

In-Vitro Antibacterial Assay of *Annona muricata* Hydroethanolic Extracts

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ABSTRACT

Annona muricata (Soursop) plant is well known to be therapeutic due to its phytochemical constituents. The antibacterial outcome of the hydro-ethanolic extracts of *Annona muricata* leaves, root and stem bark were evaluated on standard strains of *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 29522), *Pseudomonas aeruginosa* (ATCC 27853) and *Enterococcus faecalis* (ATCC 29212) using agar well diffusion method. The extracts inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis* at varied concentrations. The root extract at 25.88 ± 0.22 mm on *E. coli* was observed to have the highest zone of inhibition while the leaves extract at 11.67 ± 6.01 mm on *E. coli* revealed the least zone of inhibition. The quantitative and qualitative phytochemical assay of the leaves, stem bark and root affirmed the presence of tannins, saponins, alkaloids, flavonoids, and carbohydrate in varying percentages. The highest quantity of flavonoids and saponin were observed in the stem bark respectively at 15.57 % and 131.49 % while the leaf samples showed highest quantity for alkaloids, tannin, and carbohydrate at 10.00 % 25.43 % and 11.26 % respectively. The presence of the phytochemical compounds in varying quantities may have contributed to the antibacterial activities of *Annona muricata*. The study further affirms the potency of *A. muricata* parts in pharmacognosy and therapeutic applications.

Keywords: *Annona muricata*, bacteria, extraction, hydroethanolic, in-vitro, phytochemicals

Introduction

Herbal medicines have continued to play significant impacts in healthcare delivery. All over the world, different communities constantly explore medicinal plants for therapeutic purposes. It has become necessary to scientifically authenticate more medicinally useful products to compete with fast growing pharmaceutical market (Mishra *et al.*, 2013). A better understanding of the plant derived medicine depends mainly on two criteria that goes hand in hand. One criterion

involves the proof to show that the formulated medicine does what it is claimed to do and the other is the identification of the active compound in such plant (Rout *et al.*, 2009).

Annona muricata Linn. is a member of the family of custard apple trees called *Annonaceae*. *A. muricata* also known as sour-sop, sir sak or guanabana has been named as popular fruit tree that is cultivated throughout the tropical regions of the world (Gajalakshmi *et al.*, 2012). A wide assemblage of ethno-medicinal activities is

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attributed to different parts of *A. muricata*, and communities around Africa and South America extensively utilize this plant in their folk medicine (Moghadamtousi *et al.*, 2015). The phytochemicals observed in the plant possess antioxidant activities, which prevent or can be administered in the treatment of many diseases including cancer (Gavamukulya *et al.*, 2017). The plant is also known to have good immune modulatory properties which act by stimulating both non-specific and specific immunity (Galjalakshmi *et al.*, 2012). The leave, fruit, root, and bark extracts have been assayed against clinical fungi (Ebabhi *et al.*, 2018). The leaves against oral pathogens (Pai *et al.*, 2016) and various antibacterial reports of the various parts of the plant (Iyanda-Joel *et al.*, 2019; da Silva *et al.*, 2022). As reported by Barbalho *et al.* (2012), the decoction of the leaves contains parasiticide, anti-rheumatism and neuralgic effects when utilized for internal purposes while the cooked leaves when applied topically can fight rheumatism and abscesses. Gavamukulya *et al.* (2017) had earlier reported that more than 200 chemical compounds have been identified and isolated from this plant, the most important being alkaloids, phenols and acetogenins. This study therefore evaluated the efficacy of the combination of hydroethanolic extract of the leaves, root, and stem bark of *Annona muricata* against *Enterococcus faecalis* *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* which are known human pathogens.

Materials and Methods

Collection and identification of *Annona muricata* parts

Fresh *Annona muricata* parts (leaves, stem bark and roots) were collected from the Forestry Research Institute of Nigeria (FRIN), Ibadan. These were transported in Ziploc transparent bags to the Lagos University Herbarium for proper identification and authentication. Voucher specimen leaf (LUH 9026), stem bark (LUH 9027)

and root (LUH 9028) were deposited at the herbarium.

