



RESEARCH ARTICLE

MICROBIOLOGY

“IN VITRO EVALUATION OF ANTIBACTERIAL ACTIVITY OF HEARTWOOD EXTRACT OF ACACIA CATECHU WILLD”.**LAKSHMI T¹ *, GEETHA R.V² AND ANITHA ROY¹**¹Department of Pharmacology, Saveetha Dental College, Velappanchavady, Chennai-77²Department of Microbiology, Saveetha Dental College, Velappanchavady, Chennai.-77

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ABSTRACT

The objective of the present study was to assess the anti bacterial activity of *Acacia catechu willd* (AC). AC is a moderate sized deciduous tree growing in tropical countries commonly called “Karungali” in Tamil. It is a potent medicinal plant in the traditional Indian medicinal systems. Aqueous and ethanolic extract of Heartwood of *Acacia catechu* is tested for antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*. Disc diffusion technique was followed for screening anti bacterial activity. The discs were loaded with 50µl of aqueous and ethanolic extracts at different concentrations. Positive controls used were Amoxycillin (30 µg/disc) and Ciprofloxacin (5 µg/disc). After incubation at 37°C over night, the zone of inhibition was measured. Ethanolic extract showed significant activity than Aqueous extract against the micro organism compared to standard.

KEY WORDS

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

INTRODUCTION

Acacia catechu Willd. (AC) (Family: Fabaceae and subfamily: Mimosoideae) known as Black cutch. AC, a deciduous thorn like tree mainly found in India and also found in deciduous forests around the world. The leaves, bark, heartwood has many nutritional and medicinal uses. The foliage is softly textured, light green and oval-shaped. The branches are thin and spike like because tiny thorns grow around the exterior. AC typically reaches heights of up to 50 feet. 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. The wood contains epicatechin², Atzelchin, catechin tetramer, dicatechin, gallochin, gossypetin, phlobatannin, kaempferol, quercitrin, quercitin¹⁷. The catechin content varies from 4 to 7 per cent and is distributed throughout the heartwood from the root to the branches. Another important constituent is taxifolin. Catechin is biologically highly active. It is used as a haemostatic. Taxifolin has antibacterial¹, anti-fungal¹³, antiviral, anti-inflammatory, and antioxidant activity^{15,16}. The extract of *Acacia catechu* have been reported to have various pharmacological effects like immuno modulatory¹⁴, anti pyretic, hypoglycaemic⁷, anti diarrhoeal³, hepatoprotective activity^{3,6}. Heartwood is used to yield concentrated aqueous extract i.e. cutch. Cutch (extract) is astringent, cooling, and digestive. It is useful in cold and cough^{1,4,5,8} ulcers, boils and eruptions of the skin, bleeding piles, uterine haemorrhages, atonic dyspepsia, chronic bronchitis etc. An antibacterial mouthwash made from the extract treats gingivitis and mouth sores.

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 *Staphylococcus aureus* [Gram positive GP], *Bacillus subtilis*[GP], *E.coli*[Gram negative GN], *Psuedomonas auroginosa*[GN] and *Klebsiella pneumonia* [GN]. 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. 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 100µg, 150µg and 200 µg respectively. The discs were dried and kept aseptically.

Screening of antibacterial activity [disc diffusion technique]

Over night broth culture of the bacterial strains compared to Mac Farland's standard⁹⁻¹² 0.5 were prepared. Ten plates of Muller Hinton agar [MHA-Hi media M1084] was taken and divided into two sets of 5 each for testing ethanolic and aqueous extracts respectively. Lawn culture of the test organisms were made on the MHA 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 Ciprofloxacin (5mcg/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 determined by measuring the zone of inhibition. The results are given in the table 1.

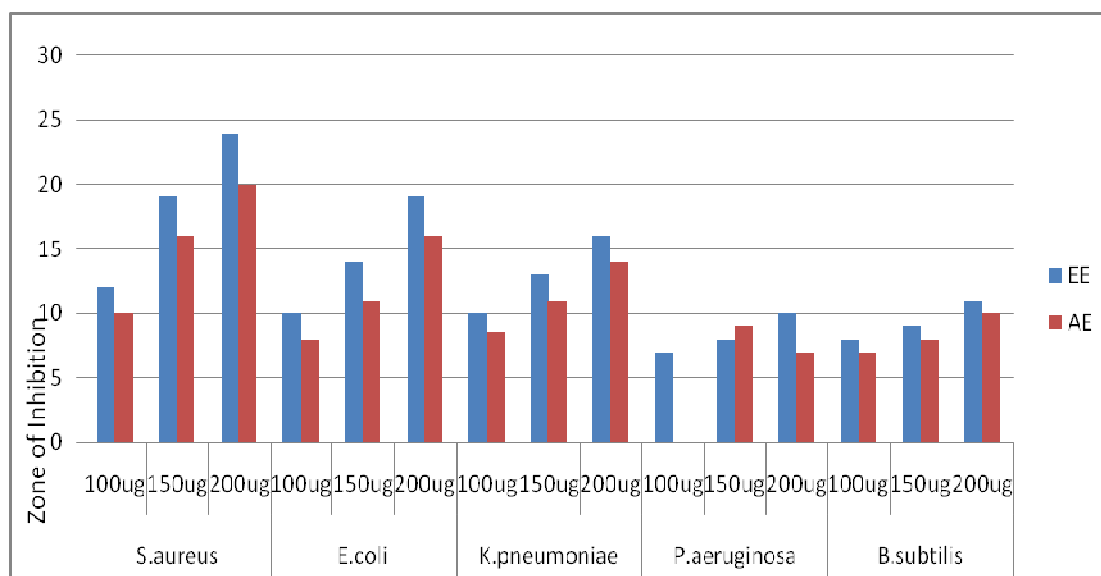
Table 1
Antibacterial Activity of the heartwood Extracts of *Acacia catechu* Willd.

