

Phytochemical Profiling and Bioactivity Assessment of *Alternanthera philoxeroides* and in Silico Evaluation of Dendryphiellin I Isolated from Its Endophytic Fungi

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How to cite this paper: Rahman, A., Islam, M.S., Begum, M.N., Zinnurine, R., Akter, T., Ahamed, K.U., Rony, S.R., Hasan, C.M. and Sohrab, M.H. (2026) Phytochemical Profiling and Bioactivity Assessment of *Alternanthera philoxeroides* and in Silico Evaluation of Dendryphiellin I Isolated from Its Endophytic Fungi. *Pharmacology & Pharmacy*, 17, 291-315.
<https://doi.org/10.4236/pp.2026.1710017>

Received: August 18, 2026

Accepted: October 20, 2026

Published: October 23, 2026

Abstract

Alternanthera philoxeroides (Mart.) Griseb., locally known as Malancha shak, is a medicinally important leafy vegetable found in Bangladesh. This study investigated the phytochemical profile and biological activities of the methanolic extract, as well as the isolation and characterization of an endophytic fungal metabolite. FTIR and NMR analyses demonstrated the presence of varied functional groups and secondary metabolites. The extract showed moderate DPPH radical-scavenging activity, with an IC₅₀ value of 127.48 ± 0.53 µg/mL, and antibacterial activity against *Bacillus cereus* (11.5 mm), *Pseudomonas aeruginosa* (12 mm), *Staphylococcus aureus* (9 mm), and *Escherichia coli* (9 mm), whereas it exhibited no activity against *Salmonella typhi* and the tested fungi. The corresponding endophytic fungus was identified as *Curvularia* sp., from which Dendryphiellin I was isolated and characterized. Molecular docking and ADMET analyses provided preliminary computational evidence of potential biological activity and drug-like properties of Dendryphiellin I.



Keywords

Alternanthera philoxeroides, Phytochemical, FTIR, NMR, Antioxidant Activity, Antimicrobial, Molecular Docking

1. Introduction

Natural products continue to be an important reservoir of lead compounds for drug discovery, and medicinal plants play a major role in the development of many clinically approved drugs [1] [2]. Plant-derived secondary metabolites function as useful pharmacophores, drug precursors, and molecular scaffolds for creating new therapeutics [3]. In addition, ethnomedicinal knowledge offers a solid scientific foundation for discovering bioactive natural products that have therapeutic promise [4]. *Alternanthera philoxeroides* (Mart.) Griseb. is a tropical perennial herb and leafy vegetable with substantial ethnomedicinal significance. In Bangladesh, it is locally known as Malancha shak and has traditionally been used to manage a range of conditions, including gynecological disorders, fever, the common cold, dysuria, eczema, snakebite, anthrax, and viral encephalitis [5]. The species is a stoloniferous aquatic plant that typically grows in floodplains and in poorly drained agricultural lands, where its hollow stems produce dense floating mats [6]. *A. philoxeroides* has also drawn interest as a promising source of bioactive compounds for pharmaceutical use, insecticidal, bioherbicidal, and other industrial purposes. Prior phytochemical studies have documented a wide range of secondary metabolites, such as alkaloids, flavonoids, phenols, terpenoids, tannins, steroids, and additional bioactive phenolic compounds. Even with its recognized traditional value and the phytochemical variety that has been reported, in-depth work that integrates compound isolation, structural characterization, biological assessment, and computational analysis is still scarce [7]-[9]. Endophytes are beneficial microorganisms that live within plant tissues and support host growth as well as stress tolerance. Successful colonization by these organisms is enabled through multiple mechanisms, including modification of the cell wall, alteration of phytohormonal signaling, secretion of effector proteins, and production of a range of active biochemical natural metabolites [10] [11].

In the present investigation, the methanolic extract of *A. philoxeroides* was examined for its phytochemical properties through FTIR and ^1H and ^{13}C NMR spectroscopy, along with antioxidant and antimicrobial assays. Moreover, an endophytic fungal strain associated with the plant was isolated and identified through molecular methods, and the secondary metabolite Dendryphiellin I was subsequently isolated from the fungal culture and characterized spectroscopically. The isolated compound was further assessed using molecular docking and ADMET analyses to investigate its potential interactions with selected protein targets related to antioxidant and antimicrobial activity, and to evaluate its predicted drug-likeness, pharmacokinetic behavior, and toxicity profile.

2. Materials and Methods

2.1. Collection, Identification, and Extraction of Plant Material

New specimens of *Alternanthera philoxeroides* were collected in September 2023 from Pabna, Bangladesh, and were taxonomically identified at the Salar Khan Herbarium, University of Dhaka (voucher no. DUSH-10819). The entire plant of *Alternanthera philoxeroides* was air-dried and then oven-dried at 40°C for 24 h. The dried material (100 g) was pulverized into a coarse powder and extracted by maceration with 250 mL of 100% methanol (1:2.5, w/v) at room temperature for 12 days, with occasional shaking. The extract was filtered and concentrated under reduced pressure to obtain 9.40 g of dried crude methanolic extract, corresponding to an extraction yield of 9.4% [12] [13].

2.2. Phytochemical Profiling

2.2.1. FTIR (Fourier Transform Infrared Spectroscopy) Analysis of the Sample

FTIR analysis of the methanolic crude extract was performed using a BRUKER ALPHA II FTIR spectrophotometer over the spectral range of 4000 - 500 cm^{-1} to identify the functional groups present [14] [15]. The dried extract was analyzed using the diffuse reflectance technique, and the resulting spectra were compared with reference data to assign functional groups [16] [17].

2.2.2. ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) Spectra of the Crude Extracts

Nuclear Magnetic Resonance (NMR) spectra were obtained on a Bruker Avance II 600 MHz spectrometer with CD_3OD (deuterated methanol) used as the solvent. Chemical shifts (δ) were expressed in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. The coupling constant (J) was reported in hertz (Hz). Spin multiplicities were labeled as singlet (s), broad singlet (brs), doublet (d), double doublet (dd), doublet of doublets (ddd), triplet (t), quintet (quint), doublet of triplets (dt), triplet of doublet doublets (tdd), and others [18].

2.3. Biological Activities of Plant Crude Extracts

2.3.1. Antimicrobial Assay

Preliminary antimicrobial activity was determined using the disc diffusion method. The crude extract (5 mg) was dissolved in 100 μL of methanol, and 10 μL (500 μg per disc) was loaded onto sterile discs. Antibacterial activity was examined against *Staphylococcus aureus* (ATCC 9144), *Bacillus cereus* (clinical isolate), *Escherichia coli* (ATCC 11303), *Salmonella typhi* (ATCC 13311), and *Pseudomonas aeruginosa* (ATCC 27833). Antifungal activity was evaluated against *Aspergillus niger* (ATCC 1004) and *Aspergillus flavus* (UCFT 02). After incubation (18 h at 37°C for bacteria and 48 h at 28°C for fungi), inhibition zones were recorded and compared with kanamycin (30 μg per disc) and ketoconazole (30 μg per disc) as positive controls, whereas methanol (10 μL per disc) was used as the negative control [19] [20].

2.3.2. Antioxidant Activity

Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical scavenging assay, according to the procedure described by Brand-Williams *et al.* (1995). We performed the DPPH assay in three independent experiments, with three replicate measurements at each concentration. The crude extract (1.6 mg) was dissolved in methanol (400 μ L) and subjected to serial dilution (0.78 - 200 μ g/mL). For each dilution, 2 mL was combined with an equal volume of DPPH solution (20 μ g/mL), incubated in the dark at room temperature for 30 min, and the absorbance was recorded at 517 nm. Ascorbic acid and butylated hydroxyanisole (BHA) were used as positive controls, while the DPPH solution mixed with solvent acted as the negative control. Methanol alone served as the solvent blank to zero the spectrophotometer before absorbance measurements at 517 nm. The IC₅₀ (concentration needed to scavenge 50% of DPPH radicals, expressed in μ g/mL) was calculated by fitting a logarithmic regression to the plot of percent inhibition against concentration in Microsoft Excel software. These figures were subsequently compared with the corresponding values for the two standard antioxidants, ascorbic acid and butylated hydroxyanisole (BHA) [21]. Radical scavenging activity was calculated as

$$\text{Scavenging activity (\%)} = \left[\frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}} \right] \times 100,$$

where A_{Blank} is the absorbance of the blank and A_{Sample} is the absorbance of the sample.

