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Inhibitory effects of *Coptis chinensis* extract on the growth and biofilm formation of *Streptococcus mutans* and *Streptococcus sobrinus*

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Streptococcus mutans and *Streptococcus sobrinus* play important roles in dental caries. *Coptis chinensis* is a natural product with antimicrobial activity against enterobacteria; however, its effects on oral streptococci are still unknown. Therefore, the effects of *C. chinensis* on the growth and biofilm formation of the representative cariogenic bacteria *S. mutans* and *S. sobrinus* were investigated for the possible use of *C. chinensis* as an anticaries agent. The *C. chinensis* extract was diluted with sterile distilled water, and 0.1–2.5% of the extract was used in the experiment. The effects of the *C. chinensis* extract on the growth and glucan formation of *S. mutans* and *S. sobrinus* were measured by viable cell counting and spectrophotometry at 650 nm absorbance, respectively. Crystal violet staining was also carried out to confirm the *C. chinensis* extract's inhibitory effect on biofilm formation. The *C. chinensis* extract significantly inhibited the growth of *S. mutans* and *S. sobrinus* at concentrations of ≥ 0.3% as compared with the control group. The viable cell count of colonies decreased by 1.7-fold and 1.2-fold at 2.5% and 1.25%, respectively, compared with the control group. The biofilm formation of *S. mutans* and *S. sobrinus* was inhibited by > 20-fold at *C. chinensis* extract concentrations of ≥ 1.25% as compared with the control group. In summary, the *C. chinensis* extract inhibited the growth and biofilm and glucan formation of *S. mutans* and *S. sobrinus*. Therefore, *C. chinensis* might be a potential candidate for controlling dental caries.

Keywords: Coptis, Growth, Biofilm, *Streptococcus mutans*, *Streptococcus sobrinus*

Introduction

According to a survey of Korean children's oral health status in 2018, more than half of 12-year-olds experienced permanent teeth caries. And the average number of caries experienced by 12-year-olds was 1.84 which is higher than the average of 1.2 Organization for Economic Cooperation and Development (OECD) member countries [1]. When bacteria that cause early dental caries are isolated, two strains of *Streptococcus mutans* and *Streptococcus sobrinus* are separated into 80% and 20%, respectively [2]. They produce glucan and fructan using sucrose as an efficient energy source by glucosyltransferase (GTFase) and fructosyltransferase, respectively. Among them, *S. mutans* has a close correlation with early dental caries in humans, and extracellular insoluble glucan which is synthesized from sucrose in food by the action of GTFase of *S. mutans*, is the cause of promoting dental caries [3]. Especially, insoluble glucan makes oral bacteria to adhere onto teeth sur-

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face and support to form microbial biofilm. Cariogenic bacteria such as *S. mutans* and *S. sobrinus* produce organic acids from glucan as well as dietary sugars. The organic acid, especially, lactic acid decomposes hydroxyapatite, a chemical component of tooth enamel. In addition, glucan makes biofilm dense and insoluble, thus, it acts as a barrier to prevent saliva penetration into biofilm resulting in reduced saliva buffering capacity and then, causes acid stagnates locally, which finally leads to dental caries [4]. Therefore, dental caries can be greatly decreased by inhibiting the growth of *S. mutans* and *S. sobrinus*. Until now, many studies have been performed to inhibit the growth of *S. mutans* and *S. sobrinus* for the control of dental caries. Several agents including antibiotics were developed but they showed adverse effects such as disturbance of intestinal flora, digestive disorders, and hypersensitivity reactions [5]. There are also several natural extracts such as *Camellia sinensis* [6–8], propolis [9], *Akebia quinata* [10,11], *Magnolia officinalis* [12,13], and Xylitol [14] which have inhibitory effects on dental caries. They show excellent antibacterial activities against cariogenic bacteria, however they still have several adverse effects [15–18]. Thus, we need to find out the effective agent to control dental caries.

Coptis chinensis (CC) which belongs to Ranunculaceae family has long been used as a herbal medicine, proving its stability [19]. CC is a perennial herb that grows in the mountainous region. It is an uneven columnar shape, 2–4 cm long, slightly curved, tastes bitter, and can remain yellowish on saliva. The roots of CC have various alkaloids such as berberine, worenine, coptisine, palmatine, and sanguinarine [20]. Among them, berberine, a major component of CC, inhibits bacterial growth by inhibiting bacterial carbohydrate metabolism and protein synthesis [19]. Traditionally, it has been used for detoxification, disinfection, treatment of eczema and burn, and hemostasis as well as removal of fever. It was also used to treat various diseases including conjunctivitis, otitis, hypertension and diabetes [21]. CC extract has significant antimicrobial activity against a variety of microorganisms including bacteria, viruses, fungi, protozoans, helminths, and Chlamydia [22–24]. However, the effect of CC on oral streptococci including *S. mutans* and *S. sobrinus* are not still investigated.

In this study at first, we tested the antibacterial activities of several extracts which have been reported to be effective for oral diseases in oriental medicine for the development of preventive agents of dental caries. Among them, CC extract showed the most excellent antibacterial effect. Thus the aim of this study was to study the anticariogenic activity of CC including the inhibitory effects on bacterial growth, glucan formation and biofilm formation.

