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CHEMICAL CONSTITUENTS OF THE ENDOPHYTIC FUNGUS *PENICILLIUM SHEARII* ISOLATED FROM *PYRROSIA LANCEOLATA* (L.) FARW.

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Summary

Chemical Constituents of the Endophytic Fungus *Penicillium shearii* Isolated from *Pyrrosia lanceolata* (L.) Farw.

Endophytic fungi are one of the typical endophytic microorganism groups that have been particularly interested in research recently because of their roles in many aspects; the most special thing is the ability to biosynthesize secondary substances with biological activity such as antioxidant, antibacterial, anti-tumor... The endophytic fungal strains were identified as *Penicillium shearii* CBS 290.48 by morphological microscopic and DNA sequencing based on the modified Sanger method. From the ethyl acetate fraction of the medium extract of *P. shearii*, four compounds were isolated and identified as ergosterol (**1**), dehydroxypaxilline (**2**), chrysophanol (**3**), and paspaline (**4**). Their structures were determined by means of spectroscopic methods (MS, 1D, and 2D NMR).

Keywords: *Penicillium shearii, Endophytic fungus, Pyrrosia lanceolata, DNA sequencing.*

1. Introduction

Endophytic fungi are one of the typical endophytic microorganisms, present in the majority of plant species and existing in many parts of plants such as roots, stems, leaves, flowers, fruits, and seeds [1]. The interaction relationship between endophytic fungi and host plants is very complex to ensure a sustainable symbiosis [2],[3]. Endogenous fungi could produce many secondary metabolites in different groups, such as alkaloids, flavonoids, glycosides, coumarins, lactones, chromones, quinones, steroids, terpenoids, xanthones, lignans, peptides, polyketides, organic acids, and phenolic compounds [4],[5],[6],[7],[8]. In addition, the compounds isolated from endophytic fungi exhibited remarkable biological activities as antioxidant [8],[9], antibacterial [10], and anti-tumor [11],[12], which is a potential source for new drug research. However, there are not many studies on endophytic fungi in Vietnam. Therefore, this study is conducted to expand research on potential substances from plant-endophytic fungi, and to contribute to the development of bioactive secondary metabolites for the treatment of several diseases. Our present study on phytochemical constituents from the ethyl acetate fraction of the endophytic fungus strains of *P. shearii* led to the isolation of four compounds. Their structures were identified as ergosterol (**1**), dehydroxypaxilline (**2**),

chrysophanol (**3**), and paspaline (**4**) (Fig. 2) by spectroscopic data and compared to those reported in the literature.

2. Experimental

2.1. Materials and chemicals

The whole plant of *Pyrrosia lanceolata* (L.) Farw. Polypodiaceae was collected in Phuoc Buu-Binh Chau town, Ba Ria-Vung Tau province (GPS N10°55′ E107°44′) in August 2019 for the isolation of the endophytic fungus strains. The voucher specimen of endophytic fungus strain (3PD3082019.VT) was deposited at the Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City (UMP), Vietnam. The solvents were used for isolation and extraction: ethanol 96%, *n*-hexane, ethyl acetate, dichloromethane, chloroform, methanol, and *n*-butanol (Scharlau, Spain).

2.2. General experimental procedures

Silica gel (40-63 µm, Merck) and Sephadex LH-20 (GE Healthcare, Sweden) were used for column chromatography (CC). TLC was performed using normal phase *silica gel* 60 F_{254} plates (Merck). Spots were detected by UV and 5% vanillin-sulfuric acid/absolute ethanol. The UV spectra were obtained on an UV-VIS SP8001 spectrophotometer (Axiom, Germany). ESI-MS spectra were obtained from a Xevo G2-XS QTOF mass spectrometer (Waters, USA). NMR spectra were measured on a Bruker Avance Neo 400 MHz spectrometer, using TMS as an internal standard. NMR solvents were purchased from Sigma-Aldrich (USA). Microorganisms were manipulated under sterile conditions in an Esco AVC-4A1 class III biological safety cabinet.

Fungal isolation and DNA taxonomic analysis.

