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Effects of Wild Pistachio Fruit Herbal Mouthwash on Gingival Healing Following Clinical Crown Lengthening Surgery

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A B S T R A C T

Objective: Chlorhexidine (CHX) is the gold standard for preventing dental plaque but is associated with undesirable side effects. This preliminary clinical study aimed to evaluate the feasibility of an herbal mouthwash derived from *Pistacia atlantica* fruit at concentrations of 1%, 5%, and 10% as an alternative to CHX, assessing postoperative healing following clinical crown lengthening surgery.

Materials and Methods: Patients were randomly allocated into five groups ($n = 10$ per group): CHX mouthwash (0.2%), herbal mouthwash (1%, 5%, or 10%), or normal saline (NS) placebo. The Modified Gingival Index (MGI) and Plaque Index (PI) were recorded on the day of surgery. After two weeks, assessments included mouthwash taste, parotid gland swelling, tongue discoloration, tooth staining index (SI), PI, and MGI. Data were analyzed using Kruskal–Wallis and Mann–Whitney tests with Bonferroni correction for multiple comparisons (adjusted significance threshold $p < 0.005$). Effect sizes (r) were calculated for significant pairwise comparisons.

Results: The herbal mouthwash showed comparable efficacy to CHX in improving MGI and PI, with no statistically significant difference between the combined herbal groups and CHX ($p = 0.002$, $r = 0.62$). CHX caused significantly greater tooth staining than all herbal concentrations ($p < 0.001$, $r = 0.74$). Taste preference significantly favored the herbal mouthwash over CHX ($p < 0.001$, $r = 0.62$). None of the CHX-associated adverse effects (tooth staining, tongue discoloration, parotid swelling, unpleasant taste) were observed in patients using the herbal mouthwash to the same extent.

Conclusion: Compared to chlorhexidine, the herbal mouthwash exhibited minimal adverse effects, including reduced parotid swelling, tongue and tooth discoloration, and improved taste acceptance. Additionally, it demonstrated comparable efficacy to chlorhexidine in improving gingival and plaque indices, suggesting that it may serve as an effective and well-tolerated alternative to chlorhexidine.

Keywords: Herbal mouthwash; Chlorhexidine; Clinical crown lengthening surgery; Dental plaque

➢ **How to cite this paper**

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Introduction

Successful wound healing requires the prevention of wound infection and bacterial contamination at the surgical site (periodontal or implant-related) and the establishment of a favorable healing environment [1–5]. In the early stages, inflammation predominates during surgical wound healing, which in turn facilitates plaque accumulation [6,7]. Limiting plaque formation accelerates wound healing and reduces postoperative complications, whereas the presence of infectious plaque in surgical areas leads to impaired or even deteriorated clinical outcomes [8]. Bacterial colonization on tooth surfaces plays a critical role in the progression of gingival inflammation and periodontitis [9].

Daily toothbrushing and dental flossing (mechanical plaque control) remain the primary methods for plaque removal. Despite the effectiveness of mechanical control, many patients report discomfort and sensitivity in surgically treated areas; therefore, various adjunctive methods, including chemical plaque control, have been introduced [10].

Chlorhexidine (CHX) mouthwash, due to its potent antimicrobial properties, is currently regarded as the gold standard in chemical plaque control. Chlorhexidine is a bis-biguanide compound with proven efficacy against both Gram-positive and Gram-negative bacteria, fungi, and certain lipophilic viruses. It enhances wound healing and reduces postoperative complications [10–12]. The antimicrobial activity of chlorhexidine is dose-dependent: at low concentrations (0.02–0.06%), it exhibits bacteriostatic effects, whereas at higher concentrations (>0.12%), it demonstrates bactericidal activity [13]. The use of 0.12–0.2% chlorhexidine mouthwashes significantly reduce plaque index, gingival inflammation, and gingival bleeding [14].

