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Original article

The effect of epigallocatechin gallate on *Streptococcus Gordonii* biofilm formation

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ABSTRACT

Background: *Dental caries, as a primary disease in dentistry, is strongly influenced by the presence of biofilms. One of the Gram-positive bacteria that acts as an initiator in the biofilm formation process is Streptococcus gordonii. As the primary catechin in green tea, epigallocatechin gallate (EGCG) is easily found in our daily lives, and it has a broad spectrum of antimicrobial effects. Several studies have revealed that EGCG inhibited the growth of Gram-positive bacteria, including inhibiting biofilm formation by damaging the bacterial cell wall and reducing glucosyltransferase activity. However, there is still limited information that explains the effect of EGCG on S. gordonii bacterial biofilms.* ***Purpose:*** *This study aims to analyze the effect of EGCG in inhibiting the formation of S. gordonii bacterial biofilms.* ***Methods:*** *This study was an in-vitro experimental laboratory study, with samples divided into five groups, namely, the group containing BHIB-bacteria, the BHIB-bacteria-5% sucrose groups, and the treatment groups containing BHIB-bacteria-5% sucrose-EGCG with concentrations of 12.5%, 6.25%, and 3.125%, respectively, incubated for 24 hours. The data was analyzed using the Kruskal–Wallis test.* ***Results:*** *There was a significant difference in the formation of biofilms in S. gordonii bacteria with the addition of 5% sucrose in BHIB compared with the group of S. gordonii bacteria in BHIB. The highest biofilm formation in the group containing bacteria-BHIB-5% sucrose, while the lowest biofilm formation occurred in the treatment group containing bacteria-BHIB-5% sucrose-12.5% EGCG with significant difference between the group.* ***Conclusion:*** *The addition of EGCG 12.5% inhibits the formation of S. gordonii biofilms.*

Keywords: *biofilm formation; epigallocatechin gallate; human disease; medicine; Streptococcus gordonii*
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INTRODUCTION

The oral cavity is an important part of the body as it serves as the main entrance for nutrients to enter the systemic body. Therefore, oral health requires increased attention.[1] However, many children face issues with their oral cavity, such as dental caries.[2] According to the World Health Organization,[3] 60–90% of school-age children suffer from dental caries, especially in developing countries.

Dental disease is undoubtedly a public health concern and one of the most prevalent diseases worldwide, particularly dental caries, which is a biofilm-associated disease.[4] Biofilm is an aggregate of bacteria with various species. Bacterial cells adhere to a surface and are enveloped in a matrix produced by the bacteria, known as extracellular polymeric substances (EPS). *Streptococcus gordonii* is a Gram-positive bacterium with a coccus-shaped alpha- hemolytic chain that plays a crucial role as a pioneer bacterium in the biofilm formation process.[5,6]

Recently, herbal medicine in dentistry has become the preference for many, with green tea being one of the choices.[7] The compositions of green tea includes polyphenols, amino acids, proteins, fats, and various minerals and carbohydrates, with the main content being

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polyphenols.[8] Almost all of the polyphenols in green tea are catechins.[9,10] The primary catechin in green tea is epigallocatechin gallate (EGCG), which offers positive health effects, such as antitumor, antioxidant and antimicrobial effects.[11,12] Furthermore, EGCG exhibits broad-spectrum antimicrobial activity. In several studies, EGCG has been shown to be more effective in inhibiting the growth of Gram-positive bacteria than that of Gram-negative bacteria.[12]

A previous study revealed that EGCG influences the expression of regulatory genes in the formation of biofilms and affects the morphology of microorganism cells.[13] Another study demonstrated that EGCG can bind to *Streptococcus mutans* glucansucrase and inhibit enzyme activity.[14] Therefore, this study aims to confirm the effect of EGCG against the formation of *S. gordonii* bacterial biofilms.

