

Effectiveness of Indigenously Prepared Punica Granatum and Camellia Sinesis Mouthwashes as an adjunct to Non Surgical Periodontal Therapy: A Clinical Trial

Dr. Harshal Rajeshbhai Champaneri,¹ Dr. Nirma Yadav,² Dr. Barkha Makhijani,³
Dr. Neema Shetty,⁴ Dr. Aditi Mathur,⁵ Dr. Balaji Manohar⁶

^{1,2}Resident, ³Senior lecturer, ^{4,5}Reader, ⁶Professor and Head
Department of Periodontics, Pacific Dental College & Hospital, Udaipur, India

ABSTRACT

Background: Chronic periodontitis is a bacterially induced chronic disease that is immune-inflammatory in nature. The incidence and progression of this disease is related to a substantial increase in gram negative anaerobic rods. The use of antimicrobial mouthwashes as an adjunct to mechanical plaque control is well established in dental practice. Punica granatum and Camellia sinesis extract are naturally occurring antimicrobial agents. The active ingredient in Punica granatum and Camellia sinesis is polyphenolic flavonoid, which is an antioxidant in nature. It inhibits the production of arachidonic acid metabolites such as pro-inflammatory prostaglandins and leukotriens, resulting in a decreased inflammatory response.

Aim: To evaluate the efficacy of indigenously prepared Punica granatum and Camellia sinesis mouthwashes to the commercially available chlorhexidine mouthwash.

Materials and Methods: Thirty subjects diagnosed with chronic generalized marginal gingivitis were selected and randomly divided into three groups. Group 1 - Camellia sinesis mouthwash group, Group 2 - Punica granatum mouthwash group and Group 3 - Chlorhexidine mouthwash group. Subjects were instructed to use the prescribed mouthwash for 14 days. Plaque index, gingival index and calculus component of periodontal index were recorded at baseline and at 14 days.

Results: The clinical study observed significant improvement in plaque and gingival status at all sites ($p < 0.05$). Camellia sinesis and Punica granatum mouthwash showed significant improvement in plaque, gingival and calculus score.

Conclusion: Camellia sinesis and Punica granatum mouthwash are beneficial in improving gingival status due to its profound styptic action, with sufficient reduction in plaque scores.

Key Words: Chlorhexidine, calculus, Camellia sinesis, gingivitis, mouthwash, plaque, Punica granatum

INTRODUCTION

In Ayurveda, dental health (dantaswasthya) is determined by two factors, the constitution (prakriti) of each person and the climatic changes resulting from the solar, lunar and planetary influences (kala parinama).¹ In dental sciences, it is

believed that dental plaque is the main etiological factor that causes caries, gingivitis and periodontal disease. Although, brushing of teeth is considered the most effective method of cleaning the teeth and controlling plaque, mouthwashes are widely used as adjuncts to toothbrushing and in delivering active agents to the teeth and gums.² Chlorhexidine is a very popular mouthwash that has been prescribed by clinicians.

Chlorhexidine is regarded as the 'gold standard' antiplaque agent and is particularly effective against gingivitis. This mouthwash is widely used as an adjunct in the treatment of periodontitis. However; most practitioners do not recommend the long-term and daily use of chlorhexidine as a mouthwash, mainly because of its side effects, such as objectionable taste, tooth discoloration, desquamation and soreness of oral mucosa. The activity of this mouthwash is pH

Correspondence:

Dr. Harshal Rajeshbhai Champaneri
Department of Periodontics,
Pacific Dental College & Hospital, Udaipur, India
email: harshalcham@gmail.com

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dependent and is greatly reduced in the presence of organic matter. The side effects caused by this mouthwash limit the acceptability by the users for the long-term use in preventive dentistry. Manufacturers have tried to modify the taste of the mouthwashes, but the bitter taste of chlorhexidine is evidently difficult to mask.²

With the continuous need to counter the adverse effects, improve the antiplaque and antigingivitis potential and to reduce the increasing microbial resistance to conventional antiseptics and antibiotics, attention is now turning to the use of natural antimicrobial compounds (herbal extracts). In 1989, a patent had been filed at the European patent office stating that the combination of herbal extracts leads to the synergistic reduction of both dental plaque and gingival bleeding.²

