Antimicrobial and Antioxidant Potential of Endophytic Fungi Isolated from Ethnomedicinal Plants of Western Ghats, Karnataka

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A total of 79 endophytic fungal isolates were obtained from four wild medicinal plants - *Tinospora cordifolia* (Willd.) Hook.f and Thomson (*Menispermaceae*), *Piper nigrum* L., *Piper longum* L. (*Piperaceae*) and *Zingiber officinale* Roscoe (*Zingiberaceae*) from Western Ghats of Karnataka and screened for antimicrobial and antioxidant potential. Among them, 16 isolates depicting good antimicrobial activity by agar plug method (2.33mm-20.66mm) and agar well diffusion method (2.66mm-21mm) against human pathogens were identified by molecular techniques and subjected to secondary metabolite extraction. The extracts were tested for their antioxidant potential by DPPH assay, ABTS assay, reducing power assay and total phenolic content. The isolate ABR4 (*Fusarium solani*) of *Tinospora cordifolia* showed remarkable antimicrobial activity against the human pathogens at tested concentrations of 20-100µg/ml. The isolate GKS (*Aspergillus terreus*) of *Zingiber officinale* demonstrated excellent antioxidant activity (IC$_{50}$ - 3.34) as compared to standard Gallic acid (IC$_{50}$ - 5.54) which has not been reported previously. The findings of the study indicate that endophytic isolates serve as a potential source of novel bioactive products.

**Keywords**: Endophytic fungi, medicinal plants, antimicrobial, antioxidant, phenolic content.

Endophytic fungi reside within the host plants without causing any apparent symptoms of disease and are considered to be a rich source of bioactive metabolites. They produce a broad array of secondary metabolites possessing antioxidant, antimicrobial, antitumor activities that have applications in medicine, agriculture and industry. The bioprospecting of these endophytic fungi offers tremendous potential to discover natural products with therapeutic value. Approximately one million endophytic species have been described. Metabolites produced by endophytes could be influenced by the chemistry of their host plants. During the long period of coevolution, some endophytes have the ability to produce similar or identical bioactive compounds as their host plants. Endophytes help the host plant to tolerate biotic and abiotic stress, increase growth rate and extent of reproduction and hence improve the resistance of host medicinal plants by secretion of bioactive metabolites. Of late, medicinal plants have been exploited for their bioactive compounds having important biological activities. There has been an upsurge of interest among the research groups to obtain endophytic species from the Western Ghats, which is well known for its rich and unique assemblage of flora and harbors several ethnomedicinal plants widely used in traditional practices. It is therefore recognized as one of the
34 hotspots of biodiversity\(^9\). The global emergence of infectious diseases due to antibiotic resistant microorganisms results in mortality and morbidity in human health which necessitates for inventive measures to tackle the problem of drug resistance. The screening of antimicrobial compounds from endophytic fungi is a promising way to deal with drug resistant human and plant pathogens and results in the discovery and development of new drugs\(^10\). The byproducts of biological reactions result in the generation of free radicals. These free radicals are highly unstable and the reactive nature of these free radicals can damage cellular macromolecules. The involvement of free radicals in the pathogenesis of a large number of diseases is well documented\(^11\). An antioxidant is capable of inhibiting the oxidation of other molecules by the initiation of oxidizing chain reactions. During the normal cellular metabolism, reactive oxygen/nitrogen species (ROS/RNS) are produced which are essential for apoptosis, cell signaling, gene expression and ion transportation. However, ROS/RNS can result in oxidative stress if accumulated in excess amounts in the body resulting in a myriad of diseases in living systems\(^12\). Recently, research is focused on endophytic fungi associated with medicinal plants as they are promising source of antioxidants and other bioactive metabolites\(^13\). Antioxidants act as scavengers and prevent cell and tissue damage. Vitamin C, Vitamin E and flavonoids are some of the naturally occurring antioxidants used for the treatment and prevention of human diseases\(^14\). In the present study, mycoendophytic isolates from four medicinal plants collected from the Bisle region, Western Ghats of Karnataka were evaluated for their antimicrobial and antioxidant potential.

**MATERIAL AND METHODS**

**Isolation and identification of endophytic fungi for antimicrobial activity**

Endophytic fungi were isolated from fresh and healthy tissues of four wild medicinal plants collected from Bisle region, Western Ghats of Karnataka and identified as *T. cordifolia* (Willd.) Hook. f and Thomson, *P. nigrum* L., *P. longum* L. and *Z. officinale* Roscoe. Herbarium of plant samples were prepared and deposited to National Ayurveda Dietetics Research Institute (Central Council for Research in Ayurveda and Siddha), Department of AYUSH, Ministry of Health and Family Welfare, Govt. of India, (New Delhi) Jayanagar, Bangalore, India. Standard protocols have been followed for the isolation of endophytic fungi as reported in our previous work\(^15\). The endophytic fungi were identified based on the cultural characteristics, morphology of the fruiting bodies and spores, using standard manuals\(^16\).

**Preliminary screening for antimicrobial activity by agar plug method**

The fungal isolates were screened for antimicrobial activity against the test human pathogenic bacteria- *Staphylococcus aureus* (NCIM No. 2079), *Bacillus cereus* (NCIM No. 2106), *Escherichia coli* (NCIM No. 2256), *Salmonella typhimurium* (NCIM No. 2501) and *Pseudomonas aeruginosa* (NCIM No. 2200) and test human pathogenic fungi *Candida albicans* (NCIM No. 3471). Cylindrical pieces were cut out from well grown culture of the endophytic fungi strain on potato dextrose agar medium (PDA). The blocks were placed on the Petri dishes deep inoculated with a fixed amount of test-microorganisms grown in nutrient broth medium for bacteria and Sabouraud Dextrose Agar medium (SDA) for yeast (10\(^6\) cells/ml). The cultures were kept for 12 hours at 2-8°C for the antibacterial substance diffusion and thereafter they were incubated for the growth of bacterial test-microorganisms at 37°C for 24 hours and incubated for 48 hours at 28°C for fungi. The antimicrobial activity was measured in mm\(^17\).

