International Research Journal of Multidisciplinary Science&Technology
www.irjmrs.com ISSN : 2455-930X

In Vitro Evaluation of Anticariogenic Activity of Acacia Catechu against Selected Microbes

Dr.Lakshmi T [1]*, Rajeshkumar S [2]

[1]Associate Professor ,Department of Pharmacology, Saveetha Dental College & Hospitals Saveetha University Chennai ,India
[2]Department of Pharmacology, Saveetha Dental College and Hospitals, SIMATS, Chennai, India
Email:Lakshmi@saveetha.com

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ABSTRACT: *In this present investigation, we evaluate the anticariogenic Activity Of Acacia Catechu extract against selected microbes. The study is to determine the anticariogenic activity Of Acacia catechu extract on selected microbes. Anticariogenic is a substance that prevents tooth decay. Khadira is the important tree which has medicinal uses. Its botanical name is Acacia catechu and it belongs to Mimosoideae family. It is used to treat a cough, sore throat, skin diseases, diabetes and urinary tract disorders. It helps in the treatment of oral problems like dental caries, gingivitis, pyorrhea. The extract used in this study have significant anticarcinogenic activity against all the bacterial strains tested. This study is to evaluate anticariogenic activity of Acacia catechu* extracton selected microbes. This may help in the development of other products with Acacia catechu as its constituent.

Keywords: Anti-cariogenic; Acacia catechu extract; Dental caries; MIC; MBC

INTRODUCTION

Our country has a rich source of traditional medicines and they are of mostly plant origin (Gupta 1994). Medicinal plants are a source of a great economic value. Plant herbs are naturally available for the synthesis of medicinal compounds (Mirghani et al., 2012, Sudhasupriya et al., 2017, Rajeshkumar and kayalvizhi 2015). The extraction and of bioactive compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value and they are easy to afford (Nithya karpagam et al., 2016). Even though there is abundant progress in the development of medical science plants serves to be an important source of drugs in many countries around the world due to its reduction in side effects. For the past two decades, the usage and reliability of herbal product have become of utmost importance, due to the side effects and complications of many synthetic and chemical medicines (Malhotra et al., 2011, Sujatha et al., 2018, Bhavani et al., 2017). Acacia catechu is called as Senegalia catechu is a deciduous, thorny tree which grows up to15 m in height. The plant is called Khair. Common names for it include her, catechu, cachou, cutch tree and a black clutch. Senegalia catechu is found in India, China, Japan and the Indian Ocean area. It contains derivatives of flavonoids, which has acquired its name to the important catechins, catecholamines of chemistry and biology (Ujwala et al., 2015, Frawley and Ranade 2001). The extract prepared from the hardwood of Acacia catechu has many uses and they are used for treating fever, diarrhea, leucorrhoea, piles, and erysipelas. The juice of its fresh bark has been used in the treatment of hemoptysis and gonorrhea (Kirtikar and Basu 1932).

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 (Jenkinson and Lamont 2005). Dental caries is a localized infectious disease that leads to loss of teeth. When sugary foods are taken in when oral hygiene is not maintained not properly it leads to the formation of a matrix over the enamel surface which will lead to demineralisation of the tooth and ultimately leads to the formation of a 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. Even though 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, diarrhoea. Due to its side effects still, there is a process going on to find medicines with naturally available products with minimal side effects. Natural products obtained from plants used in traditional medicine acts as an alternative to synthetic chemicals. About 80% of individuals from developed countries use traditional medicine which has compounds derived from medicinal plants (Chung et al., 2006, Park et al., 2003, Tenguria et al., 2012).

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), 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 microplates. 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.

The different concentrations of *Acacia catechu* extract were taken in 1 ml quantities in a U bottom microculture plates. Control well received plain Broth without the plant extract. The plates were kept in sealed covers and incubated at 37°C for 24hours and growth/no growth was detected. All the tests were done in triplicate to minimize the test error.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of herbal extract against the tested organisms was determined by Macro Broth dilution method. A series of two-fold dilution of the plant extract (0.15 mg/ml to 100 mg/ml) was made in to which 1 ml of a standardised bacterial suspension containing the organisms was made in Tryptic Soy Broth (TSB) as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1990). 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 colored 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, were noted as the MBC of the extract for that particular organism.

RESULTS AND DISCUSSION

The minimum inhibitory concentration [MIC] was determined for the extract and is given in table 1. From MIC, minimum bactericidal concentrations [MBC] also determined and are given in table 2.

Table 1									
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

Table1: Minimum inhibitory concentration of the extract

Table 2		
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

Table2: Minimum bactericidal concentration Of the extract

From the table 1, it is clear that the extract at different concentrations exhibited significant anticarcinogenic activity against all the bacterial strains tested. No Growth indicates high effectiveness of the extract whereas the 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 the 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. The extract has a very higher effect on Streptococcus salivarius, Streptococcus sanguinis and shows the minimal effect on Streptococcus mitis. Thus the result of our present study shows that the Acacia *catechu* extract used has significant anticariogenic activity.

The oral cavity is a complex ecosystem which contains highly divergent acid tolerant and acid-producing microbiota (Lakshmi T, Aravind Kumar 2011). It is a habitat for temporary and permanent microorganisms (Prajapati R, and Raol 2013). 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 (Ilakkiya Ezhil, T. Lakshmi 2017, Kamel and Mostafa 2015, Okusu et al., 1996). Some of the microorganisms used In our study play the main role in the development of dental caries. The occurrence of dental caries in developing countries like India is increasing day by day (Prajapati and Raol 2014, Saini et al., 2011). 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., a hardened form of a plaque and lead to gingivitis or periodontal disease (Lakshmi 2011, Rogers 2008). Dental plaque plays a major role in the pathogenesis of 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. Treatment becomes a critical management and patients frequently seek out complementary and alternative strategies (Matteo et al., 2017). A good level of dental treatment is required to cope up this situation which may be costly and cannot be affordable for everyone. The prevention should be done own self at the doorstep level using an herbal remedy, is the solution of choice which is economically feasible. Many plants being used in the prevention of dental caries (Prajapati and Raol 2014, Chopra 2006, Vanmathi Selvi et al., 2017). 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 this until now.

Natural products can also be a possible source for a new potential microbial agent to which pathogenic organisms are not resistant. Current researches indicate that the polyphenols, being secondary metabolites, are present in a rich amount in various plants (Reinthaler et al., 2003, Guajardo-Lara et al., 2009, Wilkerson et al., 2004). The bark of the Acacia catechu commonly known as Khair is used in traditional medicine and possess antimicrobial property. 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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