MATERIALS AND METHODS

This research constitutes a laboratory experiment with a post-test only control group design. The sample in this study was *S. gordonii (*ATCC 51656, VPI E1A-1A [PK488], Manassas, United States) available at the Research Center for the Faculty of Dental Medicine, Airlangga University. The laboratory instruments and materials included a micropipette, incubator, polystyrene test tube, inoculation loop, fridge (−80°) (Thermo Fisher Scientific, Waltham, MA, USA), anaerobic jar (Thermo Fisher Scientific, Oxoid, Waltham, MA, USA), clean bench (Sanyo, Osaka, Japan), vortex (Thermo Fisher Scientific, Waltham, MA, USA), centrifugation instrument (Thermo Fisher Scientific, Waltham, MA, USA) and an enzyme-linked immunosorbent assay (ELISA) reader (Epoch, Biotek, Germany).

This study utilized EGCG (No.cat CFN99569, 989-51-5, Chem Faces, Tokyo, Japan), 5% sucrose (TCI, Tokyo, Japan), brain heart infusion broth (BHIB) (Thermo Fisher Scientific, Oxoid, Waltham, MA USA), Tryptone Soya Broth (Sigma-Aldrich, Darmstadt, Germany), and phosphate buffer saline (PBS) (Sigma- Aldrich, Darmstadt, Germany). This work was supported by the Committee on Bioethics, Faculty of Dental Medicine, Airlangga University, Surabaya (341/HRECC.FODM/VI/2022) from the Research Center, Airlangga University, Surabaya, Indonesia.

One inoculation loop of pure *S. gordonii* bacteria was inoculated into BHIB medium and then incubated in an incubator for 24 hours at 37°C. The EGCG used in this study was in the form of a solution. Epigallocatechin gallate preparation in experiments or studies typically involves solubilizing EGCG powder in a suitable solvent. Here, powder-form EGCG was added to sterile water to obtain concentrations of 3.125%, 6.25%, and 12.5%.

S. gordonii bacteria, which had been cultured in BHIB, BHIB-5% sucrose and BHIB-5% sucrose-EGCG (3.125%, 6.25%, 12.5%) for 24 hours at 37°C were taken in 100-µL samples and then placed into a microtiter dish. The bacteria were then incubated at 37°C for 48 hours, followed by the removal of bacterial cells that did not adhere to the microtiter plate. Next, the planktonic cells were rinsed using 200 µL of PBS. Following this, all wells were stained with 200 µl of 0.1% crystal violet for 15 minutes at room temperature; subsequently, the dye was removed by rinsing twice with sterile water, PBS and 100 µl of 95% alcohol. The microtiter plate was shaken for 10 minutes using a shaker. The formed biofilm was measured using the ELISA reader at 540 nm (GloMax® Discover Microplate Reader, Promega, Madison, Wisconsin, USA).[15] The Kruskal–Wallis test was used to determine the significance of intergroup differences. Statistical significance was defined as a p-value of <0.05 (Statistical Analysis for Social Science (SPSS), IBM corporation, Illinois, Chicago, USA).

RESULTS

The results of this study indicated a significant difference in the formation of biofilms in *S. gordonii* bacteria with the addition of 5% sucrose in BHIB, measuring (25.95 mg/mL±0.694) compared with the group of *S. gordonii* bacteria in BHIB (11.92 mg/mL±3.288) (Figure 1). There was a notable difference between these groups (p<0.01), as described in Table 1. As Figure 2 and Figure 3

