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Antibacterial activity of Green Tea extract and Green Coffee extract in comparison with Chlorhexidine against Streptococcus Mutans and Lactobacillus Acidophilus- An in Vitro study

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ABSTRACT

Title: Antibacterial activity of Green Tea extract and Green Coffee extract in comparison with Chlorhexidine against Streptococcus Mutans and Lactobacillus Acidophilus- An In Vitro study

Aim: The aim of this study was to determine the *in vitro* antibacterial effect of *green tea* extract and green coffee extract in comparison with Chlorhexidine on *Streptococcus mutans* and *Lactobacillus acidophilus.*

Methodology: Ethanolic green tea and green coffee extract at ten different concentrations and 0.2% chlorhexidine were used. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and zone of Inhibition of the test and control agents against *Streptococcus mutans* and *Lactobacillus acidophilus* were investigated.

Conclusions: The antibacterial activity of chlorhexidine was highest against S. Mutans but lowest against L. Acidophilus in comparison with Green Tea and Green Coffee. Chlorhexidine gluconate 0.2% had the highest antibacterial activity against S.mutans and Green coffee 300ug/ml had the highest antibacterial activity against L. acidophilus.

Key words: green tea, green coffee, streptococcus mutans, lactobacillus acidophillus, chlorhexidine.

BACKGROUND & AIMS

Dental caries is one of the most prevalent chronic diseases of people worldwide and individuals are susceptible to this disease throughout their lifetime. Dental caries forms through many host factors, including teeth and saliva, form a complex interaction between acid-producing bacteria and fermentable carbohydrates.[1]

The formation of plaque plays an important role in the development of caries and is initiated by several strains of oral streptococci. Cariogenic microorganisms like mutans group of Streptococci and Lactobacillus acidophilus metabolize sucrose into sticky glucan and, thereby, encourage the accumulation and adherence of plaque Biofilm. The major etiological players are the two alpha hemolytic streptococci –mutans streptococci and streptococcus sobrinus.[2] These microorganisms lead to localized demineralization by degrading the dietary carbohydrates producing lactic acid, eventually, the formation of dental caries. Hence, the use of the antimicrobial agent is warranted to limit the formation of plaque and growth of cariogenic microorganisms to prevent dental caries.[3]

Chlorhexidine digluconate (CHX) remains the gold standard as ant plaque and anti-gingivitis agent as it has the ability to bind with soft and hard tissues in the oral cavity that enables it to act for a long period after application. It also has

the potential to prevent dental caries because it can suppress the growth of mutans streptococci. However, its use as anticariogenic agent remains controversial.[3] One of the strategies being developed is the use of compounds that modify either the mutans streptococci cell wall or tooth surfaces, therefore preventing bacterial colonization and enamel demineralization.[4]

In this direction, the use of natural products in the prevention and treatment of oral conditions has increased recently.[5]Tea and coffee are the most common beverages consumed by Indians on daily basis.[2]

The bioactive components of green tea are able to influence the process of caries formation at several different stages: inhibiting proliferation of the streptococcal agent, interfering with the process of adhesion to tooth enamel or acting as inhibitors of glucosyl transferase and amylase.[6]Several In-vivo and in-vitro studies have reported a reduction in bacterial counts, saliva/plaque pH and gingivitis associated with the local administration of green tea extracts.[7-11]

Coffee is a rich source of antioxidants and has antibacterial activity and has shown to inhibit the adherence of streptococcus mutans to tooth surface.[12]Studies have indicated that, due to interference with proteins of microbial membrane, coffee inhibits *S. mutans* adhesion and leads to reduction in oral acid production by the inhibition of glucosyltransferase and amylase.[13]Anila Namboodiripad and kohri observed that those who consumed coffee without milk and sugar on daily basis showed reduced cases of dental caries in comparison to other people, demonstrating the potential of coffee as an anticaries agent[14]. Signoretto *et al.* studied the microbial plaque index of people who drink coffee and suggested a significant reduction of *S. mutans* and *Lactobacillus count* in their plaque and saliva.[15]

Green coffee is characterized by being composed of antibacterial agents such as caffeine, chlorogenic acids, trigonelline and the diterpenescafestol and kahweol.[16]

Despite abundant literature on the general health benefits of drinking green tea and green coffee, studies on their effect on cariogenic microbes is limited. Therefore, the present study was carried out to determine the *in vitro* antibacterial effect of *green tea* extract and green coffee extract on *Streptococcus mutans* and *Lactobacillus acidophilus.*

