



Propolis versus chlorhexidine mouthwash following impacted mandibular third molar surgery: A randomized clinical trial

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ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 17 Jul. 2026
Revised: 12 Sep. 2026
Accepted: 14 Sep. 2026
published: 1 Oct. 2026

Keywords:
Propolis
Bee bread
Chlorhexidine
Gingival index
Periodontal index
Dry socket

ABSTRACT

Introduction: Propolis has anti-inflammatory, antimicrobial, and wound-healing properties. This study compared the effects of propolis and chlorhexidine mouthwash on the gingival index (GI), plaque index (PI), and incidence of dry socket (DS) after surgical extraction of impacted lower third molars.

Methods: This randomized, double-blind, parallel-group clinical trial included 32 patients aged 15-30 years undergoing surgical extraction of an impacted mandibular third molar. Patients were randomly allocated to receive either a propolis mouthwash (test group) or 0.2% chlorhexidine mouthwash (control group). The primary outcome was GI severity. Secondary outcomes included PI and DS severity, which were evaluated at baseline, day 7, and day 14. Statistical analyses were performed using SPSS version 26 by Fisher's exact test, the chi-Square test, and an ordinal mixed-effects model to analyze categorical variables.

Results: A total of 32 participants, with 16 in each group, completed the study. Generalized linear mixed model analysis indicated no statistically significant between-group differences in GI ($P = 0.783$) or PI ($P = 0.793$) over the 14-day follow-up. Similarly, DS severity showed no drug difference ($P = 0.906$), although both groups improved significantly over time ($P = 0.045$). Notably, tooth discoloration was descriptively more frequent and severe in the chlorhexidine group, whereas no severe discoloration occurred in the propolis group; however, the difference was not statistically significant ($P = 0.185$).

Conclusion: Propolis mouthwash demonstrated comparable clinical outcomes to chlorhexidine in reducing gingival inflammation, plaque accumulation, and DS incidence, with a potentially lower incidence of side effects. However, more studies with larger sample sizes are required to confirm the results.

Implication for health policy/practice/research/medical education:

Propolis mouthwash may have the potential to serve as a safe and effective alternative to chlorhexidine 0.2% for postoperative oral care, offering benefits in reducing gingivitis and plaque accumulation. Future studies should involve larger, multicenter randomized controlled trials with longer follow-up periods to confirm these findings and assess long-term safety and cost-effectiveness, and establish standardized formulations and dosing protocols.

Please cite this paper as: Najafi A, Shirvani F, Rabienejad N, Moslehi S, Najafi N. Propolis versus chlorhexidine mouthwash following impacted mandibular third molar surgery: A randomized clinical trial. J Herbmed Pharmacol. 2026;15(4):458-467. doi: 10.34172/jhp.54044.

Introduction

Oral and dental health are essential components of general health and quality of life, and play an important role in overall health and well-being (1,2). Inadequate oral hygiene

can lead to periodontitis, gingivitis, plaque accumulation, and dental caries, which are linked to systemic issues like diabetes, heart disease, and pulmonary infections (3). Gingival index (GI) and plaque index (PI) are key indicators

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for assessing the severity of gingival inflammation and microbial plaque accumulation, respectively (4). Dry socket (DS) is a common postoperative complication following third molar extraction, particularly in the lower jaw. It is characterized by severe pain, absence of a blood clot, and delayed healing (5). Factors such as poor surgical techniques, infection, smoking, and poor oral hygiene contribute to its development. Mouthwashes reduce the microbial load and DS (6).

Sodium hypochlorite, normal saline, and chlorhexidine mouthwash are commonly used in dentistry to remove microorganisms (7). Chlorhexidine is widely recognized as the gold standard in preventing periodontal diseases due to its extensive antimicrobial properties for managing chemical plaque control and for reducing gingival bleeding (8-10). However, the use of these solutions has disadvantages. Chlorhexidine also has various side effects, such as antimicrobial resistance, discoloration of teeth, discoloration of same-colored tooth restorations, change in sense of taste, allergies, unpleasant taste in the mouth, burning and dryness of the mouth, flaking of the gums, and negative systemic effects if swallowed (11-13). These limitations have prompted the search for safer, natural alternatives. One complementary medicine option for treating gingival inflammation and dental plaque is the use of natural products (14). In recent years, the use of herbal medicines and their derivatives has become more popular due to their fewer side effects than chemical treatments and satisfactory results in the prevention and treatment of diseases (15-18). Meanwhile, recent studies have demonstrated that propolis has also shown satisfactory results in dental procedures due to its regenerative, anti-inflammatory, and antimicrobial effects (19). Propolis is a resinous substance synthesized by honeybees from plant exudates and is notable for its rich and chemically diverse composition. Its bioactive constituents include flavonoids such as chrysin, kaempferol, quercetin, and quercetin-3-D-xyloside, as well as phenolic and aromatic acids, including caffeic and rosmarinic acids, and amino acids and vitamins (20). It exhibits potent anticancer, antimicrobial, antidiabetic, anti-inflammatory, and antioxidant properties (20,21). In this clinical trial, we aimed to compare the clinical efficacy of propolis and chlorhexidine mouthwash in reducing GI, PI, and DS after surgical extraction of impacted mandibular third molars.

