



Antibacterial Comparison of the Mango Seed Components by HPLC

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Abstract

When *Staphylococcus aureus*, *Bacillus* sp., *E. coli*, and *Pseudomonas aeruginosa* were cultured on Mueller–Hinton, the ZOI significantly increased. Compared with the other concentrations and bacteria, the percentage of *Pseudomonas aeruginosa* was 75%. For *Pseudomonas aeruginosa* and *E. coli*, the optimal minimum inhibitory concentration (MIC) was 75% at concentrations of 75% and 40 and 35 mm, respectively; for *Staphylococcus aureus* and *Bacillus* sp., the MIC was 50% at a concentration of 75%. For all the microorganisms at a concentration of 10%, the MAC was 10%. Gallic acid (43.2%), isorhamnetin (14%), kaempferol, myricetin, quercetin, coumaric acid, and ferulic acid were the components with the best extraction results at a 75% ethanolic concentration. The concentration of gallic acid, which was 43.2% at a concentration of 75%, increased with increasing ethanol concentration. 75% was the optimal extraction concentration that yielded numerous components. These findings suggest that while some substances (isorhamnetin and kaempferol) may be extracted more successfully at higher ethanol concentrations, gallic acid may be better extracted at higher ethanol concentrations. The maximum ZOI and MIC for *Pseudomonas aeruginosa* and *E. coli*, which reached 75% and 50% for *Staphylococcus aureus* and *Bacillus* sp., respectively, were enhanced by the highest concentration of gallic acid (75%) extraction liquid that can be used as an antibiotic for pathogen bacteria and bioremediation for eco-remediation.

Keywords:

Mango seed, alcohol extraction, HPLC, inhibitor

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Introduction

Mango is considered a member of the fruit family, grows in tropical sites, belongs to the order Sapindales and the family Anacardiaceae (Sharma, 2017), and normally consists of an outer layer full of fibrous material, followed by a coat that covers the seed, which contains the embryo (Ahmed, 2015). These important aspects are related to seed active compounds, which include xanthenes, flavonoids, phenolic acids, stearic compounds (24–57%) (Ballesteros-Vivas et al., 2019), oleic acids

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(34–56%), and 52–56% unsaturated fatty acids (Shehabeldin et al., 2021), all of which are known to have medical importance (Sadiea et al., 2024).

The phytochemicals in mango have antioxidant, antidiabetic, antibacterial, antiviral, anti-inflammatory, antifungal, and anticancer activities. *Pseudomonas aeruginosa* and *Bacillus* spp. are commonly found in ecosystems, hospital environments, and moist surfaces (Mousa & Abdul Hassan, 2025). *Escherichia coli* is commonly found in the intestines of humans and warm-blooded nonpathogenic animals (Toma & Nakamura 2021). Most strains of *E. coli* are nonpathogenic and play a beneficial role in the gut microbiota by assisting in the digestion and synthesis of certain vitamins (such as vitamin K). However, some strains are pathogenic and can cause a range of diseases, from mild food poisoning to severe, life-threatening infections. *Staphylococcus aureus* is among the most common bacteria responsible for human infections, is frequently found in wounds and is also responsible for abscesses characterized by the accumulation of pus (Masood et al., 2019). In general, antibiotics are used to treat wound infections; however, improper use of antibiotics can lead to bacterial resistance. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global health concern, with increasing infection rates, mortality rates, and the potential for causing bacteremia or sepsis (Li et al., 2019).

Key Contribution

In this study, the components of mango seed were investigated by high-performance liquid chromatography (HPLC), in addition to the antibacterial activity of mango seed alcohol extract at different concentrations, especially against *Staphylococcus aureus*, to identify the perfect component that can be used as an ointment against wounded bacteria such as *Staphylococcus aureus*.

Materials and Methods

Instruments and equipment

All of the instruments, equipment, solutions, and Petri dishes are available freely from the Scientific Research Commission.

Seed collection and extraction preparation:

The mango fruit was locally gathered from the market, and the seed was only removed, cleaned, dried at room temperature for 2 days, and then crushed by hand and machine to make powder. The alcohol preparation concentration (0, 10, 25, 50, and 75%) was mixed with 7.5 g/100 ml of absolute ethanol (v/v) from the previous concentration, and the mixture was kept in a shaker incubator for 3 hours. to filter by centrifugation (5000 rpm/20 min). The supernatant was concentrated by a rotary evaporator (50 °C/180 r), and each sample was powered on a Petri dish and dried under room conditions (Lim et al., 2019).