METHODS

Green tea extract (Vista,Nutrition), Green Coffee bean extract(Arogya Ayurvedic Kendra), Acetone; ethanol; n-butanol; methanol, Folin and Ciocalteaus reagent, Muller Hinton media(Hi media, Mumbai), Paper discs (Hi media,Mumbai) 2-Diphenyl-picrylhydrazyl(DPPH), Ascorbic acid, UV Spectrometer

Preparation of green tea and green coffee extract:
1.0 mg of green tea bean extract and green coffee bean extract were mixed with 150 ml of 75 % ethanol, acetone and aqueous respectively for 1 min to make extracts in respective solvents using an electronic mixer and soaked overnight at room temperature and filtered using Whatman No.1 paper. The filtered solutions were evaporated overnight and then dissolved in respective solvents. The Residue over whatman paper were mixed with 75% ethanol, soaked overnight and its extract was filtered. The Residue of ethanol was mixed with acetone and soaked overnight and the extract was filtered. The presence of natural major chemical groups such as tannins, saponins, flavanoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins and steroids were analyzed. Phytochemical screening of green tea extracts and green coffee extracts with acetone, ethanol and aqueous as solvents were done

Qualitative phytochemical screening:

The qualitative antioxidant activity of the green tea and green coffee extracts using all the three solvents were determined using DPPH. 50 µl of extracts of green tea was taken in the microtiter plate. 100 µl of 0.1% methanolic DPPH was added over the samples and incubated for 30 min in dark condition. The samples were then observed for discoloration. The antioxidant positive samples were subjected for further quantitative analysis. Same method was repeated for green coffee.

Quantitative analysis of free radical scavenging activity of green tea and green coffee:

The antioxidant activities were determined using DPPH (Sigma-Aldrich, USA) as a free radical. A volume of 100 µl of green tea extract was mixed with 2.7 ml of methanol and 200 µl of 0.1% of methanolic DPPH and incubated for 30 min. Subsequently, at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan at 517 nm. Absorption of the blank sample containing the earlier mentioned amount of methanol and DPPH solution was prepared and measured as a control. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of BHT. Free radical scavenging activity was calculated by the following formula:

Inhibition =[Absorbance of control (Ac517) Absorbance sample (As517)] x100

[Absorbance control (Ac517)]

Same method was repeated for green coffee. Antioxidant activity of green tea and green coffee extract using all the three solvents was determined. Heretofore, the extract that possessed greater antioxidant activity was used for further procedures.

Extraction of active compound:

The fine powder of the green tea and green coffee extracts were macerated with 75% of ethanol and evaporated. The residue was mixed with n-butanol and water (2:1). The upper layer of n-butanol and lower layer of water were separated and evaporated under vacuum. The upper layer of n-butanol residues was washed with petroleum ether to remove fatty components and then extracted with ethanol.

Minimum inhibitory concentration of green tea and green coffee extract:

Minimum inhibitory concentration (MIC) of green tea against both the bacteria were assessed by serial dilution method. Ten different concentrations of the green tea extract (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7% 0.8%, 0.9%, 1.0%) were incorporated into nutrient broth in different test tubes. In each test tube, 5 ml of green tea extract was added to 4.9 ml of nutrient broth and 0.1 ml of bacterial culture. A control tube containing the growth medium and the bacteria was set-up. The mixtures were incubated at 37°C for 24 h and analyzed for turbidity. The minimum concentration of green tea extract that will inhibit the growth of the microorganism was determined as MIC. Similar procedure was done to determine MIC of green coffee.

Minimum bactericidal concentration of green tea and green coffee extract:

Minimum bactericidal concentration of green tea was determined from MIC range using Spread plate method. Mueller Hinton agar in Petri dishes were sub-cultured from tubes without growth and incubated at 37°C for 24 h. The petri dishes were observed macroscopically. The highest dilution that yielded no bacterial colony on a solid medium was taken as MBC. The same method was repeated for green coffee.

Zone of inhibition by Agar Well Diffusion Method:

15-20 mL of Mueller-Hinton broth was poured on 40 glass petri plates of same size and allowed to solidify. Agar surface of each plate was streaked by a sterile cotton swab with the strain of streptococcus mutans(ATCC 25175) and lactobacillus acidophilus(ATCC 4356) (20 plates each) respectively. Each Agar plate was punched with a sterile cork borer of 4 mm size, 4 wells in each plate, and each sample was poured with micropipette in the bore. In each plate, 3 wells were filled with 100 ul of the extract (green tea or green coffee) 300ug/ml,200ug/ml and 100ug/ml respectively, and the fourth well was filled with 100ul of 0.2% chlorhexidine. The plates were allowed to standby for 30 min. The plates were incubated at 37°C for 48 h.

