

Research Article	Pak-Euro Journal of Medical and Life Sciences	
DOI: 10.31580/pjmls.v8i4.3257	Copyright © 2025 All rights are reserved by Corresponding Author	
Vol. 8 No. 4, 2025: pp. 821-832		
www.readersinsight.net/pjmls	Revised: December 25, 2025	Accepted: December 28, 2025
Submission: November 01, 2025	Published Online: December 31, 2025	

ANTIBACTERIAL POTENTIAL OF MANGIFERA INDICA SEED EXTRACTS AGAINST MULTIDRUG RESISTANT PATHOGENS

Waleed Khan¹, Umer Shah¹, Imad Ali Shah¹, Latif U Rahman¹, Ali Raza¹, Waheed Ahmed¹, Qaiser Iqbal¹, Muhammad Farooq^{2*}

¹Department Microbiology, Abdul Wali Khan University, Mardan, Pakistan

²University Institute of Diet and Human Nutritional Sciences, The University of Lahore, Lahore, Pakistan

*Corresponding Author: Muhammad Farooq E. mails: farooq.fst28@gmail.com



Abstract

In the present study, crude methanolic and aqueous extracts of *Mangifera indica* (mango) seeds were evaluated for their antibacterial and hemolytic activities against multidrug-resistant (MDR) bacterial pathogens, including vancomycin-resistant *Staphylococcus aureus* (VRSA), vancomycin-resistant *Staphylococcus epidermidis* (VRSE), and *Klebsiella pneumoniae*. Three different concentrations of the extracts (10, 20, and 30 mg/mL) were tested against these MDR strains. The highest antibacterial activity, indicated by a zone of inhibition of 34 mm, was observed with the 30 mg/mL aqueous extract against VRSE, while the lowest activity (13 mm) was recorded for the 10 mg/mL methanolic extract against *Klebsiella pneumoniae*. In the hemolysis assay, the maximum hemolytic activity (4.2%) was exhibited by the 30 mg/mL methanolic extract, whereas the minimum hemolysis (0.8%) was observed with the 10 mg/mL aqueous extract. Overall, the findings suggest that *Mangifera indica* seed extracts contain bioactive compounds with significant antibacterial and hemolytic properties. This study provides a foundation for future research aimed at isolating and characterizing these bioactive constituents for potential pharmaceutical applications. The GC-MS analysis successfully identified 18 compounds, revealing that the sample is primarily composed of fatty acids, with Oleic acid (32.46%) and Stearic acid (20.18%) being the major constituents and together accounting for over half of the composition. Significant levels of the phenolic antioxidant 1, 2, 3-Benzenetriol (Pyrogallol) (9.58%) were also found, indicating a rich mixture of lipids and bioactive phenolics.

Keywords: Antibacterial properties, Antibiotic resistance GC, *K. pneumoniae*, MDR bacteria, VRSA, VRSE

INTRODUCTION

Medicinal plants and herbs have been widely utilized across the world, historically serving as the primary sources of therapeutic agents. These natural remedies have played a crucial role in the development of modern pharmaceuticals. *Mangifera indica* (mango) is one of the most commonly consumed tropical fruits; however, its seeds, which are usually discarded as waste, contain bioactive compounds with notable antimicrobial properties. Research indicates that the seed extracts of *Mangifera indica* contain flavonoids, phenolics, tannins, and saponins all of which are responsible for their antibacterial activity. Evaluating the efficiency of these seed extracts against multidrug-resistant (MDR) bacterial strains is important to determine their potential as alternative therapeutic agents (1).

Bacterial infections continue to be a major public health problem worldwide, largely due to the increasing prevalence of MDR pathogens. Among these, *Vancomycin-resistant Staphylococcus aureus* (VRSA), *Vancomycin-resistant Staphylococcus epidermidis* (VRSE), and *Klebsiella pneumoniae* represent significant challenges because of their ability to resist conventional antibiotic treatments. The rapid emergence of such resistant pathogens has created an urgent need for new, naturally derived antimicrobial agents. One such promising candidate is *Mangifera indica* seed extract, which has demonstrated considerable antibacterial potential in previous studies (2). A substantial proportion of modern antibiotics have originated from natural or semisynthetic sources. It is estimated that around 20% of the world's plant species have undergone pharmacological or biological screening, and over 80% of the global population in developing countries relies

on herbal medicines as their primary healthcare resource (3). According to the World Health Organization (WHO), medicinal plants are among the most promising resources for discovering diverse bioactive compounds. Furthermore, WHO reports that approximately 80% of the global population still depends on herbal remedies for managing various health conditions (4). In Pakistan, particularly in rural areas, herbal medicines are widely used due to their perceived effectiveness, affordability, and minimal side effects, making them a preferred alternative to conventional therapies. Growing concerns about the side effects of synthetic antibiotics and the alarming rise of antimicrobial resistance have intensified scientific interest in exploring the antimicrobial potential of herbal extracts (5).

Mangifera indica is well recognized for its nutritional and pharmacological significance, with many of its therapeutic components concentrated in the seed. While the pulp of the fruit is commonly consumed, the seed is often discarded despite being rich in valuable phytochemicals with medicinal potential (6). Previous research has revealed that *Mangifera indica* seeds possess antimicrobial, antioxidant, and anti-inflammatory properties, making them suitable for applications in the pharmaceutical and nutraceutical industries (7). The biochemical constituents of *Mangifera indica* seed extract include polyphenols, flavonoids, tannins, and essential oils—compounds known to exhibit strong antibacterial effects. Flavonoids and phenolic acids, in particular, are known to disrupt bacterial cell membranes, deactivate bacterial enzymes, and interfere with bacterial communication systems (8). Tannins also exert antibacterial effects by coagulating microbial proteins, preventing normal bacterial function and reproduction.

The roots and seeds of *Mangifera indica* have been used traditionally to treat infections, digestive disorders, and skin diseases. Experimental studies have shown that its bioactive compounds possess antifungal and antibacterial activities against several bacterial species, including the Gram-positive *Staphylococcus aureus* and Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. In addition to its antimicrobial activity, *Mangifera indica* seed extract also exhibits antioxidant properties that help reduce oxidative stress and inflammation during infections. These dual activities highlight the potential of *Mangifera indica* seed extract as a natural antibiotic candidate, particularly in combating MDR bacterial infections (9).

