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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*

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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, combination properties. Ethanol, petroleum ether and water (distilled) 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 Dimethyl 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±0.33mm 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±0.33mm while *S. aureus* was the least susceptible with 7.00±0.58mm 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 mm for *E.*

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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 including those caused by microorganisms [11,12]. The *J. tanjorensisi*, a member of the 'Euphorbiaceae family, is 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 and cardiovascular diseases [13]. *A. muricata*, a member of the 'Annonaceae' family is 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].

Phytochemicals are secondary metabolites synthesized by plant and they include compounds such as steroids, phenolic, alkaloids, flavonoids, terpenoids, saponins and tannins. Plants compounds phenol, tannins and 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 associated with synthetic antimicrobial 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 locally collected from Sir Charlse, Owerri-road,Elele,Rivers state and properly authenticated by 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 air-dried for 3days before oven-drying at 40^{o}C temperature. 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 ethanol, petroleum ether and water in increasing order of their polarity. Extraction followed the method of Daniyan and Muhammad [19] with modification. Powdered material of *A. muricata* and *J. tanjorensis* leaves weighing 100g were introduced into extraction

chamber of sohxlet extractor (Buchi E-800) and extraction done for 48hours with temperature maintained at 45^{o}C for petroleum ether solvent, 70^{o}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

Clinical *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^{8} CFU/mL.

Agar well diffusion method as described by Esimone et al. [22] with modification, was used to carry out the antimicrobial susceptibility testing. 1g of plant extracts was dissolved in 10mL 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. (Ghamba,2014)

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

The MIC was determined using tube dilution method as described by Chikezie, [23], [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°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).MBC was determined from the tubes that had no growth. The minimum concentration at which the plates showed no microbial growth was regarded as the MBC.

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 separated by Duncan Multiple Range test (DMRT).All results at were expressed as mean±SD, while statistical decisions were taken at 95% level of significance. Statistical Packages for Social Sciences (SPSS) version 26 package

3. RESULTS

Table 1 shows quantitative phytochemicalcomposition 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
Phenols	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±0.00	31.0±0.00a
	S. aureus	7.00±0.58b	9.830±0.17b	0.00±0.00	33.0±0.00a
	P. aeruginosa	0.00±0.00	11.33±0.33c	0.00±0.00	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±0.00	33.0±0.00a
	P. aeruginosa	0.00±0.00	9.0±0.00a	0.00±0.00	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±0.00	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 ether	*E. coli*	-	+	+	+	+	100
		S. aureus	-	+	+	+	+	100
J. tajorensis	Ethanol	*E. coli*	-	-	+	+	+	50
		S. aureus	-	-	+	+	+	50
		P. aeruginosa	-	-	-	+	+	25
	Petroleum ether	*E. coli*	-	+	+	+	+	100
		S. aureus	-	+	+	+	+	100
A. muricata* + *J. tajorensis	Ethanol	*E. coli*	-	-	-	+	+	25
		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 [24] reported the presence of Flavonoid, tannin, alkaloids, glycosides, saponin, terpenoids and phenols in both methanolic and aqueous extracts of *A. muricata.* The 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. tanjorensis*, showed activities against *E. coli* and *S. aureus*. Vinothini and Growther [25] 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. [24], 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. [26] 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 Silva [27] reviewed literatures on the antimicrobial activities of *A. muricata* and reported that MIC ranged between 0.156 mg/mL and 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. [24], reported higher MICs for *A. muricata*, 400 mg/mL and 200 mg/mL, against *S. aureus* and *E. coli* MICs respectively. .Although both ethanol and 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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