



## Research Article

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### COMPARATIVE EFFICACY OF PLACEBO VS FENUGREEK SEED EXTRACT MOUTHWASH IN GINGIVITIS SUBJECTS

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#### ABSTRACT

Gingivitis is the inflammatory condition of gingiva that supports teeth. The consensus is that toxins produced by anaerobic bacteria in oral cavity, specifically those that cause plaque formation, are cause of gingivitis. One spice that can alter texture of food is *Trigonella foenum-graecum*, commonly known as fenugreek, a member of Fabaceae family. Fenugreek has many well-established pharmacological properties, such as immunomodulatory, antidiabetic, antioxidant, hypocholesterolemic, anti-inflammatory, antiulcerogenic, and antipyretic actions. Objectives: To evaluate clinical parameters at baseline and 21 days post non surgical periodontal therapy after the use of fenugreek seed extract mouthwash and to evaluate the colony count of *Porphyromonas gingivalis* in plaque samples at baseline and after 21 days the use of fenugreek seed extract mouthwash. Methodology: A total of 30 gingivitis subjects were selected and divided randomly into Group A and B. Group A, non surgical periodontal therapy followed by placebo (15). Group B, non surgical periodontal therapy followed by fenugreek mouthwash (15). The supra gingival plaque samples were collected from both the groups at baseline and 21 days post non surgical periodontal therapy. Results: Significant reduction in clinical parameters and the colony count of *Porphyromonas gingivalis* after the use of fenugreek mouthwash. Conclusion: Fenugreek seed extract mouthwash has better promising effect on inflammation in gingivitis subjects when compared to gingivitis subjects who had undergone non surgical periodontal therapy alone.

**Keywords:** Gingivitis, Non-surgical periodontal therapy, Plaque samples, *Porphyromonas gingivalis*, *Trigonella foenum-graecum*.

#### INTRODUCTION

One of the most common clinical disorders in the oral cavity, gingivitis is characterized by inflammation of the gingival tissue, which is restricted to the soft tissues of the gingival epithelium and connective tissue. Gingivitis can take many different forms according to the aetiology, severity, duration, and clinical manifestations of the illness. Dental plaque is a thin layer of bacteria that develops on the tooth's surface as a result of poor dental hygiene. It contains a lot of bacteria and causes the gingival inflammation.<sup>1,2</sup> Among the bacteria that cause gingivitis include *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Prevotella intermedia*, and *Treponema denticola*. The two main causes of the disease's advancement are plaque and inadequate oral hygiene. Most patients are either ignorant of or unable to identify gingivitis since it is typically painless, seldom causes spontaneous bleeding, and frequently exhibits minor clinical changes.<sup>3</sup>

A few strategies to inhibit plaque formation include using mouthwash, irrigants, sprays and varnishes.<sup>4,5</sup> However, the most effective method among all is to use mouthwash like Chlorhexidine gluconate (CHX). Despite its many applications and benefits, it has certain disadvantages, including dry mouth, burning sensations, taste alteration, allergic reactions, and tooth discolouration.<sup>6</sup> Growing awareness of the advantages of complementary and alternative medicine has led to an increase in the use of herbal mouthwashes. For patients who exhibit better outcomes, any herbal mouthwash with more advantageous benefits than Chlorhexidine gluconate (CHX) is necessary.

There are many herbs like coriander, mint, clove, aloe vera with anti-inflammatory, antimicrobial, antioxidant, immune suppressive and anti-bacterial properties against potent periodontal pathogens like *Porphyromonas gingivalis*, which were used as mouth wash in gingivitis patients.<sup>7,8</sup>

*Trigonella foenum-graecum*, a member of Fabaceae family is one of the spices that can change the texture of food. Fenugreek is native to Eastern Europe and parts of Asia but now widely cultivated almost all over the world for its leaves and seeds, which are commonly used as leafy vegetables and condiments. It is variously known as Fenugrec (French), Methi (Hindi), Bockshorklee (German), Fienogreco (Italian), Pazhitnik (Russian), Alholva (Spanish), Koroha (Japanese), Hulba (Arabic), Halba (Malaya), and K'u-Tou (China) and Methika (Sanskrit) is commonly known as fenugreek (English). While the leaves are consumed as green leafy vegetables, the seeds are utilized as spices all over the world.<sup>9</sup>

