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RESEARCH ARTICLE

Antimicrobial activity of *Crocus sativus* (saffron) and *Syzygium aromaticum* (clove) against *Streptococcus mutans*: An in vitro study

Crocus sativus (safran) ve *Syzygium aromaticum* (karanfil)'un *Streptococcus mutans*'a karşı antimikrobiyal aktivitesi: İn vitro bir çalışma

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Abstract

Aim: This study aimed to evaluate the antimicrobial activity of extracts and aqueous infusions derived from Crocus sativus and Syzygium aromaticum against *Streptococcus mutans* and to compare their efficacy with chlorhexidine.

Methods: Experimental groups consisted of extracts and aqueous infusions prepared from *Crocus sativus* and *Syzygium* aromaticum. A commercially available 0.2% chlorhexidine mouthwash was used as the positive control. Antimicrobial activity against *Streptococcus mutans* ATCC 25175 strain was assessed using the disk diffusion method. Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations were determined using the broth microdilution method with well plates. The normality of the data distribution was assessed using the Shapiro–Wilk test and non-parametric statistical tests were applied. $p<0.05$ were considered statistically significant.

Results: *Crocus sativus* extracts and aqueous infusions did not exhibit a measurable antimicrobial effect against *Streptococcus mutans*. In contrast, *Syzygium aromaticum* extract showed concentration-dependent antibacterial activity. The antimicrobial effect of the 25% Syzygium aromaticum extract was not significantly different from that of 0.2% chlorhexidine, whereas the aqueous infusion produced a significantly smaller inhibition zone than chlorhexidine. The extract exhibited MIC and MBC values of 15.625 mg/mL and 62.5 mg/mL, respectively, indicating bactericidal activity.

Conclusion: In this *in vitro* study, *Crocus sativus* showed no significant antibacterial activity against *Streptococcus mutans*. In contrast, both the extract and aqueous infusion of Syzygium aromaticum demonstrated bactericidal activity; the 25% extract produced inhibition zones comparable to those of chlorhexidine, whereas the aqueous infusion was effective but significantly less so than chlorhexidine. These findings highlight the potential of *Syzygium aromaticum* as a natural antimicrobial candidate for oral health applications. Further investigations using multispecies biofilm models and clinical studies are needed to confirm its therapeutic potential.

Keywords: Chlorhexidine, *Streptococcus mutans, Syzygium aromaticum, Crocus sativus,* herbal mouthwash.

Özet

Amaç: Bu çalışma, *Crocus sativus* ve *Syzygium aromaticum* bitkilerinden elde edilen ekstraktlar ve sulu infüzyonların *Streptococcus mutans*'a karşı antimikrobiyal aktivitelerini değerlendirmeyi ve etkinliklerini klorheksidin ile karşılaştırmayı amaçlamıştır.

Yöntemler: Deney grupları, *Crocus sativus* ve *Syzygium aromaticum* bitkilerinden hazırlanan ekstraktlar ve sulu infüzyonlardan oluşturulmuştur. Pozitif kontrol olarak ticari olarak temin edilen %0,2 klorheksidin gargarası kullanılmıştır. *Streptococcus mutans* ATCC 25175 suşlarına karşı antimikrobiyal aktivite disk difüzyon yöntemi ile değerlendirilmiştir. Minimum İnhibitör Konsantrasyon (MİK) ve Minimum Bakterisidal Konsantrasyon (MBK) değerleri, kuyucuklu plaklarda broth mikrodilüsyon yöntemi kullanılarak belirlenmiştir. Verilerin normal dağılıma uygunluğu Shapiro–Wilk testi ile değerlendirilmiş ve non-parametrik istatistiksel testler uygulanmıştır. $p<0,05$ değeri istatistiksel olarak anlamlı kabul edilmiştir.

