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Antibacterial Potential of *Annona muricata* (Linn.) Leaf Extract: A Promising Natural Source for Novel Antibacterial Therapies

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Authors' contributions

This work was carried out in collaboration among all authors. Author AOO designed the study and wrote the protocol. Author POM wrote the first draft of the manuscript and managed the analyses of the study. Authors EDW and JOA managed the literature searches and prepared the final draft of the manuscript for publication. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Bacterial infections pose a significant public health challenge, necessitating the search for new antimicrobial agents. This study investigated the antibacterial potential of *Annona muricata* (Linn.) leaf ethyl acetate extract against some bacterial pathogens.

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Study Design: An experimental design was adopted for this study.
Place and Duration of Study: Fresh leaves of *Annona muricata* (Linn.) were collected from Modakeke, Osun State, South-West, Nigeria. Proper identification and authentication of the leaves were carried out at the herbarium unit of the Department of Botany, Obafemi Awolowo University (OAU), Ile-Ife, Osun State, Nigeria.
Methodology: The plant material was extracted using ethyl acetate, and concentration-dependent assays determined antibacterial activity, including minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The phytochemical analysis identified bioactive compounds.
Results: The extract concentrations of 35.00 mg/ml, 17.50 mg/ml, 8.75 mg/ml, and 4.38 mg/ml demonstrated significant antibacterial activity against a total of ten bacterial strains out of the twenty-one (47.6%) tested. These strains include *Klebsiella pneumoniae, Proteus vulgaris, Staphylococcus mariliensis, Salinicoccus roseus, Enterobacter asburiae, Streptococcus canis, Streptococcus pyogenes, Streptococcus equi, Limonorella grimontii, Proteus hauseri, Citrobacter werkmanii, Staphylococcus rosterii, Versinia enteroculitica, Macrococcus bruensis, Precoccus suis, Limorella richardii, Staphylococcus aureus, Macroccocus lavnae, Salmonella enterica, Streptococcus salviohxodontae, and Klebsiella ozanae*. The range of obtained inhibition zone values varied from 70 mg/ml to 4.38 mg/ml. The minimum inhibitory concentration (MIC) values varied between 4.48 mg/ml and 35.00 mg/ml, while the minimum bactericidal concentration (MBC) values ranged from 2.19 mg/ml to 17.5 mg/ml. Susceptibility to the extract increased with higher concentrations. Comparative analysis with ampicillin indicated superior inhibitory properties. Phytochemical analysis of the extract revealed the presence of some phytochemical constituents.
Conclusion: The ethyl acetate extract obtained from *Annona muricata (*Linn.) leaf demonstrated significant antibacterial activity against tested bacterial isolates. The extract exhibited concentration-dependent inhibitory effects, indicating the presence of bioactive compounds with antibacterial properties. These findings highlight the potential of *Annona muricata* (Linn.) as a source for developing natural antibacterial agents. Further research is needed to isolate and identify the specific active compounds, understand their mechanisms of action, and assess the extract's therapeutic potential and safety profile. The study emphasizes the importance of exploring natural plant extracts for their antibacterial properties and opens avenues for future applications in medicine and pharmaceuticals.

Keywords: Annona muricata (Linn.); antibacterial; Bacterial; extract.

1. INTRODUCTION

Throughout history, disease has posed a significant challenge to human health. Medicinal plants have long been recognized for their therapeutic properties and have been used for centuries to combat the effects of disease [1]. The utilization of plant-based remedies in medical practice has evolved from traditional approaches to modern medicine, aiming to enhance the quality of life for patients worldwide. Nature-derived medicines derived from plants are often considered safer alternatives with reduced or no side effects [1]. Plants have played a crucial role in human existence since the dawn of life, and various plant species and parts have been employed to treat a wide range of diseases [2-4].

Numerous studies conducted worldwide have investigated the antibacterial properties of plants, leading to the identification of potential therapeutic alternatives [5-8]. These properties are attributed to various compounds found in plants, including quinine, tannins, alkaloids (such as anonaine and anoniine), acetogenins, flavonoids, and others. The exploration of these plant-derived products, either pure compounds or standardized extracts, offers many opportunities developing new drug leads due to the immense chemical diversity available in plants. Consequently, many medicinal plants used in traditional medicine have been scientifically evaluated and shown to possess bactericidal properties.

