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ORIGINAL ARTICLE

Phytochemical Analysis and Antioxidant Activity of Endophytic Fungi Isolated from *Dillenia indica* Linn.

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Abstract

Endophytic fungi live symbiotically inside plants and are hidden source of natural bioactive molecules. The present study was carried out to investigate the phytochemical analysis and antioxidant activity of endophytic fungi isolated from the ethnomedicinal plant *Dillenia indica* L. The ethyl acetate crude extracts of the endophytic fungal strains were preliminarily evaluated for their phytochemical analysis, and the results showed the presence of alkaloids, flavonoids, phenolics, terpene, and saponins. The crude extracts of more than 60% of the isolates showed 50–90% antioxidant activity by DPPH and H_2O_2 assay. The inhibition percentage of ethyl acetate extracts ranges from 34.05 to 91.5%, whereas IC_{50} values vary from 72.2 to 691.14%. Among all the strains, *Fomitopsis meliae* crude extract showed a maximum inhibition percentage, i.e., 91.5%, with an IC_{50} value of 88.27 µg/mL. *Chaetomium globosum* showed significant activity having an inhibition percentage of 89.88% and an IC_{50} value of 74.44 µg/mL. The total phenolic and flavonoid content in the crude extract of *Chaetomium globosum* was 37.4 mg gallic acid equivalent (GAE)/g DW and 31.0 mg quercetin equivalent (GAE)/g DW. GC–MS analysis of crude extract of *C. globosum* revealed different compounds, such as squalene; butanoic acid, 2-methyl-; hexadecanoic acid; 2-propanone, 1-phenyl-; 5-oxo-pyrrolidine-2-carboxylic acid methyl ester; 9,12-octadecadienoic acid (z)- etc. Many of these belong to phenolics, which are natural antioxidant compounds. The findings suggested that endophytic fungi associated with *Dillenia indica* L. can be a potential source of novel antioxidant compounds.

Keywords Endophytic fungi · Antioxidant compounds · *Dillenia indica* · Phytochemical analysis · *Chaetomium globosum*

Introduction

Endophytes are symbiotic microbes that live inside the tissues of plants without producing any symptoms of disease in the host plants. They have worldwide distribution and are found in all plants studied to date [1]. Endophytes and plants have a beneficial

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symbiotic association in which the host plants provide endophytes with essential nutrients. Endophytic fungi support host plants by preventing the invasion of pathogens and improving resistance and tolerance to various biotic and abiotic stresses [2]. Fungal endophytes produce a wide range of bioactive secondary metabolites having antimicrobial, antidiabetic, immunomodulatory, anticancerous, and antioxidant activities. So, studying endophytic fungi from medicinal plant species can lead to the discovery of many new medicinally important compounds [3, 4].

Free radicals, like reactive oxygen and nitrogen species, are formed in biological systems due to normal metabolic activities or external factors such as x-ray exposure, air pollution, and industrial chemicals [5]. Excessive production of reactive oxygen species causes various diseases such as aging, atherosclerosis, cancer, immunosuppression, diabetes, and neurological. Antioxidants protect cells from harm by neutralizing reactive oxygen species (ROS) [6]. Therefore, there is a need for natural antioxidant molecules. Endophytic fungi can produce secondary antioxidant metabolites that block free radical cascade. For the last few years, these fungi have been studied for their biological activities, such as antimicrobial, anticancer, antidiabetic, antiviral etc. [7]. But very few studies have demonstrated their antioxidant activity. Exploring natural substances is the most promising approach for finding novel biomolecules having broad industrial values [8]. Endophytic fungi have attracted considerable attention since the last century because of their potential to produce new bioactive molecules with a wide range of biological properties. They can be used in medical, pharmaceutical, and agronomic applications [9].

Dillenia indica is a medium-sized evergreen tree growing up to a length of about 6–15 m. Its bark is smooth, thick, red, and bearing tomentose branches, and is commonly used to make good firewood. It occurs in countries like Bhutan, India, Indonesia, Nepal, Laos, Malaysia, Myanmar, Philippines, Sri Lanka, Thailand, and Vietnam. It is native to India. In India, it is scattered in the sub-Himalayan region of Assam, Bihar, North Bengal, Orissa, Madhya Pradesh, and Gujarat. It is an important medicinal plant having antimicrobial, antioxidant, analgesic, anti-inflammatory, dysentery, antidiabetic, and antileukemic properties [10]. There was no previous report on the antioxidant activity of endophytic fungi isolated from *Dillenia indica* L. Therefore, the present study evaluated the phytochemical analysis and antioxidant activity of endophytic fungi isolated from *Dillenia indica* L.

Materials and Methods

Endophytic Fungi

The fungal endophytes used in this study were isolated and identified from different parts of *Dillenia indica* Linn. using a culture-dependent approach [11].

Preparation of Crude Extract and Metabolite Extraction

The crude extract was prepared by growing the isolated fungal strains in Potato Dextrose broth. The selected fungi were grown on Potato Dextrose Agar (PDA) plates for 4–5 days at 28 °C ± 2. Five to six fungal discs were cut from the freshly grown cultures and were inoculated in 1-L flasks containing 500 mL of Potato Dextrose broth. The flasks were incubated for 21 days at 24 °C ± 2 with intermittent shaking. After 21 days, the mycelium was separated

from the filtrate using a cheese cloth. The filtrates were extracted thrice with ethyl acetate. The obtained extracts were concentrated to dry residue using a rotatory evaporator [12].

Phytochemical Analysis of Crude Extracts

Qualitative phytochemical screening of fungal extracts was done according to standard protocols [13–19] to identify the chemical nature of the active components present in the crude extracts of endophytic fungi. All the crude extracts were checked for the presence of various secondary metabolites.

