

hydrocyanic acid, and phytic acid, which may be responsible for the various biological activities of the plant including antioxidant, antidiabetic, anti-inflammatory, and antimicrobial properties.^{17–20} Additionally, some phytochemicals with interesting bioactivities have been reported from the plant, including iloneoside (cytotoxic/anticancer), marsectohexol (α -amylase inhibitor/antidiabetic), and ajugoside (antioxidant).^{21–23}

Several studies have documented interesting biomolecules from endophytic fungi associated with *G. latifolium*. Okoye and his fellow team isolated and identified bioactive compounds, including corynesidones A, C, and D, corynethers A and B, corynether lactone A, and their derivatives, from an endophytic fungus *Corynespora cassicola* associated with the plant.^{7,8} These compounds have been reported to have interesting biological activities, including antimicrobial, cytotoxic, antioxidant, and aromatase inhibition.^{24–26} Additionally, Abba et al. reported the cytotoxic potential of secondary metabolites from several endophytic fungi of *G. latifolium*.¹¹

Antibiotic-resistant pathogenic microbes have globally become a serious threat to the healthcare systems leading to immense human and economic losses. Therefore, the demand to explore new sources for novel antimicrobial agents is an urgent need.^{27,28} Fungal endophytes proved to be a prolific source of antimicrobial compounds against various pathogenic bacteria and fungi.²⁹ On the other hand, metabolic reactions in the human body produces free radicals (reactive oxygen species, ROS) resulting in an increased oxidative stress and in turn damages the cellular components. These cellular damages can lead to various diseases such as cancers, neurodegenerative disorders, cardiovascular diseases and others.^{30–32} Therefore, there is a huge demand to attain novel antioxidant compounds combating the harmful effects of high oxidative stress yet to be of natural origin to minimize the adverse effects of synthetic antioxidants.^{33–35}

In the course of our ongoing research targeting the identification of bioactive molecules from endophytic fungi, particularly those with antimicrobial and/or antioxidant properties, we explored the total organic extract of the endophytic fungus *Nigrospora* sp. derived from the Nigerian medicinal plant *G. latifolium* Benth. (Apocynaceae) and characterized its secondary metabolites. The fermentation extract was selected based on its results, which revealed moderately active antimicrobial and antioxidant properties. Further chemical investigation of the fungal extract revealed a rare chlorinated fungal metabolite and five α -pyrone derivatives.

2. RESULTS AND DISCUSSION

2.1. Fungal Identification. This study reports the isolation of an endophytic fungus from the leaves of *G. latifolium* growing in southeastern Nigeria. Following taxonomic identification through DNA amplification and sequencing of its ITS regions, the fungus was identified as *Nigrospora* sp. The obtained nucleotide sequence was deposited in GenBank under the accession number MF327715 (Table 2).

Table 1. Primers Used for PCR⁶⁸

gene name	primer sequences (5'→ 3')	direction
ITS1	TCCGTAGGTGAACCTGCGG	forward
ITS4	TCCTCCGCTTATTGATATGC	reverse

Table 2. Identification of the Endophytic Fungus, Determined by Amplifying the ITS1 and ITS2 Regions of Its rDNA

organism	host	primer	nucleotide Sequence (FASTA format)	accession number ^a
<i>Nigrospora</i> sp.	<i>Gongronema</i> <i>latifolium</i>	ITS1/ ITS4	CTACCGGACCCGCGCCCGGGCGCCCGCGGACAAACCTGTTATCTTCGTTGATTACTGACGCTCTATTATTAATAAGTCAAAC TTTCAACAACGATCTCTGGTTCTGGCATCGATGAAGACGCAATGCGATAGTGAATTGCAGATTCAGTGAATCATCGAATCTTTGAACG CACATTGCGCCATAGTATTCTAGTGGCATGCCTTTCGAGCTCAATTTCAACCCCTAAGCAGACTTATTGTTGGCGCTACTCTGTAGTGCCT CAAAGACTTGGCGGCGCAGTAGTCTCTGAGCGTAGTAATCTTTATCTGCTCTACCGGAGCCCGCCGCGCGCCGCGGAGCA ACCAACTCTGTTATCTTCGTTGATTATCTGAGCGCTTATTTAATAAGTCAAACTTTCACAAAGGATCTCTTGGTCTGGGATCGATGAAGACGACG GAATCGGATGAATGTGAATTGCAAGATTCAGTGAATCATCGAATCTTGAACGCAATTCGCGCATTCGATAGTATTTAGTGGGATCGCTCTTCGAGCGT CATTTCAACCCCTAAGCACAGCTTATTGTTGGCGCTACTCTGTAGTGCCTCAAAGACATTTGGCGGAGCGCGAGTAGTCCTCTGAGCGTAGTAATCTTT TATCTGCTCTGTTAGGCGTGCCCGCCGCGCGTAAACCCCGCCCATTTTTTTCTGGTTGACCTGGATCGGATCGAATAACCCGCTGAACCTTAA GCATATCTGTTAGGCGTGCCCCCGCGCGCTAAACCCCGCCCATTTTTTTCTGGTTGACCTGAGTAGGAAATACCCGCTGAATCTTAAGCATAT	MF327715

⁴Details of sequence submission on the NCBI database can be found at <https://www.ncbi.nlm.nih.gov/nucleotide/>.

This is the first report of the isolation of endophytic *Nigrospora* sp. from *G. latifolium*. There is no indication that this fungus is pathogenic to the plant, as it was isolated from the internal tissues of healthy *G. latifolium* leaves using standard procedures for endophytic fungi isolation.

The fungus was subjected to solid-state fermentation, and the resulting secondary metabolites were extracted and evaluated for antimicrobial and antioxidant activities. The crude fungal extract showed moderate antibacterial activity at 1 mg/mL, producing an IZD of 2 mm against *Bacillus subtilis* and *Salmonella typhi*. However, it was inactive against the tested fungal strains (Table 3). The extract also exhibited average antioxidant activity (48% inhibition) at 500 μ g/mL (Table 4).

