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Antibacterial Activity of *Sida acuta* Burm (Malvaceae) Crude Extracts on Clinical and Typed Enteric Bacteria

Opeyemi Dorcas Bodunrin[1*] and Ebenezer Olayemi Dada[1]

[1]Department of Microbiology, Federal University of Technology, Akure, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author EOD designed the study; ODB managed the literature searches, conducted the experiment, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author EOD reviewed the manuscript. Author ODB managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was carried out to investigate the antibacterial activity of *Sida acuta* crude extracts on some enteric bacteria.
Study Design: Experimental design.
Place and Duration of Study: The study was conducted at The Federal University Technology, Akure (FUTA), Ondo State, Nigeria.
Methodology: Ethanol and cold water were used as extracting solvents. The phytochemical analysis and antibacterial activity of the crude plant extracts were carried out using standard techniques. The clinical and typed bacteria isolates used are *Escherichia coli*, *Salmonella typhimurium* and *Shigella dysenteriae.* Commercial antibiotics were used as positive reference to compare the sensitivity of the bacterial strains.
Results: The results of qualitative phytochemical screening of the ethanol and aqueous extracts of *S. acuta* revealed the presence of alkaloids; tannins, flavonoids, cardiac glycosides, phlobatanin, and terpenoid. Quantitative result showed that alkaloids and terpenoids were significantly ($p \geq$

**Corresponding author: Email: bodunrinopeyemi8@gmail.com;*

0.05.) higher in ethanol (9.41±0.06) and 7.66±0.06) compared to aqueous extract (6.61±0.05 and 5.55±0.05). The ethanol extract showed highest inhibitory effect (32.00±1.00) on clinical *S. dysentariae* and typed *S. typhimurium* at 200mg/ml. Highest inhibition observed for the aqueous extract at 200mg/ml was (24.67±1.03) and (24.33±1.03) for clinical *S. typhimurium* and *E. coli* respectively. The antibiotics sensitivity test showed that only ofloxacin was effective against the enteric bacterial isolates.

Conclusion: Findings from this study revealed the potency of *Sida acuta,* as herbal candidate for the treatment of human enteric bacteria especially on S*. dysentariae, S. typhimurium* and *E. coli* at 200mg/ml which validates the antibacterial potency of *Sida acuta* and its potential as an herbal candidate for treating human enteric bacterial infections.

Keywords: Enteric bacteria; antibacterial; phyto-chemical; antibiotics; resistance; sensitivity; Sida acuta.

1. INTRODUCTION

Enteric bacteria can be found in a variety of habitats, not just in the intestinal tract. They are said to be chemo-organotrophs (organisms which oxidize the chemical bonds in organic compounds as their energy source) and they exhibit both respiratory and fermentative metabolism [1]. WHO [2] listed some organisms which were prioritized for research to support the development of new and effective drugs. Among the organisms prioritized for research were *Escherichia coli* which is constantly associated with community and hospital-acquired extended-spectrum beta-lactamase producing *Enterobacteriaceae*, non-typhoidal *Salmonella*, *Salmonella typhi* and *Shigella* spp, which are associated with resistance to fluoroquinolone. *Salmonella typhimurium* and *Escherichia coli* are resistant to more than two antimicrobial drug classes which are defined as multi-drug resistant (MDR).

This has led to the urgent need to source for other alternatives to cure these infectious diseases that poses serious threat to the lives of millions of people [3]. Subsequently, in a bid to find natural, safe and effective alternatives, scientists have explored medicinal plants which have been found to have biological activities against some of these organisms, especially the resistant strains [4]. Sarita et al. [4], stated further that many of these plants have been used because of their antimicrobial qualities, which are attributed to phytochemicals synthesized in the secondary metabolism of these plants. Still on Sarita et al. [4], these plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found *in vitro* to have antimicrobial properties.

