Original Article

Antifungal properties of leaf extract of *Catharanthus roseus* l (g.) Don

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ABSTRACT

Catharanthus roseus is well known for its pharmacological significance. The present study aims to determine the antifungal activity of *Catharanthus roseus* against various clinically significant fungal strains (*Candida albicans, Aspergillus fumigatus, Aspergillus niger, Fusarium moniliforme).* Assessment of antifungal activity of *Catharanthus roseus* was done by paper disc diffusion method. In this study, three extraction media (Ethanol, Acetone, and Aqueous) were used. Data depicts that the pattern of inhibition largely depend upon extraction solvent. Organic extracts provided more potent antifungal activity as compared to aqueous extracts. Leaves of *Catharanthus roseus* showed significant inhibition. Leaves of *Catharanthus roseus* showed excellent activity against *Fusarium moniliforme* in ethanolic extracts. Literature survey had not reflected any activity of *Catharanthus roseus* against *Fusarium moniliforme* therefore; antifungal activity of *Catharanthus roseus* against *Fusarium moniliforme* is reported for the first time. MIC of the extract against the tested fungal strains ranges between 25µg/µl to 50 µg/µl.

Keywords: Paper disc diffusion method, Antifungal properties, *Candida albicans, Fusarium moniliforme, Aspergillus niger*.

INTRODUCTION

Catharanthus roseus L.(G.) Don. is short – lived perennial with dark green and glossy leaves belongs to family Apocyanaceae. Pharmacological studies have revealed that *C. roseus* contain more than 70 different type of alkaloids (indole alkaloids) and chemotherapeutic agents that are effective in treating various type of cancers-breast cancer, lung cancer, uterine cancer, melanomas, and hodgkin's and non-hodgkin's lymphoma[1] Traditionally, *Catharanthus roseus* L. (G.) Don. has been used in folk medicine to treat diabetes and high blood pressure. As antidiabetic remedy, it was believed to promote insulin production However, in modern medicine alkaloids and chemotherapeutic agents from

C. roseus are known for their anticancer, pain-relieving properties.

The anticancer drugs vincristine and vinblastine are synthesized from alkaloids of *Catharanthus roseus L. (G.) Don.* The plant is also known for its antihypertensive and antispasmodic properties due to presence of alkaloids like ajamalicine, serpentine and reserpine. The root bark contains the alkaloid alstonine which has been used traditionally for its calming effect and its ability to reduce blood pressure.

MATERIALS AND METHODS

This work was carried out between January 2011 to August 2011. The plant being a perennial one, there was no difficulty in getting the material for the investigation.

Microbes used

A total of four microbes *Candida albicans, Aspergillus fumigatus, Aspergillus niger, Fusarium moniliforme* are selected to assess susceptibility patterns against the phytochemical extracts. These fungal cultures were collected from S. N. medical college, Agra and authenticated by Lab of Microbiology, Dayalbagh Educational institute. All Fungal cultures were maintained in SDA slants at 28^0C.

Collection of plant material

The fully mature *Catharanthus roseus L.(G.) Don.* Plant was collected from Heera bagh park, Agra. Plant materials were washed separately under running tap water, followed by rinse using sterilized distilled water. Excess of water was removed from the plant material using filter paper before they were used for extraction.

Extract preparation

Aqueous extract

Dried plant material was grinded by using grinder, and 10 gm of grinded plant material was dissolved in 100 ml distilled water and left for 48 hrs. at room temperature and then filtered using muslin cloth. The filtrate was collected in fresh sterilized conical flask and used within 24 hrs. for evaluation of antimicrobial activity.

Solvent extract

10 gm grinded plant material was dissolved in 100 ml organic solvent (Ethanol & Acetone) and after 48 hrs. filtered with muslin cloth. Extracts were stored at 4^0C for further use.

Assessment of antifungal activity of plant extract against clinically significant fungal strains

The antimicrobial activities of plant extract of *Catharanthus roseus* L.(G.) Don. against the target fungal strains were studied in terms of –

a) Paper disc diffusion method2,3

Antimicrobial activity by disc diffusion method was carried out. Disc measuring 06 mm in diameter was pinched from Whatman no. 1 filter paper using a cork borer of fixed diameter. The discs, saturated with different extracts containing varying concentration were placed on SDA medium seeded with the test organism. Disc fed with corresponding solvent alone served as control. The plates were incubated at suitable temperature and observed for zone of inhibition after 1-3 days.

