



RESEARCH ARTICLE

# Metabolite profiling and bioactivity assessment of diverse endophytic fungi from the endangered plant, *Nilgiranthus ciliatus*

Pragyan Priyadarshini & Suma Sarojini\*

<sup>1</sup>Department of Life Sciences, CHRIST (Deemed to be University) Bangalore 560 029, Karnataka, India

\*Email: [suma@christuniversity.in](mailto:suma@christuniversity.in)



## ARTICLE HISTORY

Received: 27 September 2024

Accepted: 08 February 2025

Available online

Version 1.0 : 06 March 2025



## Additional information

**Peer review:** Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

**Reprints & permissions information** is available at [https://horizonepublishing.com/journals/index.php/PST/open\\_access\\_policy](https://horizonepublishing.com/journals/index.php/PST/open_access_policy)

**Publisher's Note:** Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Indexing:** Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See [https://horizonepublishing.com/journals/index.php/PST/indexing\\_abstracting](https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting)

**Copyright:** © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (<https://creativecommons.org/licenses/by/4.0/>)

## CITE THIS ARTICLE

Priyadarshini P, Sarojini S. Metabolite Profiling and bioactivity Assessment of diverse endophytic Fungi from the endangered plant, *Nilgiranthus ciliatus*. Plant Science Today (Early Access). <https://doi.org/10.14719/pst.5333>

## Abstract

Endophytic fungi are potential sources of bioactive compounds with therapeutic properties. This study investigated the fungal endophytes associated with *Nilgiranthus ciliatus*, an endangered medicinal plant, to discover its secondary metabolites and bioactivities. Molecular analysis revealed the prominent species to be *Aspergillus niger*, *Didymella* sp., *Trichoderma viride*, *Bipolaris zeicola* and *Nigrospora sphaerica*. Alkaloids, flavonoids, phenolics, terpenes and saponins were detected in ethyl acetate extracts employing phytochemical screening. *Didymella* sp. has showed the highest level of antioxidant activity, demonstrating strong DPPH radical scavenging and reduction capability. *T. viride* had strong antibacterial action against *Klebsiella pneumoniae* and *Escherichia coli*, meanwhile *Didymella* sp. and *N. sphaerica* were most effective against *E. coli*. GC-MS analysis uncovered many bioactive chemicals, including trans-farnesol and pentadecanoic acid, which are renowned for their antibacterial and antioxidant properties. These findings highlight the presence of the rich variety of diverse endophytic fungi harboring such medicinal plants, which offer promising applications in medicine, biotechnology and agriculture as sources of novel bioactive compounds. Further exploration and characterization of these strains could unlock valuable sustainable resources for various industries.

## Keywords

antibacterial; antioxidant; didymellanosine; endophytic fungi; *Nilgiranthus ciliatus*

## Introduction

The potential uses of endophytic fungi and their metabolites in industry, agriculture and medicine have drawn a lot of attention to the study of these organisms in recent years. *Nilgiranthus ciliatus*, a fragrant plant from the Western Ghats that is internationally threatened and has numerous uses in Ayurveda, is one such plant of interest. There are many medical uses for Sahacharadi Thailam, a well-known Ayurvedic medication prepared from this plant (1). Despite the increasing focus on the bioactive components of *N. ciliatus*, there remain notable gaps in our understanding of its endophytes and their contributions to the plant's medicinal properties. Recent phytochemical screenings of *N. ciliatus* have identified several bioactive compounds, including stigmasterol, lupeol and betulin, which exhibit promising antioxidant, antibacterial and anticancer activities (2). The significant potential of *N. ciliatus* in the creation of nanoparticles and their prospective uses as an antibiotic and pesticide have been brought to light by recent investigations. When *N. ciliatus*

leaf extracts were used to create selenium nanoparticles (SeNPs), they showed impressive inhibitory effects against a variety of diseases and pests (3). However, the degree to which endophytes contribute to the therapeutic features of *N. ciliatus*, particularly in the context of its specific environmental conditions and genetic makeup, remains largely uncharacterized. This gap in knowledge is crucial, as the interaction between endophytes and their host can considerably influence the accumulation of bioactive chemicals (4).

Numerous studies have demonstrated that endophytic fungi can maintain the health of host plants by competing with or killing different pathogenic bacteria, as well as by producing bioactive secondary metabolites that complement or mimic the activities of their host counterparts (5). Host plants provide endophytic fungi with the nutrients needed for survival and endophytic fungi reciprocate the host plants by promoting their growth, development and evolution in various ways (6). These endophytes are one of the most inventive classes which produce secondary metabolites, many of which play a crucial role in alleviating human diseases. The medicinal property of the host plants is shaped by the host genotype, environment and endophytic fungal genotype affecting the host plant (7).

Endophytes have been found to play a crucial role in the sustainable biosynthesis of pharmaceutically important secondary metabolites as well as playing a vital role in plant growth, health and development in addition to guarding the plants against several biotic and abiotic stresses (8). Endophytic fungi, living within healthy plant tissues and organs, are vital sources of natural bioactive products and new microbial resources (9). They produce a variety of bioactive secondary metabolites, including alkaloids, polysaccharides, polyketones, terpenes, sterols, anthraquinones, flavonoids, xanthines, phenols, furandione and cyclic peptides (10). This study employs a methodical approach to investigate the endophytic population within *N. ciliatus* and its implications to enhance the plant's medicinal value, consequently contributing to the long-term production of bioactive chemicals for various applications. Although the therapeutic potential of *N. ciliatus* is widely documented, the involvement of its endophytic fungus in increasing its therapeutic value through the generation of bioactive metabolites has received little attention. This study aims to provide a thorough knowledge of these interactions, laying the groundwork for future research and applications in medicine, agriculture and biotechnology.

## Materials and Methods

### Field study & collection of samples

*N. ciliatus* is indigenous to the Southern Western Ghats of India and occurs naturally in a temperature range of 20°C to 35°C during the year. In India, it is distributed mostly in the states of Kerala, Karnataka and Tamil Nadu etc. The Transdisciplinary University (TDU) in Bengaluru has conserved this endangered plant through *in vitro* nodal culture. Mature and healthy whole plants of *N. ciliatus* were carefully collected in sterile bags from TDU.

### Methodology for isolation and identification of endophytic fungi

Plant samples collected from TDU were washed and explants (including root, stem, leaf and flower) were surface sterilized by three washes with 70% ethanol for 30-45 s and 2% Sodium hypochlorite (NaOCl) solution for 30 to 60 s. On blot-drying, explants were inoculated on Sabouraud Dextrose Agar (SDA) media augmented with 1mg/ml of ampicillin to inhibit bacterial growth. 100 µl of the last wash water was spread plated onto control SDA plates to check for any contamination. All the culture plates were incubated in the dark at 27°C for 6 days to facilitate the growth and isolation of endophytic fungi (11), which were then subcultured to obtain pure cultures.

### Morphological identification of endophytic fungi

The morphological features were identified by lactophenol cotton blue staining by observing fungal hyphae. The slide culture method was done using Sabouraud dextrose agar (SDA) to facilitate the observation of hyphae and sporangia in the fungal mycelia (12).