The whole plant of *P. lanceolata* was washed with reverse osmosis water, then treated with 7% Javel (3 min), 70% ethanol (3 min) before being used to isolate the endophytic fungi strains. Three different agar media such as potato dextrose agar-PDA (with chloramphenicol 25 mg/L), starch casein-SC (with cyclohexamide 250 mg/L), and water agar-WA (with chloramphenicol 25 mg/L) were used to investigate the growth ability of endogenous fungi and incubated at room temperature for 8 days. The fungi strain was identified based on the microscopic morphology characteristics with lactophenol cotton blue (LPCB) and DNA sequencing based on the modified Sanger method [13],[14].

Scale-up cultivation and fractionation.

The fungus *P. shearii* was cultivated on 35 L of PDA agar (× 250 Erlenmeyer flasks) at room temperature for 14 days. The agar and fungal mycelia were then harvested and extracted with EtOAc (32 L). The combined organic phase and water layer were filtered and concentrated *in vacuo* at 40°C to yield EtOAc extract (PSE, 6.0 g) and water extract (PSW, 13.6 g), respectively.

2.3. Extraction and isolation

The EtOAc extract (3.0 g) was subjected to Sephadex LH-20 CC (CH_2Cl_2-MeOH, 9:1) to give 4 fractions (E1-E4). Fraction E2 (788.3 mg) was further purified by Sephadex LH-20 CC (CH_2Cl_2-MeOH, 9:1) to give 4 sub-fractions (E2.1-E2.4) and compound **1** (40 mg) was obtained from sub-fraction E2.3 (473 mg) by crystallization method. Sub-fraction E2.3 was continued to separate by silica RP C18 CC (80 g) and eluted with MeOH-H_2O (25:75, 50:50, 75:25, 85:15 and 95:5, 100:0, v/v) to obtain 12 sub-fractions (E2.3.1- E2.3.12). Compounds **2** (3.8 mg), **3** (3.2 mg), and **4** (5.1 mg) were isolated and purified by Sephadex LH-20 CC (MeOH) from sub-fractions E2.3.6 (22.4 mg), E2.3.8 (25.7 mg), and E2.3.9 (19.7 mg), respectively.

Ergosterol (1): colorless amorphous powder. ESI-MS: *m/z* 397.3 $[M+H]^+$, $C_{28}H_{44}O$. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 5.50 (1H, d, J = 5.6 Hz, H-6), 5.32 (1H, d, J = 5.6 Hz, H-7), 5.14 (1H, m, H-23), 5.12 (1H, m, H-22), 3.57 (1H, tt, J = 11.6, 4.0 Hz, H-3), 2.40 (1H, m, H-4a), 2.21 (1H, dd, J = 16.0, 11.6 Hz, H-4b), 1.99 (1H, m, H-12a), 1.97 (1H, m, H-20), 1.90 (1H, s, H-9), 1.82 (1H, m, H-1a), 1.82 (1H, d, J = 9.5 Hz, H-14), 1.81 (1H, m, H-2a), 1.79 (1H, m, H-24), 1.68 (1H, m, H-16a), 1.66 (1H, s, H-11a), 1.59 (1H, s, H-15a), 1.53 (1H, s, H-11b), 1.43 (1H, m, H-2b), 1.40 (1H, m, H-25), 1.30 (1H, s, H-15b), 1.25 (1H, m, H-1b), 1.23 (1H, m, H-16b), 1.19 (1H, m, H-17), 1.17 (1H, m, H-12b), 0.97 (3H, d, J = 6.4 Hz, H_3-21), 0.88 (3H, s, H_3-18), 0.85 (3H, t, J = 6.8 Hz, H_3-28), 0.77 (3H, d, J = 6.4 Hz, H_3-27), 0.74 (3H, d, J = 6.4 Hz, H_3-26), and 0.56 (3H, s, H_3-19). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 141.4 (C-8), 139.8 (C-5), 135.6 (C-22), 132.0 (C-23), 119.6 (C-6), 116.2 (C-7), 70.5 (C-3), 55.7 (C-17), 54.6 (C-14), 46.3 (C-9), 42.84 (C-24), 42.8 (C-13), 40.8 (C-4), 40.4 (C-20), 39.1 (C-12), 38.4 (C-1), 37.0 (C-10), 33.1 (C-25), 32.0 (C-2), 28.2 (C-16), 23.0 (C-15), 21.12 (C-21), 21.11 (C-11), 20.0 (C-27), 19.7 (C-26), 17.6 (C-28), 16.3 (C-18), and 12.1 (C-19).

