



In Vitro Evaluation of Anticariogenic Activity of Acacia Catechu Extract Against Selected Microbes

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ABSTRACT

Background: Anticariogenic is a substance that prevents tooth decay. Khadira is the important tree which has medicinal usage. Its botanical name is Acacia catechu and it belongs to Mimosoideae family. It is used to treat cough, sore throat, skin diseases, diabetes and urinary tract disorders. It helps in the treatment of oral problems like dental caries, gingivitis, and pyorrhea. Objective: The study is aimed at evaluating the anticariogenic activity of Acacia Catechu extract against selected microbes. Result: The extract used in this study has significant anticariogenic activity against all the bacterial strains tested. Conclusion: This may help in the development of other products with Acacia catechu as its constituent.

Key Words: Anti-Cariogenic, Acacia Catechu Extract, Dental Caries, MIC, MBC.

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INTRODUCTION

Our country has a rich source of traditional medicines which are mostly of plant origin [1]. Medicinal plants are source of a great economic value. Plant herbs are naturally available for the synthesis of medicinal compounds [2]. The extraction of bioactive compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value and they are easy to afford [3]. Eventhough there is an abundant progress in the development of medical science, plants serve to be an important source of drugs in many countries around the world due to having less side effects. For the past two decades, the usage and reliability of herbal product has become of utmost importance, due to the low side effects and complications of many synthetic and chemical medicines [4].

Acacia catechu which is also called Senegalia catechu, is a deciduous, thorny tree which grows up to 15 m height. The common names for it includes kher, catechu, cachou, cutch tree and black cutch. Senegalia catechu is found in India, China, Japan and the Indian Ocean area. It contains derivatives of flavonoids, and it has acquired its name from the important catechins, catecholamines of

chemistry and biology [5, 6]. The extract prepared from the hard wood of Acacia catechu has many uses and they are used for treating fever, diarrhoea, leucorrhoea, piles and erysipelas. The juice of its fresh bark has been used in treatment of haemoptysis and gonorrhoea [7].

Oral health is integral to general well-being and relates to the quality of life. The link between the activities of microbial species and oral diseases that form part of the microbiota of the oral cavity is well established [8]. Dental caries is a localized infectious disease that leads to loss of teeth. When sugary foods are taken in case the oral hygiene is not maintained properly, it leads to formation of matrix over the enamel surface which will lead to demineralisation of the tooth and ultimately to formation of cavity. Individuals heavily colonized by cariogenic bacteria are considered to be at high risk for dental caries. Streptococcus mutans is considered to be the main cause of dental caries.

Several antibacterial agents such as Chlorhexidine, Fluorides and various antibiotics are commercially available that can be used to prevent dental caries. But they have many side effects that cause nausea, vomiting, and diarrhea and accordingly an attempt has been made to find medicines with naturally available products with

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minimal side effects. Natural products obtained from plants used in traditional medicine act as an alternative to synthetic chemicals. About 80% of individuals from developed countries use traditional medicine which have compounds derived from medicinal plants [9-11].

The present investigation was undertaken for the evaluation of Anticariogenic activity Acacia catechu against the causative agent of dental caries.

MATERIALS AND METHODS

The Acacia catechu extract was obtained from Hi-Media, Mumbai. Pure strains of Streptococcus salivarius (ATCC 25975), Streptococcus sanguinis (ATCC 10557), and Streptococcus mitis (ATCC9811) were obtained from Department of Microbiology, Saveetha Dental College & Hospitals, Chennai. Tryptic Soy Broth is used as a medium for culturing those organisms.

Preparation of extract in different concentrations

The extract of 200 mg was weighed aseptically into a sterile test tube and dissolved in 2 ml of the sterile Tryptic Soy Broth (TSB). From the stock solution various concentrations of extract were prepared, viz., 100 mg/ml, 50 mg/ml, 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.24 mg/ml, 0.62 mg/ml, 0.31 mg/ml, 0.15 mg/ml and these concentrations were poured into the respective wells made in the micro plates. The tested organisms were allowed to grow in the Tryptic Soy Broth medium for 24 hours at 37°C and the concentrations were adjusted to Mac Farlands standard.

Acacia catechu extract's different concentrations were taken in 1 ml quantities in a U bottom micro culture plates. Without the plant extract, control well received plain Broth. Then, the plates were kept in sealed covers and incubated at 37°C for 24 hours and growth/no growth was detected. All the tests were done in triplicate to minimise the test error.

