



Article

Isolation and Antibacterial Characterization of Endophytic Fungi from *Rotheca myricoides* (Hochst.) Steane & Mabb. and Their Secondary Metabolites

Kiburi Alex Murithi^{1,2,3,4}, Clarence Maikuri Mang'era², Josphat Clement Matasyoh^{2,*} and Beatrice Njeri Irungu³

¹ Department of Chemistry, Faculty of Science, Egerton University, Njoro Main Campus, Njoro P.O. Box 536-20115, Kenya

² Department of Biochemistry and Molecular Biology, Faculty of Science, Egerton University, Njoro Main Campus, Njoro P.O. Box 536-20115, Kenya

³ Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute (KEMRI), Mbagathi Road, Nairobi P.O. Box 54840-00200, Kenya

⁴ Institute of Microbial Genetics, Department of Agricultural Sciences, BOKU University, Konrad Lorenz Straße 24, 3430 Tulln an der Donau, Austria

* Correspondence: jmatasyoh@egerton.ac.ke

How To Cite: Murithi, K.A.; Mang'era, C.M.; Matasyoh, J.C.; et al. Isolation and Antibacterial Characterization of Endophytic Fungi from *Rotheca myricoides* (Hochst.) Steane & Mabb. and Their Secondary Metabolites. *eMicrobe* **2026**, *2*(3), 16. <https://doi.org/10.53941/emicrobe.2026.100016>

Received: 17 July 2026

Revised: 4 September 2026

Accepted: 21 September 2026

Published: 30 September 2026

Abstract: Endophytic fungi were isolated from healthy tissues of *Rotheca myricoides*. Antibacterial activity was screened using dual-culture and disc-diffusion assays against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*. Active isolates were identified through morphological characterization and molecular sequence analysis. Six antibacterial-active isolates were selected from 62 culturable endophytic fungi and were assigned to genus, species complex, or species based on ITS or beta-tubulin sequences: *Fusarium incarnatum* agg., *Fusarium solani* agg., *Fusarium equiseti* agg., *Alternaria arborescens* agg., *Purpureocillium* sp., and *Colletotrichum acutatum*. *C. acutatum* produced the largest inhibition zones in dual culture, followed by *A. arborescens* agg. Bioassay-guided purification yielded five metabolites from *C. acutatum*, namely N-acetyltyramine (1), tyrosol (2), N-(2-phenylethyl)acetamide (3), N-acetyltryptamine (4) and indole-3-acetic acid (5). One compound, alternariol monomethyl ether (AME) (6) was obtained from *A. arborescens* agg. AME (6) exhibited the strongest antibacterial activity, with minimum inhibitory concentration (MIC) values of 31.25 µg/mL against *S. aureus* (ATCC 29203) and 62.5 µg/mL against *K. pneumoniae* (DCM 132), *P. aeruginosa* (ATCC 10145), and *E. coli* (ATCC 25922). Although the isolated compounds do not represent novel antibiotic classes and generally exhibited only moderate antibacterial activity, they expand current knowledge of the chemical diversity of Kenyan fungal endophytes and provide a basis for future studies aimed at elucidating their mechanisms of action, structure–activity relationships, and pharmacological relevance.

Keywords: *Rotheca myricoides*; endophytic fungi; *Colletotrichum acutatum*; *Alternaria arborescens* agg.; antimicrobial resistance

1. Introduction

Antimicrobial resistance (AMR) is a major global public health threat that continues to undermine the effectiveness of available antibiotics. This has limited treatment options for infectious diseases and intensified the need for novel antimicrobial scaffolds and alternative drug discovery strategies [1]. Natural products remain



Copyright: © 2026 by the authors. This is an open access article under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Publisher's Note: Scilight stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

central to antimicrobial discovery because they occupy a chemical space that has been shaped by millions of years of ecological competition, making it inherently more biologically relevant than synthetic libraries [2]. Within this context, fungi have long served as a major source of clinically important antibacterial agents. The discovery of penicillin from *Penicillium rubens* marked the beginning of the golden era of antibiotic discovery, followed by cephalosporins from *Acremonium* spp. [3]. Fungal endophytes are especially attractive, as they colonize internal plant tissue without causing visible disease and may contribute to host defence, stress tolerance and chemical adaptation through secondary metabolite production [4]. The association between endophytes and their host plants facilitates biochemical interactions that result in the production of structurally diverse secondary metabolites [5].

Studies have reported potent antibacterial activities of secondary metabolites produced by endophytic fungi isolated from medicinal plants, including *Azadirachta indica* [6], *Dioscorea bulbifera* L. [7], *Mikania cordata* [8], and several other ethnomedicinal species. Only a small fraction of the world's estimated endophytic fungal diversity has been explored, suggesting a vast and largely unexplored source of novel antimicrobial compounds [9,10]. In Kenya, endophytic fungi associated with medicinal plants have demonstrated significant antibacterial activity against clinically relevant pathogens [11,12].

Rotheca myricoides (Hochst.) Steane & Mabb. (formerly *Clerodendrum myricoides*) is a perennial medicinal shrub belonging to the family Lamiaceae [13]. The species is extensively utilized in traditional medicine for the management of tonsillitis, furunculosis, typhoid, diabetes, respiratory infections, and malaria [14,15]. Despite its ethnomedicinal importance, the endophytic fungal communities associated with *R. myricoides* remain poorly investigated and may represent a valuable source of bioactive secondary metabolites. Accordingly, the present study investigated antibacterial endophytic fungi associated with *R. myricoides*, characterized their secondary metabolites, and evaluated the antibacterial activity of the purified compounds against selected Gram-positive and Gram-negative bacterial pathogens.

2. Materials and Methods

2.1. Plant Material Collection

A single healthy individual of *R. myricoides* was selected for sampling in March 2023 from a conserved area in Nthaani Sub-location, Machakos County, Kenya (1°29'54" S, 37°31'02" E). Healthy plant tissues were collected from this individual aseptically, transported to the laboratory, and processed within 8 h for endophytic fungal isolation.

2.2. Isolation and Purification of Endophytic Fungi

Endophytes were isolated following the protocol of [16] with minor modifications. Tissues were washed, rinsed with sterile distilled water, and surface-sterilized sequentially in 70% ethanol (60–90 s) and 2% sodium hypochlorite (3–5 min), then rinsed thrice in sterile water. Sterilization efficacy was confirmed by plating the final rinse water on PDA. Sterilized tissues were cut into 2 mm × 2 mm segments and plated on PDA (with 0.03 g L⁻¹ streptomycin sulphate) and incubated at 25 ± 2 °C. Emerging hyphae (3–7 days) were sub-cultured to purity based on colony morphology. Sixty-two endophytic fungal isolates were obtained and maintained on PDA slants at 4 °C, with glycerol for long-term storage.

2.3. Primary Antibacterial Screening by Dual Culture Assay

Antagonistic activity against *Staphylococcus aureus* (ATCC 29203), *Klebsiella pneumoniae* (DCM 132), *Pseudomonas aeruginosa* (ATCC 10145), and *Escherichia coli* (ATCC 25922) was tested by dual culture assay using the method of [17]. Bacterial suspensions (0.5 McFarland, ~1.5 × 10⁸ CFU mL⁻¹) were swabbed onto Mueller-Hinton agar, and 6 mm mycelial plugs (7-day-old cultures) were placed on the inoculated plates. Plates were incubated at 32 °C for 24 h and inhibition zones measured in triplicate, with chloramphenicol as positive control and bacteria-only plates as negative control. Antibacterial activity was determined by measuring the total diameter of the inhibition zone (mm), including the 6 mm fungal plug and the surrounding clear zone. Where no clear zone was observed beyond the fungal plug, the result was recorded as n.i. (no inhibition). Ten isolates showed the strongest activity, resolving to six genetically distinct strains after molecular identification.

