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Comparative Evaluation Of Antimicrobial Efficacy of Commercially Available Toothpaste Fortified With Terminalia Chebula Extract And Chlorhexidine Against Streptococcus Mutans - An In-Vitro Study.

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Abstract

Dental plaque is an oral biofilm comprised of a diverse and complex microbial community. Hence,to maintain ideal oral environment regular plaque removal is very important. Several active antimicrobial agents are incorporated in the tooth pastes for reducing the microbial load and to prevent microbial diseases like dental caries and periodontitis.As herbal remedies have a long history of use for gum and tooth problems, the present study was designed to investigate antimicrobial efficacy of a commercially available toothpaste fortified with Terminalia Chebula extract and Chlorhexidine control and to compare between the two.

Aim and Objectives: To evaluate and compare the antibacterial efficacy of a commercially available toothpaste at 100%,50% concentrations when fortified with 25% Terminalia Chebula extract and Chlorhexidine control on Streptococcus Mutans.

Materials and Methods: The antibacterial efficacy of a commercially available toothpaste at 100%,50% concentrations when fortified with 25% Terminalia Chebula extract and Chlorhexidine control on Streptococcus Mutans was tested using agar well diffusion method.

Results: The results showed that T.Chebula extract was active against S.Mutans with ($p<0.001$).The Zones of Inhibition for group 1 is 38.4 $\pm$ 2mm, for Group 2 is 31.6

± 2mm, for Group 3 is 45.9 ± 2 mm,for group 4 is 40.3 ± 2 mm, for Group 5 is 36.1 ± 2 mm ,for Group 6 is 21.3 ± 2 mm.

Conclusion: Due to the cariostatic properties of T.Chebula, it can be involved into caries preventive regimen by selectively inhibiting cariogenic bacteria basically targeted for use in pediatric population.This study can pave the way for the development of a synergetic compound with long-lasting anticaries action on the tooth surface.

Keywords: Terminalia Chebula, Dental caries, Antibacterial activity, Zone of inhibition.

Introduction

Dental caries is a common oral bacterial pathology caused by a biofilm consisting of microorganisms present on the tooth surface. It is a disease that has been associated with Streptococcus species mainly S.Mutans.As dental plaque is an oral biofilm comprising of a diverse and complex microbial community,it is imperative for it's removal in order to maintain ideal oral environment. Since tooth brushing is the most common and effective oral hygiene aide, several active antimicrobial agents are incorporated in the tooth pastes for reducing the microbial load and to prevent microbial diseases like dental caries and periodontitis.[1] Also, there has been a change in thinking globally, with a growing tendency to "GO NATURAL" because of side effects of many synthetic antimicrobial agents.Even today, in many parts of rural India, people use various herbal tooth cleaning aides. [2] There are several toothpastes available in market that claim to be anticariogenic due to presence of several active constituents. [1] *T. chebula* belonging to the family Combretaceae is native to India, China, Malaysia, Sri Lanka, Pakistan, and Tibet.[3] In addition to treating oral disease like gingivitis and stomatitis, it is also used in the treatment of asthma, sore throat, vomiting,bleeding piles, gout, heart and bladder diseases. [4]In view of these reported medicinal values and also the plant being inexpensive and readily available, the present work was carried out to evaluate the antimicrobial potential of a commercially available toothpaste fortified with 25% T. Chebula extract against S. Mutans with Chlorhexidine control and to compare the zones of inhibition between the two.

Materials and Methods

Extract of dried fruit of Terminalia Chebula 25% concentration

Colgate Kids toothpaste (2 to 5yrs)

0.2% Chlorhexidine (ICPA)

Distilled water

Brain Heart Infusion Broth

Equipment

Vernier Calipers

Petri Dishes

Tested Microorganisms: The Microorganisms used in this study were Streptococcus Mutans (MTCC 890)

The Institutional Ethical Committee approved the study.

Figure 1: Materials Used

Methodology

A total of 42 samples will be divided into 7 groups

7 samples per each group

Group 1: 100% concentration of pure toothpaste

Group 2: 50% concentration of pure toothpaste

Group 3: 100% concentration of toothpaste fortified with 25%Terminalia Chebula Extract

Group 4: 50% concentration of toothpaste fortified with 25%Terminalia Chebula Extract

Group 5: 100% concentration of toothpaste fortified with 0.2% chlorhexidine

Group 6: 50% concentration of toothpaste fortified with 0.2% chlorhexidine.

