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Antimicrobial Efficacy of *Vaccinium macrocarpon* mouthwash against Steptococcus mutans in dental plaque of caries active children – A randomized controlled trial

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ABSTRACT

Cranberry (Vaccinium macrocarpon) extracts have been found to be rich in polyphenols specifically, including flavonoids, that have been found to exhibit beneficial properties to human health. Considering the recent inclination in the preference of people towards herbal based products and remedies, and taking into account the possible side-effects of chlorhexidine, the current study was undertaken to comparatively evaluate the antimicrobial efficacy of Vaccinium macrocarpon and chlorhexidine mouthwash on the Streptococcus mutans count in the dental plaque of caries, active children. The study follows a parallel design involving 50 children divided into two groups of 25 each - Group 1: chlorhexidine mouthwash; Group 2: cranberry mouthwash. The initial plaque samples and the samples were taken after 14 days were evaluated using a digital colony counter for determining the streptococcal colony count/ml. A statistically significant difference was found in both the groups with respect to the Streptococcal colony count when the intra-group comparison was made comparing the baseline values to the values after 14 days. However, no significant difference was seen in the percentage of reduction in the microbial CFU/ml between the two groups. Cranberry mouthwash can be considered to be an effective alternative to Chlorhexidine mouthwash, given its additional systemic effects apart from local beneficial effects in the oral region. Future scope in research should be aimed at evaluating its long term health benefits and any possible adverse effects.

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INTRODUCTION

Dental caries remains to be the most prevalent and preventable of the oral diseases affecting a major part of the world's population which is induced by dental plaque. (Marsh, 2003) Within the dental plaque, there is an interaction between the various constituents of the diet and specific bacteria resulting in the destruction of the dental hard tissues. When left unchecked, this condition may progress towards the dental pulp causing its death and eventually may lead to the spread of the infection to the periapical areas leading to undesirable consequences. (Khairnar *et al.*, 2015)

Streptococcus mutans, a major colonizer of dental plaque, is known to be the major contributor of dental caries. (Sundas and Rao, 2015) It acts by: i)effective utilization of dietary sucrose to synthesize substantial quantities of extracellular polysaccharides ii)firm adherence to glucan coated sur-

faces iii)high acidogenic as well as acid-tolerant activity. (Hamada and Slade, 1980; Gibbons, 1984; Bowen, 2002)

Currently, broad-spectrum antibiotics are the most widely used agents for combating oral infections. (Allaker and Douglas, 2009) Amongst that, Chlorhexidine has gained the reputation of being the standard gold agent majorly because of its comprehensive antimicrobial action and high substantivity. However, it's continued use can cause certain adverse effects like alteration of taste, pigmentation and the formation of supragingival calculus. (Flotra *et al.*, 1971; Khairnar *et al.*, 2015)

Recently, there is an inclination towards research regarding plant-based biological products. It was observed that nowadays people tend to prefer products which they perceive to be healthier, safer with minimal or no toxic chemical and synthetic agents. (Saini *et al.*, 2011)

Cranberries (*Vaccinium macrocarpon*) have been in use for the therapeutic purpose since the 17^{th} century, mainly to relieve conditions like scurvy and in diseases related to stomach and liver. (Bonifait and Grenier, 2010) Nowadays, cranberries are gaining popularity for their preventive role in urinary tract infections in women (Cimolai and Cimolai, 2007). It was found that the high molecular weight polyphenols of cranberry are said to exert an anti-adhesive effect on Escherichia coli pathogen thereby preventing its adhesion to the urinary tract mucosa and thus lessening the probability of urinary tract infection (Lavigne *et al.*, 2007; Tao *et al.*, 2011; Hisano *et al.*, 2012). It was also found to exhibit an anti-adhesive effect on Helicobacter pylori towards the gastric mucosa, thus preventing the development of gastric ulcers in human beings. (Burger, 2000)

Cranberries are found to contain moderate quantities of dietary fibers , vitamin C as well as essential dietary minerals, manganese, including evaluated levels of additional essential micro-nutrients. [Table 1]

Thus, observing the recent inclination in the preference of people towards herbal based products and remedies, and taking into account the possible side-effects of chlorhexidine, the current study was undertaken to determine the antimicrobial efficacy of Vaccinium macrocarpon when compared to chlorhexidine mouthwash on the Streptococcal colony count in the dental plaque of caries active children.

