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Comparative Evaluation of Antimicrobial Activity of Pomegranate, Papaya Juices, 0.12% Chlorhexidine And 2% Sodium Fluoride Against Streptococccus Mutans: An In Vitro Microbial Study.

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Abstract

Introduction: Dental caries is an infectious disease influenced by the relationship between diet and cariogenic oral microorganisms and the characteristics of the host. Caries begins in childhood and eventually affects 90% of adults.[1] Dental caries has been identified as one of the most prevalent chronic conditions and is a major problem for children all over the world.

There is evidence of successful treatment of various oral diseases in literature like bleeding gums, mouth ulcers, halitosis and tooth decay by using herbs like tulsi patra, triphala, green tea, neem, pudina, clove oil, aloe vera.

Aim: To compare the antimicrobial efficacy of Pomegranate, Papaya juice, 0.12% Chlorhexidine and 2% Sodium Fluoride against Streptococcus Mutans. An in vitro microbial study.

Methodology: Surfaces of fruits were peel sterilized with 70% alcohol and then rinsed with sterile distilled water. 100% concentrated fresh juices along with seeds of these two selected fruits were prepared in the laboratory in a sterile metal grinder just before the experiment. Sodium fluoride solution was freshly prepared. Standard strain of Streptococcus mutans (ATCC 25175) (HIMEDIA, Mumbai) was used in the study.

The experimental groups were:

- Group A: Pomegranate juice (100%)
- Group B: Papaya juice (100%)
- Group C: Chlorhexidine (0.12%)
- Group D: Sodium fluoride (2%)

The zone of inhibition was measured by Electronic calliper scale in millimeter.

Statistical analysis: Mean comparison in between the multiple groups was done using one way Analysis of Variance test (ANOVA) for mean difference in between the 4 groups.

Results: It implies that, the antimicrobial activity against Streptococcus mutans for

Chlorhexidine>Pomegranate>Sodium Fluoride=Papaya

Conclusion: It can be concluded that both fruit juices, owing to their constituent, are potent antimicrobial agents as well as 0.12% Chlorhexidine gluconate and 2% Sodium Fluoride.

Keywords: Antimicrobial, Caries, Fluoride.

Introduction

Dental caries is an infectious disease influenced by the relationship between diet and cariogenic oral microorganisms and the characteristics of the host. Caries begins in childhood and eventually affects 90% of adults.[1] Dental caries has been identified as one of the most prevalent chronic conditions and is a major problem for children all over the world.[2]

Streptococcus mutans has been implicated as the principal etiological agent in the development of dental caries in humans due to its ability to produce extracellular polysaccharides called glucans/fructans and is frequently isolated from human dental plaque.[3] High acidogenic Streptococcus mutans reach the terminal pH 3.4 within 18 hours of growth in broth medium. S.mutans synthesizes the insoluble glucans which increase the pathogenicity of oral biofilm by promoting the adherence and accumulation of cariogenic microorganism on tooth surface.[3,4]

Commercial chemical mouthwashes are available in larger number. Chlorhexidine is one of them, which is considered as a gold standard.[5,6] Chlorhexidine has been studied for nearly 40 years primarily for its ability to reduce gingivitis. It is the most effective anti-plaque agent, but it is not a magic bullet because of its side effects such as tooth staining on long-term use, enhanced supragingival calculus formation, taste disturbance and desquamation of the oral mucosa.[7]

Punica Granatum or pomegranate belongs to the Punicaceae family and is a shrub native to Asia.[8] It has a molecular weight of 1000 Daltons, pH between 3- 4.5. The ripe fruit is about five inches wide with deep red, leathery skin, grenade shape with a pointed calyx. The fruit contains many seeds which are separated by white membranous pericarp. Each seed is surrounded by tart and red juice. [7,9] Pomegranate fruits are widely consumed and available in commercial products, such as juices, jams and wines. Pomegranate preparations are used as topical applications particularly for controlling oral inflammation, as well as bacterial and fungal counts in periodontal disease and candida-associated denture stomatitis.[10]

Papain belongs to family Caricaceae. It is extracted from the latex of the leaves and fruit of the adult green papaya i.e. Carica papaya. Papain has a molecular weight of 23.406 Daltons, pH and temperature optimum between 3-

9 and 65- 80°C. Papain is a cysteine protease hydrolase enzyme. It consists of 212-218 amino acids. This enzyme is an endoprotein similar to human pepsin, which has bactericidal, bacteriostatic and anti-inflammatory activity, and is a debriding agent. Papain does not damage healthy tissue.

