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Antibacterial Effects of *Garcinia mangostana* Leaf Extracts on Bacterial Strains Isolated from Wound Infections

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Abstract: Several pathogenic bacteria exhibit resistance or have multiple adverse impacts on human health. A diverse array of plants is employed in traditional medicine for the healing of wounds and various ailments. The wound healing potential of the medicinal plant *Garcinia mangostana*, which has been traditionally used for treating wounds and preventing bacterial infections, was evaluated. The bacterial pathogens from accident wound infections were identified using conventional microbiological techniques. Antimicrobial susceptibility testing was performed using the disc diffusion technique following the Kirby-Bauer method. The objective of the present investigation was to detect the presence of bioactive chemicals in methanol extracts of *G. mangostana* plant leaves by phytochemical screening and gas chromatography-mass spectrometry (GC-MS) techniques. The analysis revealed the existence of steroids, terpenoids, and phenolic chemicals in the methanol extracts of the leaves. The GC-MS analysis detected the existence of 20 bioactive components in the methanol extracts of *G. mangostana*. Moreover, the antibacterial activity of the plant extract was assessed using the well diffusion method. The methanol extract exhibited much higher antibacterial activity compared to the conventional antibiotics against all tested species. The medicinal plants possible antibacterial activity against gram-negative and positive bacteria supports its usage as a wound healing agent. The extraction of the active compound from this plant can be utilized as the basis and direction for the development of enhanced or innovative medications in the drug industry.

Keywords: Wound infection, *Garcinia mangostana*, Plant extract, Antibacterial activity, Pathogen, Medicinal plant

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Introduction

The skin is a complicated tissue that covers the entire surface of the body. The skin serves as a protective layer, shielding the body from the external environment. It plays a crucial role in preventing the loss of water and electrolytes, limiting the penetration of chemicals, and

defending against harmful microbes (Albahri *et al*., 2023). Wounds in developed nations result in significant financial burdens, amounting to millions of dollars annually. These wounds pose a significant public health issue due to the various complications they can cause, including infection, retarded healing, and the emergence of bacteria that are resistant to multiple treatments (Daeschlein, 2013).

Typically, the duration since the injury is not the sole determinant in assessing if the wound has become infected. Firstly, it is crucial for bacteria to have sufficient time to multiply and reach a critical number. Additionally, it is necessary to decrease tissue resistance in order to facilitate bacterial multiplication. Every accidental wound contains bacteria, and infection depends on various factors. These factors include the age of the wound, the type and number of bacteria present, the presence of contributory factors like dirt, and the condition of the tissue defenses (Johnston, 1990).

It is important to note that the presence of infection in a wound can significantly impede the healing process, potentially leading to complications such as wound breakdown, herniation, and complete wound dehiscence (Alexander, 1994; Bowler *et al*., 2001). Despite the progress made in surgical techniques and wound care, wound infections remain a significant concern in healthcare settings, particularly among surgical patients. These infections are known to contribute to higher rates of illness and death (Obi, 2015). *Pseudomonas aeruginosa* infection is a severe complication of burns and wounds that can have significant negative effects on health and even lead to death (Augustine *et al*., 2015). *Staphylococcus aureus* is a highly adaptable pathogen that can cause a wide range of illnesses in humans, including both infections and soft tissue infections (Almuhayawi *et al*., 2023).

Medicinal plants have the potential to provide therapeutic agents and a diverse range of chemical constituents that could be used to develop selective drugs. Reservoirs of valuable chemical compounds can offer valuable insights and evidence for modern drug design (Legehar, 2023). The study of chemicals found in medicinal plants has played a crucial role in the development of new drugs for the treatment of various human diseases. Today, traditional medicinal practices play a crucial role in complementary or alternative medicine. While the effectiveness and mechanisms of action of these simple medicinal preparations have not been extensively studied, they can still yield valuable results which is due to their active chemical constituents (Park *et al*., 2009). In addition, numerous modern drugs are derived from natural sources. Plant-derived pharmacological compounds have gained significant recognition due to their wide range of applications. Therapeutic plants have proven to be a valuable resource for a wide range of medications. There is a growing interest in studying the active components of these plants and their effects on the body (Uraku and Nwankwo, 2015).