MATERIALS AND METHODS

The current study follows a double-blinded, parallel design group based clinical trial carried out in the Department of Pediatric Dentistry, Saveetha Dental College, Chennai, Tamil Nadu, India. The ethical clearance was acquired from the ethical committee of Saveetha Dental College, Chennai; and the entire procedures performed were in accordance with the 1964 Helsinki declaration as well as its later amendments and the ethical standards of the institutional and/or national research committee or comparable ethical standards. The informed consent was taken from all the participants' parents prior to the study. It included a sum of 50 subjects in the age range of 8–10 years. The necessities of the study are 0.2% Chlorhexidine mouthwash, 0.6% Cranberry mouthwash, disposable sterile cotton swabs, and sterile test tubes containing saline.

Individuals exhibiting good general health and minimal of 3-4 active carious lesions, acceptance to postpone any elective dental treatment such as oral prophylaxis, and acceptance to meet the criteria regarding the study visits were incorporated into the study. Individuals with severe malalignment of teeth, those wearing orthodontic appliances, removable partial dentures, full-coverage crowns; and those who are already utilizing mouthwash or dental floss and those with any medical or pharmacological history were excluded.

Study design

The sample size was taken as 25 per group with an effective sample size of 50 based upon the data obtained from a previous in-vivo study (Khairnar *et al.*, 2015) at 80% power. 50 children were randomly divided into Group A (25) - 0.2% Chlorhexidine mouthwash (Hexidine mouthwash, ICPA Health Products Ltd., Gujarat, India) and Group B (25) - 0.6% Cranberry mouthwash using lottery method. A double-blinded parallel study design was employed as the investigator was not aware of the sampling of the groups and study subjects were also not aware regarding the mouthwash they were utilizing. At the beginning of the study, plaque samples were taken for each subject before using mouthwash and baseline streptococcal CFU/ml recordings were measured.

Cranberry mouthwash preparation

Cranberry/ Vaccinium macrocarpon extract was acquired from Herbo Neutra, New Delhi, India. The Cranberry mouthwash was indigenously prepared in the Department of Biochemistry, Saveetha University. Cranberry mouthwash was formulated by the investigator using 600 mg concentration as this

specific concentration gave rise to the maximum inhibition zone against *S.mutans* among the five different concentrations which were tested in a previous study. (Sethi and Govila, 2011) For the preparation of 100 ml of Cranberry mouthwash, 600 mg of Cranberry extract was taken and dissolved in 90 ml of distilled water and 10 ml of alcohol along with 0.1 g zinc chloride, 0.1 g sodium saccharine, 0.1 g sodium benzoate, 3 ml of glycerine and 0.05 g menthol.

Method of plaque collection

After asking the patient to rinse the mouth entirely with plain water, water jet was sprayed to remove any debris attached on the surfaces of the teeth. The plaque samples were then subsequently derived from buccal surfaces of the molars using disposable sterile cotton swabs. [Figure 1] The obtained samples were transported to sterile tubes containing 1 mL of 0.15 M saline solution and stored at 2°C in ice bags in order to prevent denaturation. These specimens were then immediately transferred to the lab within 15 min of processing.

The obtained plaque samples which were dissolved in saline were then inoculated on blood agar plates followed by incubation at 37°C for 24–48 h. The numbers of streptococcal colonies were calculated utilizing a digital colony counter (Lab Hosp Colony Counter Digital LHC 06).

The individuals were then directed to rinse then mouth twice daily, once in the morning after breakfast and next at night before going to bed, for 14 days with 10 ml of the assigned mouth rinse (undiluted) for a total of 30 seconds and then instructed to expectorate the rinse. The children were provided with a measuring cup for dispensing 10 ml of the assigned mouth rinse.

After a period of 14 days, plaque samples were collected again and inoculated on blood agar plates for the determination of streptococcal colony count.

Statistical analysis

Intra-group comparison to evaluate streptococcal count before and after the use of mouthwash was made making use of paired t-test and intergroup comparison was calculated by the percentage reduction between the two groups using SPSS Software 21.0.

RESULTS

According to the current study results, there was a statistically significant difference observed in both the groups at baseline and after 14 days in respect to the mean number of CFU/ml. (P<0.05) [Table 2] However, no statistically significant difference was observed between both the groups on comparing the percentage reduction of CFU/ml at baseline and after 14 days. (P=0.09)For the Chlorhexidine group, the percentage reduction was 73% whereas for in the Cranberry mouthwash group it is a little higher, i.e., 76%. [Figure 2]

Figure 1: Method of plaque collection

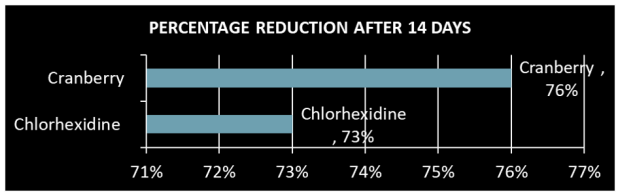

Figure 2: Percentage reduction in microbial CFU/ml scores in both the groups (P=0.09; not significant)

