Comparative Evaluation of Antimicrobial Properties of Various Oral Preparations against Lactobacillus acidophilus – A Microbiological In-vitro Study

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Abstract

Aim: The present study was undertaken to evaluate various preparations – Chlorhexidine, Alum, Amla, Aloe Vera, Papaya and Probiotic for their antimicrobial activity against Lactobacillus acidophilus and to compare the antimicrobial activity of these preparations against Lactobacillus acidophilus.

Materials And Method: Saliva samples of 30 children with nursing bottle caries or rampant caries were collected using sterile cotton swab, cultured onto the Rogosa Agar culture plates and incubated aerobically for bacterial growth to become appreciated. Agar well diffusion method was employed for antimicrobial susceptibility on Mueller Hinton agar plates. Crude form of extracts was used. Antimicrobial activity of the extracts was determined by measuring the mean zones of inhibition in mm.

Results: 0.2% Chlorhexidine showed the highest antimicrobial activity against Lactobacillus acidophilus followed by alum and amla extracts. Probiotics, aloe vera and papaya extracts were found to be ineffective against the bacteria.

Conclusion: According to the result it was concluded that oral environment could be stabilized by using these natural extracts as mouthwashes where alum proved to be most effective against the bacteria.

Keywords: Dental Caries, Lactobacillus acidophilus, Agar well diffusion method, Antimicrobials

Introduction

Dental caries is a multifactorial, chronic bacterial disease causing demineralization and destruction of the dental hard tissues, by production of acid by bacterial fermentation of the food debris accumulated on the tooth surface. Today, caries remains one of the most common chronic diseases of human beings worldwide. Approximately 36% of the population is affected in the permanent dentition while 9% of the population is affected in deciduous dentition[1].

Oral cavity shelters a vast and varied microbial flora[2], predominant microbes in deep carious lesions being S. mutans and genus Lactobacillus amongst others .

Streptococcus mutans is the main factor that initiates caries. They are the most cariogenic pathogens as they are highly acidogenic, producing short-chain acids which dissolve calcified tissues of teeth.

Lactobacilli occur in high numbers in both superficial and deep caries, though they are not suspected of being involved in bacterial invasion of unexposed dental pulp[3]. The production of acids, mainly lactic acid, lowers the pH of the oral environment, release of hydrogen peroxide and bacteriocins. Thus, Lactobacillus in addition to a cariogenic activity also plays a key role in maintaining the microecological balance in the mouth and gastrointestinal tract[1].

Chlorhexidine digluconate has been a gold standard in the field of chemotherapeutics[4]. It is currently the most potent chemotherapeutic agent against mutans streptococci and dental caries. It is available in the form of varnishes, gels and mouthwashes[5]. A 0.2% CHX solution is used as the standard international concentration[6].

The scientific name of Alum [$KAl(SO_4)_2$ $12H_2O$] is Aluminium potassium sulphate. In dentistry, alum solution has been used as a mouthwash following conventional periodontal procedures, which helps in improvement of clinical parameters of periodontal health in comparison to conventional periodontal treatment alone. Alum solution in 0.02% concentration is found to be better than other concentrations in improving periodontal health after conventional root planing[7]

Amla (Emblica officinalis) belonging to the family Euphorbiaceae, possesses anti-viral, antibacterial, anti-cancer, anti-allergy and anti-mutagenic properties. Commonly known as amla, it is highly valued in traditional Indian medicine and Unani medicine[8]. Extracts of amla pulp has been found to be highly effective in controlling growth of Streptococcus aureus, Vibrio cholerae and Pseudomonas aeruginosa in vitro[9].

Aloe Vera (Aloe barbadensis) - is a well known medicinal plant belonging to the Liliaceae family. Because of healing property of aloe vera due to presence of glucomannan, a manose rich polysaccharide, it has been used after periodontal surgery, extractions, injury by abrasion brushes and dental floss. Aloe vera tooth gel and toothpastes have been equally effective against Candida albicans, Streptococcus mutans, Lactobacillus acidophilus, Entercoccusfaecalis and Prevotella intermedia.[10]

Papaya (Carica papaya L.)(Family Caricaceae) commonly called pawpaw is a monosexual plant of Central American origin.

