Original Article

Green Tea Extracts as Natural Antimicrobial Agents: A Comparative Analysis Against Periodontal Disease-Causing Bacteria

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Green tea (*Camellia sinensis*) has been recognized for its health-promoting properties, notably its antimicrobial and antioxidant effects. This study compares the antimicrobial efficacy of green tea extracts obtained using different solvents (ethanol, methanol, acetone, and water) against periodontal pathogens such as *Porphyromonas gingivalis* and *Streptococcus sanguinis*. Bioactive compounds were analyzed using column chromatography extraction. The ethanolic extract showed the highest levels of key components like epigallocatechin gallate (EGCG) and caffeine and demonstrated significant antimicrobial activity with zones of inhibition up to 12.34 ± 1.04 mm for *Porphyromonas gingivalis*. These results suggest that green tea extracts, particularly ethanol-based extract, could be effective natural antimicrobial agents in combating periodontal disease.

Keywords: Green tea, Camellia sinensis, antimicrobial activity, periodontal disease, epigallocatechin gallate (EGCG), disk diffusion method, bioactive compounds, natural therapies.

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Introduction

Periodontal diseases, such as gingivitis and periodontitis, are chronic inflammatory conditions affecting the gums and supporting tissues of the teeth, leading to tooth loss if untreated. A significant contributor to periodontal disease is bacterial infection, with pathogens like *Porphyromonas gingivalis* and *Streptococcus sanguinis* playing key roles in disease progression (1, 2). Current treatments often rely on mechanical cleaning and antibiotic therapy, but the rise in antibiotic resistance underscores the need for alternative, natural antimicrobial agents (3, 4).

Green tea (*Camellia sinensis*) is rich in polyphenolic compounds such as catechins, which have demonstrated antimicrobial, anti-inflammatory, and antioxidant activities (5). Among these, epigallocatechin gallate (EGCG) has garnered attention for its potent inhibitory effects on bacteria associated with oral diseases (6). However, the efficacy of green tea extracts can vary based on the solvent used for extraction, which influences the concentration of bioactive components (7). This study aims to evaluate the antimicrobial potential of green tea extracts prepared using different solvents and their effectiveness against periodontal pathogens.

Materials and Methods

Sample Collection

Fresh green tea leaves were collected from Srimangal, Chattogram, and Panchagarh tea estates in Bangladesh. The leaves were air-dried at room temperature and ground into a fine powder using a mechanical grinder.

Extraction Procedure

The powdered green tea was subjected to extraction using four solvents: ethanol, methanol, acetone, and water. Each extraction was performed as follows:

- **Ethanolic Extraction**: 50 g of tea powder was extracted with 100 mL ethanol using a Soxhlet apparatus.
- **Methanolic Extraction**: 50 g of tea powder was extracted with 100 mL methanol at 70°C.
- **Acetone Extraction**: 50 g of tea powder was extracted with 100 mL acetone (50%) for 2 hours at room temperature.
- **Aqueous Extraction**: 50 g of tea powder was soaked in 100 mL distilled water at room temperature.

The extracts were filtered, concentrated under reduced pressure using a rotary evaporator, and stored at 4°C until further analysis.

Bioactive Component Analysis

Column chromatography extraction was used to quantify catechins (EGCG, EGC), caffeine, and theanine in the extracts. The results are expressed in mg/g dry weight of the tea leaves (Table 1).

Microbial Assays

Four periodontal pathogens—*Porphyromonas gingivalis*, *Actinobacillus*, *Fusobacterium*, and *Streptococcus sanguinis*—were tested for susceptibility to the green tea extracts using the disk diffusion method (8). Bacterial strains were cultured on Mueller-Hinton agar plates. Disks impregnated with 1 mg and 2 mg of the extracts were placed on the agar, and zones of inhibition were measured after 24 hours of incubation at 37°C (9).

Statistical Analysis

Results were expressed as mean ± standard deviation (SD). Statistical significance between groups was assessed using one-way ANOVA, with a p-value < 0.05 considered significant. Microbial assay was done in Disc diffusion method and MIC.

