Postprint: Screening of Bioactive Endophytic Fungi from Aegiceras corniculatum and Their Antibacterial Chemical Constituents

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Abstract

To investigate the antimicrobial value of endophytic fungi from Aegiceras corniculatum, active strains were screened based on the antimicrobial activity of their fermentation products. Chemical constituents from active strains were isolated using a bioactivity-guided fractionation approach combined with various chromatographic techniques, structures of individual compounds were identified by spectroscopic methods coupled with literature data comparison, and antimicrobial activities of individual compounds were determined by microplate assay. The results showed that: (1) The 16 endophytic fungal strains isolated from Aegiceras corniculatum belonged to 2 classes, 7 orders, 10 families, and 10 genera, with Fusarium being the dominant genus. The fermentation products of endophytic fungi GXIMD02029 and GXIMD02039 exhibited varying degrees of inhibitory effects against Bacillus subtilis, Staphylococcus epidermidis, methicillin-resistant Staphylococcus aureus, Micrococcus luteus, Actinomyces viscosus, and Staphylococcus aureus, while the fermentation product of GXIMD02038 showed inhibitory activity against methicillin-resistant Staphylococcus aureus, Micrococcus luteus, and Staphylococcus aureus. (2) Seven compounds were isolated from endophytic fungus Phomopsis sp. GXIMD02029 and identified as (15R)-acetoxydothiorelone A (1), cytosporone B (2), pestalotiopsone H (3), pestalotiopsone B (4), 4-Hydroxybenzaldehyde (5), p-Hydroxybenzoic acid (6), and N-(2-phenylethyl)acetamide (7). (3) Compounds 1 and 2 exhibited varying degrees of antimicrobial activity. The MIC values of compound 1 against Bacillus subtilis, Staphylococcus epidermidis, and methicillin-resistant Staphylococcus aureus were 16.25 $g \cdot mL^{-1}$, against Micrococcus luteus and Actinomyces viscosus were 7.8125 $g \cdot mL^{-1}$, and against Staphylococcus aureus was 31.25 $g \cdot mL^{-1}$. The MIC value of compound 2 against Micrococcus luteus was 62.5 $g \cdot mL^{-1}$, against Bacillus subtilis, Staphylococcus epidermidis, methicillin-resistant Staphylococcus aureus, and Actinomyces viscosus was 125 $g \cdot mL^{-1}$,

and against Staphylococcus aureus was 250 $g \cdot mL^{-1}$. Three active strains were screened, and compound 1 was reported for the first time to possess antimicrobial activity, providing evidence for the antimicrobial value of endophytic fungi from Aegiceras corniculatum.

Full Text

Screening of Active Endophytic Fungi Derived from *Aegiceras corniculatum* and Their Antibacterial Chemical Constituents

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Abstract: To explore the antibacterial potential of endophytic fungi derived from *Aegiceras corniculatum*, this study screened active strains based on the antibacterial activity of their fermentation extracts. Chemical constituents from active strains were isolated using bioactivity-guided fractionation combined with multiple chromatographic techniques, and their structures were elucidated by spectroscopic analysis and comparison with literature data. The antibacterial activities of pure compounds were evaluated using microplate assays. The results showed: (1) Sixteen endophytic fungal strains isolated from *A. corniculatum* were classified into 2 classes, 7 orders, 10 families, and 10 genera, with *Fusarium* being the dominant genus. The fermentation extracts of strains GXIMD02029 and GXIMD02039 exhibited varying degrees of inhibition against *Bacillus subtilis*, *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus*, *Micrococcus luteus*, *Actinomyces viscosus*, and *Staphylococcus aureus*, while GXIMD02038 showed inhibitory effects against methicillin-resistant *S. aureus*, *M. luteus*, and *S. aureus*. (2) Seven compounds were isolated and identified from the endophytic fungus *Phomopsis* sp. GXIMD02029: (15R)-acetoxydothiorelone A (1), cytosporone B (2), pestalotiopsone H (3), pestalotiopsone B (4), 4-Hydroxybenzaldehyde (5), p-Hydroxybenzoic acid (6), and N-(2-phenylethyl)acetamide (7). (3) Compounds 1 and 2 demonstrated antibacterial activity with varying potency. Compound 1 showed MIC values of 16.25 $\mu g \cdot mL^{-1}$ against *B. subtilis*, *S. epidermidis*, and methicillin-resistant *S. aureus*; 7.8125 $\mu g \cdot mL^{-1}$ against *M. luteus* and *A. viscosus*; and 31.25 $\mu g \cdot mL^{-1}$ against *S. aureus*. Compound 2 exhibited an MIC of 62.5 $\mu g \cdot mL^{-1}$ against *M. luteus*, 125 $\mu g \cdot mL^{-1}$ against *B. subtilis*, *S. epidermidis*, methicillin-resistant *S. aureus*, and *A. viscosus*, and 250 $\mu g \cdot mL^{-1}$ against *S. aureus*. This study

