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Evaluation of Antibacterial Effect of Green Tea (*Camellia sinensis*) Against Dental Pathogen *Streptococcus mutans* and *Streptococcus sobrinus*

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Abstract
Dental caries, a prevalent global health concern, is primarily caused by acidogenic oral pathogens such as *Streptococcus mutans* and *Streptococcus sobrinus*. Rising resistance to conventional antibiotics has sparked interest in natural alternatives, such as green tea (*Camellia sinensis*), renowned for its antimicrobial polyphenols. This study evaluated the antibacterial activity of green tea extract against *S. mutans* and *S. sobrinus* using total phenolic content (TPC), TQ-LCMS analysis, well diffusion, and broth macrodilution assays. TQ-LCMS confirmed catechin, epigallocatechin (EGC), and gallocatechin gallate (GCG) as active compounds, with EGC being the most abundant. TPC analysis yielded 264.11 mg GAE/g. Green tea extract produced inhibition zones of 0.65–1.2 mm (*S. mutans*) and 0.43–1.27 mm (*S. sobrinus*). MIC values were 25 mg/mL and 50 mg/mL for *S. mutans* and *S. sobrinus*, respectively. MBC values were 100 mg/mL and 50 mg/mL. These results indicate that green tea extract possesses significant antibacterial activity, supporting its potential as a natural additive in oral healthcare products to prevent dental caries.
Keywords: Green tea extract; Catechins; *Streptococcus mutans*; *Streptococcus sobrinus*; Antibacterial activity

Introduction

Dental caries is a common oral disease caused by acid-producing bacteria such as *Streptococcus mutans* and *Streptococcus sobrinus* (Borg-Bartolo *et al*., 2022). These pathogens produce acids from carbohydrate metabolism, contributing to enamel demineralization and biofilm formation (Zubaidah *et al*., 2022). The rise of antibiotic-resistant strains due to horizontal gene transfer poses a significant challenge to existing treatments, prompting the search for natural alternatives, such as green tea, which is rich in polyphenolic compounds with known antibacterial properties (Moghadam *et al*., 2020).

Oral diseases such as dental caries continue to affect populations worldwide, especially in children and young adults, and are often linked to poor oral hygiene and diet. With increasing reliance on sugar-rich foods, the oral cavity becomes a prime environment for acidogenic bacteria to thrive. *Streptococcus mutans* and *Streptococcus sobrinus* are among the most cariogenic organisms identified due to their ability to metabolize sucrose into lactic acid, which forms dental plaques and contributes to the demineralization of tooth enamel.

The widespread and sometimes inappropriate use of antimicrobial agents has led to a rise in antibiotic resistance, prompting the need for effective and natural alternatives. Natural products derived from plants are being studied extensively as alternative therapeutic agents due to their bioactive compounds that exert antibacterial, antifungal, and antioxidant properties. Among these, green tea (*Camellia sinensis*) has emerged as a promising candidate due to its broad-spectrum antimicrobial activity attributed to catechins and polyphenols.

Previous studies primarily examined epigallocatechin-3-gallate (EGCG) from green tea in isolation (Higuchi *et al*., 2024). However, few have investigated the combined antibacterial effects of green tea extract powder, which contains multiple bioactive constituents, including theaflavins, flavonols

glycosides, and caffeine. Furthermore, the effect of green tea on *S. sobrinus* remains underexplored (Cao *et al*., 2021), underscoring the need for further research.

This study focuses on identifying bioactive compounds in green tea extract powder and quantifying its antibacterial effects against both *S. mutans* and *S. sobrinus*, using well diffusion and broth macrodilution assays.

Materials and methods

Green tea extract powder was obtained from a commercial source (Smartwaynisb) and stored at a temperature below 28°C. The supplier confirmed the extract contained 34.26% tea polyphenols. For total phenolic content (TPC) analysis, the Folin-Ciocalteu method was employed. One milligram of the extract was dissolved in methanol, mixed with Folin-Ciocalteu reagent and sodium carbonate, and the absorbance at 750 nm was measured after incubation. A standard curve using Gallic acid was used to express results in mg GAE/g (Hemmati *et al*., 2021).

Triple quadrupole-liquid chromatography mass spectrometry (TQ-LCMS) analysis was conducted to identify major phenolic compounds. Approximately 500 mg of green tea extract was dissolved in 50% acetonitrile, then vortexed, sonicated, and centrifuged before being filtered. The analysis was performed using a Shimadzu TQ-LCMS system with Multiple Reaction Monitoring (MRM) mode, targeting catechin, epigallocatechin (EGC), and gallocatechin gallate (GCG) (Zhang *et al*., 2025).

