SHORT COMMUNICATION

Antimicrobial potential of endophytic fungi isolated from *Dillenia indica* L. and identification of bioactive molecules produced by *Fomitopsis meliae* (Undrew.) Murril

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ABSTRACT

The present study evaluated the antibacterial potential of endophytic fungi isolated from *Dillenia indica* L. and identified bioactive compounds responsible for their antimicrobial activity. A total of twenty-five endophytic fungi were preliminarily screened for their antibacterial activity against human pathogenic bacteria, i.e., *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* by dual culture assay. The strains having antibacterial activity were selected and crude extracts were prepared from them. The crude extracts were screened for antibacterial activity by agar well diffusion assay. The ethyl acetate extract of *Fomitopsis meliae* showed the highest activity against selected human pathogenic bacteria, with a zone of inhibition ranging from 15 to 29 mm. The Gas chromatography-Mass spectrometry (GC-MS) analysis of the ethyl acetate extract of *F. meliae* showed the presence of 40 compounds with predominance of Dodecane (8.87%), Ethyl 2-thiopheneacetate (4.12%), Tetradecane (13.86%), Hexadecane (12.05%), Octadecane (6.67%), Benzaldehyde, 4-(1-methylethyl)- (10.83%), Griseofulvin (3.31%).

1. Introduction

The emergence of new pathogenic microbes and the development of resistance by existing microbes against available commercial drugs is one of the severe problems...
faced by health services worldwide (Costelloe et al. 2010). So, finding new and effective antimicrobial agents led the researchers to explore different sources to overcome this problem (Xing et al. 2011). Endophytes are the microbes that colonise inter and intracellular spaces of plant tissues without causing any disease to the host plant (Rodriguez et al. 2009). These microbes produce various bioactive molecules, i.e., alkaloids, flavonoids, terpenoids, phenolics, steroids, quinones, isocoumarins, lignans, phenylpropanoids, and lactones (Deshmukh et al. 2014).

Medicinal plants are the most important source of natural bioactive molecules, and human beings have been dependent on medicinal plants from time immemorial for their needs (Kebede et al. 2021). The overexploitation of these plants led the decrease in their number, leading to the extinction. Thus, we need an alternative source of such bioactive molecules. Hence, attention is deflected toward endophytic microbes as they produce bioactive molecules similar to those of the host plant (Kusari et al. 2013).

From the last few years, GC-MS has been used as one of the most important techniques to identify unknown secondary metabolites in both microbes and plant species (Akbar et al. 2015).

Dillenia indica L. is a medicinal plant that belongs to the family Dilliniaceae and is being used as anti-inflammatory, analgesic, anti-diabetic, antimicrobial, anticancerous, antioxidant, antidiarrhea, and to treat several diseases such as wound healing, indigestion, influenza, asthma, dysentery, jaundice, rheumatism, etc. (Chowdhury et al., 2013; Khan et al. 2021). Due to their medicinal properties, the present study was carried out to screen secondary metabolites of endophytic fungi isolated from D. indica against drug-resistant pathogenic bacteria. The secondary metabolites responsible for antimicrobial activity were identified by GC-MS.

2. Results and discussion

A total of 25 fungal taxa were isolated from the different parts (leaves, fruits, and stem) of D. indica (PAN: 22063). The isolated fungi were preliminarily subjected to antibacterial activity on a nutrient agar medium by dual culture method. The results showed that out of 25 isolated fungi, only 15 fungi showed activity for one or more than one bacteria. The fungi having positive activity were further checked for their antibacterial activity by the agar well diffusion assay. All the selected fungi showed activity against the selected pathogenic bacteria (Supplementary material; Figure S1–S4). The results clearly showed that ethyl acetate extract has higher antibacterial activity than methanolic extract by all the fungi. The highest zone of inhibition was shown by the endophytic fungus F. meliae (NCBI Accession no. MK757169) was 29 mm against Pseudomonas aeruginosa, as shown in the Supplementary material; Figure S5.

