International Journal of Health Sciences and Research
Volume 14; Issue: 11; November 2024
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

Comparative Evaluation between Cranberry, Guava Leaf and Chlorhexidine (0.2%) Mouthwashes on Oral Microbiota and pH Among Interns in a Dental College in Rajahmundry City - A Randomized Control Trail

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DOI: https://doi.org/10.52403/ijhsr.20241123

ABSTRACT

Introduction: Oral cavity is the mirror of our body hence protecting it from various microorganisms is essential. Natural sources of medicine like Cranberry and guava leaf are gaining good attention these days, and were found to exhibit antimicrobial activity: However, their use is less explored in combating oral disease
Aim: To compare the efficiency between 0.2% Chlorhexidine, 0.6% Cranberry and 0.5% guava leaves extract mouthwashes on their antibacterial activity and salivary pH.
Materials and methods: The present study is a randomized clinical trial conducted on 30 subjects (>18years) who volunteered to participate in the study. (0.2%) Chlorhexidine, (0.6%) Cranberry, (0.5%) Guava leaf extract mouthwashes, sterile swab, and pH strips were used. The study was conducted over a period of one week. The base line pH and microbial count was calculated for all the subjects before start of the study. Using double blinding each participant was given a random mouth wash and were asked to rinse twice a day after 30 minutes of brushing. After intervention all the groups were checked for the difference in the microbial count and salivary pH.
Results: The difference in mean values before and after intervention for Cranberry (28.3%) and Guava leaf (28.5%) was very similar to chlorhexidine (25.8%), proving all the three mouthwashes are equally potent. There was no significant difference between mean salivary pH.
Conclusion: Both Cranberry and guava leaf mouthwash were equally effective as Chlorhexidine with additional benefits of being herbal. Hence, they can be used as an alternative to Chlorhexidine.

Keywords: Chlorhexidine, Cranberry, Guava leaf, Oral Microbiota, pH

INTRODUCTION

Oral biofilm formation is a daily natural process however, its presence is an important step for caries formation. It was found that streptococcus mutans are not the only basic bacterium involved in the

development of plaque, but they are also involved in the initiation of caries. Current methods of combating caries-associated bacteria are usage of physical aids. [1]

Although toothbrushing and dental flossing are the most dependable mechanical plaque removal methods, some people lack knowledge on proper plaque control. As a result, several chemical anti-plaque agents have been introduced as mouthwashes to improve oral health and to maintain good oral hygiene. Chlorhexidine is still the gold standard for its antimicrobial action and high substantiveness, but side effects, such as pigmentation, taste alteration limited its continued use. [1]

Among few plant-based compound used in folk medicine for thousands of years is the cranberry extract. Cranberry extracts are particularly rich in polyphenols, including flavonoids, which have biological properties that can be beneficial to human health. [2]

Cranberry is known for its excellent anti-oxidant properties they are mainly used to relieve scurvy and problems with the stomach and liver. Today, Cranberry juice is commonly recognized as having a preventive effect on urinary infections in women, through the ability of its high-molecular-weight polyphenols (tannins). [2]

Natural herbs have been used alone or in a combination with other products and have been scientifically proven to be safe against various oral health problems such as bleeding gums, halitosis, oral ulcers, and caries. Another such commonly available plant derived product is Guava (Psidium Guajava) plant. [1]

Guava leaves are used in the United States as an antibiotic for wounds, ulcers, and toothache in the form of dressing or decoction Guava sticks have been used since ancient times for effective teeth cleaning. In addition, they have been used as a toothpaste in folkloric practices to preserve oral hygiene. [1]

The null hypothesis tested was, there is no difference in using cranberry or guava leaf mouth wash on oral microbiota or on salivary pH.