Test for Flavonoids

One milliliter of extract was mixed with a few drops of concentrated sodium hydroxide (NaOH) solution and observed for yellow coloration, which disappeared upon the addition of dilute HCl (6N) and confirmed the presence of flavonoids [13].

Test for Phenolic Compounds (Phenolics)

To 2 mL of fungal crude extract, 1 mL of 1% ferric chloride solution was added. Blue or green color indicates the presence of phenols [14].

Test for Cardiac glycosides

To 2 mL of endophytic fungal crude extract, 1 mL of glacial acetic acid and 1–2 drops of $FeCl_3$ were added, followed by 1 mL of concentrated H_2SO_4. The appearance of a brown ring at the interface indicates the presence of cardiac glycosides [15].

Test for Tannins

To 2 mL of crude extract, 2 mL of 5% $FeCl_3$ solution was added. The formation of a yellow–brown precipitate indicates that tannins are present [16].

Test for Saponins

The dried crude extract was subjected to a frothing test by adding water. Frothing persistence indicated the presence of saponins. Later, the froth was mixed with a few drops of olive oil. The formation of emulsion indicates the presence of saponins [17].

Test for Terpenes/Terpenoids

To 2 mL of fungal crude extract, 5 mL chloroform, 2 mL acetic anhydride, and concentrated H_2SO_4 were added carefully to form the layer. The reddish-brown coloration of the interface indicates the presence of terpenes/terpenoids [18].

Test for Alkaloids

To the 2 mL filtrate, 1.5 mL of 1% HCl was added. After heating the solution in the water bath, six drops of Dragendorff reagent were added. An orange precipitate's formation indicates the alkaloids' presence [19].

Antioxidant Activity

The antioxidant activity of crude extracts was carried out using two different methods, i.e., DPPH and hydrogen peroxide (H_2O_2) scavenging assays.

DPPH Radical Scavenging Assay

The ethyl acetate crude extract was evaluated for its ability to scavenge free radical 2,2′-diphenyl-1-picryl-hydrazyl (DPPH) by a standard method with slight modifications [20]. Various concentrations (50–300 μg/mL) of fungal crude extracts were prepared and mixed with 1 mL of 0.02% methanolic DPPH solutions. The tubes were incubated in the dark at room temperature for 30 min, and absorbance was recorded at 517 nm. Ascorbic acid was used as a standard. The lower absorbance indicates higher antioxidant activity. Percentage inhibition was calculated using the formula:

$$\% \text{ inhibition} = \left[\left(A_c - A_s\right)/A_c\right] \times 100,$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample. IC_{50} was calculated by plotting a graph in an Excel sheet.

H_2O_2 Radical Scavenging Assay

The efficiency of crude fungal extracts to scavenge hydrogen peroxide was determined spectrophotometrically using the method given by Al-Owaisi et al. [21] with slight modifications [21]. A solution of hydrogen peroxide (40 mmol/L) in phosphate buffer (50 mmol/L, pH 7.4) was prepared. One milliliter of crude fungal extracts of various concentrations (50–300 μg/mL) was added to hydrogen peroxide. Both solutions were mixed and kept at room temperature for 10 min. After 10 min, absorbance at 230 nm was measured using a spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The following formula was used to determine the percentage of hydrogen peroxide scavenging:

$$\text{Scavenged } H_2O_2(\%) = \left[\left(A_i - A_t\right)/A_i\right] \times 100,$$

where A_i is the absorbance of the control and A_t is the absorbance of test samples.

Total Phenolic and Flavonoid Content

The phenolic content in the extract was determined spectrophotometrically using the Folin-Ciocalteu assay. For analysis, 100 μL of fungal extract (different concentrations) was mixed with 100 μL of 10% Folin-Ciocalteu and 1000 μL of $NaHCO_3$. The reaction mixture was

incubated at 45 °C for 30 min [22]. The absorbance was recorded at 765 nm. Gallic acid (100–500 µg/mL) was used as a standard to make a calibration curve.

For the determination of total flavonoid content, 125 µL of extract (different concentrations) was mixed with 5% sodium nitrate (50 µL) and 10% aluminum chloride (75 µL). The reaction mixture was kept for 6 min at room temperature, and 250 µL of sodium hydroxide was added. The reaction mixture was diluted with distilled water to make the volume of 10 mL. The reaction mixture was mixed thoroughly. The absorbance was recorded spectrophotometrically at 510 against blank [23]. Quercetin (5–200 µg/mL) was used as a standard to make a calibration curve.

Gas Chromatography-Mass Spectroscopy

The chemical constituents in the ethyl acetate extract were analyzed using TRACE 1300 GC, TSQ 8000 TRIPLE QUADRUOLE MS fitted with TG 5MS column, and S/SL Injector. The following were the GC conditions: 1-min split less time; 1.0 mL/min helium carrier; oven temperature from 70 to 135 °C at 2 °C/min for 10 min, then to 220 °C at 4 °C/min for 10 min, and finally to 270 °C at 3.5 °C/min for 20 min. HP-5MS capillary column (0.32 mm, 30 m, 0.25 m), GC injector at 280 °C, and MS transfer line temperature at 290 °C were employed for the analysis. The identification of the detected compounds was carried out by comparing them to mass spectra from the NIST database [24].

Results

Phytochemical Screening

The qualitative phytochemical screening of fungal extract showed the presence of various phytochemicals such as alkaloids, flavonoids, cardiac glycosides, phenolic, and terpenoids (Table 1). The results revealed that all the isolates showed the presence of phenolic, followed by flavonoids, alkaloids, cardiac glycosides, and terpenoids.

