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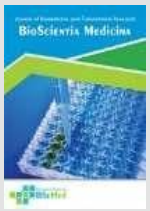

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Unveiling the Antibacterial Potential of Arumanis Mango (*Mangifera indica L.*) Leaf Extract Against *Enterococcus faecalis*: A Comparative Study with Melinjo (*Gnetum gnemon L.*)

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A B S T R A C T

Background: Root canal infections, frequently caused by the bacterium *Enterococcus faecalis* due to its resistance to conventional treatments, present a significant challenge in dental practice. Effective disinfection of the root canal system is crucial for successful endodontic therapy, necessitating the exploration of alternative irrigating solutions. This study aimed to evaluate and compare the in vitro antibacterial efficacy of Arumanis mango (*Mangifera indica* L.) leaf extract and melinjo (*Gnetum gnemon* L.) leaf extract against *Enterococcus faecalis*. **Methods:** This in vitro experimental study employed the disc diffusion method to assess the antibacterial activity of ethanolic extracts of Arumanis mango and melinjo leaves against *Enterococcus faecalis*. The extracts were tested at three different concentrations: 25%, 50%, and 75%. Chlorhexidine 0.2% and distilled water served as positive and negative controls, respectively. The diameter of the inhibition zones around the discs was measured after incubation. Phytochemical screening of both extracts was also conducted. **Results:** The phytochemical screening revealed the presence of flavonoids, alkaloids, and tannins in both Arumanis mango and melinjo leaf extracts. However, the antibacterial activity varied significantly. Melinjo leaf extract did not exhibit any measurable inhibitory effect on *Enterococcus faecalis* at any of the tested concentrations. In contrast, Arumanis mango leaf extract demonstrated significant antibacterial activity at concentrations of 50% and 75%, with mean inhibition zone diameters of 11.73 ± 0.75 mm and 12.90 ± 0.30 mm, respectively. Statistical analysis using the Kruskal-Wallis test and post-hoc Mann-Whitney U test confirmed a significant difference in the antibacterial activity between the Arumanis mango leaf extract at 50% and 75% concentrations and the melinjo leaf extract at all tested concentrations, as well as the distilled water control. The positive control, chlorhexidine 0.2%, showed the largest inhibition zone (15.10 ± 0.10 mm). **Conclusion:** The findings of this study indicate that the ethanolic extract of Arumanis mango (*Mangifera indica* L.) leaves possesses significant in vitro antibacterial activity against *Enterococcus faecalis*, suggesting its potential as a natural alternative irrigating solution in endodontic treatment. Conversely, under the conditions of this study, melinjo (*Gnetum gnemon* L.) leaf extract did not demonstrate any antibacterial effect against this specific bacterium. Further research is warranted to explore the clinical efficacy and safety of Arumanis mango leaf extract in root canal disinfection.

1. Introduction

Root canal infections represent a significant challenge in dental practice. These infections arise when bacteria infiltrate the dental pulp, typically through carious lesions, and subsequently colonize the root canal system. The consequences of such infections can be far-reaching, as these microorganisms can extend into the periapical tissues, triggering inflammatory responses that lead to the destruction of both soft and hard tissues. The ultimate

goal of root canal treatment is to eliminate infection and prevent its recurrence, thereby allowing the tooth to return to its functional state. This complex process involves a series of critical steps, including mechanical preparation, sterilization (irrigation), and hermetic obturation of the root canal system. Among the diverse array of microorganisms implicated in root canal infections, *Enterococcus faecalis* stands out as a particularly formidable challenge. This Gram-positive, facultative anaerobic bacterium is frequently associated with persistent root canal infections and treatment failures. Several factors contribute to its resilience. *Enterococcus faecalis* possesses the ability to survive in harsh environments, form biofilms, and resist common antimicrobial agents, all of which contribute to its recalcitrance to endodontic therapy. The ability of E. faecalis to form biofilms is particularly concerning, as biofilms provide a protected niche for bacteria, shielding them from the action of antimicrobial agents and host defense mechanisms. This inherent resistance underscores the need for effective strategies to combat this persistent pathogen. Disinfection of the root canal system is a cornerstone of successful endodontic therapy. Irrigation plays a crucial role in this process. Irrigating solutions are essential for flushing out debris, dissolving organic tissues, and delivering antimicrobial agents to inaccessible areas within the root canal. The complexity of the root canal system, with its intricate anatomy and the presence of anatomical variations, makes complete disinfection a challenging endeavor. Therefore, the choice of irrigating solution is of paramount importance. Conventional irrigating solutions, such as sodium hypochlorite (NaOCl) and chlorhexidine, have been widely used in endodontics due to their potent antimicrobial properties. Sodium hypochlorite, in concentrations ranging from 1% to 6%, exhibits broad-spectrum antibacterial activity and the ability to dissolve organic matter. Its ability to dissolve organic tissue is particularly advantageous in removing pulp tissue and disrupting biofilms. Chlorhexidine is also effective against E. faecalis and possesses substantivity, providing a prolonged antibacterial effect. Substantivity refers to the ability of chlorhexidine to bind to oral tissues and release slowly over time, thus prolonging its antimicrobial action.[1-4]