However, long-term use of chlorhexidine (longer than two weeks) in dental treatments is frequently associated with tooth, tongue, composite, and glass ionomer discoloration. Additionally, chlorhexidine may cause mucosal irritation, taste disturbances, and rarely unilateral or bilateral parotid gland swelling; these effects are generally transient and resolve after discontinuation of treatment [15–17]. Chlorhexidine gradually darkens upon prolonged exposure to light and air [18]. Its antimicrobial activity is pH-dependent, with an optimal range of 5.5 to 7 [19], and it is reduced in the presence of serum, blood, pus, organic materials, soaps, and other anionic compounds. Furthermore, chlorhexidine is ineffective against acid-fast microorganisms and spores [20].

The American Dental Association has approved chlorhexidine and essential oils as antiseptic agents for use in mouthwashes [21]. Given the growing interest in medicinal plants and natural compounds in the pharmaceutical industry and their favorable biocompatibility there is increasing emphasis on the use of herbal antimicrobial agents [22]. Numerous medicinal plants with anti-inflammatory, antimicrobial, and antioxidant properties have been identified [23]. Herbal products used in toothpastes and mouthwashes may be formulated from single or multiple medicinal plant extracts [24].

The genus *Pistacia* (family *Anacardiaceae*) comprises over 600 species. *Pistacia vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk*, and *P. lentiscus* are among the most well-known species, widely distributed throughout the Mediterranean region and the Middle East [25,26]. Over 40% of global pistachio production originates from Iran [27]. The long history of pistachio cultivation in Iran (3,000–4,000 years) reflects its extensive use in traditional medicine and cuisine. *Pistacia atlantica* (wild pistachio) is the most economically important species found in Iran [28]. Various traditional and industrial applications have been reported for its main components, including resin and fruit. Recent studies have documented a broad range of medicinal properties of *P. atlantica*, such as antimicrobial, antioxidant, antidiabetic, antitumor, and antihyperlipidemic activities [29].

To assist clinicians in selecting the most effective mouthwash for plaque prevention, products must be systematically evaluated, as flavoring and coloring additives may reduce antimicrobial efficacy. Considering the adverse effects associated with postoperative chlorhexidine use, the present study aimed to compare the effects of two herbal mouthwashes containing wild pistachio fruit extract with chlorhexidine on gingival healing following clinical crown lengthening surgery.

While several herbal mouthwashes (e.g., Persica, Terminalia chebula, mint-based formulations) have been compared to chlorhexidine in previous studies, most have focused on chronic gingivitis or routine oral hygiene rather than postoperative healing following periodontal surgery. Furthermore, to our knowledge, no study has specifically evaluated a *Pistacia atlantica* fruit-based mouthwash across three different concentrations (1%, 5%, and 10%) in the context of clinical crown lengthening surgery. The present study therefore provides preliminary data on the concentration-dependent effects of this specific herbal formulation on postoperative outcomes, including plaque control, gingival inflammation, and adverse effects such as tooth staining and taste preference outcomes that are particularly relevant for patient compliance following surgical procedures.

In a study conducted by Alimohammadi et al. in 2020 in Tehran, it was demonstrated that the herbal mouthwash exhibited fewer adverse effects than chlorhexidine with respect to parotid gland swelling, tongue discoloration, and tooth staining, while also showing improved taste acceptability [30-33]. Furthermore, the herbal mouthwash demonstrated effects comparable to chlorhexidine in improving gingival index (GI) and plaque index (PI), suggesting that it may serve as an appropriate alternative to chlorhexidine [34].

In another study carried out by Sadeghi et al. in 2009 in Rafsanjan, the findings indicated that the herbal mouthwashes Persica and Matrica exhibited lower efficacy than chlorhexidine in inhibiting the growth of oral bacteria. However, the authors emphasized that further studies are required to confirm these findings [35].

In a study conducted by Paknejad et al. in 2016 in Tehran, chlorhexidine was found to be more effective than Matrica (herbal mouthwash) in reducing periodontal pocket depth. Nevertheless, Matrica demonstrated superior efficacy in reducing gingival inflammation and the Papillary Bleeding Index (PBI). Consequently, Matrica was suggested as a preferable option for the treatment of gingivitis [36].

