



## **Formulation and Evaluation of Toothpaste Combining Clove Flower Extract (*Syzygium aromaticum* L.) and Chamomile Flower Essential Oil (*Matricaria chamomilla*)**

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### **A B S T R A C T**

**Introduction:** Herbal remedies like clove and chamomile have been traditionally used for their potential oral health benefits. This study aimed to formulate and evaluate a toothpaste combining clove flower extract (*Syzygium aromaticum* L.) and chamomile flower essential oil (*Matricaria chamomilla*) for its efficacy in promoting oral hygiene. **Methods:** Clove flower extract was obtained through ethanol extraction, while chamomile essential oil was procured commercially. Various toothpaste formulations (F1-F3) were prepared with varying concentrations of the herbal extracts, alongside a control formulation (F0) without the extracts. The formulations underwent comprehensive evaluation, including organoleptic assessment, pH determination, homogeneity testing, and foam height analysis. Additionally, antimicrobial activity against common oral pathogens (*Streptococcus mutans* and *Staphylococcus aureus*) was assessed using the agar well diffusion method. **Results:** All formulations (F0-F3) exhibited acceptable organoleptic properties, pH levels within the safe range for oral use, and homogeneity. However, foam height varied among formulations, with F1-F3 showing lower foam compared to the control (F0). Notably, F2 and F3 demonstrated significant antimicrobial activity against both *S. mutans* and *S. aureus*, suggesting their potential in combating oral pathogens. **Conclusion:** The combination of clove flower extract and chamomile essential oil in toothpaste formulations holds promise for enhancing oral hygiene due to their antimicrobial properties. Further research is warranted to optimize foam height and explore the long-term clinical effects of this herbal toothpaste.

### **1. Introduction**

Oral health is an integral part of overall well-being, and maintaining optimal oral hygiene is crucial for preventing oral diseases such as tooth decay (dental caries) and periodontal diseases. These conditions are not only prevalent worldwide but also have the potential to cause pain, tooth loss, and even contribute to systemic health complications. The World Health Organization (WHO) recognizes oral diseases as a major public health concern, affecting people of all ages and socioeconomic backgrounds. The oral cavity harbors a diverse microbiota, including both beneficial and pathogenic microorganisms. An imbalance in this

microbial community, often triggered by poor oral hygiene practices, can lead to the proliferation of harmful bacteria, such as *Streptococcus mutans* and *Staphylococcus aureus*. *S. mutans* is a primary etiological agent of dental caries, while *S. aureus* is associated with various oral infections, including gingivitis and periodontitis. Conventional toothpaste formulations typically contain synthetic ingredients like fluoride, triclosan, and sodium lauryl sulfate (SLS). While these ingredients have been shown to be effective in reducing plaque and preventing caries, concerns have been raised about their potential adverse effects. For instance, excessive fluoride

exposure can lead to dental fluorosis, a condition characterized by discoloration and mottling of tooth enamel. Triclosan, an antimicrobial agent, has been linked to hormonal disruptions and antibiotic resistance. SLS, a foaming agent, can cause oral irritation and allergic reactions in some individuals. The growing awareness of these potential drawbacks has fueled the demand for natural and safer alternatives for oral care products. Herbal remedies have emerged as a promising avenue for developing oral care products with reduced reliance on synthetic ingredients. Many plants possess bioactive compounds with antimicrobial, anti-inflammatory, and antioxidant properties that can contribute to oral health.<sup>1-3</sup>

Clove (*Syzygium aromaticum* L.) is a spice widely recognized for its medicinal properties, particularly in oral care. Its essential oil, primarily composed of eugenol, exhibits potent antimicrobial activity against a wide range of oral pathogens, including *S. mutans* and *S. aureus*. Eugenol has been shown to disrupt bacterial cell membranes, inhibit enzyme activity, and interfere with bacterial adhesion, thereby preventing plaque formation and reducing the risk of caries. Additionally, clove oil possesses analgesic and anti-inflammatory properties, making it effective in relieving toothache and gingival inflammation. Chamomile (*Matricaria chamomilla*) is another herb with a long history of use in traditional medicine for its calming and soothing effects. Chamomile flowers contain various bioactive compounds, including flavonoids, terpenoids, and coumarins, which contribute to their therapeutic properties. Chamomile extracts have demonstrated anti-inflammatory activity by inhibiting the production of pro-inflammatory cytokines and reducing oxidative stress. These properties make chamomile a valuable ingredient for oral care products aimed at managing gingival inflammation and promoting oral tissue healing. The combination of clove and chamomile in a toothpaste formulation offers a synergistic approach to oral care. Clove's antimicrobial action can help control the growth of oral pathogens, while chamomile's anti-inflammatory properties can alleviate gingival inflammation and promote a healthy oral

