

ORIGINAL ARTICLE

Antimicrobial Activity of Seven Herbal Toothpastes Against Oral Microorganisms

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ABSTRACT

Introduction: Various herbal toothpaste brands claim to possess anti-caries and anti-gingivitis properties. This study assessed the antimicrobial activity of seven herbal toothpastes against oral microorganisms commonly associated with oral diseases. **Materials and Methods:** Herbal toothpastes at a concentration of 50 mg/mL were tested against *Streptococcus mutans* ATCC 25175, *Streptococcus salivarius* ATCC 13419, and *Candida albicans* ATCC 10231 to determine their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. The MIC was established using a two-fold serial microdilution technique at concentrations ranging from 12.50 mg/mL to 0.01 mg/mL. The MBC values were determined through the subculturing method. **Results:** Colgate Total® exhibited the highest inhibitory effects against *C. albicans* (0.781 mg/mL), followed by *S. salivarius* (1.56 mg/mL) and *S. mutans* (1.56 mg/mL) at 24 and 48 hours of incubation. Halagel®, Herbalfresh, and Himalaya demonstrated similar inhibitory effects as Colgate Total® against *C. albicans* (0.781 mg/mL), while MorningKiss was effective against *S. salivarius* and *S. mutans* (1.56 mg/mL) at 24 hours. Most microbial effects diminished after 48 hours. However, the MBC values were higher than the MIC values for all microorganisms after 48 hours. **Conclusion:** All tested herbal toothpastes demonstrated the ability to inhibit the growth of the test microorganisms; however, their antimicrobial activities were weaker compared to Colgate Total®.

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INTRODUCTION

Dental caries and periodontitis are significant public health concerns in Malaysia. According to a national oral health survey conducted in 2017, over 70% of children aged 6 years were found to have dental caries (1). The prevalence is slightly lower but still concerning in older children and adolescents. The National Oral Health Survey of Adults (NOHSA) 2020 indicated that 85.1% of adults had experienced dental caries, with a decreasing trend for caries prevalence from 2010 (88.9%) and 2000 (90.3%) (2-4). The decreased mean DMFT score from 11.4 in 2010 to 9.7 in 2020 suggests an improvement in caries severity (2, 3). While the NOHSA 2020 and

NOHSA 2010 found that approximately 94.5% of adults had some form of periodontal disease, ranging from gingivitis (a mild form) to severe periodontitis, which can lead to tooth loss if untreated (2, 3). The prevalence of periodontitis remained high at 38.2%, but there was a decrease in shallow pockets to 23.7% and deep pockets to 14.5% (2). The common risk factors for periodontitis in Malaysia include poor oral hygiene, smoking, diabetes, and socioeconomic factors (5). In addition, the NOHSA 2020 also reported the prevalence of oral lesions (9.5%), and dental prostheses (16.3%), with partial dentures at 8.0% and full dentures at 5.0% of the Malaysian adults tested (2). Thus, public health initiatives aim to address these issues through education and improved access to dental care.

In this study, three types of oral microbiotas, such as *Streptococcus mutans*, *Streptococcus salivarius* and *Candida albicans* were selected due to their nature as normal oral microbiota, but on the other hands, can

bring harm if their numbers are increased. Cariogenic bacteria such as *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*) contribute to the demineralisation of teeth, leading to enamel and dentine destruction, followed by pulp and periapical tissue inflammation (6). While periodontitis is primarily associated with *Actinomyces*, *Aggregatibacter* (formerly *Actinobacillus*), *Streptococcus*, and *Candida* species (7, 8). Among them, *Candida albicans* is a persistent member of the oral microbiota in children with caries and exhibits significant growth in response to sucrose exposure (9, 10). It produces organic acids such as pyruvate and acetate, which play a key role in caries development (11). Beyond caries, *C. albicans* can cause oral thrush and, in immunocompromised individuals, may spread systemically or lead to chronic mucocutaneous candidiasis (12). Overgrowth is common in these patients and denture wearers with ill-fitting dentures, often resulting in denture stomatitis (13). Additionally, an *in vivo* study found that *C. albicans* promotes the colonisation of *Porphyromonas gingivalis*, contributing to severe caries onset (14). *Streptococcus salivarius*, a dominant commensal bacterium primarily found on the tongue, was used for comparative analysis in this study. It is absent in six-month-old breastfed infants but becomes prevalent in saliva following cereal and sugar intake, occasionally contributing to smooth surface caries (15). Therefore, effective control of pathogenic bacteria and dental plaque is crucial for maintaining oral health and preventing oral diseases.

