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Susceptibility Patterns of Some Enteric Bacteria to Crude and Purified Bark Extracts of *Annona muricata* L.

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Authors' contributions

This work was carried out in collaboration between both authors. Author EOD designed the study and wrote the protocol. Author KYA performed the statistical analysis, wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. Author EOD guided in the entire research and edited the final draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: This research was carried out to evaluate the susceptibility pattern of some enteric bacteria to crude and purified extracts of *Annona muricata* bark.
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 bark was done by maceration and phytochemical screening was carried out on the bark 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 were performed using tube dilution technique.

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Results: The quantitative phytochemical screening for bark extract revealed that glycosides (7.06±0.04, 34.67±0.02 and 19.35±0.01) extracted with aqueous, ethanol and methanol respectively is the most abundant phytochemical constituents. The antibacterial activities of the bark extracts revealed that aqueous showed no inhibition to none while ethanol and methanol inhibited all the test organisms. The highest value of minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) for both ethanol and methanol bark extracts was 50 mg/ml and 100 mg/ml respectively.
Conclusion: This research revealed that *A. muricata* bark extracts possesses antibacterial activity against human enteric bacteria isolates used in this study. The purified extracts of *A. muricata* bark showed higher zones of inhibition which indicates that it can compete well with standard antibiotics and it may also serve as a substitute to the commercially available antibiotics that can be used for the treatment of infections caused by enteric bacteria.

Keywords: Antibacterial activity; ethanol extract; Annona muricata bark; phytochemical constituents; enteric bacteria.

1. INTRODUCTION

Different plants and their parts are used all over the world for various purposes. Study reported that plants have an efficient constituents which are mostly used as antioxidant, antibacterial, antifungal, antiulcer, anti-inflammatory, antiviral and anticancer agents [1].

Annona muricata (L.) is referred to as graviola, guanabana or soursop in English-speaking countries and in Nigeria. It belongs to a family called Annonaceae. *A. muricata* is used in traditional medicine in many regions. It is popularly grown across the tropical regions of the world [2]. The plant is known to produce an edible fruit that is green in colour, large, heart-shaped and 15–20 cm in diameter with a white fleshy mesocarp [3]. The plant parts have been used from time immemorial, thus its ethnopharmacological use. Generally, the fruit and its juice are used to combat worms and parasitic organisms, to cool fevers, increase breast milk production after birth, and as an astringent for diarrhea and dysentery [1]. The fresh leaves when crushed are applied on skin eruption for quick healing. The leaf or bark tea or combination of both is used as a sedative and heart tonic by the indigenes of Guyana [3]. Studies have revealed that the barks, fruits, leaves and seeds of *A. muricata* consist of biological and pharmacological activities such as antimicrobial, cytotoxic, anti-parasitic and pesticidal activities [4].

Enteric bacteria are bacteria that have the ability of causing enteric diseases. Enteric bacteria pathogens are one of the major causes of food borne gastroenteritis in humans and remain an important health problem worldwide [5]. Enteric bacteria are microbes that reside in the guts of animals and humans. They can cause a mild infection, such as food poisoning or severe community infections like diarrhea [6].

Food borne diseases are important cause of morbidity and mortality worldwide. Most food borne bacterial infections cause self-limiting diarrhea, systemic infection and however, death can occur, particularly in vulnerable groups such as the elderly, people with diminished immunity or infants and young children [5]. Bacteria have accounted for more than 70% of deaths associated with food borne transmission [7].

The emergence and spread of antibiotic resistance continue to be an important global problem particularly in developing countries. The increasing drug resistance is partly due to the frequent mutation of the pathogens and partly because of the overuse or misuse of drugs [8]. Antibiotic resistance in bacteria continues to spread and cause morbidity, mortality, and increase in cost of the treatment of infectious diseases due to treatment failures [9].