Annona muricata (Linn)., belonging to the family *Annonaceae* and the genus *Annona*, is a well-known species primarily recognized for its edible fruits, commonly called ‘sour sop’. The name ’sour sop’ is derived from its slightly acidic flavor when fully ripe. In English, it is commonly known

as 'Graviola' or 'sour sop'; in some regions like India and Australia, it is also called custard apple (although this term also encompasses *Annona reticulata*, a closely related species). Spanish-speaking countries call it guanabana or guanaba, while other regions use names such as sorsaka, zuurzak, corossol, and graviola.

Annona muricata (Linn)., has a significant history of traditional use in herbal medicine. Various parts of the plant, including the bark, leaves, roots, fruit, and seeds, are used in tropical region's natural medicine [9,10]. These different plant parts possess distinct medicinal properties and are attributed to various therapeutic uses. *Annona muricata* exhibits a wide range of pharmacological activities, such as antifungal, antitumor, analgesic, hypotensive, anti-inflammatory, and immune-enhancing effects [6,9,11]. Additionally, it has been recognized for its sedative, ulcer treatment, hypotensive, nervine, and oxytocic properties [10,12]. Phytochemical analysis of *Annona muricata* has identified alkaloids, flavonoids, carbohydrates, cardiac glycosides, saponins, tannins, phytosterols, terpenoids, and proteins [13]. These findings underscore the potential therapeutic value of *Annona muricata* (Linn) in traditional medicine.

Therefore, the aim of this study was to investigate the antibacterial activities of the ethyl acetate extract of *Annona muricata* (Linn.), determine the minimum inhibitory concentration and minimum bactericidal concentration of the leaf extract, and analyze the phytochemical constituents of *Annona muricata* (Linn), thus providing baseline data for *Annona muricata* (Linn.) to be used as candidate in drug development to treat bacterial infections.

2. METHODOLOGY

2.1 Collection and Authentication of Plant Materials

Annona muricata (Linn.) fresh leaves were obtained from Modakeke, Osun State, South-West, Nigeria. The leaves were carefully identified and authenticated at the herbarium unit of the Department of Botany, Obafemi Awolowo University (OAU), Ile-Ife, Osun State, Nigeria. Subsequently, the leaves were air dried at room temperature, ground to a fine powder [14] in the drug research and production unit at the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, and appropriately stored until further use.

2.2 Source of Test Isolates

The bacterial strains used in this study were sourced from the Department of Microbiology research laboratory, OAU, Ile-Ife, Nigeria. Well isolated colonies from the test isolate cultures were sub-cultured on nutrient agar (Merck, Germany) and incubated at 37ºC for 24 hours. Colonies from the cultures were aseptically transferred to 5ml of nutrient broth in test tubes and incubated at 37ºC for 18 hours.

2.3 Serial Dilution and Colony Counting

The serial dilution was performed using standard procedures. Initially, a 1 ml sample of the test organism was added to a sterile test tube containing 9 ml of sterile broth (Becton, Dickinson and Company, USA). The test tube was thoroughly mixed to ensure proper dispersion of the organism.

From this initial dilution, 1 ml was transferred to a second sterile test tube containing 9 ml of diluent, resulting in a 1:10 dilution. This process was repeated multiple times, with 1 ml of the previous dilution being added to 9 ml of diluent each time, to achieve subsequent dilutions. After preparing the serial dilutions, 0.4 ml of each dilution was aseptically transferred onto separate Petri dishes using a sterile pipette. The dilution was evenly spread on the agar surface using a sterile glass rod. The Petri dishes were then incubated at 37^{o}C under appropriate conditions for the growth of the test organism. Following incubation, the resulting colonies on each plate were counted and recorded using an electronic colony counter. This allowed for the quantification of the test organism in the original sample and determination of the appropriate dilution for subsequent analysis or experimentation.

2.3.1 Culture and maintenance of test isolates

Pure cultures of the experimental bacteria were maintained on a nutrient agar medium. Each bacterial strain was regularly subcultured on the same medium and stored at 4ºC before use in the experiments.

