Comparing the effect of three different mouthwashes cranberry, HIORA and chlorhexidine on the management of periodontal diseases

Diksha Sayal[1], Ridhima Sood[2,*], Nidhi Yadav[3], Bhavna Jha[4], Vidya Dodwad[5]

[1,2,3]Ex-PG Student, [4]Senior Lecturer, [5]HOD, Dept. of Periodontology & Implantology, ITS Centre for Dental Studies & Research, Muradabad, Ghaziabad, Uttar Pradesh

***Corresponding Author:**
Email: soodyr@gmail.com

Abstract

Background: The aim was to compare the effect of three different mouthwashes cranberry, hiora and chlorhexidine on the management of localized periodontitis

Materials and Methods: The study model comprised of three groups and 10 subjects will be taken in each group(5 sites each) for sample collection at baseline and 21 days. Sites in each group were irrigated with chlorhexidine, hiora and cranberry mouthwashes. In each group sites with pocket depth more than or equal to 4mm and less than 7mm were selected. The plaque samples which were taken from the subjects on baseline were inoculated on blood agar plates and colony count was taken.

Results: The mean change in the GI, PI within the groups when compared from baseline to 21 days was found to be statistically significant. At 21 days the mean PD was found to be 2.90±0.57 in group 1, 3.90±0.57 in group 2 and 2.30±0.48 in group 3. The mean % difference in the microbial count(Aa) from baseline to 21 days was found to be less in group 2 (32.14%) when compared with group 1 (70.93%)and group 3(62.04%). The microbial count (Pg) from baseline to 21 days was found to be less in group 2 (24.03%) when compared with group 1 (50.98%) and group 3(66.46%).

Conclusion: Herbal products like Cranberry can prove to be effective or better alternatives to Chlorhexidine in improving the oral health with added systemic benefits and minimal side effects.

Keywords: Chlorhexidine, Cranberry extract, Prevotella intermedia, Porphyromonas gingivalis and Actinobacillus actinomycetumcomitans

Introduction

Periodontitis is an inflammatory disease of the tooth supporting tissues. The disease is initiated by an overgrowth of specific gram-negative anaerobic bacteria that leads to gingival connective tissue destruction and irreversible alveolar bone resorption.[1,2] A number of chemical agents which have antiseptic or antimicrobial action have been used with variable success to inhibit supragingival plaque formation and the development of gingivitis.[3] Chlorhexidine is the most effective antiseptic for plaque inhibition and prevention of gingivitis as well as as an adjunct treatment for periodontitis when used twice daily as mouth rinse.[4]

Hiora mouthwash is a herbal preparation, made from natural herbs with their beneficial properties like anticariogenic and antiplaque (due to *S. persica* which contains trimethyl amine, salvadorine, chlorides, high amounts of fluoride and silica, sulphur, vitamin C, small amounts of tannins, saponins, flavonoids, sterols, antibiotic (due to the presence of *Piper betle* and *Elettaria cardamomum*) and anti-inflammatory and immunity booster (due to the presence of Terminalia bellerica). *Mentha and Trachyspermum ammi* which are natural flavouring agents.[5]

Cranberry (*Vaccinium macrocarpon*), the native North American fruit has recently come into limelight owing to its numerous beneficial effects on health. In the globally functional food industry, the fruit is popularly known for its nutrient and antioxidant qualities and is referred to as a supper fruit.[6,7,8] It is a unique rich source of several classes of bioactive flavonoids including flavanols, anthocyanins and proanthocyanidins enhancing its therapeutic potential.[9]

Experimentally clinical studies revealed that the NDM fraction of cranberry hindered the colonization of by *Porphyromonas gingivalis and Fusobacterium nucleatum* in the gingival crevice.[10] It also prevented the adhesion of *P. gingivalis* to various proteins including type I collagen thus reducing bacterial coaggregation in periodontal diseases.[11] Cranberry restrains the proteolytic activity of the red complex specifically the gingipain activity of *P. gingivalis*, trypsin like activity of *Tanerella forsythia* thus restricting their growth resources from amino acids, peptides and also inhibit the tissue destruction mediated by bacterial proteinases.[12,13]

