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IN VITRO EVALUATION OF ANTI BACTERIAL ACTIVITY OF HEART WOOD EXTRACT OF *ACACIA CATECHU* ON ORAL MICROBES

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ABSTRACT

The aim of the present study was to evaluate the anti bacterial activity of Acacia catechu on selected oral microbes. Acacia catechu [AC] is a moderate thorny deciduous tree growing in tropical countries. Antibacterial activity of ethanolic and aqueous extract of heart wood of AC is screened against *Streptococcus mutans*, *Streptococcus salivarius*, *Lactobacillus acidophilus and Enterococcus faecalis* using disc diffusion technique. The Minimum inhibitory concentration [MIC] and Minimum bactericidal concentration [MBC] of the extracts were also determined. The results of this study showed that both the extracts at different concentrations exhibited anti bacterial activity against the bacterial species tested. The ethanolic extract showed higher degree of activity than aqueous extract when compared with the standards.

Key words : Acacia catechu ,Anti bacterial ,Disc diffusion , MIC,MBC.

INTRODUCTION

Herbal medicines have been used for many years. Their history can be rooted from ancient civilization wherein their role as a primary source of medication is evident. Herbs with medicinal properties are a useful and effective source of treatment for various diseases [1-3]. Many of the common drugs used by us today are derived from herbs. Their beneficial effects allied to the current worldwide "back to Nature" trend and greater attention being paid to these products. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substance from other sources including plants.[4] Currently many studies are being conducted to know these herbs in depth. Screening of medicinal plants for antimicrobial activities are important for finding potential new compounds for therapeutic use. There have been numerous reports of the use of traditional plants and natural products for the treatment of oral diseases. Many plant-derived medicines used in traditional medicinal systems have been recorded in pharmacopeias as agents used to treat infections and a number of these have been recently investigated for their efficacy against oral microbial pathogens. The present study was to evaluate the antibacterial activity of heart wood extract [ethonolic & aqueous] of Acacia catechu *willd* on selected oral microorganisms.

Acacia catechu (AC) (Family: Fabaceae and subfamily: Mimosoideae)

known as Black cutch..AC is medium sized thorny deciduous tree mainly found in India and also found in deciduous forests around the world. It grows up to 13 meters in height It is said that the name 'catechu' was given to it because its bristles resemble the claws of animals of the cat family or may be because its heart wood contains gummy extract called kath or cutch. The foliage is softly textured, light green and oval-shaped. The branches are thin and spike like due to tiny thorns grown around the exterior. The sap wood of AC is large and yellowish white and heart wood is small and red in colour. The chief constituents of the heartwood are catechin and catechutannic acid [5]. The wood contains epicatechin [5], Atzelchin, catechin tetramer, dicatechin, gallochin, gossypetin, phlobatannin, kaempferol, quercitrin, quercitin [6,7,8]. Catechin is biologically highly active. It is used as a haemostatic. Taxifolin an other important constituent has antibacterial[8], anti-fungal[12], antiviral, anti-inflammatory, and antioxidant activity[6]. The extract of Acacia catechu extract have been reported to have various pharmacological effects like immuno modulatory[13], anti pyretic [7], hypoglycaemic[7], anti diarrhoeal[7,] hepatoprotective activity[7,11]. Cutch is astringent, cooling, and digestive. It is useful in cold and cough[9,10] ulcers, boils and eruptions of the skin, bleeding piles, uterine haemorrhages, atonic dyspepsia, chronic bronchitis etc. In stomatitis, halitosis, dental caries and cavities[16], AC is used with great benefit. An antibacterial mouthwash made from the extract treats gingivitis and mouth sore

The leaves, bark, heartwood has many nutritional and medicinal uses.

MATERIALS AND METHODS

Plant material

The ethanolic and aqueous extract of heartwood of *Acacia catechu willd* was obtained from Green Chem Herbal Extract & Formulations. Bangalore.

Test microorganisms

Bacterial strains used were *Streptococcus mutans [Gram positive cocci]*, Streptococcus salivarius [Gram positive cocci], *Enterococcus faecalis [Gram positive cocci]*, *Lactobacillus acidophilus [Gram positive bacilli].*, The organisms were obtained from department of Microbiology, Saveetha Dental College and maintained in nutrient agar slope at 4°C.

METHODOLOGY

The extracts were prepared in the following concentrations in sterile water. 1mg/ml, 2mg/ml, 3mg/ml and 4mg/ml. 50µl of extract of different concentrations were loaded on sterile filter paper discs measuring 6mm in diameter, so that the concentration of the extract on each disc was 50µg, 100µg, 150µg and 200 µg respectively. The discs were dried and kept aseptically.

Screening of antibacterial activity [Disc diffusion technique]

Broth culture of the bacterial strains compared to Mac Farland's standard [17-20] 0.5 were 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 concentrations of the extract were placed on the respective plates. The plates were incubated at 37°C overnight and the zone of inhibition of growth was measured in millimeters. Standard antibiotic discs of amoxicillin

(30mcg/disc) and PenicillinG (30mcg/disc) were used as positive control. All the tests were done in triplicate to minimize the test error.

