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RESEARCH ARTICLE

Antibacterial Effects of Tea Tree Oil and Mastic Oil to *Streptococcus mutans*

Song-Yi Yang, So-Hyun Lee, On-Bi Park, Hee-Rang An, Yeong-Hyeon Yu, Eun-Bi Hong, Kyung-Hee Kang, and Hwa-Soo Koong†

Department of Dental Hygiene, Konyang University, Daejeon 35365, Korea

Background: Tea tree oil has antiviral, antimicrobial and antifungal effects and Mastic oil has antifungal and anticancer effects. For synergistic effects of oils, blending oil containing a mixture of two to three oils is recommended. This study aimed to determine the antibacterial effects of Tea tree oil, Mastic oil, and Blending oil containing the two oils in a mixture, to verify and suggest the potential use of these oils as a substance to prevent dental caries.

Methods: Tea tree oil, Mastic oil, and Blending oil with a 1:1 blend of the two oils were diluted in liquid medium to 0% (negative control), 0.5%, 1.0%, and 2.0%. *Streptococcus mutans* was applied to each experimental group of the three diluted oils and after 8 h culture, the optical density (OD) was measured and the growth inhibition rate for *S. mutans* was estimated.

Results: Tea tree oil had significantly low OD values across all concentrations ($p<0.05$) without significant variation among different concentrations ($p>0.05$). Mastic oil did not significantly vary in OD compared to the negative control across all concentrations ($p>0.05$) without significant variation among different concentrations ($p>0.05$). Blending oil, compared to the negative control, did not significantly vary in OD at 0.5% ($p>0.05$) but significant variation was found as the concentration increased ($p<0.05$). Additionally, for Tea tree oil and Mastic oil, the growth inhibition rate showed no significant variation according to concentration ($p>0.05$), whereas for Blending oil, the growth inhibition rate for *S. mutans* showed a significant difference at 1.0% ($p<0.05$) and at higher concentrations.

Conclusion: Blending oil containing a Tea tree oil and Mastic oil demonstrated a significant growth inhibition effect on *S. mutans* from the concentration of 1.0%, which suggested its potential use as an effective antibacterial agent for dental caries.

Key Words: Antibacterial effect, Blending effect, Mastic oil, *Streptococcus mutans*, Tea tree oil

Introduction

1. Background

Dental caries is a disseminated disease that frequently affects humans across the world. *Streptococcus mutans* (*S. mutans*) is known as the main causal pathogen as it forms dental plaques by engaging in the initial attachment to dental surfaces. *S. mutans* produces lactic acid to induce tooth demineralization, and it is acid-tolerant with the ability to survive in acidic conditions of pH ≤5. The strain also synthesizes extracellular polysaccharides (glucans) and intracellular polysaccharides to mediate the binding among the bacteria within dental plaques or act to supply energy sources to the bacteria. Hence, the acid produced by *S. mutans* degrades the calcium and phosphorous on the tooth to cause decalcification, which leads to dental caries[1,2].

The data of the Health Insurance Review and Assessment Service (HIRA) of the recent past four years show that the number of individuals with an experience of dental caries was 5,880,500 in 2018, 6,451,211 in 2019, 6,190,365 in 2020 and 6,361,109 in 2021, indicating an increase in proportion. Additionally, dental caries was ranked 4th in the current status of the outpatient care for high-frequency diseases in 2017, then 6th in 2018 and 4th in 2019, indicating a

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†Correspondence to: Hwa-Soo Koong, *https://orcid.org/0000-0001-6866-5066*
Department of Dental Hygiene, Konyang University, 158, Gwanjeodong-ro, Seo-gu, Daejeon 35365, Korea
Tel: +82-42-600-8445, Fax: +82-42-600-8408, E-mail: goong@konyang.ac.kr

high level of cost for the treatment of dental caries[3,4].

