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Mahogany (*Swietenia mahagoni*) Leaf Extract Exhibits Strong Antibacterial Activity Against *Enterococcus faecalis*: A Promising Natural Alternative to Chlorhexidine for Root Canal Infections

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A B S T R A C T

Enterococcus faecalis is a prevalent bacterium in root canal infections, exhibiting high resistance to conventional antibacterial agents. Mahogany (*Swietenia mahagoni*) leaf extract, rich in flavonoids, saponins, and tannins, has demonstrated promising antibacterial properties. This study investigated the antibacterial efficacy of mahogany leaf extract against *E. faecalis* and compared its effectiveness to chlorhexidine, a commonly used root canal irrigant. A laboratory experimental study was conducted using the disk diffusion method on Mueller Hinton Agar (MHA). Mahogany leaf extract was prepared at concentrations of 25%, 50%, and 70%. Chlorhexidine (0.2%) served as the positive control, and dimethyl sulfoxide (DMSO) as the negative control. The diameter of inhibition zones was measured after 24 hours of incubation. Statistical analysis was performed using One-Way ANOVA and Post Hoc LSD tests. All concentrations of mahogany leaf extract exhibited significant antibacterial activity against *E. faecalis* ($p < 0.05$). The 70% concentration produced the largest inhibition zone (20.39 ± 1.38 mm), followed by 50% (18.67 ± 1.12 mm) and 25% (17.28 ± 0.60 mm). The inhibition zones of the 50% and 70% mahogany leaf extract were comparable to those of chlorhexidine (19.40 ± 0.70 mm). In conclusion, mahogany leaf extract demonstrates strong antibacterial activity against *E. faecalis*, suggesting its potential as a natural alternative to chlorhexidine for root canal infections. Further research is warranted to explore its clinical application in endodontic treatment.

1. Introduction

Root canal infections represent a significant challenge in endodontic treatment, primarily due to the intricate nature of the root canal system and the persistent presence of pathogenic microorganisms. The primary objective of root canal therapy is to eliminate these microorganisms and prevent reinfection, thereby promoting healing and preserving the tooth. However, the complex anatomy of the root canal system, which includes irregularities, accessory canals, and dentinal tubules, provides a niche for bacterial colonization and proliferation. Among the diverse microbial communities that inhabit the root canal, *Enterococcus faecalis*, a facultative anaerobic gram-positive coccus, is frequently implicated in persistent endodontic infections and treatment failures. This bacterium possesses several virulence factors and adaptive mechanisms that contribute to its survival and persistence in the root canal environment. *E. faecalis* can withstand harsh conditions, including limited nutrient availability and wide pH variations. It is also capable of invading

dentinal tubules, which provides a protected site for bacterial colonization, shielding it from intracanal medicaments and host defense mechanisms. Furthermore, *E. faecalis* has a remarkable ability to form biofilms, complex microbial communities encased in a self-produced extracellular matrix. Biofilm formation enhances bacterial resistance to antimicrobial agents and host immune responses, making eradication challenging. The treatment of root canal infections relies on a combination of mechanical instrumentation and chemical disinfection. Mechanical instrumentation aims to remove infected tissue and shape the root canal system to facilitate disinfection. Chemical disinfection, achieved through the use of irrigants and intracanal medicaments, plays a crucial role in eliminating residual bacteria and disrupting biofilms.[1-4]

However, the effectiveness of conventional antibacterial agents is being compromised by the emergence of antibiotic-resistant bacterial strains. The increasing prevalence of antibiotic resistance among *E. faecalis* strains poses a significant challenge in the treatment of root canal infections. This phenomenon underscores the urgent need for novel therapeutic strategies to combat these infections effectively. In light of the limitations associated with conventional endodontic treatment and the growing concern over antibiotic resistance, there is a growing interest in exploring alternative therapeutic approaches. Natural products, particularly plant-derived extracts, have garnered considerable attention as potential sources of novel antimicrobial agents. These extracts often contain a complex mixture of bioactive compounds that can exert synergistic effects against target microorganisms. Plant-derived extracts offer several advantages, including a broad spectrum of activity, lower toxicity, and reduced risk of developing bacterial resistance. The diverse phytochemical constituents present in plant extracts, such as flavonoids, saponins, tannins, and terpenoids, have been shown to possess potent antimicrobial properties. These compounds can target various bacterial cellular processes, including cell wall synthesis, cell membrane integrity, enzyme activity, and nucleic acid synthesis. Mahogany (*Swietenia mahagoni*) is a tropical tree belonging to the Meliaceae family. This tree is widely distributed in Asia and is known for its various medicinal properties. Traditionally, different parts of the mahogany tree have been used to treat a variety of ailments, including fever, malaria, and diarrhea.[5-7]

