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Research Article

EVALUATION OF ANTIBACTERIAL ACTIVITY OF *CARALLUMA ADSCENDENS* ROXB. STEM

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ABSTRACT
Antibacterial activity of various extracts of stem of *Caralluma adscendens* var. fimbriata, Roxb., Family Asclepediaceae was studied against *Bacillus pumilus, Escherichia coli, Staphylococcus aureus, Shigella sonnei*. Among the various extracts petroleum ether extract is effective antimicrobial against *S. aureus* and *E.coli* while n- n-butanol extract is effective against *Shigella sonnei* and *B. pumilus* at a concentration of 5 mg/ml and 10 mg/ml. The inhibitory effect of various extracts was compared with standard antibiotic Gentamycin. MIC for both extracts was also determined.
KEYWORDS: *Caralluma adscendens* Roxb., Disc diffusion method, Antibacterial.

INTRODUCTION

Caralluma adscendens var. fimbriata, Family Asclepediaceae, is succulent plant found in Africa, India, south Europe, Ceylon and Afghanistan. In India it is found in the Kolli Hills of Tamil Nadu, in arid regions of Andhra Pradesh, Kerala and Maharashtra. It is essentially a vegetable of daily use in tribal India and also eaten during famines. It is used in preserves like chutneys and pickles[1]. The key phytochemical constituent of the herb are pregnane glycoside (25%), flavone glycosides (chemotaxonomic marker), saponin glycoside (10%), magastamine glycoside, bitters (3%), sitosterol and tomantogenin.[2,3] *Caralluma* species have shown anti- inflammatory, anti-nociceptive,[3,4] antidiabetic,[5,6] gastric mucosa protecting,[7] antiulcer and cytoprotective[8] properties.

As a result of indiscriminate use of antimicrobial drugs in the treatment of infectious diseases, microorganisms have developed resistance to many antibiotics. There is need to develop alternative antibiotic drugs from plant. One approach is to screen local medicinal plants which represent rich source of novel antimicrobial agents. The present study was carried out to investigate the antibacterial properties of *Caralluma adscendens* extracted by various solvents. Inhibitory effect by zone of inhibition and minimum inhibitory concentration (MIC) were carried out in this study.

MATERIALS AND METHODS

The Plant *Caralluma adscendens* used for the study were obtained from Satara District and Laling Ghat of Dhule District. Botanical identification was performed at Botanical Survey of India, Pune and Herbarium specimen number RCP/07C has been deposited at department of R.C. Patel College of Pharmacy, Shirpur. The stem of the plant was dried under shade and ground into powder.

EXTRACTION

The 100 g of powder was successively extracted in soxhlet apparatus with petroleum ether (60-80°C), n-butanol and methanol. Lastly powder was kept in distilled water for 3 days. All the extracts were filtered and concentrated under reduced pressure using rotary evaporator (Roteva Equitron, Mumbai) and dry extracts were obtained.

Preparation of extract solution

Dry extracts were dissolved in sterile Dimethyl sulphoxide (DMSO AR Grade) to a final concentration of 5 mg/ml for disc diffusion assay and for broth microdilution technique. All the extracts were sterilized by passing through a 0.45µm membrane filter.

MICRO-ORGANISM

The bacterial strains used in the study were *Staphylococcus aureus* NCIM 2079, *Bacilus pumilus* NCIM 2327, *Escherichia coli* NCIM 2109, *Shigella sonnei* MTCC 2957. All the bacterial strains were grown and maintained on nutrient agar slants. The inoculum size of each test strain was 1 X 10^8 bacteria /ml for disc diffusion assay which was standardized by adjusting the optical density of the bacterial suspension to turbidity corresponding to spectrophotometric absorbance = 0.5 at 540 nm.

SCREENING OF ANTI BACTERIAL ACTIVITY

Disc diffusion method was carried out to evaluate the anti bacterial activity by using Muller Hinton agar.[9, 10] Sterile filter paper disc Whatman (No.1, 6 mm) was impregnated with 100 µL of each of the extracts (5mg/ml and 10 mg/ml) to give a final concentration of 0.5 mg/ disc and 1.0mg/disc. The discs were properly placed on already seeded Muller Hinton agar plates. Sterile DMSO served as negative control. Gentamycin was used as a standard to compare antibacterial potential of extracts. All the plates were incubated for 24 hrs, at 37°C. The antibacterial activity was interpreted by determining diameter of zone of inhibition (in mm). Each extracts was assayed in triplicate.