2.4 Preparation of Plant Powder

The leaves of *Annona muricata (Linn.)* were dried under shade for 2-3 weeks and subsequently ground into fine powder using a clean mortar and pestle.

2.5 Preparation of Plant Extracts

A volume of 2.5 L of ethyl acetate was used to soak 1 kg of powdered leaves in a glass jar for five days to prepare the plant extracts. The suspension was thoroughly agitated every 12 hours. On the fifth day, the suspension was filtered using filter paper, and the solvent was evaporated to dryness at 40ºC using a rotary evaporator [15] at the Central Science Laboratory, OAU, Ile-Ife, Osun State, Nigeria. The resulting crude extract was stored in glass Petri dishes.

2.6 Reconstitution of the Plant Extract

The crude extract was reconstituted to a concentration of 35 mg/ml. Serial dilutions were then performed to obtain subsequent concentrations of 17.50 mg/ml, 8.75 mg/ml, 4.38 mg/ml, and 2.19 mg/ml, which were utilized for determining the minimum inhibitory concentration (MIC).

A total of 1.4 g of the crude extract was dissolved in a mixture of water and dimethyl sulfoxide (DMSO) in a ratio of 6:4. Two-fold serial dilutions were carried out using 5 ml of the diluted extract (stock) and 5 ml of water to obtain five dilutions. Each dilution was added to McCartney bottles containing 15 ml of sterilized nutrient agar, thoroughly mixed, and then poured into labeled Petri dishes. The Petri dishes were divided based on the number of test organisms used, and each dilution was appropriately labeled on the dishes [16].

2.7 Antibacterial Susceptibility Test

2.7.1 Agar well diffusion method

The inocula used for the test organisms were standardized using a 0.5 McFarland standard to ensure consistent cell density. This standard is a turbidity standard that corresponds to a specific optical density at a wavelength of 625 nm. By comparing the turbidity of the inoculum suspension to the 0.5 McFarland standard, the density of the inoculum was adjusted to achieve a desired cell concentration. The isolates were inoculated into nutrient broth (Becton, Dickinson and Company, USA) and incubated at 37^{o}C for 4-18 hours. The cultures were then spread on Mueller Hilton agar (EO labs, Scotland) plates. Wells were made on the plates, and varying concentrations of the leaf extract (35 mg/ml, 17.5 mg/ml, 8.75 mg/ml) were introduced separately into three wells. Ampicillin and dimethyl sulfoxide (DMSO) were introduced into wells 4 and 5, respectively, serving as positive and negative controls. The plates were incubated at 37ºC for 24 hours, and the diameters of the zones of inhibition were measured to the nearest millimeter using a transparent ruler.

2.7.2 Measurement of antibacterial activity of *Annona muricata* (Linn.) leaf extract using agar well diffusion

The antibacterial activity of the *Annona muricata (Linn.)* leaf extract was assessed by measuring the zones of inhibition using a meter rule and comparing with the standard Kirby Bauer table against the tested organisms. The results were compared to the activity of the standard antibiotic, ampicillin.

2.7.3 Determination of minimum Inhibitory concentration (MIC)

The minimum inhibitory concentration was determined using a simple serial dilution method to obtain various concentrations of the leaf extract. Nutrient agar plates containing different concentrations of the leaf extract were inoculated with the test organisms. The plates were then incubated at 28ºC for 72 hours. The lowest concentration of the extract that showed no visible growth of the organisms was defined as the MIC.

2.7.4 Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration was determined using a simple serial dilution technique to obtain different concentrations of the *Annona muricata (Linn.)* leaf extract. Nutrient agar plates containing varying concentrations of the leaf extract were inoculated with the test organisms. After incubation at 28ºC for 72 hours, the MBC was determined as the lowest concentration of the extract at which no growth of the organisms was observed.

2.8 Phytochemical Analysis

Phytochemical analysis was conducted to determine the presence or absence of secondary metabolites, including phenols, saponins, flavonoids, alkaloids, tannins, etc., in the *Annona muricata (Linn.)* leaf extract. These compounds contribute to the antibacterial properties exhibited by the leaf.

