MOLEKUL
eISSN: 2503-0310

Articles
https://doi.org/10.20884/1.jm.2025.20.3.16748

Colletotrichum sp. from Nutmeg Leaves Shows Antiproliferative Activity Against MCF-7 Cancer Cells: *In Vitro* Evaluation, Metabolite Profiling, and Docking Analysis

Siti Aisyah[1], Suryati[1]* Agus Budiawan Naro Putra[2], Kartika Dyah Palupi[2], Dwinna Rahmi[2], Muhammad Ilyas[3], Andria Agusta[2]**

[1]Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Andalas, Kampus Limau Manis, Padang 25163, Indonesia.
[2]Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN), Cibinong, 16911, Indonesia.
[3]Research Center for Biosystematics and Evolution, National Research and Innovation Agency (BRIN), Cibinong, 16911, Indonesia.

*Corresponding author email: suryati@sci.unand.ac.id
**Corresponding author email: agus135@brin.go.id

Received June 29, 2025; **Accepted** September 11, 2025; **Available online** November 20, 2025

ABSTRACT. Endophytic fungi are recognized as a valuable source of bioactive compounds with significant therapeutic potential, with species from the genus *Colletotrichum* being particularly noted for their prolific production of structurally diverse secondary metabolites. This study aimed to evaluate the antiproliferative activity of the endophytic fungus *Colletotrichum* sp., isolated from the leaves of *Myristica fragrans* Houtt., commonly known as nutmeg against MCF-7 human breast cancer cells using an integrated *in vitro* and *in silico* approach. Bioassay-guided fractionation of the fungal extract followed by LC-HRMS analysis led to the identification of three major compounds: 2-amino-1,3,4-octadecanetriol, L-α-palmitin, and muscone-like compound. The antiproliferative activity of each subfraction was assessed using the MTT assay, while molecular docking studies were conducted to evaluate their binding affinities and interaction profiles with estrogen receptor α (ERα). Among the tested subfractions, F2.3 demonstrated the strongest antiproliferative activity, reducing MCF-7 cell viability to 22.08% at a concentration of 50 μg mL^{-1}. The identified compounds demonstrated notable antiproliferative potential, with L-α-Palmitin showing the moderate binding affinity to ERα as indicated by a binding energy of -6.406 kcal mol^{-1}. Molecular docking analyses revealed key interactions, including hydrogen bonding and hydrophobic contacts, contributing to receptor-ligand stability. These findings highlight *Colletotrichum* sp. as a promising source of antiproliferative agents, thus warranting further investigation into the isolation, structural characterization, and *in vivo* validation of its active constituents for potential pharmaceutical development.

Keywords: antiproliferative, *Colletotrichum* sp., endophytic fungi, LC-HRMS, *Myristica fragrans* Houtt., secondary metabolites.

INTRODUCTION

Cancer is recognized as a primary contributor to premature death in a number of countries globally, with a particularly high burden observed in Asian nations. According to data from the World Health Organization (WHO), there were 17 million cancer cases in 2018, and it is projected to double to 34 million cases by 2070 (Soerjomataram & Bray, 2020). The high incidence of cancer underscores the urgency for scientists to discover and develop drug candidates for cancer treatment. Active compounds isolated from natural sources are considered a promising avenue for cancer therapy due to their relatively lower toxicity profile compared to synthetic sources (Abutaha et al., 2020). Of the 22.000 bioactive secondary metabolites isolated from microorganisms, approximately 38% are derived from fungi.

Endophytic fungi have emerged as a potential source for discovering novel compounds from plants (Abutaha et al., 2020). The application of endophytic fungal extracts from plants to explore secondary metabolites remains relatively limited, despite offering advantages such as minimal plant material requirements, thereby preserving the plant and ensuring efficiency. Moreover, endophytic fungi can synthesize secondary metabolites that are analogous to those produced by their host plants, as well as unique compounds that are absent in the host (Li et al., 2023).

Colletotrichum sp. is a genus of endophytic fungi commonly isolated from various plant species (Chen et al., 2020). Most of these secondary metabolites show promise as lead compounds in drug discovery efforts. Notably, the genus *Colletotrichum* is

recognized for its prolific biosynthesis of unique and structurally complex bioactive molecules. Several secondary metabolites that have been reported to originate from the *Colletotrichum* include polyketides, terpenoids (Yang et al., 2019), sesquiterpenes, phenolics, siderophores, and alkaloids (Liu et al., 2018), which exhibit various pharmacological activities such as antimicrobial (Talukdar et al., 2021), antiproliverative (Liu et al., 2018), antioxidant, and cytotoxic (Rai et al., 2023). Nutmeg, has been identified as a host plant for endophytic fungi, particularly species from the genus *Colletotrichum*. These endophytes have been isolated from different parts of the plant (Rahmi et al., 2023). Indonesia is among the world's largest producers of nutmeg. While the seeds, mace, fruit flesh, and oil are widely utilized as export commodities and industrial raw materials, the leaves of nutmeg are frequently discarded and remain underexplored. Notably, nutmeg leaves contain diverse secondary metabolites, including phenolics, flavonoids, saponins, alkaloids, terpenoids, and tannins (Ginting et al., 2017).

The detection of *Colletotrichum* in multiple tissues of Nutmeg indicates a potentially significant fungal-plant interaction that remains to be elucidated. Given the documented ability of *Colletotrichum* species to produce bioactive secondary metabolites, their occurrence in nutmeg may represent a valuable source for the exploration of novel pharmacologically relevant compounds. Endophytic fungus MF-DnT-3 was chosen based on our preliminary screening, which shows a high inhibitory effect on the growth of MCF-7 cancer cells (unpublished data). Thus, this study aimed to evaluate the antiproliferative activity of extracts from *Colletotrichum* sp. MF-DnT-3, isolated from the leaves of nutmeg, and to identify potential compounds for novel drug development. Additionally, a molecular docking approach was employed to support the bioactivity findings. Molecular docking analysis serves as a significant tool to determine the binding affinity of candidate molecules with their targets and to describe target inhibition by ligands, which is crucial in the optimization of promising compounds (Patel et al., 2024).

