Indian Journal of Experimental Biology
Vol. 58, July 2020, pp. 445-451

In vitro anticariogenic effect of gallic acid against *Streptococcus mutans*

Dimple Goyal, Shabeer A. Rather, Sukesh Chander Sharma & Akhtar Mahmood*
Department of Biochemistry, Panjab University, Chandigarh

Received 06 December 2019; *revised 21 June 2020*

Involvement of *Streptococcus mutans* in the pathogenesis of dental caries among human populations is well established. Here, we studied the effect of gallic acid, a naturally occurring polyphenol on certain cariogenic activities of *S. mutans*. Gallic acid inhibited the glycosyltransferase activity, a key enzyme of sucrose metabolism by 27-36% in *S. mutans.* Minimal inhibitory concentration (MIC) of gallic acid (136 µg/mL) inhibited the growth of *S. mutans* by 50%. About 0.4 mM of the polyphenol reduced biofilm formation by 40%, hydrophobicity 60% and acid production 36% by the organism under *in vitro* growth conditions. Fluorescence microscopy revealed that in absence of gallic acid, the cells were present as clumps, however in the presence of gallic acid (68 µg/mL), they were well segregated due to the inhibition of biofilm formation. The present findings suggest that gallic acid has cariostatic activity against *S. mutans*, which may have potential application in prevention of dental caries.

Keywords: Biofilm formation, Cariogenic factors, Cariostatic activity, Dental caries, Glycosyltransferase inhibition, Polyphenols

Dental caries is a multifactorial disease that leads to decalcification and destruction of tooth[1]. A number of Gram-positive bacteria including *Streptococcus mutans* are closely related to the formation and progression of the disease[2]. The cariogenic potential of this organism resides in its ability to produce large amounts of extracellular polysaccharides or glucans from sucrose by the action of glucosyltransferases (GTFs), coupled with its adhesion and acid producing activities. The acids so produced dissolve the tooth enamel consequently leading to localized decalcification, cavity formation and breakdown of calcified dental tissue[3]. The extracellular glucans promote the accumulation of cariogenic bacteria on the tooth surface, and are critical for the formation and structural integrity of biofilms[4-6]. Bacterial adhesion to the tooth surface involves hydrophobic interactions between complementary molecules on the microorganism and the host surface[7]. *S. mutans* has a high degree of surface hydrophobicity, and its adhesion to saliva coated surfaces is dependent upon hydrophobic interactions[8]. Thus, an important approach for the prevention of dental caries may be to inhibit the activities of GTFs, which are the crucial constituents of biofilm formation in *S. mutans*. A variety of compounds have been employed to inhibit the growth and adherence of *S. mutans* to the tooth surface in order to prevent dental caries[9,10]. However, *S. mutans* is either resistant to many of the antibacterial agents *viz*., penicillin, amoxicillin, tetracycline, cefuroxime and erythromycin[11] or they may lead to side effects including gastrointestinal problems[12].

Gallic acid (3,4,5-trihydroxybenzoic acid) is an endogenous plant polyphenol, abundantly found in a number of plants and fruits, which include oak bark, gallnuts, grapes, pomegranate and berries[13,14]. Shadi & Naczki[15] described that red fruits, black radish and onions contain gallic acid in 10s of mg per kg of the fresh weight. Tea is an important source of gallic acid containing up to 4.5 g per kg of the fresh weight[16].

Kim[17] has reported that gallic acid acts as anti-melanogenic agent by reducing tyrosine kinase activity. It is shown to impair lysozyme, chymotrypsin and amylase activities over a wide range of pH. Gallic acid is a potent inhibitor of brush border disaccharidases in mammalian intestine[18]. In the present report, we describe the effect of gallic acid on certain cariogenic parameters of *S. mutans in vitro*.

Materials and Methods

Chemicals

All the chemicals used were of analytical grade. Agar, Brain Heart Infusion Broth (BHI), exogenous dextrose, glucose, sucrose, peptone and maleic acid,

*Correspondence:
Phone: +91 172 2534136; Fax: +91 172 2541409
E-mail: akhtarmah@yahoo.com

NaCl, yeast, and ethanol were obtained from HiMedia Pvt. Ltd. (Mumbai, India). Sephadex G-200 and gallic acid was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were obtained from E. Merck Pvt. Ltd. (Mumbai, India) or Sisco Research Lab Pvt. Ltd. (Mumbai, India).

