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Comparative evaluation of antibacterial efficacy of green coffee bean extract mouthwash and chlorhexidine mouthwash against *Streptococcus mutans* and *Lactobacilli* spp. – An *in vitro* study

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Abstract:

INTRODUCTION: Dental caries can occur in an individual irrespective of age, which makes preventing the incidence of dental caries vital. The prevention of dental caries includes various types of oral hygiene measures. One such modality is the use of mouthwash. As chlorhexidine mouthwash causes several adverse effects, there is a need for herbal alternative. Green coffee bean extract is one such herbal alternative.

AIM: The aim of this study is to compare and evaluate the antibacterial efficacy of green coffee bean extract mouthwash and chlorhexidine mouthwash against *Streptococcus mutans* and *Lactobacilli* spp.

MATERIALS AND METHODS: The ethanolic extract of green coffee bean was obtained by treating it with cellulase and 30% ethanol. Minimum inhibitory concentration and minimum bactericidal concentration were obtained by the broth dilution method and culture plating method, respectively. Based on these values, 3% green coffee bean extract mouthwash was prepared, and the antibacterial efficacy against *S. mutans* and *Lactobacilli* spp. was tested using the direct contact test. The obtained data were analyzed using the Mann–Whitney U-test and Wilcoxon matched-pair test.

RESULTS: Intergroup comparison using Mann–Whitney U-test showed that green coffee bean extract mouthwash is equally effective against *S. mutans* and *Lactobacilli* spp. as compared to chlorhexidine mouthwash. Wilcoxon matched-pairs test showed that the efficacy of green coffee bean extract mouthwash reduced over a period of 10, 30, and 60 min time intervals.

CONCLUSION: Therefore, green coffee bean extract can be used as an alternative to chlorhexidine as mouthwash.

Keywords:

Antibacterial, chlorhexidine, green coffee bean, *Lactobacilli* spp., mouthwash, *Streptococcus mutans*

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Introduction

Carious destruction of tooth is considered to be a common oral disease worldwide; individuals are likely to develop this throughout their lifespan. Dental caries form through multifaceted interaction over a period of time with acid-producing microbes, fermentable carbohydrate, and various host factors such as teeth and saliva. It can arise in the early life of a child as an aggressive tooth decay that affects the primary teeth of infants

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and toddlers.[1] The prevalence of dental caries varies from 49% to 83% across different countries.[2] Caries status in India was reported to be 68.4%.[3] In 2018, irrespective of age, occurrence of dental caries was found to be high, and varied across India.[2] Increased occurrence of dental caries in children could be attributed to socioeconomic status, lack of preventive measures, and dietary changes.[3]

Acidogenic bacteria, especially *Streptococcus mutans*, in the existence of fermentable sugars cause demineralization of the teeth structure. Colonization of the oral cavity by *Lactobacilli* requires a retentive niche, which is formed by the action of *S. mutans*.[4]

Prevention treatment modalities such as oral hygiene measures, different forms of fluoride application, pit-and-fissure sealants, and use of xylitol were found to be essential.[5]

We, Indians, who were the first to use medicinal plants to treat ailments and preserve the normal harmony of the human body. Due to the Western concept of medicine, the practice of herbal plants was replaced by allopathic form drugs. These drugs are considered to be "double-edged sword" as they can also cause serious discomforts or adverse effects to the individual who is taking the drugs for any particular disease.

Hence, in the past few years, there has been a shift in the concept of treating from the western culture to the adaptation of the Eastern culture, which includes the use of herbal plants to treat diseases. One scenario is the use of chlorhexidine mouthwash for the prevention of initiation of dental caries. Chlorhexidine can cause adverse effects such as brown staining of the tooth, taste alteration, and mucosal erosions, etc., Hence, there is a need for an herbal alternative.

One such herbal alternative is the green coffee bean extract, which contains caffeine, volatile, and nonvolatile organic acids such as chlorogenic acids (CGAs) and caffeic acids, which have the antibacterial ability.[6] CGAs are phenolic compounds formed by the esterification of cinnamic acids, which has a series of health benefits like antibacterial activity against cariogenic bacteria. During roasting, there is a reduction of about 30%–50% of CGA.[7] Hence, the green coffee bean is considered to be the best source for CGA. Among, the various herbal products that are being used in dentistry, the green coffee bean has not been researched yet. Furthermore, very few studies have been carried out applying this herb as a mouthwash.