2.8.1 Test for alkaloids (Dragendorff's Test)

A solution of 0.5 g of the plant extract in 5 ml of 1% hydrochloric acid was prepared and subjected to Dragendorff's reagent. The appearance of turbidity or precipitation indicates the presence of alkaloids [17].

2.8.2 Test for terpenoids (Salkowski Test)

A mixture of 0.2 g of the extract, 2 ml of chloroform, and 3 ml of concentrated sulfuric acid was prepared. The formation of a reddish-brown interface indicates the presence of terpenoids [17].

2.8.3 Test for sterols

A solution of 0.5 g of the extract in 3 ml of chloroform was prepared and filtered. To the filtrate, 2 ml of concentrated sulfuric acid was added to form a lower layer. The chloroform layer appears red, while the acid layer exhibits a greenish-yellow fluorescence, confirming the presence of sterols [18].

2.8.4 Test for flavonoids (Shinoda Test)

A solution of 0.2g of the extract in 2 ml of methanol was prepared and heated. Magnesium metal chips were added to the mixture, followed by a few drops of concentrated hydrochloric acid. The appearance of a red or orange coloration indicates the presence of flavonoids [16].

2.8.5 Test for saponins (Hemolysis Test)

Freshly prepared 7% blood agar medium was used, and wells were dug using sterile cork borer. Methanol extracts were dispensed, with distilled water and methanol serving as negative controls, while a commercial saponins solution was used as a positive control. The plates were incubated at 35ºC for 6 hours, and complete hemolysis of the blood around the extract indicates the presence of saponins [16].

2.8.6 Test for phenols (Ferric Chloride Test)

Exactly 2 mg of the extract was dissolved in 4 ml of distilled water. A few drops of 10% ferric chloride solution were added to the solution. The observation of a blue or green coloration indicates the presence of phenols [17,18].

2.8.7 Test for phlobatannins (Hydrochloric Acid Test)

Approximately 2 mg of the extract was dissolved in 4 ml of distilled water and subjected to boiling with 1% aqueous hydrochloric acid. The formation of a red precipitate serves as evidence for the presence of phlobatannins [17,18].

3. RESULTS

3.1 Antibacterial Activity of Ethyl Acetate Extract from *Annona muricata (Linn.)*

Table 1 illustrates the antibacterial efficacy of ethyl acetate extract derived from *Annona muricata* against a panel of test bacteria. The extract concentrations of 35.00 mg/ml, 17.50 mg/ml, 8.75 mg/ml, and 4.38 mg/ml demonstrated considerable antibacterial efficacy, inhibiting the growth of ten out of the twenty-one (47.6%) tested bacterial strains. Notably, these strains encompass *Klebsiella pneumoniae, Proteus vulgaris, Staphylococcus mariliensis, Salinicoccus roseus, Enterobacter asburiae, Streptococcus canis, Streptococcus pyogenes, Streptococcus equi, Limonorella grimontii, Proteus hauseri, Citrobacter werkmanii, Staphylococcus rosterii, Versinia enteroculitica, Macrococcus bruensis, Precoccus suis, Limorella richardii, Staphylococcus aureus, Macroccocus lavnae, Salmonella enterica, Streptococcus salviohxodontae*, and *Klebsiella ozanae.*

3.2 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Annona muricata (Linn.)* Leaf Extract

Tables 2 and 3 present the MIC and MBC values of the *Annona muricata* (Linn.) leaf extract against susceptible test organisms. The MIC values ranged from 4.48 mg/ml to 35.00 mg/ml, while the MBC values ranged from 2.19 mg/ml to 17.5 mg/ml.

3.3 Phytochemical Analysis of *Annona muricata (Linn.)* Leaf Extract

Table 4 elucidates the findings of phytochemical analysis performed on the leaf extract of *Annona muricata* (Linn.). The analysis revealed the presence of diverse phytochemical constituents, including, but not limited to alkaloids, sterols, and saponins, in the leaf extract.