Bacterial strain

A lyophilized culture of *Streptococcus mutans* (MTCC 890) was obtained from microbial type culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

Growth of bacterial strain

For revival of the bacteria, the strain was inoculated into growth medium-87 media (1% dextrose, peptone, yeast extract, 0.5% BHI and 2% agar, pH 7.2-7.5) containing 0.05% Tween 80 and the inoculated broth was incubated for 18-24 h at 37°C[19]. Culture purity was checked regularly by microscopic examination.

Cell free extracts

Cells grown in batch culture (500 mL) were harvested by centrifugation at 12000 rpm for 20 min at 4°C and washed thrice with saline (0.9% NaCl). The supernatant fluid served as the cell-free extract and was used as the enzyme source[20].

Assay of glycosyltransferase activity

Glycosyltransferase activity was determined following the method of Mukasa *et al*.[21]. Reaction tube contained 200 µL of the enzyme, 0.02% sodium azide, 32.40 mg of sucrose, with or without 0.34 mg of exogenous dextran T20 having zero or different concentrations of gallic acid in 20 mM sodium maleate buffer (pH 6.0) in a total volume of 2 mL. Incubation was carried out at 37°C for 2 h. The reaction was terminated by placing the tubes in a boiling water bath for 5 min. The supernatant was removed and the tube contents were washed with sterile water. Total amounts of water-soluble and insoluble glucan were measured by the phenol-sulphuric acid method[22]. Three replicates were made for each concentration of the extracts and enzyme activity was calculated.

Determination of minimum inhibitory concentration (MIC)

MIC of gallic acid was determined by plate-hole assay as described by McRae *et al*.[23]. The MIC was the lowest concentration, which yielded a visible zone of inhibition more than 6 mm. About 100 µL of the culture was spread on BHI agar and allowed to dry for 10 min. A sterilized core-borer (6 mm diameter) was used to produce wells in the agar. Increasing concentrations of gallic acid (4.25-300 µg/mL) were dispensed into each well. All assays were performed in triplicate, by incubating overnight at 37°C under capnophilic conditions. Except otherwise stated sub minimal concentrations of gallic acid (68 µg/mL or 0.4 mM) were used in all subsequent experiments.

Sucrose-dependent and sucrose-independent adherence of *S. mutans* to smooth glass surfaces

The effect of the sub-MIC concentrations of gallic acid on the adhesion of *S. mutans* to smooth glass surface was studied following the method of Zhao *et al*.[24]. The organism was grown in a glass tube at an angle of 30° containing 10 mL of BHI medium with different concentrations of polyphenol with or without sucrose 5% (w/v) at 37°C for 24 h. The appropriate controls of BHI were run simultaneously. After incubation, the glass tubes were slightly rotated and the planktonic cells were decanted. The adhered cells were removed and agitated by adding 0.5 M of sodium hydroxide. The cells were washed and suspended in normal saline. The cell adherence was quantified spectrophotometrically by measuring OD at 600 nm. All the determinations were made in triplicate, using untreated BHI medium as the control.

$$\text{Percentage adherence} = \frac{\text{OD of adhered cells}}{\text{OD of total cells}} \times 100$$

Determination of cell-surface hydrophobicity

The cell surface hydrophobicity of *S. mutans* was determined following the microbial adhesion test as described by Martin *et al.*[25]. The cells were grown in BHI medium supplemented with 5% sucrose and different concentrations of gallic acid (4.25-68 µg/mL). The cells were taken in sterile saline (0.85%) and optical density was adjusted to 0.3 at 600 nm. 2.5 mL of the cell suspension was placed in a tube and 0.2 mL of toluene was added. After vortexing for 2 min, they were allowed to equilibrate at room temperature (25-28°C) for 15 min. After separating the organic phase, OD of the aqueous solution was measured at 600 nm. Controls with *Streptococcus mutans* alone were run simultaneously, hydrophobic index >70% was arbitrarily classified as hydrophobic.