Table 3. Antimicrobial Assay Showing the Mean IZDs (mm) $\pm$ Standard Error of Mean (SEM) Produced Against Test Organisms

test organisms	fungal extract (1 mg/mL)	positive control ciprofloxacin (5 μ g/mL)	negative control DMSO
<i>Staphylococcus aureus</i>	0 $\pm$ 0.00	5 $\pm$ 0.33	0 $\pm$ 0.00
<i>B. subtilis</i>	2 $\pm$ 0.00	8 $\pm$ 0.00	0 $\pm$ 0.00
<i>S. typhi</i>	2 $\pm$ 0.00	7 $\pm$ 0.33	0 $\pm$ 0.00
<i>Escherichia coli</i>	0 $\pm$ 0.00	8 $\pm$ 0.33	0 $\pm$ 0.00
		miconazole (50 μ g/mL)	DMSO
<i>Candida albicans</i>	0 $\pm$ 0.00	12 $\pm$ 0.89	0 $\pm$ 0.00
<i>Aspergillus niger</i>	0 $\pm$ 0.00	14 $\pm$ 0.33	0 $\pm$ 0.00

Table 4. DPPH Antioxidant Assay

Sample (500 μ g/mL)	% inhibition
fungal extract	48
quercetin	93

2.2. Structure Elucidation of 1–6. Compounds 1 and 2 coeluted and were isolated as an inseparable mixture (1:3) in form of an off-white amorphous solid using preparative high performance liquid chromatography (HPLC). The HPLC chromatogram (Figure S1) revealed a single peak that could not be clearly separated. The molecular formulas of 1 and 2 were determined to be $C_{11}H_{12}Cl_2N_2O_5$ and $C_{10}H_{12}O_4$ based on their HR-ESI-MS spectra that revealed protonated molecular ion peaks at m/z 323.0196 $[M + H]^+$ and m/z 197.0808 $[M + H]^+$ indicating six and five degrees of unsaturation, respectively. The ^1H NMR spectral data of 1 and 2 (see Supporting Information Figure S4, Table S1) revealed two different sets of proton signals that could be easily distinguished based on their different integration indices. One set revealed two aromatic resonances at δ_H 7.66/8.20 each integrated for two protons and thus indicating the presence of 1,4-disubstituted aromatic ring. In addition, the ^1H NMR spectrum of 1 revealed one oxygenated methylene group as two diastereotopic protons at δ_H 3.63 (dd, J = 10.9, 6.0 Hz) and 3.83 (dd, J = 10.9, 7.2 Hz) that were correlated via the HSQC spectrum (Figure S8) to sp^3 carbon signal (δ_C 62.2). The ^1H – ^1H COSY spectrum of 1 (Figure S5) revealed a spin system extending from the diastereotopic methylene protons to two sp^3 methines (δ_H 4.16 and 5.18). Based on the results obtained, a literature search of 1 revealed its chemical identity as 3-(*p*-nitrophenyl)-3-hydroxy-4-dichloromethoxy-isobutanamide, a dichlorinated fungal metabolite solely reported from

the soil-derived fungus *Acrodontium salmoneum* and trivially named as acrodontiolamide³⁶ that exhibited antifungal activity in particular against phyto- and entomopathogenic fungi.³⁷ To the best of our knowledge, this study is the second report of acrodontiolamide from a natural source.

The ^1H and ^{13}C NMR spectrum of 1 and 2 (Figures S4 and S6, Table S1) revealed a second set of proton and carbon signals categorized into three olefinic signals at δ_H 6.62 (td, J = 6.2, 1.4 Hz; δ_C 135.0), δ_H 6.24 (d, J = 2.1 Hz; δ_C 100.0) and δ_H 5.63 (d, J = 2.1 Hz; δ_C 89.2). In addition, three proton resonances were also identified as a hydroxymethylene moiety at δ_H 4.35 (dd, J = 6.2, 1.2 Hz; δ_C 59.6), an olefinic methyl δ_H 1.92 (d, J = 1.2 Hz; δ_C 12.5) and an oxygenated methyl group δ_H 3.90 (s; δ_C 57.0). A literature search of 2 unravelled its identity as an α -pyrone derivative hydroxypestalopyrone that was reported from an endophytic fungus *Pestalotiopsis microspora*.^{38,39}

Further chromatographic separations of the fungal extract afforded four other α -pyrone derivatives (3–6) (Figure 1) that

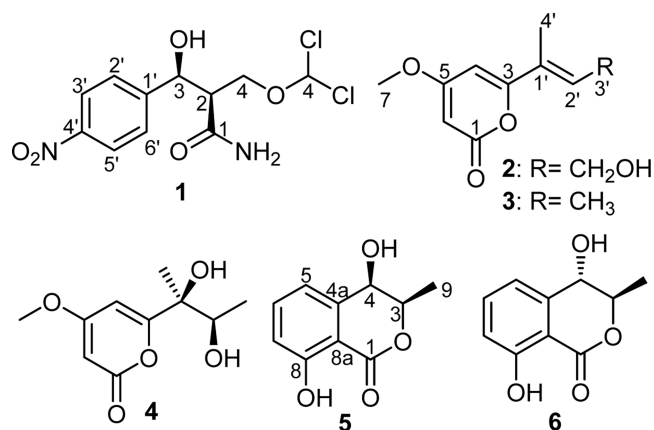


Figure 1. Chemical structures of 1–6.

were identified based on comparing their spectroscopic (1D/2D NMR) and mass spectrometric analyses with the reported literature namely; pestalopyrone (3),^{38,39} pestalotiopyrone D (4),^{40,41} *cis*-4-hydroxymellein (5),^{42,43} and *trans*-4-hydroxymellein (6).^{42,43}

Nigrospora sp. is a fungus in the class Sordariomycetes that can exist as an endophyte, saprophyte, and/or opportunistic pathogen depending upon host and environmental conditions.⁴⁴ *Nigrospora oryzae* is known to cause leaf spots on wild rice.⁴⁵

Fungi of the genus *Nigrospora* have proven to be a rich source of secondary metabolites such as nigrosporolide, nigrosporapyrone, nigrosporins, nigrospoxydons, epoxydon, deoxyabscisic acid, abscisic acid, phaseic acid, pestalopyrone, hydroxypestalopyrone, clavatul, 3-hydroxymethylphenol, and lactones. These compounds exhibit diverse biological properties, including antibacterial, antifungal, anti-inflammatory, antioxidant, cytotoxic, phytotoxic, and plant-growth regulating activities.^{38,46,47}

