Phytochemical Screening and Antibacterial Activities of Ethanolic and Aqueous Leaf Extracts of *Alchornea cordifolia* and *Sida acuta* on Organisms Isolated from Meat

Edward, K.C., Chijioke, D.U. and Friday, C.G.
Department of Microbiology, Michael Okpara University of Agriculture, Umudike
Corresponding Author: dorisukamakachijioke@gmail.com

Abstract: The ethanolic and aqueous extracts of *Alchornea cordifolia* and *Sida acuta* were screened for antibacterial activities against isolates from meat. A mortar and pestle were used to macerate the meat and a serial dilution was obtained. The diluents were cultured and biochemical tests were done. For the antibacterial activities, the disc diffusion method was employed while the macro broth dilution method was used to determine the minimum inhibitory concentrations and minimum bactericidal concentrations. Five (5) genera of bacteria, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli*, *Salmonella* spp, and *Shigella* spp were isolated from meat and used for this work. The ethanol and aqueous extracts of the two plants' leaves showed a broad spectrum of activities. The aqueous extract of *A. cordifolia* had larger zones of inhibition ranging from 3.0 mm – 17 mm and lower MIC and MBC values that ranged from 3.125 mg/mL – 6.25mg/mL, while the ethanolic extract had zones of inhibition that ranged from 4.0 mm – 15mm and MIC and MBC values that ranged from 6.25mg/mL- 12.5mg/mL. *Sida acuta* had higher zones of inhibition that ranged from 5mm- 18mm and lower MIC and MBC values that ranged from 1.563mg/mL- 3.125mg/mL in the ethanolic extract. The aqueous extract had lower zones of inhibition that ranged from 5mm- 15mm and MIC and MBC values ranging from 6.25mg/mL- 12.5mg/mL. The biochemical determinations of the plants leave revealed the presence of Tannin, Saponin, Terpenoids, Steroids, Alkaloids, Flavonoids, Phenol, and Hydrogen Cyanides in varying quantities. Steroids were absent in *Sida acuta*. This study revealed that both extracts had antibacterial activities against the test organisms hence suggesting that they could be a good source of antibiotics for treating foodborne diseases caused by these bacteria.

Keywords: Antimicrobial Resistance, Antibacterial Activity, Plant Extracts, Phytochemicals

INTRODUCTION

The global challenge of antimicrobial drug resistance in the twenty-first century is the rise of resistant bacterial strains (Furin et al., 2011). As a result, researchers thought of using natural resources like medicinal plants in the creation of new antibiotic drugs (Abdallah, 2011). Discovering novel sources of antimicrobials is crucial in the fight against dangerous pathogens (Mustafa and Mustafa, 2018).

The existence of bioactive components in plants, such as alkaloids, phenols, tannins, glycosides, saponins, terpenoids, flavonoids, steroids, hydrogen cyanide, and essential oils, is what gives them their medicinal characteristics. This calls for ongoing testing of medicinal plants to uncover potential new active principles as well as establish the scientific rationale behind their use (Karou et al., 2006). Numerous studies have been conducted to identify the various antimicrobial and phytochemical components of medicinal plants and employ them to treat microbial infections as potential substitutes for antibiotics and other chemotherapeutic agents to which many infectious microorganisms have developed resistance. The primary benefits of using plant-derived medicines are that they are relatively cheaper than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments.

MATERIALS AND METHODS

Collection, Identification, and Preparation of plant leaves: *Alchornea cordifolia* and *Sida acuta* leaves were collected from Michael Okpara University of Agriculture, Umudike, Abia State Nigeria. The plants were authenticated by a plant taxonomist in the Department of Plant Science and Biotechnology, College of Natural Science, MOUAU. The leaves were air-dried at room temperature and reduced to powder using an electric miller.

Source of Meat: Meat samples were obtained from Ndoro market, Ikwuano Local Government Area, Abia State.

Isolation and Identification of Test Organisms: The meat was macerated using a sterile mortar and pestle. 5g of macerated meat was put into 45 mL of distilled water. This was diluted serially to obtain a dilution of 10^{-2} dilution factor. A sterile wire loop was used to inoculate dilution 10^{-2} on an already prepared nutrient agar media using the streak plate method. The plates were incubated at 37^0C for 24 hours. The morphological characteristic of the colonies was observed after 24 hours of growth and discrete colonies were obtained and stored on slants at 4^0C and further characterized microscopically and biochemically.

