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Original Article

Comparison of Myrrh and Chlorhexidine Mouthwashes in Reducing Plaque, Gingivitis, and Inflammation: A 3-Arm Randomized Controlled Trial

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ABSTRACT

This study aimed to evaluate the effectiveness of 1% myrrh mouthwash compared to 0.2% chlorhexidine mouthwash in inhibiting plaque activity, alleviating gingivitis, and reducing pro-inflammatory cytokines. The clinical trial included 19 participants (10 males, 9 females), with 6 in the myrrh group, 7 in the chlorhexidine group, and 6 in the saline group. Initially, participants refrained from their usual oral hygiene routine for 2 weeks to allow experimental gingivitis to develop. After this period, they were instructed to stop brushing and use 15 ml of the assigned mouthwash twice daily for 1 minute. Clinical parameters, including the modified gingival index (MGI), plaque index (PI), pro-inflammatory interleukin (IL)-1β biomarker, and bleeding on probing (BOP), were recorded at baseline and after the intervention. Data analysis was performed using mixed ANOVA. At baseline, all groups showed similar clinical parameters ($P > .05$ for all comparisons). Post-intervention, the myrrh group showed significantly lower MGI and BOP scores compared to the saline group ($P = .016$ and $P < .001$, respectively). While the chlorhexidine group also showed lower scores in these two parameters, the difference in MGI didn't reach statistical significance ($P = .09$). No significant differences were observed between the groups in terms of mean PI and IL-1β levels. In conclusion, 1% myrrh mouthwash proved to be as effective as 0.2% chlorhexidine mouthwash in reducing gingival inflammation and BOP.

Keywords: Randomized controlled trial, Myrrh, *Commiphora myrrha*, Mouthwash, Chlorhexidine.

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Introduction

Dental plaque consists of a diverse microbial community that adheres to teeth and other hard oral surfaces, forming a sticky biofilm. These biofilms are a major cause of various oral health issues, including caries, gingivitis, and periodontitis [1, 2]. The accumulation of plaque in the gingival crevice, often because of poor oral hygiene, can lead to chronic inflammation and gradual damage to the periodontal tissues supporting the teeth [3]. Therefore, controlling dental plaque is crucial for maintaining good oral hygiene and preventing periodontal diseases. Effective plaque removal can be achieved through both chemical and mechanical methods. Mechanical plaque control involves using physical force to dislodge microbial biofilms from the tooth surface. This can be done through professional cleaning procedures like root planing and scaling, or through self-care practices such as brushing twice a day and using interdental cleaning tools [4].

Chemical plaque control is often used alongside mechanical cleaning to prevent the growth and buildup of microbial communities. Toothpaste and mouthwashes are common delivery methods for

various anti-plaque chemicals. Numerous organic and inorganic compounds serve as anti-plaque agents, including, phenolic compounds, delmopinol, quaternary ammonium compounds, essential oils (such as menthol, methyl salicylate, and thymol), chlorhexidine gluconate (CHX), and herbal extracts [5]. Among these, CHX has been extensively studied and is considered the most effective anti-plaque agent. Its efficacy has set the standard against which other anti-plaque and anti-gingivitis products are measured [6, 7]. Depending on the concentration, CHX can exhibit both bactericidal and bacteriostatic effects. However, one significant drawback of CHX is its ability to impair cell migration and survival [7, 8]. Furthermore, long-term use of CHX can lead to several side effects, including tooth discoloration, mucosal irritation, and altered taste sensation. As a result, many dental professionals now recommend using CHX only under their supervision [7-10].

