The Antibacterial Efficacy of *Annona muricata* (Linn) Leaves against Some Enteric Bacteria

ABSTRACT

Aims: This research was carried out to evaluate the susceptibility patterns of some enteric bacteria to aqueous, ethanol and methanol extracts of *Annona muricata* leaf
Study design: Experimental design
Place and Duration of Study: Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria. Between January, 2019 and May, 2019.
Methodology: Extraction of bioactive components of leaves was done by maceration and phytochemical screening was carried out on the leaf extracts to determine the bioactive components present. The bacteria isolates were subjected to antibiotic sensitivity test using standard methods while the antibacterial activity of the plant extracts on human enteric bacteria was determined using agar well dilution method. *A. muricata* bark extracts were purified using column chromatography method. The minimum inhibitory and minimum bactericidal concentrations (MIC/MBC) of the extracts was performed using tube dilution technique.
Results: The phytochemical screening of leaf extracts showed presence of saponins, tannins, flavonoids, terpenoid and glycosides. Antibacterial activities of the leaf extracts revealed that aqueous, ethanol and methanol extracts had highest zone of inhibition of 22.00±0.58mm, 24.67±0.33mm and 24.00±0.58mm respectively on *Salmonella typhi*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) for ethanol extract ranged between 25-100mg/ml and 50-200mg/ml respectively while methanol extract ranged between 25-50mg/ml and 50-100mg/ml respectively. Ciprofloxacin (CPX) was significantly (P≤0.5) the most active of all the antibiotics used against the test organisms (both clinical and typed isolates).
Conclusion: This research revealed that *A. muricata* leaf extracts possesses antibacterial activity against human enteric bacteria isolates used in this study. The commercial antibiotics were observed to be effective in inhibiting the test organisms. The purified extracts of *A. muricata* leaf showed higher inhibitory effect compared to crude extracts which can serve as ingredient for the production of novel antibacterial agents for the treatment of infections caused by enteric bacteria.

Keywords: Annona muricata leaf; phytochemical constituents; Antibacterial activity; food borne diseases; enteric bacteria.

1. INTRODUCTION

Enteric bacteria are referred to as Gram-negative bacteria that are associated with gastrointestinal flora or diseases. These bacteria are rod-shaped and possess the following characteristics; they are facultative anaerobes, catalase positives, ferment glucose, oxidase-negative and possess the enterobacterial common antigen in the cell wall [1]. They are one of the major causes of food borne gastroenteritis in humans and remain an important health problem worldwide [2].
World Health Organization defined Foodborne disease as disease that are usually either infectious or toxic in nature caused by agents that enter the body through the ingestion of food [3]. Foodborne illness is usually caused by bacteria, parasites, viruses, toxins, metals, and prions [4]. WHO estimates that worldwide foodborne and waterborne diarrhoeal diseases all together kill about 2.2 million people annually [3].

Antibacterial resistant problems associated with commonly used antibiotics as well as the re-emergence of multi-antibiotic resistant strains of pathogens has become a possible threat to public health [5]. Medicinal plants are the major bio resource of drugs for both traditional and conventional system of medicine [6]. Natural products, especially those derived from plants, have been used to help mankind sustain its health since the dawn of medicine. Over the past century, the phytochemicals in plants have been a pivotal pipeline for pharmaceutical discovery. The importance of the active ingredients of plants in agriculture and medicine has stimulated significant scientific interest in the biological activities of these substances [7].

A. muricata whole plant is a deciduous tree and medicinal plant that belongs to the member of the *Annonaceae* (custard apple) family comprising approximately 130 genera and 2300 species [8]. *A. muricata* is widely known as soursop or prickly custard apple due to the sour and sweet taste of its fruit [9]. Different parts of *A. muricata* are widely used in traditional medicine of many countries to cure various ailments and diseases. The natives of Malaysia apply the leaf juice of *A. muricata* on the head to protect against fainting and they also use the leaves to treat cutaneous (external) and internal parasites. The juicy flesh-fruit of the soursop is a remedy for rheumatism, arthritic pain, fever, heart, and liver diseases, diarrhea, dysentery, malaria, parasites, skin rashes and worms as well as increasing breast milk after childbirth. The seeds are used as an anti-anthelmintic against external and internal worms and parasites. *A. muricata* is employed in tropical Africa as insecticidal and pesticidal agents besides being used for the treatment of coughs, pain and skin diseases [10]. Studies have shown that *A. muricata* contain saponins, condensed tannins and glycosides as the major constituents and trace amounts of flavonoids which contribute immensely to the bioactivity of the plant and also to its usage in treating various diseases [11]. Plants possess active phytoconstituents that can confer antimicrobial activities against pathogenic organism with minimal or no side effect. Some pathogens use the host immune response for spreading the infection. Thus, requires the urgency in finding new methods of controlling enteric diseases and hence exploring plants as alternatives. Therefore, it is of great interest to assess the susceptibility patterns of some enteric bacteria to leaf extracts of *Annona muricata.*

