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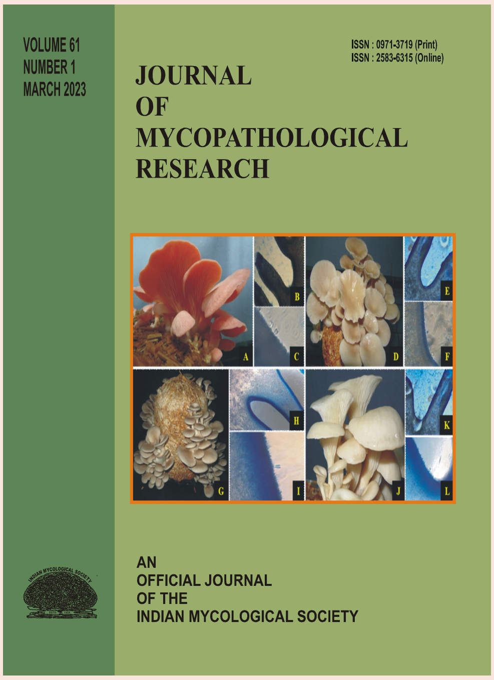

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REVIEW

Endophytic fungi as a medicinal repository of potential therapeutic compounds

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Plants have been long used for their ethnomedicinal values. However, the fungi growing in association with the plants are also found to produce metabolites of potential medicinal use explored only to a limited extend. Endophytic fungi, isolated from medicinal plants have been screened for various in-vitro biological activities. Anticancer, antimicrobial, antidiabetic, antioxidant etc. activities have been reported from these endophytic fungal isolates. Especially mangrove endophytic fungi owing to the harsh environmental conditions produce more of these metabolites and thus demand special attention of researchers. The present review makes an exhaustive study of these endophytic fungi from diverse sources, the therapeutic compounds isolated from them and their respective pharmacological activities. However, the reported data remains incomplete due to lack of *in vivo* experimentations which therefore needs further investigations in future.

Keywords: Anticancer, antimicrobial, endophytic fungi, mangrove, pharmacological activities, therapeutic compounds

INTRODUCTION

Endophytic fungi are a highly diverse group that intermittently colonize all plants asymptomatically and their interaction with the host is saprophytic, commensalistic or mutualistic. Diversity of endophytic fungi depends on the plant species and genotypes, plant tissue samples, the geography of the plant, and the season of sampling (Galindo-Solis and Fernandez 2022).

Endophytic fungi help to maintain plant health even under biotic and abiotic stress conditions. As a consequence of these interactions, they produce a variety of secondary metabolites and thus act as a reservoir of a variety of secondary metabolites with unknown natural functions but interesting bioactivities. Concerning trophic interactions, secondary metabolites may have roles in establishing beneficial endophytic interaction with the host plant. Some fungal terpenoids act as plant growth-promoting agents and as signalling molecules in the early stage of mutualistic interaction among plants and fungi. *Trichoderma viride* produced metabolites that positively influenced plant height, flowering, number of lateral roots, and biomass of *Arabidopsis thaliana*. *Talaromyces wortmannii* promotes growth and pathogen resistance of *Brassica campestris* by beta-caryophyllene production. (Galindo- Solis and Fernandez, 2022).

Mangrove-derived endophytic fungi have received wide attention due to the special living environment, which is exposed to high salinity, dampness and temperature that contribute to a different metabolism compared to traditional terrestrial microorganisms. A mangrove is an ecosystem situated in a transition area between terrestrial and marine environments, which makes it unique in parameters such as high salinity, tidal flooding, high temperatures, anaerobic soil, and amount of sludge. Thus, mangroves have become perfect ecosystems for the growth of plants and microorganisms that have ecological,

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morphological, biological, and physiological adaptations that allow them to survive under these particular conditions and these adaptive features are often imparted by the plethora of unique secondary metabolites (qualitatively and quantitatively) they produce. More than 200 species of endophytic fungi have been isolated and identified from mangroves, constituting the second-largest community of marine fungi. The main genera of endophytic fungi reported from mangroves include *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Pestalotiopsis*, *Phoma*, *Phomopsis*, *Phyllosticta*, and *Trichoderma.* (Wu *et al.* 2021).