Minimum inhibitory concentration (MIC)

Assay

MIC of Petroleum ether extract was determined against *S. aureus* and *E. coli* while MIC of n-butanol extract was determined against *B. pumilus* and *Shigella sonnei* using the two fold serial microdilution method.[11, 12] The concentration used in experiment ranging from 5 mg/ ml to 0.0781 mg/ml. The tested extracts were added to sterile micro titer plates containing Muller Hinton. In each plate diluted bacterial suspension (final inoculum of 1X10^8 bacteria/ml) were added. The bacterial suspension was used as a positive control and extracts in both were used as a negative control. The MIC values were taken as the lowest concentration of the extracts in wells of the microtiter plate that showed no turbidity after 24 hrs of the incubation at 37°C. The turbidity of the wells in microtiter plate was interpreted as visible growth of the microorganisms. Each extract was assayed in triplicate.

Table no. 1 Antimicrobial activity of various extracts of stem of *Caralluma adscendens* var. *fimbrita*

Extract	Concentration (mg/ml)	Zone of inhibition(mm)#			
		S. aureus	*E. Coli*	*S.sonnei*	*B.Pumilus*
PE	5	16	16	13	13
	10	20	22	17	16
BE	5	13	10	15	15
	10	16	14	19	19
ME	5	12	11	12	09
	10	14	15	14	10
AE	5	11	10	09	12
	10	13	13	11	14
Standard (Gentamycin)	50 (µg/ml)	17	18	15	17

PE= Petroleum ether extract, BE= n-butanol Extract, ME= Methanol extract, AE= Aqueous extract
# Values are average of three determinations

Table no. 2 Determination of MIC Values of Stem of *Caralluma adscendens* var. *fimbriata*

Concentration (mg/mL)	Pet. Ether extract		n-butanol Extract		Control	
	S. aureus	*E. coli*	*Shigella*	*B. pumilus*	Positive	Negative
5.0000	-	-	-	-	+	-
2.5000	-	-	-	-	+	-
1.2500	-	-	-	-	+	-
0.6250	-	-	-	-	+	-
0.3125	-	-	+	-	+	-
0.1563	-	-	+	+	+	-
0.0781	+	+	+	+	+	-
0.0391	+	+	+	+	+	-
0.0195	+	+	+	+	+	-
0.0098	+	+	+	+	+	-

'-'Absence of growth; '+'Presence of growth;
Positive control: Bacterial suspension +Extract and broth; Negative Control: Extracts and broth

RESULT

Various extracts of *Caralluma adscendens* were tested for antibacterial activity against bacteria, *S. aureus, B. pumilus, E. coli, Shigella sonnei* by disc diffusion method. The results were summarized in table no. 1. According to the results of zone of inhibition represented by petroleum ether and n-butanol extracts, the focus has been shifted towards determination of MIC of both extracts (table no. 2). The minimum concentration of petroleum ether extract at 0.1563 mg/ml exhibited higher activity against *S. aureus* and *E. coli* while minimum concentration of n-butanol extract at 0.6250 mg/ml exhibited antibacterial activity against *Shigella sonnei* and *B. pumilus.*

DISCUSSION

All four extracts showed antibacterial activity. Petroleum ether extract and n-butanol extract exhibited good antibacterial activity against Gram positive and Gram negative bacteria at 5 mg/ml and 10 mg/ml as compared to standard sample. Significant inhibition against *Shigella sonnei* proves antidiarrhoeal potential of n-butanol extract of *Caralluma adscendens*. Qualitative chemical investigation revealed the presence of flavonoids and saponin glycosides in n-n-butanol extract. Antibacterial activity may be attributed due to the presence of flavonoids.[13, 14] The detailed chemical nature of the active principle (s) responsible for the antibacterial property is under progress.

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