Materials and Method

The study was conducted on the patients visiting the Outpatient Department of Periodontology and Implantology, ITS-CDSR Ghaziabad was conducted in accordance with the Helsinki Declaration of 1975 revised in 2000. The study model comprised of three groups with 10 subjects in each group. 5 sites in each (total 30 sites) were taken for sample collection at baseline and 21 days. In each group sites with pocket depth more than or equal to 4mm and less than 7mm were selected. Subjects with chronic gingivitis with localized pockets PD ≥ 4, with good general health and

were in agreement to comply with the study visits were included in the study. Subjects using mouthwash or dental floss; tobacco consumers and subjects with medical or pharmacological history that could compromise the conduct of the study were excluded. Group 1 received SRP followed by sub gingival irrigation with 0.2% Chlorhexidine solution, in Group 2 SRP followed by sub gingival irrigation with Hiora solution and in Group 3 SRP followed with subgingival irrigation with 5 ml of 99.8% of cranberry extract diluted in 60 ml of distilled water was done.(Fig. 2 & 3)

Fig. 1: Cranberry, hiora and 0.2% Chlorhexidine mouthwashes loaded in syringes and the transport media

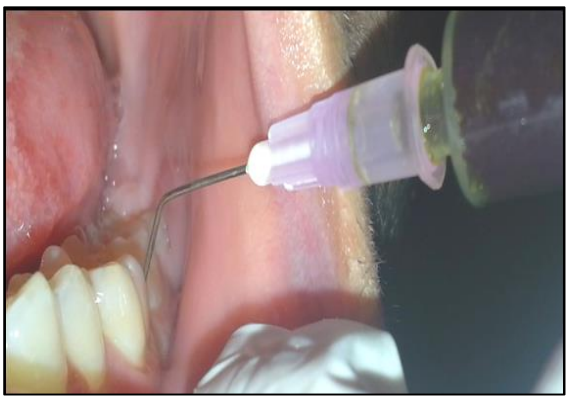


Fig. 2: Subgingival irrigation with cranberry at the selected site

Fig. 3: Subgingival irrigation with hiora and 0.2% chlorhexidine at the selected sites

All the selected patients were subjected to supragingival scaling to prevent contamination of subgingival plaque samples. The criteria used for clinical evaluation were Turesky Gilmore-Glickman modification of Quigley-Hein plaque index[14], Gingival index (Loe and Silness)[15] and Probing pocket depth (PD) was recorded as the distance between the base of the pocket and gingival margin. Measurement was recorded by using UNC-15 probe. PPD was measured at four places around each tooth namely mesiofacial, buccal, distofacial and lingual. Recordings were taken at baseline and followed by 21 days respectively.

The baseline plaque samples were obtained from the depth of the periodontal pocket using a sterlized Gracey curette parallel to the long axis of the tooth and moved coronally by scraping along the root surface. These plaque samples were placed in airtight thioglycolate broth transport media supplemented with Hemin and Vitamin K in a 5 ml test tube. The collected plaque samples were immediately sent to the lab within 48 hours of collection for microbial culture of ***Prevotella intermedia***, ***Porphyromonas gingivalis*** and ***Actinobacillus actinomycetumcomitans*** now (***Agregatibacter actinomycetemcomitans)***. The subgingival scaling was performed using ultrasonic scalers along with root planing. A syringe (2 ml 24 gauge) was taken and the needle was bent along its shank at an angle of approximately 110 degrees and

inserted in the pocket for sub gingival irrigation. Each site was irrigated with 1 ml of solution over 20 seconds. Triple irrigation regimen was repeated at an interval of 5 minutes in each respective groups on the same day at baseline.(Fig. 1)

Microbiological procedure: Samples were then mixed vortexing and 10ml of it were inoculated on to the media to see the growth of microorganisms. Supplemented Blood Agar for pigmented anerobes (Pg and Pi) was used and Dentaid media (Yeast extract, sodium fumerate, sodium formate and Vancomycin) for Aa.(Fig. 3) The media was incubated anaerobically in a modified gas pack for 5 days.(Fig. 4 & 5) The identification of the organisms were carried out by colony characters, pigmentation, Gram stain appearance and certain standard key biochemical reaction. The quantity of colonies was carried out by counting the number of each type of colonies with the digital colony counter with magnifying glass and multiplying by diluted factor i.e. 100 and expressed as colony forming units (CFU/ml).(Fig. 6)

Fig. 4: Dentaid media and bacteriods bile esculis agar for anaerobic culture

Fig. 5: Anaerobic glass jar and incubator for bacterial culture

Fig. 6: Colony counting unit and magnifier for colony count

Statistical analysis: The data were carried out using a computer software program (SPSS version 17). One way ANOVA tests were used to identify significant differences between the means of the study groups. Finally, paired t-tests and post hoc bonferroni were used to assess the significance of changes within each group between time periods. Critical p values of significance were set at 0.05 (confidence of 95%).