Extract	conc. (µg)	Zone of inhibition* (diameter-mm)				
		B1	B2	B3	B4	B5
Ethanolic	100	12	8	10	10	7
	150	19	9	14	13	8
	200	24	11	19	16	10
Aqueous	100	10	7	8	8.5	no zone
	150	16	8	11	11	7
	200	20	10	16	14	9
Amoxycillin	30mcg/disc	24	26	22	23	22
Ciprofloxacin	5mcg/disc	22	25	24	25	21

B1= *Staphylococcus aureus* B2 = *Bacillus subtilis* B3= *Escherichia coli*
B4= *Klebsiella pneumoniae* B5= *Pseudomonas aeruginosa*.

*Each value represents the mean of three determinants.

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.



Graph 1
Antibacterial Activity of the Heartwood Extracts of *Acacia catechu* Willd. (EE- ethanolic extract AE – aqueous extract.)



The ethanolic extract was more effective against *Staphylococcus aureus* with a zone of inhibition of 24 mm diameter (at conc 200 µg.) and was least effective against *Pseudomonas aeruginosa* and *Bacillus subtilis* with zone of inhibition of 10mm (at conc. 200 µg.) and 11mm (at conc. 200 µg.) respectively. Among the other bacterial species studied *E.coli* 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.).

CONCLUSION

From the results, it can be concluded that the *Acacia catechu willd* heart wood extracts has got antibacterial activity after extensive

investigation. Further work will emphasize the isolation and characterization of active principle responsible for antibacterial activity of heartwood extracts of *Acacia catechu willd*.

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REFERENCES

1. Anonymous, Indian Herbal Pharmacopoeia, Revised new edition 2002, Indian Drug Manufacturer's Association, Mumbai, 2002, 1-11.
2. Rao PR, Seshadri TR, L-Epi-catechin from *Acacia catechu*, Journal Scientist Indian Research, 7B, 1948, 59
3. 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.
4. Anonymous, The Wealth of India, Raw Material, Vol 1, CSIR, New Delhi, 2004, 11.
5. Qadry JS, Shah's and Qadry's Pharmacognosy, 12th edition, B.S Shah Prakashan, Ahmedabad, 2008, 302-303.
6. Jayasekhar P, Mohanan PV, Hepatoprotective activity of ethyl acetate extract of *Acacia catechu*, Indian Journal of Pharmacology, 29(6), 1997, 426-428
7. Singh KN, Mittal RK, Barthwal KC, Hypoglycaemic activity of *Acacia catechu*, *Acacia suma*, And *Albizia odoratissima* seed diets in normal albino rats, Indian Journal of Medical research, 64(5), 1976, 754-757.
8. Wallis TE, Textbook of Pharmacognosy, 5th Edition, CBS Publishers and Distributors, New Delhi, 2005, 461-463.
9. Collins,CH and Lyne, P.M 1976.Microbiological methods, London, Butterworths and co.288p
10. Betty A.Forbes., Daniel F.Sahm., Alice S.Weissfeld. Bailey & Scott's Diagnostic Microbiology 11th edition Mosby page 229 – 257
11. Connie R.Mahon., George Manuselis., Saunder's Diagnostic Microbiology 2nd Edition
12. J.G.Colle., A.G.Faster., B.P.Marmion., A.Simmons. Practical Microbiology [Mackie & Mc Cartney] 14th edition page 851 – 852
13. 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,1(2):197 - 198 (2008)
14. Syed Ismail and Mohammed Asad Immunomodulatory activity of *acacia*



- catechu* Indian Journal of Pharmacology 2009; 53 (1): 25–33
15. Bibhabasu Hazra, (*Bose Institute*), *Kolkata*, Rhitajit Sarkar, Santanu Biswas, Nripendranath Mandal, The Antioxidant, Iron Chelating and DNA Protective Properties of 70% Methanolic Extract of 'Katha' (Heartwood extract of *Acacia catechu*)journal of complementary & integrative medicine vol.7 / 2010 /issue 1.
16. 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.
17. Evans W.C, Trease and Evans Pharmacognosy, 15th Edition, W.B.Saunders Publishers, 227P.