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ANTIBACTERIAL EFFECTIVENESS OF ETHANOLIC EXTRACT OF ACACIA CATECHU BARK AND AZADIRACHTA INDICA LEAF AGAINST STREPTOCOCCUS MUTANS –A COMPARATIVE IN VITRO STUDY

Lakshmi.T^{*1}, Dhinahar.S²

Affiliated to:

¹Reader, Department of Pharmacology, Saveetha Dental College & Hospitals, Chennai.

²Professor, Department of Orthodontics, SRM Dental College & Hospitals, Kattankulathur.

ABSTRACT

Acacia catechu & Azadirachta indica (neem) is used as an phytomedicine since antiquity for treatment of diarrhea, bleeding gums, oro dental infections etc. In our present study the anti bacterial activity of Acacia catechu bark ethanolic extract and Azadirachta indica ethanolic leaf extract were tested against streptococcus mutans that commonly causes dental plaque/caries in fixed orthodontic appliances patients undergoing orthodontic treatment. Anti bacterial activity was examined by determining the Minimum Bactericidal Concentration (MBC) using Macro dilution broth technique. Acacia catechu ethanolic extract exhibited significant antibacterial activity against the bacterial strain tested at various concentrations when compared to Azadirachta indica leaf extract.

Keywords: Acacia catechu bark, Azadirachta indica, Anti bacterial activity, Dental Plaque, Minimum Bactericidal Concentration.

INTRODUCTION

Dental caries/plaque is an extremely prevalent infectious disease that has been shown to be associated with serious health problems. The occurrence of Streptococcus mutans makes the oral environment more conducive to caries/plaque. These cariogenic pathogens utilise dietary sucrose and produce adhesive

exo-polysaccharides and acids which lead to plaque formation and carious lesions on susceptible tooth surfaces.^[1]

Acid production by S. mutans plays an important role in the pathology of dental caries/plaques. Oral cavity is a complex

ecosystem with highly divergent acid tolerant and acid-producing microbiota. Acidogenic oral microbes are the key factor of dental plaques. The primary acid tolerant bacteria associated with Dental plaque includes streptococcus mutans that surround orthodontic appliances are a common orthodontic problem in many patients undergoing Orthodontic treatment. [2-5]

Poor oral hygiene is one of the reasons for accumulation of these microbes. The common method for maintaining good oral hygiene is brushing the teeth with the tooth powder /paste that possess antibacterial properties and can prevent the degradation of tooth enamel. Synthetic drugs commonly used contain chemical agents, which are known to produce harmful side effects on prolonged use. [6]

Recent years, the extracts of medicinal plants and herbs are becoming popular. One such phytochemical extract is Acacia catechu that is supposed to possess antiseptic, cariostatic, antibacterial, desensitizing, and gum-tightening properties. It has been claimed to be effective in the prevention of bleeding from gums, pyorrhoea, toothache, as prophylaxis against dental caries, inflamed gums with haemorrhage, and foul smell. The present study was, therefore, undertaken to evaluate the antibacterial activity of Acacia catechu ethanolic bark extract against cariogenic organism *S. mutans*.

The main objectives of the study were:

- a. To evaluate the antibacterial activity of Acacia catechu bark ethanolic extract powder against *S. mutans*.
- b. To evaluate the effects of Acacia catechu bark extract as compared to a standard *Azadirachta indica* (Neem) leaf extract.

Materials and Methods

Plant material

The Herbal extracts were obtained from Green Chem, Herbal Extracts & Formulations, Bangalore.

Test microorganism

Bacterial strains used are *Streptococcus mutans* (ATCC 25175) the organism was obtained from Department of Microbiology, Saveetha Dental College & Hospitals, Chennai.

Anti Bacterial Activity

The herbal extracts each 200mg were weighed aseptically into a sterile tube and dissolved in 2ml of sterile Tryptic soy Broth (TSB). From the stock solution various concentrations were prepared, viz., 62µg, 125 µg, 250 µg, 500 µg/100µl, 1mg, 5mg, 10mg/100µl respectively in to wells of micro plates. The tested organism was grown in (TSB) Tryptic soy broth medium [MHA-Hi media, Mumbai] for 24hrs at 37°C and concentration was adjusted to 0.5 McFarland Standard. [7-9] The above concentration of extracts were taken in 100µl quantities in a U

bottom micro culture plates. Control well received plain broth without plant extract. The plates were kept in sealed covers and incubated at 37°C overnight and growth/no growth was detected. All the tests were done in triplicate to minimize the test error.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of herbal extracts against tested microorganism was determined by macro broth dilution method.^[10] A series of two- fold dilution of each extract (62 µg/100µl to 10mg/100µl) was made in to which 100µl of the standardized bacterial suspension containing 10⁶ organisms was made in Tryptic soy broth as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1990)^[11]. The control well received plain broth without herbal extract .The plates were incubated at 37°C for 24 hours and observed for visible growth. As the extracts were colored, MIC could not be read directly by

visual methods. Hence subcultures from all the wells were made and growth/ no growth is detected. Then the MBC were obtained.

Minimum Bactericidal Concentration (MBC)

The MBCs were determined by selecting wells that showed no growth. The least concentration, at which no growth was observed, was noted as the MBC.

Result

The results obtained from the macro broth dilution method were given in table 1. The ethanolic extract of Acacia catechu bark exhibited significant antibacterial activity when compared to Azadirachta indica leaf ethanolic extract. Antibacterial activity was carried out in various concentrations. Azadirachta indica leaf extract showed no growth at concentration of 500µg/ml whereas Acacia catechu bark extract showed no growth at concentration of 62µg/ml. The least concentration at which the extract shows no growth indicates MIC/MBC.

Table 1 **Minimum Bactericidal Concentration of Acacia catechu ethanolic bark extract compared to Azadirachta indica ethanolic leaf extract against Streptococcus mutans**

Extract	62µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1mg/ml	5mg/ml	10mg/ml	control	MIC/MBC
Acaciacatechu ethanolic bark	NG	NG	NG	NG	NG	NG	NG	Turbidity	62 µg/ml
Azadirachta indica ethanolic leaf	Turbidity	Turbidity	Turbidity	NG	NG	NG	NG	Turbidity	500µg/ml

NG =No Growth (Indicates high effectiveness of the tested extract)

Turbidity = Growth (Indicates the least effectiveness of the tested extract)

Discussion

The main objective of the study is to investigate the antibacterial activity of Acacia catechu

ethanolic bark extract with comparison to Azadirachta indica (Neem) leaf extract against Streptococcus mutans.

Acacia catechu ethanolic bark extract is found to be good anti bacterial agent .the anti bacterial activity is due to the presence of secondary metabolites such as alkaloids, flavonoids, phenols, saponins, tannins and quercetin, catechins, epicatechin.^[12] Phytochemical extracts are known to be safe without producing unwanted side effects. Thus we say that Acacia catechu bark extract as a potent anti bacterial agent and can be encouraged to get included as an ingredient in oral care products like dentifrices in order to prevent the development of dental caries/plaque in fixed orthodontic appliance patients.

Summary and Conclusion

On the basis of results obtained, it can be said that performing the macro broth dilution method to detect MIC and MBC of the extract against *S. mutans* is a good method of detecting no growth of this facultative anaerobe under microaerophilic conditions for antimicrobial assay. The test herbal extract Acacia catechu ethanolic bark possesses good antibacterial activity against cariogenic bacteria *S. mutans*, and its antibacterial activity is comparable to the standard *Azadirachta indica* leaf extract.

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