Results

The mean GI scores at baseline for group 1 and group 2was 3.14±0.39 (mean±SD) and group 3 3.17±0.41. At 21 days the mean GI was found to be 1.75±0.54 in group 1, 2.02±0.30 in group 2 and 1.35±.47 in group 3 respectively. The mean GI within the groups and the difference within the groups was found to be statistically non significant at baseline in all the groups but at 21 days statistically significant results were seen. However the mean change in the GI within the groups when compared from baseline to 21 days was found to be statistically significant ($p<0.05$).(Table 1)

Table 1: Intragroup comparison of gingival index

GI	Groups	Mean	Std. Deviation	Std. Error	F-value	p-value
At Baseline	Group 1	3.14	0.39	0.12	0.019	0.981
	Group 2	3.14	0.39	0.12		
	Group 3	3.17	0.41	0.13		
At 21 days	Group 1	1.75	0.54	0.17	5.593	0.009*
	Group 2	2.02	0.30	0.10		
	Group 3	1.35	0.47	0.15		
% diff	Group 1	1.39	0.48	0.15	5.781	0.008*
	Group 2	1.12	0.32	0.10		
	Group 3	1.82	0.56	0.18		

The mean PI scores at baseline for group 1, group 2 and group 3 was 2.45±0.37 (mean± SD). At 21 days the mean PI was found to be 1.25±0.42 in group 1, 1.65±0.24 in group 2 and 1.20±0.42 in group 3 respectively. The mean difference within the groups was found to be statistically non significant at baseline in all the groups but at 21 days statistically significant results were seen. The mean change in the PI within the groups when compared from baseline to 21 days was found to be statistically significant. ($p<0.05$)(Table 2)

Table 2: Intragroup comparison of plaque index

PI	Groups	Mean	Std. Deviation	Std. Error	F-value	p-value
At Baseline	Group 1	2.45	0.37	0.12	0.000	1.000
	Group 2	2.45	0.37	0.12		
	Group 3	2.45	0.37	0.12		
At 21 days	Group 1	1.25	0.42	0.13	4.380	0.023*
	Group 2	1.65	0.24	0.08		
	Group 3	1.20	0.42	0.13		
%diff	Group 1	1.20	0.48	0.15	2.597	0.043*
	Group 2	0.80	0.42	0.13		
	Group 3	1.25	0.54	0.17		

The mean PD scores at baseline for group 1, group 2 and group 3 was 5.10±0.74. At 21 days the mean PD was found to be 2.90±0.57 in group 1, 3.90±0.57 in group 2 and 2.30±0.48 in group 3 respectively. The mean difference within the groups was found to be statistically non significant at baseline in all the groups but at 21 days statistically significant results were seen. The mean change in the PD within the groups when compared from baseline to 21 days was found to be statistically significant ($p<0.05$).(Table 3)

Table 3: Intragroup comparison of probing index

PD	Groups	Mean	Std. Deviation	Std. Error	F-value	p-value
At Baseline	Group 1	5.10	0.74	0.23	0.000	1.000
	Group 2	5.10	0.74	0.23		
	Group 3	5.10	0.74	0.23		
At 21 days	Group 1	2.90	0.57	0.18	22.329	<0.001*
	Group 2	3.90	0.57	0.18		
	Group 3	2.30	0.48	0.15		
%diff	Group 1	2.20	0.63	0.20	13.781	<0.001*
	Group 2	1.20	0.42	0.13		
	Group 3	2.80	0.92	0.29		

The post hoc comparison of the scores showed that the mean GI reduced significantly from baseline to 21 days respectively in but statistically significant mean percentage difference from baseline to 21 days was seen between group 2 and group 3. The mean PI reduced significantly from baseline to 21 days respectively in but statistically significant mean percentage difference from baseline to 21 days was seen between group 2 and group 3. ($p<0.05$) The mean PD reduced significantly from baseline to 21 days but statistically significant mean percentage difference from baseline to 21 days was seen between group 2 and group 3.(Table 4)