2.4. Morphological and Molecular Identification

Morphology was examined after 5 days using the cellotape technique with lactophenol cotton blue staining under ×100 oil-immersion light microscopy. For molecular identification, DNA was extracted from fresh (3–5-day-old) fungal colonies cultivated on MEA medium, using the Quick-DNA Fungal/Bacterial Miniprep Kit

(ZymoBIOMICS™, Irvine, CA, USA) according to the manufacturer's instructions. The ITS region was amplified with primers ITS1/ITS4 in a 20 µL PCR reaction mix containing 13.8 µL of PCR-grade water (Sigma Aldrich, Saint Louis, MO, USA), 4 µL of 5× MyTaq™ Reaction Buffer Red (Bioline, London, UK), 0.5 µL of each primer (10 pM each), 0.2 µL of MyTaq™ DNA Polymerase (Bioline, UK), and 1 µL of template DNA. PCR conditions: 94 °C for 5 min; 34 cycles of 94 °C/30 s, 52 °C/30 s, 72 °C/1 min; final extension 72 °C for 10 min. Products were verified on 1% agarose gel (100 V, 30 min). Because a usable ITS sequence could not be obtained from the isolate Rm8, part of the beta-tubulin (*tub2*) coding sequence was amplified from that isolate using the primers T10 [18] and Btb2 [19], with the same PCR mix as for ITS. Amplification was carried out using the following cycling conditions: initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 60 s, and extension at 72 °C for 60 s; followed by a final extension step at 72 °C for 10 min. PCR products were enzymatically purified (BIO-BASIC EZ-10 kit) and sequenced on an ABI 3730xl analyser (Applied Biosystems, Waltham, MA, USA). Sequence reads were edited and assembled in Geneious 8.1 (Biomatters, New York, NY, USA). Contigs were deposited in GenBank and subjected to a Blast search restricted to type material against GenBank database.

2.5. Fermentation, Extraction, Isolation and Metabolite Characterization

2.5.1. Fermentation, Extraction and Isolation

Five-day-old cultures of two endophytic fungal isolates showing the highest antibacterial activity were selected for secondary metabolite production. Agar plugs from actively growing cultures were inoculated into 50 mL of Yeast Malt Broth (YMB) and incubated at 24 ± 2 °C on a rotary shaker for 14–21 days to generate a fully colonized liquid inoculum. This inoculum served as the starter culture for large-scale solid-state fermentation on sterilized rice medium, which was incubated at 24 ± 2 °C for 30 days to promote secondary metabolite production.

Once the rice substrate was fully colonized, fermentation was terminated by adding an equal volume of acetone and extracting the culture at room temperature under ultrasonication for 30 min. The acetone extract was filtered and concentrated to dryness under reduced pressure. The resulting residue was suspended in distilled water and partitioned against ethyl acetate; the ethyl acetate fraction was collected and concentrated under reduced pressure to yield the crude extract.

Crude extracts from both *C. acutatum* and *A. arborescens* agg. were further purified by high-performance flash chromatography on an Interchim PuriFlash® 450 system, using a binary gradient of HPLC-grade water (mobile phase A) and HPLC-grade acetonitrile (mobile phase B). The gradient began at 5% B, was held isocratically for 2 min, and then increased linearly to 100% B over 60 min, with mobile phase A adjusted inversely.

Samples were loaded via dry-load column chromatography. Each crude extract was mixed with Celite® 545 (particle size 20–100 µm) in HPLC-grade acetonitrile and concentrated to dryness by rotary evaporation. A PuriFlash® Dry Load Column (PF-DLCET08/25G, Interchim; 21 mm i.d. × 130 mm) was packed to roughly two-thirds capacity with Celite® (10 g, 45 mL bed volume), onto which the dried sample was applied. A thin layer of sea sand (Macherey-Nagel™, ~0.5–1.0 cm) was placed on top of the sample bed to ensure uniform solvent flow during elution. Flash chromatographic separation was performed on an IR-C18 reversed-phase column (50 µm particle size; Interchim PuriFlash®, cartridge F0025) at a maximum pressure of 22 bar. This yielded 13 fractions from the *C. acutatum* extract (C.a F1–F13) and 7 fractions from the *A. arborescens* agg. extract (A.a F1–F7).

For analytical HPLC, each fraction was prepared at 20 mg/mL in HPLC-grade acetonitrile, sonicated to ensure complete dissolution, and centrifuged to remove insoluble particulates prior to injection (20 µL) onto a Gemini® C18 column (10 µm, 110 Å, 250 mm × 10 mm i.d.). The resulting chromatograms were used to optimize separation conditions and determine target compound retention times for preparative purification. Multiple injections were performed to obtain approximately 1–3 mg of each purified compound for LC-MS and NMR analysis. Fractions C.a F3 and C.a F9 showed the strongest antibacterial activity and were selected for further purification.

C.a F3 was purified by preparative reversed-phase HPLC (Agilent system; Gemini® NX-C18 column, 5 µm, 110 Å, 150 mm × 21.2 mm i.d.; part no. 00F-4454-P0-AX) using a binary gradient of solvent A (H₂O with 0.1% formic acid) and solvent B (acetonitrile with 0.1% v/v formic acid). Elution began at 5% B for 1 min and increased linearly to 95% B over 25 min (injection volume: 300 µL). This yielded three subfractions; C.a F3_F1 (compound 2), C.a F3_F2, and C.a F3_F3 (compound 1) with retention times of 6.313, 6.588, and 6.908 min, respectively concentrated to dryness and weighed. These subfractions yielded 1.71 mg (C.a F3_F1), 1.37 mg (C.a F3_F2), and 1.93 mg (C.a F3_F3), and were subjected to NMR and MS analysis.

C.a F9 was purified under the same preparative RP-HPLC conditions. Elution began at 15% B for 2 min and increased linearly to 95% B over 58 min (injection volume: 400 µL), yielding six subfractions (C.a F9_F1–F6) with initial masses of 12.73, 15.14, 13.58, 8.11, 12.71, and 10.51 mg, respectively. Following a further round of purification and concentration to dryness, the subfractions were reweighed: 1.361 mg (C.a F9_F1, compound 3),

2.06 mg (C.a F9_F2), 1.24 mg (C.a F9_F3), 0.75 mg (C.a F9_F4, compound 4), 1.07 mg (C.a F9_F5, compound 5), and 2.17 mg (C.a F9_F6). All subfractions were analyzed by NMR and MS.

Flash chromatography of the *A. arborescens* agg. ethyl acetate extract yielded seven fractions (A.a F1–F7), which were screened for antibacterial activity. A.a F5 showed significant bioactivity and was purified using the same analytical and preparative procedures applied to C.a F3: prepared at 20 mg/mL in HPLC-grade acetonitrile, sonicated, centrifuged, and analyzed by injection (20 µL) onto a Gemini® C18 column (10 µm, 110 Å, 250 mm × 10 mm i.d.) to optimize preparative conditions. Multiple preparative runs yielded a single pure compound (compound 6), concentrated to dryness (2.16 mg) and subjected to NMR and MS analysis.

2.5.2. Metabolite Characterization

All analytical procedures followed the method of [20] with slight modifications. HR-ESIMS data were acquired on a MaXis ESI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) coupled to an Agilent 1260 HPLC-UV system fitted with a C18 Acquity UPLC BEH column (Waters). Separation was performed at 40 °C and 0.6 mL/min flow rate, using the same solvent system (H₂O with 0.1% v/v formic acid as A; acetonitrile with 0.1% v/v formic acid as B), with a gradient of 5% B for 2 min, increasing to 100% B over 35 min, and held for 5 min. DAD-UV detection was recorded at 200–600 nm. HR-ESIMS data were acquired in both positive and negative modes.

NMR spectra (1D and 2D) were recorded on Avance III Bruker spectrometers at three field strengths; ¹H (700 MHz)/¹³C (176 MHz), ¹H (600 MHz)/¹³C (151 MHz), and ¹H (400 MHz)/¹³C (101 MHz) each fitted with a 5 mm TCI cryoprobe. Chemical shifts are reported in parts per million (ppm) and coupling constants in hertz (Hz). Samples were dissolved in deuterated solvents, with chemical shifts referenced to residual solvent signals (CD₃OD: δ_H 3.31, δ_C 49.15). Proton assignments were derived from ¹H NMR and, where necessary, HSQC spectra, while multiplicity-edited HSQC (DEPT-HSQC) experiments were used to assign carbon multiplicities.

2.6. Antibacterial Activity of Fractions and Purified Compounds

The antibacterial activity of the HPLC fractions was evaluated using the agar disc-diffusion assay as described by [21,22], with slight modifications. Fresh bacterial inoculum was prepared by suspending actively growing colonies in sterile physiological saline (0.85% NaCl) and adjusting the turbidity to the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL. The standardized bacterial suspensions were uniformly spread onto the surface of Mueller–Hinton agar (MHA) plates using sterile cotton swabs to obtain confluent growth. Sterile Whatman No. 1 filter paper discs (6 mm diameter) were prepared by punching the filter paper and sterilizing the discs in a hot-air oven at 160 °C for 1 h. Each disc was impregnated with 20 µL of HPLC fraction (2.5 mg/mL; equivalent to 50 µg/disc) dissolved in dimethyl sulfoxide (DMSO), air-dried under aseptic conditions, and placed onto the inoculated MHA plates at equidistant positions using sterile forceps. The plates were incubated at 37 °C for 18 h.