2. METHODOLOGY

2.1 Plant Collection and Identification

Fresh leaves of *A. muricata* L. were collected during the raining season from a garden at Adebayo, Ado Ekiti, Ekiti State. The leaves were identified and authenticated at the Department of Crop, Soil and Pest management, Federal University of Technology, Akure (FUTA).

2.2 Extraction of *A. muricata* bark

The leaves of *A. muricata* were washed with sterile water, air dried for three weeks and pulverized into fine powder. Fifty grams (50 g) of the powdered leaves was soaked into one liter (1L) of cold water, 100% ethanol and methanol (BDH England). The container of the mixtures were labeled and left covered for 3 days (72 hours) with intermittent agitation followed by sieving with a muslin cloth and filtered using No 1 Whatman filter paper (UK). The filtrates were vaporized to dryness using rotary evaporator (RE-52A Union laboratories England) and subsequently lyophilized to remove the extracting solvent. The crude extracts obtained was preserved in a sterile container and stored in the refrigerator at 4°C until when ready for use [5]. The crude extracts which were obtained were sterilized using 0.45µm millipore membrane filter in order to remove any contaminant that might be present before subsequent use.

The weight of the dried extracts was measured and the percentage recovery was calculated as;
Percentage recovery = (Weight of extract recovered after extraction×100%)/Initial weight of plant part

2.3 Purification of *A. muricata* Leaf Extracts

The leaf extracts were purified according to the method described by [12]. The column was packed with silica gel of 60-120 mesh. During this process of packing the gel, utmost care was taken in other to avoid

distortion and cracking of the gel. A 250ml burette was attached to a retort stand; small piece of glass wool was tucked down lightly to avoid particles from the cotton dropping into the fraction during separation into the burette with the aid of an applicator stick. For the mobile phase 1: 1: 1 proportions of three solvents were used. 100g of silica gel was mixed thoroughly with equal volume of Methanol, Ethanol and Acetone and poured into the burette. A 100ml of the solvents was used to top the silica gel for it to flow down slowly to allow the proper packing of the column. Two (2g) grams of the crude extracts was mixed with 5ml of the solvents and added carefully unto the surface of the column. More solvent was added as the fractions of the extracts were being obtained in small sterile containers.

2.4 Phytochemical Screening of *A. muricata* Leaf Extracts

The aqueous, ethanol and methanol leaf extracts of *A. muricata* were subjected to qualitative and quantitative phytochemical screening for the presence of bioactive constituents such as tannins, phenols, alkaloids, glycosides, anthroquinones, saponins and flavonoids using the method described by AOAC [13].

Test for Alkaloids: Five millimeters (5ml) of 1% aqueous hydrochloric acid (HCl) was added to 0.5g of the extracts in test tubes and put in a water bath for 2minutes, after which the mixture was filtered. 1ml of the filtrate was treated with three drops of Dragendroffs reagent. The presence of alkaloids was confirmed by the production of blue black turbid colouration.

Test for Tannins: About 0.5g of the plant extracts were stirred in distilled water and filtered. Ferric chloride (0.1 % $FeCl_3$) reagent was added to the filtrate. A blue black or blue green precipitate was taken as preliminary evidence for presence of tannin.

Test for Steroids: Two milliliters (2ml) of acetic anhydride was added to 0.5g of the extract of each sample with addition of 2ml of sulphuric acid (H_2SO_4). A colour change from violet to blue or green indicates the presence of steroids.

Test for Saponins: The ability of Saponins to produce frothing in aqueous solution was used as screening test for saponins. 0.5g of the extract was boiled with distilled water in a water bath and shaken vigorously for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Phlobatannins: Extracts of *A. muricata* was boiled with 1% HCl (aqueous). The formation of red precipitate was taken as preliminary evidence of phlobatannin.

Test for Anthraquinone: About 0.5g of the extract was shaken with 10ml of benzene, filtered and then 5ml of 10% ammonia solution was added to the filtrate and the mixture was shaken. The presence of red, pink or violet colour in the ammonia (lower) layer indicates the presence of free anthraquinone.

