

Antifungal Activity of *Geodorum densiflorum* (Lam.) Schltr. against Pathogenic Fungi

M. Keerthiga*¹ and S.P. Anand²

¹Department of Biotechnology, National College (Autonomous), Tiruchirappalli-01, Tamil Nadu, India

²PG & Research Department of Botany, National College (Autonomous), Tiruchirappalli-01, Tamil Nadu, India

Address for Correspondence

Department of
Biotechnology,
National College
(Autonomous),
Tiruchirappalli-01,
Tamil Nadu, India.

E-mail:
keerthigamanohar@gmail.com

ABSTRACT

Introduction: Nowadays scientific researches on the medicinal plants are increased because of their extraordinary applications in both human and plant disease control. Therapeutic alternatives from plants were having empirically high antifungal properties against wide range of fungi.

Aim: Present study deals with the antifungal activity of *Geodorum densiflorum* (Lam.) Schltr. an medicinal orchid against various infective fungus.

Material and Methods: Plant extract using different solvents such as water, ethanol and chloroform was evaluated against *Penicillium chrysogenum* (MTCC 6449), *Aspergillus niger* (MTCC 4325), *Rhizoctonia solani* (MTCC 4634) and *Colletotrichum falcatum* at different concentrations (25, 50, 75 and 100 mg/ml).

Result: Maximum antifungal activity was observed with ethanol extract of the orchid, which recorded excellent inhibitory activity against all four pathogenic fungi when compared with the fluconazole as standard.

Conclusion: It is hoped that *Geodorum densiflorum* will compromise the search for potent antifungal agent by young researchers.

Keywords: *Geodorum densiflorum*, Antifungal activity, Mycotoxins, Phytoalexins.

INTRODUCTION

India is rich floristic and genetically diversified in forest resources. Medicinal plants, a natural source for molecules which leads the pharmaceutical importance for the development of new drugs to control human

diseases¹. Scientific studies on the medicinal plants reported the presence of antimicrobial compounds, which are important to humans and plants. Fungi are most significant destroyer of food stuffs and storage grains,

mycotoxins produced were toxic to human and animals^{2,3}. The toxic effects of fungi are carcinogenic, genotoxic, nephrotoxic, hepatotoxic, immunosuppressive and cause reproductive disorders⁴⁻⁶. As, the humans are not healthy to fight back off infections, due to compromised immune systems, complexity of remedial procedures, advancing age of our population. Synthetic antifungal agents are azoles, imidazole (Clotrimazole, Econazole, Fenticonazole, Ketaconazole, Miconazole, Tioconazole and Sulconazole) or Triazole nucleus (Itraconazole, Voriconazole and Fluconazole)⁷. These drugs are insufficient due to their adverse side-effects and emergence of resistance to them by fungal pathogens.

The compounds of plant origin were recommended highly as antifungal agents to control numerous fungal infections and diseases than synthetic antibiotics. It is necessary to prove the potential of folk medicine against the infectious fungus scientifically. Thus we can provide new lead compounds from natural products. Naturally derived antifungal antibiotics are Amphotericin B, *Griseofulvin*, *Echinocandis*, *Caspofungin*, *Anidulafungin*, *Nystiatin* etc. Nowadays there will be increasing demand for the novel drugs to meet the need for the fungal infections. For 5,000 to 10,000 experimental lead compounds tested, Food and Drug Administration (FDA) will approve only one compound after 10 to 15 years of research and development⁸. Therefore, broad study is necessary for the development of new antifungals to overwhelm the antifungal resistance and toxicity effects of current therapies.

Geodorum densiflorum (Lam.) Schltr. belongs to the family orchidaceae is an endangered terrestrial orchid. It is having high therapeutic values used for the treatment of menstrual cycle regulation, joint pains, arthritis, diabetes, carbuncles,

wound healing, skin infection, etc.⁹. It is also reported to having biological activities such as antimicrobial and antidiabetic property¹⁰, Antioxidant and cytotoxicity¹¹, Thrombolytic activity¹², analgesic, sedative and anxiolytic properties¹³. Naturally, the orchid plants are having the ability to control the fungal infections as reported previously, this study was mainly to evaluate the antifungal efficacy of *Geodorum densiflorum* (Lam.) Schltr. an orchid against pathogenic fungus.

MATERIALS AND METHODS

Plant collection

The plants were collected from the Periyakombai hill area, Namakkal district, Tamil Nadu. Collected plants materials were sterilized and shade dried. Dried plants were grounded and the plant power was used for the extraction procedure.

Extract preparation

Two grams of the plant powder was kept in the 10 ml solvents such as water, ethanol and chloroform. They were ground well with the help of Mortar and Pestle. The plant materials were subjected to centrifugation, for 10 min (at 10000 rpm) the process was repeated for 3 times. The supernatant was collected and made to known volume, by adding respected solvents and stored for further antifungal screening purpose.

Microbial cultures and growth conditions

The plant extracts were assessed for antifungal activity against the fungal strain obtained from MTCC. The fungal strains were listed as *Penicillium chrysogenum* (MTCC 6449), *Aspergillus niger* (MTCC 4325), *Rhizoctonia solani* (MTCC 4634) and *Colletotrichum falcatum*. These fungi was grown on Potato Dextrose Agar plate at 28°C and maintained with Periodic sub-culturing at 4°C for further studies.