RESULTS

Table I shows comparison of zone of inhibition against Lactobacillus acidophilusin different concentrations of green tea, green coffee, Chlorhexidine gluconate and normal saline groups.

Kruskal Wallis test showed significant difference between zone of inhibitions of different concentrations of green tea, Chlorhexidine gluconate and normal saline groups against Lactobacillus acidophilus. After this Mann Whitney U test was applied for pairwise comparison, which showed following observations:

1. Zone of inhibition by Green tea at 300 µg/ml was significantly larger than Green tea 200 µg/ml, whichwas significantly larger than Green tea 100 µg/ml.
2. Zone of inhibition by Green tea 100 µg/ml was significantly larger than Chlorhexidine gluconate (0.2%) which was significantly larger than Normal saline.

(Green tea 300 µg/ml> Green tea 200 µg/ml > Green tea 100 µg/ml > Chlorhexidine gluconate (0.2%) > Normal saline).Similar results were found with Green Coffee against L. Acidophilus.

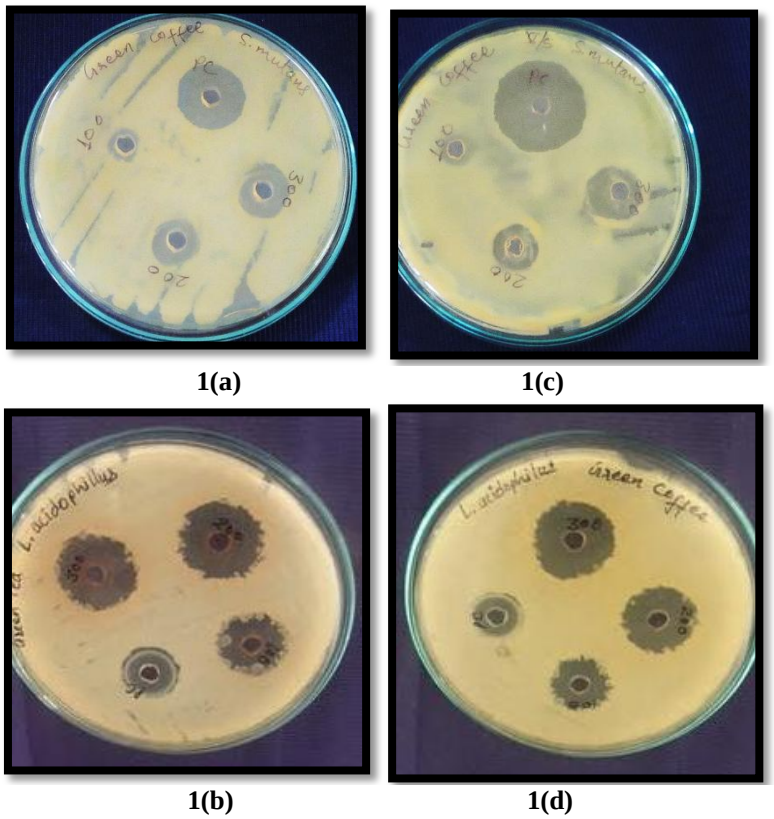
Kruskal wallis test showed significant difference between similar concentrations of green tea and green coffee (300 µg/ml, 200 µg/ml and 100 µg/ml) against Lactobacillus acidophilus. After this Mann Whitney U test was applied for pairwise comparison, which showed following observations:

1. Zone of inhibition by Green coffee 300 µg/ml was significantly larger than Green tea 300 µg/ml which was significantly larger than Green coffee 100 µg/ml
2. There was no significant difference for zone of inhibition between Green tea 300 and Green Coffee 200 µg/ml.

