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Effect of *Anacardium occidentale* Leaf Extracts on Dental Plaque Bacteria

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Abstract

Dental plaque is a slimy build-up of bacteria that forms on the surface of teeth. Dental plaque is a biofilm of microorganisms (mostly bacteria) that grows on surfaces within the mouth. Dental plaque can give rise to dental caries (tooth decay) the localized destruction of the tissues of the tooth by acid produced from the bacterial degradation of fermentable sugars and periodontal problems such as gingivitis and chronic periodontitis. Thus, the present study concentrates on isolation and identification of microorganisms present in dental plaques. The antibacterial activity of the *Anacardium occidentale* (Cashew leaves) leaf extracts were also determined. The dental swabs were collected from individual having dental plaque and streaked on nutrient agar plates. The colonies were subjected to gram staining and series of biochemical tests like indole, methyl-red, voges-proskauer, citrate utilization and triple sugar ion test. Biofilm forming ability was determined using Congo red assay and tube test using crystal violet. *Anacardium occidentale* leaf extracts were prepared using aqueous and methanol as solvents. From the analysis, the isolated pathogens were found to be *Streptococcus* sp and *Staphylococcus* sp. The organisms were found to be biofilm producers. Aqueous extracts showed 15mm and 14mm; and methnol extracts showed 15mm and 13mm against *Streptococcus* sp. and *Staphylococcus* sp. Therefore, *Anacardium occidentale* leaf extracts can be used for the treatment of dental plaques.

Keywords

Dental Plaque, *Streptococcus* sp., *Staphylococcus* sp., *Anacardium occidentale,* antibacterial activity

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INTRODUCTION

The oral cavity is the breeding ground to a wide range of gram positive and gram-negative bacteria. This dynamic micro flora changes with respect to age, hormonal status, diet, and health status of an individual. More than 700 bacterial species from healthy oral cavity have been identified. Some of these bacteria show specificity as to individual subjects, others are specific to sites within the oral cavity. Oral biofilms harbouring pathogenic bacteria are among the major virulence factors associated with dental diseases such as caries and periodontitis [1]

Dental plaque is a slimy build-up of bacteria that forms on the surface of teeth. Dental plaque is a biofilm of microorganisms (mostly bacteria) that grows on surfaces within the mouth. It is a sticky colorless deposit at first, but when it forms tartar, it

is often brown or pale yellow. It is commonly found between the teeth, on the front of teeth, behind teeth, on chewing surfaces, along the gumline, (supragingival) or below the gumline cervical margins (subgingival) [2]. Dental plaque is also known as microbial plaque, oral biofilm, dental biofilm, dental plaque biofilm or bacterial plaque biofilm. The cells divide and generate a biofilm. At first, the biofilm is soft enough to come off by using the fingernail. However, it starts to harden within 48 hours, and in about 10 days the plaque becomes dental calculus (tartar) hard and difficult to remove. Dental plaque can give rise to dental caries (tooth decay) the localized destruction of the tissues of the tooth by acid produced from the bacterial degradation of fermentable sugars and periodontal problems such as gingivitis and chronic periodontitis [3].

Bacterial plaque is one of the major causes for dental decay and gum disease. These microorganisms all occur naturally in the oral cavity and are normally harmless. However, failure to remove plaque by regular tooth-brushing allows them to proliferate unchecked and thereby build up in a thick layer, which can by virtue of their ordinary metabolism cause any of various dental diseases for the host. Those microorganisms nearest the tooth surface typically obtain energy by fermenting dietary sucrose; during fermentation they begin to produce acids [4].

Human beings and their ancestors have always been afflicted by this disease. The advent of modern or allopathic medicine turned attention of scientists increasingly from plant sources to synthetic preparations as the basis for modern drugs. However, the deleterious side-effects of many modern drugs along with the development of drug-resistant organisms have brought back into focus ethnomedicinal studies. The general antimicrobial activities of medicinal plants and plant products have been well known, and more attention have been focused on potential sources of functional substances such as antimicrobial substances [5].

The present study focuses on identification of dental plaque causing bacterial pathogens from infected tooth having dental plaque. Antibacterial activity of the *Anacardium occidentale* (Cashew leaves) leaf extracts were determined.