These phytochemicals are responsible for various biological activities. *Xylaria longipes*, *Daldinia eschscholtzii*, and *Schizophyllum commune* showed the presence of all the tested phytochemicals, whereas *Curvularia lunata*, *Alternaria alternata*, and *Diaporthe phaseolorum* produced only phenolic and flavonoids. *Chaetomium globosum* showed the presence of all the tested phytochemicals except cardiac glycosides (Fig. 1). In contrast, *Clonostachys rosea* contains alkaloids, phenolics, and flavonoids.

Antioxidant Activity

The ethyl acetate extracts of endophytic fungi isolated from *Dillenia indica* were evaluated for their antioxidant activity by two different methods. All the extracts showed varying levels of antioxidant activity. As the concentration of the crude extract increased, antioxidant activity also increased. Most isolates showed more than 50% scavenging activity at 300 µg/mL extract.

The crude ethyl acetate extract of all the endophytic fungal isolates showed activity against the DPPH radical at different concentrations. The scavenging activity was evaluated by using ascorbic acid as standard. The results showed an increase in the

Table 1 Showing the qualitative phytochemical analysis of crude extracts of different endophytic fungi

Fungi	Phytochemicals
Chaetomium globosum	Alkaloids, flavonoids, phenolics, terpenoids
Lasiodiplodia theobromae	Flavonoids, cardiac glycosides, phenolics
Schizophyllum commune	Alkaloids, flavonoids, cardiac glycosides, phenolics, terpenoids
Phomopsis sp.	Phenolics, terpenoids
Colletotrichum gigasporum	Alkaloids, phenolics
Colletotrichum gloeosporioides	Alkaloids, flavonoids, phenolics
Clonostachys rosea	Alkaloids, flavonoids, phenolics
Daldinia eschscholtzii	Alkaloids, flavonoids, cardiac glycosides, phenolics, terpenoids
Diaporthe phaseolorum	Flavonoids, phenolics
Nigrospora sphaerica	Alkaloids, flavonoids, cardiac glycosides, phenolics
Xylaria longipes	Alkaloids, flavonoids, cardiac glycosides, phenolics, terpenoids
Alternaria alternata	Flavonoids, phenolics
Curvularia lunata	Flavonoids, phenolics
Fusarium oxysporum	Alkaloids, cardiac glycosides, phenolics
Fomitopsis meliae	Flavonoids, cardiac glycosides, phenolics, terpenoids

free radical scavenging activity as the concentration of the extract increased. All the fungi showed free radical scavenging activity at varying levels. The crude extract of more than 60% of the isolates showed more than 50–90% activity. The *Fomitopsis meliae* showed a maximum inhibition percentage, i.e., 91.5%, with an IC_{50} value of 88.27 µg/mL. *Chaetomium globosum* showed significant activity having an inhibition percentage of 89.88 and an IC_{50} value of 74.44. The minimum inhibition percentage is 34.05%, shown by *Alternaria alternata*, and has an IC_{50} value of 691.14. The inhibition percentage of the screened isolates is in their decreasing order as control > *Fomitopsis meliae* > *Chaetomium globosum* > *Nigrospora sphaerica* > *Daldinia eschscholtzii* > *Diaporthe phaseolorum* > *Schizophyllum commune* > *Lasiodiplodia theobromae* > *Xylaria longipes* > *Colletotrichum gigasporum* > *Clonostachys rosea* > *Cladosprium cladosporioides* > *Colletotrichum gloeosporioides* > *Phomopsis* sp. > *Curvularia lunata* > *Alternaria alternata* (Table 2 and Figs. 2 and 3). The value of IC_{50} ranges from 72.2 to 545.68 µg/mL for DPPH assay. The lower the IC_{50} value,

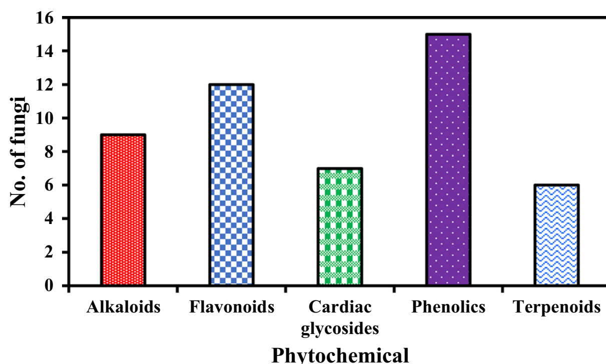

Fig. 1 Bar graph showing the number of isolates showing the presence of different phytochemicals

Table 2 Inhibition percentage and IC_{50} values of crude extracts (300 μg/mL) of different fungal taxa

Sr. no	Fungal isolates	Inhibition percentage (300 μg/mL)		IC50	
		DPPH	H_2O_2	DPPH	H_2O_2
1	*Chaetomium globosum*	89.88	81.88	74.44	104.13
2	*Lasiodiplodia theobromae*	83.55	71.9	111.89	139.45
3	*Schizophyllum commune*	84.57	74.71	97.39	110.15
4	*Phomopsis* sp.	41.77	35.61	372.23	458.32
5	*Colletotrichum gigasporum*	53.51	44.51	249.19	328.04
6	*Colletotrichum gloeosporioides*	43.37	36.37	349.47	440.47
7	*Clonostachys rosea*	46.6	41.6	305.87	306.87
8	*Daldinia eschscholtzii*	87.48	75.48	72.20	132.11
9	*Diaporthe phaseolorum*	86.31	76.31	101.7	93.67
10	*Nigrospora sphaerica*	88.1	76.1	85.04	150.89
11	*Xylaria longipes*	79.73	71.73	145.14	179.77
12	*Alternaria alternata*	34.05	28.05	545.68	691.14
13	*Curvularia lunata*	40.01	31.1	360.77	495.13
14	*Cladosprium cladosporioides*	52.99	35.99	430.94	424.41
15	*Fomitopsis meliae*	91.5	78.5	88.27	128.97
16	Control	97.8%	92.6	15.1	17.61

Control, ascorbic acid

the higher the antioxidant activity. The lowest IC_{50} value was shown by *Daldinia eschscholtzii*. Similarly, in the H_2O_2 assay, IC_{50} values range from 93.67 to 691.14 μg/mL. These isolates can be further exploited commercially to produce antioxidant molecules (Table 2 and Fig. 4).