The leaves of *Swietenia mahagoni* are rich in bioactive compounds, including flavonoids, saponins, and tannins. Flavonoids are a class of polyphenolic compounds known for their antioxidant, anti-inflammatory, and antimicrobial properties. They can disrupt bacterial cell membranes, increase membrane permeability, and inhibit bacterial enzyme activity. Saponins are glycosidic compounds that exhibit surfactant properties. They can destabilize bacterial cell membranes, leading to cell lysis and death. Tannins are complex polyphenolic compounds that can bind to proteins and other macromolecules. They can interfere with bacterial cell wall synthesis, inhibit bacterial enzymes, and disrupt bacterial adhesion. Previous studies have investigated the antibacterial activity of mahogany leaf extract against various bacterial species, including both gram-positive and gram-negative bacteria. These studies have demonstrated the potential of mahogany leaf extract as a broad-spectrum antimicrobial agent. However, the specific efficacy of mahogany leaf extract against *E. faecalis*, a key pathogen in root canal infections, requires further investigation. Chlorhexidine is a commonly used root canal irrigant known for its broad-spectrum antibacterial activity and substantivity. It is effective against a wide range of microorganisms, including *E. faecalis*. However, chlorhexidine has some limitations, including potential cytotoxicity, tooth discoloration, and hypersensitivity reactions. These drawbacks have prompted the search for alternative irrigants with comparable or superior antimicrobial efficacy and improved biocompatibility.[8-10] This study aimed to evaluate the antibacterial activity of mahogany leaf extract against *E. faecalis* and compare its

effectiveness to that of chlorhexidine.

2. Methods

This study employed a laboratory experimental design to evaluate the antibacterial activity of mahogany (*Swietenia mahagoni*) leaf extract against *Enterococcus faecalis* and to compare its effectiveness with chlorhexidine. The methodology encompasses plant material collection and extract preparation, bacterial strain and culture conditions, antibacterial activity assay, and statistical analysis.

The leaves of *Swietenia mahagoni* were the source material for the plant extract. Mature *Swietenia mahagoni* trees served as the source of the leaves, and the collection occurred in Medan, Indonesia, in October 2024. The leaves were carefully harvested and then subjected to a rigorous cleaning process. This involved washing the leaves thoroughly with distilled water to remove any surface contaminants. Following the washing step, the leaves were air-dried at room temperature for a period of 7 days. This drying process was crucial to reduce the moisture content of the leaves, which is essential for effective grinding and extraction. After the drying process was completed, the dried leaves were ground into a fine powder. A blender was used to achieve this, ensuring a consistent and homogenous powder. The powdering process increases the surface area of the plant material, which facilitates efficient extraction of the bioactive compounds. For the extraction of the bioactive compounds, 1000 grams of the powdered leaves were used. The extraction process involved maceration, where the powdered leaves were soaked in a solvent to dissolve the desired compounds. In this study, 96% ethanol was used as the solvent. One liter of 96% ethanol was added to the powdered leaves, and the mixture was allowed to stand for 24 hours at room temperature. To ensure thorough mixing and extraction, the mixture was shaken occasionally during this 24-hour period. Following the maceration period, the mixture was filtered to separate the liquid extract from the solid residue. Whatman No. 1 filter paper was used for this purpose. The residue remaining after the initial filtration still contained some extractable compounds, so it was re-extracted with another 500 ml of 96% ethanol. This second extraction step aimed to maximize the yield of the extract. The filtrates obtained from both extraction steps were combined. The combined filtrates were then subjected to a concentration process using a rotary evaporator. This process was carried out at a temperature of 40°C and under reduced pressure. The rotary evaporator is designed to remove the solvent (ethanol in this case) from the extract, resulting in a more concentrated form. The reduced pressure lowers the boiling point of the solvent, allowing for evaporation at a lower temperature, which helps to prevent degradation of heat-sensitive compounds in the extract. The resulting concentrated crude extract was collected and stored at 4°C. This storage temperature is used to preserve the stability of the extract and prevent degradation of its bioactive components until further use in the study.

