

Inhibitory activity of green tea and Black tea on *Streptococcus mutans* – A preliminary study

Sai Chinmaye Perumalla¹, Poosarla Chandrashekar², Vatsalya Kommalapati³,
Kiran Kumar Kattappagiri⁴, Lalith Prakash Chandra⁵, H Aparna Latha⁶

ABSTRACT

Introduction: Dental caries is the most common bacterial, global disease. Green tea consumption is more these days due to potential health benefits. Many studies concluded that green tea and black tea reduces oxidative stress, inflammation and also helps in the prevention of tooth decay.

Aim & Objective: Based on these literature findings, our preliminary study was designed to find out the invitro inhibitory activity of Green tea and Black tea on *Streptococcus mutans* at a basic concentration of 20mg/ml.

Materials and methods: Study group consists of 26 healthy individuals aged 18-25 with dental caries. Green tea and black tea solutions were prepared for bacterial culture. Unstimulated saliva was collected from each participant. It was diluted and incubated in brain–heart infusion broth. Samples were then plated on Mitis salivarius–Bacitracin agar and incubated anaerobically to identify *S. mutans* using Gram's stain and Catalase test. Agar well diffusion method on blood agar was used to perform the antibacterial tests. Statistical analysis was done using the paired 't' test.

Results: The inhibitory zone measured was in the range of 0.5 – 1.8mm for black tea and 0.45 – 2mm for green tea the results were not statistically significant at this basic concentration. Higher concentrations may give more positive results.

Conclusion: Current study has been done to evaluate the efficacy of green tea & Black tea with low concentrations which was not statistically significant. Studies on various concentrations are being done to evaluate their efficacies at higher concentrations.

Keywords: Green Tea, Black Tea, Saliva, *Streptococcus Mutans*.

INTRODUCTION

Dental caries is the most common bacterial, global disease. It begins early in life, progress rapidly and may affect one's quality of life. According to World Health Organization oral health report 2003, dental caries and periodontal diseases are pandemic diseases affecting all population irrespective of gender, age, or socioeconomic status. Lack of access to oral health care services and preventive measures make this a concerning problem in developing Countries¹. Dental caries is an irreversible process and is associated with streptococcus mutans indigenous to oral cavity².

Tea is the most popular beverage in the world. Green tea consumption is more these days due to potential health benefits. Many studies concluded that green tea and black tea reduces oxidative stress and inflammation. Some bioactive compounds in tea are attributed to effective bactericidal action against *Streptococcus mutans*³. Natural product, theoflavins (TFs) is the main component of black tea, which is benzoquinone compound oxidized by tea polyphenols. It is known for its widely proven anti-oxidation, antibacterial and anti-inflammatory effects.

Department and Institution Affiliation: ¹Intern, SIBAR Institute of Dental Sciences, Guntur, Andhra Pradesh, India; ²Professor, Department of Oral Pathology and Microbiology, SIBAR Institute of Dental Sciences, Guntur, Andhra Pradesh, India; ³Assistant Professor, Department of Oral Pathology and Microbiology, SIBAR Institute of Dental Sciences, Guntur, Andhra Pradesh, India; ⁴Professor & Head, Department of Oral Pathology and Microbiology, SIBAR Institute of Dental Sciences, Guntur, Andhra Pradesh, India; ⁵Professor, Department of Oral Pathology and Microbiology, SIBAR Institute of Dental Sciences, Guntur, Andhra Pradesh, India; ⁶H.Aparna Latha, Assistant Professor, Department of Oral Pathology & Microbiology, Navodaya Dental College, Raichur, India.

Corresponding author: Dr. K.Vatsalya, Assistant Professor, Department of Oral Pathology and Microbiology, SIBAR Institute of Dental Sciences, Guntur, Andhra Pradesh, India. Email: vatsalyakommalapati@gmail.com

How to cite the article: Perumalla S C, Chandrashekar P, Kommalapati V, Kattappagiri K K, Chandra L P, Latha H A. Inhibitory activity of green tea and Black tea on *Streptococcus mutans* – A preliminary study. J. Oral Maxillofac Pathol J 2025; 16(2); 202-205.