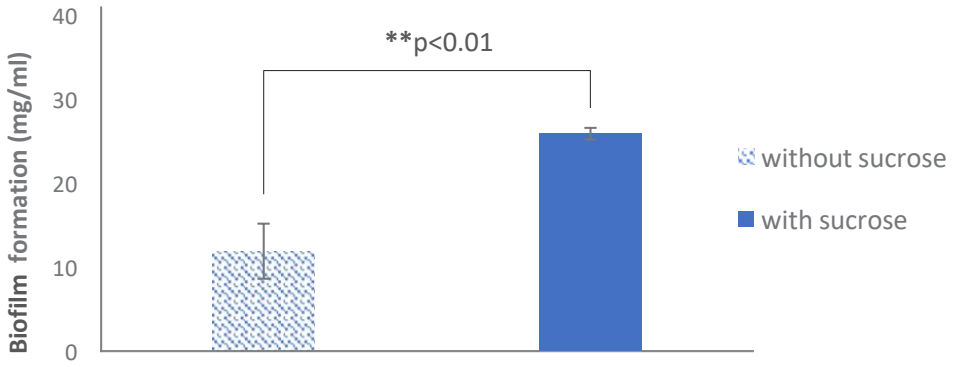

Figure 1. Biofilm formation of *Streptococcus gordonii* after incubating in brain heart infusionbroth with/without sucrose 5%.

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Table 1. The significance differences between the groups

Groups	Significance Difference
Group 1 – Group 2	<0.001
Group 1 – Group 3	0.14
Group 1 – Group 4	0.42
Group 1 – Group 5	0.310
Group 2 – Group 3	0.189
Group 2 – Group 4	0.083
Group 2 – Group 5	0.006
Group 3 – Group 4	0.676
Group 3 – Group 5	0.151
Group 4 – Group 5	0.310

Table 2. Mean and standard deviation of biofilm formation

Groups	Mean	Standard Deviation
Group 1 (Bacteria + BHIB)	11.92	3.289
Group 2 (Bacteria + BHIB + sucrose 5%)	25.94	0.694
Group 3 (Bacteria + BHIB + sucrose 5% + EGCG 3.125%)	20.24	2.446
Group 4 (Bacteria + BHIB + sucrose 5% + EGCG 6.25%)	18.54	0.797
Group 5 (Bacteria + BHIB + sucrose 5% + EGCG 12.5%)	16.48	1.262

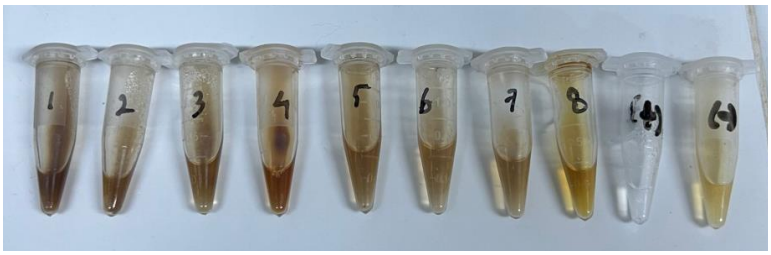

Figure 2. Dilution method to perform the Minimum Inhibitory Concentration (MIC).

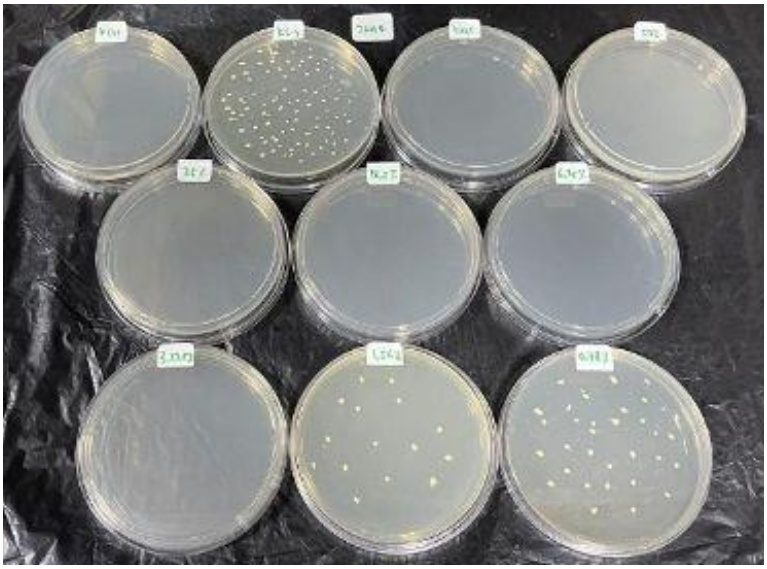

Figure 3. Colony forming of *Streptococcus gordonii* to perform the Minimum Bactericidal Concentration (MBC).

