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Antimicrobial Activities of Chloroform Extract of Whole Plant of *Sida acuta* Burm. f against Selected Clinical Isolates from Laboratory Samples of a Private Hospital in Benin City, Edo State, Nigeria

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ABSTRACT: Disease-causing microorganisms pose significant health challenges, and the search for alternative therapeutics remains a present quest. *Sida acuta* Burm. f. is a common weed plant with reported pharmacological activities. The present study was to investigate the antimicrobial activities of chloroform extract of whole plant of *S. acuta* against selected clinical isolates from laboratory samples of a private hospital in Benin City, Edo State, Nigeria using agar well diffusion and broth dilution techniques. The bacterial isolates were properly identified, using standard morphological and biochemical techniques. Extract of whole plant of *S. acuta* was prepared by cold maceration in chloroform. The zone of inhibition diameter, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined to ascertain the antimicrobial activities of the plant extract. Morphological and biochemical observations were confirmatory for *Staphylococcus aureus*, *Streptococcus* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* sp. and *Proteus vulgaris.* The highest (15.00 ± 0.29 mm) and lowest (5.50 ± 0.29 mm) zones of inhibition were recorded against *P. aeruginosa* and *E. coli* at 20 and 80 mg/mL respectively. However, no activity was recorded against *C. albicans* and *A. niger.* The extract had MIC of 62.5 mg/mL against *S. aureus*, *Streptococcus* sp. and *Klebsiella* sp. and MBC of 62.5 mg/mL against *E. coli* and *P. vulgaris.* The extract demonstrated broad-spectrum antibacterial activities against both Gram-positive and Gram-negative isolates.

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Sida acuta Burm.f. (Malvaceae) is an erect, branched small perennial herb or small shrub of about 1.5 m height. The plant has been reported to possess anti-inflammatory/analgesic (Oboh and Onwukame, 2005), antimalaria (Karou *et al*., 2003), wound healing (Adetutu *et al*., 2011), insecticidal (Adeniyi *et al*., 2010) and hepatoprotective (Sreedevi *et al.*, 1988) activities. Ethanol leaf extract of *S. acuta* has been reported to possess antimicrobial activities against some microbes from skin infections (Ekpo and Etim, 2009). The objective of the present study was to investigate the antimicrobial activities of chloroform extract of whole plant of *Sida acuta* against selected clinical isolates collected from laboratory samples of a private hospital in Benin City, Edo State, Nigeria.

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MATERIALS AND METHODS

Experimental materials: The following materials were used during the experiment: glassware, conical flask, petri dishes, slides, measuring cylinder, wire loop, spirit lamp, pasture pipette, cotton wool, matches, masking tape, aluminium foil, spatula, nutrient agar, nutrient broth, potato dextrose agar, potato dextrose broth, chloroform extract, gram staining kits, 20 % dimethyl sulfoxide, hydrogen peroxide, oxidase powder, methylated spirit and distilled water.

Preparation of extract: The whole plants of *Sida acuta* were collected at the University of Benin, Ugbowo, Benin City, Edo State, Nigeria. The plant materials were rinsed with running tap water to remove adhering particles. Thereafter, it was air-dried on the laboratory bench and then grounded to powder using an electric mill. About 500 g of the powdered whole part of the plant material was macerated with 2.5 L of 99.0 % chloroform for 96 hours at room temperature. The extract was filtered and concentrated to dryness in a water bath at 100 ^{O}C to yield 12 g of the chloroform extract, which was used for antimicrobial test.

Test organisms: To perform this work, chloroform extract of *Sida acuta* whole plant were tested on clinical isolates of *Staphylococcus aureus, Streptococcus* sp., *Escherichia coli*, Klebsiella sp., *Proteus vulgaris, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*. These microorganisms were obtained from the laboratory stock of a private hospital in Benin City, Edo State, Nigeria. They were stored in agar slants at 4 ^{0}C in the refrigerator.

Characterization of bacterial isolates: The colonies were studied for their morphological characterization, microscopic examination and then grouped on the basis of their gram reaction. Oxidase, catalase and coagulase tests were carried out.