Source of Support: Nil

Conflict of Interest: None

Green tea contains polyphenols like Catechin which has anti-cariogenic effects along with many other health benefits. Some studies showed that green tea extracts help in prevention of tooth decay and its local administration led to reduced streptococcus mutans counts^{4,5}.

AIMS AND OBJECTIVES

- To determine the prevalence and pattern of dental caries among individuals aged 18 to 25 years participating in the study.
- To isolate and identify Streptococcus mutans from unstimulated saliva samples using selective culture media and standard microbiological techniques.
- To assess the in vitro antibacterial efficacy of aqueous extracts of green tea and black tea at a concentration of 20 mg/ml against Streptococcus mutans.
- To compare the zones of inhibition produced by green tea and black tea using the agar well diffusion method.
- To analyze the statistical significance of the inhibitory effects between green and black tea using appropriate biostatistical tools.
- To explore the potential of natural polyphenol-rich beverages as alternative antimicrobial agents in oral health management.
- To generate preliminary data that could inform future studies investigating higher concentrations and clinical applicability of tea extracts in preventing dental caries.

MATERIALS AND METHODS

The present study was an observational study, and the study sample consists of 26 healthy individuals of 18-25 years age group including both genders with caries.

Inclusion Criteria

- Healthy individuals aged between 18 and 25 years.
- Presence of clinically detectable dental caries as per WHO diagnostic criteria.
- Individuals with a full complement of teeth (dentate).
- Willingness to participate and provide written informed consent.

Exclusion Criteria

- History of systemic illness or medically compromising conditions.

TABLE 1: Comparison of inhibitory zones between mean of black and green tea

Variable	Group	Mean $\pm$ SD	P-Value
Inhibitory Zone (in mm)	Black Tea	1.12 $\pm$ 0.4	0.703
Inhibitory Zone (in mm)	Green Tea	1.14 $\pm$ 0.41	0.703

Paired T test, $P \leq 0.05$ considered statistically significant.

- Use of antibiotics within 14 days prior to sample collection.
- Use of antibacterial mouthwash within 12 hours prior to saliva collection.
- Individuals with poor compliance or unwilling to participate in the study procedures.

One milliliter of unstimulated saliva was obtained in sterile plastic containers from all the individuals. Written consent for obtaining saliva sample from the individuals was obtained after giving the necessary instructions. Ethical approval for the study was obtained from the institutional review board.

Preparation of black tea and green tea solutions:

Tea solutions were made according to commercially available manufacturer formulations with a concentration of 20mg/ml. Two grams of each tea powder sample was weighed and then added to 100 ml of boiling distilled water and further boiled for 30 minutes.

Bacterial culture:

One milliliter of collected saliva was diluted in 100 ml of saline and 1 ml of this diluted saliva was added to brain-heart infusion broth and incubated for 48 h at 37°C. One microliter of this turbid broth was plated on a culture plate containing Mitis salivarius–Bacitracin (MSB) agar along with sucrose, bacitracin and potassium tellurite and again incubated anaerobically at 37°C for 48 h. Colonies were identified and confirmation for *S. mutans* was done using Gram's stain and Catalase test.

Antibacterial Test

Agar well diffusion method was used to determine the antimicrobial activity. *S. mutans* strains obtained were inoculated on blood agar plate using a sterile swab. Wells of diameter 6 mm were punched on the blood agar plate using the tip of a 1 ml micropipette. Tea extracts were inoculated into these wells. The agar plates were incubated for 24 hours after which the diameter of the zone of inhibition was measured in mm using a vernier calipers. Results were analyzed using paired 't' test.

Outcome Measurement

The primary outcome of the study was the antibacterial efficacy of green tea and black tea against Streptococcus mutans, assessed by measuring the zone of inhibition using the agar well diffusion method. The diameter of the clear zone around each well was measured in millimeters (mm) using a transparent ruler or vernier caliper after incubation. This zone of inhibition served as a quantitative indicator of the antibacterial activity of the tested tea extracts.

