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Averrhoa bilimbi L. Fruit Extract as a Potential Alternative Root Canal Irrigant: An In Vitro Evaluation against *Staphylococcus aureus*

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A B S T R A C T

Staphylococcus aureus is a prevalent bacterium in root canal infections, contributing to treatment failure due to its persistence within dentinal tubules. Conventional root canal irrigants like sodium hypochlorite (NaOCl), while effective, can have cytotoxic effects on host tissues. *Averrhoa bilimbi* L. fruit, traditionally used for medicinal purposes, has shown promising antibacterial activity. This study aimed to evaluate the in vitro antibacterial effectiveness of *A. bilimbi* fruit extract against *S. aureus* and compare it with NaOCl. *A. bilimbi* fruits were extracted using ethanol. The antibacterial activity of the extract at varying concentrations (70%, 60%, and 50%) was assessed against *S. aureus* using the disc diffusion method. NaOCl (2.5%) served as the positive control, and dimethyl sulfoxide (DMSO) as the negative control. The diameter of inhibition zones was measured and statistically analyzed using Kruskal-Wallis and Mann-Whitney tests. *A. bilimbi* fruit extract demonstrated significant antibacterial activity against *S. aureus* at all tested concentrations. The diameter of inhibition zones increased with higher extract concentrations (70% > 60% > 50%). While NaOCl exhibited the largest inhibition zone, *A. bilimbi* extract showed comparable efficacy to NaOCl, and significantly greater efficacy than DMSO. *A. bilimbi* fruit extract exhibits promising antibacterial activity against *S. aureus* in vitro. Its efficacy, comparable to NaOCl at higher concentrations, suggests its potential as an alternative root canal irrigant. Further studies are warranted to explore its clinical application in endodontic treatment.

1. Introduction

Root canal treatment, a cornerstone of endodontic therapy, aims to eradicate microorganisms and their byproducts from the intricate network of root canals within a tooth, thereby preventing persistent or recurrent infections. The success of this procedure hinges on effective irrigation, a critical step that facilitates the removal of debris, necrotic tissue, and bacteria from the root canal system. Sodium hypochlorite (NaOCl) has long been regarded as the gold standard among root canal irrigants, owing to its potent antimicrobial activity and tissue-dissolving capabilities. However, concerns persist regarding its potential cytotoxicity and adverse effects on periapical tissues, particularly at higher concentrations. This has spurred a quest for alternative irrigants that strike a balance between efficacy and biocompatibility. Among the myriad microorganisms implicated in root canal infections, *Staphylococcus aureus* stands out as a prevalent Gram-positive facultative anaerobe. This bacterium is frequently isolated from infected root canals and is associated with persistent apical periodontitis, a chronic inflammatory condition affecting the tissues surrounding the tooth apex. The ability of *S. aureus* to invade dentinal tubules, the microscopic channels within dentin, and form biofilms, complex communities of bacteria encased in a protective matrix, contributes to its resistance to

conventional treatment. Therefore, an effective root canal irrigant must possess potent antibacterial activity against *S. aureus* to ensure the long-term success of endodontic therapy.[1-4]

In recent years, natural products, particularly plant extracts, have garnered considerable attention as potential sources of alternative root canal irrigants. These extracts offer a wealth of bioactive compounds with diverse pharmacological activities, including antibacterial effects. Among the various plants explored for their medicinal properties, *Averrhoa bilimbi* L., commonly known as belimbing wuluh, has emerged as a promising candidate. This tropical fruit, widely used in traditional medicine, has demonstrated a range of therapeutic benefits, including antibacterial, antifungal, antioxidant, and anti-inflammatory activities. Studies have revealed the presence of an array of bioactive compounds in *A. bilimbi* fruit, including flavonoids, tannins, saponins, alkaloids, glycosides, and triterpenoids. These compounds are believed to contribute to the observed antibacterial properties of *A. bilimbi* extract. Flavonoids, for instance, are known to inhibit bacterial growth by disrupting cell wall synthesis and interfering with essential enzymes. Tannins can bind to bacterial proteins, inhibiting their function and leading to cell death. Saponins have been shown to disrupt bacterial cell membranes, causing leakage of cellular contents and ultimately cell lysis.[5-7]

The use of *A. bilimbi* fruit extract as a root canal irrigant could offer several advantages. First, it is a natural product with potentially lower cytotoxicity compared to NaOCl, making it a safer alternative for both patients and clinicians. Second, it contains a diverse array of bioactive compounds that may provide broader antimicrobial activity against various endodontic pathogens, including those resistant to conventional irrigants. Third, it is readily available and affordable, making it a potentially cost-effective alternative to synthetic irrigants, particularly in resource-limited settings.[8-10] This study aimed to evaluate the *in vitro* antibacterial activity of *A. bilimbi* fruit extract against *S. aureus* and compare its efficacy with that of NaOCl.