Phytochemicals found in the leaves, fruits, seeds, bark, and roots of medicinal plants offer natural protection against a range of diseases. These phytochemicals consist of primary and secondary compounds. The primary compounds found in plants are chlorophyll, proteins, and common sugars. On the other hand, secondary compounds include terpenoids, alkaloids, flavonoids, saponins, and phenolic compounds (Saranraj *et al*., 2016). Terpenoids have been found to have important pharmacological effects against inflammation, cancer, malaria, as well as viral and bacterial infections (Yang *et al*., 2020). Alkaloids, which are commonly found in medicinal plants, have various beneficial properties. They can act as anesthetics, inhibit microorganisms, and have antihypertensive and antimalarial effects (Rajput *et al*., 2022). In addition, various contemporary techniques are used to identify and measure bioactive compounds in plant materials. Gas chromatography-mass spectrometry (GC-MS) has emerged as a valuable technique for identifying non-polar components, volatile oils, fatty acids, lipids, and alkaloids (Betz *et al*., 1998).

Mangosteen, also known as the 'queen of fruits,' is an evergreen tree that belongs to the Guttiferae family. It is native to several countries including Indonesia, Malaysia, India, Myanmar, Thailand, the Philippines, and Sri Lanka. Traditional medicine in these regions utilizes both the rind and flesh of the mangosteen fruit to address various ailments, including abdominal pain, diarrhea, dysentery, wound infections, pus, and chronic ulcers. The fruit is made up of approximately 70-75% rind, 10-15% flesh, and 15-20% seeds. The high antioxidant activity of mangosteen rind is attributed to its abundant polyphenolic compounds, such as anthocyanins, tannins, xanthones, and phenolic acids. In addition to its antioxidant properties, the plant demonstrates a variety of pharmacological activities, such as antimicrobial, antimalarial, anti-inflammatory, antidiabetic, antiviral, analgesic, antihistamine, antiobesity, antidepressant, antifungal, antimutagenic, antitumor, anticancer, and antiproliferative effects (Muhamad Adyab *et al*., 2019).

This study aims to evaluate the antibacterial properties of methanol extracts from the leaves of *Garcinia mangostana* against *Pseudomonas aeruginosa and Staphylococcus aureus* bacteria that were isolated from samples of Accident Wounds (AW).

Materials and Methods

Collection of organisms

Microorganisms collected from pus samples of patients after surgery were isolated in a private hospital located in Tirupur, Tamil Nadu, India. The sample was collected using a moistened cotton swab to ensure the absence of any skin microorganisms. The swab samples were collected and placed onto MacConkey agar (Hi-Media, Bangalore), mannitol salt agar, and pseudomonas agar medium (cetrimide). The plates were subsequently placed in an incubator set at a temperature of 37◦C for a duration of 18–24 h. The blood agar plate (BAP) from Oxoid, Ltd. and the chocolate agar plate (CAP) were incubated in a moist environment with a 5% concentration of CO_2 at a temperature range of 35°C–37°C for a period of 18–22 h. Every plate was subjected to a 24-h aerobic incubation period. The plates that exhibited no growth were granted an additional 48 h to cultivate. The physiological and biochemical properties of the isolated organisms *P. aeruginosa* and *S. aureus* were determined using Bergey's manual of systematic bacteriology (Koneman *et al*., 1998).

Antibiotics sensitivity test

The susceptibility of the organisms to the antibiotics was tested using the Bauer disc-diffusion technique, as described by Bauer *et al.* (1966). Antibiotic discs with varying concentrations (µg) were utilized for commercial purposes. The study utilized a total of 5 antibiotics. A group of the isolated micro-organisms was evenly spread across the entire surface of a prepared Mueller Hilton agar plate, and the antibiotic disc was inserted into the plate using a sterilized forceps. Following incubation, the presence of zones of inhibition was noted and their diameters were measured using a caliper. The categorization of the measurement as sensitive, moderate, or resistant was determined based on the interpretative handbook for standard zone size by Majumdar (2004).

