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Evaluation of the Antibacterial Activity of Extracts of *Sida acuta* Against Clinical Isolates of *Staphylococcus aureus* Isolated from Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome Patients

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Abstract: Antimicrobial activity of aqueous and ethanol leaves extracts of *Sida acuta* against 45 clinical isolates of *Staphylococcus aureus* isolated from nasal cavity of Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) patients from University of Nigeria teaching hospital, Enugu was evaluated using agar well diffusion method. The Minimum Inhibitory Concentration (MIC) of the extracts was also determined using agar well diffusion technique and the killing rates of each extracts was also determined at different time intervals of 0-90 min. Results of the agar well diffusion study revealed that ethanol extracts produced the highest antimicrobial activity (86%), followed by hot water (61%) and cold water extracts (48%). The MIC obtained ranged from 0.9625-1.8125 μg mL^{-1} for ethanol extracts, 7.8125-31.25 μg mL^{-1} for hot water and 15.625-31.25 μg mL^{-1} for cold water extracts. The result of killing rate studies showed that the test organisms were killed within 0-10 min for ethanol and hot water extracts and 5-60 min for cold water extracts. The over all results indicated that *Sida acuta* extracts have appreciable antimicrobial activity against *S. aureus* isolated from HIV/AIDS patients. In addition to authenticating, the folkloric use of *Sida acuta* in the treatment of common diseases, the finding of this study highlights the possible usefulness of this plant material in the treatment of opportunistic infections caused by *S. aureus* in HIV/AIDS patients.

Key words: Antimicrobial activity, susceptible, screening, test organism, aqueous, ethanolic, leaf extracts

INTRODUCTION

The common use of plant materials as a curative agent against diseases remain an important part of African tradition and over 70% of the population depends directly on it for primary health care. *Sida acuta* belonging to the family malvaceae (common name in Nigeria iseketu) is a shrub indigenous to Mexico and Central America and have spread to tropics and sub tropics (Holm *et al*., 1977). It has wide application in Nigeria folk medicine. Some herbalist have claimed the traditional use of this plant to cure infections such as malaria, ulcer, fever, gonorrhea, abortion, breast cancer following inflammation, wound infections (Kayode, 2006; Edeoga *et al*., 2005). The leaf part is the most frequently used against various infections. The ethanol leaf extracts of the plant has been shown to posses' moderate activity against the venom of *Bothrox athrax*. Human Immunodeficiency Virus (HIV) is a worldwide disease that kills millions of people every year with 2 distinctive types (HIV 1 and HIV 2). HIV 2 occurs most commonly in West Africa and occasionally infections have occurred in East Africa (Iroha *et al*., 2008). However, HIV 1 transmission is slightly less easy and the progression of HIV 2 infections to AIDS may be slower. HIV/AIDS infections are known to be complicated with infections caused by a wide variety of opportunistic microorganisms ranging from bacteria to fungi due to the reduced immune system of patients. The primary objective of this study, was to evaluate the antibacterial activity of leaves extracts of *Sida acuta* against clinical isolates of *S. aureus* isolated from nasal cavity of HIV/AIDS patients in University of Nigeria teaching hospital Enugu.

MATERIALS AND METHODS

Collection of plant material: The leaves of *Sida acuta* were collected from Ikpoba Okha local government area of Edo state, Nigeria. Prof. SSC Onyekwere of the Department of Applied Biology Ebonyi State University Abakaliki taxonomically identified the plant, where a herbarium voucher specimen of *Sida acuta* leaves was deposited. The study was conducted from October 2006 to July 2007 at the Department of Applied Microbiology, Ebonyi State University, Abakaliki.

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Extraction of plant materials: The washed leaves of the plant were dried on the laboratory bench for 7 days and then ground to power using a mechanical grinder. One hundred and fifty grams of the ground leaves of the plant material were separately soaked in 300 mL of ethanol and cold water for 48 h. The same quantity (150 g) of the ground plant material was immersed in 300 mL of hot water (100°C) and allowed to stand for 5 h with occasional agitation. Each of the preparation was filtered with 150 mm size of Whatmann No 1 filter paper (Schleicher and Schwell, England) and each filtrate was evaporated to dryness in a steady air current for 24 h in a previously weighed crucible. The dried extract were, respectively sterilized in hot air ovum (Galenkamp Germany) at 70°C for 1 h and checked for sterility by streaking on sterile nutrient agar plate that was incubated at 37°C for 18-24 h.

Collection of test organisms: A total of 45 clinical isolates of *Staphylococcus aureus* were collected from nasal cavity of HIV/AIDS patient at UNTH, Enugu using sterile swab sticks. A standard strain of *Staphylococcus aureus* (NCTC 11873) was used as control.