It has been documented that *A. muricata* posseses saponins, tannins and glycosides as the major constituents and trace amounts of flavonoids which contribute immensely to the bioactivity of *A. muricata* and also its usage in treating various diseases [10]. However, there is limited research work comparing the antibacterial activity of crude and purified extracts of *A. muricata* against enteric bacteria isolates. Hence, the need to evaluate the susceptibility patterns of some enteric bacteria to crude and purified extracts of *A. muricata* bark.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Fresh bark of *A. muricata* L. was collected from a garden at Adebayo, Ado Ekiti, Ekiti State. The leaves and bark 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 leaf and bark of *A. muricata* were washed with sterile water, air dried for three weeks and pulverized into fine powder. The bark were coarsely powdered using a sterile mortar and pestle and were further pulverized to powder using an electric blender. Fifty grams (50 g) of the powdered bark was soaked into one liter (1L) of cold water, 100% ethanol and methanol. 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. The filtrates were vaporized to dryness using rotary evaporator and subsequently lyophilized to remove the extracting solvent. The crude extracts obtained was preserved in a sterile container and stored in the refrigerator at 4ºC until when ready for use [1]. The crude extracts which were obtained were sterilized using 0.45 µm 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* Bark Extracts

The plant extracts were purified according to the method described by Atta, et al. [12]. The column was packed with silica gel of 60-120 mesh. During this process of packing the gel, outmost care was taken in other to avoid distortion and cracking of the gel. A 250 ml 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 100 ml 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 (2 g) grams of the crude extracts was mixed with 5 ml 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* Bark Extracts

The aqueous, ethanol and methanol bark 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 [11].

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* Bark 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 [13].

The bacterial isolates were grown in nutrient broth and adjusted to 0.5 McFarland's standard solution. Small volume of bacterial suspensions were swabbed on each already prepared

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. 200 mg/ml of the leaf and bark 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 37ºC 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 [14].

2.7 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Bark 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 37ºC 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 37ºC for 24 hrs. After incubation, the least concentration that showed no growth was recorded as the minimum bactericidal concentration (MBC) [14].

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 37ºC for 24 hours. After incubation, clear zones of inhibition were measured in millimeter and areas without clear zones were observed. Inoculated plate without antibiotics served as control [4].

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 Bark Extracts of *A. muricata*

Table 1 revealed the percentage recovery of the bark extracts of *A. muricata* after extraction. The table showed that more extracts was recovered with methanol and ethanol than water with 42.92%, 35.18% and 7.16% respectively. Extraction of *A. muricata* bark gave a percentage yield of 42.92%, 35.18% and 7.16% for methanol, ethanol and aqueous bark extracts respectively.

Table 1. Percentage recovery of bark extracts of *A. muricata*

Solvents	Percentage (%)
Aqueous	7.16%
Ethanol	35.18%
Methanol	42.92%

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

Phytochemical screening of the aqueous, ethanol and methanolic extracts of *A. muricata* bark is presented in Table 2. The results revealed that saponin, tannin, flavonoid, terpenoid and cardiac glycosides were present in the aqueous, ethanol and methanol bark extracts of *A. muricata* while phlobatannin, alkaloid and Steroid were absent in all the plant extracts.

Table 3 showed the quantitative phytochemical (mg/100 g) composition of aqueous, ethanol and methanol crude extracts obtained from bark of *A. muricata*. The result revealed that Saponin, tannin, terpernoid, glycosides and flavonoid has the highest values (14.00±0.18, 6.42±0.05, 28.16±0.01, 34.67±0.02 and 3.72±0.01) respectively in ethanol extract. Glycosides has the highest values (7.06±0.04 and 19.35±0.01) in aqueous and methanol extracts while flavonoid has the least values (0.38±0.01, 3.72±0.01 and 1.86±0.01) in aqueous, ethanol and methanol extracts.

3.3 Antibacterial Activity of *A. muricata* Bark Extracts

Table 4 shows the susceptibility patterns of the bacteria isolates to aqueous, ethanol and methanol bark extracts (crude) of *A. muricata* at 200 mg/ml measured by zone of inhibition in Plate 1, Plate 2, Plate 3, Plate 4, Plate 5 and Plate 6.