Table 4: Intergroup comparison of various clinical parameters

Clinical Parameters	Time Interval	Intergroup Comparison		Mean Difference	p-value
Gingival Index	At baseline	Group 1	Group 2	0.00	1.000
		Group 1	Group 3	-0.03	1.000
		Group 2	Group 3	-0.03	1.000
	At 21 days	Group 1	Group 2	-0.27	0.575
		Group 1	Group 3	0.40	0.172
		Group 2	Group 3	0.67	0.008*
	%diff	Group 1	Group 2	0.27	0.614
		Group 1	Group 3	-0.43	0.144
		Group 2	Group 3	-0.70	0.007*
Plaque Index	At baseline	Group 1	Group 2	0.00	1.000
		Group 1	Group 3	0.00	1.000
		Group 2	Group 3	0.00	1.000
	At 21 days	Group 1	Group 2	-0.40	0.071
		Group 1	Group 3	0.05	1.000
		Group 2	Group 3	0.45	0.035*
	%diff	Group 1	Group 2	0.40	0.227
		Group 1	Group 3	-0.05	1.000
		Group 2	Group 3	-0.45	0.042*
Probing Depth	At baseline	Group 1	Group 2	0.00	1.000
		Group 1	Group 3	0.00	1.000
		Group 2	Group 3	0.00	1.000
	At 21 days	Group 1	Group 2	-1.00	0.001*
		Group 1	Group 3	0.60	0.059
		Group 2	Group 3	1.60	<0.001*
	%diff	Group 1	Group 2	1.00	0.009*
		Group 1	Group 3	-0.60	0.185
		Group 2	Group 3	-1.60	<0.001*

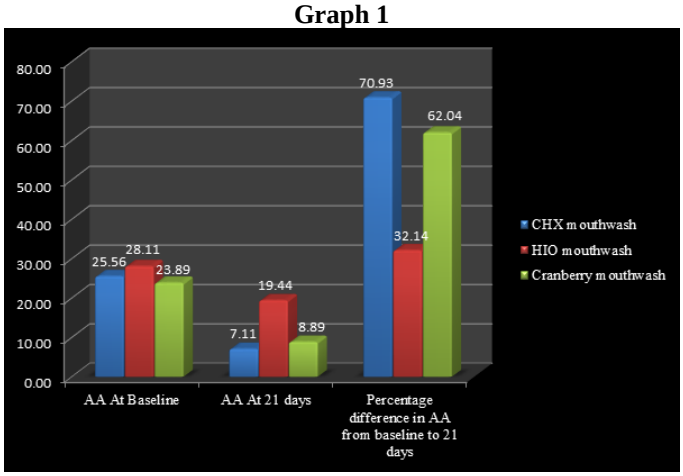
The independent t-test was used to compare the microbial count of the 3 microorganisms Aa, Pg, Pi be from baseline to 21 days and the three groups showed statistically significant decrease in all the three microbes that were assesed.(Table 5) However the mean % difference in the microbial count(Aa) from baseline to 21 days was found to be less in group 2 (32.14%) when compared with group 1 (70.93%) and group 3(62.04%) (Graph 1). The microbial count (Pg) from baseline to 21 days was found to be less in group 2 (24.03%) when compared with group 1 (50.98%) and group 3(66.46%). (Graph 2) Similarly the microbial count(Pi) from baseline to 21 days was found to be less in group 2 (32.72%) when compared with group 1 (55.22%) and group 3(60.07%).(Graph 3)

Table 5: Comparison of microbial count between the groups

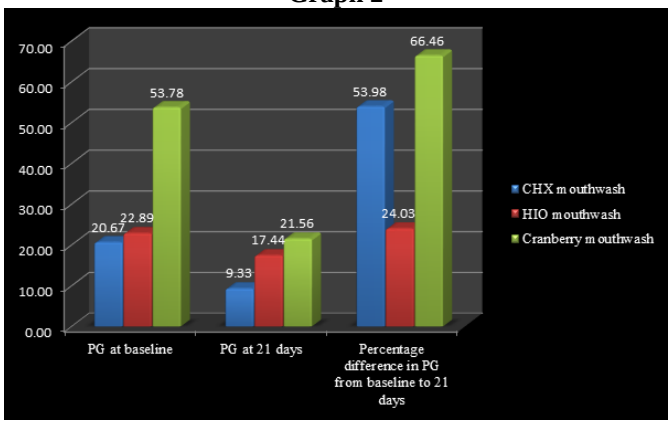
	Time interval	Micro	Mean	Std. Deviation	Mean difference	t-test value	p-value
Group 1	Baseline	Aa	25.56	6.82	18.44	9.816	< 0.001*
	21 days	Aa	7.11	2.52			
	Baseline	Pg	20.67	16.40	11.33	3.545	0.008*
	21 days	Pg	9.33	7.58			
	Baseline	Pi	41.11	12.69	23.44	6.247	< 0.001*
	21 days	Pi	17.67	6.63			
	Baseline	AA	28.11	9.47	8.67	4.333	0.003*
Group 2	21 days	AA	19.44	10.44			

