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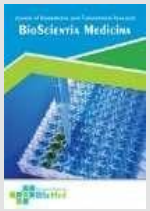

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Effectiveness of *Mangifera indica* L. Leaf Extract in Controlling *Candida albicans* Growth on Orthodontic Retainers: A Promising Approach to Improve Oral Hygiene

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A B S T R A C T

Background: Orthodontic retainers, especially thermoplastic retainers, are prone to *Candida albicans* colonization, potentially leading to oral health issues. This study investigated the effectiveness of *Mangifera indica* L. leaf extract in controlling *C. albicans* growth on orthodontic retainers and also analyzed its phytochemical constituents. **Methods:** *M. indica* leaf extract was prepared using maceration and digestion techniques. Thermoplastic retainers were contaminated with *C. albicans* and then immersed in different concentrations of *M. indica* leaf extract (25%, 50%, 75%, and 100%) for 15 minutes. Chlorhexidine digluconate 2% served as the positive control, and dimethyl sulfoxide (DMSO) was the negative control. The antifungal activity was evaluated by measuring the diameter of the inhibition zone. Phytochemical screening was conducted to identify the presence of various secondary metabolites in the extract. **Results:** All concentrations of *M. indica* leaf extract demonstrated significant antifungal activity against *C. albicans*. The 75% extract showed the highest inhibition zone, comparable to chlorhexidine digluconate 2%. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, and other bioactive compounds in the extract. **Conclusion:** *M. indica* leaf extract, particularly at 75% concentration, effectively inhibits *C. albicans* growth on orthodontic retainers, suggesting its potential as a natural alternative for maintaining oral hygiene during orthodontic treatment. The presence of various bioactive compounds in the extract contributes to its antifungal activity.

1. Introduction

The field of orthodontics has witnessed a surge in the demand for esthetic and transparent retainers, particularly thermoplastic retainers, driven by the increasing desire for discreet orthodontic treatment options. These retainers offer a comfortable and virtually invisible alternative to traditional metal braces, making them a popular choice among patients seeking to improve their smiles without compromising their appearance. However, the extended wear time of thermoplastic retainers, often for several hours a day, creates an environment conducive to microbial colonization, including the opportunistic fungus *Candida albicans*. *Candida albicans* is a commensal fungus that resides in the oral cavity of healthy individuals, typically without causing any adverse effects. However, under certain conditions, such as weakened immune systems, poor oral hygiene, or the presence of medical devices like orthodontic retainers, *C. albicans* can transition from a harmless commensal

to a pathogenic organism, leading to oral candidiasis. Oral candidiasis, commonly known as thrush, is characterized by white or red patches on the tongue, inner cheeks, and other oral tissues, causing discomfort, pain, and difficulty swallowing. In immunocompromised individuals, oral candidiasis can lead to more severe systemic infections, highlighting the importance of controlling *C. albicans* growth on orthodontic retainers.[1-4]

Maintaining good oral hygiene during orthodontic treatment is paramount to prevent microbial growth on retainers and the associated risk of oral infections. Conventional oral hygiene practices, such as brushing and flossing, may not be sufficient to eliminate *C. albicans* from the surface of thermoplastic retainers due to their porous nature and complex design. Chemical disinfectants, such as chlorhexidine digluconate, are commonly used to disinfect orthodontic retainers, but their long-term use can lead to undesirable side effects, including staining, corrosion, and an unpleasant taste. Moreover, concerns have been raised regarding the potential development of microbial resistance to chemical disinfectants, prompting the exploration of natural alternatives with antifungal properties. Nature offers a vast array of plant-derived compounds with antimicrobial properties, providing a promising avenue for the development of safe and effective alternatives to synthetic disinfectants. Among these natural resources, *Mangifera indica* L., commonly known as mango, has emerged as a potential candidate for controlling *C. albicans* growth on orthodontic retainers. Mango leaves contain a rich source of bioactive compounds, including mangiferin, flavonoids, and tannins, which have demonstrated antifungal activity against various microorganisms, including *C. albicans*.[5-7]

Mangiferin, a xanthone derivative found abundantly in mango leaves, has been shown to exhibit potent antifungal activity against *C. albicans* by inhibiting its growth and disrupting its cell wall integrity. Flavonoids, another class of bioactive compounds present in mango leaves, have also demonstrated antifungal activity against *C. albicans* by interfering with its cell membrane function and inhibiting its ability to form biofilms. Tannins, polyphenolic compounds found in mango leaves, contribute to the antifungal activity by binding to the fungal cell wall, preventing its growth and adhesion to surfaces. The antifungal properties of *M. indica* leaf extract have been investigated in several studies, demonstrating its efficacy against various fungal species, including *C. albicans*. However, the specific application of *M. indica* leaf extract in controlling *C. albicans* growth on orthodontic retainers remains largely unexplored.[8-10] This study aims to bridge this gap by investigating the effectiveness of *M. indica* leaf extract in inhibiting *C. albicans* growth on thermoplastic retainers and analyzing its phytochemical constituents.

