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Annona muricata: Comparative Assessment of the Antibacterial Activities of the Leaf and Stem Extracts against Multiple Antibiotic Resistant Clinical Isolates

O. O. Agunloye[1*] and A. K. Onifade[1]

[1]Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author OOA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AKO supervised the study. Both authors read and approved the final manuscript.

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ABSTRACT

This study was conducted to determine the antibacterial effect of crude extracts of *Annona muricata*, comparing the leaf and stem bark extracts using the same extraction solvents. The bacteria isolates were obtained from the hospital. The isolates obtained are *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis* and *Streptococcus pyogene*; they were confirmed using standard techniques. Multiple antibiotic-resistant was confirmed in the isolates after antibiotic susceptibility testing. Extracts were prepared from the leaf and stem of *A. muricata* using ethanol, chloroform, cold water and hot water. The antibacterial activities of the crude extracts were assayed using the agar well diffusion method. The phytochemical screening revealed the presence of saponins, Tannins and flavonoid especially in the ethanol extracts of both leaf and stem part of the plant. All the extract showed varying degrees of antibacterial activities. Chloroform and Hot water rated best for antibacterial activities in this

**Corresponding author: E-mail: agunloyeoluwatosin@gmail.com;*

study. *A. muricata* stem hot water extract has zones of inhibition that ranges from 16.003 ± 0.009 mm to 1.000± 0.006 mm. *A. muricata* leaf hot water extract has antibacterial effect with zones ranging from 14.500± 0.009 mm to 1.000± 0.006 mm across the isolates. Cold water extracts recorded the lowest zones of inhibition from this study for the stem and Bark antibacterial activities. The stem of *A. muricata* has higher inhibitory effect on the test isolates compared to the leaf of *A. muricata.*

Keywords: Antibacterial; resistance; A. muricata; inhibition; isolates.

1. INTRODUCTION

A. muricata commonly called Soursop or Graviola or Guanabana [1] is gaining worldwide acclaim for being a miracle tree in the field of cancer research and can pave way for research in many fields [2]. It is a flowering evergreen tree native to Mexico, Cuba, Central America and parts of India. It is a small erect evergreen tropical plant belonging to the family Annonaceae, growing 5-6 meters in height. Graviola fruit is sweet and full of health beneficial components with high moisture content. The fruit is 18 cm long and covered with spine-like structure. The pulp is soft, white and with agreeable pungent flavour [1]. The leaves, barks, fruits and roots of the *A. muricata* trees are used as ingredients in various traditional herbal medicines. The fruit and the leaves are used in traditional medicine for their tranquillizing and sedative properties. The decoction of the leaves of *A. muricata* works as a pain reliever and helps cure gall bladder diseases traditionally [3]. The miracle tree as it is widely known is a natural cancer killer [4]. The leaves can be applied topically to get rid of eczema, skin rash and swelling. Topical application of these leaves promotes fast healing of wounds and prevents infections. The fruits are used to reduce joint pain, to treat heart conditions, as a sedative and to reduce coughing or flu symptoms in herbal medicines [3]. Teas made from the leaves are traditionally used by Jamaicans as a tranquilizer, de-wormer, antispasmodic, to treat fever, dysentery, colds, for pains and as a diuretic [5].

The use of this plant in medicine has been reported by researchers to have an antimicrobial effect against common pathogen and providing solutions to problems related to human diseases [6]. Hence, Soursop with its miraculous properties was used in this study with an intention to find newer use of these miracle plants. [2] reported the effects of the extracts on some species of Bacteria and Candida. The wound healing activity of alcoholic extract of stem and bark of *A. muricata* researches was found to show the marked reduction in the area of the wound which was tested in the albino rats which proves their possible use in the healing wound. [6,7] confirmed *A. muricata* leaf methanol and Aqueous extract to have inhibitory effects against bacterial strains such as *Staphylococcus aureus ATCC29213, Escherichia coli*. Bacterial infections caused by multiple antibiotic resistant (MAR) bacteria are a growing threat worldwide [8]. Hence this study, to assess the antibacterial effect of the stem and leaf of the *A. muricata* and compare their activities against Multiple resistant clinical isolates.