Extraction of Plant Materials: 50g each of leaf powder was weighed and soaked in 500 mL of ethanol and water in other to obtain the ethanol and aqueous extracts at room temperature (28^0C) for 48 hours using a 500 mL conical flask. The conical flask was properly labeled and covered with aluminum foil to prevent contamination (Azwanida, 2015). The extracts obtained were filtered using a muslin cloth and the filtrate obtained was further purified by filtration using Whatman No.1 filter paper under aseptic conditions. The filtered extracts were concentrated in a water bath at 70^0C (Evans, 2002). The extracts were stored in sterile universal bottles at refrigerator temperature until needed.

Phytochemical Analysis of *Alchornea cordifoli*a and *Sida acuta*

Phytochemical analysis (Qualitative and Quantitative) was done to find the presence of the active constituents of the plant leaves such as Alkaloids, Flavonoids, Tannins, Steroids, Saponins, Phenols, Hydrogen cyanides, and Terpenoids, by the following procedures;

Qualitative analysis for the Presence of Phytochemicals in the Plants Studied.

Test for the Presence of Terpenoids: 0.8g of the plant samples were taken in a test tube and 10 mL of methanol was poured into it. The mixture was shaken well and filtered to take a 5mL extract of plant samples. Then 2mL of chloroform was mixed in the extract of the plant samples and 3mL of sulphuric acid was added. The formation of reddish-brown color indicates the presence of terpenoids in the selected plants. (Narayan., *et al* (2012).

Test for the Presence of Hydrogen Cyanide (HCN): The alkaline picrate technique was used to conduct this test. A corked conical flask was used to dissolve 1 g of the pulverized material in 50 ml of distilled water, and the mixture was left overnight. After filtering the extract, 1 ml of the filtrate was added to a test tube with a cork. 4 ml of alkaline picrate were incubated for 5 minutes in a water bath. The corked test tube developed a reddish-brown hue, and a spectrophotometer reading at 490 nm revealed its absorbance (Williams and Edwards, 1980).

Test for the Presence of Alkaloids: The plant extract was mixed in 1% v/v HCL, was warmed and filtered and the filtrate was treated with Mayer's reagent. The formation of yellow-colored precipitates indicates the presence of alkaloids (Sheraz *et al.*, 2018).

Test for the Presence of Flavonoid: This was done using the acid alkaline reagent test: The plant extract was treated with 2-3 drops of sodium hydroxide solution. The formation of a yellow coloration shows the presence of flavonoids. A confirmatory test was carried out by adding a few drops of concentrated hydrochloric (HCL) into the yellow solution which turned colorless. (Sheraz *et al.,* 2018).

Test for the Presence of Phenols and Tannins: 20mL of distilled water was added to a test tube, and the powdered sample of the leaves were boiled and then filtered. 3-4 drops of 0.1% v/v Ferric chloride (FeCl) were added to the filtrate which changed the color to brownish green or blue. This indicated the presence of phenols and tannins (Sheraz *et al.,* 2018)

Test for the Presence of Saponin: The presence of saponin in the test samples was determined by using the froth test: The plant extract was diluted with distilled water and shaken for 15 minutes in a

graduated cylinder. The formation of a 1cm layer of foam indicated the presence of saponin (Sheraz *et al.,* 2018).

Test for the Presence of Steroids: The presence of steroids was determined using Salkowski's test. The plant was mixed with chloroform and filtered and treated with 5-6 drops of concentrated sulphuric acid (H_2SO_4) and shaken gently and then allowed to stand. The appearance of a golden yellow color indicates the presence of steroids (Sheraz *et al.,* 2018).

Quantitative determination of the phytochemical constituents of the plants studied

Alkaloid Determination: The determination of the concentration of alkaloids in the leaf samples was carried out using the alkaline precipitation gravimetric method described by Harborne (1973). 5g of the powdered sample was soaked in 20 mL of 10% ethanolic acetic acid. The mixture stood for four (4) hours at room temperature. Thereafter, the mixture was filtered through the Whatman filter paper (Number 42). The filtrate was concentrated by evaporation over a steam bath to $^1/_4$ of its original volume. To precipitate the alkaloid, concentrated ammonia solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the precipitate was washed with 9% ammonia solution and dried in the oven at 60^{o}C for 30 minutes, cooled in a desiccator, and reweighed. The process was repeated two more times and the average was taken. The weight of the alkaloid was determined by the differences.

