ORIGINAL ARTICLE

Comparative Evaluation of Anti-Microbial Efficacy of Cranberry Extract and Chlorhexidine Mouthwash on Periodontal Pathogens: An In-vitro Study

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ABSTRACT

BACKGROUND: Chlorhexidine gluconate is considered as the gold standard among various anti-plaque agents. However, many local side effects have been reported on its long term use. Cranberry (Vaccinium macrocarpon) is rich in polyphenols, including flavonoids and proanthrocyanidins. Insufficient evidences are available to support antimicrobial property of Cranberry extract mouthwash in context to red, orange and green complexes of periodontal pathogens and even comparison of same with clinically used and accepted 0.2% Chlorhexidine.

MATERIALS AND METHODS: Sterilised nutrient agar plates were inoculated with suspensions of P. gingivalis, T. forsythia, P. intermedia and A. actinomycetemcomitans (overnight cultures grown at 37° on nutrient agar). The strains were allowed to grow in strict anaerobic condition. 1, 5, 10 and 15 mg/ml Cranberry extract, 0.2% Chlorhexidine and distilled water were added into wells. Plates were then again incubated at 37° for 24 hours. Diameter of zones of inhibition of all the plates was measured using digital vernier callipers. The mean score of zones of inhibition was calculated.

RESULTS: Results of the study showed that all four concentrations of Cranberry extract showed comparatively less significant antimicrobial property against the microorganisms, compared to 0.2% Chlorhexidine.

CONCLUSION: This study showed that 1, 5, 10 and 15 mg/ml Cranberry extract does not have significant antimicrobial efficacy against periodontopathogens, compared to that of 0.2% Chlorhexidine.

KEYWORDS Cranberry extract, Chlorhexidine, P. gingivalis, T. forsythia, A. actinomycetemcomitans, P. intermedia

INTRODUCTION

Periodontitis is one of the most prevalent oral polymicrobial infectious diseases. Various periodontopathogenic bacteria form a biofilm and adhere to the host tissues, and this comprises an essential stage in the initiation of periodontitis.1

It has been proved that dental plaque is the most important factor which initiates periodontal disease and causes its progression. If left untreated, gingivitis can progress to periodontitis and can lead to a compromised state of the periodontium. Regular mechanical plaque control is the most dependable means of achieving oral health benefits. However, studies have shown that mechanical plaque control by tooth brushing and interdental cleaning aids alone is not always completely effective as it is dependent on the dexterity and motivational level of individual.2 Hence the use of chemical plaque control agents has been suggested as adjuncts in order to better control plaque and gingival inflammation.

A number of plaque control agents have been used with different success rates for the inhibition of plaque formation and for preventing the development of gingivitis and periodontitis. These have potent anti-septic and antimicrobial properties. They include phenolic compounds, bis-biguianides, quaternary ammonium compounds, pyrimidines, halogens, oxygenating agents and heavy metal salts.3 Chlorhexidine (CHX) has been proved to be the most effective compound (gold standard) for plaque prevention and stopping formation of gingivitis when used twice daily as mouth rinse.4 When used orally, Chlorhexidine has been reported to have numerous local adverse effects like taste disturbances, tooth discoloration and mucosal erosions. Other chemical antiseptic agents have been tested but none has shown equal or better results than Chlorhexidine without eliciting unfavourable side effects.5 In order to surpass such side effects the World Health Organisation (WHO) considered researchers to explore the available use of herb and plant extracts. Hence, a rising interest has been seen in herbal/natural products having potential therapeutic uses in medicine and dentistry. The use of plants has been advocated since thousands of years in folk medicine. Many pharmaceuticals have products derived from medicinal plants. Also, many presently used antibiotics have been explored by evaluating natural products against whole organisms, which identified their bacteriostatic and bactericidal properties.6 Also evidence has shown that people

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Cranberry (Vaccinium macrocarpon) is a small plant growing in the marshlands of cold regions of northeastern North America, northern Europe and Asia. Extracts from Cranberry are distinctly rich in polyphenols, including flavonoids, which have organic properties that can improve human health. Cranberry has been used as a therapeutic agent since the 17th century. Cranberry was used for treatment of scurvy and also stomach and liver problems. Cranberry juice contains polyphenols which can hinder the adherence of Escherichia coli to the urinary tract mucosa, thus prevents urinary infections in women. Proliferation of cancerous cells can also be inhibited by Cranberry, particularly cells in prostate, bladder and mouth. The presence of proanthocyanidins in Cranberry extracts have shown to inhibit the formation of biofilms by caries causing microorganisms. Polyphenols present in Cranberry cut down the inflammatory response, and also decrease production and action of proteolytic enzymes adding to the destruction of the extracellular matrix in periodontal disease.

Several conventional anti-plaque agents are available in the market, but with the rise in resistance to antibiotics, there is considerable interest in the development of herbal alternatives for the control of infection. Before including Cranberry extract mouthwash in periodontal treatment modalities, it becomes mandatory to evaluate its anti-bacterial efficacy for periodontal pathogens and to compare the same with established local antimicrobial used that is 0.2% Chlorhexidine.

There are currently no studies assessing the efficacy of Cranberry extract with P. gingivalis, P. intermedia, T. forsythia and A. actinomyceseomycetosan, hence the present study will comprehensively report the antimicrobial potential of Cranberry extract on these key periodontal pathogens. It will also assess and compare the in-vitro efficacy of Cranberry extract with the gold standard 0.2% Chlorhexidine.