**Production and extraction of secondary metabolites**

The endophytic fungal isolates depicting good antimicrobial activity were subjected for the production of secondary metabolites. The fresh mycelia of endophytic fungi were grown on PDA plates at 28±2°C for 3-6 days and were inoculated into 1000 ml flasks containing 200gms of unpolished rice, soaked with 200 ml distilled water (autoclaved twice at 121°C for 20 min), followed by incubation for 30 days at 28±2°C\(^18\). The incubated flasks were filled with 300ml of ethyl acetate and allowed to stand for one day, shaken thoroughly and filtered. The above procedure was repeated until most of the metabolites were extracted. Finally ethyl acetate extract was treated with anhydrous Sodium sulphate to remove the moisture content and dried under rotary evaporator\(^19\).
Molecular identification of the potential endophytic fungi

The potential endophytic fungi were identified based on their ribosomal DNA (18srRNA gene) sequences. Total genomic DNA was extracted from fungal mycelia grown on PDA using the Cetyl trimethyl ammonium bromide (CTAB) method [20]. Primers ITS5 (5'-TCCTCCGCTTATTGATATGC-3') and ITS4 (5'-GAAAGTAAAAGTCGTAAGG-3') were used to amplify the 5.8S and ITS regions. The DNA fragment was amplified and sequenced [21].

Antimicrobial activity of crude extract of the endophytic fungi by agar well diffusion method

The extracted secondary metabolites from the potential endophytic fungi were dissolved in DMSO and poured into the 5mm diameter well bored into petridishes containing Nutrient Agar (NA) for test bacteria and SDA for test fungi, inoculated with a fixed amount of test-microorganisms (10^6 cells/ml). The cultures were kept at 2-8°C for 24 hours for the antimicrobial metabolite diffusion and thereafter they were incubated at an appropriate temperature for the growth of test-microorganisms. The zone of inhibition was measured in mm [22].

Evaluation of the antioxidant potential of endophytic fungi

The endophytic fungal isolates were subjected to different antioxidant assays to determine their antioxidant potential.

DPPH assay

The free radical scavenging activity of the fungal extract was measured in terms of its hydrogen donating or radical scavenging ability using the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical method [23]. The DPPH solution (0.1mM) in methanol was prepared and 1.0mg/ml of this solution was added to 3.0 ml of fungal extract and standard in solvent at different concentrations (10µM-100 µM). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture showed higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

% inhibition = control – test/control × 100

Where ‘control’ was the absorbance of the control reaction and ‘test’ was the absorbance in presence of fungal extract. The mean values were calculated from three experiments. Standard trolox solution was used as the positive control.

ABTS Radical Scavenging activity

The ABTS [2, 2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] radical scavenging activity of the fungal extract was measured [24]. ABTS radical cation was produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium per sulfate and the mixture was allowed to stand in dark at room temperature for 12-16hrs before use. Different concentrations (10-50µg/ml) of extract or standard Gallic acid (0.5ml) were added to 0.3 ml of ABTS solution and the final volume was made up with solvent to make up to 1ml. The absorbance was read at 745nm and the % inhibition of the ABTS radical was calculated using the following equation:

% inhibition = control – test/control × 100

Reducing power assay

The reductive potential of the fungal extracts were determined [25]. Various concentrations of fungal methanolic extracts were mixed with 2.5mL of 0.2 M phosphate buffer (pH 6.6) and 2.5mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Post incubation, 2.5mL of 10% trichloroacetic acid (w/v) was added and the mixture was centrifuged at 1000 rpm for 10min. The supernatant (5mL) was mixed with 5 mL deionized water and 1mL of 0.1% of ferric chloride. The absorbance was measured at 700 nm using Gallic acid as a standard. Higher absorbance value of the reaction mixture indicates greater reductive potential. The assay was carried out in triplicate and the results are expressed as mean ± standard deviation.

Determination of total phenolic content

The amount of total phenolic was determined using the Folin–Ciocalteu reagent [26]. One ml sample was dissolved in 1.5 ml distilled water and 0.5 ml Folin–Ciocalteu’s reagent. After 1 min, 1 ml of 20% sodium carbonate solution was added. The final mixture was shaken three times and incubated for 2 h in the dark at 25°C. The absorbance of the mixture was measured at 760 nm. All tests were carried out in triplicate and the results were expressed as Gallic Acid Equivalents (mg GAE/mg of dry weight of the crude extract).
RESULTS

Isolation, identification and preliminary screening of endophytic fungi

A total of 79 endophytic fungal isolates were obtained from the four medicinal plants of Bisle region from the Western Ghats of Karnataka. The medicinal uses of the plants and their herbarium accession numbers are mentioned in (Table 1). The fungal isolates were identified by morphological characteristics. A majority of the fungal endophytes belong to the phylum Ascomycetes. Also, endophytic fungi belonging to the class Hyphomycetes, Zygomycetes and Coelomycetes were also obtained. The potential endophytic fungi were identified using molecular techniques by 18s rDNA analysis, submitted to Gen bank and their accession numbers were obtained (Table 2).

The fungal endophytes were primarily screened for their antimicrobial activity against human pathogens by agar plug method wherein the zone of inhibition is detected around the endophytic fungal colony on test bacteria or test fungus swabbed plate. Agar plug diffusion method is often used to highlight the antagonism between microorganisms. Among the 79 endophytic fungal isolates, 41 isolates showed antimicrobial activity towards the tested microorganisms. The fungal isolates ABLS2, ABLS3 of T. cordifolia; PKR1, PKR3, PKKR2 of P. nigrum; PLLS6, PLPS1 of P. longum and GKS from Z. officinale exhibited good antimicrobial activity. None of the endophytic isolates of T. cordifolia depicted antibacterial activity against P. aeruginosa in the agar plug method. With regard to antifungal activity, only 2 isolates (ABL1 and ABR4) of T. cordifolia were found to be promising against C. albicans and one isolate each of P. longum (PLLS6), Z. officinale (GKS) and P. nigrum (PKR2) showed inhibitory activity against C. albicans (Table 3).

Production and extraction of secondary metabolites

The potential isolates were chosen after the preliminary screening and were selected for the production and extraction of secondary metabolites for further evaluation. The ethyl acetate extracts of the potential isolates yielded around 400mg/200g of red rice media.

Antimicrobial activity of crude fungal extract by agar well diffusion method

The potential endophytic fungal crude extracts were screened for their antimicrobial activity by agar well diffusion method. The crude extracts were added at concentrations of 20–100 µg/mL. The endophytic fungi exhibited a broad spectrum of antimicrobial activity against the test pathogens, when compared to that of standard positive control tetracycline (bacteria) and flucanazole (fungi). The zone of inhibition of test pathogens ranged from 2.66 mm to 23.33 mm at concentrations of 20–100 µg/mL of tested crude extracts (Table 4).

