

Screening of Phytochemicals and Isolation of Endophytic Fungi from Medicinal plant *Helicteres isora* L.

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Abstract: The objective of this study was to screen the phytochemicals and isolate the endophytic fungi harbored in the leaf and stem of medicinal plant *Helicteres isora* L.. Medicinal plants play a key role in traditional medicine and medicinal property is found to be because of phytochemical constituents present in the plants. Phytochemicals such as alkaloids, carbohydrates, tannins, steroids, flavonoids, saponins, terpenoids, coumarins, carboxylic acid, resins, quinone and phenolic compounds were analyzed by using ethanol as solvent. There is a correlation between phytochemicals and secondary metabolites produced by the endophytic fungi. The medicinal plants and their fungal endophytic communities produce similar therapeutic products. The results of the present study showed the presence of secondary metabolites and endophytic fungi such as *Penicillium citrinum*, *Aspergillus niger*, *Curvularia vermiformis*, *Rhizopus* sp., *Cladosporium* sp., *Alternaria alternata* and *Fusarium* sp., were isolated and identified by morphological characters.

Keywords: Endophytic fungi, *Helicteres isora* L., Phytochemicals, Secondary metabolites.

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I. INTRODUCTION

Helicteres isora L. is a medicinal plant used in traditional medicine. The root and bark are expectorant, demulcent, astringent to the bowels, antilactagogue, lessen gripping; a cure for scabies when applied topically (Unani). The juice of the root is said to have a beneficial effect in empyema and stomach infections. In the Konkan it is used in diabetes, and is a favourite cure for snake-bite. The bark is used in diarrhoea and dysentery. The fruits are made into liniment for sores of the ear, and they are administered internally for colic.[1]

Medicinal plants are known to harbour endophytic fungi that are believed to be associated with the production of secondary metabolites and phytochemicals [2]. Endophytic organisms that exist in the tissues of living plants are potential resources of novel natural products for exploitation in pharmaceutical industries[3]. Endophytes are microorganisms that are present in living tissues of various plant parts establishing natural relationship without apparently causing any symptom of diseases [4,5]. These endophytes protect their hosts from infectious agents and adverse conditions by securing bioactive secondary metabolites[6,7]. Of the 300,000 plant species that exist on earth, each individual plant is to host one or more endophytes [8,9].

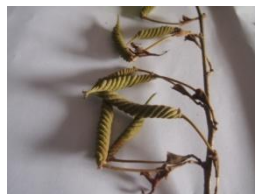
II. MATERIALS AND METHODS

2.1 Plant collection and identification

Healthy and mature leaves and stems of medicinal plant *Helicteres isora* L. were collected from different places of Udipi District of Karnataka State in plastic bags. The collected plant material was authenticated by the Taxonomist Dr. K. Gopalakrishna Bhat, Department of Botany, Poornaprajna College, Udipi, Karnataka, India. Fresh plant materials were used for preliminary phytochemical analysis and isolation of endophytic fungi.



Helicteres isora L.



Helicteres isora L. (Fruits)

2.2 Preliminary phytochemical analysis

Phytochemical analysis was done with air dried and powdered ethanolic extract of plant material, as per standard procedure [10]

2.3 Isolation of endophytic fungi

The plant materials were rinsed gently in running water to remove dust and debris. After proper washing, the leaf samples were cut into small pieces, and leaves were selected for further processing under aseptic condition. Highly sterile conditions were maintained for the isolation of endophytes. All the work was performed in the laminar air hood. Sterile glass ware and mechanical things, such as scissors, forceps, scalpel, blades were used in all experiment. Leaves were cut into 3-4mm in diameter 0.5-1cm in length with and without midrib. The isolation of endophytic fungi was done according to the standard method [11]. The surface sterilization was done by sodium hypochlorite(NaOCl) and 95% ethanol. Finally the leaves washed in sterile distilled water for 2 minutes. The plant material was blotted on sterile blotting paper. In each Petri dish 5-6 segments were placed on PDA medium.

Each Petri dish containing PDA is supplemented with antibiotics Penicillin G 100units per ml and streptomycin 100Microgram per ml. The dishes were sealed with parafilm and incubated at $27 \pm 2^{\circ}$ C for 4-6 weeks at dark. Most of the fungal growth was expected within two weeks of inoculation. The incubation period for each fungus was recorded. Observation was carried out daily until the growth of endophytic fungi was observed.

