

Evaluating the anti-plaque efficacy of Cymenol mouthwash: A Randomized Controlled Study

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Abstract

Background: Essential Oils (EOs) show strong potential in managing plaque and gingivitis, offering an effective and well-tolerated alternative to chlorohexidine (CHX). Formulations like Listerine, which contain thymol, eucalyptol, menthol, and methyl salicylate, have demonstrated notable plaque-reducing and anti-gingivitis effects with comparatively lower cytotoxicity than CHX. Among Eos, Cymenol (o-cymen-5-ol), a phenolic derivative of isopropyl cresol, exhibits antimicrobial activity primarily by disrupting bacterial cell walls and increasing membrane permeability.

Aim: To assess the antiplaque efficacy of a new mouthwash (GingiLacer) containing Cymenol and Zinc chloride as active ingredients.

Materials and methods: Thirty participants were enrolled in the study, and their baseline plaque and gingival index scores were documented following the application of an alpha two-tone solution on the teeth. All the subjects were allotted into 2 groups: 15 in Group A, which received Cymenol mouthwash and 15 in Group B, which received Listerine mouthwash after undergoing scaling. Participants were instructed to avoid any additional oral hygiene practices. On days 0 and 14, the alpha two-tone solution was applied to the teeth to highlight plaque, after which plaque and gingival indices were recorded. All the values were tabulated, and the data were evaluated using an independent t-test, paired t-test and ANCOVA.

Results: On the last day of study, the area of visible plaque was significantly lower in the Cymenol Group in comparison with Listerine; the Cymenol mouthwash was found to have reduced the area of visible plaque.

Conclusion: The use of Cymenol mouthwash as an anti-plaque agent is a valuable alternative to classical anti-plaque agents.

Keywords: Anti-plaque activity, Cymenol mouthwash, Essential oils, Gingivitis, Listerine.

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1. Introduction

The periodontium, which supports teeth, is made up of the gingiva, cementum, periodontal ligament, and alveolar bone. Age-related alterations, as well as morphologic and functional variables, might affect the periodontium [1]. Periodontal diseases (PD) are highly common across the globe and contribute to substantial health and financial challenges, greatly impacting the quality of life for

individuals who suffer from them [2]. Between 10-15% of the global adult population suffers from progressive periodontitis, which, if left unattended, results in halitosis, pain and loss of teeth. The initial signs of PD include inflammation of the gingiva and periodontium. The main contributing factor to the pathophysiology of periodontal disease is dental plaque. The development and

advancement of periodontal disease are significantly influenced by the accumulation of dental biofilm, plaque, and calculus. Effective oral hygiene and plaque removal are the most important strategies in the prevention of this disease [2,3].

Professional tooth cleaning, oral hygiene instructions, and the mechanical removal of dental biofilm are essential for preventing and managing PD, like gingivitis and periodontitis [4]. However, challenges such as time constraints, limited dexterity, and poor adherence to oral hygiene practices often impede effective plaque control. In such cases, complementary chemical hygiene methods, such as antimicrobial mouthwashes, can provide additional support. Antimicrobial mouthwashes are considered to reduce dental plaque biofilm and thus the potential to prevent plaque-induced oral diseases, particularly periodontal diseases [5]. Using chemical adjuvants in mouthwashes has been shown to strengthen demineralized tooth surfaces and reduce gum inflammation and bleeding in cases of gingivitis. These actions are crucial in managing plaque accumulation and can help stop the disease from developing at an early stage [2].

Essential oils (EOs) are among the most extensively studied active agents for anti-plaque purposes, rich in chemical compounds with antimicrobial, anti-inflammatory, and antioxidant properties. EOs have been found to enhance oral health primarily through their anti-plaque effects. Research comparing chlorhexidine gluconate (CHX) and essential oils indicates that both can be equally effective or, in some cases, EOs may perform better in improving gingival health [2]. Laboratory studies have shown that CHX can be more cytotoxic, rapidly damaging human gingival fibroblasts. Moreover, CHX is commonly linked to adverse effects such as tooth discoloration, dry mouth, and altered taste sensation. Though these side effects are reversible, they can be bothersome during prolonged use. On the other hand, EOs do not appear to share a major drawback associated with another common anti-plaque and anti-gingivitis agent, cetylpyridinium chloride (CPC), which has been linked to the potential development of antimicrobial resistance when used long-term at low concentrations. These considerations position EOs as a strong and safer alternative to both CHX and CPC [2].