HPLC Conditions

The percentage of active compounds from the different extractions was determined by separating the ethanolic extracts (50 µL), and a C18 reversed-phase column was used. The mobile phase consisted of distilled water with 1% formic acid; 80% of the final liquid was gathered with acetonitrile (20%) at a flow rate of 1 mL/min, and the detection wavelength was 350 nm/450 nm (Lim et al., 2019).

Antibacterial activity

From the microbial bank of the Scientific Research Commissions, two gram-negative bacteria were selected and positive (*Staphylococcus* spp., *Bacillus* spp., *E. coli*, and *Pseudomonas aeruginosa*), recultured and activated, and Mueller–Hinton prepared, sterilized, and powdered in dishes, after which they were kept (18–24 hours/36°C). Each bacterium was subsequently cultured on a dish, after which 0.1 ml was added, after which the diameter (mm) of the zone inhibition test (ZOI) was measured (Bhargav et al. 2016), with a turnaround time of one week (MIC & MAC).

Results

Extraction, ZOI, MIC & MAC

The extraction that was manufactured after rotary evaporation is presented in Figure 1.



Figure 1. The final solution from the extraction steps.

When the selected bacteria were encultured on Mueller–Hinton, as shown in Figure 2, when the ZOI was measured for one week, compared with the other concentrations and bacteria in Figure 3, *P. aeruginosa* reached a high level of ZOI (75%).

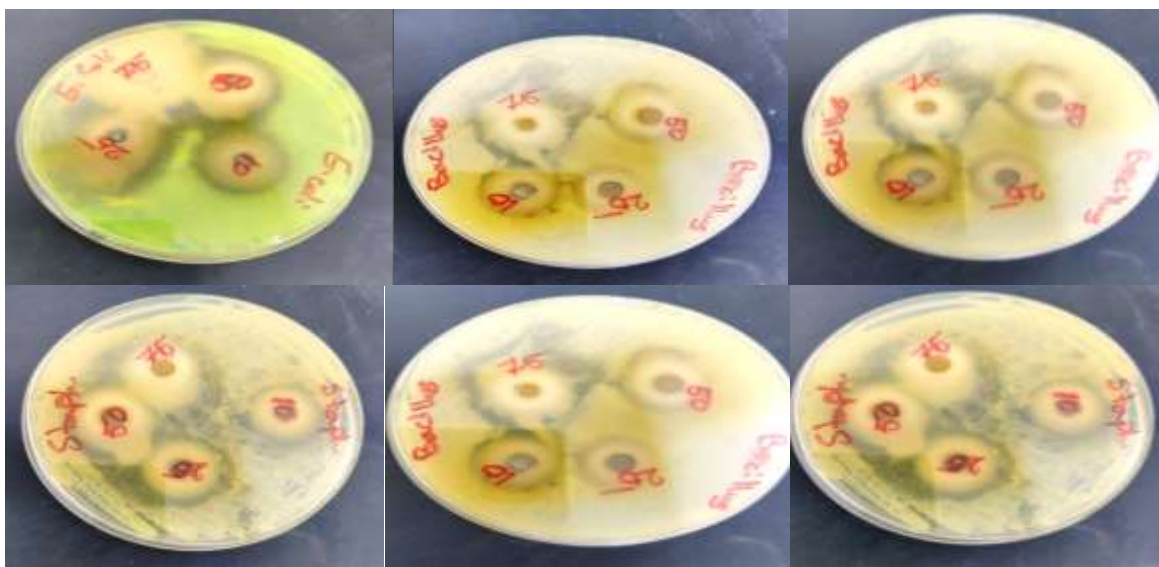


Figure 2. ZOI of *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus* spp., and *Bacillus* spp. on Mueller–Hinton

The minimum inhibition concentration (MIC) was 75% for *Pseudomonas aeruginosa* and *E. coli* and was 40 and 35 mm, respectively; thus, the MIC was 75%, whereas for *Staphylococcus aureus* and *Bacillus* sp., the inhibition started at 50% concentration, with 30 and 25 mm, respectively, of inhibition. Therefore, the MIC is 50%. The maximum inhibition concentration (MAC) was 10% for all the bacteria at a concentration of 10%.