Antifungal activity test

Test pathogenic fungi were cultured and maintained on Potato Dextrose Agar medium. Disc diffusion assay¹⁴ was performed for screening. Sterile discs of 6 mm in diameter were impregnated with 100µl of each of the extract to give a various concentration of 25, 50, 75 & 100 mg /ml and left to dry to remove residual solvent. An inoculum size 1×10^7 cell / ml of fungal suspension¹⁵ was prepared and the fungal strains were swabbed uniformly in to the sterilized medium under aseptic condition and the inoculum was allowed to dry for 5 minutes¹⁶.

Each extract was tested against fungi in triplicate with fluconazole (10 mcg / disc) as standard. The plates were kept at 4°C for 1hrs for diffusion of extract, thereafter were incubated at 28°C for fungi (48 hrs)¹⁷. The activity of the extract was assessed by measuring the zone of inhibition and recorded^{18,19}.

RESULTS AND DISCUSSION

Effect of four different concentrations (25, 50, 75 & 100 mg / ml) of three various solvent extracts (Water, Ethanol and Chloroform) of *Geodorum densiflorum* was tested against fungi. The antifungal activity of the *Geodorum densiflorum* orchid plant against some strains of fungi was shown in table 1 and figure 1. All the fungal species tested are susceptible to the ethanol and chloroform extracts of the orchid.

The ethanol extracts of *Geodorum densiflorum* plant showed maximum activity against *Rhizoctonia solani*, *Penicillium chrysogenum* and followed by *Aspergillus niger* and then *Colletotrichum falcatum*. The chloroform extracts of this plant showed maximum activity against *Rhizoctonia solani*, *Penicillium chrysogenum*, *Colletotrichum falcatum* and followed by *Aspergillus niger*. The antifungal activity of

ethanol extracts showed more effective followed by chloroform extract against all the fungal strains. The water extract doesn't show comparative effect on the pathogenic fungi with ethanol and chloroform extracts. As compared with fluconazole, the positive control ethanolic extract of *Geodorum densiflorum* showed high activity of against all the pathogenic fungi. It was evident that the inhibition of growth of fungus was more prominent with ethanol extracts than chloroform extracts. It was observed that higher concentrations of extracts were more effective on the growth inhibition of all fungi which are tested and it was also noted that from early period of incubation, the inhibition of growth occurred. The ethanolic extract of seedlings of *Cypripedium macranthos* Var. *rebunense* was containing bioactive compounds such as lusianthrin and chrysin. Lusianthrin maintains the risky symbiotic association during germination period of seeds and was found to be more potent antifungal compound than chrysin which helps to protect adult plants²⁰.

Orchidaceae family members were usually being mixotrophic, parasitic and photoassimilatory simultaneously or over timing in their life cycle. In parasitism the hyphal coils (Peletons) are present in cells of roots and rhizomes. Orchids are having their structural and physiological defences regulate fungal invasion through the plant body. The phytoalexins, fungicidal compound was supplemented with lucianthrin and chrysin^{20,21}. The compounds derived from the orchidaceae family such as *Gastrodia* Anti-Fungal Protein (GAFF, *Gastrodianin*) from a mycoheterotrophic orchid, *Gastrodia elata* Bl. *F. flavida* S^{22,23}. Orchinol isolated from *Orchis militaris*²⁴ were chemically related to loroglossol and hircinol²⁵ showed antifungal activity.

CONCLUSION

Orchidaceae are well known for its beautiful flowers and nowadays lead compounds have been isolated from the plant which is potential for various ailments. The whole study has proved scientifically for the antifungal activity of the medicinal orchid *Geodorum densiflorum* against selected fungi in a justified way. Further studies will be useful in the current search for antifungal agent from the orchid.

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Table 1. Antifungal activity of *Geodorum densiflorum* (Lam.) Schltr.

Test Microorganisms	Concentration (mg/ml)	Inhibition Zone (mm)			Fluconazole Positive Control 10 mcg/disc
		Water Extract	Ethanol Extract	Chloroform Extract	
<i>Aspergillus niger</i>	25	-	15.3±0.68	8.9±0.24	22
	50	-	25.9±0.13	14.6±0.25	
	75	-	30.2±0.48	16.2±0.35	
	100	-	38.2±0.37	18.5±0.34	
<i>Penicillium chrysogenum</i>	25	-	10.5±0.46	8.5±0.37	22
	50	-	27.2±0.71	12.5±0.49	
	75	-	32.7±0.19	16.1±0.17	
	100	-	40.1±0.28	22.3±0.54	
<i>Colletotrichum falcatum</i>	25	-	12.2±0.32	7.5±0.14	35
	50	-	28.4±0.39	16.3±0.23	
	75	-	32.5±0.27	18.2±0.24	
	100	-	38.4±0.15	20.5±0.23	
<i>Rhizoctonia solani</i>	25	-	11.6±0.12	8.3±0.29	28
	50	-	30.1±0.19	16.8±0.55	
	75	-	34.3±0.32	21.5±0.39	
	100	-	42.3±0.43	24.2±0.57	

Values are expressed as mean ± SD, n=3; (-) – no inhibition

Ethanol Extract***Rhizoctonia solani******Penicillium chrysogenum*****Chloroform Extract*****Rhizoctonia solani******Penicillium chrysogenum***

Figure 1. Antifungal activity of *Geodorium densiflorum* (Lam.) Schltr. against pathogenic fungi