2. MATERIALS AND METHODS

2.1 Isolation and Identification

Pure cultures of clinical isolates were obtained from the State Specialist Hospital, Akure, Ondo State. The bacteria isolates obtained are *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Streptococcus pyogenes.* These isolates were confirmed using standard techniques [9].

2.2 Antibiotics Susceptibility Profile

Antibiotic susceptibility testing was performed using the Kirby Bauer disk diffusion method [10] and interpretation according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [11].

2.3 Collection/Preparation of Plant

The fresh leaves and stem of *A. muricata* were collected from Elesare, Ondo state and authenticated at the Crop Soil and Pest Management Department, Federal University of Technology Akure, Ondo State. The collected plants were washed in distilled water, dried and ground. The crude extracts were obtained by soaking 100 grams of each dried powdered plant in 1000 mL of Hot water, Coldwater, Ethanol and Chloroform separately for 72 hrs, and sieved with a muslin cloth. The extract was further

concentrated by using a rotary vacuum evaporator at 45-50°C and stored.

2.3.1 Phytochemical analysis of plants

Qualitative and quantitative phytochemical analyses were carried out on the extracts using standard chemical methods as described by [12].

2.3.2 Reconstitution of extracts

The test solution of each extract was prepared by dissolving 50 mg-400 mg of the plant extracts separately in 1 mL of already prepared Tween 20(20%) to get a concentration of each extract 0.50 mg/mL- 50 mg/mL [3].

2.4 Standardization of Inoculum

The freshly prepared nutrient broth was inoculated with test organisms and incubated for 24 h at 37°C. A 0.2 mL aliquot from the cultured broth was aseptically dispensed into 20 mL of freshly prepared nutrient broth and incubated for 2 to 3 h at 37°C to standardize to 0.5 McFarland standard of Barium sulphate solution which is equivalent to 1×10^6CFU [13].

2.5 Determination of Antibacterial Activities of Extracts

2.5.1 Antimicrobial assay of crude extracts

The antimicrobial activities of the extracts were evaluated using the agar well diffusion method [14]. Standardized inoculum of each test microorganisms was spread onto sterile Mueller Hinton agar plate. The plates were allowed to gel and a sterile cork borer of diameter 8.0 mm was used to bore wells in the agar plates. Exactly 0.5 ml of 50 mg/ml (up to 400 mg/mL) each of the extract was aseptically dispensed into the wells; the plates were allowed to stand for 20 mins for proper diffusion to take place and then incubated at 37°C for 24 h.

2.5.2 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC/MBC of the organisms was determined following the method described by [15]. Varying concentrations (50-400 mg/mL) of the extracts were prepared by serial dilution using Nutrient broth as a diluent. Each of the diluted extracts was then inoculated with 100 µL of the overnight broth culture of the bacteria Isolates and incubated at 37°C for 24 hours and was observed for turbidity. The lowest concentration that showed no turbidity was taken as the MIC while the lowest concentration of the extract which showed no growth on plates after 24 hours of incubation indicates bactericidal effect and was recorded as the MBC.

2.6 Statistical Analysis

Data obtained from the experiments will be subjected to One Way Analysis of Variance (ANOVA) and means separated using Duncan's New Multiple Range Test at 95% confidence level using Statistical Packages for the Social Sciences (SPSS) version 20.0 differences between means will be considered significant at $P\leq0.05$.

3. RESULTS

Table 1a and 1b revealed the antibiotic sensitivity patterns of the isolates. *P. mirabilis,* and *Pseu. aeruginosa* were resistant to all the antibiotics used: *E. coli*, K. *pneumonie*, and *S. pyogenes* were sensitive to only one antibiotic. Table 2 shows the phytochemicals present in the extracts of *A. muricata*. Fig. 1 shows the amount of phytochemical constituent present in the extracts. In the stem extracts, Tannin is higher in ethanol extract with 1.213 ± 0.008 mg/g and least in cold water extract with 0.2000 ± 0.006 mg/g while for leaf extracts, Tannin is higher in chloroform extract with 3.406± 0.003 mg/g and least in ethanol extract with 0.803± 0.003 mg/g in the leaf extract.