The genus *Nigrospora* has been isolated from several other plants, like *Moringa oleifera*, *Paraserianthes falcata*, *Emblia officinalis*, *Combretum dolichopetalum* and *Loranthus micranthus*.^{48–51} Several biologically important compounds have been reportedly isolated from endophytic *Nigrospora* sp. such as griseofulvin and dechlorogriseofulvin (antifungal), 8-dihydroamulosin (larvicidal), mullein and its derivatives (antimicro-

bial, antiviral, and phytotoxic), altersolanol derivatives (antimicrobial, hypoglycaemic, anticancer, and cytotoxic), guaijaverin (antiplasmodial and antimicrobial), isoquercetrin, (antioxidant, anti-inflammatory, cardioprotective, anticancer, antiallergic, antiprotazoal and antidiabetic), and hyperin (antimicrobial, antioxidant, antiviral, anti-inflammatory, and anticancer).^{49–56}

In this study, a rare chlorinated fungal metabolite, acrodontiolamide (1), and five α -pyrone compounds, hydroxypestalopyrone (2), pestalopyrone (3), pestalotiopyrone D (4), *cis*-4-hydroxymellein (5), and *trans*-4-hydroxymellein (6) were isolated from fermentation extracts of *Nigrospora* sp., an endophyte of *G. latifolium*. Acrodontiolamide and the α -pyrone compounds reported in this study from *Nigrospora* sp. have been well reported in the literature to possess a wide range of biological properties, including antimicrobial and/or antioxidant properties. These compounds may have significantly contributed to the antibacterial and antioxidant activities exhibited by the fungal extract (Tables 3 and 4).

The fungal metabolite acrodontiolamide, which has been reported to show inhibitory activity against phytopathogenic and entomopathogenic fungi, was first described in 1993 from a soil-borne fungus, *A. salmoneum*.^{36,37} Similar to many other fungal secondary metabolites, acrodontiolamide has a nitro function attached to an aromatic ring. However, unlike other fungal secondary metabolites, chlorination, which normally affects the aromatic part of a molecule, was located on the compound's aliphatic moiety. Very few natural product compounds have been reported with monochlorination on the aliphatic C₃ chain, making this type of halogenation uncommon.³⁶ This is the second report of acrodontiolamide from a natural source. The α -pyrone moiety is a key structural feature of a wide range of bioactive metabolites,⁵⁷ exhibiting a variety of biological activities, including antimicrobial, antitumor, and cytotoxic activities.^{57–59} Fairlamb et al. reported the antiproliferative activities of several α -pyrone compounds against cancerous cells, with IC₅₀ values ranging from 1.8 to >50 μ M.⁵⁸ Calderón-Montañó et al. reported the cytotoxicity, due to DNA damage, of 2-pyrone against leukemia cells with an IC₅₀ of 1.26 mM.⁵⁹ Another report showed that α -pyrone compounds are effective inhibitors of 20S proteasomes, indicating their anticancer potential, with pestalotiopyrone G showing the strongest activity with an IC₅₀ of 1.2 μ M.⁴¹

The isocoumarin derivative, 4-hydroxymellein, represents a class of compounds that has been isolated from several plant species including *Uvaria hamiltonii*.⁴² The mellein derivatives obtained in this study have previously been isolated from other fungi like *Lasiodiplodia theobromae*, *Septoria nordorum*, *Xylaria* sp., and *Phomopsis* sp.^{60–63} Mellein-type pentaketides are well-known fungal metabolites with significant biological activity.^{62–64} It revealed algicidal, antibacterial, and antifungal activities against *Chlorella fusca*, *Microbotryum violaceum*, and *Bacillus megaterium*, respectively.⁶⁴

Pestalopyrone and its derivatives, pestaloside and hydroxypestalopyrone, have been isolated from *P. microspora*.³⁹ Pestalotiopyrone D, together with other α -pyrone compounds, were previously isolated from *Pestalotiopsis sydowiana*.⁴¹ These compounds are known phytotoxins that cause chlorosis of plant leaves.³⁹ They have also been isolated from *Pestalotiopsis virgatula* and have been reported to have some pharmacological activities.^{65,66} Pestalopyrone, isolated from an endophytic *Phomatospora bellaminuta*, showed activity against

Plasmodium falciparum with an IC₅₀ value of 37 μ M. This compound remains a promising prototype molecule for the development of antimalarial drugs.⁵⁸

3. CONCLUSIONS

This study describes the isolation and structure elucidation of a rare chlorinated fungal metabolite, acrodontiolamide, and five α -pyrone compounds (hydroxypestalopyrone, pestalopyrone, pestalotiopyrone D, *cis*-4-hydroxymellein, and *trans*-4-hydroxymellein), from *Nigrospora* sp., an endophytic fungus derived from the leaves of *G. latifolium*. These compounds possess a wide range of biological properties, including antimicrobial, antitumor, and cytotoxic activities, making them promising candidates for exploration in drug development.

4. MATERIALS AND METHODS

4.1. Plant Collection. Fresh leaves of *G. latifolium* were harvested from a farm at Ifite Dunu town in Dunukofia Local Government Area of Anambra State (coordinates: 6°20'N 7°00'E), southeastern Nigeria. The plant material was authenticated by a plant taxonomist, and a voucher specimen (PCG474/A/006) deposited in the herbarium of the Department of Pharmacognosy and Traditional Medicine at Nnamdi Azikiwe University, Nigeria.

4.2. Fungal Isolation and Identification. Endophytic fungal isolation was carried out using a previously described method.² The plant leaves were carefully washed in running tap water before being cut into small fragments each of about 1 cm². The leaf fragments were surface-sterilized by complete immersion in a 2% sodium hypochlorite solution, followed by 70% ethanol, and then rinsed in sterile water. The sterilized leaf fragments were placed onto Petri dishes containing malt extract agar (MEA) supplemented with chloramphenicol. The Petri dishes were incubated at 28 °C for 2–3 days, during which fungal growth from the leaf fragments was observed. To obtain pure colonies, hyphal tips from distinct colonies growing from leaf segments were transferred onto fresh MEA plates. Using a previously described method,⁶⁷ with modifications, the fungus was identified by amplifying and sequencing the internal transcribed spacer (ITS) regions of the fungal rDNA (rDNA) and comparing the resulting sequence data to those in the NCBI GenBank database. Details of the fungal identification are provided below.