Kanamycin (50 µg/disc) and DMSO (20 µL/disc) were included as positive and negative controls, respectively. Antibacterial activity was determined by measuring the total diameter of the inhibition zone (mm), including the 6 mm paper disc and the surrounding clear zone. Where no clear zone was observed beyond the paper disc, the result was recorded as n.i. (no inhibition). Fractions producing inhibition zones greater than 20 mm were considered active and were selected for further purification. All assays were performed in triplicate, and the results were expressed as mean ± standard error (SE).

The minimum inhibitory concentrations (MICs) of the purified compounds were determined using the broth microdilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) M07 guidelines [22,23]. Stock solutions of the compounds were prepared at 2 mg/mL by dissolving each compound in a minimal volume of dimethyl sulfoxide (DMSO) and adjusting the final volume with cation-adjusted Mueller–Hinton broth (CAMHB) to yield a stock solution containing 2% (v/v) DMSO. Two-fold serial dilutions were prepared in sterile 96-well microplates to yield compound concentrations of 3.906–2000 µg/mL (pre-inoculum concentrations). Bacterial inoculum was prepared from 0.5 McFarland standard suspensions and diluted in CAMHB to obtain a working suspension of approximately 1×10^6 CFU/mL. Aliquots of 100 µL of each compound dilution were then mixed with 100 µL of the bacterial inoculum in each well, giving a final assay volume of 200 µL, final compound concentrations of 1.95–1000 µg/mL, and a final inoculum density of approximately 5×10^5 CFU/mL.

Each microplate included a growth control consisting of CAMHB and bacterial inoculum without test compound, a sterility control containing CAMHB only, and a solvent control containing DMSO at the highest concentration used in the assay and bacterial inoculum without test compound. The highest test concentration (1000 µg/mL) contained a final DMSO concentration of 1% (v/v), with all subsequent dilutions containing proportionally lower concentrations of DMSO. Kanamycin and oxytetracycline were included as positive

antibiotic controls and subjected to the same two-fold serial dilution procedure as the test compounds. The reported antibiotic control MICs represent the final concentrations in the wells: 1.95 µg/mL for kanamycin against *S. aureus* and 3.91 µg/mL for oxytetracycline against *K. pneumoniae*, *P. aeruginosa*, and *E. coli*. The plates were then covered with a sterile “cling-on” sealer and incubated aerobically at 35 ± 2 °C for 18 h.

Bacterial growth was assessed by the addition of 40 µL of p-iodonitrotetrazolium chloride (INT; Sigma-Aldrich) solution (0.2 mg/mL) to each well, followed by incubation at 35 ± 2 °C for 30 min. Viable bacterial cells reduced the tetrazolium salt to a pink-red formazan product, resulting in a visible colour change from colourless to pink-red. In contrast, inhibition of bacterial growth was indicated by the absence of colour development, with the wells remaining colourless. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test compound that completely prevented colour development following INT addition [24].

2.7. Statistical Analyses

All antibacterial assays were conducted in three independent replicates ($n = 3$), with each replicate representing an independently performed experiment. Results are presented as mean $\pm$ standard error of the mean (SEM). Differences in mean inhibition-zone diameters among the tested groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey’s honestly significant difference (HSD) post hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using Stata version 18 (StataCorp LLC, College Station, TX, USA).

3. Results

3.1. Isolation and Identification of Antibacterial-Active Endophytic Fungi

Six endophytic fungal isolates were selected for further study, designated Rm1, Rm2, Rm3, Rm5, Rm6, and Rm8. These isolates were characterized using a combination of morphological and molecular approaches. Microscopic examination revealed characteristic fungal structures consistent with distinct taxa (Figure 1). Three isolates (Rm1, Rm2, Rm3) possessed septate, hyaline hyphae and falcate macroconidia, features typical of *Fusarium* species. Isolate Rm5 produced darkly pigmented, multicellular conidia with both transverse and longitudinal septa arranged in chains, consistent with *Alternaria* species. Isolate Rm6 exhibited slender, septate hyphae with elongated phialides bearing chains of unicellular, ellipsoidal conidia, characteristic of *Purpureocillium* species. Isolate Rm8 produced hyaline, aseptate, fusiform to cylindrical conidia with acute ends arising from short conidiophores, consistent with *Colletotrichum* species.

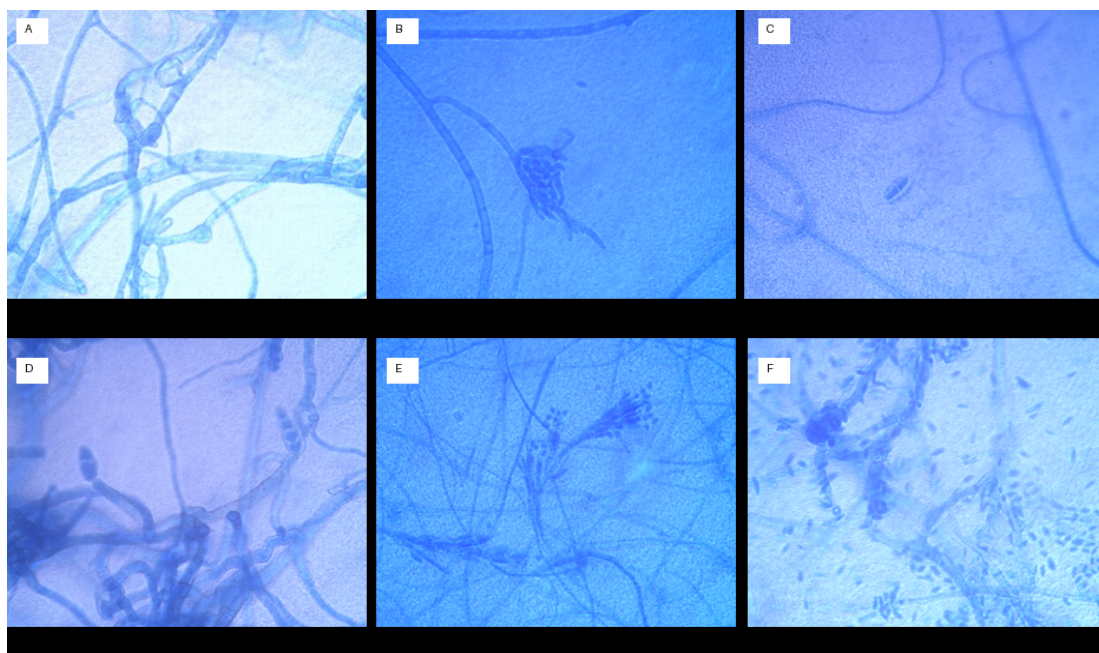


Figure 1. Microscopic morphology of the six antibacterial-active fungal endophytes isolated from *R. myricoides*. (A) *Fusarium incarnatum* agg. (B) *Fusarium solani* agg. (C) *Fusarium equiseti* agg. (D) *Alternaria arborescens* agg. (E) *Purpureocillium* sp. (F) *Colletotrichum acutatum*.

The ITS sequence of isolates Rm1, Rm2, Rm3, and Rm5 showed 100% identity with 100% query cover to one or more sequences from type material of well-known species complexes (Table 1). Rm1 could be identified as belonging to the *Fusarium incarnatum* species complex, while Rm2 and Rm3 were attributed to the *Fusarium solani* and *F. equiseti* species complexes, respectively. Rm5 matched with the *Alternaria arborescens* species complex. The ITS sequence of the isolate Rm6 showed 99.81% identity with two sequences, each from the type material of a different species (*Purpureocillium lilacinum* and *P. tiankengense*). The difference was the same in both cases, i.e., a single deletion of one C residue in a poly-C stretch. Because the ITS region was not able to differentiate between the two latter species, the identification of the Rm6 isolate was limited to genus level (*Purpureocillium* sp.). Finally, the isolate Rm8 could be identified as *Colletotrichum acutatum*, because its *tub2* sequence showed 100% identity and 100% query cover with the *tub2* sequence obtained from the culture of the epitype of *Colletotrichum acutatum*. Moreover, when the *tub2* sequence of Rm8 was blasted against GenBank, excluding *Colletotrichum acutatum*, all hits to identified species showed a similarity lower than 98%.

Table 1. Molecular identification of antibacterial-active endophytic fungi isolated from *Rothea myricoides* based on Blast searches of ITS or *tub2* (for Rm8) sequences against GenBank database.