1. (Green coffee 300ug/ml>green tea 300 ug/ml> green coffee 100 ug/ml)
2. (Green tea 300 ug/ml = green coffee 200 ug/ml)
3. Zone of inhibition by Green coffee 300 µg/ml and Green coffee 200 µg/ml were significantly larger than Green tea 200 µg/ml, which was significantly larger than Green coffee 100 µg/ml

(green coffee 300 ug/ml, green coffee 200 ug/ml >green tea 200 ug/ml> green coffee 100 ug/ml)

1. Zone of inhibition by Green coffee 300 µg/ml, Green coffee 200 µg/ml and Green coffee 100 µg/ml were significantly larger than Green tea 100 µg/ml.
(green coffee 300 ug/ml , green coffee 200 ug/ml, green coffee 100 ug/ml > green tea 100 ug/ml)



1(a) **1(c)**

1(b) **1(d)**

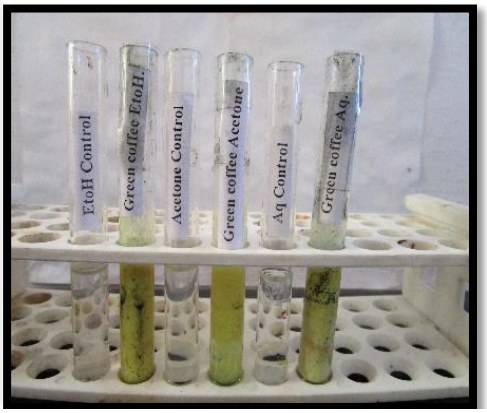
Figure_1: Zone of inhibition- green tea against S. mutans(Fig 1a), L. acidophilus(Figure 1b) and Green Coffee against S mutans(Fig 1c), and L. acidophilus(Fig 1d).

Table I: Comparison of zone of inhibition against Lactobacillus acidophillusin different concentrations of green tea, green coffee, Chlorhexidine gluconate and normal saline groups.

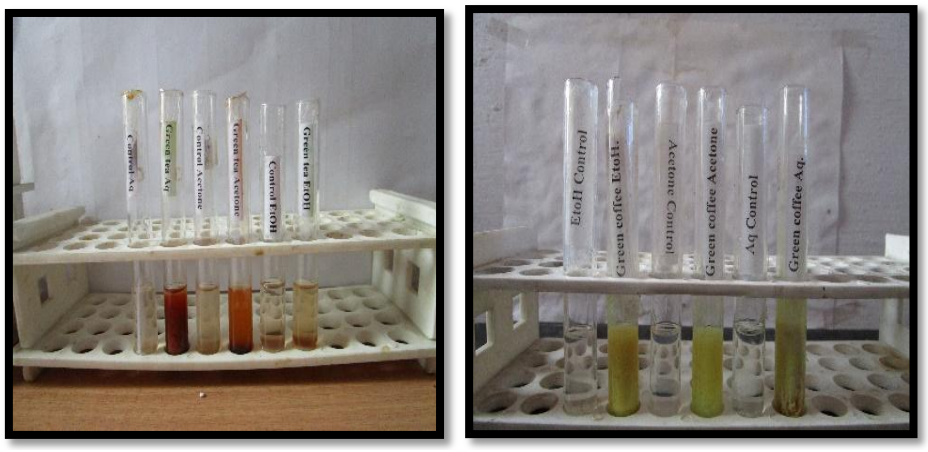
	Comparison groups (Pairs)	Mann Whitney U test
	Kruskal Wallis Test	χ^2 = 46.940, df = 4,P = 0.000 (<0.001), Sig. diff.
Comparisons of Green Tea	Green tea 300 µg/ml and Green tea 200 µg/ml	MW = 0.000, ;P= 0.000 (<0.001), Sig. diff.
	Green tea 300 µg/ml and Green tea 100 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 300 µg/ml and Chlorhexidine gluconate	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 300 µg/ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 200 µg/ml and Green tea 100 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 200 µg/ml and Chlorhexidine gluconate	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 200 µg/ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 100 µg/ml and Chlorhexidine	MW = 10.000,P= 0.002 (<0.01), Sig. diff.

	gluconate	
	Green tea 100 µg/ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Chlorhexidine gluconate ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Kruskal Wallis Test	χ^2 = 47.557,df = 4,P = 0.000 (<0.001), Sig. diff.
Comparisons of Green Coffee	Green coffee 300 µg/ml and Green coffee 200 µg/ml	MW = 1.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 300 µg/ml and Green coffee 100 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 300 µg/ml and Chlorhexidine gluconate	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 300 µg/ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 200 µg/ml and Green coffee 100 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 200 µg/ml and Chlorhexidine gluconate	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 200 µg/ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 100 µg/ml and Chlorhexidine gluconate	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 100 µg/ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Chlorhexidine gluconate ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Kruskal Wallis Test	χ^2 = 33.546,df = 3,P = 0.000 (<0.001), Sig. diff.
Comparison between Green Tea and Green Coffee	Green tea 300 µg/ml and Green coffee 300 µg/ml	MW = 4.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 300 µg/ml and Green coffee 200 µg/ml	MW = 24.000,P= 0.052 (>0.05), Not Sig.
	Green tea 300 µg/ml and Green coffee 100 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Kruskal Wallis Test	χ^2 = 35.990,df = 3,P = 0.000 (<0.001), Sig. diff.
	Green tea 200 µg/ml and Green coffee 300 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 200 µg/ml and Green coffee 200 µg/ml	MW = 1.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 200 µg/ml and Green coffee 100 µg/ml	MW = 4.500,P= 0.000 (<0.001), Sig. diff.
	Kruskal Wallis Test	χ^2 = 35.545, df = 3,P = 0.000 (<0.001), Sig. diff.
	Green tea 100 µg/ml and Green coffee 300 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 100 µg/ml and Green coffee 200 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 100 µg/ml and Green coffee 100 µg/ml	MW = 9.000,P= 0.001 (<0.01), Sig. diff.