4.3. Molecular Taxonomic Identification Protocol.

The fungal genomic DNA was extracted and purified directly from fresh, axenic mycelia using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research Corporation, USA) as described in the manufacturer's instructions. DNA amplification by PCR was performed using 'Hot StarTaq Master Mix Taq polymerase and the primer pair ITS1 and ITS4 (Invitrogen, Germany) in an iCycler (Bio-Rad, Germany) thermocycler using the following program: (i) initial denaturation 95 °C, 15 min; (ii) denaturation 95 °C, 1 min; (iii) annealing 56 °C, 1 min; (iv) extension 72 °C, 1 min; (v) final extension 72 °C, 10 min. Steps (ii–iv) were repeated 35 times. The fungal DNA was placed in a 0.2 mL PCR tube containing 25 μ L HotStarTaq Master Mix, 1.5 μ L primer mix (10 pmol/ μ L each), 1 μ L of 10–100 ng template DNA, and water until 50 μ L. The negative control, which contained all the above listed except fungal DNA, was also prepared. Sequences for the ITS1 and ITS4 primers are shown in Table 1. After PCR, 50 μ L of the PCR product was mixed with 10 μ L

of 6X gel loading dye and 25 μL of sample was loaded onto an agarose gel [1% agarose (Biozym Scientific GmbH) in 1/10 TBE buffer, 12 μL SYBR Safe DNA gel stain (supplied as a 10,000 $\times$ concentrate in DMSO) (Invitrogen)]. One well was loaded with 10 μL Quick-Load 100 bp DNA Ladder (New England Biolabs, Inc. USA). Electrophoresis was then run at 75 V for 45 min. After electrophoresis, the gel was viewed on a UV transilluminator to confirm that the PCR had been successfully carried out and that the PCR product was of the correct size, approximately 550 bp, by comparing it with the DNA ladder. The DNA fragment was then excised from the agarose gel with a clean, sharp scalpel. Pure DNA was obtained from the DNA fragment using the Zymoclean Gel DNA Recovery Kit (Zymo Research Corp, USA) according to the manufacturer's protocol. The DNA sample and ITS1 primer (7.5 μL DNA + 2.5 μL ITS1) were placed in a sterile 2 mL tube and submitted to GATC Biotech AG (Köln, Germany) for direct sequencing using Sanger sequencing technology. After sequencing, BioEdit (version 7.2.5) was used to view the sequencing chromatogram and check for quality. The sequence was then copied in FASTA format and pasted on query box in the NCBI BLAST Web site (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

4.4. Fungal Fermentation and Extraction of Secondary Metabolites. The endophytic fungus was subjected to solid-state fermentation in five 1 L Erlenmeyer flasks, each containing autoclaved rice medium (100 g of rice in 100 mL of distilled water), as previously described.^{2,3} Previous studies have validated the use of rice medium for fungal fermentation and bioprospecting of novel metabolites.^{1,10,69} This approach offers several advantages: first, solid-state fermentation in rice medium promotes high yields of microbial secondary metabolites. Second, the rice medium itself does not contribute significantly to the fungal secondary metabolite profile, making it easier to identify and isolate the distinct metabolites produced by the fungus. The flask was inoculated with 3 mm agar blocks containing the fungus and incubated at 28 $^{\circ}\text{C}$ for 30 days. At the end of the fermentation, the fungal secondary metabolites were extracted from each fermentation flask with 500 mL of ethyl acetate and concentrated under reduced pressure at 40 $^{\circ}\text{C}$ using a rotary evaporator.

4.5. Detection and Purification of Metabolites. The total fungal crude extract (5.6 g) derived from the five fermentation flasks was fractionated using vacuum liquid chromatography on silica gel 60 (Merck) with stepwise gradient elution, achieved by gradually increasing the polarity of the solvent system *n*-hexane (*n*-hex) and ethyl acetate (EtOAc) combinations in the ratios of 100:0, 70:30, 50:50, 30:70, and 0:100. Fractions were monitored using thin layer chromatography on silica gel 60 F₂₅₄ plates (Merck) with UV detection at 254 and 365 nm. Fractions containing metabolites [*n*-hex/EtOAc (50:50, 30:70, and 0:100)] were further purified using semipreparative HPLC on a Merck-Hitachi HPLC system. The system was equipped with an L-7400 UV detector, L-7100 pump, and 300 $\times$ 8 mm Eurospher-100 C₁₈ column (Knauer, Germany), with gradient or isocratic methanol (MeOH) and water (H₂O) mixture as the mobile phase (flow rate: 5 mL/min). Semipreparative HPLC of the fractions yielded the following compounds: *n*-hex/EtOAc 50:50 (1.6 g)—5 (2.3 mg) and 6 (3.2 mg); *n*-hex/EtOAc 30:70—(1.2 g): 3 (2.1 mg); *n*-hex/EtOAc 0:100 (1.9 g)—1/2 (1.5 mg), and 4 (1.2 mg).

Purity of the isolated compounds was assessed using analytical HPLC on a P580 HPLC system (Dionex Softron GmbH) equipped with a P580A LPG pump, UVD340s photodiode array detector (Dionex Softron GmbH), and a 4 $\times$ 125 mm Eurospher-10 C₁₈ separation column (Knauer, Germany) with UV detection at 235 nm. 1D and 2D NMR spectra of the compounds were recorded in deuterated chloroform or methanol, using Bruker 300 and Bruker Avance III 600 spectrometers. The spectra and their chemical shifts were referenced relative to residual solvent signals, respectively. Mass spectrometry measurements involved an HP Agilent 1100 HPLC-Thermo Quest Finnigan LCQ Deca XP LC/MS system and HR-ESI-MS UHR-QTOF maXis 4G (Bruker Daltonics). All spectroscopic measurements were performed with spectral-grade solvents.

4.5.1. Acrodontiolamide (1). Off-white amorphous solid; UV (MeOH) λ_{max} at 220.1, 309.8 nm; NMR data (¹H NMR: 600 MHz, ¹³C NMR: 150 MHz in methanol-*d*₄, see Supporting Information: Table S1) comparable to the reported literature;^{36,37} HR-ESI-MS *m/z* 323.0192 [*M* + *H*]⁺ (calcd 323.0196 for C₁₁H₁₃Cl₂N₂O₅⁺, see Supporting Information: Figure S3).

4.5.2. Hydroxypestalopyrone (2). Off-white amorphous solid; UV (MeOH) λ_{max} at 220.1, 309.8 nm; NMR data (¹H NMR: 600 MHz, ¹³C NMR: 150 MHz in methanol-*d*₄, see Supporting Information: Table S1) comparable to the reported literature;^{38,39} HR-ESI-MS *m/z* 197.0807 [*M* + *H*]⁺ (calcd 197.0808 for C₁₀H₁₃O₄⁺, see Supporting Information: Figure S3).