EXPERIMENTAL SECTION

Isolation and Identification of MF-DnT-3 from Nutmeg

Endophytic fungi were isolated from the leaves of Nutmeg (Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN), Cibinong, Indonesia). The isolate was identified based on morphological fungal characters, which is *Colletotrichum* sp. MF-DnT-3 isolated from mature leaves.

The isolated and purified endophytic fungus was identified based on morphological characteristics. Morphological identification was carried out by observing phenotypic traits both macroscopically and microscopically. For microscopic examination, a colony grown on Potato Dextrose Agar (PDA) medium was prepared using 60% (v/v) lactic acid as the mounting medium. Microscopic observation were conducted using an Olympus BX53 light microscope at up to 1000x magnification with immersion oil. Photomicrographs were taken with an Olympus DP26 camera connected to a computer. The microscopic morphological features examined included mycelia/hyphae and reproductive structures such as conidia and/or spores. Macroscopic characteristics observed included colony growth pattern or distribution, texture, color, shape, and margin morphology. Fungal identification was conducted using monographic keys based on the references Sutton (1980) and Sutton (1981).

Cultivation and Extraction of *Colletotrichum* sp. from Nutmeg

The isolates were inoculated into each of eight 500 mL Erlenmeyer flasks containing 200 mL potato dextrose broth (PDB) medium (BD Difco, Le Pont-de-Claix, France) and fermented for three weeks in the dark at room temperature (Praptiwi et al., 2018). After a fermentation period, the biomass of endophytic fungi and the growth media were extracted separately with ethyl acetate three times. The biomass was collected and blended with the growth media and mixed in an Erlenmeyer flask. Ethyl acetate was added to the Erlenmeyer flask and stirred for 30 minutes. Then, they were filtered and separated the biomass and growth media. After that, each of the biomass and growth media was extracted with ethyl acetate using a separating funnel to separate the organic and water layers. The organic layer was evaporated using a rotary evaporator (IKA, Germany) and dried with nitrogen gas. Crude extracts of the endophytic fungus MF-DnT-3 were obtained, yielding 834 mg from the growth medium and 662 mg from the biomass.

Fractionation and Isolation of Secondary Metabolites from *Colletotrichum* sp. MF-DnT-3

The crude extracts of medium (834 mg) and biomass (662 mg) were fractionated using the organic solvent such as *n*-hexane, dichloromethane, ethyl acetate, and methanol, by the liquid-liquid extraction technique with a gradient polarity approach and yielding 8 fractions(F1-F8). Subsequently, the chemical profile was examined using TLC plates with dichloromethane and methanol as the mobile phase in a 20:1 ratio. The TLC results indicated that the *n*-hexane and dichloromethane fractions exhibited nearly identical spot patterns in both the medium (F1 and F2) and the biomass (F5 and F6). Therefore, the *n*-hexane and dichloromethane fractions from the medium were combined, and those from the biomass were also merged, resulting in a total of six fractions, F1-F3 from medium and F4-F6 from biomass : F1 (212 mg), F2 (171 mg), F3 (378 mg), F4 (544 mg), F5 (102 mg), and F6 (128 mg). Furthermore, the cell growth inhibition activity of each fraction was assayed

against the breast cancer cell line MCF-7. Based on the results, F2 (171 mg) was chosen and further fractionated using column chromatography with silica gel. It was eluted with an isocratic system of *n*-hexane – ethyl acetate (HE 6:1), yielding four sub-fractions: F2.1 (10.9 mg), F2.2 (56 mg), F2.3 (3.8 mg), and F2.4 (1.9 mg). Then, the antiproliferative activity of each sub-fraction was evaluated using the same cell line.

Cell Culture and Treatment

Cell culture and treatment were done following our previous method (Putra et al., 2025). The MCF-7 (human breast cancer cell line) cells were obtained from the American Type Culture Collection (ATCC). Cells were cultured in a 10% FBS-DMEM medium supplemented with 1x antibiotic-antimycotic solution (All materials obtained from Sigma Aldrich, Saint Louis, MO, USA). Approximately 2×10^3 cells well^{-1} were seeded in a 96-well plate (Nunc, Roskilde, Denmark) and incubated in a 5% CO_2 incubator (Heracell 250i CO_2 Incubator, Thermo Scientific, Waltham, MA, USA) at 37°C for 18-24 hours. The dried sample was prepared in DMSO and diluted in a growth medium. Then, the old medium was replaced with fresh medium containing samples at a concentration of 50 μg mL^{-1}, followed by a further 48 h incubation in the same conditions as mentioned above. As positive and negative controls, 10 μg mL^{-1} of cisplatin (TCI Chemicals, Tokyo, Japan) and 1% DMSO (Sigma Aldrich) were used, respectively.

Cell Viability Assay

The cell viability was measured by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. After 48 h incubation, 100 μL of culture media containing 10 μL of MTT (5 mg mL^{-1}) reagent (Invitrogen, Eugene, OR, USA) was added to each well after the old medium was removed. Cells were then incubated in the dark for 3 h. Once formazan salt formation was observable, we carefully removed half of the medium and added 100 μL well^{-1} of DMSO, thoroughly mixing it using a plate shaker. Absorbance was measured at 570 nm using a plate reader (Multiskan GO, Thermo Scientific, Waltham, MA, USA). Cell viability (%) was calculated using the following formula: [(Absorbance of sample – Absorbance of blank)(Absorbance of negative control – Absorbance of blank)$^{-1}$] × 100% (Putra et al., 2025).

Compounds Identification using LC-HRMS

LC-HRMS analyzed the chemical composition of the endophytic fungus *Colletotrichum* sp., MF-DnT-3 subfraction. Analysis was performed using a binary pump UHPLC Vanquish (Thermo Scientific, USA) coupled with high-resolution mass spectrometry Q-Exactive Orbotrap (Thermo Scientific, USA). An analytical column of Accucore C-18 (10 mm × 2.6 mm ID × 2.1 μm) was used for metabolite separation. The column temperature was set at 40°C during analysis. The mobile phase used for analysis was water containing 0.1% formic acid (A) and methanol containing 0.1% formic acid (B) employing a gradient technique with a flow rate of 0.30 mL/min, and the total running time was 25 min. The mobile phase was set at 95% A at initial condition, then continued to 90% B at 16 min. The condition at 90% B was maintained for 10 min before going back to the initial condition. Each sample was injected at a volume of 10 μL. For mass spectrometric conditions, the sheath gas flow rate was set at 32 arbitrary units (AU) with auxiliary and sweep gas flow rates of 8 and 4 AU, respectively (Windarsih et al., 2022). Analysis was performed in positive ionization. The mass calibration was performed routinely using positive ESI calibration solutions to maintain the performance and accuracy of the mass analyzer. Mass identification was carried out using Compound Discover TM 3.2 software with filter peak extraction using the databases of MzCloud and Chemspider.

