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# Comparison of Antibacterial Efficacy of Fenugreek Seed Extract Rinse and Nigella Sativa Seed Extract Rinse against *Streptococcus* Mutant Colonies

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Dental infections were caused by the bacterium overgrowth on the surface of the tooth, and treatment should always be set up to prevent this development.Antibiotics have long been used as a conventional antibacterial medication, but their overuse has resulted in microbes gaining resistance to many of the antibiotics, trying to make many commercialized therapeutic remedies ineffectual and resulting to infection recurrence. In this regard we aim to analyze the antibacterial activity of *nigella sativa seeds'* and *fenugreek seed* extract rinses against *S. mutans'* colonies.

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**Methodology:** It was a preclinical experimental study conducted at Baqai Medical and Dental College Karachi., from January - June 2021. Calculated sample size was n = 80 Consecutive sampling technique was used. Plaque of study participants was collected on sterile strips that was transported to laboratory for culture in sterile containers.The extract of *Fenugreek seed* and *nigella sativa* seed was kept in an airtight bottle and stored in a refrigerator till usage. The extracts were diluted in distilled water in 1:4 (Extract: Distilled water). Study participants were instructed to not brush their teeth before sampling. Study participants were divided into four groups (negative control, positive control, *fenugreek seed* extract group and *Nigella sativa* group) each group had 20 participants. Diluted *Fenugreek seed* extract and *Nigella sativa* extract was given to experimental groups for rinses.
**Results:** There was significant decrease in number of colonies in positive controls, *fenugreek seed* extract group and *nigella sativa* extract group and there was no change in number of colonies in control group. Furthermore, the analysis showed significant (p-value = 0.001) difference among the groups followed by post hoc analysis. Post hoc analysis showed no difference between positive control, *fenugreek seed* extract group and *nigella sativa* group.
**Conclusion:** *Fenugreek seed* extract and *Nigella Sativa* seed extract showed comparable antibacterial properties. Also, the effect was found to be similar to commercially available mouth rinse.

*Keywords: Antibacterial properties; fenugreek; nigella sativa; extract rinse; streptococcus mutants.*

## 1. INTRODUCTION

The most frequent bacterial infections in people are dental diseases including dental decay and periodontal disease. Because of their non-life-threatening nature and widespread distribution, their importance in overall human health has been overlooked [1]. And over the last few decades, serious antibiotic-resistant microorganisms have become increasingly common [2]. Dental infections were caused by the bacterium overgrowth on the surface of the tooth, and treatment should always be set up to prevent their development. However, the financial cost of treating these oral infections can be enormous [3]. Hence this concept of bacteriologically nonspecific dental diseases provides basis for antibiotic prophylaxis in addition to daily tooth surface debridement with modern versions of traditional tools such as toothbrushes, floss, and tooth sticks [3].

Antibiotics have long been used as a conventional antibacterial medication, but their overuse has resulted in microbes gaining resistance to many of the antibiotics, that resulted in many commercialized therapeutic remedies ineffectual that led to infection recurrence. This worrisome rise in the ability of bacteria to evolve resistance has necessitated the use of plant extracts possessing antibacterial medicinal effects [4]. While up to 35% of the people already rely on therapeutic plant based seed extract to improve their living conditions, phytochemical extracts had achieved widespread recognition and are now being recommended by practitioners as a therapy alternate for antibacterial drugs [5]. Restorative benefits of *Nigella sativa* seeds (black cumin) and *Fenugreek* (*TrigonellaFoenum-gracium*) *seeds* have now been distinguished for not only its antibacterial potential but also for their antioxidant, anti-inflammatory, anti-fungal and anti-cancer characteristics, owing to a boost in botanical exploration for their therapeutic properties. *Fenugreek seeds* and *black cumin seeds* both have been discovered to be rich in a variety of metabolites [6,7].