Herbal mouthwashes have long been considered a viable alternative to CHX for reducing dental plaque and gingivitis due to their minimal side effects. Clinical trials assessing the effectiveness of these mouthwashes have shown their potential as supplementary treatments [11]. In traditional medicine, myrrh (*Commiphora myrrha*), a resin native to regions of the Middle East and North Africa, has been used for centuries to treat various inflammatory conditions [12]. Several studies have indicated its potential to address various oral health issues, such as inflamed gums, aphthous ulcers, and mucosal wounds [13, 14]. However, despite the widespread use of various herbs as mouthwash ingredients, the potential of myrrh-based formulations remains less explored. In a previous pilot study, we compared the effectiveness of a myrrh-based mouthwash to CHX and found it to slightly outperform CHX in reducing plaque and gingival inflammation [15]. Similar results have been observed in earlier research, where myrrh was as effective as CHX [16, 17]. However, these studies faced certain limitations in their design and participant selection.

The objective of this study is to further investigate the previously observed effectiveness of myrrh mouthwash by conducting additional laboratory tests, including the pro-inflammatory interleukin (IL)-1β biomarker, bleeding on probing (BOP), modified gingival index (MGI), and plaque index (PI). Our hypothesis posits that there will be no significant difference between 1% myrrh mouthwash and the commercially available 0.2% CHX mouth rinse in terms of reducing plaque accumulation, controlling gingival inflammation, and inhibiting the pro-inflammatory mediator (IL-1β).

Materials and Methods

Study design

This research was a randomized controlled clinical trial conducted at the Faculty of Dentistry, King Abdulaziz University (KAUFD), Jeddah, Saudi Arabia. We followed the guidelines outlined in the Declaration of Helsinki for biomedical research involving human participants and the CONSORT 2010 Statement for multi-arm trials reporting. Ethical approval for the study was granted by the Research Ethics Committee at KAUFD (protocol number: 058-15). The trial protocol is available on clinicaltrials.gov (NCT04723732). Written informed consent was obtained from all participants before their inclusion in the study, which took place from August 2017 to April 2018.

Patient selection

Participants were selected from individuals seeking treatment at the dental clinic of King Abdulaziz University Faculty of Dentistry (KAUFD). A poster was displayed in the waiting area to invite patients to voluntarily join the study. Interested individuals were included based on the study's inclusion and exclusion criteria. The inclusion criteria were: good periodontal health (no clinical attachment loss and less than 10% bleeding on probing), having more than twenty teeth with at least 5 teeth per quadrant, no history of systemic diseases, and no oral prophylaxis in the past six months. The exclusion criteria were: pocket depths greater than 3 mm, severe malocclusion, presence of braces or orthodontic wires, use of antibiotics or anti-inflammatory medications in the last six months, tobacco use, non-compliance with the study protocol, and pregnancy or breastfeeding.

Since there were no previous studies on the subject, the sample size was calculated using a pilot study conducted at our center with twelve participants [15]. The pilot study indicated a mean difference of 0.29 ± 0.17 between the experimental and reference groups in post-intervention values. These results were used in a statistical tool, the "sample size calculator for comparing two independent means" [18], which calculated a sample size of 6 patients per group with 80% power and a 5% significance level ($P < 0.05$, two-sided). To account for potential dropouts, 8 patients were recruited per group. A total of 24 eligible participants, consisting of 12 males and 12 females aged between 18 and 55 years, were included in the study.

Procedure

After the initial dental screening, participants completed a medical history questionnaire to verify their eligibility for the study. The first visit included oral hygiene education and professional cleaning procedures, such as oral prophylaxis or supra-gingival scaling if required, which was performed 14 days before the start of the study. During the second session, a periodontal examination was conducted to ensure the gums and periodontium were in good health. Participants were instructed to avoid brushing their teeth or performing any oral hygiene practices for the next two weeks to allow experimental gingivitis to develop.

On the third visit (day 0, the experimental period), a thorough periodontal examination was carried out to record baseline measurements of gingival health, plaque, bleeding, and inflammation. Participants were then randomly assigned to one of three groups using a simple randomization method (computer-generated random numbers), with each group consisting of eight participants: (a) normal saline, (b) 0.2% chlorhexidine gluconate mouthwash, and (c) 1% *Commiphora myrrh* mouthwash. The myrrh mouthwash was prepared according to the procedure detailed in the pilot study [15]. Chlorhexidine gluconate 0.2% (Avalon Pharma, Riyadh) and normal saline 0.9% NaCl solution, five hundred ml (Pharmaceutical Solutions Industry, Jeddah) were used as the positive and negative controls, respectively.