Collection of saliva

The saliva was collected from healthy individuals**,** who abstained from tooth brushing and eating for 4-5 h prior to collection. In order to increase saliva secretion, the subjects were allowed to chew parafilm before collection of saliva. To obtain clarified saliva it was centrifuged at 8000 g for 15 min, and stored at -80**°**C. The plate wells were coated with 100 µL of the saliva,

and incubated at 37°C for 2 h to form the salivary pellicles. After incubation, the plates were rinsed thrice with 100 µL PBS before adding the bacterial culture as described by Khan *et al.*[26].

Biofilm formation assay

The procedure of Wen *et al.*[27] was used to determine the biofilm formation using 96-well microtitre flat bottom plates. The ability of *S. mutans* to form biofilms was studied in the presence and absence of salivary pellicle. Cells grown to the mid-log phase (OD of 1 at 600 nm) were inoculated in fresh BHI medium containing sucrose 5% (w/v). The wells were first coated with saliva before adding the bacterial suspension and incubated for another 2 h at 37°C. The cells were washed three times with PBS followed by air drying for 35 min. 250 µL of bacterial suspension was added both uncoated and coated wells of plates containing varying concentrations of gallic acid (2.125-68 µg/mL) and plates were incubated for 24 h at 37°C. Blanks with media alone were run as the controls. To remove the loosely bound cells, the plates were washed thrice with 250 µL of sterile distilled water and the cells were stained with 50 µL of 0.1% crystal violet for 15 min. After rinsing twice with 250 µL of sterile water, the bound dye was removed from the stained cells using 250 µL of 99% ethanol. Plates were then placed on a shaker for 5 min to allow full release of the dye. Biofilm formed was quantified by measuring optical density at 600 nm using ELISA plate reader (BioTek).

Effect on acid production

The method of Ciardi *et al.*[28] was used to determine acid production by *S. mutans*. 5% (w/v) of sucrose in 5 mL of BHI broth containing different concentrations of gallic acid (4-68 µg/mL) were inoculated with 100 µL of 18 h cultures of *S. mutans*. To obtain a final inoculum of 1.5×10^4 CFU per mL, the cell culture was incubated at 37°C for 24 h. The pH of the bacterial medium was determined at 0 h and after 24 h of incubation. Except otherwise stated all determinations were done in triplicates and suitable controls were run simultaneously.

Fluorescent microscopy

To examine the effect of gallic acid on biofilm formation by *S. mutans,* the cells were grown on saliva-coated glass cover slips. *S. mutans* was grown in BHI supplemented with 5% sucrose in a 6-well microtiter plate[29]. All measurements were done in triplicates. Gallic acid concentration used was 68 µg/mL, and untreated cells served as the controls. After inoculation, the wells were incubated at 37°C for 24 h. The cover slips were removed from the media and gently washed with sterile PBS to remove the unattached cells. Cover slips were then stained with propidium iodide for 1h. Samples were examined under Fluorescent microscope (Axioscope Al, Carl Zeiss, Germany). The biofilm formed was scanned randomly at 4-5 different positions as described by Koo *et al.*[30].

Statistical analysis

The results were expressed as mean ± S.D. except otherwise stated. Statistical analysis of the data was performed using SPSS. A p-value <0.05 was considered significant.

Results

Effect of gallic acid on *S. mutans* growth

Using the plate hole assay, the effect of gallic acid on the growth of *S. mutans* was studied by adding different amounts of gallic acid to the growth medium. Gallic acid at a concentration of 136 µg/mL (0.8 mM) yielded inhibitory zone of 22 ± 0.09 mm corresponding to 50% inhibition of *S. mutans* growth (results not shown).

Effect of gallic acid on glycosyltransferase activity

As shown in Table-1, the activity of glycosyl-transferase was reduced by 35-40% in presence of gallic acid, which was 0.526±0.09 in the control and 0.314±0.15 in the presence of 0.4 mM gallic acid. In addition, the effect of various other polyphenols was also studied on glycosyltransferase activity. Compared to gallic acid, the enzyme inhibition by tannic acid, syringic acid and catechin was relatively small, thus further studies were carried out using gallic acid alone and are reported herein.

