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# Antibacterial Screening of Leaves Extracts of Annona muricata (Annonaceae) and Jatropha tanjorensis (Euphorbiaceae) against Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa

N. E. Ewa-Udu <sup>a</sup>, F. C. Nwanebu <sup>a\*</sup>, H. O. Stanley <sup>b</sup> and I. W. Okereke <sup>c</sup>

<sup>a</sup> Department of Microbiology, Madonna University, Elele, Rivers State, Nigeria.
<sup>b</sup> Department of Microbiology, University of Port Harcourt, Rivers State, Nigeria.
<sup>c</sup> Department of Oral Pathology and Oral Medicine, University of Nigeria, Enugu State, Nigeria.

#### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### ABSTRACT

The crude extracts of *Annona muricata* and *Jatropha tanjorensis* leaves were investigated with the aim of determining the antibacterial activity, qualitative and quantitative properties, the best solvent used for extraction, the most active ingredients and the organism that is most susceptible to them. Ethanol, petroleum ether and water (warm) were used as solvents. Agar well diffusion method was used for the susceptibility testing of extracts against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with ciprofloxacine as positive control and sterile water as negative control. Ethanol and petroleum ether extracts of the plant, either alone or in combination, showed activities against test organisms. *P. aeruginosa* was more susceptible to ethanolic extract of *A. muricata* extract with 11.33±0.33 mm zone of inhibition while *E. coli* was the least susceptible with 9.83 mm. *E. coli* was more susceptible to ethanolic extract of *J. tanjorensis* with 10.0±0.00 mm zone of inhibition while *P. aeruginosa* was the least susceptible with 9.0±0.0 mm diameter. Using petroleum ether, *E. coli* was the most susceptible to *A. muricata* extract with 7.33±0.33 while *S. aureus* was the least susceptible with 7.00±0.58 mm diameter. For *J. tanjorensis* petroleum ether

\*Corresponding author: Email: nwannekakc@gmail.com;

extract, *E. coli* was the least susceptible with 7.33.0±0.33 mm zone of inhibition while *S. aureus* was the most susceptible with 8.0±0.0.58 mm diameter. The combination of petroleum ether extracts of both plants gave zones of inhibition of 7.67±0.67 mm and 8.33±0.67 mm for *E. coli* and *S. aureus* respectively. The combination of ethanolic extracts of both plants gave zones of inhibition of 14.33±0.67 mm, 12.60±0.6 mm and 7.67±0.33 mm *E. coli*, *S. aureus* and *P. aeruginosa* respectively, which suggest a synergistic effect. The minimal inhibitory concentration of the extracts against test organisms ranged between 25 mg/mL and 100 mg/mL while the minimal bactericidal concentration ranged between 50 mg/mL and 100 mg/mL. This study reveals that the ethanol and petroleum ether extracts of *A. muricata* and *J. tanjorensis* have antibacterial effect on *E. coli*, *S. aureus* and *P. aeruginosa*.

Keywords: Annona muricata; Jatropha tanjorensis; antimicrobial; synergistic effect.

### 1. INTRODUCTION

Plants are considered natural repository of products which serve as food and medicine for man [1]. They have proven to be useful for providing chemical clues for the design and synthesis of modern drugs [2-4]. Interest in plant materials as medicinal agents are based on the presence of phytochemicals that have been proven to be efficacious in mitigating undesirable health outcomes in addition to being less toxic compared to synthetic drugs [5-7]. The most compelling reason for a second look at plants as natural remedies, stem from the rising cases of drug resistance [8].

Medicinal plants would be the unsurpassed sustainable source for a variety of drugs in the future [9]. A large proportion of the world's population relies on traditional medicine for their primary healthcare needs [9]. The plant kingdom offers a wide range of medicinal plants [10]. Plants such as Annona muricata and Jatropha tanjorensisi are among plant with evidence from ethnomedicine as suitable for the treatment of ailments those caused including microorganisms [11,12]. The J. tanjorensisi, a member of the 'Euphorbiaceae family, popularly referred to as 'Hospital Too Far' by the local folks in different parts of Nigeria because it is believed to be handy medicine [13-14]. Leaves of J. tanjorensisi are believed to be effective in the treatment of anaemia, diabetes cardiovascular diseases [13]. A. muricata, a member of the 'Annonaceae' family commonly called magic tree and its fruit, soursop. All parts of the plant are medicinal and have been reported to inhibit the growth of carcinogenic tissues and bacteria, and also possess antidiabetic. antihypertensive. analgesic, antiinflammatory and antioxidative potentials [15,16].