Molecular Docking

Molecular docking studies were conducted to explore the potential binding interactions between ligands identified from *Colletotrichum* sp. MF-DnT-3 and the human estrogen receptor α (ERα). The crystal structure of ER-α (PDB ID: 3ERT, 1A52, 1GWR) was obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (https://www.rcsb.org/). Based on the LC-HRMS analysis results, ligand structures were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Water molecules and non-essential atoms were removed from the ER-α structure using PyMOL 3.0.3. Both receptor and ligand structures were prepared for docking using AutoDock Vina Tools-1.5.7, which included the addition of polar hydrogens, merging non-polar hydrogens, and converting the file format to PDBQT. In this study, AutoDock Vina Tools-1.5.7 was selected for the molecular docking analysis because of its capability to provide accurate and efficient predictions of binding energies. The docking results allowed us to evaluate the potential interactions between the isolated compounds and the target receptors, thereby supporting the interpretation of the antiproliferative activity observed *in vitro*. The validity of AutoDock Vina has also been reported in several previous studies, demonstrating its performance comparable to that of commercial docking software.

Molecular docking was carried out using AutoDock Vina within the PyRx 0.8 software suite. During docking, the receptor was maintained as rigid, while ligands were treated as flexible. Grid parameters were defined using AutoGrid in PyRx to cover the active site of the receptor. Docking results were ranked based on binding affinity, with the most negative values indicating the strongest predicted interactions. The top-scoring ligand poses were visualized and analysed in PyMOL 3.0.3, while detailed interaction profiling, such as hydrogen binding, hydrophobic interactions, and other non-covalent contacts, was performed using

BOVIA Discovery Studio 2021. Both 2D and 3D visualizations of the ligand-receptor complexes were generated to facilitate in-depth analysis (Puspa et al., 2024).

Statistical Analysis

The statistical significance of the difference was evaluated using One-way ANOVA followed by the Tukey-Kramer test. Values with $*p < 0.05$ or $**p < 0.01$ were considered statistically significant compared to negative control (1% DMSO).

RESULTS AND DISCUSSION

Morphological Identification of Endophytic Fungus MF-DnT-3

Morphological identification of the endophytic fungus MF-DnT-3 was performed *in vitro* using cultures grown on PDA medium incubated at room temperature for 10 days. The MF-DnT-3 cultured on PDA exhibited cottony mycelia, initially white to gray, darkening with colony age. The reverse side of the colony was unpigmented. The hyphae of MF-DnT-3 were septate (monocytic), 3 – 5 μm in diameter, and hyaline (colorless). Throughout the incubation period on PDA, the MF-DnT-3 culture did not produce sexual or asexual reproductive structures, thus remaining in a sterile mycelial state (*Mycelia sterilia*).

Bioassay-Guided Isolation of *Colletotrichum* sp. from Nutmeg

The fungal isolate coded MF-DnT-3 obtained from senescent leaves of nutmeg, was identified as *Colletotrichum* sp. (**Figure 1**). To evaluate the cytotoxic activity of endophytic fungi *Colletotrichum* sp. from nutmeg, a bioassay-guided fractionation approach was used. Based on the result, the cytotoxic activity of the compounds was identified in fractions F1, F2, F3, F4, F5, and F6 (**Figure 2**). Although all fractions (F1-F6) exhibited cytotoxic activity against MCF-7 cells, fraction F2 was selected for further isolation because its TLC profile indicated a relatively smaller number of compounds compared to the other fractions. This simpler chemical complexity facilitated the purification process and allowed a more efficient identification of bioactive constituents. Therefore, the selection of fraction F2 was based not only on its biological activity but also on practical considerations to optimize the isolation process. Four subfractions were obtained: F2.1, F2.2, F2.3, and F2.4. The MTT assay results revealed that, among the four subfractions derived from the F2 fraction, F2.3 exhibited the highest antiproliferative activity (**Figure 3**).

Antiproliferative Activity

The cell growth inhibition activity of fractions from endophytic fungi, *Colletotrichum* sp. from nutmeg was assessed against MCF-7 cells. The antiproliferative potency in this study was assessed based on relative cell viability compared to the negative control (1% DMSO), with cisplatin serving as a positive control. Extracts causing ≥ 70% reduction in cell viability after 48 h treatment were considered highly cytotoxic, in accordance with established benchmarks (Sammar et al., 2019). The endophytic fungus *Colletotrichum* sp. demonstrated potent antiproliferative activity across all its fractions (**Figure 2**). These findings suggest that the endophytic fungus *Colletotrichum* sp. is likely to harbour secondary metabolites exhibiting antiproliferative properties. Cyclo (L-leucyl-L-leucyl) and Brevianamide F, isolated from the endophytic fungus *Colletotrichum gloeosporioides* residing in the *Uncaria rhynchophylla*, showed potent PI3K α inhibitory activity with an IC_{50} value of 38.1 and 4.8 μM (Yang et al., 2019). Colletotricone A is a secondary metabolite produced by *C. gloeosporioides* A12 isolated from the *Aquilaria sinensis* was cytotoxic with renge IC_{50} of 15.7 to 46.8 μM on MCF-7, NCI-H460, HepG-2, and SF-268 cell lines (Liu et al., 2018).