The addition of gallic acid 8.5-68 µg/mL to the assay system reduced glucan formation in a concentration dependent manner (Fig. 1). At a concentration of 8.5-17.0 µg/mL, gallic acid inhibited

Table 1 — Effect of gallic, tannic and syringic acids and catechins on glucosyltransferase activity of *S. mutans*

Polyphenols	Concentration (µg/mL)	Glucosyltransferase activity (µmol /mg protein)
Control	0	0.526±0.09
Gallic acid	34	0.344±0.15 (34.61%)
Tannic acid	17	0.357±0.07 (32.13%')
Syringic acid	68	0.36±0.05 (30.04%)
Catechin	68	0.384±0.09 (27%)

Values shown in parentheses are % inhibition.
[Values are Mean ±S.D; n=4]

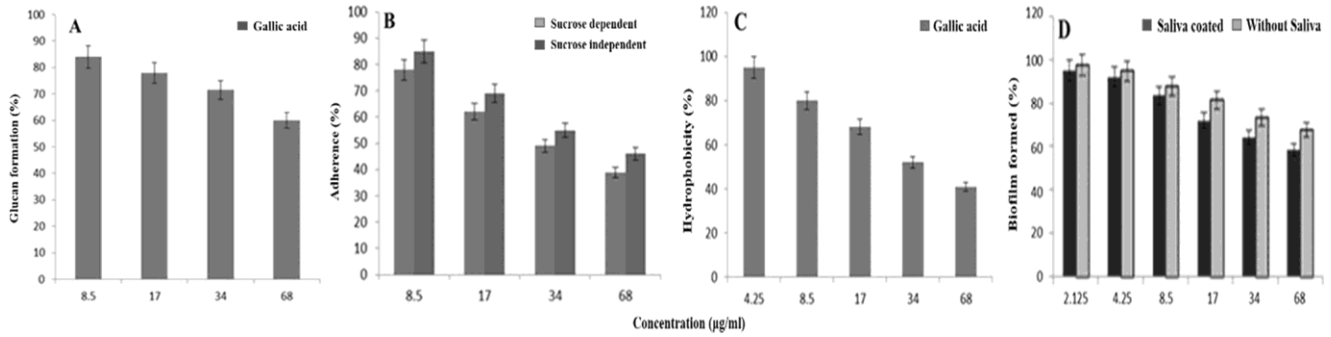

Fig. 1 — Effect of Gallic acid on (A) glucan formation in *Streptococcus mutans*; (B) the glass dependent adherence of *S. mutans* in the absence (sucrose-independent) and in presence of 5% sucrose (sucrose- dependent); (C) on cell-surface hydrophobicity of *S. mutans*; and (D) biofilm formation by *S. mutans* in the presence and absence of salivary pellicle. [All Values are mean ± SD; n=4]

glucan formation by 15-20%. Increasing the polyphenol concentration to 34-68 µg/mL in the assay system, however produced 30-40% reduction in glucan formation under the experimental conditions.

Sucrose-dependent and independent glass surface adherence of *S. mutans*

The effect of different concentrations of gallic acid (8.5-68 µg/mL) on the adherence of *S. mutans* to glass tubes was also analyzed in the presence or absence of sucrose. Addition of gallic acid to the assay system progressively inhibited both the sucrose-dependent and sucrose-independent adherence of *S. mutans* to glass surface at gallic acid concentration of 68 µg/mL by 50-60% and 40-50%, respectively (Fig. 1). The inhibition of sucrose-dependent adherence of the cells was far more pronounced than in the absence of sucrose.

Effect of gallic acid on hydrophobicity of *S. mutans*

The effect of different concentrations (4.25-68 µg/mL) of gallic acid on hydrophobicity of *S. mutans* elucidated that there was a gradual decline in cell surface hydrophobicity of *S. mutans* as the amount of gallic acid was increased in the assay tube (Fig. 1C). About 8.5-17.0 µg/mL gallic acid reduced hydrophobicity by 20-30% of the control values. However Gallic acid at a concentration of 68 µg/mL reduced the cell surface hydrophobicity by over 60% under these conditions.

Effect of gallic acid on biofilm formation by *S. mutans*

Gallic acid also impaired biofilm formation by *S. mutans* in a dose-dependent manner (Fig. 1D). About 2.12-17 µg/mL of gallic acid reduced biofilm formation by 10-15% when the glass surface was coated or not with saliva. However, gallic acid at a concentration of 68 µg/mL reduced the biofilm formation by approximately over 40% in the presence of salivary pellicle. In the absence of salivary pellicle, the biofilm reduction was around 10% of the control values.