Catalase Test Procedure

To confirm the identity of Streptococcus mutans, the catalase test was performed. A small amount of the bacterial isolate was transferred onto a clean glass slide using a sterile loop. One to two drops of 3% hydrogen peroxide (H_2O_2) were added directly onto the bacterial smear. The absence of bubble formation indicated a negative catalase reaction, consistent with catalase-negative streptococci such as *S. mutans*. The test was conducted as part of the biochemical confirmation of bacterial

isolates before further analysis.

Statistical Analysis

The collected data were compiled in Microsoft Excel and statistically analyzed using the Statistical Package for Social Sciences (SPSS) software, version 20.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were employed to summarize demographic variables and the distribution of caries among males and females. The mean zones of inhibition for green tea and black tea were compared using the paired 't' test. A p-value of ≤ 0.05 was considered statistically significant.

Sample Size Calculation

The sample size was calculated based on the formula for comparing two means in a paired design:
$$n = \frac{(dZ_{\alpha/2} + Z_{\beta} \times \sigma)^2}{d}$$

Where:

n = required sample size

$Z_{\alpha/2}$ = standard normal variate for the chosen confidence level (for 95% confidence, $Z_{\alpha/2} = 1.96$)

Z_{β} = standard normal variate for desired power (for 80% power, $Z_{\beta} = 0.84$)

σ = standard deviation

d = expected mean difference between groups (effect size)

For this preliminary study, due to limited prior data, a convenience sample of 26 individuals was selected to estimate effect sizes and variability for future larger-scale investigations.

RESULTS

The study was performed over 2 months. A total of 26 saliva samples were collected ($n=26$) in the age group of 18 to 26. Of these 26 samples '10' samples belong to the age group of 18 to 20 years. 12 samples were in the age group of 21 to 23 years and 4 samples were from the people of more than 23 years age. The gender, the male to female ratio was 9:17. Occlusal caries is present in 16 people, proximal caries in 7, and both in 3 people.

The Growth of streptococcus mutans obtained on the MSB agar plates were tested using Gram's stain and catalase test. It is a Gram-positive bacterium and gives catalase test negative. Agar well diffusion method was used to perform the antibacterial tests. Zone of inhibition was measured, which was less than 2mm in diameter.

The mean inhibitory zone measured for green tea in mm is $1.12(\pm 0.4)$. Also, the mean inhibitory zone measured for black tea in mm is $1.14(\pm 0.41)$. No statistically significance was observed. [Table 1]

DISCUSSION

Dental caries is one of the most prevalent oral diseases of public health concern affecting adolescents. The natural habitat of *S. mutans* is the oral cavity, more specifically the dental plaque, a multispecies biofilm formed on hard surfaces of the tooth. It has been largely accepted that the cariogenic potential of *S. mutans* resides in three core attributes-The ability to synthesize large quantities of extracellular polymers of glucan from sucrose that aid in permanent colonization of hard surfaces and in the development of extracellular polymeric matrix in situ. The ability to transport and metabolize a wide range of carbohydrates into organic acids (acidogenicity). The ability to thrive under environmental stress conditions particularly low pH. While *S. mutans* does not act alone in the development of dental caries, it can alter the local environment and creates a favorable niche for other acidogenic species to thrive. As a human pathogen *S. mutans* is also implicated in subacute bacterial endocarditis and a life-threatening inflammation of heart valves.

The present study examined the association between the use of specific foods and the reduction of cariogenic oral bacteria. This study was conducted to assess the anti-microbial activity of green tea and black tea on *S. mutans*. Since tea is consumed in an aqueous form this study was carried out to determine the