Given the increasing global emphasis on developing natural products as antimicrobial agents, *Mangifera indica* seed extract represents a valuable and underexplored area of research. More comprehensive studies are required to confirm its safety in clinical applications, optimize extraction methods, and understand its mechanisms of action against MDR bacteria (10). While several studies have explored plant-based antimicrobials, there is still limited information on the effectiveness of *Mangifera indica* seed extract against MDR bacterial clinical isolates. The scarcity of extensive research on this topic highlights a significant knowledge gap and presents a valuable opportunity for further scientific investigation (11). The growing emergence of antibiotic-resistant bacteria has become one of the most serious challenges in modern medicine. MDR pathogens such as VRSA, VRSE, and *Klebsiella pneumoniae* are responsible for increased mortality rates, longer hospital stays, and higher treatment failure rates. Many antibiotics that were once highly effective have become less potent as bacteria continue to evolve resistance mechanisms.

This situation emphasizes the urgent need to identify alternative antimicrobial compounds from natural sources. Despite various studies on plant-derived antimicrobials, limited research has focused on the antibacterial efficacy of *Mangifera indica* seed extract against MDR clinical isolates (12).

The current research, therefore, aims to evaluate the antibacterial potential of *Mangifera indica* seed extracts against MDR bacterial clinical isolates, particularly VRSA, VRSE, and *Klebsiella pneumoniae* (13). Identifying the phytochemicals responsible for antibacterial activity could support the development of new natural therapeutic agents for managing antimicrobial resistance (13). MDR bacteria such as VRSA, VRSE, and *K. pneumoniae* have increasingly burdened healthcare systems worldwide. Moreover, many of these pathogens form biofilms that act as protective barriers, making conventional antibiotics less effective. Given these challenges, the search for novel, safe, and cost-effective antimicrobial agents is more crucial than ever. *Mangifera indica* seed extract, being non-toxic, easily available, and inexpensive, holds significant promise as a natural alternative (14). The present study seeks to investigate its antibacterial properties against MDR bacteria to bridge the gap between natural product research and clinical microbiology. Exploring its inhibitory

mechanisms and potential pharmaceutical applications will contribute valuable insights toward developing novel plant-derived antimicrobials. As resistance to conventional antibiotics continues to rise, the importance of exploring plant-based alternatives such as *Mangifera indica* seed extract becomes increasingly evident (15).

MATERIALS AND METHODS

PLANT SELECTION AND COLLECTION

Plant samples were selected based on ethnobotanical knowledge, particularly local community recommendations and their traditional use in treating various ailments. *Mangifera indica* (mango) seeds were collected from freshly processed fruits obtained from local juice shops in Mardan, ensuring the use of mature and healthy seed samples for subsequent experimental analysis.

PLANT PROCESSING

The *Mangifera indica* seeds were thoroughly washed with clean water to remove any adhering impurities or debris. The seeds were then air-dried at room temperature until complete moisture evaporation was achieved. Once fully dried, the seeds were ground into a fine, homogeneous powder using a mechanical grinder to ensure uniform particle size and efficient extraction of bioactive compounds in subsequent procedures.

PREPARATION OF MANGIFERA INDICA SEED EXTRACTS

Extraction was carried out using a methanol and distilled water. Approximately 50 mg of *Mangifera indica* seed powder was mixed with 500 ml of methanol and 500 ml of distilled water. Then the mixtures were placed in shaker incubator to for 2 weeks. After the incubation the mixture were filtered through Whatman filter paper. Then both the filtrate (Methanol and Water) transferred into beaker and were placed in water bath at 45°C to evaporate the methanol and water, and after the evaporation, yielding 10.5 ± 0.5 g of aqueous extract and 9 ± 0.5 g of methanol extract were obtained.

PREPARATION OF STOCK SOLUTIONS

For testing, three concentrations of seed extracts (10 mg, 20 mg, and 30 mg) were prepared by dissolving the respective quantity in 1 ml of Dimethyl sulfoxide (DMSO). This yielded six different stock solutions—three methanol extracts (10 mg/ml, 20 mg/ml, and 30 mg/ml) and three aqueous extracts (10 mg/ml, 20 mg/ml, and 30 mg/ml).

COLLECTION OF CLINICAL BACTERIAL SAMPLES

Three clinical bacterial strains were collected from Mardan Medical Complex.

Sr. No.	Pathogens	Characteristics	Diseases
1	<i>Staphylococcus aureus</i>	Resistant to vancomycin, Gram positive	Bactremia (Tong <i>et al.</i> , 2025)
2	<i>Staphylococcus epidermidis</i>	Resistant to vancomycin, Gram positive	Bactremia (Kleinschmid <i>et al.</i> , 2015)
3	<i>Klebsiella pneumoniae</i>	Multi drug resistant, Gram negative	Pyrengitis (Klaper <i>et al.</i> , 2021)

REFRESHED BACTERIAL CULTURE

The bacterial samples were refreshed and sub-cultured in nutrient broth and nutrient agar to ensure active growth before antimicrobial testing.

ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS OF MANGIFERA INDICA SEED

Mueller-Hinton Agar (MHA) plates were prepared and inoculated with the bacterial strains (*Vancomycin resistance Staphylococcus aureus*, *Vancomycin resistance Staphylococcus epidermidis*, *Klebsiella pneumoniae*). Four wells were made in each plate. Three wells were filled with the extract solutions of 10 mg/mL, 20 mg/mL, and 30 mg/mL, and one well as a negative control (DMSO). A positive control antibiotic disc (vancomycin for

Vancomycin resistances staphylococcus aureus and *Vancomycin* resistance *Staphylococcus epidermidis*, cephadrine for *K. pneumoniae*) were used. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured in millimeters.

HEMOLYTIC ACTIVITY OF CRUDE EXTRACTS OF *MANGIFERA INDICA* SEED

Hemolytic activity of *Mangifera indica* seed extracts were determined by using protocol as described by (16). Further blood was collected from a healthy person under aseptic conditions in sterile EDTA tube. 3 ml of blood was poured into a sterile falcon tube. Then 9 ml of PBS was added in order to make a (1:3) blood to PBS solution. Then the tubes were mixed by inverting them a number of times to prepare a uniform mixture. Falcon tubes were centrifuge at 1500 rpm for 10 minutes. Supernatant was slowly discarded following centrifugation. Red blood cell pellet (RBCs) was resuspended in PBS. Washing was performed three times for elimination of any remaining excess plasma and soluble components. After the last wash, RBC pellet was suspended in PBS to make a uniform erythrocyte suspension. Methanol and aqueous extracts were made at three different concentrations 10 mg/ml, 20 mg/ml and 30 mg/ml the extracts. 500 µL of suspension were added into sterile Eppendorf tubes. And the pre-prepared concentration 500µl of the extract were added to it. Both aqueous and methanolic extracts were treated at each concentration. Treated sample tubes were incubated at 37°C for 1 hour in order to allow interaction among erythrocytes and plant extracts. After incubation, the samples were placed in cuvettes and absorbance was measured at 570 nm using a UV-Visible spectrophotometer. From the reading of the absorbances, cytotoxic activity was assessed for the extracts based on hemolysis. Triton X-100 was used as positive control and PBS was used as negative control. Hemolysis percentage was calculated through the following formula.

