www.ioirp.com
International Journal of Innovative Research in Technology, Science & Engineering (IJIRTSE)
ISSN: 2395-5619, Volume – 2, Issue – 3. March 2016

Preliminary Antimitotic Screening of Endophytic Fungi Isolated from Catharanthus Roseus

Vidyalakshmi L, Saurajeet Dey and Sasirekha B
Department of Microbiology, Acharya Bangalore B School, Andrahalli Main road, Magadi road, Bangalore, Karnataka, India. E-mail: sasirekha.b@acharyabbs.ac.in

Abstract - Natural product research has contributed to cancer cure in an indispensible way. Even today the top ten drugs used against cancer are either isolated or semi-synthesized from plants. Vinblastine and vincristine the wonder drugs for cancer, are being isolated from the leaves of field grown *Catharanthus roseus* plant by plant tissue culture, but their supply is limited and cannot meet the present requirements. Therefore the present aims at isolation and preliminary screening of endophytic fungi for antimitotic activity. A total of forty two endophytic fungi (SSV1 to SSV42) from the different parts of *Catharanthus roseus* plants collected from different parts of Bangalore were isolated. Most of the fungi isolated were unusual and slow growing. Out of 42 fungi isolate SSV2 ethyl acetate extract showed antimitotic activity in onion root tips.

Keywords: Endophytic fungi, *Catharanthus roseus*, antimitotic activity

INTRODUCTION

Endophytic fungi colonize living, internal tissues of the plants without causing any immediate overt negative effects [1]. Endophytic fungi are symbiotically associated with plants and can synthesize the same bioactive compounds and natural products as their host plant themselves suggesting the possibility of intergeneric genetic exchange between the plant and the fungus without causing damage to the host [2].

Catharanthus roseus (L.) which is an important medicinal plant of the family Apocynaceae is used to treat many of the fatal diseases. *C. roseus* also possess good antioxidant potential. There are about two common cultivars of *C. roseus* which is named on the basis of their flower colour that is the pink flowered 'Rosea' and the white flowered 'Alba'[3].

Plant based drugs from a microbial source like endophytic fungi will be of immediate interest to pharmaceutical industries as these will help in getting rid of the several geographical and political barriers associated with transportation of plants as well as from the various environmental conditions which can hamper the quality and production of desired compounds. Microbial fermentation has several advantages over using parts of the plants for the production of drugs and bioactive substances as this can easily be carried out in tank fermenter providing unlimited supply of drugs and negating the requirement of plant parts. Moreover, different stronger derivatives of the drugs can be obtained by altering the culture conditions. Also, the microbial extraction procedures are very easy and require less solvent in order to purify the drugs [4].

Fungi as sources for novel antifungal agents have been screened and among the many compounds isolated from fungi the echinocandins and the pneumocandins are highly potent and promising and are currently in clinical trials. Therefore the present study aimed at isolation of endophytic fungi with antimitotic activity from the different parts of *Catharanthus roseus* plant.

MATERIALS AND METHODS

Isolation of Endophytic Fungi from *Catharanthus roseus*

Sampling

Catharanthus roseus plants were collected from different areas of Bangalore, India to determine the number of genera and species of endophytes present in the plant. After plant selection, leaves, stem and buds were cut with the help of sterile scalpel and placed in sterile plastic bags to store the material at 4˚C until isolation procedure was started.

Isolation of endophytic fungi

Isolation of endophytic fungi was carried out according to the method described by Suryanarayanan *et al.* [5]. The leaves were cut into

small pieces approximately (0.2 cm x 0.2 cm) and surface sterilized with 0.01% mercuric chloride ($HgCl_2$) solution for 60 seconds and washed thoroughly with sterilized distilled water. Residual water on their surface was removed by soaking on sterile blotting paper. Small pieces of leaves were placed on the surface of potato dextrose agar (PDA) poured into Petri dishes. From the fungal population, only the slow growing and unusual fungi were considered for further study. After 10-15 days, fungi were observed growing from the leaves in the plates. Individual hyphal tips of the various fungi were removed from the PDA plates and placed again on PDA plates and incubated at room temperature for at least 10-15 days. Each fungal culture was checked for purity and maintained in agar slants. The fungal isolates were identified based on their morphological characters conidiospore structures.

Mass cultivation of endophytic fungi

The fungal endophytes were mass cultivated by inoculating agar blocks containing mycelium from 7 days old PDA slants onto 100ml potato dextrose broth in 250ml Erlenmeyer flasks. Inoculated flasks were incubated at 25° C for 7 days. After 7 days of incubation, the culture was harvested and passed hrough muslin cloth to separate the mycelia from the culture broth.

