

Antimicrobial Activity of Albizzia Lebbeck Benth against Infectious Diarrhoea

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Citation

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Abstract

Aim: Medicinal plants are an important therapeutic aid for various ailments. The invitro antimicrobial activity of Albizzia lebbeck. Benth was screened against some pathogens isolated from diarrhoeal patients. **Procedure:** The bark of the plant was powdered and extracted separately with aqueous, methanol, chloroform, petroleum ether and hexane using maceration technique. The antimicrobial activity was carried out by disc diffusion method and minimum inhibitory concentration by two-fold serial dilution method. The microorganisms tested include Enterotoxigenic E.coli, Enteropathogenic E.coli, Salmonella typhimurium, Salmonella enteritidis, Shigella dysenteria, Shigella flexneri, Candida albicans, Candida tropicalis and Candida krusei. Amikacin 10 µg/disc and fluconazole 10 µg/disc were used as standards. **Results:** Aqueous, methanol and chloroform extracts exhibited activity against E.coli and Salmonella species. Petroleum ether and hexane extracts did not exhibit any activity. None of the extracts showed activity against Shigella and Candida species activity. **Conclusion:** This study revealed that the methanol extracts of Albizzia lebbeck was effective against E.coli and Salmonella strains associated with infectious diarrhoea. Further isolation of active compound responsible for the activity could be the potential sources of new antimicrobial agents

INTRODUCTION

Diarrhoea is a major public health problem in developing countries and is said to be endemic in many regions of Asia especially in India, and is the leading cause of high degree of morbidity and mortality among paediatrics. Enteric bacteria such as Salmonella, Shigella, Proteus, Klebsiella, E.coli, Pseudomonas and Vibrio cholerae, which are commonly associated with diarrhoea, comprise the major etiologic agents of sporadic and epidemic diarrhoea both in children and adults (WHO, 1985) Multiple drug resistance among enteropathogens in various geographic regions presents a major threat in the control of diarrhoea. Therefore indigenous medicinal plants as an alternative to antibiotic are said to play a significant role. In India, since ancient times, different parts of medicinal plants have been used for this particular aspect as a remedy or home cure for diarrhoea (Almeida et al, 1995). Due to the cost effectiveness, safety, increasing failure of chemotherapy and antibiotic resistance exhibited by pathogenic microbial agents, search for plant products has increased for their potential antimicrobial activity (Hammer et al, 1999).

Albizzia lebbeck. Benth belongs to family, Mimosaceae, commonly known as women's tongue tree. It is native to tropical southern Asia, is a large, erect, unarmed, deciduous spreading tree found throughout India and has been used in Ayurveda, Sidha and Unani medicines (Kirikar and Basu 1981). Albizzia species is reported to have many important medicinal properties mainly anti-inflammatory and analgesic properties (Ayurvedic Pharmacopoeia, 2001). A. lebbeck barks has acrid taste and is used for bronchitis, leprosy, paralysis, and helminthes infections and is reported to have antidiarrhoeal activity. Barks were mainly used in dental infections (Besra et al, 2002). Decoction of the leaves and barks were used in cold and cough, respiratory problems and against bronchial asthma. The plant extract were investigated against allergic rhinitis. (Pratibha et al, 2004). Seeds were used as astringent and also given for piles and diarrhoea (Besra et al, 2002). The extracts of the pods have antidiabetic activity and also used as anticancer. The phytochemical investigations showed that the pod of the A.lebbeck contains 3', 5' Dihydroxy 4', 7 dimethoxy flavone and N-Benzoyl L phenol alanimol (Rashid et al, 2003). The plant contains saponins, tannins, macrolytic

alkaloids, and Phenolic glycosides and flavonoids (Agarwal et al, 1991; Pal et al, 1995; Ying-Tsun Ma et al, 1997; El-Mousallamy, 1998).

MATERIALS AND METHODS

PLANT MATERIAL

The bark of *Albizzia lebbeck* was collected from Miralur, a small village in Chidambaram, Tamilnadu, India and was authenticated by the Department of Botany, Annamalai University, Annamalai Nagar, Tamilnadu, India.

PREPARATION OF EXTRACTS

The barks were shade dried and powdered. The powdered bark was extracted with various solvents, aqueous, methanol, chloroform, petroleum ether and hexane using maceration techniques. Twenty-five grams of powdered bark was extracted with 125 ml of solvent with occasional shaking for 3 days at room temperature. The extracts were filtered, concentrated and dried at 50°C and the weight of each residue was recorded and percentage yield was calculated.