### Molecular identification of endophytic fungi

**DNA Isolation and Processing:** DNA was isolated from the pure cultures of all the five endophytes and its quality was evaluated on a 1.0% agarose gel, revealing a single band of high-molecular-weight DNA. The ITS region fragment was then amplified by PCR, producing a single discrete PCR amplicon band of approximately 600 bp when resolved on agarose gel. This PCR amplicon was purified to remove contaminants (13). Forward and reverse DNA sequencing reactions of the PCR amplicon were performed using ITS1 and ITS4 primers with the BDT v3.1 Cycle sequencing kit on an ABI 3730xl Genetic Analyzer. A consensus sequence of the PCR amplicon was generated from the forward and reverse sequence data using aligner software.

### BLAST analysis and phylogenetic construction

The ITS region sequence was then used to perform a BLAST search against the NCBI GenBank database. Based on the maximum identity score, the top ten sequences were selected and aligned using the ClustalW multiple alignment software program. Finally, a distance matrix and phylogenetic tree were constructed using MEGA 10 (14).

### Metabolite isolation via solvent extraction

The fresh mycelia from the pure culture of endophytic fungi were inoculated in Sabouraud dextrose broth and incubated at 25± 2°C in a rotary shaker for 10-15 days. The fungal biomass was separated by filtration through Whatman No. 1 filter paper. Solvent extraction of all five fungal endophytes was performed using a 1:1 solution of ethyl acetate: chloroform and separated using a separating funnel. The crude fungal extracts obtained were then refrigerated at 4°C until they were used for antioxidant and antibacterial assays (15).

### Qualitative tests

Concentrated residues from the ethyl acetate extracts were used for the qualitative detection of secondary metabolites, including alkaloids, flavonoids, saponins, phenols, tannins and terpenoids as per the standard protocols (15).

### Quantitative test for total phenolic content

To determine the total phenolic content, a mixture was prepared by combining 1.8 ml of Folin-Ciocalteu Reagent with 40  $\mu$ l of the sample. This mixture was then diluted with distilled water and allowed to rest at  $25^{\circ}\pm 2^{\circ}$ . After 5 minutes, a 7.5% sodium carbonate solution was added and the mixture was stirred and incubated at  $25^{\circ}\text{C}$  for 30 minutes in the dark. The absorbance was measured at 765 nm using a UV-Vis Spectrophotometer, with gallic acid serving as the calibration standard. The phenolic contents were expressed as milligrams of gallic acid equivalents (GAE) per gram of the sample, following the method described by (16).

### Estimation of total flavonoid content

For the total flavonoid content, the modified Aluminum chloride colorimetric method was used (17). Briefly, 50  $\mu$ l of methanolic extract was mixed with 150  $\mu$ l of 80% methanol, 10  $\mu$ l of 10%  $\text{AlCl}_3$  and 10  $\mu$ l of 1M Sodium acetate. The mixture was then incubated at  $25^{\circ}\pm 2^{\circ}\text{C}$  for 45 minutes and the absorbance was taken at 415 nm.

### Analysis of bioactive compounds using GC-MS chromatogram

The fungal ethyl acetate extract was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) with model, SHIMADZU GCMS-QP2010 SE (Column: Rtx-5ms 30 m  $\times$  0.25 mm I.D., 0.25  $\mu$ m). The instrument was properly connected and powered, with the GC column and inlet system set up. The GCMS solution Ver. 2.6 software was initialized. Samples were prepared according to analysis requirements (1 mg/mL sample diluted in hexane). A method was created in the GCMS solution software, setting parameters such as injection volume, temperature program and ionization mode. Samples were injected into the GC and mass spectra and chromatograms were acquired. The instrument was monitored throughout the run. The acquired data were analyzed using the software, with peaks identified and the compounds quantified and the reports were compared with the library data (18).

### Antioxidant activity assays

The antioxidant potential of the endophytic fungal extracts was analyzed using two distinct assays viz. the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and the reducing power assay. Each experiment was conducted in triplicate and the mean values were determined.

**DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity:** To assess the antioxidant activity of test samples, the change in the optical density of DPPH radicals was assessed. 0.1 mmol DPPH solution in 95% methanol was prepared. The fungal extracts were diluted to a concentration of 1 mg/mL with methanol and 0.1 mmol DPPH solution was made in 95% methanol. Subsequently, 1 mL of the 0.1 mmol DPPH solution was added to the test sample and incubated for 30 minutes at  $25^{\circ}\pm 2^{\circ}\text{C}$  in darkness. After this incubation period, the absorbance was measured at 517 nm (15). The resulting free radical scavenging activity was expressed as a percentage as follows:

Inhibition of DPPH radical (%) =  $\frac{[(\text{control absorbance} - \text{extract absorbance})/(\text{control absorbance})] \times 100}{\text{Eqn. 1}}$

**Reducing power assay:** The reductive potential of the extracts was tested using a modification of the method proposed by Chung et al.2014. In this procedure, different extracts and a standard solution (1 mg/mL) were mixed with methanol (1 mL) containing phosphate buffer (2.5 mL, 0.2 mol/L, pH 6.6) and potassium ferricyanide (2.5 mL, 1% w/v). After centrifugation for 10 minutes at 3000 rpm, the upper layer of the solution (2.5 mL) was combined with distilled water (2.5 mL) and  $\text{FeCl}_3$  (0.5 mL, 0.1% w/v). The absorbance of the resulting mixture was measured at 700 nm using a spectrophotometer. A higher absorbance value indicated greater reductive potential. All experiments were conducted in triplicate and the results were reported as mean  $\pm$  standard deviation (19).

### Screening endophytic fungi for antibacterial properties

The antibacterial properties of endophytic fungi were evaluated against four pathogenic bacteria, including two gram-positive strains [*Enterococcus faecalis* (MTCC No. 439) and *Staphylococcus aureus* (MTCC No. 3160)] and two gram-negative strains [*Escherichia coli* (MTCC No. 443) and *Klebsiella pneumoniae* (MTCC No. 190)] using a slightly modified Agar well diffusion method. Fungal extracts were dissolved in Dimethyl Sulphoxide (DMSO) to prepare stock solutions at a concentration of 5 mg/ml. Overnight grown cultures of bacteria were spread plated on Mueller Hinton Agar medium. Wells of approximately 4 mm diameter were created and filled with varying concentrations of the endophytic fungal extract. Ampicillin and DMSO served as positive and negative controls, respectively. After 24 hours of incubation at  $37^{\circ}\text{C}$ , the results were documented as the zones of inhibition in millimeters (20).

**Determination of Minimum Inhibitory Concentration (MIC):** Extracts demonstrating antimicrobial properties were further used to find out the minimum inhibitory concentration (MIC). This was achieved using the broth dilution method, where ethyl acetate extracts obtained from different endophytic fungi (5 mg/ml) were reconstituted in DMSO. Each dilution was then added into overnight grown bacterial cultures (*K. pneumoniae*, *E. coli*, *S. aureus* and *E. faecalis*) and serially diluted and incubated at  $37^{\circ}\text{C}$  for 24 hours. Bacterial growth was monitored using a spectrophotometer at 620 nm. The MIC value was identified as the lowest concentration at which no turbidity was observed. All measurements were performed in triplicate (21).

**Determination of Minimum Bactericidal concentration (MBC):** From the tubes showing no visible growth (no turbidity), 50  $\mu$ l of the culture was plated onto agar media. The plates were incubated at  $37^{\circ}\text{C}$  for 24 hours. After incubation, colonies on the plates were counted. The Minimum Bactericidal Concentration (MBC) was determined as the lowest concentration of the extract which resulted in a 99.9% reduction in colony-forming units (CFU) compared to the initial inoculum. The ratio of MBC/MIC was calculated to identify where the fungal extract is bacteriostatic or bactericidal.