2. Methods

The collection of *M. indica* leaves was carried out at a mango plantation located in Medan, Indonesia. Upon collection, the leaves were meticulously washed to remove any debris or contaminants and then subjected to a drying process under shade to preserve their bioactive constituents. The dried leaves were subsequently ground into a fine powder using a suitable grinding apparatus, ensuring a uniform particle size for efficient extraction. The powdered leaves were then subjected to a two-stage extraction process involving maceration and digestion. In the first stage, the powder was macerated with n-hexane for a period of 24 hours, followed by a second maceration with fresh n-hexane to maximize the extraction of nonpolar compounds. The residue obtained after the n-hexane extraction was carefully dried in an oven at a controlled temperature of 40°C to remove any residual solvent. The dried residue was then subjected to digestion with 96% ethanol to extract polar compounds, including mangiferin, flavonoids, and tannins, which are known for their antifungal properties. The resulting liquid extract was concentrated using a rotary evaporator, a technique that removes the solvent under reduced pressure,

leaving behind a concentrated extract rich in bioactive compounds. The concentrated extract was then diluted with distilled water to prepare different concentrations (25%, 50%, 75%, and 100%) for the antifungal activity assessment.

The concentrated *M. indica* leaf extract was subjected to phytochemical screening to identify the presence of various secondary metabolites, including alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids. These secondary metabolites are known to play a crucial role in the plant's defense mechanisms and exhibit a wide range of biological activities, including antifungal properties. The phytochemical screening was conducted using standard procedures for each class of compounds. The presence of alkaloids was detected using Dragendorff's, Mayer's, and Wagner's reagents, which form characteristic precipitates in the presence of alkaloids. Flavonoids were identified using the Shinoda test and alkaline reagent test, which produce specific color changes in the presence of flavonoids. Tannins were detected using the ferric chloride test and gelatin test, which rely on the ability of tannins to form complexes with ferric ions or proteins. Saponins were identified using the foam test and hemolysis test, which exploit their ability to form stable foams and lyse red blood cells. Glycosides were detected using Molisch's test and Fehling's test, which rely on the hydrolysis of glycosides to release reducing sugars. Terpenoids were identified using the Salkowski test and Liebermann-Burchard test, which produce characteristic color changes in the presence of terpenoids.

Thermoplastic retainers were fabricated using a standardized protocol to ensure uniformity in their dimensions and surface properties. The retainers were fabricated with a thickness of 1 mm and dimensions of 10 x 10 mm, providing a sufficient surface area for *C. albicans* colonization and subsequent antifungal activity assessment. The edges of the retainers were carefully smoothened to simulate the actual condition of retainers in the oral cavity, ensuring that the experimental conditions closely mimic the clinical setting.

The fabricated thermoplastic retainers were subjected to a series of treatments to prepare them for *C. albicans* contamination and subsequent antifungal activity assessment. The retainers were initially immersed in sterile distilled water for one day to remove any residual monomers that may interfere with *C. albicans* growth or the antifungal activity of *M. indica* leaf extract. After the removal of residual monomers, the retainers were sterilized under UV light for a total of 40 minutes, with 20 minutes of exposure per side, to eliminate any potential microbial contaminants. The sterilized retainers were then placed in Erlenmeyer flasks containing a suspension of *C. albicans* and incubated at 27°C for 48 hours to allow for *C. albicans* colonization on the retainer surface. The contaminated retainers were then subjected to treatment with different concentrations of *M. indica* leaf extract (25%, 50%, 75%, and 100%) to evaluate their antifungal activity. Chlorhexidine digluconate 2%, a commonly used disinfectant in dentistry, served as the positive control, while dimethyl sulfoxide (DMSO) served as the negative control. The retainers were immersed in 10 mL of each treatment solution for a period of 15 minutes to allow for interaction between the treatment solution and the *C. albicans* on the retainer surface.