Despite being normally present in every human oral microbiome, *Streptococcus mutans* is the microbial species most significantly linked to carious lesions [8]. Multiple types of samples were used to examine the connection between *Streptococcus mutans* and human dental decay such as samples from paraffin-stimulated saliva samples were taken from participants with age group of 0 to 30 years, other sample was from pooled occlusal and proximal plaque taken from children and young adults without any decayed or filled teeth; and samples from plaque were taken from occlusal fissures. The analysis indicated significant link between *S. mutans* in plaque accumulation and dental caries. When plaque was eliminated from single occlusal fissures, the strongest relationship (P 0.0001) was discovered [1]. The oral microbial biota constitutes highly diversified biofilm in humans and oral *streptococci* have been found in the human mouth in 25 distinct species, making for

almost 30% of the all oral bacteria [8]. Growing evidence suggested that *nigella sativa seeds* and *fenugreek seed* extracts possess antibacterial action against *S. mutans'* capacity to form biofilm and inhibit their ability to produce acid [9,10]. Experts have looked at a number of methods in which extracts from *nigella sativa seeds* and *fenugreek seeds* were successful in raising salivary pH up to 7 in approximately under ten minutes. These extracts also helped to lubricate and hydrate the oral cavity, therefore provides protection against dental caries [9,10]. All this kind of activity is important because it reduces demineralization of the tooth's outer surface. As a result, the following study's objective is to analyze the antibacterial activity of *nigella sativa seeds'* and *fenugreek seed* extract rinses against *S. mutans'* colonies.

## 2. METHODOLOGY

It was a preclinical experimental study conducted at Baqai Medical and Dental College Karachi., from January - June 2021. Calculated sample size was n = 80 Consecutive sampling technique was used. Plaque of study participants was collected on sterile strips that was 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 CO2. *Fenugreek seed* and *Nigella sativa seeds* were purchased from local market and authentication number i.e. Specimen voucher 53 and 96 was allotted. 1000-gram of both the 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$^{o}$C by using water bath. The mixture was than dried at 50$^{o}$C until a well concentrated extract was produced. The extract was kept in an airtight bottle and stored in a refrigerator till usage. The extracts were diluted in distilled water in 1:4 (Extract: Distilled water). Study participants were instructed to not brush their teeth before sampling. Study participants were divided into four groups (negative control, positive control, *fenugreek seed* extract group and *Nigella sativa* group) each group had 20 participants. Diluted *Fenugreek seed* extract and *Nigella sativa* extract was given to experimental groups 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 tukkies and Paired t test was applied as test of significance, <0.05 p-value was considered as significant at 95% confidence interval.

## 3. 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, *fenugreek seed* extract group and *nigella sativa* extract 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, *fenugreek seed* extract group and *nigella sativa* 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** | **Fenugreek** | **Nigella sativa** |
|---|---|---|---|---|
| Before | 8 x $10^4$ | 10 x $10^4$ | 9 x $10^4$ | 8 x $10^4$ |
| After | 7 x $10^4$ | 4 x $10^4$ | 5 x $10^4$ | 5 x $10^4$ |
| P value | 0.417 | 0.001* | 0.040* | 0.001* |

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

| Groups wise comparison | | | | p- value |
|---|---|---|---|---|
| Negative control | $7 \times 10^4$ | **Positive control** | $4 \times 10^4$ | 0.005* |
| Negative control | $7 \times 10^4$ | **Fenugreek seed extract** | $5 \times 10^4$ | 0.010* |
| Positive control | $4 \times 10^4$ | **Fenugreek seed extract** | $5 \times 10^4$ | 0.092 |
| Negative control | $7 \times 10^4$ | **Nigella sativa** | $5 \times 10^4$ | 0.032* |
| Positive control | $4 \times 10^4$ | **Nigella sativa** | $5 \times 10^4$ | 0.069 |
| Fenugreek seed extract | $5 \times 10^4$ | **Nigella sativa** | $5 \times 10^4$ | 1.000 |

## 4. DISCUSSION

The enhanced efficacy of novel plant-derived medications, as well as the growing interest in natural products, has sparked a surge in awareness towards medicinal plants. Natural goods have been used as an alternative to conventional treatment in the healing and treatment of numerous ailments due to concerns over the adverse effects of synthetic medicine. [11]. Due to their promising results and fewer side effects, medicinal herbs or plants are gaining considerable widespread interest in the treatment of various disorders [12]. According to the World Health Organization (WHO), herbal medicines or traditional medicine are used by 60-80% of the planet's population, particularly in poor countries, for basic health care needs [12, 13]. Furthermore, the WHO has urged emerging economies to make use of their medicinal plants as a resource for creating successful health-care programmes [13].