DISCUSSION

Cranberries (*Vaccinium macrocarpon*) belong to a class of evergreen dwarf shrubs or trailing vines which grow in the frigid regions of north-eastern North America. They are considered to be one among North America's 3 original fruits, the other two being Concord grape (*Vitus labrusca*) and blueberry (*Vaccinium* spp). Cranberries contain more than 80% water content and 10% carbohydrates and are available mainly as fresh produce, juice, dried fruit and also as encapsulated powder forms. (Bonifait and Grenier, 2010)

Single-strength cranberry juice is found to be highly acidic (pH, 2.5) and unpalatable for consumption (Raz *et al.*, 2004). The fact that cranberry juice consumption can provide any benefit to oral health is quite dubious, given the inadequate contact time between cranberry polyphenols and the oral tissues (gingival and teeth). In addition to this, the sugar added to the cranberry drinks along with the acidity of the beverages exhibits a counterproductive effect that, in turn, contributes to enamel demineralization. (Bansal *et al.*, 2015)

Table 1: The nutrient value of Cranberries per 100 g

Nutrient	Value per 100 gram
Energy	46 Kcal
Calcium	8 mg
Magnesium	6 mg
Phosphorus	13 mg
Potassium	45 mg
Sodium	2 mg
Sugars total	4.04 g
Fibre, total dietary	4.6 g
Vitamin C, Total ascorbic acid	13.3 mg
Vitamin A, IU	60 IU
Carotene, beta	36 mcg
Lutein + Zeaxanthin	91 mcg

Table 2: Baseline and final microbial CFU/ml of both the groups

Group	Baseline Mean± SD	After 14 days Mean± SD	P
Chlorhexidine	56±4.5	15±3.8	<0.001*
Cranberry	51±3.3	12±7.5	<0.001*

*Significant(P<0.05), CFU: Colony Forming Units

Cranberry extracts are found to be rich in polyphenols in particular, including flavonoids, that have been found to exhibit biological properties which prove to be advantageous to general health. (Bonifait and Grenier, 2010) Proanthocyanidins (PAC) and flavanols (FLAV), alone or in combination, are found to have an inhibitory action on the surface-adsorbed glucosyl transferases and F-AT Pases activities including the production of acids by *S. mutans* cells. (Sethi and Govila, 2011)

In the current study, cranberry extract was used for the preparation of cranberry mouthwash. Additionally, it contained distilled water, zinc chloride, glycerine—ethyl alcohol, sodium saccharin, menthol and sodium benzoate. Zinc chloride is particularly known for its anti-halitosis properties. Sodium saccharin serves as a sweetening agent and menthol act as a flavoring agent while sodium benzoate is the preservative. Glycerine acts as a humectant. Ethyl alcohol and water mixture are used as a solvent. On the other hand, chlorhexidine mouthwash contains chlorhexidine, zinc chloride, sodium fluoride as chief components dissolved in a delectable flavoured aqueous base.

According to the current study results, a comparable effect was observed with the use of both the mouthwashes on *Streptococcal mutans* CFU/ml. Thus, it thereby indicates that the Cranberry mouthwash is as efficacious as Chlorhexidine mouthwash with added systemic and local benefits as described previously.

It is said that the NDM fraction of Cranberry extract is capable of inhibiting 80-95% of streptococcal biofilm formation. (Yamanaka-Okada *et al.*, 2008) Another in-vitro study proves the anti-adhesion effect of Cranberry when used at different concentrations against S.mutans, thereby proving beneficial in the reticence of dental plaque formation. The results of our study are in accordance to a previous study (Khairnar *et al.*, 2015) done among dental students which proved that cranberry mouthwash, when used at 0.6%, was as potent as 0.2% Chlorhexidine in decreasing the S.mutans count.

One of the possible limitations of the study is the relatively small sample size. Further research and trials need to be done on a larger sample size with long term follow up to evaluate its safety and possible adverse effects. The swish and swallow method can be tested to evaluate the possibility of improving oral health as well as providing added systemic benefits such as prevention of urinary tract infection, urinary or gastric ulcers.

CONCLUSION

According to this study, it can be said that cranberry mouthwash can serve to be a better and effective

alternative to Chlorhexidine mouthwash, considering its beneficial effect both locally and systemically. Thus further trials need to be conducted to establish the long-term benefits and side effects of these herbal extracts.

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Conflict of interest

The authors declare that they have no conflict of interest for this study.

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