Dehydroxypaxilline (2): colorless amorphous powder, ESI-MS: *m/z* 442.2 $[M+Na]^+$, $C_{27}H_{33}NO_3$. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 7.79 (1H, br s, -NH-), 7.47 (1H, m, H-20), 7.33 (1H,

m, H-23), 7.13 (1H, m, H-22), 7.11 (1H, m, H-21), 5.90 (1H, s, H-11), 4.31 (1H, m, H-7), 3.75 (1H, s, H-9), 2.85 (1H, m, H-16), 2.77 (1H, dd, J = 13.2, 6.4 Hz, H-17a), 2.69 (1H, s, -OH), 2.46 (1H, m, H-13), 2.44 (1H, dd, J = 13.2, 10.6 Hz, H-17b), 2.33 (1H, m, H-6a), 2.16 (1H, m, H-5a), 1.96 (1H, m, H-6b), 1.87 (1H, m, H-15a), 1.74 (1H, m, H-5b), 1.74 (1H, m, H-14a), 1.72 (1H, m, H-15b), 1.59 (1H, m, H-14b), 1.32 (3H, s, H_3-29), 1.31 (3H, s, H_3-28), 1.10 (3H, s, H_3-25), and 1.03 (3H, s, H_3-26). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 198.3 (C-10), 168.2 (C-12), 149.2 (C-2), 140.0 (C-24), 125.0 (C-19), 121.8 (C-11), 120.9 (C-22), 119.8 (C-21), 118.6 (C-20), 118.5 (C-18), 111.6 (C-23), 82.5 (C-9), 74.7 (C-7), 72.4 (C-27), 50.5 (C-3), 48.9 (C-16), 42.6 (C-13), 42.4 (C-4), 32.9 (C-5), 29.9 (C-6), 27.3 (C-17), 26.7 (C-28), 25.5 (C-14), 24.1 (C-29), 24.0 (C-15), 16.3 (C-26), and 14.6 (C-25).

Chrysophanol (3): orange-yellow needles. ESI-MS: m/z 253.1 $[M-H]^-$, $C_{15}H_{10}O_4$. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 12.16 (1H, s, 8-OH), 12.05 (1H, s, 1-OH), 7.86 (1H, dd, J = 8.0, 1.2 Hz, H-5), 7.70 (1H, t, J = 8.0 Hz, H-6), 7.68 (1H, d, J = 2.0, H-4), 7.32 (1H, dd, J = 8.0, 1.2 Hz, H-7), 7.13 (1H, d, J = 2.0 Hz, H-2), 2.49 (3H, s, 2-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 192.6 (C-9), 182.1 (C-10), 162.8 (C-8), 162.5 (C-1), 149.4 (C-3), 137.0 (C-5), 133.7 (C-5a), 133.3 (C-4a), 124.6 (C-7), 124.4 (C-2), 121.4 (C-4), 120.0 (C-6), 115.9 (C-8a), 113.8 (C-1a), 22.3 (2-CH_3).

Paspaline (4): colorless amorphous powder. ESI-MS: m/z 442.2 $[M+Na]^+$, $C_{28}H_{39}NO_2$. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 7.75 (1H, br s, -NH-), 7.45 (1H, s, H-20), 7.31 (1H, m, H-23), 7.10 (1H, m, H-21), 7.09 (1H, m, H-22), 3.24 (1H, dd, J = 11.6, 3.2 Hz, H-9), 3.05 (1H, dd, J = 11.6, 4.0 Hz, H-7), 2.78 (1H, m, H-16), 2.70 (1H, dd, J = 13.2, 6.4 Hz, H-17a), 2.69 (1H, s, 27-OH), 2.35 (1H, dd, J = 13.2, 10.4 Hz H-17b), 1.99 (1H, td, J = 12.8, 4.0 Hz, H-5a), 1.85 (1H, m, H-11a), 1.84 (1H, m, H-6a), 1.79 (1H, m, H-14a), 1.71 (1H, m, H-6b), 1.71 (1H, m, H-15a), 1.68 (1H, m, H-10a), 1.64 (1H, m, H-5b), 1.63 (1H, m, H-14b), 1.50 (1H, m, H-13), 1.48 (1H, m, H-10b), 1.42 (1H, m, H-15b), 1.22 (3H, s, H_3-29), 1.20 (3H, s, H_3-28), 1.16 (1H, m, H-11b), 1.16 (3H, s, H_3-26), 1.05 (3H, s, H_3-25), 0.91 (3H, s, H_3-30). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 150.9 (C-2), 139.9 (C-24), 125.1 (C-19), 120.4 (C-22), 119.6 (C-21), 118.4 (C-20), 118.3 (C-18), 111.4 (C-23), 85.7 (C-7), 84.7 (C-9), 71.9 (C-27), 53.0 (C-3), 48.8 (C-16), 46.4 (C-13), 40.0 (C-4), 37.7 (C-11), 36.6 (C-12), 33.9 (C-5), 27.5 (C-17), 26.1 (C-29), 25.3 (C-14), 24.6 (C-6), 23.7 (C-28), 22.0 (C-15), 21.9 (C-10), 20.0 (C-26), 14.6 (C-25), 12.7 (C-30).