Results

Column Chromatography Analysis of Bioactive Components

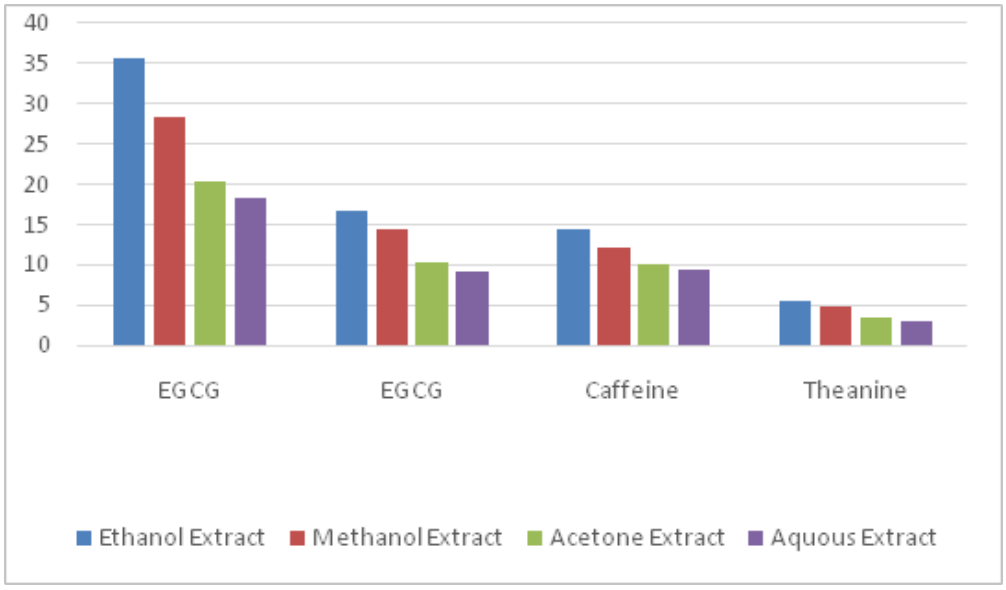
The concentrations of catechins (EGCG, EGC), caffeine, and theanine varied significantly between the different solvent extracts. Ethanol was found to be the most effective solvent for extracting these bioactive components, followed by methanol, acetone, and water (Table 1).

Table 1: Concentrations of Key Bioactive Components in Green Tea Extracts

Compound	Ethanol Extract (mg/g)	Methanol Extract (mg/g)	Acetone Extract (mg/g)	Aqueous Extract (mg/g)
EGCG	35.62 ± 2.46	28.45 ± 1.77	20.32 ± 1.55	18.40 ± 1.57
EGC	16.71 ± 1.22	14.33 ± 1.00	10.28 ± 1.21	9.12 ± 0.92
Caffeine	14.37 ± 1.09	12.05 ± 0.94	10.11 ± 0.81	9.34 ± 0.73
Theanine	5.42 ± 0.84	4.88 ± 0.76	3.33 ± 0.61	2.99 ± 0.50

The ethanolic extract exhibited the highest concentrations of EGCG (35.62 mg/g), EGC (16.71 mg/g), and caffeine (14.37 mg/g), significantly higher than those in the other solvent extracts ($p < 0.05$). These results confirm that ethanol is the most efficient solvent for extracting key bioactive components from green tea (1, 5).