As described by Rashid et al. [5], *Sida acuta* is a small, erect, perennial shrub of about 1.5m height, branching profusely from the base. Rashid et al. [5], also reported the plant as a species of flowering plant in the mallow family, Malvaceae. The common name of this plant in English is *Sida*, while the Yoruba name is Ìsékètu, the Igbo and Hausa names are Udo and Wada respectively [5]. Dicko et al. [6] reported the antioxidant and antimicrobial activities of *S. acuta* against *Shigella dysentriae, Shigella boydii, Shigella flexneri, Salmonella typhi, Salmonella paratyphi* B, *Salmonella paratyphi* C, *Klebsiella pneumoniae, Klebsiella oxytoca, Klebsiella ozenae*, *Escherichia coli* and *Staphylococcus aureus*. The aim of the research is to assess the potency of aqueous and ethanol extracts of *Sida acuta* leaves against some selected clinical and typed strains of enteric bacteria.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Fresh leaves of *Sida acuta* were collected from the Botanical Garden of Federal University Technology of Akure (FUTA) during the period of September, 2019. The plant was then authenticated at the Department of Crop, Soil and Pest Management.

2.2 Drying Procedure

The leaves were air dried at room temperature (28 ^{0}C) and ground into coarse powder using a sterile mortar and pestle. This was carried out to enhance the penetration of the extracting solvent, thus facilitating the release of active bioactive ingredients [7].

2.3 Preparation of Test Organisms

The clinical isolate and typed organisms used in this study (*Escherichia coli* ATCC 700728, *Salmonella typhimurium* ATCC 14028 and *Shigella dysenteriae* ATCC 11835) were obtained from the Department of Microbiology bacterial culture bank, Akure, Ondo State, Nigeria. All isolates were collected and inoculated into nutrient agar plate, furthered plated for confirmation on SSA and EMBA. After confirmation it was inoculated on nutrient agar slant for proper storage at 37 ˚C in an incubator for 24 hours until used.

2.4 Extraction Methods and Procedures

One hundred and seventy-seven and a half grams (177.5 g) of each ground powder was macerated successively for three days (with occasional shaking) using a cold maceration technique. Eight hundred and eighty-seven and a half millilitres (887.5 ml) of ethanol and one thousand seven hundred and seventy-five millilitres (1,775 ml) of distilled water were used as extraction solvents respectively. The macerated samples were sieved with Whatman filter paper No1 and evaporated to dryness using a steam bath. The dried extracts were weighed and stored in sterile sample bottles and kept in the refrigerator for further studies [4].

2.5 Phytochemical Screening of *Sida acuta*

The phytochemical screening of the crude extracts was carried out to detect the presence or absence of some secondary metabolites using standard method of Senthilkumar, et al. [8].

2.5.1 Qualitative phyto-chemical analysis of crude leaf extracts of *S. acuta*

The qualitative phyto-chemical screening of phytochemicals including; alkaliod, saponin, tannin, phlobatannin, anthraquinone, flavonoid, steroid, terpenoid and cardiac glycosides presence in the aqueous and ethanol extracts of *S. acuta* was carried out using standard method of Harborne, [9] and Trease et al. [10]; Sofowora, [11].

2.5.2 Quantitative phyto-chemical analysis of crude leaf extracts of *S. acuta*

The quantitative phyto-chemical determination of alkaliods, saponins, tannins, phlobatannins, anthraquinones, flavonoids, steroids, terpenoids in the aqueous and ethanol extracts of *S. acuta* was carried out using the methods as described by Harborne, [9] and Trease et al. [10]; Sofowora, [11].

2.6 *In-vitro* Antibacterial Assay

2.6.1 Preparation of crude extracts of *Sida acuta*

The method of Nwankwo and Amaechi, [12] was adopted. The crude extracts of *S. acuta* leaves was reconstituted into 1 ml of 30% dimethysulphoxide (DMSO) to obtain concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml respectively.

2.6.2 Preparation of the McFarland standard

McFarland 0.5 turbidity standard was prepared following the methods described by Cheesbrough, [13]. Fifty micrometre (50µl of 1.175% (wt/vol) dehydrate Barium Chloride ($BaCl_2.2H_2O$) solution was added to 99.5 ml of 1% (vol/vol) Sulphuric acid. The accuracy of the density of the prepared standard was examined using a spectrophotometer with a 1 cm light path. This was then agitated using a vortex mixer.

