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# AN IN-VITRO ASSESSMENT OF THE ANTIMICROBIAL EFFICACY OF FENNEL AND SESAME SEED EXTRACTS AGAINST STREPTOCOCCUS MUTANS

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## ABSTRACT

**Objectives:** Dental Caries is an infectious disease associated with numerous micro-organisms, mainly Streptococcus mutans. In this era of preventive measures, a shift towards natural/herbal products is on the rise. The aim of the study was to evaluate the antimicrobial effect of sesame and fennel seed extract against Streptococcus mutans. **Materials and Methods:** Aqueous extracts of fennel and sesame seeds were prepared. It was then subjected to microbial assays to determine the Minimum Inhibitory Concentration(MIC), Minimum Bactericidal Concentration (MBC) and Zone of Inhibition against Streptococcus mutans. Chlorhexidine and distilled water were used as a positive and negative control. **Results:** 100% and 50% concentration of fennel extracts showed inhibition of growth of Streptococcus mutans. 100% of fennel seed extract showed no growth of S mutans on the agar plate, displaying a bactericidal effect. No concentration of sesame extract showed inhibition or bactericidal effect on S mutans. 100% fennel extract showed a Zone of Inhibition (ZOI) of 18 mm. Serial dilutions did not show significant Zone of inhibitions. For sesame extracts, no concentrations showed any ZOI. **Conclusion:** Fennel extracts show promising results as an antimicrobial agent to be used in clinical conditions. However, sesame seeds used in form of an essential oil mouth rinse as supported by existing literature would yield better results.

**KEYWORDS:** Fennel, Sesame, Streptococcus mutans, MIC, MBC.

## INTRODUCTION

Dental caries is closely linked to a bacterial biofilm on tooth surfaces. Stephan established an association between production of acids from plaque and caries activity.[1] The increase in acid levels reduces the plaque pH which facilitates an ideal environment for growth of acidophilic microorganisms such as Streptococcus mutans. In dentistry, to reduce plaque formation, chemical means have been used as adjuncts to mechanical means.[2] The gold standard for chemical plaque control is Chlorhexidine. It is a bisbiguanide which has bacteriostatic activity at a concentration of 0.2% and bactericidal effect at 2% concentration. Its broad antibacterial activity, substantivity and low toxicity are well established. But the disadvantages include discoloration of teeth, mucosal erosions, bitter taste and resistant strains of bacteria.[3,4]

The world is focusing on a paradigm shift. This shift is relevant to consumers who prefer a natural substance as compared to a chemical/synthetic one. The WHO states that 80% of the world population relies on traditional medicine today and majority of the traditional therapies involve the use of plant extracts or their active constituents.[5] Various natural household products like neem[6], garlic[7], turmeric[8], cinnamon[9] and tulsi[10] have been tested in dentistry for adult and adolescent age groups. However, the taste of these agents remains questionable in children.

Foeniculum vulgare (Fennel) belongs to the Umbelliferae family and has a long history of use as a traditional medicine. In the ancient Egyptian medicine, Fennel has found mention along with ingredients as dough, honey and onions as a remedy for dental pain.[11] It is considered as one of the functional foods with digestion enhancing properties.[12] They are highly aromatic and flavorful, and commonly used as a breath freshener.

Sesamum indicum (Sesame) is a flowering plant in the Sesamum family. It is known for its antioxidant and anti inflammatory properties. In India, fennel seeds and sesame seeds are often chewed after meals along with anise seeds. In dentistry, commercial sesame oil has been used for oil pulling[13] and showed considerable reduction in plaque and S mutans count.[14] But aqueous or ethanolic extracts of sesame have not been evaluated for their antimicrobial effect.

Hence the present study was aimed at evaluating the antimicrobial effects of fennel and sesame seed extracts

against Steptococcus mutans, and if proved so to identify an inexpensive, simple and effective method to prevent and control caries.