Antioxidant potential of endophytic fungi

DPPH assay

DPPH is a stable free radical with absorption at 570nm, exhibits a deep purple color in methanol solution which gets reduced to a yellow colored product diphenyl picryl hydrazine. As antioxidants donate protons to DPPH radicals, the absorption decreases. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups. The DPPH scavenging potential of endophytic extracts may be attributed to the hydroxyl groups present in the extracts. The sample was tested against this radical at different concentrations ranging from (10 to 100µg) and the readings were observed by decreasing the absorbance taken as a measure that indicates the extent of radical scavenging property.

The antioxidant activity of the 16 potential endophytic fungi was determined and the results are tabulated in (Table 5). The ethyl acetate extracts of GKS, PKS4 and PKKR3 showed higher DPPH activity than the standard Trolox (Fig 1).

Radical scavenging activity against ABTS

ABTS is a stable free radical with the characteristic absorption at 745 nm and was used to study the radical scavenging effect of extracts. The results demonstrated that the extracts reacted with ABTS at different concentrations ranging from 200, 400, 600, 800 and 1600µg/ml respectively depending on the weight of the extracts. The readings were observed by measuring the reduction of radical cation generated by ABTS at 745 nm. The endophytic ethyl acetate extracts showed maximum decolorization at the maximum concentration of 1600µg/ml (Table 5). The extent of reduction...
in decolorization is directly proportional to the increased concentration of the extracts.

**Total phenolic content**

The Total phenolic content (TPC) was determined by the Folin Ciocalteu method. The fungal ethyl acetate extracts have been expressed as Gallic acid equivalent i.e., mg Gallic acid/g dry wt. A high phenolic content (146.33±3.78) was observed in the isolate PKKS1 (*A. terreus*) of *P. nigrum* (Table 5). Almost all the tested

**Table 1.** List of selected medicinal plants and their uses

| S. No | Medicinal Plant | Family | Herbarium Accession no. | Medicinal uses of the plants | Ref. |
|-------|-----------------|--------|-------------------------|----------------------------|------|
| 1     | *Tinospora cordifolia* (Willd.) Hook. f & Thomson | Menispermaceae | RRCBI-8976 | antipyretic, anti-asthmatic, hypoglycemic, hypotensive, analgesic, anti-inflammatory and anti-spasmodic properties | 8    |
| 2     | *Piper nigrum* L. | Piperaceae | RRCBI-MUS 135 | to treat vertigo, asthma, sinusitis, Fever, diarrhoea, arthritic disorders | 27   |
| 3     | *Piper longum* L. | Piperaceae | RRCBI-AP-2591 | Treatment of respiratory tract infections, chronic gut-related pain and arthritic conditions | 28   |
| 4     | *Zingiber officinale* Roscoe | Zingiberaceae | RRCBI-AP.4046 | Treatment of common colds, fever, to aid digestion, treat stomach upset, diarrhoea, nausea, rheumatic disorders and dizziness. | 29   |

**Table 2.** List of endophytic fungi identified by molecular methods based on 18s rDNA and their accession numbers

| Sl. no | Endophytic culture | Medicinal plant | Identification of the fungal isolate | Genbank accession number |
|--------|--------------------|-----------------|--------------------------------------|--------------------------|
| 1      | ABLS 2             | *T. cordifolia* | *Aspergillus oryzae*                 | KR181978                 |
| 2      | ABLS 3             | *T. cordifolia* | *Penicillium rubens*                 | KR181979                 |
| 3      | PLLS 6             | *P. longum*     | *Aspergillus terreus*                | KR181980                 |
| 4      | PLPS1              | *P. longum*     | *Aspergillus flavus*                 | KR181981                 |
| 5      | PKR1               | *P. nigrum*     | *Aspergillus flavus*                 | KR181982                 |
| 6      | PKR3               | *P. nigrum*     | *Sordaria tomento-alba*              | KR181983                 |
| 7      | ABS1               | *T. cordifolia* | *Aspergillus oryzae*                 | KJ729477                 |
| 8      | ABL1               | *T. cordifolia* | *Fusarium solani*                    | KJ729475                 |
| 9      | ABS3               | *T. cordifolia* | *Trichoderma asperellum*             | KJ729478                 |
| 10     | ABR4               | *T. cordifolia* | *Fusarium solani*                    | KJ729476                 |
| 11     | PKKR2              | *P. nigrum*     | *Aspergillus flavus*                 | KJ729481                 |
| 12     | PKKS1              | *P. nigrum*     | *Aspergillus terreus*                | KJ729483                 |
| 13     | PKKS2              | *P. nigrum*     | *Lasiodiplodia theobromae*           | KJ729484                 |
| 14     | PKKR3              | *P. nigrum*     | *Trichoderma virens*                 | KJ729482                 |
| 15     | PKS4               | *P. nigrum*     | *Aspergillus terreus*                | KJ729485                 |
| 16     | GKS                | *Z. officinale* | *Aspergillus terreus*                | KJ729479                 |
Table 3. List of endophytic fungi screened for antimicrobial activity by agar plug method