secondary metabolites Phytochemicals are synthesized by plant and they compounds such as steroids, phenolic, alkaloids, flavonoids, terpenoids, saponins and tannins. compounds phenol, tannins terpenoids are proven antimicrobial agents against clinical and non-clinical isolates [13-15,17]. The interest in plant derived antimicrobial compounds in medicine is because they deliver desired benefits without the side effects usually antimicrobial associated with synthetic compounds [15].

Phytomedicine has received wide reception among proponents of alternative medicines and pharmacological studies have been carried out on many medicinal plants but there still exist the problem of insufficient data regarding their efficacies [18]. This study aim to ascertain the antibacterial effect of *A. muricata* and *J. tanjorensis* extracts against three clinical isolates, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

### 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Fresh healthy leaves of A. muricata and J. tanjorensis were collected from pharmacognocy garden in Madonna University, Elele campus and Elder Ewa-udu's compound, Afikpo town in Afikpo North local government area of Ebonyi State and properly authenticated Pharmacognocy Department of Madonna University. The leaves were hand plucked aseptically and cleaned from debris using tap water and then rinsed in sterile distilled water. The leaves were oven-dried at 45°C temperature for 15 minutes. The dried leaves were grind to powder using a domestic blender. Powdered samples were weighed and stored in air-tight amber coloured glass containers, preparatory to extraction and further bioassay as per the method of Daniyan and Muhammad [19].

### 2.2 Preparation of the Leaf Extracts

The powdered material was extracted successively with water, petroleum ether and ethanol in increasing order of their polarity. Extraction followed the method of Danivan and Muhammad [19] with modification. Powdered material of A. muricata and J. tanjorensis leaves weighing 60.07g were introduced into extraction chamber of sohxlet extractor (Buchi E-800) and extraction done for 48hours with temperature maintained at 45°C for petroleum ether solvent, 70°C with ethanol solvent and at room temperature for 24hours with distilled water. The extracts produced were concentrated to dryness on water bath and then weighed.

### 2.3 Phytochemical Screening

Phytochemical screening was carried out in Pharmacognocy Laboratory Madonna University, Elele campus. Presence of phytochemicals was confirmed and quantified following methods described by Ezeonu and Ejikeme [20].

### 2.4 Test isolates

E. coli, P. aeruginosa and S. aureus were obtained from patients attending Madonna University Teaching Hospital, Elele and identified on the bases of their 16S rRNA sequences as described by Briggs et al. [21].

### 2.5 Antibacterial Susceptibility of Test Organisms to *A. muricata* Leaf and *J. tajorensis* Leaf Extracts

Standardization of the test microorganisms was done from the slant culture of the identified microorganisms (*S. aureus*, *E. coli* and *P. aeruginosa*). A colony was suspended with a sterile wire loop into a sterile Bijou bottle containing sterile distilled water and the opacity was then matched with that of 0.5Mcfarland turbidity standard, corresponding to 10<sup>8</sup> CFU/mL.

Agar well diffusion method as described by Ewa-Udu et al. [22] with modification, was used to carry out the antimicrobial susceptibility testing. 0.1g of plant extracts was dissolved in 1mL of 10% DMSO to get a stock concentration (100mg/mL). Ciprofloxacine (30mg/mL) was used as positive control. The plates (Petri dishes)

were incubated at 37° C for 24 hours. The diameter of the resulting Zones of inhibition were measured in millimeter (mm) through the base of the plates using a meter rule.

# 2.6 Determination of the Minimum Inhibitory Concentration (MIC) of *A. muricata* Leaf and *J. tajorensis* Leaf Extracts

The MIC was determined using tube dilution method as described by Ewa-Udu et al. [22]. The concentrations of extracts used were (100, mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL). Each concentration was inoculated with 0.1 mL of bacterial cell suspension and incubated at 37±2°C for 24 hours. Growth was indicated by cloudiness of the broth. The lowest concentration of the plant extracts that did not give any growth was taken as the minimum inhibitory concentration (MIC).

