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A COMPARATIVE ANALYSIS OF ANTIMICROBIAL POTENTIAL OF GREEN TEA AND GREEN *COFFEE* EXTRACT ON *STREPTOCOCCUS MUTANS* ON IN VITRO STUDY

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ABSTRACT

Background:- Tea and coffee are the two most consumed drinks in the world after water. Green tea, Camellia sinensis (Theaceae) and Green coffee, Coffea arabica (Rubiaceae) possess antimicrobial activity in adjunct to various biological properties. Through this study, we have analysed the antimicrobial properties of Green tea and Green coffee extracts in contrast to chlorhexidine which was used as the control against *Streptococcus mutans*. **Aim:** To comparatively evaluate the antimicrobial potential of Green tea and Green coffee extract at various volumes against *Streptococcus mutans*. **Methodology:** Ethanolic extracts of Green tea and Green coffee were prepared. *Streptococcus mutans* were cultured on blood agar, which was then inoculated with these samples. Antimicrobial properties were determined using disc diffusion method at three different concentrations(10µL, 20µL, 30µL). The results were compared with 0.2% chlorhexidine solution(gold standard). **Results:-** Highest zone of inhibition was found with chlorhexidine (26.6 mm) at 30 µL followed by green tea (24.4 mm) and green coffee (17.7 mm) against *streptococcus mutans*. Results were statistically analysed with the analysis of variance (ANOVA). **Conclusion:** Green tea extracts(ethanolic) showed a greater inhibitory effect on the growth of *Streptococcus mutans* than green coffee.

INTRODUCTION

Dental caries is the consequence of the interplay among the oral microbiome, the diet, the dentition and the oral environment. Bacteria are crucial to the commencement and progression of carious lesions. Without bacteria there are no caries. Among oral pathogens, Streptococcus mutans is generally regarded as the main microbial agent of dental caries furthermore additional acidogenic microorganisms may be involved.

Various plants and their products have been in use for their therapeutic value since ancient times. Tea and coffee are the two most common liquid refreshment consumed commonly across the world[4]. Tea has gained popularity amongst all sections of people and it is no elaboration to say that it is a versatile, every time favourite drink for all people.

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Green coffee (GC) bean extract with its antibacterial effects against gram-negative and gram-positive bacteria has been gaining greater attention among other herbal extracts. Green coffee beans are the crude seeds of coffee cherries that have been isolated or processed and have yet to be roasted[5].

Green tea is considered as nature's bounty, obtained from the leaves and bud of plant Camellia sinesis plant (a shrub of Theaceae family about 1-2m height)[8]. In India it was first discovered in Assam state and its farming was originally started in Darjeeling district of West Bengal[8].

Green tea contains several polyphenolic compounds in the form of catechin, epigallocatechin 3 gallate (EGCG), epicatechin gallate, epicatechin and flavanols[8]. Catechins are the most frequent and copious polyphenolic compounds.

Among the various phytomedicines that are being used in dentistry, studies on the antibacterial properties of green tea and green coffee was very scant, so this study was conducted to compare in vitro antimicrobial potential of green tea and green coffee extract at various volumes against streptococcus mu-

tans.

METHODOLOGY

The current study was an in-vitro analysis of the antimicrobial effects of green tea and green coffee extracts at various concentrations against S. mutans in contrast to 0.2% chlorhexidine, which was used as the control. This study was conducted at the Department of Microbiology, Buddha Institute of Dental Sciences & Hospital, Patna. Ethical clearance for the study was obtained from the Institutional Ethical Review Committee(Ref. No:- 74/BIDSHIEC/2024-25(2022-2023). The study was conducted over a period of 1 month(Feb-March). Blood agar culture medium was used in the study.

Collection of plant materials

Green tea leaves were obtained from local markets in Patna whereas Green coffee beans were procured from online store. The collected materials were brought to the laboratory and grounded into fine powder. Obtained powder was stored in desiccators at 4° C until use.

Saliva collection

Saliva samples were collected from the out-patient department after taking their consent. Samples were collected through sterilized cotton swab and kept in swab tube.

Preparation of standard inoculum

Streptococcus mutans was cultured in the microbiology department of Buddha Institute of Dental Sciences and Hospital, Patna. For the isolation of pure culture of S. mutans, the collected saliva sample was inoculated in selective media containing Blood agar and anaerobically incubated at 37^0 C for 48 hours.

Preparation of Ethanolic extracts

15 grams of finely powdered each of green tea and green coffee powder was used in the experiment. The powdered samples were suspended in a sterile jar containing 150 ml ethanol for 24 hours at 4° C. The suspended powdered samples were then subjected to a process of cold maceration (continuous shaking at constant intervals of time) for 48 hour. After 48 hour, the suspended samples were subjected to the process of filtration using sterile Millipore filter (0.45 µm diameter) to obtain a clear filtrate. During the filtration process, residue of green tea and green coffee was obtained. The filtrate or the solid residue so obtained was placed in the laminar flow chamber at a low temperature of less than 60° C for 3 days to facilitate evaporation of ethanol content from the filtrate which were thereafter stored in sterile airtight beakers with suitable labelling and preserved in the refrigerator at 4° C until further use. The procedure of extraction and filtration were operated at room temperature. The 100% concentration of the prepared extracts were used for the research purpose. 0.2% Chlorhexidine was used as a control.