Isolate	GenBank Accession	Sequence Length (bp)	Similarity (%)	Query Coverage (%)	Closest Blast Hits *	Blast hit Accession	BLAST-Supported Identification
Rm1	PZ433257	506	100	100	<i>Fusarium liriodendri</i> CBS 117481	JX435205	<i>Fusarium incarnatum</i> agg.
Rm2	PZ433258	527	100	100	<i>Fusarium chromolaenae</i> MFLU FT1747	PV870631	<i>Fusarium solani</i> agg.
			100	100	<i>[Neocosmospora] alboflava</i> KUNCC 3509	PV469217	
Rm3	PZ433259	507	100	100	<i>Fusarium duofalcatisporum</i> NRRL 36448	GQ505741	<i>Fusarium equiseti</i> agg.
			100	100	<i>Fusarium arcuatisporum</i> CGMCC 3.19493	PP732318	
			100	100	<i>Fusarium compactum</i> NRRL 36323	GQ505737	
			100	100	<i>Fusarium phoenicis</i> CPC 42150	PX640129	
Rm5	PZ433260	530	100	100	<i>Alternaria arborescens</i> CBS 102605	NR_135927	<i>Alternaria arborescens</i> agg.
			100	100	<i>Alternaria alstroemeriae</i> CBS 118809	NR_163686	
Rm6	PZ433261	560	99.81	100	<i>Purpureocillium lilacinum</i> NRRL 895	NR_165946	<i>Purpureocillium</i> sp.
			99.81	100	<i>Purpureocillium tiankengense</i> GZAC KY07471	NR_202650	
Rm8	PZ777401 (<i>tub2</i>)	530	100	100	<i>Colletotrichum acutatum</i> STE-U 5292	AY376558	<i>Colletotrichum acutatum</i>

* Closest hits of Blast searches restricted to type materials, with mention of the holotype voucher or ex-(epi)type cultures.

3.2. Structure Identification of the Isolated Metabolites

A total of six compounds were isolated: five from the endophytic fungus *C. acutatum* and one from *A. arborescens* agg. Their structures were elucidated by spectroscopic analysis (HRESIMS, ¹H and ¹³C NMR), physicochemical properties, and comparison with published data, and were identified as N-acetyltyramine (**1**) [25], tyrosol (**2**) [25], N-(2-phenylethyl)acetamide (**3**) [26], N-acetyltryptamine (**4**) [27,28], indole-3-acetic acid (IAA) (**5**) [29], and alternariol monomethyl ether (AME) (**6**) [30].

Five compounds were isolated from *C. acutatum*, comprising a phenolic amide (**1**), phenolic compound (**2**), an aromatic amide (**3**), and two indole derivatives (**4** and **5**). Compound **6**, isolated from *A. arborescens* agg., was identified as a dibenzo- α -pyrone polyketide (Figure 2).

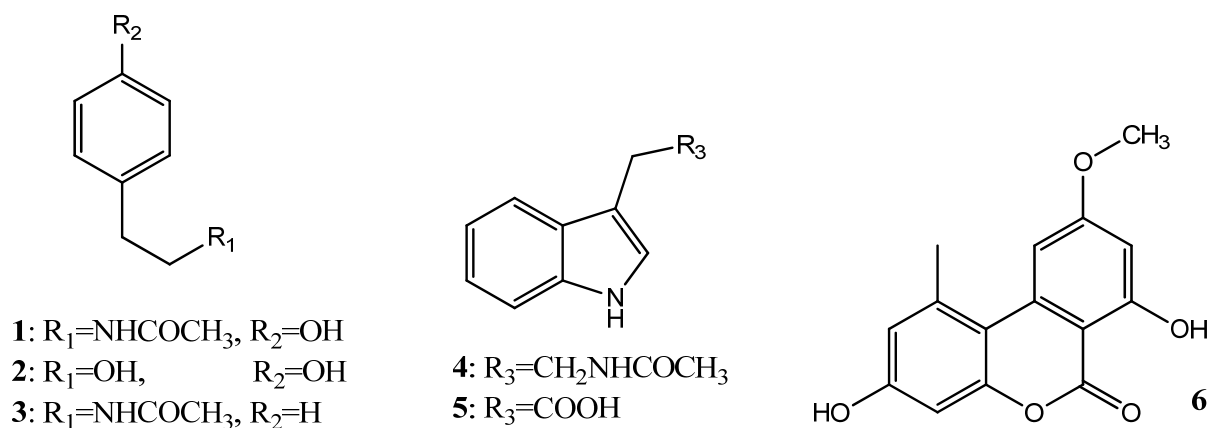


Figure 2. Chemical structures of secondary metabolites (**1–6**) isolated from *C. acutatum* and *A. arborescens* agg., endophytic fungi associated with *R. myricoides*.

Compound **1** was obtained as a white powder from the ethyl acetate extract of *C. acutatum*. Its molecular formula, $\text{C}_{10}\text{H}_{13}\text{NO}_2$ was deduced by HRESIMS at m/z 180.1017 $[\text{M}+\text{H}]^+$, (calculated for $\text{C}_{10}\text{H}_{13}\text{NO}_2$, 180.1019) implying 5 degrees of unsaturation. ^1H and ^{13}C NMR data (CD_3OD , 400 MHz, 101 MHz) δ_{H} : 7.02 (d, $J = 8.4$ Hz, H-2, H-6), 6.70 (d, $J = 8.4$ Hz, H-3, H-5), 3.32 (t, $J = 7.2$ Hz, H₂-8), 2.67 (t, $J = 7.2$ Hz, H₂-7), 1.90 (s, Me-10); ^{13}C NMR δ_{C} : 22.5 (C-10), 35.7 (C-7), 42.4 (C-8), 116.2 (C-3), 116.2 (C-5), 130.7 (C-2), 130.7 (C-6), 131.2 (C-1), 156.9 (C-4), 173.2 (C-9). After comparison with literature Compound **1** was determined as N-Acetyltyramine [25].

Compound **2** was obtained as a white to off-white crystalline solid (MeOH) from the ethyl acetate extract of *C. acutatum*. HRESIMS m/z 139.0752 $[\text{M}+\text{H}]^+$ corresponding to the molecular formula of $\text{C}_8\text{H}_{10}\text{O}_2$ (Calculated for 139.0754), implying 4 degrees of unsaturation. ^1H and ^{13}C NMR data (CD_3OD , 400 MHz, 101 MHz) δ_{H} : 7.03 (d, $J = 8.2$ Hz, H-3, H-5), 6.70 (d, $J = 8.2$ Hz, H-2, H-6), 3.68 (t, $J = 6.6$ Hz, H₂-8), 2.71 (t, $J = 6.6$ Hz, H₂-7); ^{13}C NMR δ_{C} : 39.4 (C-7), 64.6 (C-8), 116.1 (C-2), 116.1 (C-6), 130.9 (C-3), 130.9 (C-5), 131.0 (C-4), 156.8 (C-1). After comparison with literature, compound **2** was determined as 4-(2-hydroxyethyl)phenol (Tyrosol) [25].

Compound **3** was isolated as a white to off-white solid from the ethyl acetate extract of *C. acutatum*. Its molecular formula, $\text{C}_{10}\text{H}_{13}\text{NO}$ was established from HRESIMS m/z 164.1068 $[\text{M}+\text{H}]^+$, implying 5 degrees of unsaturation (calculated for 164.1070). ^1H and ^{13}C NMR data (CD_3OD , 700 MHz, 176 MHz) δ_{H} : 7.20 (d, $J = 7.0$ Hz, H-2 and H-6), 7.28 (t, $J = 7.0$ Hz, H-3 and H-5), 7.22 (t, $J = 7.0$ Hz, H-4), 3.38 (t, $J = 6.3$ Hz, H₂-8), 2.78 (t, $J = 6.3$ Hz, H₂-7), 1.90 (s, H-10); ^{13}C NMR δ_{C} : 22.6 (C-10), 36.7 (C-7), 42.3 (C-8), 127.5 (C-2), 127.5 (C-6), 129.6 (C-3), 129.6 (C-5), 129.9 (C-4). After comparison with literature, compound **3** was determined as N-(2-phenylethyl)acetamide [26].

Compound **4** was isolated as a pale-yellow oil from the ethyl acetate extract of *C. acutatum*. Its molecular formula, $\text{C}_{12}\text{H}_{14}\text{N}_2\text{O}$ was established from HRESIMS m/z 203.1178 $[\text{M}+\text{H}]^+$, implying 7 degrees of unsaturation (calculated for 203.1179). ^1H and ^{13}C NMR data (Acetone, 700 MHz, 176 MHz) δ_{H} : 7.37 (d, $J = 8.10$ Hz, H-7), 7.59 (d, $J = 7.89$ Hz, H-4), 7.01 (dd, $J = 6.96$ Hz, H-5), 7.10 (dd, $J = 6.96$ Hz, H-6), 7.16 (d, $J = 2.31$ Hz, H-2), 3.48 (t, $J = 6.3$ Hz, H₂-11), 2.92 (t, $J = 6.3$ Hz, H₂-10), 1.85 (s, H₃-14); ^{13}C NMR δ_{C} : 23.1 (C-14), 26.4 (C-10), 40.8 (C-11), 112.2 (C-7), 113.6 (C-3), 119.4 (C-4), 119.5 (C-5), 122.2 (C-6), 123.4 (C-2), 128.7 (C-9), 137.8 (C-8). After comparison with literature, compound **4** was determined as N-[2-(1H-indol-3-yl)ethyl]acetamide (N-acetyltryptamine) [27].