Detection of Biofilm production by plate method

The isolates were inoculated in brain heart infusion broth containing 2% sucrose and incubated overnight at 37◦C. They were then diluted with a 1 in 100 ratio of fresh BHI broth. 0.2 ml of the diluted cultures was carefully transferred to individual wells of sterile, polystyrene, 96 well-flat bottom microtitre plates. A control of un-inoculated broth was utilized to verify the sterility of the media and to assess any non-specific binding. The microtitre plates were incubated at 37°C for 24 h. The following day, the contents of each well were carefully removed by gently tapping the plates. The wells were rinsed four times with 0.2 ml of saline solution to eliminate any bacteria that were not attached. The

Table 1: Classification of bacterial adherence

Organism	OD value (570nm)	Adherence	Biofilm production
Blank	<0.006	Non	Non
Pseudomonas aeruginosa (AW – 1)	>0.392	Strong	High
Staphylococcus aureus (AW – 2)	>0.350	Strong	High

plate's biofilms were fixed using a solution of 2% sodium acetate and then stained with 0.1% crystal violet. The optical density value (OD) of stained adherent bacteria was measured using an ELISA reader at a wavelength of 570 nm. The experiments were conducted in triplicate and repeated three times. The data was averaged and the standard deviation was calculated. In order to account for background absorbance, the OD readings of the sterile medium, fixative, and dye were averaged and then subtracted from all the test values. All test OD values were adjusted by subtracting the mean OD value obtained from the media control well (Table 1).

Tube Method

This method is a qualitative approach for detecting biofilm, as outlined by Christensen *et al*. (1995). A loopful of organism was added to 5 ml of Trypticase soy broth, which was then incubated at 37° C for 24 h. After that, the tubes were poured off and rinsed with phosphate buffer saline (pH 7.2) before being left to dry naturally. Afterwards, the tubes were stained using a solution of crystal violet (0.1%) for a duration of 10 min. Following the staining process, the tubes were carefully rinsed with deionized water. The tubes were then left to dry naturally while inverted. The evaluation of biofilm formation was conducted by considering the control strains utilized. When a visible layer forms on the walls and base of the tube, the organisms are considered to be biofilm producing. On the other hand, if a ring forms at the interference of the liquid medium, it indicates that the organism is non-biofilm producing. The experiment was conducted in triplicates.

Plant collection and identification

The *G. mangostana* plant was sourced from a remote location in Trissur, Kerala, India. The plant was identified and authenticated at the Department of Botany at Bharathiar University in Coimbatore, Tamil Nadu. India.

Preparation of extracts

The aerial parts of the *G. mangostana* plant were carefully washed using running tap water to eliminate any dirt. They were then rinsed with distilled water and left to dry in the shade. After drying, the leaves were finely powdered using an electric blender. A thimble was created by wrapping 20 g of the powdered plant material in thick filter paper. This thimble was then placed into the extractor tube and connected to a condenser. A round-bottom flask was used to add 100 ml of methanol, which was then heated at 60°C for 20 min. Once the solvent lost its color, it was collected in the round-bottom flask and concentrated using a rotary vacuum evaporator. The extract was stored in the refrigerator for future use.

Phytochemical Screening

The plant extracts underwent qualitative phytochemical screening to identify the primary classes of compounds. These included tannins,

Table 2: Antibiotic sensitivity pattern of Gram negative and Gram positive bacterial isolates (mm)

Organism	Ciprofloxacin	Streptomycin	Ampicillin	Tetracycline	Cefmetazole
Pseudomonas aeruginosa (AW – 1)	27/S	16/R	R	R	30/S
Staphylococcus aureus (AW – 2)	29/S	13/R	R	R	34/S

saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids. The screening was conducted using standard protocols as outlined by Dubale *et al.* (2023).

Gas Chromatography Mass Spectrometry (GC -MS) analysis

The analysis performed a GC-MS study of the extract using the Agilent 7890A (GC) and Agilent 5975C (inert) MSDs, both made by Agilent Technologies and equipped with triple-axis detectors. A 30 m long and 0.25 mm thick J&W CP-Sil-8 GC column was utilized for the GC separation. With a carrier gas of methanol (5 mg/ml), helium was injected at a rate of 1 ml/min into an injection volume of 1 µl. They employed a split-mode approach (1:20) to inject the samples. The GC oven was set to heat up to 280 °C at 5 °C/min after reaching 120 °C at 10 °C/min after reaching 50 °C for 1 min (held for 2 min). The performance lasted for 65 min. To identify the compounds, spectral configurations were obtained and compared with the mass spectral database NIST -08 SPECTRAL DATA (Hasan *et al*., 2019).

