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Antibacterial Potential of Anacardium Occidentale Juice against Clinical Isolates of Streptococcus Mutans -An In Vitro Study

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Abstract

Aim:*The aim of the present study was to evaluate the antibacterial activity of Anacardiumoccidentale juice on Streptococcus mutans, the potent initiator and leading cause of dental caries worldwide.*

Background*: Tooth decay is a common problem that occurs when acids in the mouth dissolves the outer layers of the teeth. It is also known as dental decay or dental caries. Traditional system such as Homeopathy, Siddha, and Ayurveda uses plant based medicine to treat varies diseases caused by bacteria and other organisms. Anacardimoccidentalecommonly known as cashew apple possesses high nutritive values because it contains high value of Vitamin C and minerals such as, phosphorus, iron and calcium. The cashew apple shows to have several bioactivities such as antimicrobial, antioxidant, antimutagenic.*

Materials and Methods*: Antibacterial activity of this juice was screened against Streptococcus mutans, using disc diffusion technique. The extract of Anacardiumoccidetale were loaded on sterile filter paper discs measuring 6mm diameter in the following concentrations 50µl, 100µl and 200 µl respectively. The discs were dried and kept aseptically, then is placed in the agar plates. The plates were incubated at 37°C overnight and the zone of inhibition of growth was measured in millimeters. All the tests were done in triplicate to minimize the test error.*

Results*: The results of this study showed that the extracts exhibited antibacterial activity against the bacterial species tested. The extract of Anacardiumoccidentale was effective against Streptococcus mutans with a zone of inhibition of 20mm diameter (at conc200 µl.). The control (chlorhexidine) showed a zone of 29mm diameter. Dental caries is a microbial disease that results in the destruction of mineralized tissue of the teeth. Streptococcus mutans is the potent initiator and leading cause of dental caries worldwide . It is considered to be the most cariogenic of all of the oral Streptococci.*

Conclusion*: The traditional claim of Anacardiumoccidentaleas an antimicrobial have been confirmed as the extract of it showed an activity against the microorganism used in the study, the Streptococcus mutans*

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Keywords*: Anacardiumoccidentale, Cashew apple, Antibacterial activity, Dental caries, Streptococcus mutans.*

I. Introduction

Plants and plants based formulation have been used traditionally for treat of dental caries. Traditional system such as Homeopathy, Siddha, Ayurveda uses plant based medicine to treat varies diseases caused by bacteria and other organisms.Anacardimoccidentale is a member of botanical family Anacardiaceae. It is also known as cashew apple.Anacardiumoccidentale L. (Anacardiaceae), popularly known as cashew tree, is native from Brazil. The plant is mainly found along the coast of the northeastern states and is of great economic and medicinal value [1]. It possesses high nutritive values because it contents high value of Vitamin C and minerals such as, phosphorus, iron and calcium. It can be eaten as natural and also as juice, pulp, wine etc. The cashew apple shows to have several bioactivities such as antimicrobial, antioxidant,antimutagenic.The main ethnopharmacological applications of the species include the treatment of infectious and inflammatory diseases and pain conditions, such as venereal diseases, skin diseases, diarrhea, stomatitis, aphthae, bronchitis, intestinal cramps, muscle weakness, diabetes, tooth pain, weakness, inflammation, psoriasis, and cough [2].

Streptococcus mutans are gram-positive cocci shaped bacteria. The facultative anaerobes are commonly found in the oral cavity of human beings, and it is a major contributor for dental caries. The result of cariesalso affects the overall health of the individual. S. mutans are mesophilic and grows at the temperatures ranging in-betweens 18-40 degrees Celsius. Streptococcus mutans is a microorganism with cariogenic properties which breaks down glucose to produce energy and creates an acidic environment. This will demineralize the superficial structures of the tooth. The result of the conversion disintegrates the coating of the tooth then later dissolves the Calcium molecule creating a hole.S. mutans can be transmitted to all ages group of people although it is more common for infants and children [3]. The genotype transportation are responsible for the transmission of S. mutans from a mother to her children eventhough, there are genotypic variations from one population to the another. Someone with a healthy oral flora will roughly contain 10,000 CFU per ml of Streptococcus mutans in their mouth.

There are three major virulent factors which possess Streptococcus mutans. They are water insoluble glycans, acid tolerance, and production of lactic acid. A toothache is the most common symptom of dental caries. An irritation or caries infection of pulpal region of a toothis usually causes the pain. A dentist is a person who will diagnose dental caries by takingaX-Rays as the diagnosis purpose, and sometimes the X-Rays can detect the formation of a cavity before it is fully intact. There are many treatment options to treat dental caries such as: fillings, crowns and root canals. Since every human has normal flora in their oral cavity, the only prevention is to lessen the impact of acid fermentation by practicing adequate oral hygiene [4].