| S. No | Medicinal plant | Culture code | Identification of the endophytic isolate | S. aureus | B. cereus | E. coli | S. typhi | P. aeruginosa | C. albicans |
|-------|-----------------|--------------|------------------------------------------|-----------|-----------|---------|----------|--------------|------------|
| 1     | T. cordifolia   | ABL          | Aspergillus niger                         | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          | 0±0        |
| 2     | T. cordifolia   | ABL1         | Fusarium solani                           | 8.00±0.51 | 0±0       | 8.33±0.59| 0±0      | 12.33±0.78   |            |
| 3     | T. cordifolia   | ABL2         | Rhizopus sp.                              | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 4     | T. cordifolia   | ABL3         | Aspergillus flavus                         | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 5     | T. cordifolia   | ABLS1        | Mucor sp.                                 | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 6     | T. cordifolia   | ABLS2        | A. oryzae                                 | 7.33±0.29 | 6.33±0.29 | 11.00±0.89| 10.66±0.29| 0±0          |            |
| 7     | T. cordifolia   | ABLS3        | Penicillium rubens                         | 10.00±0.51| 5.66±0.29 | 6.33±0.29| 5.33±0.59| 0±0          |            |
| 8     | T. cordifolia   | ABLS4        | Mycelia sterilia                          | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 9     | T. cordifolia   | ABPS1        | Cladosporium sp.                          | 4.33±0.29 | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 10    | T. cordifolia   | ABPS2        | Aspergillus sp.                           | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 11    | T. cordifolia   | ABPS3        | Mycelia sterilia                          | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 12    | T. cordifolia   | ABS          | Curvularia sp.                            | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 13    | T. cordifolia   | ABS1         | A. oryzae                                 | 17.00±0.51| 0±0       | 11.00±0.51| 0±0      | 0±0          |            |
| 14    | T. cordifolia   | ABS2         | Mycelia sterilia                          | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 15    | T. cordifolia   | ABS3         | Trichoderma asperellum                    | 0±0       | 0±0       | 0±0     | 12.66±0.29| 0±0          |            |
| 16    | T. cordifolia   | ABSS1        | Cladosporium sp.                          | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 17    | T. cordifolia   | ABSS2        | Fusarium sp.                              | 0±0       | 0±0       | 0±0     | 7.00±0.51| 0±0          |            |
| 18    | T. cordifolia   | ABSS3        | Cladosporium sp.                          | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 19    | T. cordifolia   | ABSS4        | Aspergillus sp.                           | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 20    | T. cordifolia   | ABSS5        | A. terreus                                | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 21    | T. cordifolia   | ABR          | Phomopsis sp.                             | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 22    | T. cordifolia   | ABR1         | Mycelia sterilia                          | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 23    | T. cordifolia   | ABR2         | Colletotrichum sp.                        | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 24    | T. cordifolia   | ABR3         | Mucor sp.                                 | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 25    | T. cordifolia   | ABR4         | F. solani                                 | 0±0       | 0±0       | 6.66±0.29| 7.00±0.51| 12.33±0.78   |            |
| 26    | P. nigrum       | PKL1         | Phoma sp.                                 | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 27    | P. nigrum       | PKL2         | Paecilomyces sp.                          | 0±0       | 20.33±0.29| 0±0     | 3.33±0.29| 4.33±0.59    |            |
| 28    | P. nigrum       | PKS1         | Phoma sp.                                 | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 29    | P. nigrum       | PKS2         | Mycelia sterilia                          | 0±0       | 0±0       | 0±0     | 5.33±0.29| 19.00±0.51   |            |
| 30    | P. nigrum       | PKS3         | Colletotrichum sp.                        | 0±0       | 0±0       | 0±0     | 0±0      | 0±0          |            |
| 31    | P. nigrum       | PKKS1        | A. terreus                                | 11.00±0.51| 12.33±0.78| 0±0     | 0±0      | 0±0          |            |
| 32    | P. nigrum       | PKKS2        | Lasiodiplodia theobromae                 | 3.33±0.59 | 0±0       | 8.00±0.51| 0±0      | 0±0          |            |
|   |   |          |          |          |          |          |          |   |          |          |          |   |
|---|---|----------|----------|----------|----------|----------|----------|---|----------|----------|----------|---|
|33 | P. nigrum PKS4 | A. terreus | 3.33 ± 0.78 | 9.33 ± 0.29 | 6.00 ± 0.51 | 0° | 0° | 0° |   |
|34 | P. nigrum PKR1 | A. flavus | 5.66 ± 0.59 | 12.33 ± 0.78 | 2.33 ± 1.30 | 12.66 ± 0.59 | 14.00 ± 0.51 | 0° |   |
|35 | P. nigrum PKR2 | Penicillium sp. | 0° | 0° | 0° | 9.33 ± 0.59 | 2.33 ± 0.29 |   |
|36 | P. nigrum PKR3 | Sordaria tomento-alba | 14.00 ± 0.51 | 0° | 2.33 ± 0.29 | 7.33 ± 0.29 | 6.33 ± 0.29 | 0° |   |
|37 | P. nigrum PKKR2 | A. flavus | 16.66 ± 0.29 | 8.00 ± 0.51 | 26.00 ± 0.51 | 9.33 ± 0.59 | 0° | 0° |   |
|38 | P. nigrum PKKR3 | T. virens | 9.66 ± 0.29 | 0° | 0° | 0° | 0° | 0° |   |
|39 | P. longum PLLS1 | Mucor sp. | 7.00 ± 0.51 | 3.00 ± 0.51 | 0° | 0° | 0° | 0° |   |
|40 | P. longum PLLS2 | Penicillium sp. | 10.66 ± 0.29 | 5.66 ± 0.29 | 0° | 11.33 ± 0.51 | 0° | 0° |   |
|41 | P. longum PLLS3 | A. flavus | 14.66 ± 0.78 | 11.66 ± 0.29 | 0° | 6.33 ± 0.59 | 0° | 0° |   |
|42 | P. longum PLLS4 | Colletotrichum sp. | 6.66 ± 0.20 | 0° | 0° | 5.33 ± 0.29 | 0° | 0° |   |
|43 | P. longum PLLS5 | Penicillium sp. | 12.33 ± 0.29 | 0° | 0° | 7.33 ± 0.29 | 0° | 0° |   |
|44 | P. longum PLLS6 | A. terreus | 7.66 ± 1.19 | 3.33 ± 0.29 | 7.00 ± 0.0 | 8.33 ± 0.59 | 4.33 ± 0.29 | 3.00 ± 0.51 |   |
|45 | P. longum PLLS7 | Dreschlera sp. | 0° | 0° | 0° | 0° | 0° | 0° |   |
|46 | P. longum PLLS8 | Aspergillus sp. | 7.66 ± 0.29 | 2.33 ± 0.29 | 0° | 6.00 ± 0.51 | 0° | 0° |   |
|47 | P. longum PLLS9 | Pithomyces sp. | 6.33 ± 0.78 | 6.00 ± 0.51 | 0° | 7.33 ± 0.29 | 0° | 0° |   |
|48 | P. longum PLLS10 | Penicillium sp. | 5.33 ± 0.59 | 5.33 ± 0.59 | 0° | 0° | 0° | 0° |   |
|49 | P. longum PLLS11 | Curvularia sp. | 0° | 0° | 0° | 7.33 ± 0.78 | 0° | 0° |   |
|50 | P. longum PLLS1 | Fusarium sp. | 0° | 0° | 0° | 2.66 ± 0.29 | 0° | 0° |   |
|51 | P. longum PLLS2 | A. terreus | 13.33 ± 1.40 | 0° | 0° | 3.00 ± 0.51 | 0° | 0° |   |
|52 | P. longum PLLS3 | Curvularia sp. | 0° | 0° | 0° | 0° | 0° | 0° |   |
|53 | P. longum PLLS4 | A. terreus | 11.33 ± 0.78 | 5.66 ± 0.50 | 0° | 3.33 ± 0.78 | 0° | 0° |   |
|54 | P. longum PLLS5 | A. terreus | 6.66 ± 0.78 | 2.33 ± 0.29 | 0° | 6.33 ± 0.29 | 0° | 0° |   |
|55 | P. longum PLLS6 | Mycelia sterilia | 8.33 ± 0.78 | 0° | 0° | 4.33 ± 0.29 | 0° | 0° |   |
|56 | P. longum PLLS7 | Mucor sp. | 0° | 0° | 0° | 0° | 0° | 0° |   |
|57 | P. longum PLLS8 | Penicillium sp. | 0° | 0° | 0° | 0° | 0° | 0° |   |
|58 | P. longum PLPS1 | A. flavus | 20.66 ± 0.59 | 3.66 ± 0.29 | 6.66 ± 0.29 | 13.33 ± 0.78 | 15.33 ± 0.29 | 0° |   |
|59 | P. longum PLPS2 | Pestalotiopsis sp. | 7.00 ± 0.51 | 0° | 0° | 0° | 0° | 0° |   |
|60 | P. longum PLPS3 | Mycelia sterilia | 0° | 0° | 0° | 0° | 0° | 0° |   |
|61 | P. longum PLPS4 | Bipolaris sp. | 8.66 ± 0.78 | 0° | 0° | 9.33 ± 0.50 | 0° | 0° |   |
|62 | P. longum PLPS5 | Phomopsis sp. | 0° | 0° | 0° | 3.33 ± 0.78 | 0° | 0° |   |
|63 | P. longum PLPS6 | Penicillium sp. | 0° | 0° | 0° | 0° | 0° | 0° |   |
|64 | P. longum PLPS7 | Aspergillus sp. | 0° | 0° | 0° | 0° | 0° | 0° |   |
|65 | P. longum PLPS8 | Mycelia sterilia | 0° | 0° | 0° | 8.00 ± 0.51 | 0° | 0° |   |
|66 | Z. officinale WGL | Aspergillus sp. | 0° | 0° | 0° | 0° | 0° | 0° |   |
|67 | Z. officinale WGL1 | Rhizopus sp. | 3.00 ± 0.51 | 0° | 0° | 0° | 0° | 0° |   |
|68 | Z. officinale WGMR1 | Cladosporium sp. | 0° | 0° | 0° | 0° | 0° | 0° |   |
|69 | Z. officinale WGMR2 | Cladosporium sp. | 0° | 0° | 0° | 0° | 0° | 0° |   |
Values represent mean ± SD of three parallel experiments. In each column, mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at p < 0.05.