Materials and Methods

This *in vitro* study was conducted in the Department of Pediatric and Preventive Dentistry at KLE Academy of Higher Education, and Research's KLE VK Institute of Dental Sciences, Belagavi and the laboratory procedures were carried out in KAHER's Dr. Prabhakar Kore Basic Science Research Centre, Belagavi. The green coffee beans were procured from Coorg Coffee Supplies, Yelachenahalli, Bangalore, India (2018, 2 weeks).

The procured dried green coffee beans were grounded to a fine powder using mortar and pestle. The grounded powder was treated with 30% ethanol and cellulase. Then, the mixture was kept in water bath undisturbed for 24 h after the occasional shaking in the first 30 min. The mixture was then filtered through a sterilized Whatman No. 1 filter paper (Sigma Labsys, Karnataka, India). The obtained liquid filtrate was exposed to ultraviolet rays (Laminar air flow chamber) for 24 h and checked for sterility on nutrient agar plates and stored in labeled sterile bottles in a freezer at 4°C.[8]

The MTT(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was carried out on gingival fibroblasts obtained from American Type Culture Collection, Virginia, United States of America. At 31% concentration of green coffee bean extract, the observed viability of human gingival fibroblast cells was 99.41%, which indicates that even at 31% concentration green coffee bean extract was not cytotoxic and can be used in further *in vivo* studies. The study was performed in triplicates after consulting with statistician.

Minimum inhibitory concentration (MIC) was assessed against strains of *S. mutans* and L*actobacilli* spp. obtained from Microbial Type Culture Collection, Chandigarh, India, by broth dilution method [Figure 1].[9]

The minimum bactericidal concentration (MBC) was calculated by streaking the supernatant solutions from the microtubes used for MIC of each organism in brain heart infusion (BHI) media using a sterile streaking loop in laminar air flow chamber and incubating them for 24 h in the incubator at 37°C.[10]

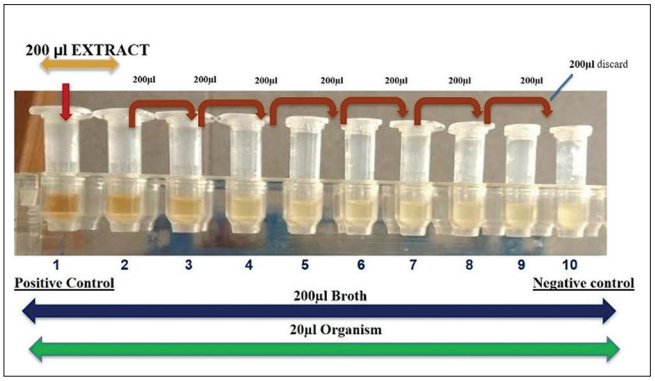


Figure 1: Schematic representation of broth dilution method

Preparation of green coffee bean extract mouthwash

MIC and MBC were observed at concentration of 2.5 µg/ml and 0.625 µg/ml against *S. mutans* and *Lactobacilli* spp., respectively. Based on MIC and MBC values, the lowest concentration at which the green coffee bean extract showed antibacterial activity against *S. mutans* and *Lactobacilli* spp. is 2.5%. Hence, 3% Green coffee bean extract mouthwash was prepared to overcome any changes in the property of extract while adding other components for mouthwash preparation. Initially, in 50 ml of distilled water sodium benzoate; methyl- and propyl-parabens were dissolved with the aid of magnetic stirrer (KI-66-02, Kumar Sales Corporation, Maharashtra, India) at room temperature to form solution 1. Then, in 50 ml of distilled water xylitol and glycerine were dissolved to get solution 2. Finally, solution 1 and solution 2 were filtered through Whatman filter paper no. 40 and filtrate was mixed with 3% green coffee bean extract and stirred in magnetic stirrer for 30 min at room temperature. The final mouthwash was poured into bottle with lid tight and stored at room temperature.[11] The final mouthwash obtained was clear and 25 ml in quantity [Table 1].

