Sabinene suppresses growth, biofilm formation, and adhesion of Streptococcus mutans by inhibiting cariogenic virulence factors

Bog-Im Park a, b, Beom-Su Kim a, Kang-Ju Kim c and Yong-Ouk You c, d

*Department of Oriental Medicine Resources, Chonbuk National University, Iksan, Republic of Korea; bCarbon Nano Convergence Technology Center for Next Generation Engineers (CNN), Chonbuk National University, Jeonju-si, Republic of Korea; cDepartment of Oral Microbiology and Immunology, School of Dentistry, Wonkwang University, Iksan, Republic of Korea; dDepartment of Oral Biochemistry, School of Dentistry, Wonkwang University, Iksan, Republic of Korea

ABSTRACT

Background: Streptococcus mutans is one of the most important cariogenic bacteria associated with dental caries. Sabinene is a major component of several herbal essential oils. However, the anti-cariogenic effects of sabinene and the underlying mechanism remain to be elucidated.

Objectives: We investigated the inhibitory effects of sabinene on the cariogenic activity and studied the underlying mechanism.

Design: S. mutans were treated with various concentrations of sabinene and the inhibitory effects were evaluated based on the bacterial growth, acid production and biofilm formation.

Real-time polymerase chain reaction (PCR) was performed for several virulence factors.

Results: The growth and adherence of S. mutans were inhibited by sabinene. Consistent with the inhibitory effects on bacterial adhesion, gbpB level significantly decreased. Acid production and biofilm formation was also inhibited. In line with the inhibitory effects of sabinene on biofilm formation and pH tolerance, real-time PCR results showed the down regulation in the expression levels of gtfB, gtfC, gtfD, vicR, bpaA, and relA. Moreover, high concentrations of sabinene exhibited bactericidal activity.

Conclusion: Together our results suggest that sabinene serves as a useful component in the inhibition of the cariogenic activity of S. mutans, indicative of its possible applications in the development of oral healthcare products.

Despite several efforts directed to prevent tooth decay, dental caries remains an unresolved serious dental issue in many countries and one of the most common health problems of the teeth. Dental caries, also known as tooth decay, is a gradual process associated with the activities of specific types of bacteria. Streptococcus mutans is the most cariogenic type of oral bacteria that play a critical role in the development of dental caries in humans [1,2].

S. mutans initially attaches to the tooth surface and produces an insoluble glucan layer. The glucan is synthesized by glucosyltransferase (GTF) and contributes to the formation of polysaccharide of the dental plaque matrix, thereby accelerating the maturation of dental plaque [2,3]. S. mutans also has the ability to metabolize the carbohydrates in foods and release organic acids such as lactic acid as byproducts. The released organic acids lower the pH of the dental plaque and dissolve tooth enamel. This sucrose-dependent mechanism is also based on GTF and involves several virulence factors associated with cariogenicity, such as glucan-binding proteins (GBPs) [4].

Although fluoride has been regarded as a universally effective compound for the inhibition of cariogenic bacterial growth [5], it is deemed cytotoxic at high concentrations [5,6]. Therefore, naturally derived products and compounds have been introduced as alternatives to prevent dental caries.

Sabinene is a chemical compound found in herbal essential oils, including the oil extracted from Chamaecyparis obtusa. C. obtusa belongs to the species Cypress that is used in construction and furniture industries because of its structural properties and natural aroma. Its essential oil has been widely used as functional additives in several industries, including cosmetics. The essential oil extracted from C. obtusa contains several types of terpenes, including limonene, bornyl acetate, and borneol, and exhibits biological properties such as antioxidant and anti-inflammatory effects [7,8]. Sabinene is a natural liquid monoterpene obtained from essential oils of various plants, including Cannabis. The chemical formula of sabinene is C10H16 and it has a bicyclic structure [9]. Several studies have reported the biological
functions of sabine, including its antifungal [10] and anti-inflammatory properties [11]. However, the effects of sabine on the cariogenic activity of \textit{S. mutans} have been incompletely characterized.

