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A Comparative Evaluation of Antimicrobial Activity 0f Magnolia Bark Extract, Triphala Extract and Commercially Available Pediatric Toothpastes: An In-Vitro Study

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Abstract

Background: Dental caries affects individuals of all ages. Lactobacilli have been recognized as key contributors to human dental caries for over a century. Magnolia bark extract and Triphala extract, renowned for their wide range of pharmacological properties, have not been extensively studied or compared against each other. Hence, this study aimed to evaluate the antimicrobial efficacy of Magnolia bark extract, Triphala extract, fluoridated, and non-fluoridated toothpastes on Lactobacilli. Materials and Method: Magnolia bark extract, Triphala extract, fluoridated, and non-fluoridated toothpastes were prepared at concentrations of 50%, 25%, and 12.5%. Antimicrobial activity was assessed using the agar well diffusion method by measuring zones of inhibition. Results: At concentrations of 50%, 25%, and 12.5%, Magnolia bark extract showed zones of inhibition of 20.33 mm, 16.66 mm, and 13 mm, respectively. Triphala extract showed zones of 17.66 mm, 13 mm, and 12.33 mm. Fluoridated toothpaste showed zones of 18 mm, 14.66 mm, and 12.33 mm, while non-fluoridated toothpaste showed zones of 15.66 mm, 12.33 mm, and 10.33 mm. Conclusion: The study findings highlight the superior antimicrobial activity of magnolia bark extract compared to triphala, fluoridated, and non-fluoridated toothpaste. Incorporating magnolia bark extract into dentifrices could prove beneficial in inhibiting caries-causing microorganisms and promoting oral health.

Keywords: Dental caries, Magnolia officinalis, Lactobacillus acidophilus, Triphala

Introduction

Dental caries remains a prevalent global oral health concern, impacting between 60% to 90% of children, often stemming from factors such as neglect, insufficient awareness of preventive measures, costly dental services, or their unavailability, especially in rural settings. This chronic and multifaceted condition, while preventable, presents a substantial public health challenge. The onset of dental caries is intricately linked to three key factors: dietary carbohydrates, cariogenic bacteria residing in plaque, and susceptible tooth surfaces. Notably, bacteria are recognized as pivotal influencers in the prevalence and occurrence of dental caries. [1,2]

In the oral cavity, *Lactobacilli* act as opportunistic invaders of pre-existing caries lesions or those in the early stages of development. The colonization process relies on three crucial conditions: 1) the presence of a retentive niche that facilitates *Lactobacilli* accumulation, leading to 2) a low pH environment and anaerobic conditions, along with 3) access to readily available carbohydrates as a source of nutrients. [3,4]

Magnolia bark extract has been used for thousands of years in traditional Chinese, Japanese, and Korean medicine, particularly from Magnolia officinalis and Magnolia obovata, which thrive in East and Southeast Asia. These species are known for their aromatic and visually striking flowers. [5,6,] The bark contains significant phenolic compounds, especially magnolol and honokiol, which are recognized for their pharmacological benefits. Research by Sarrica (2018)[7] highlights these compounds' therapeutic potential for treating various health issues, including gastrointestinal disorders, anxiety, and allergies. Due to its diverse pharmacological properties, magnolia bark extract continues to be valued in both traditional and modern medicinal applications. [7]

Herbal remedies have been essential in healthcare throughout history, valued for maintaining health and homeostasis. Triphala, a notable Ayurvedic polyherbal medicine, is made from the dried fruits of *Emblica officinalis*, *Terminalia bellirica*, and *Terminalia chebula*, all native to the Indian subcontinent. As a tridoshic rasayana, it promotes longevity and rejuvenation. Triphala is traditionally used as an antimicrobial agent in Ayurvedic medicine and has proven effective in reducing oral bacteria, dental plaque, and gingivitis. It shows notable antibacterial effects against *Streptococcus mutans*, highlighting its potential as a valuable oral antimicrobial agent. [8, 9]

Toothbrushing with dentifrices is essential for oral hygiene, removing plaque to prevent dental caries and periodontal disease. Dentifrices contain fluoride for enamel protection, abrasives for plaque and stain removal, and may include desensitizing agents and flavors for patient comfort. This combined approach, underscored by evidence-based dental science, promotes optimal oral health and overall well-being. [10]

