



Preliminary phytochemical analysis & *In vitro* Antibacterial activity of *Acacia catechu* Willd Bark against *Streptococcus mitis*, *Streptococcus sanguis* & *Lactobacillus acidophilus*.

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A B S T R A C T

The objective of our study is to investigate the *in vitro* antibacterial activity of ethanolic bark extract of *Acacia catechu willd* against selected oral microbes and to investigate the phytochemicals present in it. The inhibitory effect of the extract were tested against selected oral microbes by using the Macro broth dilution method .The ethanolic bark extract of *Acacia catechu* exhibited antibacterial activity against *streptococcus mitis* with minimum bactericidal concentration of 500µg/ml whereas the MBC for *streptococcus sanguis* and *Lactobacillus acidophilus* were found to be 1mg/ml, 5mg/ml,10mg/ml and the phytochemical analysis revealed the presence of tannins, flavonoids, amino acids , saponins, triterpenoids. The ethanolic bark extract of *Acacia catechu willd* was found to be bactericidal in action against tested bacterial strains and these action may be due to the presence of phytochemical constituents in it.

Keywords: *Acacia catechu*, Anti bacterial activity, oral microbes, MBC, Phytochemicals.

Introduction

Acacia catechu Willd (Family: *Fabaceae* and subfamily: *Mimosoideae*.) is highly valuable for its powerful astringent and antioxidant activities. The bark, wood, fruits , Gum and flowering tops of *Acacia catechu* are used for medicinal purpose. The plant is useful, internally as well as externally. Used externally as a powder by itself, it arrests the bleeding in gums. The decoction is an effective gargle in sore throat, cough and hoarseness of voice. The paste is beneficial, externally, in skin diseases and wounds.

The bath of its decoction is an effective panacea for various skin affection. In stomatitis, halitosis, dental caries and cavities, halitosis, dental caries and cavities, khadira(*Acacia catechu* in Sanskrit is known) is used with great benefit. The popular preparation Khadiradi guti in ayurveda is extremely helpful for chewing in sore throat, hoarseness of voice, and tonsillitis etc. due to vitiation of kapha doshas. It dries up the mucous secretions and regains the taste sensation.

The extracts of *Acacia catechu* exhibits various pharmacological effects like antipyretic, anti-inflammatory, antidiarrhoeal, hypoglycaemic,



hepatoprotective, antioxidant and antimicrobial activities. [1-10]

Oral microbial flora is dominated by gram positive micro organisms and hence dental plaque which is formed on the tooth surface contains gram positive *cocci* and *bacilli*. Oral health also influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases. oral bacteria like *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus oralis*, *Streptococcus intermedius*, *Streptococcus anginosus*, *Lactobacillus acidophilus*, *streptococcus salivarius*, *Streptococcus mitis*, *Streptococcus sanguis* is an potent initiator that causes dental caries/plaques. These dental plaques are more difficult to be removed in the fixed appliances patients undergoing orthodontic treatment. Hence an attempt was taken by us to evaluate the *invitro* Antibacterial activity of Ethanolic Bark extract Of *Acacia catechu* against selected oral microbes causing dental caries/plaque. moreover, The review of literature revealed that no method is yet reported for the estimation of phytochemicals in *Acacia catechu* Ethanolic Bark extract. This prompted us to conduct the preliminary study to identify the presence of phytochemical constituents in the plant extract responsible for the antibacterial action.

MATERIALS AND METHODS

Extract and Chemicals

The Ethanolic Bark extract of *Acacia catechu willd* was obtained from Green Chem, Herbal Extracts & Formulations, Bangalore.

Phytochemical analysis

Phytochemical analysis was carried out according to the methods specified by Siddiqui, [11] Edeoga [12], Iyenger(1995)[13] and Trease and evans (2002).[14] The phytochemicals analyzed were alkaloids, tannins, saponins, flavonoids, steroids, phenols, glycosides, carbohydrates, amino acids, proteins, terpenoids and phlobatannins.

Test microorganisms

Bacterial strains used were *streptococcus mitis*(ATCC 9811), *streptococcus sanguis*(ATCC 10557) and *Lactobacillus acidophilus*(ATCC 4356)The organisms were obtained from Department of Microbiology, Saveetha Dental College & Hospitals, Chennai

Anti Bacterial Assay

The plant extract 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 Macfarland

Standard.[15-17] 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 [18]. 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) [19].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 turbidity/nogrowth is detected, then the MBC was 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, were noted as the MBC.

RESULT AND DISCUSSION

Oral cavity is a complex ecosystem with highly divergent acid tolerant and acid-producing microbiota. The mouth harbors a diverse, abundant and complex microbial community. This highly diverse micro flora inhabits the various surfaces of the normal mouth. Bacteria accumulate on both the hard and soft oral tissues in biofilms.

