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PAPER

The effect of Chios mastic toothpaste on halitosis and oral hygiene in orthodontic patients: a randomized clinical trial

Antigoni Alexiou[1], **Ioulia-Maria Mylonopoulou**[1], **Spyros Papageorgiou**[2], **William Papaioannou**[3] and **Iosif Sifakakis**[1,*]

[1] Department of Orthodontics, National and Kapodistrian University of Athens, Athens, Greece
[2] Department of Biomedical Sciences, University of West Attica, Athens, Greece
[3] Department of Preventive and Community Dentistry, National and Kapodistrian University of Athens, Athens, Greece
[*] Author to whom any correspondence should be addressed.

E-mail: isifak@dent.uoa.gr

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Abstract

Chios mastic gum, derived from Pistacia lentiscus variation chia, has emerged as a significant natural remedy to improve oral health and mitigate halitosis. This study aimed to examine the effect of mastic toothpaste on halitosis, plaque, and gingival indices in adolescents undergoing orthodontic treatment with fixed appliances. This study was a double-blind, placebo-controlled, parallel-group, randomized clinical trial. Thirty-two patients were randomly divided into two groups: A) mastic-toothpaste group and B) placebo-toothpaste group. Participants in both groups used the assigned toothpaste three times daily for 14 d. The primary outcome was objective hydrogen sulfide (H_2S) levels in breath, measured using a gas chromatograph. The secondary outcomes were dimethyl sulfide and methyl-mercaptan levels, as well as the Silness and Löe Gingival Index (GI) and the Modified Silness and Löe Plaque Index (PI-M). Assessments were conducted at baseline and after two weeks. Data were analyzed using the Mann–Whitney U test and Student's t-test. A statistically significant difference was found between interventions, in favor of the mastic group's H_2S ($p = 0.001$). The H_2S median levels decreased from 158 parts per billion (ppb) to 26 ppb. Neither treatment group experienced a different decline in the levels of the other two components. Statistically significant differences were observed in the periodontal parameters, favoring the mastic group. The GI index decreased from 1.8 to 1 [$p < 0.001$, 95% CI: −0.7, −0.4], whereas the PI-M index decreased from 1.2 to 0.8 [$p < 0.001$, (95% CI: −0.5, −0.2)]. Mastic toothpaste may be an alternative option to reduce halitosis in adolescents undergoing orthodontic treatment with fixed appliances. Regular use of this toothpaste may lead to a clinically meaningful reduction in plaque and gingival indices (ClinicalTrials.gov, NCT06766097).

Abbreviations

ppb	parts per billion
GI	Silness and Löe Gingival Index
PI-M	Modified Silness and Löe Plaque Index
VSCs	volatile sulfur compounds
H_2S	hydrogen sulfide
CH_3SH	methyl mercaptan
$(CH_3)_2S$	dimethyl sulfide
P. Gingivalis	Porphyromonas Gingivalis
ICC	Intra-class correlation coefficient

1. Introduction

Halitosis, commonly known as 'bad breath,' is one of the most common reasons for visiting the dentist, followed by dental caries and periodontal disease [1]. Halitosis is estimated to affect approximately 30%–50% of the population to varying degrees [2, 3]. Halitosis is best described as physiological or pathological. Physiological halitosis, or "morning breath," results from reduced salivary flow and

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increased bacterial activity at night [4]. Additionally, lifestyle factors, such as smoking, alcohol consumption, and pungent foods, may contribute to this condition. Hormonal fluctuations, including those that occur during menstruation, can also increase halitosis [5, 6]. Pathological halitosis, which accounts for over 85% of cases, is a chronic condition that predominantly originates in the oral cavity [7]. It is most often caused by bacterial activity on the tongue, inflamed oral tissues, defective dental restorations, dental caries, or oral ulcers [8]. The malodor in this situation results from the degradation of organic components in saliva, leading to the production of volatile sulfur compounds (VSCs), including hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide [$(CH_3)_2S$] [9]. H_2S has been linked to increased gingival cell apoptosis and susceptibility to periodontitis [10]. Anaerobic bacteria commonly associated with halitosis include Capnocytophaga spp., Porphyromonas Gingivalis (P. Gingivalis), Prevotella intermedia, Aggregatibacter actinomycetemcomitans, and Fusobacterium spp [11–13].