Endophytes represent an essential source of novel bioactive molecules. Researchers are working on endophytic microbes isolated from medicinal plants for their bioactive molecules and their bioactive potential. Various fungal endophytes have been reported earlier for their broad spectrum antimicrobial activity owing to these bioactive molecules (Rao et al. 2015). 6-pentyl α pyrone isolated from endophytic fungus Trichoderma koningi showed antibacterial activity against Staphylococcus aureus, having
a MIC value of 100 μg/mL. Additionally, the compound was also reported to suppresses the production of aflatoxins by other fungi (Ismaiel and Ali 2017). The chloroform and butanol extracts of *Fomitopsis pinicola* showed significant antibacterial activity (Petrova et al. 2007).

GC-MS is a technique to detect and identify unknown volatile and semi-volatile organic compounds from a mixture of compounds based on their molecular weight and elemental composition (Hites 1997). It is ideal for identifying low molecular weight molecules that are volatile and thermally stable.

The GC-MS analysis of the crude ethyl acetate extract of *F. meliae* led to the identification of forty different compounds. Major identified compounds were Tetradecane (13.86%), Hexadecane (12.05), Benzaldehyde, 4-(1-methylethyl) (10.83), Dodecane (8.87%), Octadecane (6.67%), Ethyl 2-thiopheneacetate (4.12%), (3-Methyl-oxiran-2-yl)-methanol (3.77%), L-(++)-Ascorbic acid 2,6-dihexadecanoate (3.71%), Octadecanoic acid (3.26%) and Griseofulvin (3.31%). Other compounds were present in the extract but in low quantity. Most of these compounds have various biological activities such as antibacterial, antioxidant, antifungal, cytotoxic, etc. The compounds indentified in the present study were listed below, along with their Kovat’s retention index, molecular formula, molecular weight, and percentage (Supplementary material; Figure S6 and Table S1). Additionally the structures of most important compounds as revealed from the present study as well as from literature are shown in the Supplementary material; Figure S7.

Similar observations were made for bioactive compounds extracted from *Alternaria alternata* isolated from *Picrorhiza kurroa* (Chandra et al. 2021). Such bioactive compounds having various activities were also reported from plants. GC-MS analysis of *Symplocos racemosa* led to the identification of 57 phytochemicals having various pharmacological activities (Kar et al. 2021). Similarly, Pattnaik et al. (2017) investigated the antibacterial and antifungal activities of crude extracts of *Calotropis procera* and *Calotropis gigantea*. The crude extracts showed significant antimicrobial activity. The GC-MS analyses of these extracts showed that fatty acid ethyl ester, palmitic acid ester, linoleic acid, amino acid, palmitic acid, diterpene, triterpene, and linoleic acid were the major phytoconstituents.

Maleic hydrazide present in the extract is a phytohormone and it controls Broomrape’s parasites (Venezian et al. 2017). Dodecane having antioxidant properties was also reported in the extract of *Streptomyces cacaoi* (Nandhini et al. 2015). Tetradecane (13.86%), cis-murola-3,5-diene (0.42%), Tetradecane, 4-methyl- (0.54%) were also reported earlier to have antimicrobial activity (Arjouni et al. 2011; Rahbar et al. 2012; Kavitha and Uduman 2017). L-(++)-Ascorbic acid 2,6-dihexadecanoate is a vitamin C compound used to treat common cold, gum disease, acne, antioxidant, cardioprotective, asthma, diabetes, boost immunity etc. (http://www.webmd.com/vitamin, Hadi et al. 2016). A study reported by Semde et al. 2018 have suggested that Cis-Vaccenic acid/Omega 7 fatty acid as a compound for lowering LDL cholesterol, antimicrobial and hypolipidemic effect (Semde et al. 2018). Griseofulvin having antifungal activity was also isolated from the endophytic fungus *Xylaria* sp. (Park et al. 2005). Cyclohexadecane has also been reported to have antibacterial and antifungal activities (Habib and Karim 2016; Kumari et al. 2019).
3. Conclusions

The results clearly showed that the fungal endophytes inhabited by *Dillenia indica* L. have promising antibacterial potential against selected human bacterial pathogens. In conclusion, these endophytes can be used to isolate new antimicrobial drugs, which might help in combating drug-resistant antibiotic strains. The bioactive compounds produced by *F. meliae* have a wide range of bioactive potentials that can be used in clinical trials for further applications. To our knowledge, it is the first study on the antimicrobial activity of endophytic fungi isolated from *Dillenia indica* L. and the identification of bioactive compounds produced by *F. meliae*. Further work on these fungi is going on to isolate and purify the compounds responsible for the antimicrobial activity.

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