The study included both independent and dependent variables. The independent variables comprised age, measured as a continuous quantitative variable using a patient questionnaire, and gender, recorded as a nominal qualitative variable through clinical observation. The dependent variables included the mouthwash taste index, parotid gland swelling index, modified gingival index (MGI), tongue discoloration index, mouthwash taste preference index, plaque index (PI), tooth discoloration severity index, and tooth discoloration extent index. All dependent variables were measured as ordinal qualitative variables. The mouthwash taste index and mouthwash taste preference index were assessed using patient questionnaires, whereas the parotid gland swelling index, tongue discoloration index, tooth discoloration severity index, and tooth discoloration extent index were evaluated through clinical observation. The modified gingival index (MGI) and plaque index (PI) were determined by standardized clinical assessment procedures. This classification ensured an appropriate distinction between participant characteristics and study outcomes while facilitating the application of suitable statistical analyses.

This study aimed to evaluate the efficacy of Pistacia atlantica (wild pistachio) fruit herbal mouthwash on gingival healing following clinical crown lengthening surgery. Its clinical effectiveness and adverse effects were compared with those of 0.2% chlorhexidine and normal saline.

Material and Method

This study was designed as a randomized, single-blind, placebo-controlled clinical trial. After obtaining written informed consent, patients undergoing periodontal surgery at dental clinics in Ilam were evaluated. A total of 50 patients were enrolled and randomly assigned to five groups (n = 10 per group).

The wild pistachio extract used in this study was formulated by the Medicinal Plants Research Center of Ilam University of Medical Sciences, and herbal mouthwashes were prepared at predefined concentrations. The study groups were as follows:

Herbal mouthwash containing 1% extract

Herbal mouthwash containing 5% extract

Herbal mouthwash containing 10% extract

Chlorhexidine mouthwash (0.2%) as the positive control

Normal saline mouthwash (0.9% sodium chloride) as the placebo (negative control)

Study Design and Study Population

The present study was designed as a clinical trial in which the study population was randomly allocated into five groups (n = 10 per group): a chlorhexidine mouthwash group, three herbal mouthwash groups with concentrations of 1%, 5%, and 10%, and a normal saline group.

Randomization was performed using a computer-generated random number sequence (www.randomization.com). Allocation concealment was achieved using sequentially numbered, opaque, sealed envelopes. A research assistant not involved in outcome assessment opened the envelope and dispensed the corresponding mouthwash after the patient was enrolled.

Inclusion and Exclusion Criteria

Inclusion criteria consisted of patients requiring gingival surgery on at least one tooth and willingness to participate in the study. Exclusion criteria included antibiotic use within one month prior to enrollment; diabetes or systemic diseases; use of medications interfering with gingival healing; pregnancy; known allergies or hypersensitivity to mouthwash components; smoking; presence of pathological oral mucosal lesions; fixed or removable dental prostheses or orthodontic appliances; periodontitis at the surgical site; and tooth discoloration caused by systemic factors.

The investigator was calibrated under the supervision of a periodontics specialist. In cases where calculus or extrinsic stains were present, scaling and polishing were performed. All patients received standardized oral hygiene instructions, including the Modified Bass brushing technique and correct dental flossing.

Patients returned one week later for surgery. Prior to surgery, the Modified Gingival Index (MGI) and Plaque Index (PI) were recorded for the teeth scheduled for surgery. Due to prior scaling, polishing, and oral hygiene instruction, the baseline staining index (SI) was considered zero.

Blinding: For blinding purposes, mouthwashes were dispensed in identical bottles labeled only with codes G1–G5. The outcome assessor (the periodontist who recorded all clinical indices) was blinded to group allocation. Patients and the investigator who dispensed the mouthwashes were not blinded due to obvious sensory differences between mouthwashes (taste, color, odor). No formal assessment of blinding effectiveness (e.g., asking patients to guess their assigned rinse) was performed.

Patients were instructed to rinse with 10 mL of the assigned mouthwash for 30 seconds, twice daily (morning and evening), starting on the night of surgery and continuing for two weeks, one hour after toothbrushing. Brushing and flossing at the surgical site were prohibited for one week. To standardize the study, all patients were provided with the same soft toothbrush, dental floss, and toothpaste.

Seven days postoperatively, patients returned for suture removal and were instructed to resume brushing all teeth. On day 14, assessments included SI, MGI, PI (extent and severity), tongue discoloration, parotid gland swelling, mouthwash taste, and taste preference.