Flavonoid Determination: The flavonoid content of the sample was determined by the gravimetric method as described by Harborne (1973). 5 g of the powdered sample was placed into a conical flask and 50 mL of water and 2MHCL solutions were added. The solution was allowed to boil for 30 minutes. The boiled mixture was allowed to cool before it was filtered through the Whatman filter paper (Number 42). 10 mL of ethyl acetate extract which contained flavonoid was recovered, while the aqueous layer was discarded. A pre-weighed Whatman filter paper was used to filler the second (ethyl-acetate layer), the residue was then placed in an oven to dry at 60^o C. It was cooled in a desiccator and weighed. The number of flavonoids was determined

Determination of Phenols: The concentration of phenols in the samples was determined using Folin-Ciocalteu's reagent described by Maurya and Singh (2010).

0.2 g of the powdered sample was added into a test tube and 10 mL of methanol was added to it and shaken thoroughly the mixture was left to stand for 15 minutes before being filtered using Whatman (Number 42) filler paper. 1 mL of the extract was placed in a test tube and 1mL Folin-Ciocalteu's reagent in 5 mL of distilled water was added and color was allowed to develop for about 1 to 2 hours at room temperature. The absorbance of the developed color was measured at a 760 nm wave. The process was repeated two more times and an average was taken.

Determination of Saponins: The saponin content of the sample was determined by the double extraction gravimetric method (Harborne, 1973). 5 g of the powdered sample was 'mixed with 50 mL of 20% aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in a water bath for 90 minutes at 55^o C; it was then filtered through Whatman filter paper (Number 42). The residue was extracted with 50 mL of 20% ethanol and both extracts were poured together the combined extract was reduced to about 40 ml at 90'C and transferred to a separating funnel where 40 mL of diethyl ether was added and shaken vigorously.

Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partitioning was done repeatedly until the aqueous layer become clear in color.

The saponins were extracted, with 60 ml of normal butanol. The combined extracts were washed with a 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. It was dried at 60^0 C in the oven and reweighed after cooling in a desiccator. The process was repeated two more times to get an average. Saponin content was determined by difference.

Steroid Determination: The steroid content of the sample was determined using the method described by Harborne (1973). 5g of the powdered sample was hydrolyzed by boiling in 50 ml hydrochloric acid solution for about 30 minutes, it was filtered using Whatman filter paper (Number 12), and the filtrate was transferred to a separating funnel. An equal volume of ethyl acetate was added to it mixed well and allowed to separate into two layers. The ethyl acetate layer (extract) recovered, while the aqueous layer was discarded. The extract was dried at 100^o C for 5 minutes in a steam bath. It was then heated with concentrated amyl alcohol to extract the steroid. The mixture becomes turbid and a reweighed Whatman filler paper (Number 42) was used to filter the mixture properly. The dry extract was then cooled in a desiccator and reweighed. The process was repeated two more times and an average was obtained.

Tannin Determination: The tannin content of the leaves of the plants was determined using the Folin Dennis spectrophotometric method described by Pearson (1976). 2 g of the powdered sample was mixed with 50 mL of distilled water and shaken for 30 minutes in the shaker. The mixture was filtered and the filtrate was used for the experiment. 5mL of the filtrate was measured into a 50mL volume flask and diluted 3mL of distilled water was. Similarly, 5mL of standard tantric acid solution and 5mL of distilled water were added separately. 1mL of Folin-Dennis reagent was added to each flask followed by 2.5mL of saturated sodium carbonate solution. The content of the flask was made up to mark and incubated for 90 minutes at room temperature. The absorbance of the developed color was measured at 760 nm wavelength with the reagent blank at zero. The process was repealed two more times to get an average.

Determination of Terpenoids: The material that had been previously prepared for qualitative analysis was transferred to a cuvette assay tube for a UV spectrophotometer (95% v/v). The absorbance was measured at 538 nm using methanol as the reference material (Narayan et al, 2012).