$$\% \text{ Hemolysis} = \frac{(\text{Sample of Abs} - \text{negative control Abs}) \times 100}{(\text{Positive control Abs} - \text{negative control Abs})}$$

GAS CHROMATOGRAPHY MASS SPECTROPHOTOMETER (GC-MS) COMPOSITION OF *MANGIFERA INDICA* METHANOLIC EXTRACT

Gas chromatography–mass spectrometry (GC-MS) analysis was conducted to identify the bioactive chemical constituents present in the extract. A total of 18 peaks were detected, corresponding to various fatty acids, esters, phenols, and other organic compounds. The identification was based on mass spectral comparison with the NIST11.L library.

STATISTICAL ANALYSIS

Statistical analysis was done through SPSS software. One way ANOVA, Duncan test was done for all the triplicates results. The p-values <0.05 was considered statistically significant.

RESULTS

Mangifera indica seed was collected on the bases of informant consent. Antibacterial activity of crude extracts showed best results against all the MDR pathogens (*VRSA*, *VRSE* and *k. pneumoniae*). The crude extracts also showed best hemolytic results.

ANTIBACTERIAL ACTIVITY OF *MANGIFERA INDICA* SEED EXTRACTS AGAINST *VRSA*

The highest zone of inhibition was observed in 30 mg/ml concentration of aqueous extract against *Vancomycin Resistance Staphylococcus Epidermidis* (34 mm) and lowest zone of inhibition was observed in 10 mg/ml concentration of methanolic extract (13 mm) against *staphylococcus aureus* as shown in Fig. 1 and (Table I).

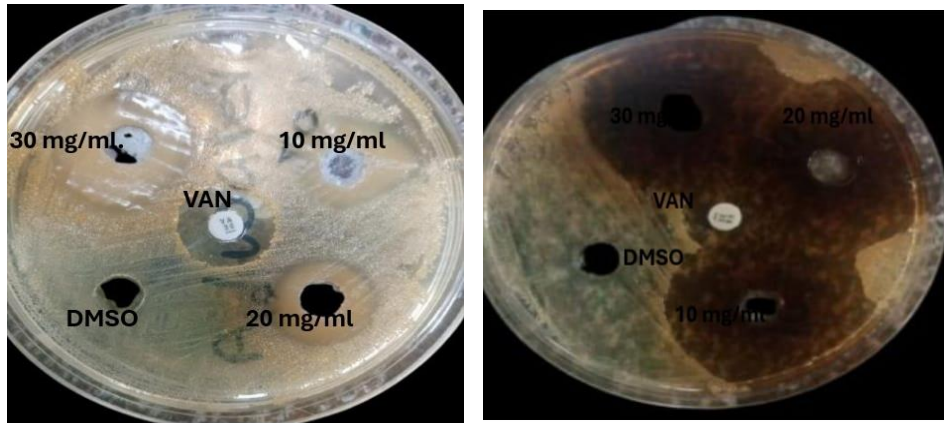


Fig. 1. *Mangifera indica* methanolic and aqueous extracts (10, 20 and 30 mg/ml) against *Vancomycin Resistance Staphylococcus Aureus*, DMSO was used as negative control while vancomycin was used as positive control.

Table I. Atibacterial activity of *Mangifera indica* seed extracts against VRSA

Bacterial strain	Solvent	Concentration (mg/ml)	Zone of inhibition crude extract (mm)	Antibiotic zone (mm)	Negative control (DMSO)
<i>Vancomycin Resistant Staphylococcus aureus</i>	Distilled	10	25±0.5	Vancomycin	0.0±0.0
	Water	20	28±0.5	12±0.5	
		30	31±0.5		
<i>Staphylococcus aureus</i>	Methanol	10	19±0.5	Vancomycin	0.0±0.0
		20	22±0.5	12±0.5	
		30	27±0.5		

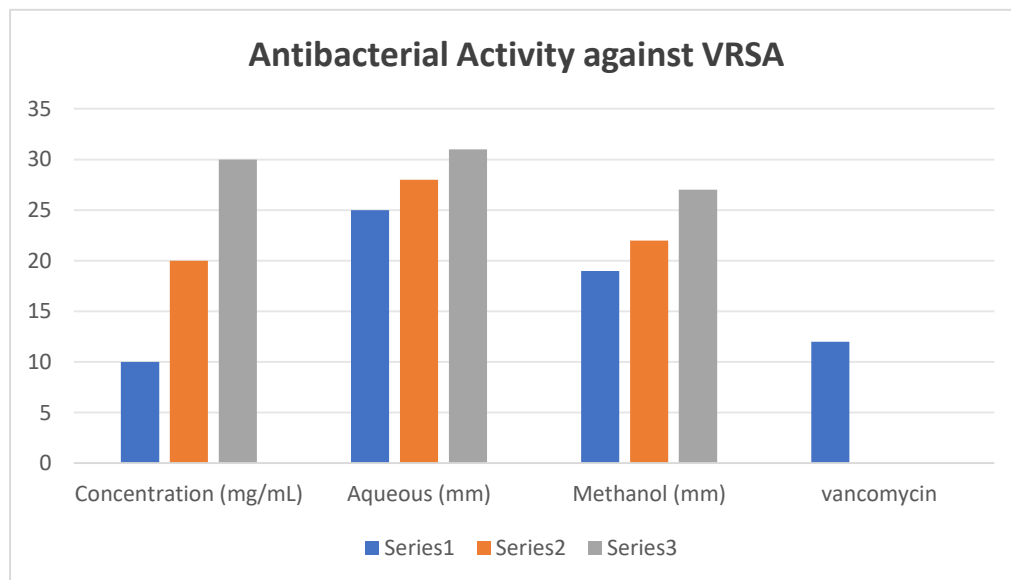


Fig. 2. *Mangifera indica* methanolic and aqueous extracts (10, 20 and 30 mg/ml) against *Vancomycin Resistance Staphylococcus Aureus*, DMSO was used as negative control while vancomycin was used as positive control (b): Antibacterial activity of *Mangifera indica* Methanol, Aqueous extracts against VRSA (10, 20, 30mg/ml) vancomycin used as a positive control

ANTIBACTERIAL AACTIVITY OF MANGIFERA INDICA SEED EXTRACTS AGAINST VRSE

Mangifera indica seed was collected on the bases of informant consent. Antibacterial activity of crude extracts showed best results against all the MDR pathogens. The highest zone of inhibition was observed in 30 mg/ml concentration of aqueous solution against *Vancomycin Resistance Staphylococcus Epidermidis* 34 mm

and lowest zone of inhibition was observed in 10 mg/ml concentration of methanolic extract 16 mm against *vancomycin resistance Staphylococcus epidermidis* as shown in Fig. 2 & 3 and Table II.