By raising awareness of the nutritional and health benefits of fenugreek, this study aims to promote its use in commercial food manufacturing. It's established medicinal qualities are explained by the literature on its health benefits and pharmacological effects, which also adds novel therapeutic effects in new indications. In addition to its well-known medicinal properties, which include carminative, gastric stimulant, antidiabetic, and galactagogue (lactation-inducer) effects, more recent studies have found hypocholesterolemic, antilipidemia, antioxidant, hepatoprotective, antifungal, antiulcer, antilithogenic, anticarcinogenic, and other medicinal effects.<sup>10</sup> Dietary fenugreek seeds contain a bioactive substance called diosgenin

(dios), a naturally occurring steroidal saponin and it has strong anti-inflammatory and antibacterial qualities against oral pathogens like *Porphyromonas gingivalis*.<sup>11</sup>

**MATERIALS AND METHODS**

**Type of study:** It is an original study which was performed in April 2025 to June 2025.

Ethical approval for the study was taken from the institutional ethics committee of P.M.N.M. Dental College and Hospital, Bagalkote, Karnataka, India (Ref. No. PMNMDCH/4259/2024-25).

A total of 30 subjects diagnosed with gingivitis aged 18-40 years, were randomly selected from the Outpatient Department of Periodontics, P.M.N.M Dental College and Hospital, Bagalkote, Karnataka, India.

The inclusion criteria includes male and female healthy subjects from 18 to 40 years of age, who did not undergo any treatment for gingivitis and were not under any medication within last 6 months which might alter the periodontal status.

The exclusion criteria includes subjects with systemic diseases that could influence periodontal conditions and who have undergone periodontal therapy in the past 6 months. Subjects on any systemic antibiotics, anti-inflammatory, hormonal therapy or corticosteroid therapy for any other reasons which affect the periodontal status were also excluded.

Keeping alpha error at 5%, β error 15%, power of the study at 85%, the sample size estimated was approximately 10 in each group. For follow-up study to avoid loss due to attrition we had taken 15 subjects in each group. Subjects were randomly divided into 2 groups, the control group (group 1 undergone scaling and root planning alone) and test group (group 2 undergone scaling and root planning followed by fenugreek seed extract mouthwash)

Chi-square tests, independent t tests, Mann-Whitney U tests for comparing two groups, and descriptive statistics with frequency, percentage, mean SD, etc. were used to evaluate the acquired data. Every participant received a thorough explanation of the purpose and methodology of the study. Every person who agreed to take part in the study provided a properly signed written informed consent form.

2500 ml of commercially available ORA- Sweet oral solution was added to 2500 ml of distilled water to obtain placebo<sup>12</sup> (Figure 1).

1000 g of fenugreek seeds were purchased from local market. After being cleaned and ground into a powder, they were soaked in 2500 ml of 90% ethanol for 30 days. Whatman filter paper was used to filter the filtrate, and a water bath was used to further treat it at 60°C. Then the mixture was dried at 50°C to get a highly concentrated extract. The extract was refrigerated in an airtight bottle till it's used. Distilled water was used to dilute the extract at a ratio of 1:4 to obtain the final mouthwash.<sup>13</sup> (Figure 2).

**Clinical examination to assess the Periodontal condition**

Every participant underwent a clinical evaluation utilizing the gingival index, plaque index, and bleeding on probing at baseline and 21 days following nonsurgical periodontal therapy. Supra gingival plaque samples were taken on day 0 and after 21 days from the buccal surface of each quadrant's first molar using a sterile curette. In a sterile Eppendorf tube, it was mixed with 500µl of reduced transport fluid (RTF). The samples were then sent to microbiological analysis for bacterial culture to determine the *Porphyromonas gingivalis* colonies.<sup>14</sup>

Oral prophylaxis was carried out after baseline data collection. Group A participants were instructed to use placebo and Group B participants to use supplied fenugreek mouthwash and avoid using any other oral hygiene products other than toothpaste and toothbrush.

**RESULTS**

The results of the study showed that there was a statistical difference in colony forming unit of *Porphyromonas gingivalis* and clinical parameters in group B compared to group A after 21 days. The scores of changes in all parameters except CFU from baseline to Day 21 in Group A and Group B did not follow normal distribution. Therefore, the non-parametric tests were applied. Otherwise parametric tests would have been applied (Table 1). The colony forming unit (CFU) of *Porphyromonas gingivalis* was significantly lower in the group who received scaling and fenugreek mouthwash than in the group that who received scaling along with placebo with mean difference of 18.90 in group A and 16.35 in group B (Table 2, Figures 3 and 4). Gingival index score showed a mean difference of 0.49 in group A from base line to day 21 and group B showed a mean difference of 0.80 from base line to day 21 (Table 3). Plaque index score showed a mean difference of 0.16 in group A from base line to day 21 and group B showed a mean difference of 0.57 from base line to day 21 (Table 4). Bleeding on probing score showed a mean difference of 0.38 in group A from base line to day 21 and group B showed a mean difference of 0.45 from base line to day 21 (Table 5).

