Research Article

IN VITRO EVALUATION OF ANTI BACTERIAL ACTIVITY OF HEART WOOD EXTRACT OF ACACIA CATECHU *WILLD* ON ENTERIC PATHOGENS

Geetha R.V[1*] Anitha Roy[2] and Lakshmi T[2]
[1]Department of Microbiology, Saveetha Dental College, Velappanchavady, Chennai-77, India.
[2]Department of Pharmacology, Saveetha Dental College, Velappanchavady, Chennai.-77, India.
***Corresponding author's E-mail:** rgeetha2010@yahoo.in

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ABSTRACT

The aim of the present study was to evaluate the anti bacterial activity of Acacia catechu *willd* on selected enteric pathogens. *Acacia catechu willd* (AC) is a moderate thorny deciduous tree growing in tropical countries commonly known as Black cutch. It has a diverse pharmacological and phytochemical importance and is a potent medicinal plant in the traditional Indian medicinal systems. Antibacterial activity of ethanolic and aqueous extract of heart wood of Aacacia catechu was screened against *Salmonella typhi, [Gram negative bacilli-GNB], Shigella flexneri [GNB], E.coli [GNB], Klebsiella pneumonia [GNB], Vibrio cholera [GNB], Pseudomonas aeruginosa [GNB] and Staphylococcus aureus, [*Gram positive cocci], using agar well diffusion technique. 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.

Keywords: *Acacia catechu willd,* Anti bacterial evaluation, Mac Farland's standard, Zone of inhibition.

INTRODUCTION

Enteric or diarrhoeal infections are major public health problems in developing countries. Enteric bacteria comprised of *Salmonella* sp., *Shigella* sp., *Proteus* sp., *Klebsiella* sp., *E. coli, Pseudomonas* sp., *Vibrio cholerae,* and *S. aureus, which* are major etiologic agents of sporadic and epidemic diarrhoea both in children and in adults.[1] Recently, it has been demonstrated that many human pathogenic bacteria have developed resistance against several synthetic drugs. Available reports on lesser efficacy and more side effects of synthetic drugs need to search for alternative medicine[2-4]. There are several reports on antimicrobial activity of crude extracts prepared from plants that inhibit various bacteria. However, a limited numbers antimicrobial activity of *in vitro* studies on herbal preparation has been published. 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.[5,6] There have been numerous reports of the use of traditional plants and natural products for the treatment of enteric diseases. The present study was to evaluate the antibacterial activity of heart wood extract [ethonolic & aqueous] of Acacia catechu *willd* on selected enteric pathogens.

Acacia catechu Willd. (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**[5].** 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.[6] The important chemical constituents reported in the heartwood are catechin, catechutannic acid, epicatechin, catechin tetramer,dicatechin, gallocatechin, kaempferol, taxifolin, isorhamnetin, (+)afzelechinn, L-arabinose, D-galactose, D-rhamnose and aldobiuronic acid.[7-10] Catechin is biologically highly active. It is used as a haemostatic. Another important constituent is taxifolin. Taxifolin has antibacterial[13], anti-fungal[16], antiviral, anti-inflammatory, and antioxidant activity[11]

The extract of Acacia catechu extract have been reported to have various pharmacological effects like immuno modulatory[15,] anti pyretic[10], hypoglycaemic[12], anti diarrhoeal[12,] hepatoprotective activity[11,12]. Cutch is astringent, cooling, and digestive. It is useful in cold and cough[5] ulcers, boils and eruptions of the skin, bleeding piles, uterine haemorrhages, atonic dyspepsia, chronic bronchitis etc. In stomatitis, halitosis, dental caries and cavities, 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 *Salmonella typhi, [Gram negative bacilli-GNB], Shigella flexneri [GNB], E.coli [GNB], Klebsiella pneumonia [GNB], Vibrio cholera [GNB], Pseudomonas aeruginosa [GNB], and Staphylococcus aureus, [*Gram positive cocci]. 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. 2mg/ml, 3mg/ml and 4mg/ml, so that 50µl of extract of different concentrations delivers 100µg, 150µg and 200 µg respectively.