### Statistical analysis

Statistical analyses were conducted using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test



(DMRT), utilizing SPSS software version 29.0.2.0 for Windows. Results were presented as mean  $\pm$  standard deviation (SD) for total phenol, total flavonoid, DPPH and ferric-reducing antioxidant power (FRAP) assays across all groups. A p-value of  $<0.05$  was considered statistically significant.

## Results

### Isolation and identification of endophytic fungi

In our study, endophytic fungi were isolated from various plant parts of *N. ciliatus* and five of them were studied in detail as they showed significant bioactivity. The colony characteristics of the five fungal strains grown in SDA are depicted in Fig. 1. Molecular characterization via 18S rRNA analysis confirmed their taxonomic identity. The sequences were subsequently submitted to GenBank to obtain the accession numbers for all five fungal endophytes viz. *Aspergillus niger* (PQ187659), *Trichoderma viride* (PQ187660), *Didymella* sp (PQ187661), *Bipolaris zeicola* (PQ187677), *Nigrospora sphaerica* (PQ187680).

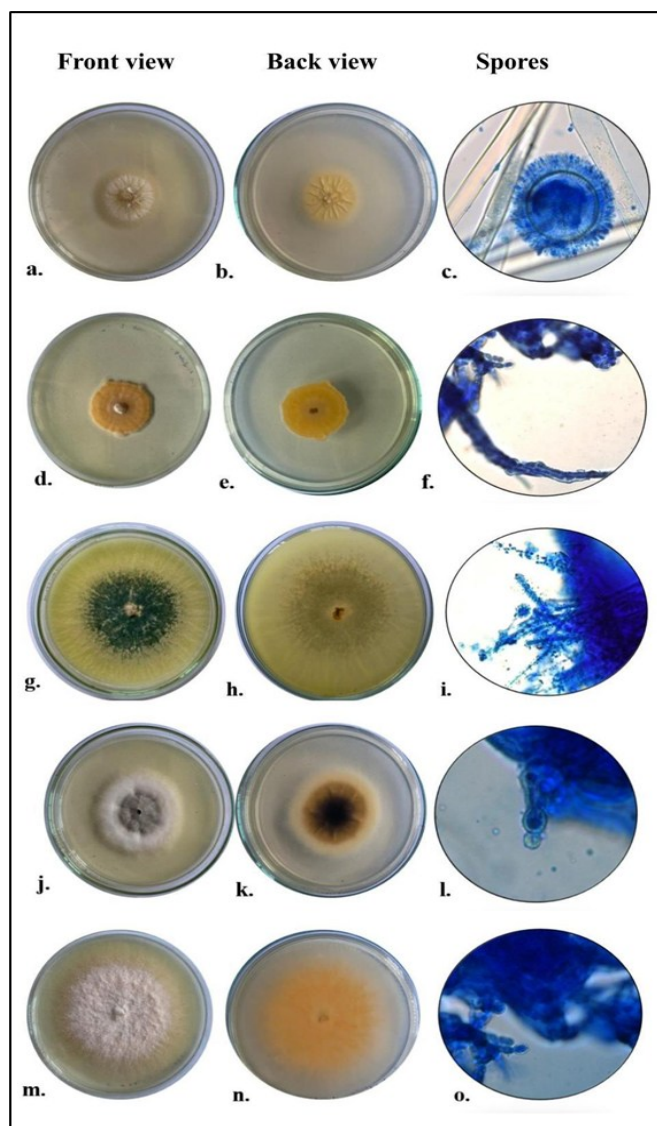
### Phytochemical screening

Phytochemical analysis of the crude fungal extract revealed the presence of alkaloids, phenols, tannins, amino acids, carbohydrates, saponins, terpenes, flavonoids and sterols as described in Table 1. These chemical constituents are responsible for the different biological activities of the extracts. This information additionally highlights the medicinal prospects of these extracts owing to the ample amount of these bioactive compounds.

### Total phenolic and flavonoid content

There was a wide range in the total phenolic concentration in the endophytic fungal extracts. The values varied from 0.684 to 1.139 mg GAE/g of dry weight. The highest concentration of phenols was observed in the extract of *Didymella* sp.  $1.139 \pm 0.02$  followed by *Bipolaris zeicola* and *Nigrospora sphaerica* ( $1.094 \pm 0.02$  and  $1.044 \pm 0.19$ ). *Trichoderma viride* and *Aspergillus niger* showed relatively less concentration of phenols i.e.  $0.928 \pm 0.15$  and  $0.684 \pm 0.02$  respectively.

The Total Flavonoid Content (TFC) of the tested fungal strains was within a range of 0.927 to 1.317 mg Quercetin Equivalents (QE) per gram of dry weight. Among the five strains, the endophytic fungus *Trichoderma viride* exhibited the highest TFC, registering at  $1.317 \pm 0.04$  mg QE/g of dry weight. This was closely followed by *Bipolaris zeicola* and *Didymella* sp., which demonstrated concentrations of  $1.289 \pm 0.05$  and  $1.195 \pm 0.03$  mg QE/g of dry weight, respectively. Meanwhile, *Nigrospora sphaerica* and *Aspergillus niger* displayed moderate flavonoid concentrations, with values reaching up to  $1.012 \pm 0.1$  and  $0.927 \pm 0.07$  mg QE/g of dry weight, respectively. These observations enhance our comprehension of the phenolic and flavonoid production abilities of these fungal strains.



**Fig. 1.** Colony morphology and hyphal features of pure cultures of endophytic fungi isolated from *Nilgiranthus ciliatus* grown in SDA (a-c. *Aspergillus niger*; d-f. *Didymella* sp.; g- i. *Trichoderma viride*; j-l. *Bipolaris zeicola*; m- o. *Nigrospora sphaerica*).

### GC-MS studies and bioactivity analyses of secondary metabolites

All five fungal extracts were analysed using GC-MS to detect important secondary metabolites. The bioactivity of the prominent chemical constituents of the ethyl acetate extracts of the endophytic fungi isolated from *Nilgiranthus ciliatus* are depicted in table 2. *Didymella* sp. produced distinct bioactive substances with antibacterial and anticancer qualities, such as didymellanosine, ascomylactam D and E and derivatives of 3,3-diindolylmethane (DIM). *Trichoderma viride* extract included isosorbide and cholesta-8,24-dien-3-ol, which have antioxidant and anti-inflammatory properties. *Nigrospora sphaerica* produces trans-farnesol, a potent antibacterial and quorum sensing inhibitor. *Bipolaris zeicola* was high in isoaromadendrene epoxide, a compound with significant antifungal properties. *Aspergillus niger* extract comprised

**Table 1.** Screening for secondary metabolites in the ethyl acetate extract of endophytic fungi of *Nilgiranthus ciliatus*

Fungal endophyte	Alkaloids	Flavonoids	Phenols	Saponins	Tannins	Terpenoids
<i>Aspergillus niger</i>	+	+	+	+	-	+
<i>Didymella</i> sp.	+	+	+	-	-	+
<i>Trichoderma viride</i>	+	+	+	-	+	+
<i>Bipolaris zeicola</i>	+	+	+	-	-	+
<i>Nigrospora sphaerica</i> .	-	+	+	+	-	+