Tables 3 -6 show the antibacterial activities of the extracts at the varying concentration on the multiple antibiotic-resistant isolates. *A. muricata* leaf hot water extract has its highest zone of inhibition on *E. coli* with 14.500± 0.009 mm and the least on *S. aureus* with 1.000± 0.006 mm. *A. muricata* leaf cold water extract has its highest zone of inhibition on *P. mirabilis* with 9.013 ±0.013 mm and was not able to inhibit *S. pyogenes* and *S. aureus*. *A. muricata* leaf chloroform extract has its highest zones of inhibition on *E. coli* with 13.010± 0.015 mm and was not able to inhibit the growth of *P. mirabilis*. *A. muricata* leaf ethanol extract has its highest inhibitory effect on *E. coli* with 16.010 ± 0.015 mm but has no effect on *E. coli, S. pyogenes*, and *P. mirabilis.*

A. muricata stem hot water extract has its highest effect on *E. coli* with 16.003 ± 0.009 mm and its least effect *S. aureus* with 1.000± 0.006 mm, *A.*

muricata stem cold water extract has its highest zone of inhibition on S. *aureus* with 7.003 ± 0.008 mm and no effect on *K. pneumoniae*. *A. muricata* stem chloroform extract has its highest zone of inhibition on *E. coli* with 16.007±0.012 mm and has no effect on *S. pyogenes, K. pneumoniae*, and *S. aureus*. *A. muricata* stem ethanol extract has its highest zone of inhibition on *S. pyogenes* with 13.003 ± 0.009 mm and has no effect on *P. mirabilis* and *K. pneumoniae.*

Table 7 shows the MIC/MBC of the plant extracts on the isolates. The MIC/MBC varies across the bacterial isolates. For hot water stem extract, the MIC on the bacterial isolates ranged from 50 mg/mL – 400 mg/mL and MBC ranged from 100 mg/mL – 400 mg/mL while for hot water leaf extract, the MIC ranged from 50 mg/mL – 400 mg/mL and the MBC ranged from 100 mg/mL – 400 mg/mL. The chloroform extract of *A. muricata* stem, the MIC on the bacterial isolates ranged from 50-100 mg/mL, while the MBC ranges from 100 mg/mL – 200 mg/mL but no inhibition on *K. pneumoniae* and *S. aureus*. While for the chloroform extract of the leaf, the MIC of the extract on the bacterial isolates ranged from 50- 400 mg/mL, while the MBC ranged from 100 mg/mL -400 mg/mL and no inhibition on *P. mirabilis*.

4. DISCUSSION

Finding from this research has shown that ethanol yielded a higher-end concentration compared to other extraction solvent used. Ethanol has the ability to extract bioactive compounds like Tannis, saponins, flavonoid, alkaloid, phenol and steroid which have higher concentrations in both parts of the plant used in this study as reported by [3]. The amounts of phytochemicals constituents present in Ethanol extracts of *A. muricata* stem are higher than that of every other extract in the study. The least amount of phytochemicals was recorded in cold water extracts of *A. muricata* especially the stem extract.

A. muricata leaf extract had an inhibitory effect on the bacterial isolates in this study. This is similar to the report of [3], where he reported the efficacy of the methanolic extract of the leaves of *A. muricata* on some clinical isolates and fungi isolates. *A. muricata* leaf hot water extract and chloroform extract showed a higher inhibitory effect compared to the ethanolic extract and cold water extract as an antibacterial agent on the isolates. [12] reported that aqueous fractions of *A. muricata* generally exhibited low activity against the test pathogens also that, *A. muricata* leaf has been found rich in phenolic compounds, flavonoids, tannins, alkaloids, saponins and cardiac glycosides as secondary metabolites. *A. muricata* leaf extract was also able to inhibit the bacterial isolates in this study. This is in agreement with [3] who reported the efficacy of the methanolic extract of the leaves of *A. muricata* on some clinical isolates and fungi isolates. *A. muricata* leaf hot water extract and chloroform extract showed a higher inhibitory effect compared to the ethanol extract and cold water extract as an antibacterial agent on the isolates. [16] reported that aqueous fractions of *A. muricata* leaf have been found rich in phenolic compounds, flavonoids, tannins, alkaloids,

