

# Antibacterial Efficacy of Fenugreek Seed Extract Rinse against *Streptococcus Mutants* Colonies

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## ABSTRACT

**Background:** Antibiotics has been widely used as standard antibacterial treatment for long but exploited use of these drugs, has led microorganisms to developed resistance against many antibiotics. While 33% of the population are dependent on the therapeutic plant extract to meet their essential need, the awareness towards these phytochemicals extracts have gained wide acceptability and are being prescribed by the physicians as a treatment option for antibacterial medications. Potential source of new antibacterial compound which are sourced through extracts of fenugreek seeds was found to have effect on six different strains of pathological bacteria. The aim of current study is to further identify the antibacterial efficacy of fenugreek seed extract mouth rinse against streptococcus mutants.

**Methodology:** It was a preclinical experimental study conducted at Shaheed Muhtarma Benazir Bhutto Medical College, Lyari Larachi, Pakistan, from January - June 2021. Calculated sample size was n=60 Consecutive sampling technique was used. Plaque of study participants was collected on sterile strips that were transported to laboratory for culture in sterile containers. For culture *S. mutant* samples were inoculated in Columbia Agar with 5% sheep blood and incubated for 48 h at 37 °C and increased level of CO<sub>2</sub>. The fenugreek seed extract was diluted in distilled water in 1:4 (Fenugreek seed extract: Distilled water). Study participants were instructed to not brush their teeth before sampling. Study participants were divided into three groups (negative control, positive control and experimental) each group had 20 participants. Diluted Fenugreek seed extract was given to experimental group for rinses, distilled water was given to negative control group and Positive controls were given a commercially available mouth rinse.

**Results:** Paired analysis depicted that there was significant decrease in number of colonies in positive controls and experimental group and there was no change in number of colonies in control group.

**Conclusion:** Fenugreek seed extract showed comparable antibacterial properties to commercially available mouth rinse and reduced the number of colonies nearly equivalent to that.

**KEY WORDS** Fenugreek seed, *Streptococcus mutants*, Antibacterial medications, Colonies

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## INTRODUCTION

Antibiotics has been widely used as standard antibacterial treatment for long but exploited use of these drugs, has lead microorganisms to developed resistance against many antibiotics due to which many conventional therapies now a days are ineffective which causes the recurrence of infections [1,2]. The alarming increase in bacterial capability of developing resistance has urge the use of plants extracts which has antibacterial property in

them [3]. While 33% of the population are dependent on the therapeutic plant extract to meet their essential need, the awareness towards these phytochemicals extracts have gained wide acceptability and are being prescribed by the physicians as a treatment option for antibacterial medications [3,4]. With increase in plant study for their medicinal properties, restorative properties of Fenugreek (*Trigonella Foenum-gracium*) seeds have been highlighted for not only its antibacterial properties but antioxidant, anti-inflammatory and anti-cancer properties were also discovered [5].

Fenugreek seeds was found to be enriched with various metabolite variety that individually or collectively attribute to have antimicrobial properties such as;

alkaloids, tannins, flavonoids, glycosides and terpenoids [5]. Potential source of new antibacterial compound which are sourced through extracts of fenugreek seeds was found to have effect on six different strains of pathological bacteria [6]. Ethanol and aqueous extract from fenugreek seed had almost similar activity on bacterial species, but the ethanol extract had most pronounced effect on both Gram-positive and Gram-negative bacterial strains [7,8].

*Streptococcus mutans* an anaerobic gram-positive bacterium is a chief etiologic agent for causing dental caries and their natural habitat is the oral cavity, specifically the dental plaque [9]. This bacteria has a biofilm dependent way of life and is a mandatory human pathogen which has a host-associated lifestyle [10,11]. The three core trait of *S. mutans* for its cariogenic potential are: (i) Potential to synthesize substantial amount of glucan from sucrose that aids in buildup of extracellular polymeric matrix which leads to permanent colonization, (ii) Capacity to transport and ability to metabolize large assortment of carbohydrates into organic acids, and (iii) They have high stress tolerance with ability to withstand and survive in tough surroundings, such as low pH [10].

*S. mutans* strains based on cell surface composition are categorized in to four different types of serological group (c, e, f and k), and 75% of the serotype c is found to be associated with dental plaque, while 20% serotype e and 5% mixture of serotype f or k are linked to dental plaque [11]. Multiple studies have shown the antibacterial activity of fenugreek seeds extracts on *S. mutans* ability to form biofilm and reduce its capability to produce acid which was directly proportional to the extracts' dosage [12]. Researchers have investigated multiple ways through which extracts from the fenugreek seeds was able to increase the salivary pH up to 7.83 within ten minutes, these extracts not only helped in lubrication and moisten of the mouth but simultaneously provided protection against dental caries [12]. All this activity is of significant importance as it decreases the demineralization of the tooth's outer surface. Therefore, the aim of current study is to further identify the antibacterial efficacy of fenugreek seed extract mouth rinse against *streptococcus mutans*.