Materials and methods

Study design and setting

The Research Council of the Faculty of Dentistry, Hamadan University of Medical Sciences, Iran, approved this randomized, double-blind, controlled clinical trial under ethical code IR.UMSHA.REC.1403.622. Moreover, the study was registered in the Iranian Registry of Clinical Trials website (identifier: [IRCT20241211064021N4](https://www.irct.ir/IRCT20241211064021N4)). This randomized, double-blind, controlled clinical trial used a block randomization allocation with equal allocation (1:1)

to the propolis and chlorhexidine groups. The study was conducted during the 2023-2024 academic year in the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Hamadan. We recruited patients eligible for surgical extraction of an impacted mandibular third molar. Baseline demographic characteristics, including age, sex, and the side of surgery, were recorded. GI, PI, and DS were evaluated preoperatively and at the scheduled postoperative follow-up visits.

Participants

A total of 32 patients aged 15-30 years who required surgical extraction of one impacted mandibular third molar were enrolled in this parallel-group randomized clinical trial. All participants had bilateral impacted mandibular third molars of similar surgical difficulty; however, only one mandibular third molar from each participant was included in the study and analyzed. Participants were assigned randomly to either the propolis group ($n = 16$) or the 0.2% chlorhexidine group ($n = 16$). All randomized participants completed the study, as shown in Figure 1.

Participants were eligible if they were aged between 15 and 30 years and had bilateral impacted mandibular third molars that required similar surgical difficulty. Surgical difficulty was classified as moderate (Pederson Difficulty Index score 5-6) based on tooth angulation, depth of impaction, and available retromolar space (22), which accounts for tooth angulation, depth of impaction, and available retromolar space. Only one mandibular third molar per participant was included in the study. Individuals were excluded if they were pregnant, had systemic conditions such as cardiovascular disease, presented with poor oral hygiene or active gingivitis/periodontitis, or had received systemic antibiotic therapy within the two weeks preceding surgery or at any time during the study period.

Sample size calculation

The sample size was determined using G*Power software (version 3.1) based on the main outcome measure, namely, gingival inflammation severity as assessed by the GI, using data from a previous study by Mojtahezade et al, which reported an effect size of 1.03 (Cohen's d) (23). With a two-sided significance level (α) of 0.05 and a statistical power ($1-\beta$) of 80%, the minimum necessary sample size was established to be 16 participants for each group, resulting in a total of 32 participants.

Randomization and blinding

Patients were randomly assigned in a 1:1 ratio to either the propolis or the 0.2% chlorhexidine group using computer-generated block randomization with random block sizes of 4 to ensure balanced allocation throughout the enrollment period. An independent statistician not involved in participant recruitment, clinical assessments,