Clinical Indices

Modified Gingival Index (MGI): Used to assess gingival inflammation. Four tooth surfaces (mesial, distal, facial, and lingual) were scored from 0 to 4. The scores were summed and divided by four to obtain the tooth score (30). All MGI assessments were performed by a single calibrated examiner to reduce inter-rater variability.

Scores were defined as:
0 – No inflammation
1 – Mild inflammation involving part of the marginal or papillary gingiva
2 – Mild inflammation involving the entire marginal or papillary gingiva
3 – Moderate inflammation with redness, edema, or hypertrophy

4 – Severe inflammation with marked redness, edema/hypertrophy, and spontaneous bleeding

Plaque Index (PI)

Measured according to Löe et al. (31) using a periodontal probe on four surfaces (facial, lingual, mesiofacial, distofacial). Scoring followed the same averaging method as MGI.

Staining Index (SI)

Assessed according to Lobene et al. (32), based on both intensity and area of staining. Scores ranged from 0 (no stain) to 3 (severe stain). The mean score of two surfaces determined the final SI value.

Mouthwash Taste

Rated on a five-point scale (very poor to very good). Higher cumulative scores indicated better taste acceptance.

Tongue Discoloration

Categorized as none, discoloration affecting less than half, or more than half of the anterior two-thirds of the tongue, based on Claydon's criteria (33).

Salivary Gland Swelling

Classified as none, unilateral, or bilateral swelling.

For direct comparison, after completing one mouthwash regimen, patients were provided the alternative mouthwash and asked to select the preferred taste after a single use. Data related to taste, tongue discoloration, salivary gland swelling, and taste preference were collected using structured questionnaires.

Preparing herbal mouthwash

The fruits of *Pistacia atlantica* were collected from the heights of the Kabirkuh mountain range in eastern Ilam Province an

authenticated by an expert at the Medicinal Plants Research Center, Ilam University of Medical Sciences (Figure 3-1).

Figure 3-1: Fruit of the wild pistachio tree collected from the slopes of the Kabirkuh mountain range in eastern Ilam Province.

The dried fruits were ground to a fine powder using a laboratory mill under shade conditions (Figure 3-2).

Figure 3-2: Wild pistachio fruit ground using a laboratory mill.

The powdered fruit was subjected to Soxhlet extraction at the Biotechnology and Medicinal Plants Research Center. For this study, an aqueous-alcoholic extract of the wild pistachio fruit was prepared according to previously published protocols (Figure 3-3) (37).

Figure 3-3: Extraction of wild pistachio fruit using a Soxhlet apparatus.

The extract obtained via Soxhlet extraction was concentrated and completely dried using a rotary evaporator available at the research center, ensuring complete removal of the solvent (Figure 3-4).

Chemical standardization disclaimer: The herbal extract was used in its crude form without further chemical standardization. While previous phytochemical studies of *Pistacia atlantica* fruit have identified phenolic compounds (e.g., gallic acid, catechin) and terpenoids as major constituents, no specific marker compounds were quantified in this batch. Therefore, the exact flavonoid or terpenoid content of the mouthwashes remains unknown, which limits reproducibility.

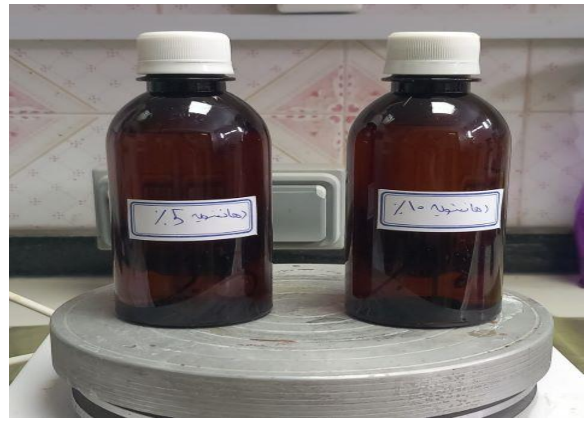
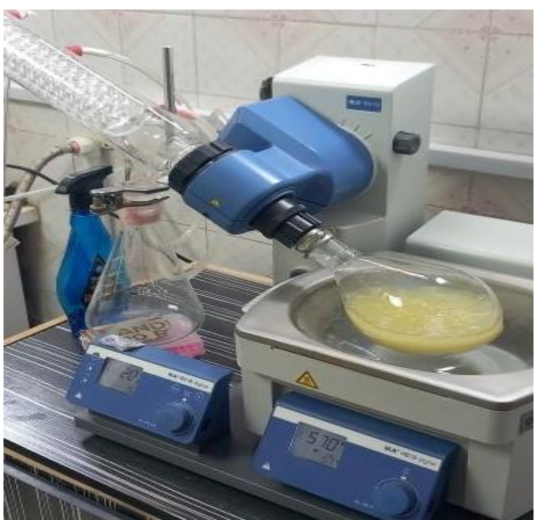