For bacterial preparation, *Streptococcus mutans* (ATCC 25175) and *Streptococcus sobrinus* (ATCC 33402) were streaked on brain heart infusion (BHI) agar and incubated. Colonies were transferred into BHI broth and cultured overnight. Gram staining was conducted using the standard protocol, which involves crystal violet, iodine, methanol, and safranin staining, followed by observation under 1000x magnification (Paray *et al*., 2023).

The antibacterial activity of green tea extract was assessed using the well diffusion assay. Bacterial lawns were spread evenly on BHI agar plates, and wells were filled with twofold serial dilutions of green tea extract ranging from 200 to 1.5625 mg/mL. Chlorhexidine and 10% DMSO served as positive and negative controls, respectively. The plates were incubated for 24 hours, and the zones of inhibition were measured in millimetres (Hattarki *et al*., 2021).

A broth macrodilution assay was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). A 400 mg/mL stock solution was prepared and diluted to final concentrations from 200 to 1.5625 mg/mL. After inoculation and a 24-hour incubation, the tubes were observed for turbidity to determine the MIC (Salman *et al*., 2022). For MBC, a loopful of broth from each clear tube was streaked on BHI agar to determine the lowest concentration with no visible bacterial growth

Results and discussion

The total phenolic content (TPC) of the green tea extract was determined to be 264.11 mg GAE/g, indicating a high concentration of phenolic compounds, which are widely recognized for their antimicrobial properties. These results are consistent with prior findings on the antibacterial effects of polyphenols (Moghadam *et al*., 2020). The TQ-LCMS analysis further validated the presence of catechin, epigallocatechin (EGC), and gallocatechin gallate (GCG), with EGC being the most abundant compound detected based on peak intensity. These catechins, particularly EGC, are known to damage bacterial membranes, interfere with enzyme function, and inhibit acid production (Han *et al*., 2021).

Table 1: Absorbance reading of each replicate of extract at 750 nm.

	Absorbance (750 nm)	Concentration (mg/mL)	Concentration (mg/g)
Blank	0	0	0
R1	0.4260	0.0677	270.9714
R2	0.4030	0.0645	257.8286
R3	0.4040	0.0646	258.4000
R4	0.4090	0.0653	261.2571
R5	0.4280	0.0680	272.1143
Average	0.4140	0.0660	264.1140