Using toothpaste is a frequent practice to prevent oral diseases and maintain oral hygiene by removing dental plaque and food debris. Toothpaste helps prevent bad breath (halitosis) and delivers active ingredients with specific purposes, such as antibacterial properties. Toothpaste components include abrasives, fluoride, surfactants, remineralisers, flavours, antimicrobial agents, preservatives, and more. Some ingredients, like hydroxyapatite nanocrystals and calcium phosphate, aid in the remineralisation of teeth (16). Nowadays, various types of herbal toothpastes are being commercialised as an alternative to fluoridated toothpaste. These herbal toothpastes, claimed by manufacturers to have antimicrobial, antiplaque properties and reduce dentin hypersensitivity, are gaining popularity. This growing user base is driven by people's preference for herbal products and their desire to avoid synthetic ingredients to minimise side effects. Additionally, many believe in the benefits of herbal products, supported by numerous studies showing positive effects against bacteria and fungi. For instance, Ozaki et al. and Al-Kholani evaluated the efficacy of Parodontax toothpaste, which contains herbal extracts like chamomile, echinacea, sage, rhatany, myrrh, and peppermint oil. They found a significant decrease in Plaque Index and Gingival Index in subjects with established gingivitis (17, 18). Similarly, Adamkova et al. confirmed that combining *Macleya*

cordata and *Prunella vulgaris* efficiently controlled gingivitis (19). Neem, propolis, and miswak are well-known herbs frequently used in herbal toothpaste. Neem (*Azadirachta indica*), in particular, is indigenous to countries like India, Myanmar, Bangladesh, Sri Lanka, Malaysia, and Pakistan, and flourishes in tropical and semi-tropical areas. Neem products have established medicinal properties, including anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, antifertility, and sedative effects. It is a significant component in Ayurvedic medicine, especially for treating skin diseases (20). Research by Mistry et al. demonstrated neem's anti-caries properties by inhibiting many oral microorganisms such as *S. mutans*, *Enterococcus faecalis*, and *C. albicans* in biofilm (21) and *Lactobacillus acidophilus* (22). Neem extract contains hydrolysable tannins that inhibit the synthesis of insoluble glucan, altering bacterial adhesion, and reducing the ability of some streptococci to colonise tooth surfaces, thereby exhibiting anti-plaque properties (23). Additionally, several studies found that miswak (*Salvadora persica*) significantly inhibits *S. mutans* (cariogenic bacterium) and *P. gingivalis* (a periodontal pathogen) (24, 25). This study supports the claim that miswak promotes good oral hygiene and reduces caries and gingivitis incidence among users. A systematic review reported that miswak effectively reduced plaque and gingivitis scores to a level comparable to toothbrushes when used exclusively (26).

To date, no study has been published on herbal toothpastes that are commercially available in Malaysia. This study aims to provide evidence supporting the antiplaque and antimicrobial properties claimed by manufacturers of herbal toothpastes available in Malaysia. Colgate Total®, containing 0.3% triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether, a safer antibacterial agent), a copolymer and fluoride was approved by the Ministry of Health Malaysia and the U.S. Food and Drug Administration (FDA), and was chosen as a positive control for comparing the antimicrobial activities of herbal toothpastes due to its proven effectiveness in reducing plaque, gingival inflammation, gingival bleeding, and coronal caries compared to a toothpaste without triclosan/copolymer (27, 28). This makes it a reliable reference point when evaluating the performance of other toothpastes. The concentration of triclosan permitted by the National Pharmaceutical Regulatory Agency (NPRA) for toothpaste and mouth rinse is 0.3% and 0.2% respectively (29). There was no evidence relating triclosan to carcinogenic effects, particularly in *in vivo* studies. The current levels of triclosan in oral hygiene products such as toothpaste and mouth rinse are safe to be used by the public (30). Therefore, this study aims to determine the antimicrobial activity of seven herbal toothpastes against dental caries contributors, such as *S. mutans* ATCC 25175, *S. salivarius* ATCC 13419, and *C. albicans* ATCC 10231, by using the broth microdilution method to determine minimum inhibitory concentration (MIC) and minimum

bactericidal concentration (MBC) values.

MATERIALS AND METHODS

Preparation of herbal toothpaste

The seven herbal toothpastes were purchased from a local supermarket in Malaysia (Table I). These included Neem (Henkel India, India), Halagel® (Halagel (M) Sdn. Bhd., Malaysia), Herbalfresh (Indonesia), Najwa (India), Oralgard (Thailand), MorningKiss (Yee Lee Trading, Malaysia) and Himalaya (Himalaya Drug Company, Bangalore). Colgate Total® (Colgate-Palmolive, New York City), containing 0.3% triclosan, a copolymer and fluoride, was used as a positive control for comparing the antimicrobial activities of the herbal toothpastes. For testing, 250 mg of each herbal toothpaste was diluted in 5 mL of distilled water and then shaken for 5 minutes using a Vortex mixer. This preparation resulted in a final concentration of 50 mg/mL of herbal toothpaste in a stock solution.

Preparation of test microorganisms

The oral microorganisms used in this study were *C. albicans* ATCC 10231, *S. salivarius* ATCC 13419 and *S. mutans* ATCC 25175 (American Type Culture Collection (ATCC), Manassas, VA 201808, USA). The bacterial stocks were stored frozen in 30% glycerol at -80°C. To restart the cultures, the samples were thawed, dispersed in Trypticase Soy Broth (TSB) (Oxoid, England), and incubated at 37°C in 5% CO₂ for 24 hours. For *C. albicans*, the sample was inoculated on Sabouraud Dextrose Agar (SDA) (Oxoid Ltd, England) supplemented with 0.1 mg/mL chloramphenicol, then incubated at 30°C in an aerobic environment for 48 hours and subsequently at room temperature for 5 days. All microorganism strains were grown and maintained on Trypticase Soy Agar (TSA) (Oxoid, England) slants for further use.