Drug discovery from natural sources has gained significant importance in recent times owing to the adverse effects of synthetic drugs. Metabolites produced by endophytic fungi belong to a variety of chemical classes including terpenoids, phenols, alkaloids, steroids, quinones,isocoumarin derivatives etc. (Wu *et al.* 2021). From preliminary screening these chemical entities have shown the potential to become novel therapeutic compounds and thus require further investigation. Large-scale cultivation of endophytic fungi may produce enormous amounts of natural products while keeping costs reasonable. Especially fungal endophytes from mangroves open up new areas of potential biotechnological exploitation owing to the unique climatic conditions; thus, isolating and cultivating these microorganisms is of great importance for the pharmaceutical industry. The high biodiversity found in the mangrove ecosystem reinforces theimportance of studying endophytic fungi, particularly to isolate new compounds. (Wu *et al.* 2021).

Cytotoxic Activity

Endophytic fungi have been a known source of anticancer agents since the discovery of the valuable drug Taxol (also known as paclitaxel, a diterpenoid) isolated for the first time from an endophytic fungus *Taxomyces andreanae* obtained from the Pacific Yew bark (*Taxus brevifolia*) Till now, more than 100 different fungal species have been identified to produce more than two hundred putative anticancer compounds reported to possess antiproliferative and/or cytotoxic properties against more than 60 different cell lines (Hridoy *et al.* 2022). Around 40% of total pharmacological arena of endophytic fungal metabolites comprises anticancer activity. Fungal extract (tetradecan) isolated from the mangrove derived endophytic fungi *Irpex hydnoides* VB4 showed very potent cytotoxic effect. An endophytic fungus *Fusarium oxysporum* was isolated from *Rhizophora annamalayana*, a mangrove plant, and analyzed for taxol production, which may be used for anticancer treatment. Cytotoxic effect of the bioactive metabolites (crude extracts) of *Pestalotiopsis microspore* VB5 at various concentrations were studied using MTT assay, and an appreciable activity was observed against Hep 2 cell lines. Three compounds named β-carboline, adenosine, and 8-hydroxyl-3, 5-dimethyl-isochroman-1-one were isolated from mangrove fungus K32. The interaction of β-carboline with calf thymus DNA was investigated by UV–vis and fluorescence spectra, resulting in the occurrence of binding reaction, which was proposed to be one possible mechanism of the antitumor activity of β-carboline (Thatoi *et al.* 2013). *Lasiodiplodia* sp., an endophytic fungus associated with *Excoecaria agallocha*, collected from Guangdong Province, China, was the source of compound 2,4-Dihydroxy-6-nonylbenzoate. It exhibited cytotoxicity against the MMQ and GH3 cell lines with IC50 values of 5.2 and 13.0 μM, respectively. Phomazines B, epicorazine A, epicorazine B, epicorazine C, exserohilone A, were isolated from an endophytic fungus, *Phoma* sp. OUCMDZ-1847 associated with the fruit of *Kandelia candel* collected in Wenchang, Hainan Province, China. These compounds cytotoxicity against the HL-60, HCT-116, K562, MGC-803, and A549 cell lines with IC50 values in the range of 0.05 to 8.5 μM. A new anthraquinone rubrumol with poly-hydroxyl groups was isolated from a halo-tolerant endophytic fungus *Eurotium rubrum*, isolated from the salt-tolerant wild plant *Suaeda salsa* L. It exhibited cytotoxic activities against A549, MDA-MB-231, PANC-1 and HepG2 human cancer cell lines, by MTT method. Using the one strain many compounds (OSMAC) approach, new diketopiperazines, spirobrocazine C and brocazine G were characterized from *Penicillium brocae* MA-

231, an endophytic fungus associated with *Avicennia marina* collected at Hainan Island, China. It showed potent cytotoxic activity against the A2780 and A2780 CisR cell lines, with IC50 values of 664 and 661 nM, respectively. This activity is higher than the cisplatin where the IC50 values were reported as 1.67 and 12.63 μM, respectively (Deshmukh *et al.* 2018). A list of more such cytotoxic endophytic metabolites is given in Table 1.