Non-fluoridated toothpaste is safer for young children who may inadvertently swallow toothpaste, as it eliminates the risk of excessive fluoride intake. For children with fluoride sensitivity or those prone to adverse reactions, such as stomach upset or oral discomfort, non-fluoridated options offer a gentler alternative. Additionally, parents who prioritize natural or organic products for their children may prefer non-fluoridated toothpaste, aligning with their lifestyle and values. [10,11]

Fluoridated toothpaste serves as a fundamental component of pediatric oral care, offering numerous benefits for children's dental health. Fluoride primarily prevents caries through its topical action. Even at concentrations as low as 1 ppm, fluoride application significantly reduces enamel solubility, hindering demineralization. By integrating into tooth surface crystals, fluoride fortifies the enamel, rendering it more resistant to acid attacks. Furthermore, fluoride promotes enamel remineralization, accelerating the ongoing process of demineralization and remineralization. Its incorporation into the mineral structure decreases susceptibility to acid erosion, thereby bolstering enamel strength. [11]

Considering that *Lactobacilli* are recognized as potential contributors to dental caries, it becomes imperative to investigate the impact of Magnolia bark extract and Triphala extract on these microorganisms. Therefore, in light of this scarcity of data, the current research endeavors to evaluate the antimicrobial efficacy of Magnolia bark extract, Triphala extract, fluoridated and non-fluoridated toothpastes on *Lactobacilli*, thus contributing to a better understanding of their potential role in dental care and disease prevention.

MATERIALS AND METHODS

The study received approval from the institutional ethical committee of Bharati Vidyapeeth Medical College and Hospital, Sangli. The study was conducted in Maratha Mandal's Central Research Laboratory, Maratha Mandal's NGH Institute of Dental Sciences & Research Centre, Belgaum, Karnataka.

The sample size was calculated using GPower software. The effect size was determined using the data obtained from a previous study conducted Thomas B (2011). [12] Keeping α error as 0.05 and 1-β error as 0.95, total sample size came to be 12. As there were 4 groups, hence the study was done in triplicates.

Groups:

Group 1 – Magnolia bark extract at 50%, 25% and 12.5% concentrations
Group 2 – Triphala Extract at 50%, 25% and 12.5% concentrations
Group 3 – Fluoridated Toothpaste at 50%, 25% and 12.5% concentrations
Group 4 – Non-Fluoridated Toothpaste at 50%, 25% and 12.5% concentrations

Stock solution preparation:

Preparation of Magnolia Bark Extract:

Magnolia Bark Extract powder (Vital Herbs, India) which consisted of 98% of magnolol and honokiol and prepared by hydroalcoholic extraction process. It was converted into solution by dissolving 10 gram powder in 10 ml of 5% dimethyl sulfoxide. [13]

Dilutions of Magnolia Bark Extract solution (Group 1) were made at 50%, 25% and 12.5% concentrations by dissolving 5ml of solution + 5ml of distilled water, 2.5ml of solution + 7.5 ml distilled water and 1.25ml of solution + 8.75 ml distilled water respectively.

Preparation of Triphala:

Triphala (Bixa Botanical, India) consisted of 100% of organic Amla, Bibhitaki and Haritaki powder. It was made into solution by dissolving 10 gram powder in 10 ml of 5% dimethyl sulfoxide. [13]

Dilutions of Triphala solutions (Group 2) were made at 50%, 25% and 12.5% concentrations by suspending 5ml of solution + 5ml of distilled water, 2.5ml of solution + 7.5 ml distilled water and 1.25ml of solution + 8.75 ml distilled water respectively.

Preparation of Aqueous Solution of toothpaste:

10 gram of each Fluoridated and Non-Fluoridated toothpaste was suspended in 10 ml of distilled water. Dilutions of Toothpaste 1 (Group 3) and 2 (Group 4) were made at 50%, 25% and 12.5% concentrations by suspending 5 ml solution + 5 ml of distilled water, 2.5 ml solution + 7.5 ml distilled water and 1.25 ml solution + 8.75 ml distilled water respectively. [12]

Agar well diffusion method:

1. Media used: Brain Heart Infusion Agar
2. Temperature: Agar plates were brought to room temperature before use.
3. Inoculum preparation: Colonies were transferred to the plates using a swab, and the turbidity was visually adjusted with broth to match a vortexed 0.5 McFarland turbidity standard.
4. Inoculation of Agar plate: After adjusting the inoculum to a McFarland 0.5 turbidity standard, a sterile cotton swab was dipped into the inoculum and rotated against the tube wall above the liquid to remove excess. The agar plate surface was then swabbed three times, rotating the plate about 60º between each streak to ensure even distribution while avoiding contact with the sides of the petri plate to prevent aerosol formation. The inoculated plates were left to stand for at least 3 minutes, but no longer than 15 minutes, before making wells.
5. Addition of compound into plate: A 5mm diameter hollow tube was heated and pressed onto the inoculated agar plate to create a well, then promptly removed. This was repeated to make three wells on each plate, resulting in four plates prepared for four groups. A micropipette was used to add 50 µl of each prepared solution into the wells.
6. Incubation: The plates were placed in the incubator within 15 minutes of applying the compound, followed by incubation at 37°C for 18-24 hours.
7. Reading plates: The evaluation of plates for readings was limited to instances where the growth lawn showed full or nearly full coverage. Measurement of the zone of inhibition was conducted meticulously, ensuring precision to the nearest whole millimeter using a measuring device. (Fig 1, Fig 2, Fig 3, Fig 4.)

Fig. 1 - Zone of Inhibition of Group 1 – Magnolia Bark Extract

Fig. 2 - Zone of Inhibition of Group 2 – Triphala Extract

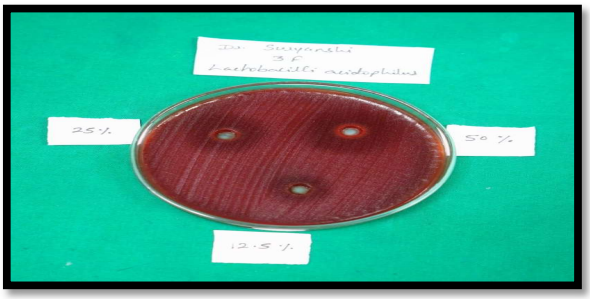


Fig. 3 - Zone of Inhibition of Group 3 – Fluoridated Toothpaste

Fig. 4 - Zone of Inhibition of Group 4 – Non-Fluoridated Toothpaste

RESULTS

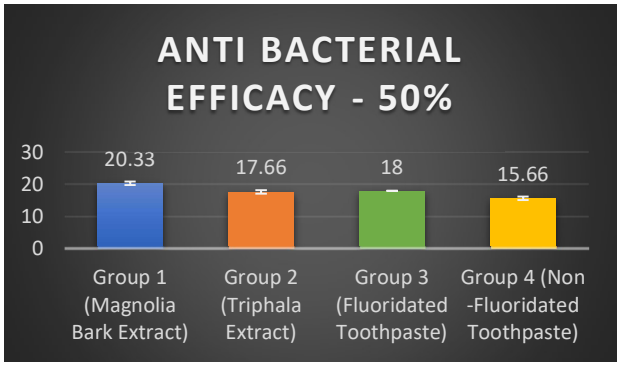
Statistical Product and Service Solution (SPSS) version 21 for Windows (Armonk, NY: IBM Corp) software was used to analyze the data. Statistical analysis was done by using tools of descriptive statistics such as mean, and standard deviation for representing quantitative data.

Inter group comparison was done using One-way ANOVA F test followed by pair-wise comparison using the Tukey post hoc test. Probability $p<0.05$, considered as significant as alpha error set at 5% with a confidence interval of 95% set in the study. The power of the study was set at 80% with a beta error set at 20%.

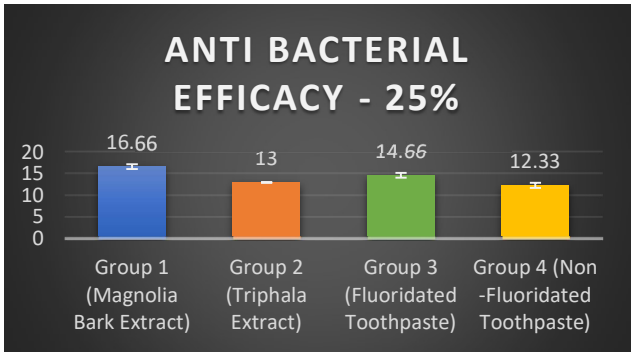
Graph 1 reveals that there is a highly statistically significant difference ($p< 0.001$) between groups 1 and 4. Maximum zone of inhibition was shown by magnolia bark extract (20.33mm) followed by fluoridated toothpaste (18mm), Tripahala extract (17.66mm) and Non fluoridated toothpaste (15.66 mm).