Determination of Hydrogen Cyanide: Nwokoro et al.'s (2009) approach was used to ascertain the hydrogen cyanide content. Picric acid: Na2CO3: H2O (1:5:200 v/w/v) was produced in a test tube containing 2mL of 2% KOH. Using three Whatman No. 1 papers, each measuring 8 by 1 cm, standard absorbance curves were created. 15 minutes were spent dipping the sheets in the alkaline picrate solution. The picrate-impregnated papers were taken out of the solution and immediately used to measure cyanide. Glass bottles were used to prepare cyanide solutions with concentrations ranging from 50 to 200 g KCN/mL. Three picrate-impregnated papers were used to instantly seal the acidified, 20% HCl solution, which had been heated to 80°C. 28 20 C) was used for the system's incubation period of 24 hours. 30 minutes of elution with a 50% ethanol solution resulted in the red-colored complex produced. Utilizing a Spectrumlab 23A spectrophotometer, the eluate absorbance was determined at 510nm.

Antimicrobial Screening of extracts: The antimicrobial test was carried out using the disc diffusion method (Clinical and Laboratory Standard Institute, 2009). Muller Hilton agar-based plates were seeded with the standard inoculum size (1×10^7 CFU/mL). Sterile filter paper discs (6 mm in diameter) were impregnated with 100µl of each of the extracts (10 mg/mL concentration) to give a final concentration of 1 mg/disc and were left to dry to remove residual solvent, which

might interfere with the determination. Extract discs were then placed on the seeded agar plates. Serial dilutions of the stock solution of the extracts were made to obtain other concentrations (100mg/mL, 50mg/mL, 25mg/mL, and 12.5mg/mL) of the extract. Water served as the negative control for aqueous extracts while ethanol served as the negative control for ethanolic extracts. Gentamicin (5mg/mL) served as a positive control for all the extracts. The plates were left to stand for 10mins for the diffusion of extract after which the plates were incubated at 37°C for 48 hours. The zones of inhibition were measured in millimeters. The above method was carried out in duplicates and the mean of the duplicate results was taken for all isolates and both extracts (CLSI, 2009)

Estimating Minimum Inhibitory Concentration (MIC): The macro broth dilution method was used to determine the minimum inhibitory concentration (MIC) of the samples (Wiegand *et al.,* 2008). A weight of 1g of the plant extracts was dissolved in 10 ml of ethanol and water (For ethanol and aqueous extracts respectively) making 100mg/mL of the extracts. A serial dilution of the plant extracts adding 1mL of the dissolved extract into 1mL of the broth (Muller Hinton Broth) medium already pipetted into the sterile screw-capped test tubes, with a dilution factor of half (50mg/mL, 25mg/mL, 12.5mg/mL, 6.25mg/mL, 3.125mg/mL and 1.5625mg/mL) was established. 1mL of the 24 hours old cultures already standardized to 0.5 McFarland standard turbidity was added to each tube. Extra tubes containing Muller Hinton Broth and bacterial inoculums only, served as a positive control, and an additional tube containing broth only was used as a negative control. The tubes were incubated at 37^0C for 18 hours and visually examined for evidence of turbidity. The lowest concentration of the plants in the series that inhibited the growth of organisms observed by the absence of turbidity was taken to be the MIC, expressed in mg/mL (Wiegand *et al.,* 2008).

Estimating Minimum Bactericidal Concentration (MBC): The minimum bactericidal concentration was obtained from the MIC. It is the minimum concentration at which the test organisms were inhibited and not viable when subcultured on fresh agar media. All the MIC were subcultured on a Muller Hinton Agar plate and the presence or absence of growth was observed. The least concentration of extract showing no visible growth on subculturing was taken as MBC.

STATISTICAL ANALYSIS

The data was analyzed using SPSS version 24. Results were expressed as means standard deviations. The data were analyzed using Analysis of Variance (ANOVA) at a ($P< 0.05$) level of significance. Duncan's multiple range test was used to separate differences in means.

RESULTS

Table 1 shows the identification and biochemical tests of the isolates from meat. The organisms isolated, identified, and used for this study include; *S. aureus, E. coli, P. aeruginosa, Salmonella* spp*,* and *Shigella* spp.

Table 2 shows the result of qualitative phytochemical screening of *A. cordifolia* and *S. acuta* leaves which shows that Tannins, Alkaloids, Saponin, Flavonoids, Steroids, Phenols, Terpenoids, and Hydrogen cyanide were present in *A. cordifolia.* These were also present in *Sida acuta* except absent Steroids.