2.4. Molecular Identification of the Fungal Isolate APLE-2

The endophytic fungus was isolated using a surface sterilization technique and subsequently identified based on macroscopic and microscopic morphological characteristics, as described in our previous study [22], followed by molecular analysis. Molecular identification was carried out through amplification, sequencing, and subsequent analysis of the internal transcribed spacer (ITS) region [23]. A 7- to 10-day-old fungal culture was used for genomic DNA extraction. The fungal biomass was scraped aseptically using a sterile surgical blade and homogenized with a pestle and mortar in liquid nitrogen. Genomic DNA was subsequently extracted using the Maxwell[®] 16 LEV Plant DNA Kit (AS1420, Promega, USA) according to the manufacturer's protocol [24]. Residual RNA contamination was removed by treatment with DNase-free RNase at 37°C for 30 min. The ITS region was amplified by polymerase chain reaction (PCR) using the universal fungal primers ITS1F and ITS4. The amplified products were subjected to Sanger dideoxy sequencing [25] [26]. The resulting sequences were manually edited using BioEdit v7.2 and compared with reference sequences deposited in the NCBI GenBank rRNA/ITS database using the BLASTn search tool for molecular identification.

2.5. Isolation and Identification of a Secondary Metabolite from the Fungal Isolate APLE-2

For large-scale production of secondary metabolites, the fungal isolate was culti-

vated on potato dextrose agar (PDA). Each Petri plate contained 1.1 g of PDA, and a total of 540 culture plates containing 569.4 g of PDA were prepared. The cultures were incubated at 28°C for 21 - 27 days. Following incubation, the fungal cultures were pooled and extracted three times with ethyl acetate (5 L total volume). The combined ethyl acetate extracts were concentrated under reduced pressure to yield 13.25 g of crude extract. The crude extract was subjected to silica gel column chromatography using silica gel (70 - 230 mesh, 283.75 g) packed in a glass column (bed height: 33 cm, internal diameter: 4.3 cm). Elution was carried out using a step gradient of *n*-hexane, *n*-hexane-dichloromethane (DCM), DCM, and DCM-methanol with increasing polarity. A total of 57 fractions (100 mL each) were collected and pooled into 15 combined fractions based on their TLC profiles. Fraction 9 (1.2 g) was re-chromatographed on a smaller silica gel column (bed height: 16 cm, internal diameter: 2 cm), yielding 23 fractions (50 mL each) that were grouped into 14 major fractions according to TLC monitoring. Fraction 5 was further purified by preparative thin-layer chromatography (PTLC; stationary phase: silica gel F₂₅₄, plate thickness: 0.2 mm) using toluene-20% ethyl acetate containing 0.5% acetic acid as the mobile phase. The desired target band ($R_f = 0.53$) was scraped from the plate and eluted sequentially with DCM, DCM-ethyl acetate (1:1, v/v, applied twice), ethyl acetate, and ethyl acetate containing 0.25% - 0.5% methanol. Evaporation of the solvent yielded compound 1 (3 mg) as a light brown to colorless oil. The structure of the isolated pure compound was elucidated through extensive 1D and 2D NMR spectroscopic analysis (¹H, ¹³C, COSY, HSQC, and HMBC) [27] [28].

2.6. Protein Preparation, Molecular Docking, and Interaction Analysis

The crystal structures of rat neuronal nitric oxide synthase (PDB ID: 6NGJ; 1.76 Å), glutathione reductase (PDB ID: 3GRS; 1.54 Å), DNA gyrase (PDB ID: 1KZN; 2.30 Å) and penicillin-binding protein 2X (PDB ID: 5OJ0; 2.66 Å) were retrieved from the Protein Data Bank based on their reported relevance to antioxidant and antimicrobial activities [29]-[32]. The proteins were prepared and energy-minimized using Swiss-PdbViewer. The isolated Dendryphiellin I and reference compounds were prepared as ligands, and molecular docking was performed using PyRx with AutoDock Vina. The grid-box dimensions and center coordinates were specified along the X, Y, and Z axes. For 6NGJ, the grid-box dimensions were 69.341 × 79.512 × 59.232 Å, with center coordinates of X = 11.464, Y = 4.904, and Z = 22.600 Å. For 3GRS, the dimensions were 95.182 × 48.242 × 69.517 Å, with center coordinates of X = 70.381, Y = 51.349, and Z = 18.712 Å. For 1 KZN, the dimensions were 46.482 × 46.891 × 41.213 Å, with center coordinates of X = 19.519, Y = 19.178, and Z = 43.213 Å. For 5OJ0, the dimensions were 64.440 × 63.051 × 108.451 Å, with center coordinates of X = 47.963, Y = 14.717, and Z = 31.4596 Å. The resulting ligand-receptor complexes were analyzed using BIOVIA Discovery Studio Visualizer to identify and characterize intermolecular interac-

tions, including conventional hydrogen bonds, carbon-hydrogen interactions, alkyl interactions, π -alkyl interactions, and other non-covalent interactions. The binding affinities obtained from AutoDock Vina were used to compare the predicted binding potential of Dendryphiellin I with that of the respective reference compounds [33]. To assess the reliability and reproducibility of the molecular docking protocol, a validation procedure was performed for the selected protein targets. For 6NGJ, 1KZN, and 5OJ0, conventional ligand redocking was performed using the experimentally determined co-crystallized ligands present in the respective crystal structures. The crystallographic ligands were removed from the corresponding protein structures and independently redocked into their original binding sites using the same docking software, search parameters, and docking protocol applied to the test compounds. The predicted binding poses were compared with the experimentally observed crystallographic poses by superimposition, and the root-mean-square deviation (RMSD) between the corresponding ligand atoms was calculated [34]. For 3GRS, validation was performed using a literature-guided binding-site and reference-ligand approach because conventional crystallographic ligand-redocking was not employed. The functionally characterized FAD/redox-active region of glutathione reductase was selected as the docking site based on previously reported structural and molecular docking studies [34] [35]. The binding-site region comprised VAL49, GLU50, SER51, HIS52, GLY56, THR57, CYS58, VAL61, GLY62, CYS63, LYS66, GLY128, HIS129, ALA130, ALA155, THR156, GLY157, TYR197, ARG291, GLY330, ASP331, LEU337, LEU338, THR339, and PRO340 [36]. The docking grid was centered at X = 59.698, Y = 52.917, and Z = 18.674 Å, with the grid dimensions set to 25 × 25 × 25 Å. Butylated hydroxytoluene (BHT), a previously reported reference antioxidant, was used as a reference ligand and docked into the selected binding region using the same docking protocol. The resulting binding mode and interactions of BHT were compared with the previously reported literature findings to support the appropriateness of the selected binding site and docking procedure [36].

2.7. Drug-Likeness, Pharmacokinetics, and Toxicological Profiling

In silico ADME and toxicity profiles of the isolated compound and reference compounds were evaluated using SwissADME and pkCSM web-based platforms, respectively. Swiss ADME was used to predict physicochemical properties, lipophilicity, gastrointestinal absorption, blood-brain barrier permeability, and drug-likeness based on Lipinski's rule of five [37] [38]. The toxicity-related pharmacokinetic parameters were further estimated using the pkCSM platform [39].

3. Results and Discussion

3.1. Phytochemical Analysis

The phytochemical profiling shows a better response for different groups of compounds in methanolic extracts. The FT-IR spectrum showed different character-

istic absorption bands (Figure 1 and Table 1). The ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) spectra of the plant crude extracts also displayed multiple chemical shifts (Figure 2 and Figure 3 and Table 2).

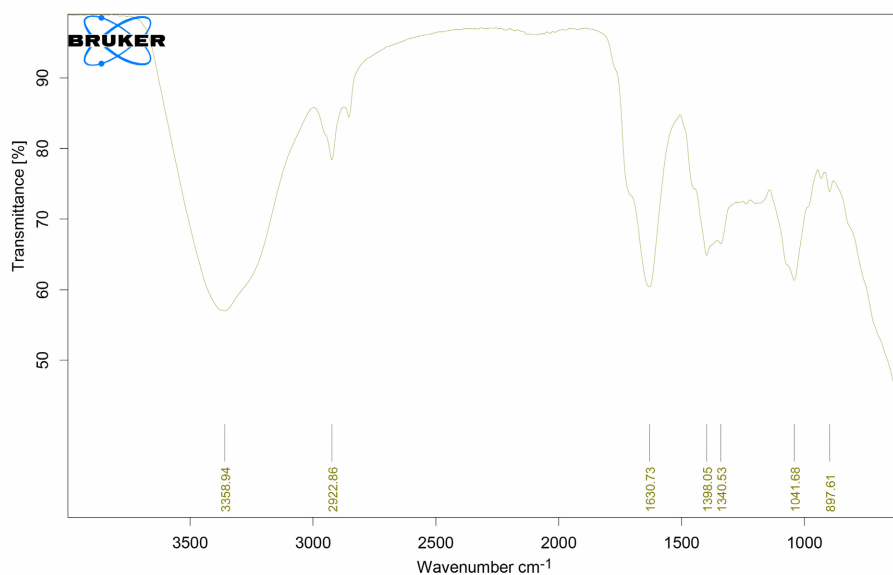


Figure 1. FTIR spectra of *Alternanthera philoxeroides*.