To prevent dental caries, a serious oral disease, removing and controlling dental plaques is the most important[5]. While tooth brushing is the most effective physical method to remove dental plaques[6], using solely the physical methods such as tooth brushing, dental flossing and interdental brushing, do not ensure the complete removal of dental plaques and it is more effective to use such chemical means as tooth paste and mouth wash in addition to physical methods[7-9]. Certain previous studies showed that toothpastes and mouth washes exhibit an antibacterial effect on oral pathogens[10]; however, these products contain sodium lauryl sulfate, phenolic compounds (Triclosan) and bisbiguanides (Chlorhexidine), the chemical substances reported to cause side effects in the oral cavity, and studies have since been actively exploring natural substances to replace those chemicals[11].

Essential oils are mainly extracted from plants as compounds exhibiting pharmacological effects in the human body. They vary in efficacy from antioxidant to antibacterial and antifungal effects based on the constituents[12,13]. Notably, the use of a mouth wash containing an essential oil has been reported to reduce Streptococcal strains as a result of the strong antibacterial effect[12]. Tea tree oil is a type of essential oil extracted from the leaves of the *Melaeuca alternifolia* tree[14], containing terpinene-4-ol, a key component of antimicrobial agents with antiviral, antibacterial and antifungal effects, and a tea tree oil component in denture cleansers and mouth washes has been shown to exert an outstanding antibacterial effect[15]. Mastic oil can be extracted from the stems or leaves of the *Pistacia lentiscus* tree, and it exerts a powerful antibacterial effect on *Helicobacter pylori* and antifungal effects as well as anticancer effects to reduce the proliferation of oral cancer cells and induce apoptosis[11,16]. Applying mastic oil in the form of mastic gum to the oral cavity has been shown to prevent the formation of dental plaques and promote the secretion of a greater amount of saliva to treat xerostomia[17].

According to a previous study[18], the use of blending oil containing a mixture of two to three oils rather than a single oil is recommended to generate synergistic effects. Nevertheless, only a few studies have investigated the antibacterial effects and synergistic effects of blending oil with a blend of tea tree oil and mastic oil.

2. Objectives

Thus, this study aimed to determine the concentration-dependent antibacterial effects of Tea tree oil, Mastic oil and Blending oil with a mixture of the two oils, on *S. mutans*, a major causal pathogen of dental caries, thereby suggesting the potential use of these oils as a substance to prevent dental caries and providing basic data for such uses. Therefore, the following null hypothesis were developed, and the present investigation was conducted: (1) The antibacterial effects of Tea tree oil, Mastic oil and Blending oil on *S. mutans* will not vary significantly; (2) The antibacterial effects of Tea tree oil, Mastic oil and Blending oil on *S. mutans* will not vary significantly according to the oil concentration.

Materials and Methods

1. Study design

1) Essential oils

To test the antibacterial effect of each non-blend oil, a 100% extract of tea tree oil from tea tree leaves (Naimee tea tree oil 100%; Niceday365 Co. Ltd., Seoul, Korea) and a 100% extract of mastic oil from mastic gums (Mastic oil; Mastic Korea Co. Ltd., Cheongju, Korea) were used. To test the antibacterial effect of the blended oil, the tea tree oil and mastic oil were mixed in 1:1 ratio (Table 1). The concentration-dependent antibacterial effects were compared by diluting the non-blend and blended oils to 0% (negative control), 0.5%, 1.0%, and 2.0% using liquid medium.

2) Test strain

The test strain in this study was *Streptococcus mutans* KCTC 3065 obtained from the Korean Collection for Type Cultures (KCTC). Prior to use, the strain was cultured in Brain Heart Infusion (BHI; Difco, Fairlawn, NJ, USA) for 24 hours in a 37±1°C incubator (C-IN incubator; CHANG SHIN Co., Seoul, Korea).