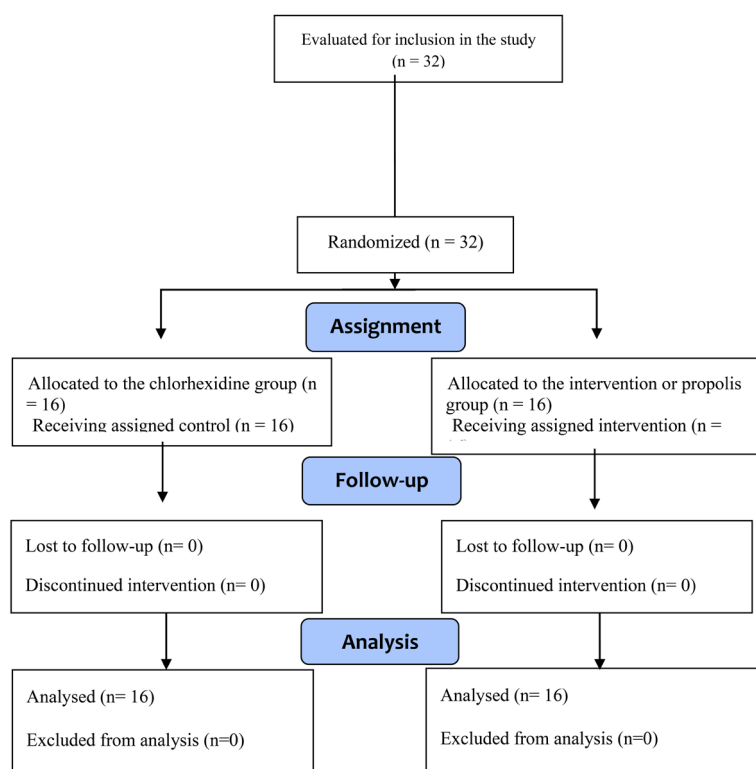


Figure 1. CONSORT diagram of the implementation process of this clinical trial.

or data analysis generated the random allocation sequence using SPSS version 26 (IBM Corp., Armonk, NY, USA). To ensure strict allocation concealment, the independent statistician placed the generated sequence in sequentially numbered, opaque, sealed envelopes. Each envelope contained a coded card indicating the treatment assignment. The envelopes were stored securely and were opened by the principal researcher only after a participant was enrolled, confirmed eligible, and had provided written informed consent. This process ensured that neither the investigators enrolling participants nor the participants themselves could foresee the upcoming allocation.

The propolis and chlorhexidine were formulated to have a similar appearance, color, and odor and were dispensed in identical, indistinguishable bottles. The allocation code was securely maintained until data collection and statistical analysis were complete. Neither the participants nor the investigators responsible for clinical assessments (including evaluation of GI, PI, and DS) were aware of the treatment allocation throughout the study.

Intervention

A single oral and maxillofacial surgeon performed all extractions under aseptic conditions, ensuring procedural consistency throughout the trial. Following surgical extraction, participants were randomly allocated in a 1:1 ratio to one of two postoperative mouthwash regimens. The test group received a commercially available propolis-based mouthwash (Misha Organic Shahdram, Tehran, Iran), derived from raw propolis sourced from Iranian

apiaries and authenticated by the manufacturer based on its botanical and physicochemical characteristics, with no alcohol or phosphate buffer additives. The control group used 0.2% chlorhexidine gluconate mouthwash (Shahr Darou Pharmaceutical Company, Tehran, Iran), the established gold-standard antimicrobial rinse in oral surgery. To maintain double-blinding, an independent coordinator not involved in clinical assessment or data analysis dispensed both formulations in identically coded containers.

Mouthwash consumption began on the evening of surgery, approximately 12 hours postoperatively. Participants rinsed twice daily with 10 mL of their assigned solution for 30 seconds, then expectorated. Eating, drinking, and water rinsing were discouraged for at least one hour after each application to prolong mucosal contact. The regimen continued for 14 days. Compliance was monitored through verbal confirmation and self-reported records at each postoperative visit. Participants who were non-adherent to the prescribed regimen or required additional antimicrobial therapy because of severe postoperative complications were considered to be excluded from the final analysis.

Oral hygiene was standardized across both groups. All participants received identical oral hygiene kits containing a soft-bristled toothbrush and fluoride toothpaste (1450 ppm) and were instructed in the modified Bass brushing technique (24). Participants were advised to brush twice daily for approximately 2 minutes (25). To protect the postoperative blood clot and avoid disruption of the

surgical wound, no toothbrushing was permitted directly at the surgical site during the first 7 postoperative days. During the first 24 hours after surgery, participants could brush the remaining teeth while avoiding the surgical area. Beginning on postoperative day 2, gentle toothbrushing of the teeth adjacent to the surgical site was permitted, particularly the mandibular second molar, where GI and PI were assessed; however, participants were specifically instructed to avoid any direct contact with the extraction wound or sutures. Interdental cleaning devices were not recommended in the surgical area during the first 7 postoperative days, although they could be used for other teeth. Following suture removal on postoperative day 7, participants resumed normal toothbrushing of the entire mouth, including the previously protected surgical area.

