

In Vitro Antimicrobial Activities against *Streptococcus Mutans*: A Comparative Study of Green Versus Black Tea Extracts and 0.2% Chlorhexidine and Fluoride

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ABSTRACT

Introduction: The aim of the present study was to evaluate and compare the antibacterial effects of black and green tea extracts with those of 0.2% sodium fluoride and chlorhexidine (CHX) mouth rinse on *SM in-vitro*.

Methods: In this *in-vitro* study the effects of fluoride and CHX mouth rinse, black and green tea extracts on *SM PTCC 1683* were evaluated and compared using the minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) technique and disk diffusion method by measuring the diameters of inhibition zones. Descriptive statistics were retrieved and data was analyzed using Mann-Whitney and Kruskal-Wallis analysis. Statistical significance level was established at $p < 0.05$.

Results: MIC and MBC of black and green tea extracts were similar (62.5 mg/mL), with 0.125 and 2 mg/mL for CHX and fluoride, respectively. The inhibition zones for pure green and black tea extracts and CHX were 16.05, 11.77 and 9.91 mm, respectively. The green tea had the larger inhibition zone than CHX and fluoride. (P value < 0.01) There was no significant difference between green and black tea. (P value = 0.23)

Conclusion: The green and black tea had similar antibacterial effect on 62.5 to 1000 mg/ml concentrations. The anti SM

activity of CHX and fluoride appears on a lower concentration than green and black tea extracts. However, the inhibitory effect of green tea at higher concentration was significantly more than CHX and fluoride. In general, the antibacterial effects of green tea, black tea and CHX were significantly higher than fluoride.

Key Words: Chlorhexidine, *Streptococcus mutans*, Green Tea, Black Tea, Sodium fluoride.


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INTRODUCTION

Streptococcus bacterial species is one of the main factors initiating dental caries.¹ Since dental caries is a multifactorial condition, preventive techniques use combined methods, including nutritional counseling, observation of oral hygiene using mechanical and chemical methods, etc. The most common chemical method is the use of mouth rinses such as sodium fluoride or chlorhexidine (CHX).² CHX nonspecifically decreases the counts of useful and pathogenic bacteria; however, it has complications such as discoloration of teeth and the tongue and irritation of the mucosa, also its use and acceptance by young children is a major challenge.³

New caries preventive techniques have focused on the use of safe natural substances with anticariogenic properties, including plant-

based extracts containing polyphenols, such as tea.⁴⁻⁷ Tea is prepared from the leaves of the plant *Camellia sinensis*. There are three kinds of tea: non-fermented (green tea), semi-fermented (oolong tea) and fermented tea (black tea).⁸⁻¹³ Studies carried out on the effect of green tea on *SM* are numerous but studies on black tea as one of the most commonly used drinks all over the world, especially in Iran, are limited and there are contradictory reports in relation to the comparison of the antibacterial effects of green and black tea.^{8,10,14} Use of tea by young children is much easier than CHX and fluoride, it is safe, and swallowing it has no systemic side effects.¹⁵ The aim of the present study was to evaluate and compare the antibacterial effects of green and black tea extracts with 0.2% CHX and fluoride on *SM in vitro*.

MATERIALS AND METHODS

This experimental design, *in vitro* lab setting study was approved by Isfahan University of Medical Science Ethics Committee; Isfahan, Iran (Project No. 3937179). It was performed in the School of Pharmacy and Dental Research Center of Dentistry Faculty from May to June 2015. Pure cultures of *SM* (PTCC 1683), were achieved from the Department of Microbiology.

In the present study, 0.2% sodium fluoride (Behsa, Arak, Iran), 0.2% CHX (Nazhou, Tehran, Iran) mouth rinses were used.

TSB (Trypticase Soy Broth) (Difco Laboratories, Detroit, MI USA) was used for serial dilutions of extracts, CHX and fluoride mouth rinse to determine the minimal inhibitory concentration (MIC). Disk diffusion method was used to determine the diameters of inhibition zones and solid blood agar culture medium (HIME DIA., India) was used to determine minimal bactericidal concentration (MBC).