Table 1. FT-IR spectrum peaks of plant crude extracts and their possible assigned functional groups [40].

Peak frequency (cm^{-1})	Possible functional groups
3358.94	Alcohols, Phenols
2922.86	Alkanes(stretch)
1707.44, 1732.50, 1709.23, 1739.39, 1737.75, 1698.73	Ketone, Carboxylic acid, Ester
1630.73	Primary and secondary amines and amides(bend)
1398.05	Fluoride
1340.53	Sulfones, sulfonyl chlorides, sulfates, sulfonamides
1041.68	Sulfoxides, Amines, Alcohols, ethers, esters, carboxylic acids, anhydrides, Fluoride
897.61	Aromatics, Alkenes

Table 2. Compounds characterized via ^1H NMR spectroscopy.

Serial No.	ppm	Compounds
1	0.835 - 1.733	Alkanes
2	1.606 - 2.632	Alkenes
3	1.889 - 1.969	Alkynes
4	2.137 - 2.911	Ketone, amide, nitriles
5	3.191 - 4.901	Ethers, esters, nitro alkanes

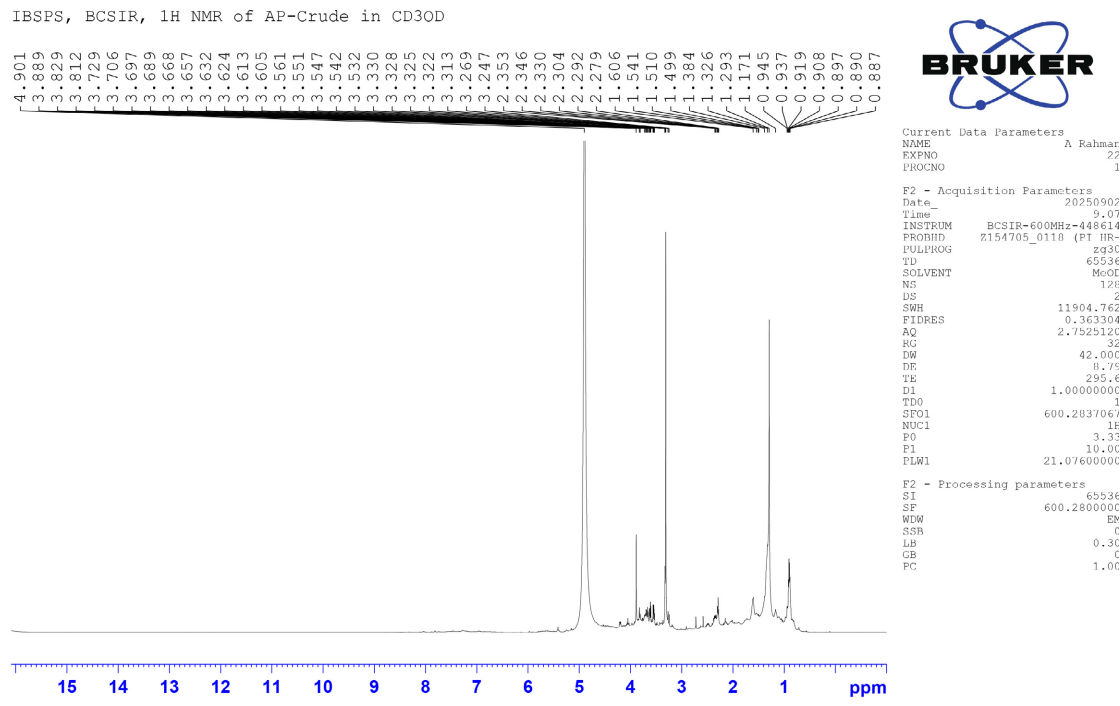


Figure 2. ¹H nuclear magnetic resonance (NMR) analysis of the plant's crude extract.

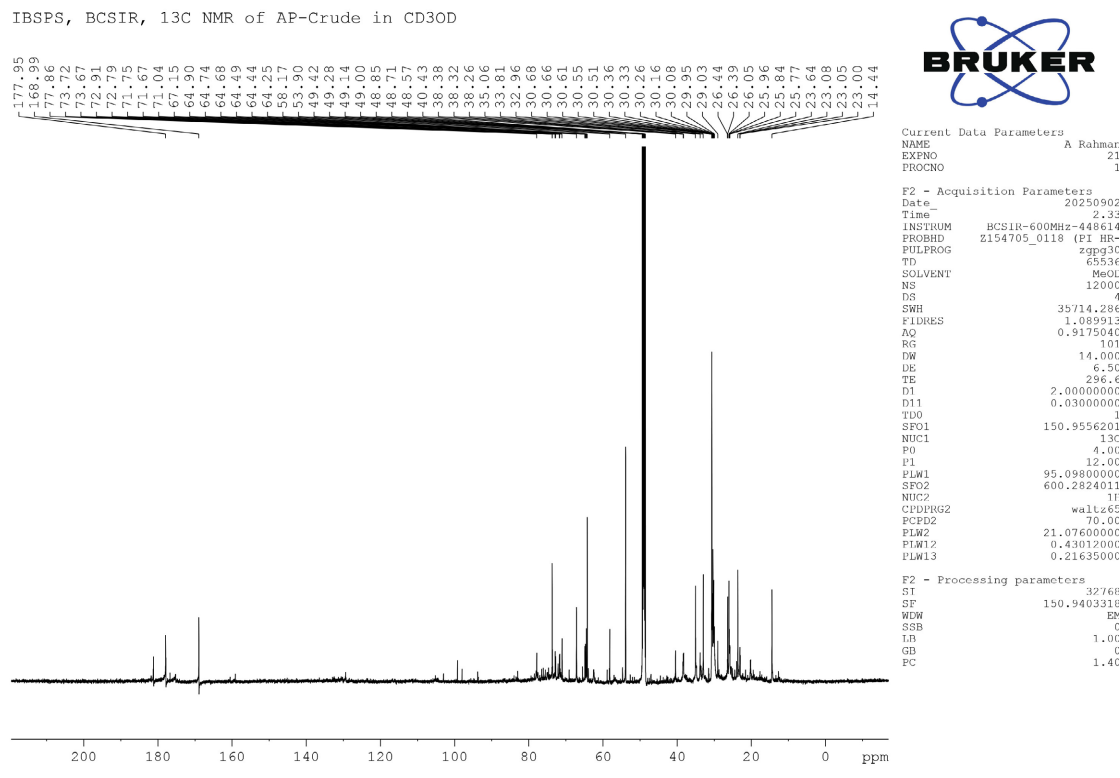


Figure 3. ¹³C NMR spectrum of plant crude extract.

3.2. Evaluation of Antimicrobial Activity

The disc diffusion method was used to evaluate antimicrobial activity. The crude

plant extract showed no activity against the bacterial strain *S. typhi* or against all fungal strains (Table 3). The antibacterial and antifungal reference compounds, kanamycin and ketoconazole, demonstrated the greatest potency against the tested bacterial and fungal strains. As anticipated, the solvent methanol produced no activity. The plant extract produced the strongest antimicrobial activity against the bacterial strains *B. cereus* (11.5 mm) and *P. aeruginosa* (12 mm), and it showed only mild activity against the bacterial strains *S. aureus* (9 mm) and *E. coli* (9 mm).

Table 3. Antimicrobial activity of crude extract of *Alternanthera philoxeroides* (Mart.) Griseb. at a concentration of 500 µg/disc.

Sample	Diameter of the inhibition zone (mm) ^a						
	<i>B. cereus</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>A. niger</i>	<i>A. flavus</i>
AP	11.5	9	12	9	-	-	-
Kanamycin (30 µg/disc)	35	46	45	32	30	nd	nd
Ketoconazole (30 µg/disc)	nd	nd	nd	nd	nd	39	40
Solvent (Methanol)	-	-	-	-	-	-	-

^a“-” indicates no activity detected and “nd” indicates not done.

3.3. Antioxidant Assay

The antioxidant activity of the methanolic crude extract of *Alternanthera philoxeroides* was evaluated using the DPPH radical-scavenging assay. The extract exhibited concentration-dependent radical-scavenging activity, with increasing inhibition observed at higher concentrations. The IC₅₀ value of the crude extract was 127.48 ± 0.53 µg/mL, whereas the reference antioxidants ascorbic acid and butylated hydroxyanisole (BHA) showed substantially stronger activity, with IC₅₀ values of 4.31 ± 0.07 and 3.40 ± 0.08 µg/mL, respectively (Figure 4). Thus, the crude extract demonstrated moderate DPPH radical-scavenging activity compared with the reference standards.