Graph 1: Concentrations of Key Bioactive Components in Green Tea Extracts



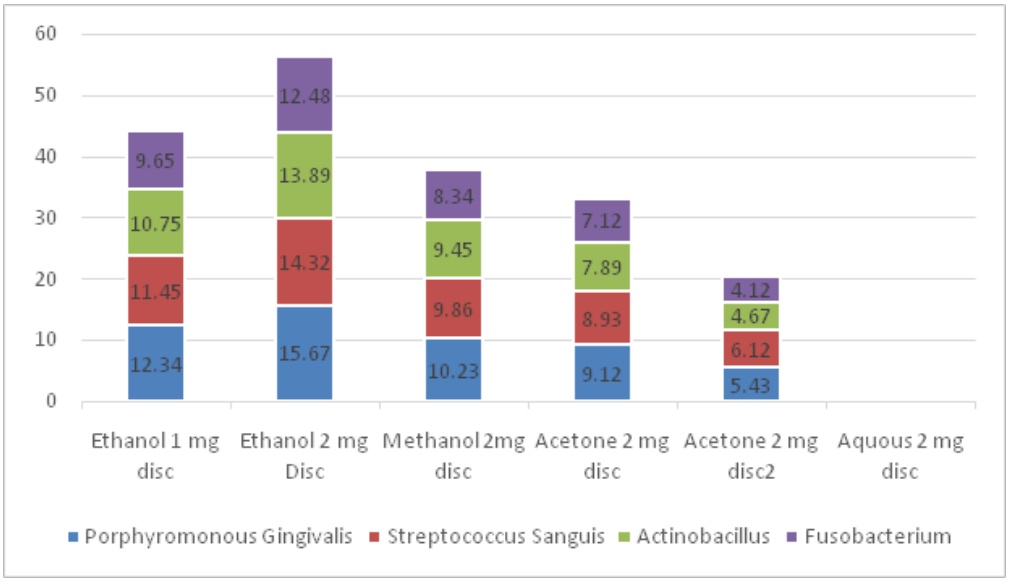
Antimicrobial Activity

The antimicrobial activity of the different extracts was assessed using the disk diffusion method. The ethanolic extract demonstrated the highest antimicrobial activity, with significant zones of inhibition against *Porphyromonas gingivalis* and *Streptococcus sanguinis* (Table 2).**Table 2: Antimicrobial Activity of Green Tea Extracts Against Periodontal Pathogens (Zone of Inhibition in mm)**

Bacteria	Ethanol (1 mg/disc)	Ethanol (2 mg/disc)	Methanol (2 mg/disc)	Acetone (2 mg/disc)	Aqueous (2 mg/disc)
Porphyromonas gingivalis	12.34 ± 1.04	15.67 ± 1.29	10.23 ± 0.87	9.12 ± 0.72	5.43 ± 0.45
Streptococcus sanguinis	11.45 ± 1.02	14.32 ± 1.17	9.86 ± 0.79	8.93 ± 0.64	6.12 ± 0.53
Actinobacillus	10.78 ± 1.13	13.89 ± 1.23	9.45 ± 0.67	7.89 ± 0.58	4.67 ± 0.34
Fusobacterium	9.65 ± 0.97	12.48 ± 1.11	8.34 ± 0.63	7.12 ± 0.56	4.12 ± 0.32

The ethanolic extract displayed the largest zones of inhibition, particularly against *Porphyromonas gingivalis* (15.67 mm at 2 mg/disc) and *Streptococcus sanguinis* (14.32 mm at 2 mg/disc) ($p < 0.05$). The methanolic and acetone extracts also showed moderate antimicrobial activity, while the aqueous extract exhibited minimal inhibition (9).

Graph 2: Zones of Inhibition for Different Extracts



Minimum Inhibitory Concentration (MIC) Analysis

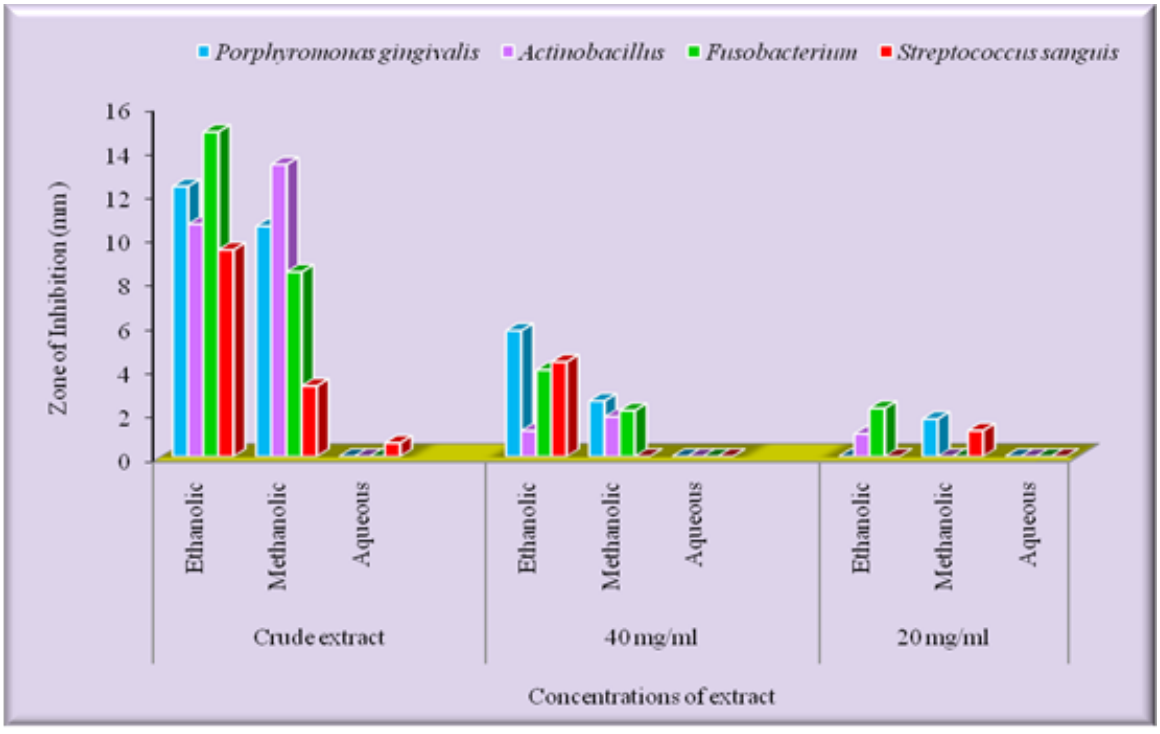
The MIC values were determined for the four periodontal pathogens tested. The ethanolic extract exhibited the lowest MIC values, indicating higher efficacy, followed by methanol, acetone, and water extracts.