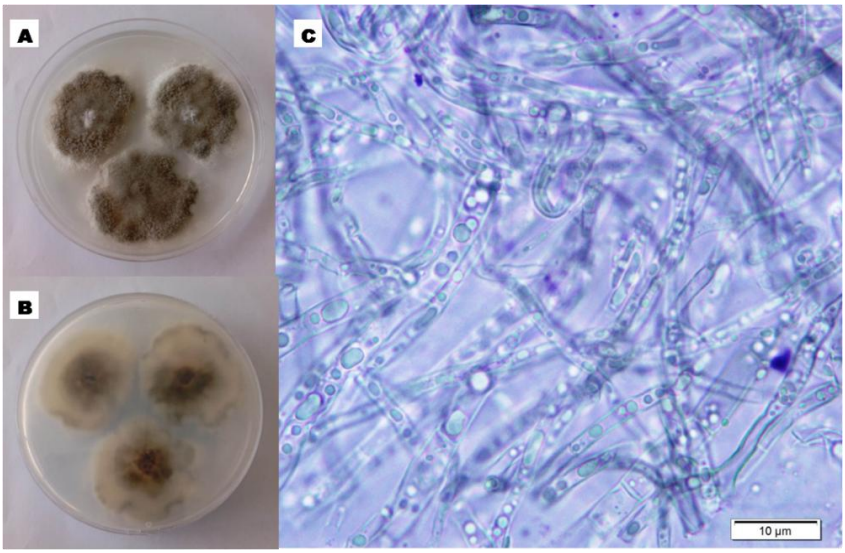


Figure 1. Morphological characteristics of the endophytic fungus MF-DnT-3. Upper colony surface (A), reverse side (B), and microscopic view at 1000x magnification (C).

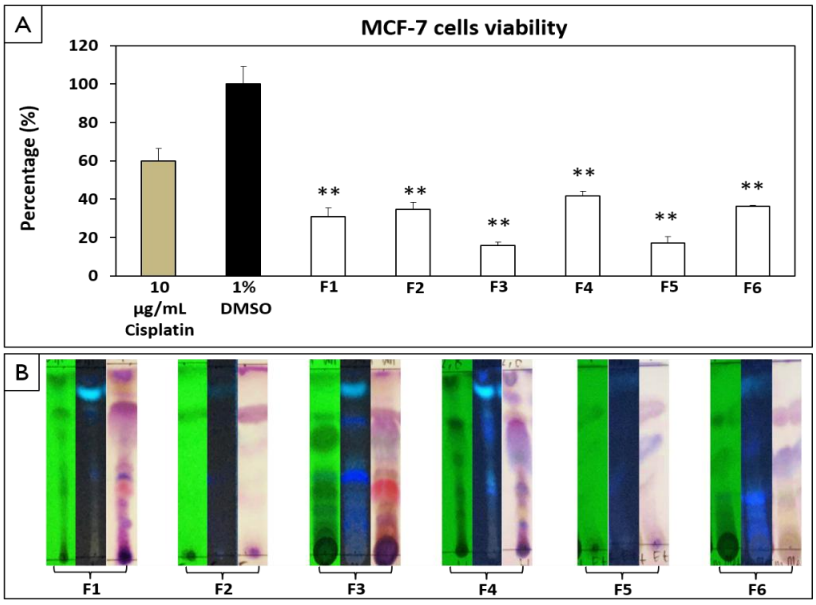


Figure 2. Viabilities of MCF-7 cells treated with or without fractions (**A**). The TLC chromatograms of *n*-hexane and dichloromethane, ethyl acetate, and methanol fractions from media (F1-F3) and biomass (F4-F6) (**B**). Statistical analysis was performed using one-way ANOVA followed by the Turkey-Kramer test. Data showing significance against the negative control (0.5% DMSO) is denoted by asterisks *p , 0.05; **p , 0.01. The TLC chromatograms under UV 254 nm, 366 nm, and visualizing reagent.

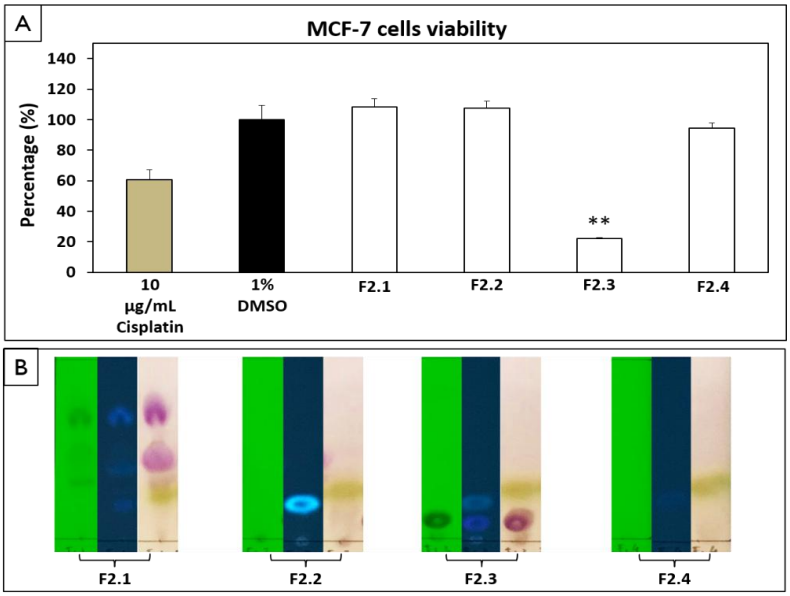


Figure 3. Viabilities of MCF-7 cells treated with or without subfractions (**A**), TLC chromatogram of subfractions from F2 (**B**). Statistical analysis was performed using one-way ANOVA followed by the Turkey-Kramer test. Data showing significance against the negative control (0.5% DMSO) is denoted by asterisks *p , 0.05; **p , 0.01. The TLC chromatograms under UV 254 nm, 366 nm, and visualizing reagent.

Among the four subfractions (F2.1, F2.2, F2.3, and F2.4), F2.3 exhibited the most significant antiproliferative activity, reducing cell viability to 22.08%, compared to 108.32%, 107.73%, and 94.43% for F2.1, F2.2, and F2.4, respectively (**Figure 3**). The bioactive components are presumably associated with chromatographic bands that fluoresce under UV light at 254 and 366 nm, which are able to develop a purple coloration upon visualization with vanillin reagent, suggesting the presence of specific secondary metabolites contributing to the observed activity.

LC-HRMS Analysis of Bioactive Fractions

To characterize the secondary metabolites in subfraction F2.3, an LC-HRMS analysis was carried out. LC-HRMS analysis revealed the presence of secondary metabolites in the subfraction. The Total Ion Chromatogram (TIC) profile obtained from the LC-

HRMS analysis revealed seven prominent peaks (**Figure 4**), indicating the presence of major secondary metabolite constituents within the subfraction. Among these, only three compounds were successfully elucidated, namely 2-amino-1,3,4-octadecanetriol, L-α-palmitin, and (±)-muscone-like compound (**Table 1**).