Bacterial adhesion is particularly important for oral bacteria. Dental plaque is the material that adheres to the teeth and consists of bacterial cells (mainly *S. mutans* and *S. sanguis*), salivary polymers and bacterial extracellular products. Plaque is a biofilm on the surfaces of the teeth. This accumulation of microorganisms subject the teeth and gingival tissues to high concentrations of bacterial metabolites which results in dental disease. If not taken care of, via brushing or flossing, the plaque can turn into tartar (its hardened form) and lead to gingivitis or periodontal disease.²¹

Acidogenic oral microbes is the key factor of Dental plaques. The primary acid tolerant bacteria associated with Dental plaque includes *Streptococcus mutans*, *Streptococcus oralis*, *Streptococcus sobrinus*, *Lactobacillus acidophilus*, *Streptococcus salivarius*, *Streptococcus mitis*, *Streptococcus sanguis*, *Streptococcus intermedius*, *Streptococcus anginosus* that surround orthodontic appliances are a common orthodontic problem in many patients undergoing Orthodontic treatment. [20,22,23]

It also has been reported that presence of fixed orthodontic appliance greatly inhibits oral hygiene and creates new retentive areas for plaque and debris, which in turn predisposes to

increased carriage of microbes and subsequent infection. Therefore, prevention of bacterial attachment to orthodontic wires is a critical concern for orthodontists.^{24,25}

The occurrence of *mutans streptococci* and *Streptococcus sobrinus* together makes the oral environment more conducive to caries/plaque. These cariogenic pathogens utilise dietary sucrose and produce adhesive exopolysaccharides and acids which lead to plaque formation and carious lesions on susceptible tooth surfaces.

Dental plaque plays the primary role in the pathogenesis of the dental caries. Dental plaque is a general term for the diverse microbial community found on the tooth surface, embedded in a matrix of polymers of bacterial and salivary origin. Plaque is an example of a biofilm; current researches are showing that the properties of bacteria associated with a surface in a biofilm can be markedly different than those of the same cells growing in liquid broth. Plaque is found preferentially at protected and stagnant surfaces, and these are at the greatest threat of disease.

The preliminary phytochemical analysis was carried out for the plant extract by standard procedure. The extract showed the presence of Tannins, phenol, saponins, carbohydrates, flavonoids, alkaloids and glycosides. The extract lacks the presence of phlobatanins, proteins and amino acids. The data is represented in Table 1. The findings of the Antibacterial study reveals that the extract at different concentrations has exhibited antibacterial activity against the bacterial strain tested. The ethanolic bark extract of *Acacia catechu* exhibited antibacterial activity against *Streptococcus mitis* with minimum bactericidal concentration of 500 µg/ml whereas the MBC for *Streptococcus sanguis* and *Lactobacillus acidophilus* were found to be 1 mg/ml, 5 mg/ml and 10 mg/ml.

The presence of No growth is an indication of high effectiveness of the extract whereas presence of Growth indicates the less effectiveness of the extract, which was represented in Table 2.

Current research indicate that the polyphenols, being secondary metabolites, are present in rich amount in various plants. Many of them possess antioxidant, anti-inflammatory and several others

therapeutic properties *Acacia catechu willd* contains many active constituents in it like Catechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, phloroglucin, protocatechuic acid, poriferasterol glucosides, poriferasterol acylglucosides, lupenone, kaempferol, dihydrokaempferol, Quercetin, Taxifolin etc.,

Table 1 Phytochemical analysis of *Acacia catechu willd* bark extract

Phytochemical constituents	<i>Acacia catechu</i> Ethanollic bark extract
Saponins	++
Tannins	++
Phenol	+
Carbohydrates	+
Proteins	-
Amino Acids	-
Flavonoids	++
Phlobatanins	-
Alkaloids	+
Glycosides	+

++ =high concentration + =moderate concentration - =Absence

TABLE – II DETERMINATION OF THE MBC

Strain	Concentrations of <i>Acacia catechu willd</i> bark extract						
	62µg/ml	125µg/ml	250µg/ml	500µg/ml	1mg/ml	5mg/ml	10mg/ml
<i>Streptococcus mitis</i>	Turbidity	Turbidity	Turbidity	NG	NG	NG	NG
<i>Streptococcus sanguis</i>	Turbidity	Turbidity	Turbidity	Turbidity	NG	NG	NG
<i>Lactobacillus acidophilus</i>	Turbidity	Turbidity	Turbidity	Turbidity	Turbidity	NG	NG

NG –No Growth(Indicates MBC)

Turbidity –Least effectiveness of extract

Conclusion

In conclusion, our study provides new scientific information about *Acacia catechu* ethanolic bark extract, based on its antibacterial potential and chemical profiling that has never been reported. The antibacterial activity of *Acacia catechu bark extract against* oral microbes may be attributed to the various phytochemical constituents present in the refined extract. The purified components have more potency with respect to inhibition of Selected oral microbes. Further work on the types of phytoconstituents and purification of individual groups of bioactive components can reveal the exact potential of the plant to inhibit several pathogenic microbes and encourage in developing a noval broad spectrum antibacterial herbal formulation in future to prevent the dental caries/plaque in fixed appliances patients undergoing orthodontic Treatment