Compound **5** was obtained as a white powder from the ethyl acetate extract of *C. acutatum*. Its molecular formula, $\text{C}_{10}\text{H}_9\text{NO}_2$ was deduced by HRESIMS m/z 176.0702 $[\text{M}+\text{H}]^+$, (calculated for $\text{C}_{10}\text{H}_9\text{NO}_2$, 176.0706) implying 7 degrees of unsaturation. ^1H and ^{13}C NMR data (MeOD, 600 MHz, 151 MHz) δ_{H} : 3.70 (H-10), 7.33 (H-7), 7.55 (H-4), 7.00 (H-5), 7.08 (H-6) and 7.16 (H-2); ^{13}C NMR, δ_{C} : 32.8 (C-10), 109.6 (C-3), 112.2 (C-7), 119.5 (C-4), 119.7 (C-5), 122.3 (C-6), 124.5 (C-2), 128.8 (C-8), 138.0 (C-9), 177.5 (C-11). After comparison with literature, compound **5** was determined as 2-(1H-indol-3-yl)acetic acid (Indole-3-acetic acid) [29].

Compound **6** was isolated as a colourless to pale-yellow crystalline solid from the ethyl acetate extract of *A. arborescens* agg. Its molecular formula, $\text{C}_{15}\text{H}_{12}\text{O}_5$ was established from HRESIMS m/z 273.0753 $[\text{M}+\text{H}]^+$, implying 10 degrees of unsaturation (calculated for 273.0757). ^1H and ^{13}C NMR data (CD_3OD , 400 MHz, 101

MHz) δ_H ; 7.30 (d, $J = 2.3$ Hz, H-10), 6.56 (d, $J = 2.3$ Hz, H-8 and H-6a), 6.62 (d, $J = 2.6$ Hz, H-4), 6.71 (d, $J = 2.6$ Hz, H-2), 3.93 (s, H₃-9'), 2.78 (s, H₃-2'); ^{13}C NMR δ_C ; 25.7 (C-2'), 56.3 (C-9'), 100.0 (C-8), 100.0 (C-6a), 102.9 (C-4), 104.9 (C-10), 110.8 (C-10b), 118.7 (C-2), 139.7 (C-1), 139.9 (C-10a), 154.5 (C-4a), 160.1 (C-3), 166.3 (C-6), 166.9 (C-7), 168.2 (C-9). After comparison with literature compound **6** was determined as alternariol monomethyl ether (AME) [30].

3.3. Antibacterial Screening of Endophytic Fungi and Their Bioactive Metabolites

The dual culture antibacterial assay of the six isolated endophytic fungi against the selected bacterial pathogens demonstrated variable levels of antagonistic activity. Among the isolates, *C. acutatum* exhibited the highest antibacterial activity, producing an inhibition zone of 14.7 mm (Table 2).

Table 2. Antibacterial activity of endophytic fungi isolated from *R. myricoides* assessed through dual culture assay.

Isolate	Endophytic Fungi	Inhibition Diameter Zones (mm)			
		<i>S. aureus</i> (ATCC 29203)	<i>K. pneumoniae</i> (DCM 132)	<i>P. aeruginosa</i> (ATCC 10145)	<i>E. coli</i> (ATCC 25922)
Rm1	<i>Fusarium incarnatum</i> agg.	* 7.3 ± 0.17 ^{ch}	n.i.	n.i.	n.i.
Rm2	<i>Fusarium solani</i> agg.	8.3 ± 0.17 ^{de}	8.0 ± 0.29 ^d	7.0 ± 0.29 ^e	7.0 ± 0.00 ^d
Rm3	<i>Fusarium equiseti</i> agg.	7.0 ± 0.29 ^{ef}	n.i.	n.i.	n.i.
Rm5	<i>Alternaria arborescens</i> agg.	11.3 ± 0.17 ^c	10.0 ± 0.29 ^c	10.0 ± 0.29 ^c	10.3 ± 0.17 ^c
Rm6	<i>Purpureocillium</i> sp.	9.0 ± 0.29 ^d	8.0 ± 0.29 ^d	8.0 ± 0.29 ^d	7.0 ± 0.29 ^d
Rm8	<i>Colletotrichum acutatum</i>	14.7 ± 0.17 ^b	12.0 ± 0.29 ^b	12.3 ± 0.17 ^b	12.0 ± 0.29 ^b
	Chloramphenicol	26.0 ± 0.29 ^a	24.0 ± 0.29 ^a	23.0 ± 0.29 ^a	22.0 ± 0.29 ^a

* Values are presented as means of three replicates ± standard error (SE). # Values in a column with same superscript are not significantly ($p \geq 0.05$) different based on Tukey HSD test. n.i. = no inhibition.

Further screening of HPLC-fractionated crude extracts, evaluated using the disc diffusion method, revealed that metabolite-rich fractions from both *C. acutatum* and *A. arborescens* agg. exhibited pronounced antimicrobial activity against the tested bacterial pathogens (Tables 3 and 4).

Table 3. Antibacterial activity of ethyl acetate HPLC fractions from *Alternaria arborescens* agg. against selected clinically important bacterial pathogens.

Fraction	Inhibition Diameter Zones (mm)			
	<i>S. aureus</i> (ATCC 29203)	<i>K. pneumoniae</i> (DCM 132)	<i>P. aeruginosa</i> (ATCC 10145)	<i>E. coli</i> (ATCC 25922)
F1	* 14.6 ± 0.17 ^{dh}	n.i.	10.0 ± 0.29 ^d	11.4 ± 0.17 ^c
F2	10.6 ± 0.17 ^f	n.i.	10.0 ± 0.00 ^d	10.0 ± 0.00 ^{de}
F3	12.0 ± 0.29 ^e	n.i.	10.6 ± 0.17 ^{cd}	10.6 ± 0.17 ^d
F4	14.6 ± 0.17 ^d	10.0 ± 0.00 ^c	10.0 ± 0.00 ^d	10.6 ± 0.17 ^d
F5	28.0 ± 0.29 ^a	18.6 ± 0.17 ^b	22.0 ± 0.29 ^a	22.0 ± 0.29 ^a
F6	12.0 ± 0.29 ^e	10.0 ± 0.00 ^c	10.6 ± 0.17 ^{cd}	10.0 ± 0.00 ^{de}
F7	16.0 ± 0.29 ^c	n.i.	14.0 ± 0.29 ^b	13.4 ± 0.17 ^b
Kanamycin	26.0 ± 0.29 ^b	24.0 ± 0.29 ^a	22.0 ± 0.29 ^a	22.0 ± 0.29 ^a
DMSO	n.i.	n.i.	n.i.	n.i.

* Values are presented as means of three replicates ± standard error (SE). # Values in a column with same superscript are not significantly ($p \geq 0.05$) different based on Tukey HSD test. n.i. = no inhibition.

Table 4. Antibacterial activity of ethyl acetate HPLC fractions from *Colletotrichum acutatum* against selected clinically important bacterial pathogens.

Fraction	Inhibition Diameter Zones (mm)			
	<i>S. aureus</i> (ATCC 29203)	<i>K. pneumoniae</i> (DCM 132)	<i>P. aeruginosa</i> (ATCC 10145)	<i>E. coli</i> (ATCC 25922)
F1	* 10.6 ± 0.17 ^{gh}	n.i.	10.0 ± 0.00 ^{ef}	10.0 ± 0.00 ^{fg}
F2	15.4 ± 0.17 ^{de}	n.i.	10.0 ± 0.00 ^{ef}	11.4 ± 0.17 ^{ef}
F3	28.6 ± 0.17 ^b	19.4 ± 0.17 ^c	22.0 ± 0.29 ^c	22.0 ± 0.29 ^b
F4	12.6 ± 0.17 ^f	n.i.	10.6 ± 0.17 ^e	12.0 ± 0.29 ^c
F5	14.6 ± 0.17 ^c	10.6 ± 0.17 ^c	10.0 ± 0.00 ^{ef}	16.6 ± 0.17 ^c
F6	11.4 ± 0.17 ^g	12.6 ± 0.29 ^d	10.0 ± 0.00 ^{ef}	12.0 ± 0.29 ^c

Table 4. Cont.