Endophytic fungi are ubiquitous in their distribution and are reported from almost every plant. The secondary metabolites from endophytic fungi from four medicinal plants from Western Ghats, Karnataka were investigated in a quest to search for novel antimicrobial and antioxidant agents. There is a dearth of information on the antioxidant activities from the medicinal plants *Piper nigrum* L, *Piper longum* L and *Zingiber officinale* Roscoe which prompted us to evaluate the antioxidant and antimicrobial potential of these medicinal plants. In this study, 79 endophytic fungal strains belonging to different taxa were obtained. Fungal endophytes are especially common among the Ascomycetes, representing at least five classes, dozens of families, and large numbers of previously unknown species. Most of the taxa were common endophytic fungi as observed earlier. Cultures which failed to sporulate or those lacking reproductive structures and which could not be identified to genus or species level without molecular analysis were grouped as Mycelia sterilia. There are a vast range of endophytic microorganisms which have been unappreciated for bioactive metabolite production or finding an alternative to the problem of drug resistance acquired by the pathogens. Recent research has focused on finding an alternative to the problem of drug resistance acquired by the pathogens. Endophytic fungi are ubiquitous in their distribution and are reported from almost every plant. The secondary metabolites from endophytic fungi from four medicinal plants from Western Ghats, Karnataka were investigated in a quest to search for novel antimicrobial and antioxidant agents. There is a dearth of information on the antioxidant activities from the medicinal plants *Piper nigrum* L, *Piper longum* L and *Zingiber officinale* Roscoe which prompted us to evaluate the antioxidant and antimicrobial potential of these medicinal plants. In this study, 79 endophytic fungal strains belonging to different taxa were obtained. Fungal endophytes are especially common among the Ascomycetes, representing at least five classes, dozens of families, and large numbers of previously unknown species. Most of the taxa were common endophytic fungi as observed earlier. Cultures which failed to sporulate or those lacking reproductive structures and which could not be identified to genus or species level without molecular analysis were grouped as Mycelia sterilia. There are a vast range of endophytic microorganisms which have been unappreciated for bioactive metabolite production or finding an alternative to the problem of drug resistance acquired by the pathogens. Recent research has focused on finding an alternative to the problem of drug resistance acquired by the pathogens.