Bulgular: *Crocus sativus* ekstraktları ve sulu infüzyonları, *Streptococcus mutans*'a karşı ölçülebilir bir antimikrobiyal etki göstermemiştir. Buna karşılık, *Syzygium aromaticum* ekstraktı konsantrasyona bağlı antibakteriyel aktivite sergilemiştir. %25'lik Syzygium aromaticum ekstraktının antimikrobiyal etkisi %0,2 klorheksidin ile istatistiksel olarak anlamlı farklılık göstermezken, sulu infüzyonun inhibisyon zonu klorheksidinden anlamlı olarak daha küçük bulunmuştur. Ekstrakt için MİK ve MBK değerleri sırasıyla 15,625 mg/mL ve 62,5 mg/mL olarak belirlenmiş olup, bu sonuç bakterisidal aktiviteyi göstermektedir.

Sonuç: Bu in vitro çalışmada, *Crocus sativus*'un *Streptococcus mutans*'a karşı anlamlı bir antibakteriyel aktivite göstermediği belirlenmiştir. Buna karşın, *Syzygium aromaticum*'un hem ekstraktı hem de sulu infüzyonu bakterisidal aktivite sergilemiş; %25'lik ekstrakt klorheksidine yakın inhibisyon zonu oluştururken, sulu infüzyon daha düşük ancak ölçülebilir bir aktivite göstermiştir. Bu bulgular, *Syzygium aromaticum*'un ağız sağlığı uygulamalarında doğal bir antimikrobiyal ajan adayı olabileceğini göstermektedir. Bununla birlikte, terapötik potansiyelinin doğrulanabilmesi için çok türlü biyofilm modelleri ve klinik çalışmalarla desteklenen ileri araştırmalara ihtiyaç vardır.

Anahtar Kelimeler: Klorheksidin, *Streptococcus mutans, Syzygium aromaticum, Crocus sativus,* bitkisel gargara.

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INTRODUCTION

Dental caries, primarily caused by microbial dental plaque, remains one of the most prevalent chronic diseases worldwide and is a significant public health concern. The oral microbiome comprises more than 700 bacterial species, with approximately 200 species colonizing the oral cavity of an individual, forming a complex microbial ecosystem that plays a critical role in oral health and disease (1). Among these, *Streptococcus mutans (S. mutans)* plays a central role in the etiology of dental caries. *S. mutans* adheres to tooth surfaces and ferments sucrose, ultimately leading to the production of extracellular polysaccharides and acids (2). Therefore, reducing the number of S. mutans or inhibiting its adhesion to tooth surfaces may decrease acid production, prevent critical reductions in plaque pH, and ultimately reduce the risk of dental caries (3).

Today, a range of mechanical, chemical, and biological preventive strategies are being developed to reduce the incidence of dental caries. Mouthwashes, used in dentistry for both preventive and therapeutic purposes, help inhibit dental plaque formation through a chemomechanical mechanism. Among these mouthwashes, chlorhexidine is considered the gold standard for reducing *S. mutans* levels (1). However, chlorhexidine has limitations, including staining of the teeth and tongue, an unpleasant taste, and increased calculus formation. In addition, as bacteria develop resistance over time, many antibacterial agents tend to lose their effectiveness (4). These challenges have led to increased interest in identifying natural antibacterial products that are safe for human use, specifically target microorganisms involved in dental caries, and have minimal side effects.

The saffron plant, scientifically known as *Crocus sativus L.*, is a small, perennial member of the Iridaceae (iris) family cultivated in various parts of the world as a traditional medicinal plant. Its dried female reproductive part (stigma), commonly referred to as saffron, is used in the food industry as a fragrant spice and coloring agent, as well as in the pharmaceutical industry for its soothing and analgesic effects in the treatment of asthma, whooping cough, and inflammation (5). Recent studies have demonstrated that the saffron plant may alleviate certain health conditions. Specifically, the flowers of *Crocus sativus* were found to contain bioactive compounds with antimicrobial properties against streptococci *(Streptococcus pyogenes)* (6). The antimicrobial activity of saffron has been reported to originate from compounds such as safranal and crocin (7). These compounds exert antimicrobial effects due to their solubility in water and volatility, which facilitate their access to microbial cells (8). The saffron plant is primarily cultivated for its stigmas in countries such as Iran, Spain, Morocco, Turkey, India, Greece, and Italy (9).