Test for Flavonoids: Ten milliliter (10ml) of ethyl acetate was heated with plant extracts in a water bath for thirty minutes. The mixture was filtered and four milliliters (4ml) of each filtrate was shaken with one milliliter (1ml) of dilute ammonia solution in a conical flask. A yellow colouration indicates the presence of flavonoids.

Test for Cardiac glycosides: The following tests were carried out to determine cardiac glycosides

Legal's test: The extract was dissolve in pyridine and a few drops of 2% sodium nitroprusside together with few drops of 20% sodium hydroxide (NaOH) were added. A deep red colouration which faded to a brownish yellow indicates the presence of cardenolides.

Lieberman's test: Two milliliter (2ml) of acetic anhydride was added to 0.5g of the extract and filter, 2ml of concentrated sulphuric acid (H_2SO_4) was to the filtrate. There was a colour change from violet to blue, green which indicate the presence of steroids nucleus i.e. aglycone portion of the cardiac glycoside.

Salkowski's test: Two milliliter (2ml) of chloroform was used to dissolve 0.2g of the extracts. Concentrated sulphuric acid was carefully added which formed a lower layer. A reddish-brown colour at the interface was observed which indicate the presence of steroidal ring.

Keller-killiani's test: Two milliliter (2ml) of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution was used to dissolve 0.2g of the plant extracts. The mixture was under layered with 1ml concentrated sulphuric acid (H_2SO_4). A brown ring obtained at the interface indicates the presence of a deoxy-sugar.

Quantitative phytochemical screening was carried out using spectrophotometer (Model 721G) [13].

2.5 Collection of Bacterial Isolates

Clinical enteric bacteria isolates (*Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris Pseudomonas aeruginosa, Salmonella typhi* and *Shigella dysenteriae*) were obtained from the stock culture of Ekiti State University Teaching Hospital, Ado Ekiti, Ekiti State and the typed enteric bacteria isolates (Escherichia coli ATCC 25922, *Klebsiella pneumonia* ATCC 13883, *Proteus vulgaris* ATCC 29905, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella typhi* ATCC 14028) was obtained from Federal Institute of Research, Oshodi (FIRO). The bacteria isolates were kept on already prepared nutrient agar slants and transported immediately to the microbiology laboratory of the Federal University of Technology, Akure, Ondo State for further analysis. These organisms were confirmed by biochemical tests.

2.6 Determination of the Antibacterial Activity of *A. muricata* Leaf Extracts

The sensitivity of each of the extracts was determined using agar well diffusion. The ethanol, aqueous and methanol extracts was reconstituted with 30% Dimethyl sulfoxide (DMSO) to obtain varying concentration [14].
The bacterial isolates were grown in nutrient broth and adjusted to 0.5 McFarland's standard solution. The standardized bacteria isolates were aseptically inoculated on the surface of already prepared sterile Mueller-Hilton agar plate by means of sterile cotton swab making sure they were evenly spread on the surface of the agar plate. This procedure was repeated by streaking two times, rotating the plate approximately 60° each time to ensure an even distribution of the inoculums. The agar wells were bored using a sterile corkborer with 6mm diameter on the solidified agar medium. 200mg/ml of the leaf extract was prepared using a reconstituting solvent of 30% Dimethyl sulfoxide (DMSO). 0.2 ml of each of the extracts was carefully added into the wells of labeled plates and holes. The plates were allowed to stand on the work bench for 1 hr to allow proper inflow of the extract into the medium before incubation. Plates were incubated in an upright position at 37°C for 24 hrs. DMSO was used as the negative control while ciprofloxacin was used as positive control. After overnight incubation, zones of inhibition formed on the surface of the plates were measured in millimeter [15].

2.7 Determination of Minimum Inhibitory Concentration (MIC) and Minimum bactericidal Concentration (MBC) of Leaf Extract

The MIC and MBC of the extracts was carried out using tube dilution technique. To 1 ml of graded concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml) of the extracts, 10 ml of 24 hrs Mueller-Hinton broth was added and a loopful of test organisms previously diluted was introduced into the tubes. Ciprofloxacin was included as positive control and distilled water as negative control in different tubes. A tube containing only nutrient broth was seeded with test organism to serve as positive control while a tube that was not inoculated served as the negative control. All the broth cultures were incubated at 37°C for 24 hrs. After incubation the tubes were examined for microbial growth by observing for turbidity using spectrophotometer. Growth inhibition was indicated by low turbidity while growth was indicated by high turbidity. From each of the set of test tubes used for the determination of MIC, a loopful of broth was collected from the tubes that do not show any visible growth and was inoculated on sterile Mueller-Hinton agar plates. The plates were incubated at 37°C for 24 hrs. After incubation, the least concentration that showed no growth was recorded as the minimum bactericidal concentration (MBC) [15].