3. Results and discussion

The endophytic fungi strain number 3PD3 showed the ability to increase biomass rapidly and to produce secretions on the surface, which is one of the important factors in the isolation of fungi. The PDA medium showed the suitability for fungi growth. Therefore, 3PD3 fungal strain and PDA medium were selected for the scale-up cultivation.

The endophytic fungus strain was investigated the microscopic characteristics by color dyeing with LPCB as shown in Fig. 1. In eight days, the colony of endophytic fungi are nearly round in shape, 41-43 mm in diameter. Spores are gray-brown, occupying most of the area of the fungi cluster. Mycelia are thin and white. The edge of the fungi cluster is white and splitless. On the surface of the fungi cluster, there are many colorless, irregular droplets of secretions, distributed throughout the cluster as shown in Fig. 1.

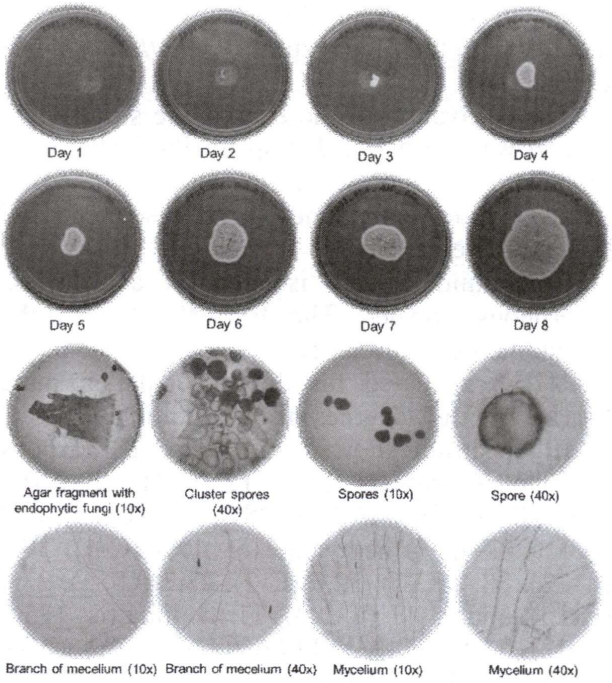

Fig. 1. The isolation of endophytic fungi strain of *Penicillium shearii* CBS 290.48 and microscopic characteristics of *P. shearii*.

Genomic DNA was extracted from the mycelia of 3PD3 and the 18s rRNA genes were amplified by PCR, which were carried out by Nam Khoa company and nucleotide sequence (5′-3′) was obtained as shown:

AGGGGGGGGATTACCCTAGTAACGGC
GAGTGAAGCGGTAAGAGCTCAAATTTGA
AAGCTGGCCCCTTCGGGGTCCGCATTGTA
ATTTGCAGAGGATGCTTCGGGAGCGGTCC
CCATCTAAGTGCCCTGGAACGGGCCGTCA
TAGAGGGTGAGAATCCCGTATGGGATGG
GGTGCCCGCGCCCGTGTGAAGCTCCTTCG
ACGAGTCGAGTTGTTTGGGAATGCAGCTC
TAATTGGGTGGTAAATTTCATCTAAAGCT
AAATATTGGCCGGAGACCGATAGCGCAC
AAGTAGAGTGATCGAAAGATGAAAAGCA
CTTTGAAAAGAGAGTTAAAAAGCACGTG
AAATTGTTGAAAGGGAAGCGCTTGCGATC
AGACTCGCTCGCGGGGTTCAGCCGGCCTT
CGGGCCGGTGTACTTCCCCGCGGGCGGGC
CAGCGTCGGTTTGGGCGGCCGGTCAAAG
GCCCCTGGAATGTAACGCCTCTCGGGGCG
TCTTATAGCCAGGGGTGCCATGCGGCCTG
CCCGGACCGAGGAACGCGCTTCGGCTCG
GACGCTGGCATAATGGTCGTAAGCGACCC
GTCTTGAAACACGGACCA.