Herbal products are being used since ancient times for the treatment of various ailments. Of late, the commercial use of these products in toothpaste and for oral irrigation delivery has increased manifold. Some of the natural or herbal products and their extracts, such as guava, pomegranate, neem, propolis, tulsi, green tea, cranberry and grapefruit, when used in mouthwashes have shown significant advantages over the chemical ones.²

Complementary medicine received a great attention during recent decades which recommends supplementation with various ingredients. Diverse modes of delivery including chewing candy, chewing gum, dentifrices and local drug delivery strips are introduced. Tea and in particular, green tea (*Camellia sinensis*) are among most popular beverages, with high daily consumption in Asia and especially in Iran. Several properties including antioxidant, anticaries, antibacterial, antiviral, antidiabetic, antimutagenic and antitumoral properties are addressed for *Camellia sinensis*.³ *Camellia sinensis* from the family of *Thea* Cease is mostly cultivated in coasts of Caspian sea, North of Iran. Its remedial effects are associated with the polyphenol contents comprising catechin (C), epicatechin (EC), gallic catechin (GC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). The latter two are mainly found in green tea rather than the black tea and are amongst the most potential contents to be reviewed for periodontal adjunct therapies in terms of their special anti-collagenase activity.^{4,6}

One of the recent herbal products is Pomegranate (*Punica granatum*). *Punica granatum* Linn belongs to family Punicaceae, mostly known as "Punica granatum." It is a shrub native from Asia where several of its parts have been used as an astringent, haemostatic and for diabetes control.⁷

The genus name, Punica, was the Roman name for Carthage, where the best Punica granatums were known to grow.⁸ *Punica granatum* is currently finding important applications in the field of dental health. Clinical studies have shown that this popular antioxidant superstar attacks the causes of tooth decay at the biochemical level, with remarkable vigour. When used regularly in combination with toothpaste that has been reinforced with bioactive botanical extracts, *Punica granatum* containing mouthwash may fight dental plaque and calculus formation by inhibiting the activities of the microorganisms.⁹

Sole treatment with scaling and root planing may lack achievement in complete eradication of red complex bacteria in deep narrow pockets, hence; adjunct chemical elimination for plaque induced periodontal diseases seems necessary. The aim of present study was to evaluate the effectiveness of indigenously prepared 10% *Camellia sinensis* and 20% *Punica granatum* mouthwash over 0.2% chlorhexidine mouthwash in 30 subjects.

The objectives were to evaluate the efficacy of 10% *Camellia sinensis* mouthwash and 20% *Punica granatum* mouthwash over 0.2% chlorhexidine mouthwash as an anti-plaque agent, to evaluate the efficacy of 10% *Camellia sinensis* mouthwash and 20% *Punica granatum* mouthwash over 0.2% chlorhexidine mouthwash as an anti-gingivitis agent and to evaluate the efficacy of 10% *Camellia sinensis* mouthwash and 20% *Punica granatum* mouthwash over 0.2% chlorhexidine mouthwash on inhibition of calculus formation.

MATERIALS AND METHODS

A single blinded controlled clinical trial was conducted in 30 subjects (18 males, 12 females), aged 20-60 years who presented with chronic generalized marginal gingivitis. Subjects with a minimum of 20 natural teeth with a Gingival index score (Loe and Silness) between 1.1 to 2.0, Plaque index score (Silness and Loe) between 1.1 to 2.0, and periodontal index (Ramjford calculus component) < 2 were included. Subjects who had undergone periodontal therapy in the past three months were excluded from the study.

Sample Preparation

Ten percent *Camellia sinensis* mouthwash: 500 g of dry green tea leaves were soaked in 500 ml of water and 500 ml of 95.5% absolute ethanol for 3 days, and then heated to 40 °C until the volume was reduced to a third. Finally green tea mouthwash 10% was prepared (10 ml of extract in 100 ml of distilled water) and dispensed in bottles containing 250 ml. (Figure 1)