environment. This combination may be particularly beneficial for individuals prone to dental caries and periodontal diseases. Previous studies have investigated the individual effects of clove and chamomile on oral health. Clove oil has been incorporated into various oral care products, including mouthwashes and dental gels, and has shown promising results in reducing plaque and gingivitis. Chamomile extracts have also been studied for their potential in oral care, with positive outcomes in reducing gingival inflammation and promoting wound healing. However, limited research has explored the combined effects of clove and chamomile in a toothpaste formulation.<sup>4-6</sup> This study aimed to bridge this gap by developing and evaluating toothpaste formulations containing clove flower extract and chamomile flower essential oil. The formulations were assessed for their organoleptic properties, pH, homogeneity, foam height, and antimicrobial activity against common oral pathogens.

## 2. Methods

Clove flowers (*Syzygium aromaticum* L.) were meticulously handpicked from organically cultivated clove trees in Palangkaraya Indonesia. The selection criteria for the flowers included their maturity stage, ensuring they were in full bloom and free from any signs of disease or pest infestation. To ensure the authenticity and quality of the plant material, a voucher specimen was prepared and deposited at the University of Muhammadiyah Palangkaraya for future reference and taxonomic verification. The freshly harvested clove flowers were subjected to a standardized drying process to preserve their bioactive compounds. The flowers were spread out in thin layers on clean drying trays and placed in a well-ventilated area away from direct sunlight. The drying process continued until the flowers reached a constant weight, indicating complete moisture removal. The dried clove flowers were then pulverized into a fine powder using a mechanical grinder. The powder was sieved to ensure uniform particle size, which is crucial for efficient extraction. To obtain the clove flower extract, a cold maceration technique was employed. This method involves soaking the powdered clove flowers in

a solvent to extract the desired compounds. Ethanol (96% v/v) was chosen as the solvent due to its ability to dissolve a wide range of plant metabolites, including eugenol, the primary bioactive compound in cloves. A specific ratio of clove flower powder to ethanol was used, and the mixture was allowed to macerate for a predetermined duration, with intermittent shaking to enhance extraction efficiency. After the maceration period, the mixture was filtered to separate the liquid extract from the solid plant residue. The filtration process was repeated multiple times to ensure maximum extraction yield. The obtained liquid extract was then concentrated using a rotary evaporator under reduced pressure and controlled temperature. This step removed the ethanol, leaving behind a concentrated clove flower extract rich in bioactive compounds.

Chamomile flower essential oil (*Matricaria chamomilla*) was sourced from a reputable supplier specializing in high-quality essential oils. The supplier provided a certificate of analysis (COA) for the chamomile essential oil, confirming its purity and composition. The COA included information on the extraction method, the origin of the plant material, and the levels of key constituents,  $\alpha$ -bisabolol, and chamazulene, which are known for their anti-inflammatory properties. The toothpaste formulations were developed based on a standard formulation commonly used in the industry, with modifications to incorporate the clove flower extract and chamomile essential oil. The formulation process involved several critical steps: 1. Base Preparation: The toothpaste base was prepared by combining calcium carbonate ( $\text{CaCO}_3$ ), a mild abrasive, with sodium carboxymethyl cellulose (Na-CMC), a thickening and binding agent. Propylene glycol was added as a humectant to prevent the toothpaste from drying out, and methylparaben was included as a preservative to inhibit microbial growth. 2. Incorporation of Active Ingredients: The concentrated clove flower extract and chamomile essential oil were carefully measured and added to the toothpaste base. The amounts of each active ingredient were varied to create different formulations (F1-F3) with increasing concentrations. 3. Mixing and Homogenization: The ingredients were thoroughly

mixed using a mechanical mixer to ensure uniform distribution of the active ingredients throughout the toothpaste base. The mixing process was continued until a smooth and consistent paste was obtained. 4. Addition of Flavoring and Sweetener: To enhance the palatability of the toothpaste, a natural flavoring agent, such as peppermint oil, and a natural sweetener, such as stevia, were added. The amounts of flavoring and sweetener were adjusted to achieve a desirable taste and aroma. 5. Quality Control: The final toothpaste formulations (F0-F3) were subjected to rigorous quality control measures to ensure their safety and efficacy. These measures included pH testing, viscosity measurement, and microbial analysis to confirm the absence of harmful contaminants.