Its mechanism of action against the bacteria and fungi may be due to the inhibition of cell wall formation in the cell resulting in a leakage of cytoplasmic constituents by the bioactive components of the extract. The latex from the leaves has been used as antihelminths & for the treatment of infections of bacterial origin[11]. Papaya aqueous leaves extract exhibit potential activity against dengue fever. Platelet counts, white blood cell counts and neutrophil counts are increased after the administration of leaves extract[13].

Probiotics were defined by FAO/WHO (The Food Agricultural Organization/World Health Organization) as live microorganisms which when administered in adequate amounts (in food or as a dietary supplement) confer a health benefit on the host[12]. Commonly, most of the species ascribed as having probiotic properties belong to the genera Lactobacillus and Bifidobacterium.

Mechanism of action explaining beneficial probiotic effects include modulation of host immune response leading to strengthening of the resistance to pathogenic challenge, alteration of the composition and metabolic activity of host microbiota at the specific location. Considering the particular activities of probiotics and their inhibitory effect on the growth of pathogens, research interest has been extended to the oral cavity where probiotics may also exert their therapeutic or preventive effect on the development and progression of common oral diseases[14].

The present study was undertaken due to the easy availability, cost-effectiveness and medicinal advantages of alum, amla, aloe vera, papaya and probiotics to a common man. Chlorhexidine was used as a positive control against which other anti-microbial agents were compared.

Materials And Methods Source Of Sample

The study was conducted on a total of 30 children in the age range from 3 to 15 years. Parents of selected patient were made aware of the experimental design and written informed consent was obtained from them before the study.

Methods

A Case history performa was prepared to record the various details of the child like personal details i.e., name, age, sex, etc; oral hygiene habits, dietary history, medical history including any recent antibiotic treatment, dental history including any recent fluoride treatment, use of any prosthetic or orthodontic appliances, natal history etc. (periods of use of feeding bottles or pacifiers of children, etc.).

Sample Collection

Saliva samples were collected using a sterile swab from:

1. Vestibular sulcus
2. Dorsum of the tongue

Plant collection

Preparation of Plant extracts

Ø Plants extracts in the present study were used in the crude form by grinding fresh plant parts.

Ø All the fresh plant parts were collected and ground finely in a grinder to obtain a homogenous paste and filtered through Whatman filter paper no.1. This filtrate was then used for antimicrobial sensitivity.

Procedure

The saliva samples were cultured onto the surface of Rogosa agar culture plates and incubated aerobically at 37°C for 48 hours. Lactobacillus acidophilus were identified by colony characteristics and the bacteria was confirmed using gram staining.

Colony characteristics of lactobacilli- Small, greyish white, smooth colonies were seen.

Report

Gram positive rods arranged in chains were seen.

1. Characterization of bacteria was done by standard biochemical methods and Isolated colonies were inoculated into the peptone water broth.
2. The bacterial isolates were inoculated into 10 ml of sterile peptone water broth and adjusted according to 0.5 Mcfarland's solution to standardize the number of colonies according to national committee on clinical laboratory standards (NCCLS) guidelines, and incubated at 37ºC for 8 hours.

Antimicrobial Susceptibility Testing

- The agar well diffusion method was employed for antimicrobial susceptibility testing according to NCCLS standards (2000).
- Sterile cotton swabs were dipped in the inoculum and streaked uniformly onto each Mueller Hinton agar plate, and the plates were left on the bench for excess fluid to be absorbed.
- Seven wells of 5mm diameter were punched on each agar plate with the help of a micropipette, 100µl of different extracts (3-plant extracts, 1-alum, 1-probiotic, 1-chlorhexidine, 1-distilled water) were dropped into each well, which filled them to the fullness, in the plate. The agar plates were incubated aerobically at 37ºC for 48 hours for the growth to become appreciable
- Antimicrobial activity of the extracts was determined by measuring the mean zones of inhibition in millimetre for the bacterial isolates. The inhibition zones with diameter less than 8 mm were considered as having no antibacterial activity. This was repeated three times to minimize the error and the mean values were calculated.
- The antimicrobial sensitivity for negative controls was also evaluated against the bacterial isolates.

