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Full Length Research Article

PHYTOCHEMICALS AND ANTIBACTERIAL EFFICACY OF *ANNONA MURICATA* (SOURSOP) STEM BARK AND LEAF EXTRACTS AGAINST SOME CLINICAL BACTERIAL ISOLATES

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ABSTRACT
The threat of drug-resistant pathogens necessitates exploring plant-derived bioactive compounds to develop potent antibacterial solutions. This study determined the phytochemicals and antibacterial activities of *Annona muricata* (soursop) stem bark and leaf extracts against some clinical bacterial isolates. The phytochemicals and antibacterial activity of leaf and stem bark extracts of *Annona muricata* were analyzed following standard methods. Invitro susceptibility of bacteria isolates to the plant extracts was evaluated using the agar well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant extracts were also determined using the broth dilution method. The result of quantitative and qualitative phytochemical composition of the leaf and stem bark extracts of *Annona muricata* showed the presence of alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, glycosides, and anthraquinones. The quantitative phytochemical composition of the leave extract revealed the presence of total alkaloids content (577.07±2.19), total flavonoids content (22.37±1.40), total tannin content (0.69±0.12), total saponin content (3.90±0.06), and total phenolic content (3.51±0.26) for leaf aqueous extract. The findings of this study revealed that *Annona muricata* (soursop) stem bark and leaf extracts were active against clinical *Salmonella*, *Escherichia coli, and Staphylococcus aureus* isolates. The diameter of zones of inhibition observed was concentration dependent for both the stem bark and leaf extracts. Diameter of zones of inhibition ranged between 0.07 ± 0.033 to 8.07 ± 0.067 mm in leaf extract and 0.07 ± 0.033 to 3.39 ± 0.140 mm lowered in stem bark extracts. In conclusion, all *Annona muricata* extracts showed potential in preventing *Salmonella*, *E. coli*, and *S. aureus* infections, with the ethanolic extract demonstrating the strongest activity, highlighting the potential of plant secondary metabolites in controlling these infections.

Keywords: *Annona muricata*, phytochemical analysis, antioxidant, antibacterial, clinical bacterial isolates

INTRODUCTION

Annona muricata, also known as soursop because of the sweet and sour taste of its fruit. It is one of the interesting medicinal plants that can provide an alternate bactericidal agent (Mutakin *et al.,* 2022). It is an evergreen tree with broad leaves those blooms. The fruit's flavor is described as a blend of strawberries and apple, with a tart citrus flavor that contrasts with an underlying creamy texture reminiscent of coconut and banana. Its aroma is comparable to that of pineapple (Morton and Miami, 2018). In tropical and subtropical regions like Southeast Asia, South America, and the African rainforests, this plant is commonly farmed. According to Moghadamtousi *et al.* (2015), the plant yields edible fruit throughout the year and is used as a traditional remedy for bacterial infections, skin conditions, respiratory disorders, fever, diabetes, high blood pressure, and cancer. The actions of various portions of *A. muricata* vary. The leaves are used to treat cancer, headaches, sleeplessness, and cystitis; the fruit is used to treat diarrhea, arthritis, and neurological problems; and the seeds fight parasite infections. Acetogenin, alkaloids, and flavonoids are the primary active ingredients in *A. muricata* (Coria-Tellez *et al.,* 2018). Plants have been the main source of medicine since prehistoric times. Nature is always a great approach to illustrate the common occurrences of coexistence. Human diseases are treated with natural compounds derived from plants, animals, and minerals (Maurya *et al.,* 2021). Since ancient times, people have most likely thought about therapeutic plants. Early humans became more or less conscious of the properties of the plants around them as they recognized and used them for food, clothing, shelter, and fuel. Medicinal plants have emerged as one of the oldest fields in countries such as China, Greece, Egypt, and India (Jamshidikia *et al.,* 2018).