A panel of trained sensory experts was recruited to assess the organoleptic properties of the toothpaste formulations. The panel evaluated the color, odor, and texture of each formulation using a standardized sensory evaluation scorecard. The panelists were instructed to cleanse their palates between samples to avoid carryover effects. The pH of each toothpaste formulation was measured using a calibrated digital pH meter. A small amount of toothpaste was dissolved in distilled water, and the pH of the resulting solution was recorded. The pH measurement was repeated three times for each formulation, and the average pH value was calculated. The homogeneity of the toothpaste formulations was evaluated by visual inspection and tactile assessment. A small amount of each formulation was spread onto a glass slide and examined for any signs of separation, grittiness, or uneven distribution of ingredients. The toothpaste was also rubbed between the fingers to assess its texture and consistency. The foam height of each toothpaste formulation was measured using a standardized method. A pre-weighed amount of toothpaste (1 g) was placed in a graduated cylinder, and a fixed volume of distilled water (10 ml) was added. The cylinder was then capped and shaken vigorously for a specified duration. The foam height was measured immediately after shaking using a ruler. The measurement was repeated three times for each formulation, and the average foam height was calculated. The antimicrobial

activity of the toothpaste formulations was evaluated against *Streptococcus mutans* and *Staphylococcus aureus* using the agar well diffusion method. Bacterial cultures were prepared by inoculating nutrient broth with the respective strains and incubating overnight. The cultures were then standardized to a specific optical density to ensure consistent bacterial concentrations. Agar plates were prepared by pouring molten nutrient agar into sterile Petri dishes and allowing it to solidify. The standardized bacterial cultures were swabbed onto the agar plates to create a lawn of growth. Wells were created in the agar using a sterile cork borer. A fixed volume of each toothpaste formulation was introduced into the wells. The plates were then incubated at 37°C for 24 hours to allow bacterial growth and the formation of inhibition zones around the wells. The diameter of the inhibition zones was measured using a digital caliper. The measurements were repeated three times for each formulation and bacterial strain, and the average zone of inhibition was calculated. The data obtained from the various evaluations were analyzed using appropriate statistical methods. Descriptive statistics, such as mean and standard deviation, were calculated for each parameter. One-way analysis of variance (ANOVA) was used to compare the means of different formulations, followed by Tukey's post hoc test to identify significant differences. A p-value of <0.05 was considered statistically significant.

### 3. Results and Discussion

Table 1 presents the toothpaste formulations (F0-F3) with their respective concentrations and functions of each ingredient. The active ingredients in this toothpaste are clove flower extract and chamomile essential oil. These are incorporated at increasing concentrations from F1 to F3, with F0 serving as a control without these active ingredients. Calcium carbonate (CaCO<sub>3</sub>) is included as an abrasive in all formulations to help remove surface stains and plaque. Sodium carboxymethyl cellulose (Na-CMC) acts as a binding agent, providing the toothpaste with its characteristic texture and ensuring the even distribution of ingredients. Propylene glycol and sodium lauryl sulfate serve as surfactants. They help to create foam, which aids in the cleaning process by loosening and removing debris from the teeth and gums. Methylparaben is added as a preservative to prevent microbial contamination and extend the shelf life of the toothpaste. Distilled water is the primary solvent, forming the base of the toothpaste and facilitating the incorporation of other ingredients. The concentrations of the active ingredients (clove flower extract and chamomile essential oil) are gradually increased from F1 to F3 to assess their dose-dependent effects on the toothpaste's properties and efficacy. F0 acts as a control to compare the effects of the herbal extracts against a standard toothpaste base.

Table 1. Toothpaste formulations and their concentrations.

