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## Comparative evaluation of efficacy of a botanical oral rinse containing *Punica granatum*, grapefruit seed extract, thymus vulgaris with chlorhexidine mouthwash on plaque and gingivitis

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### Abstract

Herbs are nature's gift to humans. They have various bioactive components which possess enormous medicinal value with least side effects. Over the recent years the use of these medicinal plants to treat oral conditions has resurfaced.

### Objectives:

1. To check the anti-inflammatory effect of a commercially available herbal mouthwash containing pomegranate, grapefruit seed extract and thymus vulgaris.
2. To compare the anti-inflammatory effect of the herbal mouthwash with that of Chlorhexidine.

**Materials and Methods:** Patients (males and females) having gingivitis and between the age 18 to 55 years were included in the study. Patients who were allergic to the herbs present in the mouthwash, those on antibiotics or any other mouthwash were excluded from the study. The plaque index (PI), Gingival Index (GI), and Sulcus bleeding index (SBI) were recorded at baseline and at 3 weeks. Scaling and root planing (SRP) was done for all patients and the patients were randomly allocated in one of the 3 groups: Group I: SRP and the test mouthwash, Group II: SRP and chlorhexidine mouthwash and Group III: SRP alone.

**Results:** Test mouthwash botanical oral rinse showed better results than control and CHX in relation to GI and SBI indices, but the results were not statistically significant. The results showed that botanical oral rinse show synergistic effect compared to CHX and control groups.

**Conclusion:** Botanical oral rinse containing *Punica granatum*, grapefruit seed extract, thymol has shown better anti-inflammatory effects than chlorhexidine though the results were not statistically significant.

**Keywords:** Grapefruit, thymus vulgaris, chlorhexidine, scaling and root planing

### Introduction

Gingivitis is a mild form of periodontal disease that affects a significant proportion of the global population. Gingivitis is caused by the accumulation of dental plaque, a biofilm that adhere to the tooth surface. If left untreated, gingivitis can progress to periodontitis, a more severe condition characterized by inflammation of the periodontium, leading to tooth loss and other systemic complications.

The gold standard antimicrobial for the prevention and treatment of gingivitis is chlorhexidine (CHX), that has been widely used in oral rinses. However, its use has been associated with certain side effects such as discoloration of teeth, alteration of taste, and increased risk of allergic reactions<sup>[1]</sup>. Use of herbs is in practice from ancient days. In dentistry use of herbs in the form of mouth wash and gels is become common. Herbs have various bioactive components which possess enormous medicinal values with least reported side effects<sup>[2]</sup>. Synthetic antimicrobial agents and antibiotics are known to cause antimicrobial resistance hence there was emergence of herbal products. Natural phytochemicals have proven to be good alternatives to such synthetic agents<sup>[3]</sup>. Natural products like curcuma zedoaria, calendula, aloe vera and other herbs have been used effectively to treat oral diseases in the past<sup>[4]</sup>.

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In recent years, there has been a growing interest in the use of botanical oral rinse as safer alternative for the prevention and treatment of oral diseases.

*Punica granatum*, commonly known as pomegranate, has been shown to possess anti-inflammatory and antimicrobial properties, making it a promising agent for the prevention of gingivitis [5]. Grapefruit seed extract which is rich in flavonoids and limonoids, has shown antimicrobial activity against oral microorganisms [6]. Thymol, a phenolic compound which is derived from *Thymus vulgaris*, has shown antimicrobial and anti-inflammatory properties, making it a potential agent for the prevention of gingivitis [7].

### Aim and Objective

**Aim:** To evaluate and compare the efficacy of a botanical oral rinse containing *Punica granatum*, grapefruit seed extract, and thymol with chlorhexidine mouthwash in reducing dental plaque and gingivitis.

### Objective

1. To evaluate efficacy of botanical oral rinse and chlorhexidine mouthwash in reducing plaque and gingivitis.
2. To find out whether the botanical mouth rinse is as effective as chlorhexidine mouthwash in reducing plaque and gingivitis

### Material and Methods

The clinical study was conducted in the Department of Periodontology and Oral Implantology, SDM Dental College and Hospital, Dharwad.

### Inclusion criteria

- 1) Individuals aged from 18 to 55yrs, both males and females.
- 2) Individuals with a minimum of 20 natural teeth.
- 3) Patients with moderate to severe gingivitis were included.

### Exclusion criteria

- 1) Pregnant or lactating women
- 2) Individuals with a history of systemic diseases affecting oral health.
- 3) History of allergy to any of the ingredients in the mouth rinses
- 4) Use of antibiotics or anti-inflammatory medications within the past three months

The patients who met the inclusion and exclusion criteria were selected for the study. Informed consent was taken from patients. Ethical clearance was taken from the institutional ethical committee.