Antimicrobial activity of leaf extracts using the Kirby–Bauer method

The antimicrobial activity of methanol extracts of *Garcinia mangostana* was tested against wound pathogens, specifically *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The screening was conducted using the Agar well diffusion method, with a concentration of 50 mg/ml of the plant extract. A standard antibacterial drug, Streptomycin and Tetracycline (10 µg/ml), was utilized. After incubation, the zone of inhibition was recorded (Abirami *et al*., 2021).

Results and Discussion

Antibiotic sensitivity test

The evaluation of the antibiotic susceptibility pattern revealed the presence of both resistance and sensitivity in the accident wound samples. The sensitivity of *S. aureus* and *P. aeruginosa* to certain antibiotics was observed in the study. The results showed that the pathogens were most sensitive to certain antibiotics, with *S. aureus* showing a sensitivity of 34 mm and *P. aeruginosa* showing a sensitivity of 30 mm. Ciproflaxin also exhibited good sensitivity, with *S. aureus* showing a sensitivity of 29 mm and *P. aeruginosa* showing a sensitivity of 27 mm (Table 2; Fig. 1). These findings suggest that these antibiotics could be effective options for treating these two pathogens. Both pathogens exhibited resistance to Streptomycin, and *S. aureus* and *P. aeruginosa* were also found to be completely resistant to Streptomycin and Tetracycline. Controlling wound infections has become increasingly difficult due to the widespread resistance of bacteria to antibiotics and the higher occurrence of infections caused by methicillin-resistant *S. aureus*, polymicrobic flora, and fungi (Yah *et al*., 2004). The susceptibility pattern of the *S. aureus* isolates in this study revealed a significant level of resistance to commonly used antimicrobial agents. This finding aligns with the research conducted by Regasa *et al*. (2015).

Biofilm formation

The biofilm formation of *S. aureus* and *P.*

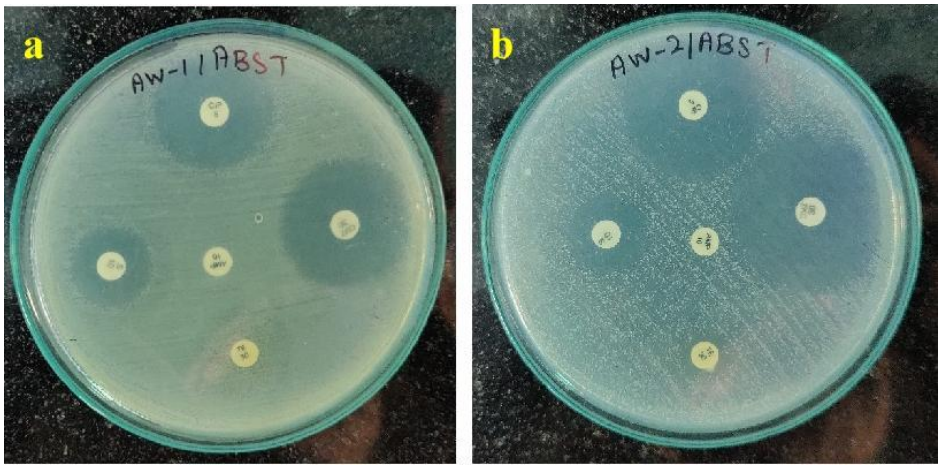

Fig. 1: Antibiotic sensitivity pattern of Gram negative and positive bacterial isolates (a) *Pseudomonas aeruginosa* (AW – 1) and (b) *Staphylococcus aureus* (AW – 2).

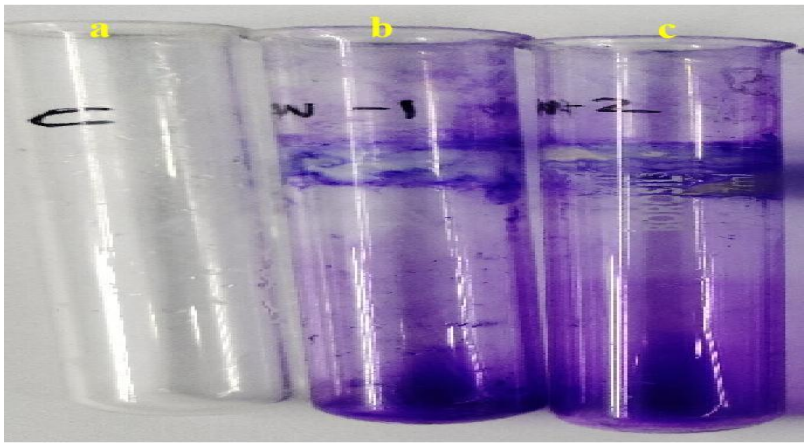

Fig. 2: Biofilm formation assay by tube method (TM). (a) control (PC); (b and c) strong biofilm producer (*Pseudomonas aeruginosa* and *Staphylococcus aureus*).