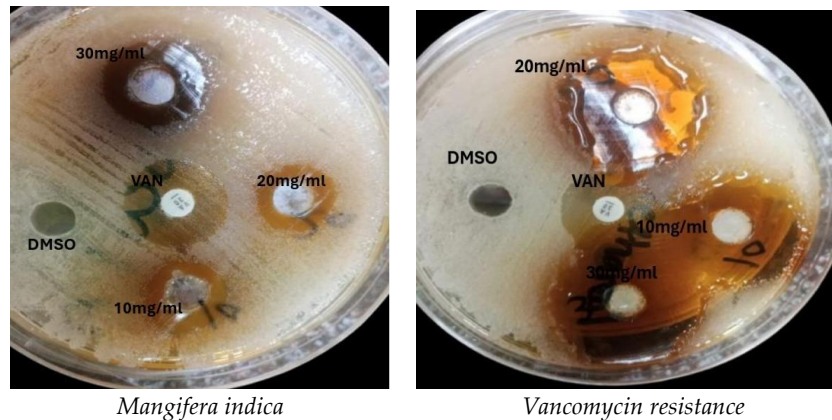


Fig. 3. *Mangifera indica* methanolic and aqueous extracts (10, 20 and 30 mg/ml) against *Vancomycin Resistance Staphylococcus Aureus*, DMSO was used as negative control while vancomycin was used as positive control.

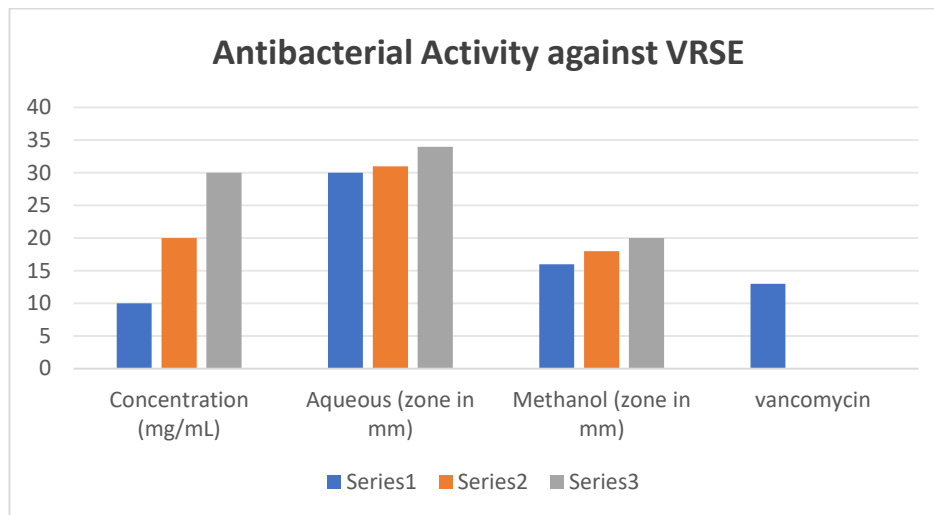


Fig. 4. Antibacterial activity of *Mangifera indica* Methanol, Aqueous extracts against VRSE (10, 20, 30mg/ml) vancomycin used as a positive control

Table II. Antibacterial activity of *Mangifera indica* seed extracts against VRSE

Bacterial strain	Solvent	Concentration (mg/ml)	Zone of inhibition crude extract (mm)	Antibiotic zone(mm)	Negative control (DMSO)
<i>Vancomycin Resistant Staphylococcus epidermidis</i>	Distilled water	10	30±0.5	Vancomycin 13±0.5	0.0±0.0
		20	31±0.5		
		30	34±0.5		
<i>Vancomycin Resistant Staphylococcus epidermidis</i>	Methanol	10	16±0.5	Vancomycin 13±0.5	0.0±0.0
		20	18±0.5		
		30	20±0.5		

ANTIBACTERIAL ACTIVITY OF MANGIFERA INDICA SEED EXTRACTS AGAINST *K. PNEUMONIAE*

Mangifera indica seed was collected on the bases of informant consent. Antibacterial activity of crude extracts showed best results against all the MDR pathogens. The highest zone of inhibition was observed in 30 mg/ml concentration of aqueous solution against *Vancomycin Resistance Staphylococcus Epidermidis* 21mm

and lowest zone of inhibition was observed in 10 mg/ml concentration of methanolic extract 13mm against *klebsiella pneumoniae* as shown in Fig. 5 & 6 and Table III.

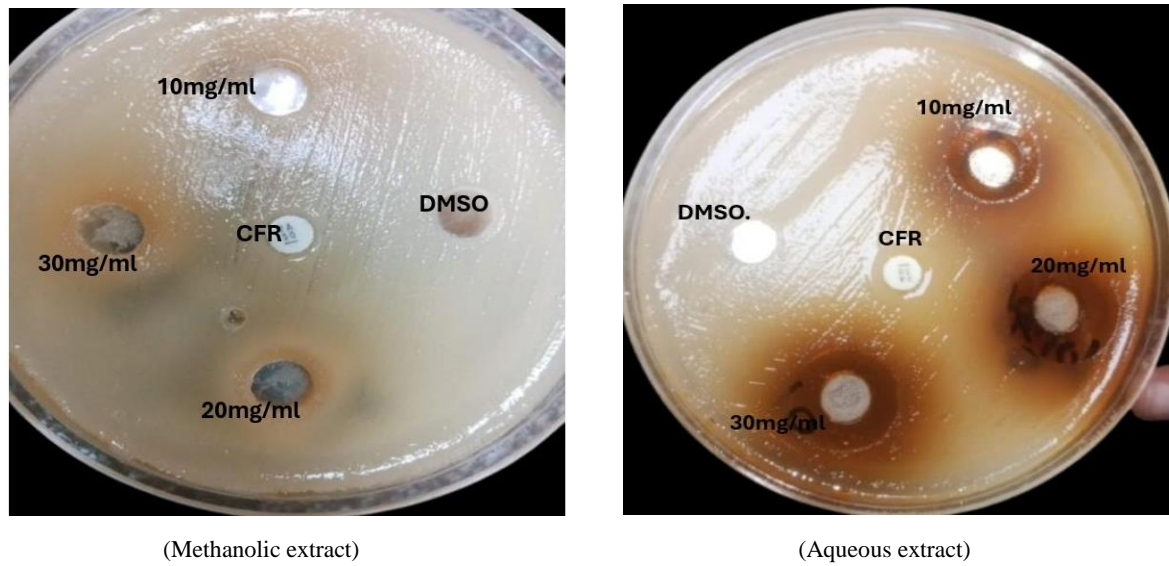


Fig. 5. *Mangifera indica* methanolic and aqueous extracts (10, 20 and 30 mg/ml) against *K. pneumoniae*, DMSO was used as negative control while cephradine was used as positive control