To prepare bacterial cell suspensions for antibacterial activity assays, each overnight culture was subcultured on Mueller Hinton Agar (MHA) (Oxoid, England) and incubated for 24 hours at 37°C in 5% CO₂ for *S. mutans* and *S. salivarius*, while *C. albicans* was incubated at 30°C in an aerobic environment. The inoculum size of each bacterial species (10⁸ cells/mL) was standardised using a spectrophotometer (Metertek SP-830, Taiwan). The turbidity of the bacterial suspension was adjusted to an optical density to 0.08 - 0.1 at 625 nm, which is equivalent of 0.5 McFarland unit (equal to 1 × 10⁸ colony-forming unit per mL; CFU/mL) and further dilute the culture 1:100 in MHB to achieve a final inoculum concentration of 1 × 10⁶ CFU/mL.

Determination of MIC and MBC values

The minimum inhibitory concentration (MIC) of the

herbal toothpastes against the tested oral microorganisms was determined using a modified broth microdilution method in 96-well microtiter plates (26), where sterile normal saline (0.85% NaCl) was used to prepare the serial 2-fold dilution to ensure proper dissolution of active compounds in each solution. First, 50 µL of sterile normal saline (0.85% NaCl) was pipetted into the first well until the 10th well. Next, 50 µL of the herbal toothpaste, prepared at a stock concentration of 50 mg/mL, was added to the first well. This mixture was serially diluted two-fold across wells 1 to 10, followed by discarding 50 µL of the mixed solution from the last well. After dilution, 50 µL of a standardised oral microorganism suspension was added to each well, resulting in a final bacterial or fungal concentration of 5 × 10⁵ CFU/mL. The total volume per well was 100 µL, and the final concentrations of the toothpaste ranged from 12.50 mg/mL to 0.02 mg/mL. Each toothpaste was tested in triplicate, and the entire experiment was repeated two times for accuracy.

For controls, herbal toothpaste in sterile normal saline (0.85% NaCl) was used as a negative control to ensure medium sterility, while fungal or bacterial suspensions in normal saline served as positive microbial growth. The microtiter plates were incubated for 24 and 48 hours at 37°C in 5% CO₂ for *S. mutans* and *S. salivarius*, and at 30°C in an aerobic environment for *C. albicans*. Turbidity or sedimentation after incubation indicated microorganism growth.

The MIC was defined as the lowest concentration of the herbal toothpaste that prevented visible bacterial growth after incubation. To determine the minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC), samples from the wells showing no cloudiness were transferred to sterile agar plates. The MBC or MFC was recorded as the lowest concentration which resulted in no visible bacterial or fungal growth on the agar plate. If bacteria or fungi were still present on the agar plates, the result was recorded as “no bactericidal effect at tested concentrations” (NB).

Statistical analysis

The assay was done in triplicate experiments and was repeated two times independently (N=6) and expressed as a median (IQR) of two biological replicates experiments.

RESULTS

Types of Herbal Toothpastes

All the toothpastes used in this research were simultaneously purchased from various local supermarkets in Kuala Lumpur. Colgate Total®, which contains 0.3% triclosan, served as a positive control for comparing the antimicrobial activity of herbal

toothpastes. The composition of herbal toothpastes used in this study either contained a single herbal extract or a blend of multiple extracts. Najwa included miswak (*Salvadora Persica*) and MorningKiss contained tea tree oil, each featuring a single herbal extract. The other herbal toothpastes had a combination of herbal extracts. Neem toothpaste included carrageenan, neem extract, spearmint, and tea tree oil. Halagel® had neem, peppermint oil, and clove oil, while Herbalfresh contained carrageenan, chamomile, herbal mint, and peppermint. Oralgard comprised chamomile, myrrh, peppermint oil, propolis, and sage oil. Himalaya toothpaste contained *Acacia arabica*, *Embelia ribes*, neem extract, and *Vitex negundo*. Manufacturers made various claims about these herbal toothpastes, such as removing bad breath (Herbalfresh, Oralgard, MorningKiss), providing natural antiseptic benefits (MorningKiss), and protecting gums (Neem, Oralgard, MorningKiss, Himalaya), as summarised in Table 1.

Table 1: Types of toothpastes tested, and ingredients found in Malaysia

Tooth-paste	Batch no.	Manu-facturer	Ingredients as listed on packages	Claims
Colgate Total®	101971-01	Colgate Palmolive, Thailand	Sodium Fluoride (1450 ppm F), Triclosan 0.3% w/w, Hydrated Silica, water, Glycerin, Sorbitol, PVM/Ma Copolymer, Sodium Lauryl Sulfate, flavor, Cellulose Gum, Sodium Hydroxide, Propylene Glycol, Carrageenan, Sodium Saccharin,	12-hour anti-bacterial protection
Neem	T07/09/B38	Henkel India, India	Calcium carbonate, water, Glycerin, Sodium Lauryl Sulphate, Spearmint, Neem extract, Carrageenan, Sodium Saccharine, Sodium Benzoate, CI 19140, CI 42090, Tea Tree Oil, Fluoride 1000pm	Protect teeth & gums
Halagel®	MAL0404-00001K	Halagel (M) Sdn Bhd, Malaysia	Calcium Carbonate, Sorbitol, water, Sodium Lauryl Sulfate, Sodium Carboxymethyl, Cellulose, Glycerin, Sodium Chloride, Peppermint oil, Neem extract, Clove oil, Menthol, Silica, Sodium Saccharin, Benzalkonium chloride, Sodium Benzoate, CI 19140, CI 42090	Fresh breath, shiny teeth
Herbal-fresh	241107	Indonesia	Calcium Carbonate, Water, Sorbitol, Silica, Sodium Lauryl Sulfate, Herbalmint, Carrageenan, Sodium Monofluoro-phosphate, Sodium Saccharin, Methyl Paraben, Chamomile extract, Peppermint.	Brighter teeth, removes bad breath

CONTINUE

Table 1: Types of toothpastes tested, and ingredients found in Malaysia (CONT.)