	Baseline	PG	22.89	14.63	5.44	4.249	0.003*
	21 days	PG	17.44	12.03			
	Baseline	PI	41.44	16.55	12.89	4.877	0.001*
	21 days	PI	28.56	15.04			
Group 3	Baseline	AA	23.89	7.41	15.00	8.050	< 0.001*
	21 days	AA	8.89	3.33			
	Baseline	PG	53.78	45.90	32.22	3.861	0.005*
	21 days	PG	21.56	22.83			
	Baseline	PI	36.44	19.26	21.67	5.455	0.001*
	21 days	PI	14.78	8.94			

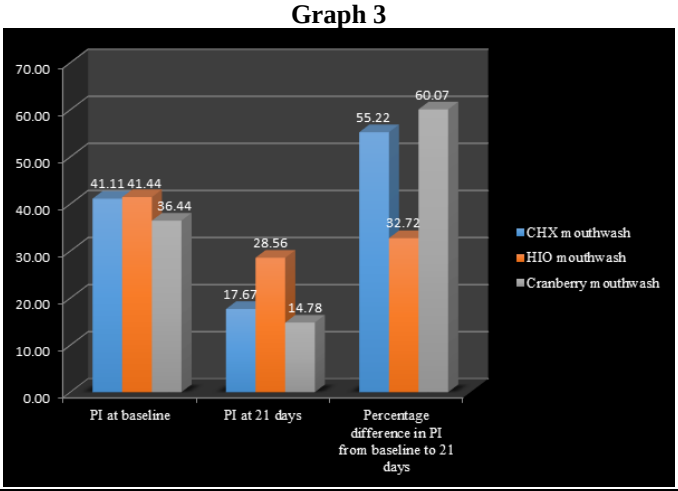
Graph 1



Graph 2



Graph 3



Discussion

The aim of the present study was to compare the effectiveness of 0.2% chlorhexidine, hiora mouthwashes and cranberry juice in chronic gingivitis patients with localised pockets.

The mean PI, GI and PD scores between the groups was found to be statistically non significant at baseline in all the groups but at 21 days statistically significant results were seen. However the mean change in the PI, GI and PD scores between the groups when compared from baseline to 21 days was found to be statistically significant.The reduction in PD scores in group 1 are in accordance with the study conducted by ***Soskolne et al[16] and Quirynen M et al.[17]***

Cranberry juice has been used in herbal medicine as an anti-infection agent. The high molecular- weight non-dialysable material (NDM) fraction constituent of the juice exhibits anti-co-aggregation activity against a variety of oral bacteria responsible for periodontitis. This provided the impetus to assess the effectiveness of Cranberry as an anti-adhesion agent against Aa, Pg, Pi according to ***Weiss etal in 1999***.[18]

Chlorhexidine solution has been identified as an appropriate chemotherapeutic agent at a concentration of 0.2%. It is effective against the sub gingival flora in deep periodontal pockets. Studies have reported the anti-microbiological and clinical effects of a single subgingival irrigation of chlorhexedine in advanced periodontal lesions. In the present study there was significant reduction in Aa (70.93% in group 1) similar results were achieved in a study conducted by ***Sanjay etal[19]*** and the microbiological study conducted by ***Daneshmand N et al***[20]. The mean percentage difference in the microbial count(Aa) from baseline to 21 days was found to be less in group 2 (32.14%) when compared with group 1 (70.93%)and group 3.

In the present study there was significant mean percentage reduction in Pg in group 3(66.46%) followed by group 1(50.98%) and least was seen in group 2. The results are in accordance with study conducted by ***Yamanaka etal in 2001[21]*** who showed that cranberry polyphenol efficiently inhibited Arg-gingipain and Lys-gingipain activities in P. gingivalis ***Kadowaki et al***[22] reported that gingipain inhibitors reduced the pathogenicity of P. gingivalis The mean percentage difference in the microbial count (Pg) baseline to 21 days was found to be less in group 2 (24.03%) when compared with group 1 (50.98%) and group 3(66.46%) whereas the mean percentage difference in the microbial count(Pi) from baseline to 21 days was found to be less in group 2 (32.72%) when compared with group 1 (55.22%)and group 3(60.07%).