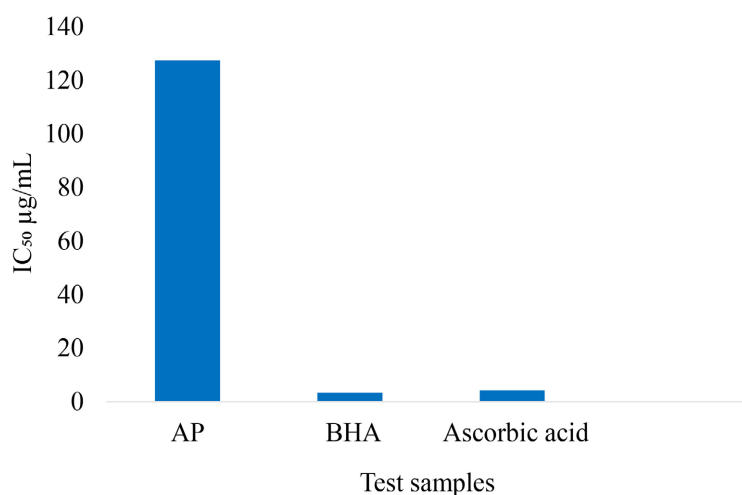


Figure 4. Free radical scavenging activity of crude extract and standards.

3.4. Molecular Identification and Phylogenetic Analysis of Fungal Isolate APLE-2

The assembled sequence was obtained from the forward-primer sequence and cleaned by removing the first 18 base pairs based on the chromatogram inspection. The cleaned sequence was then subjected to BLASTn analysis against the NCBI database, followed by phylogenetic analysis using MEGA 12, and the top-scoring hit was selected as the putative identification of the fungal strain. The fungal isolate's sequence was successfully amplified and compared against the NCBI database using the BLASTn algorithm. The sequence produced significant alignments with several species within the genus *Curvularia*, a diverse group known for its endophytic and pathogenic lifestyles in plants. The ITS rDNA sequence obtained from isolate APLE-2 was subjected to BLASTn analysis against the NCBI GenBank database. The sequence exhibited its highest similarity (96.91% identity; 99% query coverage; E-value = 0.0) to the ITS sequence of *Curvularia lunata* CBS 730.96 (type material; GenBank accession NR_138223.1). Phylogenetic analysis based on ITS sequences further confirmed the placement of APLE-2 within the genus *Curvularia*. However, APLE-2 did not form a well-supported sister relationship or cluster directly with *C. lunata* CBS 730.96, but instead occurred on a distinct lineage in the reconstructed tree. Therefore, considering both the BLASTn results and the phylogenetic placement, APLE-2 was designated *Curvularia* sp. Further multi-locus sequence analysis incorporating additional phylogenetically informative loci would be necessary to achieve robust species-level identification. Following taxonomic confirmation, the validated ITS sequence will be submitted to the NCBI GenBank database for accession number assignment (**Figure 5** and **Figure 6**).

ITS rDNA Sequence of the fungal isolate APLE-2:

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GCACGAGGTCAACGTGATAAGGAGGCTTGATGGATGCCGTTTGTCTGATGGCAAGC
GCAAAATGTGCTGCGCTGCGAAACCAGAAAGGCCGGCTGCCAATCACTTTAAGGCG
AGTCTTTGGGCGACCAAAGACAAAAACGCCCAACACCAAGCAAAGCTTGAGGGTAC
AAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTGCGTTC
AAAGATTCGATGATTCATCTGAATTCTGCAATTCACACTACGTATCGCATTCGCTGCG
TTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAAATGATTTACA
TTGTTTGTACTGACGCTGATTGCAACTGCATAAAAAGGTTTATTTGGTGGTCCTGGTG
GCGAGCGACCCGCCAGGAACAACAAGTGCGCAAAGACAAGGGTAATAAATACTC
CAGCTGCGACAGCCTTCATATTGTATTGTGTAATGATCCCTCCGACGGTCACCTACG
GAGACTTGTACAATTTTACTTCCTCAATGACAAGAA
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3.5. NMR Spectral Analysis of the Secondary Metabolite, Compound 1

The isolated compound (3 mg), with the molecular formula $C_{25}H_{32}O_5$, an exact mass of 412.22 Da, and an R_f value of 0.53, was characterized as Dendryphiellin I based on its ^1H and ^{13}C NMR spectroscopic data (**Figure 7** and **Figure 8**), which were consistent with the reported spectroscopic data for Dendryphiellin I. The ^{13}C NMR spectrum exhibited 25 carbon signals. In the ^1H NMR spectrum, an aldehy-

dic proton was observed at δ H 9.52 (1H, s), together with characteristic olefinic signals at δ H 7.30 (1H, d, J = 15.6 Hz), 5.66 (1H, d, J = 9.6 Hz), 6.85 (1H, s), 6.28 (1H, dd, J = 9.9, 5.1 Hz), 6.41 (1H, d, J = 9.6 Hz), 6.01 (1H, s) and 5.77 (1H, d, J = 15.6 Hz). The ^{13}C NMR spectrum showed characteristic signals at δ C 197.2, 192.8, 167.2, 162.0, 154.6, 149.2, and 74.9 ppm, corresponding to carbonyl, aldehyde, olefinic, oxygenated, methine, methylene, and methyl carbons (Table 4).

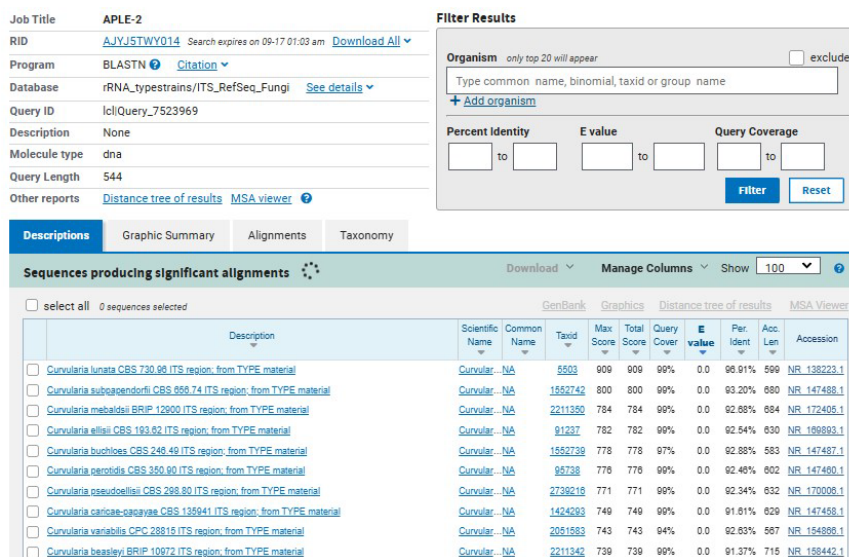


Figure 5. BLAST result of the fungal isolate APLE-2.

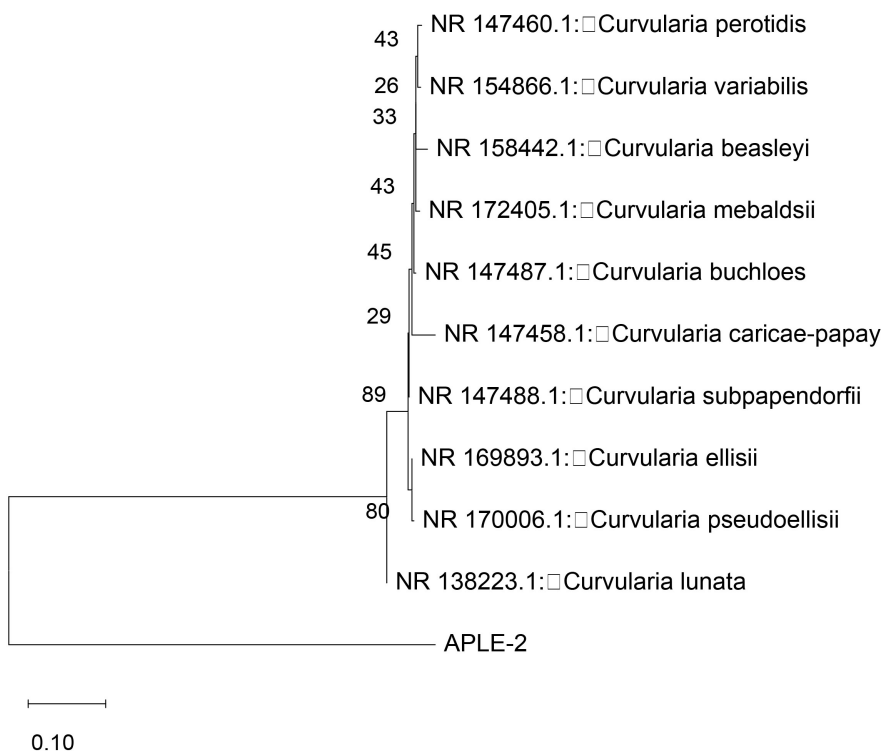


Figure 6. Phylogenetic analysis of fungal isolate APLE-2 based on ITS rDNA sequences.