**Table 1: Normality of changes in all parameters from baseline to Day 21 in Group A and Group B**

Parameters	Groups	Kolmogorov-Smirnov	df	Sig.	Shapiro-Wilk	df	Sig.
GI	Group A	0.1350	20	0.2000	0.7500	20	0.3630
	Group B	0.1750	20	0.0890	0.9040	20	0.0500*
PI	Group A	0.1780	20	0.0980	0.8390	20	0.2280
	Group B	0.2010	20	0.0350*	0.9500	20	0.3600
BOP	Group A	0.1720	20	0.1190	0.9110	20	0.0670
	Group B	0.2900	20	0.0001*	0.7630	20	0.0001*
CFU	Group A	0.1585	20	0.2000	0.9370	20	0.7010
	Group B	0.1520	20	0.2000	0.9575	20	0.3340

Table 2: Comparison of Group A and Group B with CFU counts at baseline and Day 21 treatment time points by Independent t test

Time points	Group A		Group B		t-value	P-value
	Mean	SD	Mean	SD		
Baseline	33.75	7.93	26.2	9.95	1.6859	0.0998
Day 21	14.85	4.36	9.85	4.32	3.2424	0.0025*
Difference	18.90	4.70	16.35	7.07	0.1843	0.8548

Table 3: Comparison of Group A and Group B with GI scores at baseline and Day 21 treatment time points by Mann-Whitney U test

Time points	Group A			Group B			U-value	Z-value	P-value
	Mean	SD	Mean rank	Mean	SD	Mean rank			
Baseline	1.16	0.34	23.33	1.08	0.27	17.68	142.5	1.5148	0.1298
Day 21	0.67	0.19	27.50	0.28	0.20	13.50	62.0	3.7735	0.0002*
Difference	0.49	0.41	18.18	0.80	0.28	22.83	143.5	-1.2443	0.2134

Table 4: Comparison of Group A and Group B with PI scores at baseline and Day 21 treatment time points by Mann-Whitney U test

Time points	Group A			Group B			U-value	Z-value	P-value
	Mean	SD	Mean rank	Mean	SD	Mean rank			
Baseline	1.06	0.20	21.03	0.93	0.32	19.98	189.5	0.2705	0.7868
Day 21	0.71	0.11	30.10	0.36	0.17	10.90	8.0	5.1801	0.0001*
Difference	0.35	0.16	13.53	0.57	0.32	27.48	60.5	-3.7600	0.0002*

Table 5: Comparison of Group A and Group B with BOP scores at baseline and Day 21 treatment time points by Mann-Whitney U test

Time points	Group A			Group B			U-value	Z-value	P-value
	Mean	SD	Mean rank	Mean	SD	Mean rank			
Baseline	0.96	0.35	21.83	0.87	0.28	19.18	173.5	0.7033	0.4819
Day 21	0.58	0.24	26.50	0.42	0.12	14.50	80.0	3.2325	0.0012*
Difference	0.38	0.39	17.53	0.45	0.26	23.48	140.5	-1.5960	0.1105