identified three active strains and reports for the first time the antibacterial activity of compound 1, providing a foundation for the antibacterial potential of endophytic fungi from *A. corniculatum*.

Keywords: *Aegiceras corniculatum*, endophytic fungi, antibacterial activity, chemical constituents, *Phomopsis*

Aegiceras corniculatum is an important component of mangrove ecosystems, from which various compounds including sterols, triterpenoids, quinones, flavonoids, and organic acids have been isolated. These substances exhibit diverse pharmacological activities such as anti-inflammatory, analgesic, anti-tumor, antibacterial, antioxidant, hypoglycemic, antimalarial, antidiarrheal, hepatoprotective, and anticoagulant effects (Tian et al., 2017; Bibi et al., 2019). However, extracting these bioactive compounds requires harvesting large quantities of *A. corniculatum*, which can severely damage mangrove ecosystems. To protect and rationally utilize the medicinal resources of this plant, research on its endophytic microorganisms and their metabolites has gained increasing attention in recent years. Li et al. (2020) reported that endophytic bacteria from *A. corniculatum* possess antithrombotic properties, representing a valuable resource for discovering thrombolytic agents. Currently, most bioactive metabolites have been derived from endophytic fungi associated with this plant, primarily including alkaloids, anthraquinones, polyketides, and phenolic compounds with potential antiviral and antitumor properties (Wang et al., 2014; Cadamuro et al., 2021). For instance, phenolic and benzaldehyde derivatives containing unsaturated fatty acid chains (pestalols A-E) and sterol compounds isolated from the endophytic fungus *Pestalotiopsis* sp. showed inhibitory effects against influenza A virus subtypes H3N2 and H1N1, with pestalol B and pestalol C also exhibiting cytotoxic activity against multiple tumor cell lines (Sun et al., 2014). The endophytic fungus *Fusarium incarnatum*, isolated from the fruit of *A. corniculatum*, produced three structurally unique alkaloids with weak antiproliferative and cytotoxic activities against HUVEC, K-562, and HeLa cell lines (Ding et al., 2012). Anthraquinone dimer derivatives alterporriols K-M were obtained from the endophytic fungus *Alternaria* sp. ZJ9-6B, with alterporriols K and L showing certain cytotoxic effects on tumor cells (Huang et al., 2011). Multiple tetramic acid and polyketide compounds isolated from *Penicillium* sp. endophytic fungi demonstrated significant tumor cell proliferation inhibition (Lin et al., 2008a, b). Despite the considerable medicinal potential of endophytic fungi from *A. corniculatum*, reports on their antibacterial chemical constituents remain scarce.

This study utilized *A. corniculatum* leaves collected from the Maowei Sea Kangxiling Mangrove Reserve in Guangxi to isolate endophytic fungi. Active strains were screened based on the antibacterial activity of their fermentation products. Bioactive secondary metabolites from these strains were isolated using antibacterial activity-guided fractionation, and their antimicrobial effects were evaluated using microplate assays to explore the medicinal value of these fungal

metabolites and provide resources and material basis for antibiotic development.