Despite their effectiveness, conventional irrigating solutions have certain limitations. NaOCl has potential drawbacks, including tissue toxicity, unpleasant odor, and corrosiveness. The toxicity of NaOCl to periapical tissues is a major concern, as it can cause damage to the surrounding vital structures. While chlorhexidine is generally considered biocompatible, its efficacy in dissolving organic tissue is limited. This limitation can hinder its ability to effectively remove pulp tissue and disrupt biofilms, potentially compromising its antimicrobial efficacy. The search for alternative irrigating solutions with improved efficacy and biocompatibility has led to increased interest in exploring natural products, particularly medicinal plants, as sources of antimicrobial agents in dentistry. Natural compounds are often perceived as safer, more cost-effective, and readily available compared to synthetic drugs. The use of medicinal plants for therapeutic purposes has a long history, and many traditional remedies are based on the antimicrobial properties of plant extracts. The exploration of natural products offers the potential to discover novel antimicrobial agents with unique mechanisms of action, potentially addressing the challenges posed by resistant bacteria. Indonesia, with its rich biodiversity, harbors a vast array of plants with potential medicinal properties. The country's tropical climate and diverse geographical landscape support the growth of a wide variety of plant species, many of which have been used in traditional medicine for centuries. This wealth of biodiversity presents a unique opportunity for the discovery of novel therapeutic agents.[5-7]

Among these plants, melinjo (*Gnetum gnemon* L.) is a tropical plant commonly found in Indonesia, known for its culinary uses and traditional medicinal applications. Various parts of the melinjo plant, including the leaves and seeds, contain bioactive compounds such as tannins, flavonoids, and alkaloids. These compounds have been reported to possess antibacterial properties against certain oral pathogens. The presence of these bioactive compounds suggests that melinjo may have potential as a source of antimicrobial agents for dental applications.

Arumanis mango (*Mangifera indica* L.), a popular variety of mango cultivated in tropical and subtropical regions, is widely known for its sweet fruit. However, the leaves of *Mangifera indica* L. are also a rich source of bioactive compounds, including mangiferin, flavonoids, tannins, alkaloids, steroids, and saponins. These compounds have demonstrated potential antibacterial and antibiofilm activities. The increasing cultivation of mango trees also leads to a significant amount of leaf waste, making the exploration of its potential applications even more relevant. Utilizing this waste material for medicinal purposes not only adds value to the plant but also contributes to sustainability. Given the prevalence of *Enterococcus faecalis* in root canal infections and the limitations of conventional irrigants, there is a need for novel and effective strategies to combat this persistent bacterium.[8-10] This study aimed to compare the in vitro antibacterial efficacy of ethanolic extracts from melinjo (*Gnetum gnemon* L.) leaves and Arumanis mango (*Mangifera indica* L.) leaves against *Enterococcus faecalis*.

2. Methods

This study employed an in vitro experimental laboratory design. The disc diffusion method was utilized to evaluate the antibacterial activity of the plant extracts. This method is a widely accepted technique for assessing the antimicrobial activity of various substances against microorganisms. The preparation of the plant extracts was conducted at the Phytochemistry Laboratory of Universitas Sumatera Utara. The antibacterial activity testing was performed at the Microbiology Laboratory of the Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. The study was carried out between January and February 2025.

Fresh, mature leaves of melinjo (*Gnetum gnemon* L.) and Arumanis mango (*Mangifera indica* L.) were collected from the Berastagi region, North Sumatra, Indonesia. The plant materials were authenticated by a botanist to ensure the correct species were used in the study. The leaves were washed thoroughly with distilled water to remove any surface contaminants. The washed leaves were then air-dried at room temperature. Following air-drying, the leaves were oven-dried at 40°C for 72 hours. This prolonged drying period at a controlled temperature ensured the complete removal of moisture from the plant material. The dried leaves were pulverized into a fine powder using an electric blender. The powdered leaves were stored in airtight containers to prevent moisture reabsorption and degradation of the plant material until further use.

The powdered leaves of both melinjo and Arumanis mango were subjected to extraction. For each plant, 2 kg of powdered leaves were used, making a total of 4 kg for both plants. The extraction process employed the maceration method with 70% ethanol as the solvent. Maceration is a simple and widely used extraction technique that involves soaking the plant material in a solvent to dissolve the desired compounds. The powder was soaked in ethanol at a ratio of 1:10 (w/v). This means that for every gram of powdered leaves, 10 milliliters of 70% ethanol were used. The mixture was left to stand for 72 hours at room temperature with occasional stirring. The occasional stirring ensured adequate mixing and improved the extraction efficiency. After 72 hours, the mixture was filtered through Whatman No. 1 filter paper. Filtration was used to separate the liquid extract from the solid plant material. The filtrate was then concentrated using a rotary evaporator under reduced pressure at 50°C. A rotary evaporator is a laboratory apparatus that uses vacuum and heat to remove solvents from liquid samples. The process was continued until a viscous extract was obtained. The concentrated extracts were further dried in a water bath to remove any residual solvent. The dried extracts were weighed to determine the yield of the extraction process. The dried extracts were stored at 4°C until used for antibacterial testing. Storage at low temperatures helps to preserve the stability of the extracts and prevent degradation of bioactive compounds.