Tooth-paste	Batch no.	Manu-facturer	Ingredients as listed on packages	Claims
Najwa	1396087	India	Calcium Carbonate, Sorbitol, Silica, Miswak (<i>Salvadora Persica</i>)	Maintain healthy gums
Oralgard	598005	Thailand	Propolis extract, Sage oil, Chamomile extract, Myrrh extract, Peppermint oil, Optamint	Protects enamel, deodorizes bad breath, protects gums & teeth
Morning-Kiss	9120002	Yee Lee Trading, Malaysia	Sodium Monofluorophosphate, Dicalcium Phosphate, Sorbitol, Water, Sodium Lauryl Sulfate, Xanthan gum, Sodium Benzoate, Sodium Saccharin, Tea Tree oil, Allantoin, Titanium Dioxide, Panthenol	Natural antiseptic, natural gum protector, natural breath fresher
Himalaya	I109016S	The Himalaya Drug Company Makali, Bangalore	Pomegranate (<i>Punica granatum</i>), Toothache tree, False Black Pepper (<i>Embelia ribes</i>), five-leaved Chaste Tree (<i>Vitex negundo</i>), Indian Gum-Arabic Tree (<i>Acacia arabica</i>), Triphala, Fluorspar, Neem (<i>Azadirachta indica</i>), Thymol, Aqua, Glycerin, Hydrated Silica, Menthol, Saccharin, Silica, Sodium Benzoate, Sodium Lauryl Sulfate, Sodium Sulfite, Titanium dioxide, Xanthan gum.	Maintains healthy gums and strengthens it

MIC Determination of Herbal Toothpastes

The minimum inhibitory concentration (MIC) values of the herbal toothpastes against all tested oral microorganisms after 24 hours of incubation are presented in Fig. 1. The lowest MIC values correspond to the highest bactericidal effects. Colgate Total® demonstrated superior inhibitory effects, particularly against *C. albicans*, with a MIC of 0.781 mg/mL, followed by *S. salivarius* and *S. mutans* with a MIC value of 1.56 mg/mL each.

Halagel, Herbalfresh and Himalaya showed comparable inhibition to Colgate Total® against *C. albicans* (MIC of 0.781 mg/mL), but their efficacies against *S. salivarius* and *S. mutans* were lower, with MIC values of 3.125, 3.125, and 6.25 mg/mL, respectively (Fig. 1). MorningKiss exhibited a lower inhibitory effect on *C. albicans* (MIC of 3.125 mg/mL) but matched Colgate Total®’s effectiveness against *S. salivarius* and *S. mutans* (MIC of 1.56 mg/mL). Neem, Najwa, and Oralgard had MIC values of 1.56 mg/mL against *C. albicans*, indicating lower inhibitory effects compared to Colgate Total®, yet they displayed similar inhibitory effects against *S. salivarius* (MIC of 1.56 mg/mL) but lower against *S. mutans* (MIC of 3.125 mg/mL).

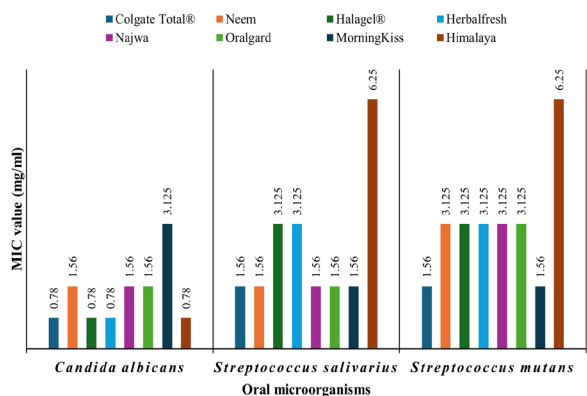


Figure 1: MIC values of herbal toothpastes against *Candida albicans*, *Streptococcus salivarius* and *Streptococcus mutans* at 24 hours of incubation. Note: The lowest MIC values indicate the highest inhibitory effect.

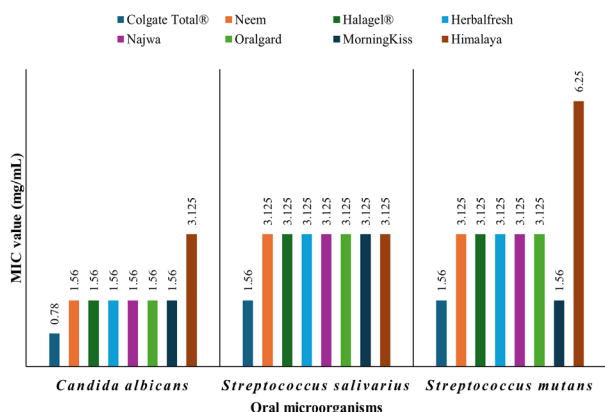


Figure 2: MIC values of herbal toothpastes against *Candida albicans*, *Streptococcus salivarius* and *Streptococcus mutans* at 48 hours of incubation. Note: The lowest MIC values indicate the highest inhibitory effect.