Materials and Methods

1. Bacterial culture

S. mutans and *S. sobrinus* were cultured aerobically in brain heart infusion (BHI) at 37℃ in a 5% CO_2 atmosphere up to the late log phase of growth.

2. Preparation and treatment of CC extract and other natural extracts

CC extract and other natural extracts used in the study were taken from the Okcheondang located in Yeongcheon. All extracts were extracted with hydrothermal water using a reflux cooling extractor with 10 times (w/v) distilled water per 100 g. The extract was filtered with a filter paper (Whatman No. 2; Sigma-Aldrich, St. Louis, MO, USA) and then concentrated with the rotary vacuum evaporator (Rotavapor R-100; Buchi, Essen, Germany) before freeze drying it (TFD5505; ilShin BioBase, Dongducheon, Korea). The concentration of the extracts was adjusted by dissolving in phosphate-buffered saline (PBS) from 0.1% (1 mg/mL) to 10% (100 mg/mL).

3. Screening experiments of herbal extracts against *S. mutans* and *S. sobrinus*

Mentha piperascens, *Ulmus macrocarpa Hance*, *Syzygium aromaticum*, *Polygonum tinctorium* and CC extract suspended in PBS at the concentration of 0 to 5% were added into a 96 well plate and the bacteria were inoculated to 1×10^5 CFU/well. After incubating at 37℃ for 24 hours, the absorbance was measured at 650 nm using a spectrophotometer (Tecan, Männedorf, Switzerland). The control group was conducted without any herbal extracts.

4. Measurement of growth of *S. mutans* and *S. sobrinus*

The equal amount of BHI broth and CC extract were added to the 96 well plate and each bacterial species was inoculated to 1×10^5 CFU/well. After incubating at 37℃ for 24 hours, absorbance was measured at 650 nm using spectrophotometer. The control group was conducted without CC extract.

5. Viable cell count

CC extract (5 mL) was added into each tube at different concentrations 0 to 2.5%. *S. mutans* and *S. sobrinus* (1×10^4 CFU/mL) grown and diluted in BHI broth were inoculated into CC extract containing tube. After 18 hours of incubation at 5% CO_2 incubator, diluted with PBS and inoculated into BHI agar to measure the number of viable cells after 48 hours of incubation.

6. Measurement of biofilm formation

Each BHI broth with 5% sucrose in a 24 well plate was inoculated with *S. mutans* and *S. sobrinus*, respectively and mixed with CC extract to the concentration of 0 to 10%. After incubation at 37℃ for 72 hours to induced biofilm production, the biofilm was washed three times with PBS and then stained with 0.1% crystal violet solution for 10 minutes. After washing three times with PBS and drying in the hood for 15 minutes, the biofilm was dissolved by adding 100% ethanol and the absorbance was measured at 570 nm using a spectrophotometer.