2.6.3 Preparation of standard inoculums for *in-vitro* assay

A method described by Lalitha [14] was adopted for the preparation of standard inoculums of the clinical isolates for the assay. The isolated test organisms were sub cultured overnight on Nutrient Agar plates after which they were inoculated in nutrient broth. The inoculum size of each test organisms was standardized by taking 1 ml of the isolated colonies from overnight bacterial growth on nutrient broth and suspending in sterile distilled water until it matched a turbidity equivalent to a 0.5 McFarland standard (approximately 10^8 CFU/ml).

2.6.4 Antibacterial sensitivity test

Antibacterial activity of the plant extracts were tested using well-in-agar method as described by Kuta et al. [7]. Agar well diffusion assay were prepared by pouring 25 ml of Mueller Hinton Agar into sterile petri-dishes. The plates were allowed to solidify. For each organism, a sterile cotton swab was dipped into the suspension, rotated several times on the inside wall of the tube above the fluid level to remove excess

inoculum from the swab. The swab was drawn over the entire surface of already prepared plates of Mueller Hinton Agar to get uniform distribution of bacterial.

The plates were bored with 5 mm cork borer with a pre-sterilized cork borer. Three of these were made in each plate at a distance of 1-2 cm from the periphery of the plates. For each plate seeded with a test organism, two different extracts namely ethanol and water. Sterile distilled water was used for the third hole as a control. To each plate, 0.2 ml of each plant extract was added aseptically into the well. The plates were allowed to stand until extracts have been completely absorbed by the medium. The plates were later incubated at 37 ºC for 24 hours. The effectiveness of these extracts was recorded by measuring the diameter of inhibition zone. Each experiment was performed in duplicate.

2.6.5 Minimum inhibitory concentration

The MIC of the extracts was determined via tube dilution method as described by Nwankwo and Amaechi, [12]. Concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml prepared from reconstituted extracts, 9 ml of Mueller Hinton Broth was then pipetted into sterile test tubes, after which 0.1 ml of prepared standard inoculum was introduced into each of these test tube, then 0.9 ml of each concentration of the extracts were pipetted into the test tubes and mixed thoroughly, and incubated at 37 ˚C for 24 hours. Tubes with low turbidity indicated growth inhibition, tubes with high turbidity indicated growth, while the concentration with no growth at all or least growth (as indicated by clear broth) was recorded as the minimum inhibitory concentration (MIC), which was then used to determine the Minimum Bactericidal Concentration (MBC).

2.6.6 Minimum bactericidal concentration (MBC)

This was determined as described by Kuta et al. [7] tube dilution technique, a loop full suspension from each of the tube that showed no growth during MIC determination. These were streaked onto extract-free Mueller Hinton agar plates and incubated at 37^0C for 24 hours. The least concentration at which no growth was observed was noted as the Minimum Bactericidal Concentration (MBC).

2.7 Determination of Antibiotic Sensitivity Pattern of Bacterial Isolates

Susceptibility of the gram-negative isolates to eight antimicrobial agents each was tested by the disc diffusion technique, following the methods described by Osungunna and Adeyemi, [15]. The gram-negative antibiotic disc employed for the assasy, contained augmentin (30 µg;ofloxacin (5 µg;gentamycin (10 µg;nalidixic acid (30 µg;nitrofurantoin (200 µg;cotrimoxazole (25 µg;amoxycillin (25 µg) and tetracycline (25 µg). A colony of each test organism was taken from a nutrient agar culture plate and inoculated into 10 ml of sterile distilled water using a sterile loop; the suspension was then thoroughly mixed with a spin mixer. The resulting suspension was adjusted to a turbidity of 0.5 McFarland standards and was evenly spread over the surface of dried Mueller Hinton agar with a sterile swab stick. The inoculated plates were incubated at 37 °C for 20 minutes for acclimatization and growth of the organisms. Antibiotic discs (ABTEK, Liverpool, UK) were then lightly but firmly pressed onto the surface of the plates using a pair of sterile forceps. The plates were then refrigerated at 4°C for thirty minutes to ensure adequate diffusion of antibiotics. *E. coli* ATCC 25922 was used as control strain. All plates were incubated at 37 °C for 18 hours. The diameters of inhibition zones were measured in millimetres and interpreted according to CLSI, [16].