## METHODOLOGY

It was a preclinical experimental study conducted at

Shaheehd Muhtarma Benazir Bhutto Medical College, Lyari Larachi, Pakistan, from January-June 2021. Calculated sample size was n=60 Consecutive sampling technique was used. Plaque of study participants was collected on sterile strips that were transported to laboratory for culture in sterile containers. For culture *S. mutans* samples were inoculated in Columbia Agar with 5% sheep blood and incubated for 48 h at 37°C and increased level of CO<sub>2</sub>.

Fenugreek seed were purchased from local market and authentication number i.e. Specimen voucher 53 was allotted. 1000-gram fenugreek seeds were soaked in 2500ml of 90% ethanol for 30 days after washing and grinding to powder. Filtrate was then filtered with Whitman filter paper that was further processed at 60°C by using water bath. The mixture was then dried at 50°C until a well concentrated extract was produced. The extract was kept in an airtight bottle and stored in a refrigerator till usage. The fenugreek seed extract was diluted in distilled water in 1:4 (Fenugreek seed extract: Distilled water). Study participants were instructed to not brush their teeth before sampling. Study participants were divided into three groups (negative control, positive control and experimental) each group had 20 participants. Diluted Fenugreek seed extract was given to experimental group for rinses, distilled water was given to negative control group and Positive controls were given a commercially available mouth rinse. Next sample of plaque was collected after two hours to observe the effects of fenugreek seed extract on bacterial colonies. ANOVA followed by post hoc tuckies and Paired t test was applied as test of significance, <0.05 p-value was considered as significant at 95% confidence interval.

## RESULTS

The mean age of the study participants was 29 ± 5 Table 1 shows the demographic data of study participants. Paired analysis depicted that there was significant decrease in number of colonies in positive controls and experimental group and there was no change in number of colonies in control group as shown in Table 2. ANOVA was applied on the results of paired analysis that showed significant (p-value=0.001) difference among the groups followed by post hoc analysis. Post hoc analysis showed no difference between positive control and experimental group. Results of post hoc analysis are depicted in Table 3.

**Table 1: Demographic data of study participants.**

Age (mean)	29 ± 5						
Gender F (P)	Male	19 (47.5%)			Female	21 (52.5%)	
Education F (P)	Matric	8 (20%)	Inter (XII)	12 (30%)	Graduate	20 (50%)	
Brushing F (P)	Daily	28 (70%)	Alternative day	8 (20%)	Once a week	4 (10%)	

**Table 2: Number of colonies before and after rinses in negative control, positive control and experimental groups.**

	Negative Control	Positive control	Experimental
Before	8 x 10 <sup>4</sup>	10 x 10 <sup>4</sup>	9 x 10 <sup>4</sup>
After	7 x 10 <sup>4</sup>	4 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>
P value	0.417	0.001*	0.040*

**Table 3: Post hoc analysis among groups.**

Groups wise comparison				p-value
Negative control	7 x 10 <sup>4</sup>	Positive control	4 x 10 <sup>4</sup>	0.005*
Negative control	7 x 10 <sup>4</sup>	Experimental	5 x 10 <sup>4</sup>	0.010*
Positive control	4 x 10 <sup>4</sup>	Experimental	5 x 10 <sup>4</sup>	0.092

## DISCUSSION

Periodontal disease and dental caries, both of which are deemed as "Pouch oral infections," are caused by dental biofilm [13,14]. Mouth rinses, when used in conjunction with mechanical tooth brushing approach and dental flossing, helps to maintain oral biologic balance, as mechanical plaque control techniques entirely cannot preserve oral health on their own [13,14]. To control supragingival plaque deposition and gingivitis, a variety of substances have been used. But most of the time chlorhexidine takes the lead because of its high substantivity and broad range of activity it is regarded as the gold standard of antibacterial rinses [15]. Studies have shown that rinsing with a chlorhexidine antimicrobial mouth rinse reduced bacterial plaque by a significant amount [16]. Besides all the beneficial qualities of Chlorhexidine mouth wash it is not a "magic solution," as it has a various side effects such as alteration in taste, irritation in mucosa, dry mouth, discoloration of teeth and cytotoxicity therefore; the World Health Organization advised researchers to investigate the potential of herbs and plant extracts for their antimicrobial effects [17,18]. Herbs have been shown to be safe against a variety of health problems when used alone or in combination [19]. Many antimicrobials derived from plants have been discovered to be useful in the treatment of periodontal disease for instance reduce inflammation, prevent dental cavities, and heal gingivitis and periodontitis [19]. The herbs used in this study were Fenugreek (*Trigonella foenumgraecum*) and the goal was to compare the antimicrobial activity of herbal mouthwash containing Fenugreek seed extract to commercially available mouthwash.