A BLAST analysis (NCBI database) on the resulting 28S rRNA gene partial sequence (Supplementary, Figures S3−S5, GenBank accession no. NG_067353.1) revealed 100.0% identity with the fungal strain *Penicillium shearii* CBS 290.48.

The cultivation of the endophytic strain of *P. shearii* was further scaled-up for the isolation of secondary metabolites. From the ethyl acetate extract, four compounds were isolated and structurally elucidated.

Compound **1** was isolated as a colorless amorphous powder. The ESI-MS showed the pseudomolecular ion peak with m/z = 397.3 $[M+H]^+$, calculated for the molecular formula $C_{28}H_{44}O$ with 7 degrees of unsaturation. The ^{13}C NMR spectrum showed signals of 28 carbons including 6 methyls (δ_C 21.12 (C-21), 20.0 (C-27), 19.7 (C-26), 17.6 (C-28), 16.3 (C-18), and 12.1 (C-19)), 7 methylene (δ_C 40.8 (C-4), 39.1 (C-12), 38.4 (C-1), 32.0 (C-2), 28.2 (C-16), 23.0 (C-15), and 21.11 (C-11)), 11 methine (δ_C 135.6 (C-22), 132.0 (C-23), 119.6 (C-6), 116.2 (C-7), 70.5 (C-3), 55.7 (C-17), 54.6 (C-14), 46.3 (C-9), 42.84 (C-24), 40.4 (C-20), and 33.1 (C-25)), and 4 non-protonated carbons (δ_C 141.4 (C-8), 139.8 (C-5), 42.8 (C-13), and 37.0 (C-10)). The ^1H NMR data showed proton signals of one hydroxymethine group at C-3 (δ_H 3.57, δ_C 70.5), 6 methyl groups, including 2 singlet signals at δ_H 0.56 (H_3-19), 0.88 (H_3-18) and 4 doublet signals at δ_H 0.97 (H_3-21), 0.85 (H_3-28), 0.77 (H_3-27), and 0.74 (H_3-26), and aliphatic protons in the region of δ_H 2.5 to 1.2 ppm. In addition, the ^1H-NMR spectrum showed the presence of 3 double bonds by the signals of 4 olefinic protons at δ_H 5.12 (1H, m, H-22), 5.14 (1H, m, H-23), 5.50 (1H, d, J = 5.6 Hz, H-6), 5.32 (1H, d, J = 5.6 Hz, H-7), and 6 olefinic carbons at δ_C 139.8 (C-5), 119.6 (C-6), 116.2 (C-7), 141.1 (C-8), 135.6 (C-22), and 132.0 (C-23). The ^1H-^1H COSY spectrum of **1** showed the correlations of four consecutive protons H_2-1/H_2-2/H-3/H_2-4, two olefinic protons H5/H-6, three aliphatic protons H-9/H11/H-12, and ten consecutive protons H-14/H-15/H-16/H-17/H-20/H-22/H-23/H-24/H-25/H_3-26/H_3-27 as depicted in Fig. 3. Based on the NMR spectral data, compound **1** was suggested to be a sterol. The HMBC spectrum showed the correlations between H-6 and C-5/C-4/C-10, between H-7 and C-5/C-8/C-9/C-14, between H_3-18 and C-1/C-5/C-9/C-10, H_3-19 and C-11/C-13/C-14/C-17, H_3-21 and C-17/C-20/C-22, H_3-26 and C-24/C-25/C-27, H_3-27 and C-24/C-25/C-26, and H_3-28 and C-23/C-24/C-25. Based on the above NMR spectra data and comparison with the literature data [15], compound **1** was identified as ergosterol, a sterol commonly found in the outer membrane of fungi.