Table 1. Diameter of zone of inhibition of *Annona muricata (Linn.)* extract on test bacteria

Tests Organisms	DMSO (mg/ml)	70 mg/ml	35 mg/ml	17.5 mg/ml	8.75 mg/ml	4.38 mg/ml	Ampicillin (mg/ml)
Staphylococcus aureus	0.00	22.00	13.00	11.00	19.00	0.00	0.00
Aerococcus suis	0.00	7.00	0.00	0.00	0.00	0.00	0.00
Limonella grimontii	0.00	12.00	0.00	0.00	0.00	0.00	0.00
Limorella richardii	0.00	11.00	0.00	0.00	0.00	0.00	0.00
Globicatella suifidifaciens	0.00	15.00	0.00	0.00	0.00	0.00	0.00
Salinincoccus roseus	0.00	13.00	0.00	0.00	0.00	0.00	10.00
Staphylococcus muscae	0.00	6.00	0.00	0.00	0.00	0.00	0.00
Proteus hauseri	0.00	12.00	6.00	0.00	0.00	0.00	0.00

Key:

- *Ampicillin = positive control*
- *DMSO = negative control*

Table 2. Minimum inhibitory concentration (MIC) of *Annona muricata (Linn.)* extract

Test Organisms	35 mg/ml	17.5 mg/ml	8.75 mg/ml	4.38 mg/ml	2.19 mg/ml
Klebsiella pneumonia	-	-	+	+	+
Proteus vulgaris	-	-	+	+	+
Staphlococcus massiiliensis	-	-	+	-	+
Streptococcus canis	-	-	+	+	+
Streptococcus equi	-	-	+	+	+
Proteus hauseri	-	-	-	+	+
Proteus vulgaris	-	-	+	+	+
Staphlococcus muscae	-	-	+	+	+
Salinicoccus roseus	-	-	+	+	+
Citrobacter werkmanii	-	-	+	-	-
Globicatella sulfifaciens	-	-	+	+	+
Yersinia enterocolitica	-	-	+	+	+
Aerococcus suis	-	-	+	+	+
Liminorella richardia	-	-	-	+	+

Key:

+ represents growth at that concentration

- represents no growth at that concentration

Table 3. Minimum bactericidal concentrations of *Annona muricata (Linn.)* extract on test organisms

Test Organisms	35 mg/ml	17.5 mg/ml	8.75 mg/ml	4.38 mg/ml	2.19 mg/ml
Klebsiella pneumonia	-	+	+	+	+
Proteus vulgaris	-	+	+	+	+
Staphlococcus mariliensis	-	+	+	+	+
Streptococcus canis	-	-	+	+	+
Streptococcus equi	-	-	+	+	+
Proteus hauserii	-	-	+	+	+
Klebsiella ozanae	-	+	+	+	+
Staphlococcus muscae	-	+	+	+	+
Salinicoccus roseus	-	+	+	+	+
Citrobacter werkmanii	-	-	+	-	+
Glabicatella sulfifaciens	-	+	+	+	+
Yersinia enterocolitica	-	-	+	+	+
Aerococcus suis	-	-	+	+	+
Limonella richardii	-	-	+	-	+
Staphylococcus muscae	-	-	+	+	+

Key
+ represents Growth
- represents Inhibition (no growth)

Table 4. Phytochemical constituents of *Annona muricata (Linn.)* leaf extract

Phytochemical constituents	Observation
Alkaloids	-
Sterols	-
Terpenoids	+
Tannins	-
Flavonoids	-
Saponins	-
Phlobatannins	+
Phenols	-

Key:
+ The constituent is present
- The constituent is absent

4. DISCUSSION

The concept of the 'magic bullet', introduced by Paul Ehrlich, marked the beginning of the antimicrobial era. However, over time, the irrational use of antimicrobial therapies has led to the development of resistance in microorganisms [19]. While antibiotics aim to eliminate or inhibit the growth of pathogenic microorganisms, they can also have detrimental effects on the host. Generalized adverse events are commonly associated with antibiotic use, necessitating the exploration of alternative treatment options and therapies.

Plants have been utilized as medicinal agents since ancient times. The use of plants as alternative medicine offers several advantages, including their diverse and flexible applications, regional availability, and affordability. One key advantage is the potential to reduce adverse reactions. In low- and middle-income countries, where plants enjoy widespread acceptance, their cost-effectiveness and relatively low technological requirements make them an ideal alternative to expensive therapies. Therefore, plant extracts have the potential to serve as superior and safer alternatives, provided they are supported by scientific evidence [20,21]. The present study investigated the antibacterial effects of the ethyl acetate extract derived from *Annona muricata* (Linn.) leaf.