**Table 2.** Bioactivity of the prominent chemical constituents of the ethyl acetate extracts of the endophytic fungi isolated from *Nilgiranthus ciliatus*

Compound name	Chemical formula	Bioactivity	References
<b><i>Aspergillus niger</i></b>			
Docosanoic acid, ethyl ester	C <sub>24</sub> H <sub>48</sub> O <sub>2</sub>	Skin barrier repair and anti-inflammatory	(22)
1-Hexacosanol	C <sub>26</sub> H <sub>54</sub> O	Antimicrobial and antioxidant	(23)
Bis(2-ethylhexyl) phthalate	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	Antibacterial and larvicidal	(24)
Gamolenic acid	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	Anti-inflammatory and for dermatological applications	(25)
<b><i>Didymella sp</i></b>			
9,12-Octadecadienoyl chloride	C <sub>18</sub> H <sub>31</sub> ClO	Repellent of mosquitoes and biting midges	(26)
Pseudo sarsasapogenin	C <sub>28</sub> H <sub>44</sub> O <sub>3</sub>	Potential in drug discovery for steroid derivatives	(27)
Pentadecanoic acid	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	Protects cardiometabolic, immune and liver health; activates AMPK; inhibits mTOR	(28)
1-Undecene	C <sub>11</sub> H <sub>22</sub>	Inhibits mycelial growth of mushrooms, antimicrobial activity	(26)
9-Octadecenal	C <sub>18</sub> H <sub>34</sub> O	Antioxidant properties	(29)
Heptasiloxane	C <sub>16</sub> H <sub>48</sub> O <sub>6</sub> Si <sub>7</sub>	Larvicidal activity against mosquitoes	(30)
<b><i>Trichoderma viride</i></b>			
Oleic Acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	Anti-inflammatory, antioxidant and potential therapeutic agent for various conditions	(31)
2,5-Hexanediol	C <sub>6</sub> H <sub>14</sub> O <sub>2</sub>	Suppresses chromatin motion and hyper-condenses chromatin in human cells	(32)
9-Octadecenal	C <sub>18</sub> H <sub>34</sub> O	Antioxidant	(29)
Trans-Z.-alpha.-Bisabolene epoxide	C <sub>15</sub> H <sub>24</sub> O	Potential use in agriculture as a biologically active natural product	(33)
<b><i>Bipolaris zeicola</i></b>			
2-tert-Butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxy benzyl)phenol	C <sub>40</sub> H <sub>58</sub> O <sub>3</sub>	Antioxidant	(34)
Malonic acid, dihydroxy-, diisobutyl ester	C <sub>11</sub> H <sub>20</sub> O <sub>6</sub>	Competitive inhibitor of succinate dehydrogenase	(35)
Threitol, 2-O-heptyl-	C <sub>11</sub> H <sub>24</sub> O <sub>4</sub>	Fungal signal promoting colonization	(36)
Sorbitol	C <sub>6</sub> H <sub>14</sub> O <sub>6</sub>	Laxative, diuretic	(37)
Cyclooctyne	C <sub>8</sub> H <sub>12</sub>	Bioorthogonal reagent	(38)
<b><i>Nigrospora sphaerica</i></b>			
trans-farnesol	C <sub>15</sub> H <sub>26</sub> O	Antimicrobial	(39)
Oleic Acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	Anti-inflammatory, anticancer	(31)
Nonadecanoic acid	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	Antibacterial	(40)
1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	Chemopreventive	(41)
n-capric acid isopropyl ester	C <sub>13</sub> H <sub>26</sub> O <sub>2</sub>	Antimicrobial	(42)

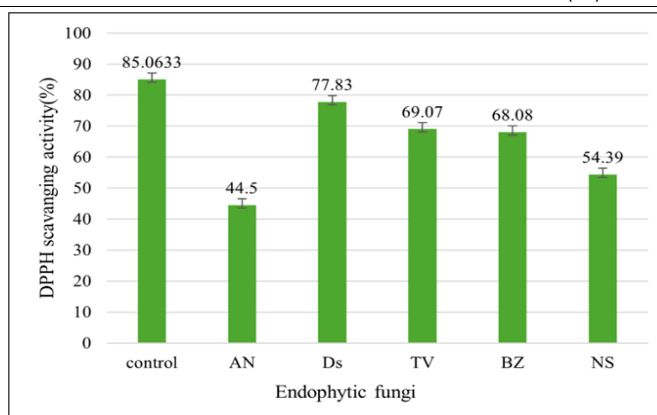
bioactive lipids such gamolenic acid and E-11-hexadecenoic acid ethyl ester. These findings emphasise the numerous and pharmacologically relevant metabolites found in *N. ciliatus* endophytic fungus, emphasising their potential as sources of new therapeutics.

### Antioxidant activity

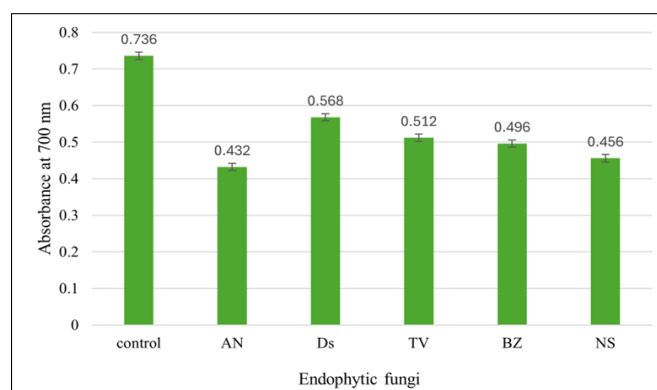
The antioxidant activity of the five fungal extracts were evaluated using two methods, yielding results ranging from 40% to 80%. *Didymella sp.* exhibited the highest antioxidant activity, with robust DPPH radical scavenging and strong reducing properties. Notably, extracts with higher antioxidant activity have higher total phenolic content, as measured by the Folin-Ciocalteu assay.

**DPPH radical scavenging activity:** DPPH radicals are stable, nitrogen-centered free radicals that produce a violet color in ethanol solution. When a substrate capable of donating a hydrogen atom is added to the DPPH solution, it is reduced to a yellow-colored product known as diphenylpicryl hydrazine. In this assay, the antioxidant nature of fungal extracts was evident by a color transition from purple to yellow. Among the samples tested, *Didymella sp* showed the highest activity of 77.83±1.2% and *B. zeicola* and *T. viride* exhibited robust antioxidant capacities of 68.08 ±1 and 69.07 ±0.55 respectively. These results highlight the varying antioxidant potential of these samples as shown in fig. 2.

**Reducing power assay:** In the reducing power assay, the ability of compounds in the fungal extracts, to reduce Fe<sup>3+</sup> to Fe<sup>2+</sup> was checked (Fig.3). *T. viride* and *Didymella sp* exhibited higher absorbance values, indicating their strong reductive potential and electron-donating capacity for neutralizing free radicals. In



**Fig. 2.** DPPH radical scavenging activity of *Nilgiranthus ciliatus* endophytic fungal ethyl acetate extracts (AN- *Aspergillus niger*, Ds- *Didymella sp*, TV- *Trichoderma viride*, BZ- *Bipolaris zeicola*, NS- *Nigrospora sphaerica*).