Participants were instructed to avoid using any additional oral hygiene products throughout the study period to maintain consistency between groups. The standardized oral hygiene instructions were reviewed and reinforced at each postoperative follow-up visit. Adherence to both the prescribed mouthwash regimen and oral hygiene instructions was reassessed through verbal questioning and self-reported records at every visit. Thus, the same standardized oral hygiene protocol was applied to both study groups throughout the study, with direct mechanical cleaning of the surgical wound withheld until suture removal on day 7. This approach was intended to minimize postoperative wound disturbance while preventing excessive plaque accumulation on the adjacent teeth and ensuring consistent assessment of GI and PI.

After surgery, all participants received the same standardized postoperative medication regimen. This included Gelofen tablets 400 mg (three tablets daily for 5 days) and amoxicillin 1.5 g (three 500-mg capsules daily) (26). Participants were instructed to take the medications as prescribed. Participants were not permitted to take additional analgesics, antibiotics, anti-inflammatory drugs, or other medications that could affect study outcomes unless clinically necessary.

Outcome measures

The primary outcome was the severity of gingival inflammation, assessed using the GI, while the PI, DS, and tooth discoloration were considered secondary outcomes. Because the study specifically aimed to evaluate local periodontal changes associated with mandibular third molar surgery, GI and PI were assessed using a localized protocol rather than a full-mouth assessment. For each participant, the mandibular second molar adjacent to the surgical site was selected as the index tooth. The gingival tissues surrounding this tooth were examined at four sites (mesial, distal, buccal, and lingual), and the scores from each site were recorded according to the respective index criteria. The mean of the four site-specific scores was calculated to obtain the participant-level GI and PI score

at each assessment time point. The same index tooth and assessment sites were used at all follow-up examinations to ensure consistency and reproducibility.

GI and PI were assessed at baseline and on postoperative days 7 and 14. The GI was evaluated according to the L  e and Silness GI, which scores gingival inflammation on a four-point ordinal scale from 0 to 3. The PI was assessed according to the Silness and L  e PI, also using a four-point scale from 0 to 3 (27). In the context of the GI, a score of 0 signifies the absence of inflammation. A score of 1 represents mild inflammation, characterized by minimal alterations in color and texture without bleeding on probing. A score of 2 denotes moderate inflammation, marked by redness, swelling, and bleeding upon probing. A score of 3 indicates severe inflammation, characterized by pronounced redness and swelling, a propensity for spontaneous bleeding, and/or ulceration.

Similarly, for the PI, a score of 0 indicates no presence of plaque. A score of 1 reflects a thin film of plaque adhering to the free gingival margin and the adjacent tooth surface, which is detectable upon probing. A score of 2 indicates a moderate buildup of soft deposits that are visible to the naked eye. Finally, a score of 3 demonstrates a substantial accumulation of soft deposits within the gingival sulcus and/or along the interface of the tooth and gingival margin. The same calibrated examiner performed all GI and PI assessments using the same examination procedure throughout the study.

DS was evaluated as a postoperative complication and was diagnosed independently of pain severity. A diagnosis of DS was made when the extraction socket showed partial or complete loss of the blood clot with exposed alveolar bone, accompanied by clinically characteristic postoperative pain occurring or increasing after the initial postoperative period, typically between 1 and 3 days after extraction. The presence or absence of DS was recorded as a dichotomous outcome (yes/no) based on these clinical criteria. Pain intensity was evaluated using a 10-cm visual analogue scale (VAS), where 0 signifies no pain, and 10 represents the worst pain imaginable. VAS scores were used to quantify pain intensity and were not considered diagnostic criteria for DS. For descriptive purposes, pain intensity was categorized as no pain (0), mild pain (1–3), moderate pain (4–6), and severe pain (7–10) (28). Tooth discoloration was evaluated using the VITA Classical Shade Guide under standardized D65 lighting by two independently calibrated examiners. Discoloration was scored on a four-point ordinal scale ranging from 0 (no discoloration) to 3 (severe discoloration) (29). Both examiners independently recorded the shade score, and the predefined scoring criteria were applied consistently across all assessment time points (29).

Statistical analysis

The data were analyzed using SPSS version 26. We also used descriptive statistics to summarize demographic and

clinical data. We assessed the normality of continuous data, and because the distribution was non-normal and sample sizes were small, we used Fisher's exact test, the Mann-Whitney test, and the chi-square test to compare categorical variables between groups. To investigate the effects of treatment group (propolis vs. chlorhexidine), time (baseline, postoperative day 7, and postoperative day 14), and the group-by-time interaction on GI, PI, and DS severity, cumulative logit models with a multinomial distribution, logit link function, and proportional odds assumption were employed for the ordinal repeated-measures outcomes. This approach was appropriate for analyzing the four-level ordinal outcomes (scored from 0 to 3) while accounting for the within-subject correlation arising from repeated measurements over time. The models included treatment group, time, and the group-by-time interaction as fixed effects to assess differences in outcome scores between the two treatment groups, changes over time, and whether temporal changes differed between the groups. A P value < 0.05 was considered statistically significant.