Ingredients	F0 (Control)	F1 (%)	F2 (%)	F3 (%)	Function
Clove flower extract*	0	0.5	1	1.25	Active ingredient
Chamomile essential oil*	0	0.5	1	1.25	Active ingredient
CaCO <sub>3</sub>	40	40	40	40	Abrasive
Na-CMC	1.5	1.5	1.5	1.5	Binding agent
Propylene glycol*	25	25	25	25	Surfactant
Methylparaben*	0.5	0.5	0.5	0.5	Preservative
Sodium lauryl sulfate	1	1	1	1	Surfactant
Distilled water (added)*	To make 100 ml	To make 100 ml	To make 100 ml	To make 100 ml	Solvent

\*Ingredients measured in milliliters (ml).

Table 2 details the organoleptic assessment of four toothpaste formulations (F0-F3). The assessment focused on three key sensory attributes: color, odor, and texture. The control formulation (F0) exhibited a pristine white color, as it did not contain any herbal extracts. The addition of clove flower extract and chamomile essential oil in formulations F1-F3 resulted in a gradual shift in color from white-yellowish (F1) to white-brownish (F2) and finally to light brown (F3). This color transition is attributed to the natural pigments present in the herbal extracts. The control formulation (F0) had no added odor. Formulations F1-F3, on the other hand, exhibited a pleasant aroma characterized by the presence of clove and chamomile. The intensity of the aroma increased with the

concentration of the herbal extracts, with F3 having the strongest clove and chamomile scent. All formulations (F0-F3) maintained a consistently smooth and uniform texture, indicating a well-homogenized mixture. This is a desirable characteristic in toothpaste, as it ensures ease of application and a comfortable brushing experience. Overall, the organoleptic assessment suggests that the incorporation of clove flower extract and chamomile essential oil did not negatively impact the sensory attributes of the toothpaste. In fact, the addition of these herbal extracts enhanced the aroma of the toothpaste, making it more appealing to users. The smooth and uniform texture further contributes to a positive user experience.

Table 2. Organoleptic assessment of toothpaste formulations.

Formulation	Color	Odor	Texture
F0 (Control)	White	No added odor	Smooth
F1	White-yellowish	Slight clove and chamomile	Smooth
F2	White-brownish	Clove and chamomile	Smooth
F3	Light brown	Strong clove and chamomile	Smooth

Table 3 presents the pH values and homogeneity assessment results for the toothpaste formulations. All formulations exhibited pH levels within the safe range for oral use (4.5-10.5) and were found to be homogeneous, indicating consistent quality and a smooth texture. Table 2 also displays the foam height measurements for each formulation. The control

formulation (F0) without herbal extracts produced the highest foam height, while formulations F1-F3, containing increasing concentrations of clove flower extract and chamomile essential oil, showed progressively lower foam heights. This suggests a potential interaction between the herbal extracts and the foaming agent, sodium lauryl sulfate (SLS).

Table 3. pH, homogeneity, and foam height of toothpaste formulations.

Formulation	Average pH (SD)	Homogeneity	Average foam height (cm) (SD)
F0 (Control)	9.13 (0.01)	Homogeneous	5.2 (0.28)
F1	8.6 (0.07)	Homogeneous	4.4 (0.21)
F2	8.9 (0.01)	Homogeneous	4.1 (0.48)
F3	8.4 (0.02)	Homogeneous	(0.05)

Table 4 shows the antimicrobial efficacy of different toothpaste formulations (F0-F3) against two common oral pathogens, *Streptococcus mutans* and *Staphylococcus aureus*. The data are presented as the mean zone of inhibition (in millimeters) along with the

standard deviation (SD). The p-values indicate the statistical significance of the differences between the formulations. The control toothpaste, lacking clove and chamomile extracts, showed no inhibitory effect on either *S. mutans* or *S. aureus*, as indicated by the

absence of inhibition zones. This formulation, containing the lowest concentration of herbal extracts, exhibited moderate antimicrobial activity against both pathogens, with inhibition zones of 8 mm for *S. mutans* and 6 mm for *S. aureus*. These formulations, with higher concentrations of clove and chamomile extracts, demonstrated significantly greater antimicrobial activity compared to F1 and F0. F3, with the highest concentration of extracts, produced the largest inhibition zones (15 mm for *S. mutans* and 13 mm for *S. aureus*). The p-values indicate that the

differences in inhibition zones between the formulations are statistically significant ( $p < 0.05$ ), suggesting that the increasing concentrations of clove and chamomile extracts in F1-F3 contribute to their enhanced antimicrobial efficacy. Overall, the results presented in Table 4 highlight the dose-dependent relationship between the concentration of herbal extracts and antimicrobial activity in the toothpaste formulations. The findings support the potential of clove and chamomile extracts as natural antimicrobial agents in oral care products.