## MATERIALS AND METHODS

The dry powder of fennel and sesame seeds were obtained from Konark Herbals and Healthcare, Mumbai. The obtained powder was weighed up to 50g and mixed with 100 ml of sterile distilled water in a round bottom flask with occasional shaking. The extract was then filtered through a muslin cloth and further through Whatman No.1 filter paper and kept in an airtight amber colored container.

The Streptococcus mutans (MTCC497) was revived and maintained at 37°C overnight on brain heart infusion agar. The growth concentration was adjusted to $10^5$ organisms/ml by using 0.5 McFarland's turbidity standard.

### Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determination for the aqueous fennel and sesame seed extracts

The MIC was performed according to the standard reference method (Clinical and Laboratory Standards Institute, 2012). The extract dilutions were made in several two-fold decreasing concentrations with BHI broth containing 5% dimethyl sulfoxide (DMSO) from 100 mg/mL to 0.097 mg/mL.0.1 milliliter of standard inoculums of the bacterial strain matching the No. 0.5 Mc Farland was seeded into 1-ml dilution. All test tubes were incubated at 37°C in anaerobic conditions in dessicators for 24 hours and observed for turbidity indicating bacterial growth. Two control tubes, positive and negative control, were also performed. Tube with no extract represented the positive control while the one without the extract and culture represented the negative control for the test. MIC was determined as the lowest concentration of the extract that showed no visible growth.

The tubes that showed no growth during MIC determination was further selected for MBC. The MBC were determined by the drop plate method. Ten microliters from the selected tubes were further sub cultured onto BHI agar and incubated at 37°C in aerobic conditions for 24 hours. The least concentration, at which no growth was observed, was noted as the MBC.

### Determination of antibacterial effect of aqueous fennel and sesame extracts

Petridishes containing BHI agar for S. mutans were inoculated with microbial strains using swab technique. Wells of 10 mm diameter were cut into solidified agar media using a sterilized standard device. 100µl of each extract was poured into the well and the plates were incubated at 37°C for 24 h. To ensure the consistency of all findings, the experiment was performed and repeated under strict aseptic conditions. The antibacterial activity of each extract was expressed in terms of the diameter of zone of inhibition (in mm) measured using a vernier caliper. All the measurements of zone of inhibition were carried out by a single examiner. A standard disc of 0.2% chlorhexidine was used as a positive control.

## RESULTS

**Table 1: Minimum inhibitory concentration of aqueous fennel and sesame seed extract against S. mutans.**

| Tube no | Concentration in % | BHI broth (ml) | Stock (ml) Extract | Culture (ml) | Result Fennel seed | Result Sesame seed |
|---|---|---|---|---|---|---|
| 1 | 100 | 3 | 3 | 0.1 | - | + |
| 2 | 50 | 3 | 3 | 0.1 | - | + |
| 3 | 25 | 3 | 3 | 0.1 | + | + |
| 4 | 12.5 | 3 | 3 | 0.1 | + | + |

Culture used: ***Streptococcus mutans***
(Key: +: growth seen, -: No growth)

