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# Comparative evaluation of Cranberry extract mouth rinse, Ozonized water and 0.2% Chlorhexidine on salivary Streptococcus mutans and Lactobacilli count: An in-vivo study

**Research Article**

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## Abstract

Background: Cariogenic bacteria has always been considered as the highest risk factor for dental caries. The eradication of microorganisms from the oral health environment is important and hence may remedies have been suggested for its removal for a better oral health. Objectives: To assess the minimal inhibitory concentration (MIC) of Cranberry extract and to determine the efficacy and compare the effect of the three products on salivary Streptococcus Mutans count and lactobacilli count. Materials and Methodology: A randomized control, double blind, parallel-group clinical trial was conducted on 75 subjects of age group of 15 years who were divided into three study groups ,Cranberry, Ozonized water and Chlorhexidine mouthwash. Microbial analysis was done by colony counter unit at baseline, 8th day and 15th day of using the mouthwashes. The inter-group variations was analysed by using One way ANOVA followed by Tukey's post hoc and Repeated measures for intra-group variations at different time intervals. Results: Streptococcus mutans and Lactobacilli colony count showed a statistically highly significant difference in reduction of colony count between the three groups (p=0.001) at 8th and 15th day of using the mouthwashes. Ozonized water showed better reduction when compared with Cranberry mouth wash in reduction of Streptococcus mutans count. Cranberry mouth wash had shown a better reduction when compared with Ozonized water in reduction of Lactobacilli colony count. Conclusion: The test mouthwash was effective in reducing the microbial count, similar to Chlorhexidine mouth wash. Recent advances may allow the dental community to be exposed to much better oral health care.

**Key Words:** Ozone water, Lactobacilli, Streptococcus, Cranberry, Chlorhexidine.

## Introduction

Oral health is a significant component of physical health which is necessary for general quality of life, self-esteem, and confidence (1).

Individuals are thought to be at greater risk of developing dental caries if they are severely infected by cariogenic bacteria (2). Although it is not possible to completely eliminate microorganisms from the oral cavity, many attempts have been made to reduce the viable counts of cariogenic organisms from the oral flora. Several antibacterial agents such as chlorhexidine, fluorides, and various antibiotics like penicillins, cephalosporins, erythromycin, tetracycline and metronidazole are used as antiplaque and anticaries agents (3). Only two antiseptic i.e bis-biguanide (0.2% chlorhexidine gluconate) and a mixture of phenol-related essential oil have amassed enough supporting evidence to be approved by the American Dental Association's Council on Dental Therapeutics (4). When used for an extended period of time, these substances have been found to produce unwanted side effects like tooth discoloration, altered taste, and many others. As a result, researchers are constantly looking for new treatments that can combat periodontal and dental disorders at the same time (1).

In dentistry, ozone has been suggested as an alternate mouth antiseptic. Ozone has numerous actions on the human body that are recognised, including immune-stimulating, analgesic, anti-hypoxic, detoxicating, and antibacterial qualities. This novel idea has spawned a number of procedures that try to preserve and protect the tooth structure by just removing the diseased and demineralized dental tissue (5). Ozone is created in nature when molecular oxygen is photo dissociated into activated oxygen atoms, that subsequently interact with other oxygen molecules to

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form ozone. This brief radical anion quickly protonates, which causes it to disintegrate into a more potent oxidant, the hydroxyl radical OH (6). It is a part of the evolving minimally invasive dentistry theme and its aim of preserving the original tissues (7). Despite having very straightforward administration forms and effective principles, this allotropic molecule has been extensively applied as a therapy component for more than 50 pathological diseases (8-12) and has been utilized in dental field.