7. Glucan synthesis test

S. mutans and *S. sobrinus* were inoculated to 1×10^5 CFU/

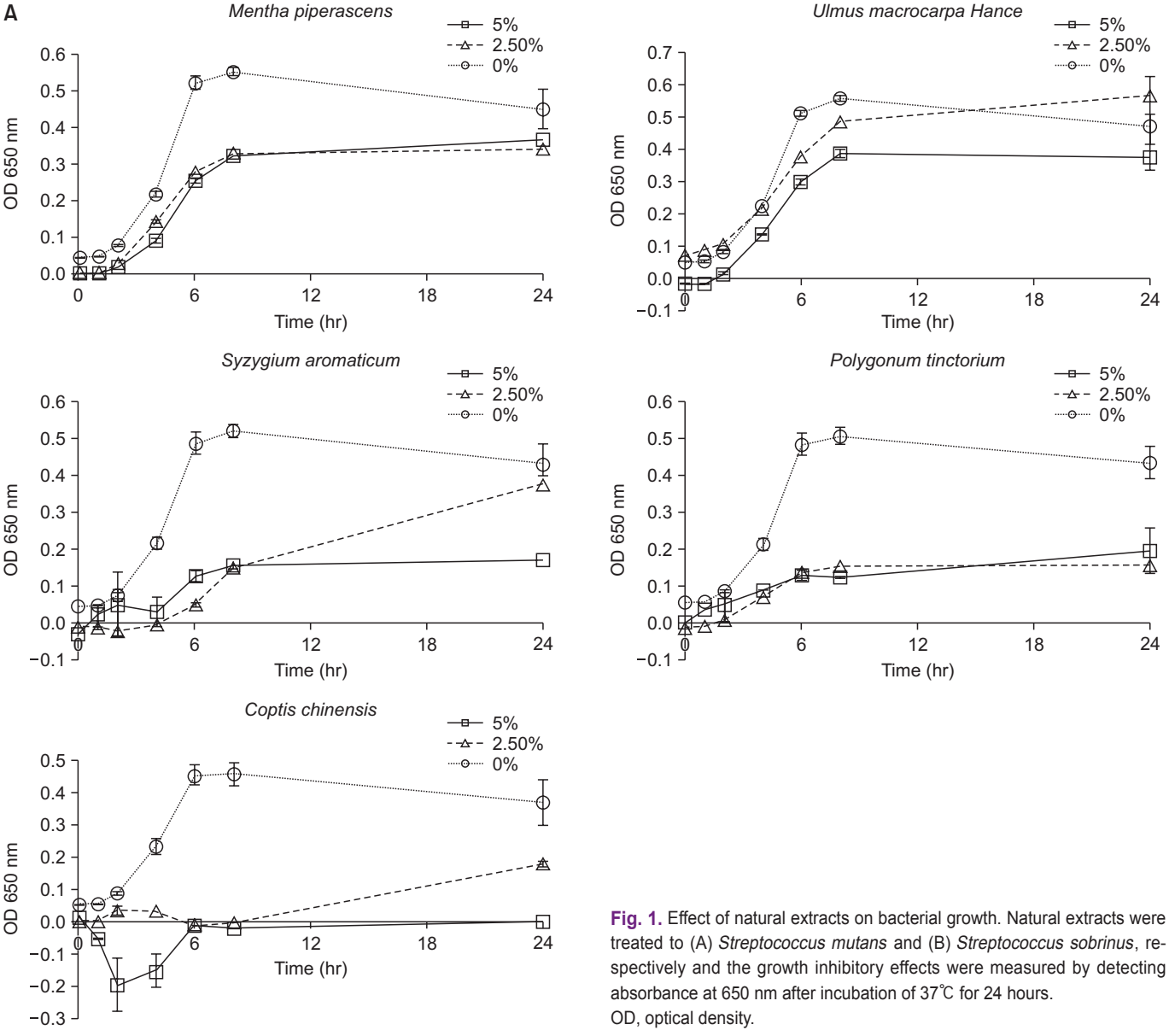

Fig. 1. Effect of natural extracts on bacterial growth. Natural extracts were treated to (A) *Streptococcus mutans* and (B) *Streptococcus sobrinus*, respectively and the growth inhibitory effects were measured by detecting absorbance at 650 nm after incubation of 37℃ for 24 hours.
OD, optical density.