The metabolism and growth of S. mutans will change the environmental conditions of the normal flora, which enables fastidious organisms to colonize and also cause the formation of dental plaques. Streptococcus mutans is a specialized microorganism which equips with the receptor that allows it to be adheres towards the surface of the tooth and createsslimy environments. After adherence to the tooth, S. mutans will began to construct a biofilmby dividing and producingmicrocolonies within the slime layer. S. mutans will start to

synthesize and grow dextran with the enzyme named dextransucrase. Dextran contains a capsule that binds to the enamel and forms a biofilm that consists of 300-500 bacterial cells. S. mutan metabolises the biofilm which causes the abilty of sucrose to cleave with the enzyme Glucansucrase.

With these products fructose is fermented as an energy source for bacterial growth while glucose is polymerized to an extracellular dextran polymer. This is important because the polymer cements S. mutans at the enamel and becomes the matrix of dental plaque. The dextrin can also depolymerize to glucose and use the monosaccharide as a carbon source, which produces lactic acid in the biofilm by decalcifying the enamel, and leads to dental caries. The combination of acid and plaque results in the causative agent of decay [5].

Studies have described the antimicrobial activity of different parts of A. occidentale, such as the fruits, stem bark, leaves and gum, but there are no reports of similar activity in the flowers [6].The most frequent antimicrobial activity was described to the barks and leaves against bacteria (Escherichia coli,Streptococcusmutans, Staphylococcusaureus, Streptococcus sobrinus, , Enterococcus faecalis among others) and fungi (Candida albicans) Similarly, antioxidant, is considered with the activities in comparing with quercetin, has been described else whereby pseudofruis,fruits and roots, but this is not applicable for the flowers [7].

With respect to the chemical components, phenolic compounds such as, cardol,scardanolsanacardic acids and 2-methycardols found in the aerial parts of the cashew fruit, was been isolated from the cashes apple fruit juice [8]. On the basis of these considerations, we have evaluate comparatively the antimicrobial activities of extracts obtained from the fruit of A. occidentale in order to determine amount of this fruit part exhibits higher activity, since most studies have evaluated different components [9].

Chlorhexidine (CHX) gluconate is known to be an effective ingredient to eliminate streptococcus mutans because of its excellent antimicrobial efficacy and substantivity characteristic. The mechanism of action of CHX is associated with intracellular component leakage by the process of adsorbing onto the cell wall of microorganisms [10]. At low concentration, it has bacteriostatic effect. At higher concentration, has bactericidal effect due to precipitation and coagulation of intracellular constituents [11].

However, herbal alternatives are always been an advantage in dentistry due to its less potential of side effects, safety concerns, and effectiveness of conventional prevention of dental caries [12]. Extracts of plant origin have been found to have therapeutic properties since thousands of years. As per WHO report, 80% of world's population relies mainly on traditional therapies. There are many advantages of using herbs as antimicrobials, they have minimal side effects, cost effective, have better patient tolerance, and are renewable in nature. Phytochemical extracts such as Vitis Viniferous-grape seed, Curcuma longa -Turmeric, Azadirachtaindica - Neem, Morindacitrifolia - Noni, Propolis, and Triphala have been reported to exert antimicrobial properties suggesting their potential to be used in dentistry [13]. However, there is a lack of sufficient supporting documentation regarding the antibacterial activity of these extracts in dentistry.

Present studies shows that it is capable of inhibiting the dental caries causing bacteria, Streptococcus mutans. Dental caries is one of the major reason of patients to meet a dentist. Dental caries occurs due to lack of oral hygiene, no proper brushing technique, no awareness, etc. Most of people from rural area are more prone to

dental caries. As a study to resist the dental caries,Anarcardiumoccidentale is chosen as its juice has the criteria of antibacterial activity.

II. Materials and Methods

Test microorganisms

Clinical isolates of S. Mutans were used in the present study. These isolates were sub cultured onto Mueller Hilton agar (Hichrome differential media). They were further confirmed by standard biochemical tests and stock culture and stored at -20 degree C in Department of Microbiology, Saveetha Dental College for futher use.

Preparation of A.Occidentale juice Extract

Ethanolic extract of AnacarduimOccidentale juice was freshly prepared by BioChemlabroratory, Bangalore and was sent to our department. The extract was kept in a refrigerator to avoid denaturing of its proactive components.

