Original Article

Comparative evaluation of the antimicrobial activity of three tea extracts against chlorhexidine, chlorine dioxide, and cariogenic bacteria: An *in vitro* study

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Abstract

Background: Tea derived from *Camellia sinensis* contains several bioactive compounds with documented antimicrobial potential. Differences in the degree of fermentation among green, black, and oolong tea may influence their antimicrobial efficacy against cariogenic microorganisms.

Aim: To compare and evaluate the anticariogenic activity of aqueous and ethanol extracts of green tea, black tea, and oolong tea with 0.2% chlorhexidine and 0.1% chlorine dioxide against *Streptococcus mutans, Streptococcus oralis*, and *Lactobacillus acidophilus*.

Materials and Methods: About 50 µL of aqueous and ethanol tea extracts, along with chlorhexidine and chlorine dioxide, were inoculated into wells prepared on agar plates smeared with the test microorganisms. Plates were incubated at 37°C for 24 h, after which the diameter of the zone of inhibition was measured. Statistical analysis was performed using the one-way ANOVA followed by Tukey's *post hoc* test.

Results: Aqueous oolong tea extract demonstrated the highest antimicrobial activity against all three microorganisms, followed by chlorhexidine, green tea, black tea, and chlorine dioxide. Ethanol extracts showed comparatively lower antimicrobial activity; however, oolong tea remained the most effective. The differences between aqueous and ethanol extracts were statistically significant ($P < 0.05$).

Conclusion: Aqueous tea extracts exhibited superior antimicrobial activity compared to ethanol extracts. Oolong tea showed greater anticariogenic potential than chlorhexidine, suggesting its possible role as a natural adjunct in caries prevention.

Keywords: Epigallocatechin; *Lactobacillus acidophilus; Streptococcus mutans; Streptococcus oralis*

INTRODUCTION

Streptococcus mutans plays a key role in the initiation of dental caries, whereas *Streptococcus oralis* and *Lactobacillus acidophilus* are implicated in the advancement of the disease process.[1-3] Mechanical plaque control alone is often inadequate in areas that are difficult to access within the oral cavity, thereby necessitating the adjunctive use of chemical plaque control agents. Chlorhexidine is widely accepted as the gold standard among these agents due to its potent antimicrobial action and substantivity, which results from its affinity for binding to both hard and soft oral tissues.[4-6] Despite its effectiveness, chlorhexidine

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use is associated with several adverse effects, such as discoloration of teeth and tongue, taste alteration, mucosal irritation, and a burning sensation.[7-9]

To address these limitations, alternative antimicrobial agents, including chlorine dioxide and naturally derived products such as tea, have been explored. Tea (*Camellia sinensis*) is commercially available in three forms depending on the degree of fermentation: Green tea (nonfermented), oolong tea (partially fermented), and black tea (fully fermented).[1,10,11] Tea is rich in bioactive constituents that have demonstrated the ability to reduce bacterial adhesion to tooth surfaces, inhibit glucosyltransferase activity – thereby limiting glucan formation – and suppress both salivary and bacterial amylase activity.[9,12-18]

In view of the growing demand for effective yet safe anticariogenic agents, the present *in vitro* study was designed to comparatively evaluate the antimicrobial effects of 0.2% chlorhexidine, 0.1% chlorine dioxide, and green, black, and oolong tea extracts against *S. mutans, S. oralis*, and *L. acidophilus.*

Null hypothesis

There is no statistically significant difference in the antimicrobial activity of aqueous and ethanol extracts of green tea, black tea, and oolong tea when compared with 0.2% chlorhexidine and 0.1% chlorine dioxide against *S. mutans*, *S. oralis*, and *L. acidophilus*.

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology of Kothiwal Dental College and Research Center (KDCRC/IERB/02/2022/24).

A total of 90 samples (plates smeared with different bacteria) were included in the study and were divided into two main groups based on the type of extract used.

Group 1: Aqueous extract group (*n* = 45)

This group consisted of samples treated with aqueous tea extracts along with chlorhexidine and chlorine dioxide.

Group 1 was further subdivided into three subgroups based on the test microorganism:

- Subgroup 1A: Plates smeared with *S. mutans* ($n = 15$)
- Subgroup 1B: Plates smeared with *S. oralis* ($n = 15$)
- Subgroup 1C: Plates smeared with *L. acidophilus* ($n = 15$).

