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In Vitro Anti Bacterial Activity of Ethanolic Bark Extract of *Acacia catechu Willd* against Enteric Pathogens

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Abstract

Aim: The Objective of Our study is to investigate the *in-vitro* antibacterial activity of ethanolic bark extract of ***Acacia catechu willd*** (*fabaceae*) against **Enteric pathogens**.

Materials and Methods: The inhibitory effect of ethanolic bark extract of *Acacia catechu willd* was tested against five Gram negative *bacilli* and one Gram positive *cocci* by using the Disc diffusion method and the MIC & MBC values were determined.

Results: The ethanolic bark extract exhibited antibacterial activity against *staphylococcus aureus and shigella dysentriae* with minimum inhibitory concentration, 1 mg/ml and minimum bactericidal concentrations of 1mg/ml.

Conclusion: The ethanolic bark extract of ***Acacia catechu willd*** was found to be **bactericidal** in action.

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

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Introduction

The use of natural products has been one of the most successful strategies for the discovery of new drugs[1]. Natural products have been used for thousands of years in folk medicine; they were believed to be the new source of antimicrobial agents[2]. Global, regional and national estimates clearly place diarrhoeal diseases as a major,

albeit a substantially neglected, public health problem. Deaths of children aged<5 years owing to diarrhoea was estimated to be 1.87 million at the global level (uncertainty range from 1.56 to 2.19 million), which is approximately 19% of total child deaths[3]. In the south-east Asian region, almost 48% of the estimated 3.07 million deaths annually are attributed to acute respiratory infections and diarrhoeal diseases with the highest burden of diarrhoeal disease in 5 countries: Bangladesh, India, Indonesia, Myanmar and Nepal where these diseases caused 60,000 deaths annually[4,5].

Diarrhoea is a syndrome that can be caused by different bacterial, viral and parasitic pathogens. Accurate understanding of the cause of diarrhoea in a given setting is an onerous task that requires systematic monitoring of the various pathogens. The availability of a well equipped clinical microbiology laboratory is a prerequisite to undertake such studies. Clinical studies conducted at the National Institute of Cholera and Enteric Diseases (NICED), which includes hospital and community, based surveillance for diarrhoea was focused on common enteric pathogens using conventional assays[6-8].

Acacia catechu Willd (Family: Fabaceae and subfamily: Mimosoideae.) is widely used in Ayurveda for many diseases and mainly for skin diseases[9]. Most of the people in Kerala use boiled Khadira water (karingali water) for drinking purpose.

There are a number of ayurvedic taila (oil) formulations which contain Khadira as one of the active ingredients[10]. *Acacia catechu* is highly valuable for its powerful astringent and antioxidant activities. It is commonly known as Katha which is an indispensable ingredient of Pan that is beetle leaf preparation chewed in India. It is useful in Dental, oral, throat infections and as an astringent for reducing oozing from chronic ulcers and wounds. The concentrated aqueous extract known as Khair gum or cutch is an astringent, cooling and digestive, beneficial in cough and diarrhea, applied externally to ulcer, boils and skin eruptions and is used extensively in Ayurvedic formulations[11]. The extracts of ***Acacia catechu*** exhibits

various pharmacological effects like antipyretic, anti-inflammatory, antidiarrhoeal, hypoglycaemic, hepatoprotective, antioxidant and antimicrobial activities[12,13-24]. Main chemical constituents of ***Acacia catechu willd*** are catechin, (-) epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallete rocatechin,phloroglucin,protocatechuic acid, quercetin, poriferasterol glucosides, poriferasterol acyglucosides, lupenone, lupeol, procyanidin AC, kaempferol, dihydrokaemferol, taxifolin, (+)-afzelchin gum and mineral[12,25-30]. The chief phytoconstituents of the heartwood are catechin and epicatechin. Catechins have siginificant antioxidant and antimicrobial effects[31]. It is considered to be the best antioxidant.

Acacia catechu is useful as a topical agent for sore gums and mouth ulcers.The bark of *Acacia catechu* in combination with other drugs is prescribed for snake bite[32]. Acacia catechu also shows hypotensive effect[33]. The decoction of bark mixed with milk is taken to cure cold and cough[34,35]. The bark is said to be effective against dysentery, diarrhoea and in healing of wounds. The seeds have been reported to have an antibacterial action[36].