Bacterial infections have been a persistent and evolving challenge to public health worldwide. However, recent years have witnessed a concerning rise in the incidence of bacterial infections and the emergence of antibiotic resistance. Recent studies have shown an alarming increase in the prevalence of bacterial infections, with particular emphasis on antibiotic-resistant strains. Gram-negative bacteria, such as *Escherichia coli, Salmonella typhi*, and *Saccharomyces cerevisiae*, have exhibited multidrug resistance, limiting the effectiveness of conventional antibiotics (Gupta *et al.,* 2020). Similarly, gram-positive bacteria such as *Staphylococcus aureus, Streptococcus pneumoniae* and *Candida albicans* have emerged as challenging healthcare-associated pathogens (Tacconelli *et al.,* 2018). These resistant strains are associated with increased morbidity, mortality, healthcare costs, and prolonged hospital stays (Tacconelli *et al.,* 2018; Gupta *et al.,* 2020). These antibiotic-resistant bacteria constitute a serious threat to the public health in developed countries as well as in resource limited setting in developing countries (Ventola, 2015). Its prevalence is dangerously escalating worldwide leading healthcare practice towards a perilous era of post-antibiotics (WHO, 2014). Deaths attributable to antibiotic resistance have been estimated to be more than 700,000 annually and a recent analysis by the projects that if left unaddressed, antibiotic-resistant infections could lead to more than 39 million human deaths between 2025 and 2050 (World Bank, 2024). The mortality associated with infections by these

difficult to treat bacteria has been shown to be more than twice that of infections in patients with susceptible bacteria (Founou *et al.,* 2017). Studies conducted in many African countries have revealed a growing prevalence of Gram-negative bacteria resistant to commonly prescribed antibiotics (Kariuki and Dougan, 2014; Tadesse *et al.,* 2017). In Nigeria, the Nigeria Centres for Disease Control and Prevention (NCDC) has equally documented a high rate of resistance to the commonly used antibiotics (NCDC, 2017). There is the fear that the lack of effective antibiotics seriously threatens further progress in many areas of medicine such as in intensive care, transplant medicine, oncology and surgery. Despite the availability of numerous antibiotics in use today, infections are still common in human populations. Developing countries are worst hit by these infections for obvious reasons. In most cases, the potent antibiotics are expensive and are not so accessible to all. Hence the pressing need to identify more antibacterial agents that could help mitigate the proliferation of disease-causing bacteria.

A lot of research has been done on natural substances in an effort to find novel drugs (Chandra *et al.,* 2017). As a source of antibiotics, antineoplastics, analgesics, and cardioprotectives, among other things, plants have in fact been utilized as medicines for over 5000 years (Brown and Wright, 2016). (Chen *et al.,* 2015). Natural substances have been used by people to prevent diseases in the recent past (Newman and Cragg, 2016). In poor nations, between 70 and 90 percent of people still use traditional remedies made from plant extracts. Humans rely on secondary metabolites, which are the most potent and promising components of plants (Robinson and Zhang, 2011).

Importantly, over half of the medications that have received FDA approval are made from natural ingredients or their derivatives (Chavan *et al.,* 2018). Generally speaking, the relationship between matter and life is one of the interactions covered by natural products. Food, illnesses, poisons, antidotes, physiological and pathological theories, and biological theories pertaining to genetics and molecular biology. The secondary metabolites and their synthetic or semisynthetic derivatives have a multitude of potential applications due to these interactions (David *et al.,* 2015).

MATERIALS AND METHODS

Collection of Plant Materials

Fresh leaves and stem bark of *Annona muricata* were collected from Kaduna Polytechnic Quarters, Kaduna South Local Government Area Nigeria.

Identification and Processing

Annona muricata plant specimen was identified at the herbarium of the Department of Biological Sciences, Nigerian Defence Academy and deposited with voucher number NDA/BIOH 2024-45 for the leaf and NDA/BIOH 2024-11 for the stem bark.

The leaves and stem bark of the plant were washed and dried under shade for 28 days. The dried samples were then ground using laboratory mortar and pestle. The powdered sample were stored in closed plastic container for further analysis.

Bacterial Culture Collection

The clinical isolates of *Salmonella*, *Escherichia coli, and Staphylococcus aureus* used in this study were collected from the Yusuf Dantsoho Memorial Hospital, Kaduna State. All samples were collected in nutrient agar slants, labelled and placed in a cold box and transported to the post graduate laboratory of the Biochemistry Department, Kaduna State University. Samples were incubated at 37 °C for 24 h.