After 48 hours of incubation, Colgate Total® retained its inhibitory effects against *C. albicans*, *S. salivarius*, and *S. mutans* with consistent MIC values of 0.781, 1.56, and 1.56 mg/mL, respectively. Neem, Najwa, and Oralgard also sustained their inhibitory effects against *C. albicans* with MIC values of 1.56 mg/mL (Fig. 2). Halagel®, Herbalfresh, MorningKiss, and Himalaya showed increased MIC values to 1.56, 1.56, 1.56, and 3.125 mg/mL, respectively, against *C. albicans*, indicating a reduction in inhibitory effects compared to the 24-hour results, except for MorningKiss. Most herbal toothpastes exhibited decreased inhibitory effects against *S. salivarius*, except for Halagel®, Herbalfresh, and Himalaya, which maintained or increased their efficiency. The inhibitory effects against *S. mutans* varied, where Halagel®, Najwa, and Oralgard maintained their effects, while Neem, Herbalfresh, and Himalaya showed increased inhibitory effects with lower MIC values, and MorningKiss displayed reduced efficacy after 48 hours (Fig. 2).

MBC Determination of Herbal Toothpastes

The minimum bactericidal concentration (MBC) values of herbal toothpastes against *C. albicans*, *S. salivarius* and *S. mutans* with a final inoculum of 10^5 cells/mL after 24 hours of incubation are shown in Fig. 3.

The lowest MBC or MFC values indicate the highest bactericidal or fungicidal (or microbial killing effects), typically occurring at or 2-3 times higher than the MIC values. Colgate Total® exhibited the highest bactericidal and/or fungicidal effects against *S. salivarius*, followed by *C. albicans* and *S. mutans*, with MBC values of 3.125, 6.25, and 6.25 mg/mL, respectively. Halagel®, Herbalfresh, and Himalaya showed the same MIC and MBC values for *C. albicans* (0.781 mg/mL), indicating they could inhibit and kill the fungus at this concentration. Similarly, Neem and MorningKiss had MBC values of 1.56 and 3.125 mg/mL, respectively, while Oralgard showed no bactericidal effect at tested concentrations (NB) result. The NB results for Herbalfresh, MorningKiss, and Himalaya against *S. salivarius* and *S. mutans* indicated that the bacteria could still grow at the highest concentration of these herbal toothpastes. This was also observed for Neem and Halagel® against *S. salivarius* and Oralgard against *C. albicans*.

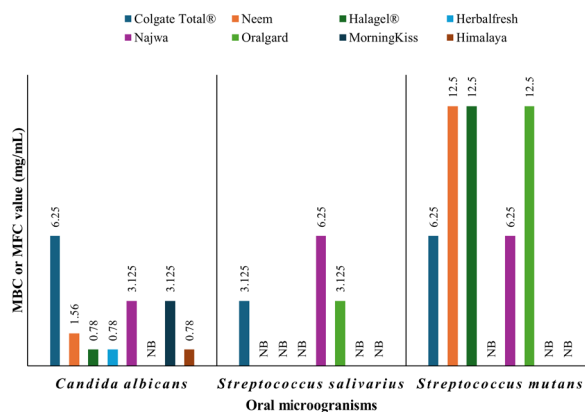


Figure 3: MFC and MBC values of herbal toothpastes against *Candida albicans*, *Streptococcus salivarius* and *Streptococcus mutans* at 24 hours of incubation. Note: The lowest MFC or MBC value indicates the highest bactericidal effect. NB = no bactericidal effect at tested concentrations indicates microbial growth.

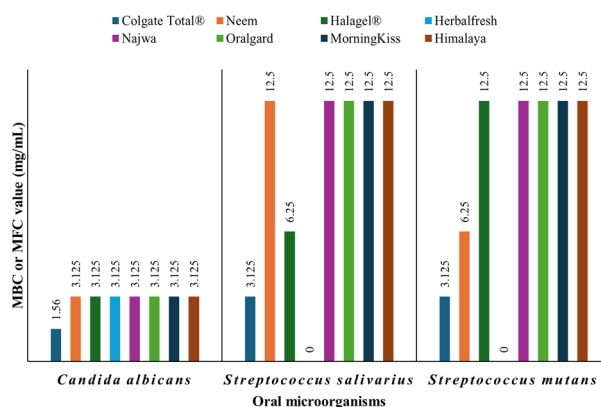


Figure 4: MFC and MBC values of herbal toothpastes against *Candida albicans*, *Streptococcus salivarius* and *Streptococcus mutans* at 48 hours of incubation. Note: The lowest MFC or MBC value indicates the highest bactericidal effect. NB = no bactericidal effect at tested concentrations indicates microbial growth.