2.8 Antibiotic Sensitivity Pattern

Antibiotic sensitivity testing was performed using disc diffusion method. Standard antibiotic discs for gram negative bacteria were used against the bacteria isolates. These antibiotics include pefloxacin 30 µg (PEF), gentamycin 30 µg (CN), augmetin 10 µg (AU), sparfloxacin 10 µg (SP), amoxacillin 30 µg (AM), chloramphenicol 30 µg (CH), ciprofloxacin 30 µg (CPX), streptomycin 30 µg (S), septrin 30 µg (SXT) and tarivid 10 µg (OFX). The inoculum was prepared by emulsifying three to four discrete colonies of each test isolate in a sterile test tube containing peptone water and incubated for 30 minutes. The suspension was adjusted to match with 0.5 McFarland turbidity standards after which the peptone water isolate suspension was poured into a freshly prepared Mueller-Hilton agar plate and swirled gently to cover the surface of the agar. Then, the antibiotic discs was placed aseptically on the surface of the inoculated plate using a sterile forceps and pressed lightly to ensure contact with the agar surface. The plate was incubated at 37°C for 24hours. After incubation, clear zones of inhibition were measured in millimeter and areas without clear zones were observed. Inoculated plate without antibiotics served as control [16].

2.9 Statistical Analysis

Data obtained were subjected to One Way Analysis Of Variance (ANOVA) while the means were compared with Duncan's New Multiple Range Test at 95% confidence interval using Statistical Package for Social Sciences version 23.0. Differences were considered significant at $p \leq 0.05$.

3. RESULTS

3.1 Percentage Recovery of Aqueous, Ethanol and Methanol Leaf Extracts of *A. muricata*

Table 1 revealed the percentage recovery of the leaf extracts of *A. muricata* after extraction. The table showed that more extracts was recovered with methanol and ethanol than water with 55.36%, 50.80% and 41.08% respectively. Methanol extract has the highest percentage yield of 55.36%.

Table 1: Percentage recovery of leaf extracts of *A. muricata*

Solvents	Percentage (%)
Aqueous	41.08%
Ethanol	50.80%
Methanol	55.36%

3.2 Qualitative and Quantitative Phytochemical Screening of *A. muricata* Leaf Extracts

Qualitative phytochemical screening of the aqueous, ethanol and methanolic extracts of *A. muricata* leaf is presented in Table 2. The results revealed that saponin, tannin, flavonoid, terpenoid and cardiac glycosides were present in the aqueous, ethanol and methanol leaf extracts of *A. muricata* while phlobatannin, alkaloid and anthraquinone were absent in all the plant extracts. However, the presence of Steroid was recorded only for methanol extract but absent in other crude extracts of the plant.

Table 3 showed the quantitative phytochemical (mg/100g) composition of aqueous, ethanol and methanol crude extracts obtained from leaf of *A. muricata*. The result revealed that there was significant difference ($P<0.05$) in the quantity of saponin, tannin, terpernoid, glycosides, flavonoid and steroid composition of crude extracts obtained from the leaves but there was no significant difference in the alkanoid and phlobatannin composition of the crude extracts. For aqueous extract, saponin had the highest quantity (56.09±0.27mg/g) while tannin had the least quantity (5.09±3.34mg/g). Glycosides had the highest quantity (19.59±0.05mg/g) in ethanol extract while Tannin had the least quantity (7.17±0.01mg/g) in methanol extract

Table 2: Qualitative phytochemical screening of aqueous, ethanol and methanol leaf extract of *A. muricata*

	Aqueous	Ethanol	Methanol
Saponin	+	+	+
Tannin	+	+	+
Phlobatannin	-	-	-
Flavonoid	+	+	+
Steroid	-	-	+
Terpenoid	+	+	+
Alkaloid	-	-	-
Anthraquinone	-	-	-
Cardiac glycosides			
Legal test	+	+	+
Keller kiliani test	+	+	+
Salkwoski test	+	+	+
Lieberman test	-	-	+

Table 3: Quantitative Phytochemical Composition of *Annona muricata* leaf crude extract