Table 3 shows the result of quantitative phytochemical screening of the *A. cordifolia* and *S. acuta* which shows that Hydrogen cyanide (HCN) ($20.00+or\ -0.00^a$) has the highest quantity in *A. cordifolia* while Tannin ($0.24+or\ -0.24^a$) had the lowest quantity. Saponin ($16.04+or-0.11^a$) has the highest quantity in *S. acuta* while Tannin ($0.25+or\ -\ 0.00^b$) had the lowest quantity. Steroids were absent in *Sida acuta*. Sample means with the same superscripts across the row do not differ significantly ($P< 0.05$)

while means with different superscripts across the row differ significantly (P<0.05)

Tables 4 and 5 show the antimicrobial activities of the ethanolic and aqueous extracts of *A. cordifolia*. The aqueous and ethanolic extracts of *A. cordifolia* showed activity against all the bacteria isolates from meat. The highest activity for the ethanolic extract was recorded against *P. aeruginosa* (15mm) at a concentration of 100mg/mL and 9.0mm at a concentration of 12.5mg/mL and the least zone of inhibition was observed against *E. coli* (4mm) at a concentration of 100mg/mL and no inhibition at 12.5mg/mL (Table 4). The aqueous extract showed activity against the isolates with higher zones of inhibition compared to the ethanolic extract. The larger zone of inhibition is seen in *Salmonella spp* (17mm) at 100mg/mL and 8.0mm at 12.5mg/mL. The lowest was observed in *Shigella spp* (3.0mm) at a concentration of 100mg/mL and no zone of inhibition at 12.5mg/mL (Table 5).

Tables 6 and 7 show the antimicrobial activities of the ethanolic and aqueous extracts of *Sida acuta*. The ethanolic extract of *S. acuta* showed higher activity against the isolates compared with the aqueous extract. The highest diameter zone of inhibition was recorded against *Shigella spp* (18mm) at 100mg/mL and 11mm at 12.5mg/mL. The lowest zone of inhibition was recorded against *E. coli,* (5mm at) 100mg/mL, and no inhibition at 12.5mg/mL (Table 6). Smaller zones of inhibition were recorded in aqueous extract against the isolates. *E. coli* and *S. aureus* have the same zones of inhibition (15mm each) at 100mg/mL concentration which is the highest zone of inhibition, while at 12.5mg/mL, they had 6mm and 7mm respectively. *Salmonella spp* showed the lowest zone of inhibition (10mm) at 100mg/mL and (5mm) at 12.5mg/mL (Table 7).

Tables 8 and 9 show the minimum inhibitory concentration and minimum bactericidal concentration of the plant extracts measured in milligrams per mille (Mg/mL). The aqueous extract of *A. cordifolia* showed lower values of MIC and MBC with *E. coli* and *Salmonella spp* having the lowest values while *S. aureus, P. aeruginosa*, and *Shigella* spp have the highest values. The Ethanol extract has higher MIC and MBC values than the aqueous extract with *S. aureus, Salmonella* spp*,* and *Shigella* spp having lower MIC values while *P. aeruginosa* and *E. coli* have the highest values (Table 8).

The ethanol extract of *Sida acuta* showed lower MIC values compared to aqueous extract with *S. aureus* and *E. coli* having the lowest values while *P. aeruginosa* has the highest value. The aqueous extract has higher MIC and MBC values with *Salmonella* spp and *Shigella* spp having the lowest values while *S. aureus*, *P. aeruginosa*, and *E. coli* have the highest values (Table 9).

Table 1: Biochemical Test of Isolates from Meat

ISOLATES	Gram Stain	Cat	Coag	TSI	Mot	Ind	Ure	Cit	H_2S	MR	VP	Oxi	GAS
E. coil	-	-	N/A	N/A	+	+	-	-	-	+	-	-	+
P.aeruginosa	-	+	N/A	K	+	-	+	+	-	N/A	N/A	+	-
S. aureus	+	+	+	A/A	-	-	N/A	N/A	N/A	N/A	N/A	N/A	-
Salmonella	-	+	-	A/A	+	+	-	+	+	+		-	+
Shigella	-	+	-	K	-	-	-	+	-	+	-	-	+

KEYS:

+ = Positive Cat = Catalase Cit = Citrate
- = Negative Coag = Coagulase H_2S = Hydrogen sulphide
N/A = Not applicable Mot = Motility MR = Methyl red
A/A = Acid IND = Indole VP = Vogues Proskauer
K = Alkaline URE = Urease Oxi = Oxidase