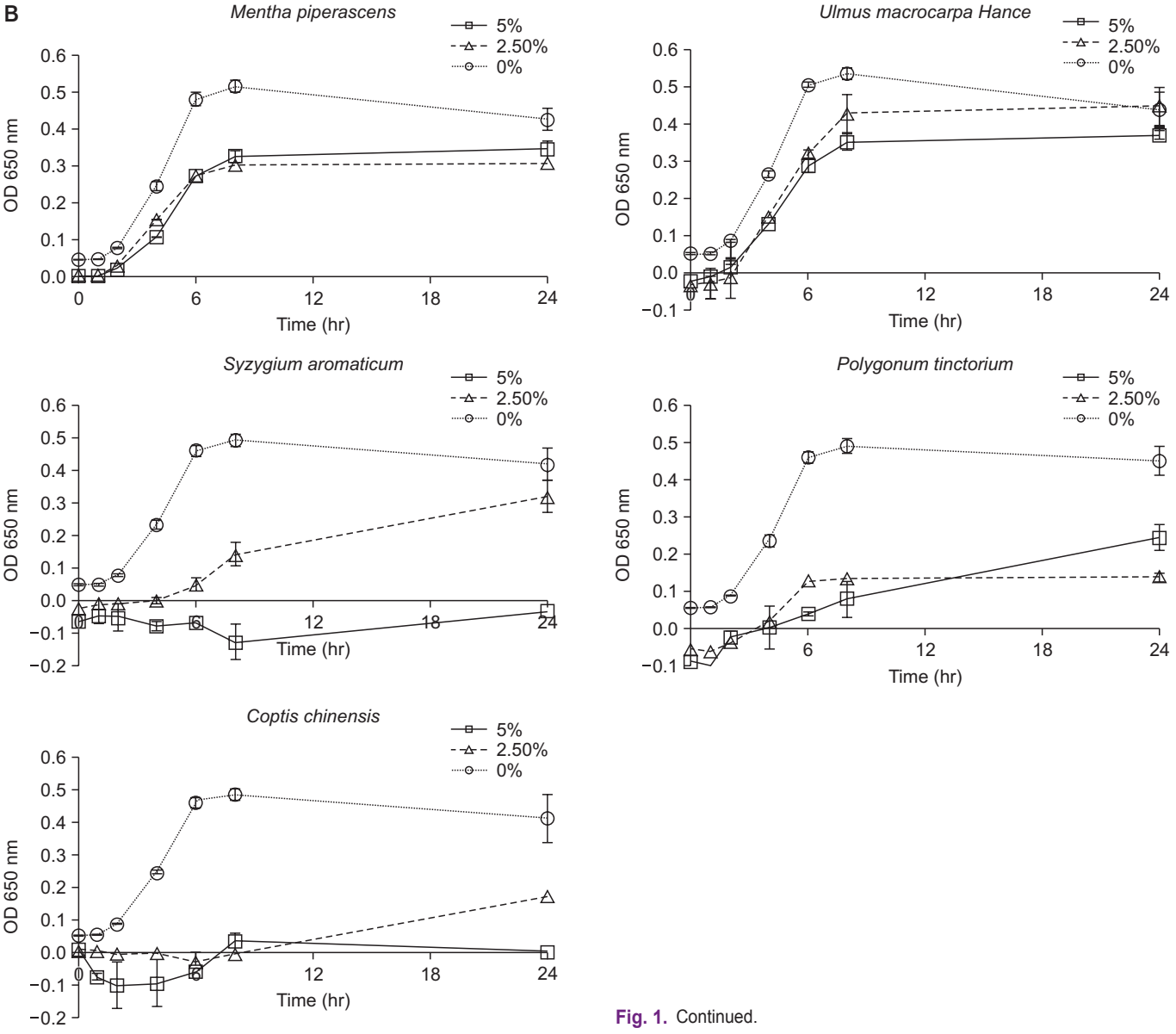

Fig. 1. Continued.

mL into glass test tubes containing BHI broth with or without 5% sucrose and then CC extract were added to the concentration of 0 to 2.5%. After incubation at 37℃ for 24 hours, 1 mL of the culture supernatant was centrifuged and 200 μL of supernatant was transferred into 96 well plate, and then the absorbance was measured at 650 nm by a spectrophotometer. The control group was performed without CC extract.

Results

1. Screening of natural extract with antibacterial activity

The extracts from *Mentha piperascens*, *Ulmus macrocarpa Hance*, *Syzygium aromaticum*, *Polygonum tinctorium*, and CC were tested for the inhibitory effect on bacterial growth. As shown in Fig. 1, CC extract most inhibited the growth of *S. mutans* and *S. sobrinus* compared to other natural extracts. Thus, CC extract was selected for further experiments.