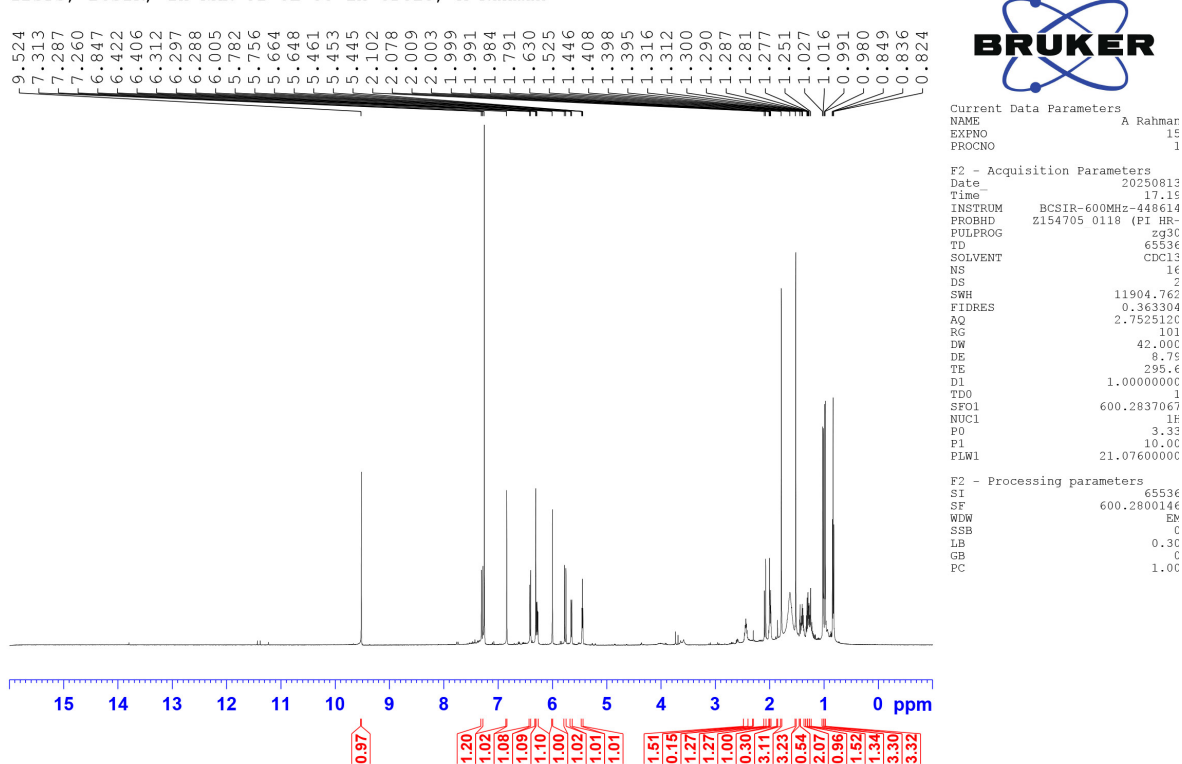
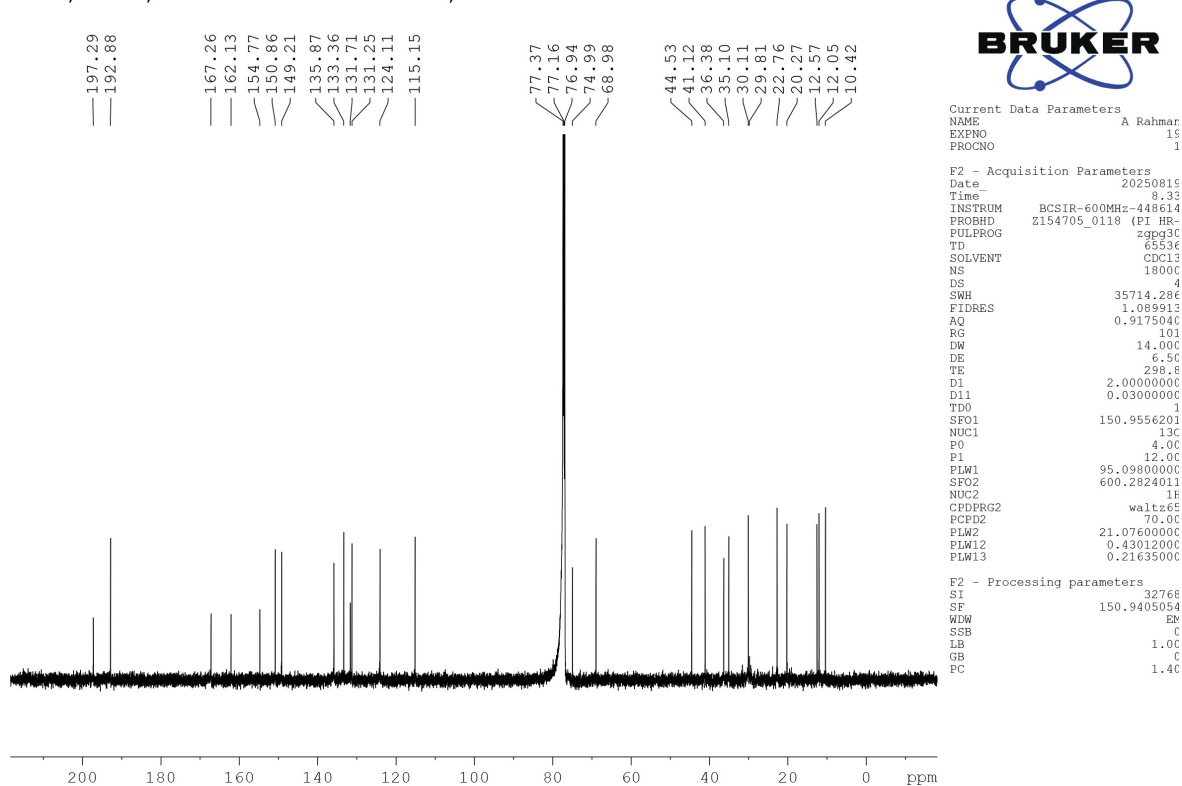
IBSPS, BCSIR, ¹H NMR of CL-55 in CDCl₃, A Rahman**Figure 7.** ¹H NMR spectrum (600 MHz, CDCl₃) of compound 1.IBSPS, BCSIR, ¹³C NMR of CL-55 in CDCl₃, A Rahman**Figure 8.** ¹³C NMR spectrum (150 MHz, CDCl₃) of compound 1.

Table 4. ^1H (600 MHz, CDCl_3) and ^{13}C (150 MHz, CDCl_3) NMR data for compound 1 with reference data [41].

No.	δ_{C} ppm	δ_{H} ppm	Type	Reference value of compound 1 in CD_3OD	
				δ_{C} ppm	δ_{H} ppm
1	197.2		C=O	198.0	
2	192.8	9.52 (1H, s)	CHO	194.9	9.51 (1H, s)
3	167.2		C	168.6	
4	162.0		C	163.5	
5	154.6		C	156.4	
6	150.8	7.30 (1H, d, J = 15.6 Hz)	CH	151.9	7.32 (1H, d, J = 15.6 Hz)
7	149.2	5.66 (1H, d, J = 9.6 Hz)	CH	150.2	5.71 (1H, d, J = 9.9 Hz)
8	135.8	6.85 (1H, s)	CH_2	136.9	6.83 (1H, brs)
		6.31 (1H, s)			6.41 (1H, brs)
9	133.3	6.28 (1H, dd, J = 9.9, 5.1 Hz)	CH	134.0	6.28 (1H, dd, J = 9.8, 5 Hz)
10	131.6			133.1	
11	131.1	6.41 (1H, d, J = 9.6 Hz)	CH	132.2	6.51 (1H, d, J = 9.8 Hz)
12	124.0	6.01 (1H, s)	CH	125.0	5.92 (1H, s)
13	115.0	5.77 (1H, d, J = 15.6 Hz)	CH	116.0	5.82 (1H, d, J = 15.6 Hz)
14	74.9	-	C	75.9	-
15	68.8	5.45 (1H, t, J = 4.8 Hz)	CH	70.6	5.46 (1H, dd, J = 5, 5 Hz)
		1.99 (1H, d, J = 14.4 Hz)			2.01 (1H, d, J = 14.3 Hz)
16	44.4	2.10 (1H, d, J = 14.4 Hz)	CH_2	46.4	2.09 (1H, d, J = 14.3 Hz)
		2.01 (1H, d, J = 14.4 Hz)			1.99 (1H, dq, J = 5.0, 7.1 Hz)
17	41.0		CH	44.8	
18	36.3	-	C	37.5	-
19	35.0	2.44 (1H, m)	CH	36.2	2.5 (1H, m)
		1.42 (1H, m)			1.44 (1H, m)
20	30.0	1.29 (1H, m)	CH_2	31.0	1.32 (1H, m)
		1.53 (3H, s)			1.56 (3H, s)
21	22.7		CH_3	23.0	
22	20.2	0.99 (3H, d, J = 6.6 Hz)	CH_3	20.5	1.01 (3H, d, J = 6.6 Hz)
23	12.5	1.79 (3H, s)	CH_3	12.5	1.82 (3H, s)
24	12.0	0.84 (3H, t, J = 7.2 Hz)	CH_3	12.3	0.87 (3H, t, J = 7.4 Hz)
25	10.3	1.02 (3H, d, J = 7.2 Hz)	CH_3	9.2	1.02 (3H, d, J = 7.1 Hz)

