#### **RESEARCH ARTICLE**

# Screening of Antifungal Activity of Endophytic Fungi From *Dioscorea bulbifera*

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#### Manuscript details:

ABSTRACT

Available online on http://www.ijlsci.in

ISSN: 2320-964X (Online) ISSN: 2320-7817 (Print)

**Editor: Dr. Arvind Chavhan** 

#### Cite this article as:

Suradkar KP, Hande DV and Kadu SR (2017) Screening of Antifungal Activity of Endophytic Fungi From *Dioscorea bulbifera, Int. J.of. Life Sciences,* Special Issue, A8: 59-62.

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Antifungal potential of endophytic fungi was studied in the present investigation. Secondary metabolites were produce by endophytic fungi isolated from *Dioscorea bulbifera* to investigate their antifungal activity against two pathogenic fungi, one as a human pathogenic (*Candida albicans* MTCC 7315) and one as a plant pathogenic (*Colletotrichum acutatum* MTCC 2213) taken from IMTECH, Chandigarh, India.

Key words; Secondary metabolites, Antifungal Potential.

## INTRODUCTION

Metabolites produced by endophytes are being recognized as versatile arsenal of antimicrobial agents. Some endophytic fungi have been known to have capabilities to produce a bioactive compounds, owing to their presumable gene recombination with their host while living and reproducing within the host tissues (Li *et al.*, 2005). 80% endophytic fungi produce biologically active compounds with antibacterial, antifungal, antioxidant, anticancerous and herbicidal properties (Schulz *et al.*, 2002). The development of new antimicrobial metabolites is important to prevail the problems related to the treatment of diseases caused by resistant pathogens (Petersen *et al.*, 2004). Thus, endophytic fungi have emerged as an alternative source to synthesize new antimicrobial compounds.

Due to the ability of production of important secondary metabolites by endophytic fungi, the study of these fungi from selected medicinal plants provide greater understanding of its diversity and potentials to synthesize bioactive metabolites. In India from long period medicinal plants have been used for the treatment of different diseases and provides specific atmosphere to endophytic fungi. Many endophytic fungi with novel and natural bioactive secondary metabolites are reported previously from medicinal plants (Strobel *et al.*, 2004). In view of these earlier observations, the present study was carried out to investigate the bioactive potential of endophytic fungi.

#### **MATERIALS AND METHODS**

*Dioscorea bulbifera* was collected from Pohara Ferest of Amravati district and brought to laboratory in sterile bags and stored at 4°C till further use. Collected samples were rinsed gently in running water to remove adhered dust and debris. Surface sterilization were done according to the method described by (Suryanarayanan *et al*, 2011). The sterilized samples were inoculated on potato dextrose agar (PDA) to isolate the endophytes.

# Solvent Extraction for Isolation of Secondary Metabolites

Liquid-liquid extraction procedure was adopted to extract the spent broth of endophytic fungal isolates. The aqueous layer was extracted using solvent ethyl acetate. This art was repeated thrice. Solvent layer and residue was separated with the help of separating funnel. Then organic layer containing compounds of interest was dehydrated with anhydrous sodium sulphate. The organic layer was then collected in a preweighed crucible. After incubating, the solvent was removed and stock solutions of extracts were preserved in DMSO and stored at 4°C till use. Antifungal activity was tested by disc diffusion method.

## **Disc Diffusion Assay**

The assay was conducted as per the procedure defined by Jorgensen and Turnidge (2007). The crude extracts were dissolved dimethyl sulfoxide (DMSO). The test organisms with the inoculum size of  $10^5$  colonyforming units (CFU)/ mL were streaked on the surface of the media Muller-Hinton agar (Hi-media) using sterile cotton swab.Sterile Whatman paper disc impregnated with 20 µl of each extracts. DMSO was applied as a negative control to detect the solvent effects. The plates were incubated at 28°C for 48 hrs. The diameter of the clear zones surrounding the disc were measured.

## **RESULTS AND DISCUSSION**

Several endophytic fungi have been isolated from a variety of plant which have proved as a rich source of biologically active metabolites. (Devi *et al.*, 2013).



Fig. 1Antifungal activity of Endophytes against Colletotrichum acutatum.A. Aspergillus stellatusB. Epicoccum nigrumC. Penicillium chrysogenumD. Stachybotrys nilgirica



Fig. 2: Antifungal activity of Endophytes against Candida albicans.A. Stachybotrys nilgiricaB. Penicillium chrysogenumC. Epicoccum nigrumD. Aspergillus stellatus

Medicinal plants have been considered potential source of endophytes synthesizing associated plant natural products (Strobel and Daisy, 2003).

In this study total nine endophytic fungi were isolated from the Dioscorea bulbifera namely Arthrinium phaeospermum, Aspergillus stellatus, Curvularia lunata, Epicoccum nigrum, Nigrospora oryzae, Penicillium Pithomyces chartarum, Phoma chrysogenum, crysanthemicola and Stachybotrys nilgirica. Crude extracts of all the isolated endophytes were extracted. Ethyl acetate crude extracts produced by all the endophytic isolates were screened for their antifungal action and most of the isolates revealed great inhibitory activity against tested pathogen. Among all tested extracts ethyl acetate crude extracts produced by Aspergillus stellatus, Epicoccum nigrum, Penicillium chrysogenum, Stachybotrys nilgirica exhibited

promising results for growth inhibition of pathogenic fungi) (*Colletotrichum acutatum* MTCC 2213) (Fig.1) and (*Candida albicans* MTCC 7315) (Fig.5 to 8).

## CONCLUSION

The results of this study demonstrate the great antifungal potential of endophytic fungi isolated from *Dioscorea bulbifera* agaist pathogenic fungi. Therefore, it suggest that these endophytes can be important sources of bioactive substances which may useful for new drug discovery.

**Conflicts of interest:** The authors stated that no conflicts of interest.

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