### 2.7 Statistical Analysis

One-way analysis of variance (ANOVA) was used to compare the mean differences between the zones of inhibition of the extracts and controls. Significant difference was taken at 95% level of confidence.

### 3. RESULTS

Table 1. shows quantitative phytochemical composition of leaf extracts. Flavonoid, tannin, alkaloids, glycosides, saponin, terpenoids and phenols were detected in ethanolic extracts of A. muricata and J. tajorensis. Flavonoid, alkaloids and terpenoids were detected in petroleum ether extracts of both plants. Tannin, alkaloids and carbohydrates were detected in water extract of A. muricata while flavonoid, alkaloids and carbohydrates were detected in water extract of J. tajorensis. Of all the phytochemicals detected in A. muricata leaf extracts, tannin had the least concentration of 0.03 mg/100g and glycosides had the highest concentration 57.18 mg/100g as detected in water and ethanol extracts respectively. For *J. tajorensis* leaf extracts, tannin had the least concentration of 2.02 mg/100g and glycosides had the highest concentration 59.35 mg/100g as detected in ethanol extracts.

### 3.1 Test Microorganisms

The test microorganisms are exact match with E. coli, P. aeruginosa and S. aureus, with

percentage similarity of 100%, on the bases of their 16S rRNA sequences.

### 3.2 Susceptibility of Test Organisms to Extracts

Table 2. shows test organisms were susceptible to extracts of A. muricata and J. tanjorensis. P. aeruginosa was more susceptible to ethanolic extract of A. muricata extract with 11.33±0.33 mm zone of inhibition while E. coli was the least susceptible with 9.83±0.17 diameter. E. coli was more susceptible to ethanolic extract of J. tanjorensis with 10.0±0.00 mm zone of inhibition while P. aeruginosa was the least susceptible with 9.0±0.0 mm diameter. Using petroleum ether, E. coli was the most susceptible to A. muricata extract with 7.33±0.33 while S. aureus was the least susceptible with 7.00±0.58 diameter. For J. tanjorensis petroleum ether extract, E. coli was the least susceptible with 7.33.0±0.33 mm zone of inhibition while S. aureus was the most susceptible with 8.0±0.0.58 mm diameter. The combination of petroleum ether extracts of both plants gave zones of inhibition of 7.67±0.67 mm and 8.33±0.67 for E. coli and S. aureus respectively. The combination

of ethanolic extracts of both plants gave zones of inhibition of 14.33±0.67 mm, 12.60±0.6 mm and 7.67 ±0.33 mm *E. coli, S. aureus* and *P. aeruginosa* respectively.

## 3.3 Minimum Inhibitory Concentrations (MIC) and Minimum Inhibitory Concentrations (MBC) of Extracts

The MICs of ethanolic extract of A. muricata against E. coli, S. aureus and P. aeruginosa were 25 mg/mL, 100 mg/mL and 50 mg/mL respectively. The MIC of petroleum ether extract of A. muricata against E. coli and S. aureus 100 mg/mL for both organisms. The MICs of ethanolic extract of J. tanjorensis against E. coli, S. aureus and P. aeruginosa were 50 mg/mL, 50 mg/mL and 25 mg/mL respectively. The MIC of petroleum ether extract of J. tanjorensis against E. coli and S. aureus was 100 mg/mL for both organisms. The MICs of the combination of ethanolic extracts of both plants against E. coli, S. aureus and P. aeruginosa were 25 mg/mL, 100 mg/mL and 25 mg/mL respectively. The MIC of petroleum ether extract of both plants against E. coli and S. aureus was 100 mg/mL (Table 3).