Preparation of extracts: Green and black tea samples (Golestan, Iran) were powdered using an electric mill in order to completely extract the constituents of the plant. Samples were pure without any aromatic or additive materials. Extracting processes were carried out in the Faculty of Pharmaceutics, Isfahan University of Medical Sciences, Isfahan, Iran, using the percolator technique by a pharmacologist. A total of 500 g of the powdered plant was placed in a percolator, at the end of which a piece of cotton had been placed. Then 70% ethanol (Merck, Germany) was continuously added to it as a solvent and as the first drops of the solvent exited the percolator, the evacuation tap was closed and the percolator was stored in the laboratory for 24 hours. After 24 hours the evacuation tap of the percolator was opened. The extraction procedure continued until the fluid exiting the percolator was without any color. The exit rate of the extract from the percolator was adjusted at one mL/min for 500 g of the plant powder. All the containers containing the extract were covered with aluminum foils to prevent the detrimental effects of light and evaporation of the solvent during the extracting procedures.

Determination of phenolic constituents in the extracts: Total phenolic content in the ethanolic tea leaf extracts of *C. sinensis* was determined by the Folin-Ciocalteu colorimetric method.¹⁶ A total of 20 mL of tea extracts, blank and standard solutions were poured into test tubes and 100 μ L of the reagent folin siocalto and 1.58 mL of distilled water were added to each test tube and properly mixed. Then 300 μ L of saturated sodium carbonate solution was added to each test tube and stored at 25°C for 120 minutes in a dark environment. Then, a UV spectrophotometer was used to determine the optical density (OD) of each sample at a wavelength of 765 nm compared to the blank. The standard concentration curve of phenolic compounds was prepared with the use of the OD of standard gallic acid versus their concentrations. The total phenolic compositions of the extracts were determined using the standard curve equation and the OD the plant extracts ($R^2=0.9899$; $Y=0.0007X+0.038$).

The overall polyphenol contents of green and black tea were 8.1 mg/ml and 6.32 mg/ml, respectively. All the pure phenolic extracts were stored in refrigerators at 4°C and transported to the Dental Research Center of Dentistry Faculty.

Determination of MIC and MBC using the agar dilution method: The agar dilution method recommended by the NCCLS standards (National Committee for Clinical Laboratory Standards) was used.¹⁷

100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56% and 0.75% concentrations of each extract were prepared. These concentrations are equal to 1000, 500, 250, 125, 62.5, 31.25, 15.625 and 7.6 mg/mL, respectively.

One mL of the microbial suspension containing 1.5×10^8 CFU/mL (0.5 McFarland turbidity standards) of bacterial sample was added to each test tube.

The test tubes were incubated at 37°C for 24 hours and the results were evaluated in terms of turbidity of the test tubes compared to the time before incubation and the positive and negative controls. After incubation, the minimum concentration of each extract that resulted in the inhibition of bacterial growth was considered as MIC.

The turbidity in each test tube indicated the inefficacy of that concentration in inhibiting bacterial growth. Subsequent to determination of MIC, approximately 50 μ L of the solutions from test tubes with no turbidity were transferred onto the solid blood agar culture plates (HIME DIA., India) and cultured with a swab using the spread plate method, followed by incubation at 37°C for 48 hours to determine the presence or absence of bacterial growth macroscopically (observing the colonies by a microbiologist). The minimum concentration in which no bacterial proliferation was detected (no colonies) was reported as MBC. Each examination was tested seven times by one microbiologist.

In relation to determination of MIC it should be pointed out that due to the turbid nature of the extracts used in the present study and the inability to determine the accuracy of turbidity or clarity of the test tubes after incubation, microbial plates were prepared from the turbid test tubes and evaluated under a microscope to evaluate the growth or inhibition of growth of bacteria.

The culture medium along with the strain was considered the negative control and the tube with *SM* and penicillin was the positive control.

Disk diffusion method to determine the diameters of the inhibition zones: Spread plate method was used to prepare a culture from the bacterial suspension at 0.5 McFarland concentrations on blood agar. After 5–10 minutes, wells measuring six mm in diameter were prepared in the culture and the bottoms of the wells were sealed with blood agar medium; the distance between the wells and the edge of the plate was 1.5 cm and the wells were 2–2.5 cm apart. Then 50–100 μ L of the pure concentrations of the extracts and 0.2% fluoride and CHX were transferred into the wells. The negative and positive control wells were filled with physiologic serum and 10 μ g of penicillin, respectively. The plates were placed in a refrigerator for 1–2 hours to provide the antimicrobial agent with the opportunity to diffuse into the environment. The plates were incubated at 37°C for 18–24 hours. Then Vernier calipers were used to determine the diameters of the zones of inhibition. This test was repeated separately seven times for each material by one microbiologist who also examined the MIC and MBC.