Figure 3-5: Prepared herbal mouthwash derived from wild pistachio fruit at the Biotechnology and Medicinal Plants Research Center.

Figure 3-4. Removal of solvents from the Soxhlet-derived extract using a rotary evaporator



Using the dried extract, herbal mouthwashes containing 1%, 5%, and 10% concentrations of *P. atlantica* fruit extract were formulated for the study (Figure 3-5).

Sample Size and Calculation

An a priori sample size calculation was performed based on the primary outcome measure (Plaque Index or Modified Gingival Index), using a two-sided significance level (α) of 5%, statistical power (1-β) of 90%, and an estimated effect size of 40% (based on expected differences between active treatments and placebo). The calculation yielded a total of 50 participants. To ensure equal allocation across five groups — chlorhexidine mouthwash (CHX), herbal mouthwash at 1%, 5%, and 10% concentrations, and normal saline (NS) placebo — each group was assigned 10 participants. Participants were recruited randomly from patients attending public and private dental clinics in Ilam Province.

Data Collection Instruments and Procedures

All study variables were obtained through direct clinical observation and structured patient questionnaires. Clinical observations and measurements were performed by trained personnel with careful adherence to standardized protocols to ensure accuracy and consistency.

Data Analysis

Normality of continuous data was assessed using the Shapiro–Wilk test. Normally distributed data were summarized as mean ± standard deviation; non-normally distributed data were summarized as median with interquartile range.

For comparisons across all five groups, the Kruskal–Wallis test was used. When a significant overall difference was detected, post-hoc pairwise comparisons were performed using the Mann–Whitney U test.

To control for family-wise error due to multiple pairwise comparisons (10 comparisons), a Bonferroni correction was applied. The adjusted significance level was set at $p < 0.005$ ($0.05 \div 10$). Unadjusted p-values are reported for transparency, with interpretation based on the corrected threshold.

Effect sizes for Mann–Whitney comparisons were calculated as $r = Z / \sqrt{N}$, where $r = 0.1$ indicates a small effect, $r = 0.3$ a medium effect, and $r = 0.5$ a large effect.

Confidence intervals (95% CI) were not calculated due to the small sample size and non-parametric distribution of the data. All statistical analyses were performed using SPSS version 25 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $p < 0.05$, with Bonferroni-corrected $p < 0.005$ for multiple comparisons.

Results

A total of 50 participants were enrolled in this study, with a mean age of 43.2 ± 10.5 years (range 24–55 years). The cohort included 29 women (60%, mean age 45.6 ± 8.5 years) and 21 men (40%, mean age 35.6 ± 12.5 years). The age and gender distribution of the study groups is presented in Table 1-4.

Table 1-4. Mean Age and Gender Distribution in Study Groups

Group	Mean Age (years ± SD)	Male (n)	Female (n)
HM 1%	38.4 ± 13.07	6	4
HM 5%	45.6 ± 14.03	4	6
HM 10%	41.06 ± 11.78	3	7
CHX	38 ± 10.56	5	5
NS	40 ± 10.56	3	7
p-value	0.605*	0.203**	0.109

HM: Herbal Mouthwash; CHX: Chlorhexidine; NS: Normal Saline

*One-way ANOVA (age)

**Fisher's exact test (gender distribution)

Statistical approach: For all pairwise comparisons, a Bonferroni-corrected significance threshold of $p < 0.005$ was used to account for 10 multiple comparisons. Effect sizes (r) are reported for significant findings.

