protective functions of the layer which will induce many cell types into the wound, to initiate host response. Despite the most favorable treatment, some wounds are slow to heal. The clinical and microbiological challenge is to identify those wounds in which healing is impaired as a result of various infections and heavy bacterial burden in which systemic or topical antimicrobial treatment will be of benefit (Akinnibosun & Pela, 2015).

The aim of this research work is to evaluate the antimicrobial and phytochemical efficacy of *Sida acuta* leaf extracts on selected wound isolates.

Materials and Methods

Collection and Identification of Plant Materials

Fresh *Sida acuta* leaves were obtained from the surroundings of homes and gardens in Malete, Kwara State, Nigeria. These have been identified at the Herbarium Section of the Department of Plant Biology, University of Ilorin, Ilorin, Kwara

state with the voucher number UILH/101/2019/1102.

Preparation of Plant Extracts

The leaves were cleansed with distilled water and air-dried in the Microbiology Laboratory of Kwara State University, Malete, Kwara state. They were then ground to powder using a mortar and pestle. The extracts were obtained sequentially with the use of three different solvents. This was done in an increasing order of polarity; n-hexane →acetone → aqueous. Exactly 150 g of the powder was soaked in 550 ml of n-hexane in a reagent bottle and well mixed before placing on a shaker for intermittent shaking at 130 revolutions per minute (rpm) for 48 h. Using a clean muslin cloth and a Whatman no1 filter paper, the solution was filtered. The filtrate was collected in a separate reagent bottle while the plant residue was spread out to air-dry on a clean foil paper in the laboratory. When the residue is completely dry, the process was repeated in order to obtain the acetone and aqueous extracts. After the last extract has been obtained, the filtrates were collected in reagent bottles while the resulting residue was discarded. The filtrates were evaporated to dryness using a rotary evaporator and then kept in the refrigerator at 4 °**C** prior to use (Jayaseelan *et al*., 2012).

Test Organisms

Bacterial cultures of the test organisms including, *Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecium, Acinetobacter baumannii, Salmonella typhi* and *Pseudomonas aeruginosa*, and the fungal isolate *Candida albicans*, were obtained from the Department of Medical Microbiology, University of Ilorin teaching hospital, Ilorin, Nigeria. Their identity was confirmed by the use of morphological and cultural method of characterization, Gram staining, Lacto-phenol cotton blue stain and some biochemical tests such as catalase test, citrate test, oxidase test and indole test as previously described by (Akinnibosun & Pela, 2015). The microbial cultures were stocked in nutrient agar and PDA slants at 4 ºC prior to use.

Phytochemical Screening of the Plant Extracts

The qualitative phytochemical screening of the plant extracts were carried out at the Biochemistry Department, Kwara state University, Malete, Kwara state whereby saponin, tannin, alkaloids and flavonoids were detected.

Antimicrobial Sensitivity Bioassay

Using the agar well diffusion method, the antimicrobial assay was performed. The organisms were inoculated in nutrient broth and potato dextrose agar respectively then incubated for 12-18 h at 37 ºC to obtain a fresh culture. Standardization of the test organisms was done by adjusting their turbidity to 0.5 McFarland Standard. Mueller Hinton agar plates were lawn cultured with the standardized microbial culture broth. On the media, (MHA plates), 6 wells were bored using 6 mm diameter of sterile cork borer, each well was filled with the 4 different concentrations of the plant extracts which were already reconstituted with 3% Di-Methyl Sufoxide (DMSO) to obtain the working concentrations of 512, 256, 128 and 64 mg/ml, positive control (Cefuroxime as the antibacterial and Fluconazole as antifungal agents) and distilled water without the plant extract served as the negative control. The plates were allowed to diffuse for about 30 min at room temperature and then incubated for 18-24 h at 37 ºC. After incubation, the plates were observed for the formation of a clear zone of inhibition around the wells which demonstrates the antimicrobial activity of the tested compounds (Sarita *et al*., 2019).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the plant extracts was carried out by diluting the extracts serially with nutrient broth in a series of sterile test tubes. In each of the tubes, the same volume of the test organisms i.e 0.5 McFarland standard was added and

incubated at 37 ºC for a period of 24 h. Controls were prepared by inoculating the tubes without the extracts but with the cell suspensions of the isolates. The test tubes were examined for the observation of turbidity of the bacterial suspension after the incubation period. The least or minimal concentration with no observable bacterial/ fungal growth when compared with the control was considered as the Minimum Inhibitory Concentration (Akinnibosun & Pela, 2015).

Determination of Minimum Bactericidal Concentration (MBC)

The positive MIC tubes were sub-cultured on nutrient agar plates with proper labels followed by incubation at 37º C for 24 h. The plates were later examined for growth of the isolates. The tube which contains the minimum concentration of the plant extract in which the growth of the organisms was completely stopped was clearly noted as the minimum bactericidal concentration (Akinnibosun & Pela, 2015).