Plant extraction

The plant samples weighing one kilogram each were washed thoroughly under a running tap in order to remove sand and debris. The leaves were dried in-between sterile blotting paper to remove water ruminant, cut into pieces, air dried for fourteen days in a room and with the aid of a hammer mill they were pulverized into fine powder. These were stored in airtight containers. *A. muricata* plant samples were extracted using 500 g of each powered sample with 2:1 v/v hydro-ethanol for 48 h period in a Soxhlet apparatus (Aldrich® Soxhlet Extraction Apparatus, Z556203, St. Louis, MO, USA). The extracts were each concentrated using a rotary evaporator under reduced pressure and temperature (40 °C). The filtrates were brought to dryness using water bath and stored in the refrigerator for further use. This was carried out using a modified procedure as described by Boakye-Gyasi *et al.* (2017).

Collection of organisms

Standard cultures of clinical isolates which include *Escherichia coli* (ATCC 2952), *Staphylococcus aureus* (ATCC 29523), *Pseudomonas aeruginosa* (ATCC 2785), *Enterococcus faecalis* (ATCC 29212) were collected from the Microbiology section of the Lagos University Teaching Hospital (LUTH), Lagos-Nigeria and maintained on nutrient agar slants at 4 °C. The isolates were confirmed using standard techniques as described by Fawole and Oso, (2004).

Standardization of inoculum

This was carried out using a slightly modified method of Cheesbrough (2006) whereby the pure culture of each organism was obtained by inoculating on freshly prepared nutrient agar plates and incubated at 37 °C for 24 h. An inoculum from the cultured plate was aseptically dispensed into 20 ml of freshly prepared nutrient broth and incubated

for 2-3 h at 37 °C to standardize to 0.5 McFarland standard.

Antibacterial activity of the crude extracts

In vitro screening for antibacterial activity was carried out using the agar well diffusion method of Valgas *et al.* (2007). Dilute test organism (1.5 ml) from the standardized broth was added into the prepared culture media, shaken gently and allowed to mix together. The mixture of the culture media and the test organism were poured accordingly into different Petri dishes and allowed to solidify. After 5 minutes, two uniformly spaced 5 mm wells were bored using a sterilized cork borer. The experimental setup was in triplicate for each plant part.

A sterile needle and syringe of different range was used to introduce the crude extracts at different concentration into each hole for different plate. The concentrations used were 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml. A conventional antimicrobial agent (Ciprofloxacin) was used as a positive control while ethanol was used as negative control. All the plates were incubated at 37°C. The antibacterial activity was determined by measuring the inhibition zone diameters (mm) after 24 hr for 3 days for each concentrate of extract and were recorded. The antibacterial testing results were statistically analyzed.

Phytochemical screening of plant extracts

The extract of *Annona muricata* plant parts (leave, stem bark and root) were screened for the presence of alkaloid, saponins, anthraquinone, steroids, tannin, flavonoid, reducing sugars, cardiac glycosides, protein, phenol and triterpenoids according to standard methods (Odebiyi and Sofowora, 1978; Sofowora, 1993; Harborne, 1998; Onwukeame *et al.*, 2007).

Statistical analysis

Results were analyzed using SPSS 20. The data was expressed as mean±SD and analyzed using

ANOVA one way analysis of variance followed by Duncan's Multiple Range Test for mean comparison.

Results

Antibacterial assay

The result of antibacterial activity of *Annona muricata* plant part (leave, stem bark and root) are presented in Figures 1- 3. The hydroethanolic leaf extract at 50mg/ml, 150mg/ml, and 200mg/ml had no significant effect on *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis* while *Pseudomonas aeruginosa* was not affected by the leaf extract (Figure 1). The leaf extract at concentration of 100 mg/ml however showed inhibitory effect on *E. coli* with a mean value of 11.67±6.01 mm. The stem bark extract inhibited the growth of *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis* at various concentration. The highest zone of inhibition at 21.67±3.33 mm was observed on *E. coli* while the least was 12.55±1.31 mm on *E. faecalis*. The zones of inhibition were comparable with that of the positive control (Figure 2). The root extract only showed significant effect on *E. coli* at 150 mg/ml and 200 mg/ml with mean zone of inhibition respectively at 21.99±1.33 mm and 25.88±0.22 mm compared to the conventional antimicrobial agent (Figure 3).