Traditional medicines are cheaper, with minimal side effect and safe. To ensure the safety of its products and practices standardization is of vital importance. The knowledge of medicinal plants came from our ancient literature. Due to over and subclinical use of antibiotics lead pathogens to gain resistance against new generation antibiotics. More over in the Indian system of medicine, most herbal practitioners formulate and dispense their own recipes; this requires proper documentation and research[37]. Hence an attempt was carried by us to evaluate the *in-vitro* Antibacterial activity of Ethanolic Bark extract of ***Acacia catechu willd*** against enteric pathogens.

Table 1: Pharmacological Activity of *Acacia Catechu Willd.*

Scientific Name	Common Name	Family	Parts used	Pharmacological activity
Acacia catechu Willd.	Katha Khadira Karungali Black cutch.	*Fabaceae* *Mimosaceae* (Touch-me-not family)	**Bark**	**Dysentery, Diarrhea and in healing of wounds, Antioxidant, healing of sore throat, gingivitis, Antidiabetic activity.**
			Leaf	Hepatoprotective, Antisecretory and Anti ulcer, Antioxidant and Antibacterial, Antimycotic activity.
			Heartwood	Anti bacterial, Anti mycotic, To treat mouthsore, gingivitis, dental caries It possess Anti oxidant and Anti diarrhoeal activity. Ethyl acetate extract of *Acacia catechu* possess Analgesic, Antipyretic Hepatoprotective, Antidiabetic activity

MATERIALS AND METHODS

Plant material

The Ethanolic Bark extract of ***Acacia catechu willd*** was obtained from Mr.R.Rajendran. Green Chem. Herbal Extract & Formulations. Bangalore.

Test microorganisms

Bacterial strains used were *Shigella dysentriae [Gram negative bacilli-GNB], E.coli[GNB], Klebsiella pneumoniae[GNB], Vibrio cholerae[GNB], Pseudomonas aeruginosa[GNB],and Staphylococcus aureus,[GPC*]. 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, 4mg/ml and 6mg/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, 200µg and 300 µg respectively. The discs were dried and kept aseptically.

SCREENING OF ANTIBACTERIAL ACTIVITY [DISC DIFFUSION TECHNIQUE]

Broth culture of the bacterial strains compared to Mac Farland's standard 0.5 was prepared[38,39]. 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. 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 Penicillin G (30mcg/disc)

were used as positive control. All the tests were done in triplicate to minimize the test error.

MINIMUM INHIBITORY CONCENTRATION (MIC)

Macro broth dilution or tube dilution method was done to determine the Minimum inhibitory concentration (MIC) of the extracts. A series of two fold dilution of each extract ranging from 8mg/ml to 0.125mg/ml was made in Muller Hinton broth as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1990). 100µl of standard inoculum of the bacterial strains matched to 0.5 Mac Farland's standard was seeded into each dilution. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and growth media without inoculum) and organism control (tube containing the growth medium and the inoculum) .The tubes were incubated at 37°C for 24 hours and checked for turbidity. MIC was determined as the highest dilution (that is, lowest concentration) of the extract that showed no visible growth[39-41].

MINIMUM BACTERICIDAL CONCENTRATION (MBC)

The MBCs were determined by selecting tubes that showed no growth during MIC determination; a loop full from each tube was sub cultured onto Muller Hinton agar plates and incubated for further 24 hours at 37°C. The least concentration, at which no growth was observed, was noted as the MBC.

RESULT AND DISCUSSION

The antibacterial activity of the Ethanolic bark extract of *Acacia catechu willd* at different concentrations was screened by disc diffusion technique and the zone of inhibition was measured in mm diameter. The results are given in the Table 2. The minimum inhibitory concentration [MIC] and minimum bactericidal concentration [MBC] were also determined for the extracts and the results are given in Table 3.

The extract at different concentrations exhibited antibacterial activity against all bacterial strains tested. The ethanolic bark extract exhibited a high degree of activity against the organism tested. The ethanolic bark extract was more effective against *Staphylococcus aureus, Shigella dysentriae,* and *E.coli* with a zone of inhibition of 22mm, 20mm and 19 mm diameter (at conc300 µg.) respectively. With *Vibrio cholerae* and *Pseudomonas aeruginosa* the zone of inhibition was found to be 17mm (at conc. 300 µg.) *Klebsiella pneumoniae* showed comparatively less inhibition zone of 14mm diameter (at conc. 200 µg.).

The ethanolic bark extract of ***Acacia catechu willd*** was found to have low MIC and MBC values of 1mg/ml & 1mg/ml for *Staphylococcus aureus* and for *Shigella dysenteriae* it was 2mg/ml & 2mg/ml. With *Pseudomonas aeruginosa, E.coli*, and *Vibrio cholerae* the MIC and MBC value were found to be 4 mg/ml & 4mg/ml and for *Klebsiella pneumoniae* it was 4mg/ml & 8mg/ml. The lower MIC and MBC value is an indication of high effectiveness of the extract whereas higher MIC and MBC indicates the less effectiveness of the extract.