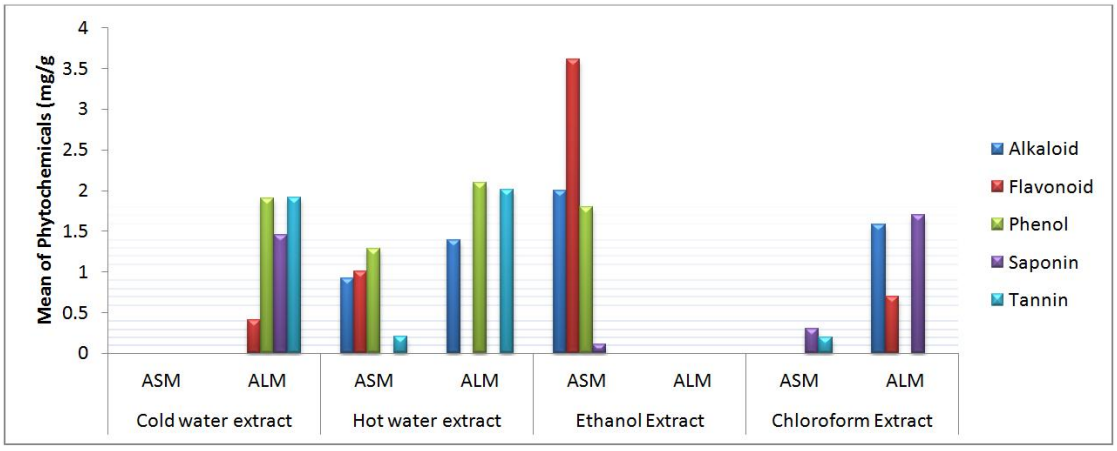

Fig. 1. Amount of phytochemical constituent present in *A. muricata* extracts
KEY: AMS- A. muricata stem, AML- A. muricata leaf

Table 1a. Antimicrobial susceptibility patterns of gram negative isolates

Isolates	CN	PEF	OFL	S	SXT	CH	SP	CPX	AM	AU
	Zones of Inhibition (mm)									
E. coli	0.000±0.000^{a}	14.007±0.012^{d}	12.997±0.009^{b}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	11.003±0.008^{c}	0.000±0.000^{a}
P. mirabilis	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}
K. pneumoniae	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	12.004±0.008^{b}	0.000±0.000^{a}
Pseu. aeruginosa	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}

Key: CN- Gentamycin 10 μg, S- Streptomycin 30 μg, PEF- Pefloxacin 10 μg, OFL- Tarivid 10 μg, SXT-Septrin 30μg, CH- Chloramphenicol 30μg, SP- Sparfloxacin 10 μg, CPX- Ciprofloxacin 10 μg, AM- Amoxicillin 30 μg, AU-Augmentin 30 μg; Data are represented as mean± SE (standard error). Each value is a mean of three (3) replicates; Values with the same superscript letters along the same column are not significantly different ($p \leq 0.05$)

Table 1b. Antimicrobial susceptibility patterns of gram positive isolates

Isolates	CN	PEF	S	SXT	CPX	AM	APX	E	Z	R
	Zones of inhibition(mm)									
S. pyogenes	0.000±0.000^{a}	12.007±0.012^{c}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	9.897±0.003^{b}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}
S. aureus	0.000±0.000^{a}	5.007±0.006^{b}	14.003±0.0074^{e}	13.967±0.003^{f}	10.993±0.012^{c}	0.000±0.000^{a}	0.000±0.000^{a}	10.997±0.003^{d}	0.000±0.000^{a}	0.000±0.000^{a}