Graph 2 shows that there is a highly significant statistical difference between Group 1 and 2 and Group 1 and 4. Maximum zone of inhibition was seen in Group 1 - 16.66 mm followed by Group 3 - 14.66 mm, Group 2 - 13mm and least zone of inhibition was seen in Group 4 - 12.33 mm.

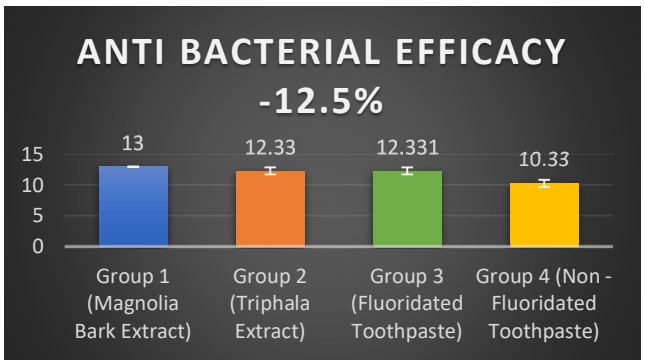
Graph 3 reveals there is highly significant statistical difference between Group 1 and 4. Group 1 showed a zone of inhibition of 13 mm followed by Group 2 and 3 with a zone of inhibition of 12.33 mm and Group 4 showed 10.33 mm of zone of inhibition.



Graph 1 - Anti-bacterial activity of all four groups at 50% concentration



Graph 2 - Anti-bacterial activity of all four groups at 25% concentration



Graph 3 - Anti-bacterial activity of all four groups at 12.5% concentration

DISCUSSION

Magnolia bark has garnered significant attention for its diverse pharmacological properties, which include anti-cancer, anti-inflammatory, antioxidant, anti-alzheimer, and anti-atherosclerosis effects. Due to these beneficial attributes, magnolia bark is utilized in the treatment of various diseases. The primary antimicrobial constituents of magnolia bark, magnolol, and honokiol, have been reported to exhibit antimicrobial effects against a range of oral bacteria, including *Staphylococcus aureus, Porphyromonas gingivalis,* and *Enterococcus faecalis.* This suggests the potential of magnolia bark and its constituents in promoting oral health and combating oral infections, highlighting its significance in both traditional and modern medicine.

In our study, we observed that magnolia bark extract exhibited significant antimicrobial activity against *Lactobacilli* at various concentrations. Specifically, magnolia bark extract demonstrated the greatest antimicrobial efficacy at all concentrations of 50%, 25%, and 12.5%, resulting in zones of inhibition measuring 20.33 mm, 16.66 mm, and 13.0 mm, respectively. These findings suggest that magnolia bark extract possesses potent antimicrobial properties against *Lactobacilli*, highlighting its potential as a natural antimicrobial agent.

Yongjin Hu (2011) investigated the antimicrobial properties of Magnolia officinalis extract against *Staphylococcus aureus* using minced mutton as a model system. Their study demonstrated that adding Magnolia officinalis extract to minced mutton significantly suppressed the growth of *S. aureus* compared to the control samples without Magnolia officinalis extract, highlighting Magnolia officinalis extract's potential as a natural preservative for meat products. [14]

Furthermore, Cagetti in 2020 explored the potential of caries-prevention benefits of a sugar-free chewing gum that contained xylitol and magnolia bark extract in high-risk adult populations. According to their research, subjects who used chewing gum that combined xylitol and Magnolia saw the least amount of new carious lesion, the least caries progression, and the fewest restorative treatments compared to those using gums containing only xylitol or polyols. These findings suggest that including magnolia bark extracts in sugar-free chewing gum formulations may enhance caries prevention and improve oral health outcomes.[15]

Campus in 2011 investigated the effects of daily consumption of sugar-free chewing gum containing magnolia bark extract on dental caries and gingivitis over a 30-day period. The results showed that the magnolia bark extract gum significantly reduced plaque acid production, *Mutans Streptococcus* salivary levels, and gingival bleeding when compared to the xylitol and control groups. [16]