Fraction	Inhibition Diameter Zones (mm)			
	<i>S. aureus</i> (ATCC 29203)	<i>K. pneumoniae</i> (DCM 132)	<i>P. aeruginosa</i> (ATCC 10145)	<i>E. coli</i> (ATCC 25922)
F7	12.0 ± 0.29 ^{fg}	10.0 ± 0.00 ^{ef}	10.0 ± 0.00 ^{ef}	10.6 ± 0.17 ^f
F8	10.0 ± 0.00 ^h	n.i.	10.0 ± 0.00 ^{ef}	10.0 ± 0.00 ^{fg}
F9	32.6 ± 0.17 ^a	24.0 ± 0.29 ^a	27.4 ± 0.17 ^a	28.0 ± 0.29 ^a
F10	16.0 ± 0.29 ^d	12.6 ± 0.17 ^d	14.0 ± 0.29 ^d	13.4 ± 0.17 ^d
F11	12.0 ± 0.29 ^{fg}	10.0 ± 0.00 ^{ef}	10.0 ± 0.00 ^{ef}	10.6 ± 0.17 ^f
F12	n.i.	n.i.	n.i.	n.i.
F13	10.6 ± 0.17 ^{gh}	n.i.	10.0 ± 0.00 ^{ef}	10.0 ± 0.00 ^{fg}
Kanamycin	26.0 ± 0.29 ^c	22.0 ± 0.29 ^b	23.0 ± 0.29 ^b	22.0 ± 0.29 ^b
DMSO	n.i.	n.i.	n.i.	n.i.

* Values are presented as means of three replicates ± standard error (SE). # Values in a column with same superscript are not significantly ($p \geq 0.05$) different based on Tukey HSD test. n.i. = no inhibition.

The MIC assays of the pure compounds (**1**, **2**, **3**, **5**, and **6**) revealed notable variation in the antibacterial potency across the tested bacterial pathogens. Compound **6** showed the highest potency, with MIC values as low as 31.25 µg/mL against *S. aureus* and 62.5 µg/mL against the other tested bacterial strains (Table 5).

Table 5. Minimum inhibitory concentrations (MIC) (µg/mL) values of pure compounds obtained from *Colletotrichum acutatum* (Rm8) and *Alternaria arborescens* agg. (Rm5) against test bacterial pathogens.

Compounds	MIC Concentrations (µg/mL)/Test Organism			
	<i>S. aureus</i> (ATCC 29203)	<i>K. pneumoniae</i> (DCM 132)	<i>P. aeruginosa</i> (ATCC 10145)	<i>E. coli</i> (ATCC 25922)
Compound 1	250.0 *	n.i.	n.i.	n.i.
Compound 2	62.5	500.0	500.0	125.0
Compound 3	n.i.	n.i.	n.i.	n.i.
Compound 4	n.t.	n.t.	n.t.	n.t.
Compound 5	62.5	250.0	250.0	125.0
Compound 6	31.25	62.5	62.5	62.5
Control ^b	1.95 (K)	3.91 (O)	3.91 (O)	3.91 (O)

* Values are minimum inhibitory concentrations (MICs, µg/mL) determined from three independent experiments (n = 3 independent experiments), n.i. = no inhibition, n.t. = not tested. ^b Positive control; O = Oxytetracycline, K = Kanamycin. Values reported for the positive controls represent the final concentrations in the wells.

4. Discussion

This study demonstrates that the sampled *R. myricoides* individual plant harbours endophytic fungal communities capable of producing secondary metabolites that exhibit in vitro antibacterial activity against clinically relevant bacterial pathogens. Active isolates were identified as belonging to the genera *Alternaria*, *Colletotrichum*, *Fusarium*, and *Purpureocillium*, taxa that have previously been reported as prolific producers of structurally diverse bioactive secondary metabolites, including compounds with documented antibacterial properties [31]. This taxonomic distribution is consistent with the broader literature on endophyte-associated antimicrobial discovery and adds to the growing body of evidence that endophytic fungi colonizing medicinal plants represent a valuable reservoir of bioactive natural products [32]. A limitation of the present study is that fungal isolation was conducted from a single individual of *R. myricoides*, and therefore the isolates recovered represent only the culturable endophytic fungi associated with the sampled individual. The absence of host-level biological replication limits the extent to which the observed fungal diversity, taxonomic composition, or metabolite production can be generalized to the broader *R. myricoides* population. Consequently, the findings should be interpreted as a characterization of the endophytic fungi and antibacterial metabolites recovered from the sampled plant rather than as a representation of the overall endophytic fungal community of *R. myricoides*. Further studies involving multiple individuals across different populations and environments are needed to determine the consistency and broader distribution of these associations.

The metabolites identified in the present study belong to chemical classes that have previously contributed important scaffolds for antibacterial drug discovery. Phenolic moieties are common structural features in numerous bioactive natural products and antibiotics. Vancomycin, a clinically important glycopeptide antibiotic contains multiple phenolic functional groups that contribute to its biological activity [33]. The antibacterial activity

observed for tyrosol (**2**) and N-acetyltyramine (**1**) supports the role of phenolic compounds as biologically active antimicrobial scaffolds. Similarly, aromatic amides have established relevance in antimicrobial chemotherapy. Chloramphenicol is characterized by a substituted aromatic amide structure that is central to its antibacterial activity [34]. The identification of N-(2-phenylethyl)acetamide (**3**) is therefore noteworthy, although its antibacterial activity in the present study was limited compared to established antibacterial agents. Indole-containing compounds also represent an important class of bioactive microbial metabolites. Indolmycin, a naturally occurring indole antibiotic exerts antibacterial activity through inhibition of bacterial tryptophanyl-tRNA synthetase [35]. N-acetyltryptamine (**4**) and IAA (**5**) further highlights the ability of endophytic fungi to produce indole-derived metabolites. IAA has also been reported to exhibit antibacterial activity against several bacterial pathogens and to suppress biofilm formation and virulence, highlighting its potential as a bioactive antimicrobial metabolite [36]. The occurrence of these compounds reinforces the importance of indole scaffolds as a source of antimicrobial chemical diversity.

AME (**6**) represents a fungal polyketide, a biosynthetic class that has yielded some of the most successful antibiotics in clinical use. Erythromycin, tetracyclines, and rifamycins are derived wholly or partly from polyketide biosynthetic pathways [37]. The antibacterial activity exhibited by AME is therefore consistent with the long-recognized importance of polyketides as a source of antimicrobial metabolites. However, unlike clinically used polyketide antibiotics, AME is a well-characterized *Alternaria*-derived mycotoxin with documented cytotoxic and toxicological effects [38]. As the present study did not evaluate mammalian cytotoxicity, haemolytic activity, or selectivity index, the observed in vitro antibacterial activity alone is insufficient to support AME's development as a therapeutic lead without further toxicological and selectivity data [39].

These findings are consistent with previous studies on Kenyan medicinal plants and their associated microbiota, which have similarly yielded structurally diverse natural products with antibacterial activity against clinically relevant pathogens [40,41]. Although few such compounds have advanced to clinical use as antibiotics, these investigations collectively underscore the value of Kenyan biodiversity, including plant-associated fungal endophytes, as a source of antimicrobial lead compounds and structurally diverse bioactive metabolites. Beyond direct bactericidal activity, plant- and fungus-derived antibacterial agents have also been reported to act through multi-target mechanisms, including membrane disruption, biofilm inhibition, and attenuation of virulence pathways, thereby complementing conventional antibiotic action against pathogens such as *S. aureus* [42].

The antibacterial metabolites produced by endophytic fungi associated with *R. myricoides* belong to chemical classes that have historically contributed important scaffolds to antibiotic discovery. Although the isolated compounds do not represent novel antibiotic classes and generally exhibited only moderate antibacterial activity, they expand current knowledge of the chemical diversity of Kenyan fungal endophytes and provide a basis for future studies aimed at elucidating their mechanisms of action, structure–activity relationships, and pharmacological relevance.