4.5.3. Pestalopyrone (3). Light yellow amorphous solid; UV (MeOH) λ_{max} at 222.1, 256.8, 311.4 nm; ¹H NMR (300 MHz in methanol-*d*₄, see Supporting Information: Table S2) comparable to the reported literature;^{38,39} HR-ESI-MS *m/z* 181.0859 [*M* + *H*]⁺ (calcd 181.0859 for C₁₀H₁₃O₃⁺, see Supporting Information: Figure S13).

4.5.4. Pestalotiopyrone D (4). Colorless oil; UV (MeOH) λ_{max} at 205.1, 281.1 nm. ¹H NMR (300 MHz in methanol-*d*₄, see Supporting Information: Table S3) comparable to the reported literature;^{40,41} HR-ESI-MS *m/z* 215.0916 [*M* + *H*]⁺ (calcd 215.0914 for C₁₀H₁₅O₅⁺, see Supporting Information: Figure S18).

4.5.5. cis-4-Hydroxymellein (5). White amorphous solid; UV (MeOH) λ_{max} 217.2, 244.1, 313.0 nm; ¹H NMR (300 MHz in chloroform-*d*, see Supporting Information: Table S4) comparable to the reported literature;^{42,43} LR-ESI-MS *m/z* 193.3 [*M*-*H*]⁻ and 195 [*M* + *H*]⁺ (see Supporting Information: Figure S23).

4.5.6. trans-4-Hydroxymellein (6). White needle-like crystals; UV (MeOH) λ_{max} 216, 246, 314 nm; ¹H NMR (300 MHz in chloroform-*d*, see Supporting Information: Table S5) comparable to the reported literature;^{42,43} LR-ESI-MS *m/z* 195.2 [*M* + *H*]⁺ (see Supporting Information: Figure S29).

4.6. Bioassays. **4.6.1. Antimicrobial Assay.** The agar-well diffusion method described by Akpotu et al.⁴ and Enyi et al.⁵ was used to assess the antimicrobial property of the crude fungal extract. The extract was tested against *S. aureus*, *B. subtilis*, *S. typhi*, and *E. coli*, *A. niger* and *C. albicans*. These laboratory-derived strains were obtained from the Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Nigeria. A 1 mg/mL solution of the fungal extract was prepared in 100% (v/v) dimethyl sulfoxide (DMSO). Then, 20 mL of molten Mueller–Hinton agar (for bacteria) and Sabouraud dextrose agar (for fungi) were poured

into sterile Petri dishes (90 mm) and allowed to solidify. Standardized concentrations (McFarland 0.5) of overnight cultures were swabbed aseptically onto the agar plates. Subsequently, 6 mm wells were made in the agar using a sterile metal cork-borer. Under aseptic conditions, 20 μ L of the extract and controls were added to each well. Positive controls for the antibacterial and antifungal tests were ciprofloxacin (5 μ g/mL) and miconazole (50 μ g/mL), respectively. DMSO (100% v/v) served as the negative control in both tests. The plates were kept at room temperature for 1 h to allow the agents to diffuse into the agar medium before incubating at 37 °C for 24 h (Mueller–Hinton agar) and 25 °C for 48 h (Sabouraud dextrose agar), respectively. Inhibition zone diameters (IZDs) were calculated by subtracting the size of the well (6 mm) from the measured zones of inhibition. This procedure was conducted in triplicate, and the mean IZDs $\pm$ standard error of mean (SEM) were calculated and recorded.

4.6.2. Antioxidant Assay. The antioxidant property of the crude fungal extract was also evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging test method previously described by Abba et al.⁶ Stock solutions of the fungal extract (1000 μ g/mL), positive control (quercetin, 1000 μ g/mL) and DPPH solution (0.2 mM) were prepared in MeOH. The sample and DPPH solution were mixed in equal volumes, resulting in final concentrations of 500 μ g/mL and 0.1 mM, respectively. The mixture was incubated for 30 min in the dark at room temperature. A UV–vis spectrophotometer was used to measure absorbance at 517 nm using MeOH as a blank. The free radical scavenging effect of the samples was computed using the formula:

$$\% \text{ inhibition} = \frac{A_0 - A_1}{A_0} \times \frac{100}{1}$$

where A_0 : absorbance of blank, A_1 : absorbance of sample.

4.7. Statistical Analysis. The mean IZDs $\pm$ standard error of mean (SEM) values obtained from the antimicrobial assay were calculated using Microsoft Excel version 16.82.

■ ASSOCIATED CONTENT

Data Availability Statement

All relevant data are included within the article and its Supporting Information. Sequencing raw data are publicly available via the NCBI GenBank database under the accession number MF327715 (<https://www.ncbi.nlm.nih.gov/nuccore/MF327715>).

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.4c09190>.

The HPLC, NMR and mass spectral data of the compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Ebada, S. S.; Eze, P.; Okoye, F. B.; Esimone, C. O.; Proksch, P. The fungal endophyte *Nigrospora oryzae* produces quercetin monoglycosides previously known only from plants. *ChemistrySelect* **2016**, *1* (11), 2767–2771.
- (2) Eze, P. M.; Ojimba, N. K.; Abonyi, D. O.; Chukwunwejim, C. R.; Abba, C. C.; Okoye, F. B. C.; Esimone, C. O. Antimicrobial activity of metabolites of an endophytic fungus isolated from the leaves of *Citrus jambhiri* (Rutaceae). *Trop. J. Nat. Prod. Res.* **2018**, *2* (3), 145–149.
- (3) Eze, P. M.; Nnanna, J. C.; Okezie, U.; Buzugbe, H. S.; Abba, C. C.; Chukwunwejim, C. R.; Okoye, F. B. C.; Esimone, C. O. Screening of metabolites from endophytic fungi of some Nigerian medicinal plants for antimicrobial activities. *Eurobiotech. J.* **2019**, *3* (1), 10–18.
- (4) Akpotu, M. O.; Eze, P. M.; Abba, C. C.; Umeokoli, B. O.; Nwachukwu, C. U.; Okoye, F. B. C.; Esimone, C. O. Antimicrobial activities of secondary metabolites of endophytic fungi isolated from *Catharanthus roseus*. *J. Health Sci.* **2017**, *7* (1), 15–22.
- (5) Enyi, E.; Eze, P. M.; Ogwaluonye, U. C.; Iloh, E. S.; Nwankwo, O. L.; Okoye, F. B. C.; Esimone, C. O. Antimicrobial, antioxidant and cytotoxic properties of secondary metabolites from endophytic fungi of *Psidium guajava*. *Pharm. Chem. J.* **2019**, *6* (1), 90–97.
- (6) Abba, C. C.; Eze, P. M.; Abonyi, D. O.; Nwachukwu, C. U.; Proksch, P.; Okoye, F. B. C.; Eboka, C. J. Phenolic compounds from endophytic *Pseudofusicoccum* sp. isolated from *Annona muricata*. *Trop. J. Nat. Prod. Res.* **2018**, *2* (7), 332–337.
- (7) Okoye, F. B. C.; Lu, S.; Nworu, C. S.; Esimone, C. O.; Proksch, P.; Chadli, A.; Debbab, A. Depsidone and diaryl ether derivatives from the fungus *Corynespora cassicola*, an endophyte of *Gongronema latifolium*. *Tetrahedron Lett.* **2013**, *54*, 4210–4214.
- (8) Okoye, F. B. C.; Nworu, C. S.; Akah, P. A.; Esimone, C. O.; Debbab, A.; Proksch, P. Inhibition of inflammatory mediators and

