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ANTIBACTERIAL EFFECT OF MOUTH RINSING WITH SOLUTION CONTAINING FENNEL SEED EXTRACT AGAINST STREPTOCOCCUS MUTANS: AN IN VIVO RANDOMIZED CONTROLLED TRIAL

Dentistry

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ABSTRACT

Aim: To assess and compare the salivary S mutans counts before and after one hour of mouth rinsing with 30 ml solutions containing 1.25% Fennel ethanol extract , 50 % Fennel aqueous extract and 0.2% Chlorhexidine mouthwash among 18–30-year-old adults. **Methodology:** A concurrent parallel randomized controlled trial was conducted involving 45 subjects randomized to 3 groups: Group A: 1.25 % Fennel ethanol extract containing mouthwash group; Group B: 50 % Fennel aqueous extract containing mouthwash group; Group B : 0.2 % Chlorhexidine mouthwash group. Salivary S Mutans counts of participants were assessed using Mitis Salivarious Bacterium (MSB) agar plates before and one hour after mouth rinsing with 30 ml of interventional mouthwashes for a minute. Statistical analysis: Intra and Intergroup comparisons of microbial counts were done using Wilcoxson signed rank test and Kruskal Wallis ANOVA respectively by setting significance level at $p \leq 0.05$. **Results:** There was significant reduction ($p \leq 0.001$) in salivary S mutans counts after using mouth rinse in all the interventional groups. The highest reduction in S mutans counts post rinsing with mouthwash was seen in Chlorhexidine group (3.93±2.73 x102 CFU) but was not significant($p=0.41$) compared to other two interventional groups. **Conclusion:** Antibacterial efficacy of 1.25% fennel seed ethanolic extract and 50% aqueous fennel seed extract containing mouthwashes against streptococcus mutans was comparable to 0.2% chlorhexidine mouthwash.

KEYWORDS

Mouthwash, Fennel seed, S mutans, Chlorhexidine

INTRODUCTION

Mouthrinses containing active therapeutic agents are being widely used in recent times as vehicles for delivering chemical agents and as a daily oral health care measure. In combination with regular mechanical methods, mouth rinses have shown to be the most feasible, secure, and efficient means of achieving an acceptable antimicrobial ecosystem.[1] One of the most commonly prescribed mouth rinse is chlorhexidine digluconate. It has bacteriostatic and bactericidal properties against a wide range of microorganisms, including gram-positive and gram-negative aerobic and anaerobic bacteria as well as fungi, including yeasts.[2] Because of its substantivity, chlorhexidine is considered the gold standard. However, it has a number of side effects, prompting researchers to look for natural alternatives with similar antibacterial properties but lower toxicity than chlorhexidine.[2]

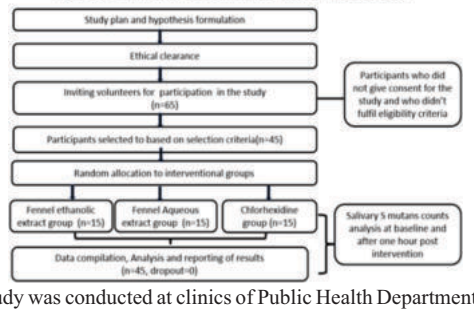
Herbal medicine is the major stay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility and few side effects. World Health Organization (WHO) encourages, recommends and promotes traditional/herbal remedies in national health care programs because the plant-based therapeutics are natural products, non-narcotic, easily bio-degradable, pose minimum environmental hazards, have less adverse effects which are easily available and affordable too.[3]

Foeniculum vulgare (Fennel) belongs to the Umbelliferae family and has a long history of use as a traditional medicine. It is considered as one of the functional foods with digestion enhancing properties.[4] They are highly aromatic and flavoured , and commonly used as a breath freshener. In India, these seeds are used for chewing alone or with betel leaves or mixed with sugar or even commercially available sugar-coated sachets. Fennel seeds are known to have anti-cancerous, anti-hirsutism, anti-inflammatory, anti- oxidant, anti-platelet, anti-thrombotic and anti-spasmodic properties.[4] Few invitro studies have demonstrated antibacterial property of fennel seed extract against streptococcus mutans.[5,6] Considering the invitro antibacterial efficacy of Fennel extracts against streptococcus mutans and relative paucity of in vivo studies testing the same , an in vivo randomized controlled trial was planned to evaluate the antibacterial effect of mouth rinsing with solutions containing 1.25% Fennel ethanol extract , 50 % Fennel aqueous extract and compare with 0.2% Chlorhexidine mouthwash on salivary Streptococcus mutans among participants aged 18-30 years visiting dental college.