MATERIALS AND METHODS

Collection of dental samples and *Anacardium occidentale* leaves

The dental swabs were collected from individual having dental plaque and inoculated into test tubes containing sterile glucose solution and incubated under sterile condition. Leaves of *Anacardium occidentale* (Cashew leaves) was collected in a sterile polythene bag. The sample was dried and grinded in a grinder. The resulted fine powdered mixture of sample was sterilized and stored in separate bottles.

Isolation and identification of microorganisms from tooth [6]

0.1ml of sample was streaked on nutrient agar plates and incubated at 37 °C for 24 hours. Then the colonies were subjected to gram staining and series of biochemical tests like indole, methyl-red, voges-proskauer, citrate utilization and triple sugar ion test. Congo red assay was performed on CRA medium and tube test using crystal violet were performed to determine the biofilm producing ability of the isolated pathogens. The cultures were grown in tryptic soy broth for 24hr. Then, the tubes are stained with crystal violet to visualize the biofilms.

Extraction of bioactive compounds from *Anacardium occidentale* leaves

Powders of *Anacardium occidentale* leaves were placed in a porous bag or “thimble” made from a strong filter paper or cellulose, which is placed, is in thimble chamber of the Soxhlet apparatus. Extraction solvent (aqueous and methanol) is heated in the bottom flask, vaporizes into the sample thimble, and condenses in the condenser and drip back. When the liquid content reaches the siphon arm, the liquid contents is emptied into the bottom flask again and the process is continued. For the study, infusion method of Soxhlet Extraction had been adopted. The dried powder was filled in the thimble and placed in the Soxhlet extractor. The extractor had been filled with solvent solution of methanol and the temperature of 60^{o}C was set and left for 6hours. The extracts were collected, and the solvents were evaporated. The dried extracts were collected and stored in sterile containers.

Antibacterial activity [7, 8]

The antibacterial efficacy of the leaf extracts was evaluated against dental plaque causing organisms by well diffusion method. Nutrient Agar was prepared and sterilized and poured into plates. (Nutrient agar Composition (for 100ml): Peptone: 0.5g; Yeast extract: 0.5g, Beef extract: 0.3g, Sodium chloride: 0.5g, Agar 1.5 g; Total pH: 7.0 ± 0.2). Overnight cultures of test pathogens were cultured and 0.1% of culture solution of each test organisms was streaked throughout the petri plate with the sterile cotton swab by rotating the plate at 60º angle for each streaking. 6mm well borer was used to bore wells on the agar surface of each NA plates. About 100µl of the extracts were loaded into the well and the plates were incubated in an incubator at 37ºC for 48h. The antibacterial activity was determined in

terms of inhibitory zones around the wells loaded with natural dyes in all the Nutrient Agar plates containing test pathogens. The obtained clear zones were observed and measured in millimetre (mm).

RESULTS

Microorganisms present in dental plaque.

About 2 organisms were isolated from the dental plaque samples and the organisms were subjected to biochemical tests for bacterial identification (Figure-1). Figure-2 and 3 shows the gram staining and IMVIC tests of the isolated organisms. Table-1 shows the identification tests results of the isolated bacteria. From the analysis the isolated bacterial colonies – 1 and 2 were found to be *Streptococcus* sp and *Staphylococcus* sp.