Following the treatment period, the retainers were rinsed with sterile distilled water to remove any residual treatment solution and then vortexed at 1500 rpm for 2 minutes to detach *C. albicans* from the retainer surface. The resulting suspension was then spread on Potato Dextrose Agar (PDA) plates, a standard growth medium for fungi, and incubated for 48 hours to allow for the growth of any surviving *C. albicans*. The antifungal activity of each treatment solution was evaluated by measuring the diameter of the inhibition zone around each retainer using a caliper. The inhibition zone represents the area where *C. albicans* growth is inhibited due to the antifungal activity of the treatment solution. The diameter of the inhibition zone is directly proportional to the antifungal activity of the treatment solution, with

larger inhibition zones indicating greater antifungal activity.

The data obtained from the antifungal activity assessment were analyzed using SPSS version 25, a statistical software package. The normality of the data was assessed using the Shapiro-Wilk test, which evaluates whether the data follow a normal distribution. One-way ANOVA, a statistical test that compares the means of three or more groups, was used to compare the antifungal activity of different concentrations of *M. indica* leaf extract. Post Hoc LSD tests, a type of multiple comparison test, were used to identify specific differences between pairs of treatment groups. The p-value, a measure of statistical significance, was used to determine whether the observed differences between groups were likely due to chance or a true effect of the treatment. A p-value less than 0.05 was considered statistically significant, indicating that the observed differences were unlikely due to chance.

3. Results

Table 1 presents the diameter of the inhibition zone (in millimeters) for different concentrations of *M. indica* leaf extract against *C. albicans*. The table also includes data for the positive control (Chlorhexidine 2%) and negative control (DMSO). All concentrations of *M. indica* leaf extract demonstrated antifungal activity against *C. albicans*, as evidenced by the presence of inhibition zones. This suggests that the extract contains compounds that can inhibit the growth of the fungus. The diameter of the inhibition zone generally increased with increasing concentrations of *M. indica* leaf extract. This indicates a concentration-dependent antifungal effect, where higher concentrations of the extract exhibit greater activity against *C. albicans*. The 75% *M. indica* leaf extract showed the highest inhibition zone (12.56 ± 0.33 mm), comparable to that of Chlorhexidine 2% (12.60 ± 0.83 mm), the positive control. This suggests that the 75% extract has a similar antifungal efficacy to Chlorhexidine, a commonly used disinfectant in dentistry. As expected, the negative control (DMSO) did not show any inhibition zone, confirming that the observed antifungal activity is due to the *M. indica* leaf extract and not the solvent used for extraction.

Table 1. Diameter of inhibition zone (mm) of *M. indica* leaf extract against *C. albicans*.

Group	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean ± SD
M. indica 25%	11.10	10.95	10.85	11.15	11.02 ± 0.17
M. indica 50%	12.05	11.60	11.95	11.80	11.82 ± 0.30
M. indica 75%	12.85	12.65	12.40	12.70	12.56 ± 0.33
M. indica 100%	11.45	11.75	11.60	11.80	11.66 ± 0.11
Chlorhexidine 2%	12.50	12.30	11.50	13.10	12.60 ± 0.83
DMSO	0	0	0	0	0 ± 0

Table 2 provides the results of the phytochemical screening of the *M. indica* leaf extract, showing the presence of various secondary metabolites and their potential implications for antifungal activity; Alkaloids: The extract tested positive for alkaloids using Bouchardat's and Dragendorff's tests. Alkaloids are known to have diverse biological activities, including disrupting fungal cell walls and interfering with cell division; Flavonoids: The presence of flavonoids was confirmed by the Mg + HCl test. Flavonoids are recognized for their ability to inhibit fungal growth by disrupting cell membranes and interfering with essential enzymes; Triterpenoids/Steroids: The Liebermann-Burchard test indicated the presence of triterpenoids/steroids. These compounds can disrupt fungal cell membrane integrity, leading to cell leakage and death; Glycosides: The Molisch's test confirmed the presence of

glycosides, which can interfere with fungal metabolism or cell signaling pathways; Saponins: The Aquades test showed the presence of saponins, known for their ability to disrupt fungal cell membranes, leading to cell lysis; Tannins: The Ferric Chloride test confirmed the presence of tannins, which can bind to fungal cell walls, inhibiting growth and adhesion.