Table 1. Phytochemicals composition of A. muricata and J. tanjorensis leaf extracts

	A. muricata			J. tanjorensis			
	Ethanol	Pet. ether	Water	Ethanol	Pet. ether	Water	
Flavonoid	17.33	8.55	AB	2.84	13.07	19.38	
Tannin	22.33	AB	0.03	2.02	AB	AB	
Alkaloids	25.16	6.50	6.7	27.28	5.03	3.18	
Glycosides	57.18	AB	AB	59.35	AB	AB	
Saponin	19.08	AB	AB	7.53	AB	AB	
Terpenoids	13.33	44.21	AB	11.18	18.77	AB	
Phenois	51.23	AB	AB	22.18	AB	AB	
Carbohydrates	AB	AB	39.60	AB	AB	14.23	

Key: AB=Absent

Table 2. Susceptibility of test organisms to *A. muricata* and *J. tanjorensis* leaf extracts at 100 mg/mL

Plant	Organism	Pet. Ether	Ethanol	Water	Positive control
A. muricata	E. coli	7.33±0.33b	8.67±0.33b	$0.00\pm0.00$	31.0±0.00a
	S. aureus	7.00±0.58b	9.830±0.17b	$0.00\pm0.00$	33.0±0.00a
	P. aeruginosa	$0.00\pm0.00$	11.33±0.33c	$0.00\pm0.00$	28.0±0.00a
J. tanjorensis	E. coli	7.33±0.33b	10.0±0.00b	0.00±0.00	31.0±0.00a
	S. aureus	8.0±0.58b	9.83±0.17b	$0.00\pm0.00$	33.0±0.00a
	P. aeruginosa	$0.00\pm0.00$	9.0±0.00a	$0.00\pm0.00$	28.0±0.00a
A. muricata + J. tanjorensis	E. coli	7.67±0.67b	14.33±0.67c	0.00±0.00	31.0±0.00a
	S. aureus	8.33±0.67b	12.60±0.6c	$0.00\pm0.00$	33.0±0.00a

Row mean with same alphabet is not significantly different (\*P>0.05)

Table 3. Minimum inhibitory concentrations of ethanolic and petroleum extracts of *A. muricata* and *J. tajorensis* on test organisms in mg/mL

Plant	Solvent	Organism	100	50	25	12.5	6.5	MIC(mg/mL)
A. muricata	Ethanol	E. coli	-	-	-	+	+	25
		S. aureus	-	+	+	+	+	100
		P. aeruginosa	-	-	+	+	+	50
	Petroleum	E. coli	-	+	+	+	+	100
	ether							
		S. aureus	-	+	+	+	+	100
J. tajorensis	Ethanol	E. coli	-	-	+	+	+	50
		S. aureus	-	-	+	+	+	50
		P. aeruginosa	-	-	-	+	+	25
	Petroleum	E. coli	-	+	+	+	+	100
	ether							
		S. aureus	-	+	+	+	+	100
A. muricata + J. tajorensis	Ethanol	E. coli	-	-	-	+	+	25
or tayor or rore		S. aureus	_	+	+	+	+	100
		P. aeruginosa	-	-	-	+	+	25
	Petroleum ether	E. coli	-	+	+	+	+	100
		S. aureus	-	+	+	+	+	100

The MBCs of ethanolic extracts of *A. muricata* against *E. coli* and *S. aureus* were 50 mg/mL and 100 mg/mL respectively. The MBC of ethanolic extracts of *J tajorensis* on *P. aeruginosa* was 100 mg/mL. The MBCs of ethanolic extracts of both plants against *E. coli* and *S. aureus* was 100 mg/mL (Table 4).

### 4. DISCUSSION

In this study, the antibacterial activities of *A. muricata* and *J. tanjorensis* leaf extracts against *E. coli, S. aureus* and *P. aeruginosa* were evaluated. Flavonoid, tannin, alkaloids, glycosides, saponin, terpenoids and phenols were detected in ethanolic extracts of *A. muricata* and *J. tajorensis*. Flavonoid, tannin, alkaloids,

glycosides, saponin, terpenoids and phenols are common phytochemicals present in A. muricata and J. tanjorensis [13,15]. Fewer phytochemical (flavonoid, alkaloids and terpenoids) were detected when petroleum ether and water were used as solvent. Contrary to the present study, Solomon-Wisdom [23] reported the presence of Flavonoid, tannin, alkaloids, glycosides, saponin, terpenoids and phenols in both methanolic and aqueous extracts of Α. muricata. antimicrobial properties or any bioactive function of medicinal plants, can be attributed to the presence and quantity of phytochemicals [2,20]. According to Coria-Tellez [16] antimicrobial properties of A. muricata leaf extracts are as a result of their alkaloids flavonoids, tannins and terpenoids contents.