3.6. Computational Studies against Antioxidant Targets

Molecular docking analysis was performed to investigate the binding potential of Dendryphiellin I against the selected antioxidant target proteins, 6NGJ and 3GRS. Dendryphiellin I exhibited binding affinities of -8.5 kcal/mol and -8.1 kcal/mol against 6NGJ and 3GRS, respectively, which were considerably higher in magnitude than those of the reference compound ascorbic acid (-6.5 and -5.6 kcal/mol, respectively) (Table 5). These results suggest that Dendryphiellin I showed a comparatively stronger predicted binding affinity toward both antioxidant targets than the reference compound. The interaction analysis showed that Dendryphiellin I established multiple non-covalent interactions within the binding sites of

both target proteins (**Table 6**). In the 6NGJ complex, the ligand was involved in conventional hydrogen bonding with TRP409, TRP587, and TYR706, with bond lengths of 2.57 - 2.96 Å. Additional alkyl, π -alkyl, and carbon-hydrogen interactions were observed with several neighboring amino acid residues, further contributing to ligand stabilization within the binding pocket. In comparison, ascorbic acid formed conventional hydrogen bonds with SER413, ARG414, ASN411, TYR698, and GLU705, with bond distances of 2.18 - 2.87 Å, together with additional carbon-hydrogen interactions.

Table 5. Binding affinities of Dendryphiellin I and reference compounds against selected antioxidant and antimicrobial target proteins.

Compound	Antioxidant targets		Antimicrobial targets	
	6NGJ	3GRS	1KZN	5OJ0
Dendryphiellin I (CID: 139590020)	-8.5	-8.1	-7.3	-8.5
Ascorbic Acid (CID: 54670067)	-6.5	-5.6	-	-
Kanamycin (CID: 6032)	-	-	-7.0	-7.4

Table 6. Non-bonding interactions of Dendryphiellin I and reference compound ascorbic acid with antioxidant target proteins 6NGJ and 3GRS.

Ligands	6NGJ				3GRS			
	Binding affinity (kcal/mol)	Residues in contact	Interaction type	Bond distance (Å)	Binding affinity (kcal/mol)	Residues in contact	Interaction type	Bond distance (Å)
Dendryphiellin I	-8.5	TRP409	CHB	2.96	-8.1	ASN294	CHB	2.21
		TRP587N:	CHB	2.91		VAL61	A	4.85
		TYR706	CHB	2.57		LYS53	A	4.68
		GLY586	CH	2.49		VAL107	A	3.54
		ALA412	A	5.03		VAL107	A	4.16
		CYS415	A	4.93		HIS52	PA	4.18
		VAL567	A	4.55		-	-	-
		CYS415	A	3.70		-	-	-
		ARG414	A	3.72		-	-	-
		PHE584	PA	4.85		-	-	-
Ascorbic Acid	-6.5	SER413	CHB	2.87	-5.6	GLY158	CHB	2.93
		ARG414	CHB	2.18		GLU50	CHB	2.16
		ASN411	CHB	2.87		GLU50	CHB	2.10
		TYR698	CHB	2.75		ALA155	CHB	2.43
		GLU705	CHB	2.37		GLY157	CH	2.11
		ALA412	CH	2.42		THR156	CH	2.18
		SER413	CH	2.62		GLU50	CH	2.61
		PHE704	CH	2.98		-	-	-

A = Alkyl, PA = Pi-Alkyl, CHB = Conventional Hydrogen Bond, CH = Carbon Hydrogen Bond.

For the 3GRS complex, Dendryphiellin I showed a conventional hydrogen-bond interaction with ASN294 (2.21 Å), along with additional alkyl and π -alkyl interactions involving residues including VAL61, LYS53, VAL107, and HIS52. Ascorbic acid, on the other hand, exhibited conventional hydrogen-bond interactions with GLY158, GLU50, and ALA155, together with carbon-hydrogen interactions involving GLY157, THR156, and GLU50. The overall interaction profiles and binding poses of Dendryphiellin I and ascorbic acid within the respective target proteins are presented in **Figure 9**.

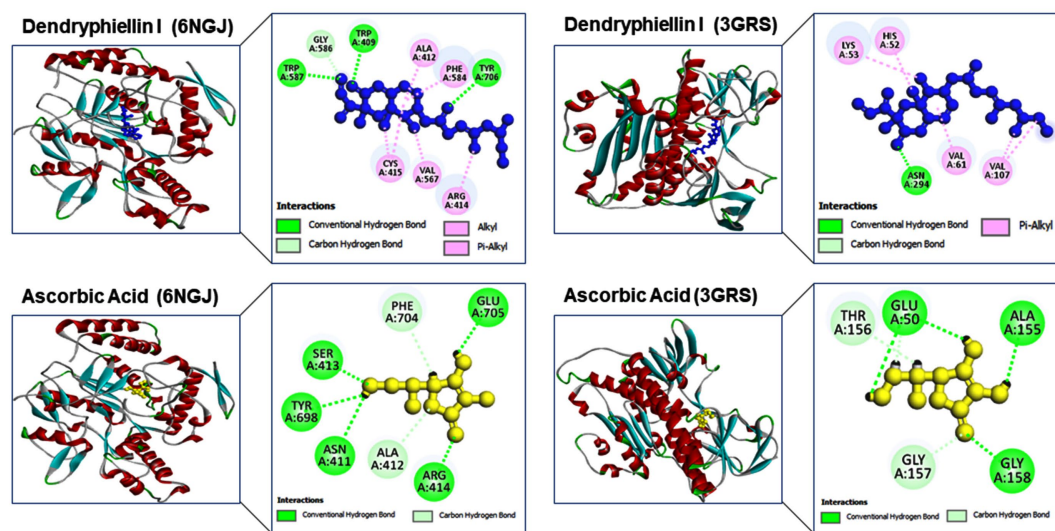


Figure 9. Molecular docking interactions and binding poses of Dendryphiellin I and the standard drug against the selected antioxidant target proteins, PDB ID: 6NGJ and PDB ID: 3GRS.

3.7. Computational Studies against Antimicrobial Targets

Molecular docking analysis was further performed to evaluate the binding potential of Dendryphiellin I against the selected antimicrobial target proteins, 1 KZN and 5OJ0. As shown in **Table 5**, Dendryphiellin I exhibited binding affinities of -7.3 kcal/mol against 1 KZN and -8.5 kcal/mol against 5OJ0. The corresponding binding affinities of the reference drug kanamycin were -7.0 and -7.4 kcal/mol, respectively. Thus, Dendryphiellin I showed slightly stronger predicted binding toward 1 KZN and a comparatively stronger binding affinity toward 5OJ0 than kanamycin.

The interaction analysis revealed that Dendryphiellin I engaged in multiple noncovalent interactions with residues within the binding sites of both antimicrobial targets (**Table 7**). Against 1 KZN, Dendryphiellin I formed conventional hydrogen-bond interactions with ALA96 at distances of 2.47 and 2.69 Å, together with carbon-hydrogen interactions involving HIS95 (2.71 - 2.97) Å and GLY119 (2.84 Å). Additional π -alkyl interactions were observed with TYR568 and PHE570, while alkyl interactions involved ALA47, ILE78, ILE90, VAL43, VAL120, and VAL167. In comparison, kanamycin formed several conventional hydrogen bonds with ASN46, GLY77, ALA96, VAL120, ASP73, THR165, and GLU50, with bond

distances ranging from 2.09 to 2.98 Å, along with additional carbon-hydrogen interactions.

For 5OJ0, Dendryphiellin I showed a conventional hydrogen-bond interaction with HIS594 (2.85 Å). It also exhibited π -alkyl interactions with TRP374, TYR568, PHE570, and HIS594 within the binding pocket. Kanamycin displayed several conventional hydrogen-bond interactions involving ASN185, ALA312, ARG186, ASP313, ALA153, LEU130, and TYR129, with bond distances ranging from 2.28 to 2.90 Å, in addition to carbon-hydrogen interactions. The predicted binding modes and intermolecular interactions of Dendryphiellin I and kanamycin with the selected antimicrobial targets are presented in **Figure 10**.

Table 7. Non-bonding interactions of the isolated compound and kanamycin with antimicrobial target proteins 1 KZN and 5OJ0.