Fluoride also has a direct inhibitory effect on the metabolic activity of cariogenic bacteria. Glycolysis is the central metabolic pathway by means of which saccharolytic microorganisms thrive. Inhibition of glycolysis by fluoride is central to the concept that the anti-microbial effect of fluoride has a role in caries prevention.

Methodology

Collection of Fruits: Pomegranate (Punica granatum) and Papaya were purchased from fruit market. All fruits were brought to Monera laboratory. All fruits were washed in running tap water. Surfaces of fruits were peel sterilized with 70% alcohol and then rinsed with sterile distilled water. 100% concentrated fresh juices along with seeds of these two selected fruits were prepared in the laboratory in a sterile metal grinder just before the experiment. (Figure 1)

Preparation of Sodium Fluoride: Sodium fluoride powder in the pure form was collected from the Department of Biochemistry. Sodium fluoride solution was freshly prepared. The solution was prepared by dissolving 20mg of sodium fluoride in 1000ml of distilled water in plastic bottle.

Preparation of Nutrient Agar Plates: The 40 grams of nutrient agar was suspended in 1000 ml distilled water. It was heated till the medium completely dissolved. It was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Then it was cooled to 45- 50°C. It was mixed well and poured into sterile Petri plates. The depth of the medium was approximately 4mm. It was then allowed for solidification. After solidification the plates were dried for 30 minutes to remove excess moisture from the surface. (Figure 2)

Cultivation of Streptococcus mutans bacterial culture: Standard strain of Streptococcus mutans (ATCC 25175) (HIMEDIA, Mumbai) was used in the study. The bacteria were reactivated and cultured in a flat bottom flask containing 50 ml of Brain Heart Infusion (BHI) broth and incubated at 37^{o}C for 7 days. Change in turbidity of the solution was an indication of viable bacterial growth. After microbial growth, the broth culture suspension in Brain Heart Infusion broth was adjusted to a turbidity equivalent to the Barium sulphate standard of 0.5 McFarland units, equivalent to 1.5 x 10^{8} colony forming units per ml (CFU/ml) with sterile BHI taken as standard. (Figure 3)

Assessment of antimicrobial activity: After incubation, 100 µl of inoculums was spread on sterile Nutrient agar plates. Wells of 6 mm size were made with sterile cup borer into Nutrient agar plates containing the bacterial inoculum. 800 µl volumes of the samples were poured into each well of inoculated plates. Sample size for each group was 30.

The experimental groups were:

- Group A: Pomegranate juice (100%)
- Group B: Papaya juice (100%)
- Group C: Chlorhexidine (0.12%)
- Group D: Sodium fluoride (2%)

The plates thus prepared were left at room temperature for 10 min so as to allow the diffusion of the extracts into the agar. The plates were then incubated for 24 hrs at 37ºC. After incubation, the plates were then observed. Antibacterial activity present on the plates was indicated by an inhibition zone surrounding the well containing the fruit juice. (Figure 4, 5, 6, 7) The zone of inhibition was measured by Electronic alliper scale in millimeter.

Result

Mean comparison in between the multiple groups was done using one way. Analysis of Variance test (ANOVA) for mean difference in between the 4 groups. Pair-wise comparison was done using Post-hoc multiple comparisons (Tukey HSD) test.

Table 1 shows the mean inhibition zone of streptococcus mutans of each group. In group I mean inhibition zone was 6.4000, in group II it was 5.3233, in group III it was 15.0833, and in group IV it was 5.2467.

Table 2 shows 95% Confidence interval of mean inhibition zone Streptococcus mutans in each group. 95% CI of zone of inhibition of Streptococcus mutans in group I was within 6.27 to 6.52, in group II, it was within 5.18 to 5.46, it was within 14.9 to 15.19 in group III and in Group IV it was within 7.26 to 8.76.