The findings from this study indicate that the *Annona muricata* (Linn.) extract had varying degrees of antibacterial activity against the tested organisms. It was most effective against *Staphylococcus aureus*, showing a concentration-dependent zone of inhibition. This is consistent with similar studies [22,23]. However, no inhibitory effect was observed for certain organisms, such as *Aerococcus suis* and *Limonella grimontii*. This result is not consistent with any published study. The study observed that the lower concentrations of the *Annona muricata* (Linn.) extract (such as 4.38 mg/ml) exhibited higher zones of inhibition compared to the higher concentrations (e.g., 35 mg/ml). This phenomenon may be attributed to several factors like dose-dependent response, saturation effect, inhibitory threshold of tested isolates, and growth conditions. It is important to note that the specific mechanisms underlying the observed variations in the zone of inhibition would require further investigation, including the identification and characterization of active compounds present in the extract.

Furthermore, this study observed varying responses of the test organisms to different concentrations of the *Annona muricata* (Linn.) extract, indicating differences in their susceptibility to the extract's inhibitory effects. Among the tested organisms, *Klebsiella pneumoniae, Proteus vulgaris, Streptococcus canis, Streptococcus equi, Proteus vulgaris, Salinicoccus roseus, Globicatella sulfifaciens, Yersinia enterocolitica*, and *Aerococcus suis* demonstrated susceptibility to the extract at concentrations of 8.75 mg/ml, 4.38 mg/ml, and 2.19 mg/ml, indicating inhibitory effects. *Staphylococcus massiiliensis, Citrobacter werkmanii*, and *Liminorella richardia* showed susceptibility at concentrations of 8.75 mg/ml and 4.38 mg/ml, but not at 2.19 mg/ml. *Staphylococcus muscae* demonstrated susceptibility only at concentrations of 8.75 mg/ml and 4.38 mg/ml. These findings are consistent similar research [24,25]. It is important to note that at the concentration of 8.75 mg/ml, *Citrobacter werkmanii* exhibited susceptibility to the *Annona muricata* (Linn.) extract, as indicated by the presence of inhibition. This suggests that the extract at this concentration is effective in inhibiting the growth of *Citrobacter werkmanii*.

However, at the lower concentration of 4.38 mg/ml, *Citrobacter werkmanii* did not show inhibition, indicating potential resistance to the extract at this specific concentration. This suggests that the susceptibility of *Citrobacter werkmanii* to the extract may vary depending on the concentration used. These findings highlight the importance of concentration-dependent effects when evaluating the antimicrobial activity of natural extracts. The observed lack of inhibition at 4.38 mg/ml for *Citrobacter werkmanii* suggests the need for further investigation to identify the concentration range at which the extract can effectively inhibit this particular pathogen. This study also observed that the lower concentrations of the *Annona muricata* (Linn.) extract (such as 2.19 mg/ml) exhibit inhibitory effects on the tested pathogens, while higher concentrations (e.g., 35 mg/ml) do not. Several factors may contribute to this observation including optimal concentration range, sensitivity variation, bacterial resistance, toxicity or adverse effects. It is important to consider that the specific inhibitory mechanisms and reasons for the observed concentration-dependent effects would require further investigation. Additional studies, including the determination of the extract's chemical composition and targeted mechanisms of action,

could provide more insights into the concentration-dependent inhibitory effects observed in the tested pathogens.

