

## DIVERSITY OF ENDOPHYTIC FUNGI FROM *HELIOTROPIUM INDICUM* L. LEAF

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Article Received on  
06 Jan. 2018,

Revised on 27 Jan. 2018,  
Accepted on 18 Feb. 2018

DOI: 10.20959/wjpr20185-11262

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### ABSTRACT

Diverse endophytes colonize internal tissue of plants. Recently, endophytic fungi residing in plants have gained undisputable attention, thus requiring their systematic identification and characterization. In this study, 179 endophytic fungi isolated from *Heliotropium indicum* leaves among 179 isolates 12 species were documented using traditional morphological methods. The isolated endophytes were identified based on the colony morphology and spore structures. The frequency of dominant genera *Colletotrichum* and *Aspergillus* is the major constituent, likewise *Alternaria alternata*, *Acremonium sps*, *Bipolaris tetramera* and *Curvularia lunata* of minimal constituent of

*Heliotropium* leaves. Result of this experiment *Heliotropium indicum* leaf consist of pharmaceutically significant fungal endophytes. Very interestingly, Three fungal endophytes (*Colletotrichum sps*) were found and highly important in the pharmaceutical research. Our study proves the promising natural product biosynthetic potential of fungi associated with *Heliotropium indicum* leaves.

**KEYWORDS:** *Heliotropium indicum*, Endophytic fungi, *Alternaria*, *Colletotrichum* bioactive metabolites.

### INTRODUCTION

Fungal endophytes are highly diverse and their presence in plants is dependent upon the host, the availability of nutrients, the environment and the community composition of other microorganisms (Porrás- Alfaro and Bayman 2011). Some endophytes exhibit specificity to

one tissue type, yet others can be found within multiple locations of the plant (Herrera *et al.* 2010). Bioactive compounds produced by endophytic fungi broadly include alkaloids, steroids, terpenoids, isocoumarins, quinones, flavonoids, phenylpropanoids, lignans, peptides, phenolics, aliphatics, and volatile organic compounds (Zhang *et al.*, 2006). But most interestingly there are several examples for endophytic fungi producing plant specific compounds. The endophytic fungus *Taxomyces andreanae* associated with *Taxus brevifolia* was shown to have the ability to form the anticancer drug taxol similar to host plant (Stierle 1995). The anti leukemia agent vincristine was reported to be synthesized by an endophytic fungus, *Mycelia sterilia* from leaves of *Catharanthus roseus* (Yang *et al.*, 2004). Asperfumin, a bioactive metabolite produced by endophytic fungi *Aspergillus fumigatus*, has shown to inhibit *Candida albicans* (Guimaraes *et al.*, 2008). As the broad applications of endophytic fungi are just begun to explore, the studies on tremendous bioactive metabolites expected from them will be very important. Enormous potential of fungal metabolites and increased demand for novel bioactive compounds signifies the exploration of endophytic fungi from *Heliotropium indicum*. *Heliotropium indicum* is an annual herb commonly known as the Cock's comb. It is usually associated with the moist rich soils of the low and tropics near rivers and lakes on the roadsides and also in waste places (Holm *et al.*, 1977).

## MATERIAL AND METHODS

### Isolation of endophytic fungi

Healthy and mature *Heliotropium indicum* leaf collected from local farms were used as source material for the isolation of fungi. Surface sterilization procedure for the isolation of endophytic fungi was carried out as described by Aravind *et al.* (2009) with minor modifications. Plant samples were washed under running tap water for 10 minutes followed by immersion in 70% EtOH for 1 minute and in NaOCl (2.5% available chlorine) for 10 minutes. Plant samples were then cut aseptically into 1 cm long segments. This was then drained and immersed in 70% EtOH again for 30 sec. Finally, the samples were rinsed with sterile distilled water several times and the final wash was plated on to media as control. The cut surfaces of the segments were grown on petridishes containing Potato Dextrose Agar media amended with penicillin antibiotics. The control and inoculated plates were incubated at 28°C for 7 days and observed for the fungal growth. The fungal isolates obtained were further purified on PDA medium. The isolates were initially subjected to staining and microscopic observation and were further identified by molecular methods.

### Identification of endophytic fungi

The fungi were identified on the basis of morphological characteristics according to Domsch *et al.*, 1980 and Aggarwal and Hasija, 1980.