1.1 Materials and Instruments

Acetonitrile (HPLC grade) was purchased from Shanghai Xingke High Purity Solvents Co., Ltd. Methanol, ethyl acetate, petroleum ether, and other reagents (analytical grade) were obtained from Guangdong Guanghua Sci-Tech Co., Ltd. DNA extraction kit (DP305) was from Tiangen Biotech (Beijing) Co., Ltd. PCR kit (2× EasyTaq PCR Super Mix) was from Beijing TransGen Biotech Co., Ltd. Primers ITS1 (5' -TCCGTAGGTGAACCTGCGG-3') and ITS4 (5' -TCCTCCGCTTATTGATATGC-3') were synthesized by Sangon Biotech (Shanghai) Co., Ltd. Silica gel for column chromatography and TLC plates were from Yantai Jiangyou Silica Gel Development Co., Ltd. Indicator strains for antibacterial assays included methicillin-resistant *Staphylococcus aureus* ATCC 43300, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 10145, *Actinomyces viscosus* ATCC 15987, *Micrococcus luteus* ATCC 49732, *Bacillus subtilis* ATCC 6051, *Staphylococcus aureus* ATCC 14222, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, and *Acinetobacter baumannii* ATCC 19606, all preserved at the Institute of Marine Drugs, Guangxi University of Chinese Medicine. Czapek medium, rose bengal agar, and MB medium were used for isolation of endophytic fungi. Rice medium (100 g rice, 1.5 g sea salt, 110 mL distilled water) was used for large-scale fermentation. *A. corniculatum* leaves were collected from the Maowei Sea Kangxiling Mangrove Reserve in Qinzhou, Guangxi in September 2020.

Instrumentation included LC-2030C 3D Plus HPLC system (Shimadzu, Japan), Avance III HD 500 MHz NMR spectrometer (Bruker), EYELA N-1300D rotary evaporator (Tokyo Rikakikai), C1000 PCR thermal cycler (Bio-Rad), Sepacore medium-pressure preparative chromatography system (Buchi, Switzerland), SHZ-CB circulating water vacuum pump (Gongyi Yuhua Instrument), KQ-250DB ultrasonic cleaner (Gongyi Yuhua Instrument), electrophoresis apparatus (Beijing Liuyi Biotechnology), gel imaging analyzer (VILBER LOURMAT), and incubator (Shanghai Jinghong Laboratory Equipment).

1.2.1 Isolation and Identification of Endophytic Fungi from *A. corniculatum*

Endophytic fungi were isolated following the method of Shan et al. (2012). Fresh leaves were rinsed three times with sterile water, immersed in 70% ethanol for 30 seconds, treated with 0.2% mercuric chloride for 20 minutes, and then rinsed five times with sterile water. Surface moisture was absorbed with sterile filter paper. Leaves were cut into approximately 0.5 cm fragments and evenly placed on MB medium, Czapek medium, and rose bengal agar, then incubated at 28°C. After colony formation, morphologically and chromatically distinct colonies were selected and subcultured on fresh medium until pure isolates were obtained. Pure strains were preserved at 4°C for subsequent use.

For molecular identification, fresh mycelia were collected and genomic DNA was extracted using the kit according to the manufacturer' s instructions. The DNA was used as template for PCR amplification with fungal universal primers ITS1 and ITS4 targeting the ribosomal DNA internal transcribed spacer region. Amplification products were verified by 1% agarose gel electrophoresis and sent to Sangon Biotech (Shanghai) for sequencing. Sequences were submitted to GenBank and analyzed using BLAST to identify similar sequences. A phylogenetic tree was constructed using the Neighbor-Joining method in MEGA X software, with evolutionary distances calculated by p-distance and bootstrap values determined from 1,000 replications.

1.2.2 Preparation of Fermentation Extracts

Endophytic fungal strains were inoculated into MB liquid medium and cultured at 28°C with shaking at 180 rpm for 3-5 days to obtain seed cultures. The seed culture was then inoculated into 1,000 mL Erlenmeyer flasks containing rice solid medium (2 flasks per strain) and incubated statically at 28°C for 30 days. The fermentation products were extracted three times with ethyl acetate under ultrasonication, and the combined extracts were concentrated under reduced pressure to obtain the crude fermentation extracts.