3) Optical density measurements

To varying concentrations of diluted oils of three types (Tea tree oil, Mastic oil, and Blending oil), *S. mutans* was inoculated, followed by 8 hours culture in a 37±1°C incubator. Next, 100 μL of the culture solution after 8 hours was aliquoted to a 96 well plate, and using a plate reader (BioTek Instruments, Inc., Wlnooski, VT, USA), the optical density (OD) was measured at 600 nm. Triplicate measurements were taken in each experiment.

4) Growth inhibition rate

Based on the OD values of the groups treated with Tea tree oil, Mastic oil, and Blending oil of the two oils at 0%, 0.5%, 1.0%, and 2.0%, the growth inhibition rate for *S. mutans* was estimated. The OD values of the negative control and experimental groups were applied to the following equation:

$$\text{Growth inhibition rate (\%)} = \frac{\text{(OD of conctol group-OD of experimental group)*100)}}{\text{(OD of control group)}}$$

2. Statistical methods

One-way analysis of variance (ANOVA) was performed using SPSS ver 28.0 (IBM Corp., Armonk, NY, USA) to compare the OD and growth inhibition rate according to the oil type and concentration. For the post-hoc test, the Tukey method was used (p=0.05).

Table 1. Preparation of Experimental Oil

Group	Tea tree oil (vol.%)	Mastic oil (vol.%)
Tea tree oil	100	
Mastic oil		100
Blending oil	50	50

Results

1. Turbidity monitoring

To determine the antibacterial effects of the Tea tree oil, Mastic oil, and Blending oil, *S. mutans* was treated with each oil at 0%, 0.5%, 1.0%, and 2.0% and after 8 hours culture, the respective solutions were visually examined. A difference in turbidity from the negative control was observed for Tea tree oil and Blending oil, but for Mastic oil, the difference was negligible (Fig. 1).

2. Optical density measurements

For the control group without the Tea tree oil treatment (0%), the OD was 0.125±0.002. With the tea tree oil treatment at 0.5%, a significant decrease in OD to 0.019±0.004 was observed ($p < 0.05$) (Table 2). At 1.0% and 2.0%, the OD was 0.017±0.004 and 0.016±0.007, respectively, indicating that the OD decreased as the Tea tree oil concentration increased ($p < 0.05$). Compared to the negative control, the OD showed a significant decrease in all concentrations of Tea tree oil ($p < 0.05$), while the OD did not vary significantly according to the concentration of Tea tree oil ($p > 0.05$) (Fig. 2).

For the control group without the Mastic oil treatment (0%), the OD was 0.111±0.005. With 0.5% Mastic oil

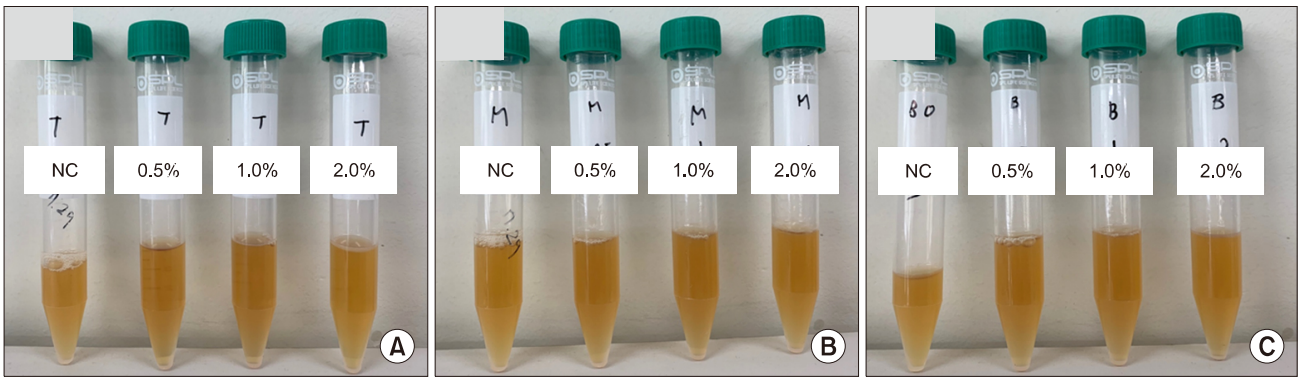

Fig. 1. Appearance of test solution at three different concentrations with *Streptococcus mutans*. (A) Tea tree oil. (B) Mastic oil. (C) Blending oil. NC: negative control.