Bacterial Strain	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
S.Mutans	7	7	7	7	7	7

Preparation of Aqueous Extract

The dried fruits were bought from a taxonomist . After washing and air drying, they were ground into a fine powder.The powder was weighed into 25g using a digital weighing machine and stored in air tight sterile containers. Cold aqueous extract was obtained by adding 100 ml of distilled water to the pre-weighed amounts of the powdered plant extract. The constituents then were stirred thoroughly and soaked separately in 250 ml conical flasks for 48 h at 4°C. Using a Whatman filter paper-1 crude aqueous extract was prepared. The prepared aqueous extract was used for antibacterial assay. [2]

Antimicrobial Assay

Initial concentration of the tooth pastes were prepared by mixing 2 gms of tooth paste with 2 ml of sterile distilled water (1:1) in order to allow for the extract to be miscible in the toothpaste(Group 1).Then, Group 2 samples were prepared by adding 4ml of distilled water to 2 gms of toothpaste(50% conc).In this study,agar well diffusion method was used to evaluate the antibacterial activity. This method depends upon the diffusion of the tested material to such an extent that growth of added microorganisms is prevented entirely in a zone around the hole containing a solution of tested material. Agar media (100 ml) was sterilized in separate conical flasks, cooled and inoculated with 0.1 ml of the respective test bacterial suspension. After thorough mixing, the inoculated medium was transferred into sterilized Petri dishes and on solidification of the liquid agar medium, wells of about 6 mm diameter were punched into it with a sterilized cork borer. Prior to the addition of the test samples,1 well was made in 1 petri dish and accordingly 7 samples for each group were prepared..The procedure was repeated for the total 42 samples. The samples were named according the above mentioned 7 groups. The prepared test samples namely the plant extracts at 25% w/v and 0.2% chlorhexidine were added to the toothpastes separately in petri-dishes at 100 and 50 % concentrations,mixed well and kept ready. A 100 µl of this prepared homogeneous mixture at different concentrations was filled in the respective wells prepared. The inoculated bacterial plates were incubated at 37°C for 24 hrs and the diameter of Inhibition zones were measured. [2]

Statistical Analysis

The Intragroup analysis was done by Unpaired t test. Intergroup analysis was done using Anova Test. There was a difference observed between seven groups and it is found to be statistically significant.Further,using Post Hoc Analysis,significant difference was observed between Groups 1,2,3,4,5,6,7 for S.Mutans with a p value of < 0.001.

Results

The results revealed that T. Chebula had good potential to be an anticariogenic agent.The zones of inhibition were larger for the mixtures fortified with T.Chebula extract when compared to plain toothpaste and toothpaste fortified with Chlorhexidine control.The Zones of inhibition of seven groups with S. Mutans in the decreasing order were 100% TP with T.Chebula extract < 50% TP with T.Chebula extract < 100% TP <100% TP +

CHX < by 50% TP < 50% TP + CHX.The results indicate that the mixtures when fortified with 25% conc of T.Chebula extract showed potential anticariogenic ability when compared to the plain toothpaste and Chlorhexidine control.

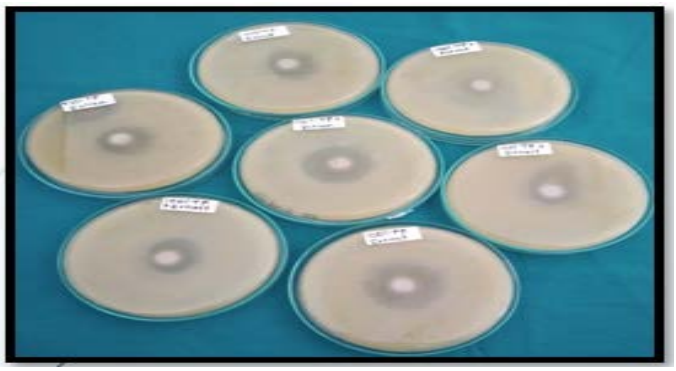


Figure 2: Inhibition Zones of TP with Extract

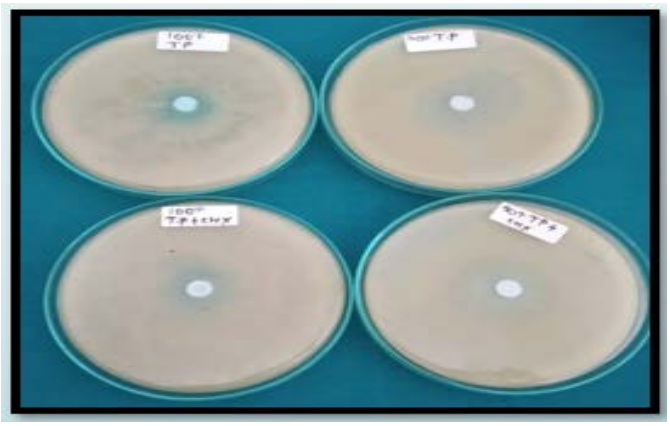


Figure 3: Inhibition Zones Of TP With Chlorhexidine

Figure 4: Total Samples

S NO	S.MUTANS
1	30.2 mm
2	32.5 mm
3	31.5 mm
4	29.0 mm
5	34.1 mm
6	32.3 mm
7	31.9 mm