Group 2: Ethanolic extract group (*n* = 45)

This group consisted of samples treated with ethanolic tea extracts along with chlorhexidine and chlorine dioxide.

Group 2 was further subdivided into three subgroups based on the test microorganism:

- Subgroup 2A: Plates smeared with *S. mutans* ($n = 15$)
- Subgroup 2B: Plates smeared with *S. oralis* ($n = 15$)
- Subgroup 2C: Plates smeared with *L. acidophilus* ($n = 15$).

Preparation of culture media

Freeze-dried strains of *S. mutans*, *S. oralis*, and *L. acidophilus* were procured from the Institute of Microbial Technology, Chandigarh, India. *S. mutans* and *S. oralis* were inoculated into Brain–Heart Infusion broth, while *L. acidophilus* was cultured in thioglycolate broth. Following incubation at 37°C for 24 h, *S. mutans* and *S. oralis* were streaked onto blood agar plates, and *L. acidophilus* was streaked onto Mueller–Hinton agar plates for subsequent antibacterial assessment.

Tea extracts preparation

Three varieties of tea – green, black, and oolong – manufactured by Vahdam Teas were used. Based on the extraction solvent, the samples were categorized into two groups: Aqueous tea extracts and ethanol tea extracts.

Group 1: Aqueous tea extracts

Ten grams of each tea sample were added to 100 mL of boiling distilled water and allowed to steep. The mixture was filtered using Whatman filter paper, and the filtrate was concentrated to 10 mL using a water bath to obtain a 100% aqueous extract.

Group 2: Ethanol tea extracts

Five grams of each tea sample were mixed with 50 mL of ethanol and ground using a mortar and pestle. The mixture was filtered through Whatmann filter paper and the filtrate was concentrated to 5 mL on a water bath to obtain a 100% ethanolic extract. All freshly prepared extracts were allowed to cool to the room temperature before antimicrobial testing.

Chlorhexidine and chlorine dioxide

Commercially available mouthrinses containing 0.2% chlorhexidine and 0.1% chlorine dioxide were used without further dilution. Chlorhexidine (0.2%) served as the positive control.

Antibacterial assay

The experiment was divided into two main groups based on the type of tea extract (aqueous and ethanolic), with each group further subdivided according to the test microorganism.

Group 1: Aqueous tea extracts

Subgroup 1: Streptococcus mutans

Fifteen blood agar plates were *uniformly* inoculated with *S. mutans* using a sterile swab. Five wells measuring 6 mm in diameter were prepared in each plate using a biopsy punch.

Each well was filled with 50 µL of 0.2% chlorhexidine, 0.1% chlorine dioxide, or aqueous extracts of green, black, and oolong tea. Plates were incubated at 37°C for 24 h, after which zones of inhibition were measured in millimeters using a zone scale [Figure 1a].

Subgroup 2: Streptococcus oralis

Fifteen blood agar plates were uniformly inoculated with *S. Oralis* using a sterile swab. Five wells measuring 6 mm in diameter were prepared in each plate using a biopsy punch. Each well was filled with 50 µL of 0.2% chlorhexidine, 0.1% chlorine dioxide, or aqueous extracts of green, black, and oolong tea. Plates were incubated at 37°C for 24 h, after which the zones of inhibition were measured in millimeters using a zone scale [Figure 1c].

Subgroup 3: Lactobacillus acidophilus

Fifteen Mueller–Hinton agar plates were uniformly inoculated with *L. acidophilus* using a sterile swab. Five wells, each measuring 6 mm in diameter, were prepared in each plate using a biopsy punch. Each well was filled with 50 µL of 0.2% chlorhexidine, 0.1% chlorine dioxide, or aqueous extracts of green, black, and oolong tea. Plates were incubated at 37°C for 24 h, after which zones of inhibition were measured in millimeters using a zone scale [Figure 1e].

Group 2: Ethanolic tea extracts

Subgroup 1: Streptococcus mutans

Fifteen blood agar plates were uniformly inoculated with *S. mutans* using a sterile swab. Five wells, each measuring 6 mm in diameter, were prepared in each plate using a biopsy punch. Each well was filled with 50 µL of 0.2% chlorhexidine, 0.1% chlorine dioxide, or Ethanolic extracts of green, black, and oolong tea. Plates were incubated at 37°C for 24 h, after which zones of inhibition were measured in millimeters using a zone scale [Figure 1b].