Results

A total of 32 individuals (16 from each group) finished the study and were considered in the final analysis. Table 1 presents the baseline demographic characteristics of the participants. There was no statistically significant difference in sex distribution between the propolis and 0.2% chlorhexidine groups ($P = 0.72$). The mean age was 23.15 ± 4.65 years in the propolis group and 24.73 ± 3.22 years in the chlorhexidine group ($P = 0.056$).

Tooth discoloration was evaluated both within and between groups (Table 2). Within-group analysis using the Friedman test revealed statistically significant changes

in discoloration scores over time within both the propolis group ($P = 0.039$) and the chlorhexidine group ($P < 0.001$), indicating that discoloration increased significantly during the follow-up period in both treatment groups. However, when comparing the two groups at each individual time point (Between-group comparisons using Fisher's Exact Test), no statistically significant between-group differences were observed at baseline ($P = 1.000$), day 7 ($P = 0.405$), or day 14 ($P = 0.185$). Although the chlorhexidine group showed a descriptive trend toward higher frequency and severity of discoloration, including one case of severe staining (score 3) on day 14, this difference did not reach statistical significance.

Table 3 presents the descriptive, cross-sectional distributions of GI, PI, and DS severity for both groups at baseline, 7 days, and 14 days postoperatively. Unadjusted Fisher's Exact tests showed no statistically significant between-group differences in the severity categories at any individual time point (all $P > 0.05$). However, to formally account for within-subject correlations over time, the primary analyses were performed using Ordinal Mixed-Effects Models (detailed in Tables 4, 5, and 6), which confirmed comparable longitudinal performance between the propolis and chlorhexidine groups.

The main effect of the drug was not statistically significant ($\beta = -0.27$, OR = 0.76, 95% CI: 0.108–5.385, $P = 0.783$), indicating insufficient evidence of a significant difference between propolis and chlorhexidine in the likelihood of being classified into higher GI categories (Table 4). Similarly, the effect of time was not statistically significant ($\beta = 0.28$, OR = 1.33, 95% CI: 0.701–2.513, $P = 0.380$), suggesting that changes over time were not significantly associated with the likelihood of being in a higher GI category. Furthermore, the drug-by-time

Table 1. Comparison of baseline demographic characteristics between the propolis mouthwash and 0.2% chlorhexidine mouthwash groups

Variable		Propolis mouthwash (n = 16)	0.2% Chlorhexidine mouthwash (n = 16)	P value
Sex	Male	8 (50.0)	9 (56.3)	0.72*
	Female	8 (50.0)	7 (43.8)	
Age (years)	Mean $\pm$ SD	23.15 $\pm$ 4.65	24.73 $\pm$ 3.22	0.056**

SD: Standard deviation. * Chi-Square test; ** Mann-Whitney test.

Table 2. Tooth discoloration assessment in both groups

Groups	Score	Before use	Day 7	Day 14	P value
Propolis	0 (No discoloration)	12 (75.0%)	10 (62.5%)	9 (56.3%)	0.039*
	1 (Very slight discoloration)	4 (25.0%)	5 (31.3%)	6 (37.5%)	
	2 (Moderate discoloration)	0	1 (6.3%)	1 (6.3%)	
	3 (Severe discoloration)	0	0	0	
Chlorhexidine	0 (No discoloration)	13 (81.3%)	7 (43.8%)	6 (37.5%)	<0.001*
	1 (Very slight discoloration)	3 (18.8%)	5 (31.3%)	4 (25.0%)	
	2 (Moderate discoloration)	0	4 (25.0%)	5 (31.3%)	
	3 (Severe discoloration)	0	0	1 (6.3%)	
P value		1.000**	0.405**	0.185**	

* Friedman test was used for within-group comparisons of discoloration scores across baseline, postoperative day 7, and postoperative day 14.

** Fisher's exact test was used for between-group comparisons of discoloration-score distributions at each corresponding time point.