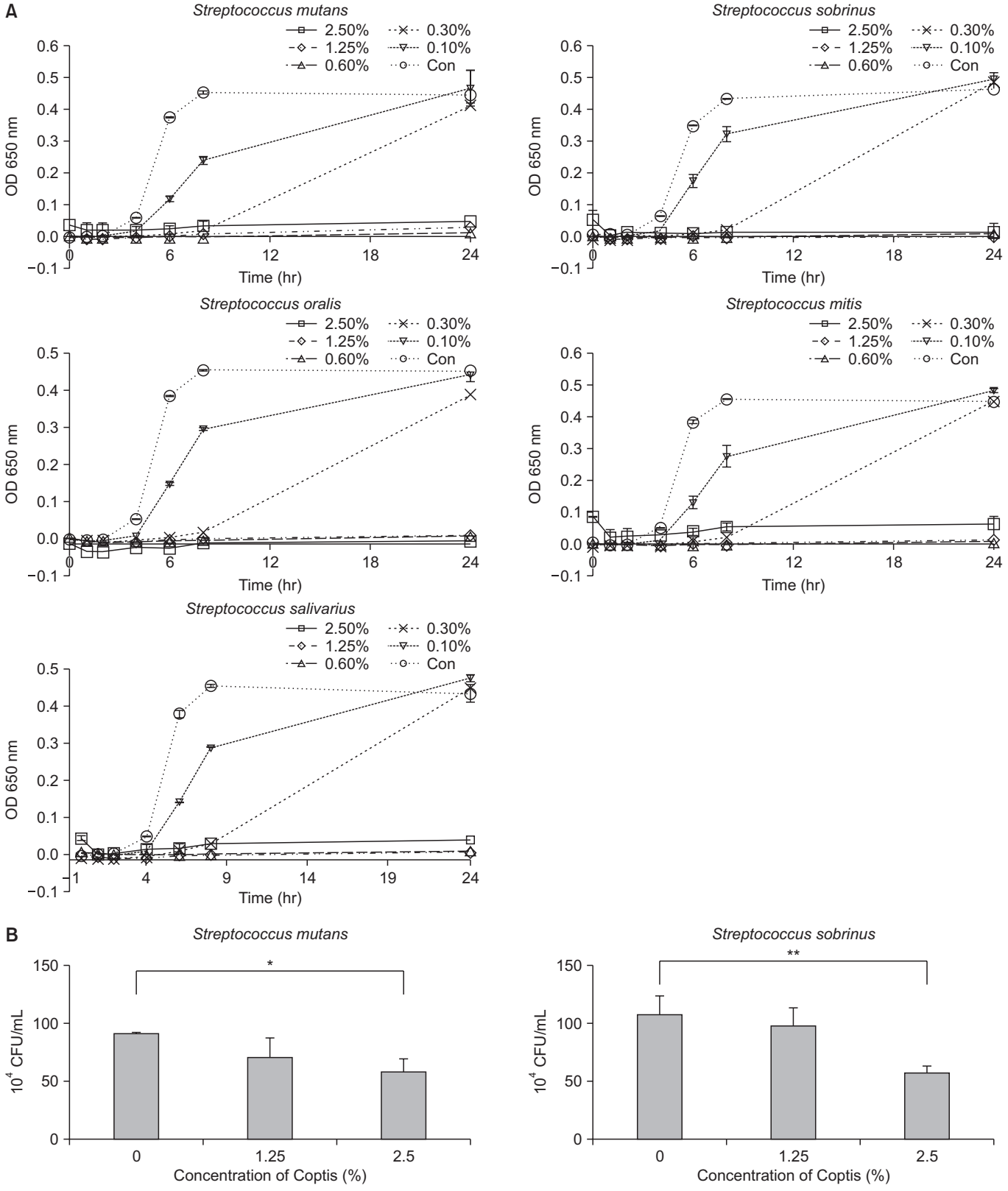

Fig. 2. Effect of *Coptis chinensis* (CC) extract on oral streptococci. (A) Oral streptococcal species were treated with CC extract solution of 0.1% to 2.5% and optical densities (OD) were measured at 650 nm after incubation of 37℃ for 24 hours. (B) *Streptococcus mutans* and *Streptococcus sobrinus* were treated with or without 1.25%, 2.5% CC extract at 37℃ overnight and viable cells were counted on brain heart infusion agar plates.
Con, control.
*$p < 0.05$, **$p < 0.01$.

2. Effect of CC extract on the growth of *S. mutans* and *S. sobrinus*

In order to examine the antibacterial activity of CC extract, *S. mutans*, *S. sobrinus*, *Streptococcus oralis*, *Streptococcus mitis*, and *Streptococcus salivarius* were treated with CC extract at 37℃ for 24 hours and the optical density was measured. All concentration of CC extract inhibited the growth of all Streptococcal species used in the experiment, and growth of all test strains was inhibited at concentrations above 0.6% (Fig. 2A). According to viable cell count, 2.5% CC extract significantly decreased the number of viable cells of *S. mutans* and *S. sobrinus* compared to control group (Fig. 2B).

3. Effect of CC extract on biofilm formation

We examined whether CC extract inhibited the biofilm formation because it is critical for causing dental caries. As shown in Fig. 3, the biofilm formation by *S. mutans* and *S. sobrinus* was decreased 20-fold more at the concentrations of 1.25 and 2.5% of CC, compared to the control.

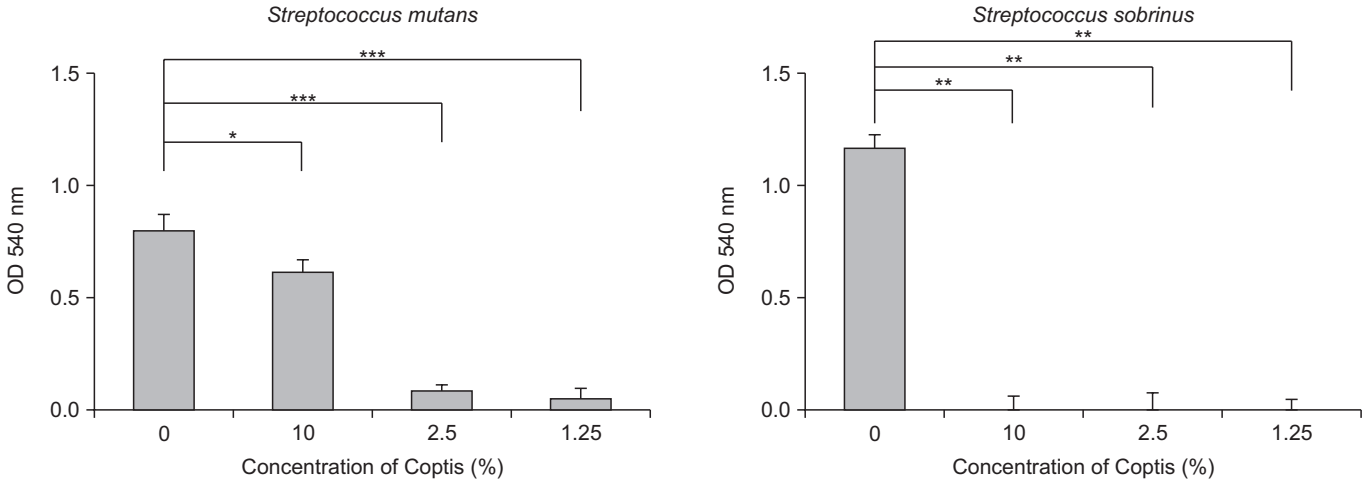

Fig. 3. Effect of *Coptis chinensis* extract on biofilm formation. The biofilm was produced by *Streptococcus mutans* and *Streptococcus sobrinus* at 37℃ for 72 hours, washed with phosphate-buffered saline, stained with crystal violet and measured by detecting optical densities (OD) at 540 nm using spectrophotometer.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