METHODOLOGY

Adults aged 18-30 years visiting Dental College and Hospital, fulfilling the eligibility criteria were included in the study. Study design was Concurrent parallel Randomized Controlled Trial (Figure 1). Sample size was calculated using G Power software. The effect size was calculated based on means and standard deviations obtained by previous invitro study done by Winnier et al.[5] Considering significance level at 0.05, Effect size -0.94,power of the study-0.8 the sample size was estimated to be 45, with 15 in each group.

Figure 1: schematic representation of study methodology



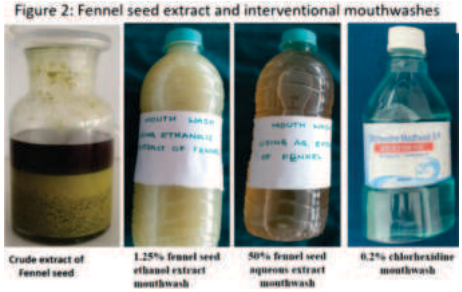
The study was conducted at clinics of Public Health Department of the College and Hospital.

Adults aged 18-30 years ,visiting the hospital who consented and had at least one cavitated active carious lesion were included in the study. Adults with significant untreated oral diseases like acute gingivitis, acute oral infections, ulcerative gingivitis or stomatitis, unable to produce adequate amount of saliva for sampling, who had low risk for dental caries (no history of restorations or presence of carious lesions) , undergoing orthodontic treatment, who were hypersensitive to oral care products and who were medically compromised and handicapped were not included in the study. Ethical clearance was obtained from the Institutional Review Board of the Dental College and Hospital, where study was conducted.(Ref No: ECR/1652/Inst/KA/2022/23-05/08-001). Voluntary informed consent was obtained from the participants prior to the start of the study after informing them about the research details through a participant information form.

Preparation Of 50 % Fennel Seed Aqueous Extract

Around 150 grams of Fennel seeds was purchased from local market and were coarsely powdered and placed inside a container, and around 750 ml of distilled water was poured on top powdered material until completely covered the material in sterile container. The container was closed and kept for at least three days. The contents were stirred periodically, and filtered, and the filtrated extract was collected, and subjected to evaporation of solvent leaving behind the crude residues. This prepared crude extracts were stored in an air-tight container at 4^0c until further use. The method followed was based on the study done by Winneier et al .[7](Figure 2)

Figure 2: Fennel seed extract and interventional mouthwashes



Preparation Of 1.25 % Fennel Seed Ethanolic Extract

150 grams of fennel seeds was purchased from local market and was coarsely powdered and placed inside a container, and around 600 ml of Ethanol was poured on top each powdered material until completely covered the material in two separate sterile containers. The containers were closed and kept for at least three days. The contents were stirred periodically, and filtered, and the filtrated extract was collected, and subjected to evaporation of the solvent leaving behind the crude residues. This prepared crude extract was stored in an air-tight container at 4 degrees Celsius until further use.The method of extract preparation was based on the study done by Wiwattanarattanabut et al .[7](Figure 2)

The below mentioned table shows the ingredients used to prepare 500 ml of Fennel seed mouthwash

Sl.no	Materials	Concentration	Quantity
1	Fennel seed ethanol /aqueous extract	0.08%	450 mg
2	Saccharin sodium	0.1%	0.5 g
3	Menthol	0.05%	0.25 g
4	Peppermint oil	0.2%	1 ml
5	Alcohol	5%	25 ml
6	Glycerin IP	10%	50 ml
7	Water	100 ml	500 ml
8	Sodium Benzoate	0.1%	0.5 g

Procedure For Preparation Of Mouthwash:

For the preparation of mouthwash, menthol, peppermint oil and sodium benzoate was added to 1 litre beaker and stirred well. To this, a mixture containing sodium saccharine, Fennel seed extract, glycerin , and distilled water was added and stirred at 4000 rpm.

Control Mouth Rinse

Commercially available 0.2% Chlorhexidine mouth rinse (Hexidine) by ICPA Health products ltd was purchased and used.

Randomization

Online software was used to generate random numbers .Concealed randomization method was followed. Random assignment of the participants to three interventional groups was done by a separate person not involved in the study using random numbers coded in sealed opaque envelopes.