The antimicrobial properties of magnolia bark extract are largely due to its high concentration of polyphenolic compounds, such as magnolol, honokiol, tetrahydromagnolol, and isomagnolol. Research has shown that phenolic compounds can inhibit microbial growth through various mechanisms. One key mechanism involves altering the permeability of microbial cell membranes, leading to the leakage of macromolecules. Once inside the cell, phenolic compounds can interact with membrane enzymes and proteins, disrupting cellular activity by causing an imbalance in proton flow. The antimicrobial effects of magnolia bark extract are primarily seen in the damage to cell membranes and cell walls, which increases membrane permeability or causes cell wall rupture, releasing cellular contents. Additionally, magnolia bark extract can disrupt structural components and alter bacterial cell morphology.[17]

In the year 2015, Chainani carried out an in-depth study aimed at examining the antimicrobial efficacy of Triphala extract when used against two specific microorganisms: *Lactobacilli* and *Candida albicans*. The results of this investigation revealed that the Triphala extract possessed significant antimicrobial properties, effectively inhibiting the growth of both *Lactobacilli* and *C. albicans*. Notably, the study highlighted that the maximum zone of inhibition achieved by the Triphala extract was measured at 22 millimeters when applied at a concentration of 6%, and a slightly smaller inhibition zone of 20 millimeters was observed at a higher concentration of 9%. [2]

Contrary to our results, a study by Shah in 2014 assessed the antimicrobial effects of extracts from Neem sticks, Tulsi leaves, and Triphala against *Streptococcus mutans* and *Lactobacillus acidophilus*. They found that while Neem and Tulsi extracts showed significant antimicrobial activity, Triphala extract did not exhibit any antimicrobial properties against either *Streptococcus mutans* or *Lactobacillus acidophilus*. In our study, however, Triphala displayed notable antimicrobial activity against *Lactobacillus acidophilus*, indicating a significant difference in findings between the two studies. [18]

In a study conducted by Thomas in 2010, the antimicrobial properties of Triphala were evaluated alongside commercially available toothpastes. Triphala displayed a significant antibacterial effect against *Streptococcus mutans*, with 50%, 25%, and 12.5% concentrations showing zone of inhibition measurements of 30mm, 28mm, and 24mm respectively. Product 1 (toothpaste 1) exhibited zone of inhibition measurements of 25mm, 20mm, and 15mm respectively, while Product 2 (toothpaste 2) demonstrated zone of inhibition measurements of 25mm, 18mm, and 12mm for *S. mutans* MTCC strain. The conclusion drawn was that Triphala displayed greater antimicrobial activity compared to both toothpaste 1 and toothpaste 2. [12]

In our study, Triphala exhibited Zone of Inhibition measurements of 17.66mm, 13mm, and 12.33mm at concentrations of 50%, 25%, and 12.5% respectively. Comparatively, Fluoridated toothpaste showed zone of inhibition measurements of 18mm, 14.66mm, and 12.33mm, while non-fluoridated toothpaste demonstrated zone of inhibition measurements of 15.66mm, 12.33mm, and 10.33mm at the same concentrations. Conclusively, Triphala showed significantly lower zone

of inhibition measurements than Magnolia, slightly lower zone of inhibition measurements than fluoridated toothpaste, and displayed higher zone of inhibition measurements than non-fluoridated toothpaste.

The inhibitory effect of Triphala can be attributed to the diverse range of phytochemicals present in its components. For example, Terminalia chebula Retz fruit contains polyphenols, flavonoids, terpenes, alkaloids, anthocyanins and glycosides. Terminalia bellirica fruit is known for its tannins, including ellagic acid, gallic acid and phylemblin while Emblica officinalis is rich in vitamin C and vitamin C complex. Additionally, various components such as cardenolide, glycosides, palmitooleolinolein-containing oil, 16-hentriacontanone, hexahydroxydiphenic acid ester, friedelin, beta-sitosterol, and chebulagic acid have been identified in the fruit extract. [14,19]