The isolates were revived from the nutrient agar slant by subculturing them on nutrient agar and incubating them for 24 h at 37°C. Then, the biochemical test carried out was Gram's reaction, Morphology, Indole, Methyl Red, Voges-Proskauer, Citrate, Catalase, Coagulase, Mobility and Oxidase.

Extract Preparation

Two hundred grams (200g) of each of the pulverized leaf and stem bark materials were cold extracted separately by maceration in 1.5 L of distilled water, ethanol and hexane and allowed to stand for 48 h with intermittent shaking. The extracts were filtered through Whatman No. 1 paper and the resultant filtrates were then evaporated using hot-air oven at 40 ^{o}C for 72 h. Afterward, it was lyophilized under vacuum at -80 ^{o}C and pressure of 0.06 mbar. The dried extracts were then stored at -20 ^{o}C for further analysis (Kong *et al.,* 2014).

Qualitative Phytochemical Analysis

The phytochemical analysis was carried out following the procedures described by Harborne, (1992). The secondary metabolites screened include:

Alkaloids

To test for alkaloid, 0.1 mg of each plant extracts were measured into 6 ml of diluted HCl. The mixtures were boiled at 85 °C, allowed to cool at 40 °C and filtered using a small 1inch sieve. The presence of alkaloids in filtrates were tested using Dragendorff's reagent, Meyer's reagent and Wagner's reagent each in separate container.

Terpenoid

In order to test for terpenoid, 1 ml of each plant extracts were dispensed into 10 ml of deionized water before adding 3 drops of ferric chloride in the solution.

Flavonoids

The flavonoid composition was determined by boiling 0.2 mg of each extract at 40°C in 10 ml of ethyl acetate for 3 minutes. It was cooled at room temperature and filtered through 1 inch sieve. Then 4 ml of the filtrate was mixed with 1ml of dilute ammonia solution and shook vigorously for about 1 min.

Saponins

Presence of saponins were determined by adding 5 ml of each plant extract into 20 ml of deionized water and shook vigorously.

Phenols

A small amount of the plant extract was taken with 1 mL of water in a test tube and 1 to 2 drops of Iron III chloride ($FeCl_3$) was added.

Tannins

A 0.5g of the powdered plant extract was mixed in test tube containing 20ml of distilled water. It was heated at 40°C for 5 mins, then filtered using 2 inches sieve and then 0.1 % $FeCl_3$ was added.

Glycosides

Glycosides was determined by adding 1 ml of concentrated H_2SO_4 to 5 ml of each of the plant extract. Afterward, 2 ml of glacial CH_3CO_2H containing 1 drop of $FeCl_3$ was then added to the mixture.

Quantitative Phytochemical Analysis

The phytochemicals constituents of the leaves and stem bark extracts of *Annona muricata* were determined following standard procedures as described below.

Determination of total phenols

Hundred milligram (100 mg) of the plant extracts were dissolved in 100 ml of triple distilled water (TDW). A 1 ml of the resultant solution was then transferred to a test tube, and 0.5 ml 2 N of the Folin Ciocalteu reagent was added. Afterward, 1.5 ml 20 % of Na_2CO_3 solution was then added and the volume was made up to 8 ml with TDW followed by vigorous shaking and finally allowed to stand for 2 h. The absorbance of the solution was taken at 765 nm. The values obtained was used to estimate the total phenolic content from a standard calibration curve prepared from various concentrations of gallic acid (Hagerman *et al.,* 2000).

Determination of total flavonoids

The method is based on the formation of the flavonoids - aluminium complex which has an absorptivity maximum at 415 nm. 100 µl of the sample extracts in methanol (10 mg/ml) was mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5 ml. The absorption at 415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of sample extracts and a drop of acetic acid, and then diluted to 5 ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in triplicates (Kumaran and Karunakaran, 2006).

Determination of total alkaloids

Five gram (5 g) of the sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harborne, 1992).

Determination of total tannins

Five hundred (500 mg) of the samples were weighed into a 50 ml plastic bottle. 50 ml of distilled water were added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 mins (Van-Burden and Robinson, 1981).