Table 3. Intergroup comparison of GI, PI, and DS severity before surgery and at 7 and 14 days postoperatively

Times	Index	Category	Propolis (n, %)	Chlorhexidine (n, %)	P value*
Before surgery	GI	Normal	9 (56.3%)	8 (50%)	0.723
		Mild inflammation	7 (43.7%)	8 (50%)	
	PI	Normal	8 (50%)	8 (50%)	1.000
		One-layer plaque	8 (50%)	8 (50%)	
7 days after surgery	DS	Normal	16 (100%)	16 (100%)	-
		Normal	0 (0%)	2 (12.5%)	
		Mild inflammation	3 (18.8%)	1 (6.3%)	
		Moderate inflammation	7 (43.8%)	7 (43.8%)	
	GI	Severe inflammation	6 (37.5%)	6 (37.5%)	0.392
		One-layer plaque accumulation	3 (18.8%)	2 (12.5%)	
		Moderate plaque accumulation	7 (43.8%)	8 (50%)	
		Severe plaque accumulation	6 (37.6%)	6 (37.6%)	
	PI	Normal	2 (12.5%)	1 (6.3%)	0.528
		Moderate	9 (56.3%)	7 (43.8%)	
		Severe	5 (31.3%)	8 (50%)	
		Normal	5 (31.3%)	6 (37.6%)	
14 days after surgery	GI	Mild inflammation	5 (31.3%)	7 (43.8%)	0.331
		Moderate inflammation	6 (37.6%)	2 (12.5%)	
		Severe inflammation	0 (0%)	1 (6.3%)	
		Normal	7 (43.8%)	9 (56.3%)	
	PI	Moderate plaque accumulation	8 (50%)	7 (43.8%)	0.518
		Severe plaque accumulation	1 (6.3%)	0 (0%)	
		Normal	10 (62.5%)	9 (56.3%)	
		Mild	6 (37.6%)	7 (43.8%)	

GI: Gingival index; PI: Plaque index; DS: Dry socket.

* Fisher's exact test.

interaction was not statistically significant ($\beta = 0.2$, OR = 1.22, 95% CI: 0.497–3.014, $P=0.657$), indicating that the pattern of change in GI over time did not differ significantly between the two groups. The threshold parameters in the ordinal mixed-effects model represent the estimated cut-points separating adjacent GI severity categories and are not treatment effects or between-group comparisons. Thus, the $P=0.002$ reported for Threshold 3 indicates statistical significance of the estimated third cut-point in the ordinal model and should not be interpreted as evidence of a significant difference between the propolis and chlorhexidine groups. The treatment-related inference is based on the P values for Drug, Time,

and Drug $\times$ Time. Overall, neither group membership nor time nor their interaction was significantly associated with the distribution of the GI categories (all $P>0.05$).

The results of the mixed model revealed no statistically significant main effect for the drug group (OR = 0.77, 95% CI: 0.11–5.45, $P=0.793$), indicating a possible comparable plaque control outcome between the propolis and chlorhexidine groups. Furthermore, no significant main effect of time (OR = 0.93, 95% CI: 0.49–1.76, $P=0.812$) or Group $\times$ Time interaction (OR = 1.20, 95% CI: 0.49–2.97, $P=0.690$) was observed. These findings indicate that the trajectory of plaque accumulation over the 14-day follow-up period was similar in both groups, with no

Table 4. Longitudinal comparison of GI severity between propolis and chlorhexidine groups over time

Variable	Coefficient	t	OR	95% CI for OR		P value
				Lower	Upper	
Threshold 1	-0.15	-0.214	0.86	0.215	3.451	0.831
Threshold 2	1.21	1.70	3.35	0.817	13.767	0.092
Threshold 3	2.43	3.26	11.40	2.590	50.195	0.002*
Drug	-0.27	-0.27	0.76	0.108	5.385	0.783
Time	0.28	0.88	1.33	0.701	2.513	0.380
Time $\times$ Drug	0.20	0.45	1.22	0.497	3.014	0.657

Drug: Propolis (reference: 0.2% chlorhexidine). CI, confidence interval; OR, odds ratio. *Significant estimated ordinal-model cut-point; this does not represent a treatment effect or a between-group comparison. Threshold parameters represent estimated cut-points between adjacent ordinal GI categories. Statistical inference regarding treatment effects is based on the Drug, Time, and Time $\times$ Drug terms.

significant between-group differences at any assessment point (Table 5).