2.8 Statistical Analysis of Data

Data obtained from this study was expressed as mean ± standard deviation and were subjected to analysis of variance (ANOVA) of the treatment means, showing significant difference ($P \leq 0.05$) and were separated using duncan multiple range test.

3. RESULTS

3.1 The Percentage Yield of *Sida acuta* Aqueous and Ethanol Extract

The percentage yield of *Sida acuta* whole plant extract with respect to the extraction solvent used is presented in Table 1. Eight grams (8 g) of ethanol extract was recovered giving a weight loss of 169.5 g and a percentage yield of 4.5%. While, fourty-two grams (42 g) of aqueous extract was recovered giving a weight loss of 135.5 g and a percentage yield of 23.7%.

Table 1. Analysis of plant weight percentage recovery

Plant used	Dry weight (g)	Extracted weight (g)	Weight loss (g)	Percentage yield (%)
Aqueous *Sida acuta*	177.5	42	135.5	23.7
Ethanol *Sida acuta*	177.5	8	169.5	4.5

3.2 Phyto-chemical Screening

Table 2 shows the phytochemical constituents of the ethanol and aqueous extracts of *S. acuta* leaves including; alkaloids, tannins, saponins, flavonoids, cardic glycosides, phlobatanin, anthraquinone and terpenoid were present in the ethanol crude extract, while steroids was absent. alkaloids, tannins, flavonoids, cardic glycosides, phlobatanin, and terpenoid were present in the aqueous crude extract, while steroids, saponins and anthraquinone was absent.

Table 2. Qualitative phyto-chemical screening of ethanol and aqueous extracts of *S. acuta*

Chemical constituents	Ethanol	Aqueous
Alkaloids	+	+
Saponins	+	-
Tannins	+	+
Steroids	-	-
Flavonoids	+	+
Cardiac glycosides	+	+
Anthraquinone	+	-
Phlobatanin	+	+
Terpenoid	+	+

Keys: + = Positive; - = Negative

3.3 The Quantitative Phyto-chemical Screening of Ethanol and Aqueous Extracts of *S. acuta*

Table 3 shows the quantity of phytochemicals present in the ethanol extracts and water extract of *S. actua* leaves. Alkaloids (6.61 ± 0.05) was the highest phytochemical in the water extract and the least was flavonoid (2.57±0.05). Alkaloids (9.41 ± 0.06) and anthraquinone (1.85 ± 0.06) were the highest and least phyto-chemical in the ethanol extract respectively.

Table 3. Quantitative phyto-chemical screening of ethanol and aqueous extracts *S. acuta*

Bioactive compounds (mg/g)	Extraction solvent	
	Aqueous	Ethanol
Alkaloids	6.61±0.05^{g}	9.41±0.06^{g}
Saponin	0.00±0.05^{a}	4.58±0.06^{c}
Tannins	4.07±0.05^{d}	5.28±0.06^{d}
Phlobatanin	3.80±0.05^{c}	3.33±0.06^{b}
Anthraquinone	0.00±0.05^{a}	1.85±0.06^{a}
Flavonoids	2.57±0.05^{b}	3.32±0.06^{b}
Cardiac glycosides	5.74±0.05^{f}	8.80±0.06^{f}
Terpenoid	5.55±0.05^{e}	7.66±0.06^{e}

Data are represented as mean ± SE (Standard Error). Values with the same superscript letters down the same column are not significantly different (p ≤ 0.05)

3.4 Antibacterial Sensitivity Pattern of the Aqueous Extract of *S. acuta* on Selected Enteric Bacteria

Amongst the clinical isolates, *Salmonella typhimurium* had the highest zone of inhibition (24.67 ± 1.03mm) at a concentration of 200 mg and the least was *Escherichia coli* (10.33 ± 0.87mm) at a concentration of 25 mg against the aqueous extract of *S. acuta.* The typed isolate,