Orthodontic treatment, particularly the use of fixed appliances, has been found to increase the risk of halitosis owing to its impact on oral hygiene and bacterial activity, which in turn promotes the production of VSCs [1, 14, 15]. Halitosis can be assessed using three main methods: organoleptic assessment, halimetry, and gas chromatography [7]. Organoleptic evaluations require professionalism to ensure consistency and reliability [1, 16]. The halimeter is more suitable for routine and rapid screening; however, the sensitivity of this method for detecting methyl mercaptan or dimethyl sulfide is reduced [11, 17]. Gas chromatography, by contrast, offers highly accurate qualitative and quantitative analysis of the individual VSCs, making it the preferred method for comprehensive diagnostic evaluation in both routine and complex cases [9, 18, 19]. Halitosis is typically defined by a total VSCs concentration of $\geqslant$150 ppb, a threshold supported across multiple clinical studies [20–22].

Chios mastic gum (CMG), derived from Pistacia lentiscus var. chia has emerged as a significant natural remedy for improving oral health and mitigating halitosis [23]. Studies have shown that chewing mastic gum may significantly benefit individuals undergoing orthodontic treatment by reducing bacterial growth [24–29]. Specifically, CMG has demonstrated inhibitory effects against oral pathogens associated with halitosis and periodontal disease, likely through its capacity to suppress bacterial proliferation, disrupt biofilm formation, and neutralize VSCs, which are key contributors to oral malodor [23, 26]. Moreover, a mastic-based mouthwash reduced H_2S concentrations and halitosis in adolescents wearing fixed orthodontic appliances within a two-week period [30]. However, the effects of mastic toothpaste in orthodontic patients remain unexplored.

This randomized, double-blind, placebo-controlled clinical trial evaluated the efficacy of a mastic toothpaste in reducing halitosis, plaque accumulation, and gingival inflammation in adolescent patients undergoing fixed appliance orthodontic treatments. The null hypothesis was that mastic toothpaste and placebo toothpaste would have similar effects on halitosis, microbial plaque accumulation, and gingival inflammation in adolescents undergoing orthodontic treatment with fixed appliances.

2. Method

2.1. Study design, participants and eligibility criteria

This was a randomized, placebo-controlled, double-blind trial with two parallel groups and 1:1 allocation ratio. The study was conducted on patients undergoing fixed appliance orthodontic treatment with conventional brackets (Copolla 2.0; Dentalline, Birkenfeld, Germany), tied with elastomeric ligatures and bands. The patients were treated by residents in the postgraduate clinic of the Department of Orthodontics of the National and Kapodistrian University of Athens, Greece (NKUA). The inclusion and exclusion criteria are listed in table 1.

Thirty-two consecutive patients who showed up for planned orthodontic visits and met the inclusion criteria were enrolled in the study. The principal investigator handled all the measurements (AA). This trial was registered at ClinicalTrials.gov (NCT06766097). The study was conducted in accordance with the SPIRIT Statement and the CONSORT guidelines were followed for this report. No changes to the protocol were made after trial commencement.

2.2. Intervention

The participants were equally and randomly divided into Group A (mastic toothpaste: Art of Nature Mastiha Toothpaste, Mastihashop, Greece) and Group B (placebo toothpaste from the same company) (table 2).

For 14 d, participants were instructed to use the toothpaste three times daily, for three minutes each time (morning, afternoon, and evening). Participants were advised to follow their regular oral care practices during the study period i.e. the oral hygiene protocol recommended at treatment initiation in the specific site. This protocol does not include the use of mouthwashes. Measurements were taken in the morning, minimum of three hours after brushing, and subjects were instructed not to eat or drink anything but water 3 h prior to testing. Moreover, participants were advised not to eat strongly flavored foods, including

Table 1. Inclusion/exclusion criteria.

Inclusion criteria	Patients aged 13–18 years
	Fixed conventional labial appliances on both dental arches
	Stainless steel brackets/bands on at least 24 teeth for more than 4 months before recruitment
	Bands on the first molars
	Estimated duration of the rest of the treatment more than 1 month
	Last extraction at least 2 months before the start of the experiment (in extraction cases)
	Good general health
	Total initial VSCs levels higher than 150 ppb
Exclusion criteria	Active caries or periodontitis (periodontal pockets $\geqslant$3 mm)
	Mastic allergy
	Dental fluorosis or dysplasia
	Craniofacial syndromes or abnormalities
	Mental health issues, tobacco use
	Chlorhexidine or other mouthwash use in the previous three weeks
	Engagement in other clinical trials

Table 2. Composition of mastic and placebo toothpastes.