Deshpande in 2014, compared the antimicrobial efficacy of two commercially used herbal and fluoridated toothpastes against salivary microflora. The results consistently showed high antimicrobial activity of the fluoride-containing dentifrice at concentrations of 25%, 50%, and 100%, with mean zone of inhibition measurements of 14.4mm, 17.5mm, and 21.8mm respectively. It was observed that the fluoride-containing dentifrice exhibited significantly higher zones of inhibition compared to the herbal toothpaste at all concentrations. [20]

Similarly, Bora in 2014 compared the antimicrobial efficacy of ten commercially available herbal and non-herbal toothpastes. Their findings suggested that fluoridated toothpaste displayed the highest antimicrobial activity at all concentrations when compared to herbal toothpaste. [21]

Al Hilu (2024) evaluated the antimicrobial activity of various toothpaste formulations against *Lactobacillus acidophilus* and *Streptococcus mutans* using the standard agar diffusion method. The study involved six different toothpaste brands: Crest 3D White, Colgate Total 12, Charcoal, Colgate Optic White, Sensodyne, and Colgate Natural Extracts.

The results revealed that all toothpaste formulas, except for TP1, exhibited varying degrees of antibacterial activity against Gram-positive bacteria. Notably, toothpaste containing sodium fluoride, such as Colgate Total White, was the only product that effectively inhibited the growth of both *Streptococcus mutans* and *Lactobacillus acidophilus*.[22]

Kumar (2015) compared the antimicrobial efficacy of two pediatric toothpastes with fluoride, Pediflor and Kidodent, against three different bacterial species in children between the ages of 4 and 6: *S. mutans*, *Lactobacilli,* and *Candida.* After using the toothpastes for 30 and 60 days, *S. mutans* and *Lactobacillu*s levels were significantly reduced by both Pediflor and Kidodent. Average salivary *Lactobacillus* and *Streptococcus* counts significantly decreased after using toothpastes with fluoride concentrations of 458 ppm and 500 ppm. Notably, Kidodent toothpaste (500 ppm), which had precisely 500 ppm of fluoride, demonstrated equivalent efficiency to Pediflor (458 ppm). [23]

Walsh (2019) in their systematic review concluded that toothpaste with higher fluoride concentrations (>1000 ppm) was more effective in preventing caries. They found no statistically significant effect when fluoride concentrations were less than 500 ppm. [24]

Conversely, a meta-analysis conducted by Wright (2014) reported that fluoridated toothpaste containing 500 ppm or more significantly reduces caries in children aged 6 years or younger compared to toothpaste with lower fluoride concentrations. [25]

In our investigation, the findings revealed that fluoridated toothpaste containing a concentration of 600 ppm exhibited a larger zone of antimicrobial activity compared to non-fluoridated toothpaste. Additionally, our study demonstrated that fluoridated toothpaste displayed superior antimicrobial effectiveness compared to both triphala and non-fluoridated toothpaste variants.

The heightened antimicrobial activity of fluoride can be attributed to its interference with crucial enzymatic processes within oral bacteria. By disrupting these metabolic activities, fluoride effectively hinders the growth and reproduction of bacteria. This interference ultimately weakens bacterial cells, making them more susceptible to the effects of other antimicrobial agents and the body's natural defense mechanisms. These results underscore the importance of fluoride in oral care products and highlight its significant role in promoting oral health by inhibiting bacterial growth and contributing to overall hygiene and protection against dental caries.

CONCLUSION

Our study findings highlight the superior antimicrobial activity of magnolia bark extract compared to triphala, fluoridated, and non-fluoridated toothpaste. Triphala exhibited greater antimicrobial activity against *Lactobacilli* than non-fluoridated toothpaste, while fluoridated toothpaste demonstrated the most substantial zones of inhibition among triphala and non-fluoridated toothpaste. These results suggest distinct antimicrobial efficacy for each study group ingredient, indicating potential avenues for further exploration of their roles in oral health care.

In vivo studies are needed to thoroughly understand the impact of antimicrobial agents on *Lactobacilli* count. Additionally, interactions with host factors, variability in microbial susceptibility, and environmental factors may influence the study outcomes.

Clinical studies consistently show that antimicrobial dentifrices inhibit oral microorganisms and improve gingival health. Magnolia bark extract's antimicrobial properties suggest it disrupts microbial cell function, inhibiting growth. Further research into natural herbal products like magnolia bark extract may reveal new bioactive compounds with therapeutic potential for combating microbial infections and promoting oral health.

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