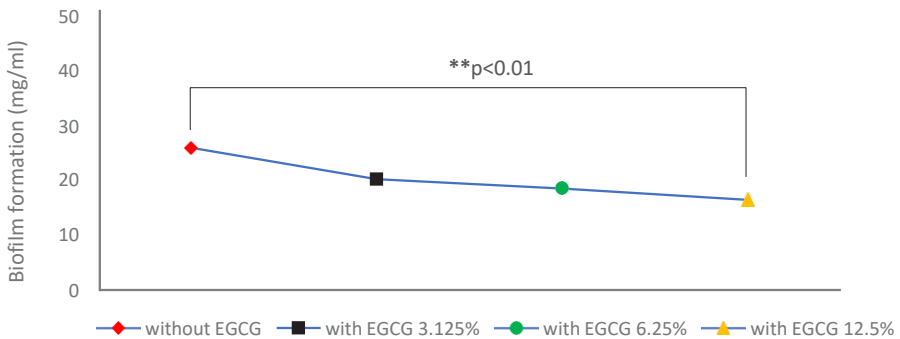

Figure 4. Effect of epigallocatechin gallate biofilm formation of *Streptococcus gordonii* afterincubating in brain heart infusion broth-sucrose 5%.

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illustrate, there was a decrease in biofilm formation with an increase in EGCG concentration. The graph highlights the highest biofilm formation in the group containing bacteria-BHIB-5% sucrose (25.95 mg/mL±0.694), while the lowest biofilm formation occurred in the treatment group containing bacteria-BHIB-5% sucrose-12.5% EGCG (16.48 mg/mL±1.262), as described in Figure 4 and Table 2. A significant difference was observed between the group containing bacteria- BHIB-5% sucrose and the treatment group containing bacteria-BHIB-5% sucrose-12.5% EGCG ($p<0.01$; Table 1).

DISCUSSION

S. gordonii plays an essential role in creating dental biofilm, which can lead to the development of dental caries. This bacterium, as an early coloniser, can aggregate with other microorganisms such as *C. albicans*, *P.gingivalis*, and other *Streptococcus* species to form biofilms on enamel surfaces.[16] *S. gordonii* can metabolise glucose by binding with the α-amylase enzyme.[17] Simultaneously, glucose metabolism leads to the production of organic acids that can demineralize the enamel structure.[18]

In addition, *S. gordonii* has a faster multiplication rate compared with other *Streptococcus* species. This bacterium also aids *S. mutans,* the primary pathogenic bacterium in dental caries,[17] by facilitating glucose metabolism, exhibiting greater resistance to acidic environments (low pH),[19] and activating the glucosyltransferase (GTF) enzyme. The ability of *S. gordonii* to facilitate and aggregate with other microorganisms in forming dental biofilm on the enamel surfaces plays a crucial role in the dental caries process.[20]

The results indicate that the formation of biofilms in the BHIB-bacteria-5% sucrose group was significantly higher than that in the BHIB-bacteria group ($p<0.01$). Thus, 5% sucrose induces the formation of *S. gordonii* biofilms. Sucrose is widely recognised as one of the most cariogenic carbohydrates among dietary sugars. It can be fermented and serves as a substrate for the synthesis of polysaccharides, particularly EPSs in dental plaque, which play a crucial role in the development of caries.[21] Previous research has demonstrated that increasing sucrose to 5% can lead to the formation of the most cariogenic biofilms.[22] Biofilm formation on tooth surfaces is associated with a high sucrose diet.[23] Sucrose is highly soluble in water and rapidly diffuses into biofilms.[24] In the presence of sucrose, bacteria synthesise soluble and insoluble glucans through the enzyme, Gtf. Synthesised glucan plays a role in mediating attachment to the tooth surface and biofilm growth by attracting planktonic cells. The accumulation of planktonic cells and EPS leads to the formation of colonies that develop into mature biofilms.[18]