treatment, the OD was 0.102±0.008 without a significant difference from the control group ($p>0.05$) (Table 3). At 1.0% and 2.0%, the OD was 0.098±0.011 and 0.101±0.005, respectively. The OD did not vary significantly according to the concentration of Mastic oil ($p>0.05$) (Fig. 3).

For the control group without the Blending oil treatment (0%), the OD was 0.114±0.005. With 0.5% Blending oil treatment, the OD was 0.108±0.006, which did not vary significantly compared to the negative control ($p>0.05$) (Table 4). At 1.0% and 2.0%, the OD was 0.080±0.006 and 0.041±0.014, respectively, indicating that the OD decreased significantly from the concentration of 1.0% ($p<0.05$) (Fig. 4).

3. Growth inhibition rate for *S. mutans* by oil concentration

The treatment of *S. mutans* with varying concentrations of Tea tree oil showed that the growth inhibition rate for *S. mutans* was 84.373±3.034% at 0.5%, 86.491±2.714% at 1.0%, and 86.779±5.283% at 2.0%, while the rate did not vary significantly across the different concentrations ($p>0.05$) (Fig. 5). The treatment of *S. mutans* with varying concentrations of Mastic oil showed that the growth inhibition rate for *S. mutans* was 8.377±5.242% at 0.5%, 11.336±11.899% at 1.0% and 9.509±2.699% at 2.0%, without significant variation across concentrations ($p>0.05$). On the contrary, the treatment of *S. mutans* with varying concentrations of Blending oil showed that the growth inhibition rate for *S. mutans* was 5.491±4.254% at 0.5%, 30.206±5.242% at 1.0% and 63.892±13.532% at 2.0%, indicating that the rate significantly increased as the concentration of Blending oil increased ($p<0.05$).

Table 2. Optical Density of Tea Tree Oil at Three Different Concentrations with *Streptococcus mutans*

Concentration (%)	Optical density (mean±SD)
0	0.125±0.002[a]
0.5	0.019±0.004[b]
1.0	0.017±0.004[b]
2.0	0.016±0.007[b]

[a,b]The same letters indicate no differences between the groups ($p>0.05$).

Table 3. Optical Density of Mastic Oil at Three Different Concentrations with *Streptococcus mutans*

Concentration (%)	Optical density (mean±SD)
0	0.111±0.005[a]
0.5	0.102±0.008[a]
1.0	0.098±0.011[a]
2.0	0.101±0.005[a]

[a]The same letters indicate no differences between the groups ($p>0.05$).

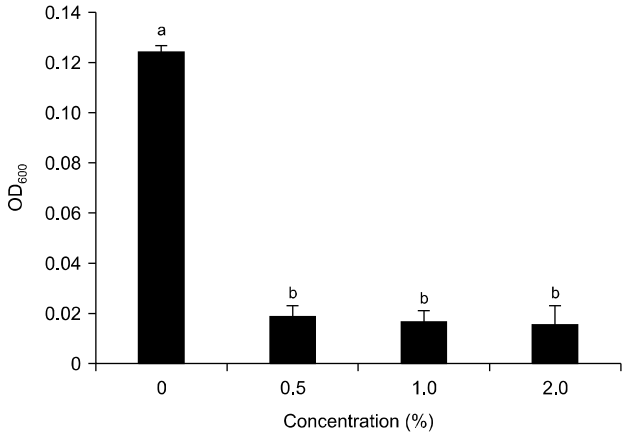

Fig. 2. Optical density (OD) of Tea tree oil at three different concentrations with *Streptococcus mutans*. The same letters indicate no differences between the groups ($p>0.05$), whereas the different letters indicate significant difference between the groups ($p<0.05$).