reactive oxygen and nitrogen species by some depsidones and diaryl ether derivatives isolated from *Corynespora cassiicola*, an endophytic fungus of *Gongronema latifolium* leaves. *Immunopharmacol. Immunotoxicol.* **2013**, *35* (6), 662–668.

(9) Abba, C. C.; Nduka, I.; Eze, P. M.; Ujam, T. N.; Abonyi, D. O.; Okoye, F. B. C. Antimicrobial activity of secondary metabolites of endophytic *Aspergillus* species isolated from *Loranthus micranthus*. *Afr. J. Pharm. Res. Dev.* **2016**, *8* (2), 136–140.

(10) Wang, H.; Eze, P. M.; Höfert, S. P.; Janiak, C.; Hartmann, R.; Okoye, F. B. C.; Esimone, C. O.; Orfali, R. S.; Dai, H.; Liu, Z.; Proksch, P. Substituted L-tryptophan-L-phenyllactic acid conjugates produced by an endophytic fungus *Aspergillus aculeatus* using an OSMAC approach. *RSC Adv.* **2018**, *8*, 7863–7872.

(11) Abba, C. C.; Eze, P. M.; Okoye, F. B. C.; Eboka, C. J. Cytotoxicity and antimicrobial activities of secondary metabolites produced by endophytic fungi isolated from *Gongronema latifolia* and *Loranthus micranthus*. *Planta Med.* **2015**, *81* (16), 105.

(12) Stierle, A.; Strobel, G.; Stierle, D. Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. *Science* **1993**, *260* (5105), 214–216.

(13) Puri, S. C.; Verma, V.; Amna, T.; Qazi, G. N.; Spiteller, M. An endophytic fungus from *Nothapodytes foetida* that produces camptothecin. *J. Nat. Prod.* **2005**, *68*, 1717–1719.

(14) Eyberger, A. L.; Dondapati, R.; Porter, J. R. Endophyte fungal isolates from *Podophyllum peltatum* produce podophyllotoxin. *J. Nat. Prod.* **2006**, *69*, 1121–1124.

(15) Kusari, S.; Lamshöft, M.; Zühlke, S.; Spiteller, M. An Endophytic fungus from *Hypericum perforatum* that produces hypericin. *J. Nat. Prod.* **2008**, *71*, 159–162.

(16) Kumar, A.; Patil, D.; Rajamohanam, P. R.; Ahmad, A. Isolation, purification and characterization of vinblastine and vincristine from endophytic fungus *Fusarium oxysporum* isolated from *Catharanthus roseus*. *PLoS One* **2013**, *8* (9), No. e71805.

(17) Ugochukwu, N. H.; Babady, N. E.; Cobourne, M.; Gasset, S. R. The effect of *Gongronema latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. *J. Biosci.* **2003**, *28*, 1–5.

(18) Ogundipe, O. O.; Moody, J. O.; Akinyemi, T. O.; Raman, A. Hypoglycemic potentials of methanolic extracts of selected plant foods in alloxanized mice. *Plant Foods Hum. Nutr.* **2003**, *58*, 1–7.

(19) Osuagwu, A. N.; Ekpo, I. A.; Okpako, E. C.; Out, P.; Ottho, E. The Biology, utilization and phytochemical composition of the fruits and leaves of *Gongronema latifolium* Benth. *Agrotechnology* **2013**, *2* (3), 115.

(20) Morebise, O. A review on *Gongronema latifolium*, an extremely useful plant with great prospects. *Eur. J. Med. Plants.* **2015**, *10* (1), 1–9.

(21) Gyebi, G. A.; Adebayo, J. O.; Olorundare, O. E.; Pardede, A.; Ninomiya, M.; Saheed, A. O.; Babatunde, A. S.; Koketsu, M. Iloneoside: a cytotoxic ditigloylated pregnane glycoside from the leaves of *Gongronema latifolium* Benth. *Nat. Prod. Res.* **2018**, *32* (24), 2882–2886.

(22) Ogunyemi, O. M.; Gyebi, A. G.; Adebayo, J. O.; Oguntola, J. A.; Olaiya, C. O. Marsectohexol and other pregnane phytochemicals derived from *Gongronema latifolium* as α -amylase and α -glucosidase inhibitors: in vitro and molecular docking studies. *SN Appl. Sci.* **2020**, *2*, 2119.

(23) Erukainure, O. L.; Ebuehi, O. A. T.; Choudhary, I. M.; Adhikari, A.; Hafizur, R. M.; Perveen, S.; Muhammad, A.; Elemo, G. N. Iridoid glycoside from the leaves of *Clerodendrum volubile* Beauv. shows potent antioxidant activity against oxidative stress in rat brain and hepatic tissues. *J. Diet. Suppl.* **2014**, *11* (1), 19–29.

(24) Talontsi, F. M.; Douanla-Meli, C.; Laatsch, H. Depsidones from an endophytic fungus *Chaetomium* sp. associated with *Zanthoxylum leprieurii*. *Z. Naturforsch. B* **2013**, *68* (11), 1259–1264.

(25) Zhao, D.-L.; Shao, C.-L.; Wang, C.-Y.; Wang, M.; Yang, L.-J.; Wang, C.-Y. Naphthalenones and depsidones from a sponge-derived strain of the fungus *Corynespora cassiicola*. *Molecules* **2016**, *21* (2), 160.

(26) Chomcheon, P.; Wiyakrutta, S.; Sriubolmas, N.; Ngamrojanavanich, N.; Kengtong, S.; Mahidol, C.; Ruchirawat, S.; Kittakoop, P. Aromatase inhibitory, radical scavenging, and antioxidant activities of depsidones and diaryl ethers from the endophytic fungus *Corynespora cassiicola* L36. *Phytochemistry* **2009**, *70* (3), 407–413.