Compound **2** was obtained as a colorless amorphous powder. The positive ESI-MS spectrum data exhibited the ion peak at m/z = 442.2 $[M+Na]^+$, which was consistent with the molecular formula $C_{27}H_{33}NO_3$, implying 12 degrees of unsaturation. The ^{13}C NMR spectrum of compound **2** showed the presence of 27 carbons, including nine methine carbons (δ_C 118.6 (C-20), 119.8 (C-21), 120.9 (C-22), 111.6 (C-23), 48.9 (C-16), 32.9 (C-5), 74.7 (C-7), 82.5 (C-9), 121.8 (C-11)), one carbonyl carbon (δ_C 198.3, C-10), five methylene carbons (δ_C 27.3 (C-17), 24.0 (C-15), 25.5 (C-14), 32.9 (C-5), 29.9 (C-6)), eight non-protonated carbons (δ_C 72.4 (C-27), 125.0 (C-19), 140.0 (C-24), 118.5 (C-18), 149.2 (C-2), 50.5 (C-3), 42.4 (C-4), 168.2(C-12)), and four methyl carbons (δ_C 14.6 (C-25), 16.3 (C-26), 24.1 (C-29), 26.7 (C-28)). The ^1H NMR spectral data showed the singlet signal at δ_H 7.79 (1H, br s) accounted for -NH-group and the signals of four aromatic protons of an indole moiety at δ_H 7.47 (1H, m, H-20), 7.11 (1H, m, H-21), 7.13 (1H, m, H-22), and 7.33 (1H, m, H-23). In addition, the carbon signals at δ_C 149.2 (C-2), 125.0 (C-19), 140.0 (C-24), 120.9 (C-22), 119.8 (C-21), 118.6 (C-20), 118.3 (C-18), and 111.6 (C-23) in the ^{13}C NMR spectrum and 4 methyl signals at δ_H 1.32

(CH_3-29), 1.03 (CH_3-26), 1.10 (CH_3-25), and 1.31 (CH_3-28) in ^{1}H NMR spectrum suggested that compound **2** was an indole diterpenoid. The COSY spectrum showed the presence of three independent spin systems incorporating all of methine and methylene protons H_2-5/H_2-6/H-7, H-13/H_2-14/H_2-15/H-16/H_2-17, and H-20/H-21/H-22/H-23. In the HMBC spectrum, the correlations between proton at δ_H 7.79 of -NH-group and C-2/C-18/C-19/C-24 of olefinic carbons in the indole framework were observed. Furthermore, the HMBC spectrum exhibited the correlations from H-11 to C-7/C-9/C-13, from H-13 to C-4/C-14/C-26, from H_2-17 to C-2/C-3/C-16/C-18, from H_3-25 to C-2/C-3/C-16/C-4, from H_3-26 to C-3/C-4/C-5/C-13, from H_3-28 to C-27/C-29/C-9, and from H_3-29 to C-27/C-28/C-9. Based on the spectroscopic data, compound **2** was identified as dehydroxypaxilline, which was isolated from the fungus *Echinopla striata* in 1988 [16],[17],[18].

Compound **3** was isolated as orange-yellow needles, and given a purple-pink color with alkaline solutions (NaOH 10%, KOH 10%). The UV spectrum (MeOH) showed the absorption at λ_{max} 225, 256, 286, and 428 nm. The ESI-MS spectral data exhibited a negative-ion peak at m/z = 253.1 $[M-H]^-$, corresponding to the molecular formula $C_{15}H_{10}O_4$ and 11 degrees of unsaturation. The ^{13}C NMR spectrum of **3** showed the presence of 15 carbon signals, including 2 carbonyl groups (δ_C 192.6 (C-9) and 182.1 (C-10)), 5 aromatic methines (δ_C 124.4 (C-2), 121.4 (C-4), 137.0 (C-5), 120.0 (C-6), and 124.6 (C-7)), 7 non-protonated carbons (δ_C 162.8 (C-8), 162.5 (C-1), 149.4 (C-3), 133.3 (C-4a), 133.7 (C-5a), 115.9 (C-8a), and 113.8 (C-1a)), and one methyl group (δ_C 22.3, 2-CH_3). The COSY spectrum exhibited the correlations between three adjacent protons H-5 (δ_H 7.86, dd, J = 8.0, 1.2 Hz), H-6 (δ_H 7.70, t, J = 8.0 Hz), and H-7 (δ_H 7.32, dd, J = 8.0, 1.2 Hz) and between H-2 (δ_H 7.13, d, J = 2.0 Hz) and H-4 (δ_H 7.68, d, J = 2.0 Hz) in *meta*-coupling. Two singlet signals at δ_H 12.16 and 12.05 were assigned as 1-OH and 8-OH. The HMBC spectrum showed the correlations from 1-OH to C-1a/C-1/C-2, from 8-OH to C-7/C-8/C-8a, from 3-CH_3 (δ_H 2.45) to C-2/C-3/C-4, from H-2 to 3-CH_3/C-4/C-1a, from H-4 to C-2/C-3/C-1a/C-4a/C-10/3-CH_3, from H-5 to C-7/C-8a/C-10, from H-6 to C-8/C-5a/C-10, and from H-7 to C-6/C-8a. The above NMR spectral features showed that compound **3** was an 9,10-dicetone anthracene derivative with one methyl substituent at C-3 and two hydroxyl groups at C-1 and C-8 of anthraquinone framework. Based on the NMR spectral data and compared to those of the reference data [19], compound **3** was determined as chrysophanol, which is first reported as the secondary metabolite from endophytic fungi *P. shearii*.