We hypothesize that sabine may exert antimicrobial activities against the cariogenic bacterium \textit{S. mutans}. In the present study, we examined the effect of sabine on the growth, adhesion, biofilm formation, and acid production of \textit{S. mutans}. We also evaluated its influence on the expression of several genes encoding virulence factors associated with bacterial adhesion and biofilm formation through real-time polymerase chain reaction (PCR).

**Materials and methods**

**Bacterial growth**

To evaluate the anti-cariogenic activity of sabine, we tested its inhibitory effects on the growth of \textit{S. mutans} (ATCC 25,175, Rockville, MD). An \textit{S. mutans} culture (0.05 mL) at a density of $5 \times 10^5$ colony-forming units (CFUs)/mL was inoculated in 0.95 mL of brain heart infusion (BHI; Difco, Detroit, MI) broth supplemented with 1% glucose and various concentrations (0.05–0.4 mg/mL) of sabine (Sigma, St. Louis, MO). After 24 h of incubation at 37°C under aerobic conditions, bacterial growth was determined based on optical density at 550 nm wavelength. In this study, sodium fluoride (NaF) was used as a positive control.

**Acid production**

We determined the influence of sabine on the acid production ability of \textit{S. mutans} using a previously described method [3]. Briefly, the sabine solution was filtered through a 0.2-μm syringe filter and 0.95 mL of phenol red broth supplemented with 1% glucose were added. This broth was used to culture \textit{S. mutans} at various concentrations (0.05–0.4 mg/mL) of sabine. After 24 h of incubation at 37°C under aerobic conditions, bacterial growth was determined using 30% acetic acid and the optical density was measured at 530 nm wavelength for quantity analysis.

**Bacterial adherence**

The effect of sabine on bacterial adherence was determined using hydroxyapatite beads (Bio-Rad, Hercules, CA.). In brief, \textit{S. mutans} in BHI was diluted to approximately $10^5$ CFU/mL. About 30 μg of hydroxyapatite beads [12] were coated with human mixed saliva for 1 h. The saliva-coated hydroxyapatite beads (S-HAs) were rinsed thrice with 10 mM potassium phosphate buffer (pH 7.0) and immersed in bacterial suspension solutions (1 × $10^7$ CFU/mL) with various concentrations of sabine. The mixtures were gently agitated for 90 min at 37°C to facilitate bacterial adhesion. After incubation, S-HAs were washed and transferred to potassium phosphate buffer in a new tube. The bacteria that adhered to S-HAs were dispersed by sonication (50 W for 30 s), and the supernatants were diluted and spread on mannitol salt agar plates supplemented with 3.2 mg/mL bacitracin. The bacterial colonies were counted after 48 h.

**Biofilm formation**

Biofilm formation was evaluated using a petri dish model and a resin teeth model according to a previously described method [13]. Briefly, various concentrations (0.05–0.4 mg/mL) of sabine were added to BHI broth supplemented with 0.1% sucrose in petri dishes, 24-well plates (Nunc, Copenhagen, Denmark), or 24-well plates containing resin teeth (Endura, Shofu Inc., Japan). The setups were inoculated with $5 \times 10^5$ CFU/mL \textit{S. mutans} culture and incubated for 24 h at 37°C. The supernatants were removed, and the dishes and plates were washed with distilled water. Biofilm formation was analyzed by staining with 0.1% safranin and subsequent graphic documentation. The stained safranin was dissolved using 30% acetic acid and the optical density was measured at 530 nm wavelength for quantity analysis.

**Scanning electron microscopy**

To confirm the formation of biofilm, 35-mm petri dishes were rinsed with distilled water, and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) was added as a fixative. The dishes were incubated at 4°C for 24 h. For dehydration, the samples were treated with a graded series of ethanol (60%, 70%, 80%, 90%, 95%, and then 100%) and subsequently freeze-dried. After sputter coating with gold, the samples were observed under vacuum conditions using a scanning electron microscope (SEM; JOM-6360, JEOL, Tokyo, Japan).