Key: CN- Gentamycin 10 μg, S- Streptomycin 30 μg, PEF- Pefloxacin 10 μg, SXT-Septrin 30μg, CPX- Ciprofloxacin 10 μg, AM- Amoxicillin 30 μg, APX-Amplicon 30μg, E- Erythromycin 10 μg, Z-Zinnclof 20 μg, R-Rocephin 25 μg; Data are represented as mean± SE (standard error). Each value is a mean of three (3) replicate; Values with the same superscript letters along the same column are not significantly different ($p \leq 0.05$)

Table 2. Qualitative phytochemical properties of *A. muricata* extracts

Phytochemical	Ethanol	Chloroform	Cold water	Hot water
		***Annona muricata* stem**		
Flavonoid	+	+	+	+
Saponin	+	-	-	-
Tannins	+	+	+	+
Alkaloid	+	+	-	-
Phenol	+	+	+	+
		***Annona muricata* leaf**		
Flavonoid	+	+	+	-
Saponin	-	-	+	-
Tannins	+	-	+	+
Alkaloid	+	-	-	+
Phenol	+	+	+	+

Key: + Present - Negative

Table 3. Antibacterial effect of ethanol extracts of *A. muricata* leaf and stem on bacterial isolates

Isolates	50 mg/mL		100 mg/mL		200 mg/mL		300 mg/mL		400 mg/mL	
	AMS	AML	AMS	AML	AMS	AML	AMS	AMS	AMS	AML
	Zones of inhibition (mm)									
E. coli	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	1.000±0.000^{b}	0.000±0.000^{a}	1.267±0.145^{c}	0.000±0.000^{a}
S. pyogenes	0.000±0.000^{a}	0.000±0.000^{a}	4.007±0.012^{b}	0.000±0.000^{a}	13.000±0.006^{c}	0.000±0.000^{a}	14.000±0.012^{e}	0.000±0.000^{a}	21.003±0.014^{e}	0.000±0.000^{a}
P. mirabilis	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}
K. pneumonia	0.000±0.000^{a}	7.003±0.009^{b}	0.000±0.000^{a}	9.997±0.008^{c}	0.000±0.000^{a}	12.003±0.009^{d}	0.000±0.000^{a}	12.507±0.012^{e}	0.000±0.000^{a}	13.007±0.01f^{c}
S. aureus	4.997±0.015^{b}	4.000±0.006^{b}	10.003±0.014^{c}	8.007±0.012^{c}	12.997±0.014^{d}	11.007±0.007^{d}	13.970±0.015^{e}	12.007±0.012^{e}	15.020±0.020^{f}	16.010±0.015^{f}
Pseu. aeruginosa	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	4.013±0.013^{b}	7.997±0.009^{b}	5.003±0.003^{c}	8.000±0.006^{b}	6.003±0.003^{d}	8.997±0.003^{c}

Key: AMS- A. muricata stem, AML- A. muricata leaf

Table 4. Antibacterial effect of hot water extracts of *A. muricata* leaf and stem on bacterial isolates