% inhibition calculated by following formula:

$$\% \text{ inhibition} = \frac{treatment - control}{treatment} \times 100$$

b) Minimum inhibitory concentration (MIC)

MIC is the lowest concentration of antimicrobials that will inhibit the visible growth of micro organism after overnight incubation. In this, a set of Petri plates with different concentrations of plant extract with

the same volume was prepared. Plates were inoculated with the tested micro organisms of standard concentration as discussed above. After incubation, plates were examined for changes.

RESULTS

The results of the experiments carried out on the antifungal activity of leaf extracts of *C. roseus* L (G) Don by using distilled water and different solvents like Ethanol, Acetone. It was resulted from the observation that-

In Ethanolic extract, *C. albicans, A. niger, F. moniliforme* gave 59% (2 cm) , 56.79% (1.62 cm), 54.36% (2.52cm) inhibition at 90µl conc., respectively while *A. fumigatus* showed 52.38% (2.1 cm) inhibition at 70 µl conc. In Acetone extract, *F. moniliforme* resulted in 59.18% (2.45 cm) inhibition at 90 µl while *A. niger, C. albicans, A. fumigatus* gave 53.70% (1.62 cm), 46.66% (1.5 cm), 35.89% (1.95 cm) inhibition at 30 µl conc. ,respectively. In Aqueous extract, *C. albicans, A. fumigatus* resulted in 56.32% (2.45 cm), 36.36% (1.1 cm) inhibition at 30 µl conc., *A.niger* gave 23.07% (1.17 cm) inhibition at 70 µl conc. while *F. moniliforme* showed nil activity. Extract of *Catharanthus roseus* tested against *C. albicans, A. fumigatus, A. niger, F. moniliforme* showed inhibition at 50µg/ µl, 25 µg/ µl, 25 µg/ µl, 25 µg/µl conc., respectively. Data depicts that the pattern of inhibition largely depend upon extraction solvent. Organic extracts provided more potent antifungal activity as compared to aqueous extracts. Literature survey has not reflected any activity of *Catharanthus roseus* against *Fusarium moniliforme* therefore, antifungal activity of *Catharanthus roseus* against *Fusarium moniliforme* is reported for the first time.

DISCUSSION

The plant of *C. roseus* has a very great medicinal value. The vast collection of literature & publications and about 295 patents dealing with the plant and its products, very well illustrated this fact [4]. *Catharanthus roseus* is mainly studied for its antibacterial properties against *Pseudomonas aeruginosa, Salmonella typhimurium, Shigella boydii, Staphylococcus aureus, Corynebacterium diphtheria*, *Escherichia coli,Bacillus cereus, Klebsiella pneumoniae, Bacillus subtilis* etc. There are also some reports on antifungal activity against *Epidermophyton flocossum, Trichophyton mentagrophytes, Aspergillus sps, Candida albicans* etc. Literature survey reflected that there is no study on antifungal properties of *Catharanthus roseus* against *Fusarium moniliforme* and most of the work done on *C. roseus* was related to their antibacterial properties and a very little literature was available related to the antifungal properties.

Satish et al (2009), evaluated 52 plant sps including *C. roseus* against different *Aspergillus sps*. One of the species tested was *A. fumigatus* but amongst the 12 plants reported to be effective against said species. *C. roseus* was not reported to be effective against the fungi. This is quite contrast to the present investigations, Leaf extract of *C. roseus* and other plant has shown considerable activity against *A. fumigatus*[5].

Kratika et al (2013), evaluated phytopotential of *C. roseus* against various pathogenic microbes. In this study, *Catharanthus* leaf extract was effective against *C. albicans* and gave considerable zone of inhibition in all extraction media i.e. Aqueous, Methanol and Chlorofom. This study supported our present investigation in which *Catharanthus* leaf extract has shown significant activity in all extracts against *C. albicans*[6].