### Reducing power assay

The reducing power of the ethyl acetate extracts of fungal strains increased with increasing concentration. The increase in absorbance of the reaction mixture implies increased reducing power. In our study, most of the endophytic fungal extracts have demonstrated potent reducing activity comparable to the standard Gallic acid (Fig. 3).
| Sl. No | Endophytic Fungi code | Medicinal plant | Conc. µg/mL | Staphylococcus aureus | Bacillus cereus | E.coli | Salmonella typhi | P. aeruginosa | C. albicans |
|-------|-----------------------|-----------------|-------------|----------------------|----------------|--------|----------------|--------------|-------------|
| 1     | ABL1                  | T. cordifolia   | 20          | 7.00±0.29            | 11.33±0.59     | 0°     | 0°             | 0°           | 0°          |
|       |                       |                 | 40          | 7.00±0.51            | 12.33±0.29     | 0°     | 0°             | 0°           | 2.56±0.03   |
|       |                       |                 | 60          | 9.66±0.51            | 12.33±0.29     | 0°     | 0°             | 0°           | 5.03±0.29   |
|       |                       |                 | 80          | 9.66±0.51            | 12.66±0.59     | 0°     | 0°             | 0°           | 7.30±0.51   |
|       |                       |                 | 100         | 11.00±0.29           | 9.66±0.29      | 0°     | 0°             | 0°           | 10.05±0.59  |
| 2     | ABS1                  | T. cordifolia   | 20          | 8.66±0.29            | 9.33±0.59      | 0°     | 0°             | 0°           | 0°          |
|       |                       |                 | 40          | 9.00±0.89            | 18.00±0.51     | 0°     | 0°             | 0°           | 0°          |
|       |                       |                 | 60          | 10.00±0.29           | 13.66±0.29     | 0°     | 0°             | 0°           | 0°          |
|       |                       |                 | 80          | 10.00±0.51           | 10.66±0.78     | 0°     | 0°             | 0°           | 0°          |
|       |                       |                 | 100         | 10.33±0.51           | 12.00±0.51     | 0°     | 0°             | 0°           | 0°          |
| 3     | ABS3                  | T. cordifolia   | 20          | 0°                   | 0°             | 5.66±0.78 | 0°             | 0°           | 0°          |
|       |                       |                 | 40          | 0°                   | 5.00±0.51      | 12.00±0.51 | 0°             | 0°           | 0°          |
|       |                       |                 | 60          | 0°                   | 7.66±0.59      | 9.33±0.29  | 0°             | 0°           | 0°          |
|       |                       |                 | 80          | 0°                   | 7.33±0.29      | 9.33±0.59  | 0°             | 0°           | 0°          |
|       |                       |                 | 100         | 0°                   | 5.33±0.29      | 10.66±0.29 | 0°             | 0°           | 0°          |
| 4     | ABR4                  | T. cordifolia   | 20          | 11.33±0.29           | 19.00±0.51     | 0°     | 7.66±0.29      | 5.66±0.29    | 3.03±0.03   |
|       |                       |                 | 40          | 10.66±0.29           | 14.33±0.59     | 0°     | 6.33±0.29      | 4.66±0.29    | 5.56±0.29   |
|       |                       |                 | 60          | 15.33±0.78           | 15.00±0.51     | 0°     | 10.33±0.78     | 7.00±0.51    | 7.50±0.29   |
|       |                       |                 | 80          | 11.00±0.51           | 13.33±0.29     | 7.33±0.29 | 10.66±0.29     | 9.00±0.51    | 9.42±0.51   |
|       |                       |                 | 100         | 9.66±0.29            | 17.33±0.59     | 11.33±0.59 | 22.33±0.29     | 13.33±0.59   | 10.55±0.29 |
| 5     | PKS4                  | P. nigrum       | 20          | 13.33±0.78           | 12.33±0.59     | 0°     | 0°             | 0°           | 0°          |
|       |                       |                 | 40          | 12.66±0.59           | 16.00±0.51     | 0°     | 0°             | 0°           | 0°          |
|       |                       |                 | 60          | 8.00±0.51            | 14.33±0.59     | 0°     | 0°             | 0°           | 0°          |
|       |                       |                 | 80          | 13.66±0.29           | 16.33±0.29     | 0°     | 7.33±0.29      | 0°           | 0°          |
|       |                       |                 | 100         | 17.33±0.59           | 21.33±0.59     | 0°     | 11.33±0.78     | 0°           | 0°          |
| 6     | PKKS1                 | P. nigrum       | 20          | 11.66±0.78           | 14.33±0.59     | 0°     | 0°             | 0°           | 0°          |
|       |                       |                 | 40          | 12.33±0.59           | 15.33±1.57     | 0°     | 0°             | 0°           | 0°          |
|       |                       |                 | 60          | 12.66±0.59           | 17.00±0.00     | 0°     | 0°             | 0°           | 0°          |
|       |                       |                 | 80          | 15.66±0.29           | 14.33±1.30     | 0°     | 0°             | 0°           | 0°          |
|       |                       |                 | 100         | 16.66±0.78           | 16.00±1.79     | 0°     | 0°             | 0°           | 0°          |
| 7     | PKKS2                 | P. nigrum       | 20          | 2.66±0.59            | 0°             | 6.66±0.59 | 0°             | 0°           | 0°          |
|       |                       |                 | 40          | 3.33±0.29            | 0°             | 5.00±0.51 | 0°             | 0°           | 0°          |
|    | 8 PKKR3 | P. nigrum |    | 20 | 5.66±0.59 | 0± | 0± | 7.66±0.29 | 0± | 0± |
|----|---------|-----------|----|----|----------|----|----|----------|----|----|
|    |         |           | 40 | 6.66±0.59 | 0± | 0± | 7.33±0.78 | 0± | 0± |
|    |         |           | 60 | 9.00±0.51 | 0± | 0± | 14.33±0.78 | 0± | 0± |
|    |         |           | 80 | 10.33±0.29 | 0± | 0± | 0± | 0± | 0± |
|    |         |           | 100| 16.66±0.78| 0± | 0± | 0± | 0± | 0± |

|    | 9 GKS   | Z. officale |    | 20 | 13.33±1.49 | 13.66±0.78 | 0± | 0± | 0± |
|----|---------|-------------|----|----|------------|----------|----|----|----|
|    |         |             | 40 | 15.00±0.51 | 16.00±0.89 | 0± | 0± | 0± |
|    |         |             | 60 | 16.00±0.89  | 18.66±0.29  | 0± | 0± | 0± |
|    |         |             | 80 | 18.33±0.59  | 13.33±0.78  | 0± | 0± | 0± |
|    |         |             | 100| 13.33±0.78  | 18.00±0.51  | 0± | 0± | 0± |

|    | 10 PKR1 | P. nigrum |    | 20 | 0±        | 4.00±0.89 | 0± | 0± | 0± |
|----|---------|-----------|----|----|-----------|----------|----|----|----|
|    |         |           | 40 | 0± | 6.00±0.51 | 0±        | 0± | 0± |
|    |         |           | 60 | 0± | 5.66±0.29 | 0± | 0± | 0± |
|    |         |           | 80 | 0± | 6.66±0.78 | 0± | 0± | 0± |
|    |         |           | 100| 0± | 7.66±0.59 | 0± | 0± | 0± |

|    | 11 PKR3 | P. nigrum |    | 20 | 0±        | 4.66±0.29 | 3.66±0.59 | 0± | 0± |
|----|---------|-----------|----|----|-----------|----------|----|----|----|
|    |         |           | 40 | 0± | 4.33±0.78 | 1.66±0.29 | 0± | 0± | 0± |
|    |         |           | 60 | 0± | 7.33±0.29 | 5.00±0.51 | 0± | 0± | 0± |
|    |         |           | 80 | 0± | 9.00±0.89 | 11.66±0.78 | 0± | 0± | 0± |
|    |         |           | 100| 0± | 13.00±0.51| 14.33±0.59 | 0± | 0± | 0± |

|    | 12 ABLS2| T. cordifolia |    | 20 | 5.00±0.89 | 5.00±0.51 | 5.66±0.29 | 6.00±0.89 | 0± |
|----|--------|---------------|----|----|-----------|----------|----|----------|----|
|    |        |               | 40 | 6.33±0.78 | 8.33±0.29 | 7.66±0.29 | 9.00±0.51 | 0± |
|    |        |               | 60 | 10.33±0.29 | 11.00±0.51 | 9.33±0.78 | 13.00±1.03 | 0± |
|    |        |               | 80 | 12.00±0.57 | 12.00±0.57 | 12.33±0.66 | 13.00±0.57 | 0± |
|    |        |               | 100| 19.00±0.51 | 16.33±0.78 | 15.33±0.59 | 16.33±0.59 | 0± |