After 48 hours of incubation, Colgate Total®'s fungicidal or bactericidal or killing effect increased against *C. albicans*, *S. salivarius*, and *S. mutans* (Fig. 4) compared to 24 hours. Most herbal toothpastes exhibited increased MBC values, indicating a decreased fungicidal effect

against *C. albicans*, except for Najwa, Oralgard, and MorningKiss. Najwa and MorningKiss maintained their fungicidal effects, while Oralgard showed an increased fungicidal effect. The bactericidal effect of most herbal toothpastes against *S. salivarius* and *S. mutans* also increased, except for Herbalfresh, Najwa, and Oralgard. Herbalfresh showed no bactericidal (NB) effect, while Najwa's bactericidal effect decreased against both *S. salivarius* and *S. mutans*. Oralgard exhibited decreased bactericidal effects against *S. salivarius* but sustained its effect against *S. mutans* (Fig. 4).

DISCUSSION

Herbal toothpastes, widely available on the market, have become popular because many consumers prefer them over modern toothpastes, which are perceived to contain potentially harmful chemicals and synthetic compounds. Herbal toothpastes are often marketed for their natural antiseptic properties, protection for gums and teeth, and ability to freshen breath naturally, which has been influenced by consumer preferences towards these products.

There has been limited research on herbal toothpastes compared to herbal extracts, and such studies have not been widely published. An *in vitro* study by Palekar et al. found varying antimicrobial activities of herbal toothpastes against *S. mutans*, *C. albicans*, *P. gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Tannerella forsythia*. They found that all the herbal toothpastes demonstrated antimicrobial activity against all tested microorganisms except for toothpaste C, to which *A. actinomycetemcomitans* and *T. forsythia* were resistant (32).

In a study by Lee et al., the antimicrobial potential of 14 herbal toothpastes was assessed using a disc diffusion method on bacteria, including *S. mutans*, *S. sanguis*, *A. viscosus*, and *C. albicans*. They found that most antimicrobial activity was observed at 24 hours, with diminished effects at 48 hours. Notably, four toothpastes did not produce any inhibition zones even at full strength, while others showed varying levels of inhibition, either at full strength or at a 1:1 dilution (33).

However, our study used the broth microdilution method, considered reliable and precise, and the antimicrobial activities of the tested toothpastes were observed at both 24 and 48 hours. This method allowed for the determination of the minimum bactericidal concentration (MBC) following the minimum inhibitory concentration (MIC) assessment. Although standard 96-well microtiter plates typically accommodate up to 400 μL per well, with 200 μL commonly used for antimicrobial testing, the 100 μL volume used in this study was sufficient and remained stable during the 48-hour incubation at 37 °C. The use of enrichment media, Mueller-Hinton broth, formulated according to NCCLS

guidelines, also provided optimal conditions for the microbial growth (34).

Our results indicated that Colgate Total® exhibited higher inhibitory effects against *C. albicans*, *S. salivarius*, and *S. mutans* at 24 and 48 hours, with consistent findings attributed to the presence of triclosan, a well-known antimicrobial agent. Triclosan has been used in skincare products, surgical scrubs, hand washes, and dental hygiene products for over 30 years. Previous *in vivo* studies demonstrated that triclosan dentifrice significantly reduced oral anaerobes compared to fluoride dentifrice (35, 36). Further evidence of triclosan's strong antimicrobial properties was seen in its effectiveness against *Candida* species. Although Colgate Total containing triclosan and fluoride have promising antimicrobial effect, it had several side effects to the host such as hormone disruption, reproductive health especially for pregnant women, antibiotic resistance, skin irritation, environmental impact, potential carcinogenic effects, and disruption of the microbiome, while fluoride affects problems with bones, teeth, and neurological development (37, 38). Thus, herbal toothpaste is a best choice to avoid all the side effects. A study reported that other toothpastes containing herbal compounds, non-fluoridated formulations, and clove oil also showed significant inhibitory zones, particularly against *C. albicans* and *C. tropicalis* (39).

In this study, Halagel®, Herbalfresh, and Himalaya demonstrated the highest inhibitory effects against *C. albicans* at 24 hours, matching the effectiveness of Colgate Total®. This suggests that these herbal products have similar inhibitory effects on *C. albicans*. The presence of peppermint in Halagel® and Herbalfresh was shown to have a positive impact on *C. albicans*. Among the 30 plant oils tested, peppermint oil was the most effective antifungal activity against *C. albicans*, as indicated by its lowest minimum fungicidal concentration (MFC) value (40). Pomegranate compound present in Himalaya toothpaste and neem compound in both Himalaya and Halagel® toothpastes, contributing to their antimicrobial properties. Endo et al. stated that punicalagin, a compound in Pomegranate peel showed strong activity against *C. albicans* and *C. parapsilosis*. Punicalagin caused a thickened cell wall, changes in the space between the cell wall and the plasma membrane, vacuoles, and a reduction in cytoplasmic content which subsequently caused irregular budding patterns and pseudohyphae seen in treated yeasts (41). A crude extract and punicalagin of Pomegranate had greater antifungal activity against *Trichophyton rubrum*, suggesting a good agent for anti-dermatophytosis, a fungal infection of the skin, hair and nails. Pomegranate, which is rich in tannins, demonstrated a fungicidal effect by disrupting cell membrane integrity and inhibiting yeast adherence (42).