Gram staining: The gram staining techniques was done on the basis of the component of the cell wall. The organisms which retained the colour of the initial stain are called gram positive bacteria while those that do not retain the primary stain when decolorized are gram negative bacteria. The non-retention of the stain is due to the cell composition. Gram staining reagents include crystal violet, iodine (mordant), 70 % alcohol (decolourizer) and neutral red (counter stain).

Procedure: A drop of sterile distilled water was placed on a clean grease free slide. The inoculating wire loop was flamed until red hot. The loop was allowed to cool and was used to pick a colony and smeared in a drop of water on the slide. The slide was heat fixed by passing it gently over flame. The smear was stained with 1 % crystal violet for a minute and washed with distilled water. Lugol's iodine was added as a mordant for a minute and rinsed with distilled water. Seventy percent (70 %) alcohol was added for 30 seconds and rinsed with distilled water. Finally, the slide was flooded with neutral red which acted as counter stain for a minute and washed off with distilled water and air dried. The slide was observed under the microscope with oil immersion and viewed with ×100 objective lens. The gram-positive organisms appeared purple while the gram-negative organisms appeared red/pink.

Oxidase test: This test was carried out to detect the production of the enzyme oxidase by the bacteria isolates. A few drops of oxidase reagent (tetramethyl-p-phenyl-diamine-dihydrochloride) were placed on a filter paper. A colony of the test organism was smeared on the filter paper. An oxidase producing organisms on the filter paper oxidized the phenyl-diamine in reagent to deep purple colour. This change in colour to deep purple within 10 seconds indicated positive result.

Catalase test: Pure culture of the test organism was placed and added to a drop of 6% hydrogen peroxide solution on a clean slide. The production of gas bubble from the surface indicated positive result. This test was used to demonstrate the presence of the enzyme catalase, which catalyzes the release of oxygen from hydrogen peroxide.

Coagulase test: A colony of the test organism was emulsified with sterile distilled water on a clean slide using a sterile wire loop. A drop of human plasma was added and mixed with emulsion. The positive coagulase organisms showed clumping while negative coagulase organisms showed no clumping. This test which distinguished pathogenic *Staphylococcus aureus* from other non-pathogen strains of *Staphylococcus* sp. and *Streptococcus* sp. was carried out to determine the enzyme coagulase.

Determination of antimicrobial activity: The chloroform extract of *Sida acuta* was tested by agar well diffusion technique as described by (Irobi *et al*., 1996). The extract of *S. acuta* was dissolved in 2 mL dimethyl sulfoxide and 8 mL of distilled water to obtain different concentrations such as 20 mg/mL, 40 mg/mL and 80 mg/mL of the extract which was used for the antimicrobial activity.

The nutrient agar and potato dextrose agar were prepared and poured into sterile petri dishes. Each test isolate was inoculated into the different agar plate by spread plate method. In each plate, four wells were

created using a 5 mm sterile cork borer and 0.5 mL of the plant extract (20 mg/mL) was pipetted into the wells to be tested in triplicate. The fourth well was left without plant extract to serve as control and same was done for other concentrations of the plant extract (40 mg/mL and 80 mg/mL) in other culture plate respectively.

The bacterial culture plates treated with plant extract were incubated at 37 ^{0}C for 24 hours and those for fungi were incubated at room temperature for 72 hours. Antimicrobial activities were determined by measuring the diameter of the zones of inhibition and the antibiotics susceptibility profiles of the bacteria isolates used were determined by using sensitivity disc. The mean zones of inhibition were calculated.

Determination of minimum inhibitory concentration (MIC): The nutrient broth was prepared and sterilized, then poured into a sterile universal container and allowed to cool. The universal container was inoculated with the clinical test isolates and few drops of different concentration (250 mg/mL, 125 mg/mL and 62.5 mg/mL) of *S. acuta* extract were placed in the nutrient broth. The containers were incubated at 37 ^{o}C for 24 hours after which they were examined for the presence of growth inhibition. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism.