**Real-time PCR analysis**

To determine the influence of sabine on gene expression, real-time PCR was performed. Sub-minimal inhibitory concentrations (0.05–0.2 mg/mL) of sabine were used to treat cultures of \textit{S. mutans} for 24 h. Total RNA was isolated from the bacteria using Trizol reagent (Gibco-BRL) and cDNA was synthesized using reverse transcriptase (Superscript; Gibco-BRL). A real-time PCR machine (ABI-Prism 7,000 Sequence Detection System, Applied Bio systems Inc., Foster City, CA) and SYBR Green detection dye (Applied Bio systems Inc) were
used. The specific primers used are listed in Table 1. In this study, 16S rRNA was used as an internal standard.

**Confocal laser scanning microscopy**

To determine the bactericidal effects of sabinene, a live/dead assay was performed. An *S. mutans* culture was diluted with BHI media to approximately $1 \times 10^7$ CFU/mL and subsequently treated with high concentrations of sabinene (0.4–3.2 mg/mL). After 30 min incubation at 37°C under aerobic conditions, the cultured bacteria were washed with phosphate-buffered saline (PBS) and treated with LIVE/DEAD Bacterial Viability Kit reagents (Molecular Probes, Eugene, OR) according to the manufacturer’s protocol. After 15 min of incubation with the stain, the bacteria were observed under a confocal laser scanning microscope (LSM 510; Zeiss, Oberkochen Germany).

**Statistical analyses**

All experiments were performed in triplicates. Statistical analysis was performed using the Student’s *t*-test and one-way analysis of variance (ANOVA) in Microsoft Excel. The data were expressed as the mean ± standard deviation. A value of *p* < 0.05 was considered statistically significant.

**Results**

**Bacterial growth inhibition by sabinene**

We evaluated the anti-cariogenic activity of sabinene as an active compound against *S. mutans*. The bacterial growth following treatment with 0.05 mg/mL (0.367 mM) sabinene was not significantly different from that observed in the control group. However, the growth of *S. mutans* was significantly inhibited (*p* < 0.05) at 0.1–0.4 mg/mL (0.734–2.936 mM) sabinene concentrations (Figure 1). Thus, sabinene inhibited the growth of *S. mutans* in a dose-dependent manner. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of sabinene were 0.2 mg/mL (1.468 mM) and > 3.2 mg/mL (34.480 mM), respectively.

**Inhibitory effects of sabinene on bacterial adherence**

The effects of sabinene on the adherence of *S. mutans* were evaluated with S-HAs. Sabinene failed to significantly inhibit the adherence of *S. mutans* at 0.05 mg/mL concentration as compared to the observation reported in the control group. However, treatment with 0.1–0.2 mg/mL sabinene resulted in a reduction in bacterial adherence by about 40% as compared with the control group. Furthermore, we observed about 60% reduction in bacterial adherence following treatment with 0.4 mg/mL sabinene (Figure 2(a)).

**Inhibitory effects of sabinene on biofilm formation**

We determined the effect of sabinene on the biofilm formation ability of *S. mutans* via safranin staining. The treatment with 0.05–0.4 mg/mL of sabinene resulted in a significant inhibition in the biofilm formation on the petri dish surface in a dose-dependent manner (Figure 3). Furthermore, the biofilm formation ability of *S. mutans* on a resin tooth surface was also inhibited after sabinene treatment