Total Phenolic and Flavonoid Content

Phenolics are primary compounds responsible for their natural antioxidant activities. In the present study, the amount of phenolic content in the extract is directly proportional to their antioxidant activity. The higher the phenolic content, the higher the antioxidant activity. The total phenolic content in the extract (300 μg/mL) was found to be 37.4 ± 0.046 mg GAE/g. The total flavonoid content in the extract was 31 ± 0.95 mg quercetin/g (Table 3).

Gas Chromatography-Mass Spectroscopy

The crude extract of the selected strain (*Chaetomium globosum*) was subjected to GC–MS analysis, revealing the occurrence of different compounds at varying retention times and peaks. Forty-six compounds were detected in the extract, and out of them, seven were the dominant compounds, namely 2,3-dihydro-3,5-dihydroxy-6-methyl-4 h-pyran-4-one; 2-propanone, 1-phenyl-; 5-oxo-pyrrolidine-2-carboxylic acid methyl ester; hexadecanoic acid, methyl ester; hexadecanoic acid; 9,12-octadecadienoic acid (z)-; and 11-octadecenoic acid, methyl ester, (Z,Z)- (Fig. 5 and Table 4).

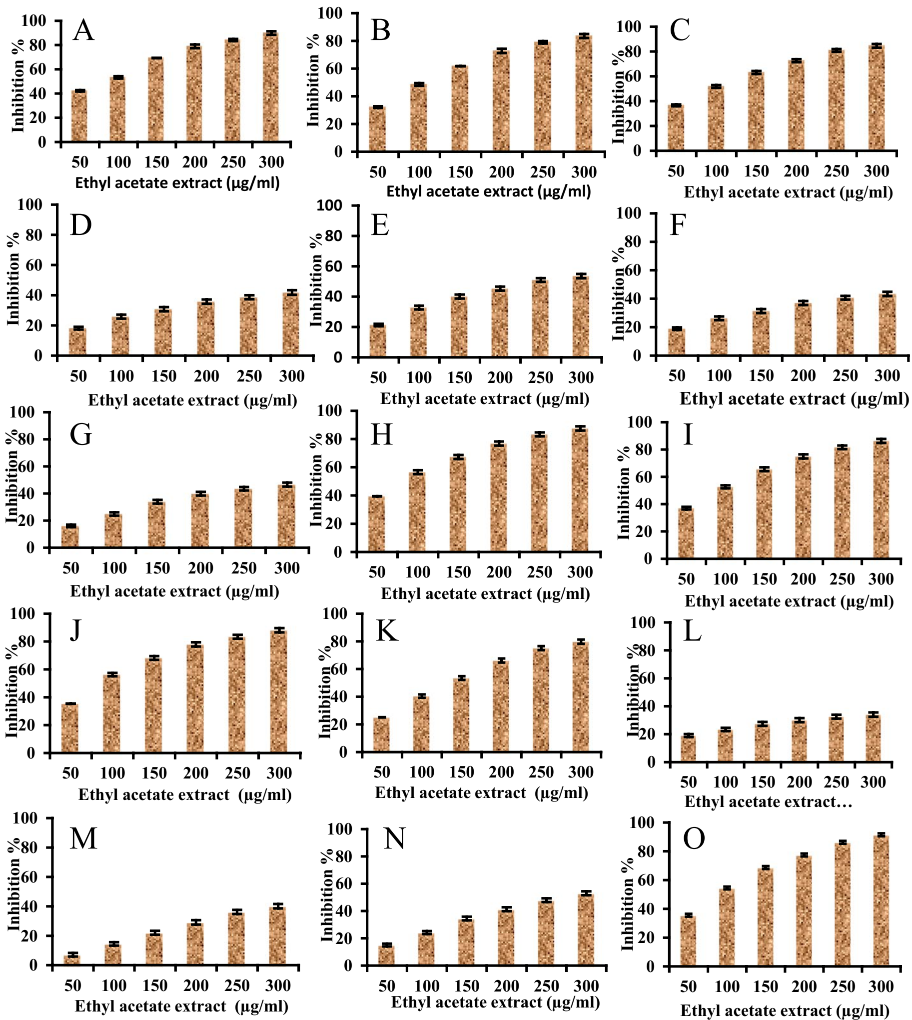

Fig. 2 Antioxidant activity of ethyl acetate extract by DPPH assay. **A** *Chaetomium globosum*, **B** *Lasiodiplodia theobromae*, **C** *Schizophyllum commune*, **D** *Phomopsis* sp., **E** *Colletotrichum gigasporum*, **F** *Colletotrichum gloeosporioides*, **G** *Clonostachys rosea*, **H** *Daldinia eschscholtzii*, **I** *Diaporthe phaseolorum*, **J** *Nigrospora sphaerica*, **K** *Xylaria longipes*, **L** *Alternaria alternata*, **M** *Curvularia lunata*, **N** *Cladosprium cladosporioides*, **O** *Fomitopsis meliae*