Isolates	50 mg/mL		100 mg/mL		200 mg/mL		300 mg/mL		400 mg/mL	
	AMS	AML	AMS	AML	AMS	AML	AMS	AML	AMS	AML
	Zones of inhibition (mm)									
E. coli	9.020±0.020^{b}	6.003±0.008^{b}	10.013±0.009^{c}	8.003±0.015^{c}	14.953±0.029^{d}	14.000±0.006^{d}	15.497±0.009^{e}	14.007±0.007^{e}	16.003±0.009^{f}	14.500±0.009^{f}
S. pyogenes	0.000±0.000^{a}	0.000±0.000^{a}	5.020±0.010^{b}	0.000±0.000^{a}	6.993±0.007^{c}	9.007±0.012^{b}	8.103±0.003^{d}	9.507±0.012^{c}	9.010±0.153^{e}	10.010±0.015^{d}
P. mirabilis	7.993±0.018^{b}	0.000±0.000^{a}	8.007±0.003^{c}	0.000±0.000^{a}	11.007±0.000^{d}	0.000±0.000^{a}	11.500±0.005^{d}	00.000±0.000^{a}	12.010±0.006^{e}	2.007±0.012^{b}
K. pneumoniae	0.000±0.000^{a}	0.000±0.000^{a}	5.003±0.003^{b}	4.997±0.014^{b}	7.007±0.012^{c}	7.010±0.010^{c}	8.007±0.012^{d}	7.503±0.008^{c}	9.013±0.018^{e}	8.010±0.010^{d}
S. aureus	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	1.000±0.006^{b}	1.000±0.006^{b}
Pseu. aeruginosa	0.000±0.000^{a}	0.000±0.000^{a}	5.013±0.013^{b}	7.003±0.008^{b}	9.000±0.006^{c}	13.003±0.003^{c}	9.007±0.012^{c}	13.003±0.009^{c}	10.017±0.017^{d}	13.507±0.012^{d}

Key: AMS- A. muricata stem, AML- A. muricata leaf

Table 5. Antibacterial effect of cold water extracts of *A. muricata* leaf and stem on bacterial isolates

Isolates	50 mg/mL		100 mg/mL		200 mg/mL		300 mg/mL		400 mg/mL	
	AMS	AML	AMS	AML	AMS	AML	AMS	AML	AMS	AML
	Zones of inhibition (mm)									
E. coli	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.983±0.017^{b}	0.000±0.000^{a}	2.003±0.003^{c}	1.000±0.000^{b}
S. pyogenes	0.000±0.000^{a}	0.000±0.000^{a}	1.007±0.012^{b}	0.000±0.000^{a}	4.003±0.000^{c}	0.000±0.000^{a}	4.507±0.012^{d}	0.000±0.000^{a}	4.997±0.009^{d}	0.000±0.000^{a}
P. mirabilis	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	7.017±0.017^{b}	0.000±0.000^{a}	8.003±0.020^{c}	0.993±0.006^{b}	9.013±0.013^{d}
K. pneumonia	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	4.993±0.007^{b}	0.000±0.000^{a}	5.017±0.016^{b}	0.000±0.000^{a}	6.993±0.012^{c}
S. aureus	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	5.003±0.015^{b}	0.000±0.000^{a}	6.003±0.015^{c}	0.000±0.000^{a}	7.003±0.008^{d}	0.000±0.000^{a}
Pseu. aeruginosa	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.997±0.008^{b}	6.993±0.007^{b}	1.000±0.010^{b}	7.003±0.014^{b}	2.013±0.009^{c}	9.007±0.018^{c}

Key: AMS- A. muricata stem, AML- A. muricata leaf

Table 6. Antibacterial effect of chloroform extract of *A. muricata* leaf and stem on bacterial isolate

Isolates	50 mg/mL		100 mg/mL		200 mg/mL		300 mg/mL		400 mg/mL	
	AMS	AML	AMS	AML	AMS	AML	AMS	AML	AMS	AML
	Zones of inhibition (mm)									
E. coli	5.003±0.014^{b}	4.010±0.010^{b}	8.017±0.009^{c}	8.007±0.012^{c}	10.000±0.006^{d}	12.993±0.012^{d}	12.003±0.003^{e}	13.010±0.015^{e}	13.010±0.015^{e}	13.010±0.015^{e}
S. pyogenes	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	7.000±0.020^{b}	0.000±0.000^{a}	11.003±0.014^{d}	11.003±0.014^{d}	11.003±0.014^{d}
P. mirabilis	5.007±0.007^{b}	0.000±0.000^{a}	6.010±0.006^{c}	0.000±0.000^{a}	6.073±0.015^{c}	0.000±0.000^{a}	6.513±0.019^{d}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}
K. pneumoniae	0.000±0.000^{a}	4.007±0.007^{b}	0.000±0.000^{a}	8.010±0.006^{c}	0.000±0.000^{a}	10.000±0.030^{d}	0.000±0.000^{a}	13.000±0.012^{f}	13.000±0.012^{f}	13.000±0.012^{f}
S. aureus	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	7.000±0.005^{c}	7.000±0.005^{c}	7.000±0.005^{c}
Pseu. aeruginosa	2.003±0.009^{b}	1.007±0.012^{b}	6.013±0.013^{c}	3.017±0.017^{c}	8.010±0.015^{d}	5.003±0.009^{d}	8.510±0.010^{e}	8.000±0.006^{f}	8.000±0.006^{f}	8.000±0.006^{f}

