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Comparative Evaluation of Antimicrobial Effects of Green and Roasted Beans of Coffee Robusta and Coffee Arabica on Streptococcus Mutans – An In Vitro Study

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KEYWORDS

Coffee phytochemicals; Streptococcus mutans; Chlorhexidine; Chlorogenic acids; Antibacterial activity; Dental biofilm

ABSTRACT:

Aim:

To comparatively evaluate the antibacterial efficacy of green and roasted Coffee Robusta and Coffee Arabica extracts against Streptococcus mutans.

Methods:

An in vitro experimental study was conducted. Aqueous extracts were prepared by decocting 20 g of finely powdered coffee in 100 mL of distilled water at 100°C for 7 minutes. Extracts were standardized at concentrations of 4 g and 10 g. Antibacterial activity was assessed using the agar well diffusion method on blood agar plates inoculated with *Streptococcus mutans*. Following 24-hour incubation at 37°C, zones of inhibition were measured in millimeters. Data were analyzed using one-way ANOVA and post-hoc Bonferroni test, with statistical significance set at $p < 0.05$.

Results:

Statistically significant differences were observed among groups at both concentrations ($p < 0.001$). Green Robusta exhibited the highest antibacterial activity (16.78 ± 0.47 mm at 4 g; 20.53 ± 0.51 mm at 10 g), followed by chlorhexidine (15.65 ± 0.62 mm; 19.65 ± 0.62 mm). All groups demonstrated a significant concentration-dependent increase in inhibition ($p < 0.01$). At 10 g concentration,

Conclusion:

Green coffee extracts, particularly Green Robusta, demonstrated significant antibacterial

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activity against *Streptococcus mutans* and showed efficacy comparable to chlorhexidine at higher concentration, indicating potential as a natural antimicrobial agent in caries prevention.

1. Introduction

Coffee is among the most extensively consumed beverages worldwide and and contains a complex matrix of phytochemicals such as chlorogenic acids, caffeine, trigonelline, diterpenes, and phenolic derivatives [1,2]. The two primary commercially cultivated species, *Coffea arabica* and *Coffea robusta*, differ significantly in chemical composition, particularly in chlorogenic acid and caffeine content [3]. These phytochemicals are known to possess antioxidant, antimicrobial, and anti-inflammatory properties [4,5].

Dental caries remains one of the most prevalent chronic disease globally [6]. The pathogenesis of dental caries involves acidogenic and aciduric bacteria, particularly *Streptococcus mutans*, which synthesizes extracellular polysaccharides via glucosyltransferase enzymes, facilitating biofilm formation and enamel demineralization [7,8]. Controlling *Streptococcus mutans* growth and biofilm activity is central to caries prevention strategies.

Chlorhexidine gluconate is widely recognized as the gold standard antimicrobial agent in dentistry due to its broad-spectrum activity and substantivity [9]. However, long-term use may result in undesirable side effects such as tooth discoloration, taste alteration, and mucosal irritation [10]. These limitations have encouraged research into plant-based bioactive alternatives.

Coffee-derived compounds, particularly chlorogenic acids and polyphenols, have demonstrated inhibitory effects against oral pathogens by disrupting bacterial membrane integrity and inhibiting enzymatic pathways associated with biofilm formation [11–13]. Roasting induces chemical transformations that may reduce certain phenolic constituents while generating melanoidins, which also exhibit antimicrobial properties [14]. Therefore, finding an interconnection between them and using one factor's potential (antimicrobial effect of coffee) to decrease the other (tooth decay), and also comparing different varieties of coffee available at varying concentrations for its antimicrobial effect was the needed for this study.

2. Alternate hypothesis

There is a difference in antimicrobial properties of coffee in different varieties of coffee extracts at varying concentration on S. mutans. The rationale for this investigation was since there are many varieties of coffee available and also it being the third most consumed beverage in the world, this study will use to determine the variety of coffee beans that shows maximum antimicrobial effect. Through this study, we have analyzed the antimicrobial properties of coffee extracts in varying concentrations on S. mutans.

3. Materials And Methods

Study design

The study was an in vitro analysis of the antimicrobial effects of various samples of different concentrations of the coffee extract on S. mutans in comparison to 0.2% chlorhexidine, which was used as the control.

Microbial Strain

A standard strain of *Streptococcus mutans* was obtained and cultured on blood agar medium at 37°C for 24 hours prior to testing.