Table 2 — Effect of gallic acid on the pH of medium in *S. mutan*

Gallic acid (µg/mL)	pH at 0 time (onset)	pH (after 24 h)
0	7.45±0.08	4.45±0.06
4	7.42±0.05	4.85±0.08
8	7.37±0.09	5.18±0.04
17	7.36±0.05	5.45±0.1
34	7.34±0.04	5.88±0.16
68	7.39±0.07	6.02±0.07

[Different concentrations of gallic acid were added to 1.5×10 CFU/ml *S.mutans* cells. The pH of the medium was recorded afte 24 h of incubation at 37°C. All the determinations were made i triplicate. Values are mean ± SD; n=4]

Effect of gallic acid on acid production by *S. mutans*

One of the characteristics of cariogenic *S. mutans* is its ability to survive in acidic environment as cells produce acid in the medium. Thus, the effect of different concentrations (0-68 µg/mL) of gallic acid on acid production by *S. mutans* was also investigated. As shown in Table-2, there was a significant change in the pH value of the medium with a gradual increase of gallic acid in the growth medium of *S. mutans*. Addition of 68 µg/mL of gallic acid elevated the pH of *S. mutans* medium from 4.43 in the control to pH 6.02, in the presence of polyphenol.

Biofilm architecture visualized by microscopy

The effect of gallic acid on biofilm architecture of *S. mutans* was also studied. As shown in Fig. 2, fluorescence microscopy revealed reduced synthesis of extracellular polysaccharides in presence of the polyphenol. For these experiments, sub-MIC concentration of gallic acid (68 µg/mL) was used. The control samples showed robust clumping of cells due

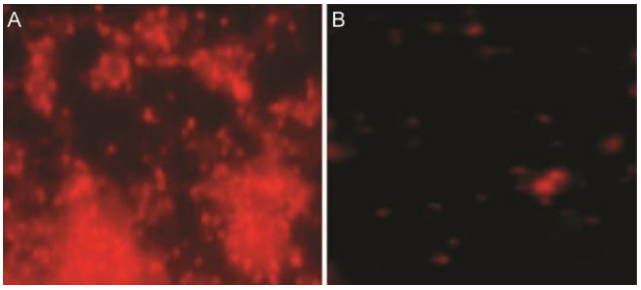

Fig. 2 — Fluorescent images of *S. mutans*. (A) control; and (B) in the presence of gallic acid (68 µg/mL) after 24 h of incubation [Magnification 400X. The assays were performed in triplicates, essentially with identical results]

to the polysaccharide matrix while cells grown in presence of gallic acid were well dispersed and separated. This implied reduced formation of extracellular matrix polysaccharides leading to impaired biofilm (plaque) formation in the presence of gallic acid.

Discussion

Streptococcus mutans optimizes the expression of various virulence traits like acidogenecity, aciduracity, extracellular polysaccharide synthesis, biofilm formation, cell-cell signalling and adherence to tooth surface. Numerous strategies including the use of antibiotics to inhibit the growth of *S. mutans* have been described[11]. A number of plant extracts have been reported to inhibit the growth of *S. mutans* but the precise nature of plant ingredients responsible for the cariostatic activity are unknown. In this study, we observed that naturally occurring plant polyphenol, gallic acid, exhibit anti-cariogenic activities against *S.mutans*. Gallic acid molecule has polyhydoxyl constellation, resembling sucrose structure, thus inhibits dextran-sucrase activity in *S. mutans*[31]. The synthesis of glucans from sucrose by GTFs is one of the most important virulence properties of *S. mutans* that contributes to the development of mature dental plaques[32]. *S. mutans* promotes plaque formation by synthesizing water-insoluble glucan[2]. On the surface of a saliva-coated tooth, GTFase can absorb in an active form and synthesize water-insoluble glucans from sucrose[32]. Present data showed that 0.4mm gallic acid inhibited nearly 35-40% of glycosyltransferase activity *in vitro*. It has been proposed that lone pair of electrons present on oxygen atom of the carboxyl group in the polyphenol may act as proton acceptor from the active site residues in the enzyme protein which forms an intermediate between the substrate and the enzyme, thus may impair the activity of disaccharidases[18]. A similar mechanism may be involved in the inhibition of GTF activity, which employs sucrose as the substrate for the synthesis of extracellular glucans.