Table 3: Minimum Inhibitory Concentration (MIC) of Green Tea Extracts (mg/mL)

Bacteria	Ethanol Extract	Methanol Extract	Acetone Extract	Aqueous Extract
Porphyromonas gingivalis	0.52 ± 0.04	0.87 ± 0.08	1.23 ± 0.12	1.56 ± 0.14
Streptococcus sanguinis	0.68 ± 0.06	0.94 ± 0.10	1.45 ± 0.12	1.78 ± 0.15
Actinobacillus	0.75 ± 0.06	1.03 ± 0.09	1.56 ± 0.13	2.01 ± 0.17
Fusobacterium	0.81 ± 0.07	1.10 ± 0.11	1.69 ± 0.13	2.11 ± 0.19

The ethanolic extract demonstrated the most effective antimicrobial properties, as evidenced by its lower MIC values across all tested bacterial strains. The methanolic extract was the second most effective, followed by acetone, with aqueous extracts showing the least antimicrobial potency ($p < 0.05$).

Graph 3: MIC Comparison of Green Tea Extracts



Discussion

The results of this study demonstrate that ethanol-based green tea extracts are the most effective in terms of both bioactive compound content and antimicrobial activity. The higher concentration of EGCG in the ethanolic extract likely accounts for its superior inhibitory effects against periodontal pathogens, as EGCG has been shown to disrupt bacterial cell membranes and inhibit biofilm formation (2, 10). The presence of caffeine may also enhance the antimicrobial action through its synergistic effects with catechins (3, 6).

The variation in antimicrobial efficacy among the different extracts can be attributed to the solvents' ability to dissolve specific bioactive compounds. Ethanol's polarity enables it to extract a broader range of compounds, including polyphenols and alkaloids, which contribute to the antimicrobial properties (7). The aqueous extract, in contrast, had lower concentrations of these compounds and showed limited antimicrobial activity (Table 2), corroborating previous research suggesting that water is less efficient at extracting catechins (8).

This study adds to the growing body of evidence supporting the use of green tea extracts as natural antimicrobial agents. Given the rising incidence of antibiotic-resistant bacteria, green tea extracts, particularly ethanol-based ones, may offer a promising alternative or adjunct therapy in the management of periodontal diseases (5, 6).

Conclusion

Green tea extracts, particularly those obtained using ethanol, exhibit potent antimicrobial activity against periodontal pathogens such as *Porphyromonas gingivalis* and *Streptococcus sanguinis*. The high concentrations of EGCG, caffeine, and theanine in the ethanolic extract likely contribute to its efficacy. These findings highlight the potential for green tea extracts to be developed into natural therapeutic agents for periodontal disease. Further research, including clinical trials, is recommended to explore the full therapeutic potential of green tea in oral health care.

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