Compound **4** isolated as a colorless flake crystal. The positive ESI-MS spectral data showed the pseudomolecular ion at m/z = 442.2 $[M+Na]^+$, corresponding to the molecular formula $C_{28}H_{39}NO_2$ with 12 degrees of unsaturation. The ^{13}C NMR spectrum showed 28 carbon signals, including 8 aromatic carbons, 3 oxygenated carbons (δ_C 85.7 (C-7), 84.7 (C-9), and 71.9 (C-27)), 7 methylene carbons (δ_C 37.7 (C-11), 33.9 C-5), 27.5 (C-17), 25.3 (C-14), 24.6 (C-6), 22.0 (C-15), and 21.9 (C-10)), 2 methine carbons (δ_C 48.8 (C-16) and 46.4 (C-13)), 3 non-protonated carbons (δ_C 53.0 (C-3), 40.0 (C-4), and 36.6 (C-12)), and 5 methyl carbons (δ_C 12.7 (C-30), 14.6 (C-25), 20.0 (C-26), 23.7 (C-28), and 26.1 (C-29)). The ^{1}H NMR data showed the singlet signals at δ_H 7.75 (1 H, br s) of –NH-group, four aromatic protons of an indole moiety at δ_H 7.45 (1H, m, H-20), 7.10 (1H, m, H-21), 7.09 (1H, m, H-22), 7.31 (1H, m, H-23), and 5 methyl signals at δ_H 1.22 (3H, s, CH_3-29), 0.91 (3H, s, CH_3-30), 1.16 ppm (3H, s, CH_3-26), 1.05 (3H, s, CH_3-25), 1.20 (3H, s, CH_3-28), which suggested that compound **4** was an indole diterpenoid. The ^{13}C NMR spectrum of **4** was similar to that of compound **2** and the differences between **4** and **2** were the addition of one methyl group at δ_C 0.91 (CH_3-30) in **4** and the change of one aromatic methine (δ_C 121.8, C-11) and one carbonyl group (δ_C 198.3, C-10) in **2** to two methylene groups (δ_C 21.9, C-10 and 37.7, C-11) in **4**. The ^{1}H-^{1}H COSY spectrum showed an additional spin system H-9/H_2-10/H_2-11 in comparison with that of compound **2**. The HMBC correlations of **4** were similar to those of **2**, except for the correlations between H_3-30 and C-7/C-11/C-12/C-13 in **4**. Based on the NMR data and compared to those of the reference data [19], compound **4** was identified as paspaline, which was isolated in 1966 from the "ergot" fungus *Claviceps paspali*.