---

**Table 1. Oligonucleotide primers that were used in this study.**

| Gene* | Primer sequences (5′-3′) |
|-------|-------------------------|
| **16S rRNA** | **Forward** CCAAGGGAGGCAGCAGTAG **Reverse** CAACAGAGCTTTACGATCCGAAA |
| gtfB (Glucosyltransferase B) | **Forward** AGCAATGCAGCCAATCTACAAAT **Reverse** ACGGAGCTTATGGACAGCCTT |
| gtfC (Glucosyltransferase S2) | **Forward** CTCAACCCACCGGACCACGTT **Reverse** ACAGCAGAAGACAGCCGAAA |
| gtfD (Glucosyltransferase-I) | **Forward** ATGGCGGTATGGACACGTT **Reverse** TTTGGCACCCTTGAAACACTT |
| bpfA (Biofilm-regulation protein) | **Forward** AATCCCGAGCATCCGCAGAAAG **Reverse** ATGCCGTTATGGACAGCCTT |
| gbp (Glucan binding protein) | **Forward** ACAGCAGAAGACAGCCGAAA **Reverse** CTCAAGGCACCGGGAGCCCC |
| relA (Guanosine tetra (penta)- phosphate synthetase) | **Forward** AAATCCAGACATCCGCAGAAAG **Reverse** ATGCCGTTATGGACAGCCTT |
| spaP (Cell surface antigen) | **Forward** GACTTTGGTAATGGTTATGCATCAA **Reverse** TTTGGCACCCTTGAAACACTT |
| vicR (Two-component regulatory system) | **Forward** GACTTTGGTAATGGTTATGCATCAA **Reverse** TTGTATCGGCCGCGATCAGGG |

*Based on the NCBI *S. mutans* genome database
To explore the effects of sabinene on the expression of the genes encoding biofilm formation-related factors, we observed the mRNA expression of \( gfbB, gftC, gftD \), and \( vicR \) (Figure 5). Real-time PCR results showed that the mRNA expression of \( gfbB \) was inhibited by 0.05–0.2 mg/mL sabinene, while \( gftC \) mRNA expression was inhibited at 0.1–0.2 mg/mL sabinene concentration. The mRNA expression levels of \( gftD \) and \( vicR \) were inhibited by 0.2 mg/mL sabinene.

**Inhibitory effects of sabinene on acid production**

The inhibitory effect of sabinene on the acid production ability of \( S. \) mutans was also determined. \( S. \) mutans was cultured in the presence of sabinene at 0.05–0.4 mg/mL concentrations and the changes in pH were determined. In the control group, the pH was significantly decreased to 5.93 ± 0.05 by \( S. \) mutans (Table 2). However, the decrease in pH was significantly inhibited following treatment of \( S. \) mutans with 0.2–0.4 mg/mL sabinene. The pH level was similar to that observed in the positive control group (0.1% NaF). Thus, sabinene may inhibit the acid production ability of \( S. \) mutans.

We explored the effect of sabinene on the expression of the genes encoding acid tolerance-related factors by evaluating the mRNA expression of \( brpA \) and \( relA \) (Figure 6). Real-time PCR results showed that \( brpA \) mRNA expression was inhibited by 0.05–0.2 mg/mL sabinene and \( relA \) mRNA expression was inhibited by 0.2 mg/mL sabinene.

**Bactericidal effects of high concentrations of sabinene**

To investigate whether high concentrations of sabinene exert bactericidal effects, we performed the live/dead assay. No significant bactericidal effect was observed upon treatment with 0.4 mg/mL (2.936 mM) sabinene. However, the bactericidal effects were evident upon treatment with sabinene at concentrations above 0.8 mg/mL (5.872 mM) (Figure 7).

**Discussion**

\( S. \) mutans is a well-known bacterial species that plays a critical role in the development of dental caries \([1,2]\). Many studies have been directed to discover the chemical compounds present in natural products to modulate the cariogenic activity of bacteria for the prevention and treatment of dental caries. In this study, we investigated the effects of sabinene on the cariogenic properties of \( S. \) mutans.

To evaluate the anti-cariogenic properties, we employed \( S. \) mutans owing to its association with the formation of dental plaques and dental caries \([1,2]\). We determined the anti-cariogenic properties of sabinene by monitoring the growth of \( S. \) mutans in the presence of sabinene and found that sabinene inhibited the growth of \( S. \) mutans.