Phytochemical screening was performed on both ethanolic extracts. This was done to identify the presence of various secondary metabolites. The secondary metabolites investigated included flavonoids, alkaloids, tannins, saponins, and steroids.

Standard qualitative methods were used for the phytochemical screening. Qualitative methods provide information on the presence or absence of these compounds.

A pure culture of *Enterococcus faecalis* was obtained from the stock culture of the Microbiology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara. The antibacterial activity of the extracts was evaluated using the disc diffusion method. Mueller Hinton Agar (MHA) medium was prepared according to the manufacturer's instructions. MHA is a microbiological growth medium commonly used for antibiotic susceptibility testing. The medium was sterilized by autoclaving at 121°C for 15 minutes. Autoclaving is a sterilization process that uses high pressure and temperature to kill microorganisms. After cooling to approximately 45-50°C, the molten agar was poured into sterile Petri dishes. The agar was poured to a depth of approximately 4 mm. Maintaining a consistent agar depth is important for ensuring uniform diffusion of substances in the disc diffusion assay. The agar was allowed to solidify. The surface of the agar was then uniformly inoculated with the prepared *Enterococcus faecalis* suspension. A sterile cotton swab was used for the inoculation. Uniform inoculation is crucial for obtaining reliable and reproducible results in the disc diffusion assay. Sterile paper discs (6 mm diameter) were used in the assay. The discs were impregnated with 20 µL of each extract concentration. The extracts were tested at three different concentrations: 25%, 50%, and 75% (w/v) in dimethyl sulfoxide (DMSO). DMSO was used as the solvent to dissolve the extracts. DMSO is a polar aprotic solvent that is commonly used to dissolve hydrophobic compounds. Discs impregnated with 20 µL of 0.2% chlorhexidine digluconate solution served as the positive control. Chlorhexidine is a well-established antimicrobial agent and was used to validate the assay. Discs impregnated with 20 µL of distilled water served as the negative control. The negative control was used to assess the effect of the solvent on bacterial growth. The impregnated discs were carefully placed on the surface of the inoculated agar plates. Adequate separation between the discs was maintained to prevent overlapping of inhibition zones. The plates were then incubated at 37°C for 24 hours. This incubation period allowed for bacterial growth and the diffusion of the test substances. After incubation, the diameter of the inhibition zone around each disc was measured. The inhibition zone represents the area of bacterial growth inhibition. The diameter of the inhibition zone was measured in millimeters using a digital caliper. A digital caliper provides precise measurements. Each concentration and control was tested in triplicate. Triplicate testing ensures the reliability and reproducibility of the results. The mean and standard deviation of the inhibition zone diameters were calculated.

The data obtained from the inhibition zone diameter measurements were analyzed using statistical software (SPSS version 25.0). The normality of the data was assessed using the Shapiro-Wilk test. The Shapiro-Wilk test is a statistical test used to determine if a data set is normally distributed. Since the data were not normally distributed, non-parametric tests were employed. Non-parametric tests are statistical tests that do not assume that the data follows a specific distribution. The Kruskal-Wallis test was used to determine if there were significant differences in the antibacterial activity among the different concentrations of the extracts and the controls. The Kruskal-Wallis test is a non-parametric test that compares the medians of two or more groups. If the Kruskal-Wallis test yielded a significant result, post-hoc Mann-Whitney U tests with Bonferroni correction were performed. Post-hoc tests are used to determine which specific groups differ significantly from each other after a significant result in the Kruskal-Wallis test. The Mann-Whitney U test is a non-parametric test that compares the medians of two groups. Bonferroni correction is a method used to adjust the significance level for multiple comparisons, reducing the risk of false positive results. A p-value of < 0.05 was considered statistically significant. This significance level is commonly used in biological research.