Minimum inhibitory concentration (MIC)

Macro Broth dilution method was used for determining the minimum inhibitory concentration of herbal extract against the tested organisms. A series of two-fold dilution of the plant extract (0.15 mg/ml to 100 mg/ml) was made into which 1 ml of standardised bacterial suspension containing the organisms in Tryptic Soy Broth (TSB) as specified by NCCLS. The control well received plain Broth without herbal extract. The plates were incubated at 37°C for 24 hours and observed for visible growth. The extracts were coloured so 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 Minimum Bactericidal Concentration (MBC) was obtained.

Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentrations(MBC) were determined by selecting wells that showed no growth. The least concentration, at which no growth was observed, was noted as the MBC of the extract for that particular organism.

RESULTS

The minimum inhibitory concentration [MIC] was determined for the extract which is given in Table 1. From MIC, minimum bactericidal concentration [MBC] was also determined which is given in Table 2.

Table 1. Minimum inhibitory concentration of the extract

Organisms	Concentration of Extract mg								
	100	50	10	5	2.5	1.24	0.62	0.31	0.15
S.salivarius	NG	NG	NG	NG	NG	G	G	G	G
S.sanguis	NG	NG	NG	NG	G	G	G	G	G
S.mitis	NG	NG	NG	G	G	G	G	G	G

Table 2. Minimum bactericidal concentration of the extract

Organisms	Concentration of Extract mg	
	MIC mg/ml	MBC mg/ml
S.salivarius	2.5	2.5
S.sanguinis	5	5
S.mitis	10	10

From Table 1, it is clear that the extract at different concentrations exhibited significant anticariogenic activity against all the bacterial strains tested. No Growth indicates high effectiveness of the extract whereas presence of growth indicates less effectiveness of the extract. The extract is very effective against Streptococcus salivarius at a minimum concentration itself comparing its activity on Streptococcus sanguinis and Streptococcus mitis. From Table 2, the MIC and MBC of the extract for Streptococcus salivarius, Streptococcus sanguinis and Streptococcus mitis were found to be 2.5mg/ml, 5mg/ml and 10mg/ml, respectively. The extract has a very higher effect on Streptococcus salivarius, Streptococcus sanguinis and shows minimal effect on Streptococcus mitis.

Thus, the result of our present study shows that the Acacia catechu extract used has significant anticariogenic activity.

DISCUSSION

Oral cavity is a complex ecosystem which contains highly divergent acid tolerant and acid-producing microbiota [12]. It is a habitat for temporary and permanent

microorganisms [13]. One of the major infectious diseases of the oral cavity throughout the world is dental caries and they are found preferentially at protected and stagnant surfaces, which are at the greatest threat of disease [14-16]. Some of the microorganisms used in our study play a main role in the development of the dental caries. The occurrence of dental caries in developing countries like India is increasing day by day [13, 17]. Dental plaque is the biofilm that adheres on the surfaces of teeth and consisting of bacterial cells (mainly *S.mutans* and *S.sanguis*), salivary polymers and bacterial extracellular products. If brushing is not proper or care should not be taken, the plaque can turn into tartar i.e., hardened form of plaque and lead to gingivitis or periodontal disease [12, 18].

Dental plaque plays a major role in the pathogenesis of the dental caries. Current researches are showing that the properties of bacteria which are associated with a surface in a biofilm that can be markedly different than those of the same cells growing in liquid broth [12]. Treatment becomes a critical management and patients frequently seek out complementary and alternative strategies [19]. A good level of dental treatment is required to cope up this situation which may be costly and cannot be affordable by everyone. The prevention should be done alone at the doorstep level using herbal remedy, but through the solution of choice which is economically feasible [13]. Many plants are used in prevention of dental caries [13, 20]. Although, many efforts have been taken to control dental caries, natural products have been used in folk medicine for several purposes during the past years. And also there were no practical set up for the issue so far.

Natural products can also be a possible source for new potential microbial agent to which pathogenic organisms are not resistant. Current researches indicate that the polyphenols, being secondary metabolites, are present in rich amount in various plants [14, 21-23]. The bark of the *Acacia catechu* commonly known as Khair, is used in traditional medicine and possesses an antimicrobial property [13].

The present study was done to evaluate the anticariogenic activity of *Acacia catechu* extract against selected microbes. The extract used at different concentrations showed varying degrees of anticariogenic activity on the microorganisms tested. Further studies are necessary to isolate the secondary metabolites and reveal the active compounds contained in the refined extract in order to test specific anticariogenic activity.

CONCLUSION

Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their

therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known. This in vitro study demonstrated that traditional medicine can be effective as modern medicine in inhibiting the pathogenic organisms. The anticariogenic activity of *Acacia catechu* extract against oral pathogens may be attributed to the various phytochemical constituents present in the refined extract. Such evaluation on natural products to cure diseases may create an alternative source of promising medicines. The result of our present study shows the extract used have significant anticariogenic activity on the cariogenic agents tested.

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