\( S. \) mutans is one of the most common causative agents of tooth decay, and it causes dental caries by attaching to and growing on the surface of teeth. \( S. \) mutans also has the ability to metabolize nutritional sugars and produce organic acids, thereby causing a pH drop in the tooth environment eventually leading to dental caries \([4]\). Inhibition of bacterial adherence is essential for the prevention of dental caries \([3]\). Herein, we evaluated the inhibitory effects of sabinene on the adherence ability of \( S. \) mutans to S-HAs. As a result, we found that the adherence of \( S. \) mutans was significantly inhibited by 0.1–0.2 mg/mL sabinene. We also explored the expression of the genes involved in the adhesion of \( S. \) mutans by analyzing the mRNA expression levels of the bacterial adhesion-related virulence genes \( gfbB \) and \( spaP \) with real-time PCR \([14]\). Real-time

---

**Figure 1.** Effects of sabinene on \( Streptococcus \) \( mutans \) growth. The bacterium was cultured in the presence of various concentrations of sabinene for 24 h at 37°C. Inhibitory activity was observed in the presence of sabinene at concentrations ranging from 0.1 to 0.4 mg/mL. Each value is expressed as the mean ± standard deviation. Significance was determined at \(*p < 0.05\) as compared with the control. We used 0.1% sodium fluoride (NaF) as a positive control.
PCR (Figure 2(b)) showed that the expression of \textit{gbpB}, but not \textit{spaP}, was inhibited after sabinene treatment. Although not significant, sabinene showed a tendency to decrease the expression of the \textit{spaP} gene, which may to be partly related to the inhibition of \textit{S. mutans} adhesion. The inhibitory effect of sabinene on \textit{S. mutans} adhesion seems to be mainly associated with the inhibition of \textit{gbpB} gene expression. Biofilm formation increases the bacterial resistance to both the host defense system and antimicrobials. In this study, biofilm formation was evaluated with safranin staining using the petri dish and tooth resin, commonly used methods for the evaluation of biofilm formation [15]; sabinene inhibited the formation of biofilm by \textit{S. mutans}. SEM results were also consistent with those of safranin staining.

\textit{S. mutans} metabolizes sugars and produces organic acids, thereby decreasing the pH of the tooth environment [16] and promoting biofilm formation [17]. Therefore, pH change is an important indicator that may be used to evaluate the efficiencies of anticariogenic agents. In the present study, the inhibitory
effect of sabinene on acid production by \textit{S. mutans} was determined and sabinene was found to significantly suppress the decrease in pH caused by \textit{S. mutans}.

Several virulence factors are associated with the adhesion and acid tolerance of \textit{S. mutans}. In general, GTF is regarded as an important factor in the formation of dental plaques by \textit{S. mutans} \cite{18}, and the genes \textit{gtfB}, \textit{gtfC}, and \textit{gtfD} are well-known virulence factors that help in the formation of extracellular polysaccharide plaque matrix \cite{19,20}, which is

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Effect of sabinene on the biofilm formation ability of \textit{S. mutans}. The biofilms that formed on the dish surface were analyzed by staining with 0.1\% safranin. The bound safranin was released from the stained bacteria with 30\% acetic acid, and the absorbance of the solution was measured at 530 nm.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{(a) Effect of sabinene on the biofilm formation ability of \textit{S. mutans} on resin tooth surfaces. (b) Scanning electron microscopy. Biofilms of \textit{S. mutans} cultured in the presence of sabinene. We used 0.1\% sodium fluoride (NaF) as a positive control. Scale bar = 10 \textmu{}m.}
\end{figure}


required for the efficient induction of dental caries [21]. The gene vicR is a modulator of other virulence factors in S. mutans, including gtfB, gtfC, gtfD, and vicR mRNA expression levels were significantly inhibited by sabine at 0.05–0.2, 0.1–0.2, 0.2, and 0.2 mg/mL, respectively. Each value is expressed as the mean ± standard deviation. Significance was determined at *p < 0.05 as compared with the control.