Determination of total saponins

Twenty gram (20 g) of the ground plant extract was weighed into conical flask and 100 cm^3 of 20 % aqueous ethanol were added. It was then heated on a hot water bath set at 55 °C for 4 h with continuous stirring. The mixture was filtered and the residue re-extracted with fresh 200 ml 20 % ethanol. The combined extracts were then evaporated to 40 ml over water bath at about 90 °C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol was added. The combined n-butanol extracts were then washed twice with 10 ml of 5 % aqueous sodium chloride and solution was evaporated in a water bath. The resultant samples were then dried in the oven to a constant weight and the saponin content calculated (Obdoni and Ochuko, 2001).

Determination of Glycosides

Cardiac glycoside content in the sample was evaluated using Buljet's reagent as described by El-Olemy (1994). 1 g of the fine powder of *Annona muricata* leaves was soaked in 10 ml of 70 % alcohol for 2 h. and then filtered. The extract obtained was then purified using lead acetate and Na_2HPO_4 solution before the addition of freshly prepared Buljet's reagent (containing 95 ml aqueous picric acid + 5 ml 10 % aqueous NaOH). The difference between the intensity of colours of the experimental and blank (distilled water and Buljet's reagent) samples gives the absorbance and is proportional to the concentration of the glycosides.

Preparation of Culture Medium

Mueller Hinton agar was prepared by weighing 38 g of the powdered agar into 1000 ml of distilled water in a clean conical flask. It was soaked for 20 mins and then covered with a cotton wool and foil paper, autoclaved at 121 °C, and 115 atmospheric pressure for 15 mins. The medium was cooled to 50 °C and 4 ml of the medium were poured into a sterile glass petri dish and allowed to solidify. The sterility of the medium was tested by allowing it to stay overnight and checking for contamination.

Preparation of Extract Stock Solution

About 1 g each of the aqueous, ethanolic and n-hexane extracts of *Annona muricata* were weighed accurately and dissolved in 10 ml each of 10 % dimethyl sulfoxide (DMSO) to give a concentration of 100 mg/ml stock solutions of each concentration. Concentrations of 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml were prepared and used for the antibacterial treatment using ciprofloxacin as standard controls for antimicrobial activity.

Preparation and Standardization of Bacteria Inoculum

A loopful of 24 h old bacteria culture was suspended in 2 ml of sterile physiological saline medium. The concentration of each bacteria suspension was adjusted to 0.5 Mc Farland turbidity standard (1.5×10^8 CFU/ml) according to the procedure of Cheesbrough (2016).

Antibacterial Activity of the Extracts of *Annona muricata* against the Clinical Isolates

Agar Well Diffusion Method

The agar well diffusion method of (Bauer *et al.,* 2020) was adopted for the determination of the antimicrobial activity of the extracts of *Annona muricata* against the clinical isolates. Using a micropipette, 100 µl of standardized bacteria inoculums were inoculated into a Mueller Hinton agar plate (in triplicates) and spread evenly over the entire surface of the plates using a sterile cotton swab stick. The plates were allowed for 10 min and seven wells equidistant to each other were aseptically made on the inoculated agar plates using 9 mm sterile cork-borer. After this, 100 µl of the various concentrations of extracts (100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml) were each dispensed into the wells. Then, ciprofloxacin (0.5 mg/ml) and 10 % DMSO were used as the positive and

negative controls agents respectively. The plates were then left for 10 minutes at room temperature for diffusion of extracts into the agar to take place and then incubated at 37 °C for 24 h. The developed diameter of zone of inhibition was measured using transparent rule and duplicate samples expressed as mean in millimeters.

Determination of Minimum Inhibitory Concentration (MIC)
Minimum Inhibitory Concentration of the extracts against the bacterial isolates was determined using broth dilution method as adopted by Andrew, (2011). The extracts concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml were prepared by serial dilutions. Afterward, 100µl each of a standardized bacteria inoculum was inoculated to the different concentrations of extracts in test tubes containing Mueller Hinton broth. The inoculated broth media were incubated at 37˚C for 24 h and examined for turbidity. The lowest concentration of extracts that showed no turbidity (inhibited the growth of a test organism) was recorded as the Minimum Inhibitory Concentration (MIC). Negative control was set up as follows Mueller Hinton broth only and Mueller Hinton broth with extract. While positive control comprised of Mueller broth with test organism and Mueller Hinton broth with antibiotics (ciprofloxacin).