Table 3 shows comparison of mean of inhibition zone of Streptococcus mutans in between the four groups by ANOVA. It was found that statistically there was highly significant ($p<0.01$) difference of mean inhibition of Streptococcus mutans in the four groups.

It implies that, the antimicrobial activity against Streptococcus mutans for.

Chlorhexidine>Pomegranate>Sodium Fluoride=Papaya

Discussion

Dental diseases are a multifactorial disease and have affected large population throughout the world. The normal oral flora comprises a large group of microorganisms including bacteria, fungi, protozoa, and viruses.[2] Occurrence of disease results from disturbance of the equilibrium of this complex ecosystem, where population shift leads to over representation of pathogenic species which results in the onset and progression of the most common oral diseases i.e., caries and periodontal disease.[11]

In the present study, to evaluate antimicrobial property of pomegranate, papaya and sodium fluoride, 100 µl of Streptococcus mutans strains inoculum was spread on sterile Nutrient agar plate. 800 µl volumes of the samples were poured into each well of inoculated plates and after 24 hours the zone of inhibition was recorded. The results of the present study showed zone of inhibition of Streptococcus mutans in all the four groups stating that, fruits juices possess substantial antimicrobial activity against Streptococcus mutans.

Results of the present study showed that pomegranate juice and papaya juice extracts were effective against the main cariogenic pathogen, S. mutans ATCC 25175 cariogenic strain. The present research was in line with other studies demonstrating antibacterial agents from natural products were effective to prevent and contrast oral and periodontal disease and tooth decay.

In our study, Pomegranate juice showed zone of inhibition ranging from 6.27 to 6.52 while papaya juice showed zone of inhibition ranging from 5.18 to 5.46 which was significantly less than Chlorhexidine range i.e. from 14.96 to 15.19, but were capable of inhibiting the growth of bacteria. In 2006, Vasconcelos LC et al investigated the antimicrobial activity of Punica granatum (pomegranate) using a therapeutic gel and miconazole against three standard streptococci strains (S. mutans ATCC 25175, S. sanguis ATCC 10577, and C. mitis ATCC 9811) and demonstrated that, there was greater efficiency of inhibiting microbial adherence in pomegranate gel than miconazole.[26] Dabholkar CS et al conducted a study to check antimicrobial activity of Pomegranate Mouthwash and Pomegranate mouthwash presented Zone of Inhibition ranging from 38.48 to 57.69%, but resistant at <10ml and for S.mutans.[12]

Our study results are also supported with Vidushi L et al study which reported highest inhibitory effect of

pomegranate aril extract against Streptococcus mutans in patients having caries.[12] Ferrazzano GF reported that the hydroalcoholic extracts of pomegranate juice and peel were able to contrast the main cariogenic bacteria involved in tooth decay i.e. S. mutans and suggested that pomegranate polyphenolic compounds could represent a good adjuvant for the prevention and treatment of dental caries.[5]

Results of our study were also support by Suramaniam P et al study, which proved that pomegranate extract had significantly higher inhibitory effect on S mutans at all concentrations ($P \leq 0.05$).[13] Mahajan et al., studied the efficacy and antimicrobial properties of five herbal mouth rinses with Chlorhexidine gluconate mouth-rinse. They concluded that pomegranate was highly effective against Streptococcus mutans which supports results of our study.[11] The present study can serve as a prime tool to analyze the antimicrobial efficacy of Pomegranate and Papaya against Streptococcus mutans. The present study is time-saving, economical, effective, and non-toxic. The samples are easily available. We had used freshly prepared fruit juice and if it is ingested accidently, it will cause no harm as it is non-toxic. Pomegranate and Papaya can be used for treating the infectious diseases caused by microbes. The synergistic effect from the association of antibiotic with Pomegranate and Papaya extracts against resistant pathogens leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment.

