



Antimicrobial Efficacy of Chemical and Herbal Agents against *Streptococcus mutans*: An in vitro study

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Abstract

Objective: To compare and evaluate antimicrobial efficacy of chlorhexidine gluconate and fennel seeds against *Streptococcus mutans*. **Material and Methods:** Three agar petri dishes for *Streptococcus mutans* were prepared. The methanolic extract of fennel seed and 0.2% chlorhexidine were inoculated on the each petri dish and incubated at 37°C for 24 hours. Zone of growth inhibition for each extract was measured in millimeters using antibiotic inhibiting zone measuring scale. Data was analyzed using SPSS statistical software. The comparison of inhibition zone against *S. mutans* using CHX and fennel seed extract was done using Mann-Whitney and Wilcoxon tests. All statistical analyses were set at a significance level of $p < 0.05$. **Results:** The mean of zone of inhibition of *S. mutans* after inoculation with 0.2% chlorhexidine and fennel seed extract are 21.44 ± 1.46 , 15.34 ± 0.74 respectively. There was statistically significant difference in the mean of inhibition zone between 0.2% chlorhexidine and fennel seed against *S. mutans* ($p = 0.047$). **Conclusion:** Chlorhexidine and fennel seed are effective in inhibiting the growth of cariogenic bacteria. Therefore, both herbal and chemical agents can be effectively used to reduce pathogenic oral microflora.

Keywords: Chlorhexidine; Seeds; *Streptococcus mutans*.

Introduction

Streptococcus bacteria are mainly responsible for the initiation of the caries lesion especially in the enamel, whereas lactobacillus is more involved in the progression of caries. Targeting Streptococcus mutans forms the most important measure for prevention of dental caries [1,2]. This can be achieved by various mechanical and chemical aids. Many chemical antiplaque agents in the form of dentifrices and mouthwashes have been tried for improvement of oral health. Out of various mouthwashes, the most persistent reduction of Streptococcus mutans have been achieved by chlorhexidine mouthwashes [3].

Chlorhexidine gluconate (CHX), a bis-biguanidine, has routinely been used as an effective antibacterial mouthwash in dentistry. CHX is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and then enters the cell through some type of active or passive transport mechanism. It has antimicrobial activity against Gram-positive and Gram-negative organisms. CHX is available in two concentrations (bacteriostatic at 0.2% and bactericidal at 2%). CHX is not recommended for long term use due to its adverse effects like staining, increase calculus deposition, taste alterations, burning sensation [4,5].

Herbal mouth rinses do not contain alcohol and/or sugar, two of the most common ingredients found in chemical mouthwashes. The problem of these ingredients present in chemical mouthwash is that the microorganism feed on these ingredients, and release by products that cause halitosis. Thus, by use of an herbal mouthwash, we can avoid these ingredients, which itself is one step forward towards better oral hygiene [6].

Fennel (*Foeniculum vulgare* Miller) is highly aromatic and flavorful herb. Fennel seed traditionally used as anti-inflammatory, analgesic and antispasmodic agent [7]. Several scientific reports have described the inhibitory effect of spices on a variety of microorganisms, although variation for resistance of different microorganisms to a given spice and of the same microorganism to different spices has been observed [8]. Many authors have described inhibitory and antimicrobial potential of fennel seed extracts and essential oil [9,10]. Hence the present study aimed to compare and evaluate the antimicrobial efficacy of CHX and fennel seed against *S. mutans*.

Material and Methods

Strains of *S. mutans* (MTCC no.497) were commercially obtained (Microbial Type Culture Collection and Gene Bank, Chandigarh, India). Salivarius Bacitracin Agar was commercially obtained from HiMedia Laboratories (Mumbai, India). Microorganism were activated 24 hrs prior to the beginning of the study to obtain a suspension 10^8 CFU /ml.

Preparation of Fennel Seed Extract

At 60°C in hot air oven, Fennel seeds were dried and fine powdered seeds were extracted with 80% methanol (1g/10ml) in a shaker at room temperature for 4 hrs. Residue was extracted with

80% methanol again for 2 hrs. Double-layered muslin cloth was used for filtration of collected extract followed by centrifugation at 5000g for 5min in order to get clear supernatant. Extract was concentrated in a vacuum evaporator and stored at -20°C. The extract was diluted appropriately for different experiments.