Table 2. Results of phytochemical screening of *M. indica* leaf extract.

Phytochemical class	Test	Result	Observation	Possible implication for antifungal activity
Alkaloids	Bouchardat's Test	+	Orange-red precipitate formed	May disrupt fungal cell wall or interfere with cell division.
	Meyer's Test	-	No precipitate formed	-
	Dragendorff's Test	+	Orange-red precipitate formed	May disrupt fungal cell wall or interfere with cell division.
Flavonoids	Mg + HCl Test	+	Yellow to red color change upon addition of HCl	May inhibit fungal growth by disrupting cell membrane or interfering with enzymes.
Triterpenoids/Steroids	Liebermann-Burchard Test	+	Green to blue color change	May disrupt fungal cell membrane integrity, leading to cell leakage and death.
Glycosides	Molisch's Test	+	Purple ring formed at the interface	May interfere with fungal metabolism or cell signaling.
Saponins	Aquades Test	+	Persistent foam formed upon shaking	May disrupt fungal cell membrane, leading to cell lysis.
Tannins	Ferric Chloride Test	+	Bluish-black color observed	May bind to fungal cell wall, inhibiting growth and adhesion.

"+" indicates the presence of the phytochemical.

Table 3 presents the statistical analysis of the antifungal activity of *M. indica* leaf extract against *C. albicans*. The table includes results from the Shapiro-Wilk test, One-Way ANOVA, and Post Hoc LSD test. The Shapiro-Wilk test showed that the data for the diameter of the inhibition zone for each extract concentration were normally distributed ($p > 0.05$ for all groups). This indicates that the data meet the assumption of normality required for parametric statistical tests. The One-Way ANOVA revealed a statistically significant difference in antifungal activity between the groups ($p = 0.000$). This suggests that at least one group has a different mean diameter of inhibition zone compared to the others. The Post Hoc LSD test was used to identify specific differences between pairs of groups. The results showed significant differences in antifungal activity between the 25% extract and all other concentrations (50%, 75%, 100%, and 2% Chlorhexidine). This indicates that the 25% extract has significantly lower antifungal activity compared to higher concentrations and the positive control. There was also a significant difference between the 50% extract and the 75% extract, as well as between the 50% extract and the 2% Chlorhexidine.

This suggests that increasing the concentration from 50% to 75% leads to a significant improvement in antifungal activity. No significant difference was found between the 50% and 100% extracts, indicating that increasing the concentration beyond 50% may not provide additional benefit. Importantly, there was no significant difference between the 75% extract and the 2% Chlorhexidine, suggesting that the 75% extract has comparable antifungal efficacy to the commonly used disinfectant.

Table 3. Statistical analysis of *M. indica* leaf extract's antifungal activity.

Statistical test	**Data analyzed**	**Result (p-value)**	**Interpretation**
Shapiro-Wilk Test	Diameter of inhibition zone for each extract concentration	$p > 0.05$ for all groups	Data is normally distributed
One-Way ANOVA	Diameter of inhibition zone across all groups	$p = 0.000$	There is a statistically significant difference in antifungal activity between the groups.
Post Hoc LSD Test	**Pairwise Comparisons**	**p-value**	**Significant Difference**
	25% vs. 50% *M. indica* extract	0.004	Yes
	25% vs. 75% *M. indica* extract	0.000	Yes
	25% vs. 100% *M. indica* extract	0.017	Yes
	25% vs. 2% Chlorhexidine	0.000	Yes
	50% vs. 75% *M. indica* extract	0.007	Yes
	50% vs. 100% *M. indica* extract	0.526	No
	50% vs. 2% Chlorhexidine	0.004	Yes
	75% vs. 100% *M. indica* extract	0.001	Yes
	75% vs. 2% Chlorhexidine	0.874	No