Within the EO category, Cymenol (o-cymen-5-ol), a natural phenolic compound derived from isopropyl

cresol, is notable for its potential oral health benefits. Cymenol acts by disrupting the integrity of the bacterial cell wall and membrane, leading to increased permeability and ultimately compromising cell function. Beyond its anti-plaque activity, Cymenol is also effective in reducing xerostomia and controlling oral malodour. Given its efficacy and minimal adverse effects, Cymenol represents a promising alternative to CHX and other agents for improving oral health [2].

The effects of Cymenol on dental plaque levels have been evaluated through plaque and gingival indices, demonstrating its potential as a favourable option in the management of oral health. Listerine antiseptic is a topical mouthwash that has demonstrated significant effectiveness in reducing pre-existing dental plaque and preventing the formation of new plaque after thorough prophylaxis. It has been proven to notably decrease the risk and progression of gingivitis [6]. Containing essential oils such as thymol, eucalyptol, menthol, and methyl salicylate, Listerine provides both antimicrobial and refreshing effects, making it a popular choice in daily oral care routines to control bacterial plaque [6]. In contrast, Chlorhexidine (CHX), a chemical antiseptic, is frequently employed in clinical settings, but prolonged use has been linked to cellular damage. Studies have also found that Chlorhexidine mouthwash results in a greater increase in micronuclei and micronucleated cells compared to Listerine, suggesting that CHX may have a higher cytotoxic potential [7]. Therefore, the present study aimed to evaluate the anti-plaque efficacy of Cymenol mouthwash in comparison with Listerine and Chlorhexidine, specifically assessing its ability to reduce dental plaque and gingival inflammation.

2. Materials and methods

This study was approved by the Institutional Ethical Committee (Ref No.: IEC/LIDS/2025) of Lenora Institute of Dental Sciences, Rajanagaram, Rajahmundry, Andhra Pradesh, India, and the study was registered in the Clinical Trials Registry of India (Reference ID: CTRI/2025/07/090355).

2.1 Inclusion and exclusion criteria

Systemically healthy adults aged between 20 and 45 years with good oral health, i.e., a minimum of 24 permanent teeth, with no evidence of periodontitis (bleeding on probing < 10%), clinically healthy patients with gingival inflammation, and teeth free of caries or restored

teeth were included in the study. Patients who had undergone scaling/or used antibiotics in the last 3 months, undergoing any other dental treatment, or patients with periodontitis and those with a habit of smoking or former smokers were excluded.

2.2 Sample size

Sample size estimation was performed using G*Power v3.1 software. A sensitivity analysis for a two-sample t-test (two-tailed, $\alpha = 0.05$, power = 80%) was conducted to determine the minimum detectable effect size with the selected sample size. For a total of 30 participants (15 per group), the analysis showed that the study could detect an effect size of Cohen's $d = 1.06$. Therefore, the statistical power is set at 80%, and the significance threshold at 0.05, ensuring sufficient sensitivity to detect clinically meaningful differences between groups.

2.3 Methodology

A total of thirty subjects after meeting the inclusion and exclusion criteria were selected and were randomly allocated into two groups; Group A (test group; $n=15$) received Cymenol mouthwash, which corresponded to the product GingiLacer (0.1% Cymenol, 0.1% zinc chloride, potassium glycyrrhizate, and fluoride salts), and Group B (control group; $n=15$) received Listerine mouthwash (0.064% thymol, 0.092% eucalyptol, 0.042% menthol, and 0.06% methyl salicylate, batch no: BKM5003). Informed consent was obtained from the participants before the start of the study. The study was carried out for a period of 14 days from date: 03-07-2025 to 26-07-2025.

Before the start of the procedure, all the teeth of the participants were stained with alpha plaque two-tone solution and plaque index (Silness and Loe, 1964) [8] and gingival index scores (Loe and Silness, 1963) [8] were recorded using the UNC-15 probe; all the findings were recorded into an Excel sheet by the study investigator. The subjects participating in the study underwent oral prophylaxis (ultrasound scaling) in the department clinic itself. The study was designed as a single-blinded clinical trial in which the participants were blinded to the intervention. To ensure blinding, all mouthwash solutions were dispensed in identical opaque bottles that concealed the colour and appearance of the contents.

