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# Clinical and Microbiological Evaluation of Cardamom Extract and Chlorhexidine on Plaque and Gingivitis in Patients Using Orthodontic Appliance

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## ABSTRACT

**Introduction:** Dental plaque has been considered as the main factor in the initiation and progression of gingival and periodontal diseases. Various mouthwashes can be advised to reduce plaque and gingivitis.

**Objectives:** This clinic microbiological study was done to evaluate the Cardamom extract and chlorhexidine on plaque and gingivitis in patients using an orthodontic appliance.

**Methods:** The study included 40 subjects, with mild to moderate gingivitis and divided equally into 2 groups with 20 subjects in each as; Group 1-chlorhexidine gluconate mouthwash and Group 2- Cardamom extract mouthwash. Both the groups were directed to use 10 ml of mouthwash with an equal dilution of water for 1 min twice a day for thirty minutes after brushing. Parameters were noted for plaque and gingival index at day 0, 14 th, and 21 st day. The N-benzoyl-L-arginine-p- nitroanilide (BAPNA) assay was used to evaluate the trypsin-like activity of "red" complex microorganisms.

**Results:** On assessment between chlorhexidine and Cardamom extract mouthwash, the percentage reduction of the Plaque Index between 0 and 21 st day were 42.7336 and 42.7697, respectively (P=0.984), the percentage reduction of Gingival Index between 0 and 21 st day were 51.757 and 50.757, respectively (P=0.934) and percentage reduction of BAPNA values at 21 days was 41.0345 chlorhexidine and, 52.178 for cardamom.

**Conclusion:** Both cardamom and chlorhexidine mouth wash was helpful in the reduction of plaque and gingivitis.

**Key Words:** Cardamom extract, Chlorhexidine gluconate, Mouthwash

## INTRODUCTION

Dental plaque has been considered as the main factor in the initiation and progression of gingival and periodontal diseases.<sup>1</sup> A direct relationship has been established between plaque levels and the severity of gingivitis. The most sensible methodology toward the prevention of periodontal diseases would be the regular removal of plaque by personal oral hygiene measures. Plaque control can be done by mechanical and chemical method. The mechanical method includes brushing, use of interdental cleaning aids, flossing, and oral prophylaxis. These methods have proved to be very time-consuming procedures. Hence, chemical plaque control

can be used as an alternative to the mechanical plaque control method.

Chlorhexidine mouthwash though very effective also has some side effects such as; oral mucosal erosion, brown discoloration of the teeth, and bitter taste.<sup>2</sup> Henceforth, there is a need for alternate medicine to prevent plaque and gingivitis. Cardamom is the dried, unripened fruit of the perennial plant species "Elettaria cardamomum". It is also precious for its medicinal properties. The major constituents of the volatile oil are cineol, limonene, terpinene, sabinene, and terpineol in the form of formic and acetic acid. Cineole the main constituent of cardamom oil is an antiseptic that can kill bad

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breath bacteria and several others.<sup>3</sup> There is a lack of studies related to the Cardamom effect on plaque and gingivitis. Hence this clinic microbiological study was done to evaluate the Cardamom extract and chlorhexidine on plaque and gingivitis in patients using an orthodontic appliance

## MATERIALS AND METHODS

This study was done on 40 subjects in the Department of Periodontology and Oral Implantology. The inclusion criteria were; age 15 years and above, subjects with mild to moderate gingivitis, having at least 20 erupted teeth and subjects using a removable orthodontic appliance. Exclusion criteria were; the history of systemic diseases, pregnant, lactating females, history of any antibiotic therapy in the past 3 months, history of oral prophylaxis within 6 months previous to the study, mouth breathing and smoking habit.

The participants were evaluated for plaque and gingival inflammation using plaque index (Turesky-Gilmore-Glickman modification of the Quigley Hein 1970) and gingival index (Loe and Silness). After noting of gingival and plaque index, thorough scaling and polishing were done to get subjects at the baseline. Oral hygiene maintenance Instructions were given.

After 1 month, 40 subjects with mild to moderate gingivitis were included in the study. The study population was divided into two equal groups with 20 samples in each group. Group-1 – advised chlorhexidine gluconate mouthwash and Group-2 - advised experimental (Cardamom extract) mouthwash.