Minimum bactericidal concentration (MBC): A loopful of the broth which did not show any visible growth after the period of incubation was streaked into freshly prepared nutrient agar, to determine their minimum bactericidal concentration (MBC) and then incubated at 37 ^{0}C for 24 hours after which it was observed for visible growth. The concentration of extract in the plate with no growth was considered as the MBC.

RESULTS AND DISCUSSION

From the study, it was seen that the shape, margin and elevation of *Staphylococcus aureus, Streptococcus* sp., *Escherichia coli,* and *Klebsiella* sp. were round, smooth and raised respectively while *Pseudomonas aeruginosa* was round, rough and flat. In addition, *Proteus vulgaris* was circular, even and raised. Furthermore, the cocci clustered cell-type gram-positive bacterium (*S. aureus*) and cocci chained cell-type gram positive bacterium (*Streptococcus* sp) were golden yellow and dark in colour (Table 1).

The microorganisms were further identified with biochemical tests. *S. aureus, E. coli, Klebsiella* sp. and *P. vulgaris* were positive in catalase test, while *S. aureus* alone was positive in coagulase test. However, all the bacterial isolates except *P. aeruginosa* were negative in the oxidase test (Table 2).

Among the three *S. acuta* chloroform extract treatment groups (20, 40 and 80 mg/mL), significant differences existed ($P< 0.05$) in diameter of zone of inhibition between 20 mg/mL (10.33 ± 4.67 mm) and 80 mg/mL (5.50 ± 0.29 mm) with respect to activity against *E. coli*. Similarly, there was a significant difference $P< 0.05$ in diameter of zone of inhibition upon treatment of *P. aeruginosa* with 20 mg/mL (15.00 ± 0.29 mm) and 40 mg/mL (10.00 ± 1.58) of the extract. However, no activity was recorded against *Candida albicans* and *Aspergillus niger*. (Table 3).

Figure 1 shows the MIC of the test isolates were 250 mg/mL, 125 mg/mL and 62.5 mg/mL for *P. vulgaris*, *E. coli* and *P. aeruginosa*, and others respectively. In determining MBC, *E. coli* and *P. vulgaris* were recorded as 62.5 mg/mL and 250 mg/mL was recorded against other microorganisms (Table 4).

Table 1: Morphological characteristics of the isolates used in the study

Organism	Shape	Margin	Elevation	Colour	Gram reaction	Cell type	Cell arrangement
Staphylococcus aureus	Round	Smooth	Raised	Golden yellow	+ ve	Cocci	Cluster
Streptococcus sp.	Round	Smooth	Raised	Dark	+ ve	Cocci	Chain
Escherichia coli	Round	Smooth	Raised	Pink	- ve	Rod	Chain
Pseudomonas aeruginosa	Round	Rough	Flat	Green	-ve	Rod	Singly
Klebsiella sp.	Round	Smooth	Raised	White	-ve	Rod	Chain
Proteus vulgaris	Circular	Even	Raised	Green	-ve	Rod	Chains

Table 2: Biochemical test on bacterial isolates used in the study

Organism	Catalase	Coagulase	Oxidase
Staphylococcus aureus	+	+	-
Streptococcus sp.	-	-	-
Escherichia coli	+	-	-
Pseudomonas aeruginosa	-	-	+
Klebsiella sp.	+	-	-
Proteus vulgaris	+	-	-

-= negative, += positive

Table 3: Zone of inhibition diameter (mm) antimicrobial activities of chloroform extract of *Sida acuta* whole plant on clinical isolates

Microorganisms	Concentration of extract		
	20 mg/mL	40 mg/mL	80 mg/mL
Staphylococcus aureus	12.33±2.17[a]	11.00±0.50[a]	14.17±3.49[a]
Streptococcus sp	9.83± 0.72[a]	8.67±0.93[a]	8.67±1.59[a]
Escherichia coli	10.33±4.67[b]	8.83±1.36[ab]	5.50±0.29[a]
Klebsiella sp	10.17±1.48[a]	10.17±0.17[a]	8.17±1.46[a]
Pseudomonas aeruginosa	15.00±0.29[b]	10.00±1.58[a]	11.50±1.00[ab]
Proteus vulgaris	11.33±1.17[a]	12.67±1.10[a]	12.67±1.17[a]
Candida albican	-	-	- -
Aspergillus niger	-	-	- -