2(a)



2(b)



2(c) **2(d)**

Figure_2: Qualitative Phytochemical analysis :
Fig 2a: Tannin test of Green Tea
Fig 2b: Tannin test of Green Coffee
Fig 2c: Flavanoid test of Green Tea
Fig 2d: Flavanoid test of Green Coffee

Table II :Comparison of zone of inhibition against Streptococcus mutansin different concentrations of green tea, green coffee, Chlorhexidine gluconate and normal saline groups.

	Comparison groups (Pairs)	Mann Whitney U test
	Kruskal Wallis Test	χ^2 = 47.284, df = 4,P = 0.000 (<0.001), Sig. diff.
Comparisons of Green Tea	Green tea 300 µg/ml and Green tea 200 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 300 µg/ml and Green tea 100 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 300 µg/ml and Chlorhexidine gluconate	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 300 µg/ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 200 µg/ml and Green tea 100 µg/ml	MW = 3.500,P= 0.000 (<0.001), Sig. diff.
	Green tea 200 µg/ml and Chlorhexidine gluconate	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 200 µg/ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 100 µg/ml and Chlorhexidine gluconate	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 100 µg/ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Chlorhexidine gluconate ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Kruskal Wallis Test	χ^2 = 47.580, df = 4,P = 0.000 (<0.001), Sig. diff
Comparisons of Green Coffee	.Green coffee 300 µg/ml and Green coffee 200 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 300 µg/ml and Green coffee 100 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 300 µg/ml and Chlorhexidine gluconate	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 300 µg/ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 200 µg/ml and Green coffee 100 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 200 µg/ml and Chlorhexidine gluconate	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 200 µg/ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.

	Green coffee 100 µg/ml and Chlorhexidine gluconate	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green coffee 100 µg/ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Chlorhexidine gluconate ml and Normal saline	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Kruskal Wallis Test	χ^2 = 36.764,df = 3,P = 0.000 (<0.001), Sig. diff.
Comparison between Green Tea and Green Coffee	Green tea 300 µg/ml and Green coffee 300 µg/ml	MW = 0.500,P= 0.000 (<0.001), Sig. diff.
	Green tea 300 µg/ml and Green coffee 200 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 300 µg/ml and Green coffee 100 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Kruskal Wallis Test	χ^2 = 34.876,df = 3,P = 0.000 (<0.001), Sig. diff.
	Green tea 200 µg/ml and Green coffee 300 µg/ml	MW = 14.500,P= 0.005 (<0.01), Sig. diff.
	Green tea 200 µg/ml and Green coffee 200 µg/ml	MW = 1.500,P= 0.000 (<0.001), Sig. diff.
	Green tea 200 µg/ml and Green coffee 100 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Kruskal Wallis Test	χ^2 = 33.198,df = 3,P = 0.000 (<0.001), Sig. diff.
	Green tea 100 µg/ml and Green coffee 300 µg/ml	MW = 0.000,P= 0.000 (<0.001), Sig. diff.
	Green tea 100 µg/ml and Green coffee 200 µg/ml	MW = 50.000,P= 1.000 (>0.05), Not Sig.
	Green tea 100 µg/ml and Green coffee 100 µg/ml	MW = 1.000,P= 0.000 (<0.001), Sig. diff.

Table II shows comparison of zone of inhibition against Streptococcus mutans in different concentrations of green tea, green coffee, Chlorhexidine gluconate and normal saline groups.