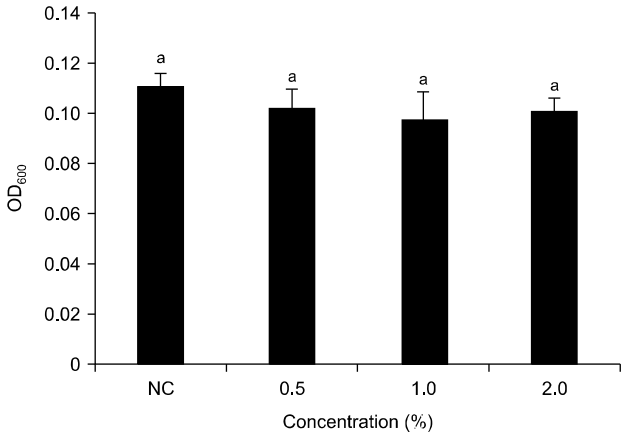

Fig. 3. Optical density (OD) of Mastic oil at three different concentrations with Streptococcus mutans. The same letters indicate no differences between the groups ($p>0.05$). NC: negative control.

Table 4. Optical Density of Blending Oil at Three Different Concentrations with *Streptococcus mutans*

Concentration (%)	Optical density (mean±SD)
0	0.114±0.005[a]
0.5	0.108±0.006[a]
1.0	0.080±0.006[b]
2.0	0.041±0.014[c]

[a,b,c]The same letters indicate no differences between the groups ($p>0.05$), whereas the different letters indicate significant difference between the groups ($p<0.05$).

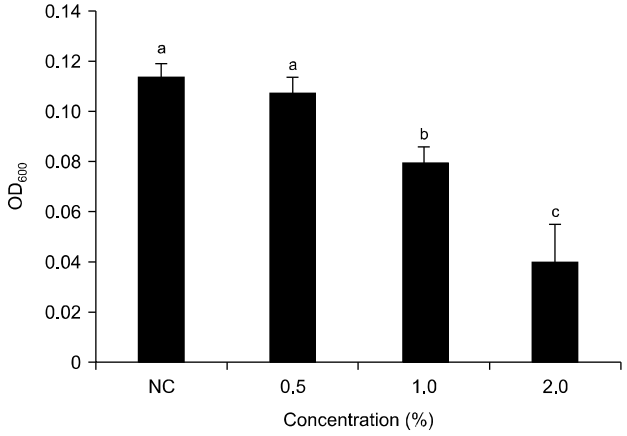

Fig. 4. Optical density (OD) of Blending oil at three different concentrations with *Streptococcus mutans*. The same letters indicate no differences between the groups ($p>0.05$), whereas the different letters indicate significant difference between the groups ($p<0.05$). NC: negative control.

4. Growth inhibition rate for *S. mutans* by oil type at identical concentrations

The growth inhibition rate for *S. mutans* among Tea tree oil, Mastic oil, and Blending oil with a mixture of the two oils was compared at oil concentrations of 0%, 0.5%, 1.0%, and 2.0%. At 0.5%, significant differences were found between Tea tree oil and Mastic oil and between Tea tree oil and Blending oil ($p<0.05$), whereas Mastic oil and Blending oil exhibited no significant variation ($p>0.05$). At 1.0%, likewise, significant differences were found between Tea tree oil and Mastic oil and between Tea tree oil and Blending oil ($p<0.05$) but no significant variation was found between Mastic oil and Blending oil ($p>0.05$). The comparison of the three experimental groups at the concentration of 2.0% showed that the growth inhibition rate varied significantly between Tea tree oil and Mastic oil