Methodology

The extract of Anacardiumoccidetalewere loaded on sterile filter paper discs measuring 6mm diameter in the following concentrations 50µl, 100µl and 200 µl respectively.The discs were dried and kept aseptically. 2% of chlorhexidine gluconate was also prepared with those concentrations as chlorhexidine is the control of this study.

Screening of antibacterial activity [Disc diffusion technique]

Broth culture of the bacterial strain compared to Mac Farland's standard0.5 was prepared. Lawn culture of the test organisms were made on the Muller Hinton agar [MHA-Hi media M1084]plates using sterile cotton swab and the plates were dried for 15 minutes. Filter paper discs loaded with different concentration ofAnacardiumoccidentaleextract and chlohexinewere placed on the plates respectively. The plates were incubated at 37°C overnight and the zone of inhibition of growth was measured in millimeters.All the tests were done in triplicate to minimize the test error [14].

Statistics

To compare the mean values between groups one way ANOVA is applied .SPSS version 22.0 is used to analyse the data. Significance level is fixed as 5% ($\alpha = 0.05$).

III. Results

The antibacterial activity of the Anacardiumoccidentale by disc diffusion technique and the zone of inhibition was measured in mm diameter. The results are given in the table 1. The extract of Anacardiumoccidentale was effective against Streptococcus mutans with a zone of inhibition of 20mm diameter (at conc200 µl.). The control (chlorhexidine) showed a zone inhibition of 29mm diameter. Dental caries is a microbial disease that results in the destruction of mineralized tissue of the teeth.Streptococcusmutans is the

potent initiator and leading cause of dental caries worldwide. It is considered to be the most cariogenic of all of the oral Streptococci.The present study was to evaluate the antibacterial activity of Anacardiumoccidentaleon caries causing organisms. The results obtained from our study shows that the cashew apple has got a very good antibacterial activity against Streptococcus mutans.

ORGANISM	concentration	Control(Chlorhexidine)	Anacardiumoccidentale
Streptococcus mutans	50µl	18	11
	100µl	24	15
	200µl	29	20

Table 1: Antibacterial activity of Anacardiumoccidentale fruit juice on Streptococcusmutans

Variables	Conc. (µl)	Mean	Std. Dev	F-Value	P-Value
Anacardium Occidentale	50	11.28	1.225	177.143	<0.001
	100	15.73	.707		
	200	20.11	1.225		
2% chlohexideine gluconate	50	18.47	2.000	393.781	<0.001
	100	24.20	1.304		
	200	29.21	.837		

Table 2: Mean zone of inhibition2% chlorexidine and anacardiumOccidentale juice extract .where 2% CHXshows highest mean zone of inhibition compared to A.occidentale extract.