Interventional Details

Group I: Participants rinsed mouth for a minute with 30 ml of solution containing 1.25 % ethanol Fennel seed extract

Group II: Participants rinsed mouth for a minute with 30 ml of solution containing 50 % aqueous fennel seed extract

Group III: Participants rinsed mouth with 30 ml of solution containing 0.2% chlorhexidine as mouth rinse for a minute

Masking

The mouth washes were bottled in identical bottles ,pre-coded and given to participants for use by an assistant who was not involved in the study.

Blinding

The present study was triple blinded as participants, investigator and analyzers were blinded to group allocation of participants.

Procedure And Instructions

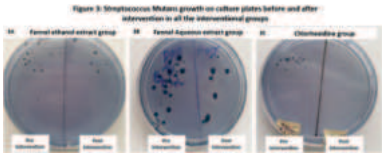
Baseline Salivary assessment of S. mutans, was done by collecting 1ml of unstimulated saliva of study participants in sterile containers. The interventional solutions were distributed to study participants. They were instructed to rinse their mouth with 30 ml of interventional solution for one minute and spit it in the disposable cup provided to them and not to eat or drink anything within 1 hour of using the interventional solution. After 1 hour of using mouthwash (Post intervention) again saliva collection was performed by the examiner.

Method of Saliva Collection

Five milliliters of unstimulated saliva was collected at baseline and one hour after intervention from the subjects by asking the subjects to bend down the head and pool the saliva in the floor of the mouth. The participant was told to spit pooled saliva into sterile containers. The samples were then sealed, labelled, coded and sent for microbiological analysis. Colony characteristics formed were studied and the number of colony forming units of S. mutans (CFU/ml of saliva) on MSB agar plates was determined using a colony counter.

Microbial Analysis Of Saliva Samples For S. Mutans Count

Saliva sample were homogenized manually by stirring with a stirrer. Hundred micro litres of saliva was diluted with 1ml of saline (1:10 dilution). Using an inoculation loop (2mm in diameter) 5 micro litre of 1:10 dilution sample was streaked on Mitis Salivarius Bacterium (MSB) agar, a selective medium for Streptococcus Mutans. This was incubated for 48 hours at 37^0C in an atmosphere of 95% nitrogen and 5% carbon dioxide. After 48 hours of incubation period S. mutans colonies appeared on the culture plate as small rough raised and adherent colonies which were expressed in terms of number of colony forming units (CFU's).(Figure 3)



Statistical Analysis

IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, N.Y., USA) was used for statistical analysis. Nonparametric tests were used for data analysis as data did not follow normal distribution. The significance level was fixed at $P < 0.05$. Intra and intergroup comparison of microbial counts was done using the Wilcoxson signed rank test and Kruskal Wallis ANOVA test respectively.

RESULT

There was significant reduction ($p<0.001$) in salivary S mutans counts after using mouth rinse in all the interventional groups. The highest reduction in S mutans counts post rinsing with mouthwash was seen in Chlorhexidine group ($3.93\pm2.73x10^2$ CFU) but was not significant($p=0.41$) compared to the reduction observed in the group 1 (3.83 ± 2.60 $x10^2$ CFU) and group 2 ($2.66\pm2.18x10^2$ CFU). The reduction in bacterial counts were more pronounced in participants who used mouthwash containing ethanolic extract of fennel seed ($3.83\pm2.60x10^2$ CFU) compared to aqueous extract (2.66 ± 2.18 x 10^2 CFU).However this was not significant ($p>0.05$) (Table 1)

DISCUSSION

There was significant reduction in salivary S mutans counts after using Fennel seed extract and chlorhexidine mouthwashes. The antibacterial effect of mouthwashes prepared from fennel seed was comparable to chlorhexidine mouthwash. This result was in accordance with few in vitro studies showing potent antibacterial property of Fennel seed extract on S mutans .[5-9]Trans-anethole is responsible for antibacterial activity of fennel seed essential oil against S. mutans.[10]Fennel seeds are concentrated source of minerals and vitamins. Its mineral and vitamin contents are calcium, phosphorous, iron, sodium, potassium, thiamine, riboflavin, niacin and vitamin C.[11] Based on study by Koudela and Petrikova, potassium, sodium, manganese and calcium content in fennel seeds were ,504.6, 29.45, 23.55 and 20.95 mg/100 g respectively and trace amounts of iron and zinc were detected correspondingly. [12] The results of the study done by Sultan et al, revealed that fennel chewing led to an increase in plaque pH, calcium and phosphate concentration. This was perhaps because of the diffusion of calcium and phosphate into plaque which may be due to the leaching of calcium from fennel seeds due to chewing which increases salivary flow and hence increases pH and calcium and phosphate concentration of plaque. [13] Shirahatti RV et al., evaluated the effect of fennel seeds on plaque and salivary pH and found that these seeds increased pH at 5 minutes, although it was not significant.[14] Ajithkrishnan CG et al., found a statistically significant rise in salivary pH immediately after chewing fennel seeds.[15] Estragole, the principal constituent of the Fennel seed extract showed the ability to disrupt the cell membrane, increase nonspecific permeability in the bacterial cell