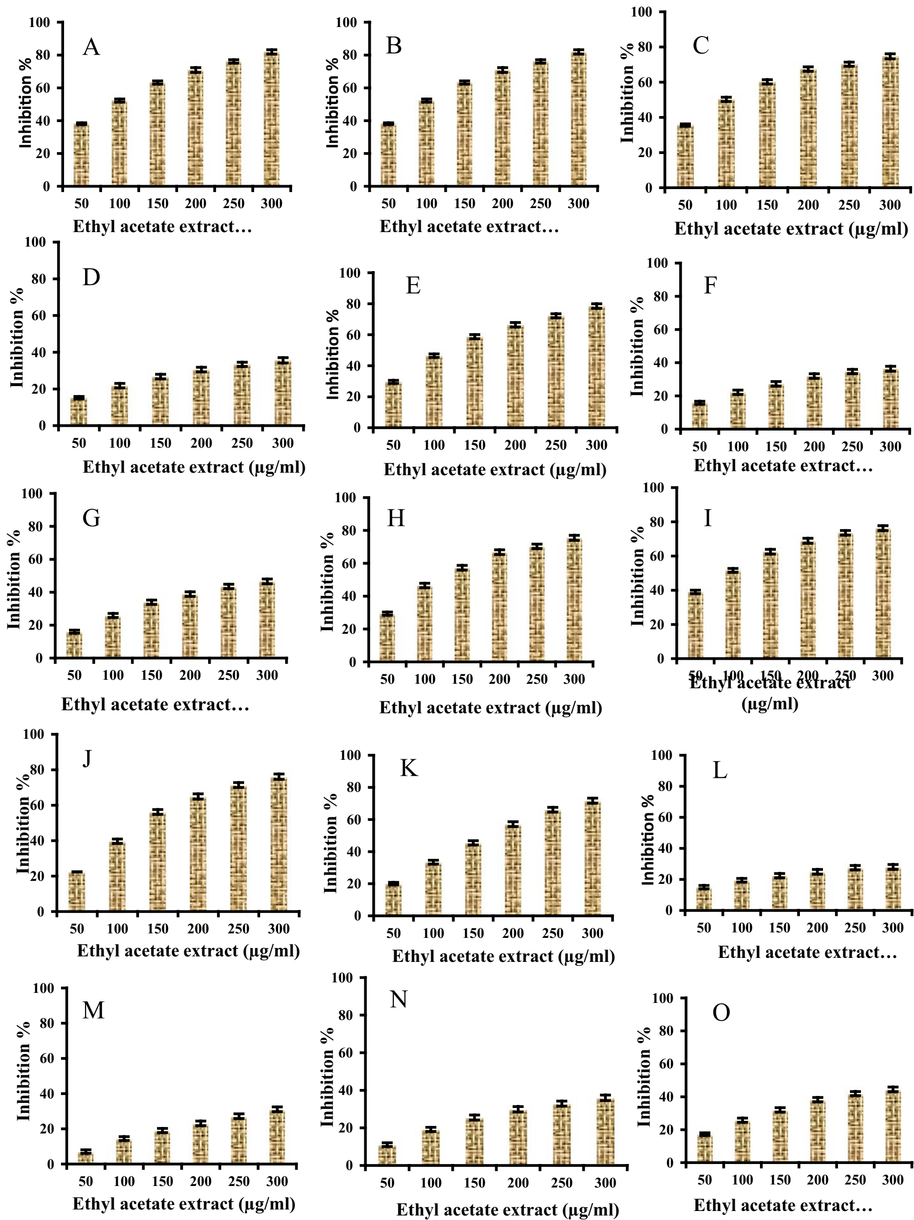

Fig. 3 Antioxidant activity of ethyl acetate extracts by H_2O_2 assay. **A** *Chaetomium globosum*, **B** *Lasiodiplodia theobromae*, **C** *Schizophyllum commune*, **D** *Phomopsis* sp., **E** *Colletotrichum gigasporum*, **F** *Colletotrichum gloeosporioides*, **G** *Clonostachys rosea*, **H** *Daldinia eschscholtzii*, **I** *Diaporthe phaseolorum*, **J** *Nigrospora sphaerica*, **K** *Xylaria longipes*, **L** *Alternaria alternata*, **M** *Curvularia lunata*, **N** *Cladosprium cladosporioides*, **O** *Fomitopsis meliae*

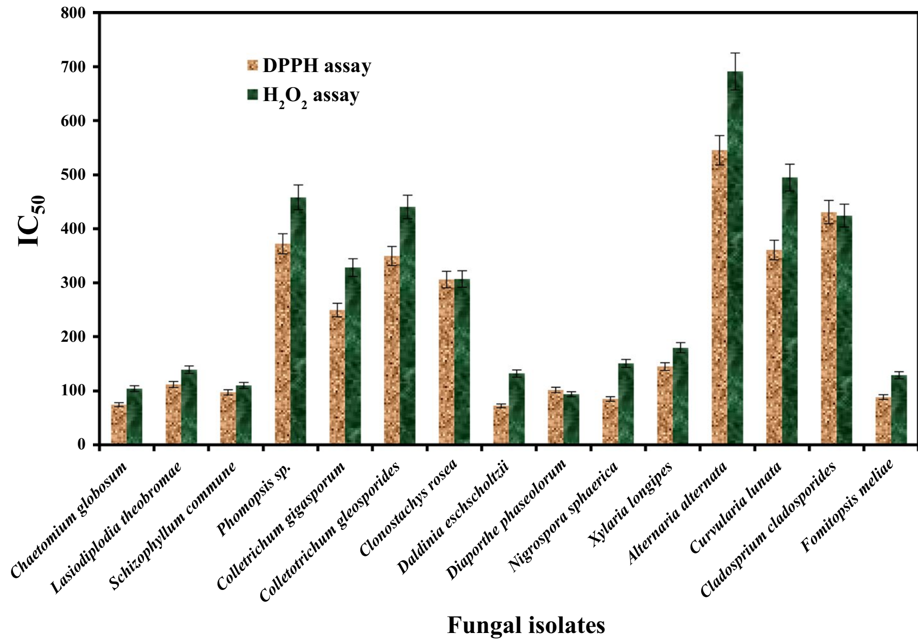

Fig. 4 IC_{50} value of fungal endophytes crude ethyl acetate extract by DPPH and H_2O_2 assay