The aqueous bark extract does not show any zone of inhibition on all the test organisms (clinical and typed isolates) while all the isolates were susceptible to ethanol and methanol bark extracts. The ethanol bark extracts showed inhibition diameter ranging from (8.67 mm to 24.33 mm) with the highest zone of inhibition on *S. typhi* (24.33 mm) and least zone of inhibition on *K. pneumoniae* (8.67 mm). The methanol bark extract ranges from (7.67 mm to 23.33 mm) with the highest zone of inhibition on *P. aeruginosa* (23.33 mm) and least zone of inhibition on *K. pneumoniae* (7.67 mm).

Table 5 revealed the result of susceptibility patterns of the bacteria isolates to ethanol and methanol bark extracts (purified) of *A. muricata* at 200 mg/ml. The purified extracts showed increase in the inhibitory effect of the plant against all isolates. The susceptibility patterns of the ethanol bark extracts showed inhibition diameter ranging from (9.00 mm to 25.00 mm) with the highest on *S. typhi* and least on *K. pneumoniae* while the susceptibility pattern of the methanol bark extract of the plant on the bacteria isolates ranges from (8.00 mm to 24.00 mm) with the highest on *P. aeruginosa* and least on *K. pneumoniae.*

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

Table 6 shows the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) o *A. muricata* bark extracts on test organisms (clinical and typed bacteria isolates).

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

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

Key: + = present, - = Negative

Table 3. Quantitative phytochemical composition of *Annona muricata* bark crude extract

Phytochemical	Aqueous (mg/100 g)	Ethanol (mg/100 g)	Methanol (mg/100 g)
Saponin	4.09±0.27^{a}	14.00±0.18^{c}	8.55±0.18^{b}
Tannin	1.76±0.00^{a}	6.42±0.05^{c}	3.82±0.01^{b}
Terpenoid	5.12±0.01^{a}	28.16±0.01^{c}	15.36±0.01^{b}
Glycosides	7.06±0.04^{a}	34.67±0.02^{c}	19.35±0.01^{b}
Flavonoid	0.38±0.01^{a}	3.72±0.01^{c}	1.86±0.01^{b}
Alkaloid	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Steroid	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Phlobatannin	0.00±0.00^{a}	0.00±0.00^{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

Table 4. Antibacterial activity of bark extracts (Crude) at 200 mg/ml

Isolates	Aqueous	Ethanol	Methanol	Control
Pseudomonas aeruginosa	0.00±0.00^{a}	19.00±0.33^{b}	23.33±0.33^{c}	31.00±0.33^{d}
Salmonella typhi	0.00±0.00^{a}	24.33±0.33^{c}	19.33±0.33^{b}	34.67±0.88^{d}
Shigella sp	0.00±0.00^{a}	20.33±0.33^{b}	22.67±0.33^{b}	42.33±1.46^{c}
Escherichia coli	0.00±0.00^{a}	15.00±0.58^{b}	16.33±0.33^{b}	24.00±0.58^{c}
klebsiella pneumoniae	0.00±0.00^{a}	8.67±0.33^{b}	7.67±0.33^{b}	24.00±0.58^{c}
Proteus vulgaris	0.00±0.00^{a}	14.00±0.58^{b}	15.00±0.58^{b}	28.33±0.58^{c}
P. vulgaris ATCC 29905	0.00±0.00^{a}	14.33±0.33^{b}	13.00±0.58^{b}	34.33±0.88^{c}
P. aeruginosa ATCC 10145	0.00±0.00^{a}	14.67±0.33^{b}	15.67±0.33^{b}	44.00±0.58^{c}
S. typhi ATCC	0.00±0.00^{a}	13.00±0.33^{b}	14.00±0.58^{b}	34.00±3.66^{c}
E. coli ATCC 25922	0.00±0.00^{a}	19.00±0.58^{c}	15.00±0.58^{c}	34.00±0.58^{d}
K. pneumoniae ATCC	0.00±0.00^{a}	15.33±0.33^{b}	14.00±0.58^{b}	28.00±0.58^{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