Antibacterial efficacy determined by direct contact test

The samples were processed by the direct contact test on 96–well microplates. Direct contact test is based on determining the turbidity of microbial growth in microplates. Strains of *S. mutans* and *Lactobacilli* spp. were grown on BHI media.[12]

Initially, 50 µL of the green coffee bean extract mouthwash was added to the wells. Then for *S. mutans*, 50 µL bacterial suspension adjusted to 0.5 McFarland scale = 1.5 × 108 colony-forming unit was placed in the wells. The same procedure was carried out for chlorhexidine mouthwash also.

After incubation for 1 h at 37°C, there will be direct contact between the respective organism and the test material. Now, 240 µL of BHI media was added in the wells. The kinetics of bacterial outgrowth in each well was measured at 630 nm using a microplate spectrophotometer (Systronics, Maharashtra, India) every 10 min, 30 min, and 60 min. Similar experimental procedures were carried out for *Lactobacilli* spp. The antibacterial test was repeated 5 times (n = 5) for each organism. The obtained results were tabulated and entered in an excel sheet [Table 2 and 3]. The statistical analysis was performed using the Mann–Whitney U test and Wilcoxon ranked pairs test using IBM Statistical Package for the Social Sciences (SPSS) software (version 20.0).

Table 1: Composition of 3% green coffee bean extract mouthwash (% w/v)

Material	Function	Formulation (%)
Green coffee bean extract	Antibacterial	3
Xylitol	Sweetening agent	2
Glycerine	Humectant	5
Sodium benzoate	Preservative (bacteriostatic)	0.1
Methyl paraben	Preservative (bactericidal)	0.05
Propyl paraben	Preservative (bactericidal)	0.01
Distilled water	Vehicle	100 ml

Table 2: Mean optical density and standard deviation of green coffee bean extract mouthwash and chlorhexidine mouthwash against *Streptococcus mutans* in direct contact test using spectrophotometer at 630 nm wavelength

Time interval (min)	Green coffee bean extract mouthwash		Chlorhexidine mouthwash	
	Mean optical density	SD	Mean optical density	SD
10	0.245	0.020	0.291	0.036
30	0.257	0.027	0.277	0.028
60	0.275	0.031	0.273	0.028

SD: Standard deviation

Table 3: Mean optical density values of green coffee bean extract mouthwash and chlorhexidine mouthwash against *Lactobacilli* spp. in direct contact test using spectrophotometer at 630 nm wavelength

Time interval (min)	Green coffee bean extract mouthwash		Chlorhexidine mouthwash	
	Mean optical density	SD	Mean optical density	SD
10	0.345	0.065	0.350	0.052
30	0.357	0.061	0.355	0.064
60	0.399	0.024	0.343	0.068

SD: Standard deviation

Results

For *S. mutans,* intergroup comparison by Mann-Whitney U-test revealed that mean optical density values of direct contact test at 10 min, 30 min, and 60 min (P = 0.0760, 0.0760, and 0.0760) showed no statistically significant difference between both mouthwashes. However, the difference of mean optical density value of direct contact test between time intervals 10 min and 30 min, 10 min and 60 min and 30 min and 60 min (P = 0.0280, 0.0280, and 0.0120) was found to be statistically significant among both mouthwashes. Intragroup comparison for green coffee bean extract mouthwash by Wilcoxon matched-pairs test revealed that statistically significant difference in mean optical density value of direct contact test was seen between 10 min and 60 min time interval (P = 0.043). However, there was no statistically significant difference observed between 10 min and 30 min and 30 min and 60 min time interval. Whereas,

intragroup comparison for chlorhexidine showed no statistically significant difference [Tables 4 and 5].

In the case of *Lactobacilli* spp., intergroup comparison by Mann–Whitney U-test revealed that mean optical density value of direct contact test at 10 min, 30 min, and 60 min (P = 0.7540, 0.9170, and 0.1170) showed no statistically significant difference between both mouthwashes. When compared, difference in mean optical density value of direct contact test between time intervals 10 min and 30 min, 10 min and 60 min (P = 0.4020 and 0.4020) showed no statistically significant difference, whereas between time interval 30 min and 60 min (P = 0.0090), a statistically significant difference was observed among both mouthwashes. Intragroup comparison for green coffee bean extract mouthwash by Wilcoxon matched-pairs test revealed that mean optical density value of direct contact test showed a statistically significant difference among 10 min and 30 min, 10 min and 60 min, and 30 min and 60 min time intervals (P = 0.0431, 0.0431, and 0.0431). For chlorhexidine mouthwash, no statistically significant difference was observed among 10 min and 30 min, 10 min and 60 min time interval, only at 30 min and 60 min, a statistically significant difference was observed (P = 0.0431) [Tables 6 and 7].