*Nigella sativa* is a top-ranked evidence-based herbal treatment that has been labeled as "wonder herb of the century. [12,14]. The organoleptic of *Nigella sativa L. seed* oil extract exhibited a yellowish brown color with a pleasant scent, and a pleasant taste. The ethanol extract of *Nigella sativa L.* was 5%, [15] some secondary metabolites, such as alkaloids, tannin, terpenoids, saponin, and steroids, were also found to be in the ethanol extract of *Nigella sativa L.* seed. All of them assist in various healthy properties along with sustainable amount of anti-bacterial properties which has been used in many dental related problems [16] With an increase in plant research for health applications, the curative benefits of *Fenugreek* (*TrigonellaFoenum-gracium*) *seeds* have also been identified [6]. *Fenugreek seeds* have also been found to be rich in a range of metabolites, including alkaloids, tannins, flavonoids, glycosides, and terpenoids, all of which exhibit antibacterial activities individually or collectively [6]. The presence of numerous secondary metabolites such as alkaloids, flavonoids, lycosides, phenols, saponins, and sterols might well be responsible for medicinal plants' therapeutic powers [17]. Thymoquinone (volatile oil of these seeds) and Melanine are two of the active components (fixed oil) with antibacterial and antimicrobial properties. In general, extracts with higher protein and carbohydrate content have superior antibacterial activity on gram positive and as well as moderate effect on gram negative bacteria [18]. Plants' microbial defense strategy involved large number of proteins and ‘puroindoline’ being the key element of a novel protein family that has shown to have antibacterial properties in *Nigella sativa L. seed* extract [19,20].

*Fenugreek* and *Nigella sativa* seed extract were used in this study, and the purpose was to analyze the antibacterial efficacy when used in the herbal mouthwash to commercially and widely available mouthwashes in the market and distilled water. All clinical markers had statistical significance in our study's intragroup comparison. The positive and experimental inter-group, post hoc analysis, revealed significant statistics. When compared to baseline values, there was a reduction in the bacterial colonies in the male and female participants who used tooth brushing technique and mouthwashes on alternate days or once a week. Results were similar to a study conducted elsewhere in which the minimum inhibitory concentration (MIC) of *Nigella sativa L. seed extract* was 380 mg/ml that

exhibited substantial amount of bacterial growth inhibition with moderate to significant impact on *Streptococcus mutans* colonies while more potently on gram positive bacteria. The diethyl ether extract of *Nigella sativa L. seeds* (25-400 g extract/disc) was found to have antimicrobial effects against not only Staphylococcus aureus but also among *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans* [21]. When used for gingivitis therapy, *fenugreek seed* extract was found to be effective against oral infections, had much less side effects, while being less pricey than synthetic therapies, which was reported in a survey [22]. Previous researches indicated that the ethanolic chemical compound of *fenugreek seeds* was much more potent than the aqueous chemical compound of the herbs because aqueous extracts had a shorter shelf life. Furthermore, the enzyme polyphenol oxidase, which degrades polyphenols in water extracts but is inactive in methanol and ethanol extracts, would have been held responsible for the aqueous extract's lower activity. Additionally, ethanol was observed to penetrate further into the cellular membrane [23].

Thymohydroquinone, derived from the volatile oil of *Nigella sativa*, has a strong antibacterial impact against gram-positive bacteria like *Staphylococcus aureus* and *Streptococcus mutants*. The concentration-dependent inhibitory activity of *N. sativa* diethyl-ether extract was noted among gram-positive bacteria *streptococcus mutans* and *S. aureus*, while also on gram-negative bacteria *Pseudomonas aeruginosa*, and *Escherichia coli* when examined [24]. While another research reported the efficacy of *Nigella sativa L. seed extract*, which was tested to antimicrobials drugs in an equality assay, and 1 part of extract activity was equal to 3 x 10-4 part of antimicrobials. This report demonstrated that the activity of the extract was still lower than that of antimicrobials drug but still effective enough to act on *Streptococcus mutans* colonies [25]. Throughout this investigation, a Plaque sample was collected after two hours of mouthwash rinse and later was compared to a commercial mouthwash readings, and both had almost the same effect on bacterial colonies. There has been no significant difference between commercial and herbal mouthwash in our study, as reflected by the findings. A similar study was done in an Indian hospital, in which the plaque index of the people participating were recorded after 30 days of using herbal *fenugreek seed* mouthwash and the other half that used commercial chlorohexidine mouthwash and yet again the comparison between these two mouthwashes in terms of mycobacterial analysis was similar [23].