This study aimed to determine the effect of EGCG on *S. gordonii* biofilm formation. Based on the results, the biofilm formation of *S. gordonii* decreases with an increase in EGCG concentration. A 12.5% concentration of EGCG significantly inhibited the biofilm formation of *S. gordonii* ($p<0.05$). Previous studies have revealed that EGCG possesses antibacterial and anti-biofilm effects against Gram-positive bacteria, such as *S. mutans*.[13,25] As a constituent of the catechins in green tea, EGCG plays a vital role in the antimicrobial effect of this type of tea. Green tea exerts antibacterial activity by acting on the protective components of saliva (secretory immunoglobulin, histatin, mucin, lysozyme, lactoferrin and oral peroxidase). In addition, EGCG inhibits bacterial attachment to the tooth surface and Gtf enzyme.[21] EGCG can inhibit the growth of both Gram-positive and Gram-negative bacteria. Several studies indicate that EGCG is more effective in inhibiting the growth of Gram-positive bacteria than Gram-negative bacteria. Other research asserts that EGCG can inhibit the growth of *Mycobacterium tuberculosis, Stenotrophomonas maltophilia, Staphylococcus aureus, Helicobacter pylori, Staphylococcus aureus* and *Streptococci spp*.[26] Previous research demonstrated that EGCG can hinder biofilm formation through three distinct mechanisms.[27] In fact, EGCG disrupts biofilm formation via several avenues, including the inhibition of bacterial growth by impeding bacterial metabolism, the promotion of bacterial aggregation, and the inhibition of Gtf activity.[27] In brief, EGCG enters the cell membrane, damaging the lipid bilayer and consequently weakening the bacterial wall, a pivotal factor in bacterial defense and growth. Furthermore, EGCG can impede the activity of the phosphoenolpyruvate-phosphotransferase system (PEP-PTS), an enzyme crucial for transporting glucose into bacteria, with the system encompassing enzymes located in both the cell membrane and cytoplasm. The inhibition of PEP-PTS activity can curtail glucose absorption into bacterial cells, thereby affecting bacterial metabolism and interfering with acid production.[27] Furthermore, EGCG hinders acidogenic and aciduric properties by suppressing the enzymatic activity of ATPase F1F0, alongside downregulating proteins in the ATP synthesized field.[28] Since ATP plays an important role as an energy source for bacteria if ATP synthesis decreases, bacterial growth will be inhibited, in severe cases, leading to bacterial cell lysis, resulting in bacterial demise.[29]

In line with previous research, it was demonstrated that EGCG disrupts biofilm formation through at least three different mechanisms, including the inhibition of bacterial growth through the inhibition of bacterial metabolism, the promotion of bacterial aggregation, and the inhibition of Gtf activity.[27] A study conducted by Zayed et al. [21] affirmed that EGCG reduces the expression of three genes that code for the Gtf enzyme. The Gtf enzyme is instrumental in converting sucrose into glucan, a foundational component of EPS crucial to biofilm formation, thereby inhibiting biofilm formation. This study limitation is only conducted in in vitro study with effect of EGCG in the *S. gordonii* bacterias with simple method.

The conclusion of the study is the addition of EGCG 12.5% inhibits the formation of *S. gordonii* biofilms.

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The results of this study offer preliminary insights into how EGCG can impede biofilms in *S. gordonii* bacteria, indicating its potential as a material for preventing dental caries. Further research is needed to determine the effect of EGCG on the formation of *Streptococcus gordonii* bacterial biofilms in the oral cavity using other methods such as scanning electron microscopy or confocal scanning laser microscopy. Further in vivo and clinical research are expected. In addition, more detailed research is expected on the effects of EGCG on Gtf enzyme activity and also on the bacterial metabolic process in *Streptococcus gordonii*.