Toothpaste	Ingredients
Mastic	Sorbitol, Glycerin, Chios Mastic Aqua (Water) 15,5%, Pistacia Lentiscus (Mastic) Gum 1%, Pistacia Lentiscus (Mastic) Gum Oil 0.2%, mastiha flavor 0,4%, Hydrated Silica, PEG-32, PVM/MA Copolymer, Titanium Dioxide, Sodium Lauroyl Sarcosinate, Elaeis Guineensis (Palm) Oil, Cocos Nucifera (Coconut) Oil, Xanthan Gum, Sodium Fluoride, Sodium Benzoate, Sodium Saccharin, Sodium Hydroxide
Placebo	Hydrated Silica, PEG-32, PVM/MA Copolymer, Titanium Dioxide, Sodium Lauroyl Sarcosinate, Elaeis Guineensis (Palm) Oil, Cocos Nucifera (Coconut) Oil, Xanthan Gum, Sodium Fluoride, Sodium Benzoate, Sodium Saccharin, Sodium Hydroxide

garlic and spices, for 24 h prior to the mastic toothpaste assessment [13].

2.3. Outcomes

Clinical measurements were conducted at baseline ($T0$) and after 2 weeks of toothpaste use ($T1$). The primary outcome was the H_2S levels in breath, and the secondary outcomes were the levels of the other two VSCs [CH_3SH and $(CH_3)_2S$], as well as the modified Plaque (PI-M) and Gingival (GI) indices [31, 32].

The three VSCs were measured using the gas chromatograph OralChroma^{TM} II (CHM-2) (NOVATRONIC Deutschland GmbH, Kölner Straße 102, D-51429 Bergisch Gladbach, Germany). Discreet 1 ml plastic syringes were used to collect the air samples. Patients were instructed to keep their lips closed and spend 30 s breathing via their nose. The plunger was then pulled back to obtain the air sample. The 0.5 ml sample was then transferred to the chromatograph for examination.

The original Silness and Löe Plaque Index (1964) [31] was not designed for orthodontic patients. The present study adopted a widely used modification of this index, in which the teeth are divided into incisal, distal, and mesial areas relative to the bracket, and plaque is graded in each area using values from 0 to 3 [30, 33]. The sum of the values for each tooth ranged from zero to nine. The mean score for each patient was calculated as the sum of all the teeth divided by the measured surfaces (table 3). The banded molars were not evaluated during PI-M measurements. Modification of this index has a much higher categorical distinctive ability in patients with a fixed orthodontic appliances [30, 33, 34].

For every individual, the mean GI score was calculated using the same approach as for the PI-M; each tooth's three designated areas of the buccal surface—mesial, cervical, and distal—were evaluated (table 4). The examination included banded molars [30, 32, 34].

2.4. Sample size calculation

The sample size was calculated based on an expected mean difference in VSCs levels of 50 ppb, a standard deviation of 60, a 95% confidence interval, and an 80% statistical power. This study showed that each group required at least 12 members [35]. Sixteen people were registered in each group to strengthen the research and help offset potential dropouts.

2.5. Randomization and concealment

Using stratified randomization, participants were paired in a 1:1 ratio for each sex to either Group A or B. Two separate random sequences of 16 letters were generated for males and females using the List Randomizer tool (www.random.org). The assigned

Table 3. Plaque index.

0	No plaque
1	Thin plaque attached to the gingival margin and adjacent areas of the tooth. Plaque is only visible after applying a revealing solution or using a probe on the tooth surface
2	Moderate accumulation of soft plaque deposits in the gingival sulcus or plaque on the gingival margin and adjacent areas of the tooth visible to the naked eye
3	Abundant soft plaque deposits in the gingival sulcus or plaque on the gingival margin and adjacent areas of the tooth

Table 4. Gingival index.

0	Normal gums
1	Mild inflammation, slight change in color and distinct change in texture, no bleeding upon probing
2	Moderate inflammation, moderate redness, and swelling, bleeding upon probing
3	Severe inflammation, visible swelling, ulceration, bleeding upon probing, and/or spontaneously

letters were printed on paper and sealed tightly inside opaque consecutively numbered envelopes, labeled M1–M16 for men and F1–F16 for women. A person unrelated to the study was assigned to open each envelope in a sequential numerical order and distribute the corresponding toothpaste to the participants. Informed consent was obtained from the parents, legal guardians, and the subjects themselves. To ensure that both participants and researchers were blinded to the treatment distribution, all patients received toothpaste in identical packaging.