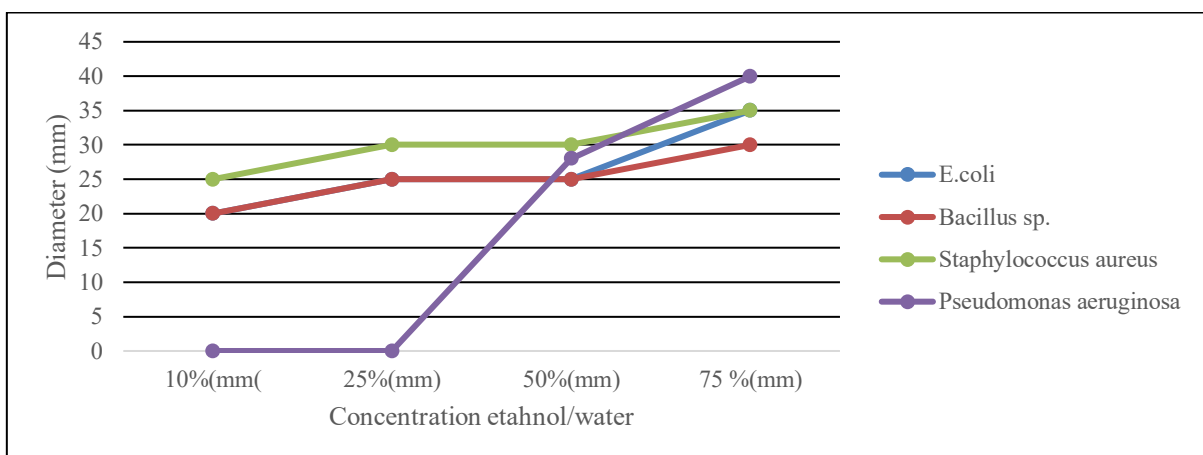


Figure 3. ZOIs of *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus* spp., and *Bacillus* spp. after 0,

10, 25, 50, and 75% ethanolic extraction.

HPLC assay

As the ethanol concentration increased (from 0% to 75%), there was a noticeable shift in the retention times and the area percentages for each compound. At higher ethanol concentrations, certain compounds, such as gallic acid, isorhamnetin, and kaempferol, are present at higher concentrations. Gallic acid was found in varying amounts across all the samples, with the highest peak in the 75% ethanol sample (43.2% area). This suggests that the presence of gallic acid increases as the ethanol concentration increases (Figure 4). Isorhamnetin is present in the 75% ethanol sample with a moderate area percentage (14.0%), indicating that it is more abundant at higher ethanol concentrations. Kaempferol and myricetin both appeared at lower concentrations in the lower ethanol samples (0%, 10%, and 25%), with their concentrations increasing in the 50% and 75% ethanol samples.

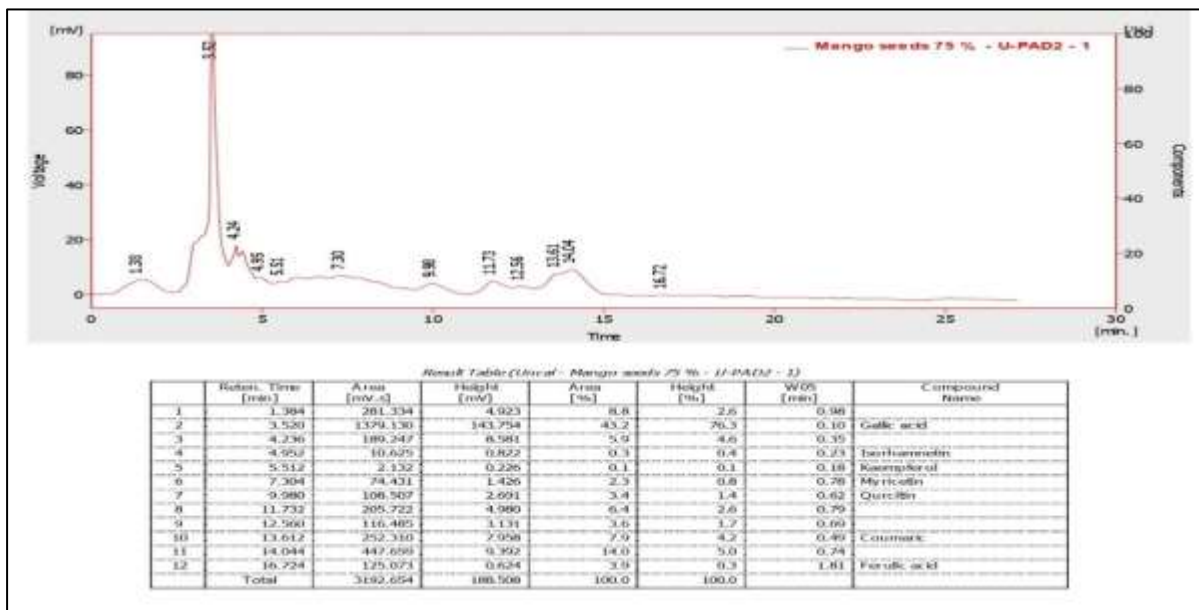


Figure 4. The concentration was 75%, and extraction components were used.