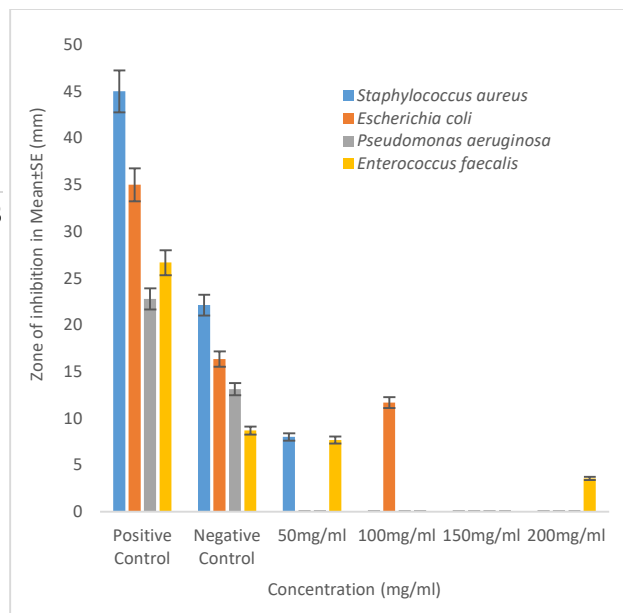


Figure 1: Antibacterial activity of hydroethanolic extract of *Annona muricata* leaves

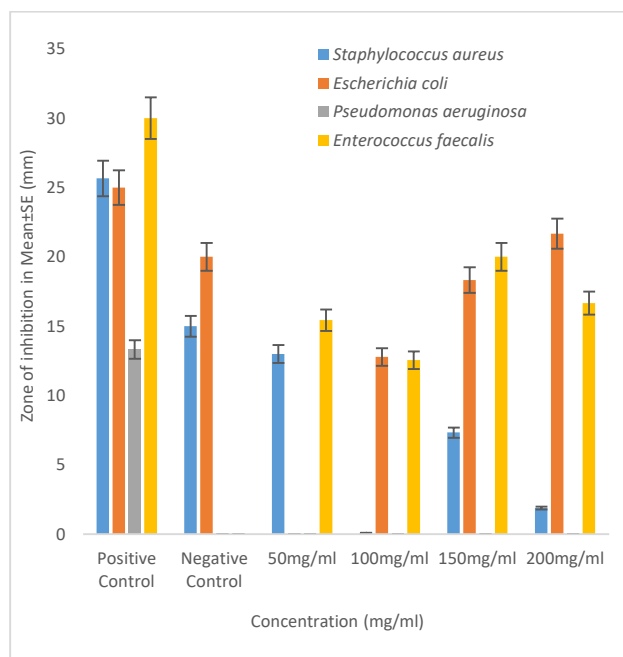


Figure 2: Antibacterial activity of hydroethanolic extract of *Annona muricata* stem bark

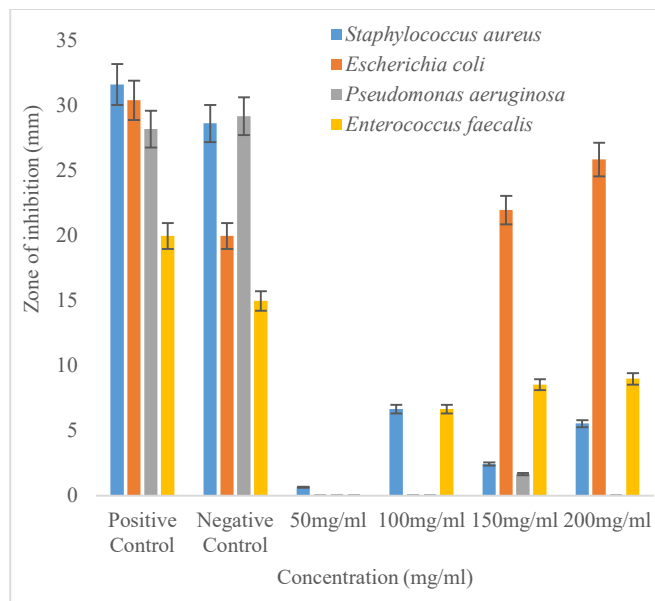


Figure 3: Antibacterial activity of hydroethanolic extract of *Annona muricata* root

Phytochemical screening

The quantitative and qualitative phytochemical screening of *Annona muricata* leaf, stem bark and root revealed the presence of Tannins, Saponins, Alkaloids, Flavonoids and Carbohydrate in varying percentages. This result is presented in Table 1 for the qualitative analysis. Saponins was observed to have the highest quantity in percentage across the plant parts while carbohydrate percentage was found to be lowest across the plant parts. The quantitative analysis is presented in Table.2.

