

**ANTIMICROBIAL ACTIVITIES OF SOME MEDICINAL PLANTS
AGAINST EXTENDED SPECTRUM BETA LACTAMASE PRODUCING
GRAM NEGATIVE ENTERIC BACTERIAL PATHOGENS**

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Summary

The objective of the study is to investigate the antimicrobial activity of methanol extracts of roots and rhizomes of *Asparagus racemosus*, tubers of *Acorus calamus* and roots and tubers of *Cyperus rotundus* against ESBL producing gram negative bacterial pathogens isolated from diarrhoeal patients. The antimicrobial activity was done by disc diffusion method at the concentration 200mg/ml. The results revealed that the plant *Asparagus racemosus* exhibits activity against all tested bacterial pathogens as compared to *Cyperus rotundus*, *Acorus calamus* did not exhibit any activity.

Key words: *Asparagus racemosus*, *Acorus calamus*, *Cyperus rotundus*, Antimicrobial activity.

Introduction

The emergence of extended-spectrum beta-lactamses (ESBLs) in gram negative bacteria is an increasing problem world wide. Several disease causing enteric bacteria has now become resistant to one or more antibiotics. The rate of development of resistance in gram negative bacteria due to production of ESBLs in 3rd generation cephalosporins (viz). Cefazidime, cefotaxime and ceftriaxone have found to be increasing ^[1]. Medicinal plants have been used for centuries for treating infectious diseases in humans because they contain compounds of therapeutic value. Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led to investigate the antimicrobial activity of medicinal plants ^[2].

Asparagus racemosus. Willd, family Liliaceae is an herb commonly known as *Shatavari*. It mostly found in several parts of India and in Srilanka. *A.racemosus* was used in Indigenous medicine. Roots of *A.racemosus* have been used as bitter tonic, emollient, constipation, galactagogue, carminative, stomachic and antiseptic [3]. *Cyperus rotundus* Linn, family Cyperaceae, is commonly known as *Mustaka*. It is habitat of South India, China, Burma and Srilanka [4]. The plant had been used in stomach disorders, diarrhoea, and dysentery. The roots and tubers are used as analgesic, antibacterial, antispasmodic, antitussive, aromatic, astringent, carminative, diaphoretic and vermifuge [5, 6]. An essential oil from the tubers has antibiotic property [7]. *Acorus calamus* Linn, family, Acoraceae, commonly known *Sweet flag* [8]. It has been found in central Asia, Europe and in North America. In India, it is found in Himalayan areas. The roots and tubers were used in the treatment of cough, fever, bronchitis, inflammation, depression, tumors, skin disease, carminative, stomachic and antidotes for several poisoning [9]. The purpose of this study was to screen the methanol extracts of these medicinal plants against ESBL producing enteric bacterial pathogens isolated from diarrhoeal patients.

Materials and Methods

Preparation of Extracts:

The plant *Asparagus racemosus* and *Acorus calamus* were procured locally and *Cyperus rotundus* was collected in and around Chidambaram. The plants were identified, confirmed and authenticated by the Department of Botany, Annamalai University, Tamilnadu, India. The plant parts were washed, shade dried, powdered and extracted separately 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 weighed and percentage yield was calculated. The dried extracts were dissolved in 5% dimethyl sulphoxide (DMSO) to the concentration 200mg/ml and finally sterilized by filtration.

Evaluation of Antimicrobial Activity:

The *invitro* screening of antimicrobial activity was carried out using *Enterotoxigenic E.coli*, *Enteropathogenic E.coli*, *Salmonella typhimurium*, *Salmonella entertidis*, *Shigella dysenteriae*, *Shigella flexineri*, *Klebsiella pneumonie*, *Pseudomonas aureginosa* isolated from diarrhoeal patients, attending Rajah Muthiah Medical College and Hospital, Annamalainagar, Tamilnadu, India. The screening of antimicrobial activity of the extracts was carried out by determining the zone of inhibition using disc diffusion method [10].

The bacterial 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 [11]. 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) was used as positive control and 5% DMSO impregnated discs was used as negative control and the zones of inhibition were recorded.

Results and Discussion

Results of antimicrobial activity has shown that the methanol extracts of *Asparagus racemosus* and *Cyperus rotundus* exhibited activity all the tested bacterial pathogens Enterotoxigenic *E.coli*, Enteropathogenic *E.coli*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella dysenteriae*, *Shigella flexineri*, *Klebsiella pneumoniae*, *Pseudomonas aureginosa*. The antibacterial activity in terms of zone of inhibition is shown in Table 1.

Table: 1 Antibacterial activity of *Asparagus racemosus*, *Acorus calamus* and *Cyperus rotundus*

Plant extracts	Conc. of disc	Zone of inhibition in mm							
		ETEC	EPEC	S.t	S.e	Sh.d	Sh.f	K.p	P.a
<i>A.racemosus</i>	4mg/ml	20	20	18	18	18	18	18	18
<i>C.rotundus</i>	4mg/ml	16	16	12	12	12	12	12	12
<i>A.calamus</i>	4mg/ml	-	-	-	-	-	-	-	-
Amikacin	10 µg	24	22	20	20	24	24	22	20
DMSO	5%	-	-	-	-	-	-	-	-

Above values are the means of three assays. -: no activity. ETEC - Enterotoxigenic *E.coli*, EPEC - Enteropathogenic *E.coli*, S.t - *Salmonella typhimurium*, S.e - *Salmonella enteritidis*, Sh.d - *Shigella dysenteriae*, Sh.f - *Shigella flexineri*, K.p - *Klebsiella pneumoniae*, P.a - *Pseudomonas aureginosa*.

The methanol extract exhibited relatively wide zone of inhibition on the three enteric bacterial pathogens as compared with chloroform and aqueous extracts.

Petroleum ether and hexane extract did not exhibit any activity. From this study it is evident that the methanol extracts of *Asparagus racemosus* and *Cyperus rotundus* has the *invitro* inhibition potential against various pathogens that cause infectious diseases. However, further identification and isolation of the single compound and *invivo* testing was required for development of novel bioactive antimicrobial compounds that are responsible for the antibacterial effects.

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