

Original Article

Antimicrobial Properties of *Eranthemum roseum* (Vahl) R.Br.

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Abstract

Antimicrobial activity of the roots of *Eranthemum roseum* (Vahl) R.Br. (Dasmuli), were tested against different bacteria (including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Shigella sonnei*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus vulgaris*, *Pseudomonas aeruginosa*) and fungi (such as *Candida albicans* and *Aspergillus niger*) by cup plate diffusion method. Minimal Inhibitory Concentration (MIC) values of each active extract were determined. The results obtained showed strong activity of the petroleum ether extract of the roots of plant against the bacteria and fungi used as test organisms.

Keywords: *Eranthemum roseum*; Dasmuli; Acanthaceae; Antimicrobial activity.

Introduction

Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide. During the past several years, there has been an increasing incidence of bacterial and fungal infections due to a growth in immunocompromised population such as organ transplant recipients, cancer and HIV/AIDS patients. The changing pattern of clinical evaluation and regulatory requirements for merits and demerits of drugs will be highlighted for future challenges and advances in antimicrobial drug development. Resurgence in the use of herbal medicines worldwide has provided an excellent opportunity to Indian companies to look for therapeutic leads from Indian ancient system of ayurveda that could be utilized for drug development.¹

Eranthemum roseum (Vahl) R.Br. (Dasmuli) (Fam.Acanthaceae) is upto 2m height and found in tropical and subtropical

parts of Asia. In India, it is found in Karnataka, Maharashtra and Chathisgarh. This shrub is cultivated in Indian gardens for its attractive foliage and flowers. Flowers are blue when they are fresh and become brown on drying. Ethanobotanically, root of *Eranthemum roseum* (Vahl) R.Br. boiled in milk is popular remedy for leucorrhoea.² Roots are also given to pregnant cattle to promote the growth of foetus.³ The present work was carried out to investigate the antimicrobial activity of different extracts of the roots of *Eranthemum roseum* (Vahl) R.Br.

Experimental

Plant material

Roots of *Eranthemum roseum* (Vahl) R.Br. were collected during June (2004) from Satpuda valley in Nandurbar district, Maharashtra in India. The plant was authenticated at Department of Botany, S.S.V.P.S's College of Science, Dhule (Maharashtra) and stored in the Herbarium of Pharmacognosy Department, R. C. Patel College of Pharmacy, Shirpur.

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Table 1. Antimicrobial activity of various extracts of roots of *Eranthemum roseum*.

ORGANISMS	DIAMETER OF ZONE OF INHIBITION (mm)					
	Extracts				Standards	
	a 2 mg/ml	b 2 mg/ml	c 2 mg/ml	d 2 mg/ml	A 20 µg/ml	B 20 µg/ml
Bacteria						
<i>Escherichia coli</i>	17	14	14	13	17	-
<i>Staphylococcus aureus</i>	16	14	23	16	17	-
<i>Bacillus subtilis</i>	14	13	10	10	17	-
<i>Shigella sonnei</i>	12	13	10	10	17	-
<i>Klebsiella pneumoniae</i>	13	9	12	10	15	-
<i>Salmonella typhi</i>	15	13	11	-	15	-
<i>Proteus vulgaris</i>	14	10	10	-	16	-
<i>Pseudomonas aeruginosa</i>	12	10	10	-	17	-
Fungi						
<i>Aspergillus niger</i>	16	14	16	21	-	17
<i>Candida albicans</i>	15	13	13	19	-	16

a= petroleum ether extract, b=chloroform extract, c= methanolic extract, d= aqueous extract.

A=standard antibacterial agent (Gentamycin, 20µg/ml)

B= standard antifungal agent (Amphotericin, 20µg/ml)

The MIC values are shown in Table 2. The presence of activity within the extracts used in the preliminary tests may well depend on the concentration of extracts

Preparation of plant extracts

For the preparation of various extracts from the roots of *Eranthemum roseum* (Vahl) R.Br., they were shade dried at room temperature and powdered by electric pulveriser. Petroleum ether, chloroform, and methanol extract obtained by successive extraction method by Soxhlet apparatus and aqueous extract by maceration method. All the extracts were concentrated under reduced pressure by rotary vacuum evaporator (Roteva) with the yield of 1.16%, 3.20%, 8.80%, and 27.20%, respectively.