Discussion

Plants harbor microbes inside their tissues, and these microbes play a significant role in their life cycle. These microbes produce various bioactive molecules having medicinal value. They are an important component of microbial diversity, and significant progress over the last 20 years in the field has proven their significance [25]. From them, endophytic fungi are one of the largest groups studied earlier for their different biological activities and have been of significant interest in recent decades [26]. These microbes produce bioactive molecules similar to their host plant. *Dillenia indica* is an important medicinal plant having antimicrobial, antidiabetic, anticancerous, antioxidant, anti-HIV, anti-inflammatory, antidiarrheal, and wound-healing properties [27, 28]. These biological activities are due

Table 3 Total phenolic and flavonoid content in the crude extract of *Chaetomium globosum*

S. no	Crude extract (μg/mL)	Total phenolic content (mg gallic acid equivalent (GAE)/g DW)	Total flavonoids content (mg quercetin equivalent (QE)/g DW
1	50	0.8 ± 0.042	0.7 ± 0.90
2	100	15 ± 0.037	13 ± 0.72
3	150	22 ± 0.046	18 ± 0.76
4	200	28.5 ± 0.040	23 ± 0.87
5	250	33.1 ± 0.042	27 ± 0.78
6	300	37.4 ± 0.046	31 ± 0.95

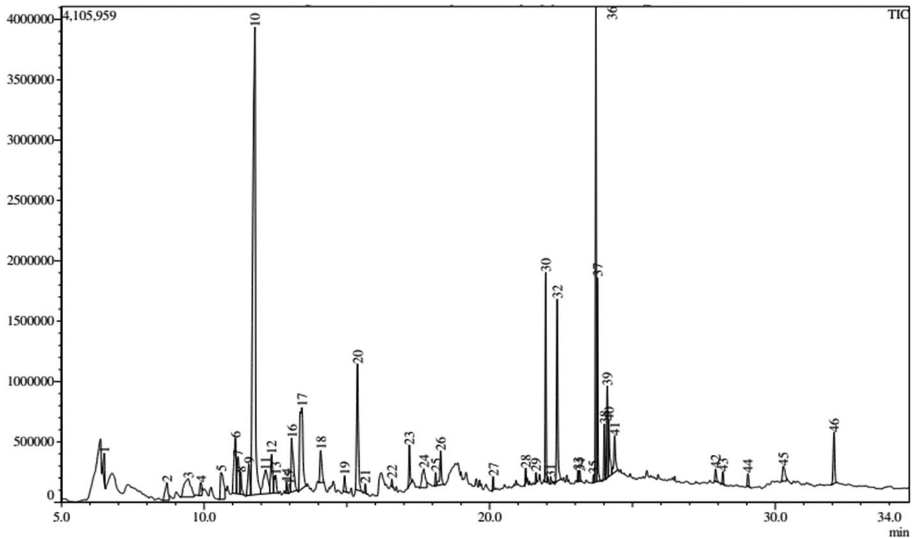

Fig. 5 GC–MS chromatogram of ethyl acetate extract of *Chaetomium globosum*

to various phytochemicals like alkaloids, flavonoids, terpenoids, cardiac glycosides, and phenolics [29].

The existence of chemical constituents in fungal crude extracts was determined using phytochemical screening as a source of potential for industrial and medical applications [30, 31]. Their existence indicates that they could be used as precursors in the development and progression of synthetic medicines. The crude ethyl acetate extracts of the selected strains showed the presence of all the phytochemicals undertaken in this study. Phytochemical screening of *Penicillium frequentans* ethyl acetate extracts revealed the existence of almost all phytochemicals [32]. The type of liquid media utilized and the environmental circumstances of the interaction affect the quantity and quality of bioactive chemicals synthesized by endophytic fungi [33]. Bioactive chemicals can be obtained from these fungi by different strategies.

In the present study, the preliminary phytochemical analysis of the crude extracts of fungal endophytes confirmed the presence of various phytochemicals such as alkaloids, flavonoids, terpenoids, phenolic, and cardiac glycosides. Phenolics, flavonoids, and terpenoids are mainly responsible for antioxidant properties. Antioxidants are shown to protect against several diseases. According to epidemiological studies, antioxidant intake is associated with a lower risk of heart disease and other ailments. That is why natural antioxidants and their role in human health and nutrition have piqued people's interest [34]. Several medicinal herbs, vegetables, fruits, spices, and fungi have been considered possible sources of natural antioxidants that are potentially safe [35]. Antioxidant activity has recently been reported in several fungal endophytes and mushrooms. Phenolics are the main chemical constituents responsible for their antioxidant activity. Earlier studies showed a direct relationship and antioxidant activity; as the concentration of phenolic increases, the antioxidant activity of the samples also increases [36].

The most efficient approach for extracting fungal secondary metabolites is ethyl acetate extraction. Low-molecular and high molecular weight phenolics are selectively extracted using ethyl acetate as an extraction solvent [37].