Determination of Minimum Bactericidal Concentration (MBC)
The positive MIC tubes were sub-cultured on nutrient agar plates with proper labels followed by incubation at 37°C for 24 h. They were examined for growth of bacteria. The tube with minimum concentration of extract in which the growth was completely stopped and noted as the minimum bactericidal concentration. The negative controls were nutrient agar only and nutrient agar with extracts only (Andrew, 2011).

Data Analysis
The data was presented as mean ± standard error of the mean (SEM). Analysis of variance (ANOVA) and visualising data in SPSS version 23.0 were used for statistical analysis, and values with $p \leq 0.05$ were used for statistical significance difference.

RESULTS

The Percentage Yield of *Annona muricata* Leaf Extract
Table 1 presents yield of leaf and stem bark extracts of *Annona muricata.* The result revealed that aqueous stem bark extract of *Annona muricata* yielded the highest percentage (50.14%), followed by the ethanolic extract (46.16%) and hexane extract (19.17%). Similarly, the aqueous leaf extract gave the highest yield (60.79%), while the hexane (26.85%) and ethanolic extracts (20.02%) produced lower yields

Table 1: Percentage yield of *Annona muricata* Stem Bark and Leaf Extract

Plant Extract	Yield of Extract (%)
Aqueous Stem Bark Extract	50.14
Ethanolic Stem Bark Extract	46.16
n-Hexane Stem Bark Extract	19.17
Aqueous Leaf Extract	60.79
Ethanolic Leaf Extract	20.02
n-Hexane Leaf Extract	26.85

Qualitative and Quantitative Phytochemical Analysis
The qualitative phytochemical composition of *Annona muricata* extracts is presented in Table 2. In this study, the efficacy of the extraction solvents in isolating bioactive compounds from *Annona muricata* varied across aqueous (AE), ethanolic (EE), and hexane (HE) extracts of both the leaf and stem bark extracts. The result showed that bioactive compounds of alkaloids, saponins, tannins, and glycosides were detected in both the leaf and stem bark extracts of *Annona muricata*. The aqueous extraction was the most efficient in retaining a broad bioactive compound, while hexane was the least effective solvents. Overall, the leaf extracts contained a broader range of bioactive compounds across extraction methods, particularly anthraquinones, while the stem bark was a stronger source of terpenoids.

Table 2: Qualitative Phytochemical Results of *Annona muricata* Extract.

Phytochemical Compounds	Leaf Extract			Stem Bark Extract		
	AE	EE	HE	AE	EE	HE
Alkaloids	+	+	+	+	+	+
Terpenoids	+	-	-	+	+	-
Flavonoids	+	+	+	+	+	-
Saponins	+	+	+	+	+	+
Phenols	+	-	+	+	-	-
Tannins	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Anthraquinones	+	+	+	+	+	-

- = Absent; + = Present. AE= Aqueous Extract, EE= Ethanolic Extract, HE= n-Hexane Extract