A total of 45 subjects with moderate to severe gingivitis were randomly divided into three groups. Plaque index (PI), Sulcus Bleeding index (SBI) and Gingival index (GI) [8, 9, 10] were recorded at baseline. Scaling and root planing (SRP) was performed for participants of all groups. All the participants were instructed to brush twice daily using a standardised brushing technique. Patients in group I and II were given the respective mouthwashes.

**Group I:** SRP + Botanical oral rinse (*Punica granatum*, grapefruit seed extract, and thymol)

**Group II:** SRP + Chlorhexidine mouthwash (0.2% concentration)

### Group III: SRP alone

The patients were instructed to rinse their mouth with 10ml of the assigned oral rinse twice daily for three weeks. Plaque index, sulcus bleeding index and gingival index were recorded after three weeks. Results obtained for the indices were statistically analysed using a software.

### Results

In the test group i.e. botanical oral rinse there was reduction in PI, GI, SBI values from baseline to post treatment recall.

Between CHX and botanical oral rinse and control groups, botanical oral rinse group showed better results in relation to plaque index but results were not statistically significant. ANOVA values between groups was 0.44.

Test mouthwash botanical oral rinse showed better results than control and CHX in relation to GI and SBI indices but the results were not statistically significant.

The results showed that botanical oral rinse show synergistic effect compared to CHX and control groups.

### Discussion

In this study, we tested the anti-inflammatory effects of a mouthwash containing three herbal components: pomegranate, grapefruit, and *Thymus vulgaris* (thyme). Individually, pomegranate and grapefruit have demonstrated multiple beneficial effects as mouthwashes. *Thymus vulgaris*, a component of Listerine mouthwash, is known for its antibacterial properties. We aimed to achieve a synergistic effect by combining all three ingredients in a single mouthwash.

Inflammation of periodontal tissues, which leads to gingivitis, occurs due to plaque and its by products. The living microorganisms in plaque release toxins, causing gingivitis. If left untreated, gingivitis can progress to periodontitis. While mechanical plaque control is crucial for plaque removal, inaccessible areas like interproximal surfaces require special care. In such cases, chemical plaque control becomes essential.

Chlorhexidine mouthwash, though considered the gold standard, has disadvantages such as staining, burning, and potential carcinogenic effects [10]. To overcome these issues, herbal mouthwashes have been explored. The major component of pomegranate, fatty acids, punicic acid, is an excellent anti-inflammatory compound that suppresses prostaglandin production [11]. Pomegranate fruit extract has a broad inhibitory effect on MMP expression and IL-1 $\beta$ -induced tissue destruction [5]. Its anti-inflammatory effect may be due to its immunoregulatory action on macrophages, T, and B lymphocytes [12]. Pomegranate extract has also shown anti-inflammatory activity by inhibiting NF-kB activity and preventing ERK-1 or ERK-2 activation. Oral ingestion of polyphenol-rich pomegranate fruit extract inhibited COX-1 and COX-2 enzymes and IL-1 $\beta$ -induced nitric oxide and PGE2 production [13].

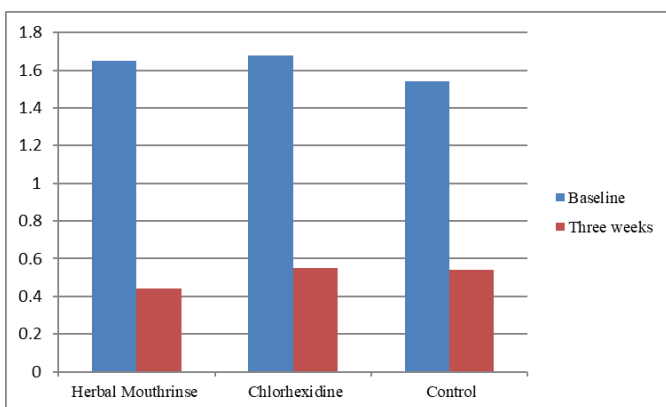
Grapefruit consumption has been observed to reduce the sulcus bleeding index. This effect is likely due to improved vitamin C supply, as it was the only intervention. Vitamin C plays a crucial role in collagen synthesis, acting as a cofactor for proline and lysine hydroxylases and influencing gene transcription. Deficient collagen production leads to higher permeability of gingival tissue, observed in vitamin C-deficient individuals [14, 15]. Legott *et al.* [16] also found that vitamin C supplementation of 250 mg/day led to decreased bleeding scores, possibly due to its role in maintaining

microvasculature structure within the sulcus. Studies have shown that periodontitis subjects produce excess ROS, thought to be a hyper-inflammatory response [17]. Grapefruit consumption may increase the antioxidant defense system of oral tissues, thereby reducing periodontal inflammation.