The assignment of mouthwashes to participants was carried out in a double-blind manner. The allocation of treatments was concealed by using anonymous, unlabeled opaque bottles. A general dentist, blinded to the treatment groups at baseline, conducted the initial dental screening, and oral hygiene procedures, and distributed the bottles. Another dentist, unaware of the randomization, performed the periodontal evaluations both before and after the intervention. Participants were instructed to refrain from any daily oral hygiene practices, such as flossing or brushing, and to use 15 ml of the assigned mouthwash twice a day for one minute. They were provided with a measuring cup and asked to shake the bottle before use, avoid other mouthwashes, and refrain from eating or drinking for 30 minutes after using the rinse. Additionally, they were encouraged to report any side effects or discomfort.

Compliance with the mouthwash regimen was monitored through follow-up sheets given to the participants, along with regular phone reminders to ensure correct use. To further verify adherence, participants were asked to return the bottles for an assessment of the remaining solution. After 14 days, the same examiner conducted a follow-up evaluation and recorded the final measurements for all clinical parameters. After the study, professional scaling, oral prophylaxis, and fluoride treatment were administered.

Outcome measures

Primary outcomes were evaluated using the modified gingival index (MGI) by Trombelli *et al.* [19], O'Leary *et al.*'s plaque index (PI) [20], Ainamo and Bay's bleeding on probing (BOP) [21], and human IL-1β ELISA kit (BioVendor R&D–Laboratory medicine a.s., Karasek, Czech Republic). Participants were monitored weekly for PI and MGI values, while BOP and IL-1β levels were assessed at baseline and after the intervention (on day 14) to detect gingival bleeding and signs of active inflammation. A standardized periodontal probe with a 0.6 mm tip and 1 mm markings was employed to measure BOP, with a probing force exceeding 0.25 N (25 g).

For IL-1β sampling, the first premolar (#12) and third molar (#16) were selected. Before gingival crevicular fluid (GCF) collection, supragingival plaque was removed from the teeth. GCF was collected using 2 × 8 mm filter paper strips, which were placed in the gingival crevice for 30 seconds. These strips were then stored in Eppendorf tubes containing 400 µl of phosphate-buffered saline (PBS) and kept on ice until frozen at −20 °C for further analysis. To elute the GCF, distilled water was applied to the strips according to the manufacturer's instructions. The BioVendor Human IL-1β ELISA kit was utilized to measure the IL-1β levels. The test was conducted following the manufacturer's product data sheet, with the absorbance of each strip measured at 450 nm using a spectrophotometer.

Intraexaminer reliability

To assess intraexaminer reliability for sulcular depth measurements, evaluations were carried out on selected patients across two separate visits, spaced one week apart. The assessment of the gingival index and plaque index was conducted using clinical scenarios and images at two distinct time intervals. The reliability of the intraexaminer measurements was determined using intraclass coefficients, yielding values of 0.88 for the gingival index, 0.92 for the plaque index, and 0.82 for sulcular depth.

Data collection and analysis

Statistical analysis was carried out using specialized software (SPSS 24, IBM Corp., Armonk, NY, USA). Data were collected before and after the intervention. Mixed ANOVA was applied for analysis, incorporating one between-subject variable (the

interventions) and one within-subject variable (time, comparing pre-mouthwash and post-mouthwash values). Mauchly's test was used to evaluate the assumption of sphericity, and if violated, adjustments were made using the Greenhouse-Geisser or Huynh-Feldt corrections. Pairwise comparisons with Bonferroni adjustment were conducted as a post-hoc test to identify significant differences between treatment groups at various time points. A P-value of less than .05 was considered statistically important for all analyses.