**DISCUSSION**

The antibacterial activity of different concentrations of Cranberry extract and 0.2% Chlorhexidine was determined by disc diffusion method. Brain heart infusion agar and blood agar was used as the culture media. Agar plates were brought to room temperature before use. Using a loop or swab, the colonies were transferred to the plates. Turbidity was visually adjusted with the broth to equal that of a 0.5 Mc Farland turbidity standard that had been vortexed. Alternatively, the suspension was standardized with a photometric device. Within 15 minutes of adjusting the inoculums to a McFarland 0.5 turbidity standard, a sterile cotton swab was dipped into the inoculums and rotated against the tube wall above the liquid to remove excess inoculum. Then swabbing the entire agar plate was done thrice, rotating plates approximately 60° to ensure even distribution. Care was taken to avoid extra hitting of the sides of the plates to prevent creating aerosols.

After the plates were inoculated, a standing time of 3 minutes, but no longer than 15 minutes was given before making wells. A hollow tube of 5 mm diameter was heated and pressed above the inoculated agar plates and was removed immediately, making a well in the plate. Likewise, four wells of 5mm diameters each were made on each plate. 50 µl of compound (1 mg/ml, 5 mg/ml, 10 mg/ml, 15 mg/ml) Cranberry extract, 0.2% Chlorhexidine and distilled water were added into the respective wells of each plate. Within 15 minutes of compound application, plates were shifted to an anaerobic jar, which was kept in an incubator, 37° C for 24-48 hours. After incubation was complete, plates were read only if the lawn of growth was confluent or nearly confluent. The diameters of zones of inhibition were measured for all the wells using a digital vernier calliper. The mean score of zones of inhibition were calculated for each solution, respectively.

**RESULTS**

The mean values of diameters of zones of inhibition of 1 mg/ml, 5 mg/ml, 10 mg/ml and 15 mg/ml Cranberry extract, 0.2% Chlorhexidine mouthwash and distilled water are shown in Table 1 (R - resistant, S - sensitive). 0.2% Chlorhexidine mouthwash showed greatest zone of inhibition against all periodontal pathogens.
Cranberry extract at a concentration of 1 mg/ml showed antibacterial activity against *P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans* and *T. forsythia* (12, 8, 12, 12 mm, respectively). At a concentration of 5 mg/ml, antibacterial activity was seen against *P. gingivalis* (8 mm) and at 10 mg/ml it was seen against *P. gingivalis* and *T. forsythia* (10 mm), whereas at 15 mg/ml concentration, antibacterial activity was not seen against any of the four periodontopathogens.

**DISCUSSION**

The purpose of this research was to evaluate the antimicrobial effectiveness of 1, 5, 10 and 15 mg/ml concentrations of Cranberry extracts and to compare it with 0.2% Chlorhexidine mouthwash against *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans*.

The results of the study showed that Cranberry extract has potent antimicrobial activity on *P. gingivalis*, *P. intermedia*, *T. forsythia* and *A. actinomycetemcomitans*, the causative bacteria for periodontal disease. Cranberry extract, having anti-infective properties, has been majorly used for urinary tract infections (UTIs). The nondialyzable material (NDM) portion of the extract shows anti-coaggregation activity against a variety of oral microorganisms. Cranberry fruit has micronutrients, vitamin C, dietary fibre and manganese, which is an important dietary mineral.

NDM has been shown to be an active constituent of Cranberry juice which reverses coaggregation of bacterial pairs. Precoating bacteria with NDM can decrease the ability of biofilm formation. Cranberry extracts such as flavonols and proanthocyanidins (PAC) can influence virulence factors in *S. mutans*. PAC and Flavonol (FLAV), alone or in combination, inhibit the surface-adsorbed glucosyl transferases and F-ATPases activities and the acid production by *S. mutans* cells.

The present study included red complex, orange complex and green complex periodontopathogens, which are mainly involved in various periodontal diseases. In this study, microorganisms were cultured in sterilized blood agar and different concentrations of Cranberry extract and 0.2% Chlorhexidine was added to examine their antimicrobial activity. It has also been concluded that though the benefits of Cranberry extract towards the periodontium are not that clear, another important finding is that its consumption is in no way harmful towards periodontal health.

The results of this study are in accordance with the study done by Polak et al. which was an animal study in which experimental periodontitis was induced in mice by *P. gingivalis* and *F. nucleatum* infection. It was found that the adhesion and coaggregation of both species of bacteria onto epithelial cells could be inhibited by NDM, an active constituent of Cranberry juice in a dose-dependent manner.

Based on the findings of this study, it is believed that further clinical trials of short and long duration hold promise for assessing the clinical efficacy of Cranberry extract in the field of periodontics.

One of the drawbacks of the present study is that the disc diffusion method, although capable of measuring varying degrees of antibacterial activity, does not help to deduce the minimum inhibitory concentration or minimum bactericidal concentration. Hence the ideal concentration of Cranberry extract for antibacterial action still remains unknown.

This was an in vitro study against all the four periodontopathogens, *P. gingivalis*, *P. intermedia*, *T. forsythia* and *A. actinomycetemcomitans*. Hence, further clinical studies evaluating the different concentrations of Cranberry extract are required to assess if the results of this study can be applied to the population.

**REFERENCES**