The quantitative phytochemical composition of *Annona muricata* extracts is presented in Table 3. The ethyl acetate leaf extract has been found to contain a total alkaloids content of (355.87±6.37), (134.00±1.15), (143.33±1.76), (577.07±2.19), (192.80±4.62), and (50.40±2.31) for Stem bark aqueous extract, stem bark ethanolic extract, stem bark hexane extract, leaf aqueous extract, leaf ethanolic extract, and leaf hexane extract respectively. The total flavonoids content was found to be (16.57±6.79), (59.52±6.80), (12.80±6.08), (22.37±1.40), (158.80±3.77), and (41.42±1.91) for Stem bark aqueous extract, stem bark ethanolic extract, stem bark hexane extract, leaf aqueous extract, leaf ethanolic extract, and leaf hexane extract respectively. The total phenolic content was also found to be (5.55±0.67), (2.62±0.50), (1.10±0.28), (3.51±0.26), (0.52±0.04), and (0.07±0.02) for Stem bark aqueous extract, stem bark ethanolic extract, stem bark hexane extract, leaf aqueous extract, leaf ethanolic extract, and leaf hexane extract respectively. The analysis revealed the total saponins content to be (2.10±0.06), (2.50±0.06), (8.10±0.06), (3.90±0.06), (19.80±0.06), and (2.40±0.06) for Stem bark aqueous extract, stem bark ethanolic extract, stem bark hexane extract, leaf aqueous extract, leaf ethanolic extract, and leaf hexane extract respectively. Finally, the total terpenoids content was (1.94±0.30), (4.71±0.55), (0.00±0.00), (0.69±0.12), (4.48±0.12), and (3.02±0.17), for Stem bark aqueous extract, stem bark ethanolic extract, stem bark hexane extract, leaf aqueous extract, leaf ethanolic extract, and leaf hexane extract respectively.

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Table 4: Quantitative Phytochemical Composition of *Annona muricata* Extracts

Plant Extracts	TPC mgGAE/g	TFC mgQE/g	TTC mgGAE/g	TSC mgDE/g	TAC mgAE/g
Stem Bark Aqueous Extract	5.55±0.67^{a}	16.57±6.79^{b}	1.94±0.30^{ab}	2.10±0.06^{abc}	355.87±6.37^{a}
Stem Bark Ethanolic Extract	2.62±0.50^{a}	59.52±6.80^{b}	4.71±0.55^{ab}	2.50±0.06^{abc}	134.00±1.15^{a}
Stem Bark Hexane Extract	1.10±0.28^{a}	12.80±6.08	0.00±0.00	8.10±0.06^{abc}	143.33±1.76
Leaf Aqueous Extract	3.51±0.26^{a}	22.37±1.40	0.69±0.12^{ab}	3.90±0.06^{abc}	577.07±2.19^{a}
Leaf Ethanolic Extract	0.52±0.04	158.80±3.77^{b}	4.48±0.12^{ab}	19.80±0.06^{abc}	192.80±4.62^{a}
Leaf Hexane Extract	0.07±0.02	41.42±1.91^{b}	3.02±0.17^{ab}	2.40±0.06	50.40±2.31

Values are expressed as mean ± SEM. Statistical significance mean difference was considered at $p<0.05$ and LSD comparison test was used for post hoc analysis. Values bearing same superscripts under the same column are significantly different.

Key: TPC: total polyphenols content; TFC: total flavonoid content; TTC: total tannins content; TSC: total saponin content; TAC: total alkaloid content; mgGAE/g: Milligrams of Gallic Acid Equivalent per gram; mgQE/g: Milligrams of Quercetin Equivalent per gram; mgDE/g: Milligrams of Diosgenin Equivalent per gram; mgAE/g: Milligrams of Atropine Equivalent per gram.

Antibacterial Susceptibility Profile

The antimicrobial susceptibility results were presented as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and zone diameters of inhibition for the test organisms, using aqueous, ethanolic, and hexane extracts of leaves and stem bark, with ciprofloxacin as the standard control, as shown in Tables 5, 6 and 7. A zone of inhibition measuring more than 8mm signifies that bacteria are susceptible to the tested antibiotics as shown in Table 6. The results of the MIC and MBC revealed a significant zone of inhibition to isolates by the plant extracts at different concentration. The extracts showed MIC and MBC activity of at 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, and the standard.

Table 5: Minimum Inhibitory Concentration (MIC) of *Annona muricata* Extracts against Bacterial Isolates

Plant part	Extraction solvent	Minimum Inhibitory Concentration (MIC) (mg/ml)		
		Salmonella sp.	*E. coli*	*S. aureus*
Leaf	Aqueous	100	100	100
	Ethanolic	100	100	100
	Hexane	50	100	100
Stem bark	Aqueous	100	100	100
	Ethanolic	50	100	100
	Hexane	100	100	100

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Table 6: Minimum Bactericidal Concentration (MBC) of *Annona muricata* Extracts against Bacterial Isolates