Figure-1: Plating of dental plaque samples

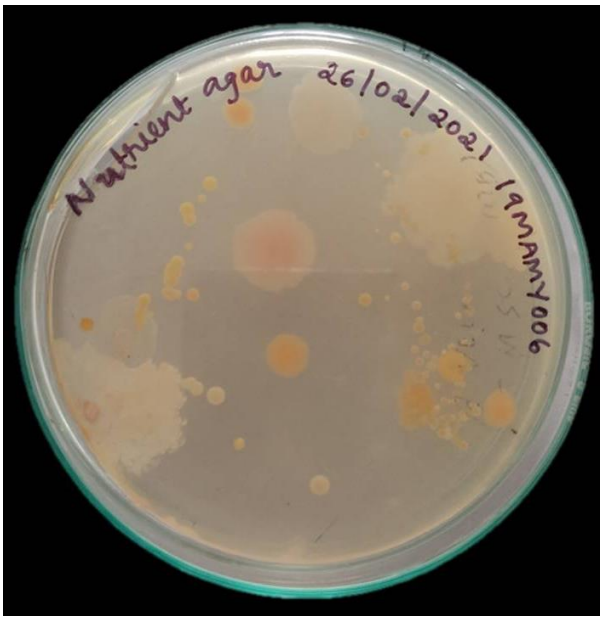
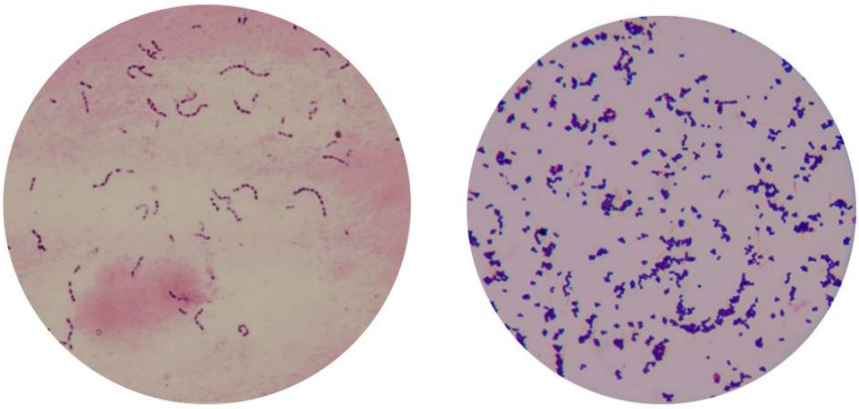
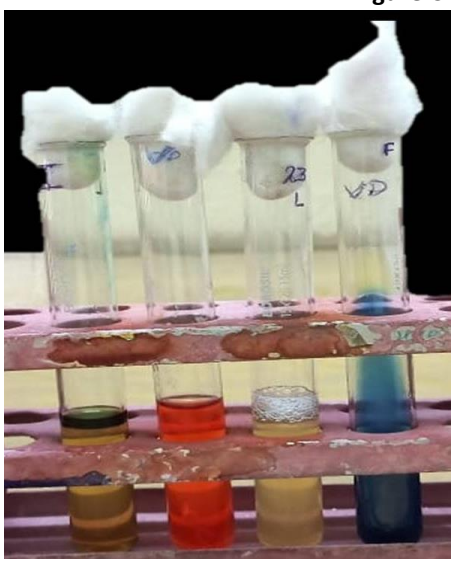


Figure-2: Gram staining



(a) Colony-1 **(b) Colony-2**

Figure-3: IMVIC tests



Colony-1

Colony-2

Figure-4: Tube test

Figure-3: Antibacterial activity of *Anacardium occidentale* leaf extracts against *Streptococcus* sp. (Colony-1)

Figure-3: Antibacterial activity of *Anacardium occidentale* leaf extracts against *Staphylococcus* sp. (Colony-2)

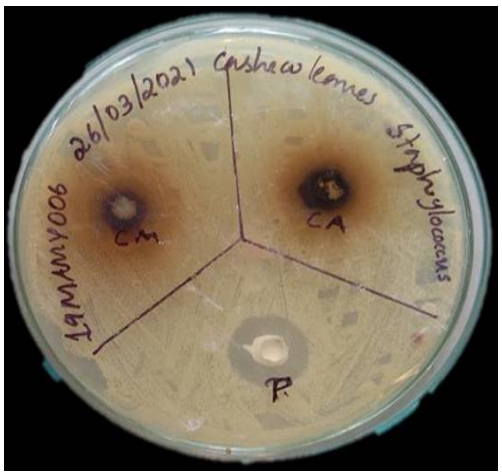


Table-1: Biochemical tests for the isolated organisms

Sl no.	Identification tests	Colony Type-1	Colony Type-2
1	Indole	Negative	Negative
2	Methyl red	Positive	Positive
3	Vogus – Proskauer	Negative	Positive
4	Citrate	Positive	Positive
5	Gram staining	Negative	Positive
6.	Morphology	Coccus	Coccus

Table-2: Antibacterial activity *Anacardium occidentale* leaf extracts against dental plaque causing bacteria

Test organism	Zone of inhibition (cm)		
	Aqueous	Methanol	Control (commercial toothpaste)
Streptococcus sp	1.5	1.5	1.5
Staphylococcus sp	1.4	1.3	1.5

Biofilm forming ability.