Thyme oil exhibits antibacterial activity and has been useful in dental practice according to Meeker H. G. (1988) [18]. One of the components of thyme, thymol, inhibits the growth of oral bacteria, reducing dental caries. Thymol is used in mouthwashes like Listerine due to its antibacterial properties [19]. Our results align with these previous studies. The test mouthwash, containing all three ingredients, demonstrated better results compared to the other mouthwash, although not statistically significant. This suggests that the synergy of the three herbs contributes to its effectiveness. However, further research with larger sample sizes and microbiological tests is necessary to conclusively prove its advantages over other mouthwashes.

**Table 1:** Mean value of Plaque Index at baseline and at 3 weeks

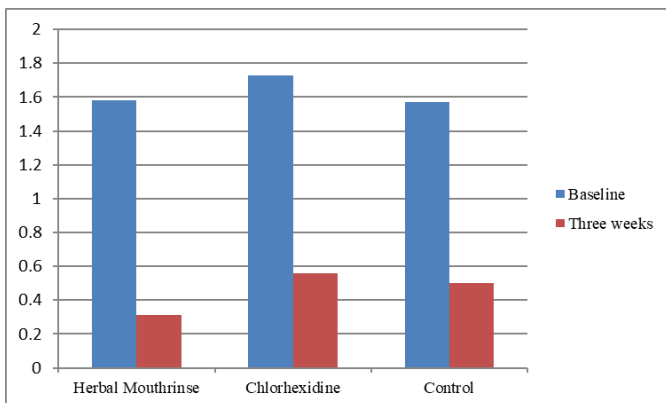
	Herbal Mouthrinse	Chlorhexidine	Control
Baseline	1.65	1.68	1.54
Three weeks	0.44	0.55	0.54



**Graph 1:** Mean value of Plaque Index at baseline and at 3 weeks

**Table 2:** Mean value of Gingival Index at baseline and at 3 weeks

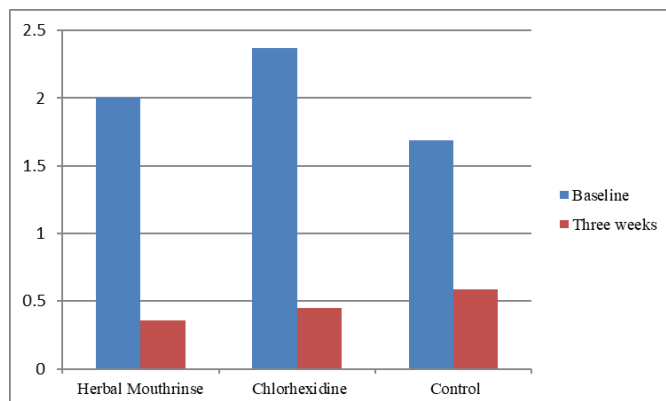
	Herbal Mouthrinse	Chlorhexidine	Control
Baseline	1.58	1.73	1.57
Three weeks	0.31	0.56	0.5



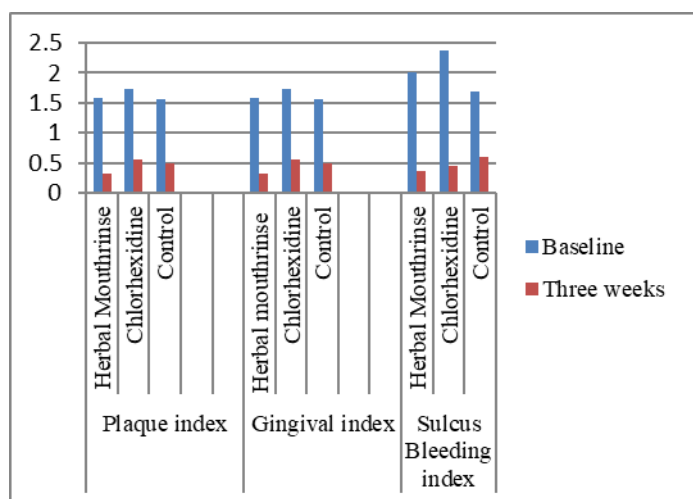
**Graph 2:** Mean value of Gingival Index at baseline and at 3 weeks

**Table 3:** Mean value of Sulcus Bleeding Index at baseline and at 3 weeks

	Herbal Mouthrinse	Chlorhexidine	Control
Baseline	2	2.37	1.69
Three weeks	0.36	0.45	0.59



**Graph 3:** Mean value of Sulcus Bleeding Index at baseline and at 3 weeks



**Graph 4:** Average value of PI, GI and SBI at baseline and after three weeks of use of mouthwash

**Conclusion**

Botanical oral rinse containing *Punica granatum*, grapefruit seed extract, thymol has shown better anti-inflammatory effects, but the results are not statistically significant. Further research is needed with a large sample size.

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