On intragroup comparison of our study, there was statistical significance in all clinical indicators while only the plaque index showed statistical similar significance among the positive and experimental intergroup when comparisons were made. There was a reduction in the plaque index of males and female using teeth brushing technique and mouthwashes, alternate days or once a week values when compared with the baseline values in both the groups. A study conducted showed fenugreek seed extract to be beneficial against oral infections when used for the gingivitis treatment, it had fewer negative

effects and less expensive than synthetic treatments [20]. Previous research found that fenugreek seed's ethanolic chemical compound were more effective than aqueous compound of the herbs because aqueous extracts had a shorter shelf life in addition to that the enzyme polyphenol oxidase, which degrades polyphenols in water extracts but is inactive in methanol and ethanol extracts, could be to blame for the lower activity of the aqueous extract [21]. Furthermore, ethanol was discovered to infiltrate the cellular membrane more deeply [21].

In this study, the sample from Plaque was taken after two hours of mouth rinse and it was compared with the reading from commercial mouthwash and the result were likewise on the bacterial colonies. As evident from the results of our study, there was no significant difference between commercial and herbal mouthwash a similar study conducted in a hospital of India where plaque index was recorded of the participants after 30 days of using herbal fenugreek seed mouth wash and other half using commercial chlorhexidine mouthwash, comparison of these both mouthwashes was similar in terms of mycobacterial analysis [21]. Along with plaque index, sulcus bleeding index scored was also decreased which was due to the anti-inflammatory activity of flavonoids and saponins components of fenugreek seed extract which reduced the synthesis of phorbol-12-myristate-13-acetate-induced inflammatory cytokines like IL-1, IL-1, IL-2, IL-6, and tumor necrosis factor (TNF) and effective against *S. mutans* was revealed by cell lysis of the bacteria by disruption of the cell membrane [21,22]. Another study backs this up that fenugreek seeds have a broad spectrum of activity, which could be related to flavonoid chemicals in the extract's capacity to combine with cell walls, inducing cell wall rupture [23]. Our findings were comparable to those of dental clinic in India, who compared 1% fenugreek seed and turmeric extract mouthwash to 2% chlorhexidine mouthwash and discovered that herbal mouthwash was just as efficient as chlorhexidine in lowering gingival and plaque index [24-26]. Another study conducted elsewhere on fenugreek seed with other herbal extracts was compared with distilled water and the test discovered that herbal mouthwash had lower plaque index than distilled water

when used on the participant thus, in contrast to the oral health efficacy of mouthwash containing fenugreek seed extract to that of a placebo in their study. Before and after the study period of plaque buildup and gingival bleeding were measured. When compared to pre-treatment data, they found that using herbal mouthwash enhanced gingival health [27,28]. Another investigation used a 4-day plaque re-growth model to compare a natural product like fenugreek seed extract, to chlorhexidine mouthwashes and they came to the conclusion that the natural product came in second place to chlorhexidine in terms of plaque inhibition [29].

Our study found that herbal mouthwash including fenugreek seed extract had similar effect as commercial mouthwash. Combining many of these herbal extracts in a single mouthwash would undoubtedly provide greater benefits in terms of eliminating oral infections. Despite its antibacterial and antiplaque properties, long-term usage of commercial mouthwashes like chlorhexidine is not recommended, as at lower concentrations, studies have revealed that chlorhexidine has no plaque inhibiting effects [24,25]. Furthermore, herbal mouthwash is less expensive than mouthwash containing 0.2 percent chlorhexidine gluconate, as a result, currently studied herbal mouthwash with fenugreek seed may be a better option to 0.2 percent chlorhexidine gluconate mouthwash in low socioeconomic populations [24].

### CONCLUSION

Fenugreek seed extract showed comparable antibacterial properties to commercially available mouth rinse and reduced the number of colonies nearly equivalent to that.

### LIMITATIONS

Only *Streptococcus mutant* colonies were checked and counted.

### ETHICAL APPROVAL

Study was approved by the ERC.

### CONFLICT OF INTEREST

None.

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