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Antifungal activity of *Leptadenia reticulata* Wight and Arn. aerial parts

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Abstract

The petroleum ether, chloroform, acetone, methanol and aqueous extracts of the aerial parts of *Leptadenia reticulata* Wight and Arn. (Asclepiadaceae) were studied for in vitro antifungal activity against *Aspergillus flavus, Aspergillus ruantti, Candida tropicalis, Candida albicans, Trichodermata viride* and *Trichodermata koningii* respectively. The methanolic extract exhibited prominent antifungal activity against all the selected strains. Minimum inhibitory concentration of the extracts was performed by broth dilution method and the zone of inhibition was studied by agar disc diffusion method at concentrations of 2, 5 and 10mg/ml in DMSO. Cotrimazole (25µg/ml) was used as reference control for antifungal studies. Results of MIC study revealed the antifungal activities of the extracts against the tested strains in between concentration ranges 50-400µg/ml. The present study indicates the potential usefulness of *L. reticulata* aerial parts as antifungal agent.

Keywords: *Leptadenia reticulata* Wight and Arn., Antifungal activity, Minimum inhibitory concentration, Zone of inhibition, Clotrimazole

Introduction

Fungal diseases are a major cause of morbidity and mortality worldwide [1]. The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad spectrum antibiotics and immunosuppressive agents [2,3]. This situation provided the impetus to the search for new antifungal substances from various sources like medicinal plants [4]. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with serious side effects [5]. Traditional medicine has made use of many different plant extracts for treatment of fungal infections and some of these have been tested for in vitro antifungal activity [6].

Leptadenia reticulate Wight and Arn. (Family-Asclepiadaceae), commonly known as “Jivanti”,

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is a much branched twinning shrub found in the sub-Himalayan tract of Punjab, U.P and through out the Deccan Peninsula up to an altitude of 900m, particularly in hedges[7,8]. The plant finds its application in various traditional system of medicine. The decoctions of the aerial parts are reported to be applied externally for skin infections and wounds [8].

Reports on the biological activity are scarce. The alcoholic extracts (50%) of the leaves and roots are reported to be active against *Micrococcus pyrogens, Bacillus megatherium, Escherichia coli, Salmonella typhi, Proteus vulgaris* and *Trychophyton rubrum.* The entire plant has been clinically tested and found useful in treatment of habitual abortion in women [8]. Aqueous extract of the aerial parts has been reported to produce prolonged and pronounced hypotensive effect in dogs [9]. The antioxidant, wound healing, galactagogue and lactogenic activity of the plant has been reported [10, 11].

The occurrence of β-sitosterol, β-amyrin, leptadenol, leptidin, n-triacontane, acetyl alcohol, stigmasterol, tocopherol, lupanol-3-O-diglucoside, leptaculatin and two flavonoids namely diosmetin and luteolin have been reported [12].

The present study was undertaken to explore the antifungal potential of the aerial parts since no such work has been reported earlier.

Materials and Methods

Plant Material

Fresh plant material (aerial parts) was collected from well-grown plants on the hills of Barunai, Khurda during May 2008 and authenticated by the taxonomists of Department of Botany, Utkal University, Bhubaneswar. After due authentication, fresh aerial parts were collected in bulk, cleaned thoroughly with distilled water and subsequently dried under shade. The shade dried leaves were pulverized in a mechanical grinder to obtain coarse powder.

Preparation of Extracts

Powdered plant material (300g) was successively extracted with 1litre each of petroleum ether (40-60°), chloroform, acetone, methanol and water in a soxhlet apparatus for 72hrs at each stage of extraction. The extractive yields of all the solvent extractions are petroleum ether-0.92%w/w, chloroform-1.5%w/w, acetone-4.65%w/w, methanol-9.8%w/w and aqueous-12.6%w/w respectively with respect to dried plant material. The liquid extracts were concentrated separately under vaccum and the resulting dried extracts were preserved in a dessicator until further use. Preliminary phytochemical tests of different extracts were performed by using specific reagents through standard procedures [13, 14].

Drug used

Cotrimazole was used as reference standard for antifungal studies.

Microorganisms used

For the present study, the microorganisms used include *Aspergillus flavus*, *Aspergillus ruantii, Candida tropicallis*, *Candida albicans, Trichodermata viride* and *Trichodermata koningii* respectively. Suitable strains of these microorganisms were procured from Centre of Biotechnology (CBT), SOA University, Bhubaneswar.