3. Results

Table 1 presents the results of phytochemical

screening performed on ethanolic extracts of Melinjo (*Gnetum gnemon* L.) and Arumanis Mango (*Mangiferaindica* L.) leaves. Phytochemical screening is a qualitative analysis used to detect the presence of various classes of secondary metabolites, which are bioactive compounds known to contribute to the medicinal properties of plants; Active Compound (Phytochemical Class): This column lists the different types of secondary metabolites tested for, including flavonoids, alkaloids, triterpenoids/steroids, glycosides, saponins, and tannins; Detection Method and Reagents: This column specifies the reagents and methods used to detect each phytochemical class. These are standard chemical tests that produce observable reactions (e.g., color change, precipitate formation) when the target compounds are present; Ethanolic Extract of Melinjo Leaves (EEDM): This column indicates the presence (+) or absence (-) of each phytochemical class in the ethanolic extract of Melinjo leaves; Ethanolic Extract of Arumanis Mango Leaves (EEDMA): This column indicates the presence (+) or absence (-) of each phytochemical class in the ethanolic extract of Arumanis Mango leaves; Flavonoids: Both Melinjo and Arumanis Mango leaf extracts tested positive (+) for flavonoids. Flavonoids are a large group of polyphenolic compounds known for their antioxidant, anti-inflammatory, and antimicrobial properties; Alkaloids: Melinjo leaf extract tested positive (+) for alkaloids using Meyer's, Dragendorff's, and Bouchardat's reagents. Alkaloids are a diverse group of nitrogen-containing compounds often possessing significant pharmacological activities. Arumanis Mango leaf extract tested negative (-) for alkaloids using Meyer's reagent, but positive (+) using Dragendorff's and Bouchardat's reagents. The difference in results using different reagents for alkaloids might be due to the varying sensitivities and specificities of the reagents to different types of alkaloids. It's possible that Arumanis Mango leaves contain alkaloids that react with Dragendorff's and Bouchardat's reagents but not with Meyer's reagent; Triterpenoids/Steroids: Both Melinjo and Arumanis Mango leaf extracts tested positive (+) for triterpenoids/steroids. These compounds have diverse biological activities, including anti-inflammatory and antimicrobial effects; Glycosides: Both Melinjo and Arumanis Mango leaf extracts tested positive (+) for glycosides. Glycosides are compounds containing a sugar moiety bonded to another molecule, and they can have various pharmacological effects; Saponins: Both Melinjo and Arumanis Mango leaf extracts tested positive (+) for saponins. Saponins are glycosides with foaming properties and have been reported to possess antimicrobial and other biological activities; Tannins: Both Melinjo and Arumanis Mango leaf extracts tested positive (+) for tannins. Tannins are polyphenolic compounds known for their astringent and antimicrobial properties.

Table 1. Phytochemical screening of ethanolic extracts of Melinjo (*Gnetum gnemon* L.) and Arumanis mango (*Mangifera indica* L.) leaves.

Active compound (Phytochemical Class)	**Detection method and reagents**	**Ethanolic extract of melinjo leaves (EEDM)**	**Ethanolic extract of arumanis mango leaves (EEDMA)**
Flavonoids	$MgHCl + H_2SO_4$	+	+
Alkaloids	Meyer's Reagent (HgCl + Distilled Water + KI)	+	-
	Dragendorff's Reagent (Bismuth subnitrate + Nitric acid + Distilled Water + Potassium iodide)	+	+
	Bouchardat's Reagent (KI + Distilled Water + Iodine)	+	+
Triterpenoids/Steroids	Lieberman-Burchard Reagent (Concentrated H_2SO_4 + Acetic Anhydride)	+	+
Glycosides	Molisch's Test (α-naphthol + Concentrated H_2SO_4	+	+
Saponins	Distilled Water (Formation of stable froth)	+	+
Tannins	$FeCl_3$ Solution	+	+

+: Detected; -: Not Detected.

Table 2 presents the results of the antibacterial activity of Melinjo and Arumanis Mango leaf extracts against *Enterococcus faecalis*, measured by the diameter of the inhibition zones in a disc diffusion assay; Group: This column categorizes the samples tested, including Melinjo Leaf Extract, Arumanis Mango Leaf Extract, Chlorhexidine 0.2% (Positive Control), and Distilled Water (Negative Control); Concentration: This column specifies the concentrations at which the plant extracts were tested (25%, 50%, and 75%). The positive and negative controls do not have varying concentrations; Replicate 1, Replicate 2, Replicate 3: These columns show the inhibition zone diameters (in mm) obtained for each of the three replicates of each test condition; Mean ± SD: This column presents the mean inhibition zone diameter and the standard deviation for each test condition; Melinjo Leaf Extract: At all concentrations tested (25%, 50%, and 75%), the Melinjo Leaf Extract showed no inhibition of *Enterococcus faecalis*. The inhibition zone diameters for all replicates were 0 mm, resulting in a mean inhibition zone of 0.00 ± 0.00 mm for each concentration. This indicates that, under the conditions of this study, the Melinjo Leaf Extract did not demonstrate any antibacterial activity against *Enterococcus faecalis*; Arumanis Mango Leaf Extract: The Arumanis Mango Leaf Extract exhibited antibacterial activity against *Enterococcus faecalis*. At 25% concentration, the mean inhibition zone diameter was 0.00 ± 0.00 mm. The antibacterial activity increased with increasing concentration. At 50% concentration, the mean inhibition zone diameter was 11.73 ± 0.75 mm. At 75% concentration, the mean inhibition zone diameter was 12.90 ± 0.30 mm. The results suggest a concentration-dependent antibacterial effect of the Arumanis Mango Leaf Extract against *Enterococcus faecalis*; Chlorhexidine 0.2% (Positive Control): The positive control, Chlorhexidine 0.2%, showed the largest inhibition zone with a mean diameter of 15.10 ± 0.10 mm. This result validates the effectiveness of the experimental setup and confirms the susceptibility of *Enterococcus faecalis* to a known antibacterial agent; Distilled Water (Negative Control): The negative control, Distilled Water, showed no inhibition of *Enterococcus faecalis* (0.00 ± 0.00 mm). This confirms that the solvent itself

did not have any antibacterial effect and that the inhibition observed with the Arumanis Mango Leaf Extract was due to the extract's components.