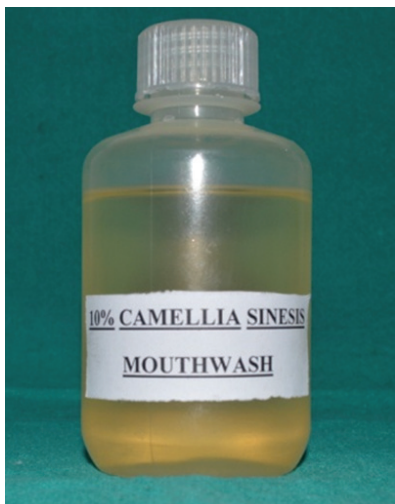


Figure 1. 10% Camellia Sinesis Mouthwash

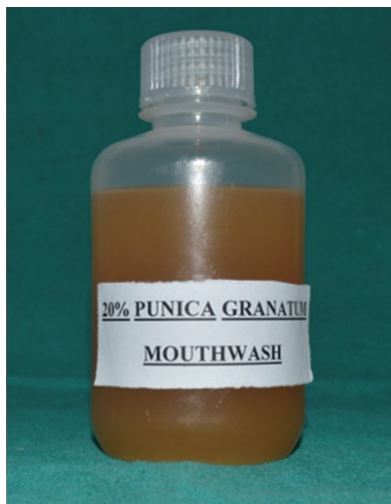


Figure 2. 20% Punica Granatum Mouthwash

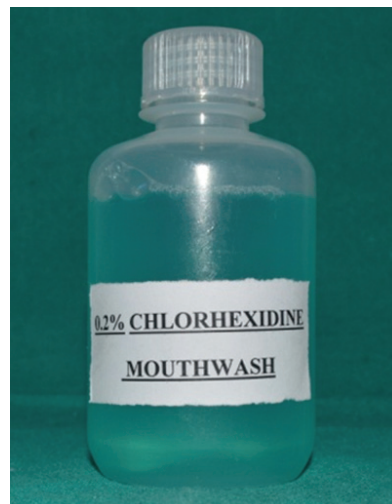


Figure 3. 0.2% Chlorhexidine Mouthwash

20% Punica granatum mouthwash: 500 g of dry rind (peel of pomegranate) were soaked in 500 ml of water and 500 ml of 95.5% absolute ethanol for 3 days, and then heated to 40°C until the volume was reduced to a third. Finally Punica granatum mouthwash 20% was prepared (20 ml of extract in 100 ml of distilled water) and dispensed in bottles containing 250 ml. (Figure 2)

The screening and clinical examinations were carried out at the Department of Periodontics, Pacific Dental College and Hospital, Debari, Udaipur. All subjects signed an informed consent form and underwent scaling and root planning.

The subjects recruited were randomly allocated into 3 groups:

Group I: 10 subjects received 10% Camellia sinesis mouthwash to rinse twice daily for 30 seconds using 10 ml solution for 14 days.

Group II: 10 subjects received 20% Punica granatum mouthwash to rinse twice daily for 30 seconds using 10 ml solution for 14 days.

Group III: 10 subjects received 0.2% chlorhexidine mouthwash to rinse twice daily for 30 seconds using 10 ml solution for 14 days.

All subjects were taught to brush their teeth twice daily by Bass method. Subjects were recalled after 14 days to evaluate clinical parameters.

STATISTICAL ANALYSIS

ANOVA test was used for statistical analysis. *p* value was adjusted at < 0.05.

RESULTS

All the 30 subjects completed the study. The present study was conducted to evaluate the effectiveness of indigenously prepared 10% Camellia sinesis and 20% Punica granatum mouthwash over 0.2% chlorhexidine mouthwash on Gingival index, Plaque index and Calculus component of periodontal index. All the clinical parameters were evaluated at baseline and after 14 days.

After professional oral prophylaxis by a single periodontist, subjects were allocated in the 3 group randomly.

Table 1 shows mean scores of all the three clinical parameters at baseline and at 14 days.

On comparison of Group I and Group II, no significant difference was seen (*p* = 0.510), suggesting 10% Camellia sinesis and 20% Punica granatum mouthwashes have similar effect on gingival index.

Table 1: Mean scores of clinical parameters

Clinical parameters	Group I		Group II		Group III	
	At base-line	14 days post-operative	At base-line	14 days post-operative	At base-line	14 days post-operative
Gingival index	1.65	0.46	1.50	0.65	1.67	1.03
Plaque index	1.47	0.36	1.50	0.67	1.49	0.89
Calculus component of periodontal index	1.86	0.10	1.74	0.20	1.62	0.30

Table 2: Comparison of Gingival index scores at 14 days

Parameters	Groups (n=10)	p value
Gingival index	Group I vs Group II	0.510
	Group I vs Group III	0.001*
	Group II vs Group III	0.027*

p<0.05; Significant

While comparing Group I and Group III, significant difference was seen ($p = 0.001$), suggesting 10% Camellia sinesis has a better effect than 0.2% Chlorhexidine mouthwash on gingival index.