Armamentarium

1. S. mutans (Mtcc 497)
2. Blood Agar Plates (BAPs)
3. Extracts of Coffee Beans
4. 0.2% Chlorhexidine Solution

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5. Whatman's No. 1 Filter Paper
6. Eppendorf tubes
7. Test Tubes
8. Flask
9. Beaker
10. Glass
11. Funnel
12. Burner
13. Tripod
14. Thermometer
15. Incubator (37°C)
16. Micropipette
17. Distilled Water
18. Stirrer
19. Cotton Swab
20. Sterile Saline

Preparation of Coffee Extracts

Coffee beans of all types were then grounded [Figure 1]. Finely powdered samples of Green Robusta, Green Arabica, Roasted Robusta, and Roasted Arabica were prepared. Twenty grams of each sample were added to 100 mL distilled water and heated at 100°C for 7 minutes [15]. The decoction was filtered using Whatman No. 1 filter paper.(Figure 2)

From the varieties ,two concentrations were standardized (Figure 3)

- 4g
- 10g

Experimental Groups

Group A – 0.2% Chlorhexidine (Control)

Group B – Green Robusta

Group C – Green Arabica

Group D – Roasted Robusta

Group E – Roasted Arabica

Figure 1: Grounded coffee beans

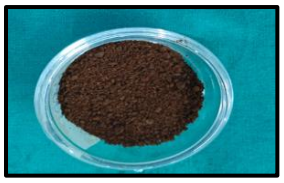
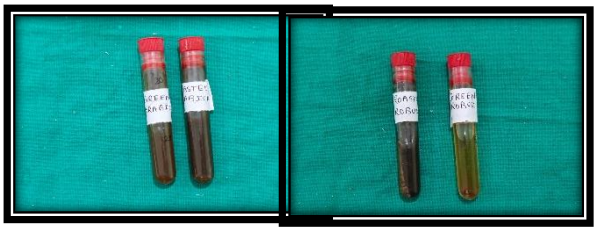


Figure 2: Extract were filtered with the help of whatsmans filter paper

Figure 3: Different extract of various concentration had been prepared



Agar Well Diffusion Method

Blood agar plates were inoculated with Streptococcus mutans using sterile cotton swabs to obtain a uniform lawn culture. Wells measuring 6–8 mm in diameter were prepared aseptically using a sterile borer. Plates were incubated at 37°C for 24 hours. The diameter of inhibition zones was measured in millimeters using a digital caliper.

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Statistical Analysis

Statistical analysis was performed using SPSS software version 25.0. Data were expressed as Mean ± Standard Deviation. Normality was assessed using Shapiro–Wilk test. Intergroup comparisons were performed using one-way ANOVA at each concentration level. Post-hoc Bonferroni test was applied for pairwise comparisons. Intra-group comparison between 4 g and 10 g concentrations was analyzed using independent t-test. Significance level was set at $p < 0.05$.

4. Results

All experimental groups demonstrated measurable antibacterial activity against Streptococcus mutans. Increasing concentration from 4 g to 10 g resulted in increased zones of inhibition across all samples. From Table 1 the results showed that Green Robusta coffee seed provide higher zone of inhibition shown in (Fig 4). Zone of inhibition was found to be dose-dependent and increase with the concentration of extract. From Table 1 the results showed that Roasted Arabica coffee seed provide zone of inhibition of 11.82 and 14.27 mm (Fig 5). zone of inhibition around the extracts of Roasted Robusta was seen 14.11and 17.21 mm (Fig 6).zone of inhibition around the extracts of Green Arabica was seen 13.43 and 15.62 (Fig 7).

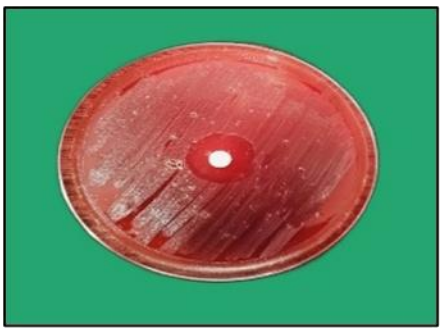

Figure 5: Zone of inhibition of Roasted Arabica

Figure 6: Zone of inhibition of Roasted Robusta

Figure 7: Zone of inhibition of Green Arabica

Table 1. Mean Zone of Inhibition (mm) at 4 g and 10 g Concentrations

Group	4 g (Mean ± SD)	10 g (Mean ± SD)
Chlorhexidine	15.65 ± 0.62	19.65 ± 0.62
Green Robusta	16.78 ± 0.47	20.53 ± 0.51
Green Arabica	13.43 ± 0.52	15.62 ± 0.48
Roasted Robusta	14.11 ± 0.39	17.21 ± 0.44
Roasted Arabica	11.82 ± 0.41	14.27 ± 0.36

Intergroup Comparison (ANOVA)

At 4g concentration:
F-value is 52.34
$p < 0.001$ (statistically significant)

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At 10 g concentration:
F-value = 68.71
$p < 0.001$ (statistically significant)

Post-hoc Bonferroni analysis demonstrated that Green Robusta showed significantly greater inhibition compared to Green Arabica and Roasted Arabica ($p < 0.05$). At 10 g concentration, no statistically significant difference was observed between Green Robusta and Chlorhexidine ($p = 0.182$).

Intra-group Comparison

All groups demonstrated statistically significant increase in inhibition when concentration was increased from 4 g to 10 g ($p < 0.01$).

DISCUSSION

Dental caries remains one of the most prevalent multifactorial infectious diseases affecting humans worldwide [1]. The initiation of caries occurs at the tooth surface and is primarily mediated by metabolic by-products of microorganisms present in dental plaque [2]. The association of *Streptococcus mutans* with dental caries was first reported by Clarke in 1924 [16]. Since then, *S. mutans* has been widely recognized as a principal etiological agent due to its acidogenic and aciduric characteristics.