Neem twigs are widely used globally as natural chewing

sticks for maintaining dental hygiene (43). Neem, scientifically known as *Azadirachta indica*, exhibits strong antimicrobial properties against microorganisms that cause infectious diseases in the oral cavity, including *C. albicans*. Studies have reported a decrease in the adhesion capacity of yeast cells to composite resin due to neem's inhibitory effects (44). Furthermore, a 7.5% aqueous neem leaf extract effectively inhibits the growth of *E. faecalis*, *S. mutans*, and *C. albicans*. Additionally, the MIC of an ethanolic neem leaf extract was found to be 1.88%, 7.5%, and 3.75% against these dental pathogens, respectively (20). The methanolic extract of neem (*A. indica*) demonstrated significant antimicrobial activity against a polymicrobial dental biofilm comprising *S. mutans*, *E. faecalis*, *S. aureus*, and *C. albicans* on extracted human teeth (21). Some studies suggest that neem may have stronger antibacterial effects compared to extracts from other sources like myrrh, catechu, cinnamon, miswak, clove, ginger, garlic, and turmeric against specific bacteria and dental caries pathogen (46-47). These findings emphasise the potential of neem as a natural and effective solution for maintaining oral health and combating oral microorganisms.

MorningKiss contains tea tree compound as its antimicrobial agent. This herbal toothpaste could inhibit all microorganisms tested even though the inhibitory effects were different among the microorganisms. Cox et al. observed the membrane toxicity of monoterpenes effects on three microorganisms, *Escherichia coli*, *Staphylococcus aureus*, and *C. albicans* (48). A significant inhibition of respiratory oxygen consumption in cultures of all three microorganisms was seen upon exposure to tea tree oil. Meanwhile, the MIC of tea tree for many microorganisms including coagulase-negative staphylococci, *Staphylococcus aureus*, *Streptococcus* spp., Vancomycin-resistant enterococci, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *C. albicans*, *Candida* spp., and *Malassezia furfur* (49).

Neem, Najwa and Oralgard had the same antimicrobial effect as Colgate Total® against *S. salivarius*. Neem toothpaste contained neem and tea tree compounds which had been discussed above in their antimicrobial potential. Meanwhile, Najwa contained miswak compound which had been well-known for its numerous benefits including its antimicrobial activities. In a study by Tasanarong et al., pre-treatment of saliva-conditioned hydroxyapatite with Neem stick extract prior to exposure to bacteria yielded significant reductions in bacterial adhesion (22). The Neem stick extract also inhibited insoluble glucan synthesis with observable Streptococci aggregation visualised under a microscope when the bacterium was incubated with Neem stick extract. These data suggest that Neem stick extract could reduce streptococci's ability to colonise the tooth surface (22).

Oralgard consists of propolis as one of its ingredients, which is bee's wax. Koru et al. studied ethanol extracts

of propolis on nine anaerobic strains to determine the MIC and MBC values. The propolis samples were collected from four regions in Turkey and from Brazil. All anaerobic tested strains were susceptible to propolis samples, and propolis from Kazan-Ankara showed the most effective MIC values (50).

Comparing MIC values determines the relative inhibitory effectiveness of Colgate Total® and herbal toothpastes. Colgate Total® consistently inhibited *C. albicans*, *S. salivarius*, and *S. mutans* at 24 and 48 hours. Neem, Najwa, and Oralgard also consistently inhibited *C. albicans* at both time points. In contrast, Halagel®, Herbalfresh, and Himalaya exhibited stronger inhibitory effects against *C. albicans* at 24 hours compared to 48 hours.

Most herbal toothpastes had better inhibitory effects at 24 hours, except Halagel®, Herbalfresh and Himalaya against *S. salivarius*. This is because Halagel® and Herbalfresh were consistent inhibitory agents against *S. salivarius*, but Himalaya was better inhibited at 48 hours incubation. Consistency in inhibitory effect was seen in Halagel®, Najwa and Oralgard against *S. mutans* but Neem, Herbalfresh, and Himalaya were better as inhibitory agents after 48 hours.

The effectiveness of each herbal toothpaste against different types of tested oral microorganisms depends on active compounds- and microbial-specifics. Table 1 showed that the individual brand of herbal toothpaste used in this study contained either one type or several mixtures of herbal extracts. Najwa and MorningKiss toothpastes contained only one type of herbal extract which is Miswak extract and tea tree oil, respectively. While Neem, Halagel and Herbalfresh toothpastes contained three mixtures of herbal extracts. Oralgard and Himalaya contained six and eight mixtures of respective types of their herbal extracts. Thus, different types of herbal extract will give different types of active compounds, which have different potency against different tested microorganisms. The better inhibitory effects after 48 hours of incubation, as shown by Himalaya toothpaste against *S. mutans* and *S. salivarius* might be due to their active compounds require prolonged contact to effectively penetrate bacterial biofilms. Additionally, extended incubation may lead to nutrient depletion, weakening the biofilm structure and making it more susceptible to the antimicrobial agents in the toothpaste. However, the reduced antifungal effectiveness of Himalaya toothpaste against *C. albicans* after 48 hours - despite initial inhibition at 24 hours might be due adaption to environmental stress by *C. albicans* by formation of mycelium or form protective biofilms reduced efficacy of the active compounds in Himalaya toothpaste.