Extraction of metabolites from endophytic fungi

Fungal metabolite from different endophytic culture broth was extracted using ethyl acetate. Equal volume of the filtrate and ethyl acetate was taken in a separating funnel and shaken vigorously for 10 min. The solution was then allowed to stand and organic solvent was collected and evaporated to dryness to yield crude extract [6].

Screening of antimitotic activity using onion root tip: The antimitotic activity was performed according to the method described by Rai *et al.*[7]. Onion bulbs were placed over coupling jars containing distilled water till roots developed actively. After reaching a length of 3- 4 cm, root tips were excised and placed in test tube containing test (crude extract) and control respectively. Water was used as control. The root tips were collected after and 48h of incubation. Root tips were macerated and placed in a fixative solution of ethanol and 45% acetic acid (3:1) for 12h. Then the root tips were hydrolysed with HCl (1N) for 15 min at 60°C and placed on a clean glass slide, followed by squashing in 2% acetocarmine stain in 45% acetic acid. The squash preparation was observed under the microscope.

RESULTS AND DISCUSSION

A total of 42 endophytic fungi were isolated from the different parts of *Catharanthus roseus* plant. Isolated endophytic fungi were grown on potato dextrose agar (PDA) at 28°C and stored on the same medium at 4°C for further use (Figure 1). From the leaves, stems and pods of *Catharanthus roseus* plant different strains of endophytic fungi isolated and identified as belonging to the genera *Alternaria, Fusarium, Aspergillus, Cladosporium, Penicillium, Rhizopus* and *Mucor* (Table 1; Figure 2, 3). Mycelia sterilia was a large group of fungi which failed to sporulate.

.

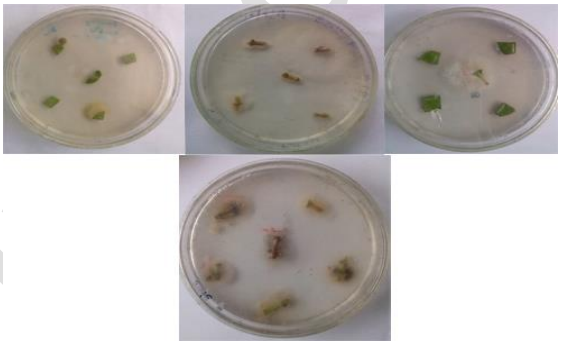

Figure 1. Isolation of endophytes from *Catharanthus roseus* plant

Figure 2. Isolate SSV2 on PDA media

Table 1: Number of endophytes isolated from different parts of *Catharanthus roseus* plant

S. No	Part	No of colonies observed

1.	Leaf	29
2.	Stem	9
3.	Pod	4

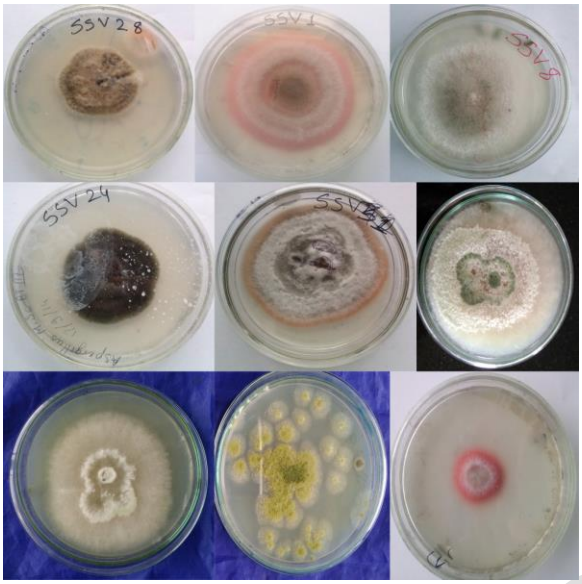

Figure 3. Isolation of endophytes from *Catharanthus roseus* plant on PDA

A number of endophytic fungi have been isolated by Kharwar *et al.* [8] from the *Catharanthus roseus* plant found in India. A rich diversity of endophytic fungi associated with the *Catharanthus roseus* was observed in this study. Fungal growth was initiated mostly within 2 days of inoculation. The difference in diversity of endophytes observed might be due to physiological differences in the interior of the stem parts. Momsia and Momsia [9] reported 7 different endophytic fungal genera, *Alternaria alternate*, *Aspergillus* sp., *Curvularia* sp., *Penicillium* sp., *Trichoderma* sp., *Helminthosporium* sp., *Fusarium* sp. in leaves and nodes of *Catharanthus roseus*.