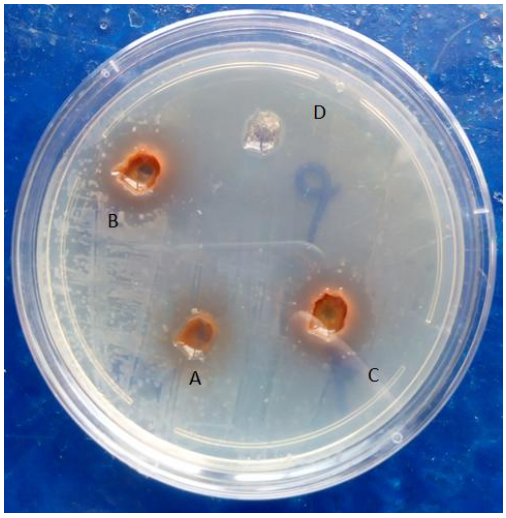

Plate 1. Zones of inhibition indicating antibacterial activities of aqueous, ethanol and methanol extracts of *A. muricata* bark on *Pseudomonas aeruginosa*

Key: A= Aqueous, B= Ethanol, C= Methanol, D= Control (Ciprofloxacin)

The MIC of both ethanol and methanol bark extract of the bacteria isolates ranged from 25 to 50 mg/ml. The MIC for ethanol bark extract of the plant showed that *P. aeruginosa, E. coli, Shigella* sp*, P. aeruginosa* ATCC 10145*, E. coli* ATCC 25922 and *S. typhi* ATCC 14028 had their MIC at 25 mg/ml while *P. vulgaris, K. pneumoniae, S. typhi, P. vulgaris* ATCC 29905 and *K. pneumoniae* ATCC 13883 had their MIC at 50 mg/ml. Similarly, the methanol bark extract of the

plant showed MIC of 25 mg/ml on *K. pneumoniae,* P. *aeruginosa, E. coli, Shigella* sp, *P. aeruginosa* ATCC 10145, *E. coli* ATCC 25922 and *S. typhi* ATCC 14028 while others had their MIC at 50 mg/ml.

Also, the (MBC) of the ethanol and methanol bark extract of *A. muricata* ranged from 50-100 mg/ml. For the ethanol bark extract, *P. vulgaris*, *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *S. typhi*, *P. aeruginosa* ATCC 10145 and *K. pneumoniae* ATCC 13883 had their MBC at 100 mg/ml while other isolates had their MBC at 50 mg/ml. Only *P. vulgaris* and *P. vulgaris* ATCC 29905 had their MBC at 100 mg/ml for methanol bark extract while others had their MBC observed at 50 mg/ml.

3.5 Antibiotic Sensitivity Pattern

Table 7 shows the 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 (29.00 mm) on *P. aeruginosa* ATCC 10145 and

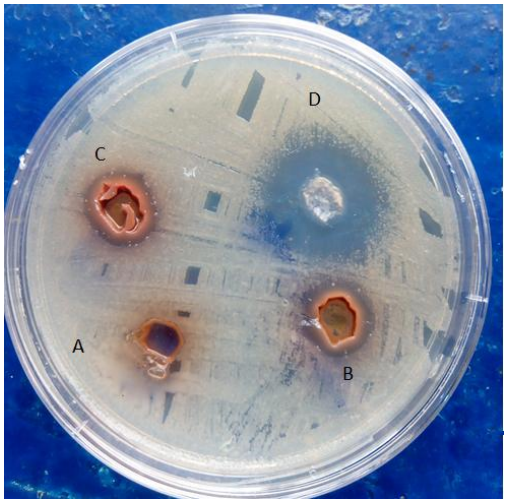

Plate 2. Zones of inhibition indicating antibacterial activities of aqueous, ethanol and methanol extracts of *A. muricata* bark on *Escherichia coli*

Key: A= Aqueous, B= Ethanol, C= Methanol, D= Control (Ciprofloxacin)