Increased Extraction with Increased Ethanol: The increase in the area percentages of compounds such as gallic acid, isorhamnetin, and kaempferol with increasing ethanol concentration suggests that ethanol is an effective solvent for extracting bioactive compounds from mango seeds. The retention times for the compounds generally increase with increasing ethanol concentration, indicating that ethanol may influence the elution time during the HPLC process (Figure 5).

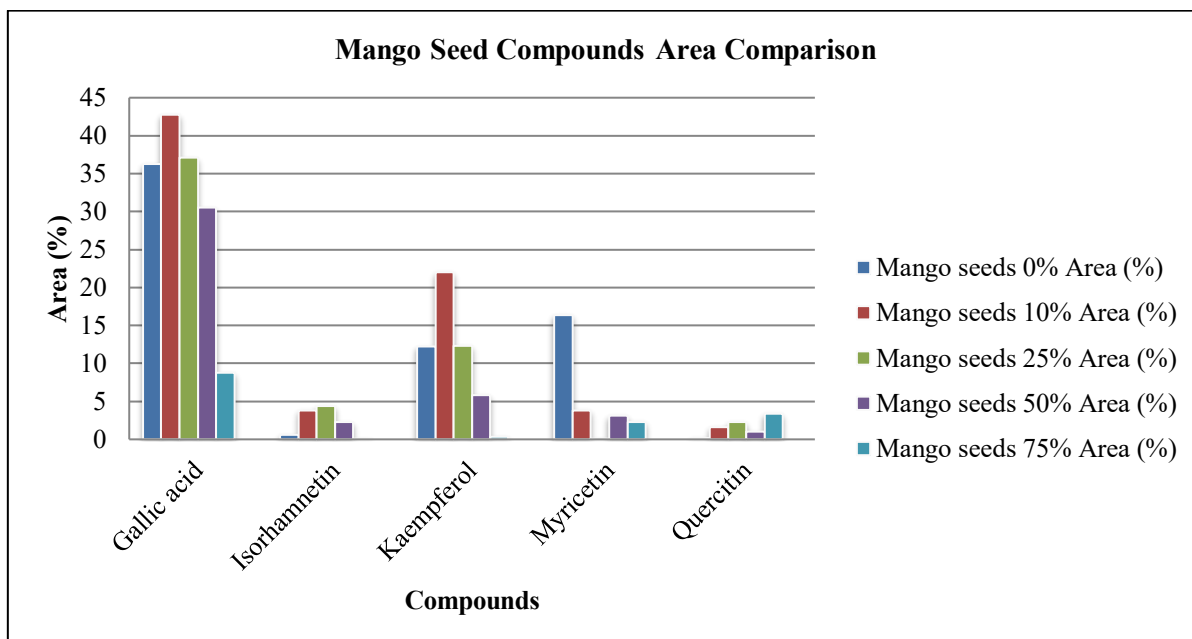


Figure 5. Area % and concentration (0, 10, 25, 50, and 75%) % of ethanolic mango seed extractions.

Discussion

Mango seeds contain many components, such as gallotannins and gallic acid, in the absence or presence of gallotannins in addition to flavonoids. Because of their structure, these compounds likely act by damaging microbial cell membranes, disrupting microbial metabolism, or binding microbial proteins, although the exact mechanisms depend on the microbe and extract preparation (Jiamboonsri et al., 2011; Kim et al., 2021; Thomas & Mitra, 2022).

Gallic acid is a naturally occurring phenolic compound widely known for its antioxidant, anti-inflammatory, and antimicrobial effects

properties (Jiamboonsri et al., 2011; Awad et al., 2012; Thomas & Mitra, 2022). Its antibacterial mechanism can involve disruption of cell membranes, inhibition of biofilm formation, and induction of oxidative stress within bacterial cells, leading to DNA damage and ultimately cell death (Kumar et al., 2019). It is known to generate reactive oxygen species (ROS), which can damage proteins, lipids, and nucleic acids within bacterial cells. Some studies have indicated that gallic acid can inhibit bacterial enzymes such as DNA gyrase (an important enzyme in bacterial DNA replication and ATP synthase), thus altering the metabolism and growth of bacteria (Choudhary et al., 2020; Yew & Patel, 2020; Thomas & Mitra, 2022). Gallic acid has also demonstrated strong activity against this ubiquitous pathogen, along with methicillin-resistant *Staphylococcus aureus* (MRSA), which is even more difficult to treat because of antibiotic resistance. It is also active against several *Bacillus* species, including *Bacillus cereus*, a foodborne pathogen.