Antifungal Activity

The antifungal activities of the extracts were tested using various pathogenic fungi. The antifungal activities of the extracts were performed by agar disc diffusion method [15,16] with the disc diameter of 6mm. The extracts were separately dissolved in DMSO at concentrations of 2, 5 and 10mg/ml. Cotrimazole (25µg/ml) in Dimethyl Sulphoxide (DMSO) served as reference control for the antifungal study. Solvent control (only DMSO) was also maintained throughout the experiment. PDA media was used for the antifungal study. The molten media was then inoculated with 200µl of the inoculums ($1x10^8$ Cfu) and pored into the sterile Petri plates. The disc was saturated with 20µl of the extracts separately, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated for 48hrs at 28°C and the zone of inhibition was measured.

Table 1: Zone of inhibition (mm) of various extracts of the aerial parts of *L. reticulate*

Sl. No	Test Substance	Conc. (mg /ml)	*Aspergillus flavus*	*Aspergillus ruantii*	*Candida tropicallis*	*Candida albicans*	*Trichodermata viride*	*Trichodermata koningii*
1	Pet. ether extract	2	-	-	-	-	-	-
		5	-	-	-	-	-	-
		10	-	-	-	-	-	-
2	Chloroform extract	2	4.0	5.5	-	-	-	-
		5	6.5	8.5	-	-	-	-
		10	11.5	13.0	-	-	-	-
3	Acetone extract	2	4.5	6.0	-	-	-	-
		5	8.0	8.5	-	-	-	-
		10	14.0	15.0	-	-	-	-
4	Methanol extract	2	6.0	5.5	5.5	5.0	4.5	5.0
		5	13.5	12.0	12.0	11.0	10.5	11.5
		10	24.0	21.5	21.0	19.5	18.5	20.0
5	Aqueous extract	2	-	-	6.0	7.0	-	-
		5	-	-	13.0	13.5	-	-
		10	-	-	22.5	23.0	-	-
6	Cotrimazole[b]	25 (µg/ml)	27.0	24.0	25.0	26.0	24.0	24.0

Minimum inhibitory concentration (MIC) of the extract was performed by broth dilution method [17] at concentrations of the extract ranging from 25µg/ml to 500µg/ml in DMSO against all the test microorganisms. The results of the zone of inhibition values were depicted in Table No.1 and MIC values in Table No.2.

Table 2:- MIC Values of different microorganisms

Sl. No.	Microorganisms	MIC Values(µg/ml)
1	*Aspergillus flavus*	200
2	*Aspergillus ruantii*	150
3	*Candida albicans*	150
4	*Candida tropicallis*	175
5	*Trichodermata viride*	200
6	*Trichodermata koningii*	300

Result and Discussion

The results of the preliminary phytochemical analysis revealed presence of steroids, sterols, triterpenoids, tannins, phenolic compounds, carbohydrates, proteins, gums, mucilages and flavonoids in different extracts.

Table No.1 depicts the antifungal activity of the extracts of *L. reticulata* aerial parts. The results of MIC study revealed the antifungal activity of the extract against the tested strains of microorganisms between concentration ranges of 150 and 300µg/ml. The results of zone of inhibition study revealed that the extracts possess antifungal activity in a concentration dependent manner against the test organisms and were comparable with the standard drug.

Percentage yield of various extracts confirm that petroleum ether extract has minimum yield (0.92% w/w) whereas aqueous extract has

maximum yield (12.6% w/w). Petroleum ether extract found to be inactive against all tested strains under study. Chloroform extract showed moderate antifungal activity over *Aspergillus flavus* and *Aspergillus ruantii*. Similar to chloroform extract, acetone extract exhibits its potency against Aspergillus species. Methanolic extract imparts significant antifungal activity against all test strains whereas aqueous extract is potent against Candida species.

There were reports in the literature that methanol is a better solvent for consistent extraction of antifungal and antimicrobial agents from medicinal plants.[18,19] The antifungal activities of medicinal plants are attributed due to the presence of flavonoids and tannins.[20,21] These reports and presence of flavonoids and tannins in the methanolic extract of aerial parts of *L. reticulate*, confirm its potential against all selected pathogens.

This suggests that the aerial parts of *L. reticulata* have a board spectrum of activity, although the degree of susceptibility could differ between different organisms. The broad spectrum of antifungal activity found in this present study may be attributed to the presence of secondary metabolites of various chemical types present in the plant material either individually or in combination. Our results indicates the potential usefulness of *L. reticulata* in the treatment of various pathogenic diseases as it may help in the discovery of new chemical classes of antibiotics that could serve as selective agents for the maintenance of human health and may provide biochemical tools for the study of fungal infectious diseases. The discovery of a potent remedy from plant origin will be a great advancement in fungal infection therapies.
There is a need to test the in-vivo activity of the extract apart from the effect on many other fungi. This plant is an ideal candidate in the search for new bioactive phytocompounds, suggesting that a more extensive biological and chemical bioassay guided fractionation is required in order to isolate and characterize such bioactive compounds.

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