(27) Ferri, M.; Ranucci, E.; Romagnoli, P.; Giaccone, V. Antimicrobial resistance: A global emerging threat to public health systems. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 2857–2876.

(28) Ayres, M. C. C.; Brandao, M. S.; Vieira-Junior, G. M.; Menor, J. C. A. S.; Silva, H. B.; Soares, M. J. S.; et al. Antibacterial activity of useful plants and chemical constituents of the roots of *Copernicia prunifera*. *Rev. Bras. Farmacogn.* **2008**, *18*, 90–97.

(29) Stierle, A.; Strobel, G. A.; Stierle, D. Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. *Science* **1993**, *260*, 214–216.

(30) Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M. T. D.; Mazur, M.; Telser, J. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* **2007**, *39*, 44–84.

(31) Agarwal, A.; Sharma, R. K.; Nallella, K. P.; Thomas, A. J., Jr; Alvarez, J.; Sikka, S. Reactive oxygen species as an independent marker of male factor infertility. *Fertil. Steril.* **2006**, *86*, 878–885.

(32) Cui, J. L.; Guo, T. T.; Ren, Z. X.; Zhang, N. S.; Wang, M. L. Diversity and antioxidant activity of culturable endophytic fungi from alpine plants of *Rhodola crenulata*, *R. angusta* and *R. schalinensis*. *PLoS One* **2015**, *10*, No. e0118204.

(33) Khalil, D. M. A.; El-Zayat, S. A.; El-Sayed, M. A. Phytochemical screening and antioxidant potential of endophytic fungi isolated from *Hibiscus sabdariffa*. *J. Appl. Biotechnol. Rep.* **2020**, *7*, 116–124.

(34) Caicedo, N. H.; Davalos, A. F.; Puente, P. A.; Rodríguez, A. Y.; Caicedo, P. A. Antioxidant activity of exometabolites produced by *Fusarium oxysporum*: an endophytic fungus isolated from leaves of *Otoba gracilipes*. *MicrobiologyOpen* **2019**, *8*, No. e903.

(35) Gunasekaran, S.; Sathivelu, M.; Arunachalam, S. In vitro antioxidant and antibacterial activity of endophytic fungi isolated from *Mussaenda luteola*. *J. Appl. Pharm. Sci.* **2017**, *7*, 234–238.

(36) De Gusmão, N. B.; Kaouadji, M.; Seigle-Murandi, F.; Steiman, R.; Thomasson, F. Acrodontiolamide, a chlorinated fungal metabolite from *Acrodontium salmoneum*. *Spectrosc. Lett.* **1993**, *26*, 1373–1380.

(37) Steiman, R.; Benoit-Guyod, J. L.; Guiraud, P.; Seigle-Murandi, F. Evaluation of acrodontiolamide, a chlorinated compound produced by *Acrodontium salmoneum* de Hoog for cytotoxicity and antimicrobial activity. *Pharmazie* **1995**, *50* (10), 693–695.

(38) Trisuwan, K.; Rukachaisirikul, V.; Sukpondma, Y.; Preedanon, S.; Phongpaichit, S.; Rungjindamai, N.; Sakayaroj, J. Epoxydons and a pyrone from the marine-derived fungus *Nigrospora* sp. PSU-F5. *J. Nat. Prod.* **2008**, *71* (8), 1323–1326.

(39) Lee, J. C.; Yang, X.; Schwartz, M.; Strobel, G.; Clardy, J. The relationship between an endangered North American tree and an endophytic fungus. *Chem. Biol.* **1995**, *2* (11), 721–727.

(40) Xu, J.; Aly, A. H.; Wray, V.; Proksch, P. Polyketide derivatives of endophytic fungus *Pestalotiopsis* sp. isolated from the Chinese mangrove plant *Rhizophora mucronata*. *Tetrahedron Lett.* **2011**, *52*, 21–25.

(41) Xia, X.; Kim, S.; Liu, C.; Shim, S. H. Secondary metabolites produced by an endophytic fungus *Pestalotiopsis sydowiana* and their 20S proteasome inhibitory activities. *Molecules* **2016**, *21* (7), 944.

(42) Asha, K. N.; Chowdhury, R.; Hasan, C. M.; Rashid, M. A. Steroids and polyketides from *Uvaria hamiltonii* stem bark. *Acta Pharm.* **2004**, *54* (1), 57–63.

(43) Rukachaisirikul, V.; Buadam, S.; Sukpondma, Y.; Phongpaichit, S.; Sakayaroj, J.; Hutadilok-Tawatana, N. Indanone and mellein derivatives from the *Garcinia*-derived fungus *Xylaria* sp. PSU-G12. *Phytochem. Lett.* **2013**, *6* (1), 135–138.

(44) Zafri, A. S.; Muhamad, R.; Wahab, A.; Mokhtar, A. S.; Hata, E. M. First report of leaf blight on *Parthenium hysterophorus* caused by *Nigrospora sphaerica* in Malaysia. *Plant Dis* **2021**, *105* (10), 3297.