Adherence of *S. mutans* to the tooth surface is an important step in the formation of dental plaques[32]. The present findings revealed that the adherence of *S. mutans* to glass surface was reduced by 50-60 % at all concentrations of gallic acid used (8.5-68 µg/ mL). There was a marked inhibition of *S. mutans* adherence to glass surface by sub-MIC concentrations of gallic acid, as compared to the control. The sucrose-dependent adherence was reduced more (50-60%) than the sucrose independent adherence process (40-50%). Krepsky *et al*.[33] have suggested that hydrophobicity is one of the important properties which allow the microorganism to adhere to both living and non-living surfaces. The cell surface of *S. mutans* is more hydrophobic than that in other *Streptococcus* species. Hence, changes in cell surface hydrophobicity may affect its ability to adhere to the matrix surface. Yamashita *et al*.[34] have reported that the agents, which inhibit hydrophobicity, also reduce the incidence of caries formation. The present findings showed that hydrophobicity of *S. mutans* cells was considerably reduced by increasing concentration of gallic acid in the growth medium. Further, the cell-surface hydrophobicity is also associated with cell-surface proteins[35]. Thus, it is likely that the observed reduction in hydrophobicity could be due to the binding of the polyphenols to proteins associated with the cell surface. Yamanaka-Okada *et al*.[36] have reported that proanthocyanidins present in cranberry reduced hydrophobicity of *S. mutans* and *S. sorbinus*. This may be due to binding and/or masking of hydrophobic proteins present on the cell surface of *S. mutans* by the proanthocyanidins present in cranberry.

The acidogenecity and acidurance are other physiological factors associated with the cariogenic potential of *Streptococcus mutans*. Thus, the incidence of caries formation can be reduced by reducing the acid production or by inhibiting the activity of enzymes associated with the growth and glycolysing systems of *S. mutans*[37]. The present data showed that acid production by *S. mutans* was markedly inhibited in presence of gallic acid. Although the exact mechanism of acid tolerance is unknown, but it is possible that the flavonoids and tannins can bind and precipitate various proteins of *S. mutans* thus affecting its ability to remove

protons from the cytoplasm by the action of F-ATPase or ATPase. The present findings further corroborate the contention that the polyphenols have anti-growth property for *S. mutans*.

Fluorescence microscopic analysis revealed that control cells were present as clumps due to secretion of extracellular polysaccharide complex containing insoluble glycans. However, in presence of gallic acid, the bacterial cells were present in isolation, which suggested the inhibition of biofilm formation under these conditions. These observations are also in agreement to data on the inhibition of biofilm formation by polyphenols in *S. mutans*. Thus, the effectiveness of polyphenols in inhibiting the growth of *S. mutans* is presumably due to the inhibition of biofilm formation as result of the inactivation of dextransucrase and glycosyltransferase[31,32] under these conditions. Since extracellular polysaccharides are produced by the bacterium for its adherence to tooth surface, the inhibition of formation of these structures by gallic acid, could be useful in the cure and prevention of dental caries. A similar phenomenon was also observed by Rukayadi *et al.*[38] who reported that the compound macelignan isolated from *Myristica fragrans* showed antibiofilm formation activity against *S. mutans* and *S. sanguis*. It may be interesting to examine the effect of gallic acid on other oral bacteria to establish the specificity of this phenomenon.

Conclusion

The present data shows that gallic acid, a naturally occurring constituent of edible fruits and vegetables, is a potent inhibitor of *Streptococcus mutans* growth *in vitro*. The cariostatic action of this compound is apparently due to inhibition of glycosyltransferase activity in *S. mutans*. The polyphenol also inhibited (a) biofilm formation, (b) hydrophobicity, and (c) acid production in *S. mutans*. Thus, gallic acid a naturally occurring compound could have potential application in the prevention of dental caries. However, its efficacy and specificity affecting other oral microorganisms needs investigation.

Acknowledgement

One of the authors, DG was recipient of JRF from UGC, New Delhi, and SAR was supported by Indian Council of Medical Research, New Delhi as SRF.

Conflict of interest

Authors declare no conflict of interests.

References

1 Dhamo B, Elezi B, Kragt L, Wolvius EB, & Ongkosuwito EM, Does dental caries affect dental development in children and adolescents? *Bosnian J Basic Med Sci*, 18 (2018) 198.