Subgroup 2: Streptococcus oralis

Fifteen blood agar plates were uniformly inoculated with *S. oralis* using a sterile swab. Five wells, each measuring 6 mm in diameter, were prepared in each plate using a biopsy punch. Each well was filled with 50 µL of 0.2% chlorhexidine, 0.1% chlorine dioxide, or Ethanolic extracts of green, black, and oolong tea. Plates were incubated at 37°C for 24 h, after which zones of inhibition were measured in millimeters using a zone scale [Figure 1d].

Subgroup 3: Lactobacillus acidophilus

Fifteen Mueller–Hinton agar plates were uniformly inoculated with *L. acidophilus* using a sterile swab. Five wells, each measuring 6 mm in diameter, were prepared in each plate using a biopsy punch. Each well was filled with 50 µL of 0.2% chlorhexidine, 0.1% chlorine dioxide, or aqueous extracts of green, black, and oolong tea. Plates were incubated at 37°C for 24 h, after which the zones of inhibition were measured in millimeters using a zone scale [Figure 1f].

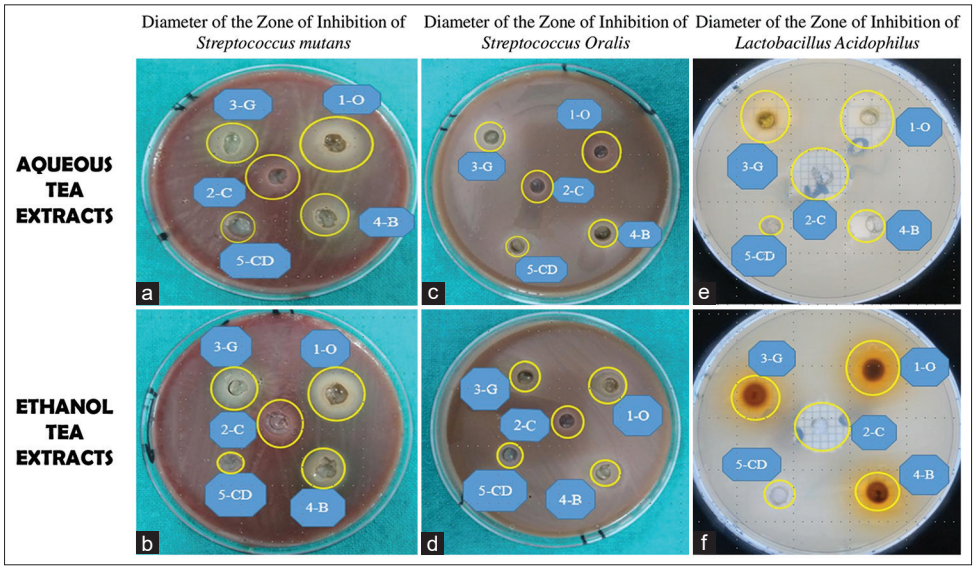


Figure 1: Represents the diameter of the zone of inhibition of *Streptococcus mutans*, *Streptococcus oralis* and *Lactobacillus acidophilus* by different anticariogenic agents: O-Oolong tea, C: Chlorhexidine, G: Green tea, B: Black tea, CD: Chlorine dioxide (a) Diameter of the Zone of Inhibition of Streptococcus mutans by Aqueous Extract Group, (b) Diameter of the Zone of Inhibition of Streptococcus mutans by Ethanol Extract Group, (c) Diameter of the Zone of Inhibition of Streptococcus Oralis by Aqueous Extract Group, (d) Diameter of the Zone of Inhibition of Streptococcus Oralis by Ethanol Extract Group, (e) Diameter of the Zone of Inhibition of Lactobacillus Acidophilus by Aqueous Extract Group, (f) Diameter of the Zone of Inhibition of Lactobacillus Acidophilus by Ethanol Extract Group

Statistical analysis

All the data were collected and analyzed. The statistical software SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) is used for the analysis of data. The descriptive statistics, such as mean, S.D., and S.E.M. of the data, were calculated. The normality of data was tested by the Shapiro–Wilk test and found to be normally distributed. The significance of the means of the diameter of zone of inhibition between two groups was tested by an Independent *t*-test and intergroup comparison of the mean of the diameter of zone of inhibition among *S. mutans*, *S. oralis*, and *L. acidophilus* of Aqueous Tea and Ethanol tea extracts in a solution by the one-way ANOVA followed by Tukey's HSD test. The 95% confidence interval and 5% level of significance were used for the analysis of data.