**Fig. 3.** Ferric-reducing antioxidant power (FRAP) activity of ethyl acetate extracts of the endophytic fungi of *Nilgiranthus ciliatus* (AN- *Aspergillus niger*, Ds - *Didymella sp*, TV - *Trichoderma viride*, BZ - *Bipolaris zeicola*, NS - *Nigrospora sphaerica*).

contrast, *B. zeicola* and *N. sphaerica* demonstrated moderate reducing activity, while *A. niger* exhibited the least activity among all the fungal extracts. These measurements were performed in triplicates.

### Screening endophytic fungi for antibacterial properties

When the endophytic fungal isolate extracts were checked for their antibacterial activity against two Gram positive bacteria - [*Enterococcus faecalis* (MTCC439) and *Staphylococcus aureus* (MTCC3160)] and two gram-negative bacteria [*Escherichia coli* (MTCC 443) and *Klebsiella pneumoniae* (MTCC 190)], *Didymella* sp. exhibited the highest inhibition (17 mm) against *E. coli*, followed by *Trichoderma viride* (14 mm) against *K. pneumoniae* as shown in Table 3. Minimum Inhibitory Concentration (MIC) values of the ethyl acetate extracts of the endophytic fungi isolated from *Nilgiranthus ciliatus* are depicted in table 4. The MIC values against *K. pneumoniae* ranged from 0.06 to 0.5 mg/ml, with *Bipolaris zeicola* having the lowest MIC (62.5 µg/ml), indicating resilient potency. *T. viride* had a MIC of 0.25 mg/ml for both *K. pneumoniae* and *E. coli*, whereas *Didymella* sp. and *Nigrospora sphaerica* had a MIC of 0.125 mg/ml for *E. coli*. Colony counts revealed greater resistance against *K. pneumoniae* and *E. coli*, implying that the extracts were more effective against Gram-negative bacteria. The MBC/MIC ratio for all extracts was less than 4, demonstrating their bactericidal properties.

## Discussion

*Nilgiranthus ciliatus* is a shrub native to the Western Ghats with a lot of medicinal properties attributed to it, especially in curing diseases of the nervous and muscular systems. Since it depicts a plethora of bioactivity, it could be harboring a lot of microbial endophytes, which may play a role in imparting these properties. Hence the present study was intended to isolate and analyze the fungal endophytic flora colonizing this much sought-after medicinal plant.

After the isolation and initial screening of *N. ciliatus* for bioactive fungal endophytes, five were chosen for further studies. The highest concentration of phenolics was seen in the extract of *Didymella* sp., followed by *B. zeicola* and *N. sphaerica*. The results obtained from our research align with recent studies that have reported a high amount of phenolic

content in certain groups of endophytic fungi. Interestingly, *T. viride*, which exhibited a moderate concentration of phenols, showed the highest TFC. This specifies that the biosynthesis of phenolic compounds and flavonoids may not be directly correlated. Different fungal strains may contain distinct metabolic pathways, benefitting the production of one type of compound over the other (43). Current research has shown that endophytic fungi are a significant source of natural products with remarkable bioactivities. The secondary metabolites of these fungi have been found to contain numerous antioxidant molecules (9) (44).

*B. zeicola* and *Didymella* sp. showed elevated levels of flavonoids, indicating their promise as suppliers of these important substances. There have been reports of a variety of plant pathogenic and saprobic species associated with a wide range of hosts in *Didymella* sp. (45). This aligns with recent research that has found multiple antioxidant compounds, such as flavonoids, in the secondary metabolites of endophytic fungi (46). *Aspergillus niger* showed moderate levels of phenols and flavonoids. This supports prior studies which have found a large variety of bioactive compounds in *A. niger* (47). The examination of bioactive compounds in endophytic fungi demonstrates a wide variety of substances such as alkaloids, phenols, tannins, amino acids, carbohydrates, saponins, terpenes, flavonoids and sterols (43). *A. niger* is recognized for synthesizing various secondary metabolites that have been linked to diverse biological functions (47). On the other hand, *Trichoderma viride* is famous for its biocontrol abilities (44). The findings of our report align with these previous studies, suggesting the potential of *A. niger*, *Didymella* sp. and *T. viride* as sources of bioactive compounds. Our report's results are in agreement with prior studies, indicating that *A. niger*, *Didymella* sp. and *T. viride* have the potential to be used as sources of bioactive compounds. Positive outcomes were demonstrated for the existence of alkaloids, phenols, tannins, amino acids, carbohydrates, saponins, terpenes, flavonoids and sterols. This highlights the need for more research and understanding of these fungal strains for their possible uses in multiple sectors such as pharmaceuticals, agriculture and food.

Research into the antioxidant properties of the five fungal extracts, using two different methods, revealed a range of 40

**Table 3.** Antibacterial activity of ethyl acetate extracts of the endophytic fungi of *Nilgiranthus ciliatus*

Endophytic fungi	Zone of inhibition (in mm)					Ampicillin	DMSO
	<i>E. faecalis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>			
<i>Aspergillus niger</i>	10	10	12	11		19	-
<i>Didymella</i> sp.	11	13	17	10		18	-
<i>Trichoderma viride</i>	9	14	11	12		19	-
<i>Bipolaris zeicola</i>	11	15	13	12		19	-
<i>Nigrospora sphaerica</i>	9	10	11	10		17	-

**Table 4.** Minimum Inhibitory Concentration (MIC) of the ethyl acetate extracts of the endophytic fungi isolated from *Nilgiranthus ciliatus*

Endophytic fungi	Minimum Inhibitory Concentration (MIC) (mg/mL) of extract							
	<i>E. faecalis</i>		<i>S. aureus</i>		<i>E. coli</i>		<i>K. pneumoniae</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>Aspergillus niger</i>	0.25	0.5	0.25	1	0.25	0.5	0.25	0.5
<i>Didymella</i> sp.	0.25	0.5	0.125	0.25	0.125	0.25	0.5	1
<i>Trichoderma viride</i>	0.5	1	0.5	1	0.25	0.5	0.25	0.5
<i>Bipolaris zeicola</i>	0.25	0.5	0.12	0.25	0.25	0.5	0.0625	0.125
<i>Nigrospora sphaerica</i>	0.5	1	0.25	1.0	0.125	0.25	0.25	0.5



to 80%. This aligns with recent studies finding multiple antioxidant molecules in the secondary metabolites of endophytic fungi (48). Moreover, it was noted that the fungal extracts with antioxidant properties also had a significant amount of overall phenols. Our research indicates that *B. zeicola* displays powerful antioxidant and antibacterial properties; important bioactive compounds discovered were isoaromadendrene epoxide and 9-octadecen-12-ynoic acid methyl ester. The findings align with recent studies on various *Bipolaris* species, which are recognized for their wide range of biological effects. Analyzing the mitochondrial genome of *B. zeicola* improves our knowledge of its genetic characteristics. Furthermore, substances such as 2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxy benzyl)phenol, isoaromadendrene epoxide, 9-octadecen-12-ynoic acid methyl ester and hexahydrofuro[3,2-b]furan-3,6-diol were detected. These possess antioxidant, anti-inflammatory, antimicrobial, anticancer and neuroprotective qualities, showcasing their potential for therapy (49). This was established utilizing a test reliant on the Folin-Ciocalteu reagent. The alteration in correlation hints at a possible link between the phenolic content and antioxidant activity of the extracts (50). *Didymella* sp. showed the highest DPPH radical scavenging activity of  $77.83 \pm 1.2\%$ ; its GC-MS analysis identified compounds such as didymellanosine and ascomylactam D and E. Didymellanosine, isolated from *Didymella* sp. IEA-3B.1 showed strong activity against lymphoma and leukemia cell lines (45). This suggests that *Didymella* sp. has a high capacity for neutralizing free radicals (51). *Nigrospora sphaerica* showed moderate levels of antioxidants and had important antibacterial effects. Compounds such as trans-farnesol and oleic acid were identified through GC-MS analysis. Prior research has shown the antimicrobial capabilities of *N. sphaerica*, including bioactive substances like phomalactone which have a wide-ranging antimicrobial effect. Furthermore, *N. sphaerica* found in *Dillenia indica* exhibits notable antioxidant and antimicrobial characteristics (52).