aeruginosa generated through the application of the crystal violet staining method. In glass test tubes, we let the microorganisms *S. aureus* and *P. aeruginosa* form separate biofilms. Next, we stained the remaining biofilm with crystal violet and measured and studied it. As seen in Figures 2 b and c, the bacterial strains were able to form biofilms on the glass test tube surface. There was no biofilm formation in the absence of bacterial strains (Fig. 2a).

The phytochemical research approach is thought to be useful in identifying the bioactive profiles of plants with medicinal value.

Primary Phytochemical Screening

The qualitative approximation of phytochemical components in the methanol extracts of *G. mangostana* had shown the existence of steroids, alkaloids, quinoines, proteins, saponins, flavonoids, glycosides, tannins, and phenol (Table 3; Fig. 3).

These secondary metabolites have been extensively studied for their diverse biological and therapeutic properties, as indicated by Cavazos *et al*. (2021). This suggests that this particular species holds great potential for medicinal applications. Mangosteen species contain a variety of bioactive substances, such as mangostin, tannins, xanthones, xanthenes, anthocyanins, flavones, phenolic compounds, and phytosterols (Wittenauer *et al*., 2012; Cunha *et al*., 2014; Abubakeer, 2015; Fayaz Pasha *et al*., 2015). In a

Table 3: Phytochemical screening of methanol extracts of *G. mangostana*

Chemical constituent	**Methanol leaf extract *Garcinia mangostana***
Tannins	+
Flavonoids	+
Saponins	+
Steroids	+
Glycosides	+
Phenols	+
Alkaloids	-
Quinoines	-
Proteins	+

Fig. 3: Phytochemical screening of methanol extracts of *G. mangostana.*

study by Ritthiwigrom *et al*. (2013), various compounds were found in *Garcinia cowa*, including phloroglucinols, flavonoids, xanthones, terpenoids, alkaloids, saponins, glycosides, carbohydrates, phenolics, tannins, phytosterols, garcinol, anthocyanins, and hydroxycitric acid. Similarly, *Garcinia indica* was also found to contain garcinol, anthocyanins, and hydroxycitric acid, along with flavonoids and xanthones. Flavonoids are found in abundance in photosynthesizing cells, making them prevalent throughout the plant kingdom. Previous studies have demonstrated that flavonoids have antimicrobial properties against pathogenic microbes. Terpenoids are a diverse group of natural products that have a variety of biological activities. They can act as antibiotics, anti-inflammatory agents, anti-HIV and anti-tumor substances, hypotensive agents,