MATERIALS & METHODS

- **Study setting**: The present study is a parallel group clinical trial carried out in Department of Public Health Dentistry, Lenora institute of dental sciences Rajahmundry.
- **Study sample:** It includes a total of 30 subjects who are enrolled in the undergraduate course and volunteered to participate in the study (aged > 18 years). The ethical clearance was obtained from the institutional ethical committee board and prior informed consent was obtained from all the participants.
- **Materials:** Materials used were 0.2% Chlorhexidine mouthwash, 0.6% Cranberry mouthwash, 0.5% Guava leaf extract mouth wash, disposable sterile cotton swabs, and pH paper strips.
- **Inclusion criteria:** Subjects with good general health, above 18 years of age and agreement to comply with the follow ups were included in the study.
- **Exclusion criteria:** Subjects with severe mal-alignment of teeth, orthodontic appliances, fully crowned teeth, removable partial dentures; subjects already using mouthwash or dental floss; tobacco consumers, and subjects with medical or pharmacological history that could compromise the conduct of the study were excluded.
- **Randomization, sequence generation, and blinding**: Randomization of the eligible subjects was done and were allocated into three equal groups where (A1) represents the participants who received guava leaves extract mouthwash (A2) represents the cranberry mouth wash group, and (A3) represents chlorhexidine group.
- The double blinding technique was used in the study. The mouthwashes were freshly prepared and poured into opaque brown glass bottles, and they were

labelled with nonidentifiable numbers to ensure discretion.

- **Study design**: The study was conducted for a period of one week. The base line pH and streptococcal colonies were counted for all the subjects before start of the study.
- Each participant was given a bottle of randomly chosen mouthwash. The participants were asked to rinse twice a day after 30 minutes of tooth brushing with 10 ml of the mouthwash for one week. The participants were asked to retain the mouthwash in their mouth for at least 1min before spitting it, and not to consume any food or drink for at least 30min after the use of mouthwash. The participants were told to store the mouthwash in the refrigerator to extend its shelf life. Then, the participants presented to the clinic after one week, the necessary samples were collected.
- Figure [1] shows a detailed illustration of study methodology and the flow of participants through the trial.
- The subjects were instructed to withdraw the use of mouthwashes and report immediately if they experienced ant side effects due to the use of mouthwashes.
- This trial was conducted and reported according to the consolidated statement of reporting guidelines
- **Method of collection of Sample**: The patient was asked to rinse thoroughly with plain water, and a jet of water spray was used to eliminate any debris present on the tooth surface.
- The plaque samples were subsequently obtained from buccal surfaces of premolars and molars of subjects using disposable sterile cotton swabs.
- **Outcome Assessment**: The samples were transferred to a sterile tube containing 1 ml of 0.15 M saline solution. These specimens were stored in ice bags at 2°C to prevent denaturation and transported to the lab within 15 min. The plaque samples, dissolved in saline, were inoculated on blood agar plates and incubated in an incubator at 37°C for 24–48 h. Numbers of streptococcal colonies were counted using the (CFU) colony forming unit.
- The salivary pH was measured immediately to avoid any changes in pH with time, using salivary pH strips.
- After one week of intervention all the three groups are to be checked for the difference in the salivary pH and streptococcal count in the oral cavity

- **STATISTICAL ANALYSIS**: for the all the participants the base line data and after intervention data (after one week) was subjected to analysis by using SPSS version 26.0 and the statistical tests applied included paired t test and repeated measures ANOVA for intra group comparison.

RESULT

In the present study among the volunteered participants there are 20 female participants and 10 male participants. The mean age of the study population was 22. At baseline, mean no. of CFU/ml for Cranberry group were 45.3 and after use of mouthwash for I week were 17.09. Baseline mean CFU/ml for Chlorhexidine group were 42.5 and after use of mouthwash for 1 week were 16.4. At baseline, mean no. of CFU/ml for Guava leaf group were 45.5and after use of mouthwash for I week were 17.09. For Chlorhexidine group, mean reduction in CFU/ml was 26.1 that is, 66% reduction, mean reduction in CFU/ml in Cranberry group was 28.2 that is, 70 %reduction whereas Guava leaf group, mean reduction in CFU/ml was 28.4 that is, 69 % reduction was seen. This Intragroup comparison, however, did not show any statistically significant difference between Chlorhexidine and Cranberry mouthwash and guava leaf mouthwash on microbial CFU/ml 1