During the reducing power test, *T. viride* and *Didymella* sp. both showed significant ability to reduce and donate electrons to counteract free radicals. Specifically, *T. viride* has been found to possess notable antioxidant characteristics. *Trichoderma* is a group of fungi that has a variety of uses in farming. In 2022, a review emphasized the characteristics and functions of secondary metabolites found in *T. harzianum*. This species is recognized for its production of diverse bioactive secondary metabolites, which are valuable for developing novel herbicides and antibiotics (53). Multicomponent microbial inoculants with *Trichoderma* were discovered to help control pathogens and promote plant growth (54). These studies indicate that *Trichoderma* species not only efficiently manage plant pathogens but also positively influence seed germination, seedling emergence and overall plant growth. In our research, *T. viride* displayed a 14mm zone of inhibition against *K. pneumoniae* and a MIC of  $0.25 \text{ mg.ml}^{-1}$  against both *K. pneumoniae* and *E. coli*, highlighting its antibacterial effectiveness. Another endophytic fungus, *Didymella* sp., showed antimicrobial properties. Previous research on this endophytic fungus derived from the medicinal plant *Zanthoxylum simulans* showed that *Didymella* was among the six significant fungal

genera with antimicrobial properties (55). Our research found that the ethyl acetate extract of *Didymella* sp. demonstrated a significant 17 mm zone of inhibition against *E. coli* and an MIC of  $0.125 \text{ mg.ml}^{-1}$  against *E. coli* and *S. aureus*, suggesting its promise as a supplier of antibacterial substances. *A. niger* is a famous type of fungus that has been researched for its capability to create bioactive substances. In 2023, a research paper identified six substances from *A. niger*, with some displaying potential as antibiotics against *Staphylococcus aureus* and *Bacillus subtilis* (18). Another study in 2024 reported the antioxidant and antibacterial activities of endophytic fungi isolated from the roots of *Taxus wallichiana*, including *Aspergillus* sp. (56). In our study, *Aspergillus niger* showed the least activity towards almost all the strains at  $0.5 \text{ mg.ml}^{-1}$  concentration, suggesting that its antibacterial potential may vary depending on the specific strain and extraction method used.

The present study could thus reveal the endophyte richness of the endangered plant *Nilgiranthus ciliatus*. The five prominent endophytic fungi viz. *Trichoderma viride*, *Didymella* sp., *Bipolaris zeicola*, *Nigrospora sphaerica* and *Aspergillus niger*, isolated from this plant, has multiple bioactivities including the demonstrated antioxidant and antibacterial activities. These findings emphasize the importance of exploring endophytic fungi as a source of new antimicrobial compounds.

## Conclusion

Our investigation on the diverse endophytic fungi isolated from *N. ciliatus* has demonstrated substantial bioactive potential with *Didymella* sp. exhibiting the highest phenolic content and DPPH radical scavenging activity and *Trichoderma viride* showing the highest total flavonoid content and significant reducing power. The results align with recent research on similar endophytes, highlighting endophytic fungi as promising sources of novel bioactive compounds. Since, *N. ciliatus* from which these endophytes were isolated has been implicated in multidimensional roles in curing diseases of the neuromuscular system, more detailed studies on the effect of these fungal metabolites on the proteins involved in these diseases should be analyzed in future. A comprehensive characterization of these bioactive compounds and elucidation of their modes of action will throw more light on ways to make use of these bioresources. Additionally, understanding the biotechnological applications of these endophytic fungi, such as the development of novel antimicrobial agents and natural antioxidants, could be a significant advancement in both medical and agricultural fields.

## Acknowledgements

The authors greatly acknowledge the Department of Life Sciences for the infrastructural facilities provided for the study and the Centre for Research, CHRIST (Deemed to be University), Bangalore, for the seed money grant.

## Authors' contributions

SS conceptualized the study and PP performed the isolation and characterization, including bioactivity studies. SS and PP wrote and edited the manuscript.