Discussion

Green coffee bean extract was prepared as an ethanolic extract to obtain the maximum number of bioactive components present in it. In this study, green coffee beans were preferred over-roasted coffee beans as the roasting process causes a reduction in the bioactive components present in the coffee beans. Alcoholic extraction, pressurized liquid extraction and supercritical CO_2 extraction are extraction techniques that have the ability to increase target molecule specificity and reduce waste solvent production.[13] Among these techniques, alcoholic extraction is used in the extraction of chemically active components from plant products. In the current study, ethanolic extraction was carried out along with cellulase, which is

Table 4: Intergroup comparison of mean optical density value of green coffee bean extract mouthwash and chlorhexidine mouthwash against *Streptococcus mutans* at different time intervals

Time points (min)	Materials	Mean	SD	SE	Mean rank	*U*	*Z*	*P*
10	Green coffee bean extract mouthwash	0.245	0.020	0.009	3.80	4.00	−1.7760	0.0760
	Chlorhexidine mouthwash	0.291	0.036	0.016	7.20			
30	Green coffee bean extract mouthwash	0.257	0.027	0.012	3.80	4.00	−1.7760	0.0760
	Chlorhexidine mouthwash	0.277	0.028	0.012	7.20			
60	Green coffee bean extract mouthwash	0.275	0.031	0.014	5.80	11.00	−0.3130	0.7540
	Chlorhexidine mouthwash	0.273	0.028	0.012	5.20			
10-30	Green coffee bean extract mouthwash	−0.012	0.012	0.005	3.40	2.00	−2.1930	0.0280*
	Chlorhexidine mouthwash	0.014	0.016	0.007	7.60			
10-60	Green coffee bean extract mouthwash	−0.012	0.012	0.005	3.40	2.00	−2.1930	0.0280*
	Chlorhexidine mouthwash	0.014	0.016	0.007	7.60			
30-60	Green coffee bean extract mouthwash	−0.018	0.015	0.007	3.10	0.50	−2.5140	0.0120*
	Chlorhexidine mouthwash	0.004	0.005	0.002	7.90			

Mann–Whitney U-test, *P<0.05. SD: Standard deviation, SE: Standard error

Table 5: Intragroup comparison of mean optical density value of green coffee bean extract mouthwash and chlorhexidine mouthwash against *Streptococcus mutans* at different time intervals

Material	Time (min)	Mean	SD	Mean different	SD different	Percentage of change	*Z*	*P*
Green coffee bean extract mouthwash	10	0.245	0.020	−0.012	0.012	−4.98	1.7529	0.0796
	30	0.257	0.027					
	10	0.245	0.020	−0.030	0.012	−12.24	2.0226	0.043*
	60	0.275	0.031					
	30	0.257	0.027	−0.018	0.015	−6.92	1.8257	0.0679
	60	0.275	0.031					
Chlorhexidine mouthwash	10	0.291	0.036	0.014	0.016	4.81	1.4832	0.1380
	30	0.277	0.028					
	10	0.291	0.036	0.018	0.016	6.18	1.8257	0.0679
	60	0.273	0.028					
	30	0.277	0.028	0.004	0.005	1.44	1.8257	0.0679
	60	0.273	0.028					

Wilcoxon matched pairs test, *P<0.05. SD: Standard deviation

Table 6: Intergroup comparison of mean optical density value of green coffee bean extract mouthwash and chlorhexidine mouthwash against *Lactobacilli* spp. at different time intervals

