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# COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF 0.2% CHLOROHEXIDINE GLUCONATE AND FENNEL SEED EXTRACT AGAINST STREPTOCOCCUS MUTANS AND LACTOBACILLUS ACIDOPHILUS-AN IN VITRO STUDY.

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## ABSTRACT

**Objectives**: The aim of this present study was to evaluate and compare antimicrobial efficacy of chlorohexidine gluconate and fennel seeds against streptococcus mutans and lactobacillus acidophilus. **Study design**: A total of six agar petri dishes for Streptococcus mutans and Lactobacillus acidophilus were prepared. The methanolic extract of fennel seed and 0.2% chlorohexidine were incoluted on the each petri dishes prepared separately for tested species and incubated at 37ºC for 24 hours. Zone of growth inhibition for each extract was measured in millimeters using antibiotic inhibiting zone measuring scale. Each extract was tested three times. The results was subjected to statistical analysis. **Results:** 0.2% Chlorohexidine showed the maximum zone of inhibition against S.mutans and Lactobacillus acidophilus. Fennel seeds also inhibited the growth of micro organism but to a lesser degree than 0.2% chlorohexidine gluconate. **Conclusion:** Chlorohexidine and fennel seed are effective in inhibiting the growth of cariogenic bacteria like Streptococcus mutans and Lactobacillus. Therefore, both herbal and chemical agents can be effectively used to reduce pathogenic oral microflora.

**KEYWORDS:** Chlorhexidine gluconate, Fennel seeds, Streptococcus mutans, Lactobacillus acidophilus, Inhibition zone.

## INTRODUCTION

The tooth surface is unique in that it is the only body part that is not subjected to metabolic turnover. It is however subjected to various infection due to factor that favours microbial growth.[1] This microbial growth leads to one of the most wide spread diseases of tooth, i.e dental caries. Streptococcus bacteria are mainly responsible for the initial phase of the caries lesion specially in the enamel (initiation), whereas lactobacillus is more involved in the progression of caries. Targeting Streptococcus mutans forms the most important measure for prevention of dental caries.[2] This can be achieved by various mechanical and chemical aids. Many chemical antiplaque agents in the form of varnishes, dentrifices and mouthwashes have been tried for improvement of oral health. Mouth washes have been found to be one of the safe and effective delivery systems as antimicrobial and antiplaque agents. Various mouth washes, the most persistent reductance of Streptococcus mutans have been achieved by chlorohexidiene mouth washes.[3] Chlorhexidine gluconate (CHX), a bis-biguanidine, has routinely been used as an effective antibacterial solution 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. Because of its chemical composition, it has antimicrobial activity against Gram-positive and Gram-negative organisms. CHX is used in two concentrations (0.2% and 2%) and its mode of antibacterial activity is related to concentration. CHX is bacteriostatic at a concentration of 0.2% and bactericidal at a concentration of 2%.[4] However it is not recommended for long term use due to its numerous adverse effects like tooth and restoration staining, soft tissue staining, increase calculus deposition, unpleasant taste alterations, burning sensation, desquamation and mucosal irritation.[5]

This necessitates the development of innovative strategies. One such strategy would be to verify the enormous wealth of medicinal plants abundantly available. In nature Herbal medicine is both promotive and preventive in its approach. Natural herbs like triphala, tulsi patra, jyestiamadh, neem, clove oil, fennel seed, pudina, ajwain and many more used either as whole single herb or in combination have been scientifically

proven to be safe and effective medicine against various oral health problems.