|    | 13 ABLS3| T. cordifolia |    | 20 | 3.66±0.29 | 4.66±0.59 | 3.33±0.29 | 8.00±0.51 | 0± |
|----|--------|---------------|----|----|-----------|----------|----|----------|----|
|    |        |               | 40 | 6.33±0.29 | 6.33±0.59 | 8.33±0.78 | 11.66±0.78 | 0± |
|    |        |               | 60 | 7.33±0.29 | 6.33±1.19 | 8.33±0.59 | 12.33±1.30 | 0± |
|    |        |               | 80 | 10.66±0.51 | 9.33±0.29 | 13.33±0.78 | 18.33±0.29 | 0± |
|    |        |               | 100| 11.33±0.59 | 12.66±0.29 | 11.33±0.59 | 21.00±0.51 | 0± |

|    | 14 PLLS6| P. longum |    | 20 | 6.33±0.59 | 3.00±0.51 | 3.33±0.59 | 5.00±0 | 4.33±0.78 |
|----|--------|-----------|----|----|-----------|----------|----|----------|----|
|    |        |           | 40 | 7.66±1.19 | 5.66±0.29 | 5.66±0.59 | 6.66±1.19 | 6.00±0 |
|    |        |           | 60 | 11.60±0.59 | 6.66±0.78 | 5.00±1.03 | 10.33±0.59 | 5.66±0.78 |
|    |        |           | 80 | 13.00±0.51 | 13.00±0.89 | 7.66±1.19 | 12.00±1.03 | 8.66±1.57 |
|    |        |           | 100| 14.33±0.78 | 17.33±0.59 | 14.00±1.03 | 20.00±1.03 | 8.66±1.19 |
Results of the antimicrobial activity of plant extracts are presented in Table 4. The fungal endophytes of *T. cordifolia* revealed good antimicrobial activities which can be utilized for the production of bioactive metabolites. Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. The endophytic fungus *F. solani* (ABR4) of *T. cordifolia* inhibited both gram positive and gram negative organisms with effective inhibition. Also, ABR4 has demonstrated significant inhibitory activity against *C. albicans* at effective concentrations (20 µg/mL) indicating the ability of the endophyte to be utilized for pharmaceutical preparations. The extracts of *P. longum*-PLLS6 and PLPS1 demonstrated effective antibacterial activity by agar well diffusion to all the tested bacterial pathogens at concentrations of 20-100 µg/mL (Table 4). Several studies have reported novel compounds with antimicrobial activity from endophytic fungi. Endophytic fungi with antimicrobial, anti-cancer and anti-malarial activities were isolated from Thai medicinal plants. A majority of microorganisms remain unexploited for diverse and valuable bioactive metabolites. The extracts of ABLS2, ABLS3 of *T. cordifolia* and PKKR2 of *P. nigrum* exhibited antibacterial activity against the tested bacteria except *P. aeruginosa* when tested by agar well diffusion method. Further, the endophytic isolates ABL1, ABS1, PKS4, and PKKS1 and GKS exhibited activity only against *S. aureus* and *B. cereus* at concentrations of 20-100 µg/mL; whereas PKR3 and ABS showed activity against *B. cereus* and *E. coli* demonstrating that it can be an effective antibacterial against both gram positive and gram negative organisms at the tested concentrations. Moreover, the extract ABL1 of *T. cordifolia* revealed antimicrobial activity by inhibiting *C. albicans* at concentrations of 40-100 µg/mL. The extract of PKR1 of *P. nigrum* exhibited significant inhibitory activity against *S. aureus* and *B. cereus* at 100 µg/mL. ABL1, ABLS2, ABLS3 and PKR3 of *T. cordifolia* and PKKR2 of *P. nigrum* exhibited effective antibacterial activity against the tested bacteria except *P. aeruginosa*. The endophytic fungus *F. solani* (ABR4) of *T. cordifolia* inhibited both gram positive and gram negative organisms with effective inhibition. Also, ABR4 has demonstrated significant inhibitory activity against *C. albicans* at effective concentrations (20 µg/mL) indicating the ability of the endophyte to be utilized for pharmaceutical preparations. The extracts of *P. longum*-PLLS6 and PLPS1 demonstrated effective antibacterial activity by agar well diffusion to all the tested bacterial pathogens at concentrations of 20-100 µg/mL. Several studies have reported novel compounds with antimicrobial activity from endophytic fungi. Endophytic fungi with antimicrobial, anti-cancer and anti-malarial activities were isolated from Thai medicinal plants. A vast majority of microorganisms remain unexploited for diverse and valuable bioactive metabolites.

| 15 | PLPS1 | *P. longum* | 20 | 6.66±0.59 | 2.66±0.29 | 4.66±0.29 | 6.33±0.59 | 7.00±0.0 | 0*  |
| 40 | 11.33±0.59 | 5.66±0.78 | 5.00±0.89 | 7.00±0.78 | 9.00±0.51 | 12.66±0.29 | 0*  |
| 60 | 13.33±0.78 | 7.00±0.89 | 5.66±0.78 | 5.00±0.89 | 7.00±0.51 | 12.66±0.29 | 0*  |
| 80 | 17.66±0.78 | 9.33±0.29 | 10.66±0.29 | 13.66±0.78 | 14.00±0.51 | 17.66±0.59 | 0*  |
| 100 | 21.00±0.89 | 13.66±0.78 | 15.00±0.51 | 17.33±0.29 | 17.66±0.59 | 0*  |

| 16 | PKKR2 | *P. nigrum* | 20 | 12.33±0.59 | 7.00±0.51 | 4.33±0.78 | 11.66±0.59 | 0*  |
| 40 | 14.00±0.89 | 7.33±0.59 | 5.66±0.29 | 11.33±0.78 | 0*  |
| 60 | 17.00±0.51 | 6.66±1.30 | 16.66±0.59 | 5.66±0.29 | 11.33±0.78 | 0*  |
| 80 | 16.33±0.66 | 8.66±0.33 | 18.00±0.57 | 13.33±0.88 | 0*  |
| 100 | 18.33±0.66 | 8.66±0.66 | 18.33±0.88 | 14.66±0.66 | 0*  |

| 17 | Positive control | Tetracycline | 20 | 21.33±0.88 | 19.33±0.59 | 20.33±0.89 | 23.33±0.88 | 20.33±0.89 | 0*  |
| 18 | Positive control | Fluconazole | 20 | - | - | - | - | - | 13.58±0.58 |