Kruskal wallis test showed significant difference between zone of inhibitions of different concentrations of green tea, Chlorhexidine gluconate and normal saline groups against Streptococcus mutans. After this Mann Whitney U test was applied for pairwise comparison, which showed following observations:

1. Zone of inhibition by Chlorhexidine gluconate (0.2%) was significantly larger than Green tea 300 µg/ml which was significantly larger than Green tea 200 µg/ml.
2. Zone of inhibition by Green tea 200 µg/ml was significantly larger than Green tea 100 µg/ml which was significantly larger than Normal saline.

(Chlorhexidine gluconate (0.2%) > Green tea 300 µg/ml> Green tea 200 µg/ml > Green tea 100 µg/ml > Normal saline)

Similar results were found with Green Coffee against Streptococcus mutans as green tea.

1. Zone of inhibition by Green tea 300 µg/ml was significantly larger than Green coffee 300 µg/ml, Green coffee 200 µg/ml and Green coffee 100 µg/ml.
   (green tea 300 ug/ml > green coffee 300 ug/ml, green coffee 200 ug/ml, green coffee 100 ug/ml)

Table III: Mean, standard deviation, median, minimum and maximum values of zone of inhibition against Lactobacillus acidophillus and Streptococcus mutans in different concentrations of green tea, green coffee, Chlorhexidine gluconate and normal saline groups.

Groups		Zone of inhibition (mm)	
		Lactobacillus acidophillus	Streptococcus mutans
Green tea 300 µg/ml	Mean ± SD	21.60 ± 0.84	19.20 ± 1.03
	Median	22.00	19.50
	Min- Max	20.00-23.00	17.00-20.00
Green tea 200 µg/ml	Mean ± SD	17.60 ± 0.84	13.80 ± 1.14
	Median	18.00	14.00
	Min- Max	16.00-19.00	12.00-16.00
Green tea 100 µg/ml	Mean ± SD	13.40 ± 1.08	11.00 ± 1.16
	Median	13.00	11.00

	Min- Max	12.00-15.00	9.00-13.00
Green coffee 300 µg/ml	Mean ± SD	24.00 ± 0.94	15.40 ± 0.97
	Median	24.00	15.50
	Min- Max	22.00-25.00	14.00-17.00
Green coffee 200 µg/ml	Mean ± SD	20.60 ± 1.08	11.00 ± 0.82
	Median	21.00	11.00
	Min- Max	19.00-22.00	10.00-12.00
Green coffee 100 µg/ml	Mean ± SD	15.40 ± 0.97	7.60 ± 0.97
	Median	15.50	7.50
	Min- Max	14.00-17.00	6.00-9.00
Chlorhexidine gluconate (0.2%)	Mean ± SD	11.80 ± 0.63	25.70 ± 0.95
	Median	12.00	26.00
	Min- Max	11.00-13.00	24.00-27.00
Normal saline	Mean ± SD	0.00 ± 0.00	0.00 ± 0.00
	Median	0.00	0.00
	Min- Max	0.00-0.00	0.00-0.00

DISCUSSION

Dental caries is defined as an infectious, microbiologic disease that is characterized by demineralization of the inorganic portion and the destruction of the organic substances of teeth. Fermentation of carbohydrates by bacteria mainly mutans group of Streptococci, results in a decrease in the pH of plaque that causes demineralization of enamel leading to formation of dental caries [17]About 700 different species of bacteria that have been identified from the human oral microbiome, mutans group of streptococci is believed to be the main factor that initiates caries and very important factor for enamel decay, whereas, Lactobacilli is responsible for further caries progression, especially in the dentin.[18,19]

Chlorhexidine is a broad spectrum antibiotic that kills Gram-positive and Gram-negative bacteria. At lethal concentrations chlorhexidine causes irreparable damage to the cell membrane of target microbes, and at sub lethal concentrations chlorhexidine can interfere with the sugar transport and acid production of the cariogenic streptococci strains, providing a bacteriostatic effect.[20] Many longitudinal studies have proved that there is direct relation between the S. mutans level in plaque and saliva and incidence of caries. Chlorhexidine can interfere with the metabolic activity of S. mutans by abolishing activity of phosphonyl pyruvate. Chlorhexidine in the form of mouthwash and gel has found to be effective in reducing the level of microorganisms but faster recovery of microorganisms to original level was a frequent observation[21]

The Dicatonic positively charged CHX is attracted to the negatively charged bacterial cell wall with specific and strong adsorption to phosphate containing compounds that alters the integrity of the bacterial cell membrane.CHX then binds to the phospholipids in the inner membrane and there is leakage of low molecular weight compounds like potassium ions. By increasing the concentration of CHX there is progressive damage to the membrane. There is coagulation and precipitation of the cytoplasm by the formation of phosphate complexes which include adenosine triphosphate and nucleic acids. Cytoplasms of the cells are chemically precipitated, creating a Bactericidal stage which is irreversible[22]