Phytochemical	Aqueous (mg/g)	Ethanol (mg/g)	Methanol (mg/g)
Saponin	56.09±0.27^{c}	16.82±0.27^{a}	26.82±0.27^{b}
Tannin	5.09±3.34^{b}	3.34±0.01^{a}	7.17±0.01^{c}
Terpenoid	16.42±0.04^{c}	13.36±0.04^{b}	10.12±0.04^{a}
Glycosides	10.59±0.05^{a}	19.59±0.05^{c}	16.96±0.05^{b}
Flavonoid	11.34±0.02^{b}	6.95±0.02^{a}	13.04±0.03^{c}
Alkanoid	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Steroid	0.00±0.00^{a}	6.74±0.02^{b}	0.00±0.00^{a}
Phlobatannin	0.00±0.00^{a}	0.00±0.0^{a}	0.00±0.00^{a}

Data are represented as mean ± standard error (n=3). Superscript of the same alphabet in the same row are not significantly different ($P<0.05$) while different alphabet denotes significant difference

3.3 Antibacterial Activity of *A. muricata* Leaf Extracts

Table 4 shows the susceptibility patterns of the bacteria isolates to aqueous, ethanol and methanol leaf extracts (crude) of *A. muricata* at 200mg/ml.

Aqueous leaf extract does not show zone of inhibition on all the test organisms (both clinical and typed) except for *S*. *typhi*, *E*. *coli* and *K*. *pneumoniae* with the highest zone of inhibition on *S. typhi* (22.00 mm) with least inhibitory activity on *E. coli* (12.33mm). Ethanol leaf extract showed inhibition diameter ranging from (14.33mm to 24.67mm) with the highest inhibitory activity on *S. typhi* (24.67 mm) and least inhibitory activity on *P*. *aeruginosa and E*. *coli* (14.33mm) respectively. Ethanol leaf extract did not inhibit *Shigella sp*. All the bacteria isolates (both clinical and typed) were susceptible to methanol extract with zones of inhibition ranging from (14.00mm to 24.00mm). Methanol leaf extract was more effective on the test organisms than cold water and ethanol extracts.

Table 5 revealed the result of susceptibility patterns of the bacteria isolates to ethanol and methanol leaf extracts (purified) of *A. muricata* at 200mg/ml. The purified leaf extracts showed increase in the inhibitory effect of the plant against all isolates. There was a significant increase in the ethanol and methanol activities of the leaf extracts on the bacteria isolates compared to the crude extracts.

The purified ethanol extract showed highest inhibitory effect against *S*. *typhi* with zone of 26.00 mm while purified methanol extract inhibited the bacteria isolates with a varied range from (18.33 mm to 26.00 mm) with the highest zone on *K*. *pneumoniae* and least zone on *E*. *coli* (18.33 mm).

Table 4: Antibacterial Activity of Leaf Extract (Crude) at 200mg/ml

Isolates	Aqueous	Ethanol	Methanol	Control
Pseudomonas aeruginosa	0.00±0.00^{a}	14.33±0.33^{b}	15.33±0.33^{c}	34.33±0.33^{d}
Salmonella typhi	22.00±0.58^{a}	24.67±0.33^{b}	24.00±0.58^{b}	39.00±0.58^{c}
Shigella sp	0.00±0.00^{a}	0.00±0.00^{a}	14.67±0.33^{b}	34.00±0.58^{c}
Escherichia coli	12.33±0.33^{a}	14.33±0.33^{b}	17.33±0.33^{c}	21.00±0.58^{d}
Klebsiella pneumoniae	20.33±0.33^{a}	22.33±0.67^{b}	24.00±0.58^{b}	35.00±0.58^{c}
Proteus vulgaris	0.00±0.00^{a}	15.00±0.58^{b}	14.00±0.58^{b}	31.00±0.58^{c}
Typed *Proteus vulgaris*	0.00±0.00^{a}	17.33±0.58^{b}	21.67±0.33^{c}	38.33±0.88^{d}
Typed *Pseudomonas aeruginosa*	0.00±0.00^{a}	19.33±0.33^{b}	22.00±0.58^{c}	35.00±0.58^{d}
Typed *Salmonella typhi*	0.00±0.00^{a}	17.33±0.33^{b}	18.33±0.67^{b}	29.00±0.58^{c}
Typed *Escherichia coli*	0.00±0.00^{a}	17.00±0.58^{b}	17.33±0.33^{b}	28.33±0.33^{c}
Typed *Klebsiella Pneumoniae*	0.00±0.00^{a}	18.33±0.58^{b}	19.67±0.33^{c}	26.33±0.33^{d}

Data are represented as mean ± standard error (n=3). Superscript of the same alphabet in the same row are not significantly different ($P<0.05$) while different alphabet denotes significant difference.