Mouthwash taste quality: The Kruskal–Wallis test revealed a statistically significant difference in taste quality between groups ($p = 0.005$). Pairwise comparisons (Mann–Whitney with Bonferroni correction) showed significant differences between the combined herbal mouthwash (HM) groups (HM 1%, 5%, and 10% pooled) and chlorhexidine (CHX) ($p = 0.002$, $r = 0.62$), and between HM 1% and HM 10% ($p = 0.003$, $r = 0.58$).

Parotid gland swelling and Modified Gingival Index (MGI): No significant differences were observed among groups for parotid gland swelling ($p = 0.225$) or MGI ($p = 0.656$). However, within each group, MGI scores decreased significantly from baseline to follow-up (Wilcoxon signed-rank test, $p < 0.05$ for all groups), indicating improved gingival inflammation over time regardless of mouthwash type.

Tongue discoloration: Pairwise comparisons showed significant differences between HM (combined) and CHX ($p < 0.001$, $r = 0.71$), HM 1% and HM 10% ($p = 0.001$, $r = 0.65$), CHX and normal saline (NS) ($p = 0.002$, $r = 0.59$), and NS and HM 10% ($p = 0.003$, $r = 0.55$).

Taste preference: Taste preference index was assessed for all active mouthwashes (HM 1%, HM 5%, HM 10%, and CHX). Pairwise comparisons showed that HM 10% was significantly preferred over CHX ($p < 0.001$, $r = 0.62$). No significant differences were observed among the three HM concentrations ($p > 0.005$ for all pairwise comparisons).

Plaque Index (PI): Kruskal–Wallis analysis showed a significant difference only between CHX and NS ($p = 0.001$). The Mann–Whitney test confirmed this difference ($p = 0.001$, $r = 0.55$). PI decreased significantly in all five groups after mouthwash use (Wilcoxon test, $p < 0.01$ for all groups).

Tooth staining index (severity and extent): Significant differences were observed between HM (combined) and CHX ($p < 0.001$, $r = 0.74$), HM 1% and HM 10% ($p = 0.001$, $r = 0.63$), CHX and NS ($p < 0.001$, $r = 0.70$), and HM 10% and NS ($p = 0.002$, $r = 0.58$).

Table 4-2. Mean Rank of Measured Indices Across Study Groups

Groups	Mouthwash Taste Index	Parotid Swelling Index	Modified Gingival Index	Tongue Discoloration Index	Taste Preference Index	Plaque Index (PI)	Tooth Staining Severity	Tootl Stainin Exter
HM 1%	41.13a	29.50a	35.5a	22.12c	24.48a	28.03ab	16.1b	16.3b
HM 5%	42.14a	30.12a	35.3a	21.5c	25.51a	27.56ab	16.89b	16.71
HM 10%	45.33a	34.18a	37.42a	25.62a	27.6a	26.45ab	17.29b	16.97
CHX	22.15b	35.02a	32.54a	46.19b	14.22c	45.63a	47.56a	46.62
NS	29.51a	29.02a	28.35a	21.43c	–	18.05c	21.38b	19.44
p-value	0.005	0.225	0.657	<0.001	<0.001	0.001	<0.001	<0.00

Note: Different superscript letters (a, b, c) within the same column indicate statistically significant differences (Mann-Whitney U test with Bonferroni correction, $p < 0.005$).Taste preference index was assessed only for active mouthwashes (HM 1%, 5%, 10% and CHX) and not for the normal saline (NS) placebo group, as NS has no distinct taste and was not considered a relevant comparator for taste preference.

Kruskal–Wallis test and pairwise comparisons using Mann–Whitney with Bonferroni correction.