- (45) Liu, Y. L.; Tang, J. R.; Li, Y.; Zhou, H. K. First report of leaf spot caused by *Nigrospora oryzae* in wild rice in China. *Plant Dis* **2021**, 105 (10), 3293.
- (46) Kim, J. C.; Choi, G. J.; Park, J. H.; Kim, H. T.; Cho, K. Y. Activity against plant pathogenic fungi of phomalactone isolated from *Nigrospora sphaerica*. *Pest Manag. Sci.* **2001**, 57, 554–559.
- (47) Mehta, G.; Islam, K. Enantioselective total synthesis of epoxyquinone natural products (–)-phyllostine, (+)-epoxydon, (+)-epiepoxydon and (–)-panepophenanthrin: access to versatile chiral building blocks through enzymatic kinetic resolution. *Tetrahedron Lett.* **2004**, 45, 7683–7687.
- (48) Amin, N. Investigation of culture filtrate of endophytic fungi *Nigrospora* sp. isolate RS10 in different concentrations towards root-knot nematode *Meloidogyne* spp. *Ind. J. Sci. Technol.* **2013**, 6 (9), 1–5.
- (49) Zhao, J. H.; Zhang, Y. L.; Wang, L. W.; Wang, J. Y.; Zhang, C. L. Bioactive secondary metabolites from *Nigrospora* sp. LLGLM003, an endophytic fungus of the medicinal plant *Moringa oleifera* Lam. *World J. Microbiol. Biotechnol.* **2012**, 28, 2107–2112.
- (50) Rathod, D. P.; Dar, M. A.; Gade, A. K.; Rai, M. K. Griseofulvin producing endophytic *Nigrospora oryzae* from Indian *Embilica officinalis* Gaertn: A new report. *Austin J. Biotechnol. Bioeng.* **2014**, 1 (3), 1–5.
- (51) Uzor, P. F.; Ebrahim, W.; Osadebe, P. O.; Nwodo, J. N.; Okoye, F. B.; Müller, W. E. G.; Lin, W.; Liu, Z.; Proksch, P. Metabolites from *Combretum dolichopetalum* and its associated endophytic fungus *Nigrospora oryzae*: Evidence for a metabolic partnership. *Fitoterapia* **2015**, 105, 147–150.
- (52) Feng, S.; Wang, W. Bioactivities and structure-activity relationships of natural tetrahydroanthraquinone compounds: A review. *Front. Pharmacol.* **2020**, 11, 799.
- (53) Houël, E.; Nardella, F.; Jullian, V.; Valentin, A.; Vonthron-Sénécheau, C.; Villa, P.; Obrecht, A.; Kaiser, M.; Bourreau, E.; Odonne, G.; Fleury, M.; Bourdy, G.; Eparvier, V.; Deharo, E.; Stien, D. Wayanin and guaijaverin, two active metabolites found in *Psidium acutangulum* Mart. ex DC (syn. *P. persoonii* McVaugh) (Myrtaceae) antimalarial decoction from the Wayana Amerindians. *J. Ethnopharmacol.* **2016**, 187, 241–248.
- (54) Prabu, G. R.; Gnanamani, A.; Sadulla, S. Guaijaverin—a plant flavonoid as potential antiplatelet agent against *Streptococcus mutans*. *J. Appl. Microbiol.* **2006**, 101 (2), 487–495.
- (55) Valentová, K.; Vrba, J.; Bancířová, M.; Ulrichová, J.; Křen, V. Isoquercitrin: pharmacology, toxicology, and metabolism. *Food Chem. Toxicol.* **2014**, 68, 267–282.
- (56) Karioti, A.; Bilia, A. R. Hypericins as potential leads for new therapeutics. *Int. J. Mol. Sci.* **2010**, 11 (2), 562–594.
- (57) Schäberle, T. F. Biosynthesis of α -pyrones. *Beilstein J. Org. Chem.* **2016**, 12, 571–588.
- (58) Fairlamb, I. J. S.; Marrison, L. R.; Dickinson, J. M.; Lu, F. J.; Schmidt, J. P. 2-Pyrones possessing antimicrobial and cytotoxic activities. *Bioorg. Med. Chem.* **2004**, 12, 4285–4299.
- (59) Calderón-Montaño, J. M.; Burgos-Morón, E.; Orta, M. L.; Pastor, N.; Austin, C. A.; Mateos, S.; López-Lázaro, M. Alpha, beta-unsaturated lactones 2-furanone and 2-pyrone induce cellular DNA damage, formation of topoisomerase I- and II-DNA complexes and cancer cell death. *Toxicol. Lett.* **2013**, 222, 64–71.
- (60) Aldridge, D. C.; Galt, S.; Giles, D.; Turner, W. B. Metabolites of *Lasiodiplodia theobromae*. *J. Chem. Soc. C.* **1971**, 1623–1627.
- (61) Devys, M.; Barbier, M.; Bouquet, J. F.; Kollmann, A. Isolation of the new (–)-(3R,4S)-4-hydroxymellein from the fungus *Septoria nodorum* Berk. *Z. Naturforsch. C* **1992**, 47 (9–10), 779–781.
- (62) Rukachaisirikul, V.; Arunpanichlert, J.; Sukpondma, Y.; Phongpaichit, S.; Sakayaroj, J. Metabolites from the endophytic fungi *Botryosphaeria rhodina* PSU-M35 and PSU-M114. *Tetrahedron* **2009**, 65, 10590–10595.
- (63) Weber, D.; Gorzalczy, S.; Martinc, V.; Acevedo, C.; Sterner, O.; Anke, T. Metabolites from endophytes of the medicinal plant *Erythrina crista-galli*. *Z. Naturforsch. C J Biosci.* **2005**, 60 (5–6), 467–477.
- (64) Hussain, H.; Krohn, K.; Draeger, S.; Meier, K.; Schulz, B. Bioactive chemical constituents of a sterile endophytic fungus from *Melilotus dentatus*. *Rec. Nat. Prod.* **2009**, 3 (2), 114–117.
- (65) Rönsberg, D.; Debbab, A.; Mándi, A.; Wray, V.; Dai, H.; Kurtán, T.; Proksch, P.; Aly, A. H. Secondary metabolites from the endophytic fungus *Pestalotiopsis virgatula* isolated from the mangrove plant *Sonneratia caseolaris*. *Tetrahedron Lett.* **2013**, 54, 3256–3259.
- (66) Cao, S.; Clardy, J. New naphthoquinones and a new δ -lactone produced by endophytic fungi from Costa Rica. *Tetrahedron Lett.* **2011**, 52 (17), 2206–2208.
- (67) Wang, S.; Li, X.; Teuscher, F.; Li, D.-L.; Diesel, A.; Ebel, R.; Proksch, P.; Wang, B. Chaetopyranin, a benzaldehyde derivative, and other related metabolites from *Chaetomium globosum*, an endophytic fungus derived from the marine red alga *Polysiphonia urceolata*. *J. Nat. Prod.* **2006**, 69 (11), 1622–1625.
- (68) Kumar, M.; Shukla, P. K. Use of PCR targeting of internal transcribed spacer regions and single-stranded conformation polymorphism analysis of sequence variation in different regions of rRNA genes in fungi for rapid diagnosis of mycotic keratitis. *J. Clin. Microbiol.* **2005**, 43 (2), 662–668.
- (69) Wang, H.; Umeokoli, B. O.; Eze, P.; Heering, C.; Janiak, C.; Müller, W. E.; Orfali, R. S.; Hartmann, R.; Dai, H.; Lin, W.; Liu, Z.; Proksch, P. Secondary metabolites of the lichen-associated fungus *Apiospora montagnei*. *Tetrahedron Lett.* **2017**, 58 (17), 1702–1705.



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