Table III. Antibacterial activity of *Mangifera indica* seed extracts against *K. pneumoniae*

Bacterial strain	Solvent	Concentration (mg/ml)	Zone of inhibition of crude extract (mm)	Antibiotic zone (mm)	Negative control (DMSO)
<i>Klebsiella pneumoniae</i>	Distilled Water	10	18±0.5	Cephradine	0.0±0.0
		20	20±0.5	2±0.5	
		30	21±0.5		
	Methanol	10	13±0.5	Cephradine	0.0±0.0
		20	18±0.5	2±0.5	
		30	20±0.5		

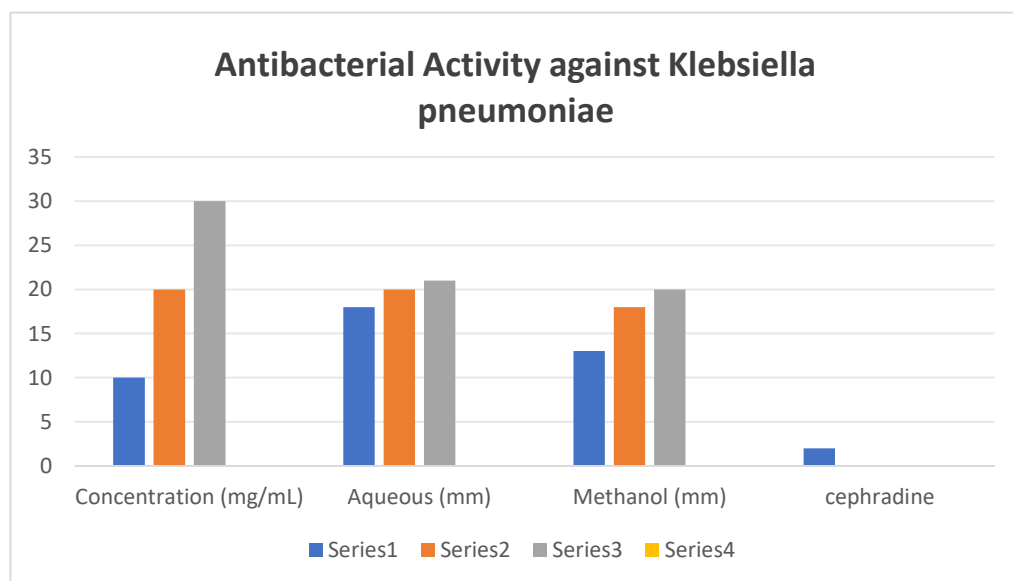


Fig. 6. Antibacterial activity of *M. indica* methanolic and aqueous extracts against *K. pneumoniae* (10, 20 and 30 mg/ml) cephradine use as a positive control

HEMOLYTIC ACTIVITY OF *MANGIFERA INDICA* SEED EXTRACTS

Hemolytic activity of *Mangifera indica* seed crude methanol extract (30mg/ml) showed the highest hemolysis (4.17%), while the minimum hemolysis was (2.17%) shown by aqueous extract as shown in Fig. 7 and Table IV.

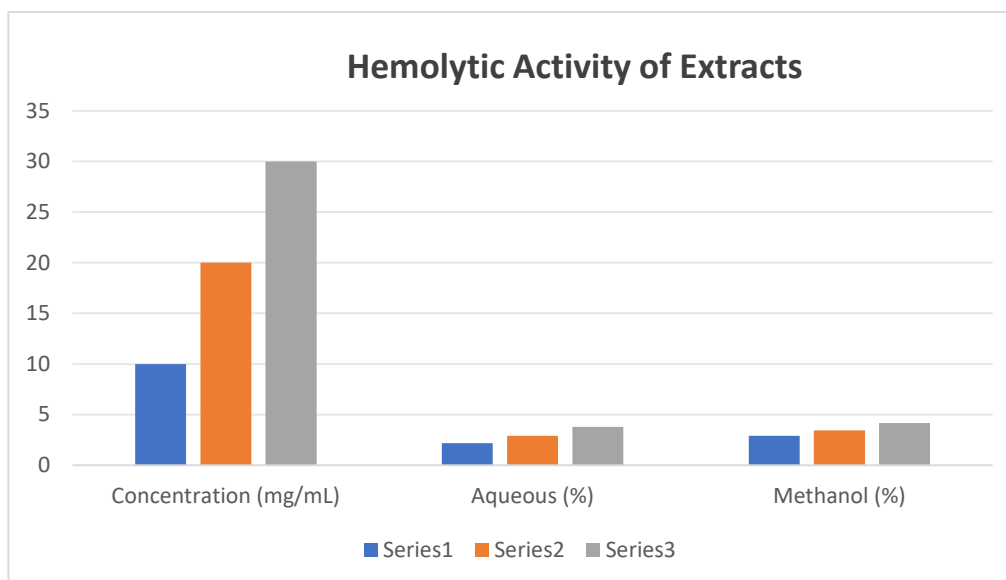


Fig. 7. *Mangifera indica* seed extracts (Methanolic and aqueous) hemolytic activity. Triton X was used as a positive control and PBS as a negative control

Table IV. Hemolytic activity of *Mangifera indica* seed extracts

Plant used	Type of extract	Concentration (mg/ml)	% Hemolysis
<i>Mangifera indica</i>	Distilled water	10	2.17±0.5
	Distilled water	20	2.91±0.5
	Distilled water	30	3.80±0.5
	Methanol	10	2.91±0.5
	Methanol	20	3.43±0.5
	Methanol	30	4.17±0.5
	Negative control (PBS)		0.52
	Positive control (Triton-X)		100

GC-MS ANALYSIS SUMMARY OF IDENTIFIED COMPOUNDS

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis successfully identified 18 different compounds in the sample, detailed in Table V. The compounds are diverse, encompassing fatty acids and their methyl esters, phenolic compounds, and other various organic molecules. The analysis revealed that the sample is predominantly composed of Oleic acid (cis-9-Octadecenoic acid), which is the most abundant compound, accounting for 32.46% of the total area. This monounsaturated omega-9 fatty acid is a key component. The second most prominent compound is Stearic acid (Octadecanoic acid), a saturated fatty acid, at 20.18%. Together, these two major fatty acids constitute over half of the identified compounds (52.64%). Significant Compound Groups Fatty Acids and Esters: This group forms the largest part of the identified components. Besides Oleic acid and Stearic acid, other significant fatty acids/esters include Palmitic acid (6.71%) and its ester Methyl palmitate (1.01%). Other fatty acid esters like Oleic acid, methyl ester (4.05%), Methyl stearate (2.23%), and Linoleic acid, methyl ester (0.52%) were also detected. Phenolic Compounds: Phenol, 3-pentadecyl- was repeatedly detected (1.04%, 6.69%, 4.61%), highlighting its presence.