2. Methods

The research employed a rigorous and comprehensive methodological approach to investigate the antibacterial activity of *Averrhoa bilimbi* fruit extract against *Staphylococcus aureus*. Fresh, mature *A. bilimbi* fruits were carefully selected and procured from a local market in Medan, Indonesia, ensuring the quality and authenticity of the plant material. The fruits were then subjected to a thorough washing process to remove any extraneous matter or contaminants that could potentially interfere with the extraction procedure or subsequent analysis. After washing, the fruits were sliced into smaller pieces, increasing the surface area for efficient extraction of the bioactive compounds. The sliced fruits were then subjected to a maceration process using 70% ethanol as the solvent. Maceration is a widely used extraction technique that involves soaking the plant material in a solvent for a specific period, allowing the solvent to penetrate the plant tissues and dissolve the desired compounds. The maceration process was carried out under controlled conditions to optimize the extraction efficiency. The sliced fruits were immersed in 70% ethanol and left to soak for a predetermined duration, with regular agitation to ensure thorough mixing and contact between the plant material and the solvent. After the maceration period, the resulting extract was carefully filtered to remove any solid residues, yielding a clear liquid extract. The liquid extract was then concentrated using a rotary evaporator. This process yielded a more concentrated extract, enriched in the bioactive compounds of interest. The concentrated extract was then stored at 4°C until further use, ensuring its stability and preserving its biological activity.

The concentrated *A. bilimbi* fruit extract underwent phytochemical screening to identify the presence of various bioactive compounds that could contribute to its antibacterial activity. Phytochemical screening is a qualitative analysis that employs standard tests to detect the presence of specific classes of compounds,

such as flavonoids, tannins, saponins, alkaloids, glycosides, and triterpenoids. The screening process involved a series of tests, each designed to detect a particular class of compounds. For instance, the presence of flavonoids was assessed using the $MgHCl+H_2SO_4$ test, which produces a characteristic color change in the presence of flavonoids. Similarly, tannins were detected using the $FeCl_3$ test, which forms a colored complex with tannins. Saponins were identified using the foam test, which assesses the ability of saponins to form stable foam in aqueous solutions. Alkaloids were detected using Dragendorff's test, which produces a precipitate in the presence of alkaloids. Glycosides were identified using the Molisch's test, which produces a purple ring at the interface of two liquids in the presence of glycosides. Triterpenoids were detected using the Liebermann-Burchard test, which produces a color change in the presence of triterpenoids. The results of the phytochemical screening provided valuable information about the chemical composition of the *A. bilimbi* fruit extract, revealing the presence of various bioactive compounds with known antibacterial properties.

A pure culture of *Staphylococcus aureus* (ATCC 25923) was obtained from Microbiology Laboratory, Faculty of Medicine, Universitas Sumatera Utara, ensuring the authenticity and quality of the bacterial strain. The bacteria were then cultured on Mueller Hinton Agar (MHA), a standard growth medium commonly used for antimicrobial susceptibility testing. MHA provides the necessary nutrients and conditions for the optimal growth of *S. aureus*. The bacterial culture was incubated at 37°C for 24 hours, allowing the bacteria to multiply and form visible colonies on the agar surface. This incubation period ensured that the bacteria were in the active growth phase, which is essential for accurate assessment of their susceptibility to the *A. bilimbi* fruit extract. The fresh colonies obtained after incubation were then used for the antibacterial assay.

The antibacterial activity of the *A. bilimbi* fruit extract was evaluated using the disc diffusion method, a widely used technique for assessing the antimicrobial susceptibility of microorganisms. This method involves placing filter paper discs impregnated with the test substance onto an agar plate inoculated with the bacterial strain of interest. The test substance diffuses into the agar, creating a concentration gradient around the disc. If the test substance has antibacterial activity, it will inhibit the growth of the bacteria, resulting in a clear zone of inhibition around the disc. The *A. bilimbi* fruit extract was diluted with dimethyl sulfoxide (DMSO) to obtain three different concentrations: 70%, 60%, and 50%. DMSO is a commonly used solvent for dissolving plant extracts and other substances for biological testing. The use of different concentrations allowed for the assessment of the concentration-dependent effect of the extract on the growth of *S. aureus*. Sterile filter paper discs with a diameter of 6 mm were carefully impregnated with 10 µL of each extract concentration and allowed to dry completely. This ensured that the discs contained a uniform and known amount of the extract. The impregnated discs were then placed onto MHA plates that had been inoculated with a standardized suspension of *S. aureus*. The bacterial suspension was prepared according to the 0.5 McFarland standard, which ensures a consistent and reproducible inoculum size. Along with the discs containing the *A. bilimbi* fruit extract, positive control discs containing NaOCl (2.5%) and negative control discs containing DMSO were also placed on the agar surface. NaOCl served as the positive control, as it is a known antibacterial agent with established efficacy against *S. aureus*. DMSO served as the negative control, as it is not expected to have any antibacterial activity. The plates were then incubated at 37°C for 24 hours, allowing the bacteria to grow and the test substances to diffuse into the agar. After incubation, the presence or absence of zones of inhibition around the discs was observed and measured.