REFERENCES

1. Wicaksono DP, Washio J, Abiko Y, Domon H, Takahashi N. Nitrite production from nitrate and its link with lactate metabolism in oral Veillonella spp. Appl Environ Microbiol. 2020; 86(20): e01255-20.
2. Dianawati N, Setyarini W, Widjiastuti I, Ridwan RD, Kuntaman K. The distribution of Streptococcus mutans and Streptococcus sobrinus in children with dental caries severity level. Dent J. 2020; 53(1): 36–9.
3. Van Chuyen N, Van Du V, Van Ba N, Long DD, Son HA. The prevalence of dental caries and associated factors among secondary school children in rural highland Vietnam. BMC Oral Health. 2021; 21(1): 349.
4. Chen X, Daliri EB-M, Kim N, Kim J-R, Yoo D, Oh D-H. Microbial etiology and prevention of dental caries: Exploiting natural products to inhibit cariogenic biofilms. Pathogens. 2020; 9(7): 569.
5. Mosailova N, Truong J, Dietrich T, Ashurst J. Streptococcus gordonii : A rare cause of infective endocarditis. Case Rep Infect Dis. 2019; 2019: 7127848.
6. Toma S, Behets C, Brecx MC, Lasserre JF. In vitro comparison of the efficacy of peri-implantitis treatments on the removal and recolonization of Streptococcus gordonii biofilm on titanium disks. Materials (Basel). 2018; 11(12): 2484.
7. Furquim dos Santos Cardoso V, Amaral Roppa RH, Antunes C, Silva Moraes AN, Santi L, Konrath EL. Efficacy of medicinal plant extracts as dental and periodontal antibiofilm agents: A systematic review of randomized clinical trials. J Ethnopharmacol. 2021; 281: 114541.
8. Nugraha AP, Narmada IB, Sitasari PI, Inayati F, Wira R, Triwardhani A, Hamid T, Ardani IGAW, Djaharu'ddin I, Rahmawati D, Iskandar RPD. High mobility group box 1 and heat shock protein-70 expression post (-)-Epigallocatechin-3-gallate in East Java green tea methanolic extract administration during orthodontic tooth movement in Wistar rats. Pesqui Bras Odontopediatria Clin Integr. 2020; 20: e5347.
9. Ramadhani Y, Rahmasari RRP, Prajnasari KN, Alhakim MM, Aljunaid M, Al-Sharani HM, Tantiana T, Juliastuti WS, Ridwan RD, Diyatri I. A mucoadhesive gingival patch with Epigallocatechin-3-gallate green tea (Camellia sinensis) as an alternative adjunct therapy for periodontal disease: A narrative review. Dent J. 2022; 55(2): 114–9.
10. Ismiyatin K, Subiyanto A, Suhartono M, Sari PT, Widjaja OV, Sari RP. Efficacy of topical hydrogel Epigallocatechin-3-gallate against neutrophil cells in perforated dental pulp. Dent J. 2020; 53(2): 88–92.
11. Dai W, Ruan C, Zhang Y, Wang J, Han J, Shao Z, Sun Y, Liang J. Bioavailability enhancement of EGCG by structural modification and nano-delivery: A review. J Funct Foods. 2020; 65: 103732.
12. Nikoo M, Regenstein JM, Ahmadi Gavlighi H. Antioxidant and antimicrobial activities of (-)-Epigallocatechin-3-gallate (EGCG) and its potential to preserve the quality and safety of foods. Compr Rev Food Sci Food Saf. 2018; 17(3): 732–53.
13. Schneider-Rayman M, Steinberg D, Sionov RV, Friedman M, Shalish M. Effect of epigallocatechin gallate on dental biofilm of Streptococcus mutans: An in vitro study. BMC Oral Health. 2021; 21(1): 1–11.