4. Discussion

The antifungal activity of *M. indica* leaf extract can be primarily attributed to its diverse array of bioactive compounds, including mangiferin, flavonoids, and tannins. These compounds have demonstrated remarkable antimicrobial properties against a broad spectrum of microorganisms, including the opportunistic fungus *C. albicans*. Mangiferin, a prominent xanthone derivative found abundantly in mango leaves, has garnered significant attention for its potent antifungal activity against *C. albicans*. This bioactive compound exerts its antifungal effects by primarily targeting the fungal cell wall, a crucial structure that provides structural integrity and protection to the fungal cell. Mangiferin disrupts the integrity of the fungal cell wall by interfering with its synthesis and function. Specifically, mangiferin inhibits the activity of key enzymes involved in cell wall biosynthesis, such as glucan synthase and chitin synthase. These enzymes are responsible for the production of glucans and chitin, the major structural components of the fungal cell wall. By inhibiting these enzymes, mangiferin weakens the cell wall, making it more susceptible to osmotic stress and lysis. The fungal cell wall is a dynamic structure that undergoes constant remodeling to maintain its integrity and adapt to environmental changes. Mangiferin's ability to interfere with cell wall biosynthesis disrupts this delicate balance, leading to structural defects and compromised functionality. The weakened cell wall

becomes more vulnerable to external stressors, such as osmotic pressure, ultimately leading to cell lysis and death. In addition to its effects on cell wall biosynthesis, mangiferin can also directly interact with the fungal cell wall, causing structural damage and alterations in its permeability. Mangiferin's ability to bind to cell wall components, such as chitin and glucans, can disrupt the intricate network of these structural molecules, leading to weakened cell wall integrity. The disruption of cell wall integrity can have profound consequences for the fungal cell. The cell wall acts as a barrier, protecting the cell from environmental insults and maintaining its shape. Damage to the cell wall can lead to the leakage of intracellular components, such as ions, metabolites, and proteins, disrupting cellular homeostasis and ultimately resulting in cell death. The ability of mangiferin to interfere with fungal cell wall synthesis and function makes it a promising candidate for the development of novel antifungal agents. Its natural origin and minimal toxicity further enhance its potential as a safe and effective alternative to conventional antifungal drugs. Many conventional antifungal drugs have limitations, such as toxicity, drug resistance, and narrow spectrum of activity. Mangiferin's unique mechanism of action and favorable safety profile make it an attractive candidate for the development of new antifungal therapies. Further research is needed to fully explore its potential and optimize its therapeutic applications. Flavonoids, a diverse group of polyphenolic compounds widely distributed in plants, have emerged as versatile bioactive compounds with a wide range of biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. In the context of antifungal activity, flavonoids have demonstrated remarkable efficacy against *C. albicans* by disrupting its cell membrane function and interfering with its ability to form biofilms. The fungal cell membrane is a semipermeable barrier that plays a critical role in regulating the passage of molecules into and out of the cell. It maintains cellular homeostasis by controlling the influx of nutrients and the efflux of waste products. Flavonoids can disrupt the integrity of the fungal cell membrane by altering its fluidity and permeability. The cell membrane is composed of a lipid bilayer with embedded proteins that perform various functions, such as transport, signaling, and enzymatic activity. Flavonoids can interact with the lipid bilayer, altering its physical properties and disrupting the function of membrane proteins. This disruption can impair nutrient uptake, waste removal, and overall cellular processes, leading to cell death. In addition to their direct effects on cell membrane function, flavonoids can also interfere with the ability of *C. albicans* to form biofilms. Biofilms are complex communities of microorganisms that adhere to surfaces and are notoriously resistant to antimicrobial agents. They are a major cause of persistent infections and are implicated in various medical device-associated infections. Flavonoids can inhibit biofilm formation by interfering with the adhesion of *C. albicans* to surfaces and disrupting the communication signals that coordinate biofilm development. Adhesion is a critical step in the colonization and proliferation of *C. albicans* on host tissues and medical devices, such as orthodontic retainers. Flavonoids can interfere with the adhesion process by binding to cell surface adhesins or by altering the surface properties of the substrate. Furthermore, flavonoids can also disrupt quorum sensing, a communication system that bacteria use to coordinate their behavior, including biofilm formation. Quorum sensing involves the production and detection of signaling molecules that regulate gene expression in response to population density. By interfering with quorum sensing, flavonoids can disrupt the coordinated behavior of *C. albicans* cells, inhibiting biofilm formation and reducing its virulence. The disruption of cell membrane integrity and biofilm formation can significantly impair the survival and virulence of *C. albicans*. By targeting these critical aspects of fungal biology, flavonoids offer a promising approach for the development of novel antifungal agents. The increasing prevalence of antifungal resistance and the limitations of