Discussion

This study evaluated the effects of a Pistacia atlantica-based herbal mouthwash compared to chlorhexidine (CHX) and normal saline (NS) following clinical crown lengthening surgery. Outcomes included taste preference, parotid gland swelling, tongue discoloration, tooth staining (SI), plaque index (PI), and modified gingival index (MGI). Although CHX showed greater PI improvement than NS, its effect did not differ statistically from the herbal mouthwash. This suggests comparable antiplaque efficacy between the herbal formulation and CHX. Laugisch et al. similarly found no significant difference between a combined 0.05% CHX–herbal extract and 0.1% CHX alone, supporting our finding that alternative agents can achieve similar plaque control (38). In contrast, Kamali reported significantly better PI improvement with 0.2% CHX than with Persica (containing Salvadora persica, yarrow, and mint). Alai et al. also found that mint mouthwash resulted in 1.4× higher plaque formation than CHX. These discrepancies likely arise from differences in herbal composition, study duration, postoperative hygiene protocols, and baseline plaque levels. Importantly, our study used a Pistacia atlantica extract, which contains tannins and polyphenols known to inhibit bacterial adhesion — a mechanism distinct from that of Persica or simple mint formulations (39,40). All five mouthwashes, including NS, showed similar MGI improvement. This finding indicates that mechanical irrigation and routine oral hygiene may contribute as much to early gingival healing as active chemical agents. Jamalpour et al. and Gupta et al. both reported comparable anti-inflammatory effects between herbal mouthwashes and CHX, aligning with our results. However, Gupta et al. found that both active groups differed significantly from NS a contrast with our study. One explanation is that our patients maintained their usual brushing habits, potentially minimizing the additional benefit of active ingredients over saline (41,42). Participants consistently preferred the taste of the herbal mouthwash over CHX. This is consistent with Alai et al. and Gupta et al., who reported better taste acceptance for herbal formulations. However, Abedipour et al. found no taste difference between Persica and CHX in chemotherapy patients, which may reflect altered taste perception in that population rather than a true contradiction with our findings (40,42,43). CHX caused significantly greater staining severity and extent than the herbal mouthwash or NS. Discoloration from CHX is attributed to chemical changes in plaque proteins and the formation of iron sulfide complexes (44). Alai et al. reported 2.8× higher staining with CHX than with mint mouthwash, and Kamali found 6.6× higher staining compared to Persica both supporting our results. Laugisch et al., however, observed no difference in staining between 0.05% CHX–herbal extract and 0.1% CHX alone. This discrepancy is likely explained by the lower CHX concentration (0.05%) in their study, which may reduce staining without fully compromising efficacy (38,39,40). The comparable efficacy of the Pistacia atlantica mouthwash to CHX in PI and MGI, despite CHX's known broad antimicrobial activity, suggests that the herbal formulation acts through complementary mechanisms. Pistacia atlantica contains phenolic compounds (e.g., gallic acid, flavonoids) and terpenoids with antioxidant and antiadhesive properties. These may reduce bacterial colonization without disrupting the oral microbiota as profoundly as CHX. Additionally, the absence of significant differences in MGI between NS and active rinses implies that mechanical debridement and saline irrigation may be sufficient for early gingival healing in low-inflammation settings. Future studies should directly quantify biofilm composition and inflammatory cytokines to clarify these pathways. Several limitations should be considered when interpreting these findings. First, this study was not fully blinded. The mouthwashes differed visibly and sensorially CHX has a distinct bitter taste and clear appearance, the herbal mouthwash had a characteristic color and odor, and NS is colorless and tasteless. Therefore, true participant blinding was unlikely. While the outcome assessor was blinded, we did not formally assess blinding effectiveness. This introduces potential performance and detection bias, particularly for subjective outcomes. Second, the sample size was very small (n = 10 per group). No formal power analysis was conducted prior to the study. With only 10 participants per group, statistical power to detect clinically meaningful differences is low, and the risk of type II error (false negative) is high. This study should be viewed as preliminary, and larger confirmatory trials are needed. Third, the herbal mouthwash was not chemically standardized for its bioactive constituents. No specific marker compounds (e.g., gallic acid, catechin, or total flavonoid content) were quantified. Without standardization, reproducibility across studies is limited. Fourth, no microbiological assessment was performed. Therefore, we cannot determine whether observed improvements resulted from antimicrobial activity, antiadhesive effects, or mechanical irrigation. Fifth, clinical attachment level (CAL) was not measured. CAL is a critical periodontal parameter that reflects true periodontal support. Its absence limits our ability to fully assess periodontal health outcomes beyond inflammation and plaque control. Sixth, several outcome measures were inherently subjective, including MGI, taste perception, tongue discoloration, and tooth staining. Subjective outcomes are vulnerable to bias, particularly without full blinding. Seventh, potential noncompliance with home mouthwash instructions cannot be ruled out. Participants assigned to CHX may have reduced use voluntarily due to progressive tooth discoloration, which could have attenuated CHX efficacy relative to the herbal rinse. Eighth, recruitment of participants posed a challenge. Some volunteers required verbal reassurance regarding the safety of the herbal mouthwash, which may have introduced selection bias. Finally, this study did not evaluate bleeding on probing (BOP) in healthy teeth or a dedicated wound healing index (WHI). These indices could provide complementary information on soft tissue healing.