2.4 Identification of endophytic fungi

The isolated fungi were Identified based on the morphology of surface texture, pigmentation and spores at the hyphal tips which were used to identify the endophytic fungi at species level using standard manual[12,13]. The microscopic examination was also done to study their reproductive spores[14].The identified fungal isolates from the respective plant tissue segments were isolated and then subcultured in a Petri dish which contains sterile PDA media. To preserve as a pure culture, the endophytic fungi was inoculated in PDA slant and incubated at 4° C.



Endophytic fungal growth on PDA medium.

2.6 Calculation of colonizing frequency

Colonizing frequency was calculated as per standard procedure[15].

$$\text{Colonization frequency} = \frac{\text{Number of segments colonized by fungi}}{\text{Total number of segments observed}} \times 100$$

2.7 Relative Percentage Occurrence (RPO) of Different Fungi is calculated by using the following slightly modified formula [16]

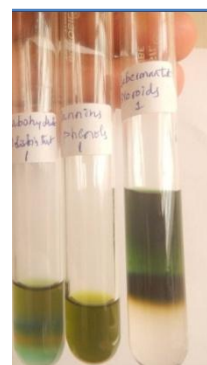
$$\text{RPO} = \frac{\text{Density of colonization of one group}}{\text{Total Density of colonization}} \times 100$$

III. RESULTS

Traditional medicinal plant *Helicteres isora* possess phytochemicals such as alkaloids, carbohydrates, tannins and phenolic compounds which, is shown in table 1. In the present study, 12 leaf and 12 stem segments of *Helicteres isora* were processed for isolation of endophytic fungi. A total of 7 fungi were isolated out of which four fungal forms belong to the class Ascomycetes, two belong to Hypomyctes and one Zygomycetes, which is shown in Table 2. Colonization frequency percentage and Relative Percentage Occurrence(RPO) are also given in Table 3, 4 and Figures 1 and 2 respectively.

Table 1: Results of preliminary phytochemical tests

Test for	Present/Absent
Alkaloid	+
Steroid	-
Carbohydrate	+
Tannin	+
Flavanoids	-
Saponins	-
Terpenoid	-
Coumarins	-
Phenol	+
Carboxylic acid	-
Resins	-
Quinone	-



Carbohydrate(+), Phenol(+), Steroid(-)

(+): Present (-):Absent

Table 2: Endophytic fungi isolated from different parts of *Helicteres isora* L.

S.No.	Plant Parts	Name of the Endophytic fungi	Class
1	Leaf	<i>Alternaria alternata</i>	Ascomycetes
2	Leaf	<i>Penicillium citrinum</i>	Ascomycetes
3	Leaf	<i>Aspergillus niger</i>	Ascomycetes
4	Leaf	<i>Cladosporium</i> sp.	Ascomycetes
5	Leaf	<i>Rhizopus</i> sp.	Zygomycetes
6	Stem	<i>Curvularia vermiformis</i>	Hyphomycetes
7	Stem	<i>Fusarium</i> sp.	Hyphomycetes

Table 3: Name and colonizing frequency of Endophytic fungi isolated from *Helicteres isora* L.

S.No.	Name of the Endophytic fungi	Isolate from	Total number of segments observed	Number of segments colonized	% Frequency of colonization
1	<i>Alternaria alternata</i>	Leaf	12	3	25%
2	<i>Penicillium citrinum</i>	Leaf	12	2	16.6%
3	<i>Aspergillus niger</i>	Leaf	12	4	33.3%
4	<i>Cladosporium</i> sp.	Leaf	12	1	8.3%
5	<i>Rhizopus</i> sp.	Leaf	12	2	16.66%
6	<i>Curvularia vermiformis</i>	Stem	12	2	16.66%
7	<i>Fusarium</i> sp.	Stem	12	3	25%

Fig.1: Colonization Frequency (%) of Endophytic fungi isolated from *Helicteres isora* L.