Disc diffusion method

Six discs of diameter 3 mm were seeded over the petri dishes containing Blood Agar by means of a sterile tweezer and were pressed down lightly to ensure complete contact between the disc and media surface. Various volumes of green tea and green coffee extracts (10µL, 20µL, 30µL) and control were added on each disc separately and spread in triplicates by using sterile micropipette on blood agar plate. The tests were repeated in triplicates against the bacteria to overcome any technical errors that might occur during a single attempt. The procedure was performed under aseptic conditions. The culture plates were aerobically incubated at 37 °C for 24 to 48 hours and subsequently, they were observed for clear zone formed around the discs. The antimicrobial activity against the test isolates were then determined by measuring the diameters of the zone of inhibition to the nearest millimeter (mm) with the help of sterilised digital Vernier Calliper.

Statistical analysis

Data was analysed using SPSS version 26. The p value was set at 0.05 to be significant, and p value less than 0.01 was considered as highly significant. Repeated measures Analysis of Variance(ANOVA) Parametric test was used to analyze antimicrobial effect of different groups at different volumes.

Fig. 1 Shows the inoculation of saliva sample in petri plate containing Blood Agar

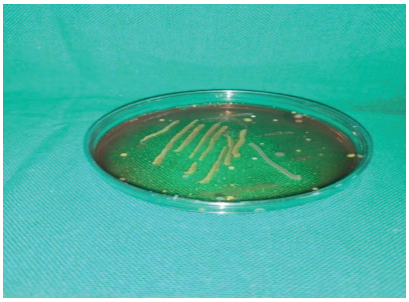

Fig. 2 Shows bacterial colony of Streptococcus mutans

Fig.4 Shows filtration of suspended Green tea po wder in Ethanol

Fig. 3 Shows filtration of suspended Green coffee powder in Ethanol Green tea powder in Ethanol

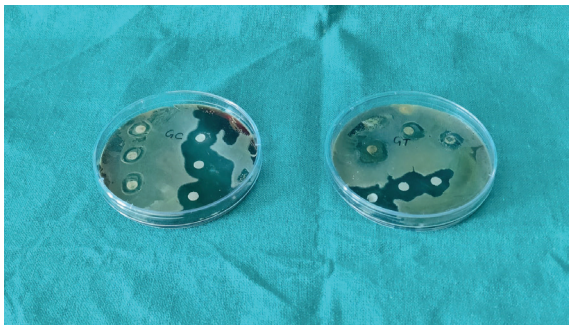

Fig. 5 Shows clear zone of inhibition formed by 10% ethanolic extract of green coffee and green tea at various volumes against *Streptococcus mutans*

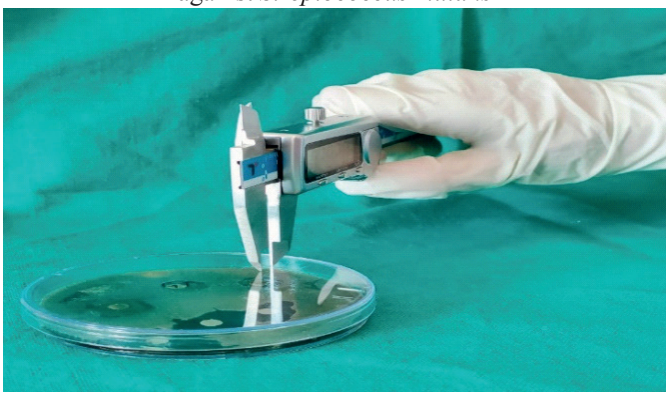

Fig. 6 Shows measurement of zone of inhibition formed by 10% ethanolic extract of green tea extract against S*treptococcus mutans* with the help of Vernier caliper

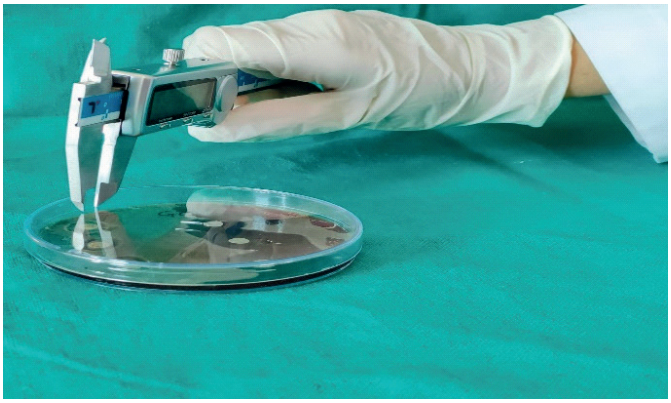

Fig. 7 Shows measurement of zone of inhibition formed by 10% ethanolic extract of green coffee against *Streptococcus mutans* with the help of Vernier caliper