1,2,3-Benzenetriol (Pyrogallol), a strong antioxidant, was also found in a notable concentration (9.58%). Minor Components: Other compounds present in smaller amounts include Glycerol monooleate (3.19%), Bis(2-ethylhexyl) phthalate (0.75%), and fundamental organic molecules like Glycerin (Glycerol) (0.65%).

DISCUSSION

Medicinal plants have increasingly gained attention in recent years for their diverse bioactive compounds and broad therapeutic potential, particularly their antimicrobial, antioxidant, and cytotoxic properties. These natural products are recognized as rich sources of pharmacologically active compounds that can serve as alternatives to synthetic drugs, especially in the face of rising antimicrobial resistance (17-18). Among these, *Mangifera indica* (mango) has emerged as a promising candidate due to its significant antimicrobial potential. The phytochemical constituents of *M. indica*—including flavonoids, tannins, saponins, and polyphenols—are widely reported to contribute to its antibacterial activity and overall biosafety profile (19). In the present study, aqueous (distilled water) and methanolic extracts of *Mangifera indica* seed kernels were evaluated for their antibacterial and cytotoxic (hemolytic) activities against multidrug-resistant (MDR) bacterial isolates. The targeted MDR strains included *Klebsiella pneumoniae*, *Vancomycin-resistant Staphylococcus epidermidis* (VRSE), and *Vancomycin-resistant Staphylococcus aureus* (VRSA). Three concentrations (10, 20, and 30 mg/mL) of each extract were tested to assess their inhibitory potential. The results revealed variable antibacterial activity among the extracts and concentrations tested. The aqueous extract at 30 mg/mL exhibited the highest antibacterial activity against VRSE, producing a maximum zone of inhibition of 34 mm. Conversely, the lowest activity was observed with the methanolic extract at 10 mg/mL against *Klebsiella pneumoniae*, which produced a 13 mm inhibition zone (19).

This may be attributed to the better solubility and stability of polar bioactive compounds in aqueous media, which enhance their diffusion and interaction with bacterial membranes. The results of this study are in agreement with several previous reports. Sadiea et al. observed that ethanol extracts of *Mangifera indica* seed kernel produced a 30 mm inhibition zone against *Staphylococcus aureus* (20). Similarly, in another study, (21, cited in the original text but not the reference list) reported moderate inhibitory effects of *M. indica* extracts against *S. aureus* (22). Collectively, these findings support the current study and confirm that *Mangifera indica* seed extracts exhibit promising antibacterial potential against a variety of bacterial strains, including those resistant to conventional antibiotics (22).

Table V. GC-MS identified compounds

No.	RT (min)	Phytochemicals compound	Area%	Match	CAS No.	Molecular formula
1	2.661	1,3-Dihydroxyacetone dimer	1.04	64	62147-49-3	C6H12O6
2	4.223	Glycerin (Glycerol)	0.65	59	56-81-5	C3H8O3
3	11.088	1,2,3-Benzenetriol (Pyrogallol)	9.58	95	87-66-1	C6H6O3
4	11.782	Methoxycarbonylmethyl disulfanyl-acetic acid methyl ester	2.01	99	1665-64-1	C6H10O4S2
5	16.951	Methyl palmitate	1.01	98	112-39-0	C17H34O2
6	17.353	Palmitic acid (n-Hexadecanoic acid)	6.71	99	57-10-3	C16H32O2
7	18.503	Linoleic acid, methyl ester	0.52	99	112-63-0	C19H34O2
8	18.586	Oleic acid, methyl ester	4.05	99	1937-62-8	C19H36O2
9	18.851	Methyl stearate	2.23	99	112-61-8	C19H38O2
10	19.029	Oleic acid (cis-9-Octadecenoic acid)	32.46	99	112-80-1	C18H34O2
11	19.251	Stearic acid (Octadecanoic acid)	20.18	99	57-11-4	C18H36O2
12	21.657	Cyclopropaneoctanal, 2-octyl-	0.62	68	56196-06-6	C18H34O
13	21.891	Phenol, 3-pentadecyl-	1.04	80	501-24-6	C21H36O
14	22.164	Bis(2-ethylhexyl) phthalate	0.75	91	117-81-7	C24H38O4
15	23.277	Glycerol monooleate	3.19	95	111-03-5	C21H40O4
16	23.335	Phenol, 3-pentadecyl- (repeat)	6.69	72	501-24-6	C21H36O
17	23.487	Methyl carbamic acid, 3-methylphenyl ester	2.65	38	1129-41-5	C9H11NO2
18	25.298	Phenol, 3-pentadecyl- (repeat)	4.61	64	501-24-6	C21H36O

The increasing global problem of antibiotic resistance has necessitated the exploration of natural bioactive compounds as potential alternatives to synthetic antimicrobial drugs (21). Mango seed kernels, which are typically discarded as waste during fruit processing, are thus emerging as a valuable and sustainable source of antimicrobial agents (23). The antibacterial activity observed in *M. indica* extracts is likely due to the synergistic effects of its phytochemical constituents. Flavonoids and phenolic acids are known to disrupt bacterial cell membranes, interfere with enzymatic activity, and inhibit nucleic acid synthesis, while tannins cause protein precipitation and damage bacterial cell walls, leading to cell lysis. These mechanisms collectively contribute to the strong antibacterial potential of *Mangifera indica* seed extracts (24). In addition to antibacterial activity, the present study also assessed the cytotoxicity of both aqueous and methanolic extracts through a hemolysis assay to evaluate their biosafety. The aqueous extract showed significantly lower hemolytic potential compared to the methanolic extract (24).