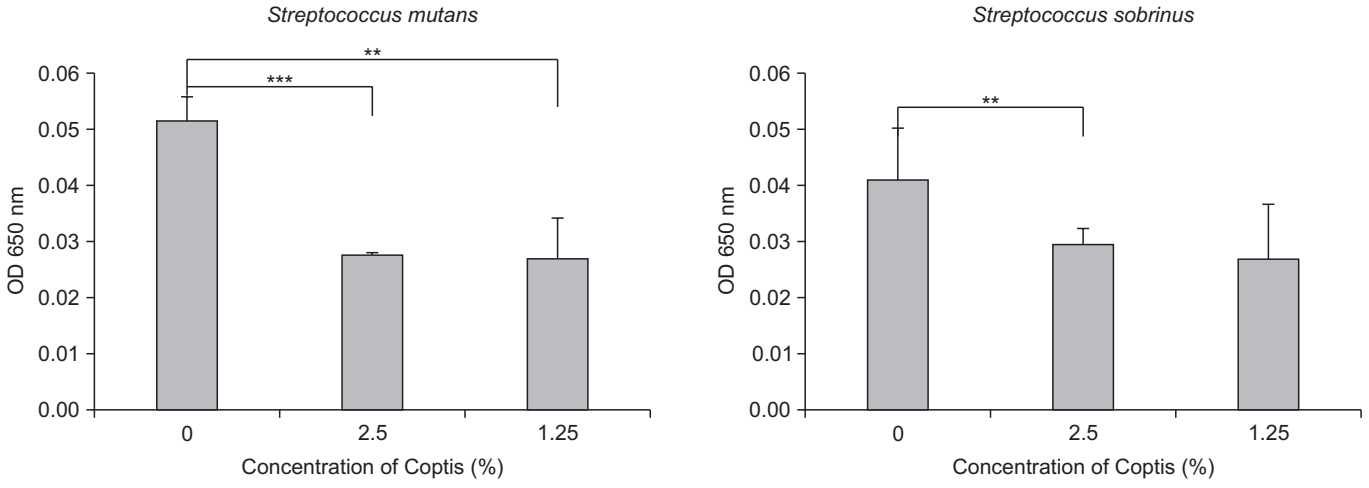

Fig. 4. Effect of *Coptis chinensis* (CC) extract on glucan synthesis. *Streptococcus mutans* and *Streptococcus sobrinus* were grown in BHIS broth with or without CC extract at 37℃ overnight. The culture solution was centrifuged and the optical densities (OD) of the culture supernatants were measured at 650 nm to determine glucan synthesis.
**$p < 0.01$, ***$p < 0.001$.

4. Effect of CC extract on glucan synthesis

We examined the effect on glucan synthesis which is an essential component for biofilm development. CC extract inhibited glucan synthesis (Fig. 4). Especially, 1.25% and 2.5% of CC extract significantly inhibited glucan synthesis by both *S. mutans* and *S. sobrinus* compared to the control.

Discussion

CC is a traditional Chinese herbal medicine that has been used to treat diabetes for more than 1,000 years [25]. It has also been used to treat gastroenteritis and diarrhea [26]. In particular, Illustrated Book of Korean Medical Herbs describes the various applications of the efficacy of the CC in relation to infectious diseases [27]. CC has been reported to possess the bactericidal activity in skin infection [27]. Although the anticariogenic effect of CC extract on *S. mutans* has been previously reported [19], the effects on biofilm formation or glucan synthesis associated with *S. sobrinus* have not been fully investigated.

CC is composed of several active ingredients such as coptisine, epiberberine, berberine, and palmatine [28]. Among these, coptisine, epiberberine, and plamatine were reported to inhibit *Helicobacter pylori* urease and to exhibit antimicrobial effect against *H. pylori* [29-31]. In this study, CC extract showed strong inhibitory effect on the growth of *S. mutans* and *S. sobrinus* in a dose-dependent manner. In particular, the number of viable bacteria was significantly reduced when *S. mutans* and *S. sobrinus* were exposed to CC extract with concentration of 2.5% for 18 hours. Therefore, these results suggested that the antibacterial effect of CC extract can also be used to inhibit dental caries by *S. mutans* and *S. sobrinus*.

Next, we examined the effects of CC extract on biofilm formation produced by *S. mutans* and *S. sobrinus*. *S. mutans* and *S. sobrinus* play an important role in oral biofilm formation [32]. Bacterial populations form biofilms adhering to teeth surfaces and to synthesizing bacterial membranes composed of polysaccharides. Biofilm significantly increases resistance to various environmental stresses such as nutrient depletion, acidity changes, and osmotic pressure compared to floating bacteria, and affect oral health [33-36]. Therefore, it is necessary to control the biofilm formation by *S. mutans* and *S. sobrinus* to effectively prevent dental caries. In this experiments, as shown in Fig. 3, we examined the inhibitory effect of CC extract on the biofilm formation by *S. mutans* and *S. sobrinus*.

Finally, we investigated the effects of CC extract on glucan synthesis by *S. mutans* and *S. sobrinus*. Glucan promotes bacterial attachment to teeth and interrupts their separation owing to physical forces such as masticatory movements. *S. mutans* attaches to the tooth surface via sucrose-dependent and sucrose-independent pathways [30-32]. Sucrose-dependent adhesion is mediated by glucan binding proteins and glucans produced by GTFase enzymes [37]. The present results showed that CC extract reduced glucan synthesis, suggesting that CC extract could contribute to inhibiting GTFase activty. This result is similar to previous reports that other oriental medicines such as *Aconitum koreanum* extract and *Radix pulsatillae* extract, inhibited glucan synthesis and further showed anti-caries effects [4,38]. Taken together, CC extract suppressed the synthesis of biofilm and glucan which is critical for dental plaque formation and dental caries development. This might be caused by the inhibitory effect of CC extract on the growth of *S. mutans* and *S. sobrinus*.