Table 5: Antibacterial Activity of Leaf Extract (Purified) at 200mg/ml

Isolates	Ethanol	Methanol	Control
Pseudomonas aeruginosa	16.33±0.33[a]	18.33±0.67[a]	35.00±1.00[b]
Salmonella typhi	26.00±0.58[a]	25.67±0.33[a]	38.33±0.33[b]
Shigella sp	9.33±0.33[a]	16.33±0.33[b]	34.33±0.88[c]
Escherichia coli	15.33±0.67[a]	18.33±0.33[b]	21.33±0.88[c]
klebsiella pneumoniae	24.00±0.58[a]	26.00±0.58[a]	35.33±0.88[b]
Proteus vulgaris	18.00±0.58[a]	16.67±0.88[a]	32.00±0.58[b]
Typed *Proteus vulgaris*	19.67±0.33[a]	23.33±0.33[b]	38.00±0.58[c]
Typed *Pseudomonas aeruginosa*	21.00±0.58[a]	24.00±0.58[b]	34.67±0.33[c]
Typed *Salmonella typhi*	18.00±0.58[a]	19.33±0.67[a]	28.67±0.88[b]
Typed *Escherichia coli*	17.67±0.67[a]	19.33±0.33[a]	27.00±0.58[b]
Typed *Klebsiella Pneumoniae*	20.33±0.88[a]	20.67±0.88[a]	27.33±0.33[b]

Data are represented as mean ± standard error (n=3). Superscript of the same alphabet in the same row are not significantly different (P<0.05) while different alphabet denotes significant difference.

3.4 Determination of Minimum Inhibitory Concentration (MIC) and Minimum bactericidal Concentration (MBC) of Leaf Extracts

Table 6 shows the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of *A. muricata* leaf extracts on test organisms (clinical and typed bacteria isolates).
The MIC of ethanol extract ranged from 25 to 100 mg/ml while that of methanol extract ranged from 25 to 50 mg/ml. The MIC for ethanol extract of the plant showed that *P*. *aeruginosa* and *K*. *pneumoniae* had the highest MIC at 100 mg/ml while methanol extract of the plant showed the lowest MIC of 25 mg/ml on *E*. *coli, Shigella* sp*, S*. *typhi*, *P*. *vulgaris* ATCC 29905 and *K*. *pneumoniae* ATCC 13883.

Also, the (MBC) of the ethanol and methanol leaf extract of *A. muricata* ranged from 50-200mg/ml.
The MBC for ethanol extract of the plant showed that *P*. *vulgaris, P*. *aeruginosa* ATCC 10145, *K*. *pneumoniae* ATCC 13883 and *S*. *typhi* ATCC 14028 had the lowest MBC at 50 mg/ml while methanol extract showed the highest MBC at 200 mg/ml on *K*. *pneumoniae*

Table 6: Minimum inhibitory MIC (mg/ml) and minimum bactericidal concentration MBC (mg/ml) for leaf extract (mg/ml)

	MIC		MBC	
Organisms	**Ethanol**	**Methanol**	**Ethanol**	**Methanol**
Proteus vulgaris	25	50	50	100
Klebsiella pneumoniae	100	50	200	200
Pseudomonas aeruginosa	100	50	200	100
Escherichia coli	50	25	100	50
Shigella sp	50	25	200	50
Salmonella typhi	25	25	100	50
Typed *Proteus vulgaris*	50	25	100	50
Typed *Pseudomonas aeruginosa*	25	50	50	100
Typed *Klebsiella pneumoniae*	25	25	50	100
Typed *Escherichia coli*	50	50	100	100
Typed *Salmonella typhi*	25	50	50	100

3.5 Antibiotic Sensitivity Pattern

Table 7 shows the antibiotics sensitivity patterns of the Gram negative bacterial isolates to conventional antibiotics. All the isolates were susceptible to ciprofloxacin (CPX) and Pefloxacin (PEF) with the highest diameter zone of inhibition (29.00mm) on *P*. *aeruginosa* ATCC 10145 and *Shigella* Sp (25.67mm) respectively. *S*. *typhi* showed total resistance to Gentamycin (CN) while others were susceptible with highest diameter zone of inhibition (19.67mm) on *E*. *coli.* Only *K*. *pneumoniae* was resistant to Tarivid (OFX) while others were susceptible with the highest diameter zone of inhibition (23.67mm) on *Shigella* Sp.

