In Vitro Evaluation of Antienterobacterial Activity of *Khadira* Extract on Selected Microbes

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Abstract:

Aim: To evaluate the antienterobacterial activity of Khadira extract on selected microbes.

Objective: The study is to determine the antienterobacterial activity of *Khadira* extract against selected bacterial pathogens. **Background:** *Khadira* if a famous Ayurvedic herb. Its botanical name is *Acacia catechu* and it belongs to *Mimosoideae* family. Gums of *Khadira* are used for medicinal purpose. The paste is used in treating in skin diseases and wounds. It is helpful for chewing in sore throat, hoarseness of voice, tonsillitis and also dries up the mucous secretions and helps in regaining the taste sensation. **Results:** The extract used in this study have significant antienterobacterial activity against all the bacterial strains tested. **Conclusion:** This study is to evaluate antienterobacterial activity of *Khadira*(*Acacia catechu*) extract on selected microbes. This may help in the development of other products with *Khadira* extract as its constituent.

Keywords: Khadira, Antienterobacterial, Enteric pathogens, MIC, MBC

Introduction:

In most of the countries particularly in developing countries, infectious diseases represent an important cause of mortality and morbidity among general population[1][2]. The situation is alarming in developing as well as developed countries are due to undiscriminating use of antibiotics[3][4][5]. Enteric bacterias like *Pseudomonas sp., Shigella sp., Proteus sp., Klebsiella sp.,* causes major public health problems and are the etiological agents of epidemic diarrhoea in both adult and children.[6][7]

Due to lesser efficacy and more side effects of synthetic drugs available in markets, there is need to search for alternate drugs. Natural products are believed to be the new source of antienterobacterial agents[6][8][9][10]. Traditionally used medicinal plants have known therapeutic properties[3][11]. *Acacia catechu* commonly called as *Karungali* in Tamil and *Khadira* in Sanskrit is an evergreen tree with lots of nutritional and medicinal value. People living in Kerala consume *Acacia catechu* water to get relief from digestive disorders. It is widely used in ayurveda and it has various pharmacological properties like antioxidant, chemoprotective, anti-inflammatory, antidiarrhoeal, anticancer and antimycotic activities[12]. It's heartwood contains gummy extract called kath or cutch. The methanolic extract of this plant possesses antimicrobial activity against different species of pathogenic and non-pathogenic microorganisms. The branches of this plant are being used as chewing sticks in various parts of the world, due to its antimicrobial effect, and hence it is considered as a valuable ingredient which is used in dental care preparations. In foods and beverages, catechu is used as a flavouring agent. And also, it contains catechin which is biologically highly active[6] and it plays an important role in antioxidant property and they are very famous for its tanning and astringent effect. The compounds that were isolated and characterized from *Khadira* includes quercetin, kaempferol, catechin, epicatechin, rutin, isorhamnetin, afzelechin, epiafzelechin, mesquitol, ophioglonin, aromadendrin and phenols. Catechins, rutin and isorhamnetin exhibit antioxidant property by scavenging free-radicals. The ethanolic bark extract of *khadira* is capable of controlling the production of auto antigen and helps in inhibiting the protein denaturation in rheumatoid arthritis. It is most commonly used in the treatment of sore throat[13].

One of the active ingredients in a number of ayurvedic taila formulations contain *Khadira*[14]. It is beneficial in cough and diarrhoea, applied externally to ulcer, boils and skin eruptions[15]. The bark of this in combination with other drugs is prescribed for snake bite, and it is mixed with milk to cure cough and cold[15][16][17]. It also has hypotensive effect[18].

Since medically marketed products are of much cost and have greater side effects, we took a step to evaluate the benefits of product obtained from plants in order to reduce the side effects and to gain better beneficence. The main aim of the study is to evaluate the antienterobacterial effect of Acacia catechu extract on enteric pathogens.