Extract	Conc [µg]	Zone of inhibition [in mm diameter]					
		B1	B2	B3	B4	B5	B6
Ethanolic	100	12	11	10	9	10	14
	200	15	14	13	11	12	17
	300	20	19	17	14	17	22
Penicillin G	30mcg/ disc	24	21	22	19	20	26
Amoxycillin	30mcg/ disc	25	20	20	21	22	24

Table 2: Anti Bacterial activity of *Acacia catechu willd* bark Extract. (Disc Diffusion Method)

B1-*Shigella dysenteriae*, B2-*E.coli,* B3-*Vibrio cholerae*, B4-*Klebsiella pneumoniae,* B5-*Psuedomonas aerugenosa*, B6- *Staphylococcus aureus*

Table 3: Anti Bacterial Activity of *Acacia catechu willd* bark Extract. (MIC& MBC)

Bacterial species	MIC (mg/ml)	MBC(mg/ml)
Shigelladysenteriae	2	2
E.coli	4	4
Vibrio cholerae	4	4
Klebsiella pneumoniae	4	8
Pseudomonas aerugenosa	4	4
Staphylococcus aureus	1	1

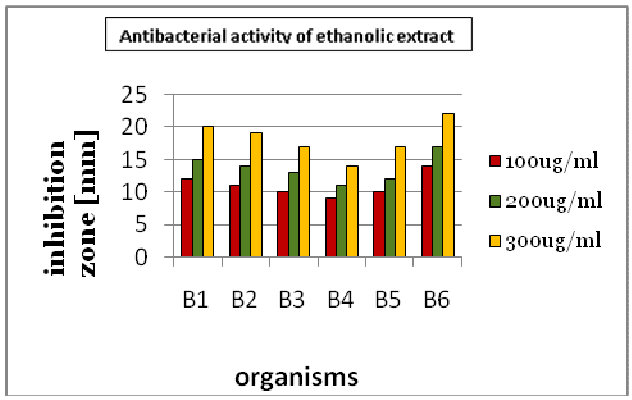

Fig: 1 Anti Bacterial Activity of *Acacia catechu willd* Bark extract

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

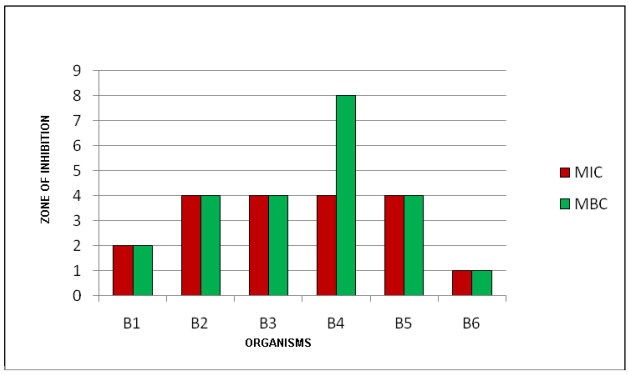

Fig 2: Anti Bacterial Activity of *Acacia catechu willd* Bark extract (MIC &MBC)

B1-*Shigella dysenteriae*, B2-*E.coli*, B3-*Vibrio cholerae*, B4-*Klebsiella pneumoniae*,B5- *Psuedomonas aerugenosa*, B6- *Staphylococcus aureus*

CONCLUSION

Majority of the diarrhoeal patients were infected with more than one pathogen. Polymicriobial infection (20-50%) seems widespread in many developing countries .This trend in diarrhoea is a clear indication that the source of infection is related to grossly contaminated food and water, affecting the patients from low-income group and those who are living in unhygienic environments. If a vaccine is available against *V. cholerae* O1, rotavirus and shigellae, the overall hospitalization due to diarrhoea can be considerably reduced. Rotavirus and cholera vaccines are now available as prescription product in India for the first time after a hiatus of 30 years. Not much progress has, however, been made with a *Shigella* vaccine. It would be interesting to see how these vaccines would ameliorate the burden of enteric infections in settings of diarrhoea in endemic areas all over India.

Hence Our finding suggest that the antibacterial effects of the ethanolic bark extract of ***Acacia catechu willd***, is an indication of its broad spectrum antibacterial potential which may be helpful in the management of enteric infections. However, further studies are necessary to isolate and reveal the active compound(s) contained in the refined extract *Acacia catechu willd* and to establish the mechanism of action.

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CONFLICT OF INTEREST: NIL

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