2 Alam MK, Zheng L, Liu R, Papagerakis S, Papagerakis P & Geyer CR, Synthetic antigen-binding fragments (Fabs) against *S. mutans* and *S. sobrinus* inhibit caries formation. *Sci Rep*, 8 (2018) 10173.

3 Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, Abranches J, & Brady LJ, The Biology of *Streptococcus mutans*. *Microbiology Spectrum*, 7 (2019) 1128.

4 Klein MI, Hwang G, Santos PH, Campanella OH & Koo H, *Streptococcus mutans*-derived extracellular matrix in cariogenic oral biofilms. *Front Cell Infect Microbiol*, 5 (2015) 10.

5 Castillo Pedraza MC, Noves TF, Faustoferri RC, Quivey RG, Jr Terekhov A, Hamaker BR, & Klein MI, Extracellular DNA and lipoteichoic acids interact with exopolysaccharides in the extracellular matrix of *Streptococcus mutans* biofilms. *Biofouling*, 33 (2017) 722.

6 Wen ZT, Liao S, Bitoun JP, De A, Jorgensen A, Feng S, Xu X, Chain P, Caufield PW, Koo H & Li Y, *Streptococcus mutans* displays altered stress responses while enhancing biofilm formation by *Lactobacillus casei* in mixed-species consortium. *Front Cell Infect Microbiol*, 7 (2017) 524.

7 Weiss E, Rosenberg M, Judes H & Rosenberg E, Cell-surface hydrophobicity of adherent oral bacteria. *Curr Microbiol*, 7 (1982) 125.

8 Westergren G & Olsson J, Hydrophobicity and adherence of oral *streptococci* after repeated subculture *in vitro*. *Infect Immun*, 40 (1983) 432.

9 Onisi M, Ozaki F, Yoshino F & Murakami Y, An experimental evidence of caries preventive activity of non-fluoride component in tea. *J Dent Health*, 31 (1981) 158.

10 Ooshima T, Fujiwara T, Takei T, Izumitani A, Sobue S & Hamada S, The caries inhibitory effects of GOS-sugar *in vitro* and in rat experiments. *Microbiol Immunol*, 32 (1988) 1093.

11 Qiu, W, Zhou, Y, Li Z, Huang T, Xiao Y, Cheng L, Peng X, Zhang L, & Ren B, Application of Antibiotics/Antimicrobial Agents on Dental Caries. *BioMed Res Int*, 2020 (2020) 5658212.

12 Bhattacharya S, Virani S, Zarvo M & Hass JG, Inhibition of *Streptococcus mutans* and other oral *streptococci* by Hop (*Humulus lupulus L.*) constituents. *Econ Bot*, 57 (2003)118.

13 Carig A, Antimicrobial resistance, danger signs all around. *Tenness Med*, 91(1998) 433.

14 Ma J, Luo XD, Protiva P, Yang H, Ma C, Basile MJ, Weinstein IB & Kennelly EJ, Bioactive novel polyphenols from the fruit of *Manilkara zapota* (Sapodilla). *J Nat Prod*, 7 (2003) 983.

15 Shahidi F & Naczki M, Food penolics, sources, chemistry, effects, applications, (Technomic Publishing Co. Lancaster, UK), 1995.

16 Tomas-Barberan FA & Clifford MN, Dietary hydroxybenzoic acid derivatives and their possible role in health protection. *J Sci Food Agric*, 80 (2000) 1024.

17 Kim YJ, Antimelanogenic and antioxidant properties of gallic acid. *Biol Pharm Bull*, 30 (2007) 1052.

18 Rohn S, Rawel HM & Kroll J, Inhibitory effects of plant phenols on the activity of selected enzymes. *J Agric Food Chem*, 50 (2000) 3566.

19 Gupta N, Gupta S & Mahmood A, Gallic acid inhibits brush border disaccharidases in mammalian intestine. *Nutr Res*, 27 (2007) 230.

20 Goyal D, Sharma S & Mahmood A, Inhibition of dextran-sucrase activity in *Streptococcus mutans* by plant phenolics. *Indian J Biochem Biophys*, 50 (2013) 48.

21 Mukasa H, Shimamura A & Tsumori H, Effect of salts on water-insoluble glucan formation by glucosyltransferase of *Streptococcus mutans*. *Infect Immun*, 23 (1979) 564.