1.2.3 Antibacterial Activity Screening of Fermentation Extracts

Antibacterial activity was evaluated using the agar diffusion method with filter paper discs. Fermentation extracts were dissolved in DMSO at an initial concentration of 50 $mg \cdot mL^{-1}$. Positive controls (ampicillin sodium and ciprofloxacin) were prepared in DMSO at 0.1 $mg \cdot mL^{-1}$, with DMSO serving as negative control. Three microliters of each test solution were applied to sterile filter paper discs (6 mm diameter), which were then placed on LB agar plates seeded with indicator bacteria. After incubation at 37°C for 16 hours, inhibition zone diameters were measured using the cross-streak method. Strains producing antibacterial substances were selected based on inhibition zone size.

1.2.4 Scale-up Fermentation and Metabolite Isolation from *Phomopsis* sp. GXIMD02029

The fermentation of *Phomopsis* sp. GXIMD02029 was scaled up to 100 flasks using the method described in section 1.2.2. The fermentation products were extracted five times with ethyl acetate and concentrated under reduced pressure to yield 95 g of crude extract. The extract was subjected to normal-phase silica gel column chromatography (200-300 mesh) eluted with a dichloromethane-methanol gradient (100:0 to 0:100, V/V) to obtain four fractions (Fr. 1-4) based on TLC analysis. Fr. 2 was further separated by reversed-phase chromatography using a methanol-water gradient (30:70 to 70:30, V/V) to yield subfractions Fr.2.1-2.4. Fr.2.1 was purified by two rounds of silica gel column chromatography to obtain compound **5** (40.0 mg). Fr.2.2 was separated by reversed-phase column chromatography (methanol-water 30:70 to 70:30, V/V) followed by prepar-

ative HPLC (acetonitrile-water 25:75, V/V) to afford compound **7** (184.4 mg). Fr.2.3 was decolorized by gel filtration and then purified by preparative HPLC (acetonitrile-water 55:45, V/V) to yield compound **1** (7.6 mg). Fr.2.4 was separated by reversed-phase chromatography with isocratic elution (methanol-water 80:20, V/V) to give Fr.2.4A and Fr.2.4B. Fr.2.4A was further purified by gel filtration and preparative HPLC (acetonitrile-water 60:40, V/V) to obtain compounds **3** (11.3 mg) and **2** (293.5 mg). Fr.2.4B was purified by preparative HPLC (acetonitrile-water 70:30, V/V) to yield compound **4** (13.6 mg). Fr.3 was purified by gel filtration followed by preparative HPLC (acetonitrile-water 70:30, V/V) to obtain compound **6** (67.7 mg).

1.2.5 Determination of Minimum Inhibitory Concentration (MIC)

Pure compounds were dissolved in DMSO at an initial concentration of 10 $mg \cdot mL^{-1}$. Positive controls (ampicillin sodium and ciprofloxacin) were prepared as 1 $mg \cdot mL^{-1}$ DMSO solutions. Indicator bacteria were cultured in LB medium at 37°C with shaking to logarithmic phase, and cell density was adjusted to 10^6 $CFU \cdot mL^{-1}$. In a 96-well microplate, 190 µL of LB broth and 10 µL of test compound were added to the first column, while 100 µL of LB broth was added to columns 2-9. After mixing, 100 µL was transferred from column 1 to column 2, and serial two-fold dilutions were performed through column 8. Subsequently, 100 µL of bacterial suspension was added to wells in columns 1-8, column 9 received no bacteria as negative control, and column 10 received 100 µL LB broth plus 100 µL bacterial suspension as blank control. Final compound concentrations ranged from 250 to 1.953125 $\mu g \cdot mL^{-1}$. Each concentration was tested in triplicate, and experiments were repeated three times. After incubation at 37°C for 24 hours, the MIC was determined as the lowest concentration showing no visible bacterial growth (clear wells).

2.1 Strain Identification Results

Based on colony morphology and color characteristics, 16 endophytic fungal strains were selected for genomic DNA extraction and PCR amplification, yielding single fragments of 500-600 bp. BLAST analysis results are summarized in Table 1 . The 16 endophytic fungi were classified into 2 classes, 7 orders, 10 families, and 10 genera, with *Fusarium* as the dominant genus (25% of identified strains). A phylogenetic tree was constructed using the Neighbor-Joining method with highly similar reference sequences (Figure 1 [Figure 1: see original paper]), showing that all 16 strains formed terminal clusters with their respective similar strains with bootstrap support >50%.