Table 2 Quantitative Phytochemical Analysis of *Alchornea cordifolia* and *Sida acuta* Leaves

Phytochemical components	*Sida acuta*	*Alchornea cordifolia*
Tannin	++	++
Alkaloid	++	++
Saponin	++	++
Flavonoid	++	++
Steroid	--	++
Phenol	++	++
Terpenoids	++	++
Cyanide	++	++

KEYS
++ = Presence; -- =Absence

Table 3 Quantitative Phytochemical Screening Analysis of *Alchornea cordifolia* and *Sida acuta* Plant Leaves

	Alkaloids	Saponins	Flavonoids	Steroids	Tannin	Phenol	Terpenoids	HCN
Alchornea	3.18	11.83	9.44	5.71	0.24	2.38	18.45	20.00
cordifolia	$\pm$0.05^{de}	$\pm$0.26^{bc}	$\pm$0.31^{cd}	$\pm$8.08^{cde}	$\pm$0.24^{d}	$\pm$0.05^{de}	$\pm$0.72^{a}	$\pm$0.00^{a}
Sida	5.17	16.04	12.49	5.41	0.25	2.42	13.33	4.00
acuta	$\pm$0.04^{b}	$\pm$0.11^{a}	$\pm$0.10^{a}	$\pm$7.65^{b}	$\pm$0.00^{b}	$\pm$0.56^{b}	$\pm$0.436^{a}	$\pm$0.00^{b}

Sample means with the same superscripts across the row do not differ significantly ($P \leq 0.05$) while means with different superscripts across the row differ significantly ($P \leq 0.05$).
KEY: HCN= Hydrogen Cyanide

Table 4 Antibacterial Activities (Zone inhibition (mm)) Values of Ethanol Extract of *A. cordifolia* against Isolates

Isolates	Concentrations (mg/mL)				Controls (5mg/mL)	
	100	50	25	12.5	Gentamicin	Ethanol
S. aureus	8.0	6.0	3.0	1.0	30.0	2.0
E. coli	4.0	2.0	1.0	NI	20.0	3.0
Salmonella spp	13.0	11.0	9.0	6.0	25.0	5.0
P. aeruginosa	15.0	13.0	10.0	9.0	25.0	4.0
Shigella spp	5.0	3.0	1.5	1.0	29.0	6.0

KEY: NI= No inhibition

Table 5 Antibacterial Activities (Zone inhibition (mm)) Values of Aqueous Extract of *A. cordifolia* against Isolates

Isolates	Concentrations (mg/mL)				Controls (5mg/mL)	
	100	50	25	12.5	Gentamicin	Water
S. aureus	11.0	8.0	5.0	3.0	30.0	NI
E. coli	16.0	13.0	9.0	7.0	27.0	NI
Salmonella spp	17.0	15.0	11.0	8.0	20.0	NI
P. aeruginosa	10.0	7.0	5.0	2.0	30.0	NI
Shigella spp	3.0	1.5	1.0	NI	27.0	NI

KEY: NI= No inhibition

Table 6 Antibacterial Activities (Zone inhibition (mm)) Values of Ethanol Extract of *Sida. acuta* against Isolates

Isolates	Concentrations (mg/mL)				Controls (5mg/mL)	
	100	50	25	12.5	Gentamicin	Ethanol
S. aureus	6.0	4.0	3.0	NI	26.0	2.0
E. coli	5.0	3.0	2.0	NI	30.0	3.0
Salmonella spp	15.0	13.0	11.0	8.0	25.0	5.0
P. aeruginosa	13.0	11.0	9.0	5.0	20.0	4.0
Shigella spp	18.0	15.0	13.0	11.0	20.0	6.0

KEY: NI= No inhibition

Table 7 Antibacterial Activities (Zone inhibition (mm)) Values of Aqueous Extract of *Sida. acuta* against Isolates

Isolates	Concentrations (mg/mL)				Controls (5mg/mL)	
	100	50	25	12.5	Gentamicin	Ethanol
S. aureus	15.0	11.0	9.0	7.0	35.0	NI
E. coli	15.0	13.0	10.0	6.0	25.0	NI
Salmonella spp	10.0	7.0	6.0	5.0	29.0	NI
P. aeruginosa	13.0	10.0	8.0	6.0	25.0	NI
Shigella spp	14.0	13.0	10.0	7.0	30.0	NI

KEY: NI= No inhibition

Table 8 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ethanol and Aqueous Leave Extracts of *Alchornea cordifolia* Against Test Organism.