ISOLATION OF ENTERIC PATHOGENS

One hundred paediatric patients with complaints of diarrhoea, attending Rajah Muthiah Medical College, Chidambaram, during June' 2008 was screened for enteric pathogens. The specimens were examined by Gram's stain and were inoculated in Sabouraud's dextrose agar with chloramphenicol for fungi and Blood agar, MacConkey agar for bacterial pathogens. The confirmation of enteric pathogens was done based on standard techniques (Collee et al, 1996). The stock cultures for bacteria and fungi were maintained in nutrient agar and Sabouraud's dextrose agar. Serotyping for *E.coli* and *Salmonella* strains were done in Central Research Institute, Kasauli, Himachal Pradesh, India.

SCREENING OF ANTIMICROBIAL ACTIVITY

The antimicrobial screening was evaluated against Enterotoxigenic *E.coli*, Enteropathogenic *E.coli*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella dysenteriae*, *Shigella flexneri*, *Candida albicans*, *Candida tropicalis* and *Candida krusei*, isolated from diarrhoeal patients. The activity of the above mentioned extracts were tested by disc diffusion method (Kirby-Bauer, 1996). The extracts were freshly reconstituted with 5% dimethyl sulphoxide (DMSO) at a concentration of 200 mg/ml. A sterile 6mm disc which were impregnated with 20µl of the extracts and placed aseptically over Muller-Hinton agar plates for bacteria and Sabouraud's dextrose agar plates for *Candida*, which were

previously inoculated with the test strains. Amikacin 10 µg/disc and fluconazole 10 µg/disc were used as positive control for bacteria and *Candida* respectively and 5% dimethyl sulphoxide (DMSO) impregnated disc was used as negative control. The plates were incubated at 37°C for 24 to 48 hours. Each experiment was carried out in triplicate. The diameters of zone of inhibition surrounding the discs were recorded.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

The extracts that showed antimicrobial activity by disc diffusion method were subjected to minimum inhibitory concentration (MIC) assay by using two-fold serial dilution method. MIC's were interpreted as the lowest concentration of the sample, which showed clear fluid without development of turbidity. All the MIC tubes, which did not show any turbidity, were streaked over the Muller-Hinton agar plates for bacteria and Sabouraud's dextrose agar plates for *Candida*. The minimum bactericidal concentration was recorded as the lowest concentration (MBC) that did not permit any visible growth on the plates after the period of incubation. Observation and results are given in Table 1 and Table 2.

PHYTOCHEMICAL EVALUATION

The methanol extract of *A. lebbeck* was evaluated for the presence of alkaloids, amino acids, saponins, tannins phenolic compounds, flavones and for carbohydrates by modified method of Brindha et al, 1981.

RESULTS AND DISCUSSION

Total of one hundred diarrhoeal paediatric patients was screened and 52 (91.2%) bacterial pathogens and 5 (8.8%) *Candida* was isolated from 57 (57%) patients. The bacterial pathogens include Enteropathogenic *E.coli* 14 (24.5%), Enterotoxigenic *E.coli* 25 (43.8%), *Salmonella typhimurium* 6 (10.5%), *Salmonella enteritidis* 4 (7%), *Shigella dysenteriae* 3 (5.2%), *Shigella flexneri* 2 (3.5%), *Candida albicans* 3 (3.5%), *Candida tropicalis* 1 (1.7%) and *Candida krusei* 1 (1.7%) and multiple pathogens 15 (26.3%). The antimicrobial assay showed that the aqueous, methanol and chloroform extracts of *Albizzia lebbeck* barks exhibited activities against Enterotoxigenic *E.coli*, Enteropathogenic *E.coli*, *Salmonella typhimurium*, *Salmonella enteritidis*. Petroleum ether and hexane extracts did not exhibit any activity. The inhibitory zones produced by methanol extract against *E.coli* and *Salmonella* spp. were higher than chloroform and aqueous extract. No activity was observed

against *Shigella* sp and *Candida* sp. Minimum inhibitory concentration of active extracts is shown in Table. 2. The MIC values observed for methanol extract was 2.5 mg/ml for *E.coli* strains and 5 mg/ml for *Salmonella* sp. The MBC values observed for *E.coli* was 5 mg/ml and for *Salmonella* sp was 10 mg/ml. Aqueous and chloroform extracts exhibited MIC and MBC values for *E.coli* and *Salmonella* sp. were 5 mg/ml and 10 mg/ml respectively.

