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Research Article

Harnessing Endophytic Fungi from *Terminalia chebula*: A Dual Approach to Antimicrobial and Phytochemical Exploration

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ABSTRACT

Endophytic fungi isolated from *Terminalia chebula* exhibit potent antimicrobial activity, showcasing their potential to develop novel therapeutic agents. Additionally, these fungi produce diverse phytochemicals, which may contribute to their medicinal properties and offer new avenues for natural product research. This research study investigated the endophytic fungi isolated from *T. chebula* and assessed their antimicrobial and phytochemical potential. A total of 28 endophytic fungal species were isolated from leaf and bark tissues. Antimicrobial assays revealed significant inhibitory effects against pathogenic fungi *Candida albicans* - MTCC 183 & *Saccharomyces cerevisiae* - MTCC 174 and bacteria *Escherichia coli* - MTCC 2939, & *Bacillus subtilis* - MTCC 1305, with endophytic *Alternaria sp.* exhibiting maximum inhibition zone for antifungal activity and endophytic *Aspergillus flavus* demonstrating potent activity for their antibacterial analysis. A wide array of phytochemical compounds like alkaloids, flavonoids, phenols, steroids, terpenoids, tannin, saponin, etc, were observed whereas, phytochemical screening indicated the presence of alkaloids and flavonoids in all selected endophytes. These findings suggest that *T. chebula* harbors a rich endophytic community with the potential for producing novel antimicrobial compounds.

Keywords: Endophytic fungi, Isolation, Antimicrobial, *Terminalia chebula*, Phytochemical.

INTRODUCTION

Endophytes are microorganisms that reside within healthy plant tissues inter and intracellularly without causing any disease symptoms to the host.[1] This symbiotic relationship offers a unique advantage to both partners. The plant can benefit from the endophyte's ability to enhance nutrient acquisition, stress tolerance, and defense against pathogens.[2] They often establish mutualistic relationships, influencing the plant's growth, stress tolerance, and production of bioactive compounds.[3]

In recent years, there has been much focus on the microbial inhabitants of plants, in particular, endophytic fungi, as researchers recognize their potential contributions to the host plant's health and their possible applications in various fields. In turn, the endophyte gains access to nutrients and a protected environment within the host plant.

Recent research focuses on fungal endophytes, an ecological group presumed to harbor many hitherto unknown fungal species.[4,5] A source of important natural bioactive compounds[6] and industrial enzymes.[7]

Endophytic fungi of *Terminalia chebula* Retz. which is valued for its proven versatile pharmacological potentials is selected for the present study. After discovering the anti-cancerous drug Taxol, its importance is well established from the endophytic fungi *Taxomyces andreanae*.[8] *T. chebula*, the revered "king of medicine," is a treasure trove of medicinal properties. And it seems the magic extends beyond the plant itself to its microbial inhabitants.[9]

The present research paper is intended to explore the interesting world of fungal endophytes of *T. chebula* from different sites in the Patna region. In light of the above, this study aims to examine the phytochemicals of different endophytic fungi associated with *T. chebula* of the Patna region and further evaluate their antimicrobial potential. Our findings may form a basis for further studies on endophytic fungi from these medicinal plants for use in medicine in the view of developing novel potential drugs.

MATERIAL AND METHODS

Study Plant

T. chebula Retz., family Combretaceae is commonly known as "Haritaki" or "Chebulic myrobalan.

Plant Material Collection

Healthy, fresh, and symptomless leaves and inner tissue of the bark of *T. chebula* were collected from two regions of the Patna district. Plant samples were placed in sterile polythene bags and, transported in the icebox to the laboratory, and processed within 24 to 48 hours of collection.

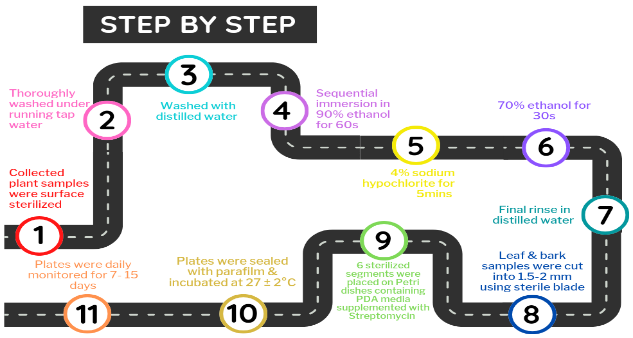

Fig. 1: Step by step process for isolation of endophytic fungi

Isolation of Endophytic fungi from freshly collected samples

Isolation of Endophytic fungi from *T. chebula* was carried out using the protocol described by[10] with slight modifications (Fig. 1).