Statistical Analyses

All experiments were carried out in triplicates. The Statistical Package for Social Scientists (SPSS, Version 23.0) was used for the analysis of the data obtained. One way analysis of variance (ANOVA) was used to obtain variables and to test if there is significant difference in the effect of each extracts of *Sida acuta* leaf

Results

Table 1: Microorganisms implicated in wound infections used as test organisms

Test organisms
Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecium, Acinetobacter baumannii, Salmonella typhi, Pseudomonas aeruginosa and Candida albicans

Table 2: Qualitative phytochemical analyses *Sida acuta* leaf extracts

Phytochemical components	Extracts	
	Acetone	Aqueous
Tannin	++	+++
Saponin	+	+++
Flavonoids	++	++
Alkaloids	++	++

Key: +++ high amount; ++ moderate amount; + trace/minute amount

Table 3: Antimicrobial effect of n-hexane extract of *Sida acuta* leaf on the test organisms using agar-well diffusion method

	Concentration and Zone of inhibition (mm)			
Organisms	512 mg/ml	256 mg/ml	128 mg/ml	64 mg/ml
Staphylococcus aureus	2.50±0.71	2.0±0.71	0.00±0.00	0.00±0.00
Klebsiella pneumoniae	8.65±0.64	3.00±0.71	2.50±0.00	2.00±0.00
Enterococcus faecium	3.00±0.71	0.00±0.00	0.00±0.00	0.00±0.00
Acinetobacter baumannii	9.80±0.42	4.35±1.63	0.00±0.00	0.00±0.00
Salmonella typhi	8.60±0.57	8.25±0.35	7.00±0.00[b]	4.25±0.35
Pseudomonas aeruginosa	3.35±0.21	0.00±0.00	0.00±0.00[a]	0.00±0.00
Candida albicans	3.35±0.212	0.00±0.00	0.00±0.00[a]	0.00±0.00

Table 4: Antimicrobial effect of acetone extract of *Sida acuta* leaf on the test organisms using agar-well diffusion method

	Concentration and Zone of inhibition (mm)			
Organisms	512 mg/ml	256 mg/ml	128 mg/ml	64 mg/ml
Klebsiella pneumoniae	12.75±0.35	9.10±0.14	1.35±0.21	0.00±0.00
Enterococcus faecium	8.60±0.57	6.80±0.42	3.00±0.00	3.00±0.00
Acinetobacter baumannii	6.75±0.35	5.30±2.55	1.50±2.12	1.50±2.12
Salmonella typhi	12.10±0.14	10.75±0.35	8.60±0.57	6.75±0.35
Pseudomonas aeruginosa	9.60±0.57	8.60±0.85	7.20±0.00	6.25±0.35
Candida albicans	12.60±0.56	8.65±0.63	7.00±1.06	8.63±2.69

Table 5: Antimicrobial effect of aqueous extract of *Sida acuta* leaf on the test organisms using agar-well diffusion method

	Concentration and Zone of inhibition(mm)			
Organisms	512 mg/ml	256 mg/ml	128 mg/ml	64 mg/ml
Klebsiella pneumoniae	17.5±2.12	10.85±2.33	8.0±2.12	7.70±2.12
Enterococcus faecium	19.00±0.70	16.00±0.00	16.00±0.00	13.15±0.07
Acinetobacter baumannii	19.0±0.00	14.05±0.07	14.25±0.35	7.50±2.12
Salmonella typhi	25.00±1.40	20.00±0.70	18.25±1.06	16.25±2.47
Pseudomonas aeruginosa	17.35±0.21	14.75±1.06	13.75±0.35	9.60±0.57
Candida albicans	14.35±0.21	11.60±0.85	11.50±0.71	9.00±0.00

Table 6: Effects of the positive and negative control on the test organisms

Organisms	Positive control (CFX-AB/FCZ-AF) (mm)	Negative control (STD) (mm)
Staphylococcus aureus	27.00±1.32	-
Klebsiella pneumonia	27.32±1.50	-
Enterococcus faecium	19.00±0.72	-
Acinetobacter baumanni	25.00±1.49	-
Salmonella typhi	26.00±1.29	-
Pseudomonas aeruginosa	25.52±1.30	-
Candida albicans	-	-

Key: CFX=Cefuroxime, FCZ=Fluconazole, AB=Antibacterial agent, AF=Antifungal agent, STD=Sterile Distilled Water, -=No zone of inhibition

Table 7: Minimum inhibitory and cidal concentrations of *Sida acuta* leaves extracts

Microorganisms	Concentrations(mg/ml)					
	Aqueous		Acetone		N-hexane	
	MIC	MCC	MIC	MCC	MIC	MCC
S. aureus	10.0	12.5	7.5	7.5	12.5	-
K. pneumonia	12.5	12.5	10.0	12.5	12.5	-
E. faecium	7.5	10.0	10.0	10.0	12.5	-
A.baumanni	12.5	20.0	12.5	20.0	10.0	-
S. typhi	10.0	-	10.0	-	10.0	-
P. aeruginosa	10.0	-	7.5	10.0	10.0	-
C. albicans	25.0	-	25.0	-	7.5	-

Discussion

This study revealed the effects of aqueous, acetone and n-hexane extracts of *Sida acuta* leaf on some selected clinical isolates associated with wound infections; *Staphylococcus aureus, Enterococcus faecium, Klebsiella pneumoniae, Acinetobacter baumannii, Salmonella typhi, Pseudomonas aeruginosa* and *Candida albicans* as wounds offer bacteria an attractive environment in which they can potentially flourish whereby causing significant damage if left untreated (Table 1).