Table 4. Antibacterial activity of aqueous extract of *S. acuta* on test organisms

Test organisms	Concentration (mg)			
Bacterial isolates	**200mg**	**100mg**	**50mg**	**25mg**
Shigella dysentariae (A)	24.00±1.03^{a}	18.67±0.75^{a}	16.33±0.54^{b}	10.67±0.87^{a}
Shigella dysentariae ATCC 11835 (B)	23.00±1.03^{a}	20.67±0.75^{a}	14.33±0.54^{a}	11.67±0.87^{a}
Escherichia coli (A)	24.33±1.03^{a}	20.33±0.75^{a}	18.00±0.54^{b}	10.33±0.87^{a}
Escherichia coli ATCC 700728 (B)	23.33±1.03^{a}	20.00±0.75^{a}	16.67±0.54^{b}	12.67±0.87^{a}
Salmonella typhimurium (A)	24.67±1.03^{a}	20.00±0.75^{a}	17.00±0.54^{b}	11.00±0.87^{a}
Salmonella typhimurium ATCC 14028 (B)	24.00±1.03^{a}	20.00±0.75^{a}	19.67±0.54^{c}	12.33±0.87^{a}

Keys: A = Clinical Isolate, B = Typed isolate. Data are represented as mean ± SE (Standard Error). Values with the same superscript letters down the same column are not significantly different (P ≤ 0.05)

Salmonella typhimurium ATCC 14028 had the highest zone of antibacterial activity at (24.00 ± 1.03mm) in 200 mg concentration and the least was *Shigella dysentariae* ATCC 11835 (11.67 ± 0.87mm) at 25 mg concentration for the aqueous extract of *S. acuta* as shown in Table 4.

3.5 Antibacterial Sensitivity Pattern of the Ethanol Extract of *S. acuta* on Selected Enteric Bacteria

The ethanol extract of *S. acuta* for the clinical isolates showed high antibacterial activity at 200 mg concentration for *Shigella dysentariae* (32.00±1.00mm) and least at 25 mg concentration both in *Salmonella typhimurium* and *Shigella dysentariae* with (12.00±0.84mm), while for the typed isolate, the highest antibacterial activity was observed at 200 mg concentration for *Salmonella typhimurium* ATCC 14028 at (32.00±1.00mm) and the least was observed at 25 mg concentration for *Salmonella typhimurium* ATCC 14028 (10.33±0.84mm) as illustrated in Table 5.

3.5.1 Minimum inhibitory concentration of aqueous extract of *S. acuta*

The MIC of the aqueous extract on the clinical isolates of *Shigella dysentariae, Escherichia coli* and *Salmonella typhimurium* was 30.00 mg/ml, 50 mg/ml and 30 mg/ml respectively, while on the typed isolate the MIC recorded for *Shigella dysentariae* ATCC 11835*, Escherichia coli* ATCC 700728 and *Salmonella typhimurium* ATCC 14028 was 200.00 mg/ml, 50 mg/ml and 30 mg/ml respectively as shown in Table 6.

3.5.2 Minimum inhibitory concentration of ethanol extract of *S. acuta*

The MIC of the ethanol extract on the clinical isolates of *Shigella dysentariae*, *Escherichia coli* and *Salmonella typhimurium* was 30.00 mg/ml, 12.5 mg/ml and 6.25 mg/ml respectively, while on the typed isolate the MIC recorded for *Shigella dysentariae* ATCC 11835*, Escherichia coli* ATCC 700728 and *Salmonella typhimurium* ATCC 14028, was 30.00 mg/ml, 15 mg/ml and 6.25 mg/ml respectively as illustrated in Table 7.