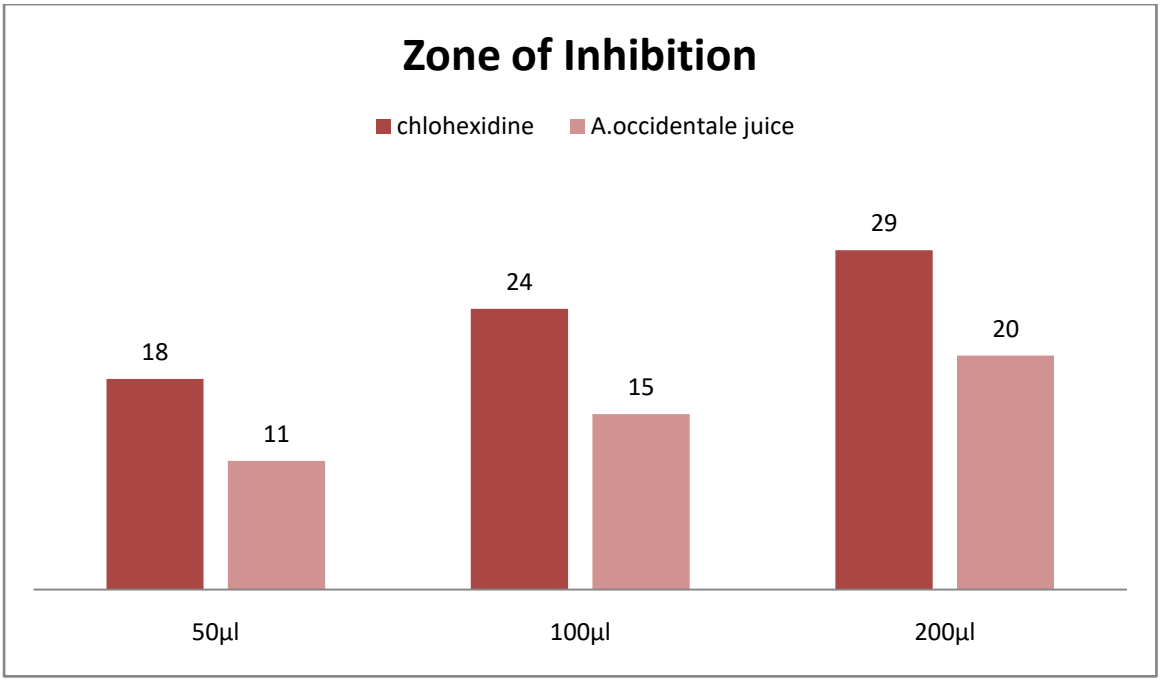

Figure 1:shows zone of inhibition of chlorhexidine and A. occidentale against S. mutans

IV. Discussion

Dental caries is an infectious, transmissible disease of oral cavity affecting people of all age group. Mutans group of Streptococci in particular S. mutans are considered as principal agents causing the disease [15]. Various parts such as fruit, nut shell, stem bark of A. occidentale have shown to possess inhibitory activity against S. mutans. The phenolic compounds isolated from the fruit of A. occidentale showed inhibition of S. mutans showed the bactericidal activity of anacardic acids from A. occidentale against S. mutans [16].

Pereira et al. [17] showed in vitro inhibition of clinical isolates viz., Streptococcus mitis, Streptococcus mutans and Streptococcus sanguis by stem extract of A.occidentale. Melo et al. showed the efficacy of stem bark extract of A. occidentale extract against S. mutans. Akinjogunla et al. [18] showed the efficacy of ethanolic stem extracts of A. occidentale against S. mutans associated with dental caries. In the present study, the condensed juice extract of cashew apple demonstrated inhibitory activity against clinical isolates of S. mutans recovered previously from dental caries subjects.

S.Mutan is a Gram positive opportunistic human pathogen. It is one of the most important bacteria isolated from burn patients and is a common cause of community and hospital acquired infections involving skin infections and septicemia. Multidrug resistant strains of S. Mutan are being reported frequently from burn patients by Beheshti and Zia [19]. It has been found that A. occidentale possess inhibitory efficacy against S. Mutans. Satish et al has reported that aqueous extract of the fruit of A. occidentale showed inhibitory activity against S. Mutans. They also found higher inhibitory activity of methanol extract of A. occidentalefruitthan ethyl acetate. Petroleum ether and chloroform extracts were not effective. Nut shell liquid of A. occidentale was shown to possess inhibitory activity against Methicillin resistant S. Mutans reported by Parasa et al [20].

In another study by Vinayaka et al, [21]ethanolic extract of leaf and bark of A. occidentalewereinhibitorier against S.Mutans than that of extract of its juice. In our study, the condensed juice of cashew apple was found to possess marked inhibitory activity against S. Mutans isolates recovered from dental caries subjects.

V. Conclusion

The traditional claim of *Anacardiumoccidentale*as an antimicrobial have been confirmed as the extract of it showed an activity against the microorganism used in the study,the*Streptococcus mutans*.The use of flora in dentistry should be based on evidence of effectiveness and safety. The anti-bacterial activities could be enhanced if active components are purified and adequate dosage determined for proper administration.

Understanding the consistency, dose–response and biological plausibility of the association between the chemical and herbal (cashew apple extract) product is based on the totality of the human intervention studies. These outcomes are substantiated by experimental studies that elucidate the molecular mechanisms of the herbal extract and some of its individual components. In this regard, the knowledge on the mode of action of phenolic components in general parallels and supports as antimicrobial agent are more refined and prepared specifically insights into the physiological processes has been described in the studies on the herbal product that embody the specific monomeric and oligomeric phenolic extract. In the quest for unravelling the molecular action of herbal extracts that could be developed as an ideal antimicrobial agent in the following generation.

Studies are ongoing to enhance our existing knowledge and understanding in the intricately related fields of opportunistic micro flora diseases. The prepared pleiotropic effects of this herbal extract explain why and how it can be applied as an antimicrobial remedy as well as a non-toxic material in the field of dentistry.

Within the limitations of the study, it can be concluded that anacardiumoccidentale juice extract demonstrated antimicrobial activity against S. Mutans. Since the study is qualitative analysis further testing needs to be done to final quantitative analysis of the antimicrobial activity of this extract. Henceforth, further dilution studies needs to be carried out to find out the better activity. This study warrants the use of herbal based non-irritant non-toxic antimicrobial agent in place of chemical ones.

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