The bacterial strain used in this study was *Enterococcus faecalis*. Specifically, the *Enterococcus faecalis* strain used was ATCC 29212. This strain was obtained from the Microbiology Laboratory, Faculty of Medicine, Universitas Sumatera Utara. The bacteria were cultured on Mueller Hinton Agar (MHA) plates. MHA is a microbiological growth medium that is commonly used for antimicrobial susceptibility testing. The bacteria were incubated on the MHA plates at a temperature of 37°C for 24 hours. This incubation period allows for sufficient bacterial growth. The incubation was carried out under anaerobic conditions. Anaerobic conditions are necessary because *Enterococcus faecalis* is a facultative anaerobic bacterium, meaning it can grow in both the presence and absence of oxygen, but some strains may prefer or tolerate anaerobic conditions better. The use of anaerobic conditions in this study likely aimed to mimic the conditions found in root canal infections, which are often characterized by low oxygen levels.

The disk diffusion method was employed to evaluate the antibacterial activity of the mahogany leaf extract. This method is a widely used technique for

assessing the in vitro effectiveness of antimicrobial agents against bacteria. The mahogany leaf extract, which was initially in crude form, was dissolved in dimethyl sulfoxide (DMSO). DMSO is a polar aprotic solvent that is often used to dissolve compounds that are poorly soluble in water. It is commonly used in antimicrobial assays as a solvent control because it generally does not exhibit significant antibacterial activity at low concentrations. The extract was prepared in DMSO to achieve three different concentrations: 25%, 50%, and 70%. These varying concentrations were used to assess the concentration-dependent effect of the extract's antibacterial activity. Chlorhexidine (0.2%) was used as the positive control in this assay. Chlorhexidine is a broad-spectrum antimicrobial agent that is commonly used in endodontics as a root canal irrigant. It is known for its effectiveness against *Enterococcus faecalis* and other root canal pathogens. The use of chlorhexidine as a positive control allows for a comparison of the antibacterial activity of the mahogany leaf extract with a known effective antimicrobial agent. DMSO, the solvent used to dissolve the extract, was used as the negative control. This control is crucial to ensure that any observed antibacterial activity is due to the extract and not the solvent itself. Sterile filter paper disks were used as the carriers for the test solutions. The filter paper disks were 6 mm in diameter. These disks were impregnated with 20 µl of each test solution. This precise volume was applied to each disk to ensure consistency in the amount of test substance delivered. The test solutions included the three concentrations of mahogany leaf extract (25%, 50%, and 70%), the positive control (chlorhexidine 0.2%), and the negative control (DMSO). The filter paper disks, each impregnated with a different test solution, were then placed on MHA plates. These MHA plates had been previously inoculated with *Enterococcus faecalis*. The inoculation process involves spreading a standardized suspension of the bacteria evenly over the surface of the agar plate, creating a bacterial lawn. After the disks were placed on the inoculated agar plates, the plates were incubated at 37°C for 24 hours. This incubation period allows time for the test substances to diffuse from the disks into the surrounding agar and for any antibacterial activity to manifest as zones of inhibition. As mentioned earlier, the incubation was carried out under anaerobic conditions. Following the incubation period, the plates were examined for zones of inhibition. A zone of inhibition is a clear area around the disk where bacterial growth has been inhibited or prevented by the antimicrobial agent. The diameter of the inhibition zones around each disk was measured in millimeters. A digital caliper was used for these measurements to ensure accuracy and precision. The diameter of the zone of inhibition is a quantitative measure of the antibacterial activity of the test substance. A larger zone of inhibition indicates greater antibacterial activity.

The data obtained from the antibacterial activity assay were subjected to statistical analysis to determine the significance of the results. The statistical analysis was performed using SPSS software, version 28. Prior to conducting the main statistical tests, the normality of the data was assessed. The Shapiro-Wilk test was used to determine whether the data were normally distributed. This test is appropriate for small sample sizes and is commonly used to check for normality in statistical analysis. Assessing the normality of the data is important because it determines the type of statistical tests that can be appropriately used. One-Way ANOVA (Analysis of Variance) was used to compare the mean inhibition zone diameters among the different treatment groups. One-Way ANOVA is a statistical test that is used to compare the means of three or more independent groups. In this study, the treatment groups were the different concentrations of mahogany leaf extract, the positive control (chlorhexidine), and the negative control (DMSO). This test allows for determining whether there are any statistically significant differences in antibacterial activity among the different treatments. If the One-Way ANOVA indicated that there were significant differences among the groups, Post Hoc LSD (Least Significant Difference) tests were performed. Post Hoc tests are used to make pairwise

comparisons between the group means to identify which specific groups are significantly different from each other. The Post Hoc LSD test is a commonly used test for this purpose. These tests help to pinpoint exactly where the differences lie. In all statistical tests, a p-value of less than 0.05 was considered statistically significant. The p-value is the probability of obtaining the observed results (or more extreme results) if there were no real effect. A p-value of less than 0.05 indicates that the results are unlikely to have occurred by chance, and therefore, there is evidence to reject the null hypothesis and conclude that there is a statistically significant difference or effect.

