



ANTIMICROBIAL INVESTIGATION OF AERIAL PARTS AND ROOTS OF *HELIOTROPICUM ELIPTICUM* LEDEB.

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ABSTRACT

The present study deals with the antimicrobial studies of ethanolic extract of aerial parts and roots of *Heliotropium elipticum* Ledeb. The ethanolic extracts of both the parts were examined for antibacterial and antifungal studies by using Disc diffusion method. *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Pseudomonas aeruginosa* as Gram -ve and *Staphylococcus aureus* as Gram +ve bacteria were selected for antibacterial efficacy and *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme* and *Rhizoctonia bataticola* fungi were selected for antifungal efficacy. In case of antibacterial efficacy the ethanolic extract of aerial parts showed trace activity against *S.aureus* and *P. aeruginosa* and the ethanolic extract of roots exhibited trace activity against *K.aerogenes* only. In case of antifungal efficacy, extract of both the parts inhibited growth of almost all the test fungi. Out of selected fungi, the growth of *R.bataticola* inhibited the most by ethanolic extract of aerial parts and root extract showed maximum activity against *A.flavus*.

KEYWORDS: *Heliotropium elipticum* Ledeb., Aerial parts, Roots, Ethanolic extract, Antimicrobial activity.

INTRODUCTION

The plant *Heliotropium elipticum* Ledeb. syn. *Heliotropium eichwaldi* Steud. belongs to the family Boraginaceae. The genus comprises mainly herbs and shrubs. It has about 45 species, 25 of which are found in India. Some of these find use as traditional medicines.^[1,2] The plant *H.indicum* and *H. strigosum* are considered as diuretic, useful in the inflammation of eyes.^[3] *H. indicum* also said to cause permanent sterilization^[4] and methanolic extract of leaves of this plant was found to be active against some fungi.^[5,6] *H. elipticum* is a herb found wildy in various parts of India. It is employed as an emetic and in snake bite and the extract was found to produce hypotensive effect in mice.^[7,8]

Keeping the medicinal importance in view, the antibacterial and antifungal investigation of the ethanolic extract of aerial parts and roots of *Heliotropium elipticum* were undertaken.

MATERIALS AND METHODS

Plant Materials

The plant material (aerial parts and roots) of *H. elipticum* Ledeb. were collected from Durgapura farm, near Jaipur and were identified for authenticity in the department of botany, University of Rajasthan, Jaipur (Herbarium sheet No. RUBL 2776).

Extraction

Powdered aerial parts and roots of the plants were extracted on a steam bath for 8 X 3 hrs. with ethanol separately. Later, each of these extract was filtered, the residue re-extracted (2 X) for complete exhaustion, the extracts were pooled individually and concentrated under reduced pressure and stored in dark colored bottle at 4°C in a refrigerator.

Sources of Test Organisms

Bacteria

Pure culture of all test organisms, namely *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Pseudomonas aeruginosa* as Gram -ve and *Staphylococcus aureus* as Gram +ve bacteria, the human pathogens were obtained through the courtesy of SMS Medical College, Jaipur, which were maintained on Nutrient Broth Medium.

Fungi

The pure cultures of test fungi, namely *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme* and *Rhizoctonia bataticola* were obtained from the Seed Pathology Laboratory, Department of Botany, University of Rajasthan, Jaipur, which were maintained on Potato Dextrose Agar (PDA) medium.

Culture of Test Microbes

For the cultivation of bacteria, Nutrient Broth Medium (NBM) was prepared using 8% Nutrient Broth (Difco) in distilled water and agar-agar and sterilized at 15 lbs for 25-30 min. The agar test plates were prepared by pouring -15 ml of NBM into the petri-dishes (10 mm) under aseptic conditions. The peptone saline solution was prepared (by mixing 3.56 g KH_2PO_4 + 7.23 g NaH_2PO_4 + 4.30 g NaCl + 1.00 g peptone in 1000 ml distilled water, followed by autoclaving) and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24 hrs.