Table 2. The pH changes in cultures of S. mutans incubated with various concentrations of sabine.

| Conc. (mg/mL) | pH (before incubation) | pH (after incubation) |
|---------------|-------------------------|-----------------------|
| Control       | 7.07 ± 0.05             | 5.93 ± 0.05           |
| 0.05          | 7.13 ± 0.05             | 5.30 ± 0.17*          |
| 0.1           | 7.10 ± 0.00             | 5.93 ± 0.30           |
| 0.2           | 7.17 ± 0.05             | 6.70 ± 0.17*          |
| 0.4           | 7.10 ± 0.00             | 6.60 ± 0.17*          |
| 0.1% Sodium fluoride (NaF) | 7.07 ± 0.05      | 6.87 ± 0.05*          |

Each pH value is represented as the mean ± standard deviation. *p < 0.05 as compared with the control group after incubation.

In addition, brpA and relA play critical roles in biofilm formation and acid tolerance of S. mutans [22–24]. In this study, sabine significantly inhibited the expression of gtfB, gtfC, gtfD, and vicR. Furthermore, it also inhibited the expression of brpA and relA. Therefore, sabine inhibits biofilm formation by repressing gtfB, gtfC, gtfD, and vicR and suppresses acid production through the inhibition of brpA and relA expression.

To evaluate its bactericidal activity, sabine was used to treat cultures of S. mutans at high concentrations, and a live/dead assay was performed. Figures 1 and 7 show that sabine exerts bacteriostatic effects at...
low concentrations (0.1–0.4 mg/mL) and bactericidal activities at higher concentrations (0.8 mg/mL). Taken together, we conclude that sabinene exhibits anti-cariogenic properties by controlling several virulence factors (Figure 8).

In summary, we observed the inhibitory effects of sabinene on the growth, acid production, biofilm formation, and adherence of *S. mutans*. These anti-cariogenic properties are regulated by several genes encoding virulence factors. Our findings suggest that sabinene has the potential to be used for the prevention of dental caries caused by *S. mutans*. Our study highlights the possible application of sabinene in oral healthcare products such as toothpaste; further studies are warranted to test these applications. Many types of bacteria cause other diseases in the oral cavity.
Aggregatibacter actinomycetemcomitans is known among several other bacteria to cause periodontitis. Therefore, further studies should also explore the effects of sabinene on the growth of A. actinomycetemcomitans and other periodontopathogens.

**Authors’ contributions**

The study design and sample collections were performed by YOY, BSK, and KJK. All laboratory work was completed by YOY and BIP. BSK, KJK, and YOY wrote the paper. All authors contributed to the final manuscript.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This paper was supported by Wonkwang University in 2017.

**ORCID**

Bog-Im Park [http://orcid.org/0000-0002-9421-1794](http://orcid.org/0000-0002-9421-1794)

Beom-Su Kim [http://orcid.org/0000-0001-6562-4373](http://orcid.org/0000-0001-6562-4373)

Kang-Ju Kim [http://orcid.org/0000-0001-6525-3744](http://orcid.org/0000-0001-6525-3744)

Yong-Ouk You [http://orcid.org/0000-0002-7754-3033](http://orcid.org/0000-0002-7754-3033)

**References**

[1] Wiater A, Choma A, Szczodrak J. Insoluble glucans synthesized by cariogenic streptococci: a structural study. J Basic Microbiol. 1999;39:265–273.

[2] Abdus Salam M, Matsumoto N, Matin K, et al. Establishment of an animal model using recombinant NOD.B10.D2 mice to study initial adhesion of oral streptococci. Clin Diagn Lab Immunol. 2004;11:379–386.

[3] Matsumoto M, Minami T, Sasaki H, et al. Inhibitory effects of oolong tea extract on caries-inducing properties of mutans streptococci. Caries Res. 1999;33:441–445.

[4] Kohler B, Birkhed D, Olsson S. Acid production by human strains of Streptococcus mutans and Streptococcus sobrinus. Caries Res. 1995;29:402–406.

[5] Guha-Chowdhury N, Iwami Y, Yamada T, et al. The effect of fluorhydroxyapatite-derived fluoride on acid production by streptococci. J Dent Res. 1995;74:1618–1624.