Participants were instructed to use the mouthwash as per protocol without knowledge of the specific formulation they received. The opaque bottles prevented participants from visually distinguishing

between the test and control mouthwash, thereby minimizing bias related to product identification. Only the study coordinator responsible for dispensing the mouthwash was aware of the group assignments, while the participants remained unaware throughout the study duration.

Then the participants in group A (test group) were advised to use Cymenol mouthwash, and participants in group B (control group) were advised to use Listerine mouthwash. Each mouthwash (Cymenol and Listerine) was provided to participants in an opaque bottle with instructions about the necessary volume. The participants are instructed to rinse 10 ml of mouthwash for 60 s, two times a day. Apart from brushing, participants were instructed not to use any other oral hygiene measures like flossers, interdental cleaners, or oral irrigators. On day 14 of the study, an alpha two-tone solution was applied to teeth for staining dental plaque, and plaque was assessed using plaque index [8] and gingival index [8].

2.4 Statistical analysis

The data collected from the study were compiled, tabulated, and analyzed statistically by Paired t-test, Independent t-test and ANCOVA. Data were analyzed using IBM SPSS Statistics (Version 20, IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean \pm standard deviation (SD).

Normality of continuous variables was assessed using the Shapiro–Wilk test and visual inspection of Q–Q plots. Homogeneity of variances was evaluated with Levene's test. Between-group comparisons at 2 weeks were performed using analysis of covariance (ANCOVA), with baseline values entered as covariates. Adjusted mean differences (AMD) between groups were reported with 95% confidence intervals (CIs) and corresponding p-values. Effect sizes were calculated as partial eta squared (η^2) from the ANCOVA model, and standardized effect sizes (Cohen's d_{adj}) were estimated as the adjusted mean difference divided by the root mean square error of the model. All analyses were conducted under the intention-to-treat (ITT) principle; per-protocol sensitivity analyses yielded consistent results. Statistical significance was set at $\alpha = 0.05$ (two-tailed).

3. Results

Table 1 depicts the intra and inter-group comparison of mean Plaque index scores. At

baseline, the mean PI scores for Groups A and B were 1.64 and 1.55, respectively, with no statistically significant difference between the groups ($p = 0.55$). However, at 2 weeks of follow up there was a decrease in mean plaque index scores, and the difference observed was statistically significant. ($p \leq 0.001$).

Table 2 depicts the intra and inter-group comparison of mean Gingival index scores. At baseline, the mean GI scores for Groups A and B were 1.26 and 1.27, respectively, with no statistically significant difference between the groups ($p = 0.951$). However, at 2 weeks of follow up there was a decrease in mean gingival index scores, and the difference observed was statistically significant. ($p \leq 0.001$).

The primary outcome was pre-specified as Plaque Index (PI) at 2 weeks (day 14), adjusted for baseline PI. The secondary outcome was the Gingival Index (GI) at 2 weeks, adjusted for baseline GI. After adjusting for baseline values, both Plaque Index (PI) and Gingival Index (GI) at 2 weeks were significantly lower in the Cymenol group compared with the Listerine group. For PI, the adjusted mean difference was -0.58 (95% CI -0.81 to -0.36 ; $p < 0.001$), with a large effect size ($d_{adj} = -1.98$; partial $\eta^2 = 0.52$), indicating that Cymenol produced a significant reduction in

plaque levels relative to Listerine. Similarly, for GI, the adjusted mean difference was -0.54 (95% CI -0.72 to -0.37 ; $p < 0.001$), also with a very large effect size ($d_{adj} = -2.31$; partial $\eta^2 = 0.60$), showing greater improvement in gingival health in the Cymenol group (Tables 3 and 4).

Table 1. Comparison of Plaque index (PI) scores between the study groups at baseline and 2-weeks follow-up.

PI	Group A (Mean \pm SD)	Group B (Mean \pm SD)	p-value	t-value (df)
Baseline	1.64 \pm 0.47	1.55 \pm 0.35	0.553	0.600 (28)
2-Weeks	0.55 \pm 0.26	1.09 \pm 0.40	$\leq 0.001^*$	-4.426 (28)
p-value	$\leq 0.001^*$ ($t = 9.563$, df = 14)	$\leq 0.001^*$ ($t = 5.955$, df = 14)		

*Statistically significant.
SD: Standard Deviation.