3.6 Minimum Bactericidal Concentration of Aqueous and Ethanol Extract of *S. acuta*

The MBC of the aqueous extract on the clinical isolates of *Shigella dysentariae*, *Escherichia coli* and *Salmonella typhimurium* was 60.00 mg/ml, 100 mg/ml and 30 mg/ml respectively, while on the typed isolate the MBC recorded for

Table 5. Antibacterial activity of ethanol extract of *S. acuta* on test organisms

Test organisms	Concentration (mg)			
Isolates	**200mg**	**100mg**	**50mg**	**25mg**
Shigella dysentariae (A)	32.00±1.00^{b}	23.00±1.12^{a}	15.00±0.86^{a}	12.00±0.84^{ab}
Shigella dysentariae ATCC 11835 (B)	28.00±1.00^{a}	22.33±1.12^{a}	16.00±0.86^{ab}	12.00±0.84^{ab}
Escherichia coli (A)	27.33±1.00^{a}	24.00±1.12^{a}	18.00±0.86^{bc}	13.00±0.84^{ab}
Escherichia coli ATCC 700728 (B)	30.33±1.00^{ab}	23.33±1.12^{a}	18.00±0.86^{bc}	13.33±0.84^{b}
Salmonella typhimurium (A)	30.33±1.00^{ab}	26.00±1.12^{a}	21.00±0.86^{d}	12.00±0.84^{ab}
Salmonella typhimurium ATCC 14028 (B)	32.00±1.00^{b}	26.00±1.12^{a}	20.67±0.86^{cd}	10.33±0.84^{a}

Keys: A = Clinical Isolate, B = Typed isolate. Values with the same superscript letters down the same column are not significantly different (P ≤ 0.05). Data are represented as mean ± SE (Standard Error).

Table 6. Minimum inhibition concentration (MIC) of aqueous extract

Test organisms	Concentration (mg/ml)							
	6.25	**12.50**	**15.00**	**30.00**	**50.00**	**60.00**	**100.00**	**200.00**
Shigella dysentariae (A)	+	+	+	-	-	-	-	-
Shigella dysentariae ATCC 11835 (B)	+	+	+	+	+	+	+	-
Escherichia coli (A)	+	+	+	+	-	-	-	-
Escherichia coli ATCC 700728 (B)	+	+	+	+	-	-	-	-
Salmonella typhimurium (A)	+	+	+	-	-	-	-	-
Salmonella typhimurium ATCC 14028 (B)	+	+	+	-	-	-	-	-

Keys: - = No growth; + = Growth; A = Clinical Isolate; B = Typed isolate

Table 7. Minimum inhibition concentration (MIC) of ethanol extract of *S. acuta*

Test organisms	Concentration (mg/ml)							
	6.25	12.50	15.00	30.00	50.00	60.00	100.00	200.00
Shigella dysentariae (A)	+	+	+	-	-	-	-	-
Shigella dysentariae ATCC 11835 (B)	+	+	+	-	-	-	-	-
Escherichia coli (A)	+	-	-	-	-	-	-	-
Escherichia coli ATCC 700728 (B)	+	+	-	-	-	-	-	-
Salmonella typhimurium (A)	-	-	-	-	-	-	-	-
Salmonella typhimurium ATCC 14028 (B)	-	-	-	-	-	-	-	-

Keys: - = No growth; + = Growth; A = Clinical Isolate; B = Typed isolate

Table 8. Minimum Bactericidal Concentration (MBC) of aqueous and ethanol extract of *S. acuta*

Test organisms	Concentration of extracts (mg/ml)	
	Aqueous	Ethanol
Shigella dysentariae (A)	60	60
Shigella dysentariae ATCC 11835 (B)	-	30
Escherichia coli (A)	100	30
Escherichia coli ATCC 700728 (B)	50	60
Salmonella typhimurium (A)	30	30
Salmonella typhimurium ATCC 14028 (B)	60	25

Keys: - = Not available; A = Clinical Isolate; B = Typed isolate

Escherichia coli ATCC 700728 and *Salmonella typhimurium* ATCC 14028 was 50 mg/ml and 60 mg/ml respectively as illustrated in Table 8.