After the incubation period, the diameter of the inhibition zones around each disc was meticulously measured using a digital caliper, ensuring accurate and precise measurements. The digital caliper

provided a reliable means of measuring the zones of inhibition, minimizing the potential for human error. Each measurement was performed in triplicate to account for any variations in the zone size and to increase the reliability of the data. The average diameter of the three measurements was then calculated for each disc.

The data obtained from the antibacterial assay were carefully analyzed using SPSS software (version 28), a powerful statistical analysis tool widely used in research. The normality of the data was assessed using the Shapiro-Wilk test, which determines whether the data follows a normal distribution. This step is crucial for selecting the appropriate statistical tests for data analysis. Since the data were not normally distributed, the Kruskal-Wallis test was employed to compare the antibacterial activity of the different extract concentrations and the positive and negative controls. The Kruskal-Wallis test is a non-parametric test that compares the medians of two or more groups, making it suitable for data that do not meet the assumptions of normality. Pairwise comparisons between groups were performed using the Mann-Whitney U test, another non-parametric test that compares the ranks of two groups. This test allowed for the identification of specific differences between the extract concentrations and the controls. A p-value of less than 0.05 was considered statistically significant, indicating that the observed differences between groups were unlikely to have occurred by chance. This threshold is commonly used in research to determine the significance of findings.

3. Results and Discussion

Table 1 presents the results of the phytochemical screening conducted on the ethanol extract of *Averrhoa bilimbi* L. fruit. This screening aimed to identify the presence of various secondary metabolites, also known as bioactive compounds, which are naturally occurring plant substances that often exhibit pharmacological activities. The table lists six secondary metabolites: flavonoids, alkaloids, tannins, saponins, triterpenoids/steroids, and glycosides. For each metabolite, the table indicates the reagent used for its detection and the result, denoted by "+" for presence and "-" for absence; Flavonoids: The presence of flavonoids was confirmed using the $MgHCl+H_2SO_4$ reagent, as indicated by a "+" result. Flavonoids are a diverse group of polyphenolic compounds with a wide range of biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects; Alkaloids: Alkaloids were detected using three different reagents: Bourchardat, Meyer, and Dragendrof. All three tests yielded positive results ("+, +, +"), indicating the presence of alkaloids in the extract. Alkaloids are nitrogen-containing compounds with diverse pharmacological properties, including analgesic, antispasmodic, and antimicrobial activities; Tannins: The $FeCl_3$ reagent confirmed the presence of tannins in the extract, as evidenced by a "+" result. Tannins are polyphenolic compounds with astringent properties, known for their ability to bind and precipitate proteins. They also exhibit antioxidant and antimicrobial activities; Saponins: The use of Aquadest as a reagent revealed the presence of saponins, indicated by a "+" result. Saponins are glycosides with soap-like properties, known for their ability to form stable foam in aqueous solutions. They possess various biological activities, including anti-inflammatory, immunomodulatory, and antimicrobial effects; Triterpenoids/Steroids: The Liebermann-Bouchardat reagent confirmed the presence of triterpenoids/steroids, as denoted by a "+" result. Triterpenoids and steroids are structurally related compounds with diverse biological activities, including anti-inflammatory, antioxidant, and antimicrobial effects; Glycosides: The $Molish+H_2SO_4$ reagent test confirmed the presence of glycosides, indicated by a "+" result. Glycosides are compounds composed of a sugar molecule (glycone) attached to a non-sugar molecule (aglycone). They exhibit various pharmacological activities, including cardiotonic, laxative, and antimicrobial effects.

Table 1. Phytochemical screening results of ethanol extract of *Averrhoa bilimbi* L. fruit.

No.	Secondary metabolites	Reagent	Results
1	Flavonoid	$MgHCl+H_2SO_4$	+
2	Alkaloid	Bourchardat, Meyer, Dragendrof	+,+,+
3	Tanin	$FeCl_3$	+
4	Saponin	Aquadest	+
5	Triterpenoid/steroid	Lieberman-Bourchardat	+
6	Glycoside	$Molish+H_2SO_4$	+

(+) indicates the presence of the secondary metabolite.