conventional antifungal drugs have spurred the search for new therapeutic options. Flavonoids' diverse mechanisms of action and favorable safety profile make them attractive candidates for the development of new antifungal therapies. Further research is needed to fully explore their potential and optimize their therapeutic applications. Tannins, another class of polyphenolic compounds found in *M. indica* leaf extract, are well-known for their astringent properties and ability to bind to proteins and other macromolecules. In the context of antifungal activity, tannins have demonstrated efficacy against *C. albicans* by binding to its cell wall, preventing its growth and adhesion to surfaces. The fungal cell wall is a complex structure composed of various polysaccharides, proteins, and lipids. It provides structural support, protection against osmotic stress, and mediates interactions with the environment. Tannins can bind to these components, forming insoluble complexes that disrupt the structural integrity of the cell wall. The binding of tannins to cell wall components can alter the physical properties of the cell wall, such as its rigidity and permeability. This disruption can lead to cell lysis and death, as the cell becomes unable to maintain its structural integrity and protect itself from external stressors. Furthermore, tannins can also interfere with the adhesion of *C. albicans* to surfaces. Adhesion is a critical step in the colonization and proliferation of *C. albicans* on host tissues and medical devices, such as orthodontic retainers. By binding to the fungal cell wall, tannins can prevent its attachment to surfaces, thereby inhibiting its growth and spread. The adhesion of *C. albicans* to surfaces is mediated by various adhesins, which are cell surface proteins that recognize and bind to specific receptors on the substrate. Tannins can interfere with this process by binding to the adhesins or by altering the surface properties of the substrate, making it less conducive to fungal attachment. The inhibition of fungal growth and adhesion can help prevent the colonization and proliferation of *C. albicans* on orthodontic retainers, reducing the risk of oral candidiasis and other associated complications. Oral candidiasis, also known as thrush, is a common fungal infection of the mouth caused by *C. albicans*. It can cause discomfort, pain, and difficulty swallowing, and in severe cases, it can lead to systemic infections. The use of tannins as antifungal agents can help prevent oral candidiasis and maintain oral health, particularly in individuals who wear orthodontic retainers or other medical devices. The presence of multiple bioactive compounds with antifungal properties in *M. indica* leaf extract suggests a potential synergistic interaction between these compounds. Synergism occurs when the combined effect of two or more compounds is greater than the sum of their individual effects. In the case of *M. indica* leaf extract, the synergistic interaction between mangiferin, flavonoids, and tannins may enhance its overall antifungal activity against *C. albicans*. For example, mangiferin's ability to disrupt cell wall integrity may make the fungal cell more susceptible to the membrane-disrupting effects of flavonoids. Similarly, tannins' ability to bind to the fungal cell wall may enhance the penetration of other antifungal compounds into the cell. The synergistic interaction between these bioactive compounds may contribute to the observed potent antifungal activity of *M. indica* leaf extract against *C. albicans*. Further research is needed to fully elucidate the complex interplay between these compounds and their combined effects on fungal growth and survival.[11-15]

The use of *M. indica* leaf extract as a natural antifungal agent for orthodontic retainers offers several advantages over conventional chemical disinfectants. These advantages stem from its natural origin, safety profile, accessibility, cost-effectiveness, minimal side effects, and contribution to sustainable oral hygiene practices. *M. indica* leaf extract is a natural product derived from the leaves of the mango tree (*Mangifera indica* L.), a plant widely cultivated for its delicious fruit. Mangoes have been consumed for centuries and are a staple food in many tropical and subtropical regions. The leaves of the mango tree have also been traditionally used for various medicinal purposes, including the treatment of infections. The