3. Results and Discussion

Table 1 presents the diameter of inhibition zones (in millimeters) for mahogany leaf extract at different concentrations, chlorhexidine (positive control), and DMSO (negative control) against *Enterococcus faecalis*. The table clearly demonstrates that mahogany leaf extract exhibits antibacterial activity against *E. faecalis* at all tested concentrations (25%, 50%, and 70%). This is evident by the mean inhibition zone diameters, which range from 17.28 mm to 20.39 mm. A concentration-dependent relationship is observed with the mahogany leaf extract. As the concentration of the extract increases, the mean diameter of the inhibition zone also increases. The 70% concentration of mahogany leaf extract produced the largest inhibition zone (20.39 ± 1.38 mm), followed by the 50% concentration (18.67 ± 1.12 mm) and then the 25% concentration (17.28 ± 0.60 mm). When comparing the mahogany leaf extract to the positive control, chlorhexidine (0.2%), it is observed that the inhibition zone of the 70% mahogany leaf extract (20.39 ± 1.38 mm) is slightly larger than that of chlorhexidine (19.40 ± 0.70 mm). The 50% concentration's inhibition zone (18.67 ± 1.12 mm) is comparable to chlorhexidine. The negative control, DMSO, showed no inhibition zone (0.00 ± 0.00 mm), indicating that the solvent itself did not exhibit any antibacterial activity. This confirms that the inhibition observed in the other groups is due to the antibacterial activity of the mahogany leaf extract and chlorhexidine.

Table 1. Diameter of inhibition zones (mm) of mahogany leaf extract and controls against *E. faecalis*.

Treatment group	Mean ± SD
Mahogany leaf extract 25%	17.28 ± 0.60
Mahogany leaf extract 50%	18.67 ± 1.12
Mahogany leaf extract 70%	20.39 ± 1.38
Chlorhexidine 0.2%	19.40 ± 0.70
DMSO	0.00 ± 0.00

Table 2 presents the results of the Post Hoc LSD (Least Significant Difference) test, which was used for multiple comparisons of the inhibition zone diameters. This test helps to determine which specific groups are significantly different from each other after the One-Way ANOVA indicated an overall difference among the groups; Mahogany 25% vs. Mahogany 50%: The mean difference in inhibition zone diameters is -1.38 mm, and the p-value is 0.013. This indicates a statistically significant difference between the 25% and 50% concentrations of mahogany extract. The negative value suggests that the 50% concentration had a larger inhibition zone; Mahogany 25% vs. Mahogany 70%: The mean difference is -3.10 mm, and the p-value is 0.000. This shows a highly significant difference between the 25% and 70% concentrations, with the 70% concentration having a larger zone of inhibition; Mahogany 25% vs. Chlorhexidine: The mean difference is -2.12 mm, and the p-value is 0.000. This indicates a significant difference between the 25% mahogany extract and chlorhexidine, with chlorhexidine showing a larger inhibition zone; Mahogany 25% vs. DMSO: The mean difference is 17.28 mm, and the p-value is 0.000. This

demonstrates a highly significant difference between the 25% mahogany extract and DMSO, with the mahogany extract showing a much larger inhibition zone, as expected since DMSO is the negative control; Mahogany 50% vs. Mahogany 70%: The mean difference is -1.72 mm, and the p-value is 0.003. This shows a significant difference between the 50% and 70% concentrations, with the 70% concentration having a larger inhibition zone; Mahogany 50% vs. Chlorhexidine: The mean difference is -0.73 mm, and the p-value is 0.168. This indicates that there is no statistically significant difference between the 50% mahogany extract and chlorhexidine. Their inhibition zones are comparable; Mahogany 50% vs. DMSO: The mean difference is 18.67 mm, and the p-value is 0.000. This shows a highly significant difference between the 50% mahogany extract and DMSO; Mahogany 70% vs. Chlorhexidine: The mean difference is 0.98 mm, and the p-value is 0.069. This indicates that there is no statistically significant difference between the 70% mahogany extract and chlorhexidine. Their inhibition zones are comparable; Mahogany 70% vs. DMSO: The mean difference is 20.39 mm, and the p-value is 0.000. This shows a highly significant difference between the 70% mahogany extract and DMSO; Chlorhexidine vs. DMSO: The mean difference is 19.40 mm, and the p-value is 0.000. This demonstrates a highly significant difference between chlorhexidine and DMSO.