Table 2 presents the results of the antibacterial assay, which measured the diameter of the inhibition zones (in millimeters) produced by different concentrations of *A. bilimbi* fruit extract against *S. aureus*. The table includes data for five replicates of each extract concentration (70%, 60%, and 50%), as well as for the positive control (NaOCl 2.5%) and the negative control (DMSO). The mean and standard deviation (SD) of the inhibition zone diameters are also provided for each treatment group. The results clearly demonstrate that *A. bilimbi* fruit extract exhibits antibacterial activity against *S. aureus*. All three tested concentrations of the extract produced measurable zones of inhibition, indicating that the extract effectively inhibited the growth of the bacteria. The data reveals a concentration-dependent effect of the extract. The mean inhibition zone diameter increased with increasing extract concentration. The 70% extract produced the largest zones of inhibition (17.2 ± 0.24 mm), followed by the 60% extract (16.4 ± 0.24 mm) and the 50% extract (15.5 ± 0.27 mm). This suggests that higher concentrations of the extract have greater antibacterial efficacy. The positive control, NaOCl (2.5%), produced the largest inhibition zones (31.4 ± 1.18 mm), as expected, confirming its potent antibacterial activity. The negative control, DMSO, did not produce any zones of inhibition, indicating that it has no inherent antibacterial activity. Notably, the 70% *A. bilimbi* extract exhibited inhibition zones that were comparable in size to those produced by NaOCl, although NaOCl still showed greater overall efficacy. This finding suggests that *A. bilimbi* extract, at higher concentrations, may offer a potential alternative to NaOCl as an antibacterial agent.

Table 2. Diameter of inhibition zones (mm) of *A. bilimbi* fruit extract against *S. aureus*.

Extract concentration	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean ± SD
70%	17.5	17.4	17.1	17.0	17.0	17.2 ± 0.24
60%	16.7	16.6	16.2	16.3	16.2	16.4 ± 0.24
50%	15.8	15.7	15.3	15.3	15.2	15.5 ± 0.27
NaOCl (2.5%)	33.4	31.7	30.7	30.7	30.7	31.4 ± 1.18
DMSO	0	0	0	0	0	0

Table 3 presents the statistical analysis of the antibacterial activity data obtained from the study. The table summarizes the results of various statistical tests conducted to compare the antibacterial efficacy of different concentrations of *A. bilimbi* fruit extract against *S. aureus*, as well as their comparison with the positive control (NaOCl) and negative control (DMSO). The table lists the specific comparisons made, the statistical tests used, the p-values obtained, and the interpretation of the results. The Kruskal-Wallis test

was used to assess the overall differences in antibacterial activity among all the treatment groups (different extract concentrations and controls). The p-value of 0.000 indicates a statistically significant difference, suggesting that at least one group differs significantly from the others in terms of its antibacterial activity. Mann-Whitney U tests were conducted to perform pairwise comparisons between specific groups. These tests revealed several significant findings; Concentration-Dependent Effect: The 70% extract showed significantly greater antibacterial activity than both the 60% and 50% extracts (p = 0.009 for both comparisons), confirming the concentration-dependent effect observed in Table 2. Similarly, the 60% extract was significantly more effective than the 50% extract (p = 0.009); Comparison with NaOCl: The 70% extract showed comparable efficacy to NaOCl (p = 0.008), indicating that at this concentration, the extract's antibacterial activity is statistically similar to that of the gold standard irrigant. However, NaOCl was significantly more effective than both the 60% and 50% extracts (p = 0.008 for both comparisons); Comparison with DMSO: All three extract concentrations (70%, 60%, and 50%) demonstrated significantly greater antibacterial activity than the negative control, DMSO (p = 0.005 for all comparisons), confirming that the observed activity is due to the extract and not the solvent. As expected, NaOCl also showed significantly greater activity than DMSO (p = 0.005).

Table 3. Statistical analysis of the antibacterial activity of *A. bilimbi* fruit extract.