Materials And Methods:

The Acacia catechu extract was obtained from Hi-Media, Mumbai. Pure strains of Salmonella typhi(ATCC 19430), Shigella boydii(ATCC 25922), Vibrio cholerae(ATCC 6395) 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 micro plates. 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 extract were taken in 1 ml quantities in a U bottom micro culture plates. Control well received plain Broth without the plant extract. The plates were kept in sealed covers and are incubated at 37°C overnight and growth/no growth was detected. All the tests were done in triplicate to minimise the test error.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of *Acacia catechu* 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 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. As the extracts were coloured, 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) were 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 the table 1. From MIC, minimum bactericidal concentration [MBC] also determined and are given in the table 2. The concentration at which no growth indicates high effectiveness of the extract whereas presence of growth indicates less effectiveness of the extract against the organisms tested.

Table 1									
Organisms	Concentration Of Extract mg								
	100	50	10	5	2.5	1.24	0.62	0.31	0.15
S.typhi	NG	NG	NG	G	G	G	G	G	G
Shi.boydii	NG	NG	NG	NG	G	G	G	G	G
V.cholerae	NG	NG	G	G	G	G	G	G	G



Table 1: Minimum Inhibitory Concentration Of Extract

 Table 2 : Minimum Bactericidal Concentration Of Extract

The extract at different concentrations exhibited significant antienterobacterial activity against all the bacterial strains tested. The *Khadira* extract exhibited a high degree of activity against the organisms tested. The Acacia catechu extract was more effective against *Shigella boydii*, *Salmonella typhi*, *Vibrio cholerae*. The MIC and MBC of the extract for *Shigella boydii*, *Salmonella typhi* and *Vibrio cholerae* was found to be 5mg/ml, 10mg/ml and 50mg/ml. The extract shows very higher effect on *Shigella boydii* and *Salmonella typhi* at a concentration of 5mg/ml and 10mg/ml. And also the extract show very lesser effect against *Vibrio cholerae* at a concentration of 50mg/ml.

A source of great economic value are medicinal plants. Plant herbs are naturally gifted for the synthesis of medicinal compounds[19]. They are in use as medicinal resource since prehistoric age[20]. Natural products obtained from plants have shown to possess abundant pharmacological properties such as antimicrobial, anti-inflammatory, and cytostatic effects. It also contains active principles like cyanidol ,tannins,flavonoids and polyphenols. The presence of bioactive compounds comprising saponins, tannins, flavonoids, alkaloids, present in plants is attributed for their effectiveness against pathogenic organisms[21]. *Acacia catechu* is commonly known as *Mimosa catechu*, whose phytoconstituents possess several pharmacological properties. It may serve as a novel source of anti-HIV-1 active compounds. The active compounds obtained from stem bark of *Acacia catechu* responsible for anti-HIV-1 activity[22]. Along with other therapeutic applications, the Ayurvedic Pharmacopoeia of India indicates the use of stembark of *Acacia catechu* in treating acute diarrhoea and helminthiasis.

By using synthetic antienterobacterial agents that are not based on existing agents is one of the best way to prevent antibiotic resisitance of pathogenic organisms. Some studies shown that medicinal plants are more effective in treating infectious diseases than the synthetic antibiotics[6][23][24]. And also the medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases[25]. There can also be a possible source for new potential agent to which the pathogenic organisms are not resistant. Most popular observations on the efficacy and use of medicinal plants contribute to the disclosure of their therapeutic properties, so that they are most frequently prescribed, even if their chemical constituents are not completely known[26].

The present study was done to evaluate the antienterobacterial activity of *Acacia catechu* extract against pathogens that causes enteric infection. The reducing capacity of a compound may serve as a significant indicator of its potential antienterobacterial activity. The extract used in our study showed varying degrees of antienterobacterial activity on the microorganisms which were tested. Further work is also needed to isolate the secondary metabolites from this extract studied in order to test specific antienterobacterial activity.

Conclusion:

Folk medicine an economically safe alternative to treat infectious diseases. In many developing countries Polymicriobial infection (20- 50%) seems as an widespread. In diarrhoea, it is a clear indication that the source of infection is related to grossly contaminated with food and water, affecting the patients from low-income group and those who are living in poor hygiene environments. Our findings in our study suggest that the antienterobacterial effects of the *Acacia catechu* extract, is an indication of its broad spectrum antienterobacterial potential which may be helpful in the management of enteric infections. However, further studies are necessary to isolate and reveal the active compounds that are present in the refined extract and also to establish its mechanism of action.

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