Table 2. Post Hoc LSD test results for multiple comparisons of inhibition zone diameters.

Comparison	**Mean difference**	**p-value**
Mahogany 25% vs. Mahogany 50%	-1.38	0.013
Mahogany 25% vs. Mahogany 70%	-3.10	0.000
Mahogany 25% vs. Chlorhexidine	-2.12	0.000
Mahogany 25% vs. DMSO	17.28	0.000
Mahogany 50% vs. Mahogany 70%	-1.72	0.003
Mahogany 50% vs. Chlorhexidine	-0.73	0.168
Mahogany 50% vs. DMSO	18.67	0.000
Mahogany 70% vs. Chlorhexidine	0.98	0.069
Mahogany 70% vs. DMSO	20.39	0.000
Chlorhexidine vs. DMSO	19.40	0.000

Table 3 presents the results of the phytochemical analysis of mahogany leaf extract. This analysis aimed to identify the presence of various plant secondary metabolites, which are known to contribute to the medicinal properties of plants. The table lists the phytochemicals tested, the qualitative tests used to detect them, and the results of those tests; Flavonoids: The presence of flavonoids was confirmed by positive results in the Shinoda test, Alkaline Reagent test, and Lead Acetate test. Flavonoids are a class of polyphenolic compounds known for their antioxidant, anti-inflammatory, and antimicrobial properties; Saponins: Saponins were detected by positive results in the Foam test, Froth test, and Liebermann-Burchard test. Saponins are glycosidic compounds that exhibit surfactant properties and have been shown to possess antimicrobial activity; Tannins: The presence of tannins was confirmed by positive results in the Ferric Chloride test, Gelatin test, and Lead Acetate test. Tannins are complex polyphenolic compounds that can bind to proteins and have astringent and antimicrobial properties; Terpenoids: Terpenoids were detected by positive results in the Salkowski test, Liebermann-Burchard test, and Noller's test. Terpenoids are a diverse group of compounds known for their various biological activities, including antimicrobial and anti-inflammatory effects.

Table 3. Phytochemical analysis of mahogany leaf extract.

Phytochemical	Qualitative test	Results
Alkaloids	Mayer's Test	-
	Wagner's Test	-
	Dragendorff's Test	-
Flavonoids	Shinoda Test	+
	Alkaline Reagent Test	+
	Lead Acetate Test	+
Saponins	Foam Test	+
	Froth Test	+
	Liebermann-Burchard Test	+
Tannins	Ferric Chloride Test	+
	Gelatin Test	+
	Lead Acetate Test	+
Terpenoids	Salkowski Test	+
	Liebermann-Burchard Test	+
	Noller's Test	+

The results of this study unequivocally demonstrate that mahogany (*Swietenia mahagoni*) leaf extract possesses significant antibacterial activity against *Enterococcus faecalis*. This finding is a cornerstone of the investigation, providing initial evidence that supports the potential of this natural extract as a source of antimicrobial agents effective against a bacterium of critical importance in endodontic infections. The observation of clear inhibition zones in the disk diffusion assay serves as a direct visual and quantitative indicator of the extract's capacity to impede bacterial growth. All concentrations of the extract tested, specifically 25%, 50%, and 70%, exhibited inhibitory effects on the growth of *E. faecalis*. This consistency across the tested concentrations underscores the inherent antibacterial potential of the mahogany leaf extract. It suggests that the bioactive components responsible for the antimicrobial action are present in sufficient quantities within the extract to elicit a measurable response across a range of concentrations. The fact that even the lowest concentration tested (25%) demonstrated a noticeable inhibitory effect is particularly noteworthy, as it implies a relatively high potency of the extract. The formation of clear inhibition zones is a fundamental observation in the disk diffusion assay, and it provides crucial information about the interaction between an antimicrobial agent and bacteria. In this context, the inhibition zones represent areas on the Mueller Hinton Agar (MHA) plates where bacterial growth has been prevented or inhibited by the diffusion of the mahogany leaf extract. The absence of bacterial growth within these zones indicates that the extract contains components that are capable of interfering with essential bacterial processes, ultimately leading to cell death or growth arrest. The mechanisms by which the mahogany leaf extract inhibits *E. faecalis* growth are multifaceted and likely involve the interaction of various bioactive compounds with different bacterial targets. While this study did not delve into the specific mechanisms of action, it is plausible that the extract's components disrupt bacterial cell membranes, interfere with metabolic pathways, inhibit enzyme activity, or damage bacterial DNA. Further investigations, such as those involving electron microscopy, enzyme assays, and molecular techniques, could elucidate the precise mechanisms by which the extract exerts its antibacterial effects. The observation of inhibition zones, regardless of the precise mechanisms involved, is a critical first step in validating the antibacterial potential of a substance. It provides a clear indication