FIG 1: ROGOSA AGAR

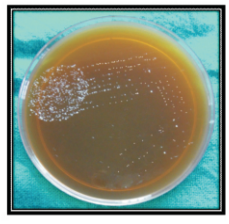

FIG 2: LACTOBACILLUS ACIDOPHILUS GROWTH ON ROGOSA AGAR

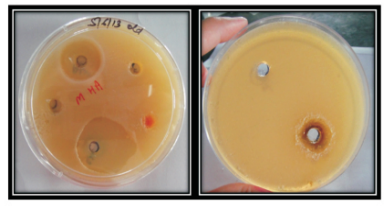

FIG 3: ZONE OF INHIBITION FOR TEST AGENTS AFTER SUSCEPTIBILITY TESTING

Observation And Results

Some of the test agents exhibited antimicrobial activity while some showed negligible effect against Lactobacillus acidophilus. 0.2% Chlorhexidine (used as positive control) showed the maximum antimicrobial efficacy against the bacteria, followed by .02M Alum solution. Next in the order was amla. Aloe vera, Probiotic and Papaya showed no antimicrobial activity.

Table No.1: Mean Diameter of Zones of Inhibition of various Test Agents used

Test Agents	N	Mean Diameter of Zones of Inhibition (in mms) with S.D (Mean±S.D; Range)
Chlorhexidine	30	28.23±2.38; 25.0 - 33.5
Alum	30	19.85±2.16; 17.5 - 23.0
Amla	30	15.62±1.28; 13.0 - 17.5
Aloe vera	30	0.00±0.00; 0.0 - 0.0
Probiotic drink	30	0.00±0.00; 0.0 - 0.0
Papaya	30	0.00±0.00; 0.0 - 0.0
Distilled Water	30	0.00±0.00; 0.0 - 0.0

Distilled water was used negative control.
N = Number of sample size
Range = Minimum diameter of the zones in millimetre - Maximum diameter of the zones in millimetre observed
Mean = Arithmetic mean of the diameter of the zones of activity.

Table No. 2: Comparative evaluation of mean diameter of zones of inhibition of various test agents with respect to negative control (Distilled water) using independent t-test

Test Agents	Mean	S. D.	P-value
Chlorhexidine	28.23	2.38	0.000*
Negative Control (Distilled Water)	0	0	
Alum	19.85	2.16	0.000*
Negative Control (Distilled Water)	0	0	
Alum	15.62	1.28	0.000*
Negative Control (Distilled Water)	0	0	
Aloe vera	0	0	1.000
Negative Control (Distilled Water)	0	0	
Probiotic drink	0	0	1.000
Negative Control (Distilled Water)	0	0	
Papaya	0	0	1.000
Negative Control (Distilled Water)	0	0	

S.D = Standard Deviation.
This table illustrates the Mean and Standard deviation of diameter of zones of inhibition of test agents in comparison to negative control using T-test. The mean diameter of zone of inhibition was found to be significantly (p-value<0.05) more among Chlorhexidine, Alum and Amla in comparison to the Negative Control (Distilled Water).