2.2 Screening Results for Active Strains

The antibacterial activities of fermentation extracts are presented in Table 2 . Strains GXIMD02029 and GXIMD02039 exhibited inhibitory effects against *Bacillus subtilis*, *Staphylococcus epidermidis*, methicillin-resistant *Staphylococ-*

cus aureus, *Micrococcus luteus*, *Actinomyces viscosus*, and *Staphylococcus aureus*. Strain GXIMD02038 showed inhibitory activity against *M. luteus*, *A. viscosus*, and *S. aureus*. No antibacterial activity was observed for other strains. None of the fermentation extracts demonstrated inhibitory effects against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, or *Escherichia coli*.

2.3 Isolation and Structural Identification of Metabolites from *Phomopsis* sp. GXIMD02029

Seven compounds were isolated from the fermentation extract of *Phomopsis* sp. GXIMD02029 using silica gel column chromatography, reversed-phase chromatography, gel filtration, and preparative HPLC. These were identified as (15R)-acetoxydothiorelone A (**1**), cytosporone B (**2**), pestalotiopsone H (**3**), pestalotiopsone B (**4**), 4-Hydroxybenzaldehyde (**5**), p-Hydroxybenzoic acid (**6**), and N-(2-phenylethyl)acetamide (**7**). Their structures are shown in Figure 2 [Figure 2: see original paper].

Compound 1: White powder. ^{1}H NMR (500 MHz, CD_3OD) δH: 6.20 (1H, d, J = 2.2 Hz, H-4), 6.27 (1H, d, J = 2.2 Hz, H-6), 3.58 (2H, s, H-2), 2.91 (2H, t, J = 7.5 Hz, H-10), 4.11 (2H, q, J = 7.0 Hz, H-17), 2.01 (3H, s, H-20), 1.28-1.68 (8H, m, H-11–H-15), 1.20 (3H, d, J = 6.5 Hz, H-16), 1.25 (3H, t, J = 7.0 Hz, H-18); ^{13}C NMR (125 MHz, CD_3OD) δC: 173.6 (C-1), 40.5 (C-2), 137.0 (C-3), 111.7 (C-4), 161.3 (C-5), 102.7 (C-6), 159.8 (C-7), 121.2 (C-8), 208.8 (C-9), 45.0 (C-10), 25.4 (C-11), 30.2 (C-12), 26.3 (C-13), 36.8 (C-14), 72.4 (C-15), 20.2 (C-16), 61.8 (C-17), 14.5 (C-18), 172.7 (C-19), 21.2 (C-20). These data were consistent with literature values (Luo et al., 2018), identifying compound **1** as (15R)-acetoxydothiorelone A.

Compound 2: Light yellow powder. ^{1}H NMR (500 MHz, CD_3OD) δH: 6.25 (1H, d, J = 2.5 Hz, H-4), 6.18 (2H, d, J = 2.5 Hz, H-6), 4.09 (2H, q, J = 7.5 Hz, H-17), 3.56 (2H, s, H-2), 2.89 (2H, t, J = 7.5 Hz, H-10), 1.60 (2H, m, H-11), 1.26-1.34 (8H, m, H-12–H-15), 1.22 (3H, t, J = 7.5 Hz, H-18), 0.88 (3H, t, J = 7.5 Hz, H-16); ^{13}C NMR (125 MHz, CD_3OD) δC: 173.5 (C-1), 40.5 (C-2), 137.0 (C-3), 102.7 (C-4), 161.3 (C-5), 111.7 (C-6), 159.8 (C-7), 121.2 (C-8), 209.0 (C-9), 45.2 (C-10), 25.5 (C-11), 30.4 (C-12), 30.2 (C-13), 32.9 (C-14), 23.6 (C-15), 14.4 (C-16), 61.8 (C-17), 14.5 (C-18). These data matched literature values (Brady et al., 2000), identifying compound **2** as cytosporone B.