Clove *(Syzygium aromaticum)* is an evergreen plant native to regions around Iraq and the Mediterranean and can grow to a height of 8–12 meters (10). Clove was traditionally used to treat toothaches, insect bites, gastroenteritis, and intestinal parasites. In dentistry, it is widely used as a sedative and as a component in cement materials (11). The essential oil of clove has been reported to possess antimicrobial activity, with eugenol identified as its major antimicrobial component which acts by denaturing microbial proteins (12). Although numerous previous studies have demonstrated the antibacterial effects of essential oils and extracts from rosemary, eucalyptus, and clove (13), available research investigating the use of these agents in mouthwash form as antimicrobial agents specifically targeting S. mutans remains scarce.

For many years, antibiotics and other antimicrobial agents have been used to treat infectious diseases. However, the emergence of antimicrobial resistance among pathogenic bacteria has become an increasingly common problem. Consequently, researchers have turned to plant-based sources in search of new agents with potential antimicrobial chemotherapeutic effects (14). Various studies have shown that natural plant extracts, due to their richness in phenolic compounds, may possess strong antimicrobial properties (15). With growing public concern over chemical preservatives, scientific attention has shifted toward natural alternatives.

Therefore, the aim of the present study was to evaluate the antimicrobial activity of extracts and aqueous infusions derived from Crocus sativus and Syzygium aromaticum against S. mutans and to compare their effects with those of chlorhexidine. The null hypothesis was that no significant differences would exist between

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chlorhexidine and the tested herbal preparations in terms of antibacterial activity against S. mutans.

METHODS

Study Design and Ethical Approval

This *in vitro* experimental study was conducted at the Department of Pediatric Dentistry, the Department of Medical Microbiology, and the Department of Organic Chemistry at Karabük University. Ethical approval was obtained from the Ethics Committee of Karabük University (Approval No: 2024/1758).

Plant Material and Origin

Crocus sativus stigmas were collected during the flowering period from a cultivated agricultural field in Karabük. The plant material was authenticated by a botanist in the Department of Biology, Faculty of Arts and Sciences, Karabük University. After harvesting, the stigmas were dried at room temperature (22 °C) in a dark, well-ventilated environment.

Syzygium aromaticum (clove) dried flower buds were obtained from a licensed commercial herbal supplier and originated from Zanzibar, Tanzania. All plant materials were stored in airtight containers protected from light and humidity until use.

Preparation of Herbal Extracts

The dried plant materials were ground separately into a fine powder using a sterile mortar and pestle. Each plant material (*Crocus sativus* stigmas and *Syzygium aromaticum* flower buds) was extracted separately using a Soxhlet apparatus with ethanol (analytical grade) as the solvent. Approximately 5 g of each powdered plant material was placed into individual extraction cartridges, and extraction was carried out for 6–8 hours until the solvent in the siphon tube became colorless. The extraction process was repeated three times to ensure adequate yield.

After extraction, the solvent was removed under reduced pressure using a rotary evaporator at 40–45 °C, and the crude extracts were obtained. The dried extracts were weighed, and the extraction yield was calculated as the percentage of dry extract obtained relative to the initial plant material weight (w/w %).

The dried crude extracts were reconstituted in sterile distilled water. For each extract, 5 g, 10 g, and 25 g of dried extract were dissolved and the final volume was adjusted to 100 mL to obtain 5%, 10%, and 25% (w/v) solutions, respectively (16). The solutions were freshly prepared before each experiment (Figure 1).