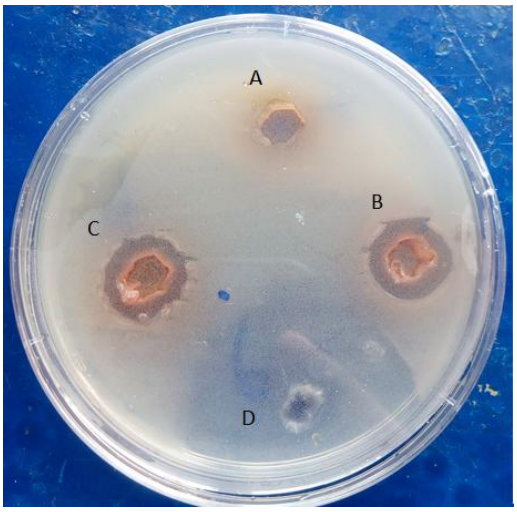

Plate 3. Zones of inhibition indicating antibacterial activities of aqueous, ethanol and methanol extracts of *A. muricata* bark on *Salmonella typhi*

Key: A= Aqueous, B= Ethanol, C= Methanol, D= Control (Ciprofloxacin)

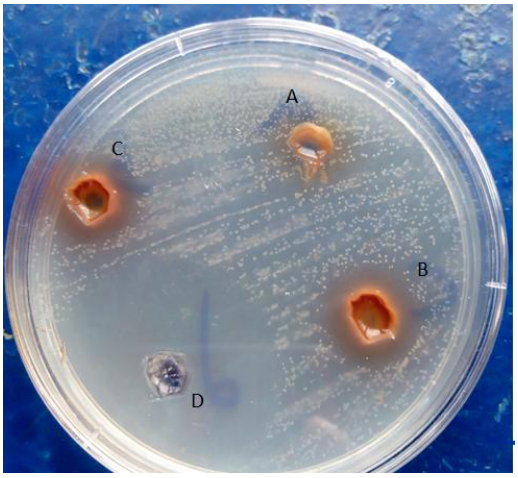

Plate 4. Zones of inhibition indicating antibacterial activities of aqueous, ethanol and methanol extracts of *A. muricata* bark on *Shigella sp*

Key: A= Aqueous, B= Ethanol, C= Methanol, D= Control (Ciprofloxacin)

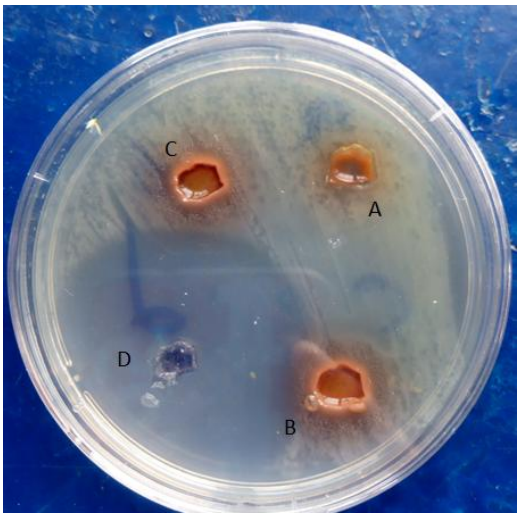

Plate 5. Zones of inhibition indicating antibacterial activities of aqueous, ethanol and methanol extracts of *A. muricata* bark on *Proteus vulgaris*

Key: A= Aqueous, B= Ethanol, C= Methanol, D= Control (Ciprofloxacin)

Shigella Sp (25.67 mm) respectively. *E. coli*, *S. typhi*, and *P. aeruginosa* ATCC 10145 were resistant to streptomycin (S) while others were susceptible with highest diameter zone of inhibition (22.67 mm) on *Shigella* Sp. only *S. typhi* was resistant to sparfloxacin (SP) while others were susceptible with diameter zone of inhibition ranging from (13.67 mm to 25.67 mm). Highest zone of inhibition was observed on *E. coli*.