Table 4 List of bioactive compounds detected in the crude ethyl acetate extract

Peak	Name	R. time	Formula	Mol. weight	Area%
1	Butanoic acid, 2-methyl-	6.499	$C_5H_{10}O_2$	102	0.67
2	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	8.700	$C_6H_8O_4$	144	1.11
3	1,2,3-Propanetriol	9.441	$C_3H_8O_3$	92	2.77
4	4-Oxopentanoic acid	9.889	$C_5H_8O_3$	116	0.59
5	1-(6-Oxabicyclo[3.1.0]hex-1-yl) ethanone	10.591	$C_7H_{10}O_2$	126	2.03
6	Butanedioic acid, monomethyl ester	11.093	$C_5H_8O_4$	132	2.60
7	Butanedioic acid, monomethyl ester	11.187	$C_5H_8O_4$	132	1.95
8	4-Methyloxazole	11.292	C_4H_5NO	83	0.67
9	2-Acetyl-2-hydroxy-.gamma	11.568	$C_6H_8O_4$	144	1.45
10	2,3-Dihydro-3,5-dihydroxy- 6-methyl-4 h-pyran-4-one	11.772	$C_6H_8O_4$	144	26.26
11	1,2,3,4-Butanetetrol, [S-(R*,R*)]-	12.142	$C_4H_{10}O_4$	122	2.52
12	2-Buten-1-amine, N-butyl-, (E)-	12.368	$C_8H_{17}N$	127	1.70
13	(S)-(+)-2',3'-Dideoxyribonolactone	12.508	$C_5H_8O_3$	116	0.93
14	Dianhydromannitol	12.885	$C_6H_{10}O_4$	146	0.24
15	1,2-Ethanediol, 1-(2-furanyl)-	12.983	$C_6H_8O_3$	128	0.31
16	5-Hydroxymethylfurfural	13.070	$C_6H_6O_3$	126	2.64
17	2-Propanone, 1-phenyl-	13.426	$C_9H_{10}O$	134	5.64
18	Heptanoic acid, 6-oxo-	14.085	$C_7H_{12}O_3$	144	1.26
19	Dl-erythro-pentonic acid, 2-deoxy-3-o-methyl-, gamma-lactone	14.918	$C_6H_{10}O_4$	146	0.54
20	5-Oxo-pyrrolidine-2-carboxylic acid methyl ester	15.374	$C_6H_9NO_3$	143	4.86
21	(E,S)-2-Hexenoic acid, 4-amino-5-methyl-, methyl ester	15.643	$C_8H_{15}NO_2$	157	0.20
22	1-Dodecanol	16.572	$C_{12}H_{26}O$	186	0.13
23	6-Methoxy-3-methylbenzofuran	17.186	$C_{10}H_{10}O_2$	162	1.04
24	d-Mannitol, 1,4-anhydro-	17.694	C_6H12O_5	164	1.45
25	1-Hexadecanol	18.111	$C_{16}H_{34}O$	242	0.29

Table 4 (continued)

Peak	Name	R. time	Formula	Mol. weight	Area%
26	Carbamic acid, (3-methylphenyl)-, ethyl ester	18.282	$C_{10}H_{13}NO2$	179	1.19
27	Tetradecanoic acid	20.127	$C_{14}H_{28}O_2$	228	0.29
28	Pentadecanoic acid	21.260	$C_{15}H_{30}O_2$	242	0.30
29	9,17-Octadecadienal, (Z)-	21.620	$C_{18}H_{32}O$	264	0.20
30	Hexadecanoic acid, methyl ester	21.964	$C_{17}H_{34}O_2$	270	4.05
31	Cyclopentadecanone, 2-hydroxy-	22.133	$C_{15}H_{28}O_2$	240	0.14
32	Hexadecanoic acid	22.367	$C_{16}H_{32}O_2$	256	5.06
33	9-Octadecynoic acid	23.098	$C_{18}H_{32}O_2$	280	0.23
34	10,10-Dimethoxy-3,7-dimethyl-deca-2, 6-dien-1-ol	23.167	$C_{14}H_{26}O_3$	242	0.23
35	1-Hexadecanol	23.634	$C_{16}H_{34}O$	242	0.15
36	9,12-Octadecadienoic acid (z)-	23.721	$C_{19}H_{34}O_2$	294	9.91
37	11-Octadecenoic acid, methyl ester, (Z,Z)-	23.778	$C_{19}H_{36}O_2$	296	4.15
38	Octadecanoic acid, methyl ester	24.015	$C_{19}H_{38}O_2$	298	1.03
39	10,12-Hexadecadien-1-ol	24.124	$C_{16}H_{30}O$	238	2.72
40	13-Docosenoic acid	24.175	$C_{22}H_{42}O_2$	338	1.62
41	Octadecanoic acid	24.376	$C_{18}H_{36}O_2$	284	1.38
42	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	27.917	$C_{19}H_{38}O_4$	330	0.33
43	1,2-Benzenedicarboxylic acid	28.171	$C_{24}H_{38}O_4$	390	0.33
44	17-(1,5-Dimethyl-hex-4-enyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-Tetradecahydro-1 h-cyclopenta	29.044	$C_{27}H_{44}O$	384	0.36
45	9,12-Octadecadienoic acid (z,z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	30.292	$C_{21}H_{38}O_4$	354	0.80
46	Squalene	32.058	$C_{30}H_{50}$	410	1.70

Several studies have found that most degenerative diseases in human beings are caused due to the production of harmful free radicals [38]. As a result, antioxidants have a wide range of uses in treating conditions involving free radicals. Therapeutic bioactive molecules from plants have been used to combat oxidative stress. Instead of plants themselves, microbes associated with plants have now been proven to be a potential source of bioactive chemicals with antioxidant activity investigated in drug formulation [39]. In this study, endophytic fungi isolated from the *Dillenia indica* were found to have antioxidant properties. The bioactive molecules present in their extracts are responsible for these bioactivities. The phenolics are mainly accountable for antioxidant activity, supporting the previous findings [40, 41]. The phenolic content of the ethyl acetate extract was higher than the flavonoid content, which could explain the extract's significant scavenging and reducing abilities.