*Significant $P < 0.05$,

** Highly significant $P < 0.01$

*** Very highly significant $P < 0.001$

NS not significant $P > 0.05$.

RESULTS

The antimicrobial efficacy of aqueous and ethanol extracts of green tea, black tea, and oolong tea was compared with 0.2% chlorhexidine and 0.1% chlorine dioxide against *S. mutans, S. oralis*, and *L. acidophilus* using the agar well diffusion method. The mean diameter of the zones of inhibition (in mm) was recorded and statistically analyzed.

Comparison between aqueous and ethanol extracts

Overall, aqueous tea extracts demonstrated significantly greater antimicrobial activity than ethanol extracts against all three microorganisms. No statistically significant difference was observed between aqueous and ethanol preparations of chlorhexidine and chlorine dioxide ($P > 0.05$).

Streptococcus mutans

Among the aqueous extracts, oolong tea showed the highest mean zone of inhibition (19.00 ± 1.00 mm), followed by chlorhexidine (17.13 ± 1.12 mm), green tea (15.60 ± 1.50 mm), black tea (14.27 ± 1.28 mm), and chlorine dioxide (8.73 ± 1.03 mm) [Table 1].

In the ethanol extract group, oolong tea again demonstrated the highest inhibition (18.13 ± 1.18 mm), followed by chlorhexidine (17.07 ± 1.16 mm), green tea (14.13 ± 1.06 mm), black tea (12.13 ± 1.18 mm), and chlorine dioxide (8.67 ± 1.29 mm) [Table 1].

Intergroup comparison revealed that the aqueous extracts of oolong tea, green tea, and black tea exhibited significantly higher antimicrobial activity than their corresponding ethanol extracts ($P < 0.01$), whereas no significant difference was observed for chlorhexidine and chlorine dioxide ($P > 0.05$) [Figure 2a-c].

Streptococcus oralis

In the aqueous extract group, the highest mean zone of inhibition was observed with oolong tea (18.73 ± 1.49 mm), followed by chlorhexidine (17.60 ± 1.35 mm), green tea (15.93 ± 1.10 mm), black tea (14.53 ± 0.99 mm), and chlorine dioxide (8.73 ± 1.03 mm) [Table 1].

In the ethanol extract group, oolong tea again showed maximum inhibition (18.13 ± 1.55 mm), followed by chlorhexidine (17.53 ± 1.46 mm), green tea (14.80 ± 0.86 mm), black tea (13.60 ± 0.91 mm), and chlorine dioxide (8.67 ± 1.05 mm) [Table 1].

Statistical analysis showed that the aqueous extracts of green tea and black tea produced significantly larger zones of inhibition than their ethanol counterparts ($P < 0.01$). Differences for oolong tea, chlorhexidine, and chlorine dioxide were not statistically significant ($P > 0.05$) [Figure 2a-c].

Lactobacillus acidophilus

For *L. acidophilus*, aqueous oolong tea demonstrated

Table 1: Distribution of mean±standard deviation of diameter of zone of inhibition of *Streptococcus mutans*, *Streptococcus oralis*, and *Lactobacillus acidophilus* of two groups among five solutions

Test solution	Group	*n*	*Streptococcus mutans* (mean±SD)	SEM	*Streptococcus oralis* (mean±SD)	SEM	*Lactobacillus acidophilus* (mean±SD)	SEM
Oolong tea	1 (Aqueous)	15	19.00±1.000	0.258	18.73±1.486	0.384	19.20±1.373	0.355
	2 (Ethanolic)	15	18.13±1.187	0.307	18.13±1.552	0.401	18.00±1.464	0.378
Chlorhexidine	1 (Aqueous)	15	17.13±1.125	0.291	17.60±1.352	0.349	17.20±1.320	0.341
	2 (Ethanolic)	15	17.07±1.163	0.300	17.53±1.457	0.376	17.13±1.457	0.376
Green tea	1 (Aqueous)	15	15.60±1.502	0.388	15.93±1.100	0.284	16.00±1.195	0.309
	2 (Ethanolic)	15	14.13±1.060	0.274	14.80±0.862	0.223	15.07±0.704	0.182
Black tea	1 (Aqueous)	15	14.27±1.280	0.330	14.53±0.990	0.256	14.80±1.207	0.312
	2 (Ethanolic)	15	12.13±1.187	0.307	13.60±0.910	0.235	13.80±0.775	0.200
Chlorine dioxide	1 (Aqueous)	15	8.73±1.033	0.267	8.73±1.033	0.267	8.73±1.100	0.284
	2 (Ethanolic)	15	8.67±1.291	0.333	8.67±1.047	0.270	8.67±0.900	0.232