Recommendations

Based on the findings and limitations of this study, the following recommendations are made for future investigations: Blinding and study design: Future studies should employ double- or triple-blind randomized controlled trial designs, with formal assessment of blinding effectiveness (e.g., asking participants to guess their assigned rinse). Sample size: Future research should include larger sample sizes based on a priori power calculations to ensure adequate statistical power and reduce the risk of type II error. Herbal standardization: Future studies should chemically standardize the *Pistacia atlantica* extract by quantifying specific marker compounds (e.g., total phenolic content, flavonoids, gallic acid, catechin) to ensure reproducibility across batches and studies. Microbiological assessment: Future investigations should include quantitative microbiological analysis (e.g., bacterial colony counts or biofilm composition) to elucidate the antimicrobial mechanisms of the herbal mouthwash. Objective periodontal measures: Future studies should measure clinical attachment level (CAL), bleeding on probing (BOP), and wound healing index (WHI) to provide more comprehensive periodontal outcomes beyond gingival and plaque indices. Combination therapy: Future studies should evaluate the potential synergistic effects of combining the herbal mouthwash with low-concentration chlorhexidine to reduce adverse effects while maintaining efficacy. Other herbal formulations: Future research could investigate other medicinal plants (e.g., *Mentha spicata*, *Salvadora persica*, *Terminalia chebula*) in mouthwash formulations for comparison with *Pistacia atlantica*. Longer follow-up: Future studies should include longer follow-up periods to assess the durability of clinical outcomes and potential late-onset adverse effects.

Conclusion

This preliminary study evaluated the effects of a Pistacia atlantica-based herbal mouthwash compared to chlorhexidine (CHX) and normal saline (NS) following clinical crown lengthening surgery. The herbal mouthwash demonstrated comparable efficacy to CHX in improving plaque index (PI) and modified gingival index (MGI), while exhibiting significantly fewer adverse effects, including reduced parotid swelling, tongue discoloration, tooth staining, and improved taste acceptance. However, these findings must be interpreted with caution due to several important limitations: the small sample size (n = 10 per group), lack of full blinding, absence of chemical standardization of the herbal extract, no microbiological assessment, and failure to measure clinical attachment level (CAL). Therefore, this study should be considered preliminary rather than definitive. Within these limitations, the Pistacia atlantica mouthwash appears to be a well-tolerated alternative to chlorhexidine for postoperative oral hygiene following crown lengthening surgery. Larger, rigorously designed trials are necessary to confirm these findings.

Study Setting

This study was conducted at the School of Dentistry, Ilam University of Medical Sciences.

Ethical Considerations

The fruit of *Pistacia atlantica* is commonly consumed as food, and the plant's resin has long-standing applications in traditional medicine. It is widely used in various forms and has multiple applications in the food industry. Based on its extensive use and previous studies, the plant has no known toxic effects on humans. In this study, the fruit extract was used in the form of a mouthwash, and therefore its effects were assessed topically.

Nonetheless, all study participants provided written informed consent, and the intervention was fully explained to them. The study was approved by the Ethics Committee of Ilam University of Medical Sciences under code IR.MEDILAM.REC.1403.114. Additionally, the trial was registered in the Iranian Registry of Clinical Trials under code IRCT20241126063859N1.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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