In the present investigation, assessment of antifungal activity of *C. roseus*

leaf extract was done by paper disc diffusion method and maximum inhibition was observed in *F. moniliforme* i.e. 2.52 cm (54.36%) at 90 µl conc. in ethanolic extract followed by *C. albicans* i.e. 2.45 cm (56.32%) at 90 µl conc. in aqueous extract followed by *A. fumigatus* i.e 2.1 cm (52.38%) at 70 µl conc. in ethanolic extract followed by *A. niger* i.e. 1.62 cm (56.79%) at 90 µl conc. in Ethanolic extract. Control serve as negative control and Antifungicide as positive control. The concentration of antifungicide is 1 mg/ml. *Catharanthus* extract has shown significant inhibition against selected microbes. Data depicts that the pattern of inhibition largely depend upon extraction solvent. Ethanol solvent is a better extraction solvent as compared to acetone. Literature survey reflected that Antifungal activity against plant pathogenic fungi *F. moniliforme* is also reported for the first time.

CONCLUSION

The plant extract proved effective against selected fungal strains *A. fumigatus, C. albicans, A. niger, F. moniliforme.* Ethanol solvent is a better extraction solvent as compared to acetone. MIC ranges between 25µg/µl to 50µg/µl. Control serve as negative control and antifungicide as positive control. Maximum inhibition was observed in ethanolic leaf extract of *C. roseus* against *F. moniliforme* i.e. 2.45 cm (59.18 %) at 90 µl conc. Antifungal activity against plant pathogenic fungi *F. moniliforme* is also reported for the first time.

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Table 1. Activity of *C. roseus* leaf against different fungal cultures by Paper disc diffusion method

Conc.		Zone of inhibition (In cm)											
		Candida albicans			*Aspergillus fumigatus*			*Aspergillus niger*			*Fusarium moniliforme*		
		Acetone	Ethanol	Aqu.	Acetone	Ethanol	Aqu.	Acetone	Ethanol	Aqu.	Acetone	Ethanol	Aqu.
C	Mean±S.D. %inhibition	0.8 ± 0 0%	.82 ± .04 0%	1.07 ± .08 0%	1.25 ± .05 0%	1 ± 0 0%	0.7 ± 0 0%	.75 ± .05 0%	0.7 ± 0 0%	.9 ± 0 0%	1 ± 0 0%	1.15 ± .05 0%	0 ± 0 0%
30	Mean±S.D. %inhibition	1.5 ± .07 46.66%	1.52 ± .04 46.05%	1.95 ± .05 45.12%	1.95 ± .11 35.89%	1.07 ± .04 6.54%	1.1 ± .07 36.36%	1.45 ± .05 48.27%	1.07 ± .04 34.57%	1.07 ± .08 15.88%	1.65 ± .30 39.39%	1.22 ± .08 5.73%	0 ± 0 0%
70	Mean±S.D. %inhibition	1.17 ± .04 31.62%	1.12 ± .04 26.78%	2.45 ± .11 56.32%	1.72 ± .19 27.32%	2.1 ± .08 52.38%	.95 ± .05 26.31%	1.62 ± .10 53.70%	0.9 ± .07 22.22%	1.17 ± .19 23.07%	1.5 ± .08 33.33%	2.22 ± .10 48.19%	0 ± 0 0%
90	Mean±S.D. %inhibition	1.42 ± .04 42.66%	2.00 ± .07 59.00%	2.30 ± .07 53.47%	1.55 ± .05 19.35%	1.85 ± .11 45.94%	1.02 ± .04 31.37%	1.02 ± .04 26.47%	1.62 ± .04 56.79 %	0 ± 0 0%	2.45 ± .05 59.18%	2.52 ± .17 54.36%	0 ± 0 0%
Anti	Mean±S.D. %inhibition	0 0%	0.8 ± .43 0.2 %	2.62 ± .04 59.16%	1.40 ± .12 15.00%	1.17 ± .08 14.52%	1.15 ± .05 39.13%	1.55 ± .08 51.61%	.77 ± .04 9.09%	1.25 ±.04 28.00%	1.5 ± 0 33.33%	.87±.04 0.32%	0 ± 0 0%

Mean of all readings ± Standard Deviation

Fig 1-6. Antifungal activity of *C. roseus* leaf extract against different fungal strains by paper disc diffusion method (in different solvents)