Results and Discussion

A total of 24 participants were initially enrolled in the study, but five were later excluded due to failure to attend follow-up visits (**Figure 1**). The final study sample consisted of 19 individuals, comprising 10 males and 9 females, distributed as follows: myrrh group (n = 6), CHX group (n = 7), and saline group (n = 6). The participants' mean age was 30 years (± 10.55). At baseline, there were no significant differences among the groups.

Table 1 provides the mean values for MGI, BOP, PI, and IL-1β in each group before and after the intervention. Mixed ANOVA results, shown in **Table 2**, revealed a statistically significant variation in MGI and BOP scores (P = .014 and P < .001, respectively) when both intra- and inter-group variations were analyzed (time * treatment interaction). However, no significant differences were observed in PI and IL-1β across treatment groups or over time.

Pairwise comparisons of outcome measures at the two-time points are presented in **Table 3**. No statistically significant differences were found in mean MGI, BOP, PI, or IL-1β between treatment groups at baseline (P > .05 for all comparisons). After the intervention (time point 2), the myrrh group exhibited significantly lower mean MGI and BOP scores than the control group (mean difference = 1.121, P = .016; mean difference = 44.173, P < .001, respectively). The CHX group also demonstrated lower mean MGI and BOP scores than the control, though the difference in MGI wasn't statistically significant (P=0.09). No significant differences in PI or IL-1β levels were detected among treatment groups at either time point (P > 0.05 for all comparisons).