Statistical Analysis: Statistical analysis was done using the software SPSS (version 20, Chicago, IL, USA). Descriptive statistics were retrieved and data of the inhibition zones was compared using Kruskal–Wallis followed by Mann–Whitney U test for pairwise comparison. Statistical significance level was established at $p < 0.05$.

Table1: MIC, MBC and Inhibition Zone of extracts, Chlorhexidine and Sodium fluoride 0.2%.

	MIC (mg/ml)	MBC (mg/ml)	Inhibition Zone of pure concentration mean (mm)
Green Tea	62.5	62.5	16.05
Black Tea	62.5	62.5	11.8
Chlorhexidine 0.2 %	0.125	0.125	9.92
Sodium fluoride 0.2%	2	2	0

Minimal inhibitory concentration (MIC); Minimal bactericidal concentration (MBC);
mg/ml: milligram /milliliter; mm: millimeter

Table 2: Mean of Inhibition zone (mm) in different concentrations of green and black tea, fluoride and chlorhexidine

Materials	Concentrations					Penicillin (positive control)
	100 %	50 %	25 %	12.5 %	6.25 %	
Green Tea	16.05	14.05	11.98	9.93	7.98	15.12
Black Tea	11.8	10.1	8.02	5.87	4.05	15.12
Fluoride 0.2%	0	0	0	0	0	15
Chlorhexidine 0.2%	9.92	7.95	5.96	3.96	1.92	15

Table 3: Inhibition zone analysis in different concentrations of green and black tea, fluoride and chlorhexidine.
Small letters represent a comparison of various concentrations of each substance (vertical comparison).
Large letters indicate comparison of different materials in each concentration (horizontal comparison)

Concentration%	Green tea	Black tea	Fluoride 0.2%	Chlorhexidine 0.2%
6/25	Aa	Aa	Ca	Ba
12/5	Aa	Aa	Ca	Ab
25	Aa	Aa	Aa	Ac
50	Ac	Ab	Ba	Ad
100	Ab	Ac	Ca	Ae

RESULTS

Table 1 summarizes the MIC, MBC and the mean diameters of the inhibition zones for black and green tea extracts, CHX and fluoride mouth rinse. The MIC, MBC results were the same in all seven times of examinations.

MIC and MBC: The green and black tea had an antibacterial effect on 62.5 to 1000 mg/ml concentrations. MIC for green and black tea was achieved at 62.5 mg/mL, i.e. the minimal concentration of green and black tea that inhibited bacterial growth was 62.5 mg/mL. At concentrations less than 62.5 mg/mL the test tubes were not clear compared to the time before incubation and turbidity was observed due to bacterial growth. At concentrations higher than 62.5 mg/mL the test tubes were clear compared to the time before incubation, indicating that the concentration was able to inhibit bacterial growth.

However, the MIC and MBC of tea extracts were higher than CHX (0.125 mg/mL) and fluoride (2 mg/mL). After determination of MIC for each extract and transfer of each clear test tube onto the solid blood agar plates to determine MBC, the following results were observed. By observing or not observing any bacterial colonies on the blood agar culture media, MBC for tea extracts was determined at 62.5 mg/mL, i.e. the minimum concentration of the material that destroyed the bacteria was 62.5 mg/mL, at which no bacterial colonies were observed.

The MIC and MBC of CHX were similar (0.125 mg/mL). MIC and MBC of fluoride were similar as well. (2 mg/mL)

Considering seven measurements made for MIC and MBC and the similarity of all the values in each sample (with a variance of zero for the values), there were not significant differences between MIC and MBC of each sample ($P=1$). The correlation coefficient for MIC and MBC values in all the materials was the same ($r=1$, $P<0.01$).

Results of the disk diffusion method: The diameter of the inhibition zone (mean) for pure green and black tea (100%) was 16.05mm, 11.8mm respectively with 9.92 mm for 0.2%CHX mouth rinse. Bacterial growth was not inhibited around the fluoride mouth rinse and the diameter of inhibition zone was zero.