### Cardamom extract preparation

Fresh cardamom belongs to *Elettaria cardamomum* L. Maton spices was obtained from a local market. Cardamom fruits were cleaned and then ground to a fine powder in a mechanical grinder. Ten grams of this finely powdered cardamom was mixed with 100 ml of sterile deionized water and kept in a water bath in a round-bottomed flask at 55°C–60°C for 5 h and then filtered through sterile filter paper (Whatman®, UK). The aqueous extract was decanted, clarified by filtration through a muslin cloth, and evaporated in a porcelain dish at 40°C, which resulted in the dried extract. The chemical composition of cardamom powder was found out using standard methods. The investigation comprises the valuation of moisture, crude fat, crude protein, crude fibre and ash on a dry weight basis. Likewise, nitrogen-free extract (NFE) was deliberated conferring to the following expression:  $NFE = 100 - (\% \text{ moisture} + \% \text{ crude protein} + \% \text{ crude fat} + \% \text{ crude fibre} + \% \text{ ash})$ .<sup>4</sup>

For 21 days, both the groups were directed to rinse the mouth for one minute, 30 min after brushing twice daily using 10 ml of mouthwash with the equivalent dilution of water. The

findings were noted for plaque and gingival index at day baseline (0), at 14<sup>th</sup> day, and after 21<sup>st</sup> day.

The microbial evaluation was done using an N-benzoyl-L-arginine-p-nitroanilide assay. For microbiological evaluation, a total of 40 subjects (twenty from each group) were included. The supragingival plaque samples were obtained from the buccal surfaces of tooth numbers 16 and 36 with help of a sterile Gracey curette on baseline (0) and at 21<sup>st</sup> day. The supragingival plaque samples were transported in transport media-Tris-HCl (pH 6.8) for microbiological evaluation. The plaque samples were evaluated for assessing trypsin-like protease activity of the “Red Complex” microorganisms namely *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*.<sup>5</sup>

The plaque collected was placed in pre-weighed coded microcentrifuge tubes. A 1 ml quantity of a solution comprising the enzyme-substrate (N-benzoyl-L-arginine-p-nitroanilide, BAPNA; Sigma, St. Louis, Missouri, USA) was then mixed into the tubes. Then this suspension was positioned in an ultrasound bath for 10 min with 2-s cycles and 2-s intermissions at 17 W using a 100 W ultrasonic processor. After 17 h of incubation at 37°C, the reaction was halted with ice and by the addition of 100 ml of glacial acetic acid. The absorbance was read at 405 nm. The results were given in nanomoles of product per minute per milligram of dental plaque wet weight.<sup>6</sup>

The obtained data were statistically evaluated using paired t-test (Intragroup comparisons) and intergroup comparisons of post-treatment by unpaired t-test. A  $P < 0.05$  was measured as a statistically significant difference.

## RESULTS

Total 40 subjects were included for clinical study with 20 samples in each group. At baseline, there was no statistically substantial alteration amongst the two groups with respects to plaque index and gingival index (Tables 1-4). The Plaque index (PI) was decreased during the study in both groups, but there was no statistically significant difference between the two groups (Tables 1,2).

### Plaque Index

For Chlorhexidine mouthwash (Group 1) the mean plaque index (PI) was  $3.203 \pm 0.4678$ ,  $1.757 \pm 0.4124$  and  $1.146 \pm 0.2345$  at day 0, 14 days and at day 21. The difference of mean plaque index between 0 and 14<sup>th</sup> day was 1.761 and between 0 and 21<sup>st</sup> day was 2.505 and it was statistically significant ( $P < 0.001$ ) (Table 1). For Cardamom extract mouth wash (group 2) the mean PI value was  $3.209 \pm 0.3478$ ,  $1.7655 \pm 0.2744$  and  $0.970 \pm 0.2509$  at day 0, 14 days at day 21 respectively. The difference of mean plaque index between 0 and 14<sup>th</sup> day was

1.600 and between 0 and 21<sup>st</sup> the day was 2.458 which was statistically significant ( $P<0.001$ ) (Table 1). On the comparison between chlorhexidine with cardamom extract mouthwash, the percentage reduction of PI between 0 and 14<sup>th</sup> day were 42.7336 and 42.7697, respectively ( $P=0.984$ ). The percentage reduction of PI between 0 and 21<sup>st</sup> day were 63.087 and 67.548 respectively ( $P=0.968$ ) (Table 2).

### Gingival index

Gingival index score was decreased during the study in both groups, but no statistically substantial variance was observed among the two groups (Table 3, 4). For Chlorhexidine mouthwash (group 1) the mean GI was  $1.797\pm 0.134$ ,  $0.843\pm 0.141$  and  $0.679\pm 0.134$  at 0 days, 14 days, and at 21 days respectively. The difference of mean gingival index between 0 and 14 the day was 1.191 and between 0 and 21<sup>st</sup> the day was 1.246 which was statistically significant ( $P<0.001$ ) (Table 3). For cardamom extract mouthwash (group 2) the mean GI value was  $1.757\pm 0.125$ ,  $0.786\pm 0.165$  and  $0.640\pm 0.124$  at 0,  $0.833\pm 0.199$  at 0 days, 14 days, and at 21 days respectively. The difference of mean plaque index between 0 and 14<sup>th</sup> day was 1.040 and between 0 and 21<sup>st</sup> the day was 1.324 which was statistically significant ( $P<0.001$ ) (Table 3).