Petri dishes containing solid agar was punched with 7mm diameter wells. The inoculums were spread on to the agar plates using sterile swabs. Extract of fennel seed and 0.2% chlorhexidine were inoculated in the wells of petri dishes and then incubated at 37°C for 24 hours. After incubation period, zone of growth inhibition for each agent was measured in millimeters using antibiotic scale. Each agent was tested three times.

Statistical Analysis

Data was analyzed using SPSS version 20.0 statistical software (SPSS Inc., Chicago, Ill, USA). The comparison of inhibition zone against *S. mutans* using CHX and fennel seed extract was done using Mann-Whitney and Wilcoxon tests. All statistical analyses were set at a significance level of $p < 0.05$.

Results

The mean and standard deviation of zone of inhibition of *S. mutans* after inoculation with CHX and fennel seed extract were 21.44 ± 1.46 and 15.34 ± 0.74 respectively (Table 1). There was statistically significant difference in the mean of inhibition zone between CHX and fennel seed against *S. mutans* ($p=0.047$).

Table 1. Comparison of mean and standard deviation of inhibition zone of *S. mutans* with 0.2% Chlorhexidine gluconate and fennel seed extract.

Species	Agent	N	Mean	Median	SD	p-value
Streptococcus mutans	Chlorhexidine Gluconate	3	21.44	21.00	1.46	0.047*
	Methanolic Extract of Fennel Seed	3	15.34	15.00	0.74	

*Statistically Significant; SD= Standard Deviation.

Discussion

The association between *Streptococcus* species and dental caries has been well documented with *S. mutans* playing an important role in caries initiation [11,12]. *S. mutans* metabolized the sucrose to form dextrin polymer, and combined with salivary protein to create a sticky and colorless film on tooth surfaces. The control of dental caries is hidden in the inhibition of each step in the process of caries initiation and progression [13]. Various chemical antimicrobial agents have been used as antiplaque agents but because of the resistance, toxic and harmful side effects of few common agents, there is a need for alternative agents, which are easily affordable, non-toxic and effective. Natural plant extracts or herbal agents could be used as effective antiplaque agents.

Out of various antiplaque agents, chlorhexidine (CHX) is considered the gold standard agent for its clinical efficacy in plaque control. It has broad antibacterial spectrum, with strong affinity for epithelial tissue and mucous membranes of oral cavity. Chlorhexidine is substantive, thus reducing levels of microorganisms in saliva up to 90% for several hours [14].

Some authors reported and studied the antimicrobial properties of fennel seed extracts [15]. It was previously reported that presence of tannins, flavanoids, saponins and sterols with absence of anthocyanes and alkaloids after phytochemical tests on the stem, root and seeds of the plant fennel [16]. In the present study, chlorohexidine was found to be more effective against *S.mutans* via agar diffusion method when compared to methanolic extract of fennel seed. There was statistically significant difference in the mean of inhibition zone between CHX and fennel seed against *S. mutans*.

The comparison of antibacterial effect of 0.2% chlorohexidine, 0.05% sodium fluoride mouthwash and 2% povidone iodine mouthwash against *S.mutans* was previously reported and the authors concluded that 0.2% Chlorohexidine showed the maximum antibacterial effect on *S. mutans* which was consistent with our study result [17].

The antimicrobial efficacy of herbal and chlorhexidine mouth rinses were evaluated against *Streptococcus mutans* and it was concluded that chlorhexidine mouth rinse provided better results in its antimicrobial efficacy against *Streptococcus mutans* [18]. Some authors have evaluated the antibacterial effect of 2% concentration methonolic extract of fennel seed on streptococcus mutans and concluded that methonolic extract of fennel seed showed poor response which was not similar to our study results [19]. Five minutes chewing of fennel seeds showed a rise in salivary PH, this can prevent demineralization and have an anti-cariogenic effect [20].

Only few studies have been done and reported on antimicrobial effects of herbal products against oral microorganism, it is better that the effect of herbal extracts or agents on oral microbes that have cariogenic activity be studied.

Conclusion

The antimicrobial efficacy of chlorhexidine was more than herbal agent (Fennel seed extract) against *Streptococcus mutans*. Fennel seed extract used as an herbal agent can be used as an alternative antibacterial agent to reduce pathogenic oral microflora. Further researches are still required to check the antimicrobial efficacy of herbal mouth rinse.

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