Mathur *et al* have reported a number of local side-effects with CHX use such as discoloration of teeth, altered taste sensation, mucosal irritation, parotid swelling, and enhanced supra-gingival calculus formation due to precipitation of salivary proteins and organic salts, which limits its use as a therapeutic agent.[23]

The most important compounds of green tea are the polyphenols. Polyphenols exist in many plants such as fruits, vegetables, teas and cocoa. Green tea compounds, especially polyphenols impart many health benefits like antioxidant, anti-inflammatory, hypoglycemic, and hypolipidemic properties, depression of hypertension, and other biological properties; which can be related to tea's abilities on metal chelating, free radical scavenging, and antioxidant activity. Flavonoids are a major group of polyphenols. The main flavonoids in green tea are catechins (flavoa-3-ols), such as epicatechin, epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), gallocatechin (GC), gallocatechin gallate (GCG), catechin, and catechin gallate (CG)[2]. Tannin and catechins of green tea are able to inhibit enzymatic activity of amylase which is responsible for caries incidence by hydrolysis of starch in foods to lower molecular weight carbohydrates.[24]

There is convincing evidence that the bioactive components of green tea are able to influence the process of caries formation at several different stages: they may inhibit proliferation of the streptococcal agent, interfere with the process of adhesion to tooth enamel or act as inhibitors of glucosyl transferase and amylase.[7]Catechins, which are a major part of green tea, have an affinity for proteins, that is speculated to inhibit the enzymes such as amylases and glucosyl transferase, and the adherence of S. mutan to the tooth structure.[25]

Green coffee is another popular drink. The anticariogenic potential of coffee is related to its capacity to alter the biosynthesis of extracellular polysaccharides (mainly mutans), avoiding the adhesion of streptococci.[26] It has also been suggested that the coffee active molecules may adsorb to host surfaces, preventing the tooth receptor from interacting with bacterial adhesions and preventing both reversible and irreversible Streptococcus mutans adherence to tooth surfaces.[4]

Phytochemicals play an important role in preparations as antibacterial agents as well as antioxidants.[26] The qualitative phytochemical screening of green tea and green coffee revealed the presence of tannins, saponins, flavonoids, quinones, glycosides, cardiac glycosides, terpenoids, phenol, coumarins, steroids, alkaloids, and betacyanins. The presence of these phytochemicals were tested in aqueous, acetone and ethanol solvent of the extract of green tea and green coffee.

The antibacterial activity of tannin is due to the interaction with proline-rich proteins or cell-surface lipoteichoic acid and Inhibition of glucosyltransferase activity and bacterial growth by their strong iron-binding capacity. Alkaloids interfere with the division of cells thus inhibiting their growth.The flavonoids possess anti-glucosyltransferase activity and can inhibit adherence of microbes.[27,28]

The tannin, phenol and flavonoid content were found to be 136 ug, 286 ug and 106 ug respectively for green tea and for green coffee 13, 13.3 and 93.3 ug respectively. In a study done by Anita P et al[27], the tannin, phenol and flavonoid content for green tea was 243 mg/g, 96mg/g and 7.5mg/g respectively and by Subramaniam P et al[29], the tannin, phenol and flavonoid content was .308mg/g, 0.0105mg/g and 350mg/g respectively. In a study by Subhashini et al[28], green tea has 168.8mg of total phenol (GAE) and 353 mg of flavonoids (ECE). This variation in the quantitative phytochemicals might be ascribed to the source of green tea from which the extract is prepared. To the best of our knowledge, no previous study has till now evaluated the qualitative and quantitative phytochemical analysis of green coffee, therefore its results cannot be compared. However, in comparison with green tea, it has shown lower total phenolic, alkaloid and flavonoid content.

The highest antimicrobial activity of tea is due to presence of catechins and polyphones which damages bacterial cell membrane.[4]The slow release of catechins, the aflavins and tannins from tea leaves is likely to contribute to the caries preventive effects of the non-fluoride component of tea that has been demonstrated in several human clinical trials and animal studies.[30]The antioxidant activity of green tea and green coffee were highest in ethanolic extract in comparison with aqueous and acetone extract. This result was in agreement with Anita P et al[27] and Subramaniam P et al[29]. Hence, further tests were done with ethanolic extract of green tea and green coffee. The antioxidant activity was more in ethanolic extract of green tea than the ethanolic extract of green coffee. This might be due to its lower phenolic, flavonoid and tannin content than green tea.