SD: Standard deviation, SEM: Standard error of the mean

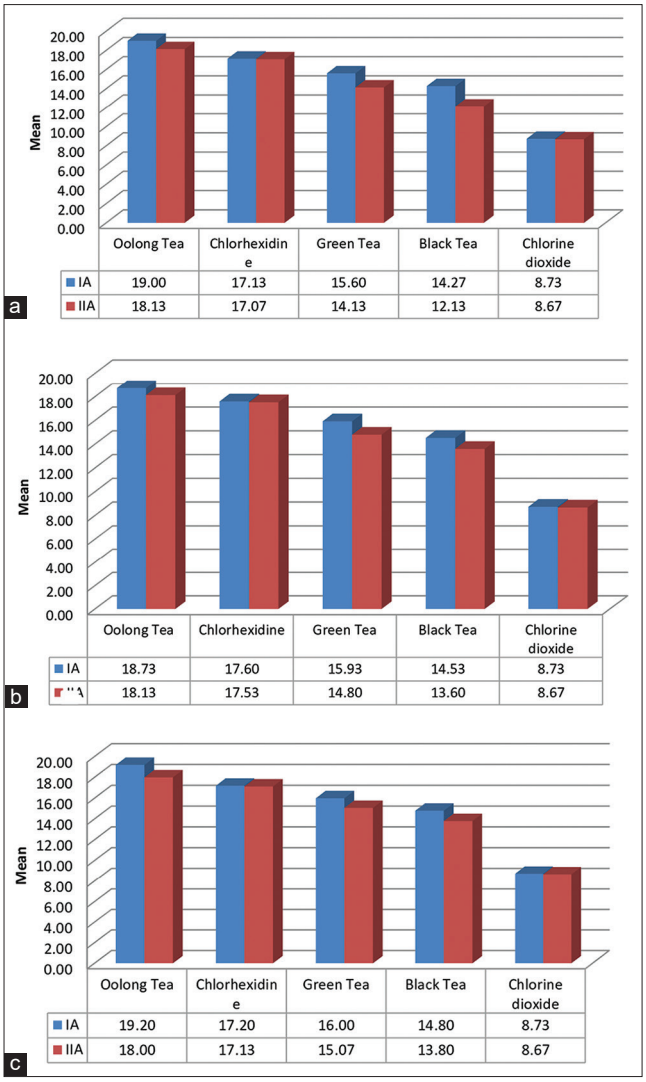


Figure 2: Represents graphs depicting inter-group comparison of the diameter of the zone of inhibition of *Streptococcus mutans, Streptococcus oralis,* and *Lactobacillus acidophilus* between groups among five solutions. (a) Inter-group comparison of the diameter of the zone of inhibition of *Streptococcus mutans* between groups among five solutions, (b) Inter-group comparison of the diameter of the zone of inhibition of *Streptococcus oralis* between groups among five solutions, (c) Inter-group comparison of the diameter of the zone of inhibition of *Lactobacillus acidophilus* between groups among five solutions

the highest antimicrobial activity (19.20 ± 1.37 mm), followed by chlorhexidine (17.20 ± 1.32 mm), green tea (16.00 ± 1.19 mm), black tea (14.80 ± 1.21 mm), and chlorine dioxide (8.73 ± 1.10 mm) [Table 1].

Similarly, in the ethanol extract group, oolong tea showed the highest inhibition (18.00 ± 1.46 mm), followed by chlorhexidine (17.13 ± 1.46 mm), green tea (15.07 ± 0.70 mm), black tea (13.80 ± 0.78 mm), and chlorine dioxide (8.67 ± 0.90 mm) [Table 1].