Conclusion

From the results obtained, the following conclusions were drawn:

1. Fruit juices, 0.12% Chlorhexidine, and 2% Sodium Fluoride under evaluation showed reduction in the growth of Streptococcus mutans. Hence it can be concluded that both fruit juices, owing to their constituent, are potent antimicrobial agents as well as 0.12% Chlorhexidine gluconate and 2% Sodium Fluoride are exhibiting good antimicrobial efficacy against Streptococcus mutans.
2. The reported antimicrobial efficacy of fruit juices can be utilized in eliminating Streptococcus mutans in the form of mouth wash or gel.

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Legends Tables and Figures

Table 1: Mean Inhibition Zone of Streptococcus Mutans of Each Group

Group	Contents	N	Mean	S.D.
Group I	Pomegranate juice	30	6.4000	0.32800
Group II	Papaya juice	30	5.3233	0.36736
Group III	Chlorhexidine (0.12%)	30	15.0833	0.30522
Group IV	Sodium Fluoride (2%)	30	5.2467	0.24598

N: number of samples; S.D.: Standard Deviation

Table 2: 95% Ci Of Mean Inhiition Zone Of Streptococcus Mutan Of Each Group

Groups	Contents	N	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
Group I	Pomegranate juice	30	6.2775	6.5225
Group II	Papaya juice	30	5.1862	5.4605
Group III	Chlorhexidine (0.12%)	30	14.9694	15.1973
Group IV	Sodium Fluoride (2%)	30	5.1548	5.3385

N : Number of samples 7.2657

Table 3: Comparison of Mean Inhiition Zone Of Streptococcus Mutans In Between Groups By Anova

	Sum of Squares	df	Mean Square	F	P value
Between Groups	2024.349	3	674.783	6812.430	.000
Within Groups	11.490	116	.099		
Total	2035.839	119			

df: Degree of freedom

F: F value as per Fisher test

P value: value of significance

Figure 1: Incubator

Figure 2: Four Experimental Groups

Figure 3: Broth culture suspension of Streptococcus mutans in Brain Heart Infusion broth adjusted to a turbidity equivalent to the 0.5 McFarland standard, equivalent to 1.5 x 10^8 CFU/ml, with sterile BHI taken as standard

Figure 4: Zone of inhibition of Group I (Pomegranate)

Figure 5: Zone of inhibition of Group II (Papaya juice)

Figure 6: Zone of inhibition of Group III (Chlorhexidine)

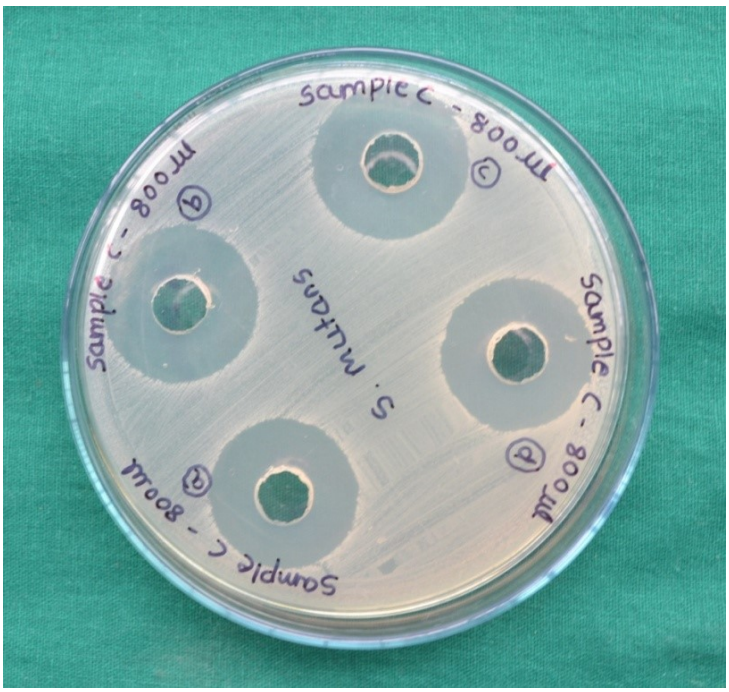

Figure 7: Zone of inhibition of Group IV (Sodium Fluoride)