Comparison of MBC values among herbal toothpastes is about understanding the ability to kill fungus (fungicidal)

and bacteria (bactericidal) at 24 and 48 hours. The fungicidal and bactericidal effects of Colgate Total® were improved after 48 hours against *C. albicans*, *S. salivarius* and *S. mutans*. Most herbal toothpastes exhibited a reduction in fungicidal effects after 48 hours of incubation against *C. albicans*. They are also more effective as bactericidal at 24 hours except for Neem, Halagel, Herbalfresh, MorningKiss and Himalaya. While, Najwa had consistent bactericidal effects at 24 hours against *S. salivarius* and *S. mutans*. Meanwhile, Neem had a better bactericidal effect against *S. mutans* at 48 hours.

The MBC values of herbal toothpastes against *S. salivarius* and *S. mutans* showed that most herbal toothpastes were better as bactericidal agents at 48 hours except Halagel, Herbalfresh, Najwa and Oralgard. Herbalfresh was more consistent with the result even though the result shows no bactericidal effect at tested concentrations (NB). In the meantime, Najwa was a good bactericidal agent at 24 hours compared to the 48 hours result. On the other hand, Oralgard was a better bactericidal agent against *S. salivarius* at 24 hours but consistent against *S. mutans*.

There is no clear increasing or decreasing order of antimicrobial activities of herbal toothpastes based on their inhibitory or bactericidal effects due to different activities or varied results on each tested microorganism. However, certain herbal toothpastes can be suggested if based on inhibitory and bactericidal effects, separately. Based on the inhibitory effect which is MIC values against *C. albicans*, Colgate Total® was the most superior to all herbal toothpastes, but Halagel®, Herbalfresh and Himalaya showed higher inhibition than other herbal toothpastes at 24 hours of incubation.

The MIC test against *S. salivarius* demonstrated that Colgate Total® was the most effective inhibitory agent, maintaining consistent results at both 24 and 48 hours. It was followed in effectiveness by Neem, Najwa, Oralgard, and MorningKiss, based on MIC values at 24 hours. Similarly, in the antimicrobial activity against *S. mutans*, Colgate Total® emerged as the most effective toothpaste, surpassing other herbal toothpaste brands at both 24 and 48 hours. MorningKiss, Neem, and Herbalfresh followed in terms of effectiveness.

Regarding fungicidal or bactericidal effects, Halagel®, Herbalfresh, and Himalaya ranked among the top toothpastes based on MBC values for *Candida albicans* at both 24 and 48 hours. Colgate Total® emerged as the most effective toothpaste against *S. salivarius*, followed by Oralgard. It also proved superior against *S. mutans*, with Neem and Najwa following in effectiveness.

Based on the overall results, Colgate Total® was the most effective toothpaste against *S. salivarius* and *S. mutans*. While Halagel®, Herbalfresh, and Himalaya were more effective against *Candida albicans*. Among

herbal toothpastes, Oralgard is recommended for *S. salivarius* and Neem for *S. mutans*. It is advisable to choose toothpaste based on its bactericidal effect, as it can eliminate pathogenic microorganisms. Thus, the herbal toothpastes like Halagel®, Herbalfresh and Himalaya, Oralgard and Neem are the best choice to be used as alternative antimicrobial agent due to potential side effects of the chemical toothpaste containing fluoride and triclosan, like Colgate Total®.

However, a clear hierarchy of antimicrobial activity based on MIC and MBC values could not be established due to variability in MIC values among the microorganisms tested for each toothpaste and unavailable MBC values for some toothpastes. For example, *C. albicans* was more susceptible to Halagel®, Herbalfresh, and Himalaya, but this was not the case for *S. salivarius* and *S. mutans*. The lack of MBC values for some herbal toothpastes suggests that these products may slow down but not entirely eliminated the microorganisms. Therefore, only tentative recommendations can be made regarding the relative reliability of herbal toothpastes.

This study has several limitations. The concentrations of active phytochemicals in each toothpaste were unknown, preventing accurate quantification of bioactive compounds (e.g., flavonoids, tannins, essential oils). Consequently, it was difficult to establish dose–response relationships or compare efficacy across formulations. Additionally, the *in vitro* setup does not reflect the complex oral environment, including saliva flow, pH variation, mucosal interaction, and microbial diversity. These factors limit the clinical relevance and direct applicability of the findings.

CONCLUSION

Colgate Total® demonstrated the strongest and most consistent antimicrobial activity against *Candida albicans*, *S. salivarius*, and *S. mutans* at both 24 and 48 hours, outperforming all herbal toothpastes tested. Among the herbal formulations, Halagel®, HerbalFresh, and Himalaya were comparably effective against *C. albicans*, while MorningKiss matched Colgate's efficacy against *S. salivarius* and *S. mutans* at 24 hours but showed weaker bactericidal action based on MBC values. Oralgard was most effective against *S. salivarius*, whereas Neem and Najwa performed best against *S. mutans* within the herbal group. Notably, most herbal toothpastes exhibited reduced antimicrobial effects after 48 hours, likely due to phytochemical degradation or microbial adaptation, highlighting the superior time-stable efficacy of Colgate Total®.

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