Conclusion

It is suggests that herbal products like Cranberry can prove to be effective or better alternatives to Chlorhexidine in improving the oral health with added systemic benefits and minimal side effects. Further scope lies in the long-term evaluation of the advantages and side effects of such herbal extracts. Newer techniques need to be developed to incorporate the concentrated polyphenolic fraction in toothpastes, mouth rinses, and other oral hygiene products.

References

1. Newman MG, Carranza FA, Takei H, Klokkevold PR. Carranzas clinical Periodontology 10th ed. Elsevier health sciences;2006.
2. Marsh PD. Dental plaque as a biofilm and microbial community-implication for health and diseases. BMC Oral Health 2006;6:814.
3. Mandel ID. Chemotherapeutic agents for controlling plaque and gingivitis. J Clin Periodontol 1988;15:488-98.
4. Ribeiro LG, Hashizume LN, Maltz M. The effect of different formulations of chlorhexidine in reducing levels of mutans streptococci in the oral cavity: A systematic review of the literature. J Dent 2007;35:359-70.
5. Rose Kanwaljeet Kaur, M P Singh, Rohit Chopra, Archana Bhatia. Evaluation of Efficacy of Three Commercially Available Herbal Mouthwashes in Treatment of Chronic Gingivitis: A Comparative Clinical Study. Int J Dent Med Res 2014;14:42-46.
6. Newsroom - 'Super fruits' the future of health. Hort Research. (Last retrieved on 2009 Nov 13).
7. Eck P. The American cranberry. New Brunswick, NJ: Rutgers University Press; 1990.
8. Bodet C, Chandad F, Grenier D. Cranberry components inhibit interleukin-6, interleukin-8, and prostaglandin E2 production by lipopolysaccharide-activated gingival fibroblasts. Eur J Oral Sci 2007;115:64–70.
9. Bodet C, Chandad F, Grenier D. Anti-inflammatory activity of a high-molecular-weight cranberry fraction on macrophages stimulated by lipopolysaccharides from periodontopathogens. J Dent Res 2006;85:235-9.
10. Yamanaka-Okada A, Sato E, Kouchi T, Kimizuka R, Kato T, Okuda K. Inhibitory effect of cranberry polyphenol on cariogenic bacteria. Bull Tokyo Dent Coll 2008;49:107-12.
11. Labrecque J, Bodet C, Chandad F, Grenier D. Effects of a high molecular-weight cranberry fraction on growth, biofilm formation and adherence of Porphyromonas gingivalis. J Antimicrob Chemother 2006;58:439-43.
12. Bodet C, Piche M, Chandad F, Grenier D. Inhibition of periodontopathogen-derived proteolytic enzymes by a high-molecular-weight fraction isolated from cranberry. J Antimicrob Chemother 2006;57:685-90.
13. Turesky S, Gillmore ND, Glickman 1. Reduced plaque formation by the chloromethyl analogue of vitamin C. J Periodontol 1970;41:41.
14. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963;21:533-551.
15. Soskolne WA, Heasman PA, Stabholz A, Smart GJ, Palmer M, Flashner M, Newman HN. Sustained local delivery of chlorhexidine in the treatment of periodontitis: A multi-center study. J Periodontol 1997;68(1):32-38.
16. Quirynen. M, Avontroodt. P, Peeters. W. Effect of different chlorhexidine formulations in mouthrinses on de novo plaque formation. J Clin Periodontol 2001;28;1127-1136.
17. Weiss EI, Lev-Dor R, Kashman Y, Goldhar J, Sharon N, Ofek I. Inhibitory effect of a high-molecular-weight constituent of cranberry on adhesion of oral bacteria. Crit Rev Food Sci Nutr 2002;42:285–29.

18. Devki B, Sanjay J and Sangeeta M. The comparative effects of 0.12% chlorhexidine and herbal oral rinse on dental plaque induced gingivitis. A randomized clinical trial J Indian Soc Periodontal 2015;19(4):393-395.
19. Daneshmand N, Jorgensen MG, Nowzari H, Morrison JL, Slots J. Initial effect of controlled release chlorhexidine on subgingival microorganisms. J Periodontal Res. 2002;37(5):375-9.
20. Yamanaka A, Kimizuka R, Kato T, Okuda K. Inhibitory effects of cranberry juice on attachment of oral streptococci and biofilm formation. Oral Microbiol Immunol 2004;19:150–154.
21. Kadowaki T, Baba A, Abe N et al. Suppression of pathogenicity of Porphyromonas gingivalis by newly developed gingipain inhibitors. Mol Pharmacol 2004;66:1599–1606.