The compound 2-amino-1,3,4-octadecanetriol significantly inhibits the epithelial-mesenchymal transition (EMT) process, as evidenced by the upregulation of E-cadherin and the downregulation of vimentin, fibronectin, and ZEB1 expression levels. Moreover, this compound suppresses the self-renewal capacity of breast cancer by reducing the $CD44^{high}/CD24^{low}$ subpopulation and inhibiting spheroid formation and growth, thereby impairing the stem-like characteristics of these cells. In addition, 2-amino-1,3,4-octadecanetriol enhances the sensitivity of cancer stem cell (CSC)-like populations in basal-type breast cancer cells, effectively blocks the metastasis potential of breast cancer cells *in vivo*, and directly binds to EGFR, thereby suppressing EMT via the deactivation of its downstream signalling pathway (Kang et al., 2017). This compound also induces apoptosis through two mechanisms. First, by directly activating caspase-8 in a death receptor-independent manner, as it does not involve classical receptors such as Fas, DR4/DR5, or TNFR, nor the adaptor protein FADD, suggesting an alternative pathway for caspase-8 activation. Second, the compound disrupts mitochondrial integrity by promoting Bax translocation to the mitochondria, leading to cytochrome C release and the subsequent activation of caspase-9 and caspase-3, which are hallmark events of apoptotic cell death (Park et al., 2003).

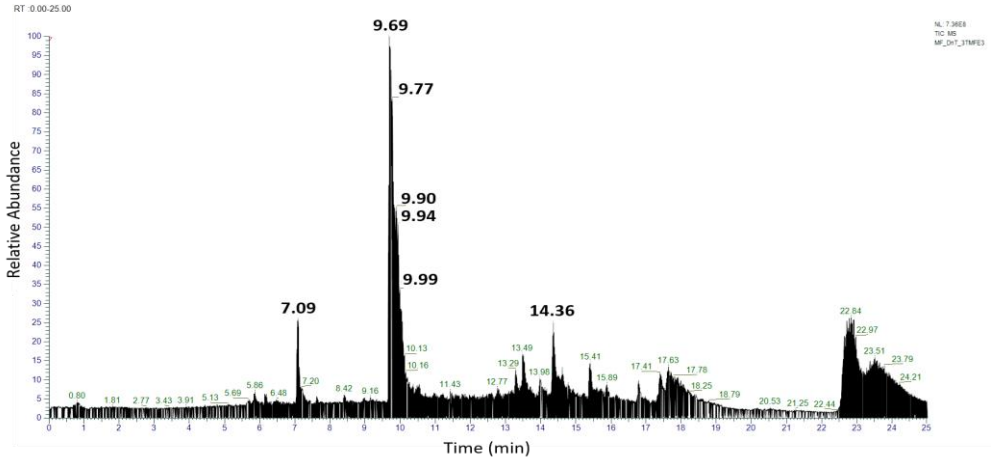


Figure 4. LC-HRMS analysis of F2.3 Subfractions

Table 1. Eight major compounds in F2.3 subfractions detected by LC-HRMS

Propose compounds	Rt (min)	Molecular Formula	Molecular weight (m/z)	Fragment Ions	Ionization	Mass error (ppm)
Undefined	7.09	$C_{34}H_{67}N_{10}PS$	678.50	340.26, 351.25, 679.51, and 701.49	$[M+2H]^{+2}$, $[M+H+Na]^{+2}$,$[M+H]^{+1}$, and $[M+Na]^{+1}$	4,8
Undefined	9.69	$C_{17}H_{36}N_2O$	284.28	285.29	$[M+H]^{+1}$	-0.11
Undefined	9.77	$C_{14}H_{31}NO$	229.24	230.25	$[M+H]^{+1}$	0.06
2-Amino-1,3,4-Octadecane triol	9.90	$C_{18}H_{39}NO_3$	317.29	318.30 and 340.28	$[M+H]^{+1}$ and $[M+Na]^{+1}$	-0.56
Undefined	9.94	$C_{16}H_{35}NO_3$	289.26	290.27	$[M+H]^{+1}$	0.13
Undefined	9.99	$C_{18}H_{39}NO_4$	333.29	334.30	$[M+H]^{+1}$	-0.15
L-α-Palmitin	14.36	$C_{19}H_{38}O_4$	330.28	331.28, 313.27, and 353.27	$[M+H]^{+1}$, $[M+H-H_2O]^{+1}$, and $[M+Na]^{+1}$	-0.66
Muscone-like compound	14.36	$C_{16}H_{30}O$	238.23	239.24 and 256.26	$[M+H]^{+1}$ and $[M+NH_4]^{+1}$	0.48

The MF-DnT-3 cultured on PDA exhibited cottony mycelia, initially white to gray, darkening with colony age. The reverse side of the colony was unpigmented. The hyphae of MF-DnT-3 were septate (monocytic), 3 – 5 μm in diameter, and hyaline (colorless). Throughout the incubation period on PDA, the MF-DnT-3 culture did not produce sexual or asexual reproductive structures, thus remaining in a sterile mycelial state (*Mycelia sterilia*).

Bioassay-Guided Isolation of *Colletotrichum* sp. from Nutmeg

The fungal isolate coded MF-DnT-3 obtained from senescent leaves of nutmeg, was identified as *Colletotrichum* sp. (**Figure 1**). To evaluate the cytotoxic activity of endophytic fungi *Colletotrichum* sp. from nutmeg, a bioassay-guided fractionation approach was used. Based on the result, the cytotoxic activity of the compounds was identified in fractions F1, F2, F3, F4, F5, and F6 (**Figure 2**). Although all fractions (F1-F6) exhibited cytotoxic activity against MCF-7 cells, fraction F2 was selected for further isolation because its TLC profile indicated a relatively smaller number of compounds compared to the other fractions. This simpler chemical complexity facilitated the purification process and allowed a more efficient identification of bioactive constituents. Therefore, the selection of fraction F2 was based not only on its biological activity but also on practical considerations to optimize the isolation process. Four subfractions were obtained: F2.1, F2.2, F2.3, and F2.4. The MTT assay results revealed that, among the four subfractions derived from the F2 fraction, F2.3 exhibited the highest antiproliferative activity (**Figure 3**).