## Compliance with ethical standards

**Conflict of interest:** The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this research article.

**Ethical issues:** None

## References

- Rameshkumar R, Largia MJV, Satish L, Shilpha J, Ramesh M. *In vitro* mass propagation and conservation of Nilgiranthus ciliatus through nodal explants: A globally endangered, high trade medicinal plant of Western Ghats. *Plant Biosys.* 2017;151(2):204–11. <https://doi.org/10.1080/11263504.2016.1149120>
- Kavitha K, Sujatha K, Manoharan S, Ramaprabhu SR. Preliminary phytochemical analysis and in-vitro hypoglycemic potential of Nilgiranthus ciliatus Nees. *Int J Pharma Sci Res.* 2015;6(8):3299–305.
- Meenambigai K, Kokila R, Chandhirasekar K, Thendralmanikandan A, Kaliannan D, Ibrahim KS, et al. Green synthesis of Selenium nanoparticles mediated by Nilgiranthus ciliatus leaf extracts for antimicrobial activity on foodborne pathogenic microbes and pesticidal activity against *Aedes aegypti* with molecular docking. *Biol Trace Elem Res.* 2022;200(6):2948–62. <https://doi.org/10.1007/s12011-021-02868-y>
- Srinivasan M, Padmaja B, Nair SC. Phytochemical identification of Nilgiranthus ciliatus by GC-MS analysis and its DNA protective effect in cultured lymphocytes. *As J Biomed Pharmed Sci.* 2013;3:14–17.
- Sivakumar B, Rao NR, Poornamath BP, Jayaram S, Sarojini S. Multifarious pigment producing fungi of Western Ghats and their potential. *Plant Sci Today.* 2022;9(3):733–47. <https://doi.org/10.14719/pst.1759>
- Biswas S, Sarojini S. Fungal endophytic species *Fusarium annulatum* and *Fusarium solani*: identification, molecular characterization and study of plant growth promotion properties. *Plant Sci Today.* 2024;11(1):466–72. <https://doi.org/10.14719/pst.2688>
- Anand K, Kumar V, Prasher IB, Sethi M, Raj H, Ranjan H, et al. Bioactive molecules from fungal endophytes and their applications in pharmaceutical industries: challenges and future scope. *J Basic Microbiol.* 2023;63(7):690–708. <https://doi.org/10.1002/jobm.202200696>
- Kumari P, Deepa N, Trivedi PK, Singh BK, Srivastava V, Singh A. Plants and endophytes interaction: a secret wedlock for sustainable biosynthesis of pharmaceutically important secondary metabolites. *Microb Cell Fact.* 2023;22(1):226. <https://doi.org/10.1186/s12934-023-02234-8>
- Biswas S, Philip I, Jayaram S, Sarojini S. Endophytic bacteria *Klebsiella* spp. and *Bacillus* spp. from *Alternanthera philoxeroides* in Madiwala Lake exhibit additive plant growth-promoting and biocontrol activities. *J Genet Eng Biotechnol.* 2023;21(1):153. <https://doi.org/10.1186/s43141-023-00620-8>
- Yu L, Du F, Chen X, Zheng Y, Morton M, Liu F, et al. Identification of the biosynthetic gene cluster for the anti-mrsa lysocins through gene cluster activation using strong promoters of housekeeping genes and production of new analogs in *Lysobacter* sp. 3655. *ACS Synth Biol.* 2020;9(8):1989–97. <https://doi.org/10.1021/acssynbio.0c00067>
- Qiu M, Xie RS, Shi Y, Zhang H, Chen HM. Isolation and identification of two flavonoid-producing endophytic fungi from *Ginkgo biloba* L. *Ann Microbiol.* 2010;60(1):143–50. <https://doi.org/10.1007/s13213-010-0016-5>
- Lu Y, Chen C, Chen H, Zhang J, Chen W. Isolation and identification of endophytic fungi from *Actinidia macrocarpa* and investigation of their bioactivities. *Evid Based Complement Alternat Med.* 2012;2012:382742. <https://doi.org/10.1155/2012/382742>
- Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol.* 1980;16(2):111–20. <https://doi.org/10.1007/BF01731581>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol.* 2018;35(6):1547–49. <https://doi.org/10.1093/molbev/msy096>
- Yadav M, Yadav A, Yadav JP. *In vitro* antioxidant activity and total phenolic content of endophytic fungi isolated from *Eugenia jambolana* Lam. *Asian Pac J Trop Med.* 2014;7S1:S256–61. [https://doi.org/10.1016/S1995-7645\(14\)60242-X](https://doi.org/10.1016/S1995-7645(14)60242-X)
- Murray MJ. Total phenols in gasoline and in cresylic acids. *Anal Chem.* 1949;21(8):941–45. <https://doi.org/10.1021/ac60032a016>
- Sembiring EN, Elya B, Sauriasari R. Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of *Caesalpinia bonduc* (L.) Roxb. *Pharmacogn J.* 2017;10(1):123–27. <https://doi.org/10.5530/pj.2018.1.22>
- Bora P, Devi NN. Exploration of the chemical constituents and its antioxidant, antibacterial activities of endophytic fungi isolated from the medicinal plant *Dillenia indica*. *Arch Microbiol.* 2023;205(2):67. <https://doi.org/10.1007/s00203-023-03407-0>
- Chung IM, Ali M, Praveen N, Yu BR, Kim SH, Ahmad A. New polyglucopyranosyl and polyarabinopyranosyl of fatty acid derivatives from the fruits of *Lycium chinense* and its antioxidant activity. *Food Chem.* 2014;151:435–43. <https://doi.org/10.1016/j.foodchem.2013.11.061>
- Mitscher LA, Park YH, Clark D, Clark GW 3rd, Hammesfahr PD, Wu WN, et al. Antimicrobial agents from higher plants. An investigation of *Hunnemannia fumariaefolia* pseudoalcohols of sanguinarine and chelerythrine. *Lloydia.* 1978;41(2):145–50.
- Rojas R, Bustamante B, Bauer J, Fernández I, Albán J, Lock O. Antimicrobial activity of selected peruvian medicinal plants. *J Ethnopharmacol.* 2003;88(2-3):199–204. [https://doi.org/10.1016/S0378-8741\(03\)00212-5](https://doi.org/10.1016/S0378-8741(03)00212-5)
- Moore EM, Wagner C, Komarnytsky S. The enigma of bioactivity and toxicity of botanical oils for skin care. *Front Pharmacol.* 2020;11:785. <https://doi.org/10.3389/fphar.2020.00785>
- Teixeira B, Marques A, Ramos C, Serrano C, Matos O, Neng NR, et al. Chemical composition and bioactivity of different oregano (*Origanum vulgare*) extracts and essential oil. *J Sci Food Agric.* 2013;93(11):2707–14. <https://doi.org/10.1002/jsfa.6089>
- Javed MR, Salman M, Tariq A, Tawab A, Zahoor MK, Naheed S, et al. The antibacterial and larvicidal potential of Bis-(2-ethylhexyl) phthalate from *Lactiplantibacillus plantarum*. *Molecules.* 2021;27:220. <https://doi.org/10.3390/molecules27212200>
- Avato P, Tava A. Rare fatty acids and lipids in plant oilseeds: occurrence and bioactivity. *Phytochem Rev.* 2022;21(2):401–28. <https://doi.org/10.1007/s11101-021-09770-4>
- Lo Cantore P, Giorgio A, Iacobellis NS. Bioactivity of volatile organic compounds produced by *Pseudomonas tolaasii*. *Front Microbiol.* 2015;6:1082. <https://doi.org/10.3389/fmicb.2015.01082>
- García-Merinos JP, Yépez R, Ramírez-Lozano CM, Rincón S, Ochoa ME, López Y, et al. Smilagenin transformation products under