that the substance has the capacity to directly or indirectly interfere with bacterial viability. In the context of this study, the clear inhibition zones observed with mahogany leaf extract provide a strong rationale for further investigation into its potential as an antimicrobial agent for endodontic applications. Beyond the mere presence of inhibition zones, the study also revealed a crucial relationship between the concentration of the mahogany leaf extract and the extent of its antibacterial activity. The antibacterial activity of the extract was found to be concentration-dependent. This concentration-dependent relationship is a common characteristic of antimicrobial agents, including plant extracts. It is a fundamental principle in pharmacology and microbiology that the effectiveness of a substance in inhibiting or killing microorganisms is often directly related to its concentration at the site of action. In this study, as the concentration of the mahogany leaf extract increased, the diameter of the inhibition zones also increased. This observation signifies that higher concentrations of the extract resulted in a greater inhibitory effect on *E. faecalis* growth. The 70% concentration of the extract produced the largest inhibition zone, followed by the 50% concentration, and then the 25% concentration. This pattern is consistent with the expectation that a greater amount of the active antimicrobial components will exert a more pronounced effect on bacterial cells. The concentration-dependent relationship observed with mahogany leaf extract has several important implications. First, it suggests that the antibacterial effect is not an all-or-nothing phenomenon. Rather, there is a graded response, with increasing concentrations leading to increasing inhibition. This allows for the possibility of titrating the concentration of the extract to achieve a desired level of antibacterial activity. Second, the concentration-dependent relationship provides insights into the potency of the extract. The fact that even the lower concentrations exhibited measurable inhibition suggests that the active components are effective at relatively low concentrations. However, the greater inhibition observed at higher concentrations indicates that increasing the concentration enhances the overall antibacterial effect. Third, the concentration-dependent relationship has practical implications for the potential clinical application of the extract. It suggests that higher concentrations may be more effective in eradicating *E. faecalis* in root canal infections. However, it is also important to consider potential toxicity and biocompatibility issues when using higher concentrations of any antimicrobial agent. The fact that the 70% concentration of the extract produced the largest inhibition zone is particularly noteworthy. This observation suggests that this concentration is the most effective among those tested in inhibiting the growth of *E. faecalis* under the conditions of the study. It implies that the 70% concentration delivers the highest amount of active antimicrobial components to the bacterial cells, resulting in the greatest inhibitory effect. However, it is crucial to recognize that the optimal concentration for clinical use may differ from the optimal concentration observed in vitro. Factors such as the complex environment of the root canal system, the presence of dentinal tubules, and the interaction with host tissues can influence the effectiveness of an antimicrobial agent. Therefore, further studies are needed to determine the optimal concentration of mahogany leaf extract for endodontic applications. The observation that the 25% concentration of the extract produced the smallest inhibition zone, while still demonstrating measurable antibacterial activity, is also significant. This finding suggests that even at relatively low concentrations, the extract possesses some degree of inhibitory effect on *E. faecalis*. This is important because it indicates that the extract may have a relatively high potency, as it can elicit a response even at lower concentrations. However, it is also important to consider that the antibacterial activity observed at the 25% concentration may not be sufficient for effective eradication of *E. faecalis* in all clinical situations. The severity of the infection, the bacterial load, and the presence of biofilms can all influence the effectiveness of an antimicrobial agent.

Therefore, higher concentrations may be necessary to achieve a clinically significant antibacterial effect. The concentration-dependent relationship observed in this study is a common characteristic of antimicrobial agents, including plant extracts. This type of relationship is not unique to mahogany leaf extract and has been reported for a wide range of natural and synthetic antimicrobial substances. It reflects the fundamental principle that the effectiveness of an antimicrobial agent is often directly related to its concentration at the site of action. The fact that this concentration-dependent relationship is observed with mahogany leaf extract further supports its potential as a source of antimicrobial agents. It suggests that the extract behaves in a manner consistent with other known antimicrobial substances and that its effectiveness can be modulated by adjusting its concentration. The observation of a concentration-dependent relationship also has implications for the standardization and formulation of mahogany leaf extract for endodontic use. It highlights the importance of controlling the concentration of the extract in order to ensure consistent and predictable antibacterial activity. Standardized extraction procedures and formulations are necessary to ensure that clinicians can reliably obtain a product with a defined concentration of active antimicrobial components.[11-15]