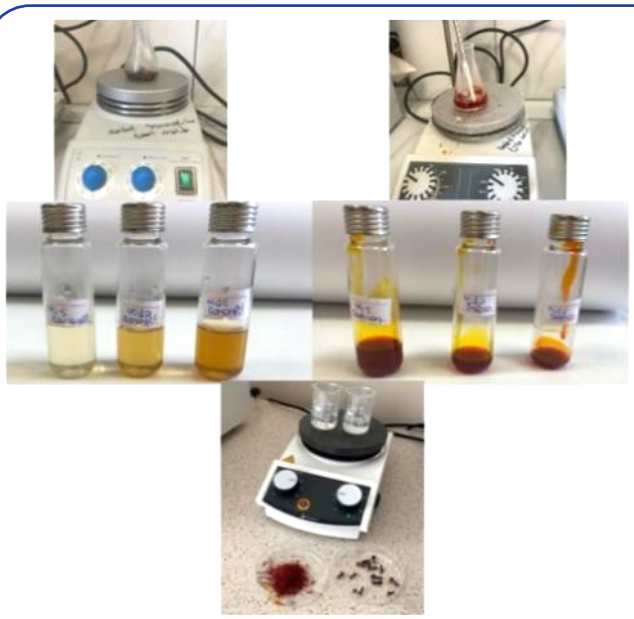

Figure 1. Preparation of herbal extracts and aqueous infusions

Preparation of Aqueous Infusions

Aqueous infusion of *Crocus sativus* was prepared by adding 100 mL of boiling water to 10 g of dried stigmas, and aqueous infusion of Syzygium aromaticum was prepared by adding 100 mL of boiling water to 10 g of flower buds. After 30 minutes of infusion, the infusions were filtered through 0.45 µm and 0.22 µm filters (17). The infusions were stored at 4 °C and used within 24 hours.

Control Solution

Commercial 0.2% chlorhexidine digluconate mouthwash (Klorhex gargara®, Drogsan, Turkey), which reflects concentrations commonly used in clinical practice, was used as the positive control.

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Preparation of Culture Media for Bacterial Cultivation

S. mutans was cultured in Tryptic Soy Broth (TSB, Merck, Germany) and on Tryptic Soy Agar (TSA, Merck, Germany); Mutans-Sanguis Agar (Merck, Germany) was additionally used for the disk diffusion assay. All media were prepared according to the manufacturer's instructions and sterilized in an autoclave at 121 °C for 15 min. The agar media were then poured into Petri dishes (approximately 20 mL per plate) and allowed to solidify.

Preparation of Bacterial Suspension

The S. mutans ATCC 25175 strain was selected because it is widely used as a reference strain in the literature, has well-characterized properties, and provides consistent, reproducible results under laboratory conditions. The strain used in this study was obtained from the Department of Medical Microbiology Laboratory at Karabük University S. mutans (ATCC 25175) was activated by incubation in Tryptic Soy Broth (TSB) and on Tryptic Soy Agar (TSA) at 37 °C for 24 h under anaerobic conditions. The resulting colonies were assessed for viability and purity before subsequent use.

Determination of Inhibition Zones by Disk Diffusion Method

The commercially obtained *S. mutans* (ATCC 25175) strain was activated by incubation in TSB and TSA media at 37°C for 24 h under anaerobic conditions. At the end of the incubation, the resulting colonies were suspended in 0.1 M PBS buffer, and the bacterial density was adjusted to a 0.5 McFarland standard. Then, 100 µL of the prepared *S. mutans* suspension was transferred onto Mutans-Sanguis Agar and evenly spread across the surface using a sterile glass rod. Standard blank antibiotic discs were placed on the Petri dishes, and 12.5 µL of each of the following solutions was applied per disc: Crocus sativus extract (5%, 10%, 25%), aqueous infusion of Crocus sativus, Syzygium aromaticum extract (5%, 10%, 25%), aqueous infusion of Syzygium aromaticum, and chlorhexidine solution. The prepared Petri dishes were incubated at 37°C for 24 h under anaerobic conditions. At the end of the incubation period, the inhibition zone was measured from the edge of the 6 mm disc to the outer edge of the clear zone, and values are reported as the net inhibition zone, excluding the disc. All antimicrobial assays were performed in triplicate, and the mean inhibition zone diameters were calculated for statistical analysis.