Intergroup comparison demonstrated that aqueous extracts of oolong tea, green tea, and black tea were significantly more effective than ethanol extracts ($P < 0.05$), while chlorhexidine and chlorine dioxide showed no significant difference between the two groups ($P > 0.05$) [Figure 2a-c].

DISCUSSION

The present study evaluated the anticariogenic activity of 0.2% chlorhexidine, 0.1% chlorine dioxide, and aqueous and ethanolic extracts of green, black, and oolong tea against *S. mutans*, *S. oralis*, and *L. acidophilus*. Tea has been demonstrated to exert anticariogenic activity both *in vitro* and *in vivo*, including direct bactericidal effects, inhibition of bacterial adherence to teeth, suppression of glucosyltransferase activity, and inhibition of human and bacterial amylases.[2,7,8]

Green tea polyphenols primarily contain catechins, such as epicatechin, epigallocatechin (EGC), and EGC gallate (EGCG).[8] EGCG has been shown to disrupt bacterial cell membranes and prevent DNA supercoiling, leading to bacterial death, while EGC interacts with proteins, altering their tertiary structure. Other bioactive compounds, including alkaloids, flavonoids, and tannins, contribute to antimicrobial activity through inhibition of bacterial adhesion, glucosyltransferase activity, and iron-binding mechanisms.[8,9,18]

In the current study, all three types of tea exhibited inhibitory activity against *S. mutans*, *S. oralis*, and *L. acidophilus*. Interestingly, oolong tea extracts demonstrated a greater inhibitory effect compared to chlorhexidine, green tea, and black tea. This enhanced activity may be attributed to the higher content of alkaloids, tannins, saponins, and flavonoids in oolong tea, as supported by previous studies using spectrophotometric analysis.[1,7,8] The synergistic effect of monomeric polyphenols in oolong tea has also been suggested as a mechanism for its potent bactericidal activity.[1]

Consistent with prior reports, green tea demonstrated superior activity over black tea, likely due to its higher content of simple catechins, whereas black tea contains mostly polymerized catechins (theaflavins and thearubigins) formed during fermentation, resulting in reduced inhibitory activity against *S. mutans*.[1,8] In addition, aqueous extracts of tea exhibited greater anticariogenic activity than ethanolic extracts, possibly due to the higher solubility of bioactive phytochemicals in water.[8]

Chlorine dioxide was less effective than chlorhexidine, in agreement with previous studies highlighting the superior substantivity and antimicrobial potency of chlorhexidine.[4] These results emphasize that although chemical agents remain highly effective, natural products such as tea may serve as complementary or alternative agents in caries

prevention due to their favorable safety profile and bioactive properties.[3,9,18-22]

Limitations of the study

Being an *in vitro* study, the results may not accurately replicate the complex conditions of the oral cavity, where factors such as saliva, dietary habits, biofilm architecture, host immune response, and oral clearance mechanisms can influence antimicrobial efficacy.

The agar well-diffusion method used in this study assesses antimicrobial activity based on diffusion characteristics of the test agents, which may not directly correlate with their clinical effectiveness or substantivity in the oral environment.

Only standard laboratory strains of *S. mutans, S. oralis,* and *L. acidophilus* were evaluated; hence, the results may differ when tested against clinical isolates exhibiting variable virulence and resistance patterns.

The study assessed the zone of inhibition alone and did not determine minimum inhibitory concentration, minimum bactericidal concentration, or time-dependent antimicrobial effects, which could provide a more comprehensive evaluation of antimicrobial potency.

In addition, the chemical composition of tea extracts was not analyzed, and variations in polyphenol concentration due to processing methods or batch differences were not accounted for.

Finally, the short incubation period and absence of evaluation of long-term antimicrobial or anti-biofilm effects limit the extrapolation of the obtained evidence to clinical use.

CONCLUSION

Within the limitations of this study, it can be concluded that the aqueous tea extracts showed higher antimicrobial activity compared to the ethanol tea extracts.

Both the aqueous and ethanol extracts of oolong tea showed higher antimicrobial activity compared to chlorhexidine, followed by green tea, black tea, and chlorine dioxide.

Since people all over the world drink tea daily, promotion of oolong tea may help wither down the prevalence of caries, as antimicrobial activity can also influence caries progression to some extent.

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Conflicts of interest

There are no conflicts of interest.

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