Additionally, this study observed the bactericidal activity of the *Annona muricata* (Linn.) extract at different concentrations against a range of test organisms. At the highest concentration of 35 mg/ml, the extract exhibited bactericidal activity against *Klebsiella pneumoniae, Proteus vulgaris, Staphylococcus mariliensis, Proteus hauserii, Klebsiella ozanae, Salinicoccus roseus, Glabicatella sulfifaciens*, and *Yersinia enterocolitica*. This indicates that the extract at this concentration was able to not only inhibit the growth of these bacteria but also kill them. At the concentration of 17.5 mg/ml, the extract showed bactericidal activity against *Proteus vulgaris, Staphylococcus mariliensis, Streptococcus canis, Streptococcus equi, Klebsiella ozanae, Salinicoccus roseus, Glabicatella sulfifaciens*, and *Yersinia enterocolitica*. This suggests that the extract at this concentration was able to inhibit the growth of these bacteria and effectively kill them. At 8.75 mg/ml, the extract exhibited bactericidal activity against *Staphylococcus mariliensis, Streptococcus canis, Streptococcus equi, Proteus hauserii, Klebsiella ozanae, Salinicoccus roseus, Glabicatella sulfifaciens*, and *Yersinia enterocolitica*. This indicates that the extract at this concentration was able to inhibit the growth of these bacteria and exert bactericidal effects, a result in tandem with similar studies [21,23]. However, at the lower concentration of 4.38 mg/ml, the extract showed bactericidal activity against *Staphylococcus mariliensis, Streptococcus canis, Streptococcus equi, Klebsiella ozanae, Salinicoccus roseus, Glabicatella sulfifaciens*, and *Yersinia enterocolitica*. It did not exhibit bactericidal activity against *Citrobacter werkmanii* and *Limonella richardii.* At the concentration of 17.5 mg/ml, the *Annona muricata* (Linn.) extract did not exhibit bactericidal activity against *Citrobacter werkmanii* and *Limonella richardii*. This indicates that the extract at this concentration was not effective in completely killing these two bacterial strains. Similarly, at the concentration of 8.75 mg/ml, the extract did not show bactericidal activity against *Citrobacter werkmanii* and *Limonella richardii*. This suggests that the extract at this concentration was unable to fully inhibit the growth of these bacterial strains. At the lower concentration of 4.38 mg/ml, the extract also did not exhibit bactericidal activity against *Citrobacter werkmanii* and *Limonella richardii*. This implies that the extract at this concentration was insufficient to completely inhibit the growth or kill these bacteria. The results for *Citrobacter werkmanii* and *Limonella richardii* indicate that these bacterial strains may have a higher tolerance or resistance to the *Annona muricata* (Linn.) extract at the tested concentrations. Further studies may be required to explore the reasons behind the lack of effectiveness of the extract against these specific bacterial strains and to investigate alternative concentrations or treatment approaches that may yield better results.

The phytochemical analysis of *Annona muricata* (Linn.) leaf extract revealed the presence and absence of certain phytochemical constituents. Alkaloids, tannins flavonoids, saponins, and sterols were not detected in the extract, indicating their absence in the tested sample, a result not consistent with similar studies [13,26]. However, terpenoids, phlobatannins, and phenols were observed in the extract, with is consistent with a similar study [13]. Terpenoids are a diverse group of compounds known for their various biological activities, including antimicrobial, antioxidant, and anticancer properties [27]. The presence of terpenoids in the leaf extract suggests its potential therapeutic value. Phlobatannins, another class of phytochemicals, were present in the extract. Phlobatannins are known for their antioxidant and antimicrobial properties [28]. Their presence indicates that the *Annona muricata* (Linn.) leaf extract may possess these beneficial properties.

5. CONCLUSION

The findings of this study demonstrate the significant antibacterial activity of the ethyl acetate extract obtained from *Annona muricata* (Linn.) leaf. The extract exhibited promising inhibitory effects against a panel of tested bacterial isolates, highlighting its potential as an alternative therapeutic agent for combating bacterial infections.

The results revealed that the susceptibility of the bacterial isolates to the *Annona muricata* (Linn.) leaf extract was concentration-dependent, with higher concentrations showing increased inhibitory effects. This suggests that the extract contains bioactive compounds that possess antibacterial properties.

Furthermore, the study provides valuable insights into the potential application of *Annona muricata* (Linn.) leaf extract in the development of natural

antibacterial agents. These findings support further research to isolate and identify the specific active compounds responsible for the observed antibacterial activity and to explore their mechanisms of action.

This study highlights the importance of investigating natural plant extracts for their antibacterial properties and underscores the potential of *Annona muricata* (Linn.) as a source of bioactive compounds for the development of novel antimicrobial agents. Further studies are warranted to elucidate the therapeutic potential and safety profile of the extract, paving the way for its future application in the field of medicine and pharmaceuticals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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