Identification of Endophytic Fungi

Morphological identification of isolated endophytic fungi was made based on the morphology of cultural characteristics and direct microscopic observations of hyphae and reproductive structures using relevant keys and standard taxonomic fungal manuals.[11-15]

Extraction of Fungal Secondary Metabolites

Following standard methods,[16] fungal isolate was transferred into 100 mL potato dextrose broth medium (PDB) in 250 mL Erlenmeyer flasks and kept at 28°C for 21 days in a shaking (150 rpm) incubator and extracted by using ethyl acetate. Filtrate was mixed with an equivalent volume of ethyl acetate and placed on a vortex shaker for 10 minutes. Then, it remained for 5 minutes until the two clear separate layers were achieved. The separating funnel separated the ethyl acetate layer from the extract. The collected phase was evaporated using an oven at 60°C. Finally, DMSO (dimethyl sulfoxide) at 1-mg/mL of concentration was used to dissolve the fungal crude extract and then stored at −20°C until further experiments.

Antimicrobial Activity Assays

Antifungal assays

- *Well diffusion method*

The antifungal activity of each crude metabolite obtained from the selected endophytic fungus was screened against clinical pathogens using the well diffusion assay.[17]

For the susceptibility test, 20 mL SDA medium was poured into Petri plates for *Candida albicans* - MTCC 183 & *Saccharomyces cerevisiae* - MTCC 174 under aseptic conditions, and 100 μL of fungal suspension was evenly spread. All the culture plates were allowed to dry for about 5 minutes. Wells were bored in the center of the agar surface using a cork borer and wells were filled with 100 μL of endophytic fungal secondary metabolites, respectively. For positive control, Fluconazole, whereas 10% DMSO, was used as a negative control. Finally, the inoculated plates were incubated for 24 to 48 hours at 30 ± 2°C. The inhibition zone was measured in mm.

Antibacterial Assays

Agar plug method

Following the methodology outlined by,[18] a screening of antibacterial activity was conducted. The test organism used was *Escherichia coli* MTCC 2939, and *Bacillus subtilis* MTCC 1305. Nutrient agar (NA) medium was poured into petri plates and inoculated with 100 μL of a bacterial suspension (1.5×10^8 cfu/mL), spread uniformly across the medium using a sterile cotton bud. Mycelial discs (6 mm) from each endophytic fungal isolate, which had been cultivated on potato dextrose agar (PDA) for 7 to 10 days, were obtained from the actively growing margins of the isolates using a sterile cork borer. These discs were then placed on the surface of the NA medium that had been seeded with the test organisms. Control discs (6 mm) of PDA without any fungal colonies were used as negative controls. The plates were sealed with parafilm and incubated at 37°C for 24 hours. After incubation, antibacterial activity was assessed by visualizing and measuring the diameter of the inhibition zones surrounding the fungal plugs. A clear zone indicates antibacterial activity. The experiment was performed in triplicate to evaluate the antibacterial activity.

Phytochemical screening

Qualitative phytochemical screening was performed following the standard methods.[19-21]

RESULTS AND DISCUSSION

Isolated Endophytic Fungi

A total of 297 colonies were successfully isolated from 324 segments of leaves and living bark tissue of *T. chebula* trees from two different locations in the Patna region. Out of 297 colonies, 28 different species and many genera were identified and 10 different endophytic fungal secondary metabolites were selected for their bioactive analysis. These isolates were identified on their morphological and microscopic characteristics which are as follows: *Aspergillus flavus, A. niger, A. fumigatus, A. oryzae, A. candidus, A. nidulans, A. versicolor, Alternaria* sp, *Cladosporium* sp, *Curvularia* sp, *Epicoccum, Penicillium notatum, P.*

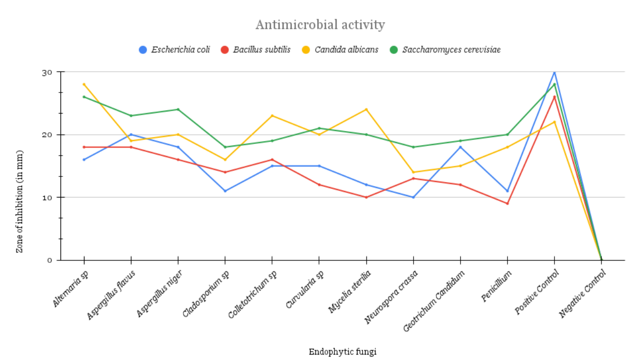

Fig. 2: Antimicrobial activity of the endophytic fungi isolated from *T. chebula* against bacterial and fungal test organisms

Table 1: Phytochemical screening of selected fungal metabolites of endophytic fungi isolated from T. chebula