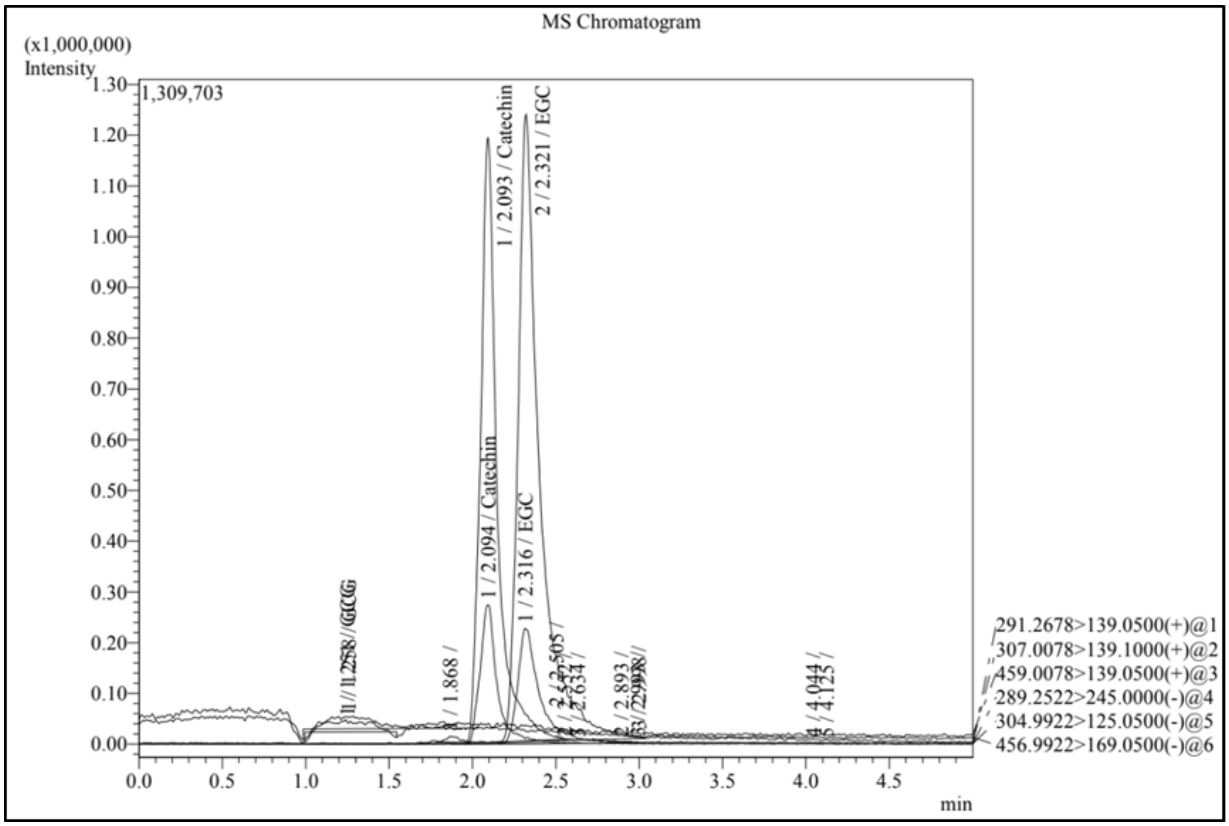


Figure 1 MS chromatogram of green tea extract.

Gram staining confirmed the morphology of both *S. mutans* and *S. sobrinus* as Gram-positive cocci, with *S. sobrinus* appearing slightly larger in size (Tripathi & Sapra, 2023). This basic identification was crucial to ensure the strains used were consistent with known oral pathogens.

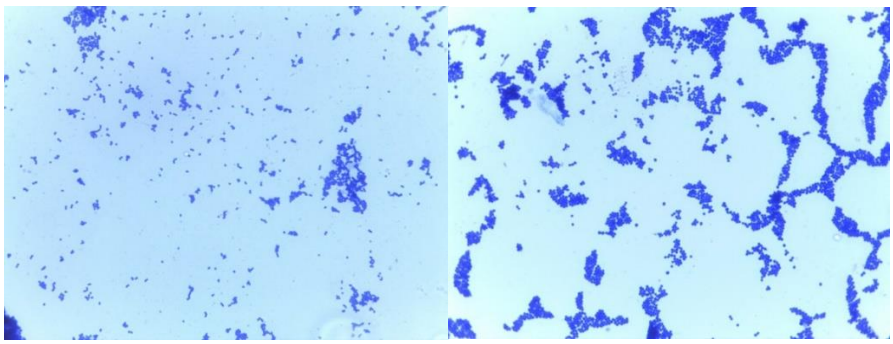


Figure 2 Gram staining of *S. mutans* (left) and *S. sobrinus* (right) observed under 1000x magnification with oil immersion.

The well diffusion assay demonstrated that green tea extract inhibited the growth of both *S. mutans* and *S. sobrinus* in a concentration-dependent manner. Catechins in green tea can disrupt bacterial cell membranes and inhibit glucosyltransferase, an enzyme critical for biofilm formation in Streptococcus species (Zhang *et al*., 2021). The highest concentration of extract (200 mg/mL) produced the largest zones of inhibition (1.20 mm for *S. mutans* and 1.27 mm for *S. sobrinus*), whereas lower concentrations showed progressively smaller zones or no visible inhibition. Notably, *S. sobrinus* demonstrated a detectable inhibition zone even at 6.25 mg/mL, a level at which *S. mutans* exhibited no measurable inhibition. This observation suggests that *S. sobrinus* may possess a greater sensitivity to certain antibacterial constituents in the green tea extract at lower concentrations.

Table 2: Zone of inhibition (mm) and mean ± SD (mm) of green tea extract against *S. mutans*.

Sample	Concentration (mg/mL)	Zone of Inhibition (mm)	Mean ± SD (mm)
Positive Control (Chlorhexidine)	-	1.1, 1.1, 1.1, 1.1	1.10 ± 0.00
Negative Control (10% DMSO)	-	-	-
Green Tea Extract	200	1.2, 1.2, 1.2, 1.2	1.20 ± 0.00
Green Tea Extract	100	1.0, 1.0, 1.0, 1.0	1.00 ± 0.00
Green Tea Extract	50	0.6, 0.7, 0.6, 0.7	0.65 ± 0.06
Green Tea Extract	25	-	-
Green Tea Extract	12.5	-	-
Green Tea Extract	6.25	-	-
Green Tea Extract	3.125	-	-
Green Tea Extract	1.5625	-	-