Compound 3: White powder. ^{1}H NMR (500 MHz, CD_3OD) δH: 6.76 (1H, d, J = 2.4 Hz, H-6), 6.67 (1H, d, J = 2.4 Hz, H-4), 5.99 (1H, s, H-10), 4.12 (2H, q, J = 7.2 Hz, H-17), 4.06 (2H, s, H-2), 2.60 (2H, t, J = 7.6 Hz, H-12), 1.72 (2H, m, H-13), 1.37 (4H, m, H-14 and H-15), 1.23 (3H, t, J = 7.2 Hz, H-18), 0.92 (3H, t, J = 7.0 Hz, H-16); ^{13}C NMR (125 MHz, CD_3OD) δC: 173.5 (C-1), 42.0 (C-2), 138.8 (C-3), 119.6 (C-4), 163.4 (C-5), 102.9 (C-6), 161.3 (C-7), 115.7 (C-8), 181.4 (C-9), 110.4 (C-10), 170.5 (C-11), 34.5 (C-12), 27.6 (C-13), 32.3 (C-14), 23.4 (C-15), 14.3 (C-16), 61.7 (C-17), 14.5 (C-18). These data were

consistent with literature values (Luo et al., 2018), identifying compound **3** as pestalotiopsone H.

Compound 4: Light yellow powder. ^{1}H NMR (500 MHz, CD_3OD) δH: 6.77 (1H, d, J = 2.3 Hz, H-8), 6.68 (1H, d, J = 2.3 Hz, H-6), 6.00 (1H, s, H-10), 4.13 (2H, q, J = 7.5 Hz, H-19), 4.07 (2H, s, H-2), 2.61 (2H, t, J = 7.5 Hz, H-12), 1.72 (2H, m, H-13), 1.26-1.43 (8H, m, H-14–H-17), 1.21 (3H, t, J = 7.5 Hz, H-20), 0.90 (3H, t, J = 7.0 Hz, H-18); ^{13}C NMR (125 MHz, CD_3OD) δC: 173.4 (C-1), 42.1 (C-2), 138.7 (C-3), 119.5 (C-4), 163.3 (C-5), 102.9 (C-6), 161.2 (C-7), 115.7 (C-8), 181.4 (C-9), 110.4 (C-10), 170.5 (C-11), 34.5 (C-12), 27.9 (C-13), 30.1 (C-14), 30.0 (C-15), 32.8 (C-16), 23.7 (C-17), 14.50 (C-18), 61.7 (C-19), 14.40 (C-20). These data matched literature values (Beekman et al., 2013), identifying compound **4** as pestalotiopsone B.

Compound 5: White powder. ^{1}H NMR (500 MHz, CD_3OD) δH: 9.74 (1H, s, CHO), 7.75 (2H, d, J = 8.4 Hz, H-2, H-6), 6.90 (2H, d, J = 8.4 Hz, H-3, H-5); ^{13}C NMR (125 MHz, CD_3OD) δC: 130.2 (C-1), 133.4 (C-2, C-6), 116.8 (C-3, C-5), 165.1 (C-4), 192.8 (CHO). These data were consistent with literature values (Shataer et al., 2020), identifying compound **5** as 4-hydroxybenzaldehyde.

Compound 6: Light yellow powder. ^{1}H NMR (500 MHz, CD_3OD) δH: 7.86 (2H, d, J = 8.7 Hz, H-2, H-6), 6.81 (2H, d, J = 8.7 Hz, H-3, H-5); ^{13}C NMR (125 MHz, CD_3OD) δC: 122.7 (C-1), 133.0 (C-2, C-6), 116.0 (C-3, C-5), 163.4 (C-4), 170.2 (COOH). These data matched literature values (Shataer et al., 2020), identifying compound **6** as p-hydroxybenzoic acid.