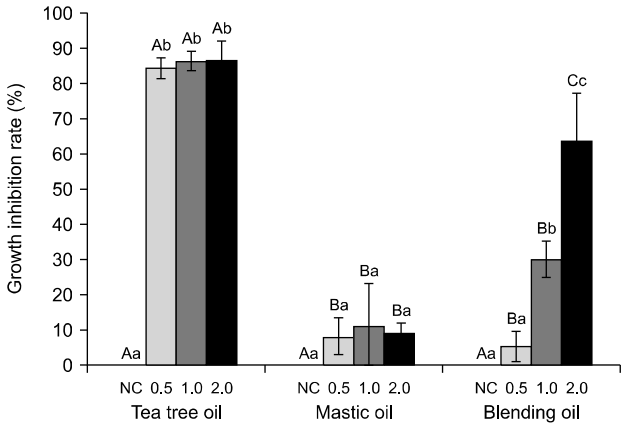

Fig. 5. Growth inhibition rate by each concentrations of three-types oils. Capital letters indicate growth inhibition rates for each oil of the same concentration. The same lowercase letters indicate no differences in growth inhibition rate between three different concentrations within each oil ($p>0.05$). The same uppercase letters indicate no differences in growth inhibition rate between three different oil within each concentration ($p>0.05$). NC: negative control.

($p<0.05$), between Tea tree oil and Blending oil ($p<0.05$), and between Mastic oil and Blending oil ($p<0.05$).

Discussion

Dental caries and periodontal disease are the representative oral diseases caused by a variety of pathogenic bacteria inhabiting the oral cavity[19]. Dental caries and periodontal disease are thus known as the two major oral diseases, experienced by approximately 60% of the global adult population, and dental caries between the two is the most well-known oral disease that threatens the oral health of humans[12]. The mechanisms of the dental caries incidence vary from the intraoral environment to the saliva and food intake, and the most important cause is the bacteria *S. mutans* found within dental plaques[20]. *S. mutans* is an oral bacteria with an excellent ability to attach to dental surfaces. The bacteria produce a large amount of acids including lactic acid, and it is acid-tolerant to be able to survive even in low pH conditions so that its continuous metabolic activities including the destruction of tooth ultimately lead to dental caries[21].

Hence, dental plaques can be viewed as the cause of dental caries and periodontal disease and it is critical that dental plaques are removed and controlled as the most

important cause of oral diseases, not only for oral hygiene but also for the prevention of oral disease and maintenance of oral health[22]. Dental plaques can be controlled via physical and chemical methods, and tooth brushing as a physical means is limited in completely removing dental plaques[23]. Thus, to resolve the limitation and maximize the effects of such physical methods as tooth brushing in removing dental plaques, oral health care products such as dentifrices and mouth washes as chemical means are used simultaneously[24]. These chemical products exert antibacterial effects on intraoral bacteria although they can also cause various side effects in the oral cavity[12]. This prompted the research to apply natural compounds from plants and antibacterial plant extracts in oral health care products to substitute the chemical agents that can cause side effects in the oral cavity[25].

Essential oils are organic substances obtained through extraction from various parts of plants[25] that have long been used in cosmetic, food and beverage products. Since the 19th century, essential oils have been applied as a temporary filler and an agent in root canal and periodontal treatments[26]. Notably, essential oils have been shown to exhibit powerful antibacterial effects without the problem of resistance as in conventional antibiotics[27].

Tea tree oil has antiviral and antibacterial effects[28], with an outstanding antifungal effect on *Candida albicans*, a causal pathogen of denture stomatitis[29]. Tea tree oil has also been reported to induce apoptosis when applied to oral squamous cell carcinoma[30]. In addition, the group with tea tree oil showed a preventive effect on oral disease compared to the control in a study on the use of tea tree oil in oral cleaning for patients undergoing chemotherapy[31].

Mastic oil is a plant-origin natural substance with antimicrobial, antifungal and anti-inflammatory effects that have been proven through various studies[25], while it has been reported as an effective anticancer agent that can be used in the treatment of oral cancer[16]. A previous study has also shown that the experimental group with one-week mastication of mastic gum without physical oral care displayed lower gingival and plaque indices than the control group to indicate an anti-plaque effect of mastic gum[32].