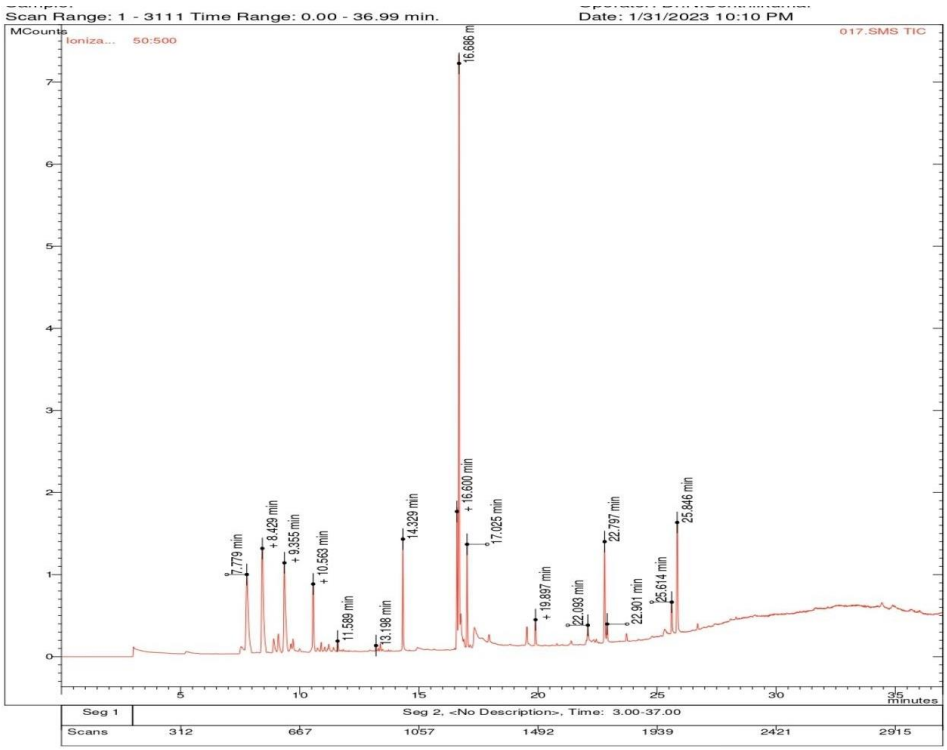

Fig. 4: GC-MS spectra of methanol extract from the leaves of *Garcinia mangostana* plant.

sweeteners, insecticides, anti-feedants, phytotoxic agents, perfumery intermediates, and plant growth hormones (Alsultan *et al*., 2017).

GC-MS analysis

The chemical profile of the extract of *G. mangostana* was analyzed using gas chromatography-mass spectrometry (GC-MS) (Fig. 4). Gas chromatography-mass spectrometry (GC-MS) is a highly useful technique that allows for the separation and identification of volatile and semi-volatile compounds. Our study provides strong evidence for the reliability of GC-MS, as it successfully extracts compounds in their pure form. The tool's effectiveness, when used with the right solvents, improves the overall product output. Table 4 illustrates a detailed account of the various bioactive compounds found in the methanol extracts of *G. mangostana* leaves, as identified through GC-MS analysis. Through GC-MS analysis, a total of 20 compounds were identified, each with unique retention times, peak areas, and mass spectra. Several studies have been conducted on different *Garcinia* species by various researchers, including Zadernowski *et al*. (2009), Madappa and Bopaiah (2012), Ogunmoyole *et al*. (2012), Widowathi *et al*. (2014) and Li and Xu (2015).

Antibacterial effect of Methanol leaf extract Garcinia mangostana

The Methanol leaf extract of *G. mangostana* was tested for its antibacterial activity against *P. aeruginosa* and *S. aureus* using the Agar well diffusion method. The methanol leaf extract *G. mangostana* demonstrated significant anti-bacterial efficacy against *P. aeruginosa* and *S. aureus* at a concentration of 50 mg/ml. The antibacterial zone of inhibition for *P. aeruginosa* was the largest, measuring 14 mm, while *S. aureus* had a slightly smaller zone of 10 mm. It is worth mentioning that it exhibited more potent antibacterial activity compared to the standard antibacterial drug (Table 5; Fig. 5). Additionally, it demonstrated antimicrobial activity against bacterial pathogens involved in wound healing.

Table 4: Chemical profile of Methanol leaf extract *Garcinia mangostana*

S. No.	NAME OF THE COMPOUND	RETENTION TIME (RT)	AREA
1	Copaene	7.779	6.03E+06
2	Bicyclo[5.2.0]nonane, 2-meth	8.429	6.90E+06
3	1,4,7,-Cycloundecatriene, 1,	8.909	820392
4	Naphthalene, 1,2,3,4,4a,5,6	9.103	1.02E+06
5	Naphthalene, 1,2,3,5,6,7,8,8	9.355	5.50E+06
6	Naphthalene, 1,2,3,5,6,8a-he	9.63	360093
7	Naphthalene, 1,2,3,4-tetrahy	9.718	491569
8	Caryophyllene oxide	10.563	2602000
9	12-Oxabicyclo[9.1.0]dodeca-3	10.899	277376
10	7-Octadecyne, 2-methyl-	13.198	127620
11	Hexadecanoic acid, methyl es	14.329	2886000
12	9,12-Octadecadienoic acid (Z	16.6	3.71E+06
13	9-Octadecenoic acid, methyl	16.686	1.76E+07
14	9-Octadecenoic acid, methyl	16.761	337984
15	Heptadecanoic acid, 15-methy	17.025	2.77E+06
16	11-Eicosenoic acid, methyl e	19.535	496920
17	Eicosanoic acid, methyl este	19.897	816387
18	9-Octadecenoic acid, 1,2,3-p	22.093	513514
19	Docosanoic acid, methyl este	22.797	3.26E+06
20	Phthalic acid, 6-ethyloct-3-	22.901	614741