2.6. Statistical analysis

The ICC was used to evaluate inter- and intra-observer reliability. To assess intra-observer reliability, VSCs measurements were repeated in 10 patients by the principal investigator. Inter-observer error was also evaluated by repeating the measurement in 10 patients by the second author (IM) and the principal investigator. Based on the data distribution, the data were statistically evaluated using the Mann–Whitney *U* test for the VSCs levels and Student's *t*-test for the oral hygiene indices. Statistical significance was set at $P < 0.05$. Blinding was used to evaluate results. IBM SPSS Statistics 23.0 was used for all statistical tests.

3. Results

3.1. Participant flow

Sixty-seven patients were assessed for eligibility, and 32 met all inclusion criteria and were registered in the study, which started in July 2024 and ended in November 2024 (figure 1).

3.2. Baseline data

The baseline data are presented in table 5. The mean age (±standard deviation) was 14.94 (±1.39) and 14.88 (±1.54) years for the mastic and placebo group respectively.

3.3. Outcomes and estimation

ICC indicated excellent agreement of 0.94 (intra-observer) and 0.92 (inter-observer). The VSC results were not normally distributed. Within the mastic group (group A), the median total VSCs levels dropped from 257.50 ppb to 71.50 ppb. Specifically, H_2S concentrations dropped from 158.50 ppb to 16.00 ppb; CH_3SH dropped from 55.00 ppb to 23.50 ppb; and $(CH_3)_2S$ dropped from 16.00 ppb to 12.00 ppb. In the placebo group (Group B) the median VSCs levels dropped from 236.00 ppb to 174.00 ppb, H_2S levels decreased from 98.50 ppb to 83.00 ppb, CH_3SH dropped from 73.50 ppb to 52.00 ppb, and $(CH_3)_2S$ levels decreased from 32.00 ppb to 24.50 ppb. The treatment effect was statistically significant for total VSCs and H_2S levels in favor of the mastic toothpaste group ($p = 0.001$).

Oral hygiene measures showed a normal distribution. Group B's score dropped from 1.12 to 1.02, and Group A's mean score on the PI-Mdropped from 1.23 0.78. In Group A, the GI mean score dropped from 1.81 to 1.08; in Group B, it decreased from 1.72 to 1.53. For GI and PI-M scores, the treatment effect was statistically significant ($p < 0.001$), independent of the mastic toothpaste group (table 5).

4. Discussion

Thus, the null hypothesis of this study was partially rejected. After 2 weeks of mastic toothpaste use in orthodontic patients, significant reductions in H_2S levels and oral hygiene indices were observed, in favor of the mastic toothpaste. However, the use of mastic toothpaste had no impact on CH_3SH or $(CH_3)_2S$. Although $(CH_3)_2S$ is a minor contributor to intra-oral halitosis [36], it was included in the present investigation to provide a comprehensive evaluation of the toothpaste's efficacy. These findings align with previous research that highlighted the antimicrobial efficacy of mastic against P. Gingivalis and its role as a natural alternative to chemical mouthwashes [37]. A recent trial concluded that mastic mouthwash