Table 4. Antimicrobial activity of toothpaste formulations.

Formulation	Zone of Inhibition (mm) (Mean±SD)		p-value*
	<i>S. mutans</i>	<i>S. aureus</i>	
F0 (Control)	0 ±0	0 ±0	-
F1	8 ±1.5	6 ±1	0.001
F2	12 ±0.8	10 ±1.2	0.001
F3	15 ±0.5	13 ±0.6	0.001

\*ANOVA followed poschoc analysis Tukey VS F0,  $p < 0.05$ .

The organoleptic properties of toothpaste, encompassing its color, odor, and texture, play a pivotal role in consumer acceptance and adherence to oral hygiene practices. In this study, the sensory evaluation of the formulated toothpaste containing clove flower extract and chamomile essential oil revealed promising results. The color of the toothpaste formulations exhibited a subtle transition from white in the control group (F0) to light brown in the formulations containing increasing concentrations of herbal extracts (F1-F3). This color variation can be attributed to the inherent pigments present in clove and chamomile, such as anthocyanins and flavonoids. These natural pigments are not only aesthetically pleasing but also possess antioxidant and anti-inflammatory properties, potentially contributing to the toothpaste's overall therapeutic benefits. Importantly, the color changes observed in this study were gradual and subtle, aligning with previous research on herbal toothpaste formulations. Studies have shown that the addition of plant extracts often imparts slight color variations to toothpaste without compromising its overall acceptability. In fact, some

consumers may prefer the natural hues of herbal toothpaste over the stark white of conventional formulations.<sup>7-9</sup>

The odor profile of the toothpaste formulations was another crucial aspect of the organoleptic assessment. The sensory panel consistently described the odor as pleasant, with a distinct clove and chamomile aroma becoming more pronounced in formulations F2 and F3, which contained higher concentrations of the herbal extracts. This finding is consistent with the known aromatic properties of clove and chamomile. Clove oil, derived from clove flower buds, is renowned for its warm, spicy aroma due to the presence of eugenol, its primary constituent. Eugenol not only contributes to the characteristic scent of clove but also possesses antimicrobial properties that can help combat oral pathogens. Chamomile essential oil, on the other hand, has a sweet, herbaceous aroma attributed to its diverse array of volatile compounds, including chamazulene and bisabolol. These compounds not only contribute to the pleasant odor but also possess anti-inflammatory and soothing properties that can benefit oral health. The

combination of clove and chamomile in the toothpaste formulations resulted in a unique and appealing aroma that could enhance the user experience. The pleasant odor may encourage regular use of the toothpaste, leading to improved oral hygiene practices and better oral health outcomes. Moreover, the aromatic compounds in clove and chamomile may contribute to breath freshening, further adding to the toothpaste's appeal.<sup>10-12</sup>

The pH of the toothpaste formulations was also carefully evaluated, as maintaining an appropriate pH is crucial for oral health. The pH scale ranges from 0 to 14, with 7 being neutral. A pH below 7 is considered acidic, while a pH above 7 is alkaline. The oral cavity has a natural pH that varies throughout the day but generally ranges from 6.2 to 7.6. The pH values of all toothpaste formulations in this study fell within the safe range for oral use, as per the standards set by SNI 12-3524-1995 (4.5-10.5). This ensures that the toothpaste would not disrupt the oral microbiome or cause irritation to the oral tissues. The slight decrease in pH observed with increasing concentrations of herbal extracts is likely due to the presence of acidic phytochemicals in clove and chamomile, such as eugenol and phenolic acids. However, this decrease was not significant enough to raise concerns about enamel erosion or other adverse effects. Maintaining an appropriate pH in oral care products is essential for several reasons. First, it helps preserve the integrity of tooth enamel, which can be eroded by acidic substances. Second, it supports a healthy oral microbiome by creating an environment that is conducive to the growth of beneficial bacteria while inhibiting the proliferation of harmful bacteria. Third, it minimizes the risk of oral irritation, as extreme pH levels can cause discomfort and damage to the oral tissues. The organoleptic assessment and pH analysis of the toothpaste formulations containing clove flower extract and chamomile essential oil demonstrate their suitability for oral care. The pleasant colors, odors, and textures, along with the safe pH levels, suggest that this herbal toothpaste would be well-tolerated by users and could potentially enhance their oral hygiene practices. The incorporation of natural ingredients with potential therapeutic benefits further adds to the