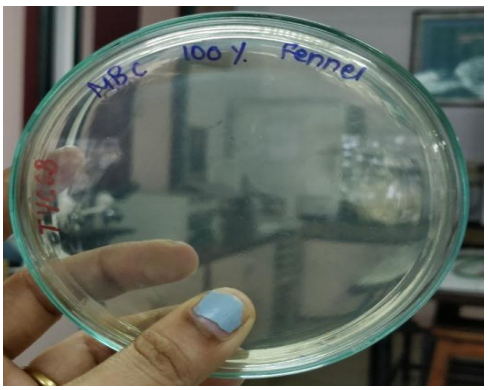


**Figure 1: Minimum Bactericidal concentration of aqueous fennel seed extract (100%) against S. mutans.**

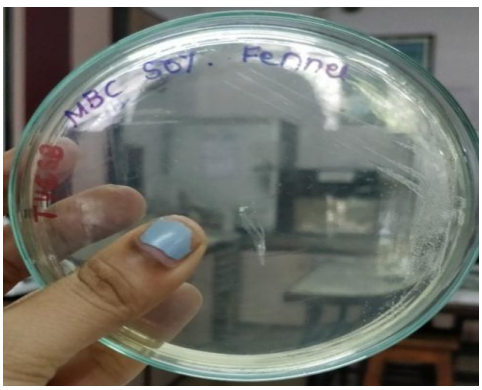


**Figure 2: Minimum Bactericidal concentration of aqueous fennel seed extract (50%) against S. mutans.**

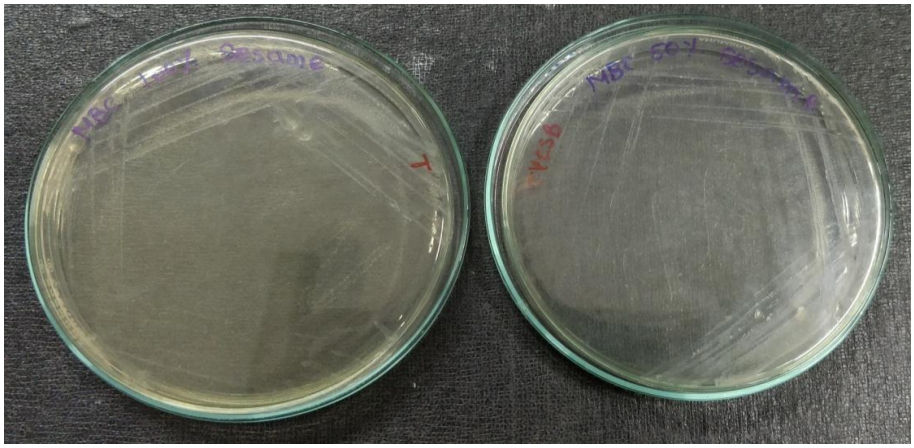

**Figure 3: Minimum Bactericidal concentration of aqueous sesame seed extract (100% & 50%) against S. mutans.**

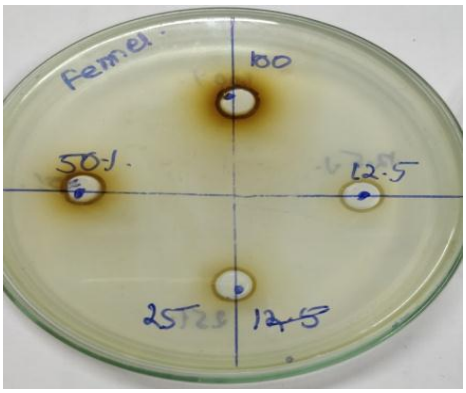


**Figure 4: Zone of Inhibition of aqueous fennel seed extract against S. mutans.**

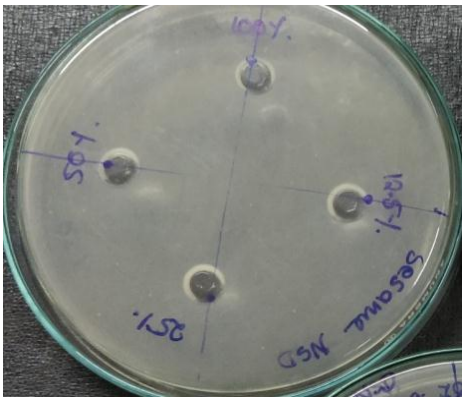


**Figure 5: Zone of Inhibition of aqueous sesame seed extract against S. mutans.**

Aqueous fennel seed extract showed inhibitory action against Streptococcus mutans at both 100% and 50% concentration, whereas aqueous sesame seed extract showed no inhibition against Streptococcus mutans at any concentration (Table 1). The aqueous fennel seed extracts showed a bactericidal effect at 100% concentration (Fig 1), but at 50% concentration bacterial growth was evident on the agar plate (Fig 2). Aqueous sesame seed extracts did not show bactericidal activity against Streptococcus mutans at any concentration (Fig 3). The zone of inhibition displayed by 100% fennel seed extract was 18mm (Fig 4), as compared to chlorhexidine which was used as a control and showed a zone of inhibition of 19mm. Subsequent dilutions of fennel seed extracts did not show a significant value for zone of inhibition. At no concentration did the sesame seed extracts show a zone of inhibition (Fig 5).