Table 3: Comparison of Mean diameter of zone of inhibition of various test agents using ANOVA test with Post-hoc Bonferroni comparison

Test Agents	Zone of inhibition (in mm)			F-value	P-value	Post Hoc Bonferroni comparison
	No.	Mean	S.D.			
Chlorhexidine	30	28.23	2.38	2287.511	0.000*	Chx, Alum, Amla > Aloe vera, Probiotic drink, Papaya Chx >Amla, Alum Alum >Amla
Alum	30	19.85	2.16			
Amla	30	15.62	1.28			
Aloe vera	30	0	0			
Probiotic drink	30	0	0			
Papaya	30	0	0			

*** The mean difference is significant at the 0.05 level.**

Discussion

Alum showed excellent antibacterial activity with mean inhibition zones of 19.85±2.16 against Lactobacillus acidophilus. The present study is a premier study and first of its kind to evaluate the antibacterial effect of alum against Lactobacillus acidophilus. The results in the present study are in accordance with the results demonstrated by Mourughan K et al (2004) which showed that 0.02M alum mouth rinse produced significant reductions in Streptococcus mutans levels in children.

Papaya showed negligible antibacterial activity with mean inhibition zones of 0.00±0.00 against Lactobacillus acidophilus. It is in contrary with the study by Doughari et al (2007) where the organic extracts of papaya were more effective than aqueous extracts and the methanol extracts demonstrated the highest activity against some gram positive and gram negative bacteria.

Probiotics exhibited negligible antibacterial activity against Lactobacillus acidophilus. The study simulates a study by EsberCaglar (2008) where the probiotic ice-cream containing Bifidobacteriumlactis showed unaltered effects on salivary lactobacilli levels, though, salivary mutans Streptococci levels showed significant reduction[114].

This study has shown that, crude forms of Amla and Alum possess reasonable antibacterial activity against Lactobacillus acidophilus and have high potential as an effective antibacterial agent. Thus, these products can be considered to be having beneficial potentials in the field of dentistry as oral healthcare products such as toothpastes and mouthwashes.

This observation provides an insight into the usage of these extracts in traditional medicine as an important and accessible caries-preventive agent against Lactobacillus acidophilus.

Conclusion

Considering the aims and objectives of the study, the following conclusions were made.

The extracts showed significant activity against the investigated microbial strains, which is promising. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any adverse effects that are often associated with synthetic compounds; hence these plants may yield significant novel antimicrobials.

All the crude extracts showed varying degrees of antimicrobial activity against the strains of Lactobacillus acidophilus.

These oral preparations could be a source of new antibiotic compounds which could be more effective against multidrug resistant strains of bacteria and fungus, which is indicative of the fact that medicinal extracts can prove to be alternative source of remedy against oral bacteria such as Lactobacillus acidophilus. If similar results are confirmed in clinical trials, the plant extracts can be used to produce new, useful and economic mouthwashes.

However, further studies on these medicinal plants are necessary to determine their active constituent-activity relationship. The antibacterial activities could be enhanced if active components are purified and adequate dosage is determined for proper administration. This may be the first preliminary report on the antimicrobial activity of these medicinal extracts in India.

Clinical Significance

Oral environment stabilization has been used to reduce the number of pathogenic microorganisms in the mouth, preventing the installation of or progression of diseases, especially in pediatric patients. Over the ages, the whole dental fraternity has been relying on some modes of prevention of Dental caries such as various gels, varnishes, mouthwashes, chemicals etc. Besides a number of side effects associated with them, these chemotherapeutic drugs are unaffordable to a large mass of population in developing countries. As synthetic mouthwashes and gels like Chlorhexidine have the risk of ingestion in very small children, hence a natural alternative is contemplated which is safe, economical and feasible.

The observations in the present investigation can facilitate in forming the basis for further phytochemical studies to isolate active compounds, elucidate the structures, evaluate them against wider range of bacterial strains, dental plaque and in vivo models and can also be tested for their safety and efficacy to find new therapeutic principles against infectious disease. Further investigations are warranted to determine whether mouth-rinses and other oral preparations with antibacterial effects might be determined from these extracts.

Although most of the extracts were less potent than Chlorhexidine, still the extracts were relatively effective antimicrobial agent in inhibiting the growth of oral bacteria in vitro. Furthermore, it can be processed in the form of a natural antimicrobial mouth rinse alternative for patients who wish to avoid alcohol, artificial preservatives, artificial flavors and colors.

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