However, for the cultivation of fungi, Potato Dextrose Agar (PDA) medium was prepared by mixing 1000 ml potato infusion prepared from 200 g potatoes, 20 g agar and 20 g glucose, followed by autoclaving; the test fungi were incubated at 27°C for 48 hrs and the cultures were maintained on the same medium by regular sub-culturing.

To prepare the test plates, in both bacteria and fungi, 10-15 ml of the respective medium was poured into the petri-dishes and used for screening.

For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown agar slant, while for fungicidal efficacy, a uniform spread of the test fungi was made using sterile swab.

Bactericidal and fungicidal assay

For both bactericidal and fungicidal assays Disc diffusion method was adopted, because of reproductivity and precision.^[9] The different test organisms were preceded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whatman No. 1 paper (6 mm in diameter), which were containing 500 µg and 1000 µg of the test extracts and control amikacin (10 µg/ml) or mycostatin (100 units /ml) as reference drugs separately. These plates were initially placed at low temperature for 1 h, so as to allow the maximum diffusion of the compound from the discs into the agar plate and later, incubated at 37°C for 24 h in case of bacteria and 48 h for fungi, after which the zones of inhibition could be easily observed.

RESULTS AND DISCUSSION

In case of antibacterial efficacy, the ethanolic extract of aerial parts inhibited the growth of *S.aureus* and *P. aeruginosa* to some extent at both the concentrations (1000 µg/disc and 500 µg/disc) but the ethanolic extract of roots showed trace activity against *K.aerogenes* at the concentration 1000 µg/disc only. The extracts failed to inhibit the growth of rest of the bacteria. As shown in Table 1.

Table 1. Bactericidal activity

Plant part	Dose µg/disc	<i>E.coli</i>		<i>K.aerogenes</i>		<i>P. vulgaris</i>		<i>P. aeruginosa</i>		<i>S.aureus</i>	
		IZ*	AI*	IZ	AI	IZ	AI	IZ	AI	IZ	AI
Aerial parts	1000	-	-	-	-	-	-	4.00	0.18	3.00	0.13
	500	-	-	-	-	-	-	3.00	0.13	3.00	0.13
Roots	1000	-	-	4.00	0.18	-	-	-	-	-	-
	500	-	-	-	-	-	-	-	-	-	-

IZ: inhibition zone (in mm) including the diameter of disc (6mm)
 AI: activity index=(inhibition zone of sample/inhibition zone of standard)
 Standard : Amikacin = 10 µg/ml.

(±) Trace activity: (-) No activity

In case of fungicidal activity, ethanolic extract of aerial parts as well as roots inhibited the growth of all the test fungi. The growth of *R. bataticola* inhibited the most by ethanolic extract of

Table 2. Fungicidal activity

Plant part	Dose µg/disc	<i>A. flavus</i>		<i>A. niger</i>		<i>F. moniliforme</i>		<i>R. bataticola</i>	
		IZ*	AI*	IZ	AI	IZ	AI	IZ	AI
Aerial parts	1000	14.00	0.64	15.00	0.65	10.00	0.43	20.00	0.91
	500	8.00	0.36	8.00	0.36	8.00	0.36	14.00	0.64
Roots	1000	15.00	0.65	10.00	0.43	11.00	0.48	8.00	0.36
	500	10.00	0.43	10.00	0.43	8.00	0.36	7.00	0.30

IZ: inhibition zone (in mm) including the diameter of disc (6mm)
 AI : activity index=(inhibition zone of sample/inhibition zone of standard)
 Standard: Mycostatin = 100 units/disc.

aerial parts (IZ=20 mm at 1000 µg/disc, IZ=14 mm at 500 µg/disc) and both the ethanolic extracts (aerial parts and roots) were significantly active against *A. flavus* (IZ=14 mm at 1000 µg/disc, IZ= 8 mm at 500 µg/disc in case of aerial parts and IZ=15 mm at 1000 µg/disc, IZ= 10 mm at 500 µg/disc in case of roots). As shown in Table 2.

CONCLUSION

The present analysis clearly revealed that both the plants parts are significantly active against fungi. The aerial parts showed pronounced activity and can be used as safe and harmless substitute of traditional antifungal agents. Also, further researches are needed in order to analyze the active substances.

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