Determination of Minimum Inhibitory Concentrations

The minimum inhibitory concentration (MIC) values were determined using the broth microdilution (BMD) method in sterile 96-well microplates according to the principles outlined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, version 10.0). *S. mutans* colonies grown on TSA were incubated anaerobically at 37°C for 24 h and, then suspended in TSB to adjust the bacterial density to 0.5 McFarland standard. Each well contained Tryptic Soy Broth as the growth medium, the test agent at the relevant concentration, and the bacterial inoculum, with a final volume of 100 µl per well.

For the plant preparations (*Crocus sativus* and *Syzygium aromaticum* extracts and aqueous infusions), the initial concentration in the first well was 250 mg/mL, and serial two-fold dilutions yielded 125, 62.5, 31.25, 15.625, 7.8125, 3.906, 1.953, 0.976, 0.488, and 0.244 mg/mL. For chlorhexidine, the initial concentration was 1 mg/mL (1000 µg/mL), with two-fold dilutions yielding 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.906, 1.953, and 0.976 µg/mL.

Except for the last two wells, 10 µL of microorganism inoculum were added to all wells. One well that contained only the culture medium and microorganism was designated as the microorganism growth control. Another well that contained only the culture medium and the extract was used as the contamination control. After anaerobic incubation of the prepared microplate at 37°C for 24 h, the lowest concentration at which microbial growth was inhibited was determined as the MIC value. Starting from the well where the MIC was identified, samples from all wells showing no bacterial growth were inoculated onto Mutans-Sanguis agar and incubated anaerobically at 37°C for 24 h to determine the minimum bactericidal concentration (MBC) value (see Figure 2).

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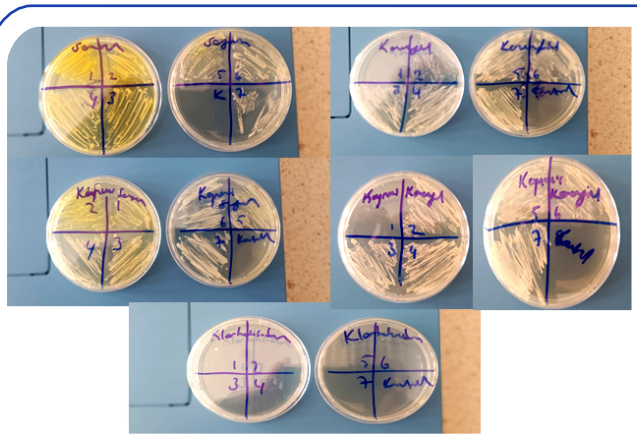

Figure 2. Determination of MIC and MBC values

Table 1. **Comparing inhibition zones between *Syzygium aromaticum*, *Crocus sativus* and chlorhexidin**

Concentration	Syzygium Aromaticum		Crocus Sativus	
	Mean (mm)	St. Dev	Mean (mm)	St. Dev
5 %	4.20[a]	1.17	0	0
10 %	6.60[b]	1.78	0	0
25 %	11.40[c]	3.47	0	0
Infused solution	6.40[b]	2.76	0	0
Chlorhexidin (0.2 %)	12.40[c]	2.31	0	0
p	**0.032**		-	

Different lowercase letters in the same column indicate a statistically significant difference. Inhibition zone diameters are reported as net values, excluding the 6 mm disc.

Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive data were presented as mean ± standard deviation (SD). The normality of inhibition zone diameter data was assessed using the Shapiro–Wilk test. Since the data were not normally distributed, non-parametric statistical methods were used. Differences among the inhibition zone diameters of the tested groups were evaluated using the Kruskal–Wallis test. When a statistically significant difference was detected, pairwise comparisons were performed using Dunn's post hoc test with Bonferroni correction. All disk diffusion assays were performed in triplicate, and the mean values were used for statistical analyses. A p-value < 0.05 was considered statistically significant. MIC and MBC values obtained by the broth microdilution method were reported descriptively.