Fungal isolates	*Alkaloids*	*Flavonoids*	*Phenols*	*Saponins*	*Steroids*	*Tannins*	*Terpenoids*	*Cardiac glycosides*
Alternaria sp	+	+	+	+	-	+	+	+
A. flavus	+	+	+	-	-	+	+	+
A. niger	+	+	+	-	+	+	+	+
Cladosporium sp	+	+	+	+	-	+	-	+
Colletotrichum sp	+	+	+	-	-	+	+	-
Curvularia sp	+	+	+	-	-	+	+	-
M. sterilia	+	+	+	-	-	+	+	+
N. crassa	+	+	-	-	-	-	+	-
G.candidum	+	+	+	+	-	+	+	+
P. chrysogenum	+	+	+	+	-	+	+	+

*** + Presence of compound, - Absence of compound*

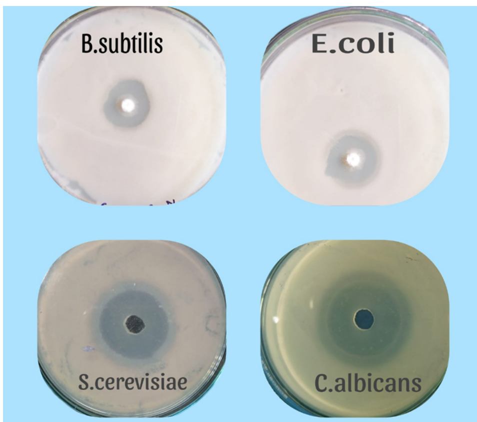

Fig. 3: Antimicrobial activity: Zone of inhibition against *E. coli, B. subtilis*, *C. albicans* and *S. cerevisiae*

chrysogenum, Colletotrichum sp*, Neurospora sitophila, Phoma* sp*, Mycelia sterilia, Bipolaris* sp*, Rhizoctonia* sp*, Mucor* sp*, Geotrichum candidum, Neurospora crassa, Nigrospora, Rhizopus* sp*, Stemphylium, Trichoderma* sp. and some unidentified colonies.

Species of *Aspergillus, Neurospora, Penicillium, Alternaria, Phomopsis, Phoma*, and Mycelia sterilia were the most frequent colonies. Most of the discovered endophytic fungi belong to different taxonomic groups, including Ascomycetes, Coelomycetes, Hyphomycetes, and Mucormycetes.

Antimicrobial Activities

The antimicrobial activity of isolated endophytic fungi was tested for their antibacterial and antifungal activity of selected endophytes. The extracted fungal secondary metabolites were tested for their potential to inhibit the actively growing mycelial discs (6 mm) of each endophytic fungal isolate for 7 to 10 days old grown on PDA media for antibacterial activity against test fungal and bacterial pathogens.

Among the endophytic fungi tested for antifungal activity, the extract of *Alternaria* sp exhibited maximum inhibition of 28 and 26 mm of inhibition zone against *C. albicans* and *S. cerevisiae* (Fig. 2). Followed by *A. niger, Curvularia* sp, *Cladosporium* sp, and *P. chrysogenum*. Whereas, *Neurospora crassa* exhibited a minimum inhibition zone of 14 and 18 mm. Fluconazole was used as the positive control for the study.

The actively growing endophytic fungi discs of 6mm were cut and tested for their antibacterial activity. *A. flavus* exhibited maximum inhibition of 20 and 18 mm of inhibition zone against *E. coli* and *Bacillus subtilis*. Streptomycin was used in the study for positive control. The finding of the study can be attributed to the promising antimicrobial potential of the ef, as mentioned earlier, which is almost at par with the standard antibiotic streptomycin used in the research (Figs 2 and 3).

Phytochemical Screening

Phytochemical screening of endophytic fungal secondary metabolites extracted from the isolates of leaves and inner tissues of the bark of *T. chebula* was done to test for the presence of various bioactive compounds. Alkaloids, flavonoids, phenols, saponins, steroids, tannins, terpenoids, and cardiac glycosides were tested and all the compounds are present, which is shown in (Table 1). In the present research study, phytochemical analysis of all the selected endophytic fungi showed the presence of alkaloids and flavonoids.[22]

CONCLUSION

With the successful isolation of a rich microbial diversity of endophytic fungi from *T. chebula* of the Patna region, 28 fungal species were identified. Interestingly, many isolates showed notable antimicrobial activity, including *Alternaria* sp. and *A. flavus,* demonstrating a high level of pathogen inhibition. Notably, the fact that these endophytes contain flavonoids and alkaloids highlights their potential as sources of bioactive substances. These findings provide important new information about endophytic fungi and their potential applications in biotechnology. Hence, from the present findings, endophytic fungi and their crude secondary metabolites have the potential for a wide spectrum of antimicrobial and phytochemical activity. Further, investigation will be continued study in this area at an advanced molecular and to develop new therapeutic agents, future research should focus on isolating and identifying the precise compounds causing these antimicrobial effects and the creation of novel pharmaceuticals for human consumption.

CONFLICTS OF INTEREST

The authors share no conflict of interest.

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