Microbiological media: Nutrient agar, nutrient broth and Mueller Hinton agar (Oxoid UK) used were prepared according to manufacture's specifications.

Susceptibility studies using agar-well diffusion method: Each of the test organisms were grown in a nutrient broth for a period of 24 h at 37°C. The 24 h broth culture was serially diluted to 0.5 MacFarlands equivalent turbidity standard. Sterile Mueller Hinton agar plates were prepared and each of the test organisms was streaked on the surface of the respective agar plates. These were allowed for 45 min to pre-diffuse and a 6 mm sterile cork borer was used to bore holes on the agar plate. One hundred micro litre of the respective extracts of ethanol, cold and hot water extracts were introduced into the wells and allowed for 45 min to diffuse into the agar. A 25 mg mL^{-1} of Lincomycin was used as control antibiotic. The inoculated Mueller Hinton agar plates were incubated at 37°C for 24 h and the inhibition zone diameter was measured using meter rule. Minimum Inhibition Concentration (MIC) of Five clinical isolates of the test organisms that showed the highest zone of inhibition was determined using standard agar diffusion technique.

Determination of Minimum Inhibitory Concentration (MIC) using agar diffusion method: A stock solution of 500 mg mL^{-1} of hot and cold water extracts and a 50 mg mL^{-1} of stock concentration of ethanol extracts were prepared by first reconstituting in 20% DMSO with sterile water and was serially diluted in 2 folds to achieved different concentrations of the extracts. A 20 mL sterile Mueller Hinton agar was poured into agar plates and allowed to stand for 1 h to gel. Five selected clinical isolates of *S. aureus* that had the highest zones of inhibition on each of the extracts were seeded on the surfaces of the respective agar plates. The plates were allowed to stand for about 15-30 min to pre-diffuse. Wells of 6 mm diameter were then bored on the agar plates using sterile cork borer and 100 μL of each diluted extract were introduced into the wells. All the plates were incubated at 37°C for 24 h and the least concentration of each extracts that inhibited microbial growth after the incubation period was taken as the MIC.

Killing rate kinetics: Five selected clinical isolates of *S. aureus* that had the highest zones of inhibition were subjected to killing rate studies. A 0.1 mL of each test organism was inoculated into a double strength nutrient broth and incubated at 37°C for 24 h. Another fresh 5 mL of double strength nutrient broth was prepared and 0.1 mL of the 24 h old culture of the test organisms were inoculated and incubated at 37°C for 3 h in order to activate and obtain fresh inoculums of the test organisms. These were subsequently diluted to achieve 0.5 MacFarland equivalent standard and then inoculated into test tubes containing fresh 5 mL of 25 mg mL^{-1} of each extracts. A 0.1 mL of each mixture was taken at various time intervals of 0, 5, 10, 30, 60, 70, 80 and 90 min. This was diluted in sterile normal saline and spread plated in an over dried sterile agar plate. This was incubated at 37°C for 18-24 h.

RESULTS

The results of the screening showed that 86% clinical isolates of *S. aureus* tested were susceptible to ethanol extracts of *Sida acuta*, 61% to hot water extracts, 48% to cold water extracts and 80% to Lincomycin (Fig. 1). The MIC results of the 5 selected strains ranged from 0.9625-1.8125 μg mL^{-1} for ethanol extract, 7.8125-31.25 μg mL^{-1} for hot water and 15.625-31.25 μg mL^{-1} for cold water extract. The MIC of the control antibiotic was within the ranges of 7.8125-31.25 μg mL^{-1}. The MIC result of ethanol extract on the standard strains was 6.125 μg mL^{-1}, for hot water is 31.25 μg mL^{-1}, for cold water is 62.5 μg mL^{-1} and for Lincomycin is 1.99 μg mL^{-1} (Table 1). The killing rate studies of the ethanol extracts showed that test organisms No. 1 was killed from 10 min, No. 2 from 30 min, No. 3 from 5 min and No. 4 and 5 from 0 min. For hot water extract isolates No. 1 was killed from 10 min, No. 2 and 3 from 5 min and No. 4 and 5 were from 0 min. For cold water extracts isolates No. 1 was killed from 30 min, No. 2 from 10 min and No. 3-5 were from 5 min (Table 2-4).