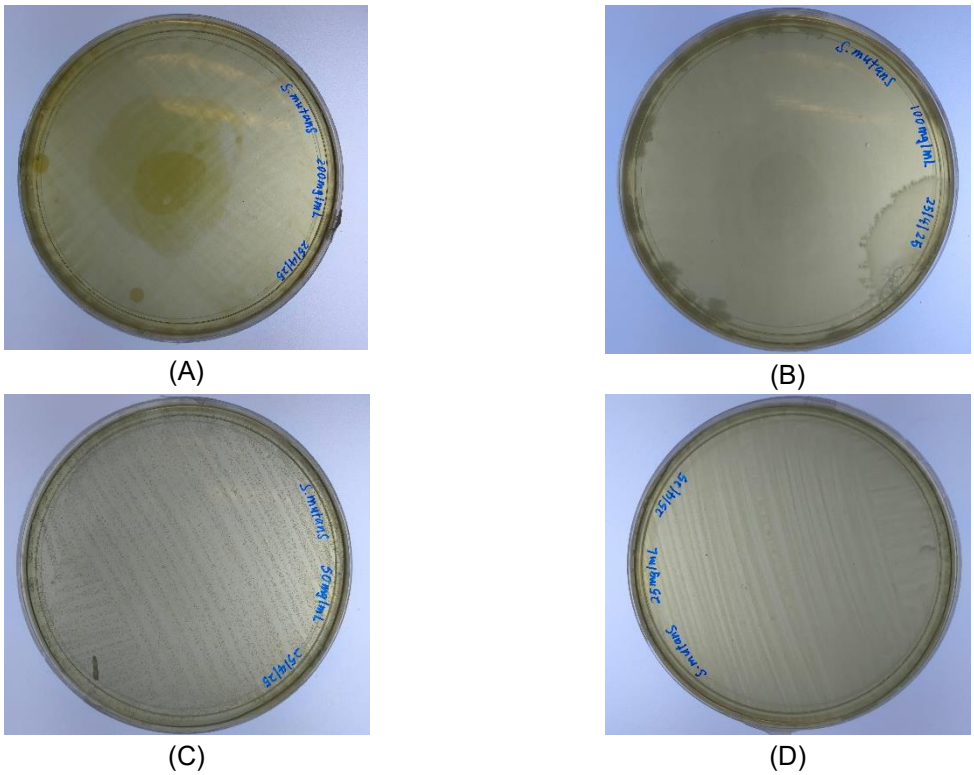
Table 3: Zone of inhibition (mm) and mean ± SD (mm) of green tea extract against *S. sobrinus*.

Sample	Concentration (mg/mL)	Zone of Inhibition (mm)	Mean ± SD (mm)
Positive Control (Chlorhexidine)	-	0.8, 0.7, 0.7, 0.6	0.70 ± 0.08
Negative Control (10% DMSO)	-	-	-
Green Tea Extract	200	1.3, 1.3, 1.2	1.27 ± 0.06
Green Tea Extract	100	1.2, 1.1, 1.2	1.17 ± 0.06
Green Tea Extract	50	0.7, 0.8, 0.8, 0.9	0.80 ± 0.08
Green Tea Extract	25	0.7, 0.6, 0.6, 0.7	0.65 ± 0.06
Green Tea Extract	12.5	0.5, 0.6, 0.6, 0.6	0.58 ± 0.05
Green Tea Extract	6.25	0.4, 0.4, 0.4, 0.5	0.43 ± 0.05
Green Tea Extract	3.125	-	-
Green Tea Extract	1.5625	-	-

Broth macrodilution assays further substantiated these findings. The minimum inhibitory concentration (MIC) for *S. mutans* was determined to be 25 mg/mL, while the MIC for *S. sobrinus* was higher, at 50 mg/mL. Conversely, the minimum bactericidal concentration (MBC) for *S. mutans* was 100 mg/mL, while for *S. sobrinus*, it was 50 mg/mL. These variations in MIC and MBC values suggest species-specific differences in susceptibility, which could be due to variations in cell wall composition or stress response mechanisms. These results are consistent with previous studies that have reported the bacteriostatic and bactericidal effects of EGC and related catechins against oral pathogens, reinforcing the potential of green tea as an effective natural alternative or adjunct to conventional antimicrobial agents in oral healthcare (Chouhan, 2025).

Table 4: MIC of green tea extract against *S. mutans* based on visual turbidity.