The MBC of the ethanol extract on the clinical isolates of *Shigella dysentariae*, *Escherichia coli* and *Salmonella typhimurium* was 60.00 mg/ml, 30 mg/ml and 30 mg/ml respectively, while on the typed isolate the MBC recorded for *Shigella dysentariae* ATCC 11835, *Escherichia coli* ATCC 700728 and *Salmonella typhimurium* ATCC 14028 was 30.00 mg/ml, 60 mg/ml and 25 mg/ml respectively as also demonstrated in Table 8.

3.7 Antibiotics Sensitivity Test

Table 9 shows the result of the sensitivity of test bacterial isolates to conventional antibiotics. The zones of inhibition are recorded in mm. The isolate showed total resistance to all tested conventional antibiotics, with no zone of inhibition recorded. Only clinical *Salmonella typhimurium* ATCC 14028 isolate was sensitive to gentamycin (10.50±0.20), while highest sensitive to ofloxacin (21.50±0.41) was recorded in clinical *Salmonella typhimurium* ATCC 14028 isolate, while *Escherichia coli* ATCC 700728 (0.00±0.41) was completely resistant. All isolates showed no zone of inhibition against all other tested conventional antibiotics except ofloxacin.

4. DISCUSSION

The result of the phytochemical analysis of aqueous and ethanol extract of *Sida acuta* in this study agrees with the phytochemical screening of the aqueous leaf extract by Senthilkumar et al. [8], which showed the presence of alkaloids, steriods, flavonoids, phenols, terpenoids, and cardiac glycosides. However, there was absence of tannins, saponins, anthroquinones and phlobatannins. Raimi, [17] also reported the presence of alkaloids, flavonoids, terpenoids and phenolics which is analogous to the findings from this study. In contrast to these studies, Ajeet and Navneet, [18], observed the presence of tannins, saponins, alkaloids, flavonoids, terpenes and phenolics in *S. acuta* leaves also in line with this study. While examining the phytochemical constituents of the chloroform and ethanol extract of the plant, Palaksha and Ravishankar, [19], observed the presence of carbohydrates, alkaloids, phyto-sterols, saponins and fixed oils. Similarly, the study of Richa and Sharma, [20] indicated the presence of high amounts of alkaloids, flavonoids, terpenoids and glycosides in methanol leaf extract of the plant which aligns with the observations of this study.

The sensitivity of the bacterial isolates to ethanol and water extracts of *S. acuta* was reported in

Table 9. Antibiotic sensitivity pattern of the test organisms

	Antibiotics (mm)							
Isolates	**AUG** **S = ≥18** **I =14-17** **R = ≤13**	**OFL** **S = ≥16** **I =13-15** **R = ≤12**	**GEN** **S = ≥15** **I =13-14** **R = ≤12**	**NA** **Not applicable**	**N** **S ≥ 17** **I =15-16** **R ≤ 14**	**COT** **S ≥ 16** **I =11-15** **R ≤ 10**	**AMX** **S = ≥17** **I =14-16** **R = ≤13**	**TET** **S = ≥23** **I =14-22** **R = ≤13**
Shigella dysentariae (A)	0.00±0.0	19.50±0.41^{b}	0.00±0.20^{a}	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
Shigella dysentariae ATCC 11835(B)	0.00±0.0	18.50±0.41^{b}	0.00±0.20^{a}	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
Escherichia coli (A)	0.00±0.0	0.00±0.41^{a}	0.00±0.20^{a}	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
Escherichia coli ATCC 700728 (B)	0.00±0.0	0.00±0.41^{a}	0.00±0.20^{a}	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
Salmonella typhimurium (A)	0.00±0.0	21.50±0.41^{c}	10.50±0.20^{b}	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
Salmonella typhimurium ATCC 14028 (B)	0.00±0.0	19.50±0.41^{b}	0.00±0.20^{a}	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0