Fruit extracts or phytochemicals that prevent the development of biofilms or oral infections have recently gained attention due to their multiple health benefits, and one such fruit is the cranberry a native of North America (13). Proanthocyanidins, which are part of the larger class of polyphenols known as flavanols, make up this substance. The inactivation of the enzymes glucosyl and fructosyl was seen in the cranberry fraction. These enzymes necessary for the production of fructan and glucan which enables streptococci in adhering to the tooth's surface, preventing the development of plaque. Due to its anti-adhesive and other anti-microbial characteristics, cranberries have demonstrated therapeutic potential in conditions including caries and periodontitis (14). The current study is planned with the aim to evaluate and compare the effects of Cranberry extract, 0.2% Chlorhexidine and Ozonated water on salivary *Streptococcus mutans* and *lactobacillus acidophilus* count.

## Materials and Methods

The present study is a randomized controlled double blind parallel clinical trial conducted over duration of 3 months in the public health dentistry department. It involved 75 participants who were children aged 15 years were selected randomly from a residential school. Ethical clearance for study was obtained from the Institutional Ethical Committee of bearing a Reference Number CODS/IEC/ 1839/2016-2017 and the preparations for the study were done during the months of January to March 2018, which included purchase of test products, preparation of test formulations, selection of subjects for the final study, approval from the concerned authorities and acquiring written informed consent from the subjects to be examined. The permission was also obtained from school authorities. A specially prepared and pretested format in English language was used, which was specifically made for keeping track of all the important facts about general information and microbial count.

**Procurement of test materials**

The mouthwashes used in this study are 10% Cranberry extract mouthwash,0.2% Chlorhexidine mouthwash as bench mark control and Ozonized water. 0.2%Chlorhexidine (0.2% Chlorhexidine gluconate) is a bis-biguanide mouthwash which is commercially available and were procured from the market. A Ozonizer machine was used to produce ozonized water in which the ozone gas was mixed with 1 litre of sterile distilled water for 20 minutes to form ozonated water. Cranberry extract mouthwash was prepared using following procedure.

**Preparation of Cranberry Extract Mouthwash**

**Preparations of extract**

The Department of Pharmacognosy at the Bapuji College of Pharmacy in Davangere produced the cranberry extract. Plain decoction was used to make hot water extract. For this, 1.5 litres of sterile distilled water were added to a beaker with 250gm of dried cranberry fruit. This was gradually heated in a water bath until the menstrual fluid was reduced to less than one-fourth of its initial volume. To create a dry version of the extract, the water content was entirely evaporated, and the remaining liquid was filtered using Whatman's filter paper No.1.

In Vitro Antibacterial Sensitivity of Crude Extract against Streptococcus mutans and Lactobacilli.

Minimum Inhibitory Concentration (MIC) was demonstrated at the Department of Microbiology, Maratha mandal College and Hospital, Belgaum.1 ml of Brain Heart Infusion broth poured into each of the test tubes (1). MIC of Cranberry Extract against *Streptococcus mutans* and *Lactobacilli* was observed to be 100 mg /ml.

**Formulation of Mouthwash**

Cranberry extract mouthwash was prepared in the Department of Pharmaceutics, Bapuji College of Pharmacy, Davangere in the concentration of 10%. The mouthwash formulation consisted of the 10% Cranberry extract, distilled water, menthol, glycerine, preservative (benzyl alcohol), prussian blue (coloring agent) and peppermint oil (flavouring agent). The prepared mouthwash was then transferred to amber colour plastic bottles.

**Study design**

After the information regarding number of students and willingness for the study was obtained, *Davan Institute* was selected to conduct the study. Sample size acquired for every group was 25, therefore the effective sample size was 75 depending on the data from the pilot study and fixing at 5% (P 0.05%), at 20%, and power at 80%. Using lottery technique, sample size of 75 subjects was randomly distributed into groups of 25 people. Products were distributed to participants in accordance with the given code.