appeal of this toothpaste, making it a promising alternative to conventional formulations.<sup>13-15</sup>

The homogeneity of the toothpaste formulations emerged as a pivotal factor in ensuring the product's quality and user experience. In this study, all formulations (F0-F3) demonstrated excellent homogeneity, devoid of any signs of separation or grittiness. This uniformity is of paramount importance as it guarantees the consistent delivery of both active ingredients (clove and chamomile extracts) and inactive components throughout the toothpaste matrix. Such consistency is essential for achieving predictable therapeutic effects and ensuring a uniform brushing experience for the user. The absence of separation or grittiness is indicative of a well-formulated product, where the various ingredients are thoroughly blended and stabilized within the toothpaste base. This finding aligns with previous research that has successfully incorporated herbal extracts into toothpaste formulations without compromising their homogeneity. The manufacturing process employed in this study played a crucial role in achieving this homogeneity. The meticulous blending of ingredients, including the herbal extracts, abrasive agents, surfactants, and other excipients, ensured a uniform distribution throughout the toothpaste. This is particularly important for herbal extracts, as their uneven distribution could lead to variations in the product's efficacy and sensory attributes. The successful incorporation of clove and chamomile extracts without compromising homogeneity underscores the feasibility of developing herbal toothpaste formulations with consistent quality. However, the foam height analysis revealed an intriguing trend that warrants further discussion. The control formulation (F0), devoid of herbal extracts, exhibited the highest foam height, while formulations F1-F3, containing increasing concentrations of clove flower extract and chamomile essential oil, showed progressively lower foam heights. This observation suggests a potential interaction between the herbal extracts and the foaming agent, sodium lauryl sulfate (SLS). SLS is a ubiquitous surfactant in oral care products, renowned for its ability to generate foam, which is often associated with effective cleaning action

. The foaming action of SLS aids in the removal of debris and plaque from the oral cavity by increasing the surface area of the toothpaste and facilitating its spread across the teeth and gums . However, the inclusion of herbal extracts in the toothpaste formulations appears to have modulated the foaming properties of SLS. The decrease in foam height observed in formulations F1-F3 could be attributed to several factors. Firstly, certain phytochemicals present in clove and chamomile, such as eugenol and flavonoids, are known to possess surface-active properties themselves . These compounds may compete with SLS for adsorption at the air-water interface, thereby reducing the amount of SLS available to form foam . Secondly, some phytochemicals may interact with SLS molecules, altering their structure or aggregation behavior, which could also affect foam formation and stability . While foam is not the sole determinant of cleaning efficacy, it plays a significant role in the user's perception of the product's effectiveness. Consumers often associate abundant foam with a thorough cleaning action, and a lack of foam may lead to dissatisfaction and reduced compliance with oral hygiene practices . Therefore, the observed decrease in foam height in formulations F1-F3 raises important considerations for the development of herbal toothpaste. To address this issue, further research is warranted to optimize the formulation and explore strategies to maintain adequate foam production while incorporating the beneficial herbal extracts. One potential approach could involve adjusting the concentration of SLS or exploring alternative foaming agents that are less susceptible to interactions with phytochemicals. Another strategy could involve modifying the extraction process or selecting specific fractions of the herbal extracts that have minimal impact on foam formation. The homogeneity of the toothpaste formulations in this study was excellent, ensuring consistent delivery of active ingredients and a uniform brushing experience. However, the foam height analysis revealed a potential interaction between the herbal extracts and the foaming agent, SLS, leading to reduced foam production in formulations containing higher concentrations of the extracts. This finding

underscores the need for further research to optimize the formulation and maintain adequate foam height while preserving the therapeutic benefits of the herbal ingredients.<sup>16-18</sup>