Figure 1

Table. 1. Antimicrobial activity of . Benth bark extracts by disc diffusion assay.

Solvent extracts	Conc./disc	Zone of inhibition in mm								
		ETEC (25*)	EPEC (14*)	S.t (6*)	S.e (4*)	Sh.d (3*)	Sh.f (2*)	C.a (3*)	C.t (1*)	C.k (1*)
Aqueous	4 mg	2+	2+	1+	1+	-	-	-	-	-
Methanol	4 mg	4+	4+	4+	4+	-	-	-	-	-
Chloroform	4 mg	3+	3+	3+	3+	-	-	-	-	-
Petroleum ether	4 mg	-	-	-	-	-	-	-	-	-
Hexane	4 mg	-	-	-	-	-	-	-	-	-
Amikacin	10 µg	4+	4+	4+	4+	4+	4+	nt	nt	nt
Fluconazole	10 µg	nt	nt	nt	nt	nt	nt	4+	4+	4+
DMSO	-	-	-	-	-	-	-	-	-	-

Above values are the means of three assays. *indicates number of isolates; - indicates no activity; nt – not tested, 1+ indicates zone of inhibition in average of 7 to 10mm; 2+ indicates zone of inhibition in average of 11 to 14mm; 3+ indicates zone of inhibition in average of 15 to 18mm; 4+ indicates zone of inhibition in average of 19 to 22mm. ETEC- Enterotoxigenic *E.coli*, EPEC- Enteropathogenic *E.coli*, S.t - *Salmonella typhimurium*, S.e - *Salmonella enteritidis*, Sh.d - *Shigella dysenteria*, Sh.f - *Shigella flexineri*, C.a- *Candida albicans*, C.t- *Candida tropicalis* and C.k- *Candida krusei*.

The results reveal that the methanol extract of *Albizzia lebbeck* was effective against *E.coli* and *Salmonella* strains associated with infectious diarrhoea. A study by Srinivasan et al shows the aqueous extract possesses antibacterial activity against *E.coli* and *Salmonella* spp (Srinivasa et al, 2001). Preliminary phytochemical screening of the methanol extract showed presence of alkaloids, saponins, tannins, carbohydrates and amino acids. In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous system of medicine or indirectly in the pharmaceutical preparations (Yoganarasimhan, 2000). Ayurveda and Siddha are the two Indian traditional systems of medicine practiced in India wherein many herbs were used as therapeutics. There are

few reports on the use of plants in traditional healing by tribal and indigenous communities in Tamilnadu for treating chronic diarrhoea (Durai Pandiayan et al, 2006). Plant based remedy for gastrointestinal problem is been practicing from ancient times. The vast knowledge about the use of plant against diarrhoeal disease has been accumulated in areas where the use of plants is till in use. Therefore it is essential to explore the potential use of these medicinal plants.

Figure 2

Table. 2 Antimicrobial activity of . Benth bark extracts by two-fold serial dilution method

Extracts		MIC and MBC values are in mg/ml			
		ETEC	EPEC	S.t	S.e
Aqueous	MIC	5	5	5	5
	MBC	10	10	10	10
Methanol	MIC	2.5	2.5	5	5
	MBC	5	5	10	10
Chloroform	MIC	5	5	5	5
	MBC	10	10	10	10

MIC- Minimum inhibitory concentration, MBC- Minimum bactericidal concentration, ETEC- Enterotoxigenic *E.coli*, EPEC-Enteropathogenic *E.coli*, S.t-*Salmonella typhimurium*, and S.e - *Salmonella enteritidis*.

SUMMARY

In rural India, 70 percent of the population is dependent on the traditional system of medicine. To determine the potential and to promote the use of herbal medicine, it is essential to intensify the study of medicinal plants. *Albizzia lebbeck* is a popular tree, which is used by traditional practitioner to cure various ailments. Present study revealed the activity of methanol extract against some enteric pathogens. The detailed chemical nature of the active principles responsible for antibacterial activity is not known. Hence, further studies should be carried out to elucidate the active principles responsible for antimicrobial in *Albizzia lebbeck*.

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