Time interval (min)	Materials	Mean	SD	SE	Mean rank	*U*	*Z*	*P*
10	Green coffee bean extract mouthwash	0.346	0.065	0.029	5.20	11.00	−0.3130	0.7540
	Chlorhexidine mouthwash	0.350	0.052	0.023	5.80			
30	Green coffee bean extract mouthwash	0.357	0.061	0.027	5.40	12.00	−0.1040	0.9170
	Chlorhexidine mouthwash	0.355	0.064	0.028	5.60			
60	Green coffee bean extract mouthwash	0.400	0.024	0.011	7.00	5.00	−1.5670	0.1170
	Chlorhexidine mouthwash	0.344	0.068	0.030	4.00			
10-30	Green coffee bean extract mouthwash	−0.012	0.008	0.004	4.70	8.50	−0.8380	0.4020
	Chlorhexidine mouthwash	−0.005	0.013	0.006	6.30			
10-60	Green coffee bean extract mouthwash	−0.012	0.008	0.004	4.70	8.50	−0.8380	0.4020
	Chlorhexidine mouthwash	−0.005	0.013	0.006	6.30			
30-60	Green coffee bean extract mouthwash	−0.042	0.047	0.021	3.00	0.00	−2.6190	0.0090*
	Chlorhexidine mouthwash	0.012	0.007	0.003	8.00			

Mann–Whitney U-test, *P<0.05. SD: Standard deviation, SE: Standard error

Table 7: Intragroup comparison of mean optical density value of green coffee bean extract mouthwash and chlorhexidine mouthwash against *Lactobacilli spp*. at different time intervals

Material	Time (min)	Mean	SD	Mean different	SD different	Percentage of change	*Z*	*P*
Green coffee bean extract mouthwash	10	0.346	0.065	−0.012	0.008	−3.41	2.0226	0.0431*
	30	0.357	0.061					
	10	0.346	0.065	−0.054	0.053	−15.68	2.0229	0.0431*
	60	0.400	0.024					
	30	0.357	0.061	−0.042	0.047	−11.86	2.0227	0.0431*
	60	0.400	0.024					
Chlorhexidine mouthwash	10	0.350	0.052	−0.005	0.013	−1.54	1.2136	0.2249
	30	0.355	0.064					
	10	0.350	0.052	0.006	0.018	1.83	0.7303	0.4652
	60	0.344	0.068					
	30	0.355	0.064	0.012	0.007	3.32	2.0222	0.0431*
	60	0.344	0.068					

Wilcoxon matched pairs test, *P<0.05. SD: Standard deviation

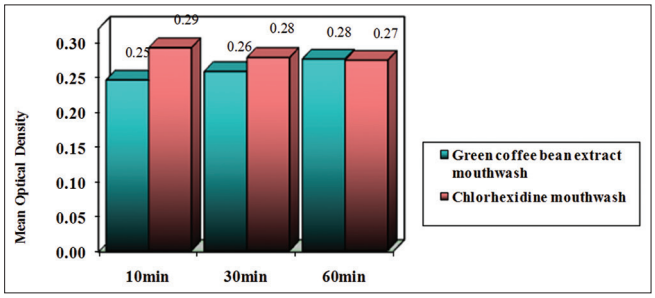
usually the enzyme of choice when it comes to breaking down plant cell walls.[14]

MIC is defined as the lowest concentration of the antimicrobial agent that prevents visible growth of a micro-organism under defined conditions. There are various methods to assess MIC like agar diffusion method and broth dilution method. Limitation of MIC method is that it does not give an indication of the mode of action (cidal or static) of the antimicrobial agent.[9] Hence to evaluate the mode of action of green coffee bean extract MBC was carried out.

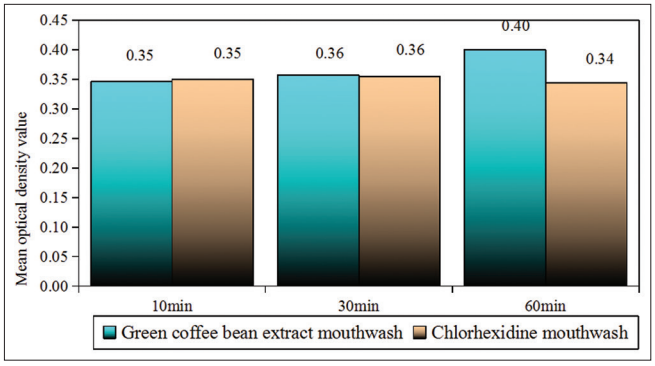
MBC is the minimum concentration of an antimicrobial drug that is bactericidal. It is determined by re-culturing (subculturing) broth dilutions that inhibit the growth of a bacterial organism (i.e., those at or above the MIC). The broth dilutions are streaked onto agar and incubated for 24 h–48 h. MBC is the lowest broth dilution of antimicrobial that prevents growth of the organism on the agar plate. Failure of the organism to grow on the plate implies that only nonviable organisms are present.[15] Based on MIC and MBC values, 3% green coffee bean extract mouthwash was prepared so that it will be effective against *S. mutans* and *Lactobacilli* spp. and to overcome any changes in the property of extract while adding other components for mouthwash preparation.