- lewis acid catalysis in acetic anhydride and synthesis of 23-Acetyl-Spirostanols. *Nat Prod Commun.* 2023;18(11):1934578X231212341.
28. Venn-Watson S, Schork NJ. Pentadecanoic Acid (C15:0), an Essential fatty acid, shares clinically relevant cell-based activities with leading longevity-enhancing compounds. *Nutrients.* 2023;15(21):4607. <https://doi.org/10.3390/nu15214607>
  29. Yakubu OE, Boyi RHN, Shaibu C, Abah MA, Akighir J. Antioxidant parameters and GC-MS phytochemical analysis of *Hymenocardia acida* stem bark ethanolic extract. *Trends Appl Sci Res.* 2019;14(4):263–70. <https://doi.org/10.3923/tasr.2019.263.270>
  30. Vasumathi D, Senguttuvan S. The major phyto-compounds heptasiloxane, hexadecamethyl- and 1,1-dimethylethyl 3-phenyl-2-propenoate derived from *Indigofera tinctoria* medicinal flora tested against various target medical and agronomic pests. *Indian J Sci Technol.* 2023;16(16):1178–86. <https://doi.org/10.17485/IJST/v16i16.310>
  31. Tanvir R, Javeed A, Rehman Y. Fatty acids and their amide derivatives from endophytes: new therapeutic possibilities from a hidden source. *FEMS Microbiol Lett.* 2018;365(12): fny114 [S1]. <https://doi.org/10.1093/femsle/fny114>
  32. Itoh Y, Iida S, Tamura S, Nagashima R, Shiraki K, Goto T, et al. 1,6-hexanediol rapidly immobilizes and condenses chromatin in living human cells. *Life Sci Alliance.* 2021;4(4): e202001005 [S2]. <https://doi.org/10.26508/lisa.202001005>
  33. Cutler HG. Biologically active natural products: potential use in agriculture. New York: American Chemical Society; 1988. 483 p. <https://doi.org/10.1021/bk-1988-0380>
  34. Khatib FN, Wilberforce T, Ijaodola O, Ogungbemi E, El-Hassan Z, Durrant A, et al. Material degradation of components in polymer electrolyte membrane (PEM) electrolytic cell and mitigation mechanisms: A review. *Renew Sustain Ener Rev.* 2019;111:1–14. <https://doi.org/10.1016/j.rser.2019.05.007>
  35. Adir N, Bar-Zvi S, Harris D. The amazing phycobilisome. *Biochim Biophys Acta Bioenerg.* 2020;1861(4):148047. <https://doi.org/10.1016/j.bbambio.2019.07.002>
  36. Wong JWH, Plett KL, Natera SHA, Roessner U, Anderson IC, Plett JM. Comparative metabolomics implicates threitol as a fungal signal supporting colonization of *Armillaria luteobubalina* on eucalypt roots. *Plant Cell Environ.* 2020;43(2):374–86. <https://doi.org/10.1111/pce.13672>
  37. González-Vázquez P, Larrañeta E, McCrudden MTC, Jarrahan C, Rein-Weston A, Quintanar-Solares M, et al. Transdermal delivery of gentamicin using dissolving microneedle arrays for potential treatment of neonatal sepsis. *J Control Release.* 2017;265:30–40. <https://doi.org/10.1016/j.jconrel.2017.07.032>
  38. Fang Y, Hillman AS, Fox JM. Advances in the synthesis of bioorthogonal reagents: s-tetrazines, 1,2,4-triazines, cyclooctynes, heterocycloheptynes and trans-cyclooctenes. *Top Curr Chem.* 2024;382(2):15. <https://doi.org/10.1007/s41061-024-00455-y>
  39. de Castilho ARF, Rosalen PL, de Souza Araújo IJ, Kitagawa IL, Costa CAG de A, Janal MN, et al. Trans, trans-farnesol, an antimicrobial natural compound, improves glass ionomer cement properties. *PLoS One.* 2019;14(8):e0220718. <https://doi.org/10.1371/journal.pone.0220718>
  40. Ye K, Ai HL, Liu JK. Identification and bioactivities of secondary metabolites derived from endophytic fungi isolated from ethnomedicinal plants of tujia in hubei province: a review. *Nat Products Bioprospect.* 2021;11(2):185–205. <https://doi.org/10.1007/s13659-020-00295-5>
  41. Kumar A, Kaur S, Dhiman S, Singh PP, Bhatia G, Thakur S, et al. Targeting akt/nf-kb/p53 pathway and apoptosis inducing potential of 1, 2-benzenedicarboxylic acid, bis (2-methyl propyl) ester isolated from *onosma bracteata* wall. against human osteosarcoma (mg-63) cells. *Molecules.* 2022;27(11):3478. <https://doi.org/10.3390/molecules27113478>
  42. Mohan B. Identification of phytoconstituents in ethanolic and aqua-ethanolic extracts of *Solanum indicum* L. through GC-MS. *Chem Sci Rev Lett.* 2020;9:693–99. <https://doi.org/10.22271/tpi.2020.v9.i7g.4973>
  43. Asogwa IS, Ikechukwu, Onah A, Agbo AC, Ibezim EC, Attama AA. Molecular characterization and evaluation of phytochemical constituents of endophytic fungi derived from *Mitracarpus scaber* Zucc. (Rubiaceae). *Int J Sci Res Arch.* 2024;11(2):267–78. <https://doi.org/10.30574/ijrsra.2024.11.2.0376>
  44. Jayaram S, Biswas S, Philip I, Umesh M, Sarojini S. Differential laccase production among diverse fungal endophytes in aquatic plants of Hulimavu Lake in Bangalore, India. *J Pure Appl Microbiol.* 2023;17(1):298–308. <https://doi.org/10.22207/JPAM.17.1.19>
  45. Ariantari NP, Ancheeva E, Frank M, Stuhldreier F, Meier D, Gröner Y, et al. Didymellanosine, a new decahydrofluorene analogue and ascolactone C from *Didymella* sp. IEA-3B.1, an endophyte of *Terminalia catappa*. *RSC Adv.* 2020;10(12):7232–40. <https://doi.org/10.1039/C9RA10685E>
  46. Luo X, Hu Y, Xia J, Zhang K, Ma L, Xu Z, et al. Morphological and phylogenetic analyses reveal three new species of *Didymella* (Didymellaceae, Pleosporales) from Jiangxi, China. *J Fungi.* 2024;10(1):75. <https://doi.org/10.3390/jof10010075>
  47. Šimonovičová A, Vojtková H, Nosalj S, Piecková E, Švehlákova H, Kraková L, et al. *Aspergillus niger* Environmental isolates and their specific diversity through metabolite profiling. *Front Microbiol.* 2021;12:658010. <https://doi.org/10.3389/fmicb.2021.658010>
  48. Gulcin İ, Alwasel SH. DPPH radical scavenging assay. *Processes.* 2023;11(8):2248. <https://doi.org/10.3390/pr11082248>
  49. Khan NA, Asaf S, Ahmad W, Jan R, Bilal S, Khan I, et al. Diversity, lifestyle, genomics and their functional role of *Cochliobolus*, *Bipolaris* and *Curvularia* species in environmental remediation and plant growth promotion under biotic and abiotic stressors. *J Fungi.* 2023;9(2):3390. <https://doi.org/10.3390/jof9020254>
  50. Kumar S, Sandhir R, Ojha S. Evaluation of antioxidant activity and total phenol in different varieties of *Lantana camara* leaves. *BMC Res Notes.* 2014;7:560. <https://doi.org/10.1186/1756-0500-7-560>
  51. Vu THN, Pham NS, Le PC, Pham QA, Quach NT, Nguyen, et al. Distribution, cytotoxicity and antioxidant activity of fungal endophytes isolated from *Tsuga chinensis* (Franch.) Pritz. in Ha Giang province, Vietnam. *Ann Microbiol.* 2022;72(1):36. <https://doi.org/10.1186/s13213-022-01693-5>
  52. Kumar V, Prasher IB. Phytochemical analysis and antimicrobial potential of *Nigrospora sphaerica* (Berk. & Broome) Petch, a fungal endophyte isolated from *Dillenia indica* L. *Adv Trad Med.* 2023;23(2):525–37.
  53. Guo R, Li G, Zhang Z, Peng X. Structures and biological activities of secondary metabolites from *Trichoderma harzianum*. *Mar Drugs.* 2022;20(11):701. <https://doi.org/10.3390/md20110701>
  54. Kredics L, Büchner R, Balázs D, Allaga H, Kedves O, Racić G, et al. Recent advances in the use of *Trichoderma*-containing multicomponent microbial inoculants for pathogen control and plant growth promotion. *World J Microbiol Biotechnol.* 2024;40(5):162. <https://doi.org/10.1007/s11274-024-03965-5>
  55. Kuo J, Chang CF, Chi WC. Isolation of endophytic fungi with antimicrobial activity from medicinal plant *Zanthoxylum simulans* Hance. *Folia Microbiol.* 2021;66(3):385–97. <https://doi.org/10.1007/s12223-021-00854-4>
  56. Adhikari P, Pandey A. Phytochemicals and antioxidants and antibacterial activities of *Taxus wallichiana* Zucc. root associated endophytic fungi. *Biologia.* 2024;79(6):1895–907. <https://doi.org/10.1007/s11756-024-01650-4>