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Research Article

EVALUATION OF ANTIMICROBIAL EFFICACY OF ECBOLIUM VIRIDE (FORSSK.) ALSTON ROOT EXTRACTS

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ABSTRACT

The present study was focused to screen the antimicrobial activity of hexane, ethyl acetate and methanol extracts from the roots of *Ecbolium viride* (Forssk.) Alston. The extracts were treated against nineteen bacterial and twelve fungal species using disc diffusion method followed by determination of minimum inhibitory concentration (MIC) by broth dilution method. The results of the study revealed that ethyl acetate extract possesses higher degree of antimicrobial activity than other extracts. The highest antibacterial activity (MIC-0.039 mg/ml) was exhibited against *Staphylococcus aureus* (ATCC 25923), while the highest antifungal activity (MIC-0.25 mg/ml) was exhibited against *Malassezia pachydermatis.* Thus the ethyl acetate extract showing significant antibacterial and moderate antifungal activity could be used in the treatment of infectious diseases.

Keywords: Green Shrimp; Antimicrobial; Zone of inhibition.

INTRODUCTION

In traditional medicine, plants serve as precious source of natural products for sustaining human health[1]. Infectious diseases are still a major threat to public health, besides the tremendous growth in human medicine. Though pharmacological industries have produced a number of novel antibiotics, some pathogens rapidly become resistant to many of the first discovered effective drugs[2]. Therefore the use of antimicrobial compounds from unexplored plants may inhibit bacterial growth by different mechanisms than those presently used as antimicrobials and have a significant clinical value in the treatment of resistant microbial strains.

Ecbolium viride (Forssk.) Alston (Acanthaceae) is a perennial woody undershrub (also known as Green Shrimp) found in plains of India and also in Arabia, Malaysia, Sri Lanka and Tropical Africa. In folk medicine, aqueous extract of dried roots of the plant are used for treating jaundice, rheumatism and menorrhagia[3, 4]. Phytochemical screening has shown the presence of alkaloids, carbohydrates, glycosides, tannins and saponins[5]. The roots of *E. viride* have been reported to have anthelmintic[6], antioxidant[7], anti-inflammatory[8], anti-hepatotoxicity[9,10], antiplasmodial, antitrypanosomal and antimalarial activity[11]. Methanolic extract from aerial parts of the plant exhibited antibacterial activity[12]. However, there is no report on the antimicrobial activity of root extracts. In the present study, we have investigated the antimicrobial potential of organic solvent extracts from the roots of *E. viride* against human pathogenic bacteria and fungi.

MATERIALS AND METHODS

Plant material

Roots of *E. viride* were collected during summer (June) of 2011 from Srirangam, Trichy, Tamil Nadu, India. It was authenticated by Dr. P. Jayaraman, Scientist from the Plant Anatomy Research Centre (PARC) Tambaram, India. A voucher specimen of the plant (PARC/2012/1152) was deposited in the herbarium of Department of Plant Biology and Biotechnology, Loyola College, Chennai, India.

Preparation of Crude extract

The roots were cleaned, shade dried at room temperature and then milled to a fine powder in manual mill and stored in closed containers in the dark until further use. The extraction was carried out with different solvents in the increasing order of polarity, namely: hexane, ethyl acetate and methanol by soaking the material in respective solvents (1:3 w/v) for 48 h at room temperature. The extract was filtered through Buchner funnel with Whatman number 1 filter paper and the filtrate was condensed in the rotary evaporator (Equitron, India). The extraction with different solvents was carried out sequentially.