The anti-inflammatory activity of flavonoids and saponins components of *fenugreek seed* extract 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 confirmed by cell lysis when along with the plaque index when results from sulcus bleeding index was scored less [26]. Another study confirmed that *fenugreek seeds* have a wide range of activity, possibly due to flavonoid compounds in the extract's ability to interact with cell walls, which eventually triggers the disintegration of the bacterial cell wall [27]. While methanol extract, n-Hexane extract, and water decoction of *Nigella sativa* seed were found to have the strongest antibacterial potential in a proven study especially towards gram positive bacteria and less potent among gram negative bacteria yet the potency of n-hexane, methanol, and water decoction sufficiently support the use of *Nigella sativa seed* alone or it's extract both in treatments directed at boosting good oral hygiene. This may lead to a significant shift away from synthetic drugs and toward actives originating from nature [28]. A study conducted in Romania, aim to compare the effects of a commercial mouthwash and herbal infusion on the production of dental plaque and gingival irritation. The study's participants were split into two groups: the CM group, who used a commercial fluoride-containing mouthwash, and the IM group, who used herbal extract infusion. At baseline and on follow-up observation sessions, the Silness–Loe plaque index (PI) and the Loe–Silness gingival index (GI) were measured. Both mouthwashes demonstrated good outcomes in terms of PI and GI reduction. The overall PI value determined in the CM group was fewer than in the IM group during all monitoring sessions, owing to the fluoride in the commercial rinse. As the CM group's average GI value was slightly greater that indicated more gingival inflammation in the participants than the IM groups, thus IM group had a better average in GI aspect. Therefore to sum up, herbal mouthwashes are very much effective in inducing optimal oral prevention through the maintenance of healthy dental health [29].

In contrast to the oral health efficacy of *fenugreek seed* extract mouthwash against

distilled water which was used as a placebo in our study, had similar results in an investigation conducted previously that also showed herbal mouthwash had a lower plaque index than distilled water when used on participants [30,31]. Plaque accumulation and gingival bleeding were examined before and on completion of the experiment's time-frame, and the researchers discovered that using herbal mouthwash enhanced gingival health when compared to preliminary data [31]. A further article compared an organic ingredients such turmeric concentrate, *fenugreek seed*, and *black cumin seed* extract to chlorhexidine mouth rinses using a 4-day plaque re-growth model, and observed that the organic remedy came in second place to chlorhexidine in regards to plaque inhibition [31].

Herbal mouthwash with *fenugreek seed* extract or *black cumin* extract exhibited a similar impact as commercial mouthwash in our investigation. Incorporating several of these plant extracts in a singular mouthwash would surely enhance the efficacy of the mouth rinses in terms of preventing oral infections. Chlorhexidine gluconate is a microbicidal mouthwash that has been used to treat infections caused by bacteria especially when it comes to treating infections caused by S. mutans and other common dental pathogens, N. sativa oil extract outperforms chlorhexidine gluconate [24]. Long term use of commercial mouthwashes like chlorhexidine is not advocated despite it's fast acting antibacterial and antiplaque capabilities, as investigations have shown that chlorhexidine has no plaque reducing effects at lower doses [32]. Subsequently herbal mouthwash is affordable than mouthwash having 0.2 percent chlorhexidine gluconate, therefore organic plant based mouth rinses might be a better option for people living in an country with striving economy [32].

## 5. CONCLUSION

Fenugreek seed extract and Nigella Sativa seed extract showed comparable antibacterial properties. Also, the effect was found to be similar to commercially available mouth rinse and reduced the number of streptococcus mutants' colonies nearly equivalent to that.

## 6. LIMITATIONS

Only Streptococcus mutant colonies were checked and counted.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

Study was approved by the ERC and preserved by author (s).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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