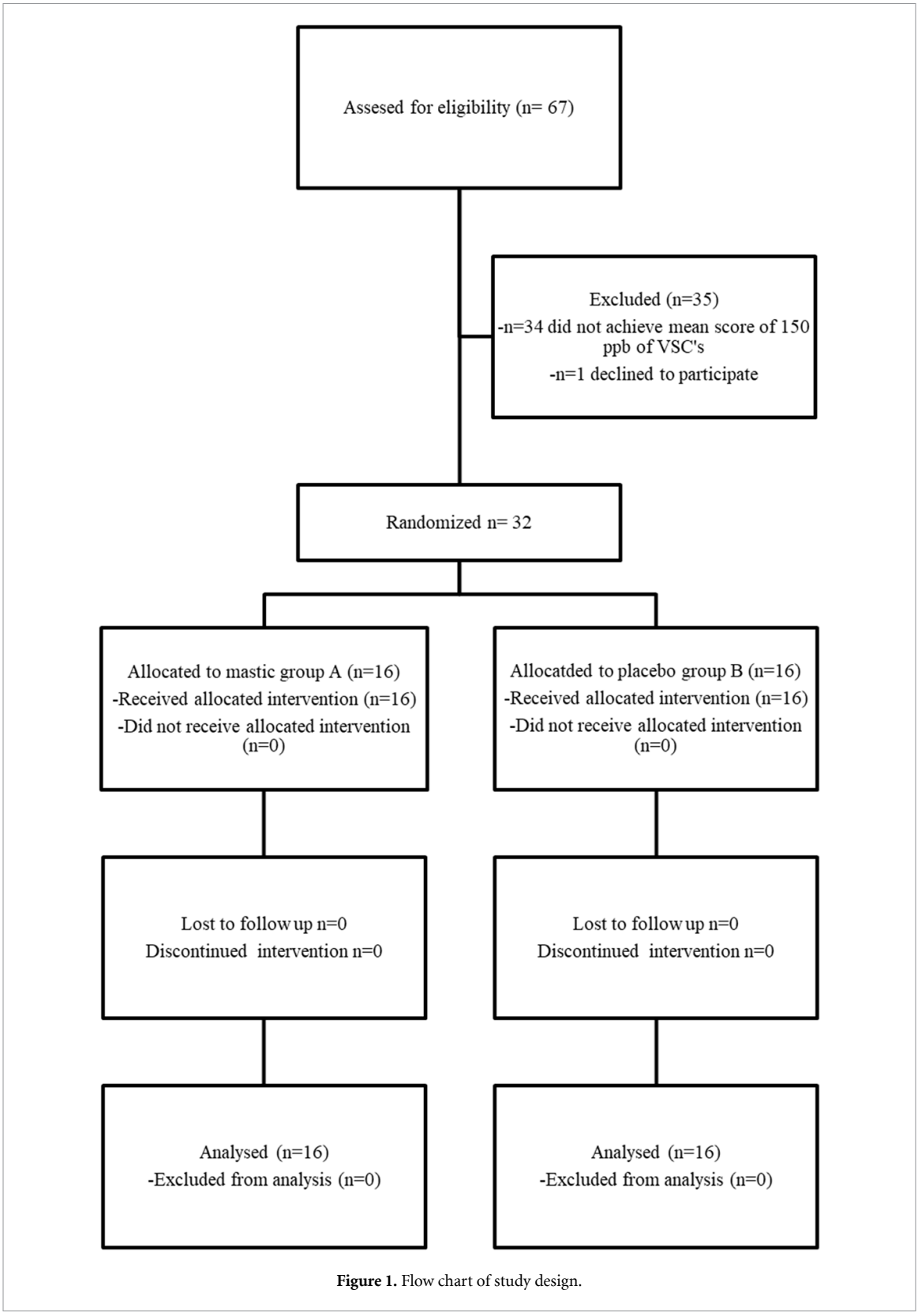


Figure 1. Flow chart of study design.

may significantly reduce VSCs levels in orthodontic patients, further supporting the role of mastic in halitosis management [30].

The two-week application of mastic toothpaste resulted in a significant improvement in oral hygiene indices among orthodontic patients. Participants were advised to follow their regular oral care practices during the study period, guaranteeing that any possible effects of toothpaste were not hidden by improvements in their oral hygiene habits, thus mitigating the impact of changes in oral hygiene routines. The bioactive components of mastic,

Table 5. H_2S, CH_3SH, $(CH_3)_2S$, total VSCs levels and periodontal indices scores at T0 and T1 for the mastic group (group A) and the placebo group (group B) (median and mean values, IQR and SD, effect sizes).

		A: Mastic group		B: Placebo group			
		Median	IQR	Median	IQR	*p*-value*	*U*
H_2S	T0	158.50	195.00	98.50	140.00		
	T1	16.00	59.00	83.00	114.00	0.001	43.50
CH_3SH	T0	55.00	80.00	73.50	93.00		
	T1	23.50	37.00	52.00	83.00	0.897	124.00
$(CH_3)_2S$	T0	16.00	42.00	32.00	45.00		
	T1	12.00	23.00	24.50	38.00	0.323	101.50
Total VSCs	T0	257.50	260.00	236.00	164.00		
	T1	71.50	96.00	174.00	192.00	0.001	235.00
		Mean	SD	Mean	SD	*p*-value**	*t*
PI-M	T0	1.23	0.33	1.12	0.28		
	T1	0.78	0.30	1.02	0.27	<0.001	−5.55
GI	T0	1.81	0.36	1.72	0.44		
	T1	1.08	0.33	1.53	0.38	<0.001	−6.92