Table 1: Phytochemical component of *Annona muricata* leave, stem bark and root

Phytochemicals	<i>Annona muricata</i>		
	Leave	Stem bark	Root
Tannins	+	+	+
Phlobtannin	-	-	-
Saponin	+	+	+
Flavonoid	+	+	+
Steroid	-	-	-
Terpenoid	-	-	-
Alkaloid	-	+	-
Carbohydrate	+	+	+
Phenol	-	-	-

Negative (-): absent Positive (+): present

Table 2: Percentage component of compound present in *Annona muricata* leaves, stem bark and root

<i>Annona muricata</i> parts	Phytochemicals				
	Flavonoid	Saponin	Alkaloids	Tannin	Carbohydrate
Stem bark	15.57%	131.49%	6.04%	15.56%	2.42%
Root	7.85%	126.91%	4.39%	7.01%	6.47%
Leaf	7.45%	123.83%	10.00%	25.43%	11.26%

Discussion

This present study on the hydroethanolic extract of *Annona muricata* stem bark, leaves and root on some clinical bacterial isolates showed significant antibacterial activities against *Escherichia coli* particularly the stem bark and root extracts. Minimal effect at different concentrations of the extracts was observed on the other isolates assayed. Using different solvents (hot water, cold water, ethanol and chloroform) for extraction, Agunloye and Onifade (2020) noted that *A. muricata* leaf extract had an inhibitory effect on bacterial isolates. Although, *E. coli* (Gram negative) was susceptible to the extracts, *Pseudomonas aeruginosa* was observed to be resistant to the various plant part extracts at the different concentrations. This could still be attributed to cell wall in gram negative bacteria that act as barrier for diffusion of the extracts into them thus, rendering them ineffective as reported in the findings of Viera *et al.* (2010) and Pai *et al.*, (2016). Kaczmarck (2020) also stated that due to the nature of the cell wall of Gram-negative bacteria, they are slowly affected by antibacterial agents. This is coupled with resistance of some bacteria to foreign bodies. In Gram-positive bacteria, the activity of tannins is rapid (Kaczmarck, 2020). The observed results in the current study could be attributed to the active constituents which includes tannin, Saponins, flavonoids and alkaloids present in the plant parts.

Antibacterial activity of the phytochemicals of the stem bark of *A. muricata* has previously been documented by Oloyede, *et al.* (2010). Kaczmarek (2020) attributed the antibacterial effectiveness of some phytochemicals like tannins to their ability to pass through the bacterial cell wall up to the internal membrane, interference with the metabolism of the cell, and as a result leading to their destruction. Several reports also have shown that Saponins which is also abundant in *A. muricata* act as antibacterial agents (Arabski *et al.*, 2012 and Khan *et al.*, 2018) However, the corollary to the findings is that the plant parts would serve a useful purpose in the treatment of infections associated with the test organisms. Plant based antimicrobials have enormous therapeutic potentials which can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu, 1993).

The result of the phytochemical screening shows that *A. muricata* is generally rich in phytochemicals which includes alkaloids, flavonoid, saponins, tannins, etc. which may justify their uses in ethnomedicine. Sofowora (2008) reported that *A. muricata* leaf has been found to be rich in phenolic compounds, flavonoids, tannins, alkaloids, saponins and cardiac glycosides as secondary metabolites. Ebabhi *et al.*, (2018) also reported that *A. muricata* is a source of various phytochemicals like alkaloid, flavonoid, phenols, tannins which lay credence to the wide use of this plant as antimicrobial agent for the treatment of many disease conditions. Sour sop pulp and drink contain appreciable amount of micronutrients and can be incorporated into the diets for the vulnerable groups to improve their nutritional status (Onyechi *et al.*, 2012).

Conclusion

The mode of extraction using hydro-ethanol as solvent for extraction in this study for antibacterial activity on clinical bacteria has not been exploited for broader comparative studies. It is difficult

drawing a conclusion as *A. muricata* is known to contain an array of bioactive components whose efficacies have variously been confirmed. However, it can be concluded from this study that *A. muricata* root, stem bark and leaves hydroethanolic extracts are potent against clinical agents like *Escherichia coli* and *Enterococcus faecalis*. Thus, further laying credence to the use of *A. muricata* plant parts in pharmacognosy and therapeutic studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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