Supplementary Materials

The additional data and information can be downloaded at: <https://media.scilit.com/articles/others/2609301622352244/eMicrobe-26070170-SM-FC.zip>. Figure S1: HRESIMS spectrum of compound **1**; Figure S2: ¹³C NMR (101 MHz, MeOD) spectrum of **1**; Figure S3: ¹H NMR (400 MHz, MeOD) spectrum of compound **1**; Figure S4: HSQC (MeOD) spectrum (¹H: 400 MHz, ¹³C: 101 MHz) of compound **1**; Figure S5: COSY (400 MHz, MeOD) spectrum of compound **1**; Figure S6: HMBC (MeOD) spectrum (¹H: 400 MHz, ¹³C: 101 MHz) of compound **1**; Figure S7: HRESIMS spectrum of compound **2**; Figure S8: ¹³C NMR (101 MHz, MeOD) spectrum of compound **2**; Figure S9: HSQC (MeOD) spectrum of compound **2**; Figure S10: ¹H NMR (400 MHz, MeOD) spectrum of compound **2**; Figure S11: COSY (400 MHz, MeOD) spectrum of compound **2**; Figure S12: HMBC (MeOD) spectrum (¹H: 400 MHz, ¹³C: 101 MHz) of compound **2**; Figure S13: HRESIMS spectrum of compound **3**; Figure S14: ¹³C NMR (176 MHz, MeOD) spectrum of compound **3**; Figure S15: HSQC (MeOD) spectrum (¹H: 700 MHz, ¹³C: 176 MHz) of compound **3**; Figure S16: ¹H NMR (700 MHz, MeOD) spectrum of compound **3**; Figure S17: COSY (700 MHz, MeOD) spectrum of compound **3**; Figure S18: HMBC (MeOD) spectrum (¹H: 700 MHz, ¹³C: 176 MHz) of compound **3**; Figure S19: HRESIMS spectrum of compound **4**; Figure S20: ¹³C NMR (176 MHz, Acetone) spectrum of compound **4**; Figure S21: HSQC (Acetone) spectrum (¹H: 700 MHz, ¹³C: 176 MHz) of compound **4**; Figure S22: ¹H NMR (700 MHz, Acetone) spectrum of compound **4**; Figure S23: COSY (700 MHz, Acetone) spectrum of compound **4**; Figure S24: HMBC (Acetone) spectrum (¹H: 700 MHz, ¹³C: 176 MHz) of compound **4**; Figure S25: HRESIMS spectrum of compound **5**; Figure S26: ¹³C NMR (151 MHz, MeOD) spectrum of compound **5**; Figure S27: ¹H NMR (600 MHz, MeOD) spectrum of compound **5**; Figure S28: HSQC (MeOD) spectrum (¹H: 600 MHz, ¹³C: 151 MHz) of compound **5**; Figure S29: COSY (600 MHz, MeOD) spectrum

of compound **5**; Figure S30: HMBC (MeOD) spectrum (^1H : 600 MHz, ^{13}C : 151 MHz) of compound **5**; Figure S31: HRESIMS spectrum of compound **6**; Figure S32: ^{13}C NMR (101 MHz, MeOD) spectrum of compound **6**; Figure S33: HSQC (MeOD) spectrum of compound **6**; Figure S34: ^1H NMR (400 MHz, MeOD) spectrum of compound **6**; Figure S35: COSY (400 MHz, MeOD) spectrum of compound **6**; Figure S36: HMBC (MeOD) spectrum (^1H : 400 MHz, ^{13}C : 101 MHz) of compound **6**. Table S1: Antibacterial screening of 62 endophytic fungal isolates via dual culture assay.

Author Contributions

J.C.M.: Conceptualization, Methodology, Funding acquisition, Supervision, Writing—review & editing. B.N.I.: Validation, Funding acquisition, Supervision, Writing—review & editing. C.M.M.: Supervision, Writing—review & editing. K.A.M.: Investigation, Methodology, Formal analysis, Writing—original draft, Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

Funding

The authors declare that financial support was received for this research. This work was carried out with the aid of a grant from UNESCO-TWAS and the Swedish International Development Cooperation Agency (Sida) under the Research Grant Agreement (RGA) for Individual Scientists No. 22-239 RG/CHE/AF/AC_G, FR 3240325139. Additional support was provided by the European Union's Horizon 2020 research and innovation program (RISE) under the Marie Skłodowska-Curie grant agreement No. 101008129, project acronym "Mycobiomics" and by the Fogarty International Center and National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number D43 TW0011813. The views expressed herein do not necessarily represent those of UNESCO-TWAS, Sida, National Institutes of Health or its Board of Governors.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

All data generated or analyzed during this study are included in this published article and its Supplementary Materials.

Acknowledgments

The authors are grateful to Olivier Raspé for his invaluable assistance with the analysis and interpretation of molecular sequence data, which greatly contributed to the taxonomic identification of the fungal isolates. We also thank Christian Puhr and Lirim Kastrati for conducting the antimicrobial bioassays, and Marcus Pruckner, Maria Doppler, and Markus Bacher for recording HPLC, LC-HRMS, and NMR data at the Institute of Microbial Genetics, BOKU University, Tulln an der Donau, Austria. We further acknowledge Esther Surges and Kirsten Harmrolfs for recording NMR data, and Aileen Gollasch for recording HRMS data at the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research, Germany. The authors are especially grateful to Joseph Strauss (Institute of Microbial Genetics, BOKU University, Tulln an der Donau, Austria) for facilitating the research visit and providing access to LC-HRMS and NMR instrumentation. Patrick B. Mutiso (Department of Biology, University of Nairobi, Kenya) is acknowledged for the identification and authentication of the medicinal plant material. This research was supported by a grant from UNESCO-TWAS and the Swedish International Development Cooperation Agency (Sida) under the Research Grant Agreement (RGA) for Individual Scientists No. 22-239 RG/CHE/AF/AC_G, FR 3240325139. Additional support was provided by the European Union's Horizon 2020 Research and Innovation Programme through the Marie Skłodowska-Curie Research and Innovation Staff Exchange (RISE) project "Mycobiomics" (Grant Agreement No. 101008129).

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

References

- Okeke, I.N.; de Kraker, M.E.A.; Van Boeckel, T.P.; et al. The scope of the antimicrobial resistance challenge. *Lancet* **2024**, *403*, 2426–2438. [https://doi.org/10.1016/s0140-6736\(24\)00876-6](https://doi.org/10.1016/s0140-6736(24)00876-6).
- Wright, G.D. Opportunities for natural products in 21st century antibiotic discovery. *Nat. Prod. Rep.* **2017**, *34*, 694–701. <https://doi.org/10.1039/c7np00019g>.
- Newton, G.G.F.; Abraham, E.P. Cephalosporin C, a New Antibiotic containing Sulphur and D- α -Aminoadipic Acid. *Nature* **1955**, *175*, 548. <https://doi.org/10.1038/175548a0>.
- Kumari, P.; Deepa, N.; Trivedi, P.K.; et al. Plants and endophytes interaction: A “secret wedlock” for sustainable biosynthesis of pharmaceutically important secondary metabolites. *Microb. Cell Fact.* **2023**, *22*, 226. <https://doi.org/10.1186/s12934-023-02234-8>.
- Venieraki, A.; Dimou, M.; Katinakis, P. Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as their hosts. *Hell. Plant Prot. J.* **2017**, *10*, 51–66. <https://doi.org/10.1515/hppj-2017-0006>.
- Rana, K.L.; Kour, D.; Kaur, T.; et al. Endophytic fungi from medicinal plants: Biodiversity and biotechnological applications. In *Microbial Endophytes*, 1st ed.; Woodhead Publishing: Cambridge, UK, 2020; pp. 273–305. <https://doi.org/10.1016/b978-0-12-819654-0.00012-9>.
- Sharma, S.; Dhar, M.K.; Kaul, S. Antagonistic, plant growth promoting and extracellular hydrolytic enzyme activity of fungal endophytes of *Dioscorea bulbifera* L. *Biocatal. Agric. Biotechnol.* **2023**, *50*, 102694. <https://doi.org/10.1016/j.bcab.2023.102694>.
- Jayatilake, P.L.; Munasinghe, H. Antimicrobial Activity of Cultivable Endophytic and Rhizosphere Fungi Associated with “Mile-a-Minute”, *Mikania cordata* (Asteraceae). *BioMed Res. Int.* **2020**, *2020*, 5292571. <https://doi.org/10.1155/2020/5292571>.
- Arnold, A.E.; Lutzoni, F. Diversity and host range of foliar fungal endophytes: Are tropical leaves biodiversity hotspots? *Ecology* **2007**, *88*, 541–549. <https://doi.org/10.1890/05-1459>.
- Gouda, S.; Das, G.; Sen, S.K.; et al. Endophytes: A treasure house of bioactive compounds of medicinal importance. *Front. Microbiol.* **2016**, *7*, 1538. <https://doi.org/10.3389/fmicb.2016.01538>.
- Obare, R.M.; Indieka, S.A.; Matasyoh, J. Antibacterial Activity of Endophytic Fungi Isolated from Leaves of Medicinal Plant *Leucas martinicensis* L. Growing in a Kenyan Tropical Forest. *Afr. J. Biochem. Res.* **2020**, *14*, 81–91. <https://doi.org/10.5897/ajbr2020.1055>.
- Wacira, T.N.; Makonde, H.M.; Bosire, C.M.; et al. Molecular Characterization and Antibacterial Potential of Endophytic Fungal Isolates from Selected Mangroves along the Coastline of Kenya. *Int. J. Microbiol.* **2024**, *2024*, 1261721. <https://doi.org/10.1155/2024/1261721>.
- Bashwira, S.; Hootele, C. Myricoidine and dihydromyricoidine, two new macrocyclic spermidine alkaloids from *Clerodendrum myricoides*. *Tetrahedron* **1988**, *44*, 4521–4526. [https://doi.org/10.1016/s0040-4020\(01\)86153-6](https://doi.org/10.1016/s0040-4020(01)86153-6).
- Keter, L.K.; Mutiso, P.C. Ethnobotanical studies of medicinal plants used by traditional health practitioners in the management of diabetes in Lower Eastern Province, Kenya. *J. Ethnopharmacol.* **2012**, *139*, 74–80. <https://doi.org/10.1016/j.jep.2011.10.014>.
- Moshi, M.J.; Otieno, D.F.; Weisheit, A. Ethnomedicine of the Kagera Region, north western Tanzania. Part 3: Plants used in traditional medicine in Kikuku village, Muleba District. *J. Ethnobiol. Ethnomed.* **2012**, *8*, 14. <https://doi.org/10.1186/1746-4269-8-14>.
- Wanga, L.A.; Wagara, I.N.; Mwakubambanya, R.; et al. Antimicrobial activity of metabolites extracted from *Zanthoxylum gillettii*, *Markhamia lutea* and their endophytic fungi against common bean bacterial pathogens. *Afr. J. Biotechnol.* **2018**, *17*, 870–879. <https://doi.org/10.5897/ajb2018.16463>.
- Srivastava, A.; Raveesha, K.A. Antimicrobial potential of fungal endophytes isolated from leaves of *Prosopis juliflora* (Sw.) DC. An important weed. *Int. J. Pharm. Pharm. Sci.* **2015**, *7*, 128–136.
- O'Donnell, K.; Cigelnik, E. Two Divergent Intragenomic rDNA ITS2 Types within a Monophyletic Lineage of the Fungus *Fusarium* Are Nonorthologous. *Mol. Phylogenet. Evol.* **1997**, *7*, 103–116. <https://doi.org/10.1006/mpev.1996.0376>.
- Glass, N.L.; Donaldson, G.C. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microbiol.* **1995**, *61*, 1323–1330. <https://doi.org/10.1128/aem.61.4.1323-1330.1995>.
- Schrey, H.; Scheele, T.; Ulonska, C.; et al. Alliaceane-Type Secondary Metabolites from Submerged Cultures of the Basidiomycete *Clitocybe nebularis*. *J. Nat. Prod.* **2022**, *85*, 2363–2371. <https://doi.org/10.1021/acs.jnatprod.2c00554>.