Table 2. Mean inhibition zone diameters (mm) of Melinjo and Arumanis mango leaf extracts against *Enterococcus faecalis*.

Group	Concentration	Replicate 1	Replicate 2	Replicate 3	Mean ± SD
Melinjo Leaf Extract	25%	0	0	0	0.00 ± 0.00
	50%	0	0	0	0.00 ± 0.00
	75%	0	0	0	0.00 ± 0.00
Arumanis Mango Leaf Extract	25%	0	0	0	0.00 ± 0.00
	50%	11.3	12.6	11.3	11.73 ± 0.75
	75%	12.9	13.2	12.6	12.90 ± 0.30
Chlorhexidine 0.2% (Positive Control)	-	15.2	15.0	15.1	15.10 ± 0.10
Distilled Water (Negative Control)	-	0	0	0	0.00 ± 0.00

Table 3 presents the results of a post-hoc analysis using the Mann-Whitney U test. This test was conducted to perform pairwise comparisons between the different extracts and concentrations after a significant result was obtained from a Kruskal-Wallis test (which would have been used to compare multiple groups initially). The Mann-Whitney U test is a non-parametric test used to compare two independent groups. In this case, it's used to determine which specific pairs of extracts and concentrations showed statistically significant differences in their antibacterial activity against *Enterococcus faecalis*; Group 1 (Extract and Concentration): This column lists the first extract and its concentration in each pairwise comparison; Group 2 (Extract and Concentration): This column lists the second extract and its concentration in each pairwise comparison; p-value: This column shows the calculated p-value for each comparison. The p-value is a measure of the probability of observing the results if there were no real difference between the two groups; Significance: This column indicates whether the comparison is statistically significant or not, based on the p-value. A p-value less than the significance level (typically 0.05) is considered statistically significant, meaning that the observed difference is unlikely to be due to chance; Melinjo (*Gnetum gnemon* L.) Extract Comparisons: Comparisons within Melinjo extract at different concentrations (25%, 50%, 75%) show "Not Significant" differences (p-value = 1.000). This confirms that varying the concentration of Melinjo extract did not result in a statistically significant difference in antibacterial activity, which aligns with the observation that Melinjo extract showed no inhibition. When Melinjo extract at any concentration is compared to Arumanis Mango extract at 50% or 75% concentration, the comparisons are "Significant" (p-values = 0.034 or 0.037). This indicates that Arumanis Mango extract at 50% and 75% had significantly greater antibacterial activity than Melinjo extract at any concentration. Melinjo extract at all concentrations compared to Chlorhexidine 0.2% are "Significant" (p-values = 0.037), showing that Chlorhexidine had significantly greater antibacterial activity than Melinjo extract. Melinjo extract compared to Aquadest (distilled water) is "Not Significant" (p-value = 1.000), which is expected as neither showed inhibition; Arumanis Mango (*Mangifera indica* L.) Extract Comparisons: Comparisons of Arumanis Mango 25% extract to 50% and 75% extracts are "Significant" (p-values = 0.034 and 0.037), indicating that higher concentrations of Arumanis Mango extract had significantly greater antibacterial activity than the 25% concentration. The comparison between

Arumanis Mango 50% and 75% extracts is "Not Significant" (p-value = 0.072). While there was a trend of increased activity with higher concentration, the difference between 50% and 75% was not statistically significant. Arumanis Mango extracts at 25%, 50%, and 75% concentrations all showed "Significant" differences when compared to Chlorhexidine 0.2% (p-values = 0.037, 0.046, and 0.050), indicating that Chlorhexidine had significantly greater antibacterial activity than all concentrations of Arumanis Mango extract. Arumanis Mango extracts at all concentrations were "Significant" when compared to Aquadest (p-values = 0.034, 0.037), confirming that Arumanis Mango extract had a significant antibacterial effect compared to the negative control; Chlorhexidine 0.2% vs. Aquadest: The comparison between Chlorhexidine 0.2% and Aquadest is "Significant" (p-value = 0.037), as expected, confirming the significant antibacterial effect of the positive control compared to the negative control.

Table 3. Post-hoc analysis of antibacterial activity using the Mann-Whitney U test.