Compound 7: White powder. ^{1}H NMR (500 MHz, $CDCl_3$) δH: 7.16-7.30 (5H, m, H-2–H-6), 3.49 (2H, t, J = 7.0 Hz, H-8), 2.80 (2H, t, J = 7.0 Hz, H-7), 1.92 (3H, s, H-10); ^{13}C NMR (125 MHz, $CDCl_3$) δC: 138.9 (C-1), 128.6 (C-2, C-6), 128.7 (C-3, C-5), 126.5 (C-4), 35.6 (C-7), 40.6 (C-8), 170.1 (C-9), 23.2 (C-10). These data were consistent with literature values (Vaca et al., 2020), identifying compound **7** as N-(2-phenylethyl)acetamide.

2.4 Antibacterial Activity of Pure Compounds

Preliminary screening by filter paper disc diffusion showed that compounds **1** and **2** exhibited inhibitory activity at 30 μg per disc against *Bacillus subtilis*, *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus*, *Micrococcus luteus*, *Actinomyces viscosus*, and *Staphylococcus aureus*. Therefore, their MIC values were determined and are presented in Table 3 . Compound **1** showed MIC values ranging from 7.8125 to 31.25 $\mu g \cdot mL^{-1}$ against the six tested pathogens, indicating moderate antibacterial activity. Compound **2** exhibited weaker activity with MIC values of 62.5-250 $\mu g \cdot mL^{-1}$.

Discussion

The 16 endophytic fungal strains isolated from *A. corniculatum* leaves belonged to 2 classes, 7 orders, 10 families, and 10 genera, consistent with previously

reported endophytic fungal taxa from this plant. Li et al. (2016) reported that endophytic fungi in *A. corniculatum* branches were more diverse than those in leaves, with *Phyllosticta* and *Leptosphaerulina* being dominant in leaves. Gong et al. (2014) identified *Colletotrichum* and *Pestalotiopsis* as dominant endophytes in branches. Deng et al. (2010) noted that dominant endophytic fungi varied among leaf veins, bark, and stems across different seasons. In our study, *Fusarium* was the dominant genus, differing from previous reports, possibly due to variations in plant parts sampled and environmental factors such as precipitation, temperature, humidity, and light intensity during collection.

The genus *Phomopsis* produces metabolites with significant pharmaceutical and agricultural applications. These fungi generate structurally diverse secondary metabolites including polyketides, terpenoids, macrolides, and alkaloids with cytotoxic, antibacterial, antiviral, antioxidant, and anti-inflammatory activities (Xu et al., 2021). Phomoxanthone A, a polyketide from *Phomopsis*, shows promise as an anti-drug-resistant cancer therapeutic (Chen et al., 2022). In this study, we screened endophytic fungi based on antibacterial activity of their fermentation products and identified GXIMD02029, GXIMD02038, and GXIMD02039 as active strains. *Phomopsis* sp. GXIMD02029 demonstrated particularly strong antibacterial activity, prompting detailed investigation of its secondary metabolites using activity-guided fractionation.

The seven compounds isolated from *Phomopsis* sp. GXIMD02029 were primarily polyketides. Compound **1** has been previously isolated from *Phomopsis* sp. and *Diaporthe* sp. SCSIO 41011 (Luo et al., 2018; Santos et al., 2021), but its antibacterial activity was not reported. Our study demonstrates for the first time that compound **1** inhibits *Bacillus subtilis*, *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus*, *Micrococcus luteus*, *Actinomyces viscosus*, and *Staphylococcus aureus*. Beau et al. (2012) reported that compound **2** had an MIC of 72 $\mu mol \cdot L^{-1}$ against methicillin-resistant *S. aureus*. Huang et al. (2008) found compound **2** inhibited *Candida albicans* and *Fusarium oxysporum* with MIC values of 64 $\mu g \cdot mL^{-1}$, but showed no activity against *Salmonella enteritidis*, *S. aureus*, or *E. coli* at 128 $\mu g \cdot mL^{-1}$. Our results for compound **2** are consistent with literature reports but expand its antibacterial spectrum. Beekman et al. (2013) reported compound **4** had an IC_{50} of 421 $\mu g \cdot mL^{-1}$ against *S. epidermidis*; however, we observed no activity in our preliminary screening and did not determine its MIC. This study provides microbial resources for isolating antibacterial secondary metabolites and offers scientific evidence for exploring antibacterial agents from *A. corniculatum* endophytic fungi, contributing to their potential applications in antimicrobial development.

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