Antiproliferative Activity

The cell growth inhibition activity of fractions from endophytic fungi, *Colletotrichum* sp. from nutmeg was assessed against MCF-7 cells. The antiproliferative potency in this study was assessed based on relative cell viability compared to the negative control (1% DMSO), with cisplatin serving as a positive control. Extracts causing ≥ 70% reduction in cell viability after 48 h treatment were considered highly cytotoxic, in accordance with established benchmarks (Sammar et al., 2019). The endophytic fungus *Colletotrichum* sp. demonstrated potent antiproliferative activity across all its fractions (**Figure 2**). These findings suggest that the endophytic fungus *Colletotrichum* sp. is likely to harbour secondary metabolites exhibiting antiproliferative properties. Cyclo (L-leucyl-L-leucyl) and Brevianamide F, isolated from the endophytic fungus *Colletotrichum gloeosporioides* residing in the *Uncaria rhynchophylla*, showed potent PI3K α inhibitory activity with an IC_{50} value of 38.1 and 4.8 μM (Yang et al., 2019). Colletotricone A is a secondary metabolite produced by *Colletotrichum gloeosporioides* A12 isolated from the *Aquilaria sinensis* was cytotoxic with renge IC_{50} of 15.7 to 46.8 μM on MCF-7, NCI-H460, HepG-2, and SF-268 cell lines (Liu et al., 2018).

L-α-Palmitin, an inactive form of palmitic acid, exhibits significant anticancer activity through multiple molecular mechanisms across various cancer types, including gastric, hepatic, breast, cervical, and colorectal cancers. This compound induces apoptotic cell death by activating the tumor suppressor protein p53 and caspase-3, inhibits cell proliferation and cell cycle progression, suppresses epithelial-mesenchymal transition (EMT) and metastasis, and modulates key oncogenic signalling pathways such as PI3K/Akt/mTOR and JAK2/STAT3 (Wang et al., 2023).

Muscone-like compound exhibits potent anticancer activity against breast cancer by targeting tumor angiogenesis, specifically, it suppresses cancer cell proliferation and migration, inhibits tumor-associated angiogenesis, downregulates VEGFA expression, and attenuates the activation of the VEGFR2/PI3K/Akt/MAPK signalling pathway in endothelial cells (Wang et al., 2024). Based on these findings, the three compounds identified in subfraction F2.3 of the endophytic fungus *Colletotrichum* sp., isolated from the leaves of nutmeg, exhibit antiproliferative activity against cancer cells, particularly against MCF-7 human breast cancer cells.

Molecular Docking

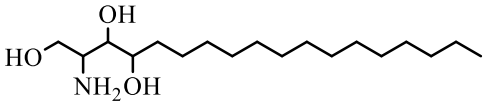
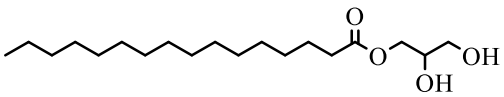
Molecular docking, a computational approach grounded in structural biology, has become an essential tool in modern drug discovery, enabling the prediction of binding affinities and interaction profiles between candidate ligands and target proteins. This technique provides critical insights into the molecular basis of potential therapeutic activity and supports the rational design of bioactive compounds (Puspa et al., 2024). This study employed molecular docking to investigate the inhibitory potential of compounds identified in endophytic fungus *Colletotrichum* sp., isolated from leaves of Nutmeg, against human estrogen receptor α, a key target in breast cancer therapy. The crystal structure of ERα (PDB ID: 3ERT, 1A52, 1GWR) was used as the target protein. Among the three target proteins, 1GWR exhibited the strongest binding affinities to the ligands: 2-amino-1,3,4-octadecanetriol (6.393 kcal/mol) and L-α-palmitin (-6.406 kcal/mol). The observed negative binding energy values indicate thermodynamically favourable interactions between the ligands and the ERα binding pocket. Importantly, more negative binding energy values correspond to stronger binding affinities, reflecting enhanced ligand-receptor interaction stability, and validation is considered to be quite feasible if the root mean square deviation value from the re-docking result is below 2 Å (Ernawati et al., 2024).

Molecular docking analysis revealed that 2-amino-1,3,4-octadecanetriol exhibited favourable binding interactions within the active site of the estrogen receptor α (PDB ID: 1GWR). As shown in **Figure 5**, the ligand formed two conventional hydrogen bonds with residues Gly421 and His524, which are known to

contribute to ligand stabilization and specificity. Additionally, multiple hydrophobic interactions, including alkyl and π-alkyl contacts with Leu387, Met343, and Phe404, further supported the ligand's stable accommodation within the binding pocket. The 3D and surface representations confirmed that the ligand was well-positioned in the receptor's binding site, exhibiting shape complementarity and spatial fit.

Figure 6 shows that the L-α-palmitin formed a conventional hydrogen bond with residue His524, which is known to play a critical role in ligand anchoring within the receptor's binding site. In addition, extensive alkyl and π-alkyl interactions were observed with multiple nonpolar residues, including Leu346, Phe404, Leu387, and Met343, suggesting that hydrophobic forces significantly contribute to the binding affinity. The 3D structural model and surface representation confirm that the ligand fits snugly within the receptor pocket, indicating shape complementarity and a favourable spatial orientation.