Ligands	1 KZN				5OJ0			
	Binding affinity (kcal/mol)	Residues in contact	Interaction type	Bond distance (Å)	Binding affinity (kcal/mol)	Residues in contact	Interaction type	Bond distance (Å)
Dendryphiellin I	-7.3	ALA96	CHB	2.69	-8.5	HIS594	CHB	2.85
		ALA96	CHB	2.47		TRP374	PA	4.97
		HIS95	CH	2.97		TRP374	PA	4.99
		HIS95	CH	2.71		TYR568	PA	4.46
		GLY119	CH	2.84		PHE570	PA	5.45
		ALA47	A	3.89		HIS594	PA	4.56
		ILE90	A	4.76		-	-	-
		ILE90	A	5.02		-	-	-
		ILE78	A	4.97		-	-	-
		ILE78	A	5.35		-	-	-
		VAL120	A	4.61		-	-	-
		VAL167	A	4.52		-	-	-
		VAL43	A	5.42		-	-	-
Kanamycin	-7.0	ASN46	CHB	2.42	-7.4	ASN185	CHB	2.35
		GLY77	CHB	2.70		ALA312	CHB	2.70
		ALA96	CHB	2.86		ARG186	CHB	2.32
		VAL120	CHB	2.09		ASP313	CHB	2.28
		ASP73	CHB	2.98		ALA153	CHB	2.81
		THR165	CHB	2.63		LEU130	CHB	2.90
		GLY77	CHB	2.63		TYR129	CHB	2.79
		GLU50	CHB	2.76		LEU130	CH	2.58
		ALA96	CH	3.02		ASN185	CH	2.43
		THR165	CH	2.81		ASP313	CH	2.62

A = Alkyl, PA = Pi-Alkyl, CHB = Conventional Hydrogen Bond, CH = Carbon Hydrogen Bond.

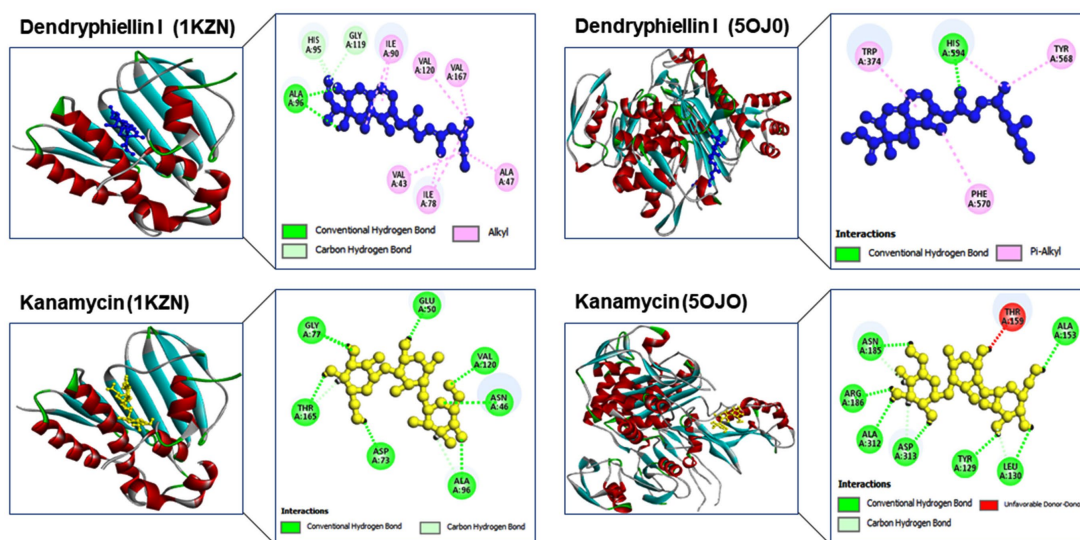


Figure 10. Molecular docking interactions and binding poses of Dendryphiellin I and the standard drug against the selected antibacterial target proteins, PDB ID: 1 KZN and PDB ID: 5OJ0.

3.8. Docking Protocol Validation

Docking protocol validation was assessed by redocking the experimentally determined co-crystallized ligands into their corresponding binding sites. The calculated RMSD values were 2.40 Å, 3.60 Å, and 2.03 Å for 6 NGJ, 1 KZN, and 5OJ0, respectively (**Figure 11**). Although these values were slightly above or, in the case of 1 KZN, clearly above the commonly applied 2.0 Å threshold, the redocking results provided partial structural support for the selected docking protocols. The higher RMSD observed for 1 KZN indicates limited reproduction of the crystallographic ligand orientation and was therefore not interpreted as definitive validation. For 3 GRS, conventional redocking validation was not performed because an experimentally resolved co-crystallized ligand suitable for this purpose was not available. Instead, the docking site was defined based on the reported FAD/redox-active region of glutathione reductase, and BHT was docked as a reference antioxidant ligand. The resulting pose was located within the reported functional region, providing structural support for the selected binding site rather than formal validation of the docking protocol. Accordingly, the docking results were interpreted as predictive/supportive evidence and were considered together with the experimental findings rather than as definitive proof of binding.

3.9. Drug Likeness and ADMET Analysis of Selected Compounds

The predicted drug-likeness and ADMET profiles of Dendryphiellin I and the reference compounds are presented in **Table 8**. Dendryphiellin I showed high gastrointestinal absorption and no predicted blood-brain barrier permeability. It complied with Lipinski's rule of five without violations and showed a bioavailability score of 0.55. However, it exhibited relatively high lipophilicity (XLOGP3 = 5.14) and was predicted to inhibit CYP3A4. In comparison, ascorbic acid also

showed high gastrointestinal absorption and no Lipinski violations, whereas kanamycin showed low gastrointestinal absorption and two Lipinski violations. Dendryphiellin I showed a lower predicted aqueous solubility (ESOL logS = -5.11) than ascorbic acid (logS = 0.23) and kanamycin (logS = 1.93), indicating comparatively lower solubility. Its predicted skin permeability (log Kp = -5.17) was higher than that of ascorbic acid (-8.54) and kanamycin (-14.19). None of the tested compounds were predicted to cause hepatotoxicity or inhibit hERG I/II channels.

Overall, Dendryphiellin I exhibited drug-likeness comparable to ascorbic acid, while showing a high predicted gastrointestinal absorption profile and better compliance with Lipinski's rule than kanamycin.

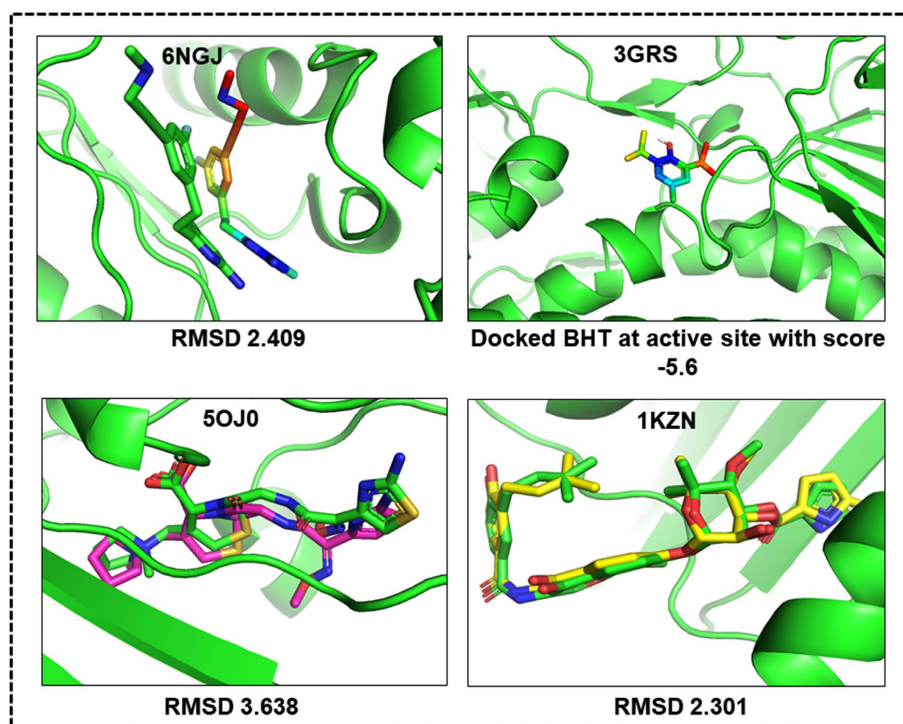


Figure 11. Docking validation of the selected protein targets.

Table 8. Drug-likeness and ADMET analysis of studied compounds.