RESULTS

In the present study, on comparing the mean zone of inhibition of green tea and green coffee extracts with 0.2% chlorhexidine gluconate against Streptococcus mutans, it was found that at different volumes (10µL, 20µL and 30µL), chlorhexidine showed the highest zone of inhibition (20.733±0.1527 mm, 22.500±0.2000 mm and 26.600±0.2645 mm respectively) followed by green tea (18.366±0.2081 mm, 20.566±0.2516 mm and 24.466±0.2516 mm respectively) and green coffee (12.700± 0.2000 mm, 15.400±1.9924 mm and 17.733±0.1527 mm respectively) and this was found to be statistically significant ($p=0.001^{**}$) (Graph 1).

Table 1 Tukey's Post Hoc analysis for pair-wise comparison of the antimicrobial effect of Green tea and Green coffee extracts with 0.2% Chlorhexidine gluconate (CHX) at various volumes against Streptococcus mutans.

Groups (I)	Groups (J)	Mean Difference (I-J) at various volumes					
		10µL	p-value	20µL	p-value	30µL	p-value
Green Tea	Green coffee	5.66667*	0.000**	5.16667*	0.004**	6.73333*	0.000**
	0.2% Chlorhexidine gluconate	-2.36667*	0.000**	-1.93333	0.185(NS)	-2.13333*	0.000**
Green coffee	Green Tea	-5.66667*	0.000**	-5.16667*	0.004**	-6.73333*	0.000**
	0.2% Chlorhexidine gluconate	-8.03333*	0.000**	-7.10000*	0.001**	-8.86667*	0.000**
0.2% Chlorhexidine gluconate	Green Tea	2.36667*	0.000**	1.93333*	0.185(NS)	2.13333*	0.000**
	Green Coffee	8.03333*	0.000**	7.10000*	0.001**	8.86667*	0.000**

Table 1 Shows Tukey's Post Hoc analysis for pair-wise comparison of the antimicrobial effect of green tea and green coffee extracts with 0.2% chlorhexidine gluconate (CHX) at various volumes (10 µL, 20 µL and 30 µL) against Streptococcus mutans.

DISCUSSION

In the oral cavity, there are many microorganisms that can cause disease and one of the diseases that can arise is dental caries. Bacterial growth and metabolism promote changes in the oral environment. Hence, it is important to prevent bacterial growth and colonization.

Recently, herbal substitutes have gained affirmation with no detrimental properties. In this regard, green coffee bean extract with its antibacterial effects against gram-negative and gram-positive bacteria (Pane et al)[11] has been gaining greater popularity among other herbal extracts.

Another herbal substitute is green tea which is considered refreshing and produces an overall feeling of satisfaction. According to Menendez A[10], chlorhexidine was effectual against Streptococcus mutans in dental plaque, hence it was considered as a positive control in the present study to evaluate the effect of green tea and green coffee extract on salivary Streptococcus mutans count.

From the results of the study, it was concluded that greater the concentration of extract, the greater the diameter of zone of inhibition was seen against Streptococcus mutans, which is in line with a study conducted by Muttaquin Z et al[3].

In this study, the mean of the chlorhexidine showed greater reduction of Streptococcus mutans followed by green tea and green coffee at different volumes and this difference was statistically significant($p=0.000^{**}$) which is in line with a study conducted by Yadav et al[16], Akhlaghi et al[14].

It was also observed that although the extracts were having less antimicrobial property than chlorhexidine, still they were relatively effective in inhibiting the growth of oral bacteria. Researchers need to have a fresh look in the area of green medicine as there may be many potential herbs which may possess significant antimicrobial properties.

CONCLUSION

In conclusion green tea and green coffee have in vitro antibacterial activity against the cariogenic bacteria Streptococcus mutans. Therefore, they can be used in the recent era of complementary medicine as an example of plant remedies with medicinal properties. This will allow it to have potential for herbal alternative for caries prevention. And also, there are studies which are suggesting its safe use.

RECOMMENDATIONS

The results of this research will serve as a basis for the future application as a safe and effective anti-caries agent. However, more in-detail investigation with different extraction solvents, roasting degrees, and different varieties of tea and coffee products is required.

Analysis of the extract of green tea and green coffee should be done for more activities like anti-inflammatory, anti-oxidant properties. Also, these extracts can possibly be incorporated into chewing gums, toothpastes, mouthwashes for its preventive actions.

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CONFLICTS OF INTEREST

There are no conflicts of interest real or perceived, financial or non-financial in this article.

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