### Statistical Analysis

The percentage of Colonization frequency (%) were calculated based on the standard methods.

### Colonization frequency (CF %)

The colonization frequency (CF %) of a single endophytic fungal species in the leaf segments were calculated by using the following formula (Suryanarayanan *et al.* 2003).

$$\text{CF (\%)} = \frac{\text{Number of segments colonized by fungi}}{\text{Total number of segments observed}} \times 100$$

## RESULTS

**Isolation of endophytic fungi:** After several rounds of standardization of surface sterilization procedure, the isolation resulted in the purification of 12 endophytic fungi *Colletotrichum sps*, *Alternaria sps*, from *Heliotropium indicum* leaves (Table 1). The absence of growth in the control plate ensured the proper surface sterilization of the used plant tissue and confirmed the isolated microbes as endophytes. The isolates were initially distinguished by the difference in colony characters and further by morphological features using staining techniques. The isolates with distinct characters were selected, purified and sub-cultured for maintenance as pure culture on PDA slants for further studies.

### Morphological Identification of Endophytic fungi

The colonies appearing on petriplates were sub-cultured into the tube containing potato dextrose agar medium for identification. Fungi were again cultured from slant to petriplates containing potato dextrose agar medium without antibiotic for 7 days. Morphological identification was done according to the standard taxonomic key included colony diameter, texture, color and the dimensions and morphology of hyphae and conidia.

**Source of fungal Endophytes from *Heliotropium indicum* leaves:** A total of 179 isolates were obtained from 189 segments of plant parts viz., leaf *H. indicum*. Among them total of 12 species of fungal endophytes were isolated from the *Heliotropium indicum* leaves such as, *Alternaria alternata*, *Aspergillus brevipes*, *Aspergillus sps*, were recorded as most common

species and occurred in two segments(Fig.1). *Acremonium sps*, *Bipolaris tetramera*, *Curvularia lunata*, were recorded as occasional and occurred in only one sample *Fusarium oxysporum*, *Colletotrichum acutatum*, *Colletotrichum coccodes*, *Colletotrichum falcadum*, *Camarosporium palliatum*, *Phoma sps*. were recorded as most common and occurred in four segments(Table.1). In leaves, endophytic fungal colonization was dominated by *Aspergillus* two sps and *Colletotrichum* three sps whereas *Alternaria* and *Curvularia* showed low percentage of colonization. In leaves *Aspergillus brevipes*, *Aspergillus sps* and *Colletotrichum* showed maximum percentage of colonization frequency, while *Alternaria* and *Phoma* showed minimum percentage of colonization. Number in parentheses designates the exact number of samplings in which the respective fungus was recorded.

**Table. 1. Endophytic fungi isolated from leaves of *Heliotropium indicum*.**

Sl. No	Endophytic fungi	Colony frequency (%)
1.	<b>Hyphomycetes</b> <i>Alternaria alternata</i>	2.5
2.	<i>Aspergillus brevipes</i>	2.3
3.	<i>Aspergillus sps</i>	2.8
4.	<i>Acremonium sps</i>	5.7
5.	<i>Bipolaris tetramera</i>	8.6
6.	<i>Curvularia lunata</i>	11.4
7.	<i>Fusarium oxysporum</i>	1.8
8.	<b>Coelomycetes</b> <i>Colletotrichum acutatum</i>	8.6
9.	<i>Colletotrichum coccodes</i>	3.1
10.	<i>Colletotrichum falcadum</i>	5.7
11.	<i>Camarosporium palliatum</i>	2.8
12.	<i>Phoma sps</i>	3.6