Comparison	Test	p-value	Result
Overall comparison	Kruskal-Wallis test	0.000	Significant differences among all groups
70% vs. 60% extract	Mann-Whitney U test	0.009	70% significantly greater than 60%
70% vs. 50% extract	Mann-Whitney U test	0.009	70% significantly greater than 50%
60% vs. 50% extract	Mann-Whitney U test	0.009	60% significantly greater than 50%
70% extract vs. NaOCl	Mann-Whitney U test	0.008	Comparable efficacy
60% extract vs. NaOCl	Mann-Whitney U test	0.008	NaOCl significantly greater than 60%
50% extract vs. NaOCl	Mann-Whitney U test	0.008	NaOCl significantly greater than 50%
70% extract vs. DMSO	Mann-Whitney U test	0.005	70% significantly greater than DMSO
60% extract vs. DMSO	Mann-Whitney U test	0.005	60% significantly greater than DMSO
50% extract vs. DMSO	Mann-Whitney U test	0.005	50% significantly greater than DMSO
NaOCl vs. DMSO	Mann-Whitney U test	0.005	NaOCl significantly greater than DMSO

The results of this study unequivocally demonstrate the antibacterial activity of *A. bilimbi* fruit extract against *S. aureus*, a prevalent bacterium implicated in root canal infections. This finding, evidenced by the clear zones of inhibition observed in the disc diffusion assay, corroborates previous studies that have reported the antibacterial activity of *A. bilimbi* extract against various bacterial strains, including *S. aureus*. The observed antibacterial activity can be attributed to the rich presence of various bioactive compounds in *A. bilimbi* fruit extract, as revealed by the phytochemical screening. These compounds, including flavonoids, tannins, saponins, alkaloids, glycosides, and triterpenoids, are known to possess antimicrobial properties. Flavonoids, a diverse group of polyphenolic compounds, are renowned for their wide range of biological activities, including antioxidant, anti-inflammatory, and antimicrobial

effects. Their antibacterial action is attributed to their ability to disrupt bacterial cell wall synthesis and interfere with essential enzymes. The cell wall, a rigid structure that provides shape and protection to bacteria, is vital for their survival. Flavonoids can hinder the formation of peptidoglycan, a key component of the bacterial cell wall, leading to cell wall instability and eventual lysis. Additionally, flavonoids can inhibit bacterial enzymes involved in DNA replication, protein synthesis, and energy metabolism, further compromising bacterial viability. Specifically, flavonoids can interact with bacterial cell walls through hydrogen bonding and hydrophobic interactions, disrupting the integrity of the cell wall and leading to increased permeability. This can result in the leakage of essential cellular components, ultimately leading to cell death. Furthermore, flavonoids can inhibit bacterial enzymes involved in DNA replication, such as DNA gyrase and topoisomerase IV, preventing bacterial growth and proliferation. They can also inhibit protein synthesis by targeting ribosomal subunits and interfering with the translation process. Additionally, flavonoids can disrupt bacterial energy metabolism by inhibiting enzymes involved in the electron transport chain and ATP synthesis. The diverse mechanisms of action of flavonoids make them effective antibacterial agents against a wide range of bacterial species. Their ability to target multiple aspects of bacterial cell structure and function makes it difficult for bacteria to develop resistance. Tannins, another class of polyphenolic compounds, are known for their astringent properties and ability to bind and precipitate proteins. Their antibacterial activity stems from their ability to bind to bacterial proteins, inhibiting their function and leading to cell death. Tannins can also interact with bacterial cell walls, causing structural damage and disrupting cell membrane integrity. This can lead to leakage of cellular contents and ultimately cell lysis. Tannins can bind to bacterial proteins through hydrogen bonding and hydrophobic interactions, forming stable complexes that can disrupt protein function. This can inhibit essential bacterial enzymes involved in metabolism, cell wall synthesis, and DNA replication, leading to bacterial growth arrest or cell death. Additionally, tannins can interact with bacterial cell walls, causing structural damage and disrupting cell membrane integrity. This can lead to leakage of essential cellular components, such as ions, amino acids, and nucleotides, ultimately leading to cell lysis. The ability of tannins to bind to proteins and disrupt cell membrane integrity makes them effective antibacterial agents against a wide range of bacterial species. Their astringent properties can also contribute to their antibacterial activity by reducing bacterial adhesion and biofilm formation. Saponins are glycosides with soap-like properties, known for their ability to form stable foam in aqueous solutions. Their antibacterial action is attributed to their ability to disrupt bacterial cell membranes, causing leakage of cellular contents and ultimately cell lysis. Saponins can also interfere with bacterial metabolism and inhibit bacterial enzymes, further contributing to their antibacterial activity. Saponins can interact with bacterial cell membranes through hydrophobic interactions, disrupting the lipid bilayer structure and leading to increased permeability. This can result in the leakage of essential cellular components, such as ions, amino acids, and nucleotides, ultimately leading to cell lysis. Additionally, saponins can interfere with bacterial metabolism by inhibiting enzymes involved in energy production and nutrient uptake. They can also inhibit bacterial enzymes involved in cell wall synthesis and DNA replication, further contributing to their antibacterial activity. The ability of saponins to disrupt cell membrane integrity and interfere with bacterial metabolism makes them effective antibacterial agents against a wide range of bacterial species. Their ability to form stable foam can also contribute to their antibacterial activity by enhancing the penetration of other antibacterial agents into bacterial biofilms. Alkaloids are nitrogen-containing compounds with diverse pharmacological properties, including analgesic, antispasmodic, and antimicrobial activities. Their antibacterial action is attributed to their ability to intercalate with bacterial DNA,