Determination of minimum inhibitory concentration

Macro broth dilution or tube dilution method was done to determine the Minimum inhibitory concentration (MIC) of the extracts17-20. A series of two fold dilution of each extract ranging from 4mg/ml to 0.125mg/ml was made in Muller Hinton broth as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1998). 100µl of standard inoculum of the bacterial strains matched to 0.5 Mc Farland's standard were seeded into each dilution. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and growth media without inoculum) and organism control (tube containing the growth medium and the inoculum) .The tubes were incubated at 37°C for 24 hours and checked for turbidity. MIC was determined as the highest dilution (that is, lowest concentration) of the extract that showed no visible growth.

Minimum Bactericidal Concentration (MBC)

The MBCs were determined by selecting tubes that showed no growth during MIC determination; a loop full from each tube was sub cultured onto Muller Hinton agar plates and incubated for further 24 hours at 37oC. The least concentration, at which no growth was observed, was noted as the MBC.

RESULT AND DISCUSSION

The antibacterial activity of the extracts (Ethanolic and Aqueous) at different concentrations was screened by disc diffusion technique and the zone of inhibition was measured in mm diameter. The results are given in the table 1. The minimum inhibitory concentration [MIC] and minimum bactericidal concentration [MBC] were also determined for the extracts and the results are given in table 2.

The ethanolic extract was more effective against *Streptococcus mutans* with a zone of inhibition of 24 mm diameter (at conc 200 µg.) and was least effective against *Lactobacillus acidophilus* with zone of inhibition of 16 mm (at conc. 200 µg.) and among the other bacterial species studied *Streptococcus salivarius* showed a zone of inhibition of 19mm diameter (at conc. 200 µg.) and *Enterococcus faecalis* showed inhibition zone of 22 mm diameter (at conc. 200 µg.).

The MIC and MBC values of ethanolic extract was found to be low compared to aqueous extract. The ethanolic extract was found to have Low MIC and MBC values of 0.5mg/ml &0.5mg/ml and 0.5mg/ml & 1mg/ml for *Streptococcus mutans* and *Enterococcus faecalis* respectively. With *Lactobacillus acidophilus* ethanolic extract showed a higher MIC and MBC value of 2mg/ml & 2mg/ml and for *Streptococcus salivarius* it was 1mg/ml and1mg/ml. The lower MIC and MBC value is an indication of high effectiveness of the extract whereas higher MIC and MBC indicates the less effectiveness of the extract.

Table 1 : Anti bacterial activity of heartwood extract of *Acacia catechu*

Extract	Conc [µg]	Zone of inhibition [in mm diameter]			
		B1	B2	B3	B4
Ethanolic	50	12	10	7	11
	100	15	12	10	14
	150	19	15	13	17
	200	24	19	16	22
Aqueous	50	10	8	-	8
	100	12	10	9	11
	150	17	12	11	14
	200	21	16	14	19
Penicillin G	30mcg/disc	24	21	22	26
Amoxycillin	30mcg/disc	25	23	20	24

B1- Streptococcus mutans, B2- Streptococcus salivarius, B3- Lactobacilus acidophilus
B4- Enterococcus faecalis

Table :2 MIC and MBC of Ethanolic and Aqueous extracts on different bacterial species

Organisms	Extract	MIC(mg/ml)	MBC(mg/ml)
Streptococcus mutans	Ethanolic	0.5	0.5
	Aqueous	1	1
Streptococcus salivarius	Ethanolic	1	1
	Aqueous	2	2
Lactobacillus acidophilus	Ethanolic	2	2
	Aqueous	4	4
Enterococcus faecalis	Ethanolic	0.5	1
	Aqueous	2	2

Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable global infectious diseases. Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases. The association between oral diseases and the oral microbiota is well established. Dental caries is a microbial disease that result in the destruction of mineralized tissue of the teeth. Streptococcus mutans is the potent initiator and leading cause of dental caries world wide [21]. It is considered to be the most cariogenic of all of the oral Streptococci. Another organism that is

important in the development of caries is Lactobacillus acidophilus. This bacteria is not important in the initiation of caries but in the continuation.[21] The other problem faced in the field of dentistry is failure of endodontic treatment. Enterococcus faecalis is the commonly isolated bacteria from the infected root canal and being considered a possible cause of failure in root canal treatment [22]. It is one of the most resistant species that can best adapt to and tolerate the ecologically demanding conditions in the filled root canal.

The present study was to evaluate the antibacterial activity of Acacia catechu against caries causing organisms and organism associated with endodontic infections. The bacterial strains used in this study were strongly associated with caries, *Streptococcus.mutans* being the most cariogenic bacteria. *Streptococcus salivarius* and *Lactobacillus acidophilus* were also found to cause caries. *Enterococcus faecalis* is responsible for most of the endodontic infections. The results obtained from our study shows that ethnolic extract has got a very good antibacterial activity against the selected oral pathogens.

CONCLUSION

The present results therefore offer a scientific basis for traditional use heartwood extract of Acacia catechu on oral pathogens. The anti-bacterial activities could be enhanced if active components are purified and adequate dosage determined for proper administration. The dentist needs to be informed regarding the herbal and over-the-counter products that may impact the delivery of safe and effective dental treatment. The use of herbs in dentistry should be based on evidence of effectiveness and safety.

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