Antimicrobial Activity

Drug resistance among microorganisms is a major concern and the search for novel antibacterial or antifungal drugs remains challenging and ongoing. It is believed that endophytes could represent a potential source of natural products with notable antimicrobial activities based on previous scientific evidence. Five fungal endophytes with different morphology were isolated from *Rhizophora mucronata* leaves (Tarman *et al.* 2013). The ethyl acetate extracts considerably inhibited the growth of *Pseudomonas aeruginosa* and *Escherichia coli*, while *Salmonella typhi* was the most resistant bacterium to the extracts tested. A new benzofuranone named sonneratione was isolated from the endophytic fungus *Aspergillus niger*, obtained from the leaves of *Sonneratia apetala* which showed considerable antimicrobial activity against *Micrococcus luteus*, *Staphylococcus aureus* and *Candida albicans* (Nurunnabi *et al.* 2019). A compound 2-deoxy-sohirnone C was isolated from the fungus *Penicillium* sp. GD6

Table 1: Cytotoxic effects of metabolites from endophytic fungi

Isolated Metabolite	Name of fungus	Host	Reported Activity
Pyrrocidine A*	*Cylindrocarpon* sp.	*Sapium ellipticum*	Cytotoxic against ovarian cancer cell line A2780 with IC_{50} of 1.7μM
Bostrycoidin*	*Fusarium solani*	*Cassia alata*	Cytotoxic against vero cell line
Bafilomycin D*	*Streptomyces* sp. YBQ59	*Cinnamomum cassia*	Showed activity against EGFR-TKI resistant A549 cells with IC_{50} 6.7μM
Demethylincisterol A_3*	*Pestalotiopsis* sp.	*Rhizophora mucronata*	Showed activity against A549, Hela, HepG
Macrophin*	*Phoma* m	*Glycyrrhiza glabra*	Cytotoxic against MDA-MB-231, T47D, MCF-7
Giluterrin*	*Aspergillus t* P63	*Axonopus leptostachyus* roots	Cytotoxic against 786-0 and PC-3 cell lines with IC_{50} 22.93 and 48.55 μM respectively
Shearilicine*	*Penicillium* sp. (strain ZO-R1-1)	*Zingiber officinale*	Active against L5178Y cell line with IC_{50} 3.6 μM.
Asperchalasins A-F*	*Aspergillus* sp.	*Pinellia ternata*	Cytotoxic against A-549 cell line
Colletotricone A*	*Colletotrichum* g *A12*	*Aquilaria sinensis*	Inhibited growth of MCF-7, NCI-H460, HepG-2, SF-268 tumor cells
Emeridones B, D, F*	*Emericella* sp. TJ29	*Hypericum perforatum* root	Cytotoxic against SMMC-7721 and SW-480 cell lines
Chetoseminudin F*	*Chaetomium* spp SYP-F7950	*Panax notoginseng* root	More cytotoxic than paclitaxel against MDA-MB-231 cells with IC_{50} 26.49 μM.
Myrothecins D-G*	*Myrothecium* r IFB-E008, IFB-E009	*Trachelospermum jasminoides*	Cytotoxic against K562 and SW1116 cell lines with IC_{50} 56 and 16μM respectively
Mollicellin G*	*Chaetomium* sp. Eef-10	*Eucalyptus exserta*	Cytotoxic against HepG2 and Hela cell lines with IC_{50} 19.64 and 13.97 μM respectively.
Foedanolide**	*Pestalotiopsis foedan*	*Bruguierasexangula*	Cytotoxic against Hela, A-549, U-251, MCF-7
Penicibrocazine A**	*Penicillium brocae* MA-231	*Avicennia marina*	Cytotoxic against SW1990, SW480, SGC-7901