Table 7: Antibiotics sensitivity patterns showing diameter of zone of inhibition

Bacteria	PEF	OFX	S	SXT	CH	SP	CPX	AM	AU	CN
EcC	25.33±0.58^{g}	21.33±0.67^{e}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	25.67±0.67^{h}	24.00±0.58^{fg}	17.50±0.58^{e}	14.33±0.33^{c}	19.67±0.33^{e}
KpC	20.33±0.58^{de}	0.00±0.00^{a}	14.67±0.58^{c}	0.00±0.00^{a}	0.00±0.00^{a}	15.67±0.67^{c}	14.00±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	17.33±0.33^{d}
PaC	16.67±0.58^{c}	20.33±0.58^{e}	20.00±0.58^{e}	21.67±0.33^{f}	20.00±0.58^{d}	21.67±0.88^{f}	23.00±0.58^{f}	15.00±0.58^{d}	19.67±0.67^{d}	18.00±0.58^{d}
StC	20.33±0.58^{de}	20.00±0.58^{d}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	24.67±0.33^{fg}	0.00±0.00^{a}	0.00±0.00	0.00±0.00^{a}
SsC	25.67±0.58^{g}	23.67±0.57^{f}	22.67±0.33^{g}	14.67±0.33^{c}	23.00±0.58^{e}	17.00±0.58^{de}	27.00±0.33^{h}	16.00±0.58^{de}	19.33±0.33^{d}	19.33±0.33
PvC	14.67±0.58^{b}	14.33±0.58^{b}	15.00±0.58^{c}	20.33±0.33^{e}	0.00±0.00^{a}	14.33±0.33^{b}	18.33±0.89^{c}	12.00±0.58^{b}	0.00±0.00^{a}	10.33±0.33^{b}
KpT	19.00±0.58^{de}	19.33±0.57^{d}	14.67±0.33^{c}	0.00±0.00^{a}	0.00±0.00^{a}	14.33±0.33^{b}	19.00±0.89^{d}	0.00±0.00^{a}	0.00±0.00^{a}	16.67±0.33^{d}
PvT	21.00±0.58^{d}	16.33±0.58^{c}	17.00±0.58^{e}	18.33±0.67^{d}	0.00±0.00^{a}	18.33±0.33^{e}	21.33±0.67^{e}	17.00±0.58^{e}	0.00±0.00^{a}	14.00±0.33^{c}
PaT	23.33±0.67^{f}	15.00±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	15.00±0.58^{c}	13.67±0.33^{b}	29.00±0.58^{i}	11.00±0.58^{b}	12.00±0.58^{b}	14.33±0.58^{c}
EcT	23.33±0.58^{f}	20.33±0.58^{de}	16.00±0.58^{e}	14.67±0.33^{c}	15.00±0.58^{c}	22.33±0.33^{g}	27.00±0.58^{h}	17.00±0.58^{e}	13.67±0.33	17.33±0.88^{d}
StT	15.00±0.58^{b}	15.00±0.58^{b}	13.33±0.33^{b}	11.67±0.33^{b}	13.67±0.33	13.67±0.33^{b}	17.00±0.58^{c}	13.33±0.33^{c}	0.00±0.00^{a}	13.67±0.33^{c}

Data are represented as mean ± standard error (n=3). Superscript of the same alphabet in the same row are not significantly different (P<0.05) while different alphabet denotes significant difference.

Legend: PEF= Pefloxacin, OFX: Tarivid, S: Streptomycin, SXT= Septrin, CH= Chloramphenicol, SP= Sparfloxacin, CPX= Ciprofloxacin, AM= Amoxicillin, AU= Augmetin, CN= Gentamycin. EcC= *E. coli,* KpC= *K. pneumoniae,* PaC= *P. aeruginosa,* StC= *S. typhi,* SsC= *Shigella. sp,* PvC= *P. vulgaris,* KpT= *K. pneumoniae* ATCC 13883, PvT= *P. vulgaris* ATCC 29905, PaT= *P. aeruginosa* ATCC 10145, EcT= *E. coli* ATCC 25922, StT= *S. typhi* ATCC 14028

4. DISCUSSION

Annona muricata are extensively used to combat worms and parasitic organisms, to cool fevers, and as an astringent for diarrhea and dysentery [5]. Findings from this study revealed that methanol had the highest extraction yield (55.36%). This agrees with the findings of [17] who reported highest yield in methanol bark extract of *Annona muricata.*

Phytochemical screening of the crude extracts of *A. muricata* leaf revealed the presence of some bioactive components such as saponin, tannin, flavonoid, terpenoid and cardiac glycosides. This is in agreement with the work of Muhammad *et al*., [18] who reported similar bioactive compounds in the leaves of *Carica papaya*. The presence of saponin, tannin, flavonoid, terpenoid and cardiac glycosides may be attributed to different solvents used in the extraction of the bioactive components [19] and probably account for the antibacterial activity of the extracts.