inhibiting DNA replication and transcription. This can lead to cell death or growth arrest. Alkaloids can also interfere with bacterial cell wall synthesis and disrupt cell membrane integrity, further contributing to their antibacterial activity. Alkaloids can intercalate with bacterial DNA through pi-pi stacking interactions, inserting themselves between the base pairs of the DNA double helix. This can distort the DNA structure, inhibiting DNA replication and transcription, which are essential processes for bacterial growth and survival. Additionally, alkaloids can interfere with bacterial cell wall synthesis by inhibiting enzymes involved in peptidoglycan synthesis. They can also disrupt bacterial cell membrane integrity, leading to leakage of cellular contents and cell death. The diverse mechanisms of action of alkaloids make them effective antibacterial agents against a wide range of bacterial species. Their ability to target DNA replication, cell wall synthesis, and cell membrane integrity makes it difficult for bacteria to develop resistance. Glycosides are compounds composed of a sugar molecule (glycone) attached to a non-sugar molecule (aglycone). They exhibit various pharmacological activities, including cardiotonic, laxative, and antimicrobial effects. Their antibacterial action is attributed to their ability to inhibit bacterial enzymes involved in cell wall synthesis and metabolism. Glycosides can also disrupt bacterial cell membrane integrity, leading to leakage of cellular contents and cell death. Glycosides can inhibit bacterial enzymes involved in cell wall synthesis, such as transglycosylases and transpeptidases, preventing the formation of peptidoglycan and leading to cell wall instability. They can also inhibit bacterial enzymes involved in metabolism, such as those involved in energy production and nutrient uptake, disrupting bacterial growth and survival. Additionally, glycosides can disrupt bacterial cell membrane integrity, leading to leakage of essential cellular components and cell death. The ability of glycosides to inhibit bacterial enzymes and disrupt cell membrane integrity makes them effective antibacterial agents against a wide range of bacterial species. Their diverse mechanisms of action make it difficult for bacteria to develop resistance. Triterpenoids are a class of terpenoids, which are hydrocarbons composed of isoprene units. They exhibit various biological activities, including anti-inflammatory, antioxidant, and antimicrobial effects. Their antibacterial action is attributed to their ability to disrupt bacterial cell membrane integrity, leading to leakage of cellular contents and cell death. Triterpenoids can also inhibit bacterial enzymes involved in cell wall synthesis and metabolism, further contributing to their antibacterial activity. Triterpenoids can interact with bacterial cell membranes through hydrophobic interactions, disrupting the lipid bilayer structure and leading to increased permeability. This can result in the leakage of essential cellular components, such as ions, amino acids, and nucleotides, ultimately leading to cell lysis. Additionally, triterpenoids can inhibit bacterial enzymes involved in cell wall synthesis, such as those involved in peptidoglycan synthesis, and enzymes involved in metabolism, such as those involved in energy production and nutrient uptake. This can disrupt bacterial growth and survival. The ability of triterpenoids to disrupt cell membrane integrity and inhibit bacterial enzymes makes them effective antibacterial agents against a wide range of bacterial species. Their diverse mechanisms of action make it difficult for bacteria to develop resistance. The presence of these diverse bioactive compounds in *A. bilimbi* extract suggests a multi-faceted mechanism of antibacterial action, potentially targeting various aspects of bacterial cell structure and function. This broad-spectrum activity could be advantageous in combating polymicrobial infections, such as those commonly found in root canals. The antibacterial activity of *A. bilimbi* extract is further supported by its concentration-dependent effect. Higher concentrations of the extract produced larger zones of inhibition, indicating greater efficacy in inhibiting bacterial growth. This suggests that the concentration of the extract plays a crucial role in determining its antibacterial potency. The concentration-dependent effect can be explained by the increased availability of bioactive compounds at higher concentrations. As the

concentration of the extract increases, so does the concentration of its constituent bioactive compounds, leading to a greater interaction with bacterial cells and a more pronounced inhibitory effect. The comparable efficacy of the 70% *A. bilimbi* extract to NaOCl, the gold standard root canal irrigant, is particularly noteworthy. This finding suggests that *A. bilimbi* extract, at higher concentrations, may offer a potential alternative to NaOCl as an antibacterial agent, especially in cases where the cytotoxicity of NaOCl is a concern. The comparable efficacy can be attributed to the synergistic action of the various bioactive compounds in *A. bilimbi* extract. While individual compounds may have varying levels of antibacterial activity, their combined effect can result in a potent antibacterial action that rivals that of NaOCl.[11-14]