In summary, our study showed that CC extract inhibited the growth of *S. mutans* and *S. sobrinus*, the biofilm formation and glucan synthesis by *S. mutans* and *S. sobrinus*. Therefore, CC extract might be suggested as a potential candidate for the control of dental caries through the antibacterial effects.

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Conflicts of Interest

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

References

1. Categorical data by Korean Ministry of Health and Welfare [Internet]. Sejong: MOHW; [cited 2019 Jun 2]. Available from: http://www.mohw.go.kr/react/jb/sjb1101vw.jsp?SEQ=89&MENU_ID=03320101&page=1&PAR_MENU_ID=03#
2. Yoo YK, Ro JS, Kim JS, Chang KW. The antibacterial effects

of some propolis constituents against *S. mutans*, Lactobacilli and Actinomyces. J Korean Acad Oral Health 1996;20:65–74.
3. Hamada S, Koga T, Ooshima T. Virulence factors of *Streptococcus mutans* and dental caries prevention. J Dent Res 1984;63:407–11. doi: 10.1177/00220345840630031001.
4. Kang SY, An SY, Lee MW, Kwon SK, Lee DH, Jeon BH, Kim KJ, You YO. Effects of aconitum koreanum extract on the growth, acid production, adhesion and insoluble glucan synthesis of *Streptococcus mutans*. J Physiol Pathol Korean Med 2015;29:27–32. doi: 10.15188/kjopp.2015.02.29.1.27.
5. Chen CP, Lin CC, Namba T. Screening of Taiwanese crude drugs for antibacterial activity against *Streptococcus mutans*. J Ethnopharmacol 1989;27:285–95. doi: 10.1016/0378-8741(89)90003-2.
6. Sakanaka S, Kim M, Taniguchi M, Yamamoto T. Antibacterial substances in Japanese green tea extract against *Streptococcus mutans*, a cariogenic bacterium. Agric Biol Chem 1989;53:2307–11. doi: 10.1271/bbb1961.53.2307.
7. Kubo I, Muroi H, Himejima M. Antimicrobial activity of green tea flavor components and their combination effects. J Agric Food Chem 1992;40:245–8. doi: 10.1021/jf00014a015.
8. Otake S, Makimura M, Kuroki T, Nishihara Y, Hirasawa M. Anticaries effects of polyphenolic compounds from Japanese green tea. Caries Res 1991;25:438–43. doi: 10.1159/000261407.
9. Steinberg D, Kaine G, Gedalia I. Antibacterial effect of propolis and honey on oral bacteria. Am J Dent 1996;9:236–9.
10. Chang KW, Oh IS, Lee JH. Effect of dietary erythritol supplemented with chitosan, extracts of Akebia and extracts of Ishige on the growth of mutans streptococci. J Korean Acad Oral Health 1997;21:545–52.
11. Chang KW, Kang DO, Kim HG. Antibacterial effect and adsorption inhibition of oral streptococci to saliva- coated hydroxyapatite beads with Akebia quinata extract. J Korean Acad Oral Health 1997;21:675–84.
12. Jang BS, Son SH, Chung CP, Bae KH. The effects of honokiol and magnolol on the antimicrobial, bacterial collagenase activity, cytotoxicity and cytokine production. J Periodontal Implant Sci 1993;23:145–58.
13. Bae KH. The antibacterial activites of components isolated from the stem bark of Magnolia Obovota against a cariogenic bacterium, *Streptococcus mutans* OMZ 176. Symp Org Chem Abstr 1987;176:34.
14. Shin KH, Yang KH, Choi NK, Kim SM, Oh JS. The effect of xylitol on the lactose fermentation of Streptococcus. J Korean Acad Pediatr Dent 2004;31:202–11.
15. de Groot AC. Propolis: a review of properties, applications, chemical composition, contact allergy, and other adverse effects. Dermatitis 2013;24:263–82. doi: 10.1097/DER.0000000000000011.
16. Schumer W. Adverse effects of xylitol in parenteral alimentation. Metabolism 1971;20:345–7. doi: 10.1016/0026-0495(71)90096-5.
17. Abshenas J, Babaei H, Zare MH, Allahbakhshi A, Sharififar F. The effects of green tea (Camellia sinensis) extract on mouse semen quality after scrotal heat stress. Vet Res Forum 2011;2:242–7.
18. Poivre M, Duez P. Biological activity and toxicity of the Chinese herb Magnolia officinalis Rehder & E. Wilson (Houpo) and its constituents. J Zhejiang Univ Sci B 2017;18:194–214. doi: 10.1631/jzus.B1600299.
19. Jang GH, Ahn BY, Oh SH, Choi DS, Kwon YJ. Anticariogenic effects of Coptis chinensis Franch extract. Korean J Food Sci Technol 2000;32:1396–402.
20. National Institute of Food and Drug Safety Evaluation [Internet]. Cheongju: National Institute of Food and Drug Safety Evaluation; 2018 [cited 2018 Dec 16]. Available from: https://www.nifds.go.kr/brd/m_184/view.do?seq=13
21. Muluye RA, Bian Y, Alemu PN. Anti-inflammatory and antimicrobial effects of heat-clearing Chinese herbs: a current review. J Tradit Complement Med 2014;4:93–8. doi: 10.4103/2225-4110.126635.
22. Cernáková M, Kostálová D. Antimicrobial activity of berberine--a constituent of Mahonia aquifolium. Folia Microbiol (Praha) 2002;47:375–8. doi: 10.1007/BF02818693.
23. Freile ML, Giannini F, Pucci G, Sturniolo A, Rodero L, Pucci O, Balzareti V, Enriz RD. Antimicrobial activity of aqueous extracts and of berberine isolated from Berberis heterophylla. Fitoterapia 2003;74:702–5. doi: 10.1016/s0367-326x(03)00156-4.
24. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564–82. doi: 10.1128/CMR.12.4.564.
25. Zhen Z, Chang B, Li M, Lian FM, Chen L, Dong L, Wang J, Yu B, Liu WK, Li XY, Qin PJ, Zhang JH, Tong XL. Anti-diabetic effects of a Coptis chinensis containing new traditional Chinese medicine formula in type 2 diabetic rats. Am J Chin Med 2011;39:53–63. doi: 10.1142/S0192415X11008646.
26. Hung TM, Lee JP, Min BS, Choi JS, Na M, Zhang X, Ngoc TM, Lee I, Bae K. Magnoflorine from coptidis rhizoma protects high density lipoprotein during oxidant stress. Biol Pharm Bull 2007;30:1157–60. doi: 10.1248/bpb.30.1157.
27. Ahn DK. Iiiustrated book of Korean medicinal herbs. Seoul:

Kyohaksa; 1998.

28. Liu S, Zhang X, Qiu F, Miao P, Shen S, Zhu L, Zeng J, Jiang J. Metabolic interaction of the active constituents of coptis chinensis in human liver microsomes. Evid Based Complement Alternat Med 2015;2015:802903. doi: 10.1155/2015/802903.
29. Li C, Huang P, Wong K, Xu Y, Tan L, Chen H, Lu Q, Luo C, Tam C, Zhu L, Su Z, Xie J. Coptisine-induced inhibition of Helicobacter pylori: elucidation of specific mechanisms by probing urease active site and its maturation process. J Enzyme Inhib Med Chem 2018;33:1362-75. doi: 10.1080/14756366.2018.1501044.
30. Tan L, Li C, Chen H, Mo Z, Zhou J, Liu Y, Ma Z, Xu Y, Yang X, Xie J, Su Z. Epiberberine, a natural protoberberine alkaloid, inhibits urease of Helicobacter pylori and jack bean: susceptibility and mechanism. Eur J Pharm Sci 2017;110:77-86. doi: 10.1016/j.ejps.2017.02.004.
31. Zhou JT, Li CL, Tan LH, Xu YF, Liu YH, Mo ZZ, Dou YX, Su R, Su ZR, Huang P, Xie JH. Inhibition of helicobacter pylori and its associated urease by Palmatine: investigation on the potential mechanism. PLoS One 2017;12:e0168944. doi: 10.1371/journal.pone.0168944.
32. Kuramitsu HK, He X, Lux R, Anderson MH, Shi W. Interspecies interactions within oral microbial communities. Microbiol Mol Biol Rev 2007;71:653-70. doi: 10.1128/MMBR.00024-07.
33. Kim HE. Change of paradigms in caries-associated bacteria in the caries process: ecological perspectives. J Dent Hyg Sci 2014;14:87-93.
34. Pratt LA, Kolter R. Genetic analyses of bacterial biofilm formation. Curr Opin Microbiol 1999;2:598-603. doi: 10.1016/s1369-5274(99)00028-4.
35. Stoodley P, Sauer K, Davies DG, Costerton JW. Biofilms as complex differentiated communities. Annu Rev Microbiol 2002;56:187-209. doi: 10.1146/annurev.micro.56.012302.160705.
36. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet 2001;358:135-8. doi: 10.1016/s0140-6736(01)05321-1.
37. Kim KJ, Park BI, Min JH, Chae MS, Lim JY, Son HJ, Lee GH, An SY, Jeon BH, Choi NY, You YO. Inhibitory effects of radix Pulsatillae extract on insoluble glucan synthesis and adhesion of *Streptococcus mutans*. J Physiol Pathol Korean Med 2016;30:27-32. doi: 10.15188/kjopp.2016.02.30.1.27.
38. Rozen R, Bachrach G, Bronshteyn M, Gedalia I, Steinberg D. The role of fructans on dental biofilm formation by *Streptococcus sobrinus*, *Streptococcus mutans*, Streptococcus gordonii and Actinomyces viscosus. FEMS Microbiol Lett 2001;195:205-10. doi: 10.1111/j.1574-6968.2001.tb10522.x.