Apart from this, all herbal mouth rinses do not contain alcohol and/or sugar, two of the most common ingredients found in most other over-the-counter products. The problem of these ingredients is that the microorganisms that cause bad breath and halitosis love to feed on these ingredients and release by products that cause halitosis. Thus, by use of a herbal mouth rinse, we can avoid these ingredients, which itself is one step forward towards better oral hygiene and better health.[6]

Fennel (Foeniculumvulgare Miller) is highly aromatic and flavorful herb with culinary and medicinal uses. Fennel fruit is a dry seed, traditionally used as anti-inflammatory, analgesic, carminative, diuretic 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] Recently there has been considerable interest in the antimicrobial potential of fennel seed extracts and essential oil.[9,10,11] Therefore, the chemical and herbal agents used in this study have also shown to posses antimicrobial activity due to the active ingredients they posses. Hence the present study aimed to asses and compare the antimicrobial efficacy of Chx and fennel seed against S. mutans and L.acidophillus.

## MATERIALS AND METHOD

Strains of S.mutans (MTCC no.497)and Lactobacillus Acidophillus (MTCC no.10307) were commercially obtained (microbial type culture collection centre, Chandigarh. For Mitis Salivarius Bacitracin Agar and for Lactobacillus acidophilus Rogosa agar was commercially obtained from HIMEDIA. 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

Fennel seeds were dried at 60ºC in hot air oven till constant weight was attained. Finely 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. Collected extract was filtered through double layered muslin cloth followed by centrifugation at 5000g for 5min in order to get clear supernatant. Extract was concentrated in a vacuum evaporator and stored at -20ºC for further use. The extract was diluted appropriately for different experiments.

The solid agar was punched with 7mm diameter wells. The inoculums were spread on to the agar plates using sterile swabs. The methanolic extract of fennel seed and 0.2% chlorohexidine were inoculated in the wells of petri dishes prepared separately for tested species. (Fig.1&2). The plates were then incubated at 37ºC for 24 hours. After incubation, zone of growth inhibition for each extract was measured in millimeters using antibiotic scale. Each extract was tested three times.(Fig. 3&4).

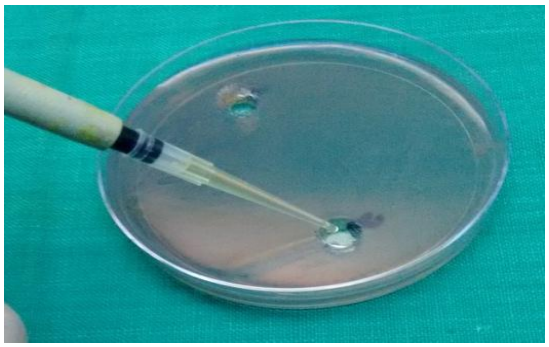

**Fig .1- Inoculation of test substance against Streptococcus Mutans.**

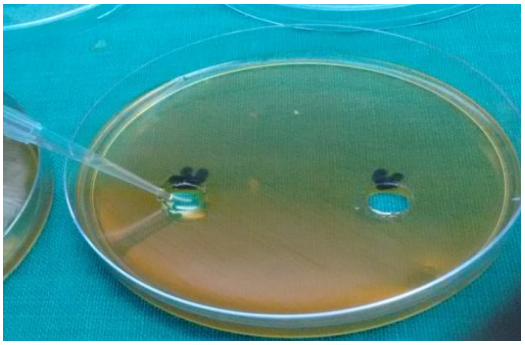

**Fig. 2- Inoculation of test substance against Lactobacillus acidophilus**

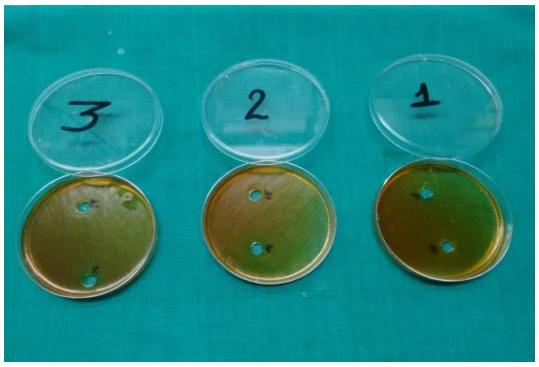


**Fig.3- Antimicrobial activity of test ubstances against Lactobacillus Acidophilus**

**Fig. 4- Antimicrobial activity of test substances against Streptococcus Mutans**

**Statistical Analysis**

Data was analyzed using SPSS version 20.0 statistical software (SPSS Inc., Chicago, Ill). The comparison of inhibition zone against S. mutans and Lactobacillus acidophilus count using Chx and fennel seed extract was done using Mann Witneys test Wilcoxen test. All statistical analyses were set at a significance level of $p<0.05$.