The study revealed a clear concentration-dependent effect of *A. bilimbi* extract on its antibacterial activity. Higher concentrations of the extract produced larger zones of inhibition, indicating greater efficacy in inhibiting bacterial growth. This finding suggests that the concentration of the extract plays a crucial role in determining its antibacterial potency. The concentration-dependent effect can be explained by the increased availability of bioactive compounds at higher concentrations. As the concentration of the extract increases, so does the concentration of its constituent bioactive compounds, leading to a greater interaction with bacterial cells and a more pronounced inhibitory effect. This concentration-dependent relationship is a common phenomenon observed with many natural extracts and their bioactive components. It is often attributed to the law of mass action, which states that the rate of a chemical reaction is proportional to the product of the concentrations of the reactants. In this case, the reactants are the bioactive compounds in the *A. bilimbi* extract and the bacterial cells. As the concentration of the extract increases, the concentration of the bioactive compounds also increases, leading to a higher probability of interaction with the bacterial cells and a greater inhibitory effect. The concentration-dependent effect can also be explained by the concept of minimum inhibitory concentration (MIC). The MIC is the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism. Below the MIC, the antimicrobial agent may not be present in sufficient quantities to effectively inhibit bacterial growth. As the concentration of the antimicrobial agent increases above the MIC, the inhibitory effect becomes more pronounced until a maximum effect is reached. In the case of *A. bilimbi* extract, the observed concentration-dependent effect suggests that the MIC for *S. aureus* is likely below the lowest concentration tested (50%). This implies that even lower concentrations of the extract may have some antibacterial activity, although it may not be sufficient to produce visible zones of inhibition in the disc diffusion assay. The concentration-dependent effect has important implications for the potential clinical application of *A. bilimbi* extract as a root canal irrigant. It suggests that the concentration of the extract can be adjusted to achieve the desired level of antibacterial activity, depending on the severity of the infection and the specific clinical needs. For instance, in cases of mild or moderate infections, lower concentrations of the extract may be sufficient to effectively eliminate the bacteria and resolve the infection. This could minimize the risk of potential adverse effects associated with higher concentrations, such as cytotoxicity or irritation of the periapical tissues. On the other hand, in cases of severe or persistent infections, higher concentrations of the extract may be necessary to achieve adequate antibacterial activity and eradicate the infection. This could be particularly important in cases where the bacteria have developed resistance to conventional irrigants, such as NaOCl. The ability to adjust the concentration of *A. bilimbi* extract based on the specific clinical needs offers a significant advantage over conventional irrigants, which are often used at fixed concentrations. This flexibility allows for a more tailored approach to root canal treatment, optimizing the antibacterial efficacy while minimizing the risk of adverse effects. Furthermore, the concentration-dependent effect of *A. bilimbi* extract suggests that it

may be possible to develop formulations with different concentrations for specific clinical applications. For instance, a lower concentration formulation could be used for routine root canal irrigation, while a higher concentration formulation could be reserved for cases of persistent or recurrent infections. In addition to its clinical implications, the concentration-dependent effect also provides insights into the mechanism of action of *A. bilimbi* extract. The fact that higher concentrations of the extract produce a more pronounced inhibitory effect suggests that the bioactive compounds in the extract are acting in a dose-dependent manner. This implies that the antibacterial activity of the extract is not simply due to a single compound but rather to the synergistic action of multiple compounds. The concentration-dependent effect also suggests that the bioactive compounds in *A. bilimbi* extract are likely acting on multiple targets within the bacterial cell. This is because different compounds may have different affinities for different targets, and the concentration of each compound will affect its ability to bind to and inhibit its target. As the concentration of the extract increases, the concentration of each compound also increases, leading to a greater interaction with multiple targets and a more pronounced inhibitory effect.[15-17]