A key and critical aspect of this study was the comparison of the antibacterial activity of mahogany leaf extract with that of chlorhexidine. Chlorhexidine, in this context, served as a widely used positive control, a benchmark against which the efficacy of the experimental extract could be rigorously evaluated. The selection of chlorhexidine as a positive control is of paramount importance due to its established role and widespread application in endodontic treatment. It is a well-recognized antimicrobial agent known for its broad-spectrum activity and effectiveness against a variety of oral microorganisms, including *Enterococcus faecalis*. Therefore, using chlorhexidine as a comparator provides a clinically relevant context for interpreting the antibacterial potential of mahogany leaf extract. The results of the study revealed a significant finding that the 50% and 70% concentrations of mahogany leaf extract exhibited antibacterial activity comparable to that of 0.2% chlorhexidine. This determination of comparability is based on the quantitative assessment of the inhibition zones produced by these treatments. Specifically, the statistical analysis of the data indicated that there was no statistically significant difference in the size of the inhibition zones produced by the 50% and 70% extracts and chlorhexidine. This lack of statistical significance is a crucial point. In scientific research, statistical significance is a measure of the likelihood that an observed effect is not due to chance. A statistically significant difference suggests that the observed effect is likely a real effect of the treatment. Conversely, a lack of statistical significance, as observed in the comparison between the 50% and 70% mahogany leaf extract and chlorhexidine, implies that the differences in the mean inhibition zone diameters are not large enough to be considered anything other than random variation. In practical terms, this means that, within the limitations of this in vitro study, the antibacterial activity of the 50% and 70% mahogany leaf extracts is statistically indistinguishable from that of 0.2% chlorhexidine. This finding carries substantial weight, as it suggests that mahogany leaf extract, at these concentrations, has the potential to be as effective as chlorhexidine in inhibiting the growth of *E. faecalis in vitro*. The implications of this finding are far-reaching. Chlorhexidine has been a cornerstone of endodontic disinfection for many years. Its effectiveness in reducing bacterial load within the root canal system is well-documented. However, chlorhexidine is not without its drawbacks. Concerns have been raised regarding its potential cytotoxicity, its ability to cause tooth discoloration, and the occurrence of hypersensitivity reactions in some patients. These limitations have fueled the search for alternative antimicrobial agents that can provide comparable efficacy with improved biocompatibility and reduced adverse effects. The finding that mahogany leaf extract, at certain concentrations,

demonstrates antibacterial activity comparable to chlorhexidine opens up the possibility of a natural alternative for endodontic disinfection. If further research confirms its efficacy and safety in more clinically relevant settings, mahogany leaf extract could potentially offer a valuable option for clinicians seeking alternatives to chlorhexidine. It is important to delve deeper into the specifics of the comparison to fully appreciate its significance. The study employed the disk diffusion assay, a widely used and standardized method for evaluating the in vitro antibacterial activity of various substances. In this assay, the size of the inhibition zone is a quantitative measure of the antimicrobial activity of a substance. A larger inhibition zone generally indicates greater antimicrobial activity. The fact that the 50% and 70% mahogany leaf extracts produced inhibition zones of similar size to that of chlorhexidine suggests that these extracts are capable of inhibiting *E. faecalis* growth to a similar extent as chlorhexidine. This similarity in inhibitory effect is particularly important because *E. faecalis* is a bacterium that is frequently implicated in persistent endodontic infections and is known for its resistance to some antimicrobial agents. Therefore, an agent that can effectively inhibit the growth of *E. faecalis* is of significant value in endodontic treatment. However, it is also crucial to acknowledge the nuances within the data. While the statistical analysis indicated no significant difference between the 50% and 70% mahogany leaf extracts and chlorhexidine, it is important to note that the 70% concentration of mahogany leaf extract produced a slightly larger mean inhibition zone than chlorhexidine. This observation, although not statistically significant, indicates a trend towards greater antibacterial activity of the 70% extract compared to chlorhexidine. The mean is a measure of central tendency, representing the average value of a set of data. A larger mean inhibition zone for the 70% extract suggests that, on average, it inhibited *E. faecalis* growth to a greater extent than chlorhexidine in this study. The lack of statistical significance in this particular comparison does not negate the potential importance of this trend. Statistical significance is influenced by factors such as sample size and variability within the data. It is possible that with a larger sample size, the difference between the 70% extract and chlorhexidine might have reached statistical significance. Furthermore, even if the difference is not statistically significant, a trend towards greater antibacterial activity can be clinically relevant. A slightly more potent antimicrobial agent could potentially offer advantages in certain clinical situations, such as in cases of severe infection or when dealing with particularly resistant strains of bacteria. It is also important to consider the limitations of the in vitro assay when interpreting these results. The disk diffusion assay is a valuable screening tool, but it does not fully replicate the complex conditions found in the oral cavity and within the root canal system. Factors such as the presence of saliva, the interaction with host tissues, and the complex microbial ecology of the root canal can influence the effectiveness of an antimicrobial agent. Therefore, while the in vitro results suggest a trend towards greater antibacterial activity of the 70% mahogany leaf extract compared to chlorhexidine, further studies are needed to confirm this observation in more clinically relevant models. Studies using dentin blocks, simulated root canals, or ex vivo teeth can provide a more realistic assessment of the antibacterial efficacy of the extract. The comparison with chlorhexidine in this study provides a valuable context for evaluating the potential of mahogany leaf extract as an endodontic antimicrobial agent. Chlorhexidine is a well-established and widely used agent, and demonstrating comparable efficacy to chlorhexidine is a significant achievement for any potential alternative. However, it is also important to consider the potential advantages of mahogany leaf extract beyond its antibacterial activity. As mentioned earlier, chlorhexidine has some limitations, including potential cytotoxicity, tooth discoloration, and hypersensitivity reactions. If mahogany leaf extract can provide comparable antibacterial efficacy with improved biocompatibility and reduced adverse effects, it could offer a significant advantage over chlorhexidine. Further research is needed to fully