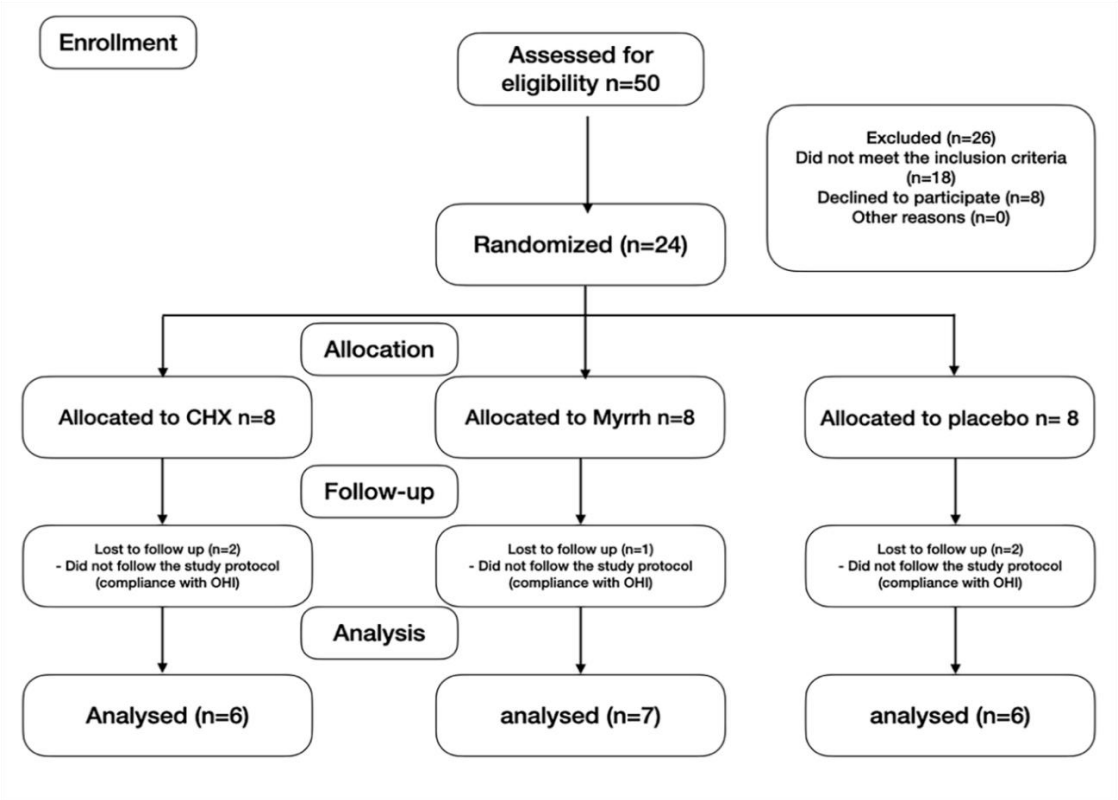

Figure 1. CONSORT 2010 flow diagram participants

Table 1. Descriptive statistics for various measurements

Groups	MGI Mean (SD)		BOP Mean (SD)		PI Mean (SD)		IL-1β average[a] Mean (SD)	
	Baseline	14 Days	Baseline	14 Days	Baseline	14 Days	Baseline	14 Days

Control	3.45 (0.96)	3.91 (0.46)	74.99 (8.83)	83.61 (8.49)	88.23 (28.36)	67.44 (39.18)	74.9 (20.3)	124.3 (88)
CHX	3.5 (0.3)	3.05 (0.53)	71.63 (13.68)	39.44 (16.1)	73.37 (33.3)	86.56 (19.03)	80.8 (29.5)	70.3 (30.1)
Myrrh	3.68 (1.18)	2.79 (0.8)	63.05 (17.61)	40.55 (13.5)	74.46 (34.71)	65.62 (31.83)	90.9 (17.8)	98.4 (46.4)

MGI = Modified gingival index, BOP = Bleeding on probing, PI = Plaque index, IL-1β = interleukin-1β, and SD = Standard deviation.

[a]IL-1β averaged over teeth 12 and 16.

Table 2. Mixed ANOVA results for MGI, BOP, PI, and IL-1β

Measurements	Source	Type III SS	df	Mean square	F	*P*	Non-centrality parameter	Observed power
MGI	Treatment	1.50	2	0.75	0.78	0.47	1.57	0.16
	Time	0.82	1	0.82	3.01	0.10	3.01	0.37
	Time * Treatment	3.03	2	1.52	5.58	.014*	11.16	0.78
BOP	Treatment	5545.83	2	2772.91	9.71	.002*	0.55	19.43
	Time	2227.89	1	2227.89	25.96	<.001*	0.62	25.96
	Time * Treatment	2752.49	2	1376.25	16.03	<.001*	0.67	32.07
PI	Treatment	721.74	2	360.87	0.29	0.75	0.04	0.58
	Time	283.79	1	283.79	0.37	0.55	0.02	0.37
	Time * Treatment	1788.78	2	894.39	1.15	0.34	0.13	2.31
IL-1β average[a]	Treatment	3901.64	2	1950.82	0.75	0.49	0.09	1.49
	Time	2262.23	1	2262.23	1.60	0.22	0.09	1.60
	Time * Treatment	5701.12	2	2850.56	2.02	0.17	0.20	4.04

[a]IL-1β averaged over teeth 12 and 16
* Statistically significant (P < .05)

Table 3. Pairwise comparisons of MGI, BOP, PI, and IL-1β scores at two-time points

Outcome measure	Time	(I) Treatment	(J) Treatment	Mean difference (I-J)	Std. Error	*P*	Lower 95% CI	Upper 95% CI
MGI	1	Control	CHX	-0.05	0.53	1	-1.46	1.36
		Control	Myrrh	-0.23	0.51	1	-1.60	1.13
		CHX	Myrrh	-0.18	0.51	1	-1.55	1.18
	2	Control	CHX	0.86	0.36	0.09	-0.11	1.83
		Control	Myrrh	1.12*	0.35	.016*	0.19	2.05
		CHX	Myrrh	0.26	0.35	1	-0.67	1.19
BOP	1	Control	CHX	3.36	8.15	1	-18.42	25.14
		Control	Myrrh	11.94	7.85	0.443	-9.04	32.93
		CHX	Myrrh	8.59	7.85	0.871	-12.40	29.57
	2	Control	CHX	44.17	7.58	<.001*	23.92	64.42
		Control	Myrrh	43.06	7.30	<.001*	23.55	62.57
		CHX	Myrrh	-1.11	7.30	1	-20.63	18.40
PI	1	Control	CHX	14.86	18.70	1	-35.13	64.86
		Control	Myrrh	13.77	18.02	1	-34.41	61.95
		CHX	Myrrh	-1.09	18.02	1	-49.27	47.09
	2	Control	CHX	-19.12	18.01	0.912	-67.26	29.01
		Control	Myrrh	1.83	17.35	1	-44.56	48.21
		CHX	Myrrh	20.95	17.35	0.735	-25.44	67.33
IL-1β	1	Control	CHX	-5.92	13.16	1	-41.08	29.25
		Control	Myrrh	-15.94	12.68	0.68	-49.83	17.95
		CHX	Myrrh	-10.02	12.68	1	-43.91	23.86
	2	Control	CHX	54.00	34.21	0.402	-37.45	145.45
		Control	Myrrh	25.98	32.97	1	-62.15	114.10
		CHX	Myrrh	-28.02	32.97	1	-116.15	60.10