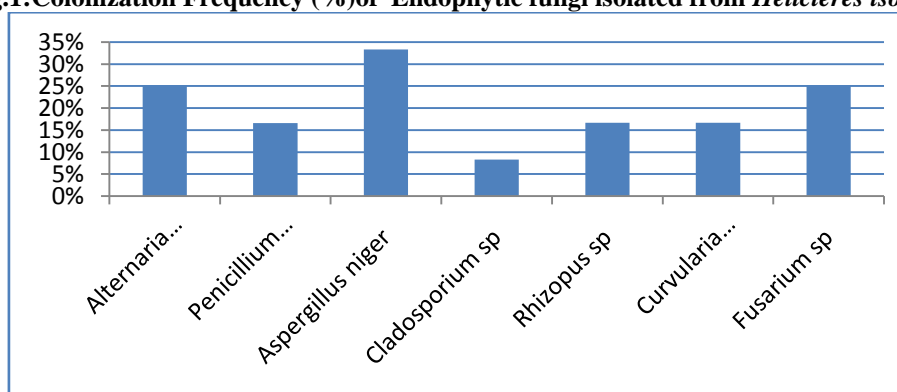
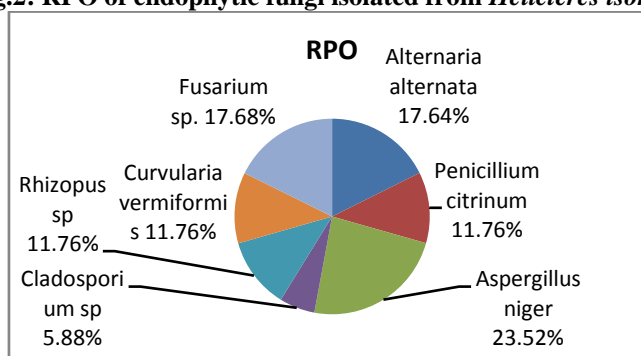


Table 4: RPO of Endophytic fungi isolated from *Helicteres isora* L.

S.No.	Name of the Endophytic fungi	Isolate from	RPO
1	<i>Alternaria alternata</i>	Leaf	17.64%
2	<i>Penicillium citrinum</i>	Leaf	11.76%
3	<i>Aspergillus niger</i>	Leaf	23.52%
4	<i>Cladosporium</i> sp.	Leaf	05.88%
5	<i>Rhizopus</i> sp.	Leaf	11.76%
6	<i>Curvularia vermiformis</i>	Stem	11.76%
7	<i>Fusarium</i> sp.	Stem	17.64%

Fig.2: RPO of endophytic fungi isolated from *Helicteres isora* L.



IV. DISCUSSION

Medicinal plants contain a wide variety of phytochemicals such as phenolic compounds, flavanoids, lignin, quinones, coumarins, which are having therapeutic values. In addition to plants, endophytes residing in medicinal plants are considered as rich sources of bioactive metabolites of multifold importance in medicine, agriculture and industries[17]. Preliminary screening of leaves of medicinal plant *Moringa oleifera* with different solvent methods showed the presence of alkaloids [18]. Many Endophytic fungi have been reported to produce novel anti bacterial, anti fungal, anti viral, anti inflammatory and anti tumor compounds. In various studies, the population and biodiversity of the fungal endophytes have been found to be varying as the environmental conditions under which the host is growing. The host plant composition, as well as seasonal variation affects the endophytic population[19].

The phytochemicals such as phenolic compounds, flavonoids and tannins etc generally occur in most of the medicinal plants. The medicinal plants and their fungal endophytic communities produce similar therapeutic products. Medicinal plants are known to harbour endophytic fungi that are believed to be associated with the production of pharmaceutical products[20]. The phenolic compounds and flavonoids isolated from endophytes of *Calotropis procera* are found to be similar and responsible for antioxidant properties[21].

V. CONCLUSION

Traditional medicinal plant *Helicteres isora* L. contains phytochemicals such as carbohydrates, tannins, phenolic compounds and alkaloids. A total of 7 endophytic fungi were isolated from this plant may also have similar chemical compounds. As there is correlation between the metabolites produced by the host plant and its endophytes, further investigation of these endophytic fungi for bioactive compounds may lead to the discovery of new drugs. The use of herbal crude drugs, in tracts and their remedies have significantly increased throughout the world. Efforts must be made to ensure safe, effective and affordable treatments for wide range of diseases by traditional methods which use locally available medicinal plants. The scientific and authentic researches on these aspects are to be done in order to exploit traditional knowledge of medicinal plants.

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