**The three intervention groups were**

- *Group I*–10% Cranberry Extract Mouthwash
- *Group II* – Commercially available 0.2% Chlorhexidine Mouthwash (control)
- *Group III*– Ozonized water

The test materials were then put into 250 ml bottles that had a similar amber colour to prevent the subjects from knowing which product they were using. The bottles were then numerically coded by the assistant, who then gave it to the volunteers, guaranteeing double blindness for both the subjects and the researcher. Saliva samples were taken from

each individual at the beginning of the study period to determine their baseline recording of streptococcal and lactobacilli CFU/ml prior to using mouthwash. To reduce bias, the examiner was unaware of the way the subjects were grouped based on the products. Throughout the whole period of the investigation, the investigator conducted all microbiological procedures while remaining blind to the culture plates of various groups. In the inclusion criteria the participants with good general health, aged 15 years, DMFT score ≤ 3, mean Gingival Index score ≥ less than 2, mean Plaque Index score ≥ 1 less than 3, agreement to follow with research visits when required and to postpone any dental procedures involving oral prophylaxis were involved in the study.The participants which were excluded from the study were those with severe dental misalignment, ortho appliances, fully crowned teeth, removable partial dentures, those who already used mouthwash or dental floss, smokers, and whose medical histories might affect the progression of the study.

**Materials used in clinical examination**

Plane mouth mirrors, tweezers, explorer, kidney trays, gauze and cotton, chip blower, disposable gloves and mouth masks, bacillol® 25 Spray,0.075% basic fuchsin, vaccine carrier with freezing mixture for transport and storage of saliva.

**Method of conducting study**

**Training and calibration of examiner:**

All the examinations and laboratory analyses were performed by a single investigator and data recording was done by the assistant who was familiar with the proforma. The kappa coefficient value for inter-examiner variability and intra-examiner variability with respect to salivary *Streptococcus mutans* and *lactobacilli* count was 0.82 and 0.90 respectively.

**Field examination and instruction:**

As per the guidelines for American Dental Association, intraoral Type III Clinical examination was performed in the morning hours at baseline, eight day and 15th day morning to collect saliva (15).

**Allocation of test products and instructions:**

All the participants were given a kit comprising of a number coded bottle of one of the test mouthwashes, a measuring cup, a tooth paste, a soft brush and a compliance check list. Subjects were asked to rinse using 10ml of particular mouthwash for 30 seconds twice daily before breakfast and at bedtime throughout the first 15 days period. 2ml of unstimulated Salivary samples were collected on the 8th day and 15thand the participants were instructed by the examiner to stop the usage of the mouthwash thereafter. The samples were then immediately placed in the vaccine carrier to minimize degradation of salivary proteins until further processing *(16).*Analysis of the salivary samples was done within 4 hours.

**Microbial analysis**

Culture media preparation:

Mitis salivarius bacitracin (MSB) agar was used to culture Streptococcus mutans, while Rogosa SL Agar (RSL) agar was used to grow Lactobacilli. The agar media was prepared as per the manufacturer's recommendations (*Hi media, Mumbai*).Serial dilution of the salivary samples was done to obtain a dilution of 1:1000. All the colonies, colony forming units/ml for each microorganism was recorded using digital colony counter.

**Statistics**

The Statistical Package for Social Sciences version 22.0 was utilized to examine data that had been compiled in an Excel sheet from Microsoft. One-way Analysis of Variance (ANOVA) was used to compare two groups at the same time interval, followed by Tukey's Post hoc test, and repeated measures ANOVA was used to compare two groups at different time intervals, followed by pairwise comparisons. Statistics were considered to be p value 0.05.

## Results

A total sample size included 75 study subjects with 25 in each group. On gender distribution, 39 (52%) males and 36 (48%) females were included in the study.

***Streptococcus Colony count***

The mean *Streptococcus mutans* colony counts on the 8th day in Group I (Cranberry mouthwash), Group II(chlorhexidine mouth wash), and Group III (Ozonized water), was 200 ± 28, 211 ± 62, 227 ± 58 respectively. One way ANOVA revealed no statistically significant difference in colony counts between the test groups (p= 0.19). The mean *Streptococcus mutans* colony counts on the 15th day in Group I, II and III were 178 ± 28, 168 ± 56, 208 ± 60 respectively and a statistically significant difference was observed (p=0.017).(Graph1) On Intra group and Inter group comparison between mean *Streptococcus mutans* colony counts in Group I, Group II and Group III at baseline, on 8th day, 15th day had shown a statistically significant difference($p<0.05$). The mean colony counts were highest in Group III followed by Group I and Group II.