Based on estimated marginal means
*The mean difference is significant at the .05 level.

[b]Adjustment for multiple comparisons: Bonferroni.

This randomized, double-blind clinical trial aimed to evaluate and compare the effectiveness of 1% myrrh mouthwash and 0.2% CHX mouthwash in reducing plaque accumulation, controlling gingival inflammation, inhibiting the inflammatory mediator IL-1β, and improving BOP. Both myrrh and CHX demonstrated effectiveness in decreasing gingival inflammation and BOP when compared to the control solution (0.9% normal saline). However, no significant differences were detected among the three groups concerning PI and IL-1β levels.

The results of this study align with previous research indicating that myrrh mouthwash contributes to gingival inflammation reduction [15-17, 22, 23]. However, the findings differ slightly from those of Bassiouny and Al-Barrak [16] and prior research conducted by our team, where better outcomes were observed, though they didn't reach statistical significance. In the present study, a significant reduction in gingival swelling was noted in the myrrh group relative to the control. Comparable findings were reported in a recent study by Alotaibi *et al.* [17], which documented a significantly lower level of gingival swelling in the myrrh group at the final assessment. Nonetheless, the study by Alotaibi *et al.* [17] reported a greater reduction in gingival inflammation in the CHX group compared to the myrrh group, a finding that contrasts with the current study, where myrrh exhibited superior gingival inflammation control over CHX when compared to the control. These discrepancies may be attributed to differences in study design, the gingival index utilized, and the method of myrrh mouthwash formulation. Additionally, Alotaibi *et al.* [17] employed a commercially available myrrh mouthwash in individuals with gingivitis or mild periodontitis, whereas the present study tested a customized formulation in an experimental gingivitis model.

The findings related to PI in this research differ from the results observed in our pilot study. Although neither myrrh nor CHX produced a significant impact on PI compared to the control, an increase in the mean PI value was noted in the CHX group following the intervention, whereas both the myrrh and control groups exhibited a decrease. This rise in PI despite CHX usage may be attributed to the experimental nature of the research, as CHX was applied to plaque-covered surfaces while participants abstained from any mechanical plaque control for two weeks. A similar outcome was reported in an experimental model by Zanatta *et al.* [24], where 0.12% CHX mouthwash demonstrated minimal antiplaque effectiveness on structured biofilm after 21 days of plaque accumulation. Other factors that could contribute to the observed differences between groups include the limited number of participants in each group and the possibility of unintentional mechanical plaque removal.

In addition to gingival and plaque indices, BOP is a well-established marker for evaluating gingival inflammation and periodontal health [25]. The marked reduction in BOP observed in this study further reinforces the efficacy of myrrh-based mouthwashes in mitigating gingival inflammation and potentially slowing the advancement of periodontal disease. These results are in agreement with a prior double-blinded study by Saeedi *et al.* [26], in which the application of myrrh-based toothpaste on bleeding gingiva led to a significant reduction in gingival bleeding compared to the control group. Furthermore, recent research by Al Eid [27] investigating wound healing after dental extraction reported that participants who used myrrh mouthwash exhibited fewer signs of inflammation and postoperative bleeding than those in the control group.