Properties	Attributes	Dendryphiellin I (CID:139590020)	Ascorbic Acid (CID: 54670067)	Kanamycin (CID: 6032)
Physicochemical properties	M. W. (g/mol)	412.52	176.12	484.50
	Heavy atoms	30	12	33
	Arom. heavy atoms	0	0	0
	Rotatable bonds	8	2	6
	H-bond acceptors	5	6	15
	H-bond donors	1	4	11
	Molar Refractivity	118.32	35.12	105.60

Continued

Lipophilicity	Log Po/w(XLOGP3)	5.14	−1.64	−6.95
Water Solubility	Logs (ESOL)	−5.11 (Low predicted aqueous solubility)	0.23 (Highly soluble)	1.93 (Highly soluble)
Pharmacokinetics	GI absorption	High	High	Low
	BBB permeant	No	No	No
	CYP1A2 inhibitor	No	No	No
	CYP2C19 inhibitor	No	No	No
	CYP2C9 inhibitor	No	No	No
	CYP2D6 inhibitor	No	No	No
	CYP3A4 inhibitor	Yes	No	No
	Log Kp (skin permeation) (cm/s)	−5.17	−8.54	−14.19
Drug-likeness	Lipinski	Yes, 0 violations	Yes, 0 violations	No, 2 violations
	Bioavailability Score	0.55	0.56	0.17
Med. Chemistry	Synthetic accessibility	5.63	3.47	6.44
Toxic properties	Max. tolerated dose in human, Log (mg/kg/day)	−0.083	1.598	0.93
	Hepatotoxicity	No	No	No
	hERG I inhibitor	No	No	No
	hERG II inhibitor	No	No	No
	Oral Rat Acute Toxicity (LD50) (mol/kg)	1.839	1.063	2.295

4. Discussion

The FT-IR and NMR spectroscopic profiles of the methanolic extract of *Alternanthera philoxeroides* indicated the presence of structurally diverse secondary metabolites. The prominent FT-IR bands at 3358.94, 2922.86, and 1698.73 - 1739.39 cm^{-1} were attributable to hydroxyl, aliphatic C-H, and carbonyl functional groups, respectively. The absorption at 1630.73 cm^{-1} was suggestive of amine/amide or conjugated functionalities, whereas the band at 897.61 cm^{-1} could be assigned to C-H bending vibrations associated with aromatic or alkene moieties [40]. These observations indicate the presence of chemically diverse constituents in the crude extract; however, FT-IR analysis alone does not permit definitive identification of individual metabolites. In the ^{13}C NMR spectra, signals observed at δ 110.0 - 170.0 ppm were indicative of aromatic carbons associated with various classes of secondary metabolites. Signals in the δ 80.0 - 150.0 ppm range corresponded to olefinic carbons. Additionally, sharp resonances at δ 3.20 - 4.00 ppm in the ^1H NMR and δ 50.0 - 65.0 ppm in the ^{13}C NMR suggested the presence of methoxy functionalities in the metabolites, and some of the observed ^{13}C NMR resonances were compatible with structural features reported for isocoumarin- and steroid-related compounds [42]. The ^1H and ^{13}C NMR spectra further indicated the presence of

aliphatic, olefinic, oxygenated, and carbonyl-containing metabolites. Several chemical-shift regions observed in the crude extract were consistent with structural features reported for different classes of natural products. In particular, resonances in the aromatic and oxygenated carbon regions may be associated with flavonoid-type metabolites [43]. However, because the spectra were obtained from a crude extract containing multiple constituents, these observations should be regarded as tentative indications of metabolite classes rather than definitive compound identification.

Previous studies have reported antibacterial and antioxidant activities associated with *A. philoxeroides* and its endophytic fungal isolates. In our previous study, the fungal crude extract APLE-2, isolated from *A. philoxeroides*, exhibited stronger DPPH radical-scavenging activity ($IC_{50} = 47.83 \mu\text{g/mL}$) and antibacterial activity against *B. cereus*, *S. aureus*, *P. aeruginosa*, *E. coli*, and *S. typhi*, with inhibition zones of 18.5, 26, 29.5, 17.5, and 17 mm, respectively [22] [44] [45]. These findings indicate that the endophytic fungal isolate may represent an additional source of biologically active secondary metabolites. The close agreement between the experimental ^1H and ^{13}C NMR data and previously reported spectral data supported the identification of the isolated compound as Dendryphiellin I [41]. The identification was further supported by the characteristic aldehydic and olefinic proton signals and the corresponding carbon resonances observed in the present study.

The in silico study provided preliminary molecular support for the potential biological activities of Dendryphiellin I isolated from the endophytic fungus *Curvularia* sp. Dendryphiellin I showed favorable predicted binding affinities for the selected antioxidant-related targets 6NGJ and 3GRS, with scores of -8.5 and -8.1 kcal/mol, respectively, compared with -6.5 and -5.6 kcal/mol for ascorbic acid. The ligand formed several non-covalent interactions, including conventional hydrogen bonds, alkyl, π -alkyl, and carbon-hydrogen interactions, which may contribute to stabilization of the ligand-protein complexes. These findings provide preliminary molecular-level support for the potential involvement of Dendryphiellin I in antioxidant-related mechanisms. However, the previously reported DPPH radical-scavenging activity was obtained from the crude fungal extract (APLE-2; $IC_{50} = 47.83 \mu\text{g/mL}$) and therefore cannot be attributed exclusively to Dendryphiellin I. Against the selected antibacterial targets 1 KZN and 5OJ0, Dendryphiellin I exhibited binding affinities of -7.3 and -8.5 kcal/mol, respectively, compared with -7.0 and -7.4 kcal/mol for kanamycin. The docking analysis revealed that Dendryphiellin I formed several stabilizing interactions with residues lining the target binding pockets, including hydrogen bonds, alkyl, π -alkyl, and carbon-hydrogen interactions. These predicted interactions are in agreement with the previously reported antibacterial activity of Dendryphiellin I [46], providing preliminary computational evidence for its potential antibacterial properties. Collectively, the docking results indicate that Dendryphiellin I may contribute to the biological activity of *Curvularia* sp. Nevertheless, experimental studies with the purified compound are necessary to confirm these predictions and elucidate the mechanisms underlying its activity. ADMET profiling further suggested favorable

drug-like properties, including compliance with Lipinski's rule of five and high predicted gastrointestinal absorption. However, its relatively low predicted aqueous solubility, high lipophilicity, and potential CYP3A4 inhibitory activity require further investigation.

Overall, *A. philoxeroides* exhibited considerable phytochemical diversity, together with moderate antioxidant and selective antibacterial activities, while its endophytic fungus *C. lunata* yielded Dendryphiellin I. The predicted binding affinity, molecular interactions, and selected favorable ADMET characteristics indicate that Dendryphiellin I merits further investigation as a potentially bioactive fungal metabolite. However, these computational findings should be regarded as preliminary and require confirmation through appropriate *in vitro* and *in vivo* experimental studies.

5. Conclusion

The methanolic extract of *Alternanthera philoxeroides* displayed a broad phytochemical profile, as shown by FTIR and $^1\text{H}/^{13}\text{C}$ NMR analyses, and it exhibited moderate antioxidant activity together with selective antibacterial activity. An endophytic fungal isolate (APLE-2) isolated from *A. philoxeroides* was identified as *Curvularia* sp.; from this fungus, Dendryphiellin I was obtained and structurally characterized. Molecular docking studies demonstrated that Dendryphiellin I showed more favorable predicted binding affinities to the selected protein targets associated with antioxidant and antimicrobial functions than the corresponding reference compounds. Further *in silico* ADME evaluation indicated favorable gastrointestinal absorption and compliance with Lipinski's rule of five. Nevertheless, its relatively high lipophilicity, restricted predicted aqueous solubility, and potential CYP3A4 inhibitory activity suggest that additional pharmacokinetic and toxicological assessment is required. Taken together, Dendryphiellin I represents a potentially bioactive fungal metabolite with predicted antioxidant- and antimicrobial-related potential, warranting further experimental validation.

Acknowledgements

The authors are grateful to the Bangladesh Council of Scientific and Industrial Research (BCSIR) for providing research facility and Abdur Rahman a research fellowship for conducting the research.

Author Contributions

Abdur Rahman: Investigation, conception and design of the study, laboratory work, writing the original draft; **Md. Saiful Islam:** Data acquisition, conceptualization, formal analysis; **Mst. Nadira Begum:** Data curation, validation, writing the original draft, editing, and reviewing; **Rabita Zinnurine:** Investigation, data curation, software, validation; **Tawhida Akter:** Software, formal analysis, validation, editing, and reviewing; **Kutub Uddin Ahamed:** Conceptualization, methodology, and reviewing; **Satyajit Roy Rony:** Conceptualization, formal analysis, pro-

ject administration, resources, software; **Choudhury Mahmood Hasan:** Conceptualization, methodology, validation, formal analysis; **Md. Hossain Sohrab:** Design of the study, funding acquisition, methodology, validation, writing, & editing.

Conflicts of Interest

The authors declared no conflicts of interest among the authors.

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