In the serial exhaustive (sequential) extraction of the test plant extracted with n-hexane, acetone and water, the lowest yield was observed in n-hexane and aqueous extraction has the highest yield. Therefore, the amount of yield extracted is high in polar solvents than the non-polar solvents. The polarity of the different solvents may be responsible for variations in solubility of active phyto-components of the plant, hence, difference in efficacy levels (Ahmed *et al.,* 2017). Difference in yield may be as a result of extraction capacities of the different solvents used as reported by Zakariyah *et al.* (2017). Method of extraction is important in antimicrobial investigations as this determines to a large extent the outcome of the study (Anyanwu & Okoye, 2017). The solvents of choice in this study of sequential extraction system for the extraction of *Sida acuta* leaves are water and acetone. Thereafter, the extracts were made to undergo sterility test and all proved sterile as there was no growth observed after incubating for 24 h.

Phytochemical screening of the aqueous and acetone extract of *Sida acuta* as shown in Table 2 revealed alkaloids, saponins, tannins and flavonoids to be present in the plant extracts. In aqueous extract of the plant, it was observed that saponins and tannins were in high quantity while alkaloids and flavonoids were in moderate amount. These results obtained are in general agreement with the reports of Raimi *et al.* (2014). The presence of these active components in studied part of the plants may be responsible for its antimicrobial sensitivity on the test organisms as reported by some scientists (Kolapo *et al.,*2009; Zakariyah *et al.,*2017). From the results of this study, it was observed that *Sida acuta* extracts compared favorably with the standard antimicrobial agents (Table 6).

The sensitivity of the test organisms to n-hexane, acetone and aqueous extract of *Sida acuta* leaf is shown in Tables 3, 4 and 5. Antimicrobial activities of n-hexane extract of *Sida acuta* leaf was mildly significant against the test organisms, this might be due to the method of extraction and the morphological characteristics of the test isolates as observed in the results of Ayanwale *et al.* (2019). At the concentration of 512 mg/ml (Table 4), it was observed that the n-hexane extract of *Sida acuta* leaf was mildly effective against *Acinetobacter baumannii, Klebsiella pneumoniae* and *Salmonella typhi* being 9.80±0.42

mm, 8.65±0.64 mm and 8.60±0.57 mm respectively and insignificant against other test organisms at lower concentrations
as they show resistance and no zones of inhibitions was observed. The aqueous extract of *Sida acuta* leaf was the most effective against all the test organisms at all concentrations (Table 5), *Salmonella typhi* had the highest zone of inhibition being 25.00±1.40 mm while the lowest zone of inhibition was observed in *Candida albicans* being 14.35±0.21 mm at 512 mg/ml. The antibacterial activity of the aqueous extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa* is in agreement with the findings of Senthilkumar *et al.* (2018). The acetone extract of *Sida acuta* leaf was almost as significant as the aqueous extract at the same concentrations except against *Staphylococcus aureus* and *Acinetobacter baumanni* where it is mildly significant with 5.85±0.50 mm and 6.75±0.35 mm respectively at 512 mg/ml. This result does not agree with the findings of Chinelo & Miracle (2018) may be due to the different methods of plant extraction and variability in the concentrations of extract. In comparison among the positive controls and the three plant extracts, *Staphylococcus aureus* and *Salmonella typhi* were susceptible to cefuroxime and has the highest zones of inhibitions at 27.00±1.32 mm and 26.00±1.29 mm respectively while *Candida albicans* which shows resistance to fluconazole as no zone of inhibition was observed. This work is in contrast with the result reported by Ghaddar *et al.* (2020).The wide range of activity shown by the samples in this study appears to provide an acceptable explanation of 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 antimicrobial drugs of natural origin for the treatment of wounds and infections caused by the test isolates.

Conclusion

This study has revealed that the leaves of *Sida acuta* have chemicals (compounds) of intermediate polarity and possess potentially active antimicrobial agents that are inhibitory to the test organisms. The significant activity of the plant extracts against the selected clinical isolates confirms its traditional usefulness in wound infection therapy. Also, it was revealed in this study that the solvents used for extraction plays a vital role in the level of activity displayed by the plant.

Conflict of Interest

Authors declare no conflict of interest.

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