Ethanolic Extract.					
	S. aureus	*P. aeruginosa*	*E. coli*	*Salmonella* spp	*Shigella* spp
MIC (mg/mL)	6.25	12.5	12.5	6.25	6.25
MBC (mg/mL)	12.5	25	25	12.5	12.5
Aqueous Extract					
	S. aureus	*P. aeruginosa*	*E. coli*	*Salmonella* spp	*Shigella* spp
MIC (mg/mL)	6.25	6.25	3.125	3.125	6.25
MBC (mg/mL)	12.5	12.5	6.25	6.25	12.5

Table 9 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ethanol and Aqueous Leave Extracts of *Sida acuta* Against Test Organisms

Ethanolic Extract					
	S. aureus	*P. aeruginosa*	*E. coli*	*Salmonella* spp	*Shigella* spp
MIC (mg/mL)	12.5	12.5	12.5	6.25	6.25
MBC (mg/mL)	25	25	25	12.5	12.5
Aqueous Extract					
	S. aureus	*P. aeruginosa*	*E. coli*	*Salmonella spp*	*Shigella spp*
MIC (mg/mL)	1.563	6.25	1.563	3.125	3.125
MBC (mg/mL)	3.125	12.5	3.125	6.25	6.25

DISCUSSION

The bacteria isolated from the meat samples included; *S. aureus*, *E. coli, P. aeruginosa, Salmonella spp,* and *Shigella spp.* These isolates represented clinically significant pathogens and are known to cause the majority of foodborne diseases and food contamination. They are capable of elaborating severe virulence factors. This result is similar to the work of Hooda *et al.,* (2010) in Singapore, who isolated similar pathogens from ground pork meat.

In this study, the phytochemical screening of the leaves of the plants revealed the presence of phenolic compounds, alkaloids, steroids, tannins, terpenoids, saponins, flavonoids, and hydrogen cyanide at varying percentages with hydrogen cyanide having the highest percentage and tannin with the least percentage in *A. cordifolia* while saponin has the highest percentage in *Sida acuta* with tannin having the lowest percentage. These secondary metabolites might be responsible for the antibacterial activity of the extracts and they exert their antimicrobial activities through different mechanisms. Phenols are generally protoplasmic poisons toxic to all types of cells. Precipitation of proteins occurs with high concentrations of phenol, while at low concentrations it denatures proteins without coagulating them. It freely penetrates the tissue because of its denaturing activity (Adeshina *et al*., 2012). The presence of flavonoids suggests that they can be used as antispasmodic and antioxidant, and confirms the reason for the use of the plant in the treatment of spasmodic bronchitis and other microbial infections. Flavonoids are also known to be scavengers of superoxide anions (Nat and Saikia, 2007). Antioxidant and antimicrobial properties of various plant extracts are of great interest because of their use as natural additives and replacements for synthetic ones (Satyender *et al*., 2017).

In this work, the diameter zones of inhibition showed that the aqueous extract of *A. cordifolia* had more activity than the ethanol extract. The degree of activity varied with the isolates and the extracts. This variation of activity could be due to the differences in the solubility of the secondary metabolite in the different solvents used and also the structural or morphological variability of the tested isolates thus, larger zones of inhibition were produced by the susceptible organisms than the resistant ones. It could also be due to the polarity of the solvents; water is more polar and dissolves more of the secondary metabolites. This result is different from the work of Adeshina *et al.,* (2012) which showed that the ethyl acetate fraction (non-polar solvent) of methanol extract of *A. cordifolia* leaf was relatively more active than the aqueous fraction (polar solvent) against type isolates of *E. coli, S. aureus, P. aeruginosa,* and *Candida albicans*. The observed differences may be a result of variations in plants' location and method of extraction. The result is similar to the findings of Mohammed *et al.,* (2012) who reported that the water extract of *Alchornea cordifolia* exerted the highest activity against *S. aureus* isolated from wound samples more than the ethanol extract. The work of Gatsing *et al*., (2010) in Cameroon showed that the aqueous leaf extract of *A. cordifolia* was more active than the 75% methanol and ethanol extracts against *E. coli, P. aeruginosa, K. pneumoniae,* and *S. aureus*. The result is also in line with the work of Osumah *et al.,* (2012) which showed that the aqueous root extract of *A. cordifolia* had more activity than the ethanol extract against *E. coli, P. aeruginosa, S. aureus,* and *S. typhi* from fecal material and wounds.