Values are expressed in mean ± SEM; n = 3; Means with similar superscripts along a row are not significantly different (P> 0.05); - = No activity

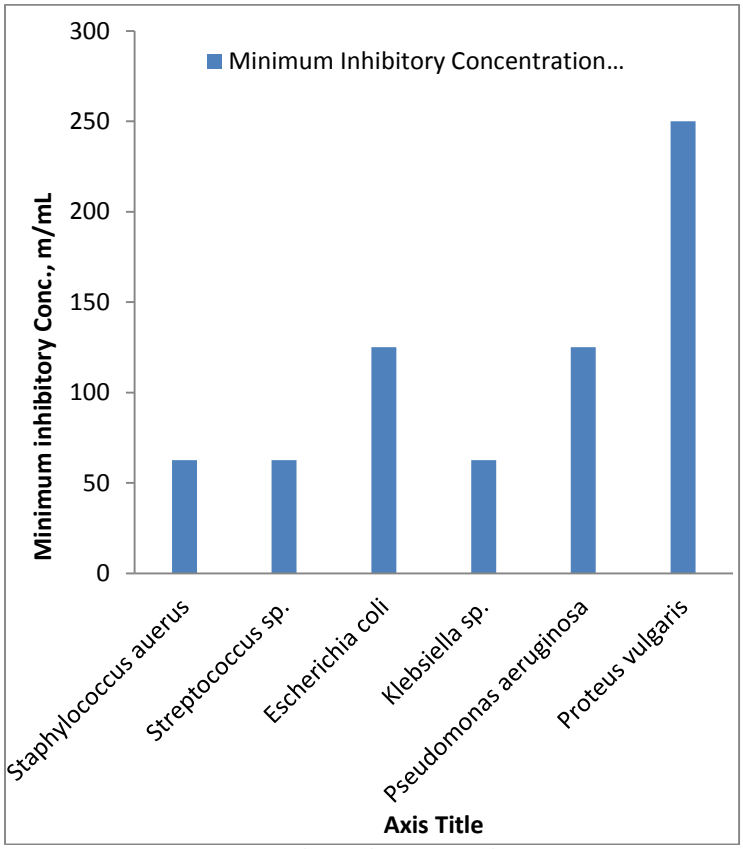

Fig 1: Minimum inhibitory concentration (mg/mL) of chloroform extract of *Sida acuta* whole plant against clinical isolates

According to Habib *et al*. (2015), the bacterial species, *Staphylococcus aureus* was isolated and characterized as Gram-positive, cocci, spherical, round in shape and possessed grape-like structure and the cells were arranged in irregular clusters. In addition, the species produced white to yellowish white and golden yellow colonies in nutrient agar. The present study which showed the organism type, cell type, shape, arrangement and colour of *S. aureus* as Gram-positive, cocci, round, cluster and golden yellow respectively, corroborates this claim. Furthermore, the present study showed that *Streptococcus* sp is a Gram-positive, cocci, round, and chain-like bacterium in alignment with (Public Health England, 2016) who stated that *Streptococcus*, *Enterococcus* and *Lactococcus species* are paired round or ovoid cells, short or long-chain Gram positive bacterium.

Table 4: Minimum Bactericidal Concentration (MBC) of chloroform extract of *Sida acuta* whole plant against selected clinical isolates

Microorganism	MBC (mg/mL)
Staphylococcus aureus	250
Streptococcus sp.	250
Escherichia coli	62.5
Klebsiella sp.	250
Pseudomonas aeruginosa	250
Proteus vulgaris	62.5