**RESULT**

The mean and standard deviation of zone of inhibition of S. mutans and L.acidophillus after inoculation with Chx and fennel seed extract are shown in Table 1. There was statistically significant difference in the mean of inhibition zone between Chx and fennel seed against S. mutans($p=0.05$) and Lactobacillus acidophillus ($P=0.046$).Chlorohexidine gluconate and fennel seed extract have shown inhibition against both the species but more against Lactobacillus acidphillus as compared to Streptococcus mutans (Fig.5). Amongst the agents used Chx proved to better antimicrobial agent against both the species compared to methnolic extract of fennel seed.

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

| Species | Agent | N | Mean | Median | Std Deviation | P-value |
|---|---|---|---|---|---|---|
| Streptococcus Mutans | Chlorohexidine gluconate | 3 | 22.33 | 22.00 | 1.53 | 0.05 |
| | Methanolic extract of fennel seed | 3 | 16.83 | 17.00 | 0.76 | |
| | Total | 6 | 19.58 | 19.25 | 3.20 | |
| Lactobacillus Acidophillus | Chlorohexidine gluconate | 3 | 23.17 | 23.00 | 0.76 | 0.046 |
| | Methanolic extract of fennel seed | 3 | 17.33 | 17.00 | 0.58 | |
| | Total | 6 | 20.25 | 20.25 | 3.25 | |

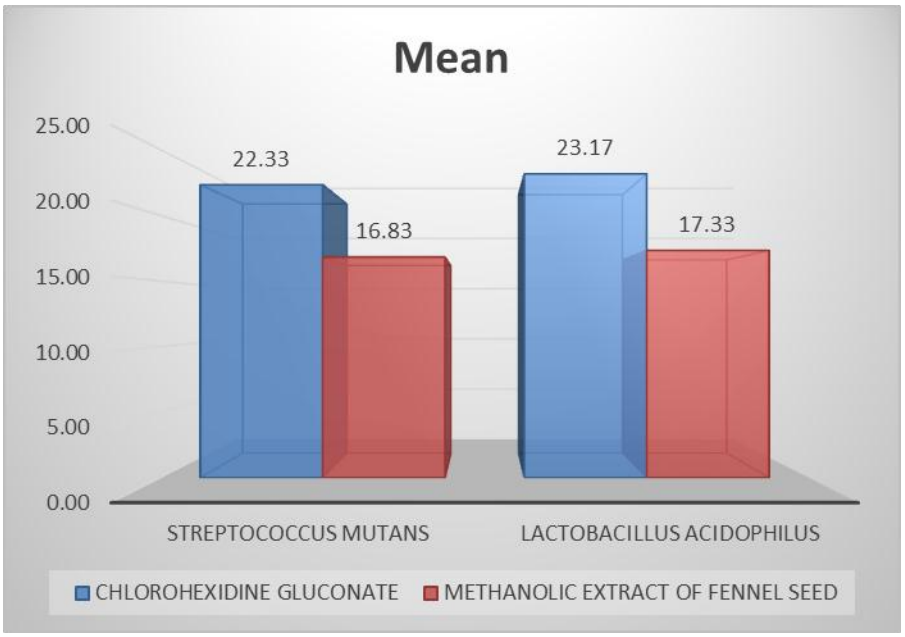


**Fig.5: Graph showing mean of inhibition zone by fennel seed extract and Chx against S.mutans and Lactobacillus acidophilus**