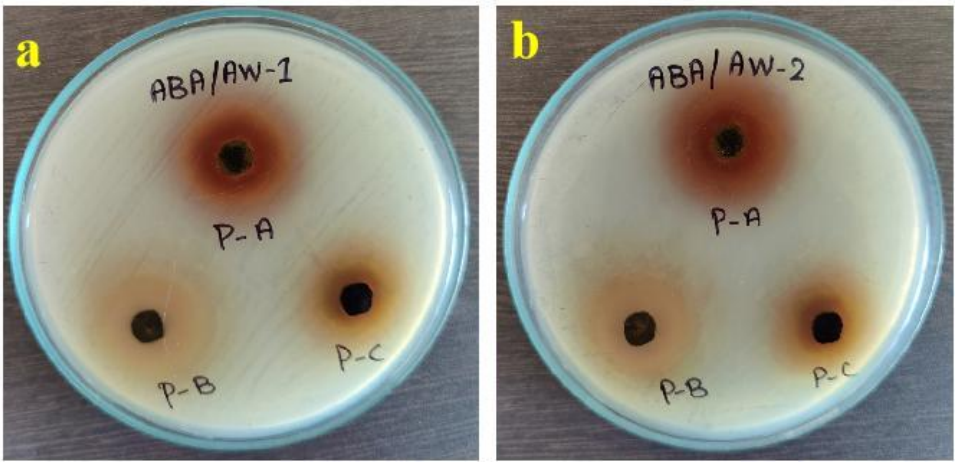

Fig. 5: Activity of methanol leaf extract *Garcinia mangostana* against the tested microbial species, (a) *Pseudomonas aeruginosa* (AW – 1) and (b) *Staphylococcus aureus* (AW – 2).

Table 5: Inhibition zone of the Methanol leaf extract *Garcinia mangostana* activity against the tested pathogenic microbe

Organism	ZONE OF INHIBITION (mm)		
	Methanol leaf extract *Garcinia mangostana* (P.A)	**Streptomycin (P.B)**	**Tetracycline (P.C)**
Pseudomonas aeruginosa (AW – 1)	14	16	7
Staphylococcus aureus (AW – 2)	10	15	5

Medicinal plants contain a wide variety of phytochemicals, which are structurally diverse molecules. These phytochemicals have been found to possess potent antimicrobial properties against a broad spectrum of bacteria (Gutierrez *et al*., 2013; Ubhrani *et al*., 2023). According to a recent report, the antibiofilm test revealed that the extract from mangosteen leaves demonstrated the highest efficacy in inhibiting biofilm formation, preventing cell attachment, and destroying biofilm (Gubali, *et al*., 2024). Previous studies have also documented the effectiveness of mangosteen pericarp extracts in combating methicillin-resistant *S. aureus* (Pohan *et al*., 2022). The findings of this study are consistent with previous research which has shown that *Garcinia* species have natural compounds that are highly effective against *S. aureus* bacteria (Lakshmi *et al*., 2011).

Conclusion

The methanol extracts of *G. mangostana* leaves showed antimicrobial activity against *S. aureus* and *P. aeruginosa* bacterial isolates that are commonly found in accident wound infections. The methanol leaf extract of *G. mangostana* showed significant activity and exhibited bactericidal effects against the bacterial strains that were tested. On the other hand, extracts of *G. mangostana* demonstrated a moderate level of activity against the majority of the tested strains at a concentration of 50 mg/ml. The findings of this study provide support for the long-standing assertion that this medicinal plant possesses antibacterial properties that can be effective in treating wound infections. Additional investigations should be conducted before suggesting their application.

Ethical Statement

The study involves only bacterial culture and not contain any human/animal sample.

Author Contributions

Purushothaman T.: Conceptualization, writing original draft; *Saranya N.*: Supervision; *Karukuvelraja R.* and *Vinitha Ebziba C.*: editing and validation. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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