At the highest tested concentration (30 mg/mL), the aqueous extract caused only 3.3% hemolysis, whereas the methanolic extract showed 4.2% hemolysis. At the lowest concentration (10 mg/mL), the aqueous extract exhibited just 0.8% hemolysis, while the methanolic extract showed 1.3%. According to (25) cited in the original text but not the reference list), plant extracts exhibiting less than 5% hemolysis are considered hemocompatible and non-toxic, indicating their suitability for therapeutic applications. The hemolysis percentages observed in this study were all below this threshold, demonstrating that both extracts—particularly the aqueous extract—are biocompatible and safe for potential pharmaceutical use. The lower cytotoxic profile of the aqueous extract may be attributed to its reduced concentration of organic solvents and its high polarity, which prevents the accumulation of membrane-disrupting compounds. This makes the aqueous extract a safer choice for further pharmaceutical development (25).

Moreover, its higher antibacterial efficacy combined with low hemolytic activity enhances its potential as a natural therapeutic agent. The findings of this study are consistent with the growing body of evidence supporting the potential use of *Mangifera indica* seed kernel extracts in biomedical and pharmaceutical formulations. The combination of strong antibacterial activity against MDR strains and low cytotoxicity suggests that aqueous *M. indica* seed extract could serve as an effective and safe antimicrobial alternative. Its ability to inhibit resistant pathogens such as VRSA, VRSE, and *K. pneumoniae* underscores its relevance in addressing the global challenge of antibiotic resistance (26).

Overall, the results of this study demonstrate that *Mangifera indica* seed kernel extracts possess potent antibacterial properties and acceptable biosafety profiles. The aqueous extract, in particular, stands out as a promising candidate for the development of new antibacterial agents, especially for combating multidrug-resistant bacterial infections (27). The extract's natural origin, wide availability, cost-effectiveness, and minimal toxicity further support its potential for pharmaceutical applications. Future research should focus on isolating and characterizing the specific bioactive compounds responsible for the antibacterial activity, as well as exploring their mechanisms of action and possible synergistic effects with existing antibiotics with continued investigation, *Mangifera indica* seed extract could become a valuable component in the formulation of novel, natural, and effective antimicrobial therapeutics. The Gas Chromatography-Mass Spectrometry (GC-MS) analysis successfully identified 18 different compounds in the sample. The compounds are diverse, encompassing fatty acids and their methyl esters, phenolic compounds, and other various organic molecules. The analysis revealed that the sample is predominantly composed of Oleic acid (cis-9-Octadecenoic acid), which is the most abundant compound, accounting for 32.46% of the total area (28). This monounsaturated omega-9 fatty acid is a key component. The second most prominent compound is Stearic acid (Octadecanoic acid), a saturated fatty acid, at 20.18%. Together, these two major fatty acids constitute over half of the identified compounds (52.64%). Significant Compound Groups Fatty Acids and Esters: This group forms the largest part of the identified components. (29). Besides Oleic acid and Stearic acid, other significant fatty acids/esters include Palmitic acid (6.71%) and its ester Methyl palmitate (1.01%). Other fatty acid esters like Oleic acid, methyl ester (4.05%), Methyl stearate (2.23%), and Linoleic acid, methyl ester (0.52%) were also detected. (30). Phenolic Compounds: Phenol, 3-pentadecyl- was repeatedly detected (1.04%, 6.69%, 4.61%), highlighting its presence. 1,2,3-Benzenetriol (Pyrogallol), a strong antioxidant, was also found in a notable concentration (9.58%). Minor Components: Other compounds

present in smaller amounts include Glyceryl monooleate (3.19%), Bis(2-ethylhexyl) phthalate (0.75%), and fundamental organic molecules like Glycerin (Glycerol) (0.65%) (30).

CONCLUSION

The present study demonstrated that *Mangifera indica* (mango) seed extracts possess strong potential against multidrug-resistant (MDR) bacterial pathogens. Both methanolic and aqueous extracts exhibited significant antibacterial and hemolytic activities, with the aqueous extract showing superior antibacterial effects and lower cytotoxicity. These findings suggest that *Mangifera indica* seeds contain potent bioactive compounds responsible for their antimicrobial action. The low hemolytic activity also indicates their biosafety and compatibility for therapeutic use. Overall, this study highlights the promising role of *Mangifera indica* seed extracts as a natural source of antibacterial agents, paving the way for the isolation and characterization of these bioactive compounds for future pharmaceutical and biomedical applications. The GC-MS analysis successfully identified 18 compounds, revealing that the sample is primarily composed of fatty acids, with Oleic acid (32.46%) and Stearic acid (20.18%) being the major constituents and together accounting for over half of the composition. Significant levels of the phenolic antioxidant 1, 2, 3-Benzenetriol (Pyrogallol) (9.58%) were also found, indicating a rich mixture of lipids and bioactive phenolics.

Conflict of interest:

There is no conflict of interest among authors regarding this article.

Authors` contribution:

WK, US, I AS, LU R, Writing, review and editing. A R, WA, help in Q I, review and editing Introduction MF Writing, all authors have reviewed and consented to the final version of the manuscript for publication.

References:

1. Prastiyanto ME, Darmawati SR, Mukaromah AH. Antibacterial activity of seed kernel extracts of seven mangoes (*Mangifera indica*) cultivars native to Indonesia against MDR-*Pseudomonas aeruginosa* isolated from wounds. *Biodiversitas Journal of Biological Diversity*. 2022;23(11).
2. Sadie RZ, Mozumder A, Mou MJ, Hasan SN, Sikder C, Akter S, Hossain MG. Evaluation of the antibacterial potential of *Mangifera indica* seed kernels in Bangladesh. *Frontiers in Tropical Diseases*. 2024;5:1473494.
3. Kiiro T. Factors Influencing Monitoring of Usage of Herbal Medicine in Kenya: a Case of Meru County, Kenya (Doctoral dissertation, University Of Nairobi). 2015.
4. El-Azazi M, El-Shafie AS, Al-Mulla R, Hassan SS, Nimer HI. Enhanced adsorptive removal of rifampicin and tigecycline from single system using nano-ceria decorated biochar of *Mangifera indica* seed kernel. *Helion*. 2023;9(5).
5. Bukhari S, Chiragh S, Tariq S, Alam MA, Wazir MS, Suleman M. In vitro activity of *Vaccinium macrocarpon* (cranberry) on urinary tract pathogens in uncomplicated urinary tract infection. *Journal of Ayub Medical College Abbottabad*. 2015; 27(3):660-663.
6. Angamuthu S, Thang Swamy S, Raju A, Husain FM, Ahmed B, Al-Shabib NA, Alomar SY. Biogenic Preparation and Characterization of Silver Nanoparticles from Seed Kernel of *Mangifera indica* and Their Antibacterial Potential against *Shigella spp.* *Molecules*. 2023;28(6):2468.
7. Zaatar S, Tahan M, Rostami O, Neshanic A, Farsi Ani H, Shahsavari A, Badghis MH. Antibacterial activity of *Mangifera indica* seed extracts combined with common antibiotics against multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates. *Bioscience Journal*. 2023;39(e39058):1981-3163.
8. Choudhary P, Devi TB, Tushir S, Kasane RC, Popa Trao DS, K N. *Mangifera indica* seed kernel: A bountiful source of nutritional and bioactive compounds. *Food and Bioprocess Technology*. 2023; 16(2):289-312.
9. Diop M, Thioune O, Neat C, Maton M, Riviere C, Martel B, Blanchemain N. In vitro evaluation of antibacterial activity of a plant extract-loaded wound dressing. *Journal of Drug Delivery Science and Technology*. 2022;67:102950.