***Lactobacilli Colony Count***

The mean *Lactobacilli* colony counts on the 8th day in Group I (Cranberry mouthwash), Group II(chlorhexidine mouth wash), and Group III (Ozonized water), was 193 ± 49, 190 ± 21, 235 ± 55 respectively. A statistically highly significant difference in counts between the test groups (p= 0.001) was observed. The mean colony count at 8th day were highest in Group III followed by Group I and Group II. The mean *Lactobacilli* colony counts in saliva on the 15th day in Group I, II and III were 174 ± 47, 151 ± 29 and 212 ± 49 respectively and a statistically significant difference in the counts between the test groups (p=0.001).(Graph

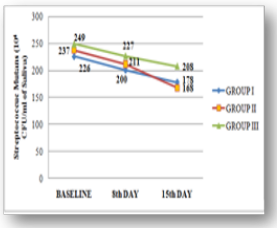
2) The mean colony counts at 15th day was highest in Group III followed by Group I and Group II. The Intra group comparison between mean *Lactobacilli* colony counts in Group I, Group II and Group III at baseline, on 8th day, 15th day had shown a statistically significant difference in the colony counts ($p<=0.05$). Overall, all the three mouthwash groups had shown percentage reduction in the mean counts as the study progressed from baseline to 8th and 15th day.

***Inter group comparison at baseline, 8th day and 15th day***

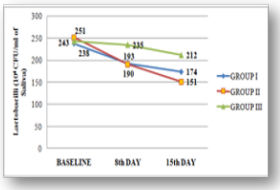
On Streptococci Colony Count (Graph 3) CHX Group had maximum reduction of 11.02 and 32.53 in the counts on 8th and 15th day respectively followed by Ozone group showed better reduction on 8th day (8.93) and (16.25) on 15th day compared to Cranberry Group i.e. CHX>Ozone Group> Cranberry Group.

On Lactobacilli colony Count (Graph 4) CHX Group had maximum reduction of 21.09 and 50.05 in the colony counts on 8th and 15th day respectively. Followed by Cranberry Group showed better reduction on 8th day (11.36) and on the 15th day than Ozone Group.i.e CHX> Cranberry group > Ozone Group.

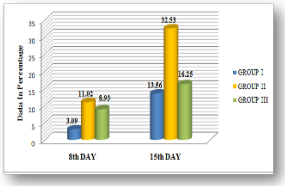
**Graph 1: Inter group Comparison of Streptococcus mutans count CFU/ml of saliva at baseline, 8th day and 15th day.**



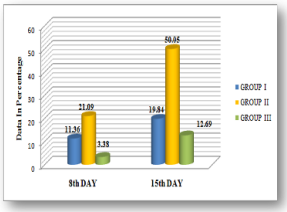

**Graph 2: Inter group Comparison of Lactobacillus count CFU/ml of saliva at baseline, 8th day and 15th day.**




**Graph 3: Inter group Mean Reduction of *Streptococcus mutans colony count* at 8th day and 15th day**




**Graph 4: Inter group Mean Reduction *of Lactobacilli colony count* at 8th day and 15th day**




## Discussion

The mouthwashes have been used for both medical and cosmetic purposes for centuries but the usage of the components has only recently been the subject of scientific research and clinical testing (17). It should have an optimum anti plaque action, biofilm penetration ability, good oral substantivity and low toxicity. The critical challenge of synthetic antimicrobial drug resistance has prompted interest in alternative natural remedies. The recovery of Streptococcus mutans from clinical samples varies significantly between chair side culture tests and classic culture-based media (3). For more than a century, lactobacilli have been connected to tooth cavities. This prompts to consider the effects of lactobacilli in the mouth and its effects on the gastrointestinal health (18). Therefore, the conventional method of isolating microorganisms from the oral cavity and the conventional technique of medium culture were adopted in the current study.