21. Imtiaj, A.; Jayasinghe, C.; Lee, G.W.; et al. Antibacterial and antifungal activities of *Stereum ostrea*, an inedible wild mushroom. *Mycobiology* **2007**, *35*, 210–214. <https://doi.org/10.4489/myco.2007.35.4.210>.
22. *CLSI M07-A10*; Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard—10th Edition. Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2015.
23. *CLSI M100-S25*; Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2015.
24. Sarker, S.D.; Nahar, L.; Kumarasamy, Y. Microtitre Plate-Based Antibacterial Assay Incorporating Resazurin as an Indicator of Cell Growth, and Its Application in the In Vitro Antibacterial Screening of Phytochemicals. *Methods* **2007**, *42*, 321–324. <https://doi.org/10.1016/j.jymeth.2007.01.006>.
25. Masi, M.; Castaldi, S.; Sautua, F.; et al. Truncatenolide, a Bioactive Disubstituted Nonenolide Produced by *Colletotrichum truncatum*, the Causal Agent of Anthracnose of Soybean in Argentina: Fungal Antagonism and SAR Studies. *J. Agric. Food Chem.* **2022**, *70*, 9834–9844. <https://doi.org/10.1021/acs.jafc.2c02502>.
26. Tie, Q.; Wang, M.; Huang, X.; et al. A new indole alkaloid from *Cladosporium* sp. SCSIO41205. *Nat. Prod. Res.* **2025**, *39*, 275–278. <https://doi.org/10.1080/14786419.2023.2261610>.
27. Li, Y.; Li, X.F.; Kim, D.S.; et al. Indolyl alkaloid derivatives, *N*₆-acetyltryptamine and oxaline from a marine-derived fungus. *Arch. Pharm. Res.* **2003**, *26*, 21–23. <https://doi.org/10.1007/bf03179925>.
28. Zhang, W.; Wei, S.; Zhang, J.; et al. Antibacterial activity composition of the fermentation broth of *Streptomyces djakartensis* NW35. *Molecules* **2013**, *18*, 2763–2768. <https://doi.org/10.3390/molecules18032763>.
29. Zhang, B.X.; Li, P.S.; Wang, Y.Y.; et al. Characterization and synthesis of indole-3-acetic acid in plant growth promoting *Enterobacter* sp. *RSC Adv.* **2021**, *11*, 31601–31607. <https://doi.org/10.1039/d1ra05659j>.
30. Li, R.; Su, Z.; Sun, C.; et al. Antibacterial insights into alternariol and its derivative alternariol monomethyl ether produced by a marine fungus. *Appl. Environ. Microbiol.* **2024**, *90*, e00058-24. <https://doi.org/10.1128/aem.00058-24>.
31. Hashem, A.H.; Attia, M.S.; Kandil, E.K.; et al. Bioactive Compounds and Biomedical Applications of Endophytic Fungi: A Recent Review. *Microb. Cell Fact.* **2023**, *22*, 107. <https://doi.org/10.1186/s12934-023-02118-x>.
32. Ortega, H.E.; Torres-Mendoza, D.; Cubilla-Rios, L. Antibacterial compounds isolated from endophytic fungi reported from 2021 to 2024. *Antibiotics* **2025**, *14*, 644. <https://doi.org/10.3390/antibiotics14070644>.
33. Levine, D.P. Vancomycin: A History. *Clin. Infect. Dis.* **2006**, *42*, S5–S12. <https://doi.org/10.1086/491709>.
34. Ehrlich, J.; Bartz, Q.R.; Smith, R.M.; et al. Chloromycetin, a New Antibiotic from a Soil Actinomycete. *Science* **1947**, *106*, 417. <https://doi.org/10.1126/science.106.2757.417>.
35. Werner, R.G.; Thorpe, L.F.; Reuter, W.; et al. Indolmycin Inhibits Prokaryotic Tryptophanyl-tRNA Ligase. *Eur. J. Biochem.* **1976**, *68*, 1–3. <https://doi.org/10.1111/j.1432-1033.1976.tb10758.x>
36. Jayaraman, S.; Adhilaxmi Kannan, M.; Rajendhran, N.; et al. Indole-3-acetic acid impacts biofilm formation and virulence production of *Pseudomonas aeruginosa*. *Biofouling* **2023**, *39*, 800–815. <https://doi.org/10.1080/08927014.2023.2269537>.
37. Katz, L.; Khosla, C. Antibiotic Production from the Ground Up. *Nat. Biotechnol.* **2007**, *25*, 428–429. <https://doi.org/10.1038/nbt0407-428>.
38. Vilela, R.S.; Pina-Martins, F.; Ventura, C. From Food to Humans: The Toxicological Effects of *Alternaria* Mycotoxins in the Liver and Colon. *J. Xenobiot.* **2025**, *15*, 205. <https://doi.org/10.3390/jox15060205>.
39. Marin, D.E.; Bulgaru, V.C.; Pertea, A.; et al. Alternariol Monomethyl-Ether Induces Toxicity via Cell Death and Oxidative Stress in Swine Intestinal Epithelial Cells. *Toxins* **2024**, *16*, 223. <https://doi.org/10.3390/toxins16050223>.
40. Wanga, L.A.; Indieka, A.S.; Matasyoh, J.C. Phenolic Compounds from *Calpurnia aurea* subsp. *aurea* (Aiton) Benth and Their Antimicrobial, Antidermatophytic, Antiproliferative and Cytotoxic Activities. *Microbe* **2025**, *8*, 100421. <https://doi.org/10.1016/j.microb.2025.100421>.
41. Adepiti, A.O.; Nyamboki, D.K.; Bedane, K.G.; et al. Secondary Metabolites from the Stem Bark of *Alstonia boonei* and the Seeds of *Picralima nitida* with Antibacterial Activities. *Nat. Prod. Res.* **2025**, *39*, 4751–4756. <https://doi.org/10.1080/14786419.2024.2435050>.
42. Nahar, L. Plant-Derived Natural Products for Treating *Staphylococcus aureus* Skin Infections: An Update. *eMicrobe* **2026**, *2*, 9. <https://doi.org/10.53941/emicrobe.2026.100009>.