Values represent mean±SD of three experiments. In each column, mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at p<0.05.
antibacterial activity against *B. cereus* only and was ineffective against the other test pathogens. The endophytic fungi (SRVK 113 and SRVK 147) from *T. cordifolia* revealed good antibacterial activity against *B. subtilis* and *S. aureus* but did not show inhibitory activity against *E. coli* and *P. aeruginosa*. Also, none of the isolates showed antifungal activity against *C. albicans* and *A. niger*. The extract PKKS2 showed activity against *S. aureus* and *S. typhimurium* indicating a strong antibacterial

### Table 5. Antioxidant activity and total phenolic content of selected fungal endophytes isolated from four medicinal plants

| Sl. No | Endophytic fungal extract | DPPH scavenging assay (%) | ABTS (IC\textsubscript{50}) (µg/ml) | Total phenol content (mg/ml) | Reducing power (µg/ml) |
|--------|---------------------------|---------------------------|-----------------|-----------------|-----------------|
| 1      | ABR4                      | 35.35±1.19                | 339             | 101±2.64        | 0.96±0.02       |
| 2      | PLPS1                     | 62.82±1.65                | 79              | 117.3±2.08      | 0.87±0.02       |
| 3      | PKKR2                     | 87.46±1.33                | 19.85           | 118.3±4.16      | 0.96±0.01       |
| 4      | PKKS2                     | 89.68±0.31                | 496             | 115±3.60        | 0.91±0.02       |
| 5      | PKS4                      | 91.22±0.36                | 116             | 129.6±1.52      | 0.91±0.009      |
| 6      | GKS                       | 92.60±0.47                | 3.34            | 125.3±1.52      | 0.96±0.02       |
| 7      | PLLS6                     | 78.52±1.14                | 58.51           | 132.6±2.08      | 0.95±0.03       |
| 8      | PKKS1                     | 89.75±1.54                | 248             | 146.3±3.78      | 0.86±0.02       |
| 9      | ABS3                      | 63.63±1.37                | 97.48           | 130.3±2.51      | 0.95±0.02       |
| 10     | ABL1                      | 87.90±1.11                | 283             | 94.0±2.00       | 0.95±0.01       |
| 11     | PKKR3                     | 91.29±1.96                | 567.14          | 116.0±1.00      | 0.95±0.006      |
| 12     | ABLS3                     | 85.10±1.12                | 915             | 144.6±4.16      | 0.92±0.02       |
| 13     | ABS1                      | 85.74±1.64                | 412             | 101.6±2.08      | 0.90±0.01       |
| 14     | ABLS2                     | 73.23±0.69                | 342             | 96.6±3.21       | 0.92±0.01       |
| 15     | PKR1                      | 66.15±2.23                | 164             | 94.6±3.05       | 0.80±0.03       |
| 16     | PKR3                      | 65.65±1.70                | 128             | 76.6±2.08       | 0.78±0.02       |
| 17     | Standard                  | 80.19±1.05*               | 5.54*           | ——              | 0.97±0.01*      |

*Standard: Trolox*/*Gallic acid*

Total phenolic content is expressed in mg Gallic acid equivalent (GAE) g/dw

Each result is expressed as mean ± S.D. (n = 3)

![Fig. 1. DPPH activity of ethyl acetate extracts of selected endophytic fungi](image-url)

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potential of the extracts of *P. nigrum*. Also, PKKR3 from *P. nigrum* depicted inhibitory activity against *S. aureus* only at all tested concentrations but showed no activity against the other bacterial and fungal pathogens. However, little work has been done on the antimicrobial activity of endophytic fungal isolates from *P. nigrum*, *P. longum* and *Z. officinale* necessitating a need for the present study. The study demonstrates that crude extracts of endophytic fungi demonstrate effective antimicrobial activity which indicates the presence of an active principle in high concentrations which results in the biological activities of the fungal endophytes. Also, the isolates which demonstrated low anti-microbial activities may have the active principle in smaller amounts and may depict higher activity when purified. Another possibility can be that these extracts may show antimicrobial activity against other microbes which were not tested in the present study.

Different assays were employed to assess the comparative antioxidant potential of the endophytic fungal extracts. The total phenol content and the antioxidant activity are strongly linked as phenols possess strong scavenging activity for free radicals due to their hydroxyl...
groups and hence the phenolic content of the endophytic fungal extracts may contribute for their antioxidant nature. The total phenol content of the ethyl acetate extract in terms of GAE indicates high antioxidant potential of the extract. In our study, the isolates PKKS1 and ABLS3 presented with highest phenolic content followed by PLLS6, ABS3, PKS4, GKS, PKKR2, PLPS1 and PKKR3 which can be powerful antioxidants. In addition, all endophytic extracts were found to possess significant amount of phenolic content. DPPH assay serves as a quick and reliable factor to assess the antioxidant activity of natural products. In our study, the sample was tested at different concentrations of 10 - 100µg and the readings were observed by a decrease in the absorbance indicating the extent of radical scavenging property in comparison to the standard Trolox. The endophytes GKS and PKS4 (A. terreus) have demonstrated efficient DPPH activity as compared to the standard trolox and be considered as powerful antioxidant agents. All the endophytic fungi have shown antioxidant activity to some extent which is in agreement with the study made by Duan et al. The endophytic extracts were tested for their radical scavenging activity against ABTS which is a reliable test of total antioxidant capacity. The results showed that most endophytic fungi have exhibited good antioxidant capacity especially the isolate GKS (A. terreus) from Z. officinale which has demonstrated strongest antioxidant activity when compared to the standard Gallic acid. This endophytic fungus will be further screened for bioactive compounds. To the best of our knowledge, this is a first report signifying the remarkable antioxidant potential of an endophytic fungal extract of A. terreus from Z. officinale which has demonstrated higher level of reducing potential with increase in concentration. The endophytic fungi have shown high phenolic content with good reducing power as well as DPPH and ABTS scavenging activity as compared to the standard Gallic acid which can be utilized to yield high amount of bioactive components with good antimicrobial and antioxidant activities.

CONCLUSION

Microbial infections pose a significant clinical threat with associated morbidity and mortality due to the development of microbial resistance to the existing antimicrobials. Therefore, the discovery of novel antimicrobial agents from endophytic fungi continues to be developed. The present study demonstrates that the fungal endophytes from medicinal plants produce bioactive compounds which can be both antimicrobial and antioxidant in nature. In our study, the endophyte ABR4 (F. solani) of T. cordifolia showed effective antimicrobial activity to the test microorganisms. Also, the endophytic A. terreus (GKS4) of Z. officinale showed highest antioxidant activity when compared to standard Gallic acid. Further investigations are needed to discover bioactive compounds from these endophytic fungi.

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