10. Hasashi S, Hasashi B. Film dressings from Thai *Mangifera indica* seed kernel extracts versus Nan crystalline silver dressings in antibacterial properties. *Journal of Pharmacy & Pharmaceutical Sciences*. 2024; 27:12674.
11. Athiappan M. Antibiofilm Efficacy of *Mangifera indica* Kernel Methanol Extract against *Staphylococcus aureus*. *Indian Journal of Natural Sciences*. 2022;13(71).
12. Fosun L, Pinhead Z, Pinhead SM. The Potency of Tedizolid, Linezolid, and Vancomycin against Extensively Drug-Resistant *Staphylococcus Aureus* Clinical Isolates. *Zahedan J Res Med Sci*. 2022; 24(4).
13. Custodio MM, Sparks J, Long TE. Disulfiram: a repurposed drug in preclinical and clinical development for the treatment of infectious diseases. *Anti-infective agents*. 2022;20(3):34-45.
14. Abraham JS, El-Fakharany EM, El-Gendi H, Saleh AK, El-Marandi YA. Therapeutic perspectives of *Mangifera indica* L. peel extract: phytochemical profile, antimicrobial, anticancer, and antiviral efficacy. *Biomass Conversion and Biorefinery*. 2025:1-24.
15. Xu J, Wei Z, Fang W, Wu J, Wang Y, Chen S. KKL-35 inhibits growth of *Staphylococcus aureus* by systematically changing bacterial phenotypes. *Archives of Microbiology*. 2024; 206(8):350.
16. King A, Chakrabarty S, Zhang W, Zeng X, Ohman DE, Wood LF, Wynne KJ. High antimicrobial effectiveness with low hemolytic and cytotoxic activity for PEG/quaternary copolyoxetanes. *Biomacromolecules*. 2014;15(2):456-467.
17. Asenjo A, Oteo-Iglesias J, Also JI. What's new in mechanisms of antibiotic resistance in bacteria of clinical origin? *Intermediates infections y microbiologic clinical (English ed.)*. 2021;39(6):291-299.
18. Iskandar K, Moliner L, Hallet S, Sartell M, Hardcastle TC, Haque M, Roques C. Surveillance of antimicrobial resistance in low-and middle-income countries: a scattered picture. *Antimicrobial Resistance & Infection Control*. 2021;10:1-19.
19. Choudhary P, Devi TB, Tushir S, Kasane RC, Popa Trao DS, K N. *Mangifera indica* seed kernel: A bountiful source of nutritional and bioactive compounds. *Food and Bioprocess Technology*. 2022;16(2):289-312.
20. Sadie RZ, Mozumder A, Mou MJ, Hasan SN, Sikder C, Akter S, Hossain MG. Evaluation of the antibacterial potential of *Mangifera indica* seed kernels in Bangladesh. *Frontiers in Tropical Diseases*. 2024;5:1473494.
21. Sharma A, Mistry V, Kumar V, Tiwari P. Production of effective Phyto-antimicrobials via metabolic engineering strategies. *Current Topics in Medicinal Chemistry*. 2022;22(13):1068-1092.
22. Alqahtani YS, Bahari A, Mirajkar KK, Basavaraj RR, Mitra SSS, Shaikh IA. In vitro antibacterial activity of green synthesized silver nanoparticles using *Mangifera indica* aqueous leaf extract against multidrug-resistant pathogens. *Antibiotics*. 2022;11(11):1503.
23. Hayat Z, Riaz T, Saleem K, Akram K, Ur Rehman H, Azam M. Optimization of gallic acid-rich extract from *Mangifera indica* Seed kernels through ultrasound-assisted extraction. *Separations*. 2023; 10(7):376.
24. Angamuthu S, Thang Swamy S, Raju A, Husain FM, Ahmed B, Al-Shabib NA, Alomar SY. Biogenic Preparation and Characterization of Silver Nanoparticles from Seed Kernel of *Mangifera indica* and Their Antibacterial Potential against *Shigella spp.* *Molecules*. 2023;28(6):2468.
25. Mirghani ME, Yosuf F, Kabbashi N, Vejjayan J, Yosuf Z. Antibacterial activity of mango kernel extracts. *J. Appl. Sci*. 2009;9:3013–3019.
26. Tong SY, Fowler VG, Skalla L, Holland TL. Management of *Staphylococcus aureus* bacteremia: a review. *JAMA*. 2025.
27. Sharma A, Mistry V, Kumar V, Tiwari P. Production of effective Phyto-antimicrobials via metabolic engineering strategies. *Current Topics in Medicinal Chemistry*. 2022;22(13):1068-1092.
28. Zahoor S, Anwar F, Qadir R, Soufan W, Sakran M. Physicochemical attributes and antioxidant potential of kernel oils from selected mango varieties. *ACS omega*. 2023;8(25):22613-22622.
29. Gopu C, Chirumamilla P, Daravath SB, Vankudoth S, Taduri S. GC-MS analysis of bioactive compounds in the plant parts of methanolic extracts of *Momordica cymbalaria* Fenzl. *J. Med. Plants Stud*. 2021; 9(3):209-218.
30. Jafari M, Gharachorloo M, Hemmaci AH. The stabilizing effect of three varieties of crude mango seed kernel oil on tallow. *Journal of Food Biosci Technol*. 2014;4(1):31-36.
31. Hayat Z, Riaz T, Saleem K, Akram K, Ur Rehman H, Azam M. Optimization of gallic acid-rich extract from mango (*Mangifera indica*) Seed kernels through ultrasound-assisted extraction. *Separations*. 2023;10(7):376.