Test microorganisms

In vitro antimicrobial activity was examined against ninteen bacterial and twelve fungal species which include gram negative bacteria viz., *Escherichia coli* (ATCC 25922), *Erwinia amylovora* (MTCC 2760), *Klebsiella pneumoniae* (ATCC 15380), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (MTCC 1771), *Proteus mirabilis* (ATCC 49565), *Salmonella paratyphi-B* and *Vibrio cholerae* (ATCC 14035). Gram positive: *Bacillus subtilis* (ATCC 441), *Enterococcus faecalis* (ATCC 29212), *Enterobacter aerogens* (MTCC 111), *Micrococcus luteus* (ATCC 4698), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (MTCC 3615) and *Shigella flexneri* (MTCC 1457). Clinical isolates: *Escherichia coli* (ESBL-3904), *Klebsiella pneumoniae* (ESBL - 3971), *Staphylococcus aureus* (MRSA - methicillin resistant) and *Enterococcus durans* (P502). Fungal strains: *Candida albicans* (MTCC 227), *Candida krusei, Candida tropicalis*, *Microsporum gypseum, Malassezia pachydermatis*, *Trichophyton rubrum* 57/01, *Trichophyton mentagrophytes* 66/01, *Epidermophyton flocussum* 73/01, *Scopulariopsis* sp. 101/01, *Aspergillus flavus*, *Botyritis cinerea* and *Curvularia lanata* 46/0. All the microbial cultures were obtained from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.

Culture media and growth conditions

Bacterial inoculum was prepared by growing the cells in Mueller Hinton Broth (MHB) (Himedia, India) for 24 h at 37 °C. The cell suspension was diluted with sterile MHB to provide initial cell count of 10^8 CFU/ml. The filamentous fungi were grown on Sabouraud Dextrose Agar (SDA) slants at 28 °C for 10 days and the spores were collected using sterile double distilled water and homogenized.

Antibacterial activity by disc diffusion method

Antibacterial activity of different extracts was carried out by the disc diffusion method[13]. Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Himedia, India). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at three different concentrations (1.25, 2.5 and 5.0 mg/disc) for the crude extract. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Ciprofloxacin (5 µg/disc) served as the positive control. The plates were incubated for 24 h at 37 °C for bacteria. Zone of inhibition were recorded in millimeters and the experiment was repeated thrice.

Antifungal activity and minimum inhibitory concentration (MIC)

The antifungal activity and MIC test were performed according to the standard reference methods for bacteria[13], filamentous fungi[14] and yeasts[15]. The extracts were dissolved in water + 2 % dimethyl sulfoxide (DMSO). The initial concentration of extract was 5 mg/ml for bacteria, 1 mg/ml for fungi and it was serially diluted two fold. Each well was inoculated with 100 µl of suspension containing 10^8 CFU/ml of bacteria and 10^4 spores/ml of fungi, respectively. The antibacterial agent ciprofloxacin and antifungal agent fluconazole were included in the assay as positive controls. For fungi, the plates were incubated for 24, 48 or 72 h at 28 °C upto 9 days for dermatophytes while bacterial plates were incubated for 24 h at 37 °C. MIC was defined as the lowest extract concentration showing no visible fungal growth after incubation time. Tested broth of 5 µl was placed on the sterile MHA plates for bacteria and incubated at respective temperature. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate. All the experiments were performed in triplicates and the results were averaged.

RESULTS AND DISCUSSION

The different extracts of *E. viride* roots showed varying degree of antibacterial and antifungal activity. The results indicated that ethyl acetate extract showed strong antibacterial activity when compared to other solvent extracts. The maximum zone of inhibition was observed against the gram positive bacterium *S. aureus* (31 mm) followed by gram negative bacterium *E. amylovora* (24 mm) at 5 mg/disc (Table 1). Similarly numerous studies have shown the effectiveness of ethyl acetate extract against a wide range of bacteria and fungi [2,16,17]. Ethyl acetate extract showed an MIC value of 0.039 mg/ml against *S. aureus* and 0.078 mg/ml against *E. amylovora* (Table 2). Minimum inhibitory concentration of different extracts of *E. viride* against fungi indicates that ethyl acetate extract has highest antifungal activity 0.25 mg/ml against *M. pachydermatis* (Table 3). The lower MIC value is an indication of high effectiveness of the extract whereas higher MIC indicates less effectiveness of the extract. Hence the current study on roots of *E. viride* showed potential antimicrobial activity and therefore further investigation is in progress to characterize the active principle, for the formulation of new drugs.