The current literature on aromatherapy and clinical specialists recommends the blending of essential oils with two or more other essential oils or mediating compounds so as to enhance the effects of each oil in the blend[18]. Nevertheless, there has been a general lack of studies examining the synergistic effects of Tea tree oil and Mastic oil in a blend regarding their antibacterial effects on *S. mutans*. Thus, this study set out to define the antibacterial effects of Tea tree oil, Mastic oil and Blending oil with a mixture of the two oils on *S. mutans*, after the dilution to 0%, 0.5%, 1.0%, and 2.0%.

1. Key results

In this study, two essential oils; Tea tree oil and Mastic oil, were mixed in 1:1 volume ratio to produce Blending oil, and the antibacterial effect of each oil on *S. mutans* was verified by culturing the bacteria in liquid broth with each oil. The negative control without any oil (0%) and experimental groups of each oil at 0.5%, 1.0%, and 2.0% were cultured in a 37±1°C incubator for 8 hours, and the OD of bacterial suspension at 600 nm was measured.

The Tea tree oil groups showed OD values considerably lower than the negative control without oil, to indicate significant antibacterial effects ($p < 0.05$) although no significant concentration-dependent variation was observed ($p > 0.05$). The Mastic oil groups showed OD values lower than the negative control but no statistically significant variation was found ($p > 0.05$). The Blending oil groups containing a 1:1 blend of Tea tree and Mastic oils showed OD values lower than the negative control. Notably, a significant difference was observed at 1.0% and at higher concentrations ($p < 0.05$).

2. Interpretation

The growth inhibition rate for *S. mutans* was estimated based on the measured OD values of each experimental group, and a significant inhibitory effect was shown by Tea tree oil across all concentrations ($p < 0.05$), whereas Mastic oil showed no significant variation in the inhibition rate according to concentration ($p > 0.05$). Blending oil also exhibited a significant inhibitory effect on *S. mutans* from 1.0% ($p < 0.05$). Comparing the growth inhibition rate for *S. mutans* of Tea tree oil, Mastic oil and Blending oil at identical concentrations showed that, at 0.5% and 1.0%, a significant difference was found between Tea tree

oil and Mastic oil ($p < 0.05$) and between Tea tree oil and Blending oil ($p < 0.05$) but not between Mastic oil and Blending oil ($p > 0.05$). At 2.0%, the growth inhibition rate varied significantly across all three oils ($p < 0.05$).

3. Comparison with previous studies

The results of this study indicated that antibacterial effects of Tea tree oil on *S. mutans* were significant across all tested concentrations; 0.5%, 1.0%, and 2.0% ($p < 0.05$), which coincided with a previous study[25] reporting an antibacterial effect of Tea tree oil at 30 vol% and 50 vol% on *S. mutans*. In contrast, Mastic oil in this study did not vary significantly in antibacterial effects across the concentrations of 0.5%, 1.0%, and 2.0%, which disagreed with the previous study[25] reporting an antibacterial effect of Mastic oil at 0.1% and 0.2% on *S. mutans*. This may be attributed to the short time of culture at 8 hours, compared to the previous study[25] where the time of culture was set as 16 hours before the measurement of OD.

4. Limitations

The limitations in this study are first, the antibacterial effects of Tea tree oil, Mastic oil, and Blending oil with a mixture of the two oils were evaluated solely on *S. mutans*. To resolve this limitation, a follow-up study should evaluate the antibacterial and antifungal effects of Tea tree oil, Mastic oil and Blending oil on *S. mutans* as well as other bacterial and fungal strains causing dental caries, with an aim to verify the potential use of these natural substances as an antimicrobial agent. Furthermore, the natural substances should be characterized via analyzing the chemical composition and bioactive compounds in Tea tree oil and Mastic oil to identify the minimum concentration and minimum treatment time for the antibacterial effects and examine the variation in antibacterial effects accordingly.