Biofilm forming ability was determined by congo red assay (CRA) and tube test using crystal violet. After incubation, black coloured colonies were observed for both the organisms. Biofilm formation was visible on crystal violet staining and the tubes were shown in the Figure-4.

Antibacterial analysis

Aqueous and methanol extracts of *Anacardium occidentale* were determined against dental plaque causing organisms. Table-2 shows the inhibitory zones of aqueous and methanolic extracts. Aqueous extracts showed 15mm and 14mm against *Streptococcus* sp. and *Staphylococcus* sp. Methanol extracts showed 15mm and 13mm against *Streptococcus* sp. and *Staphylococcus* sp. Aqueous extracts showed higher inhibition than methanolic extracts (Figure-5 and 6).

DISCUSSION

Dental plaque is a biofilm of microorganisms that grows on surfaces within the mouth. In the present study, the dental plaque causing organisms were isolated and identified. The isolated organisms were found to be *Streptococcus* sp. and *Staphylococcus* sp. Only a few specialized organisms, primarily streptococci are able to adhere to oral surfaces such as the mucosa and tooth structure. Mutans streptococci can colonize the tooth surface and initiate plaque formation by their ability to synthesize extracellular polysaccharides from sucrose, using glucosyltransferase [5]. This sucrose

dependent adherence and accumulation of cariogenic streptococci is critical to the development of pathogenic plaque. *Streptococcus mutans* survives in an extremely diverse, high cell density biofilm on the tooth surface. These bacteria are strongly associated with caries formation. All *Streptococcus mutans* serotypes such as *Streptococcus sobrinus* (serotypes d, g and h) have been shown to have significant potential to cause caries, but because of their significant genetic and biochemical differences, they should not be referred as simply as the single species *S. mutans*. *S. mutans* and lactobacilli are acidogenic and acid uric bacteria and seem to be the primary organisms associated with caries in humans [9].

Staphylococci are mainly harboured on the skin, as well as skin glands and mucous membranes in humans. Although these micro-organisms are transiently resident in the oral cavity [13, 14], during the course of our previous studies the occurrence of *Staphylococcus epidermidis* was found significantly high in saliva from healthy adults [15]. There are several early reports of *S. aureus* isolation from the healthy oral cavity but detailed information on the oral distribution of *Staphylococci* is lacking [10].

Aqueous and methanolic extracts of *Anacardium occidentale* leaves were examined for antibacterial activity against dental plaque forming organisms. The presence of bioactive ingredients in the cashew leaves like carbohydrates, tannins, saponins, resins, alkaloids and flavanoids add to their antimicrobial activities. Phytochemical screening analysis on *Anacardium occidentale* leaves have showed the presence of high concentration of tannins in the aqueous extract and its absence in the alcoholic leaf extract [11, 12]. This could probably account for the effective action of aqueous form compared to the methanolic extract in the inhibitory activity against *Staphylococcus* sp and *Streptococcus* sp. On the contrary, Ayepola and Ishola, 2009 evaluated the anti-microbial property of *A. occidentate* leaves. Leaf extracts were found to be highly active against selected pathogens like *Bacillus subtilis, Klebsiella pneumonia* and *E. coli*. These results well correlate with the present study.

CONCLUSION

The dental swabs were collected from individual having dental plaque and inoculated into test tubes containing sterile glucose solution and streaked on nutrient agar plates. The colonies were subjected to gram staining and series of biochemical tests like indole, methyl-red, voges-proskauer, citrate utilization and triple sugar ion test. Congo red assay was performed on CRA medium and tube test using crystal violet were performed to determine the biofilm producing ability of the isolated pathogens. *Anacardium occidentale* leaf extracts were prepared using aqueous and methanol as solvents. The antibacterial efficacy of the leaf extracts was evaluated against dental plaque causing organisms by well diffusion method. About 2 organisms were isolated from the dental plaque and the isolated bacteria were found to be *Streptococcus* sp and *Staphylococcus* sp. The organisms were found to be biofilm producers. Aqueous extracts showed higher inhibition than methanolic extracts. Therefore, the cashew leaf extracts can be used for treatment of dental plaques.

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