Source : * Hridoy *et al.* 2022; ** Bedi *et al.* 2017

Table 2: Antimicrobial activities of metabolites isolated from endophytic fungi

Isolated Metabolite	Fungus Name	Host	Reported Activity	References
Pyranonigrin A, Fonsecin	*A. tubingensis* (NA103)	*Malus domestica*	Inhibited growth of *A. niger* (MIC 6.4 and 5.1µg/ml respectively), *F. solani*	Mohamed *et al.* 2022
Talaromyones B	*Talaromyces stipitatus* SK-4	*Acanthus ilicifolius*	Potent antibacterial activity against *Bacillus subtilis*	Narayanan *et al.* 2022
Pestalotiopisorin B	*Pestalotiopsis* sp. HHL101	*Rhizophora stylosa*	Exhibit average antibacterial inhibition of *Escherichia coli* and *Pseudomonas aeruginosa*	Narayanan *et al.* 2022
Colletotric B	*Phoma* sp. SYSU-SK-7.	*Kandelia candel*	Efficient antimicrobial inhibition of MRSA, *C.albicans* and *P. aeruginosa*	Narayanan *et al.* 2022
Cladospolide G	*Cladosporium cladosporioides* MA-299	*Bruguiera gymnorrhiza*	Strong inhibitory activity against plant pathogen *Fusarium oxysporum*	Narayanan *et al.* 2022
Asperophiobolins H	*Aspergillus* sp. ZJ-68	*Kandelia candel*	Showed significant inhibition of *Mycobacterium tuberculosis* protein tyrosine phosphatase B	Narayanan *et al.* 2022
Herbarin A	*Alternaria brassisicola* MP-408	*Malus halliana*	Showed antifungal activity against *Trichophyton rubrum* and *Candida albicans*	Patil and Maheshwari2020 20
Diaporone A	*Diaporthe* sp	*Pteroceltis tatarinowii*	Showed modest antibacterial activity against *B. subtilis*	Guo *et al.* 2019

obtained from *Bruguiera gymnorhiza* and tested against *S. aureus* resulting in an MIC value of 80 µg/mL. (Jeewon *et al.* 2019). Oxysporone and xylitol, isolated from the endophytes *Pestalotia* sp. (from *H. fomes*) and *Pestalotia* sp. respectively showed anti-MRSA activity (Nurunnabi *et al.* 2018). Investigation of endophytic fungus *Rhizoctonia* sp. yielded rhizoctonic acid with anti-*Helicobacter pylori* activity, the causative bacteria of peptic ulcer (Selvakumar and Panneerselvam 2018). Two antimicrobial agents cytosporone B and C were isolated, from the same genus *Phomopsis* sp.; they inhibited two fungi *Candida albicans* and *F. oxysporum* with the MIC value ranging from 32 to 64 mg/ml (Huang *et al.* 2008). Table 2 enlists some similar endophytic metabolites that have promising antimicrobial activity.

Other Biological Activities

Endophytic fungal metabolites are also found to exhibit some other biological activities (listed in Table 3) which include anti-diabetic, cholinergic and anti-oxidant (Narayanan *et al.* 2022). Tejesvi *et al.* (2008) reported antihypertensive activity of *Pestalotiopsis* sp. More than 60% inhibition of angiotensin converting enzyme was observed.

CONCLUSION

Endophytic fungi act as a huge incompletely explored domain of bioresource that acts as a potential source of novel therapeutic compounds. Limited work has been carried out till date with translational approach. The works reported so far, all deal with in-vitro screening. Further in-vivo investigation (efficacy and safety compared to already available synthetic drugs) using animal models is essential in order to pave the path for drug development. Moreover, conservation of ecosystem especially mangroves is a necessity in this regard. Also, efforts must be put to culture these fungi artificially with similar bioactive constituents and scale up the process in due course of time.

Table 3: Different biological activities of metabolites from Endophytic fungi

Isolated Metabolite	Fungus Name	Host	Reported Activity
Farinomalein H	*Phomopsis* sp. SYSU-QYP-23	*Kandelia candel*	Showed inhibitory action against NO generation in LPS stimulated RAW 264.7 cells
Guanxidone A	*Aspergillus* sp. GXNU-A9	*Acanthus ilicifolius*	Showed anti-inflammatory property by reducing the NO generation in LPS influenced RAW 264.7 macrophage
Furobenzotropolones B	*Epicoccum nigrum* MLY3	*Bruguieara gymnorrhiza*	Showed DPPH radical scavenging activity
Penicimarin G	*Penicillium* sp. MGP11	*Xylocarpus granatum*	Showed significant antioxidant property
Ent-cladospolide F	*Penicillium* sp. YYSJ-3	*Heritiera littoralis*	Showed antidiabetic activity by inhibiting α-glucosidase
Asperchalazine I	*Mycospaerellea* sp. SYSU-DZG01	*Bruguiera gymnorrhiza*	Showed antidiabetic activity by inhibitingα-glucosidase.
Isoaustinol	*Aspergillus* sp. 16-5c	*Sterculia apetala*	Inhibited acetylcholinesterase
1,2-dehydro-terredehydroaustin	*Aspergillus terreus* H010	*Kandelia obovata*	Inhibited acetylcholinesterase

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