Direct contact test is the most commonly used and effective antibacterial test, which involves the direct interaction between the test material and micro-organism. The direct contact between the test material and the micro-organism is interpreted using spectrophotometer at 630 nm wavelength.[16] The present study evaluated the antibacterial efficacy of chlorhexidine and green coffee bean extract mouthwash against *S. mutans* and *Lactobacilli* spp. at 10, 30, and 60 min time interval in 96 well microplates.

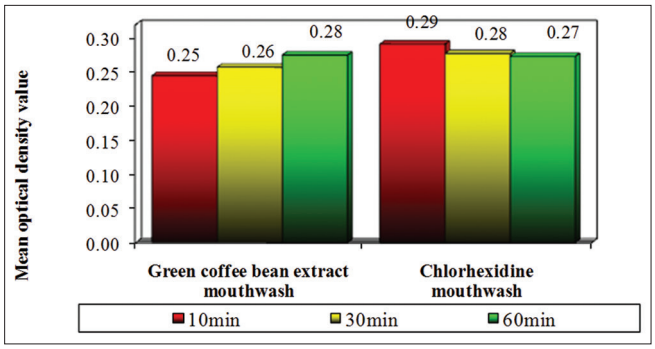
The intergroup comparison between green coffee bean extract mouthwash and chlorhexidine mouthwash showed no statistically significant difference against *S. mutans* and *Lactobacilli* spp. at 10, 30, and 60 min time intervals, which clearly denotes that green coffee bean extract mouthwash is equally effective against these



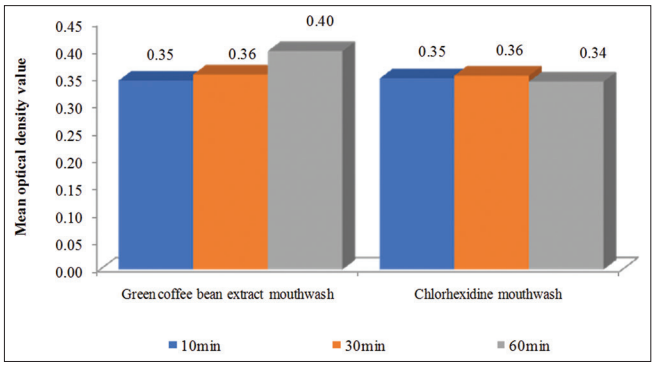
Graph 1: Mean optical density value of green coffee bean extract mouthwash and chlorhexidine mouthwash against *Streptococcus mutans* at different time intervals



Graph 2: Mean optical density value of green coffee bean extract mouthwash and chlorhexidine mouthwash against *Lactobacillus* spp. at different time intervals



Graph 3: Mean optical density value of green coffee bean extract mouthwash and chlorhexidine mouthwash against *Streptococcus mutans* at different time intervals



Graph 4: Mean optical density value of green coffee bean extract mouthwash and chlorhexidine mouthwash against *Lactobacilli* spp. at different time intervals

bacteria as compared to chlorhexidine mouthwash [Graph 1 and 2].

Intragroup comparison of green coffee bean extract mouthwash showed that the effect of the mouthwash was decreasing from 10, 30 to 60 min time interval, the difference is statistically significant, whereas for chlorhexidine mouthwash, the effect remains unchanged. Hence, the difference in the mean optical density value between the time interval 10–30 min, 30–60 min and 10–60 min was statistically higher for chlorhexidine mouthwash as compared to green coffee bean extract mouthwash against *S. mutans*. Whereas, for *Lactobacilli* spp. only at 30–60 min time interval, the difference is a statistically higher value for chlorhexidine mouthwash as compared to green coffee bean extract mouthwash [Graph 3 and 4].