On the comparison between chlorhexidine and cardamom extract mouthwash, the percentage reduction of GI between 0 and 14<sup>th</sup> day were 51.757 and 50.757, respectively ( $P=0.934$ ). The percentage reduction of PI between 0-21<sup>st</sup> day were 63.045 and 62.153 respectively ( $P=0.354$ ) (Table 4).

Table 5 showed the percentage of BAPNA values for Microbial evaluation. At 0-14 days, the value was 28.5789 for chlorhexidine and 29.4656 for cardamom and at 21 days it was 41.0345 chlorhexidine and, 52.178 for cardamom.

## DISCUSSION

In dentistry, Chlorhexidine has been considered a gold standard for the prevention of gingivitis and plaque. A greater amount of plaque reductions were observed with chlorhexidine gluconate mouth wash.<sup>6-9</sup> The results indicated that daily 2 times mouth rinses with 0.2% chlorhexidine gluconate efficiently prevented plaque formation.<sup>6</sup> In our study, chlorhexidine mouthwash showed a significant reduction in mean plaque score at 0, 14<sup>th</sup> and 21<sup>st</sup> day from baseline.

In studies done by Van der Weijden et al.<sup>10</sup> Grundemann et al.<sup>11</sup>, Leyes et al.<sup>12</sup> showed a significant reduction of gingival inflammation with chlorhexidine mouthwash which is similar to our findings. Our clinical study was supported by the microbiological assessment which strongly proved the efficacy of experimental mouthwash. The microbial assessment was done by the BAPNA assay which quantitatively assesses trypsin-like enzyme activity of important periodon-

tal pathogens like *T. forsythia*, *Treponema denticola*, and *P. gingivalis* in the plaque sample.<sup>5</sup>

Ribeiro et al. evaluated the effect of mechanical supragingival plaque control on clinical and biochemical factors of chronic periodontitis. The biochemical assessment was performed using the BAPNA test. The results indicated a reduction in plaque score from  $51.44\pm 20.78$  to  $38.64\pm 12.34$  ( $P=0.04$ ). Similarly, a decrease in BAPNA values was observed in the present study. Tarte et al. assessed the efficacy of Cardamom and Chlorhexidine Extract in the reduction of bacterial Load in Dental Aerosols. They concluded that both were effective in the reduction of bacterial load.<sup>13,14</sup> Meenapriya and Geetha evaluated the antimicrobial effectiveness of herbal mouthwash over chlorhexidine and concluded that herbal products exhibited less antimicrobial efficacy compared to the commercially available chlorhexidine mouthwash.<sup>15</sup> Prasad et al. assessed the Anti-Plaque Efficacy of Herbal and 0.2% Chlorhexidine Gluconate and found similar antiplaque activity.<sup>16</sup>

When studied microbiologically it was found that reduction in mean BAPNA values (that permits the detection of microorganisms possessing trypsin-like enzymes such as *T. forsythensis*, *Treponema denticola* and *P. gingivalis*) was found to be similar in both the groups (Group-1 and 2) after 21 days. On microbiological evaluation, both the mouthwashes were observed equally effective.

## CONCLUSION

From the present findings, it can be concluded that Cardamom has an equivalent effect to chlorhexidine mouthwash in the reduction of plaque and gingivitis. Microbiologically, both the mouthwashes were equally efficient. Chlorhexidine gluconate, as well as cardamom extract mouthwash, can be effectively used as an adjunct to mechanical plaque control in the prevention of plaque and gingivitis.

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**Author Contribution**

Dr. SNS- Manuscript writing

Dr. SB- Editing

Dr. VK- Evaluation

Dr. PS- Editing

Dr. JKS- Analysis

Dr. AK- Data collection, Investigation

## REFERENCES

1. Fine HD. Chemical agents to prevent and regulate plaque development. *Periodontology* 2000 1995;8:87-107.
2. Jan L. Textbook of Clinical Periodontology and Implantology Dentistry. 4<sup>th</sup> ed. Blackwell Munksgaard. 2003;4:477.