Myrrh has been proposed as a potential modulator of inflammatory pathways [22, 23, 28-31]. Research has indicated its anti-inflammatory properties in carcinoma cells [22, 32], with its mechanism of action believed to involve the suppression of key inflammatory mediators such as IL-1β, IL-6, tumor necrosis factor-α (TNF-α), nitric oxide (NO), and prostaglandin E2 (PGE2). This effect was highlighted in an animal model of cecal ligation and puncture (CLP) conducted by Kim *et al.* [28], where myrrh administration was associated with a decline in CLP-induced mortality and an inhibition of lipopolysaccharide (LPS)-stimulated peritoneal macrophages. CLP is widely recognized as the standard animal model for sepsis, as it closely replicates the physiological responses seen in human sepsis. The procedure involves cecal perforation to induce peritonitis, triggering an exaggerated immune response that can progress to septic shock [33]. In the present study, no notable impact of myrrh mouthwash was detected on IL-1β levels, which slightly differs from the findings of Kim *et al.* [28]. However, it is important to note that while Kim *et al.* reported suppression of IL-1β and IL-6 in CLP-induced inflammatory mediator production, the same effect was not observed in LPS-induced peritoneal macrophages. Given that our study did not utilize a sepsis model, the results align with the findings of Kim *et al.* Overall, further research is warranted to explore the anti-inflammatory properties of myrrh and its role in modulating various inflammatory mediators.

Myrrh has been recognized for its antibacterial properties, with numerous studies over the years highlighting its effectiveness against infectious diseases [12, 34-38]. Research by Rahman *et al.* [35] demonstrated that various strains of *Klebsiella pneumoniae*, *Salmonella enterica*, and *Staphylococcus aureus* exhibited sensitivity to *Commiphora molmol*. Additionally, its antimicrobial activity extends to oral microorganisms. A recent investigation by Sambawa *et al.* [36] indicated that myrrh's antibacterial potential was relatively comparable to that of CHX. Beyond its antimicrobial effects, myrrh has also been linked to accelerated wound healing. Findings by Al Eid [27] revealed that the use of myrrh mouthwash contributed to improved healing following tooth extraction. In summary, the combined anti-inflammatory, antibacterial, and wound-healing effects of myrrh may account for its notable impact on reducing gingival inflammation in this study.

Myrrh mouthwash shows promise as a potential alternative to CHX mouthwash for managing gingival inflammation, thanks to its minimal side effects, availability, and ease of preparation. However, in this research, myrrh was applied for a brief period and at a low concentration (1%), so the potential side effects of its long-term use need further investigation. Additionally, several limitations were present in this study. First, the small sample size limits the generalizability of the results. A larger randomized trial with a longer follow-up period would provide a more accurate and comprehensive comparison between myrrh and CHX. Second, the study tested myrrh mouthwash in an experimental gingivitis model over a short time frame. Inducing experimental gingivitis is challenging, as it involves suspending oral hygiene, which is not acceptable in typical settings. Third, the primary focus of this study was on the impact of myrrh on gingival inflammation, leaving its effects on other periodontal parameters unexplored. More research with a larger sample size and extended follow-up is needed to validate these findings and explore the effectiveness of higher concentrations of myrrh (e.g., 2% or 3%).

Conclusion

Despite the limitations of this preliminary study, it can be concluded that myrrh-based mouthwash is as effective as 0.2% CHX mouthwash in reducing gingival inflammation and BOP. Given the potential side effects of long-term CHX use, myrrh mouthwash presents a viable alternative. Nevertheless, further studies are necessary to confirm its effectiveness on a broader scale.

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Conflict of Interest: None

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Ethics Statement: The study received ethical approval from the Research Ethics Committee at KAUFD (protocol number: 058-15). The trial protocol is available on clinicaltrials.gov (NCT04723732).

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