

In vitro* Antimicrobial Activity and Phytochemical Analysis of *Ficus religiosa* L. and *Ficus bengalensis* L. against Diarrhoeal Enterotoxigenic *E. coli

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Issued 01 April 2009

Abstract

The barks of *Ficus religiosa* L. and *Ficus bengalensis* L., which belongs to family Moraceae, were investigated for *in vitro* antibacterial activity and phytochemical analysis. The various solvents extract like aqueous, methanol, chloroform, petroleum ether and hexane were screened for antibacterial activity against Enterotoxigenic *E. coli* isolated from diarrhoeal patients. The preliminary phytochemical analysis of the methanol extracts of both the plants showed the presence of carbohydrates, flavonoids, aminoacids, steroids, saponins and tannins. The extracts were subjected for antibacterial activity against Enterotoxigenic *E. coli* (ETEC) at 200mg/ml concentration by disc diffusion method. The results of antibacterial activity revealed that methanol extracts of both the plants barks exhibits good activity compared to chloroform and aqueous extracts. Petroleum ether and hexane extracts did not show any activity. The antibacterial activities of extracts were compared with standard antibiotics.

Key words: *Ficus religiosa*, *Ficus bengalensis*, diarrhoea, Disc diffusion Assay, medicinal plants.

Introduction

Diarrhoea is one of the most common causes of morbidity and mortality among infants and children in developing countries. Although commensal representatives found in the intestinal flora of humans are non-pathogenic, certain strains are highly pathogenic. Enterotoxigenic *E. coli* (ETEC) is the most prevalent among the various types of diarrhoeagenic *E. coli* in India (Taneja *et al.*, 2004). The increasing antibiotic resistance to commonly used antibiotics exhibited by diarrhoeal pathogens has led to the screening of several medicinal plants for their potential antimicrobial activity (Mukherjee, *et al.*, 1998). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Majorie Murphy Cowan.1999). So, the use of and search for drugs and dietary supplement derived from plants have accelerated in recent years.

Ficus religiosa. L. belongs to the family Moraceae, is commonly known as Peepal tree, and has many medicinal properties. The barks have been used for diarrhoea, dysentery, leucorrhoea, menorrhagia, for vaginal and other urogenital disorders. *Ficus bengalensis* belongs to the family Moraceae, which is commonly known

as Banyan tree. It is used in Ayurveda for treatment of diarrhoea, piles, teeth and skin disorders (Warrier *et al.*, 1995). The present study was aimed to carry out the preliminary phytochemical analysis and to screen invitro antibacterial activity against diarrhoeal Enterotoxigenic *E. coli* (ETEC) isolated from patients.

Materials and Methods

The barks of *F. religiosa* and *F. bengalensis* were collected in and around Chidambaram, Tamilnadu, India and identified, confirmed and authenticated by the Department of Botany, Annamalai University, Tamilnadu, India. The barks were washed, shade dried and extracted with aqueous, methanol, chloroform, petroleum ether and hexane for 48 hours with occasional shaking in a beaker. The extracts were filtered. The filtrate was dried at 50 to 60 °. The extracts were dried and percentage yield was calculated and subjected to preliminary phytochemical analysis. The invitro screening of antibacterial activity was carried out using three Enterotoxigenic *E. coli* (ETEC), isolated from diarrhoeal patients, attending Rajah Muthiah Medical College and Hospital, Annamali Nagar, Tamilnadu India.

The antibacterial screening of the extracts were carried out by determining the zone of inhibition using disc diffusion method (Sahoo *et al.*, 2006). The strains were grown to logarithmic phase in nutrient broth and the inoculum was prepared by adjusting the turbidity of bacterial suspension to 0.5 McFarland's tube with nutrient broth (Mc Farland *et al.*, 1987).

The dried extracts were dissolved in 5% Dimethyl sulphoxide (DMSO) to the concentration 200mg/ml and finally sterilized by filtration. The sterile discs (6mm in diameter) were impregnated with 20 µl of the above extracts to achieve desired concentration of 4mg/ml. The extract discs were placed on Muller-Hinton agar plates (Himedia), which were previously inoculated with test strains and incubated at 37°C for 24 hours. Amikacin disc (10µg) and 5% DMSO impregnated discs were used as positive and negative controls respectively and the zones of inhibition were recorded.

Results

Preliminary phytochemical analysis of the methanol extracts of the barks of *F. religiosa* and *F. bengalensis* showed the presence of carbohydrates, flavonoids, aminoacids, steroids, saponins and tannins. The antibacterial activity in terms of zone of inhibition is shown in Table 1.

Table. 1 Zone of inhibition of *Ficus religiosa*. L and *Ficus bengalensis* L.

Solvent extracts	Conc. of disc	<i>Ficus religiosa</i>			<i>Ficus bengalensis</i>		
		ETEC 1	ETEC 2	ETEC3	ETEC 1	ETEC 2	ETEC3
Aqueous	4mg/ml	8mm	8mm	10mm	8mm	12mm	10mm
Methanol	4mg/ml	12mm	12mm	14mm	16mm	14mm	14mm
Chloroform	4mg/ml	10mm	10mm	12mm	12mm	12mm	12mm
Petroleum ether	4mg/ml	-	-	-	-	-	-

Hexane	4mg/ml	-	-	-	-	-	-
Amikacin	10 µg	24mm	22mm	20mm	20mm	24mm	24mm
DMSO	5%	-	-	-	-	-	-

Discussion and Conclusion

The use of plants and plant preparations has been in existent since prehistory. The World Health Organization (WHO) reported that about 80% of the world's population depend mainly on traditional medicine and the traditional treatment involve mainly the use of plant extracts (WHO, 1993). In the present study, among the various solvent extracts, methanol extract found to be more active against all the Enterotoxigenic *E. coli*, which is isolated from diarrhoeal patients. These findings suggest new pathway in elucidating a potent antimicrobial agent from *Ficus religiosa* L. and *Ficus bengalensis* L. in general in *Ficus* species. *In vivo* experiments are needed to confirm these findings.

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