## DISCUSSION

The past few decades have showcased a renewed interest in the study of non-chemotherapeutic agents in the medical and dental field. Ajith Krishna et al reported that chewing fennel seeds increased salivary pH for upto 5 minutes after chewing.[15] Swathi et al observed increase in salivary and plaque pH after chewing fennel seeds.[5,16] Sultan et al reported increase in the plaque calcium, plaque phosphate and plaque pH levels after chewing of both these seeds.[5] Since fennel and sesame are commonly used economical Indian household products with the reported ability to improve the pH, we attempted to evaluate their antimicrobial activity on Streptococcus mutans.

The fennel and sesame seed dry powderused in the present study were obtained from Konark Herbals and Healthcare, Mumbai. The obtained powder was90% water soluble, and hence aqueous extracts of the products were prepared.

Prajapathi and Raol evaluated aqueous, methanolic, acetone and petroleum ether extract of fennel seed on a clinical strain and standard strain of Streptococcus mutans and reported a zone of inhibition of less than 13 mm with all types of extracts.[17] Bhatnagar et al assessed the antimicrobial efficacy of methanolic extract of fennel seed on standard strain of S mutans and reported a zone of inhibition of 17mm.[18] On the contrary, Chavan at al assessed the effect of aqueous fennel extract on salivary S mutans and reported an inhibition zone of 0.7mm.[19] In the present study, we had used an aqueous extract of Fennel dry power and obtained a zone of inhibition of 18 mm against S mutans. With Chlorhexidine, the inhibition zone obtained in our study was 19 mm indicating that aqueous extract of fennel powder may inhibit bacterial growth equal to chlorhexidine. While evaluating the

antibacterial activity, an organism was interpreted as highly susceptible if the diameter of inhibition zone was more than 19 mm, intermediate if diameter was 15-18 mm and resistant if diameter was less than 13 mm.[20]

Asokan et al isolated sesamin and sesamolin from sesame oil and reported no antibacterial effects against S mutans.[21] In the present study also, we observed no inhibition zone even at 100% concentration of sesame extract. However, Banjar et al evaluated the antimicrobial activity of a commercially purchased sesame oil on unstimulated saliva samples of cariogenic adult volunteers and reported a zone of inhibition of 20 mm with 40 mg/ml serial dilution of sesame oil.[22] Anand et al studied the in vitro antibacterial efficacy of sesame oil on a control strain and clinical strain of Streptococcus mutans and reported a inhibition zone of 10 mm and 9 mm respectively, contrary to our results.[23]

From the results of the present study, it may be concluded that aqueous fennel extracts have antibacterial action comparable to chlorhexidine in vitro. Sesame aqueous extract does not seem to inhibit bacterial growth as much as commercially available sesame oil. Thus sesame essential oil used as a mouth rinse would yield better results in inhibition of Streptococcus mutans as supported by existing literature.[14,21]

Further studies incorporating fennel as principal component in mouthrinse may be evaluated invitro and in clinical conditions as an alternative to chlorhexidine.

## LIMITATIONS

Only aqueous extracts of fennel and sesame was tested. In addition, alcoholic and oil extracts could have been evaluated for efficacy against S mutans. There is a lack of sufficient standardization of methods in literature for comparison, since there are variations in the extracts used (aqueous, alcoholic or oil), the methodology employed and the strain of microorganism used.

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