5. Generalizability

This study aimed to determine the effects of Tea tree oil, Mastic oil, and Blending oil containing a blend of the two oils to inhibit the growth of *S. mutans*, a well-known causal pathogen of dental caries as one of the serious oral diseases. The ultimate goal was to verify the potential use of these oils to complement for the side effects and drawbacks of such chemical agents as dentifrices and mouth washes. The results of this study demonstrated that Tea tree oil had an antibacterial effect on *S. mutans* at relatively low concentrations of 0.5%, 1.0%, and 2.0% in comparison to a previous study[15] examining the antibacterial effects of Tea tree oil on *S. mutans* at 30 vol.% and 50 vol.%. The significance of this study also lies in testing Blending oil with a mixture of Tea tree oil and Mastic oil and verifying positive antibacterial effects from the concentration of 1.0%.

Comparing the antibacterial effects of Tea tree oil, Mastic oil, and Blending oil on *S. mutans* showed that Tea tree oil exhibited significant differences at all three concentrations of experimental groups; 0.5%, 1.0%, and 2.0%, compared to the negative control at 0% ($p < 0.05$); Mastic oil did not vary significantly across all concentrations ($p > 0.05$); Blending oil varied significantly from the concentration of 1.0% ($p < 0.05$). Therefore, the first null hypothesis in this study; 'The antibacterial effects of Tea tree oil, Mastic oil, and Blending oil on *S. mutans* will not vary significantly', was rejected. Comparing the *S. mutans* growth inhibition effects of Tea tree oil, Mastic oil and Blending oil at identical concentrations showed that, at 0.5% and 1.0%, a significant difference was found between Tea tree oil and Mastic oil and between Tea tree oil and Blending oil ($p < 0.05$) but not between Mastic oil and Blending oil ($p > 0.05$), whereas at 2.0%, significant differences were found across all three oils ($p < 0.05$). Therefore, the second null hypothesis in this study; 'The antibacterial effects of Tea tree oil, Mastic oil, and Blending oil on *S. mutans* will not vary significantly according to the oil concentration', was also rejected. The positive antibacterial effects of Blending oil containing a blend of Tea tree oil and Mastic oil were thus verified for concentrations $\geq 1.0\%$ regarding a main causal pathogen of dental caries *S. mutans*. Based on the findings of this study, blending oil is predicted to serve useful as a material for the prevention of dental caries.

6. Suggestions

The results of this study collectively suggested that Blending oil with a mixture of Tea tree oil and Mastic oil had inhibitory effects on the growth of *S. mutans* from the

concentration of 1.0%, based on which it is predicted that the respective oil could be applied in oral care products such as dentifrices and mouth washes with the basic data provided by this study.

Notes

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

Not applicable.

Author contributions

Conceptualization: So-Hyun Lee. Data acquisition: On-Bi Park, Hee-Rang An, and Eun-Bi Hong. Formal analysis: Yeong-Hyeon Yu and Kyung-Hee Kang. Funding: Song-Yi Yang. Supervision: Kyung-Hee Kang. Writing–original draft: So-Hyun Lee and Song-Yi Yang. Writing–review & editing: Hwa-Soo Koong.

ORCID

Song-Yi Yang, *https://orcid.org/0000-0001-6174-6947*
So-Hyun Lee, *https://orcid.org/0000-0001-9791-4092*
On-Bi Park, *https://orcid.org/0000-0001-5865-3264*
Hee-Rang An, *https://orcid.org/0000-0001-5625-4403*
Yeong-Hyeon Yu, *https://orcid.org/0000-0003-2938-5138*
Eun-Bi Hong, *https://orcid.org/0000-0003-4466-1700*
Kyung-Hee Kang, *https://orcid.org/0000-0003-3439-1049*
Hwa-Soo Koong, *https://orcid.org/0000-0001-6866-5066*

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Data availability

The data and materials of this article are included within the article. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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