4. DISCUSSION

Antimicrobial compounds are amply available in medicinal plants as documented [15]. Thus, this work offers a guide to the extraction, phytochemical screening, purification and antibacterial activity of *A. muricata* bark extracts. The high percentage recovery of methanol and ethanol compared to aqueous extract could be due to methanol's and ethanol's ability to dissolve more of the active components of the plant than water [16].

Phytochemical screening of the crude extracts of *A. muricata* bark revealed the presence of some bioactive components such as saponin, tannin, flavonoid, terpenoid and cardiac glycosides. This is in agreement with the work of Vijayameena [17] who reported similar bioactive compounds in

the same plant. These compounds are known to exhibit medicinal, physiological, biological and therapeutic properties [18]. It has been reported that flavonoids are free radical scavengers that prevent oxidative cell damage [19,20]. Tannin are used as astringents, against diarrhoea as diuretics, against stomach and duodenal tumours, [20]. Terpenoids are lipophilic compounds with bacterial cell memebrane disruption potential [21]. Cardiac glycosides are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure [22]. The presence of cardiac glycosides in this study is in agreement with the findings of Solomon-Wisdom [23] who reported the presence of cardiac glycosides in the aqueous and methanolic extracts of *A. muricata.* Abundant presence of tannin, saponin, flavonoid, tepernoids and cardiac glycosides in ethanolic bark extract of *A. muricata* compared to other solvents conformed to the report of Vimala [24] who stated that ethanolic extract of *A. muricata* has higher composition of secondary metabolite such as flavonoid, tannin and saponin. Result of this study have shown that aqueous is not a preferable solvent for the extraction of phytochemicals from *A. muricata* bark. Similarly, Salisu [25] reported that a lesser polar solvent (ethanol and methanol) extracts more phytochemicals from the stem bark of the plant.

The susceptibility patterns of clinical and typed enteric bacteria isolates to bark extracts (crude) of *A. muricata* at 200mg/ml showed variations in the zone of inhibition for each extracts. Ethanol bark extract demonstrated a higher activity on the test organisms than aqueous and methanol extracts. The poor activities of the aqueous extract against the bacteria isolates observed in this study is in agreement with the study of Busani [26] who documented that aqueous extract of plants generally exhibit little or no antimicrobial activities against micro-organisms. Clinical isolates were observed to be more susceptible to both ethanol and methanol

Table 5. Antibacterial activity of bark extract (Purified) at 200 mg/ml

Isolates	Ethanol	Methanol	Control
Pseudomonas aeruginosa	21.67±0.33^{a}	24.00±0.57^{b}	31.33±0.88^{c}
Salmonella typhi	25.00±0.58^{b}	21.00±0.58^{a}	35.00±1.15^{c}
Shigella sp	21.67±0.88^{a}	23.67±0.67^{a}	31.00±0.57^{b}
Escherichia coli	15.67±0.33^{a}	16.33±0.33^{a}	24.67±1.20^{b}
klebsiella pneumonia	9.00±0.58^{a}	8.00±0.58^{a}	26.58±0.88^{b}
Proteus vulgaris	17.00±0.58^{a}	17.33±0.33^{a}	28.00±0.58^{b}
P. vulgaris ATCC 29905	15.67±0.33^{a}	14.33±0.33^{a}	34.67±1.20^{b}
P. aeruginosa ATCC 10145	16.33±0.33^{a}	16.67±0.88^{a}	38.67±0.88^{b}
S. typhi ATCC	16.00±0.58^{a}	15.00±0.57^{a}	35.33±0.88^{b}
E. coli ATCC 25922	21.33±1.20^{b}	16.00±1.15^{a}	35.67±1.45^{c}
K. pneumoniae ATCC	17.33±0.88^{a}	16.00±0.58^{a}	27.00±0.58^{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

Table 6. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of bark extracts (mg/ml)