Table 1- Diameter Of Zones- 100% conc of TP

SNO	S.MUTANS
1	45 mm
2	35 mm
3	34.4 mm
4	38.6 mm
5	40.1 mm
6	39.6 mm
7	36.6 mm

Table 2- Diameter Of Zones - 50% conc of TP

S NO	S.MUTANS
1	49.8 mm
2	46.2 mm
3	44.3 mm
4	49.6 mm
5	45.6 mm
6	42.4 mm
7	43.8 mm

Table 3- Diameter Of Zones-100%TP+Extract

S NO	S.MUTANS
1	38.5 mm
2	42.5 mm
3	39.1 mm
4	37.3 mm
5	40.1 mm
6	41.6 mm
7	43.6 mm

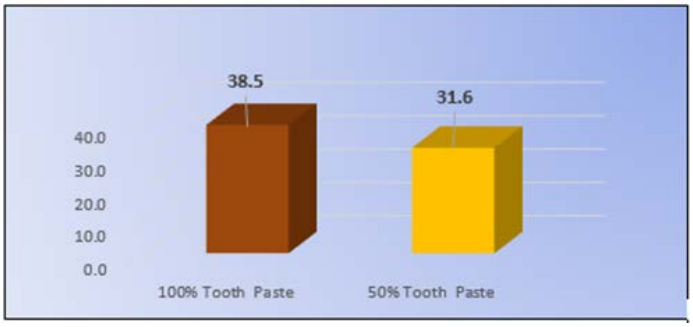
Table 4 - Diameter Of Zones -50% TP+Extract

S NO	S.MUTANS
1	40.6 mm
2	35.0 mm
3	36.2 mm
4	35.8 mm
5	32.9 mm
6	39.0 mm
7	33.8 mm

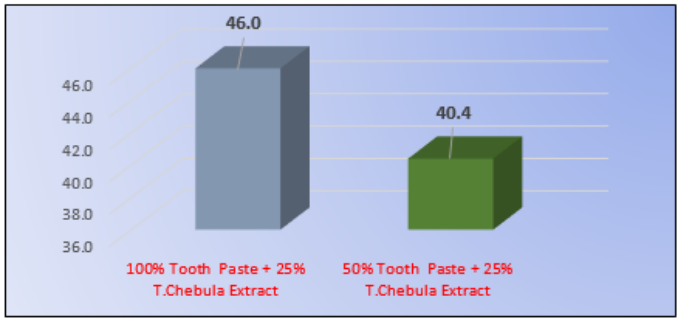
Table 5- Diameter Of Zones -100% TP+CHX

S.NO	S.MUTANS
1	19 mm
2	21.2 mm
3	11.5 mm
4	26.4 mm
5	20.1 mm
6	29.0 mm
7	21.9 mm

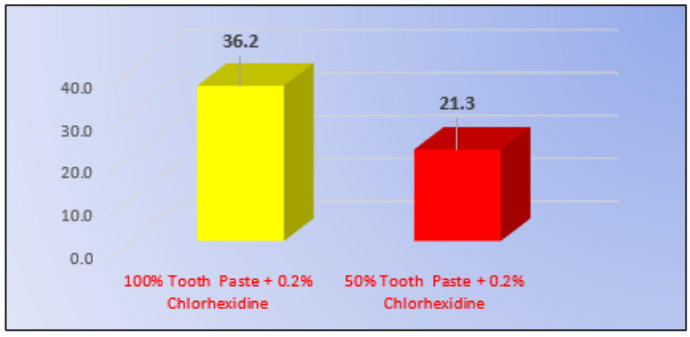
Table 6 - Diameters Of 50%TP+0.2%CHX



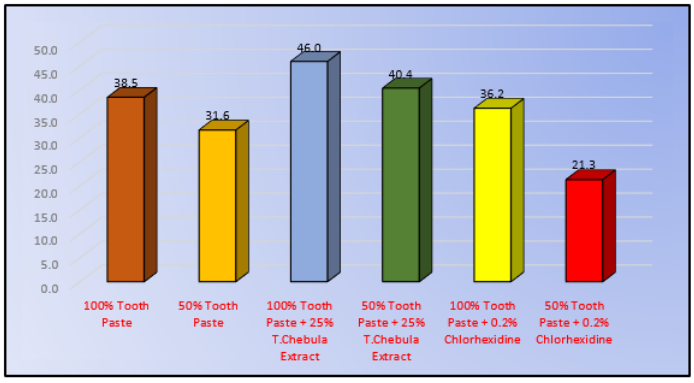
Graph 1-Comparative Antibacterial activity of 100% and 50% Concentrations of pure toothpaste against S.Mutans



Graph 2- Comparative Antibacterial activity of 100% and 50% Concentrations of toothpaste fortified with T.Chebula extract against S.Mutans



Graph 3- Comparative Antibacterial activity of 100% and 50% Concentrations of toothpaste fortified with 0.2% Chlorhexidine against S.Mutans.