The comparison of the diameter of inhibition zone in each concentration (62.5-100mg/ml) of green tea and CHX revealed significant differences. (P value=0.01) Furthermore the difference between various concentrations of green tea and fluoride was significant. (P value<0.001) All concentrations (62.5-1000mg/ml) of black tea showed significant difference with fluoride. (P value=0.001) The difference between each concentration of chlorhexidine and fluoride was significant. (P value=0.03) The results of the inhibition zone at 62.5 to 1000 mg/ml concentrations of each substance have been summarized in Table 2.

In this concentration range, the diameter of inhibition zone of the green tea was larger than the black tea; however there was no significant difference. (P value =0.23) There was no significant difference between inhibition zone of the black tea and CHX as well. (P value =0.08)

DISCUSSION

In the present study the green and black tea extracts had an antibacterial effect on 62.5 to 1000 mg/ml concentrations. The results showed that the MIC and MBC of green and black tea extracts were similar (62.5 mg/mL). In other words, green and black tea cannot completely inhibit *SM* until they reach a concentration of 62.5 mg/mL. However, the MIC and MBC of tea extracts were higher than CHX (0.125 mg/mL) and fluoride (2 mg/mL). It means that the anti *SM* activity of CHX and fluoride appears on a lower concentration than green and black tea extracts. These findings are in agreement with Thomas et al. ⁶ Base on MIC analysis against *SM*, they confirmed that chlorhexidine mouth rinse was found to be the most effective as compared to sodium fluoride, fluoride with essential oils and green tea. (P < 0.001)

The antibacterial effects of green and black tea extracts were found to be similar in the present study. Contradictory, Naderi et al ¹⁸ reported that the MIC of green and black tea was 150 and 50 mg/ml, respectively. They concluded that the anti *SM* activity of black tea appears on a lower concentration than green tea. The conflicting results of two studies could be due to differences in the type of tea (Lahijan vs Golestan,Iran) and the strain of *SM*.

The diameters of inhibition zones for pure green and black tea (16.05 and 11.8 mm) were higher than CHX (9.91 mm) and fluoride(0mm), which means bacterial growth in the presence of pure tea extract was less than that of CHX.

In the present study, 0.2% fluoride mouth rinse was unable to inhibit bacterial growth in the plate well technique, which might be attributed to the exposure time and the bacterial strain involved.

The inhibition zone analysis showed that the anti-*SM* effect of green tea on higher concentration (6.25-100%) was significantly more than 0.2% CHX and fluoride. At these concentrations, the anti *SM* activity of green and black tea was similar and there was no significant difference. In general, the antibacterial effects of green tea, black tea and CHX were significantly higher than fluoride.

These findings are in agreement with the previous studies which concluded that the green tea has an inhibitory effect on *SM*.^{19,20} Subramaniam et al²¹ demonstrated that the extract of green tea showed greater zone of inhibition than CHX and black tea. Anita et al ⁷reported that MIC and MBC of green tea extract on *SM* was found to be 0.2% and 0.8% respectively. The mean zone of inhibition for green tea was 18.33 mm.

Studies carried out on the effect of green tea on *SM* are numerous but studies on black tea as one of the most commonly used drinks all over the world, especially in Iran, are limited and there are contradictory reports in relation to the comparison of the antibacterial effects of green tea and black tea.^{11,13}

In the present study, the inhibitory effects of green and black tea were similar. On the contrary, Smullen et al²² showed that green tea has a great effect on inhibiting *SM* compared to black tea because green tea has a higher content of catechin. Rasheed and Haider²³ suggested that the antibacterial effect of black tea was

not reported to be higher than that of green tea and as a result of fermentation, the antibacterial activity of black tea changes.

Contrary to the studies above, Hamidi et al¹¹ and Naderi et al¹⁸ reported a higher antimicrobial activity of black tea on oral *SM* and inhibition of biofilm formation than that of green tea.