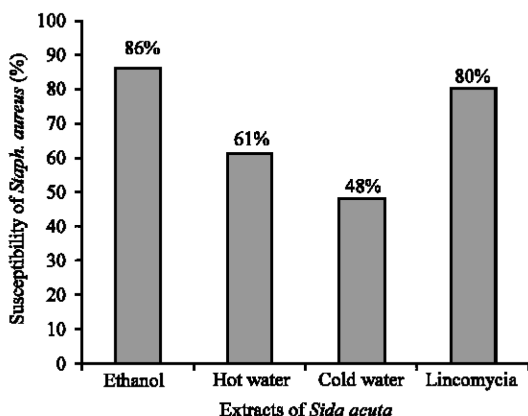

Fig. 1: Antimicrobial susceptibility patterns of 45 clinical isolates of *S. aureus* to Ethanol, hot water, cold water extracts of *Sida acuta* and lincomycin

Table 1: Minimum Inhibitory Concentration (MIC) μg mL^{-1} of ethanol, hot water, cold water extract and lincomycin of 5 selected clinical isolates of *S. aureus*

	Extract			
Org. No.	Ethanol	Hot water	Cold water	Lincomycin
01	1.8125	31.25	31.25	15.625
02	0.9625	7.8125	15.625	7.8125
03	1.8125	7.8125	15.625	7.8125
04	1.8125	7.8125	nd	31.25
05	0.9625	nd	nd	15.625
Control strain 06	6.125	31.25	62.5	1.99

Nd: not determined

Table 2: Result of killing rate kinetic studies of ethanol leaf extracts of *Sida acuta*

	Time interval (min)							
Org. No.	0	5	10	30	60	70	80	90
01	+	+	-	-	-	-	-	-
02	+	+	+	-	-	-	-	-
03	+	-	-	-	-	-	-	-
04	-	-	-	-	-	-	-	-
05	-	-	-	-	-	-	-	-

-: No microbial growth; (+): Microbial growth

Table 3: Result of killing rate kinetic studies of hot water leaf extracts of *Sida acuta*

	Time intervals (min)							
Org. No.	0	5	10	30	60	70	80	90
01	+	+	-	-	-	-	-	-
02	+	-	-	-	-	-	-	-
03	+	-	-	-	-	-	-	-
04	-	-	-	-	-	-	-	-
05	-	-	-	-	-	-	-	-

(-) No microbial growth; (+) Microbial growth

Table 4: Result of killing rate studies of cold water leaf extracts of *Sida* acuta

	Time intervals (min)							
Org. No.	0	5	10	30	60	70	80	90
01	+	+	+	-	-	-	-	-
02	+	+	-	-	-	-	-	-
03	+	-	-	-	-	-	-	-
04	+	-	-	-	-	-	-	-
05	+	-	-	-	-	-	-	-

(-) No microbial growth; (+) Microbial growth

DISCUSSION

The folkloric use of *Sida acuta* in the treatment of a wide range of human diseases has been previously reported by Oboh *et al*. (2007), Agayi *et al*. (2008), Kayode (2006) and Simplice *et al*. (2007). The present study showed that *Sida acuta* has appreciable *in vitro* inhibitory activity against *S. aureus* isolates. This is consistent with the report of Anani *et al*. (2000), in which methanolic extract of *Sida acuta* was shown to be active against isolates of *S. aureus* among others. Of interest however, is that the *S. aureus* isolates used in the present study were obtained from the nasal cavity of HIV/AIDS patients. This probably infers that this plant material could serve as a potential candidate for the treatment of opportunistic infections caused by *S. aureus* among HIV/AIDS patients.

It is important to note that while 86% of the *S. aureus* isolates used in this study were susceptible to ethanol extract of the plant material, 80% were to lincomycin (control). Similarly, whereas the Minimum Inhibitory Concentration (MIC) of the ethanol extract ranged from 0.962-1.8125 μg mL^{-1} that of the control antibiotic was 7.8125-31.25μg mL^{-1}. This is quite encouraging, since crude ethanol extract was used in this study. It is obvious that further refinement of the crude extract may yield improved result. The isolation and purification of the active principles of the plant material used in this study, along with the screening of the specific components against *S. aureus* isolates is hereby advocated.

The killing rate study showed that ethanol and hot water extracts killed the test isolates faster than the cold water extracts. Generally, ethanol proved to be the best solvent for the extraction of the active ingredients from the plant material going by its faster rate of kill and higher inhibitory activity against the test isolates and the least MIC value. That the hot water extract produced higher inhibitory activity than the cold water in this research is consistent with previous report that hot water extracts contains higher quantities of plant constituents. The antibacterial activity of this plant material has been attributed to the presence of alkaloids, polyphenols and steroidal compounds (Dinan *et al*., 2001).

CONCLUSION

The findings of this study therefore, justifies the ethno medicinal use of *Sida acuta* against some infectious diseases. The further exploitation for the probable development of active agent against *S. aureus*, especially for HIV/AIDS patients is emphasized.

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