Organisms	MIC		MBC	
	Ethanol	Methanol	Ethanol	Methanol
Proteus vulgaris	50	50	100	100
Klebsiella pneumoniae	50	25	100	50
Pseudomonas aeruginosa	25	25	100	50
Escherichia coli	25	25	100	50
Shigella sp	25	25	50	50
Salmonella typhi	50	50	100	50
P. vulgaris ATCC 29905	50	50	100	100
P. aeruginosa ATCC 10145	25	25	50	50
S. typhi ATCC	50	50	100	50
E. coli ATCC 25922	25	25	50	50
K. pneumoniae ATCC	25	25	50	50

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= Amoxacillin, 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

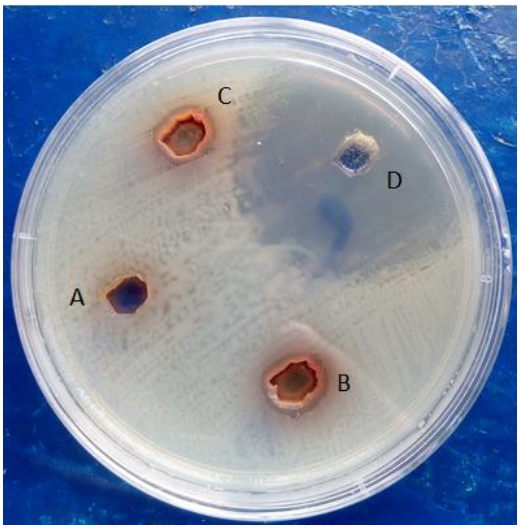

Plate 6. Zones of inhibition indicating antibacterial activities of aqueous, ethanol and methanol extracts of *A. muricata* bark on *klebsiella pneumoniae*

Key: A= Aqueous, B= Ethanol, C= Methanol, D= Control (Ciprofloxacin)

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. This is in contrast with the work done by Ogundare and Oladejo [27] who reported that the clinical isolates were more resistant to *Persea americana* extracts than the typed isolates. The demonstration of antibacterial activity of bark extracts of *A. muricata* against both clinical and typed isolates used in this study provides a scientific proof of its usage in the treatment of enteric bacterial infections. The purified extracts of *A. muricata* bark showed that there was increase in the inhibitory activities of ethanol and methanol bark extracts on the test organisms. This may be as a result of the inert impure substances present in the crude extracts which could have inhibited its antibacterial activity [28]. The result of this study is in line with the study of Oseni [29] who attested that the purified *Euphorbia hirta* extracts showed significant 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.

Findings from this study showed that the MIC of bark extracts against clinical and typed isolates was found to be (25 mg/ml) while the MBC was found to be (50 mg/ml). This result indicates that the ethanol and methanol extracts of the plant were bacteriostatic at lower concentration and bactericidal at higher concentration.

The commercial antibiotics used in this study were observed to be effective in inhibiting the test organisms. Of all the antibiotics used, ciprofloxacin (CPX) was the most effective against the test organisms (both clinical and typed isolates). The high inhibition by ciprofloxacin on clinical and typed isolate is expected because it is usually the recommended drug of choice in the treatment of enteric diseases. The high inhibition values of the antibiotics could be as a result of the purified state of the antibiotics as reported by Doughari, et al. [14] that the state of administration of an antimicrobial agent affects the effectiveness of such agent, and that antibiotics are in a refined state and plant extracts in crude state.

5. CONCLUSION

This study has revealed *A. muricata* bark extracts to be rich in flavonoids, tannins, saponins and cardiac glycosides as secondary metabolites which was responsible for the various antibacterial activities exhibited. Glycosides has the highest value in all the extracts. Clinical isolates were more susceptible to the plant than the typed isolates. This study confirmed that among the different solvents used, ethanol extract showed the highest antibacterial activity. The purified bark extracts of *A. muricata* had higher antibacterial activity on

the test organisms. Commercial antibiotics were effective in inhibiting the test organisms. However, the purified ethanol bark extracts can serve as a substitute to the commercially available antibiotics 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 bark extracts and also ascertain the biosafety of the plant part.

CONSENT AND ETHICAL APPROVAL

It is not applicable

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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