Key: AMS- A. muricata stem, AML- A. muricata leaf

Table 7. Minimum inhibitory concentration and minimum bactericidal concentration of *A. muricata* leaf and stem extracts

Isolates	Ethanol				Chloroform				Hot water				Coldwater			
	AMS		AML		AMS		AML		AMS		AML		AMS		AML	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
	(mg/mL)															
E. coli	300	400	0	0	50	100	50	100	50	100	50	100	400	400	400	400
S. pyogenes	100	200	0	0	100	200	200	400	100	200	200	400	100	200	0	0
P. mirabilis	0	0	0	0	50	100	0	0	50	100	400	400	400	400	200	400
K. pneumoniae	0	0	50	100	0	0	50	100	100	200	100	200	0	0	200	400
S. aureus	50	100	50	100	0	0	300	400	400	400	400	400	200	400	0	0
Pseu. aeruginosa	200	400	200	400	50	100	50	100	100	200	100	200	200	400	200	400

Key: AMS- A. muricata stem, AML- A. muricata leaf

saponins and cardiac glycosides as secondary metabolites. [1,17] reported that *A. muricata* is a source of various phytochemicals like Tannin, alkaloid, flavonoid, phenol and also justify the use of the plant as bactericidal agent for the treatment of so many diseases.

A. muricata stem also has an antibacterial effect on the test bacterial isolates. *A. muricata* stem is known to contain higher tannins than the leaves. Tannins are organic substances of diverse composition with pronounced astringent properties that promote the healing of wounds and inflamed mucous membranes [3]. Externally, the leaves of *A. muricata,* promote fast healing of wounds and prevent infections. *A. muricata* stem ethanolic extract rated best in antibacterial effectively compared to the other extraction solvent used. This work is in agreement with the work of [18] who in their study reported that methanol and aqueous extract of *A. muricata* inhibited all the tested organisms. Hot water extract of *A. muricata* stem was able to inhibit *K. pneumoniae* while it was resistant to the other extraction solvent used. *Proteus mirabilis* which was resistant to all the antibiotics was sensitive to the higher concentration of hot water and chloroform extract of *A. muricata* stem. [6] reported that *Annona muricata* possesses anti-inflammatory and anti-bacterial effects.

Ethanol extract of the test plants from this study only has antibacterial activity at higher concentration. This is in contrast to the report of [19] that ethanolic and methanolic extract of *A. muricata L.* shows significant antibacterial activity against bacteria. It is also in contrast with [20] who reported that ethanol extract of *A. muricata* L. leaves was screened for its anti-microbial activity against the five different Gram +ve and Gram –ve bacteria species in agar disc diffusion method. *A. muricata* hot water extracts were able to record zones across the various concentrations for all the extracts. Hot water extract of the stem part of the plant has lower MIC/MBC as compared to the leaf extracts of the plant.

In comparison, the extracts of *A. muricata* stem had better antibacterial activity on the multiple resistant clinical isolates used in this study than the *A. muricata* leaf extracts using the same extraction solvent.

5. CONCLUSION

This study has shown ethanol as the best extraction solvent for maximum extract yield also, Hot water and chloroform extract as best for antibacterial activities. The effectivity of the extracts is corresponding to the increase in concentration. There is a higher possibility of using extracts of *A. muricata* L (Stem and leaf) as antibiotic sources to control multiple antibiotic resistance in clinical isolates.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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