Table 2. Molecular docking of three compounds with the protein ER-α

Ligands	Structure	PDB ID	Binding Affinity (kcal/mol)	RMSD (Å)
2-Amino-1,3,4-Octadecanetriol		3ERT	-6.011	0.000
		1A52	-6.252	0.000
		1GWR	**-6.393**	0.000
L-α-Palmitin		3ERT	-5.903	0.000
		1A52	-6.201	0.000
		1GWR	**-6.406**	0.000

Note: Bold texts indicate the best binding score

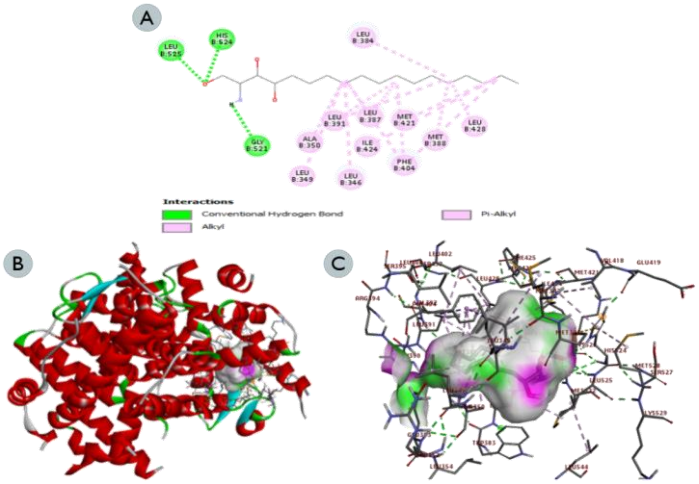


Figure 5. Ligand (2-amino-1,3,4-octadecanetriol) and receptor (1GWR) interactions. (A) 2D interactions diagram, (B) 3D representation of the receptor-ligand complex, and (C) surface view of the binding pocket.

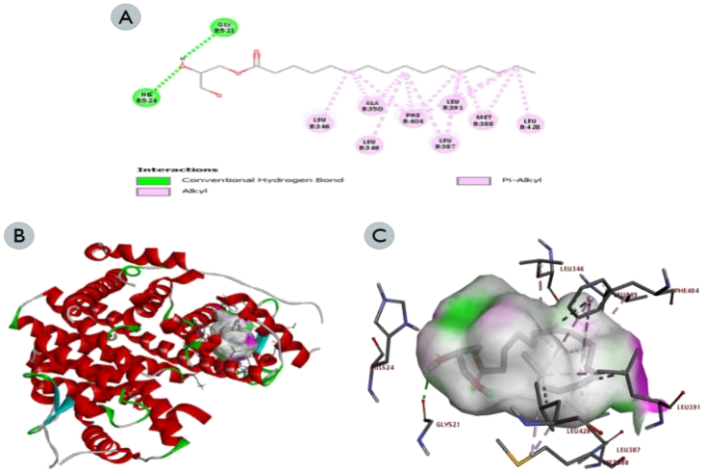


Figure 6. Ligand (L-α-palmitin) and receptor (1GWR) interactions. (A) 2D interactions diagram, (B) 3D representation of the receptor-ligand complex, and (C) surface view of the binding pocket

CONCLUSIONS

A study on the antiproliferative activity of the endophytic fungus *Colletotrichum* sp., isolated from the leaves of nutmeg, revealed that the fungal fractions ability to inhibit the MCF-7 cell growth. Among the tested subfractions, F2.3 demonstrated the strongest antiproliferative activity, reducing MCF-7 cell viability to 22.08% at a concentration of 50 μg mL^{-1}. LC-HRMS analysis of the subfraction F2.3 identified three major compounds, namely 2-amino-1,3,4-octadecanetriol, L-α-palmitin, and (±)-muscone-like compound, all of which have been reported elsewhere to possess potent antiproliferative activity. Molecular docking studies further confirmed that these compounds possessed very low binding affinity values, indicating strong interactions with the target protein. These findings highlight the potential of *Colletotrichum* sp. as a promising source of bioactive compounds for drug development. Further research focused on the isolation and structural characterization of the active constituents, as well as *in vivo* evaluation, will be essential to validate and advance the pharmaceutical applications of these bioactive compounds.

ACKNOWLEDGEMENTS

The authors acknowledge the facilities, scientific and technical support from Advanced Characterization Laboratories Yogyakarta, National Research and Innovation Agency through E-Layanan Sains BRIN for LC-HRMS analysis.

DECLARATION

Funding

This research received funding from the Joint Research Collaboration of the Rumah Program Organisasi Riset Kesehatan (Research Organization for Health), National Research and Innovation Agency (BRIN), Indonesia, during the fiscal years 2024 and 2025.

Competing interests

The authors declare no competing interests.

REFERENCES

Abutaha, N. M., Farooq, M. F., Nasr, F. A., & Ahmed, F. A. (2020). Bioassay-guided fractionation of endophytic fungal extract of *Fusarium solani* (Saccardo) against cancer cell lines and zebrafish embryo. Indian Journal of Pharmaceutical Education and Research *54*(2), 301–308. https://doi.org/10.5530/ijper.54.2s.87

Chen, J., Akutse, K. S., Saqib, H. S. A., Wu, X., Yang, F., Xia, X.,Gurr, G. M. (2020). Fungal endophyte communities of crucifer crops are seasonally dynamic and structured by plant identity, plant tissue and environmental factors. *Frontiers in Microbiology*, *11*(July), 1–13. https://doi.org/10.3389/fmicb.2020.01519

Ernawati, T., Artanti, N., & Kumiawan, Y. D. (2024). *In silico* and *in vitro* anti-cancer activity against breast cancer cell line MCF-7 of amide cinnamate derivatives. *Journal of Applied Pharmaceutical Science*, *14*(3), 102–107. https://doi.org/10.7324/JAPS.2024.162177

Ginting, B., Hadijah, S., & Helwati, H. (2017). Isolation of antioxidant compounds of n-hexane extract of nutmeg (*Myristica fragrans* Houtt) Leaves. *Jurnal Kimia Mulawarman*, *15*(1), 46. https://doi.org/10.30872/jkm.v15i1.584

Jie, H., Li, F., Liu, Q., Zheng, T., Tan, H., Feng, X., … Wang, T. (2025). Elucidating metabolites and biosynthetic pathways during musk maturation: insights from forest musk deer. *Frontiers in Pharmacology*, *16*(April), 1–11. https://doi.org/10.3389/fphar.2025.1503138

Kang, H. M., Son, H. S., Cui, Y. H., Youn, B. H., Son, B., Kaushik, N. K., … Lee, S. J. (2017). Phytosphingosine exhibits an anti-epithelial-mesenchymal transition function by the inhibition of EGFR signaling in human breast cancer cells. *Oncotarget*, *8*(44), 77794–77808. https://doi.org/10.18632/oncotarget.20783

Li, Z., Xiong, K., Wen, W., Li, L., & Xu, D. (2023). Functional endophytes regulating plant secondary metabolism: current status, prospects and applications. *International Journal of Molecular Sciences*, *24*(2). https://doi.org/10.3390/ijms24021153

Liu, H. X., Tan, H. B., Chen, Y. C., Li, S. N., Li, H. H., & Zhang, W. M. (2018). Secondary metabolites from the *Colletotrichum gloeosporioides* A12, an endophytic fungus derived from *Aquilaria sinensis*. *Natural Product Research*, *32*(19), 2360–2365. https://doi.org/10.1080/14786419.2017.1410810

Park, M. T., Kang, J. A., Choi, J. A., Kang, C. M., Kim, T. H., Bae, S., … Lee, S. J. (2003). Phytosphingosine induces apoptotic cell death via caspase 8 activation and Bax translocation in human cancer cells. *Clinical Cancer Research*, *9*(2), 878–885.