Table 6 reveals no statistically significant main effect for the drug group (OR = 0.87, 95% CI: 0.09-8.71, $P=0.906$), indicating comparable DS outcomes between the propolis and chlorhexidine groups. However, a significant main effect of time was observed (OR = 2.08, 95% CI: 1.02-4.24, $P=0.045$), indicating that DS severity changed significantly over the follow-up period in both groups. The Group $\times$ Time interaction was not statistically significant (OR = 0.96, 95% CI: 0.35-2.65, $P=0.937$), suggesting that the trajectory of DS resolution over time was similar in both groups, with no differential drug effect.

Discussion

In this randomized, double-blind trial, both propolis mouthwash and 0.2% chlorhexidine showed no statistically significant differences in gingival and plaque indices over the 14-day follow-up, with neither agent demonstrating a statistically significant advantage over the other. DS severity was likewise not significantly different between groups, suggesting no detectable difference in socket healing outcomes within this timeframe. Tooth discoloration showed a descriptive trend toward greater frequency and severity in the chlorhexidine group, including one case of severe discoloration by day 14, whereas no severe discoloration was observed in the propolis group; however, the between-group differences were not statistically significant. This descriptive pattern is consistent with the well-established staining

potential of chlorhexidine, although the present study did not demonstrate a statistically significant difference in discoloration between the two treatments. These results are consistent with previous studies highlighting the antimicrobial and anti-inflammatory properties of propolis. Santos et al and Zuhendri et al reported that propolis contains flavonoids, phenolic acids, and essential oils that contribute to its therapeutic effects in oral health. Its ability to inhibit bacterial growth and modulate inflammatory responses makes it a promising alternative to chemical agents such as chlorhexidine (30,31). Additionally, Halboub et al reported meaningful reductions in plaque and gingival inflammation with propolis mouthwash, along with a favorable safety profile with minimal adverse effects. Given that chlorhexidine, despite its widespread use, carries well-documented drawbacks including taste alteration and tooth staining, the authors concluded that propolis-based formulations represent a safe and viable alternative (32). Salari et al reported that both chlorhexidine and propolis mouthwashes produced significant reductions in PI and GI among patients with chronic gingivitis, a finding broadly consistent with our own results. Chlorhexidine demonstrated superior efficacy in plaque reduction, yet the two agents performed comparably with respect to gingival inflammation. These findings provide contextual support for the potential clinical utility of propolis, although the present study does not establish equivalence or non-inferiority to chlorhexidine. In light of the well-recognized adverse effects associated with prolonged

Table 5. Longitudinal comparison of PI severity between propolis and chlorhexidine groups over time

Variable	Coefficient	t	OR	95% CI for OR		P value
				Lower	Upper	
Threshold 1	-0.77	-1.089	0.46	0.114	1.885	0.279
Threshold 2	0.79	1.114	2.19	0.540	8.929	0.268
Threshold 3	1.76	2.400	5.79	1.353	24.811	0.018*
Drug	-0.26	-0.264	0.77	0.109	5.453	0.793
Time	-0.08	-0.239	0.92	0.488	1.758	0.812
Time $\times$ Drug	0.18	0.400	1.20	0.485	2.967	0.690

Drug: Propolis (reference: 0.2% chlorhexidine). CI: confidence interval; OR: odds ratio. *Significant estimated ordinal-model cut-point; this does not represent a treatment effect or a between-group comparison. Threshold parameters represent estimated cut-points between adjacent ordinal GI categories. Statistical inference regarding treatment effects is based on the Drug, Time, and Time $\times$ Drug terms.

Table 6. Longitudinal comparison of DS severity between propolis and chlorhexidine groups over time

Variable	Coefficient	t	OR	95% CI for OR		P value
				Lower	Upper	
Threshold 1	1.65	2.048	5.19	1.051	25.684	0.043
Threshold 2	3.29	3.821	27.01	4.869	149.837	< 0.001
Threshold 3	-0.14	-0.119	0.871	0.087	8.705	0.906
Drug	0.73	2.035	2.078	1.018	4.242	0.045
Time	-0.04	-0.079	0.961	0.348	2.652	0.937
Time $\times$ Drug	0.18	0.400	1.20	0.485	2.967	0.690

Drug: Propolis (reference: 0.2% chlorhexidine). CI: confidence interval; OR: odds ratio.

chlorhexidine use, propolis emerges as a natural, well-tolerated alternative for plaque and gingivitis management (33). Koshy et al conducted a double-blind randomized trial of 28 patients comparing 5% propolis paste against a zinc oxide eugenol sponge for the management of DS following wisdom tooth extraction. Outcomes, including pain (VAS), bone visibility, and socket odor, were assessed, with the propolis group demonstrating faster pain resolution and greater patient comfort, free of any allergic or inflammatory reactions. The between-group difference was statistically significant in favor of propolis (34).