Chromosomal aberrations are changes in the structure of chromosomes resulting from breaks or exchange of chromosomal materials [10]. On studying the antimitotic activity of ethyl acetate extract of fungal isolates, SSV2 isolate showed inhibition of mitosis. After 48hr of treatment, complete dissolution of cytoplasm, nucleus was intact (Figure 4). Channabasava and Govindappa [11] reported *in vitro* antimitotic, antiproliferative activity by endophytic fungi, *Aspergillus niger*.

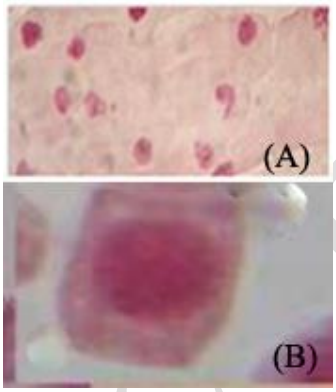

Figure 4. Chromosomal aberrations induced in *Allium cepa* by crude extract of SSV2

Similar results were observed with plant extracts of *Ocimum gratissimum*, *Morinda lucida* [12], endophytes *Fusarium oxysporum*, *Trichothecium* sp lectin [13] and *Aspergillus niger* lapachol [11].

CONCLUSION

Discovery of endophytic fungi in plant tissues opened up new avenues in search for metabolically active compounds. *Catharanthus roseus* harbours diverse species of endophytic fungi and Isolate SSV 2 crude extract displayed antimitotic activity. Further investigation of this study will focus on the bioactive secondary metabolites of SSV2 fungi.

ACKNOWLEDGMENT

We would like to express our sincere gratitude to our institution Acharya Bangalore B School for allowing us to do our project in the college.

REFERENCE

[1]. S.P. Wasser, "Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides". Appl. Microbiol. Biotechnol., 2002, 60, 258-274.

[2]. R. X. Tan, W.X. Zou, "Endophytes: a rich source of functional metabolites". Nat. Prod. Rep.,2001, 18: 448-459.

[3]. C. A. Jaleel, R. Panneerselvam, "Variations in the antioxidative and indole alkaloid status in differentparts of two varieties of *Catharanthus roseus*: An important folk herb". Chinese Journal of Pharmcology and Toxicology. 2007; 1(6): 487- 494.

[4]. A. Kumar,D, Patil, P.R. Rajamohanan, A. Ahmad A (2013) Isolation, Purification and Characterization of Vinblastine and Vincristine from Endophytic Fungus*Fusarium oxysporum* isolated from *Catharanthus roseus*. PLoS ONE,2013, 8(9), e71805.

[5]. T.S. Suryanarayanan, G. Venkatesan, T.S. Murali, "Endophytic fungal communities in leaves of tropical forest trees: Diversity and distribution patterns. Current Science, 2003,85(4),489-492.

[6]. N. S. Raviraja, G.L. Maria, K.R. Sridhar," Antimicrobial evaluation of endophytic fungi inhabiting medicinal plants of the Western Ghats of India". English Life Sci., 2006, 6515-20.

[7]. K.M.L. Rai, Y.B. Basavaraju, B. Sadashivamarthy, " Biological assay and antimitotic activity of novel analogues of β-apopicropodophyllin". Indian J. Pharmaceutical Sci., 69, 116-118.

[8]. R.N. Kharwar, V.C. Verma, G. Strobel, D. Ezra. The endophytic fungal complex of *Catharanthus roseus* (L.) G.Don. Current Science, 2008, 95 (2), 229-233.

[9]. P. Momsia, T. Momsia, "Isolation, Frequency distribution and diversity of novel fungal endophytes inhabiting leaves of *Catharanthus roseus*". International Journal of LifeScience, Biotechnology and Pharm Research, 2013 2(4), 83-87.

[10]. A.O. Sultan, T.A. Celik, "Genotoxic and antimutagenic effects of *Capparis spinosa* L. on the *Allium cepa* L. root tip meristem cells". Caryologia,2009, 62(2),114–123.

[11].Channabasava, M. Govindappa, "First report of anticancer agent, lapachol producing endophyte, *Aspergillus niger* of *Tabebuia argentea* and its *in vitro* antimitotic, antiproliferative and DNA fragmentation assay". Bangladesh Journal of Pharmacology, 2014, 9, 129-139.

[12]. B.M. Oyedare,A.A. Bakare, A. Akinboro, " Genotoxicity assessment of water extracts of *Ocimum gratissimum*, *Morinda lucida* and *Citrus medica* using the *Allium cepa* assay". Boletín Latinoamericano y del Caribe de Plantas Medicinalesy Aromáticas, 2009, 8, 97-103.

[13]. T.S Sadananda, M. Govindappa , Y.L Ramachandra, "Antibacterial activity of *Viscum album* endophytic fungal lectin'. International Journal of Biological & Pharmaceutical Research,2013, 4, 1033-1042.