RESULTS

The antimicrobial effects of Syzygium aromaticum and Crocus sativus extracts at different concentrations, as well as their aqueous infusions against *Streptococcus mutans* ATCC 25175 were evaluated based on inhibition zone diameters (mm) (Table 1).

For *Syzygium aromaticum* extracts, inhibition zone diameters increased with concentration. The mean inhibition zone diameter was 4.20±1.17 mm at 5%, 6.60±1.78 mm at 10%, and 11.40±3.47 mm at 25%. A statistically significant difference was observed among the tested concentrations (p=0.032), indicating a dose-dependent antimicrobial effect. The aqueous infusion of Syzygium aromaticum produced a mean inhibition zone diameter of 6.40±2.76 mm, comparable to that of the 10% extract but significantly smaller than that of chlorhexidine (12.40±2.31 mm). The positive control, 0.2% chlorhexidine, showed a mean inhibition zone diameter of 12.40±2.31 mm. Although the 25% extract exhibited a lower mean inhibition zone than chlorhexidine, the difference was not statistically significant (p=0.07), suggesting a comparable antibacterial effect under the tested conditions.

The relationship between the concentration of the *Syzygium aromaticum* extract and the inhibition zone diameter was assessed using a logarithmic scale. The log dose–response analysis revealed a nonlinear, concentration-dependent increase in inhibition zone diameter with increasing concentrations (Figure 3).

No inhibition zones were observed for Crocus sativus extracts at any concentration or for the aqueous infusion. Therefore, under the experimental conditions of the present study, Crocus sativus did not exhibit antimicrobial activity against Streptococcus mutans ATCC 25175.

The MIC and MBC values obtained by the broth microdilution method are presented in Table 2. For *Syzygium aromaticum* extract, the MIC and MBC values were 15.625 mg/mL and 62.5 mg/mL, respectively. The

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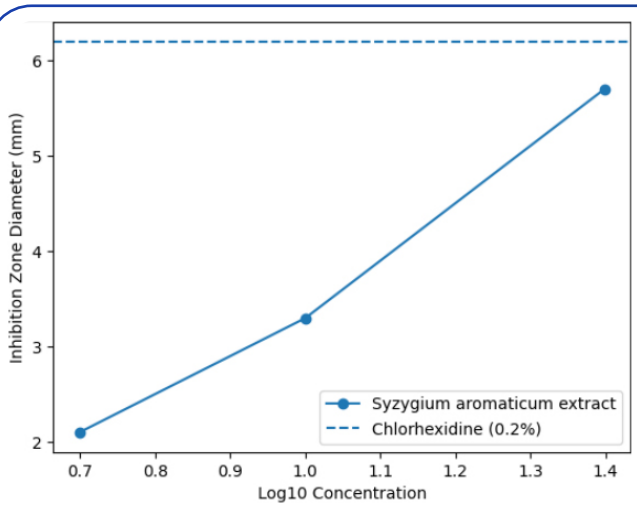

Figure 3. Log-Dose response of *Syzygium aromaticum* against *S. mutans*

MIC and MBC values of the aqueous infusion were 31.25 mg/mL and 62.5 mg/mL, respectively. For chlorhexidine, the MIC and MBC values were 0.976 µg/mL and 31.25 µg/mL, respectively.

Table 2. MIC and MBC values

Variable	MIC	MBC
Syzygium aromaticum extract	15.625 mg/mL	62.5 mg/mL
Infused Syzygium aromaticum solution	31.25 mg/mL	62.5 mg/mL
Chlorhexidin	0.976 µg/mL	31.25 µg/mL

MIC and MBC values could not be determined for *Crocus sativus* extract or its aqueous infusion because bacterial growth occured at all tested concentrations.