MIC is the lowest concentration of an extract or drug that will inhibit the visible growth of a microorganism after overnight incubation[31]The MIC for green tea was 0.8% for S.mutans and 0.9% for L.acidophilus whereas, the MIC for green coffee was 0.9% for S.mutans and 0.7% for L.acidophilus. The MBC for green tea was 0.8% and 0.9% for S.mutans and L. acidophilus respectively, whereas, the MBC for green coffee was 0.9% for both S. mutans and L.acidophilus. In a study by Tahir et al[32], the MIC and MBC for green tea was 0.7%. The MBC of S. mutans of present study was similar to the MBC by Anita P et al[27]. The lower MIC in our study can be ascribed to the lower tannin content in the sample studied. In a study by H. Barosso et al, Assuri TEA presented an inhibitory action when applied at a level of 12.5 mg/mL or higher against S. mutans. [33]No study has been conducted to assess the antibacterial effect of green coffee extract on the most common cariogenic pathogens so far.Therefore, the minimum inhibitory concentrations (MICs) and minimum bactericidal concentration (MBC) of green coffee bean extract cannot be compared with any other study.

Chlorhexidine was taken as the positive control. Its zone of inhibition was greater than even 300ug/ml of green tea and green coffee against streptococcus mutans. However, the result was not similar against Lactobacillus acidophilus, for which the zone of inhibition of chlorhexidine was smaller than that of green tea and green coffee. This difference in the mean zone of inhibition of Chlorhexidine between S. mutans and L. acidophilus is in agreement with Evans et al .[34]The results are contradictory to Anita P et al[27], where the zone of inhibition by green tea against S. mutans at 300ug/ml was greater than 0.2% chlorhexidine, and also contradictory to Subramaniam P et al[29], who showed that aqueous extract of green tea showed greater zone of inhibition than chlorhexidine. Their results might be due to the difference in the extract preparation.

Both green tea and green coffee have shown to inhibit the S. mutans and L. acidophilus strain. While Green tea showed greater zone of inhibition against Streptococcus mutans, the zone of inhibition of L. Acidophilus was greater in the green coffee group. Bacteria have different degrees of sensitivity to antimicrobial compounds. Lactobacillus species may have the highest sensitivity to antimicrobial compounds in coffee extract such as caffeine, volatile and non-volatile organic acids, phenols and aromatic compounds like phenolic compounds, aldehydes, ketones, sulphuric compounds

and esters. The acids in coffee extract such as caffeic acid, low chain fatty acids and chlorogenic acid have antibacterial activities.[35]

The differences in the diameter of the inhibition zone at each concentration may be due to a large difference in the active substances contained in Green tea and Green coffee extracts that are antibacterial, such as caffeine, trigonelline, caffeic acid, and chlorogenic acid. The mean zone of inhibition of L. Acidophilus by green tea is larger than 0.2% chlorhexidine at all the 3 concentrations (300ug/ml,200ug/ml,100ug/ml). Also, the Zone of inhibition by Green tea and green coffee at 300 µg/ml was significantly larger than 200ug/ml and 100 ug/ml against S.mutans and L. acidophilus. This shows that the bioactivity of green tea and green coffee increases as the concentration increases.

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Considering that green tea and green coffee are constituted by several substances such as caffeine, alkaloids, tannin, flavonoids, and that large quantities can be obtained at low cost, they are highly beneficial as anticariogenic agents. However, these results were obtained from preliminary screening in invitro circumstances. Therefore, further in vivo studies should be performed with the aim of investigating the effect of green tea and green coffee as a mouthwash.

CONCLUSION

Phytochemicals were found to be present in both green tea and green coffee extract. Quantitative phytochemical screening of green tea extract revealed higher concentration of total tannin, phenol, and flavonoid content than green coffee extract. Antioxidant activity and free radical scavenging activity was found to be present in both green tea and green coffee extract.

The antibacterial activity of Green tea extract and Green coffee extract was highest at 300ug/ml against S.mutans and L. Acidophilus. The antibacterial activity of chlorhexidine was highest against S. Mutans but lowest against L. Acidophilus in comparison with Green Tea and Green Coffee.

Chlorhexidine gluconate 0.2% had the highest antibacterial activity against S.mutans and Green coffee 300ug/ml had the highest antibacterial activity against L. acidophilus.

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