Table 2. Comparison of Gingival index (GI) scores between the study groups at baseline and 2-week follow-up.

GI	Group A (Mean \pm SD)	Group B (Mean \pm SD)	p-value	t-value (df)
Baseline	1.26 \pm 0.34	1.27 \pm 0.42	0.951	-0.062 (28)
2 Weeks	0.42 \pm 0.21	0.97 \pm 0.45	$\leq 0.001^*$	-4.264 (28)
p-value	$\leq 0.001^*$ ($t = 9.856$, df = 14)	$\leq 0.001^*$ ($t = 7.603$, df = 14)		

*Statistically significant.
SD: Standard Deviation.

Table 3. Plaque Index (PI) at baseline and 2 weeks (ANCOVA adjusted for baseline).

Groups	Baseline PI (Mean \pm SD)	Adjusted PI at 2 weeks (95% CI)	AMD (Groups A-B)	95% CI (AMD)	p-value	d_adj	Partial η^2
Cymenol (Group A)	1.64 \pm 0.47	0.53 (0.37-0.69)					
Listerine (Group B)	1.55 \pm 0.35	1.11 (0.96-1.27)	-0.58	-0.81 to -0.36	<0.001	-1.98	0.52

Table 4. Gingival Index (GI) at baseline and 2 weeks (ANCOVA adjusted for baseline)

Groups	Baseline PI (Mean \pm SD)	Adjusted PI at 2 weeks (95% CI)	AMD (Groups A-B)	95% CI (AMD)	p-value	d_adj	Partial η^2
Group A (Cymenol)	1.26 \pm 0.34	0.42 (0.30-0.55)					
Group B (Listerine)	1.27 \pm 0.42	0.97 (0.84-1.09)	-0.54	-0.72 to - 0.37	<0.001	-2.31	0.60

4. Discussion

Chlorhexidine (CHX) has traditionally been considered the gold standard in chemical plaque control due to its broad-spectrum antimicrobial activity and high substantivity, which allows it to remain effective within the oral cavity for extended

durations. However, its clinical utility is significantly constrained by several well-documented adverse effects, including tooth discolouration, dysgeusia, and mucosal irritation, particularly with long-term use. These limitations have prompted considerable interest in identifying alternative agents with comparable antimicrobial efficacy but improved safety and patient acceptability profiles.

Essential oil-based mouthrinses, such as Listerine, have emerged as promising alternatives, demonstrating notable antiplaque and anti-inflammatory properties. Although their clinical efficacy may be marginally lower than that of CHX, several studies have reported comparable improvements in gingival indices. Moreover, essential oils are generally associated with fewer side effects, enhancing their suitability for long-term use in daily oral hygiene regimens. Importantly, *in vitro* studies indicate that CHX exerts a more rapid and pronounced cytotoxic effect on human gingival fibroblasts, a drawback not observed with essential oils.

More recently, natural phenolic compounds such as *o*-cymen-5-ol (cymenol) have gained attention as alternative antimicrobial agents in oral care formulations. Cymenol is believed to act by disrupting bacterial cell walls and increasing membrane permeability. In addition to its antiplaque efficacy, *in vivo* investigations have demonstrated that cymenol can reduce gingival inflammation, alleviate symptoms of xerostomia, decrease oral malodour, and provide substantivity for up to four hours post-application.

Furthermore, recent formulations combining *o*-cymen-5-ol with zinc salts have shown enhanced clinical performance, with reported reductions in plaque accumulation, gingival bleeding, and halitosis. Notably, *o*-cymen-5-ol has demonstrated superior biofilm penetration compared to agents such as triclosan and cetylpyridinium chloride, potentially contributing to its enhanced antimicrobial action and clinical effectiveness.

The present study is a single-centre, parallel-design, single-blinded, simple randomized, controlled, clinical study that evaluated the antiplaque effectiveness of Cymenol mouthwash to maintain gingival health when used as a part of an oral hygiene regimen compared to Listerine mouthwash. In this study, the participants were blinded and were given mouthwashes in an opaque bottle. The study was carried out among individuals exhibiting mild to moderate gingivitis, representative of the broader general population.