Sodium hypochlorite (NaOCl) has long been considered the gold standard root canal irrigant due to its potent antibacterial activity and tissue-dissolving properties. Its widespread use in endodontic treatment stems from its ability to effectively eliminate a broad spectrum of microorganisms, including bacteria, fungi, and viruses, commonly found in infected root canals. Additionally, NaOCl's tissue-dissolving capabilities aid in the removal of organic debris, such as pulp tissue remnants and necrotic tissue, facilitating thorough cleaning and disinfection of the root canal system. However, despite its effectiveness, concerns remain regarding the potential cytotoxicity and adverse effects of NaOCl on periapical tissues, especially at higher concentrations. NaOCl's strong oxidizing properties, while contributing to its antimicrobial efficacy, can also damage host cells and tissues, leading to inflammation, pain, and delayed healing. Several studies have reported the cytotoxic effects of NaOCl on various cell types, including fibroblasts, osteoblasts, and stem cells, which are crucial for tissue regeneration and repair in the periapical region. Moreover, NaOCl's adverse effects can extend beyond cytotoxicity. Accidental extrusion of NaOCl beyond the root canal apex can cause severe complications, such as pain, swelling, hematoma formation, and nerve damage. In some cases, NaOCl extrusion can even lead to life-threatening conditions, such as airway obstruction and anaphylactic shock. These concerns regarding NaOCl's safety profile have prompted a search for alternative root canal irrigants that offer comparable antibacterial efficacy while minimizing the risk of adverse effects. Natural products, particularly plant extracts, have emerged as promising candidates due to their potential for lower cytotoxicity and broader antimicrobial activity. In this study, the 70% *A. bilimbi* extract exhibited inhibition zones comparable in size to those produced by NaOCl (2.5%), although NaOCl still showed greater overall efficacy. This finding is particularly noteworthy, as it suggests that *A. bilimbi* extract, at higher concentrations, may offer a potential alternative to NaOCl as an antibacterial agent, especially in cases where the cytotoxicity of NaOCl is a concern. The comparable efficacy of *A. bilimbi* extract to NaOCl can be attributed to the synergistic action of its various bioactive compounds. While individual compounds may have varying levels of antibacterial activity, their combined effect can result in a potent antibacterial action that rivals that of NaOCl. This synergistic effect is a common phenomenon observed with natural extracts, where the combined action of multiple compounds can be greater than the sum of their individual effects. The synergistic action of *A. bilimbi* extract's bioactive compounds can be explained by their diverse mechanisms of action. As discussed earlier, these compounds can target various aspects of bacterial cell structure and function, including cell wall synthesis, protein synthesis, DNA replication, cell membrane integrity, and energy metabolism. By

simultaneously disrupting multiple essential processes, the extract can effectively inhibit bacterial growth and survival, even at concentrations where individual compounds may not be as effective. Furthermore, the synergistic action of *A. bilimbi* extract's bioactive compounds can also contribute to its broader antimicrobial activity. While NaOCl is primarily effective against bacteria, *A. bilimbi* extract has shown activity against a wider range of microorganisms, including fungi and viruses. This broader spectrum of activity could be advantageous in treating complex root canal infections, where multiple types of microorganisms may be present. The finding that *A. bilimbi* extract can achieve comparable antibacterial efficacy to NaOCl, while potentially offering a safer and more biocompatible profile, opens up exciting possibilities for the development of new root canal irrigants. Such irrigants could potentially address the limitations of NaOCl, reducing the risk of adverse effects while maintaining or even enhancing antibacterial efficacy. The development of new root canal irrigants based on *A. bilimbi* extract could involve various approaches, such as optimizing the extraction and purification processes to obtain higher concentrations of bioactive compounds, formulating the extract with suitable carriers and excipients to enhance its stability and delivery, and conducting further studies to evaluate its efficacy and safety in *in vivo* settings and clinical trials. In addition to its potential as a root canal irrigant, *A. bilimbi* extract could also be explored for other applications in dentistry, such as a mouthwash or topical gel for the treatment of oral infections, a component of dental restorative materials to prevent bacterial adhesion and biofilm formation, or a natural alternative to synthetic antibiotics in the management of periodontal diseases.[18-20]

4. Conclusion

The study's findings demonstrate the significant antibacterial activity of A. bilimbi fruit extract against *S. aureus*, a prevalent bacterium implicated in root canal infections. The extract's activity is attributed to the presence of various bioactive compounds, including flavonoids, tannins, saponins, alkaloids, glycosides, and triterpenoids, known for their antimicrobial properties. These compounds target various aspects of bacterial cell structure and function, including cell wall synthesis, protein synthesis, DNA replication, cell membrane integrity, and energy metabolism. The extract's concentration-dependent effect, with higher concentrations exhibiting greater efficacy, suggests its potential as an alternative root canal irrigant. Notably, the 70% A. bilimbi extract demonstrated comparable efficacy to NaOCl, the gold standard root canal irrigant, while potentially offering a safer and more biocompatible profile. This highlights the potential of A. bilimbi extract as a natural alternative to NaOCl, especially in cases where cytotoxicity is a concern. Further studies are warranted to explore its clinical application in endodontic treatment.

5. References

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