**Filtration**                      **Placebo**

Figure 1: Method of preparation of placebo

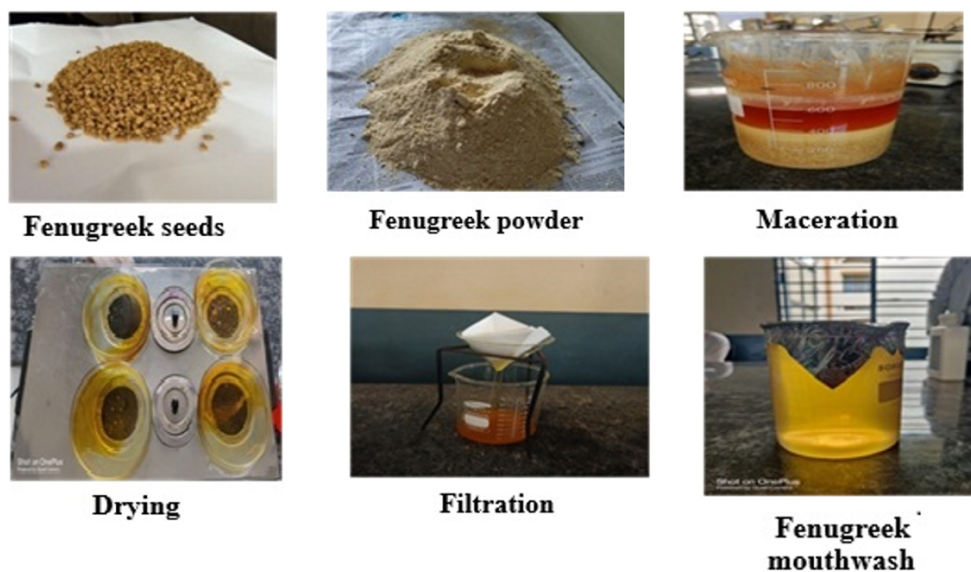


Figure 2: Method of preparation of fenugreek seed extract mouthwash

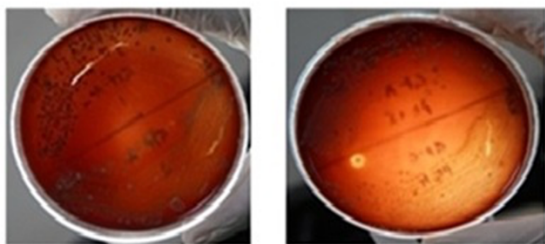


Figure 3: Group A showing CFU count at Baseline and day 21

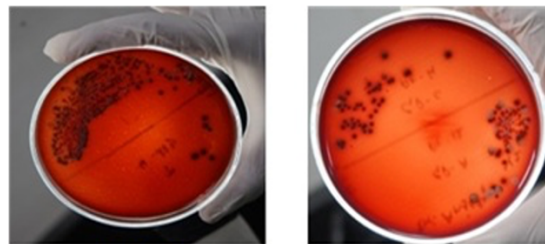


Figure 4: Group B showing CFU count at Baseline and day 21

## DISCUSSION

Bacterial infection is the most common cause of gingivitis, an inflammatory condition of the gingival tissue. The junctional epithelium does not migrate because there is no loss of attachment, unlike in periodontitis. Caries, gingivitis, and periodontitis are all known to be mostly caused by dental plaque. To inhibit the developing disease progression, effective plaque control is crucial. Combining mechanical plaque removal with a chemical plaque management treatment, such as mouthwash, can have a better antibacterial effect throughout the oral cavity. One of the most regularly prescribed chemical plaque control treatments in dentistry is Chlorhexidine. It's mainly utilized due to its antiseptic and antibacterial properties. Compared to other antimicrobials (like antibiotics), chlorhexidine gluconate has a faster rate of killing and inactivates bacteria with a wider spectrum.<sup>15</sup> Mouthrinses with anti-plaque properties are essential for controlling the progression of gingivitis by reducing plaque.

E. Polizzi *et al.* in their studies detailed about the side effects of chlorhexidine-based mouthwashes including burning sensation, dry mouth, and minor discoloration.<sup>16-17</sup>

As consumers are aware of the advantages of complementary and alternative medicine, hence there has been a recent rise in the use of herbal mouthwash. Among which fenugreek is one of them which has anti-inflammatory and antibacterial properties in different forms in gingivitis and periodontitis. The literature contains a wealth of information about the pharmacological effects and health advantages of fenugreek due to its established medicinal qualities and additional therapeutic effects in more recent indications. Divya Jyothi *et al.* conducted a study on formulation and evaluation of an herbal anti-inflammatory gel containing *Trigonella foenum graecum* seed extract and concluded that fenugreek has high potential as an anti-inflammatory agent when formulated as an herbal gel for topical use and can be effective in acute inflammatory disorders.<sup>18</sup>

Neethu Varghese *et al.* had investigated the clinical evaluation of fenugreek toothpaste and regular toothpaste in control of gingivitis in which it was evident that fenugreek toothpaste reduced the clinical characteristics of gingivitis, making it an effective treatment. This has implied that fenugreek toothpaste is important for lowering gingival inflammation. When individuals with persistent gingivitis used fenugreek toothpaste, their gingival health significantly improved.<sup>19</sup>

Syeda Amber Zaidi *et al.* conducted a study on comparison of flax seeds extract and fenugreek seed extract rinse against streptococcus mutans colonies where it was found that both the rinses and the intragroup comparison of the positive and negative controls revealed a substantial difference in the number of bacterial colonies. Herbal extracts have equivalent potential to reduce *Streptococcus mutans* colonies, as evidenced by the negligible comparison between the positive control and flax and fenugreek seed extracts. Hence, they concluded that

mouthwashes containing extracts from flax and fenugreek seeds decreased the number of bacterial colonies just as much as mouthwashes that are commercially available.<sup>20</sup>

The objective of this study was to evaluate the fenugreek seed extract mouthwash's antibacterial and anti-inflammatory properties in gingivitis patients. The supragingival plaque collected in this investigation was transferred in RTF, quickly transported to the microbiology laboratory, centrifuged, and diluted in a 1:10 ratio. Kanamycin blood agar was used to cultivate *Porphyromonas gingivalis*, which were then incubated in a CO<sub>2</sub> jar for 48–72 hours. Following the incubation time, the number of colonies and bacterial growth were measured. With a P value of 0.0001, the CFU count revealed a mean difference of 18.90 in group A and 18.55 in group B from baseline to 21 days.