From the present study, it was seen that organism type, shape, and colour of *Escherichia coli* was Gram-negative, rod-shaped and pink respectively. This

agrees with findings of Zinnah *et al*. (2007) which showed that *E. coli* (Gram-negative) isolates were rod-shaped and pink in colour. Diggle and Whiteley (2020) stated that *Pseudomonas aeruginosa* is a heterotrophic, motile, Gram-negative rod-shaped bacterium. Similarly, the present study showed organism type and shape of *P. aeruginosa* as Gram-negative and rod shape respectively. In a study carried out by Rawy *et al*. (2020), morphological characteristics check of bacterial isolates by Gram-staining showed that *Klebsiella* spp. were Gram-negative and rod-shaped bacteria. Another study carried out by Lenchenko *et al*. (2020), showed that microorganism cultures of *K. pneumoniae* on meat peptone agar formed white colonies. These findings corroborate with those of the present study which showed that *Klebsiella* sp is a Gram-negative, rod-shaped and white bacterium.

According to Sahil B. (2018), *Proteus vulgaris* is a short, straight rod-shaped (bacillus) bacterium singly arranged, paired, or in short chains. It is circular in Eosin-methylin blue (EMB) and MacConkey agar media. This aligns with the present study, which showed that *P. vulgaris* is circular, rod-shaped and chain-like in cell arrangement. A study carried out by AL-Joda and Jasim (2021) showed that *S. aureus*, *E. coli*, *Klebsiella* sp. and *P. vulgaris* were positive in catalase test, while *S. aureus* alone was positive in coagulase test. This was corroborated by the present study where *S. aureus* was positive for both catalase and coagulase tests while *E. coli*, *Klebsiella* sp. and *P. vulgaris* were positive for catalase test only.

Streptococci and organisms with morphological similarity usually test negative to catalase (Public Health England, 2016). This statement was corroborated by the present study where *Streptococcus* sp. tested negative to catalase. Furthermore, findings made by AL-Joda and Jasim (2021) using bacteria also showed *P. aeruginosa* tested positive in oxidase test while the others tested negative. This aligns with the present study in which *P. aeruginosa* tested positive for oxidase.

A study done by Sagar (2022) showed that *P. aeruginosa* tested positive for both catalase and oxidase tests but negative for coagulase test. The present study which showed that *P. aeruginosa* was positive for oxidase test corroborates that claim. However, it negates the other claim as *P. aeruginosa* tested negative for both catalase and coagulase tests in the present study. This may be due to difference in strains of bacterial species in accordance with Rocha *et al*. (2022) whose findings showed that *Bacillus thuringiensis* isolates (S2566 and S1576) showed protein molecular weight bands of 70 and 75 kDa respectively in addition to 130 and 65 kDa which were common to all *B. thuringiensis* species.

More so, the present study showed that there was a significant difference ($P< 0.05$) in diameter of zone of inhibition between 20 mg/mL (10.33 ± 4.67 mm) and 80 mg/mL (5.50 ± 0.29 mm) of chloroform extract of *S. acuta* with respect to activity against *E. coli* (Table 3). Similarly, there was a significant difference $P< 0.05$ in diameter of zone of inhibition upon treatment of *P. aeruginosa* with 20 mg/mL (15.00 ± 0.29 mm) and 40 mg/mL (10.00 ± 1.58) of the extract (Table 3). This is consistent with a previous study by Akilandeswari *et al*. (2010) who stated that chloroform and ethanol leaf extract of *S. acuta* showed appreciable antibacterial activity against *E. coli* and *P. aeruginosa*. However, no activity was recorded against *Candida albicans* and *Aspergillus niger*. This contradicts a finding by Alka *et al*. (2012) which showed that flavonoids extracted from *S. acuta* has antifungal activity. This may be due to inability of chloroform to extract flavonoids from the plant sufficiently.

According to Krishnaiah *et al*. (2009), phytochemicals are natural bioactive compounds found in plants. Herbs and spices are known to produce certain bioactive compounds which react with other organisms in the environment to exhibit antioxidant activity and inhibit bacterial and fungal growth (Aqil *et al*., 2006). The antibacterial activities in the study are as a result of the phytochemicals present in the plant.

Conclusion: The present study showed that chloroform extracts of whole plant of *S. acuta* possess concentration dependent and broad-spectrum antibacterial activities. However, it had no detectable activity against *C. albicans* and *A.niger*.

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