The MBC/MIC ratios calculated for the *Syzygium aromaticum* extract and the aqueous infusions of Syzygium aromaticum solution were 4 and 2, respectively.

DISCUSSION

Removing dental plaque, the primary etiological factor for dental caries and gingivitis, plays a crucial role in maintaining oral and periodontal health (18). In routine oral hygiene, particularly for high-risk individuals, chemical agents are commonly used as adjuncts to mechanical plaque control. Among these, chlorhexidine remains the gold standard mouthwash because of its broad-spectrum antimicrobial activity (19). However, its long-term use is associated with undesirable effects including tooth discoloration, taste alteration, mucosal irritation, and potential microbial resistance (20). These limitations have driven the ongoing search for alternative natural agents that provide comparable antimicrobial efficacy with improved safety profiles.

In this context, plant-derived products rich in bioactive compounds have gained increasing attention. Numerous medicinal plants and spices contain phenolic compounds, essential oils, and flavonoids that exhibit antimicrobial, anti-inflammatory, and antioxidant properties (21–23). Both Crocus sativus and Syzygium aromaticum have been traditionally used in herbal medicine, and their bioactive constituents suggest potential relevance for oral health applications. Saffron contains carotenoids such as crocin and safranal, which have been associated with antioxidant and antimicrobial effects (24), whereas clove is rich in eugenol, a phenolic compound known for its strong antimicrobial and analgesic properties (25,26).

In the present study, the antimicrobial activity of both plant species against Streptococcus mutans was investigated in extract and aqueous infusion forms. The findings showed that Crocus sativus, in both preparations, exhibited no detectable antimicrobial activity, as evidenced by the absence of inhibition zones and undetermined MIC/MBC values. In contrast, Syzygium aromaticum demonstrated clear antimicrobial activity, which increased in a concentration-dependent manner and produced inhibition zone values approaching those of chlorhexidine under in vitro conditions. Accordingly, the null hypothesis was rejected for Crocus sativus and for the aqueous infusion, but not for the 25% Syzygium aromaticum extract, which did not differ significantly from chlorhexidine.

The absence of antimicrobial activity of Crocus sativus in this study contrasts with previous reports demonstrating antimicrobial effects of saffron extracts against microorganisms such as Streptococcus pyogenes and various Gram-positive and Gram-negative bacteria (6,24,27). These discrepancies may be attributed to differences in extraction methods, solvent polarity, tested microbial species, and concentrations of active constituents. In particular, the relatively low yield of phenolic compounds in aqueous infusion and possible

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degradation of active components may explain the lack of activity against S. mutans observed in the present study.

Syzygium aromaticum, used for over 2,000 years in India and China, has also been employed to combat dental caries and eliminate halitosis (28). Because of its effects on dental plaque, gingival inflammation, and oral flora, *Syzygium aromaticum* has attracted attention as a potential natural agent for oral health protection (24). Eugenol, one of the main components of *Syzygium aromaticum*, has been previously reported to have strong antimicrobial, analgesic, antioxidant, and anti-inflammatory effects (29). Its antibacterial activity, along with that of other phenolic constituents, is mainly attributed to disruption of bacterial cell membranes, increased membrane permeability, and interference with essential enzymatic systems (28). In the dental literature, clove oil has also been investigated for its effects on oral biofilm formation, gingival inflammation, and cariogenic bacteria (26). Consistent with these findings, the present study demonstrates that both extracts and aqueous infusions of Syzygium aromaticum exhibit measurable antibacterial activity against *S. mutans.*

The inhibition zone results showed a clear dose-dependent increase in the antibacterial activity of Syzygium aromaticum extracts. At higher concentrations, the antimicrobial effect approached that of chlorhexidine, indicating a strong biological potential under in vitro conditions. This was further supported by MIC and MBC findings, in which the extract exhibited lower inhibitory and bactericidal concentrations compared with the aqueous infusion. This difference may be attributed to the higher concentration and more efficient extraction of bioactive compounds, particularly eugenol and other phenolic constituents, in the ethanolic extract.