Plant part	Extraction solvent	Minimum Bactericidal Concentration (MBC) (mg/ml)		
		Salmonella sp.	*E. coli*	*S. aureus*
Leaf	Aqueous	25	50	25
	Ethanolic	50	100	100
	Hexane	100	100	100
Stem bark	Aqueous	50	50	50
	Ethanolic	50	100	50
	Hexane	100	100	100

Table 7: Anti-bacterial Activity of *Annona muricata* Extracts against Bacterial Isolates

Bacterial isolates	Plant part	Extraction solvent	Mean Diameter of Zone of Inhibition (mm) / Extract Concentration (mg/ml)				
			100	50	25	12.5	Ciprofloxacin
***Salmonella* sp.**	**Leaf**	Aqueous	6.50 ± 0.000	0.07 ± 0.067	0.57 ± 0.067	0.07 ± 0.033	5.47 ± 0.033
		Ethanolic	2.50 ± 0.050	2.07 ± 0.033	1.57 ± 0.000	1.50 ± 0.033	3.47 ± 0.333
		Hexane	2.00 ± 0.000	2.75 ± 0.033	1.75 ± 0.120	1.70 ± 0.031	4.97 ± 0.000
	Stem bark	Aqueous	1.30 ± 0.020	0.97 ± 0.167	0.88 ± 0.006	0.17 ± 0.024	5.47 ± 0.033
		Ethanolic	1.80 ± 0.130	1.80 ± 0.284	1.44 ± 0.674	1.20 ± 0.000	3.47 ± 0.333
		Hexane	0.91 ± 0.011	0.75 ± 0.131	0.52 ± 0.110	0.20 ± 0.231	4.97 ± 0.000
E. coli	**Leaf**	Aqueous	4.57 ± 0.010	4.43 ± 0.007	3.57 ± 0.067	2.77 ± 0.000	7.00 ± 0.401
		Ethanolic	3.25 ± 0.047	2.75 ± 0.007	2.57 ± 0.367	2.70 ± 0.043	3.77 ± 0.045
		Hexane	2.65 ± 0.037	1.95 ± 0.017	2.55 ± 0.037	2.30 ± 0.010	2.77 ± 0.102
	Stem bark	Aqueous	2.66 ± 0.030	2.52 ± 0.457	2.18 ± 0.160	1.98 ± 0.080	7.00 ± 0.401
		Ethanolic	2.75 ± 0.171	2.54 ± 0.517	2.33 ± 0.097	1.76 ± 0.343	3.77 ± 0.045
		Hexane	2.66 ± 0.025	2.35 ± 0.114	2.13 ± 0.777	1.30 ± 0.911	2.77 ± 0.102
S. aureus	**Leaf**	Aqueous	8.07 ± 0.067	6.50 ± 0.000	6.50 ± 0.010	5.10 ± 0.023	7.47 ± 0.032
		Ethanolic	5.07 ± 0.037	4.80 ± 0.030	3.95 ± 0.044	3.50 ± 0.032	4.47 ± 0.333
		Hexane	3.17 ± 0.007	2.85 ± 0.120	2.50 ± 0.041	1.80 ± 0.005	3.97 ± 0.300
	Stem bark	Aqueous	3.39 ± 0.140	3.11 ± 0.130	2.99 ± 0.110	2.98 ± 0.124	7.47 ± 0.032
		Ethanolic	4.97 ± 0.007	4.71 ± 0.231	4.70 ± 0.443	4.12 ± 0.621	4.47 ± 0.333
		Hexane	2.97 ± 0.107	2.79 ± 0.209	1.99 ± 0.543	1.77 ± 0.455	3.97 ± 0.300