Table 1: Antimicrobial activity of *Ecbolium viride* root extracts against the bacterial strains

Tested organisms	Disc diffusion method (inhibition zone, mm)									
	Hexane (mg/ml)			Ethyl acetate (mg/ml)			Methanol (mg/ml)			Ciprofloxacin (µg/ml)
	1.25	2.5	5.0	1.25	2.5	5.0	1.25	2.5	5.0	5.0
Gram positive										
Bacillus subtilis	12	14	18	11	16	20	-	12	14	25
Enterococcus faecalis	-	-	-	-	-	-	-	-	-	25
Enterobacter aerogens	-	-	9	-	-	-	-	-	-	30
Staphylococcus aureus	16	21	29	17	24	31		10	15	30
Staphylococcus epidermidis	-	-	-	-	-	9	-	-	-	26
Shigella flexneri	-	-	10	-	9	12		-	-	26
Micrococcus luteus	-	-	-	-	-	-	-	-	-	20
Gram negative										
Escherichia coli	-	-	-	-	-	-	-		-	15
Erwinia amylovora	8	10	12	18	20	24	-		-	30
Klebsiella pneumoniae	11	12	15	14	16	18	-	-	10	25
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	20
Proteus vulgaris	-	-	-	9	10	11	-	-	-	20
Proteus mirabilis	-	8	10	14	18	21	-	9	11	18
Vibrio cholerae	-	-	-	10	12	14	-	8	9	19
Salmonella paratyphi-B	-	-	-	-	-	-	-	-	-	18
Clinical isolates										
Escherichia coli	-	-	-	-	-	-	-	-	-	18
Klebsiella pneumoniae	-	-	-	8	10	12	-	-	9	21
Staphylococcus aureus	-	-	-	-	9	12	-	-	8	25
Enterococcus durans	-	-	-	-	-	-	-	-	-	19

Values are the mean of three repeat experiments; (–) no activity; Ciprofloxacin-standard antibacterial agent

Table 2: The minimum inhibitory concentrations (MIC) of *Ecbolium viride* root extracts against the bacterial strains

Tested organisms	Minimum inhibitory concentration			
	Hexane (mg/ml)	Ethyl acetate (mg/ml)	Methanol (mg/ml)	Ciprofloxacin (µg/ml)
Gram positive				
Bacillus subtilis	0.625	0.312	2.5	0.195
Enterococcus faecalis	-	-	-	6.25
Enterobacter aerogens	2.5	-	-	0.39
Staphylococcus aureus	0.078	0.039	2.5	0.39
Staphylococcus epidermidis	-	2.5	-	6.25
Shigella flexneri	2.5	2.5	-	0.195
Micrococcus luteus	-	-	-	-
Gram negative				
Escherichia coli	-	-	-	-
Erwinia amylovora	0.625	0.078	-	0.195
Klebsiella pneumoniae	0.312	0.156	2.5	0.39
Pseudomonas aeruginosa	-	-	-	100
Proteus vulgaris	-	1.25	-	nt
Proteus mirabilis	1.25	0.312	2.5	nt
Vibrio cholerae	-	0.625	1.25	0.39
Salmonella paratyphi-B	-	-	-	-

Clinical isolates				
Escherichia coli	-	-	-	0.195
Klebsiella pneumoniae	-	0.625	2.5	0.39
Staphylococcus aureus	-	2.5	5	0.39
Enterococcus durans	-	-	-	nt

Values are the mean of three repeat experiments; nt: not test; (–) no activity; Ciprofloxacin-standard antibacterial agent

Table 3: The minimum inhibitory concentrations (MIC) of *Ecbolium viride* root extracts against selected fungi

Tested organisms	Minimum inhibitory concentration			
	Hexane (mg/ml)	Ethyl acetate (mg/ml)	Methanol (mg/ml)	Flucanazole (µg/ml)
Dermatophytes				
Candida albicans	0.5	0.5	1	-
Candida krusei	-	-	-	-
Candida tropicalis	-	-	-	-
Microsporum gypseum	-	-	-	nt
Malassezia pachydermatis	0.25	0.25	-	12.5
Trichophyton rubrum	-	-	-	25
Trichophyton mentagrophytes	-	-	-	25
Epidermophyton flocussum	-	0.5	-	12.5
Scopulariopsis sp.	0.5	0.5	1	6.25
Aspergillus flavus	-	-	-	25
Opportunistic pathogens				
Botyritis cinerea	-	-	-	nt
Curvularia lanata	-	-	-	6.25

Values are the mean of three repeat experiments; nt: not test; (–) no activity; Flucanazole standard antifungal agent

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