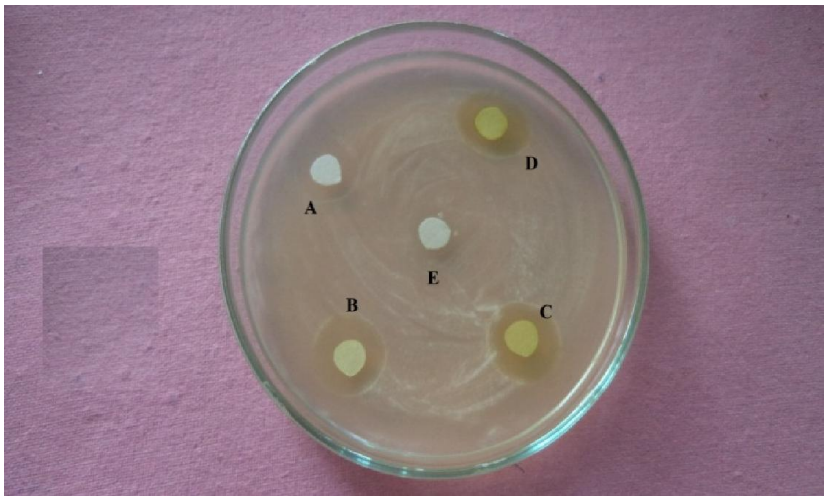

Figure 1. Leaf extract (Ethanol) against *A. fumigatus*

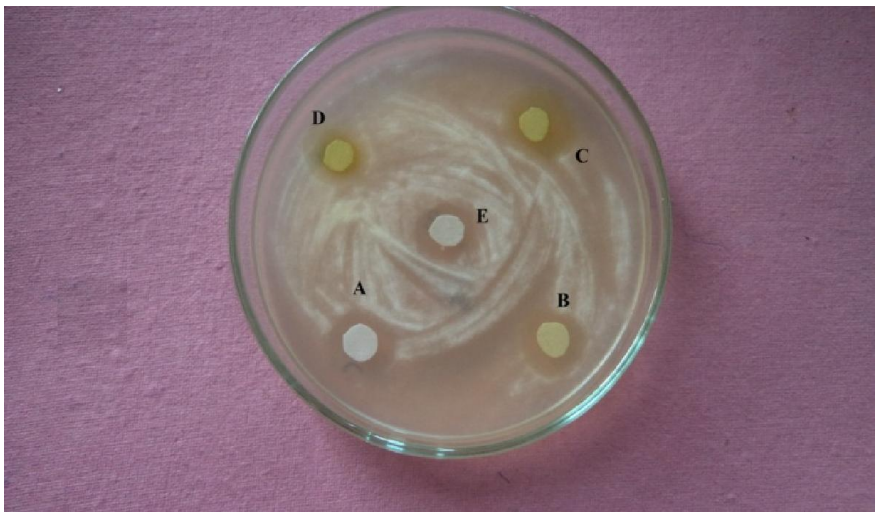

Figure 2. Leaf extract(Ethanol) against *A. niger*

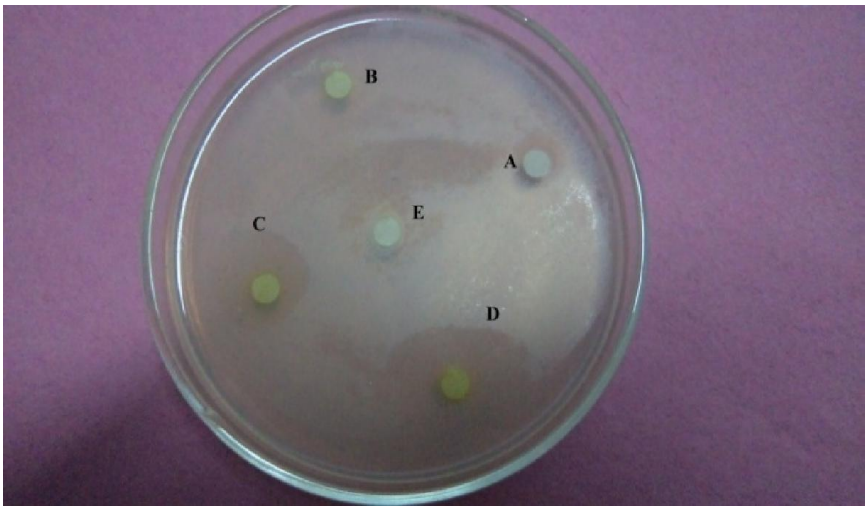

Figure 3. Leaf extract (Ethanol) against *F. moniliforme*