Test microorganisms

Strains, including fungi and bacteria were obtained from National Chemical Laboratories (NCL), Pune and Microbial Type Culture Collection (MTCC), Chandigarh.

Escherichia coli NCIM 2109, *Staphylococcus aureus* NCIM 2079, *Bacillus subtilis* NCIM 2250, *Shigella sonnei* MTCC 2957, *Klebsiella pneumoniae* NCIM 2719, *Salmonella typhi* MTCC 735, *Proteus vulgaris* NCIM 2813, *Pseudomonas aeruginosa* NCIM 2036, *Candida albicans* MTCC 227 and *Aspergillus niger* NCIM 545 were used as test

organisms.

Method of preparation of test organism suspension

Test organism was maintained on slants of medium containing 300 mg of manganese sulphate per liter and transferred to fresh slant once a week. Then the slants were incubated at temperature 32°C for 24 h. Organism was then washed by using 3 ml saline solution from agar slant onto a large agar surface of medium such as Roux bottle containing 250 ml agar. It was incubated for 24 h. Using 50 ml saline solution, the growth from the nutrient surface was washed. Then organism was stored under refrigeration. Inoculum was adjusted at 530 nm, leading to transmission equivalent to 1×10^8 cells/ml.

Antimicrobial assay

Antimicrobial activity of the above mentioned extracts was determined, using a modified cup plate method (Kirby-Bayer method)⁴. Muller Hinton agar was used for the growth of bacterial strains and Potato Dextrose agar was used for the growth of fungi. In case of spore producing organisms,

Table 2. Minimum inhibitory concentrations of extracts of the roots of *Eranthemum roseum* for various microorganisms.

ORGANISMS	CONCENTRATION (mg/ml)			
	Extracts			
	Petroleum ether mg/ml	Chloroform mg/ml	Methanol mg/ml	Aqueous mg/ml
Bacteria				
<i>Escherichia coli</i>	0.2	0.8	0.8	0.8
<i>Staphylococcus aureus</i>	0.6	1.0	0.2	0.4
<i>Bacillus subtilis</i>	1.4	1.6	1.0	2.0
<i>Staphylococcus</i>	0.6	1.4	1.0	2.0
<i>Zitobacterium parvum</i>	1.4	2.0	1.6	1.6
<i>Salmonella typhi</i>	0.6	1.2	1.8	-
<i>Proton vulgaris</i>	1.2	2.0	1.0	-
<i>Psuedomonas aeruginosa</i>	1.4	2.0	1.0	-
Fungi				
<i>Aspergillus niger</i>	0.4	1.0	0.8	0.2
<i>Candida albicans</i>	0.6	1.2	0.8	0.2

sporulated culture was also grown on Potato Dextrose agar. Plant extracts were dissolved in DMSO at a concentration of 2 mg/ml. The standard antibacterial solution containing 20 µg/ml Gentamycin and Amphotericin were prepared. Each plate was inoculated with 20 µl microbial suspension having a concentration of 1×10^8 cells/ml. 0.1 ml extract was added to each cup. The plates containing bacteria were incubated at 37°C for 24h and those containing fungi were incubated at 25°C for 7 days. The positive antimicrobial activity was read based on growth inhibition zone and compared with the standard drug. In order to determine the minimum inhibitory concentration values (i.e. minimum concentrations of agents showing growth inhibition zone when examined visually), extracts were dissolved in DMSO to make a concentration of 100 mg/ml. The extracts were then diluted in a simple dilution manner to make concentrations in the range of 0.2-3 mg/ml and 3 mg/ml. 0.1ml extracts were then added to each cup. All the tests were repeated in triplicates.⁵

Results and Discussion

From the results, it could be concluded that the *Eranthemum roseum* root extracts may be

useful as a broad-spectrum antimicrobial agent following extensive investigation. These results may provide a basis for the isolation of compounds of biological interest from *Eranthemum roseum* for its potent activity.

As shown in Table 1, the petroleum ether extracts of *Eranthemum roseum* roots exhibited activity against all set of microorganisms used. Aqueous and methanolic extracts showed more potent activity against *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* than that of the standard drug solution. All extracts of the roots of plant were also tested for their minimum inhibitory concentrations (MIC).

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