The significance of differences between groups was assessed using the Mann–Whitney *U* test (*p*-value*) and Student's t-test (*p*-value**). IQR, interquartile range; SD, standard deviation; H_2S, hydrogen sulfide; CH_3SH, methyl mercaptan; $(CH_3)_2S$, dimethyl sulfide; VSCs, volatile sulfur compounds.

including triterpenic acids, flavonoids, and polyphenols, are well documented for their antibacterial, anti-inflammatory, and antioxidant properties [26, 29]. Mastic gum inhibits the proliferation of key bacterial species implicated in periodontal disease and VSC production, such as P. gingivalis, Fusobacterium nucleatum, and S. mutans [26]. Additionally, its anti-inflammatory effects involve the modulation of pro-inflammatory cytokines, including tumor necrosis factor-α and interleukin-1β, which are associated with gingival inflammation [29]. Moreover, its antioxidant properties help neutralize free radicals, thereby protecting oral tissues from damage associated with periodontal diseases. The efficacy of mastic-based oral hygiene products in reducing plaque scores and improving oral hygiene indices makes them particularly beneficial for individuals undergoing orthodontic treatment [23].

Throughout our 14 day study period, no adverse effects were observed among participants. This is consistent with existing research showing that Chios mastic, owing to its natural bioactive compounds, has a favorable safety profile. Studies have demonstrated that mastic extract exhibits low cytotoxicity on oral cell lines, particularly when compared to conventional antiseptics such as chlorhexidine and hydrogen peroxide [26]. Its antimicrobial properties and biocompatibility have also been well documented [23, 25]. These findings support the safe use of mastic in adolescent populations and highlight its potential for long-term application in oral hygiene routines. Not only mastic, but several natural compound-based toothpastes, containing Carica papaya leaf, Scutellaria baicalensis extracts and others, may considerably improve oral hygiene and reduce oral malodor [38–41].

Despite these promising findings, this study has several limitations. The relatively small sample size and short follow-up period limited the ability to assess the long-term effects and potential adverse reactions associated with mastic toothpaste use. In addition, the study was conducted within a single institution and focused on an adolescent population, which may restrict the generalizability of the results. The participants were advised to follow the regular oral hygiene practices, recommended in the specific postgraduate clinic at treatment initiation. However, these practices may have varied among them, confounding the present results. Furthermore, differences in oral microbial composition and patient compliance may influence outcomes in adult populations. Microbial profiling at baseline would provide valuable insights, strengthen the internal validity of future studies and could be a meaningful direction for further research. Additionally, the study did not employ a cysteine challenge prior to gas chromatography analysis, which could have enhanced the possibility of detecting VSCs during halitosis diagnosis. Future studies may benefit from incorporating cysteine pre-administration to better control inter- and intra-individual variability [42].

The present study is the first to assess the effects of mastic toothpaste on halitosis in adolescents undergoing fixed orthodontic appliance therapy. We used the latest generation of gas chromatograph OralChroma, which exhibits enhanced sensitivity, particularly for H_2S [18]. Our findings suggest that mastic toothpaste can be an effective adjunct for managing halitosis and improving oral hygiene in these patients. Long-term studies are needed to evaluate its sustained effectiveness, potential interactions with the oral microbiome, and benefits for

broader populations, including individuals with periodontal disease or systemic conditions that affect oral health.

5. Conclusions

After two weeks of use, mastic toothpaste reduced objective hydrogen sulfide levels in oral breath and halitosis, as well as plaque and gingival indices, in adolescents undergoing orthodontic treatment with fixed appliances. This intervention did not affect the concentration of methyl mercaptan or dimethyl sulfide.

Data availability statement

The data cannot be made publicly available upon publication because they contain sensitive personal information. The data that support the findings of this study are available upon reasonable request from the authors.

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Conflict of interest

The authors declare that there is no conflict ofinterest.

Ethical statement

This study was approved by the Ethics Committee of of the School of Dentistry, NKUA, Greece (613/09.11.2023).

ORCID iDs

Antigoni Alexiou 0000-0003-3331-1633
Ioulia-Maria Mylonopoulou 0000-0002-8275-6690
Spyros Papageorgiou 0000-0002-6267-1112
William Papaioannou 0000-0001-5146-4399
Iosif Sifakakis 0000-0001-9686-7423

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