By taking into consideration the bacterial count, all the three mouthwashes showed

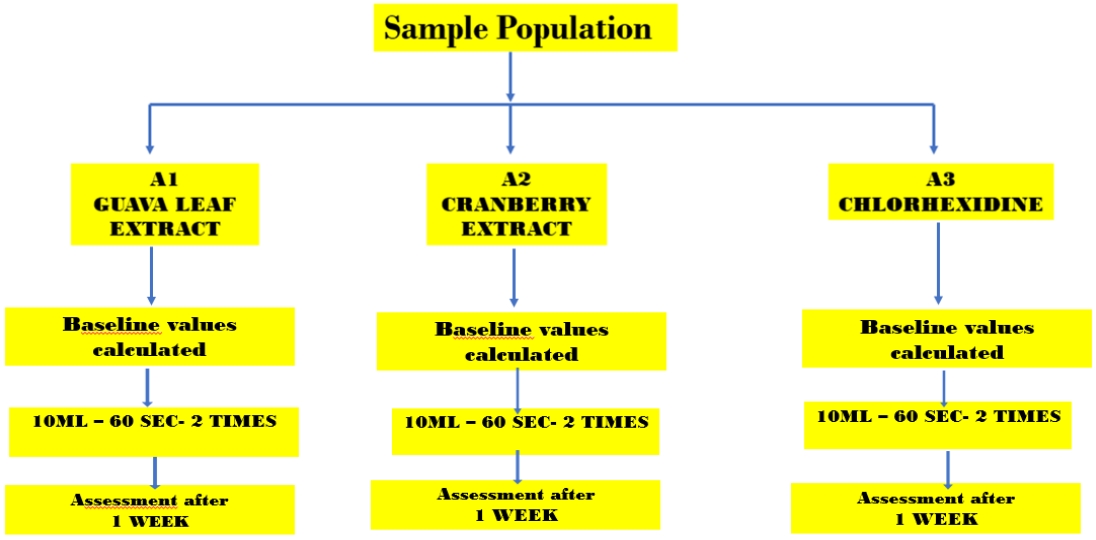
significant antibacterial effects. However, no statistically significant difference was found between them against the streptococcus +mutans. At baseline, mean pH value for Cranberry group was 6.5 and after use of mouthwash for I week was 6.2. Baseline mean pH value for Chlorhexidine group was 6.2 and after use of mouthwash for 1 week was 6.0. At baseline, mean Ph value for Guava leaf group was 6.3 and after use of mouthwash for I week was 6.1. For Chlorhexidine group, mean reduction in pH value before and after intervention was 0.2 for, Cranberry group was 0.3, whereas for Guava leaf group, was 0.2. This Intergroup comparison, however, did not show any statistically significant difference between Chlorhexidine and Cranberry and guava leaf mouthwashes on microbial CFU/ml and pH values

By taking into consideration the bacterial count, all the three mouthwashes showed significant antibacterial effects. However, no statistically significant difference was found between them against the streptococcus mutans and pH count. [Table 1]

Table 1 Comparison of mean CFU and salivary pH among three groups before and after intervention

		Sample (n)	Mean	Std. deviation	Std.error	F	Sig
CFU baseline	CHX	10	42.5200	4.91501	1.55426	1.109	.344
	CRANBERRY	10	45.3500	5.93488	1.87677		
	GUAVA LEAF EXTRACT	10	45.5100	4.14232	1.30992		
	Total	30	44.4600	5.06997	.92564		
CFU post intervention	CHX	10	16.4200	1.96288	.62072	.398	.676
	CRANBERRY	10	17.0900	1.72398	.54517		
	GUAVA LEAF EXTRACT	10	17.0900	2.11106	.66757		
	Total	30	16.8667	1.89852	.34662		
pH baseline	CHX	10	6.2000	.42164	.13333	1.016	.375
	CRANBERRY	10	6.5000	.52705	.16667		
	GUAVA LEAF EXTRACT	10	6.3000	.48305	.15275		
	Total	30	6.3333	.47946	.08754		
pH post intervention	CHX	10	6.0000	.00000	.00000	1.080	.354
	CRANBERRY	10	6.2000	.42164	.13333		
	GUAVA LEAF EXTRACT	10	6.1000	.31623	.10000		
	Total	30	6.1000	.30513	.05571		

Figure 1 showing a detailed illustration of study methodology and the flow of participants through the trial.