A significant difference ($p = 0.027$) was also found on comparing gingival index scores of Group II an Group III, suggesting 20% Punica granatum has better effect than 0.2% Chlorhexidine mouthwash.

Group I also showed significantly greater reduction in plaque index score as compared to Group II ($p = 0.026$) and Group III ($p = 0.001$).

Group II and Group III showed similar reduction in plaque index score ($p = 0.163$). 10% Camellia sinesis showed better results in plaque reduction as compared to 20% Punica granatum and 0.2% Chlorhexidine mouthwash.

There was no statistically significant difference in the Calculus component of periodontal disease index when inter-group comparisons was made at the 14th day, suggesting all mouthwashes had similar effect on calculus formation.

DISCUSSION

Though around 6000 plants in India are used in herbal medicines, little research has been conducted on efficacy, safety and properties of herbal products. Over the decades, very few studies have been conducted to show the clinical efficacy of Punica granatum and Camellia sinesis. In the present study, it was demonstrated that hydroalcoholic extract from Camellia sinesis leaves and Punica granatum rind (peel) exerted a significant reduction in clinical parameters.

Analysis of plaque index values suggests that all three mouthwashes were helpful in reducing plaque accumulation over the 14 days study period, but Punica granatum mouthwash reduced plaque scores to a greater extent as compared to chlorhexidine. These findings are contrary to the studies by Haffajee et al.¹⁰, Ahuja et al.⁸ and Overholser et al.¹¹ who showed that Chlorhexidine was better in reducing plaque score than Punica granatum. The difference in the results may be because of difference in the methodology

Table 3: Comparison of Plaque index scores at 14 days

Parameters	Groups (n=10)	p value
Plaque index	Group I vs Group II	0.026*
	Group I vs Group III	0.001*
	Group II vs Group III	0.163*

p<0.05; Significant

Table 4: Comparison of Calculus component of periodontal index scores at 14 days

Parameters	Groups (n=10)	p value
Calculus component of periodontal index	Group I vs Group II	1.000
	Group I vs Group III	1.000
	Group II vs Group III	1.000

p<0.05; Significant

employed, i.e., instead of Punica granatum whole fruit, only the peel of Punica granatum was taken for extract preparation in our study. In a clinico-microbiological study, Menezes et al.¹² showed that after 1 minute of rinsing, greater reduction in plaque score was observed with Punica granatum (84%) mouth wash as compared to Chlorhexidine mouthwash (79%), which was in accordance to our results. In our study, Camellia sinesis mouthwash reduced plaque score to a greater extent as compared with Punica granatum and chlorhexidine mouthwash. These findings are in agreement with the studies by Jenabian et al.⁶ and Moghbel et al.¹³

Analysis of gingival index scores revealed that Punica granatum was more efficient than chlorhexidine in reducing gingival index score. Similar results were also reported by Haffajee et al.¹⁰ and Ahuja et al.⁸ A study by Salgado et al in 2006 on 10% Punica granatum gel does not support our finding as this gel was not efficient in preventing supragingival dental plaque formation and gingivitis.¹⁴ Camellia sinesis mouthwash reduced gingival score to a greater extent when compared to Punica granatum and chlorhexidine mouthwash. These findings are also in agreement with the studies by Jenabian et al.⁶ and Moghbel et al.¹³

Analysis of calculus component of periodontal index showed no statistical significance amongst all three groups.

In an urge of looking for better antiplaque and antigingivitis agents with limited side effects as compared to chlorhexidine, various herbal products have been tried with fruitful results. Punica granatum and Camellia sinesis are recent herbal product used in field of dentistry. So, more clinical and microbiological studies on a long-term basis are required to elicit the precise effectiveness of this product.

CONCLUSION:

In the absence of vigilant oral care, plaque and calculus will build up resulting in gingivitis and possibly progressing to periodontitis. So, various herbal products have been tried and have shown promising results with minimal side effects. Also, their additional effect on inflammatory pathways

and antioxidant potential make them eligible to be used as effective antigingivitis agents. Our study concluded that Camellia Sinesis mouthwash and Punica Granatum mouthwash are better antigingivitis and antiplaque agent than chlorhexidine mouthwash.

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