Group 1 (Extract and Concentration)	**Group 2 (Extract and Concentration)**	**p-value**	**Significance**
Gnetum gnemon L. (25%)	*Gnetum gnemon L. (50%)*	1.000	Not Significant
Gnetum gnemon L. (25%)	*Gnetum gnemon L. (75%)*	1.000	Not Significant
Gnetum gnemon L. (25%)	*Mangifera indica L. (25%)*	1.000	Not Significant
Gnetum gnemon L. (25%)	*Mangifera indica L. (50%)*	0.034	Significant
Gnetum gnemon L. (25%)	*Mangifera indica L. (75%)*	0.037	Significant
Gnetum gnemon L. (25%)	Chlorhexidine 0.2%	0.037	Significant
Gnetum gnemon L. (25%)	Aquadest	1.000	Not Significant
Gnetum gnemon L. (50%)	*Gnetum gnemon L. (75%)*	1.000	Not Significant
Gnetum gnemon L. (50%)	*Mangifera indica L. (25%)*	1.000	Not Significant
Gnetum gnemon L. (50%)	*Mangifera indica L. (50%)*	0.034	Significant
Gnetum gnemon L. (50%)	*Mangifera indica L. (75%)*	0.037	Significant
Gnetum gnemon L. (50%)	Chlorhexidine 0.2%	0.037	Significant
Gnetum gnemon L. (50%)	Aquadest	1.000	Not Significant
Gnetum gnemon L. (75%)	*Mangifera indica L. (25%)*	1.000	Not Significant
Gnetum gnemon L. (75%)	*Mangifera indica L. (50%)*	0.034	Significant
Gnetum gnemon L. (75%)	*Mangifera indica L. (75%)*	0.037	Significant
Gnetum gnemon L. (75%)	Chlorhexidine 0.2%	0.037	Significant
Gnetum gnemon L. (75%)	Aquadest	1.000	Not Significant
Mangifera indica L. (25%)	*Mangifera indica L. (50%)*	0.034	Significant
Mangifera indica L. (25%)	*Mangifera indica L. (75%)*	0.037	Significant
Mangifera indica L. (25%)	Chlorhexidine 0.2%	0.037	Significant
Mangifera indica L. (25%)	Aquadest	1.000	Not Significant
Mangifera indica L. (50%)	*Mangifera indica L. (75%)*	0.072	Not Significant
Mangifera indica L. (50%)	Chlorhexidine 0.2%	0.046	Significant
Mangifera indica L. (50%)	Aquadest	0.034	Significant
Mangifera indica L. (75%)	Chlorhexidine 0.2%	0.050	Not Significant
Mangifera indica L. (75%)	Aquadest	0.037	Significant
Chlorhexidine 0.2%	Aquadest	0.037	Significant

4. Discussion

The ethanolic extract of Arumanis mango leaves demonstrated notable antibacterial activity against *E. faecalis*. The observation of antibacterial activity in the Arumanis mango leaf extract is consistent with a broader body of research that has explored the antimicrobial potential of various *Mangifera indica* preparations. This suggests that the *Mangifera indica* species, and Arumanis mango in particular, possesses constituents capable of interfering with bacterial viability and growth. The concentration-dependent increase in antibacterial activity, where higher extract concentrations yielded larger inhibition zones, is a crucial aspect of these findings. This dose-response relationship strongly suggests that the antibacterial effect is directly linked to the concentration of active compounds present in the extract. It implies that greater availability of these compounds enhances their

interaction with the bacterial cells, leading to a more pronounced disruption of essential bacterial processes. This observation aligns with fundamental principles in pharmacology and microbiology, where increased drug or antimicrobial agent concentrations generally correlate with increased efficacy, up to a certain threshold. The antibacterial activity observed can be attributed to the presence of various bioactive compounds identified in the phytochemical screening. These compounds, including flavonoids, alkaloids, tannins, and saponins, are known to exert their antibacterial effects through a variety of mechanisms. This multifaceted attack on bacterial cells is a key feature of many plant-derived antimicrobials, potentially contributing to their broad spectrum of activity and reduced likelihood of resistance development compared to single-target antibiotics. Specifically, mangiferin, a xanthone abundant in mango leaves, has been shown to inhibit bacterial replication. This interference with a fundamental process like replication can have a profound impact on bacterial growth and survival. Flavonoids, another group of compounds found in the extract, can alter the permeability of the cytoplasmic membrane. The disruption of membrane integrity leads to the leakage of intracellular constituents, compromising essential metabolic functions and ultimately leading to cell death. Tannins, due to their lipophilic nature, can readily bind to the bacterial cell wall, causing damage. This interaction can disrupt the structural integrity of the cell wall, interfering with its function as a protective barrier and leading to cell lysis. Alkaloids, yet another class of compounds present in the extract, can interfere with the formation of the peptidoglycan layer in the bacterial cell wall. Peptidoglycan is a crucial component of the bacterial cell wall, providing structural support and rigidity, and its disruption can compromise cell viability. Saponins, also detected in the extract, can increase membrane permeability, disrupting cell stability. This destabilization of the cell membrane can lead to the leakage of intracellular contents and ultimately cell death. The diverse mechanisms of action exhibited by these various phytochemicals likely contribute to the observed antibacterial effect of the Arumanis mango leaf extract. It is plausible that these compounds act synergistically, enhancing the overall antibacterial effect beyond what would be expected from the additive effects of individual compounds. The fact that Arumanis mango leaf extract exhibited antibacterial activity against *E. faecalis* is particularly relevant in the context of endodontic infections. *E. faecalis* is a persistent and challenging pathogen in root canal infections. Its ability to form biofilms and resist common antimicrobial agents makes it a significant obstacle in achieving successful endodontic treatment outcomes. The potential of Arumanis mango leaf extract to inhibit the growth of *E. faecalis* suggests that it could be explored as a natural alternative irrigating solution in endodontic treatment. This is particularly significant given the limitations associated with conventional endodontic irrigants, such as sodium hypochlorite and chlorhexidine, which, despite their efficacy, can exhibit toxicity or have limited ability to penetrate complex root canal anatomies and biofilms.[11-13]