Values are expressed as mean ± standard error of mean

DISCUSSION

From the finding of this study, *Annona muricata* is typically rich in phytochemicals, such as tannins, alkaloids, flavonoids, and saponins, which may support their applications in ethnomedicine. According to Sofowora (2008), secondary metabolites of *A. muricata* leaf include flavonoids, tannins, alkaloids, saponins, and cardiac glycosides in addition to phenolic compounds. *A. muricata* is a source of several phytochemicals, including alkaloids, flavonoids, phenols, and tannins, (Ebabhi *et al.,* 2018). These findings support the plant's widespread usage as an antibacterial agent for the treatment of a variety of medical ailments. The findings of this study showed that the quantities of bioactive compounds from the families of phytochemicals, such as flavonoids, phenols, tannins, saponins, and polyphenols, varied between the two plant extracts. Research has linked these phytochemicals to medicinal plants that have been shown to have antibacterial properties (Gavamukulya *et al.,* 2015; Ebana *et al.,* 2016; Pai *et al.,* 2016). In line with a prior study by Oloyede *et al.* (2010) that found that *A. muricata* methanol extract contains biologically active chemical substances like saponins, tannins, essential oils, flavonoids, alkaloids, and other chemical compounds that have preventive and curative properties, the results of this study showed that the phytochemicals in the stem bark of the plant have antibacterial action on the clinical isolates. Some phytochemicals, like tannins, have antibacterial qualities because they can enter the internal membrane of bacteria and pass through their cell walls, as reported by Kaczmarck (2020). The bacteria eventually die as a result of this disruption to their metabolism. Saponins, which are also abundant in *A. muricata*, have been shown in numerous investigations to possess antibacterial qualities (Khan *et al.,* 2018). It follows that the plant parts would be useful in the treatment of bacterial illnesses. Kanife *et al.* (2023) assert that plant-based antimicrobials have great therapeutic potential and can achieve their intended purpose with fewer adverse effects than synthetic antimicrobials. According to research, polyphenols inhibit the virulence factors of harmful microorganisms (Pai *et al.,* 2016). This study showed that aqueous, ethanol, and hexane extracts of *Annona muricata*'s stem bark and leaves have strong antibacterial activity against a few clinical isolates of Salmonella species, *Staphylococcus aureus*, and *Escherichia coli*. This is consistent with the results of Agunsoye and Onifade (2020), who found that after employing several extraction solvents, bacterial isolates were significantly inhibited by *A. muricata* leaf extract in aqueous, ethanol, and hexane forms. Although *E. coli* was responsive to the extracts, *Pseudomonas aeruginosa* was found to be resistant to the different plant part extracts at the different concentrations. The gram-negative bacteria's cell walls, which operate as a barrier to the extracts' diffusion into them and rendered them ineffective, as observed in the findings of Viera *et al.* (2010) and Pai *et al.* (2016), may still account for this. Furthermore, due to the structure of their cell walls, antibacterial medications have a delayed effect on Gram-negative bacteria (Kaczmarck, 2020). This is in addition to the fact that some bacteria are resistant to external chemicals. Gram-positive bacteria exhibit rapid tannin action (Kaczmarck, 2020). The study's reported findings could be attributed to the active chemicals present in plant components, including tannin, saponins, flavonoids, and alkaloids.

Significant sensitivity to varying doses of the *A. muricata* aqueous leaf extract was demonstrated by the *S. aureus* strains in the investigation; this sensitivity was slightly lower than that of the ciprofloxacin-treated control. This result is in line with other findings and offers more proof that *A. muricata* aqueous leaf extracts are useful in treating *S. aureus* caused infections and disorders (Abdulsalami *et al.,* 2016). Aqueous and ethanol extracts of a variety of plant species, including *Annona muricata*, shown strong antibacterial action against particular gram-positive and gram-negative bacteria, according to Bussmann *et al.* (2010). 81% of the ethanol extracts inhibited *Staphylococcus aureus*, and 36% of them had a minimum inhibitory concentration range of 0.008-256 $mgmL^{-1}$. The current investigation confirms the effectiveness of the extracts tested, particularly the two extracts that showed the maximum antibacterial activity ethanolic stem bark extract and aqueous leaf extract.

Conclusions

In this study, the stem bark and leaf extracts of *Annona muricata* (soursop) was active against clinical *Salmonella* species, *Staphylococcus aureus*, and *Escherichia coli* isolates. Moreover, the plant extracts were rich with tannins, alkaloids, flavonoids, saponins, and polyphenols that contributes to its effectiveness against bacterial infections. Therefore, *A. muricata* is a valuable resource for developing plant-based antimicrobials to address the growing concern of antibiotic resistance.

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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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