Patel, F., Upadhyay, K., Mammen, D., Robin, E., Ramachandran, A. V., & Baxi, D. (2024). Phytochemical composition and antiproliferative activity of *Opuntia elatior* Mill.: *In vitro* and in silico studies on breast cancer cell line MCF-7. *Journal of Applied Biology and Biotechnology*, *12*(1), 117–127. https://doi.org/10.7324/JABB.2024.144233

Praptiwi, Raunsai, M., Wulansari, D., Fathoni, A., & Agusta, A. (2018). Antibacterial and antioxidant activities of endophytic fungi extracts of medicinal plants from Central Sulawesi. *Journal of Applied Pharmaceutical Science*, *8*(8), 069–074. https://doi.org/10.7324/JAPS.2018.8811

Puspa, V. R., Adista, M. A., & Djufri. (2024).

Phytochemical, antioxidant, in silico, and *in vitro* anti-breast cancer effects of *Erigeron sumatrensis* from Gayo Highlands (Indonesia) against MCF-7 cells. *Biodiversitas*, *25*(12), 5123–5137. https://doi.org/10.13057/biodiv/d251250

Putra, A. B. N., Palupi, K. D., Fathoni, A., Oktavia, L., Septisetyani, E. P., Nurkanto, A., … Agusta, A. (2025). *In vitro* antiproliferative activity, docking study, and plausible mode of action of (+)-2,2′-epicytoskyrin A against breast cancer MCF-7 and T47D cell lines. *Beni-Suef University Journal of Basic and Applied Sciences*, *14*(1). https://doi.org/10.1186/s43088-025-00632-y

Rahmi, D., Praptiwi, Simajuntak, P., Ilyas, M., & Agusta, A. (2023). The antioxidant activity of endophytic fungal extract of nutmeg plant (*Myristica fragrans* Houtt.) from Cimande West Java, Indonesia. *IOP Conference Series: Earth and Environmental Science*, *1271*(1). https://doi.org/ 10.1088/1755-1315/1271/1/012063

Rai, N., Gupta, P., Verma, A., Tiwari, R. K., Madhukar, P., Kamble, S. C., … Gautam, V. (2023). Ethyl acetate extract of colletotrichum gloeosporioides promotes cytotoxicity and apoptosis in human breast cancer cells. *ACS Omega*, *8*(4), 3768–3784. https://doi.org/10.1021/acsomega.2c05746

Sammar, M., Abu-Farich, B., Rayan, I., Falah, M., & Rayan, A. (2019). Correlation between cytotoxicity in cancer cells and free radical-scavenging activity: *In vitro* evaluation of 57 medicinal and edible plant extracts. *Oncology Letters*, *18*(6), 6563–6571. https://doi.org/10.3892/ol.2019.11054

Soerjomataram, I., & Bray, F. (2020). Planning for tomorrow: global cancer incidence and the role of prevention 2020–2070. *Nature Reviews Clinical Oncology*. https://doi.org/10.1038/s41571-021-00514-z

Sutton, B. C. (1980). The coelomycetes, fungi imperfecti with pycnidia. *Acervuli and Stromata*, 470-472.

Sutton, B. C. (1981). The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata, *Scientific Research, 696*.

Talukdar, R., Padhi, S., Rai, A. K., Masi, M., Evidente, A., Jha, D. K., … Tayung, K. (2021). Isolation and characterization of an endophytic fungus *Colletotrichum coccodes* producing tyrosol from *Houttuynia cordata* Thunb. Using ITS2 RNA secondary structure and molecular docking study. *Frontiers in Bioengineering and Biotechnology*, *9*(June), 1–12. https://doi.org/10.3389/fbioe.2021.650247

Wang, D., Liu, X., Hong, W., Xiao, T., Xu, Y., Fang, X., … Meng, X. (2024). Muscone abrogates breast cancer progression through tumor angiogenic suppression via VEGF/PI3K/Akt/MAPK signaling pathways. *Cancer Cell International*, *24*(1), 1–18. https://doi.org/10.1186/s12935-024-03401-6

Wang, X., Zhang, C., & Bao, N. (2023). Molecular mechanism of palmitic acid and its derivatives in tumor progression. *Frontiers in Oncology*, *13*(August), 1–10. https://doi.org/10.3389/fonc.2023.1224125

Wang, Y., Sun, M., Chang, F., Wang, J., Wang, Y., Tang, J., … Wan, Y. (2022). The essential differences in microbial and chemical components of musk of different qualities secreted by captive male forest musk deer (*Moschus berezovskii*). *Microbial Biotechnology*, *15*(6), 1783–1794. https://doi.org/10.1111/1751-7915.14002

Windarsih, A., Riswanto, F. D. O., Bakar, N. K. A., Yuliana, N. D., Dachriyanus, & Rohman, A. (2022). Detection of pork in beef meatballs using LC-HRMS based untargeted metabolomics and chemometrics for halal authentication. *Molecules*, *27*(23), 1–14. https://doi.org/10.3390/molecules27238325

Yang, Z. D., Li, Z. J., Zhao, J. W., Sun, J. H., Yang, L. J., & Shu, Z. M. (2019). Secondary metabolites and PI3K inhibitory activity of *Colletotrichum gloeosporioides*, a fungal endophyte of *Uncaria rhynchophylla*. *Current Microbiology*, *76*(7), 904–908. https://doi.org/10.1007/s00284-019-01707-7