The cariogenicity of *S. mutans* is largely attributed to three major virulence traits: its ability to synthesize extracellular glucans via glucosyltransferase enzymes, facilitating adherence and biofilm maturation; its capacity to ferment dietary carbohydrates into organic acids; and its tolerance to low pH environments [18]. Acid production lowers the local pH at the enamel surface, initiating demineralization of the highly mineralized enamel structure [2]. Although caries is environmentally driven, enamel composition and structure may influence disease susceptibility and progression [3].

Chlorhexidine has long been considered the gold standard antimicrobial mouthrinse. Axelsson and Lindhe demonstrated its significant efficacy in reducing plaque accumulation and gingival inflammation [17]. Menendez et al. reported that chlorhexidine exhibits potent inhibitory effects against *S. mutans* within dental plaque, justifying its use as a positive control in antimicrobial studies [18].

Natural products have gained increasing attention as alternative antimicrobial agents. Coffee contains bioactive compounds such as chlorogenic acids, caffeic acid, trigonelline, caffeine, α-dicarbonyl compounds, and protocatechuic acid, many of which exhibit antibacterial properties [16,19]. Experimental evidence suggests that coffee polyphenols exert bacteriostatic activity against *S. mutans*, particularly 5-caffeoylquinic acid and caffeic acid derivatives [16,19]. Furthermore, inhibition by coffee has been partially attributed to gelatin-precipitable tannins and monomeric polyphenolic constituents [20].

The antimicrobial mechanism of coffee appears to involve interference with bacterial membrane proteins, inhibition of glucosyltransferase activity, and suppression of amylase-mediated carbohydrate metabolism [21-23]. Kashket et al. demonstrated that polyphenols significantly inhibit glucosyltransferase synthesis by cariogenic bacteria [21]. Additionally, Antonio et al. reported that caffeic acid and 5-caffeoylquinic acid contribute to reduced adherence and metabolic activity of *S. mutans* [20].

Green coffee demonstrated superior antibacterial activity compared to roasted variants in the present investigation. This finding may be attributed to the higher concentration of chlorogenic acid in green coffee beans, which undergo partial degradation during roasting. Chlorogenic acid, an ester of caffeic acid and quinic acid, plays a critical role in antimicrobial activity [24]. Zhang et al. suggested that polyphenol interactions with enamel organic matrices may reduce mineral loss by forming protective complexes through hydrogen bonding, ionic, or hydrophobic interactions [24].

The greater zone of inhibition observed in Green Robusta compared to Roasted Arabica aligns with findings reported by Yadav et al., who observed a statistically significant reduction in *S. mutans* colony count with green coffee extract comparable

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to chlorhexidine [25]. Moreover, in vivo investigations indicate that coffee-based rinses may be safe and economical for daily use [18].

Although chlorhexidine remains highly effective, its long-term use is associated with adverse effects such as tooth staining, altered taste perception, and mucosal irritation [25]. Ethanol-containing mouthrinses have also been discussed in relation to potential mucosal risks [26]. Therefore, exploration of plant-derived antimicrobials remains clinically relevant.

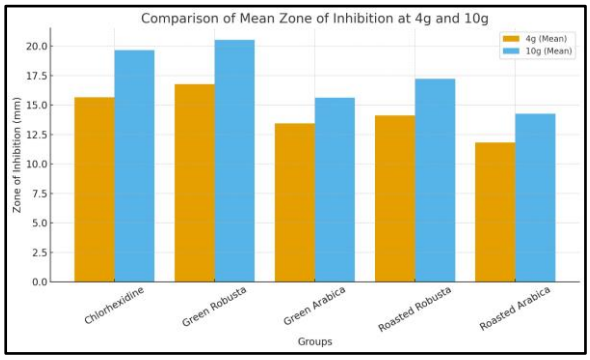
In addition to its antimicrobial potential, coffee constituents such as chlorogenic acid and caffeine contribute to its sensory properties and may facilitate incorporation into oral healthcare formulations such as toothpaste or mouthrinses [27].

Overall, the present findings support the hypothesis that green coffee, particularly Robusta species, exhibits enhanced anticariogenic potential compared to roasted variants, likely due to preservation of phenolic bioactive compounds.

Conclusion

Green *Coffea robusta* demonstrated the highest antibacterial activity against *Streptococcus mutans*. Antimicrobial efficacy increased significantly with concentration. At 10 g concentration, Green Robusta exhibited activity comparable to chlorhexidine.(Table 2) These findings support further investigation into coffee-derived phytochemicals as potential natural adjuncts in preventive dentistry.

Table 2 Mean Zone of inhibition (mm)



Limitations

A wide range of variety of coffee beans are available but this study is restricted to two varieties of coffee beans, i.e., Arabica and Robusta for comparison for its antimicrobial effect. This study is only limited to action on S. mutans, whereas other micro-organisms are also responsible for the progression of caries. Results that are obtained are only in vitro so future research on coffee consumption can be done in vivo.

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Conflict of Interest

There is no conflict of interest .

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