In contrast to the findings for Arumanis mango, the ethanolic extract of melinjo (*Gnetum gnemon* L.) leaves did not demonstrate any antibacterial activity against *Enterococcus faecalis* under the conditions of this study. This absence of observed antibacterial effect is a noteworthy finding, especially when considered in the context of some previous research that has reported antibacterial activity of melinjo leaf extract against other bacterial species. The discrepancy between the present study's results and those of prior investigations highlights the complex interplay of factors that influence the antimicrobial potential of plant extracts. This finding contrasts with some previous research that has reported antibacterial activity of melinjo leaf extract against other bacterial species, such as *Streptococcus mutans*, *Escherichia coli*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. This variation in antibacterial activity across different bacterial species underscores the specificity that antimicrobial agents can exhibit. A compound or extract effective against one bacterium may not necessarily be effective against another. This specificity can be attributed to differences in bacterial cell structure, physiology, and resistance

mechanisms. The absence of antibacterial activity against *E. faecalis* in this study might be attributed to several factors, each of which plays a crucial role in determining the overall antimicrobial efficacy of a plant extract. These factors include but are not limited to, the specific extraction method employed, the concentration of the extract tested, and the inherent resistance of *E. faecalis* to the bioactive compounds present in melinjo leaves. The extraction method used to prepare plant extracts can significantly influence the composition and concentration of bioactive compounds obtained. Different solvents and extraction techniques can selectively extract different compounds, or vary the yield of extraction. In this study, an ethanolic extract of melinjo leaves was used. Ethanol is a commonly used solvent for extracting a wide range of compounds, but it may not be optimal for extracting compounds that are effective against *E. faecalis* from melinjo leaves. It is plausible that a different solvent, such as methanol, water, or a combination of solvents, could have yielded extracts with different chemical profiles and potentially different antibacterial activities. Similarly, alternative extraction techniques, such as maceration, Soxhlet extraction, or ultrasound-assisted extraction, could also influence the extraction efficiency and the types of compounds extracted. Therefore, the choice of extraction method is a critical factor in determining the antimicrobial potential of plant extracts. Furthermore, the concentration of the extract tested in this study might not have been sufficient to elicit an antibacterial effect against *E. faecalis*. Antimicrobial agents typically exhibit a dose-response relationship, where higher concentrations lead to greater antimicrobial activity. It is possible that the concentrations tested in this study, while sufficient to demonstrate activity against other bacteria in previous studies, were not high enough to inhibit the growth of *E. faecalis*. Some studies have indicated that melinjo extract might require higher concentrations to exhibit antibacterial effects against certain bacteria. For instance, a study by Jurdillah et al. (2022) found that melinjo peel extract showed antibacterial activity against *Propionibacterium acnes* only at a high concentration of 80%. This observation underscores the importance of testing a range of concentrations when evaluating the antimicrobial potential of plant extracts. The inherent resistance of *E. faecalis* to the bioactive compounds present in melinjo leaves is another possible explanation for the lack of antibacterial activity. *E. faecalis* possesses several mechanisms to resist antimicrobial agents, and these resistance mechanisms contribute to its persistence in endodontic infections and its ability to withstand antimicrobial treatments. These mechanisms include the formation of biofilms, the presence of efflux pumps, and the ability to alter its metabolic pathways. Biofilms, which are structured communities of bacteria encased in a self-produced matrix, provide a protective barrier against antimicrobial agents, preventing them from reaching the bacterial cells. Efflux pumps actively pump antimicrobial agents out of the bacterial cell, reducing their intracellular concentration and effectiveness. *E. faecalis* can also alter its metabolic pathways to survive in the presence of antimicrobial agents. It is possible that the bioactive compounds present in melinjo leaves are not effective in overcoming these resistance mechanisms in *E. faecalis*, leading to its continued growth and survival even in the presence of the extract.[14-17]