GI index score in this study revealed a mean difference of 0.49 in group A from baseline to day 21 and a mean difference of 0.64 in group B from baseline to 21 days, with a P value of 0.0001.

Similarly Shahabe Saquib Abullais *et al.* conducted a study on comparative evaluation of 3 commercial mouthwash formulations on clinical parameters of chronic gingivitis subjects in which three periodontal clinical parameters PI, GI and BI were recorded at baseline and on days 7, 14, and 28. A double-blinded intervention (a coded mouthwash bottle) was conducted after an oral hygiene maintenance program. Analysis of variance (ANOVA) with multiple t tests was used for both intra-group and inter-group comparisons. At P<0.05 or below, all likely values were deemed to have different levels of significance. Study concluded that Manuka mouthwash is just as effective as CHX mouthwash for treating chronic gingivitis since no variations in any clinical parameters were seen at any point.<sup>21</sup>

PI index score in this study showed a mean difference of 0.35 in group A from baseline to day 21 and group B showed a mean difference of 0.7 from baseline to 21 days with a P value of 0.0001.

Similarly, Shilpa Gunjal *et al.* conducted a study on comparative effectiveness of Propolis with chlorhexidine mouthwash on gingivitis – a randomized controlled clinical study where patients were assigned into 3 groups and segregated as Groups A, B and C were treated with 0.2% Propolis, Chlorhexidine, and Placebo mouthwash following the non-surgical periodontal therapy. Supragingival plaque and gingival inflammation were assessed by full mouth Plaque index (PI) and gingival index (GI) at baseline and after 21 days for both the groups. At baseline, intergroup comparison revealed no statistically significant difference between Groups A, B and C (p>0.05). On day 21, one-way ANOVA revealed statistically significant difference between the three groups for PI (p,0.01) and hence concluded that propolis mouthwash showed notable improvements in plaque reduction and gingival health. As a result, it could serve as a successful herbal mouthwash substitute for mouthwash containing chlorhexidine.<sup>22</sup>

Bleeding on probing score in this study showed a mean difference of 0.38 in group A from base line to day 21 and group B showed a mean difference of 0.57 from base line to day 21 with a P value of 0.0002. All the results indicate the statistical difference in the various clinical parameters and CFU among both the groups at baseline and after 21 days.

Similarly Mohammadreza Talebi Ardakane *et al.* conducted a study on effect of herbal mouth washes on periodontal patients with plaque induced gingivitis: A cross over clinical trial in which patients who had undergone scaling and root planning were prescribed with 0.2% Chlorhexidine mouthwash (group 1) and herbal mouthwash (group 2) twice a day for 14 days. Both groups have received saline mouthwash for subsequent 14 days. Clinical parameters GI, PI, PPD and BOP were evaluated on baseline and after 14 days. Both groups have exhibited statistically significant improvements in periodontal indices compared to baseline ( $p < 0.005$ ) however there were no significant differences in these 2 groups and concluded that after 14 days, the experimental herbal mouthwash improved the periodontal condition in plaque-induced gingivitis, and its effects on GI, PI, PPD and BOP were similar to those of 0.2% CHX mouthwash.<sup>23</sup>

## CONCLUSION

Herbal mouthwashes have recently demonstrated their efficacy in dentistry by enhancing better clinical outcomes. The anti-inflammatory and antibacterial properties of fenugreek seed extract mouthwash in lowering a number of clinical parameters and *Porphyromonas gingivalis* colony count in gingivitis patients have been described in this study. The aforementioned advantages of fenugreek seeds have been improved a number of clinical outcomes.

## ACKNOWLEDGEMENT

MIC of the fenugreek extract against periodontal pathogen *Porphyromonas gingivalis* (0.2µl/ml) was determined in Department of microbiology laboratory, Maratha Mandal Dental College and Hospital, Belgaum district, Karnataka, India. Preparation of placebo and fenugreek mouthwash was done in Hanagal Shree Kumareshwar College of Pharmacy, Bagalkote district, Karnataka, India.

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