Chlorhexidine induces the formation of indentation spots on the cell membrane of bacteria by increasing the permeability of the membrane, through which there is leaching out of the cellular contents is seen leading to the death of the bacteria. When bacteria are treated with Chlorhexidine it is seen that there is uptake of chlorine from chlorhexidine and loss of phosphorus from the bacterial cell. Furthermore, it is seen that the concentration of chlorhexidine inside the cell increased with time.[17] Whereas, CGA, ester of caffeic acid, provoke irreversible permeability changes in the cell membrane, causing cells to lose the ability to maintain membrane potential and leakage of cytoplasm macromolecules including nucleotide.[18] Chlorhexidine mouthwash and green coffee bean extract mouthwash show almost similar mechanism of inhibiting the growth of bacteria, hence Green coffee bean extract mouthwash can be used as an herbal alternative of chlorhexidine mouthwash.

Chlorhexidine is a symmetrical cationic molecule which consists of two 4-chlorophenyl rings and two biguanide groups connected by a central hexamethylene chain. It is a strong base and is most stable in the form of its salts.[19] Although effective, it has certain side effects like brown discoloration of the teeth, oral mucosal erosion, and altered taste sensation.[20]

Jensen stated that the discoloration is mainly due to the adsorption of chlorhexidine to the surface of hydroxyapatite crystals that alters the binding ability of the crystals. The apatite treated with chlorhexidine binds considerable amounts of certain dyes which are normally used in foodstuffs and drinks (aldehydes and ketones) and the untreated apatite possesses only a minor, if any, affinity for these dyes. This binding of the dyes is mediated by an interaction between the anionic groups of the dye molecules and the cationic groups of the chlorhexidine molecules.[21]

Chlorhexidine also may produce taste disturbances by binding to a specific sodium receptor molecule in the taste buds which is uniquely different from receptors for sweet, bitter, and sour stimuli.[19]

Herbal extracts for tooth cleansing and as an antimicrobial plaque agent have been successfully used in dentistry. Herbal mouth rinses are gaining special attention in recent times because they are nonchemical and nonsynthetic.[22] Studies on the antimicrobial and anticariogenic properties of coffee species are scarce in literature despite it being the best known and one of the most popular drinks in the world. In this study, the green coffee bean extract was chosen as it is an herbal product which has shown several beneficial biological properties.

The reason for reduction in the growth of *S. mutans* and *Lactobacilli* spp. in the green coffee group can be attributed to the CGA (an active component) and polyphenols, the primary constituents of green coffee bean extract. CGA is structurally composed of an ester of caffeic acid with the 3-hydroxyl group of quinic acid. Its level is high in the green coffee as compared to the roasted form of coffee. CGA inhibits bacterial pathogens by increasing outer membrane permeability, inducing the efflux of potassium from the cell, and ultimately leading to the break in the membrane and leakage of the cytoplasmic contents, including nucleotides.[18]

Limitations

The study results should be interpreted in the light of a few limitations that it was an *in vitro* study. The result could be better trusted on

- If an *in vivo* study could have been carried out
- The antibacterial property against other pathogenic oral micro-organisms should be evaluated
- The longer duration of use of green coffee bean extract mouthwash also has to be evaluated for any long-term side effects.

Conclusion

Dental caries is the most common oral disease which occurs due to the number of factors like diet, bacteria, host, and time. *S. mutans* and *Lactobacilli* spp. are the most notorious micro-organisms which play a pivotal role in causing tooth decay.

Mouthwash is one of the means of causing reduction in the growth of oral bacteria. Chemical mouthwashes, as they show adverse effects, they are being substituted with herbal mouthwashes. These oral hygiene measures need to be promoted, especially for children who are susceptible to oral diseases and have poor compliance with oral hygiene methods. 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.

The conclusion drawn from the present study is that both chlorhexidine and green coffee bean extract mouthwash showed antimicrobial efficacy against *S. mutans* and *Lactobacilli* spp. Furthermore, green coffee bean extract mouthwash proved to have similar antimicrobial efficacy like chlorhexidine mouthwash.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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