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Biological screening of *Eclipta prostrata* Linn., for antifungal activity

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Abstract –Present study conduct to evaluate the antifungal activity of the shootparts of *Eclipta prostrata* Linn. The ethanol extract and its fractions: pet. ether, benzene, ethyl acetate were subjected to evaluate their antifungal activity. The agar well diffusion method was used to assess the activity against five fungi; *Aspergillus flavus, Aspergillus niger, Candida albicans, Penicillium chrysogenum, Trichophyton rubrum*. Selected fungus belongs to the division Ascomycota. The results, evaluated as the diameter of the inhibition zone of microbial growth, showed that the ethanolic extract as well as the mostly fractions of the aerial parts node, internode, leaf, shoot tip of *Eclipta prostrata* Linn. exhibited strong activity against *C. albicans, P. crysogenum*, and *T. rubrum***.**

Keywords: Antifungal activity, Ascomycota, *Eclipta prostrate* Linn., Herbal medicine.

1. Introduction

Plants have always been a subject of interest for human being as traditional medicine due to environmental friendly, cultural acceptance, affordability and minimum side effect. People in different regions of the world uses the same or similar plants in traditional medicines to treat chronic and infectious diseases since a long time. **S**cientists and researchers found plant based active biomolecules as key role for drugs discovery and advancement. The therapeutic assessment of various plants parts should be examined with their potential use and significance in different products.

Eclipta prostrata (L.) L. was earlier known as *Eclipta alba* (L.) Hassak, is an medicinal herbaceous plant placed to the family Asteraceae. It is commonly known as Bhringraj, Bhumiraj, False daisy or Ink plant [1]. It is widely used in treating various noninfectious diseases of cutaneous and alimentary canal diseases in India. It is primarily found in the tropic of cancer to the tropic of Capricorn line like India, Sri Lanka, Myanmar, and Pakistan. *Eclipta prostrata* possess a vast variety of bioactive chemicals saponins, alkaloids, triterpenes, flavonoids, phenolic acid.

Most of the chemical evaluation are suggested for complete plant or aerial parts. Due to high secondary metabolites, scientist and researchers check water, metholic and ethanolic extract to different microbial activity. Its water extract exhibited antioxidant activity [2,3,4], and the most potent inhibitory activity against HIV-I entegrase [5]. The extract of *Eclipta*

prostrata Linn. also exhibited anticancer activities. It applied externally as antiseptic to ulcers and wounds in cattle.

Screening of antifungal activities of the ethanolic extract of *Eclipta prostrata* Linn's aerial portion were used of in this study. The extracts exhibiting significant activities were fractionated in pet. ether, benzene and ethyl acetate. These fractions were further screened for antifungal activities.

Agar-well diffusion method was used to screen the test fungi viz. *Aspergillus flavus, A. niger, Candida albicans, Penicillium chrysogenum, Trichophyton rubrum* with ketoconazole as the reference marker.

2. Experimental

2.1. Test extracts Preparation

For antimicrobial activity, aerial part of *Eclipta prostrata* Linn. were extracted with ethanol. The ethanolic extract was concentrated in vacuo, fractionated with pet. ether, benzene, ethyl acetate and the residue was re-extracted (2 x 8 hr) for complete exhaustion. Further the extracts/fractions were pooled individually and dried in vacuo.

All the extracts were stored at 4°C in a refrigerator until screened for a particular activity. However, their final concentration was prepared in the respective solvents, before use.

2.2. Source of test organisms

Pure cultures of test fungi namely *Aspergillus flavus, Aspergillus niger, Candida albicans, Penicillium chrysogenum* and *Trichophyton rubrum* obtained from S.M.S. lab. Jaipur (Rajasthan) were cultured on Sabouraud Dextrose Broth (SDB) at 37°C for 48 hr.

2.3. Cultures of test microbes

Stock cultures were maintained at 4°C on slops of nutrient agar. Active cultures for experiments were prepared by transferring an inoculating loop of cultures from the stock cultures to test tubes of SD Broth for fungi which were incubated without agitation for 24 hr at 25°C. The Agar-well diffusion method was used to screen for the antifungal activity.

3. Antifungal assay

For antifungal assay Agar- well diffusion method was adopted, because of its reproductively and precision. Sterilized petri plates were arranged to pour media which molten in microwave. 20 to 25 ml molted media poured into plates. After 8 to 10 minutes medium gets solidify. 50 µl suspension was used to spread upon petri plates by the using glass spreader and dried for 10 minutes. The wells (6 mm diameter) were punched in the plates using a sterile stainless steel borer. The test extract and control (ketoconazole) was loaded in 6 mm well and the samples was allowed to dispensed for 40 min. After 36 hours of incubation period at the temperature of 37°C plates were kept and inhibition zones were formed around the well. Well transparency measured in millimeter scales.

4. Result and discussion

In the present study, five different microbial species were used to screen the possible antifungal activity of plant extract. Fungi are found to occur everywhere in the environment which are unavoidable and cause infection in plants as well as animals. There are ~20 fungi

that cause >99% of human fungal infections, although ~ 600 different fungi have been reported to cause infection. Amongst these fungal pathogens, species of *Candida* and *Aspergillus* are the most common causing invasive life threatening infections. Hence, all screening assays include these fungi. In spite of a large number of antifungal drugs in the market, there remains a need of drugs which are more effective and exhibit broad spectrum efficacy.

The results of antifungal activities of the selected plant extract have been presented in Table 1. The ethanolic extract as well as the mostly fractions of the aerial part of *Eclipta prostrata* Linn. showed strong activity against *Candida albicans*, *Trichophyton rubrum*, and *Trichophyton rubis* (Table 1). The maximum activity being observed in the ethanolic extract against *C.albicans*. Thus, different extracts and fractions from *Eclipta prostrata* Linn. Showed effectiveness against mostly selected fungi, suggesting a potential use of this plant as an antimicrobial agent.

Table: 1. Antifungal Activity of *Eclipta Prostrata* Linn.,

Plant species	**Type of extract/ fractions**	**Dose (mg/ per disc)**	**Test Microbes**									
			Penicillium chrysogenum		***Candida albicans***		***Trichophyton rubrum***		***Aspergillus niger***		***Aspergillus flavus***	
			IZ^{+}	**AI***	**IZ**	**AI**	**IZ**	**AI**	**IZ**	**AI**	**IZ**	**AI**
Eclipta prostrata	EtOH	4	12.06	0.40	13.12	0.59	12.00	0.57	9.20	0.34	10.04	0.37
	Pet. Ether	4	11.48	0.39	11.06	0.50	10.90	0.51	8.98	0.33	11.21	0.42
(Whole plant)	C_6H_6	4	11.74	0.40	10.94	0.50	9.92	0.47	9.00	0.33	9.29	0.35
	EtOAc	4	10.04	0.34	12.09	0.54	10.00	0.47	9.10	0.33	8.98	0.34

$^{+}$IZ = Inhibition zone (in mm) including the diameter of disc (6 mm);

*AI = Activity index = Inhibition zone of sample/Inhibition zone of standard;

Standard : Ketoconozole;

(-) = No activity.

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