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

**Original Research Article**


## 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 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 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, 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



**Introduction**

Plants are considered natural repository of products which serve as food and medicine for man of human infections [1]. They have proven to be useful for providing chemical clues for the design and synthesis of modern drugs [4-6]. 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 [7-9]. The most compelling reason for second look at plants as natural remedies, stem from the rising cases of drug resistance [10].

Medicinal plants would be the unsurpassed sustainable source for a variety of drugs in the future [11]. A large proportion of the world's population relies on traditional medicine for their primary healthcare needs [11]. The plant kingdom offers a wide range of medicinal plants [12]. 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. 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*, is a member of the 'Annonaceae' family is commonly called magic tree and its fruit, soursop. All parts of the plant are medicinal 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.*

**Materials and Methods**

**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 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 oven-dried at 45$^{o}$C temperature for 15 minutes. The dried leaves were grind to powder using a domestic blender. Powdered samples were measured and stored in air-tight amber coloured glass containers, preparatory to extraction and further bioassay as per the method of Daniyan and Muhammad [19].

**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 Daniyan 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$^{o}$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.

**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].

**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].

**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 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.

**Determination of the minimum inhibitory concentration (MIC) of *Annona muricata* leaf and *Jatropha 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).

**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.

**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.

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**

**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.

**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.

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)

**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).

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).

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*+ | Ethanol | *E. coli* | - | - | - | + | + | 25 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *J. tajorensis* | | | | | | | |
| | | *S. aureus* | - | + | + | + | + | 100 |
| | | *P. aeruginosa* | - | - | - | + | + | 25 |
| | Petroleum ether | *E. coli* | - | + | + | + | + | 100 |
| | | *S. aureus* | - | + | + | + | + | 100 |

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 |



**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 *A. muricata.* The antimicrobial properties or any bioactive function of medicinal plants, can be attributed to the presence and quantity of phytochemicals [4,20]. According to Coria-Tellez [16] antimicrobial properties of *A. muricata* leaf extracts are as a result of their alkaloids flavonoids, tannins and terpenoids contents.

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 [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 Silva [26] 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. [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 and petroleum ether extracts of both plants showed activities against test organisms, only ethanolic extracts were bactericidal.

## 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*.

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