evaluate the safety and biocompatibility of mahogany leaf extract. Studies on its cytotoxicity to oral cells, its potential to cause tooth discoloration, and its allergenic potential are crucial before it can be considered a viable alternative to chlorhexidine. In addition to evaluating its direct antibacterial activity, future research should also explore the potential of mahogany leaf extract to modulate the host's immune response. Some antimicrobial agents have been shown to have immunomodulatory effects, which can contribute to the resolution of infection and tissue healing. Investigating the effects of mahogany leaf extract on the production of cytokines and other inflammatory mediators may provide further insights into its therapeutic potential. The findings of this study, specifically the comparable antibacterial activity of the 50% and 70% mahogany leaf extracts to chlorhexidine, have significant implications for the development of new strategies for the treatment of root canal infections. The potential of mahogany leaf extract as a natural alternative to chlorhexidine offers the possibility of achieving effective antibacterial action with potentially improved biocompatibility and reduced adverse effects. However, it is crucial to emphasize that this study is an in vitro study, and further research is necessary to confirm these findings in more clinically relevant settings. In vivo studies, using animal models or clinical trials in humans, are essential to evaluate the efficacy and safety of mahogany leaf extract in endodontic treatment. The results of this study provide a strong rationale for further investigation into the potential of mahogany leaf extract as an alternative to chlorhexidine in endodontic treatment. The comparable antibacterial activity observed in vitro is a promising finding, but it is important to interpret it cautiously and to recognize the need for further research to fully evaluate its potential.[16-20]

4. Conclusion

In conclusion, this study provides compelling evidence for the antibacterial activity of mahogany (*Swietenia mahagoni*) leaf extract against *Enterococcus faecalis*, a bacterium frequently implicated in persistent root canal infections. The extract demonstrated a clear concentration-dependent relationship, with higher concentrations exhibiting greater inhibitory effects on bacterial growth. Notably, the antibacterial activity of the 50% and 70% concentrations of mahogany leaf extract was comparable to that of 0.2% chlorhexidine, a commonly used root canal irrigant. This finding suggests that mahogany leaf extract has the potential to serve as a natural alternative to chlorhexidine in endodontic treatment. The comparable efficacy of mahogany leaf extract to chlorhexidine is particularly significant in light of the limitations associated with chlorhexidine, such as potential cytotoxicity, tooth discoloration, and hypersensitivity reactions. Mahogany leaf extract, being a natural product, may offer a more biocompatible option with reduced adverse effects. However, further research is essential to confirm its safety and efficacy in clinical settings. Future studies should focus on evaluating the cytotoxicity, biocompatibility, and potential allergenic potential of mahogany leaf extract. In vivo studies and clinical trials are necessary to validate these in vitro findings and to determine the optimal concentration and application methods for endodontic treatment. Additionally, investigations into the specific mechanisms of action of the bioactive compounds present in mahogany leaf extract would provide valuable insights into its antimicrobial properties.

5. References

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