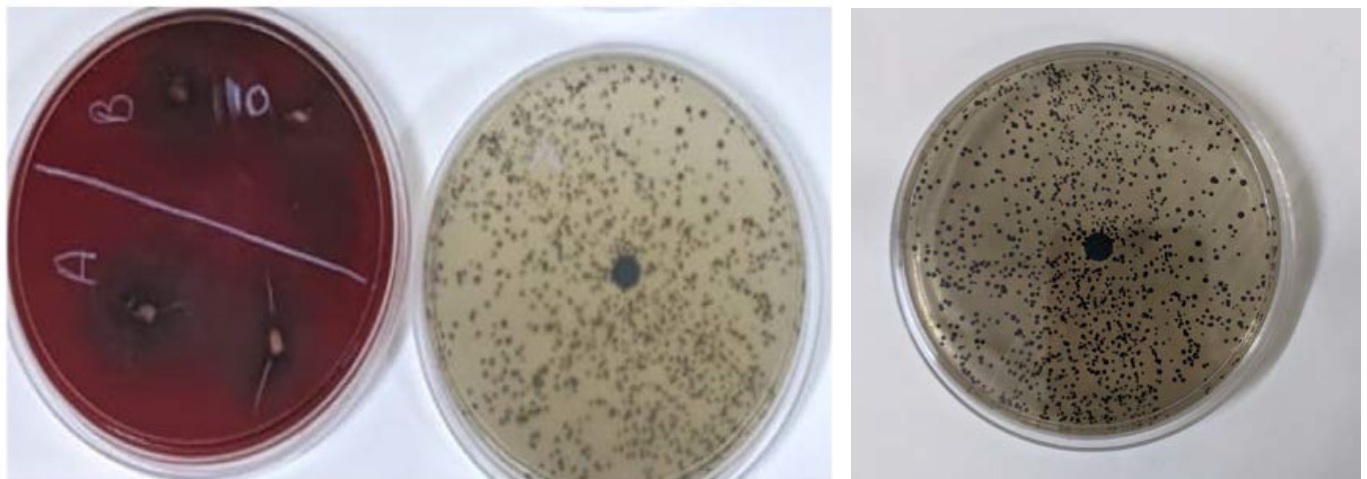


Fig.: (a) The culture of *Streptococcus mutans* obtained on MSB agar plates and the inhibitory zones on the Blood agar. (b) Microscopic view of *S. mutans* after performing Gram's stain.

effect of aqueous effects of tea on *S. mutans* growth. Minimum inhibitory concentration was determined by agar well diffusion method in plates of blood agar.

The present study includes age group of 18 to 25 which is the most prevalent group. Dental caries was observed in 9 males and 17 females with higher prevalence in females. The caries in occlusal aspect was comparatively higher than proximal aspects. Mustafa Demirci et.al in 2010⁶, Sergei N. Drachev et.al in 2017⁷, John R Lukacs in 2010⁸.

The findings of current study showed that the green tea and black tea has a tendency to reduce *S. mutans* counts. Varying concentrations of the solutes of green tea and black tea need to be considered to estimate percentage of inhibition respectively. It was observed that the inhibition of growth of *S. mutans* is insignificant at a concentration of 20mg/ml Abdel Rahman M Ramadan et.al 2019⁹. Although the reduction rates were not statically significant, these results still may provide evidence for the presence of anti-microbial compounds in tea that are useful to control bacteria associated with dental caries. These compounds can degrade the cell wall, disrupt the cytoplasmic membrane, damage membrane proteins and interfere with membrane integrated enzymes which may eventually lead to cell death. Mina Jazaeri et.al in 2015¹⁰, Zohaib Khurshid et.al in 2016¹¹, Vishal Arya et.al in 2016¹², Minan Al-Ezzi et.al in 2018¹³, Ranu Putra Armidin in 2019¹⁴.

Among 26 individuals participated in this preliminary study, 9 were males in comparison with 17 females. Maximum number of individuals are seen in age group of 21 to 23 followed by 18 to 20 years. Among 9 males, proximal caries is seen in 5 individuals followed by occlusal caries in 4 individuals. Among 17 females, maximum number of individuals show occlusal caries. Distribution of caries is seen mostly in females compared to males. *Streptococcus mutans* growth was obtained in all the 26 MSB agar culture plates. The growth of *S. mutans* was confirmed by grams stain and catalase test. It is a Gram-positive bacteria and catalase test is negative. On inoculation of tea extracts by agar well diffusion method into blood agar Zones of inhibition for green tea and black tea on blood agar was found to be less than 2mm which is insignificant at this basic concentration. Higher concentrations may provide more positive results.