natural origin of *M. indica* leaf extract makes it an attractive alternative to synthetic chemical disinfectants, which are often associated with concerns about toxicity and environmental impact. Unlike synthetic chemicals, *M. indica* leaf extract is readily biodegradable and does not pose a threat to the environment. Furthermore, *M. indica* leaf extract is generally considered safe for human use. It has been extensively studied for its various biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties, and has shown no significant toxicity or adverse effects. The safety profile of *M. indica* leaf extract makes it a suitable candidate for oral hygiene applications, where it comes into contact with delicate oral tissues. Mango leaves have been used in traditional medicine for centuries to treat various ailments, including infections, inflammation, and pain. The leaves contain a variety of bioactive compounds, including mangiferin, flavonoids, and tannins, which are responsible for their medicinal properties. In Ayurveda, the traditional Indian system of medicine, mango leaves are used to treat diabetes, diarrhea, and dysentery. They are also used as a gargle to treat sore throats and mouth ulcers. In traditional Chinese medicine, mango leaves are used to treat asthma, bronchitis, and cough. The traditional uses of mango leaves provide evidence for their safety and efficacy in treating various health conditions. The use of *M. indica* leaf extract as an antifungal agent builds upon this traditional knowledge and offers a scientifically validated approach to harnessing its medicinal properties. Synthetic chemical disinfectants are often persistent in the environment and can accumulate in soil and water, posing a threat to ecosystems and human health. In contrast, *M. indica* leaf extract is readily biodegradable, meaning it can be broken down by microorganisms into harmless substances. This makes it an environmentally friendly alternative to synthetic disinfectants. The biodegradability of *M. indica* leaf extract is due to its natural origin and the presence of enzymes in the environment that can break down its components. The breakdown products of *M. indica* leaf extract are not harmful to the environment and can be readily assimilated into natural cycles. The safety of *M. indica* leaf extract for human use has been extensively studied. In vitro and in vivo studies have shown that the extract has no significant toxicity or adverse effects. In vitro studies have evaluated the cytotoxicity of *M. indica* leaf extract on various cell lines, including human cells. These studies have consistently shown that the extract is not cytotoxic at concentrations that are effective against *C. albicans*. In vivo studies have evaluated the safety of *M. indica* leaf extract in animal models. These studies have shown that the extract is well-tolerated and does not cause any significant adverse effects, even at high doses. The extensive safety data on *M. indica* leaf extract supports its use in oral hygiene applications. Its natural origin, biodegradability, and safety profile make it an attractive alternative to synthetic chemical disinfectants. Mango trees are widely cultivated in tropical and subtropical regions, making *M. indica* leaves readily available and cost-effective. The abundance of mango trees ensures a sustainable supply of *M. indica* leaves, making it a viable option for the large-scale production of antifungal agents. Mango trees are one of the most widely cultivated fruit trees in the world. They are grown in over 100 countries, with major production centers in India, China, Thailand, Indonesia, and Mexico. The widespread cultivation of mango trees ensures a readily available and sustainable supply of *M. indica* leaves. The leaves of the mango tree are a byproduct of mango cultivation. They are typically discarded or used as animal feed. The use of *M. indica* leaves for the production of antifungal agents adds value to this byproduct and promotes sustainable agriculture. The cost-effectiveness of *M. indica* leaf extract is another significant advantage. Compared to synthetic chemical disinfectants, which can be expensive to produce and purchase, *M. indica* leaf extract is relatively inexpensive. This makes it an attractive option for individuals and healthcare systems seeking affordable and effective antifungal agents. The cost of producing *M. indica* leaf extract is relatively low due to

the abundance of mango leaves and the simple extraction process. The extract can be produced using readily available equipment and does not require specialized expertise. The low cost of *M. indica* leaf extract makes it an accessible option for individuals and communities with limited resources. It can help to improve oral hygiene and reduce the burden of oral infections in underserved populations. The accessibility and affordability of *M. indica* leaves make it a sustainable and economically viable option for the development of antifungal agents. It offers a cost-effective and environmentally friendly alternative to synthetic chemical disinfectants, particularly in regions where mangoes are widely cultivated. The production of *M. indica* leaf extract can create economic opportunities for local communities. It can provide employment and generate income, contributing to the economic development of rural areas. Unlike chemical disinfectants, which can cause staining, corrosion, and an unpleasant taste, *M. indica* leaf extract is not known to have any significant side effects. This is a major advantage, as it makes *M. indica* leaf extract a more appealing option for long-term use in oral hygiene. Chemical disinfectants, such as chlorhexidine gluconate, are commonly used for oral hygiene purposes, but they can have undesirable side effects. Chlorhexidine, for example, can cause staining of teeth and tongue, alter taste perception, and promote the formation of dental calculus. These side effects can discourage individuals from using chemical disinfectants regularly, compromising their oral hygiene. Staining of teeth and tongue is a common side effect of chlorhexidine. The staining is usually brown or yellow and can be difficult to remove. It can be unsightly and affect self-esteem, particularly in individuals who are concerned about their appearance. Alteration of taste perception is another side effect of chlorhexidine. It can cause a bitter or metallic taste in the mouth, which can make food and drinks less enjoyable. This can lead to decreased appetite and nutritional deficiencies. Promotion of dental calculus formation is another concern with chlorhexidine. Dental calculus, also known as tartar, is a hard deposit that forms on teeth. It can contribute to gum disease and tooth decay. Chlorhexidine can promote the formation of dental calculus by increasing the adherence of bacteria to teeth. In contrast to chemical disinfectants, *M. indica* leaf extract does not cause any significant side effects. It has a mild, pleasant taste and does not stain teeth or tongue. This makes it a more acceptable option for individuals seeking to maintain good oral hygiene without the discomfort or inconvenience of side effects. The absence of undesirable side effects makes *M. indica* leaf extract a more appealing option for long-term use in oral hygiene. It can be used regularly without concerns about staining, corrosion, or unpleasant taste, promoting consistent oral hygiene practices and reducing the risk of oral infections. The use of *M. indica* leaf extract as an antifungal agent can contribute to sustainable oral hygiene practices by reducing the reliance on synthetic chemicals. Synthetic chemical disinfectants are often derived from petroleum products and require energy-intensive manufacturing processes, contributing to environmental pollution and greenhouse gas emissions. The production of synthetic chemical disinfectants involves the use of fossil fuels and other non-renewable resources. The extraction, processing, and transportation of these resources contribute to environmental pollution, including air pollution, water pollution, and soil contamination. Furthermore, the manufacturing processes for synthetic chemical disinfectants often involve the use of hazardous chemicals and generate significant amounts of waste. These chemicals and waste products can contaminate the environment and pose risks to human health. In contrast to synthetic chemical disinfectants, *M. indica* leaf extract is a natural product derived from a renewable resource. Its production does not involve the use of harmful chemicals or generate significant environmental waste. The sustainable and eco-friendly nature of *M. indica* leaf extract aligns with the growing demand for environmentally conscious products. The cultivation of mango trees is a sustainable agricultural practice. Mango trees are