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References

- [1]. Anonymous, Indian Herbal Pharmacopoeia, Indian Drug Manufacturer's Association, Mumbai, 2002, 1-11.
- [2]. Singh KN, Lal B, Note on traditional uses of Khair (*Acacia catechu Willd.*) by inhabitants of shivalik range of western Himalaya, Ethnobotanical Leaflets, 10, 2006, 109-112.
- [3]. Qadry JS, Shah's and Qadry's Pharmacognosy, 12th edition, B.S Shah Prakashan, Ahmedabad, 2008, 302-303.
- [4]. Lakshmi.T, Geetha R.V, Anitha Roy. *In vitro* Evaluation of Anti bacterial Activity of *Acacia catechu willd* Heartwood Extract.” International journal of Pharma and Biosciences. 2011;2(2); B188-192.
- [5]. Geetha R.V, Anitha Roy, Lakshmi.T *In vitro* evaluation of Anti bacterial activity of heartwood extract of *Acacia catechu* on oral microbes.

- International journal of current research and review .2011;3(6);P.4-9.
- [6]. Geetha R.V, Anitha Roy, Lakshmi T . In vitro evaluation of anti bacterial activity of heart wood extract of *Acacia catechu willd* on enteric pathogens.International journal of pharmaceutical sciences review and research.2011;9(2);P.147-149.
- [7]. Anitha Roy, Geetha R.V, Lakshmi T. *In Vitro* Evaluation of Anti Mycotic Activity of Heartwood Extract of *Acacia Catechu* Willd .Journal of pharmacy research .2011;4(7)
- [8]. Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohoni DP, Biyani MK, Mohan H, Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine, 63(1), 2003, 97-104.
- [9]. Sharma P, Dayal R, Ayyer KS, Chemical constituents of *Acacia catechu* leaves, Indian Journal of Chemical Society, 1997;P.60.
- [10]. Rao PR, Seshadri TR; L-Epi-catechin from *Acacia catechu*. *J. Scient. Ind. Res.*, (7B); 59 ;1948.
- [11]. Siddiqui S, Verma A, Rather AA, Jabeen F, Meghvansi MK, Preliminary Phytochemicals Analysis of Some Important Medicinal and Aromatic Plants, *Advances in Biological Research*, 3, 2009, 188-195.
- [12]. Edeoga HO, Okwu DE, Mbaebie BO, Phytochemical constituents of some Nigerian medicinal Plants, *African Journal of Biotechnology*, 4, 2005, 685-688.
- [13]. Iyengar, M.A., 1995. *Study of Crude Drugs*. 8th ed., Manipal Power Press, Manipal, India. pp 2.
- [14]. Evans, W.C., 2002. *Trease and Evan's Pharmacognosy*. 5th ed., Haarcourt Brace And Company, pp 336.
- [15]. Siddiqui, A.A., Ali, M., 1997. *Practical Pharmaceutical chemistry*. 1st ed., CBS Publishers and Distributors, New Delhi, pp 126-131.
- [16]. J.H Jorgenson & John turniegd Susceptibility test methods dilution and disc diffusion methods,Manual of Clinical microbiology ASM Press Washington vol 1 ,9th edition pg no.1153-1172.
- [17]. Betty A.Forbes., Daniel F.Sahm., Alice S.Weissfeld. Bailey & Scott's Diagnostic Microbiology 11th edition Mosby page no 229 – 257.
- [18]. Ananthanarayan R and Paniker's: *Textbook of Microbiology: 8th edition: Publishers University Press: Hyderabad 2009: 618.*
- [19]. Jennifer MA: *Determination of Minimum Inhibitory Concentrations: Journal of Antimicrobial Chemotherapy 2001; 48, (SI): 5 -16.*
- [20]. National Committee for Clinical Laboratory Standards: *Methods for Disc Susceptibility Tests for Bacteria that Grow Aerobically: NCCLS Document M2-A7: National Committee for Clinical Laboratory Standards: Wayne, USA: 2000;*
- [21]. Rogers A H (editor). (2008). *Molecular Oral Microbiology*. Caister Academic Press. ISBN 978-1-904455-24-0.
- [22]. Anhoury P et al Microbial profile on metallic and ceramic bracket materials. *Angle Orthodontist 2002;72:338-343.*
- [23]. Bos R, van der Mei HC, Busscher HJ Physico-chemistry of initial microbial adhesive interactions—its mechanisms and methods for study. *FEMS Microbiology Reviews 1999;23:179-230.*
- [24]. Diamanti-Kipioti A, Gusberti FA, Lang NP Clinical and microbiological effects of fixed orthodontic appliances. *Journal of Clinical Periodontology 1987;14:326-333.*
- [25]. Atack NE, Sandy jr ,Addy M Periodontal and microbiological changes associated with the placement of orthodontic appliances. A review. *Journal of Periodontology 1996;67:78-85.*