Table 4. Minimum Bactericidal Concentrations (MBC) of ethanolic extracts of *A. muricata* and *J tajorensis* on test organisms in mg/mL

Plant	Solvent	Organism	100	50	25	MBC(mg/mL)
A. muricata	Ethanol	E. coli	-	-	+	50
		S. aureus	-	+	+	100
J. tajorensis	Ethanol	P. aeruginosa	-	-	+	50
A. muricata+ J. tajorensis	Ethanol	E. coli	-	+	+	100
-		S. aureus	-	+	+	100

Ethanol and petroleum ether extracts of A. muricata, either alone or in combination with J. taniorensis, showed activities against E. coli and S. aureus. Vinothini and Growther [24] in their study reported extracts of A. muricata were also active E. coli and S. aureus, among other bacteria and fungi. In the present study, ethanolic extract of A. muricata showed the highest antibacterial activity against P. aeruginosa with 11.33±0.33 mm zone of inhibition, followed by S. aureus with 9.830 mm, while E. coli was the least susceptible with 9.83±0.17 mm. Using petroleum ether, E. coli was the most susceptible to A. muricata extract with 7.33±0.33 while S. aureus was the least susceptible with 7.00±0.58 diameter. The zones of inhibition of extracts obtained from ethanol and petroleum ether were not significantly different (p>0.05). However, when compared to standard antibiotic (30 mg/mL of ciprofloxacine), the zones of inhibition of extracts showed significant difference (p<0.05). Solomon-Wisdom et al. [23], reported that methanolic extract of *A. muricata* had high antibacterial activity towards S. aureus, with 20.5 mm and E. coli with 16.5 mm, at 400 mg/mL and 200 mg/mL MICs respectively.

Ethanol and petroleum ether extracts of J. tanjorensis showed activities against all test organisms. E. coli was more susceptible to ethanolic extract of J. tanjorensis with 10.0±0.00 mm zone of inhibition and the less susceptible with 7.33.0±0.33 mm petroleum ether extract. P. aeruginosa was also susceptible to ethanolic extract of J. tanjorensis with 9.0±0.0 mm diameter but not to petroleum ether extract. J. tanjorensis petroleum ether extract was active against S. aureus with 8.0±0.0.58 mm diameter zone of inhibition. The combination of petroleum ether extracts of both plants gave zones of inhibition of 7.67±0.67 mm and 8.33±0.67 for E. coli and S. aureus respectively. The zones of inhibition of extracts obtained from ethanol and petroleum ether were not significantly different (p>0.05). However, the combination of ethanolic extracts of both plants gave zones of inhibition of 14.33±0.67 mm, 12.60±0.6 mm and 7.67 ±0.33 mm E. coli, S. aureus and P. aeruginosa respectively, which is significantly different (p<0.05) from zones of inhibition from single extract. Oboh and Masodje et al. [25] also reported that S. aureus and E. coli were susceptible to ethanol extract of J. tanjorensis.

The MICs of *A. muricata* extracts against *E. coli*, *S. aureus* and *P. aeruginosa* ranged between 25 mg/mL and 100 mg/mL and 50 mg/mL. Similarly,

the MICs of *J. tanjorensis* extract against test organisms ranged between 25 mg/mL and 100 mg/mL. The effective concentrations of extracts in this study are higher relative to other reports in literatures, as at lower concentrations (12.5 and 6.25 mg/mL), there was no activity observed. da [26] reviewed literatures antimicrobial activities of A. muricata and reported that MIC ranged between 0.156 mg/mL 1.024 mg/mL against S. aureus, and 0.256 mg/mL and 1.024 mg/mL against E. coli. However, Solomon-Wisdom et al. [23], reported higher MICs for A. muricata, 400 mg/mL and 200 mg/mL, against S. aureus and E. coli MICs respectively. Although both ethanol petroleum ether extracts of both plants showed activities against test organisms, only ethanolic extracts were bactericidal.

#### 5. CONCLUSION

The antibacterial properties of *A. muricata* and *J. tanjorensis* were demonstrated in this study. Both plants showed activities against *E. coli*, *S. aureus* and *P aeruginosa*, and act synergistically against *E. coli* and *S. aureus*.

#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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