**Cranberry Fruit**

Because they are so rich in nutrients and antioxidants, cranberries are a nutritious food. They are frequently called a "super food." They are high in vitamin C, vitamin A, and vitamin K and low in calories. Proanthocyanidins (PACs), an antioxidant that may aid in the prevention of a number of diseases and improve oral health, are also present in them. In cranberries, four types of phenols have been found. Anthocyanins (A) and flavan-3-ols are phenolic acids. By influencing the colonisation of dental surfaces and the creation of acids by cariogenic bacteria, these polyphenols in cranberries may inhibit the development of dental caries (4). Cranberry can therefore prevent S. mutans from sticking to the tooth biofilm.

**Ozonized Water**

In specialties like surgery, dermatology, cosmetics, and dentistry, ozone therapy has become a standard component of the treatment of infections (19).Ozone damages cells' cytoplasmic membranes due to ozonolysis of double bonds, and it also causes intracellular contents to change as a result of secondary oxidants' actions. These effects give ozone its anti-microbial impact. This activity does not harm the cells in the human body; instead, it is non-specific and only affects microbial cells (20). Ozone's potent ability to inactivate bacteria and makes it a possible addition to traditional treatment methods. Results of the current study showed that 0.2% chlorhexidine mouthwash was better than Cranberry extract mouthwash, which could be reinforced by the findings of an in vivo study conducted in 2015, by Preeti Guniyal et al (21), whereby it was seen that 0.2% Chlorhexidine mouthwash had superior efficacy than Cranberry extract on *Streptococcus mutans* colony count. Current study is contradictory to the outcomes of a study conducted by Mahesh.R.khairnar et al (22) in 2015, where it was observed that *cranberry* extract showed no significant difference in the growth inhibitory effect on *Streptococcus mutans* when compared to 0.2% Chlorhexidine mouthwash. The results of the present study showed that 0.2% Chlorhexidine mouthwash was better than ozonized water could be reinforced by the findings of an in vitro study conducted in 2016, by

Marcos ximens et al (23), in which the 0.2% Chlorhexidine mouthwash had shown a superior efficacy than Ozonized water on *Lactobacilli* colony count. Similar results were observed in a study conducted by H.Sancakli et al (24) in 2018 that demonstrated the application of ozone water had the potential to exert higher antibacterial effects which was contradictory to the studies conducted by D Savitri et al (25) wherein, it was established that there was no statistical difference on the basis of antimicrobial efficacy. When compared within the group of test mouthwash i.e Ozonized water, it has shown more effect in reducing *S.mutans* count whereas Cranberry extract mouth wash had shown effective reduction in reducing *lactobacilli* count at the 15th day. The oxidation potential of ozonized water has proven to reduce the microbial growth in the oral cavity and similarly cranberry mouth wash has shown a better efficiency in increasing the antioxidant potential with the presence of flavonoids. The test groups have been efficient in reducing the *S.mutans* and *Lactobacilli* count. The current study was limited to the evaluation of salivary *Streptococcus mutans* and *lactobacilli* colony counts due to lack of time and financial constraints, still further research is required in testing the efficacy of these mouthwashes in the various domains of microbiological aspects associated with dental caries and periodontal diseases. To study the proper long-term effect for continuing use, still more distant studies should be performed to evaluate the antimicrobial, antigingivitis and antiplaque effects of these mouthwashes for establishing superiority as using mouthwashes.

## Conclusion

Cranberry and Ozonized water can prove to be a substitute to chlorhexidine in reducing the cariogenic bacteria in oral cavity. Hence, both the products offers a simple adjunctive therapy for managing oral health issues along side convention methods thereby increasing their quality of life. An extensive research is required for long-term evaluation of beneficial effects of such herbal extracts.

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