Another gram (+) genus that is slightly sensitive to gallic acid is *Enterococcus* sp., one of the most common strains that is resistant to several antibiotics. It also has activity against *Escherichia coli* (*E. coli*), one of the most common causes of urinary tract infections (UTIs), foodborne illnesses and other conditions. *Pseudomonas aeruginosa* is notorious for its resistance to antibiotics and ability to survive in hospitals. Gallic acid has also been shown to be growth-inhibiting. *Salmonella* spp. have been shown to have antibacterial activity against *Salmonella* serovars that are also causes of gastroenteritis and food poisoning. *Klebsiella pneumoniae*, another gram-negative bacteria that can cause pneumonia and infections in immunocompromised patients, is also sensitive to gallic acid (Choudhary et al., 2020; Thomas & Mitra, 2022).

Concentration dependent activity of gallic acid. Antibacterial effects are usually stronger at higher concentrations. The MIC, which is the lowest concentration that can inhibit the growth of bacteria, changes according to the type of bacteria employed and according to the extraction solvent and purity of gallic acid used (Choudhury & Rane, 2023). Its MIC against *S. aureus* and *E. coli* was approximately 128 µg/mL and higher (256 µg/mL) (Liu et al., 2011). Furthermore, the results of the present study revealed that the 75% ethanolic concentration resulted in high levels of GA, which resulted in higher ZOIs and MICs for both of these bacteria. These minimum inhibitory concentration values indicate that gram-negative bacteria require high concentrations of gallic acid for effective inhibition owing to the increased

complex outer membrane structure compared with that of gram-positive bacteria (Liu et al., 2011; Choudhury & Rane, 2023).

In combination with other antimicrobial agents, gallic acid is also the subject of research. It has also been demonstrated to synergize with the use of antibiotics such that it can aid in boosting the action of currently used antibiotics (Choudhury & Rane, 2023). For example, gallic acid effectively decreased the MICs of penicillin, amoxicillin, and chloramphenicol against the

drug-resistant bacteria (Sang et al., 2024). It is considered a potential replacement or supplement to improve the treatment of infections, including those caused by resistant bacteria, because of its antimicrobial activity. It can also play a natural preservative role in topical formulas or wound covers (Khoshi et al., 2025). Gallic acid, which is prominently found in mango seed extracts, has been studied for its potential application as a natural food preservative because of its ability to inhibit bacterial growth and spoilage (Lee & Je, 2023).

Conclusion

Enculturing *Staphylococcus aureus*, *Bacillus* sp., *E. coli*, and *Pseudomonas aeruginosa* with Mueller–Hinton improved the ZOI significantly for *Pseudomonas aeruginosa*, which reached a high level (75%) compared with the other concentrations and bacteria. The best MICs were 75% at a concentration of 75% for *Pseudomonas aeruginosa* and *E. coli* and 40 and 35 mm, respectively, whereas for *Staphylococcus aureus* and *Bacillus* sp., the MICs were 50% for the 75% concentration. The MAC was 10% for all the bacteria at a concentration of 10%. The best extraction results were obtained at a 75% ethanolic concentration, and the components were gallic acid (43.2%), isorhamnetin (14%), kaempferol, myricetin, quercetin, and coumaric and ferulic acid. An increase in ethanol concentration increased the concentration of gallic acid, which was 43.2% for gallic acid at a concentration of 75%. The best extraction concentration that provides many components was 75%. These findings indicate that gallic acid might be better extracted at higher ethanol concentrations, whereas other compounds (isorhamnetin and kaempferol) may be extracted more effectively at higher ethanol concentrations. The highest concentration of gallic acid (75%) in the extraction liquid improved its relationship with the highest ZOI and MIC for *Pseudomonas aeruginosa* and *E. coli*, which reached 75% and 50%, respectively, for *Staphylococcus aureus* and *Bacillus* sp.

Ethical and Environmental Considerations

The results of the study do not involve human and/or animal rights.

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Author Contributions

All the authors contributed equally.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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