membrane, and enable it to easily pass through the lipopolysaccharide membrane of the Gram-negative bacteria. In addition, fenchone, limonene, and other compounds in the fennel extract were found to accelerate the healing of acute and chronic wounds by protecting the injury site from infection, inhibiting inflammatory cells, and increasing connective tissue formation in the repaired tissue. [15]Results of the study done by Rashid et al, supports the use of fennel extract in oral health care products as it is rich in fenchone and limonene which exhibit antibacterial property against periodontal and caries pathogens.[16] Study by Yavagal et al showed that ,eating stevia-coated fennel seeds resulted in a significant increase in salivary pH from the baseline compared to sucrose-coated and roasted fennel seeds, and hence, could serve as an anticariogenic mouth freshener. [17] In the present study, 50% aqueous extract and 1.25 % ethanol extracts were tested since these concentrations showed antibacterial effects against S mutans in vitro studies[5,7] In this study, Chlorhexidine was chosen as the reference substance because it is widely recognized as the benchmark antibacterial agent. It achieves its antibacterial action by binding to bacterial cell wall. At lower concentrations (0.12%), it exerts a bacteriostatic effect by disrupting the bacterial osmotic balance and causing the release of cytoplasmic components. Conversely, at higher concentrations (0.2%), it has a bactericidal effect by promoting the coagulation of cytoplasmic proteins. Additionally, it possesses the property of substantivity by adhering to the tissues within the oral cavity. Despite its notable efficacy in managing dental caries, Chlorhexidine is associated with several side effects, including numbness, alterations in taste perception, tooth discoloration, and the potential for bacterial resistance if used over an extended period. [18] The findings of the study should be considered under the background of few limitations. certain bacterial species such as Streptococcus sobrinus, Streptococcus anginosus and Phytobacter have the same morphological appearance as Streptococcus mutans on the Mitis Salivarius agar (MSA) media.[19] This may lead to false positive differentiation for these species as Streptococcus mutans in the clinical studies as suggested by Zeng et al. [20]Testing on Caries biofilm models would result in better clinical applicability of the results. The molecular characterization of active ingredients of the extract were not done which would provide more insights into the antibacterial property of the extracts. However, the findings of this research emphasize that the utilization of natural mouthwashes for the prevention of dental caries might prove to be more efficient and associated with fewer adverse effects than synthetic mouthwashes.

Table 1: Comparison of S mutans Colonies (Mean number of colony forming units X (102) across groups using Mouthrinses containing ethanol and aqueous extracts of Fennel seed extract and Chlorhexidine

	0.25 % Fennel seed Ethanol extract Mouth rinse		50 % Fennel seed Aqueous extract Mouth rinse		Chlorhexidine Mouth rinse		Kruskal Wallis Anova (H) P value
	Mean ±SD	Median Range	Mean ±SD	Median Range	Mean ±SD	Median Range	
Baseline	6.6± 6.15[a]	3 1-20	4.73± 3.59[b]	4 1-14	4.53± 3.2[c]	4 1-11	p=0.99
Post Intervention	2.6± 2.59[a]	0 0-12	2.06± 1.86[b]	1 0-6	0.73± 0.79[c]	1 0-2	p=0.17
Mean Difference	3.83± 2.60	2 1-14	2.66± 2.18	3 0-9	3.93± 2.73	4 1-9	p=0.41
Wilcoxon Signed rank test :a- z=-3.44,p=0.001,b- z=-3.33,p=0.001,c - z=-3.41,p=0.001							

CONCLUSION

Antibacterial efficacy of mouth rinsing with 1.25% Fennel seed ethanolic extract and 50% fennel seed aqueous extract containing mouthwash against streptococcus mutans was comparable to 0.2% chlorhexidine mouthwash.

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Conflict of Interest: Authors declare no conflict of interest

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