22 Dubois M, Gilles KA, Hamilton JK, Rebers PA & Smith F, Colorimetric method for determination of sugars and related substances. *Anal Chem*, 28 (1956) 350.

23 McRae JM, Yang Q, Crawford RJ & Palombo EA, Antibacterial compounds from *Planchonia careya* leaf extracts. *J Ethnopharmacol*, 116 (2008) 554.

24 Zhao W, Li W, Lin J, Chen Z & Yu D, Effect of sucrose concentration on sucrose-dependent adhesion and glucosyltransferase expression of *S. mutans* in children with severe early-childhood caries (S-ECC). *Nutrients*, 6 (2014) 3572.

25 Martin MA, Pfaller MA, Massanari RM & Wenzel RP, Use of cellular hydrophobicity, slime production and species identification markers for the clinical significance of coagulase negative Staphylococcal isolates. *Am J Infect Control*, 17 (1989) 130.

26 Khan R, Zakir M, Khanam Z, Shakil S & Khan AU, Novel compound from *Trachyspermum ammi* (Ajowan caraway) seeds with ant-biofilm and antiadherence activities against *Streptococcus mutans*: a potential chemotherapeutic agent against dental caries. *J Appl Microbiol*, 109 (2010) 2151.

27 Wen ZT, Suntharaligham P, Cvitkovitch DG & Burne RA, Trigger factor in *Streptococcus mutans* is involved in stress tolerance, competence development, and biofilm formation. *Infect Immun*, 73 (2007) 219.

28 Ciardi JE, Rosenthal AB & Bowen WH, Rapid quantitative determination of the effect of antiplaque agents and antisera on the growth, acid production and adherence of *Streptococcus mutans*. *J Dent Res*, 60 (1981) 756.

29 Lynch DJ, Fountain TL, Mazurkiewicz JE & Banas JA, Glucan-binding proteins are essential for shaping *Streptococcus mutans* biofilm architecture. *FEMS Microbiol Lett*, 268 (2007) 15.

30 Koo H, Vacca SAM, Bowen WH, Rosalen PL, Cury JA & Park YK, Effects of *Apis mellifera propolis* on the activities of *streptococcal* glucosyltransferases in solution and adsorbed onto saliva-coated hydroxyapatite. *Caries Res*, 34 (2000) 418.

31 Matsumoto M, Minami T, Sasaki H, Sobue S, Hamada S & Ooshima T, Inhibitory effects of oolong tea extract on caries-inducing properties of *mutans streptococci*. *Caries Res*, 33 (1999) 441.

32 Akira S, Takashi K & Hisataka K, Reduction of *Streptococcus mutans* adherence and dental biofilm formation by surface treatment with phosphorylated polyethylene glycol. *Antimicrob Agents Chemother*, 51 (2007) 3634.

33 Krepsky N, Rocha Ferreira RB, Ferreira Nunes AP, Casado Lins UG, Costa e Silva Filho F, de Mattos-Guaraldi AL & Netto-dosSantos KR, Cell-surface hydrophobicity and slime production of *Streptococcus epidermidis* Brazilian isolates. *Curr Microbiol*, 46 (2003) 280.

34 YamashitaY, Bowen WH, Burne RA & Kuramitsu HK, Role of *Streptococcus mutans* gtf genes in caries induction in the specific pathogen free rat model. *Infect Immun*, 61(1993) 3811.

35 Hasan S, Danishuddin M, Adil M, Singh K, Verma KP & Khan AU, Efficacy of *E. officinalis* on the cariogenic properties of *Streptococcus mutans*: A novel and alternative approach to suppress quorum-sensing mechanism. *PLoS One*, 7 (2012) 1.

36 Yamanaka-Okada A, Sato E, Kouchi T, Kimizuka R & Kato T, Inhibitory effect of cranberry polyphenol on cariogenic bacteria. *Bull Tokyo Dental Coll*, 49 (2008) 107.

37 Georgios A, Vassiliki T & Sotirios K, Acidogenicity and acidurance of dental plaque and saliva sediment from adults in relation to caries activity and chlorhexidine exposure. *J Oral Microbiol*, 7 (2015) 26197.

38 Rukayadi YY, Kim KH & Hwang JK, *In vitro* anti-biofilm activity of macelignan isolated from *Myristica fragrans Houtt* against oral primary colonizer bacteria. *Phytother Res*, 22 (2008) 308.