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Antibacterial studies on medicinal plants used in urinary disorders

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ABSTRACT

Two plants species, namely Tribulus terrestris Linn (Fam. Zygophyllaceae) and Tribulus alatus Delile (Fam. Zygophyllaceae) used in urinary disorders in India were tested for their antibacterial activity against both Gram-positive and Gram-negative bacteria using agar disc diffusion method. The efficacy of the extracts was compared to the standard antibiotics.

Keywords: *Tribulus terrestris*, *Tribulus alatus*, antibacterial activity, agar disc diffusion assay.

INTRODUCTION

Natural products perform various functions and many of them have interesting and useful biological activities. There are more than 35,000 plants species being used in various human cultures around the world for medicinal purpose. Biologically active compounds present in medicinal plants have always been of great interest to scientists working in this field [1]. The present study was designed to evaluate the antibacterial activity of some important medicinal plants such as *Tribulus terrestris* Linn, locally known as 'Siru Nerungil' in Tamil and *Tribulus alatus* Delile (Fam. Zygophyllaceae), locally known as 'Peru Nerungil' in Tamil used in urinary disorders in Ayurveda[2].

MATERIALS AND METHODS

Plant material

The fresh fruit of *Tribulus terrestris* was collected from Kovilpatti area of Tuticorin District and fruit of *Tribulus alatus* was collected from Viralimalai area of Pudukottai District, Tamil Nadu, India in the month of November 2010. The specimens were identified by a taxonomist Dr. V. Chelladurai, Retired Research Officer (Botany), Survey of Medicinal Plants Unit, CCRAS, Palayamkottai, Tamil Nadu, India.

Extraction of plant material

The fresh fruit samples were washed, dried and ground to fine powder using blender. About 25 g of ground powder was taken in stoppered flask and successively extracted with ethanol, chloroform and water or 5 days with occasional shaking. After 5 days extract was filtered and evaporated on a water bath [3].

Test microorganisms and microbial culture

Eight bacterial strains were used in this study, two gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and six gram-negative (*Enterobacter spp, Aeromonas spp, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Citrobactor spp*) obtained from Department of Microbiology, Sri Paramakalyani College, Alwarkurichi, Tamil Nadu, India. The bacterial strains were cultivated at 37^0C and maintained on nutrient agar slant at 4^0C [4].

Preparation of antimicrobial disc

Sterile discs were procured from Hi-fi media and used for the preparation of antimicrobial disc. The extracts of the medicinal plants were incorporated to the sterile disc. Each sterile disc was incorporated individually with the volume equivalent to 2 mg/ml dose of the extracts using a calibrated micropipette. Precautions were taken to prevent the overflow of the solvent from the outer surface of the disc. To ascertain this, the discs applied in small quantities and the discs were allowed for air–drying followed by another dose of the extract [5].

Assay of Antibacterial activity

The bacterial cultures were smeared on the sterile, air-dried nutrient agar plates using sterile cotton swab. Sterile discs loaded with known quantity of antibacterial compounds were placed on the surface of the nutrient agar petriplate with the help of flame sterilized forceps. Control discs were placed in the nutrient agar plates incorporating at the solvents only. Then the petriplates were incubated at 37^0C for 24 hrs. The zone of inhibition was observed and measured with the help of a Vernier caliper [6].

RESULTS

Table 1 Antibacterial activity of standard antibiotics

Name of Microorganisms	Zone of inhibition (diameter) in mm						
	Cr	**Cx**	**F**	**Va**	**Ak**	**Cf**	**Fr**
Staphylococcus aureus	33	21	21	17	30	35	22.5
Bacillus cereus	-	-	18.5	-	22	29	-
Enterobacter spp	-	-	15	23	40	-	20
Aeromonas spp	-	-	18	25	30	42	26
Klebsiella pneumoniae	-	-	-	-	-	-	-
Escherichia coli	19	-	21	17	22.5	35	20
Pseudomonas aeruginosa	-	-	18.5	-	22	29	-
Citrobactor spp	-	-	18	25	30	42	26

Cr = Cephaloridine *Ak = Amikacin*
Cx = Cloxaccilin *Cf = Ciprofloxacin*
F = Framycetin *Fr = Furozolidone*
Va = Vancomycin

Table 2 Antibacterial activity of *Tribulus terrestris* Extracts

Name of Microorganisms	Zone of inhibition (diameter) in mm		
	Water	Ethanol	Chloroform
Staphylococcus aureus	-	-	-
Bacillus cereus	-	11	-
Enterobacter spp	-	15	-
Aeromonas spp	-	-	-
Klebsiella pneumoniae	-	-	14
Escherichia coli	-	18	-
Pseudomonas aeruginosa	-	-	-
Citrobactor spp	-	-	-

Table 3 Antibacterial activity of *Tribulus alatus* Extracts

Name of Microorganisms	Zone of inhibition (diameter) in mm		
	Water	Ethanol	Chloroform
Staphylococcus aureus	-	-	
Bacillus cereus	-	11	-
Enterobacter spp	-	15	-
Aeromonas spp	-	9	-
Klebsiella pneumoniae	-	-	-
Escherichia coli	-	12	-
Pseudomonas aeruginosa	-	-	-
Citrobactor spp	-	11	12.5

DISCUSSION

No inhibitions were observed with the water extracts of both *Tribulus terrestris* and *Tribulus alatus* fruits samples. The ethanol and chloroform extracts of the fruits of the plants possesses antibacterial properties. In the antibacterial studies on the ethanol extracts of both *Tribulus terrestris* and *Tribulus alatus* fruits showed sensitive inhibition (more than 12mm) in 2 mg/ml dose against *Enterobacter spp* and *Escherichia coli*. The ethanol extract of *Tribulus terrestris* showed intermediate inhibition (less than 12 mm) against *Bacillus cereus* and the chloroform extract showed moderate inhibition of 14mm in 2 mg/ml dose against *Klebsiella pneumoniae*. The ethanol extracts of *Tribulus alatus* fruits showed intermediate inhibition against *Bacillus cereus, Aeromonas spp* and *Citrobactor spp.* The chloroform extracts of *Tribulus alatus* fruits showed significant activity against *Citrobactor spp*. The highest antibacterial activity of 18 mm in *Tribulus terrestris* against *Escherichia coli* and least activity recorded in *Aeromonas spp* in ethanol extracts of *Tribulus alatus.*

The results of present investigation clearly indicate these plants fruits have antibacterial activity. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drug.

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