The major difference between the green tea and black tea is in their content of catechin which is a type of polyphenol. These black and large materials are converted to theaflavin and thearubigin during the manufacturing of black tea, referred to as fermentation.⁹

A cup of green tea which has been prepared in a conventional manner contains 0.5–1 g of catechin, while black tea contains one-third of the amount mentioned above. The antibacterial effect of black tea is attributed to the presence of theaflavin and gallic acid in its chemical structure.¹²

The volatile components of tea that are found in small amounts (10–20 ppm) have antimicrobial activity and are found in higher concentrations in black tea compared to green tea.¹⁴

Studies have shown that tea, especially green tea, has a role in preventing dental caries through inhibition of bacterial proliferation, prevention of bacterial adhesion to tooth enamel, inhibition of bacterial enzymes glycosyl transferase and amylase.^{5,14} Other pieces of evidence have shown that catechin in tea alters the phenotype of *SM* and prevents its adhesion to tooth surfaces.²²

According to the above explanations, similar antibacterial effects of green and black tea as a results of the present study, seems to be reasonable.

It is difficult to directly compare the results of different studies due to differences in methods used to analyze the results, the strains of *SM*, the presence of sucrose in the culture broth, the sources available, the types of the materials and manufacturers and the techniques used to prepare extracts. As discussed above, in the present study the routine tea samples available in Iran were evaluated. Anyway during the process of preparing extracts all or some of the their active ingredients might become inactivated or the concentration of active ingredient might be different due to differences in the location, season or cultivating conditions, affecting the efficacy of tea extract.

Jazaeri et al⁴ demonstrated that the anti-cariogenic effect of fluoride-chlorhexidine was the higher than green tea in an *in vitro* study. Although green tea showed higher cariostatic effects than normal saline, in comparison with other mouth rinses, it is less effective.

One of the limitations of the present study was that the biofilm counterpart was not performed and only the anti-*SM* activity of materials was considered.

However an *in vitro* study showed that the polyphenols in tea have no effect on remineralization of enamel and the anticariogenic effect of tea is only related to its antibacterial activity.²⁴

Based on the results of the present study and by taking account of the fewer side effects of green and black tea compared to CHX mouth rinses, it appears that tea extracts can be used as economical and suitable adjuvant to synthetic compounds for dental caries prevention, especially in developing economies.^{16,25} *In vivo* studies in this area are further required as limited data are available regarding the biofilm counterpart. Hence, further studies are required for understanding antimicrobial effect of tea extract in biofilm.

CONCLUSION

The green and black tea had similar antibacterial effect on 62.5 to 1000 mg/ml concentrations. Based on MIC/MBC analyses, the anti *SM* activity of CHX and fluoride appears on a lower concentration than green and black tea extracts. However, the inhibition zone analysis showed that the anti-*SM* effect of green tea on higher concentration (6.25-100%) was significantly more than chlorhexidine and fluoride. At these concentrations, the black tea inhibited *SM* more than fluoride. In general, the antibacterial effects of green tea, black and chlorhexidine were significantly higher than fluoride.