This in vitro study was subject to certain limitations. Firstly, the sample size was limited to 26 participants within a narrow age group (18–25 years), which may not adequately represent broader population demographics. Secondly, only a single, basic concentration (20 mg/ml) of green tea and black tea was tested, restricting the ability to observe potential dose-dependent antimicrobial effects. The absence of a positive control such as chlorhexidine further limits the comparative strength of the findings. Since the study was conducted under laboratory conditions, the complex dynamics of the oral cavity, including saliva flow, pH variations, and microbial interactions,

were not replicated. Additionally, bacterial identification was performed using conventional culture methods and biochemical tests without molecular confirmation, which may influence specificity. Finally, the short duration of sample observation and lack of follow-up precluded assessment of long-term antimicrobial effects.

CONCLUSION

This preliminary study observed the inhibitory effect of green tea and black tea at concentration of 20mg/ml on *streptococcus mutans*, was found to be statistically insignificant. However higher concentration of solutes from both the compounds, the anticariogenic properties and effects of flavonoids shall be helpful to the population at the macroscopic level of utility.

REFERENCES

1. Mehta A. Trends in dental caries in Indian children for the past 25 years. *Indian J.Dent Res* 2018; 29:323-8.
2. Ramadan AM, Bakeer HA, Mahrous MS, Hifnawy TM. Influence of black tea on *Streptococcus mutans* and *Lactobacillus* levels in saliva in a Saudi cohort. *J Taibah Univ Med Sc* 2019;14(2):179-186.
3. George DE, Shetty R, Shetty PJ, Gomes LA. An In vitro Study to Compare the Effect of Different Types of Tea with Chlorhexidine on *Streptococcus mutans*. *J Clin Diagn Res.* 2017;11(9): ZC05–ZC07.
4. Neturi RS, R S, B VS, Y SS, T CS, P SK. Effects of Green Tea on *Streptococcus mutans* Counts – A Randomized Control Trail. *J Clin Diagn Res.* 201;8(11): ZC128-30.
5. Ahmed SI, Sudhir KM, Reddy VCS, Kumar RVSK, Srinivasulu G. Green Tea in the Prevention of Dental Caries-A Systematic Review. *Int Arch BioMed Clin Res.* 2017;3(1):1-6.
6. Rapp L, Maret D, Diemer F, Lacoste Ferré MH. Dental Caries in Geriatric Dentistry: An Update for Clinicians. *Int J Oral Dent Health* 2019; 5:080.
7. Drachev, S.N, Brenn T & Trovik, T.A. Dental caries experience and determinants in young adults of the Northern state Medical University, Arkhangelsk, North west Russia: A cross sectional study. *BMC Oral health* 2017; 17:136.
8. Lukacs JR. Sex differences in dental caries Experience: clinical evidence, complex etiology. *Clin Oral Investig.* 2011; 15(5):649-56.
9. Ramadan AM, Bakeer HA, Mahrous MS, Hifnawy TM. Influence of black tea on *Streptococcus mutans* and *Lactobacillus* levels in saliva in a Saudi cohort. *J Taibah Univ Med Sci.* 2019 ;14(2):179-186
10. Jazaeri M, Pakdek F, Rezaei-Soufi L, Abdolsamadi H, Rafieian 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
11. Khurshid Z, Zafar MS, Zohaib S, Najeeb S, Naseem M. Green Tea (*Camellia Sinensis*): Chemistry and Oral Health. *Open Dent J.* 2016; 10:166-73.
12. Arya V, Taneja L, Srivastava A, Nandlal S. Anticariogenic Activity of Black Tea - An In vivo Study. *J Clin Diagn Res.* 2016 Mar;10(3): ZC74-7.
13. Al-Ezzi M, Al-Mizrakchi A, Alwaheb A, Baysan A, Seoudi N, Tappuni AR. Black and green tea antimicrobial effect on *Mutans streptococci* and *Lactobacilli*. *Journal of Oral and Dental Research.* 2018 ;5: 1
14. Armidin RP, Yanti GN. Effectiveness of Rinsing Black Tea Compared to Green Tea in Decreasing *Streptococcus mutans*. *Open Access Maced J Med Sci.* 2019 14;7(22):3799-3802.