In this study, it was also observed that the ethanolic extracts of *Sida acuta* had significantly higher antibacterial activity than the aqueous extract. This difference is attributed to the solubility of the active component in different solvents. It was observed that different isolates exhibited varying degrees of resistance to the ethanolic extract of the *Sida acuta.*

This result supports the findings of Anani *et al*. (2010), who noted that methanolic extract of *Sida acuta* had a significant activity on *S. aureus, E. coli, B. subtilis,* and *Mycobacterium phlei* as against no inhibition effect recorded on *Streptococcus faecalis* and *Klebsiella pneumoniae*. Similar results were obtained by Saganuwan and Gulumbe (2006) with a methanolic extract of *Sida acuta*. This difference in susceptibility can be attributed to two factors. The inherent resistant factor of the different species of the isolates and the previous exposure of the organism to other antimicrobial drugs or agents, as a result of drug abuse in the population.

The diameters zone of inhibition of *A. cordifolia* and *S. acuta* showed a concentration-dependent result and the result also showed that the zone of inhibition values of the extracts was far lesser than that of the positive control gentamicin. This may be attributed to the fact that conventional antibiotics are usually prepared from synthetic materials using reproducible manufacturing techniques and procedures, while herbal medicinal plants products are still crude, prepared from plant and animal origins, and are subjected to contamination and deterioration most of the time (EL – Mahmood and Ameh, 2007).

In *A. cordifolia*, the MIC and MBC values were generally lower for the aqueous extract against the test isolates compared to those of the ethanol extract. *E. coli* and *Salmonella spp* were more susceptible to the aqueous extract which showed the lowest MIC and MBC values of 3.125mg/mL – 6.25mg/mL in both organisms while *S. aureus*, *Salmonella spp,* and *Shigella spp* showed lower MIC and MBC values between 6.25mg/mL- 12.5mg/mL in the ethanolic extract. This result is in contrast with the work of Osumah *et al*., (2012) who showed that the root and stem bark extracts of *A. cordifolia* had more activity against *S. aureus* isolated from fecal and wound samples. However, in *Sida acuta,* the MIC and MBC values were generally lower for the ethanolic extract against all the test organisms compared to the aqueous extract. *S. aureus* which showed MIC and MBC values of 1.5625mg/mL- 3.125mg/mL was more susceptible to the ethanol extract while *Salmonella* and *Shigella* were more susceptible to the aqueous extract with MIC and MBC values between 6.25mg/mL-12.5mg/mL. This result is in agreement with the work of Ekpo and Etim, (2009) who showed that the ethanol and aqueous leaf extracts of *Sida acuta* had more activity against *S. aureus* isolated from skin infections.

The differences in the susceptibilities of the isolates to the plant extracts can be related to the cell wall composition of the organisms. Gram-positive bacteria have cell walls composed of peptidoglycan with teichoic acid in between, therefore they are more susceptible than Gram-negative bacteria that have their cell wall surrounded by bi-lipid layers of Gram-negative lipopolysaccharides and lipoproteins, which prevent ready penetration of antibiotics through their cell wall. The lower MIC confirms the high activity of the extracts at low concentrations. The high activity of antibacterial agents at low concentrations is very essential for chemotherapeutic purposes because of their toxicity to the patient system.

The observed low MIC values from the extracts could be because leaves contain the secondary metabolites responsible for the antibacterial activity of the extracts. Many researchers have reported the presence of secondary metabolites in *A. cordifolia* and *Sida acuta.* The secondary metabolites reported by several researchers possessed appreciable inhibitory activities against various organisms (Cushine and Lamb, 2005).

CONCLUSION

This study has revealed that the leaves of *Alchornea cordifolia* and *Sida acuta* have several active agents that are inhibitory to

microorganisms, especially the organisms (*S. aureus, E. coli, P. aeruginosa, Salmonella* spp, and *Shigella* spp) used in this work. The significant activity of the ethanolic and aqueous leaf extracts of the plants against microorganisms from meat justified their use in the treatment of some bacterial diseases in folkloric herbal medicine. This indicates that *Alchornea cordifolia* and *Sida acuta* leaves can be a new source of antibiotics. This report has also revealed that the solvents used for extraction play a very important role in its level of activity.

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