Keys: A = Clinical isolate; B = Typed Isolate; AUG = Augmentin (30 µg;OFL = Ofloxacin (5 µg;GEN = Gentamycin (10 µg;NA = Nalidixic acid (30 µg;N = Nitrofurantoin (200 µg;COT = Cotrimoxazole (25 µg;AMX = Amoxycillin (25 µg;TET = Tetracycline (25 µg). Values with the same superscript letters down the same column are not significantly different (P ≤ 0.05)

this work with data obtained showing that the inhibitory effects of the crude extracts on the various investigated bacteria were dose-dependent. This observation is in agreement with the findings of Kuta et al. [7] and Akinnibosun and Itedjere, [21]. The ethanol extract was found to be more sensitive to the bacterial organisms at different concentrations than the aqueous extract ($p > 0.05$). These results are consistent with those of Dicko et al. [6] who reported that whole plant of *S.acuta* had been found to have microbiocide activity against *S. dysenteriae, S. parathyphi B* due to the phyto-chemical bioactive components of the etanol extract. The sensitivity of the both the typed and clinical bacterial isolates associated with diarrhea which demonstrated high inhibition at higher concentration is in alignment with the work of Chinelo and Egenti, [22] as they observed that the crude leaf extract of *S. acuta* had the highest inhibition against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* being 7.11±0.04, 6.89±0.02 and 7.63±0.04mm respectively at 62.5 mg/ml. At 125 mg/ml, the leaf showed the highest inhibition against *S. aureus*, *Salmonella typhi* and *E. coli* being 8.19±0.05, 7.93±0.05 and 8.67±0.03mm respectively. The high sensitivity of the crude extract of *S. acuta* observed in this study is in alliance with the observations of Ignacimuthu et al. [23] and Kumar et al. [24] who reported *S. acuta* to possess anti-diarrheagenic and anti-dysentery properties coupled with the leaf juice being active for vomiting and gastric disorders albeit at high concentrations demonstrated in this study.

The minimum inhibitory concentration and minimum bactericidal concentration also revealed the potency of the extract against bacteria strains of the ethanol extract compared to aqueous extract used in this study which bears resemblance with the Nwankwo and Amaechi, [12] and Kuta et al. [7] respectively. The MIC and MBC findings can be attributed to the high alkaloid content in both the aqueous and ethanol extract of *S. acuta* in this study, a claim in agreement with the observations of Chinelo and Egenti, [22].

The susceptibility of antibiotic resistant bacterial strains to the high doses of the crude plant extract is quite interesting and these plant extracts can be used as an alternative in the treatment of diseases caused by these implicated bacterial organisms as reported by Osungunna and Adeyemi, [15]. The resistance rate observed in this study against 3 or 4 classes of commercial antibiotics employed in this study is rising among the *Enterobacteriaceae* isolates used in this study as they are multidrug resistant; a notion supported by Segar et al. [25]; Leski et al. [26] and Parajuli et al. [27]. Observations of this study which shows sensitivity of typed and clinical isolates of *Shigella dysentariae* and *Salmonella typhimurium* to ofloxacin might be due to no change in the target enzymes and drug entry with efflux in the chemotherapeutics of bacterial organisms associated with diarrhea infections contrary to the claims of Livermore et al. [28] on the wide spread use of fluoroquinolones to the rapid emergence of diarrheagenic bacteria resistance worldwide.

The resistance of most of the isolates to the commercial antibiotics employed could be to the resistant nature of these bacteria acquired via plasmid transfer or chromosomally-mediated Osungunna and Adeyemi, [15] and Coutinho and Siqueira-Junior, [29]. Drug abuse and indiscriminate misuse of antibiotics among the general population has favoured the emergence of resistant strains as multidrug resistance was observed for most of the test bacteria as they were resistant to more than one drug [30]. 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 [31,32].

5. CONCLUSION

Sida acuta has shown to be a biologically-safe, eco-friendly, active drug and more effective than most antibacterial chemotherapeutic agent. Usually, medicinal plants contain numerous phytochemical compounds, which are very much necessary to control the growth of the microorganisms. Scientists have realized an immense potential in natural products from medicinal plants to serve as alternate source of combating infections in human beings which may also have lower cost and lesser toxicity. Therefore, based on the results it can be concluded that both extract of *S. acuta* may hold enormous resource of pharmaceutical properties.

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DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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