Graph 4-Comparative Antibacterial activity of Group 1,Group 2,Group 3,Group 4,Group 5 and Group 6 against S.Mutans.

Discussion

S.Salivarius, S. Mitis, and S. Oralis were identified as the first and most dominant oral microbes to colonize the oral cavities of new-born infants. With the eruption of primary teeth, the number and complexity of the micro-flora in the oral environment increase.[5] Once colonized , these pioneer bacteria adhere and produce acidic environment . [1] This progression involves initial adherence of bacteria to the salivary pellicle and subsequent accumulation by growth and inter-bacterial adherence. Ultimately,the tooth surface gets coated with a dense,complex micro-community that ends up in the destruction of hard enamel tissue. [6]

Many studies reveal that streptococcus Mutans have shown to be highly associated with caries in humans. [7] In

many individuals, the customary oral hygiene method of tooth brushing is, by itself, usually insufficient over a long period to provide a level of plaque control consistent with oral health. Consequently, the incorporation of chemical agents with anti-plaque or antimicrobial activity into dental products has been proposed as a potential prophylactic method of reducing plaque-mediated disease. [8] Hence, maintenance of an ideal oral environment by regular plaque removal is mandatory.Consequently, the incorporation of chemical agents with anti-plaque or antimicrobial activity into dental products has been proposed as a potential prophylactic method of reducing plaque-mediated disease. [1] They also prevent bacterial aggregation and release endotoxins. [8] There are several toothpastes available in market that claim to be anticariogenic due to presence of several active constituents. Some contain chemical components like triclosan and fluoride whereas others contain herbal components; there are few which contain both. [1] There is an increasing tendency in the medical field to opt for therapeutic agents from natural sources to reduce the resistance to antibiotics which is a worldwide problem.Researchers stated that plant extracts show target sites other than those used by antibiotics, which will be active against drug-resistant pathogens..T. Chebula is one of the exceptions, in that its extract was being used for the prevention of dental caries. [9] They also prevent bacterial aggregation and reduce the rate of multiplication. [8] The aim of this study is to compare the antibacterial activity between the plain toothpaste, toothpaste fortified with the herbal extract with the control being Chlorhexidine so as to find out the efficacy of T. Chebula on dental caries causing pathogens.In the present study, Colgate kids toothpaste was chosen as the basic medium in which the experimental and control solutions were added at different concentrations of the toothpaste. 100 and 50 percent dilutions of toothpaste were considered in this study so as to evaluate the diffusion potential of the tested plant extracts in different concentrations. Fluoridated toothpastes are given only as pea sized amounts below six years of age due to fluoride poisoning and also we cannot control the ingestion of toothpastes while brushing in children unless thoroughly supervised. [10] If the same toothpaste is fortified with a herbal extract with known therapeutic values,then with the same amount of toothpaste a synergistic antibacterial action can be achieved on the oral microbes with no side effects. *T. Chebula* acts by preventing plaque formation on the surface of tooth by inhibiting sucrose-induced adherence and glucan-induced aggregation, two processes which foster the colonization of *S. Mutans* on the surface of tooth.[2]

Chlorhexidine (control) used in this study, is a proven chemical antimicrobial agent which shows bactericidal action by altering the integrity of the bacterial cell membrane thereby leading to coagulation and precipitation of the cytoplasm.[11] Also, Hugo et al in his article “Some aspects of the mode of action of chlorhexidine” demonstrated that Chlorhexidine at low concentrations is a potent membrane active agent against both gram-positive and gram-negative bacteria. It abolishes the activity of the phosphoenolpyruvate-phosphotransferase sugar transport system, and thereby markedly inhibits acid production in oral streptococci cariogenic bacteria in subjects with a high risk of developing caries. [12]

The results revealed that highest antibacterial activity was shown with group 3 followed by group 4 meaning the samples fortified with the Extract showed better inhibition zones when compared to the plain toothpaste (Group 1 and 2) when compared to the samples fortified with Chlorhexidine control (Group 5 and 6) against S.Mutans

which is a strong positive point for this study. This study paves the way for further research if toothpastes can be fortified with pure natural products which have minimal or no side effects.But,as this is a preliminary In vitro study using the combination of a kids toothpaste and T.Chebula extract ,further randomized clinical trials can provide insight of the overall effect of the dentifrice.

Conclusion- Indian medicinal plant extracts may show the way to the development of a synergetic compound with a long-lasting anticaries action on the tooth surface.Due to the cariostatic properties of Terminalia Chebula, it can be involved into the caries preventive regimen by selectively inhibiting cariogenic bacteria basically targeted for use in the pediatric population.

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