The findings of this study demonstrate that combining professional dental cleaning with oral hygiene education significantly reduced gingival inflammation. The study design also facilitated the evaluation of the test product's ability to maintain the improved gingival health throughout the trial period.

At baseline, the mean PI scores for Groups A and B were 1.64 and 1.55, respectively, with no statistically significant difference between the groups ($p = 0.55$). However, at the 2-week follow-up, a decrease in the mean plaque index scores was observed in both groups with a statistically significant difference ($p \leq 0.001$), but Group A had a significantly greater reduction than Group B ($p \leq 0.001$), indicating superior plaque control. At baseline, no significant difference was observed in GI between Group A and B ($p=0.951$). Both groups showed significant GI reduction at 2 weeks ($p \leq 0.001$), with Group A showing a significantly greater reduction than Group B ($p \leq 0.001$), indicating better gingival health in Group A.

Ashish Kakar *et al.* (2011) [9] observed that the use of Cymenol as an ingredient in dentifrice resulted in a reduction in plaque accumulation on tooth surfaces from baseline to 2 weeks. However, their study reported a less pronounced anti-plaque effect of Cymenol mouthwash when compared to the outcomes observed in the present study. Similar studies conducted by Albert-Kiszely A *et al.* (2007) [10] and Teng F *et al.* (2016) [11], have demonstrated that in patients with gingivitis, the test rinse (essential oil) significantly reduced the re-accumulation of supragingival plaque by depleting the abundant and prevalent members of the supragingival plaque microbiome following dental prophylaxis.

In addition to its role in minimizing plaque buildup and gingival inflammation, the *o*-cymen-5-ol/zinc chloride formulation proved more effective than the sodium fluoride control in lowering and regulating levels of hydrogen sulfide and methyl mercaptan over 12 hours. This finding was reported in a study by Payne *et al.* (2011) [12]. In a study by Kato T *et al.* (1990) [13], Listerine demonstrated strong bactericidal activity against microorganisms found in both saliva and dental plaque. Conversely, Brex M *et al.* (1990) [14] reported minimal or no antibacterial effect in live subjects. Despite these differing results, multiple experimental gingivitis studies have shown that Listerine can notably suppress plaque formation and, in some cases, reduce existing plaque deposits. According to research by Grossman E *et al.* (1989) [15], and Axelsson P *et al.* (1987) [16], Listerine demonstrated a retardation of plaque accumulation primarily when used as a supplement to regular tooth-cleaning measures over extended periods. This suggests that Listerine may be particularly effective against young, sparse plaque.

Additionally, as documented in the study by Netuschil L *et al.* (1995) [17], plaque regrowth in the Listerine group was inhibited for up to 2 days, though no statistical difference from the control group was observed by day 3. Pascual J *et al.* (2023) [18] reported that a mouthwash containing a blend of o-cymen-5-ol and zinc salts effectively inhibited or slowed the growth of specific common oral microorganisms in a selective manner. The reduction in the relative abundance of microbes is primarily attributed to the inhibitory effects of the compounds in the mouthwash formulation.

Since the present study demonstrated a reduction in gingival inflammation and antiplaque effectiveness of cymenol mouthwash only over 14 days, further research is needed to assess its effectiveness over a longer duration. This study was affected by certain methodological and design-related limitations that could influence how the findings are interpreted and applied to broader populations. The single-centre nature of the study and the inclusion of a narrowly defined, healthy participant group may reduce the external validity of the results. Additionally, the sample size was very limited, which may restrict the statistical power and generalizability of the findings. The evaluation of index values may also be subject to examiner variability, potentially affecting the reliability and consistency of clinical measurements. Furthermore, the lack of microbiological assessments and the absence of post-treatment follow-up limit the ability to evaluate the durability and long-term effects of the intervention.

5. Conclusion

The results showed in favour of O-Cymen-5-ol, a phytochemical, which is an effective antimicrobial agent that promotes good oral health. Apart from that, they are also proven to be effective in reducing plaque accumulation and improving gingival health, so the natural oral health products can be effective in reducing plaque accumulation and improving the gingival health over a period of 14 days, suggesting it may serve as a safer and effective alternative for short-term plaque control.

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