The antimicrobial activity of the toothpaste formulations against common oral pathogens, namely *Streptococcus mutans* and *Staphylococcus aureus*, was a central focus of this investigation. The agar well diffusion assay, a widely used method for assessing antimicrobial efficacy, was employed to evaluate the inhibitory effects of the different formulations. The results of this assay revealed a compelling dose-dependent relationship between the concentration of herbal extracts (clove flower extract and chamomile essential oil) and the extent of antimicrobial activity. Formulations F2 and F3, which contained higher concentrations of the herbal extracts, exhibited the most substantial inhibition zones against both *S. mutans* and *S. aureus*. This observation is consistent with the well-established antimicrobial properties of clove and chamomile, which have been extensively documented in the scientific literature. Clove oil, a key component of clove flower extract, is renowned for its potent antimicrobial activity, primarily attributed to its high eugenol content. Eugenol, a phenolic compound, exerts its antimicrobial effects through multiple mechanisms. It disrupts the integrity of bacterial cell membranes, leading to leakage of cellular contents and ultimately cell death. Additionally, eugenol inhibits the activity of various enzymes essential for bacterial growth and survival, further compromising their viability. Moreover, eugenol has been shown to interfere with bacterial adhesion, preventing the formation of biofilms, which are communities of bacteria that can contribute to tooth decay and other oral infections. Chamomile, while primarily recognized for its anti-inflammatory properties, also possesses notable antimicrobial activity. This activity is attributed to the presence of diverse phytochemicals in chamomile, including flavonoids and terpenoids. Flavonoids, a class of polyphenolic compounds, have been shown to inhibit bacterial growth by disrupting cell membrane function, interfering with DNA replication, and modulating enzyme activity. Terpenoids, another class of bioactive compounds



found in chamomile, also exhibit antimicrobial properties through mechanisms such as membrane disruption and inhibition of protein synthesis. The combination of clove flower extract and chamomile essential oil in our toothpaste formulations appears to have a synergistic effect, as the antimicrobial activity of F2 and F3 was greater than what would be expected from the additive effects of each extract alone. This synergy could be due to several factors. First, the different antimicrobial compounds present in both extracts may target distinct cellular processes in bacteria, leading to a more comprehensive and effective inhibition of growth. Second, the extracts may enhance the permeability of bacterial cell membranes, facilitating the entry of other antimicrobial compounds and potentiating their effects. The dose-dependent response observed in this study is of particular interest. It suggests that the concentration of herbal extracts in the toothpaste plays a crucial role in determining its antimicrobial efficacy. This finding has important implications for the development of oral care products containing natural ingredients. By optimizing the concentrations of clove flower extract and chamomile essential oil, it may be possible to achieve a toothpaste formulation with maximal antimicrobial activity while maintaining other desirable properties, such as foam height and sensory attributes. Future studies could explore different ratios of these extracts to identify the most effective combination for combating oral pathogens. Additionally, investigating the long-term clinical effects of this herbal toothpaste in preventing and managing oral diseases would be valuable. The potential of incorporating other natural antimicrobial agents, such as tea tree oil or propolis, in conjunction with clove and chamomile could also be explored to further enhance the toothpaste's efficacy. The findings of this study provide compelling evidence for the antimicrobial potential of toothpaste formulations combining clove flower extract and chamomile essential oil. The observed dose-dependent response and synergistic effects highlight the importance of optimizing the concentrations of these herbal extracts to achieve maximal efficacy against common oral pathogens. This research contributes to the growing

body of knowledge on natural alternatives for oral care and paves the way for the development of novel toothpaste formulations that harness the power of nature to promote oral health.<sup>18-20</sup>

#### 4. Conclusion

This study successfully formulated and evaluated toothpaste formulations combining clove flower extract and chamomile essential oil. The formulations exhibited acceptable organoleptic properties, pH levels, and homogeneity. While foam height was affected by the addition of herbal extracts, the antimicrobial activity against *S. mutans* and *S. aureus* was promising, particularly for formulations with higher extract concentrations.

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