The susceptibility patterns of clinical and typed enteric bacteria isolates to leaf extracts (crude) of *A. muricata* at 200mg/ml showed variations in the zone of inhibition for each extracts. Methanol leaf extract was found to be more effective in inhibiting the test organisms than aqueous and ethanol extract. This result is in line with Adamu *et al*. [20] who reported a significant increase in microbial activities of methanol extract of *A. muricata*. Clinical isolates were observed to be more susceptible to both ethanol and methanol extracts of the plant than the typed isolates. This may be because the clinical isolates have not been previously exposed to antibacterial agents that could have generated resistance to the extracts and antibiotics used in this study [17]. Ethanol and methanol crude extracts of *A. muricata* leaf showed antibacterial activity against several of the test organisms used in this study. This may be due to the fact that water contains a lot of organic and inorganic compounds which may or may not interact to inhibit their overall activities [16]. The responses of the organisms to the crude extracts as revealed by the observed zone of inhibition might be due to the presence of metabolites revealed through the phytochemical screening, which possess pharmacological activities responsible for the use of plants in traditional phytomedicine to treat diseases caused by enteric bacterial [21].

The purified extracts of *A. muricata* leaf showed that there was increase in the inhibitory activities of ethanol and methanol leaf extracts on the test organisms. This result is in line with the study of Dada and Akinde [17] who attested that the purified leaf extracts *A. muricata* showed higher antibacterial effect on tested bacterial isolates compared to the crude extracts. This result suggests that they have remarkable therapeutic action in the treatment of enteric diseases.

The minimum inhibitory concentration (MIC) of leaf extracts against clinical and typed isolates was found to be (25mg/ml) while the MBC was found to be (50mg/ml). The result of this study revealed that the minimum bactericidal concentration (MBC) of the plant extracts indicated higher concentrations than that of the MIC. This observation is based on the fact that the concentration of the extracts required to completely eliminate an organism must be higher than the concentration required to inhibit the growth [22]

The commercial antibiotics were observed to be effective in inhibiting the test organisms (both clinical and typed isolates). Ciprofloxacin (CPX) was the most effective against the test organisms (both clinical and typed isolates) out of all the antibiotics used. The high inhibition values recorded by antibiotics than the plant extracts on clinical and typed isolate may be due to its purified nature, as reported by [15] that antibiotics are in a refined state while plant extracts are still in crude state. Also, the small molecular size possessed by antibiotics as reported by Mailard [23] aids their solubility in diluents as this could enhance their penetration through the cell wall into the cytoplasm of the organism.

When susceptibility patterns of the bacteria isolates were compared to standard antibiotics and extracts of *A. muricata*, it was deduced that for both clinical and typed isolates, the activity of the extracts will compete favourably with the standard antibiotics in the treatment of enteric bacterial infection. However, the differences observed between the activities of the standard antibiotics and extracts of *A. muricata* might be due to the proportion of the bioactive components present in the extracts.

5. CONCLUSION

This study has revealed *A. muricata* leaf extracts to be rich in flavonoids, tannins, saponins, terpenoid and cardiac glycosides as secondary metabolites which is responsible for the various antibacterial activities exhibited. The result showed that ethanol and methanol extracts exhibited considerable inhibitory activity against the test organisms used as demonstrated by the diameter zones of inhibition. Among the different solvents used, methanol extract showed the highest antibacterial activity**.** Ciprofloxacin had inhibitory effect on the tested bacterial isolates. The purified leaf extracts of *A. muricata* had higher antibacterial activity on the test organisms. However, the purified ethanol leaf extracts can serve as ingredient for the production of novel antibacterial agents which can be used for the treatment of infections caused by enteric bacteria. Thus, the need for identification of the active components contained in the leaf extracts and also ascertain the biosafety of the plant part.

CONSENT (WHERE EVER APPLICABLE)

It is not applicable

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

It is not applicable

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