[6] Jeng JH, Hsieh CC, Lan WH, et al. Cytotoxicity of sodium fluoride on human oral mucosal fibroblasts and its mechanisms. Cell Biol Toxicol. 1998;14:383–389.

[7] Joo SS, Yoo YM, Ko SH, et al. Effects of essential oil from Chamomile herbs on the development of atopic dermatitis-like skin lesions and the suppression of Th cytokines. J Dermatol Sci. 2010;60:122–125.

[8] Singh BK, Tripathi M, Chaudhari BP, et al. Natural terpenes prevent mitochondrial dysfunction, oxidative stress and release of apoptotic proteins during nimesulide-hepatotoxicity in rats. PLoS One. 2012;7:e34200.

[9] Marchini M, Charvoz C, Dujourdly U, et al. Multidimensional analysis of cannabis volatile constituents: identification of 5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane as a volatile marker of hashish, the resin of Cannabis sativa L. J Chromatogr A. 2014;1370:200–215.

[10] Cao Y, Zhang H, Liu H, et al. Biosynthesis and production of sabinene: current state and perspectives. Appl Microbiol Biotechnol. 2018;102:1535–1544.

[11] Valente J, Zuzarte M, Goncalves MJ, et al. Antifungal, antioxidant and anti-inflammatory activities of Oenanthe crocata L. essential oil. Food Chem Toxicol. 2013;62:349–354.

[12] Hay DI, Gibbons RJ, Spinell DM. Characteristics of some high molecular weight constituents with bacterial aggregating activity from whole saliva and dental plaque. Caries Res. 1971;5:111–123.

[13] Petersen FC, Pecharki D, Scheie AA. Biofilm mode of growth of Streptococcus intermedius favored by a competence-stimulating signaling peptide. J Bacteriol. 2004;186:6327–6331.

[14] Nobbs AH, Lamont RJ, Jenkinson HF. Streptococcus adherence and colonization. Microbiol Mol Biol Rev. 2009;73:407–450.

[15] Conrady DG, Brescia CC, Horii K, et al. A zinc-dependent adhesion module is responsible for intercellular adhesion in staphylococcal biofilms. Proc Natl Acad Sci U S A. 2008;105:19456–19461.

[16] Frostell G. Dental plaque pH in relation to intake of carbohydrate products. Acta Odontol Scand. 1969;27:3–29.

[17] Miranda PSD, Lannes-Costa PS, Pimentel BAS, et al. Biofilm formation on different pH conditions by Streptococcus agalactiae isolated from bovine mastitic milk. Lett Appl Microbiol. 2018;67:235–243.

[18] Gibbons RJ, Houte JV. Bacterial adherence in oral microbial ecology. Annu Rev Microbiol. 1975;29:19–44.

[19] Shemesh M, Tam A, Steinberg D. Expression of biofilm-associated genes of Streptococcus mutans in response to glucose and sucrose. J Med Microbiol. 2007;56:1528–1535.

[20] Jenkinson HF, Lamont RJ. Streptococcal adherence and colonization. Microbiol Mol Biol Rev. 2009;73:407–450.

[21] Yamashita Y, Takehara T, Kuramitsu HK. Molecular characterization of a Streptococcus mutans mutant altered in environmental stress responses. J Bacteriol. 1993;175:6220–6228.

[22] Steinberg D, Moreinos D, Featherstone J, et al. Genetic and physiological effects of noncoherent visible light combined with hydrogen peroxide on Streptococcus mutans in biofilm. Antimicrob Agents Chemother. 2008;52:2626–2631.

[23] Xu X, Zhou XD, Wu CD. The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of Streptococcus mutans. Antimicrob Agents Chemother. 2011;55:1229–1236.

[24] Lemos JA, Brown TA Jr., Burme RA. Effects of RelA on key virulence properties of planktonic and biofilm populations of Streptococcus mutans. Infect Immun. 2004;72:1431–1440.