- Souissi M, Azelmat J, Chaieb K, Grenier D. Antibacterial and anti-inflammatory activities of cardamom (*Elettaria cardamomum*) extracts Potential therapeutic benefits for periodontal infections. *Anaerobes* 2019;5:234-237.
- Abdullah, Asghar A, Butt SM, Shahid M, Huang Q. Evaluating the antimicrobial potential of green cardamom essential oil focusing on quorum sensing inhibition of *Chromobacterium violaceum*. *J Food Sci Technol* 2017;54(8):2306–2315.
- Syed SA, Gusberti FA, Loesche WJ, Lang NP. Diagnostic potential of chromogenic substrates for rapid detection of bacterial enzymatic activity in health and disease-associated periodontal plaques. *J Periodontol Res* 1984;19:618-621.
- Loe H, Schiott C. The effect of mouth rinse and topical application of chlorhexidine on the development of dental plaque and gingivitis. *J Periodontal Res* 1970;5:79–83.
- Lindhe J, Hamp SE, Loe H, Schiott CR. Influence of topical applications of chlorhexidine on chronic gingivitis and gingival wound healing. *Scand J Dent Res* 1970;78:471-478.
- Ernst CP, Prockl K, Willershemsen B. The effectiveness and side effects of 0.1% chlorhexidine mouth rinse. *Quint Int* 1998;29:443-448.
- Francetti L, del Fabbro M, Testori T, Weinstein RL. Chlorhexidine spray versus chlorhexidine mouthwash in the control of dental plaque after periodontal surgery. *J Clin Period* 2000;27:425-430.
- Van der Weijden GA, Timmer CJ, Timmerman MF, Reijerse E, Mantel MS, et al. The effect of herbal extract in an experimental mouthrinse on established plaque and gingivitis. *J Clin Period* 1998;25:399-403.
- Grundenmann LJ, Timmerman MF, Ijseman Y, van der Velden U, van der Weijden GA. Reduction of stain, plaque and gingivitis by mouthrinsing with chlorhexidine and sodium perborate. *Ned Tijdschr Tandhe* 2002;109:225-229.
- Leyes Borraro JL, Garcia VL, Lopez CG, Rodriguez-Nuñez I, Garcia FM, Gallas TM, et al. Efficacy of chlorhexidine mouth rinses with and without alcohol: A clinical study. *J Periodontol* 2002;73:317-321.
- Ribeiro ED, Bittencourt S, Nociti-Junior FH, Sallum EA, Sallum AW, et al. The effect of one session of supragingival plaque control on clinical and biochemical parameters of chronic periodontitis. *J Appl Oral Sci* 2005;13:275–279.
- Tarte PV, Moolya N, Rajhans N, Mhaske N. Comparative Evaluation of the Cardamom and Chlorhexidine Extract as an Ultrasonic Coolant for Reduction of Bacterial Load in Dental Aerosols. *Inter J Dent Sci Innove Res* 2020;240–247.
- Meenapriya M, R. V. Geetha RV. Comparative evaluation of antimicrobial efficacy of herbal mouthwash versus commercially available mouthwash 2% chlorhexidine. *Drug Inven Tod* 2019;11(10):2687-2689.
- Varma R, Prasad KA, John S, Deepika V, Dwijendra KS, Ram Reddy B, et al. Anti-Plaque Efficacy of Herbal and 0.2% Chlorhexidine Gluconate Mouthwash: A comparative Study. *J Inter Oral Health* 2015;7(8):98-102.

**Table 1: Plaque index**

Interval	Chlorhexidine (Group 1)				Cardamom extract (Group 2)			
	Mean PI±SD	Difference form base line	t value	P value	Mean PI±SD	Difference form base line	t value	P value
0 day	3.203±0.467	-	-		3.209±0.347	-	-	
14 day	1.757±0.412	1.761	30.16	0.000	1.765±0.274	1.600	22.02	0.000
21 day	1.146±0.234	2.505	51.30	0.000	0.970±0.250	2.458	32.74	0.000

PI- plaque index

**Table 2: Percentage reduction of plaque index**

	0-14 days	0-21 days
Cardamom		67.548
Chlorhexidine	42.7336	63.087
Difference between the groups		
t value	0.007	0.467
P value	0.984 NS	0.968 NS

**Table 3: Gingival index**

Interval	Chlorhexidine				Cardamom			
	Mean GI±SD	Difference form base line	t value	P value	Mean GI±SD	Difference form base line	t value	P value
0 day	1.797±0.134	-	-		1.757±0.125	-	-	
14 day	0.843±0.141	1.191	31.14	0.000	0.786±0.165	1.040	20.04	0.000
21 day	0.679±0.134	1.246	36.28	0.000	0.640±0.124	1.324	25.67	0.000

GI- gingival index

**Table 4: Percentage reduction of gingival index**

	0-14 days	0-21 days
Cardamom	50.757	62.153
Chlorhexidine	51.757	63.045
Difference between the groups		
t value	0.085	0.345
P value	0.934 NS	0.354 NS

**Table 5: Percentage reduction of BAPNA values**

	0-14 days	0-21 days
Cardamom	29.4656	52.178
Chlorhexidine	28.5789	41.0345
Difference between the groups		
t value	0.285	1.4766
P value	0.634	0.154

Unpaired t test, BAPNA-N-benzoyl- L-arginine, P-nitroanalide