perennial plants that can produce fruit for many years. They require minimal inputs, such as water and fertilizer, and can grow in a variety of climates and soil conditions. The extraction of *M. indica* leaf extract is a relatively simple process that does not require the use of harsh chemicals or generate significant waste. The extract can be produced using water or ethanol as a solvent, both of which are environmentally friendly. Furthermore, the use of *M. indica* leaf extract can promote local economic development, particularly in regions where mangoes are widely cultivated. The cultivation and processing of *M. indica* leaves can create employment opportunities and generate income for local communities. The production of *M. indica* leaf extract can be integrated into existing mango cultivation practices, providing an additional source of income for farmers. It can also create new jobs in rural areas, contributing to the economic development of these communities. The sustainable and eco-friendly nature of *M. indica* leaf extract makes it an ideal choice for individuals seeking to reduce their environmental impact and support sustainable practices. It offers a responsible and ethical alternative to synthetic chemical disinfectants, contributing to a healthier planet and a more sustainable future.[16-20]

5. Conclusion

The study's findings demonstrate that *M. indica* leaf extract, particularly at a 75% concentration, effectively inhibits *C. albicans* growth on orthodontic retainers. This natural extract offers a promising alternative to conventional disinfectants, particularly chlorhexidine digluconate, which can cause staining, corrosion, and an unpleasant taste. The antifungal activity of *M. indica* leaf extract is attributed to its rich content of bioactive compounds, including mangiferin, flavonoids, and tannins. These compounds work synergistically to disrupt fungal cell wall integrity, cell membrane function, and biofilm formation, ultimately leading to fungal cell death. In addition to its antifungal efficacy, *M. indica* leaf extract offers several advantages. It is a natural, readily biodegradable product derived from a renewable resource, making it an environmentally friendly option. The extract is generally safe for human use, with no significant toxicity or adverse effects reported. Furthermore, it is readily available and cost-effective, particularly in regions where mangoes are widely cultivated. The use of *M. indica* leaf extract as an antifungal agent for orthodontic retainers can contribute to sustainable oral hygiene practices by reducing reliance on synthetic chemicals and promoting the use of natural, renewable resources. Further research is needed to fully explore the potential of *M. indica* leaf extract and optimize its therapeutic applications. Future studies could investigate the long-term efficacy and safety of *M. indica* leaf extract in clinical settings, comparing its effectiveness to conventional disinfectants. Additionally, research could focus on developing standardized protocols for the preparation and application of *M. indica* leaf extract to ensure consistent efficacy and safety. The potential of *M. indica* leaf extract as a natural antifungal agent for orthodontic retainers is promising, offering a sustainable and effective approach to improve oral hygiene during orthodontic treatment.

6. References

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