The findings of this study are consistent with previous studies on endophytic fungi and their antioxidant activities. The antioxidant potential has been reported for the number of endophytic fungi isolated from medicinal plants. Like our studies, *Nigrospora sphaerica* isolated from *Euphorbia hirta* showed antioxidant activity with 96.80% inhibition [42]. Crude extracts of endophytic fungi (*Fusarium oxysporum*) isolated from *Otoba gracilipes* showed 51.5% scavenging by DPPH assay. [43]

Huang et al. screened the endophytic fungi isolated from *Nerium oleander* for antioxidant activity and the results revealed that most of the strains (75%) showed moderate activity. The highest activity was shown by the *Chaetomium* sp. (150.79 μmol trolox/100 mL culture) [44].

Endophytic fungus *Phyllosticta* sp. isolated from *Guazuma tomentosa* showed antioxidant activity by ABTS and DPPH assay with an IC_{50} value of 580.02 ± 0.57 μg/mL and 2030.25 ± 0.81 [45].

Fungal endophytes isolated from *Justicia gendarussa* were screened for free radical scavenging activity by DPPH assay. *Pseudopestalotiopsis camelliae-sinensis* showed significant antioxidant activity, whereas *Colletotrichum gloeosporioides*, *Fusarium solani*, and *Colletotrichum tropicale* exhibited moderate activity and *Colletotrichum siamense* have minimal activity. *Diaporthe pseudomangiferae* did not show antioxidant activity [46]. Selim et al. [47] found that *Chaetomium globosum*, isolated from the medicinal plant *Adiantum capillus-veneris*, exhibits high antioxidant activity in addition to a broad range of in vitro bioactivity (antimicrobial, antiviral, and antineoplastic) and is abundant in secondary metabolites [47].

Various scientific studies revealed that antioxidant molecules could boost many immune responses, strengthening innate and acquired immunity and retaining the structural strength of immune cells [48]. On the other side, free radicals and reactive oxygen were shown to have several beneficial roles in the immune system. These beneficial functions include mediating critical phagocytic cell recruitment, attachment, stimulation, and phagocytosis processes [49]. Additional signal transduction associated with immune responses is regulated by ROS [50]. Based on previous studies, antioxidants may play a dual role in immune function and actions, which might describe the antioxidant and immunosuppressive relationships that exhibit fungi metabolites.

The GC–MS analysis of the ethyl acetate extract of *Chaetomium globosum* (having significant antioxidant activity) showed the presence of various bioactive molecules having pharmaceutical applications such as antimicrobial, antioxidant, and anticancer properties, some used in industry. The GC–MS study of the ethyl acetate extract of *Chaetomium globosum* showed the presence of many phenolic and flavonoids. The phenolic compounds are responsible for the antioxidant activities reported earlier in several studies [51, 52]. Major compounds were 2,3-dihydro-3,5-dihydroxy- 6-methyl-4 h-pyran-4-one; 11-octadecenoic

acid, methyl ester, (Z, Z)-; hexadecanoic acid; 5-oxo-pyrrolidine-2-carboxylic acid methyl ester; 2-propanone, 1-phenyl-; 1,2,3-propanetriol; 1-(6-oxabicyclo[3.1.0]hex-1-yl) ethanone; butanedioic acid, monomethyl ester; 1,2,3,4-butanetetrol, [S-(R*,R*)]-; 5-hydroxymethylfurfural; and 10,12-hexadecadien-1-ol.

Some metabolites have different biological activities and are isolated from other microbes and plants. For example, butanoic acid, 2-methyl- isolated from *Pseudoalteromonas haloplanktis* has antimicrobial activity [53]. Octadecanoic acid has anti-inflammatory and hepatoprotective activities [54, 55]. Squalene is a well-known natural antioxidant molecule. It also has anticancer activity [56, 57]. 11-Octadecenoic acid, methyl ester, (Z, Z)-, isolated from *Jatropha curcas* and *Andrographis paniculata*, possess antioxidant and antimicrobial activities. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, also reported from *Melia azedarach*, has antioxidant, anti-inflammatory, and anthelmintic activities [58]. 1,2-Benzenedicarboxylic acid has antimicrobial and antifoul properties.

Endophytes are a promising source of bioactive molecules, having various biological activities such as antimicrobial, antioxidant, antidiabetic, anti-inflammatory, and anticancerous [59]; this study revealed that endophytic fungi might be a good source of antioxidants.

This work will serve as a platform for future research into the bioactive compounds produced by these endophytes.

Conclusion

Endophytic fungi isolated from the *Dillenia indica* have the potential to possess a myriad of phytochemicals such as alkaloids, flavonoids, phenolics, terpene, and saponins. This study revealed that fungal isolates strains, such as *Chaetomium globosum*, possess phytochemicals rich in phenolics and flavonoids, which seem to be the principal contributors to antioxidant properties in extracts from the strain. *Chaetomium globosum* is a good source of antioxidants. These molecules exhibit significant antioxidant activity and can be used as therapeutic alternatives if further research is undertaken. As a result, fungal endophytes associated with *Dillenia indica* provide an alternative avenue for developing drug-like metabolites, which could be used to find some new antioxidant drugs.

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Author Contribution VK carried out experimental work and prepared the manuscript. IBP supervised the work and refined the manuscript.

Data Availability Data is included in this article.

Code Availability Not applicable.

Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication The work is original; there is no plagiarism, and it has not been published anywhere.

Conflict of Interest The authors declare no competing interests.

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