When the repeated-measures nature of the data was taken into account using generalized linear mixed models with cumulative logit links, no significant differences were observed between the propolis and chlorhexidine groups over the 14-day follow-up period for GI (OR=0.76), PI (OR=0.77), or DS severity (OR=0.87). However, a significant effect of time was observed for DS severity (OR=2.08), indicating that DS severity changed significantly over the follow-up period in both treatment groups. This temporal pattern is consistent with the expected healing process following tooth extraction and suggests that the improvement in DS severity was related primarily to the progression of healing rather than to differences between the two mouthwash interventions (35). Overall, the mixed-effects analysis provided a more appropriate assessment of the longitudinal data by accounting for the correlation between repeated measurements within the same participants.

The severity distribution of DS in this study did not differ significantly between the two groups, and the group-by-time interaction was also not statistically significant. Although the descriptive distributions at individual time points suggested some differences between groups, these should not be interpreted as evidence of a superior healing effect of propolis. DS is a common postoperative complication, particularly in lower third molar extractions, and is influenced by factors such as surgical trauma, infection, smoking, and poor oral hygiene. The anti-inflammatory and wound-healing properties of propolis may contribute to its protective effect against alveolar osteitis, as suggested by González-Serrano et al (35). Moreover, due to its antioxidant and anti-inflammatory effects, it improved periodontal health and maintained bone health by suppressing osteoclast formation and enhancing osteoblast development (36).

The findings on tooth discoloration align with prior literature, indicating that chlorhexidine interacts with dietary chromogens and salivary proteins, leading to extrinsic staining. As a natural compound, propolis may have a more favorable discoloration profile; however, this study did not demonstrate a statistically significant between-group difference in discoloration. This is particularly important for young patients and those concerned about dental appearance.

Overall, the results of this study suggest that propolis

mouthwash may be a potential alternative to chlorhexidine, especially for patients seeking natural, biocompatible, and aesthetically acceptable oral care solutions. While chlorhexidine remains effective, its side effects may limit its use in certain populations.

Limitations of the study

The study's limitations include a relatively modest sample size and a brief follow-up duration, which may constrain the generalizability of the findings and limit the ability to detect subtle differences between groups. Future studies with larger cohorts and longer observation periods are recommended to validate these findings further and explore the long-term effects of propolis on periodontal health and postoperative recovery. Additionally, potential risk factors for DS, including smoking status, Pederson difficulty score, tooth angulation, impaction depth, and operative duration, were not assessed in this study and should be assessed in future studies.

Conclusion

Longitudinal analysis confirmed that the propolis-based herbal mouthwash produced clinical outcomes comparable to 0.2% chlorhexidine, with both agents demonstrating similar trajectories of improvement in gingival inflammation, plaque accumulation, and DS severity following impacted mandibular third molar extraction. No statistically significant between-group differences were identified across the 14-day follow-up period. Taken together, these findings may indicate that propolis is an effective alternative to chlorhexidine, offering comparable therapeutic benefits with possible low side effects. Larger, longer-term trials are nonetheless needed to confirm these findings.

Acknowledgements

This article was derived from a research project approved by the Research and Technology Department at Hamadan University of Medical Sciences. Hereby, the researchers gratefully thank the patients who participated in this study.

Authors' contribution

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Conflict of interests

The authors declare there are no conflicts of interest.

Declaration of AI-assisted tools in the writing procedure

During the preparation of this manuscript, the authors used Grammarly and OpenAI's ChatGPT for refining grammar and improving sentence structure and consistency in this study. After its use, the authors thoroughly reviewed, verified, and revised all AI-assisted content to ensure accuracy and originality. The authors take full responsibility for the integrity and final content of the published article.

Ethical considerations

The research procedures were approved by the Hamadan University ethics committee (ethical code: IR.UMSHA.REC.1403.622). The research was registered in the Iranian Registry of Clinical Trials website (identifier: [IRCT20241211064021N4](https://www.irct.ir/IRCT20241211064021N4)). Furthermore, this study was conducted in accordance with the ethical principles of the 1975 Declaration of Helsinki regarding the involvement of human subjects. Informed consent was obtained from the patients or their parents. For participants younger than 18 years, written informed consent was obtained from a parent or legal guardian, and assent was obtained from the participant.

Funding/Support

This study was funded by the Deputy of Research and Technology of Hamadan University of Medical Sciences.

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