**DISCUSSION**

The oral cavity is a habitat for temporary and permanent microorganisms. Some of these microorganisms play a main role in the development of the dental caries. The association between Streptococcus species and dental caries has been well documented with S.mutans playing a vital role in caries formation and progression.[12] It produces an enzyme dextran-sucrase, which converts the sucrose of food to dextrin polymer and dextrin then combines with salivary proteins to create a sticky, colorless film (plaque) on tooth surfaces. The plaque provides the asylum for the activities of Lactobacilli to produce lactic acid, which dissolves the enamel by decalcifying it. Hypothetically, the control of dental caries is hidden in the inhibition of each step in the process of caries formation.[13] A wide variety of synthetic antimicrobial agents have been used over the years as antiplaque agents but because of the increased

antibiotic resistance to these antimicrobial agents, toxic and harmful side effects of few common antibacterial agents, there is a need for alternative agents which are affordable, non-toxic and equally effective. It has been found that natural plant extracts could be used as effective antiplaque agents.

Out of all the antiplaque agents, chlorhexidine is considered the gold standard agent for its clinical efficacy in chemical plaque control. It has broad antibacterial activity, with very low toxicity and strong affinity for epithelial tissue and mucous membranes. Besides its antiplaque effect, chlorhexidine is substantive, thus reducing levels of microorganisms in saliva up to 90% for several hours.[14]

Antimicrobial properties of fennel seed extracts have been studied by Gulfraz.[15] Chauche et al[16] reported the phytochemical tests on the stem, root and seeds of the plant fennel. The presence of flavanoids, tannins, saponins, sterols, coumaries, essential oils and absence of anthocyanes and alkaloids was reported. In the present study chlorohexidine was found to be more effective against both S.mutans and Lactobacillus acidophilus 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(p=0.05) and Lactobacillus Acidophillus (P=0.046). Chlorohexidine gluconate and fennel seed have shown inhibition against both the species but more against Lactobacillus acidphillus as compared to Streptococcus mutans. Amongst the agents used Chx proved to better antimicrobial agent against both the species compared to methnolic extract of fennel seed .Karthik V et al[17] assed and compared the antibacterial effect of 0.2% chlorohexidine and .05% sodium fluoride mouthwash, 2%povidone iodine mouthwash against S.mutans and L.acidophillus and concluded that 0.2% Chlorohexidine showed the maximum antibacterial effect on both the species which was consistent with our study result.

Nagappan N et al[18] evaluated the antimicrobial efficacy of herbal and chlorhexidine mouth rinses against Streptococcus mutans and demonstrated that, compared to herbal mouth rinse, chlorhexidine mouth rinse provided better results in its antimicrobial efficacy against Streptococcus mutans. Prajapati RA et al[19] conducted a study to evaluate the antibacterial effect on streptococcus mutans and concluded that even at 2% concentration methonolic extract of fennel seed showed poor response which was contrary to our study result where fennel seed though inhibited the bacterial species but not completely. Similar to our study results Ajithkrishnan CG et al[20] recorded and compared the salivary pH at baseline, immediately and five minutes after chewing the fennel seeds and the outcome was that Chewing of seeds showed a rise in salivary pH, which can prevent demineralization and have an anti-cariogenic effect.

To the best of our knowledge, as few studies have been done on antimicrobial effects of herbal products against oral pathogens, it is better that the effect of herbal extracts on other oral bacteria that have cariogenic activity be studied. Because of the antimicrobial effects of some herbal extracts, which have minimal side effects in comparisons with chemical drugs, more in vivo and in vitro investigations on the efficacy of herbal extracts on oral microbial flora should be studied.

## CONCLUSION

Within the limitation of the study it demonstrated that, compared to herbal mouth rinse, chlorhexidine mouth rinse provided better results in its antimicrobial efficacy against Streptococcus mutans and Lactobacillus Acidophillus but fennel seed extract used as a herbal agent can be used as a alternative antibacterial agent to reduce pathogenic oral microflora therefore further researches are still required to check the antimicrobial efficacy of herbal mouth rinse in greater depth and in vivo clinical testing is essential to confirm the in vitro results.

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