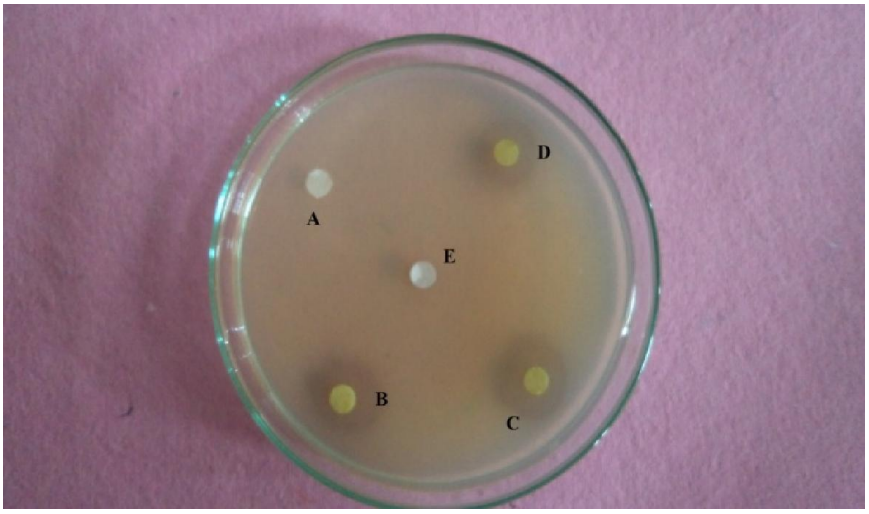

Figure 4. Leaf extract (Acetone) against *F. moniliforme*

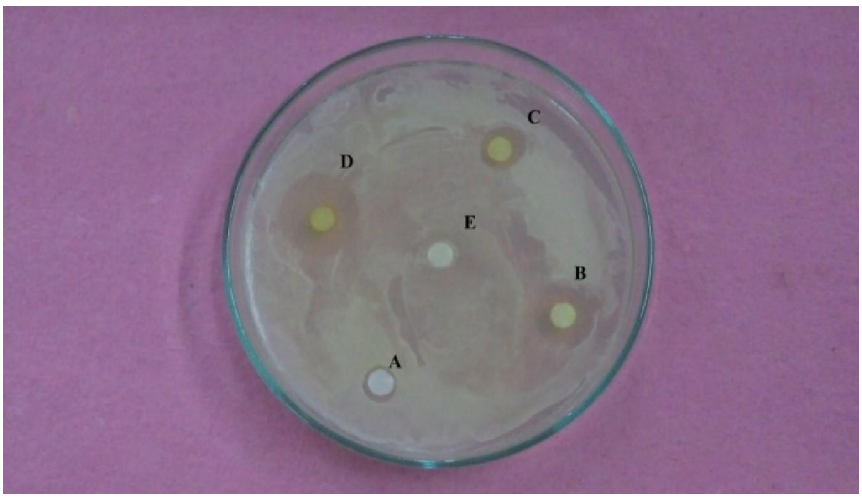

Figure 5. Leaf extract (Ethanol) against *C. albicans*

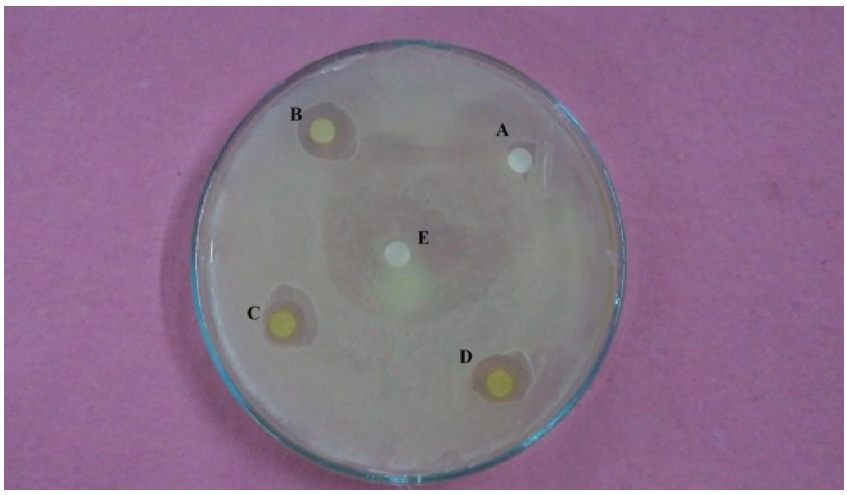

Figure 6. Leaf extract (Acetone) against *C. albicans*