14. Hairul Islam MI, Arokiyaraj S, Kuralarasan M, Senthil Kumar V, Harikrishnan P, Saravanan S, Ashok G, Chellappandian M, Bharanidharan R, Muralidaran S, Thirugnanasambantham K. Inhibitory potential of EGCG on Streptococcus mutans biofilm: A new approach to prevent cariogenesis. Microb Pathog. 2020; 143: 104129.
15. Hasan S, Danishuddin M, Khan AU. Inhibitory effect of zingiber officinale towards Streptococcus mutans virulence and caries development: In vitro and in vivo studies. BMC Microbiol. 2015; 15(1): 1–14.
16. Wang X, Li X, Ling J. Streptococcus gordonii LuxS/autoinducer-2 quorum-sensing system modulates the dual-species biofilm formation with Streptococcus mutans. J Basic Microbiol. 2017; 57(7): 605–16.
17. Soekanto SA, Alatas SS, Ristanti R, Gultom FP, Sahlan M. Efficacy of propolis fluoride in inhibiting the formation of Streptoccocus mutans, Streptococcus gordonii, and Streptococcus sanguinis biofilm. In: AIP Conference Proceedings. 2019. p. 030008.
18. Scharnow AM, Solinski AE, Wuest WM. Targeting S. mutans biofilms: a perspective on preventing dental caries. Med Chem Comm. 2019; 10(7): 1057–67.
19. Zubaidah N, Dianawati N, Ridwan RD, Shirakawa T, Kuntaman K, Setiawatie EM, Tanzil MI, Kunarti S. The clinical pattern and prevalence of Streptococcus mutans and Streptococcus sobrinus among adult and children patients with dental caries. Pesqui Bras Odontopediatria Clin Integr. 2022; 22: 1–11.
20. Li J, Wu T, Peng W, Zhu Y. Effects of resveratrol on cariogenic virulence properties of Streptococcus mutans. BMC Microbiol. 2020; 20(1): 1–11.
21. Zayed SM, Aboulwafa MM, Hashem AM, Saleh SE. Biofilm formation by Streptococcus mutans and its inhibition by green tea extracts. AMB Express. 2021; 11: 73.
22. Cai J-N, Choi H-M, Jeon J-G. Relationship between sucrose concentration and bacteria proportion in a multispecies biofilm. J Oral Microbiol. 2021; 13: 1910443.
23. Maske TT, van de Sande FH, Arthur RA, Huysmans MCDNJM, Cenci MS. In vitro biofilm models to study dental caries: a systematic review. Biofouling. 2017; 33(8): 661–75.
24. Nuraini P, Pradopo S, Pronorahardjo AS. Sucrose and xylitol-induced Streptococcus mutans biofilm adherence. Pesqui Bras Odontopediatria Clin Integr. 2020; 20: 1–5.
25. Nuraini P, Moeharyono Puteri M, Pramesty E. Anti-biofilm activity of epigallocatechin gallate (EGCG) against Streptococcus mutans bacteria. Res J Pharm Technol. 2021; 14(9): 5019–23.
26. Bartosikova L, Necas J. Epigallocatechin gallate: A review. Vet Med (Praha). 2018; 63(10): 443–67.
27. Han S, Abiko Y, Washio J, Luo Y, Zhang L, Takahashi N. Green tea-derived epigallocatechin gallate inhibits acid production and promotes the aggregation of Streptococcus mutans and mon-mutans Streptococci. Caries Res. 2021; 55(3): 205–14.
28. Górniak I, Bartoszewski R, Króliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev. 2019; 18(1): 241–72.
29. Tan J, Wang D, Cao H, Qiao Y, Zhu H, Liu X. Effect of local alkaline microenvironment on the behaviors of bacteria and osteogenic cells. ACS Appl Mater Interfaces. 2018; 10(49): 42018–29.

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