Although chlorhexidine remained substantially more potent on a mass basis, the comparable inhibition zone diameters observed at higher concentrations of Syzygium aromaticum suggest that this natural agent may represent a promising alternative or adjunct in caries prevention strategies. The bactericidal nature of Syzygium aromaticum was further confirmed by MBC/MIC ratios of ≤4, indicating true bactericidal activity under the tested conditions. According to the literature, agents with an MBC/MIC ratio ≤4 are classified as bactericidal, whereas ratios >4 indicate bacteriostatic activity (30). In the present study, both the extract and infusion of Syzygium aromaticum exhibited bactericidal potential against the tested microorganism. However, these findings are limited to in vitro conditions and may vary depending on the microbial species and clinical environment.

Importantly, the aqueous infusion of Syzygium aromaticum also demonstrated measurable antibacterial activity. This finding is clinically relevant, as aqueous preparations may offer a simpler, safer, and more accessible alternative to solvent-based extracts for potential oral health applications.

Limitations of the Study

This study has several limitations. First, the antimicrobial activity of Crocus sativus and Syzygium aromaticum was assessed under in vitro conditions, which do not fully replicate the complex biological and ecological environment of the oral cavity. Second, only a single reference strain (*Streptococcus mutans* ATCC 25175) was used; therefore, strain-dependent variability among clinical isolates was not captured, which may limit external validity.

Third, antimicrobial efficacy was evaluated exclusively against planktonic cells without biofilm-based models, despite the biofilm-associated nature of dental caries, which restricts the translational relevance of the findings. Fourth, the phytochemical composition of the extracts was neither standardized nor quantitatively characterized, preventing correlation between antimicrobial activity and specific bioactive constituents and limiting reproducibility across preparations.

Potential effects on host cells, oral pH modulation, and the broader oral microbiome were not investigated. Accordingly, while *Syzygium aromaticum* demonstrated concentration-dependent activity against S. mutans, its clinical safety, ecological impact, and therapeutic applicability remain to be established. Further studies incorporating standardized phytochemical profiling, biofilm models, and in vivo or clinical validation are warranted.

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CONCLUSION

This study demonstrated that Crocus sativus exhibited no meaningful antibacterial activity against *Streptococcus mutans*, indicating limited relevance for oral antimicrobial applications under the tested conditions. In contrast, the 25% Syzygium aromaticum extract produced inhibition zones comparable to chlorhexidine in vitro (one of the current gold-standard agents in chemical plaque control) whereas its aqueous infusion exhibited measurable but significantly lower antibacterial activity; both preparations showed bactericidal properties. This finding is notable, as it suggests that *Syzygium aromaticum* may possess clinically relevant antimicrobial potential and could represent a promising plant-based alternative or adjunct in oral care formulations. Nevertheless, these results are limited to in vitro conditions, and translation into clinical efficacy requires confirmation through standardized biofilm models, in vivo studies, and controlled clinical trials.

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Ethical Approval: The study was approved by the Karabük University Ethics Committee (decision no: 2024/1758, date: 06 May 2024).

Author Contributions: Idea/Concept: M.G.T., M.Y.C., S.S.; Design: M.G.T., M.Y.C., S.S.; Supervision: M.G.T., M.Y.C., S.S.; Resources: M.G.T., M.Y.C., S.S.; Materials: M.G.T., M.Y.C., S.S.; Data Collection and/or Processing: M.G.T., M.Y.C., S.S.; Analysis and/or Interpretation: M.G.T., M.Y.C., S.S.; Literature Review: M.G.T., M.Y.C., S.S.; Written by: M.G.T., M.Y.C., S.S.; Critical Review: M.G.T., M.Y.C., S.S.

Conflict of Interest: The authors declare that there is no conflict of interest.

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