Assay for antibacterial activity using agar well diffusion method

The screening of antibacterial activity of plant extracts was carried out using the agar well diffusion method. The bacterial strains were inoculated into tubes of nutrient broth and incubated at 37°C overnight. Each of the cultures were then adjusted to 0.5 McFarland turbidity standard.[17-20] 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. A sterile cork borer was then used to make wells (6mm diameter) for different concentrations of the extracts on each of the plates containing cultures of the different bacterial strains. 50µl of the varying concentrations (100,150, 200µg) of the extracts were introduced into the wells with the help of micropipettes. The culture plates were allowed to stand on the working bench for 30 min for pre-diffusion and were then incubated in upright position at 37°C for 24 h. After 24 hrs, antibacterial activity was determined by measurement of diameter of zones of inhibition (mm). Standard antibiotic discs of amoxicillin (30mcg/disc) and Ciprofloxacin (30mcg/disc) were used as positive control. All the tests were done in triplicate to minimize the test error.

RESULTS AND DISCUSSION

The antibacterial activity of the extracts (Ethanolic and Aqueous) at different concentrations was screened by agar well diffusion technique. The zone of inhibition was measured in mm diameter and the results are given in table 1.

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

Extract	Conc [µg]	Zone of inhibition [in mm diameter]						
		B1	B2	B3	B4	B5	B6	B7
Ethanolic	100	9	15	14	10	10	7	12
	150	14	20	21	14	13	8	19
	200	19	25	25	19	16	10	24
Aqueous	100	8	9	11	8	8	-	10
	150	11	12	13	11	11	7	16
	200	14	15	17	16	14	9	20
Ciprofloxacin	30mcg/disc	24	21	22	22	23	23	24
Amoxycillin	30mcg/disc	25	23	20	24	25	25	22

B1-Salmonella typhi, B2- Shigella flexneri, B3-Vibrio cholerae, B4- E.coli, B5- Klebsiella pneumoniae, B6- Psuedomonas aerugenosa, B7- Staphylococcus aureus.

Both the extracts at different concentration exhibited antibacterial activity against all bacterial strains tested. Ethanolic extract exhibited comparably a high degree of activity than the aqueous extract. The ethanolic extract was more effective against Shigella flexneri, Vibrio cholerae and *Staphylococcus aureus* with a zone of inhibition of 25mm, 25mm and 24 mm diameter (at conc200 µg.) respectively and was least effective against *Pseudomonas aeruginosa* with zone of inhibition of 10mm (at conc. 200 µg.) Among the other bacterial species studied *E.coli* and Salmonella typhi showed a zone of inhibition of 19mm diameter (at conc. 200 µg.) and *Klebsiella pneumoniae* showed inhibition zone of 16mm diameter (at conc. 200 µg.).

One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics.[22,23] It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases.[24] They can also be a possible source for new potent antimicrobial agent to which pathogen strains are not resistant.

The present study was to evaluate the antibacterial activity of Acacia catechu *willd* against enteric pathogens. All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested. It was evident that antimicrobial activity was more apparent in ethanol than aqueous extracts. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity.

CONCLUSION

This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. It is clear from the results that, the extract acts as a good source of antimicrobial agent against various bacterial pathogens tested and exhibited broad spectrum of antibacterial activity.