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REFERENCES

1. Ansai T, Tahara A, Ikeda M, Katoh Y, Miyazaki H, Takehara T. Influence of colonization with mutants streptococci on caries risk in Japanese preschool children: 24 month survival analysis. *Pediatric dentistry*. 2000; 22(5):377-80.
2. Tinanoff N, Kanellis MJ, Vargas CM. Current understanding of the epidemiology, mechanism and prevention dental caries in preschool children. *Pediatr Dent* 2002; 24:543-51.
3. Harini PM, Aneundi RT. Efficacy of a probiotic and chlorhexidine mouth rinses: a short-term clinical study. *J Indian Soc Pedod Prev Dent* 2010 28(3):179-82.
4. Jazaeri M, Pakdek F, Rezaei-Soufi L, Abdolsamadi H, Rafeian N. Cariostatic effect of green tea in comparison with common anticariogenic agents: an in vitro study. *J Dent Res Dent Clin Dent Prospects*. 2015;9(1):44-8.
5. Siddiqui MW, Sharangi AB, Singh JP, Thakur PK, Ayala-Zavala JF, Singh A, et al. Antimicrobial Properties of Teas and Their Extracts in vitro. *Crit Rev Food Sci Nutr*. 2016;56(9):1428-39
6. Thomas A, Thakur S, Mhambrey S. Comparison of the antimicrobial efficacy of chlorhexidine, sodium fluoride, fluoride with essential oils, alum, green tea, and garlic with lime mouth rinses on cariogenic microbes. *J Int Soc Prev Community Dent* 2015;5(4):302-8.
7. Anita P, Sivasamy S, Madan Kumar PD, Balan IN, Ethiraj S. In vitro antibacterial activity of *Camellia sinensis* extract against cariogenic microorganisms. *J Basic Clin Pharm*. 2014;6(1):35-9.
8. Lauten JD, Boyd L, Hanson MB, Lillie D, Gullion C, Madden TE. A clinical study: Melaleuca, Manuka, Calendula and green tea mouth rinse. *Phytother Res*. 2005; 19(11):951-7.
9. Kawarai T, Narisawa N, Yoneda S, Tsutsumi Y, Ishikawa J, Hoshino Y and et al. Inhibition of *Streptococcus mutans* biofilm formation using extracts from Assam tea compared to green tea. *Arch Oral Biol*. 2016;68:73-82.
10. Thomas A, Thakur SR, Shetty SB. Anti-microbial efficacy of green tea and chlorhexidine mouth rinses against *Streptococcus mutans*, *Lactobacilli* spp. and *Candida albicans* in children with severe early childhood caries: A randomized clinical study. *J Indian Soc Pedod Prev Dent*. 2016;34(1):65-70.
11. Hamdi K, Shoa Hasani AR, Ordouzadeh N, Ghaemi A. The effect of black and greentea extracts on dental-plaque forming *Streptococci*. *Shahrekord Uni Med Sci J* 2008;10(3):1-8.
12. Mahmood T, Akhtar N, Khan BA. The morphology, characteristics, and medicinal properties of *Camellia sinensis*' tea. *J Med Plants Res* 2010; 4(19): 2028-33.
13. Hassani AS, Amirzofari N, Ordouzadeh N, Hamdi K, Nazari R, Ghaemi A. Volatile components of *Camellia sinensis* inhibit growth and biofilm formation of oral streptococci in vitro. *Pak J Biol Sci* 2008 15;11(10):1336-41.
14. Zhang J, Kashket S. Inhibition of salivary amylase by black and green teas and their effects on the intra oral hydrolysis of starch. *Caries Res* 1998; 32:233-238.
15. Tehrani MH, Asghari GH, Hajiahmadi M. Comparing *Streptococcus mutans* and *Lactobacillus* colony count changes following green tea mouth rinse or sodium fluoride mouth rinse use in children (Randomized double-blind controlled clinical trial). *Dent Res J (Isfahan)*. 2011; 8(Suppl1): S58–S63.
16. McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. *Food Chem* 2001;73: 73-84.
17. Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that National Committee for Clinical Laboratory grow aerobically. Approved standard M7-A6. National Committee for Clinical Laboratory Standards. Wayne: Pa; 2003.
18. Naderi N J, Niakan M, Kharazi Fard MJ, Zardi S. Antibacterial Activity of Iranian Green and Black Tea on *Streptococcus Mutans*: An In Vitro Study. *J Dent (Tehran)*. 2011; 8(2): 55–59.
19. 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(9):2307-11
20. You SQ. Study on feasibility of Chinese green tea polyphenols (CTP) for preventing dental caries. *Zhonghua Kou Qiang Yi Xue Za Zhi* 1993;28(4):197-9, 254.
21. Subramaniam P, Eswara U, Maheshwar Reddy KR. Effect of different types of tea on *Streptococcus mutans*: an in vitro study. *Indian J Dent Res* 2012;23(1):43-8.
22. Smullen J, Koutsou G, Foster H, Zumbé A, Storey D. The antibacterial activity of plant extracts containing polyphenols against *Streptococcus mutans*. *Caries Res* 2007;41(5):342-9.
23. Rasheed A, Haider M. Antibacterial activity of *Camellia sinensis* extracts against dental caries. *Arch pharm Res* 1998;21(3):34852.
24. Li JY, Zhan L, Barlow J, Lynch RJ, Zhou XD, Liu TJ. Effect of tea polyphenol on the demineralization and remineralization of enamel in vitro. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2004; 35(3):364-6.
25. Hambire CU, Jawade R, Patil A, Wani VR, Kulkarni AA, Nehete PB. Comparing the antiplaque efficacy of 0.5% *Camellia sinensis* extract, 0.05% sodium fluoride, and 0.2% chlorhexidine gluconate mouthwash in children. *J Int Soc Prev Community Dent*. 2015;5(3):218-26.

Conflict of Interest: None Declared.

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