The phytochemical screening of the melinjo leaf extract in our study did reveal the presence of flavonoids, alkaloids, and tannins, which are generally known for their antimicrobial properties. This finding indicates that the melinjo leaf extract contains compounds that, in other contexts, have demonstrated the capacity to inhibit or kill microorganisms. The detection of these phytochemicals suggests that the extract possesses the fundamental building blocks for potential antimicrobial activity. The presence of flavonoids, alkaloids, and tannins in the melinjo leaf extract suggests that it possesses some potential for antimicrobial activity. These compounds have been extensively studied for their antimicrobial properties, and their mechanisms of action are relatively well understood. This extensive body of research provides a foundation for understanding how these compounds might exert antimicrobial effects. Flavonoids, for example, are a diverse group of polyphenolic

compounds found in plants. Their antimicrobial mechanisms are multifaceted, and include the ability to disrupt bacterial cell membranes. This disruption can lead to increased permeability, leakage of essential intracellular components, and ultimately cell death. Flavonoids can also inhibit bacterial enzymes, interfering with crucial metabolic pathways and hindering bacterial growth and survival. Tannins, another class of polyphenolic compounds, can bind to bacterial cell walls. This interaction can alter the structural integrity of the cell wall, disrupting its function as a protective barrier and leading to cell damage or lysis. Furthermore, tannins can interfere with bacterial metabolism by binding to and inactivating bacterial enzymes. This interference with metabolic processes can inhibit bacterial growth and contribute to cell death. Alkaloids, a diverse group of nitrogen-containing compounds, can disrupt various cellular processes in bacteria. Their mechanisms of action are varied and depend on the specific type of alkaloid, but they can include intercalation into DNA, inhibition of protein synthesis, and interference with cell division. These disruptions of essential cellular processes can lead to inhibition of bacterial growth and ultimately cell death. However, the fact that the melinjo leaf extract did not exhibit antibacterial activity against *E. faecalis* in this study highlights the complexity of the relationship between phytochemical composition and antimicrobial activity. The mere presence of compounds known for their antimicrobial properties does not guarantee that an extract will exhibit antimicrobial activity against a specific microorganism. This underscores the importance of considering factors beyond just the presence of certain compounds. The antimicrobial activity of a plant extract is a complex phenomenon influenced by a multitude of factors. The concentration of the bioactive compounds is critical even if antimicrobial compounds are present, their concentration may be too low to elicit a measurable effect against a particular bacterium. The specific types of compounds within a class (e.g., different types of flavonoids or alkaloids) can also influence activity, as different compounds may have varying potencies and specificities. Interactions between compounds within the extract are also important, synergistic interactions can enhance antimicrobial activity, while antagonistic interactions can diminish it. Furthermore, the susceptibility of the target microorganism plays a crucial role. Different bacteria have different levels of resistance to antimicrobial agents due to variations in their cell structure, physiology, and resistance mechanisms. A compound effective against one bacterium may not be effective against another. It is also important to note that the phytochemical screening methods used in this study are qualitative, providing information only on the presence or absence of certain compounds. Qualitative analysis, while useful for identifying the broad classes of compounds present, does not provide information about the concentration of these compounds. Quantitative analysis would be necessary to determine the exact concentration of each compound in the extracts. Techniques such as chromatography (e.g., HPLC, GC-MS) could be used to quantify the levels of specific compounds in the extract. This quantitative data could provide valuable insights into the potential antimicrobial activity of the extract and help to explain why the melinjo leaf extract did not exhibit activity against *E. faecalis*. Knowledge of the concentrations of individual compounds would also allow for a better understanding of potential synergistic or antagonistic interactions between compounds.[18-20]

5. Conclusion

This study provides compelling evidence for the antibacterial potential of Arumanis mango (*Mangifera indica* L.) leaf extract against *Enterococcus faecalis*, a bacterium frequently implicated in persistent root canal infections. The extract demonstrated significant in vitro antibacterial activity, particularly at concentrations of 50% and 75%, suggesting a concentration-dependent effect. This activity can likely be attributed to the presence of various bioactive compounds, including flavonoids, alkaloids, and tannins, known for their antimicrobial properties. These compounds likely exert their antibacterial effects through diverse mechanisms, such as disrupting bacterial membranes, interfering with cell wall synthesis, and inhibiting bacterial enzymes.

Conversely, the ethanolic extract of melinjo (*Gnetum gnemon* L.) leaves did not exhibit any antibacterial activity against *Enterococcus faecalis* under the conditions of this study. While phytochemical screening of melinjo leaf extract did reveal the presence of flavonoids, alkaloids, and tannins, the absence of antibacterial activity underscores the complexity of the relationship between phytochemical composition and antimicrobial activity. Factors such as the extraction method, extract concentration, and inherent bacterial resistance may have contributed to this result. The findings suggest that Arumanis mango leaf extract holds promise as a potential natural alternative irrigating solution in endodontic treatment, particularly for combating *Enterococcus faecalis* infections. Further research, however, is necessary to validate its clinical efficacy and safety in root canal disinfection.

6. References

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