## DISCUSSION

The extensive medicinal use of *H. indicum* is not paralleled by adequate scientific data there is therefore, the need to add to the existing scientific data to provide. Leaves have been used as cold infusion, enema to stop abdominal pains and treat cataract. The juice from the leaves is squeezed into the eye to stop dizziness; decoction of the whole plant is used to treat convulsion in children; the roasted aerial parts of the plant together with certain ingredients are used as an enema for expulsion of clotted blood in a women who have recently given birth, the poultice of the leaves mixed with honey is externally applied to the penis to restore virility, Irvine (1960). Previous researches on this plant includes wound healing activity of *H. indicum* (Reddy *et al.*, 2002) had reported that chloroform extract of *H. indicum* dose dependently inhibited the carrageenan induced rat paw oedema and also showed anti-

nociceptive activity in rats. Kugelman *et al.* (1976) isolated N-oxide of the alkaloid indicine from *H. indicum* observed that it has significant anti-tumor activity. Crude hexane extract of *H. indicum* had antimicrobial activity against *Mycobacterium tuberculosis* (H37Ra) (Machinan *et al.*, 2005). The petroleum ether and chloroform fractions of ethanolic extract of *H. subulatum* showed strong antibacterial activity against *E. coli*, *S. aureus*, *Streptococcus pneumonia* and *Bacillus subtilis*. That strong antibacterial activity might be due to the purification of five pyrrolizidine alkaloids from this plant (Singh *et al.*, 2002). The methanolic extract of aerial parts of *H. indicum* has broad spectrum antibacterial activity against *S. aureus*, *S. pneumonia*, *Salmonella typhi*, *E. coli* and *Klebsiella pneumonia* (Oluwatoyin *et al.*, 2011). The antibacterial significance of plant extracts is attributed mainly to the presence of terpenoids (Urzúa *et al.*, 2007). The sterols and triterpenoids such as sitosterol, stigmasterol, amyirin, friedelanol, cycloartenone, amyirin acetate and friedelin isolated from ethanol extract of the whole plant of *H. ellipticum* exhibited strong antibacterial activity against *E. coli*, *S. aureus* and *K. pneumonia* (Jain *et al.*, 2009). Filifolinol, one of the geranyl aromatic derivatives isolated from *H. sclerocarpum* and *H. filifolium* also showed significant antibacterial activity against *S. aureus*, *Bacillus cereus*, *B. subtilis* and *Micrococcus luteus* (Modak *et al.*, 2009). In addition to the genus *Heliotropium*, other plant species also exhibited antibacterial potential because the most important fungal isolates (*Colletotrichum*) were present the leaf samples of *Heliotropium indicum*. Our study is the first evidence presence of valuable endophytes are shown in the leaf samples. *H. indicum* is the one of the most important medicinal plants because of the presence of medicinally important endophytic fungus. Among these isolates, *Colletotrichum* sps has been reported as common endophytes. *Alternaria* is common saprobe found on many plants and other substrata worldwide, including pine needles (Grunden *et al.*, 2001). Altenusin had displayed broad antimicrobial activity against several additional multidrug-resistant bacterial and fungal strains. These reports strongly support the view that the endophytic fungi isolated from *Heliotropium indicum* are promising sources of antimicrobial agents. The results of the bioactivity suggest the presence of diverse metabolites in the fungal isolates obtained. These microbes with their metabolite richness and diversity clearly indicate promising applications of endophytic fungus obtained in the study. Musetti *et al.*, (2008) reported that three dipeptides, belonging to the family of diketopiperazines (DKPs) were extracted from broth culture of the grapevine endophyte *Alternaria* species and were tested against *Plasmopara viticola* on leaves of grapevine plants grown in greenhouse. Moreover, altersetin purified from an endophytic *Alternaria* sp. displayed potent activity against pathogenic Gram-positive

bacteria (Hellwig *et al.*, 2002). Thus, from the above contributions, researchers have come to know that the identification, isolation and purification of different groups of fungal endophytes from *H. strigosum* should reveal that this plant has a perceptible future role in the field of medical industry.

Diversify the endophytic fungi associated from the leaves of *Heliotropium indicum* it's a rare reports are find the diversity of endophytic fungi in *Helitropium* species. Even though much more species of endophytic fungi can be expected from the plant, the conditions and media used in the current study might have favoured the growth of the species obtained. Even these fungi can be a novel source for the production of compounds which can have a diverse implication. Currently we are working on the characterization of the biologically active metabolites from the isolated endophytic fungi.

## CONCLUSION

In conclusion, scientists and pharmacologists should pay serious attention to the screening of this plant by using some other scientific bioassay methodologies which might serve as a source for the identification, purification and isolation of beneficial bioactive constituents that seems to be helpful in the synthesis of new therapeutic agents of desired interest. Therefore, in the future, *Heliotropium* will be used globally as a source of safer phytomedicines.

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