Sample	Concentration (mg/mL)	Visual observation
Positive Control (Chlorhexidine)	-	Clear
Negative Control (10% DMSO)	-	Turbid
Green Tea Extract	200	Clear
Green Tea Extract	100	Clear
Green Tea Extract	50	Clear
Green Tea Extract	25	Clear
Green Tea Extract	12.5	Turbid
Green Tea Extract	6.25	Turbid
Green Tea Extract	3.125	Turbid
Green Tea Extract	1.5625	Turbid



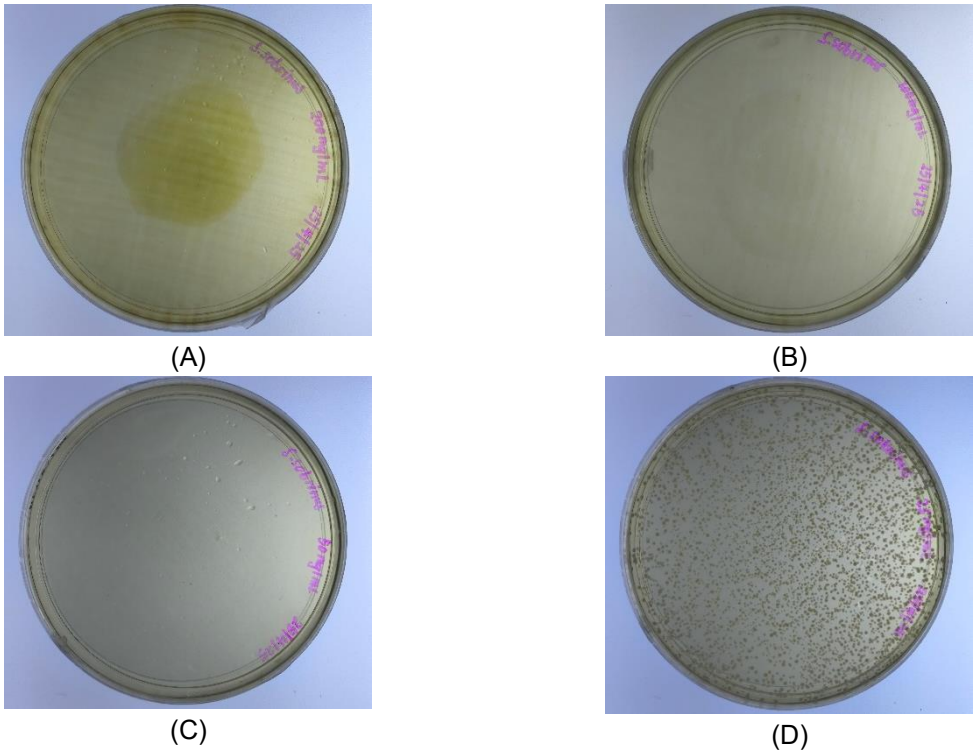
(A) (B)

(C) (D)

Figure 3 Observation of green tea extract against *S. mutans* based on colony formation. (A) 200 mg/mL and (B) 100 mg/mL showed no colony growth, while (C) 50 mg/mL and (D) 25 mg/mL showed visible colony formation.

Table 5: MIC of green tea extract against *S. sobrinus* based on visual turbidity.

Sample	Concentration (mg/mL)	Visual observation
Positive Control (Chlorhexidine)	-	Clear
Negative Control (10% DMSO)	-	Turbid
Green Tea Extract	200	Clear
Green Tea Extract	100	Clear
Green Tea Extract	50	Clear
Green Tea Extract	25	Turbid
Green Tea Extract	12.5	Turbid
Green Tea Extract	6.25	Turbid
Green Tea Extract	3.125	Turbid
Green Tea Extract	1.5625	Turbid



(A) (B)

(C) (D)

Figure 4 Observation of green tea extract against *S. sobrinus* based on colony formation. (A) 200 mg/mL, (B) 100 mg/mL, and (C) 50 mg/mL showed no colony growth, while (D) 25 mg/mL showed visible colony formation.

Conclusion

This study demonstrated that green tea (*Camellia sinensis*) extract possesses significant antibacterial activity against key oral pathogens, *Streptococcus mutans* and *Streptococcus sobrinus*. TQ LCMS and total phenolic content analyses confirmed the presence of major bioactive compounds, particularly epigallocatechin (EGC), catechin, and gallocatechin gallate (GCG), with a high phenolic concentration of 264.11 mg GAE/g. Both well diffusion and broth macrodilution assays revealed concentration-dependent inhibitory and bactericidal effects, with *S. sobrinus* exhibiting greater sensitivity at lower extract concentrations. The MIC and MBC values further support the efficacy of the extract as a natural antimicrobial agent. These findings highlight the potential of green tea extract as a safe and sustainable additive in oral healthcare formulations such as mouthwash and toothpaste, contributing to improved dental hygiene and offering an alternative to synthetic antibiotics in managing dental caries.

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