DISCUSSION

Dental caries is considered a major oral health problem in industrialized countries, and bacteria in dental plaque are considered one of the key factors for its development. So, mouthwashes are incorporated in dental practice to be used with toothbrushing to control the cariogenic plaque effectively. [4]

According to literature, chlorhexidine is one of the most effective antiplaque agents to date, and it is commonly used in patients. However, modern trends have witnessed a popular preference toward the use of naturally occurring herbal products in the field of medicine and dentistry mainly to avoid the side effects that might occur from the long-term use of chemical agents. [3]

In the present study both guava leaf and cranberry have shown excellent anti-bacterial effect in the oral cavity equally potent as chlorhexidine with guava leaf showing 69% reduction, Cranberry showing 70% reduction and chlorhexidine showing 66% reduction in CFU count respectively.

Guava and cranberry are among the most potent medicinal plants that have shown positive effects on health, as they have proved their antioxidant, antimicrobial, antispasmodic, anticancer, antihyperglycemic, analgesic, and anti-stomach-ache effects according to the study conducted by Parvez et.al.[5]

According to an analysis done by Ratnakaran et. al the study showed that guava leaves were rich in several bioactive compounds that were responsible for the antibacterial activity. Moreover, guava has been shown to be effective in treating oral diseases such as caries, ulcers, gingivitis, and toothache. [6]

The study conducted by Bonifait L et.al provided the impetus to assess the effectiveness of Cranberry as an anti-adhesion agent against S. mutans falling in line with the results of the present study. Many in vitro studies have reported the potent antibacterial effect of guava leaves and cranberry extracts on streptococcus mutans. [3]

few other in vitro studies conducted by BR CS, Nagarajappa R assessed the antibacterial activity and effect on salivary pH, especially in comparison to chlorhexidine and the results of the current study were in line with their results. [8]

The current study was also used to assess taste alteration and flavor acceptance for guava leaves mouthwash and cranberry extract mouthwash in comparison to chlorhexidine which was proven to have had an altered taste, and the users also reported that they felt an aftertaste while using the mouthwash.

Nevertheless, in case of both guava leaf and cranberry extract all the participants reported good satisfaction and no alteration in taste or left any aftertaste after subsequent use. This signifies that both the herbal mouthwashes were palatable thereby easily acceptable.

In the current study although they have shown significant regression in microbial activity of the oral flora clinically, statistical significance was not seen due to small sample size and limited duration i.e., for a period of one week which are considered as limitations and hence further studies are warranted on a larger population and with spaced intervention checks for statistically proving the effectiveness of both the herbal mouthwashes against chlorhexidine.

CONCLUSION

This study, therefore, suggests that herbal products like Cranberry extract and guava leaf extract can prove to be effective as suitable alternatives to Chlorhexidine in improving the oral health and that all the three mouthwashes exhibit similar antibacterial activity against streptococcus mutans with cranberry and guava leaf having additional benefits of being herbal with no side effects unlike chlorhexidine.

Declaration by Authors

Ethical Approval: Approved
Acknowledgement: None
Source of Funding: None

Conflict of Interest: The authors declare no conflict of interest.

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How to cite this article: Lahari. G, Hidayathulla Shaikh, Md. Zabirunnisa Begum, G. Anvesh, Shashank. B. Comparative evaluation between cranberry, guava leaf and chlorhexidine (0.2%) mouthwashes on oral microbiota and pH among interns in a dental college in Rajahmundry city - a randomized control trail. *Int J Health Sci Res.* 2024; 14(11):204-209. DOI: *https://doi.org/10.52403/ijhsr.20241123*

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