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REFERENCES

1. WHO, 5th Programme Report, Programme for control of diarrhoeal diseases, Geneva. WHO Bulletin, (1985).63: 557-772.
2. Vickers A and Zollman C, ABC of complementary medicine: herbal medicine. BMJ. (1999). 319:1050 -1053.
3. De Smet PAGM, Herbal remedies. New Engl J Med. (2002).347:2046–2056
4. Dawson W, Herbal medicine and the EU directive, J R Coll Physicians Edinb. (2005).35: 25-27
5. Anonymous., *The Wealth of India, Raw materials*, CSIR, New Delhi, Vol. I-A (Rev.), 24-30, 1985.
6. *Pharmacopoeia of India*, Ministry of.Comparative pharmacognostical and antimicrobial studies of *acacia* species (Mimosaceae) Saini et al. Journal of Medicinal Plants Research Vol. 2(12), pp. 378-386, December, 2008
7. 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.
8. Bimla, Meera, Chander, Jagdish, Kalidhar SB, A Review on the chemistry and bioactivity of Acacia Spp., Journal of Medicinal and Aromatic Plants Science, 27, 2005, 51-90.
9. Sharma P, Dayal R, Ayyer KS, Chemical constituents of *Acacia catechu* leaves, Indian Journal of Chemical Society, Page No. 60, 1997.
10. Rao PR, Seshadri TR, L-Epi-catechin from *Acacia catechu*, Journal Scientist Indian Research, 7B, 1948, 59
11. V. Gayathri devi, Anitha John, R.Sreekala devi, V. A. Prabhakaran .Pharmacognostical studies on *acacia catechu* willd and identification of antioxidant principles. International journal of pharmacy and pharmaceutical sciences. vol 3, suppl 2, 2011
12. Ray D, Sharatchandra KH, Thokchom IS, Antipyretic, antidiarrhoeal, hypoglycaemic and Hepatoprotective activities of ethyl acetate extract of *Acacia catechu Willd.* In albino rats, Indian Journal of Pharmacology, 38(6), 2006, 408-413.
13. Bhawna sunil and Bharti P Dave. In vitro anti microbial activity of Acacia catechu and its phytochemical Analysis. Indian Journal of Microbiology, Vol 50 Number 4, 369-374
14. Jayasekhar P, Mohanan PV, Hepatoprotective activity of ethyl acetate extract of *Acacia catechu*, Indian Journal of Pharmacology, 29(6), 1997, 426-428
15. Syed Ismail and Mohammed Asad., Immunomodulatory activity of Acacia catechu. Indian journal of Pharmacology 2009:53 (1), 25 -33
16. Nagaraja T.G, S.V. Sarang and D. C. Jambhale Evaluation of anti-mycotic activity of Acacia catechu Willd. (Mimosaceae) Anti-mycotic activity of *Acacia catechu* Journal of Biopesticides, (2008)1(2):197 – 198.
17. Collins, CH and Lyne, P.M 1976.Microbiological methods, London, Butterworths and co.288p
18. Betty A.Forbes., Daniel F.Sahm., Alice S.Weissfeld. Bailey & Scott's Diagnostic Microbiology 11th edition Mosby page 229 – 257
19. Connie R.Mahon., George Manuselis., Saunder's Diagnostic Microbiology 2nd Edition.
20. J.G.Colle., A.G.Faster., B.P.Marmion., A.Simmons. Practical Microbiology [Mackie & Mc Cartney] 14th edition page 851 – 85
21. L.P. Samaranayake.,Brain M.Jones., Essential Microbiology for Dentistry. Second edition page 217 – 223.
22. Sheetal verma and S.P.Singh., Current and future status of herbal medicines. Veterinary world, Vol.1(11): 347 – 350.
23. R.Guo.,P.H.Canter.,E Ernst., A systematic review of randomised clinical trials of individualised herbal medicine in any indication .Post graduate medical journal. 2007 ;83 633-637
24. Si-Yuan Pan, Si-Bao Chen, Hong-Guang Dong, Zhi-Ling Yu, Ji-Cui Dong, Zhi-Xian Long,Wang-Fun Fong, Yi-Fan Han, and Kam-Ming Ko.,New perspectives on Chinese herbal medicine research & development. Evidence Based Complementary and alternative medicine volume 2011.,11 pages.

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