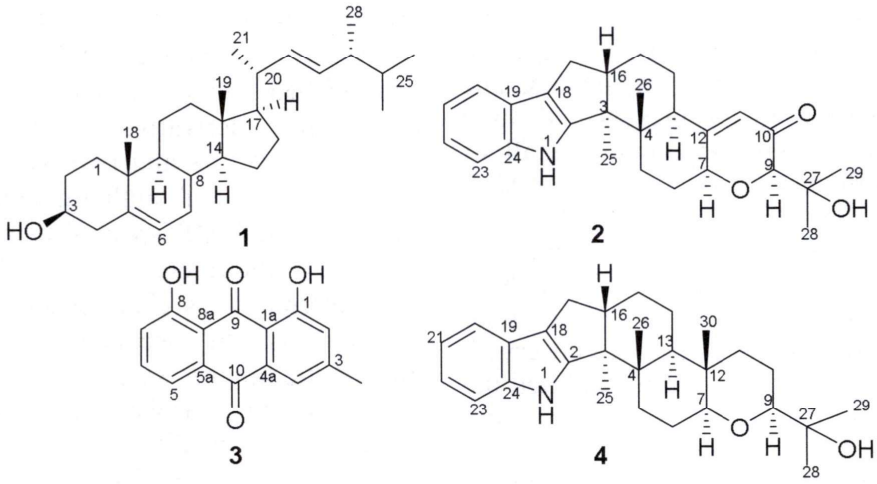

Fig. 2. Chemical structures of compounds **1-4**

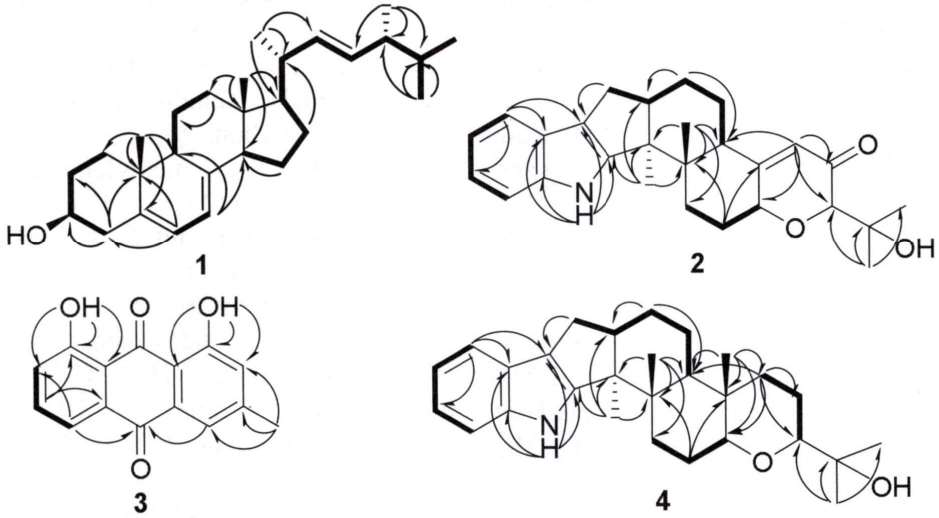

Fig. 3. The key HMBC (⟶) and COSY (▬) correlations of compounds **1-4**

4. Conclusion

In summary, the endophytic fungi from the plant *P. lanceolata* (L.) Farw were isolated and identified as *Penicillium shearii* based on the microscopic morphological characteristics and DNA sequencing by the modified Sanger method. Four compounds were isolated from the ethyl acetate fraction of the endophytic fungi *Penicillium shearii*. Their structures were identified as ergosterol (**1**), dehydroxypaxilline (**2**), chrysophanol (**3**), and paspaline (**4**). The endophytic fungus strains of *P. shearii* are first isolated and reported in the fern of *P. lanceolata* and the compounds **1** and **3** are first reported from *P. shearii*.

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PHENOLIC COMPOUNDS FROM *CURCULIGO ORCHIOIDES*

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Summary

Phenolic Compounds from *Curculigo orchioides*

Six compounds were isolated from ethyl acetate and *n*-butanol fractions from the rhizome of *Curculigo orchioides* Gaertn. (Hypoxidaceae). Their chemical structures were identified as curculigoside A (**1**), curculigoside B (**2**), orcinol *β*-D-glucoside (**3**), orcinol gentiobioside (**4**), glucosyringic acid (**5**) and vanillic acid 4-*O*-*β*-D-glucoside (**6**) by NMR, MS spectroscopic data and comparison with the reported literature. Compound **6** was isolated for the first time from this plant.

Keywords: *Curculigo orchioides, Curculigoside, Glucosyringic acid, Orcinol, phenolic compounds, Vanillic acid.*

1. Introduction

Curculigo orchioides Gaertn. is a perennial herb belonging to the family Hypoxidaceae. The rhizomes of *Curculigo orchioides* have been used in traditional medicine of Asian countries including Vietnam, for the treatment of impotence, rheumatism, hepatitis, and heart diseases… [1],[2]. This medicinal plant has shown a wide range of pharmacological activities such as antioxidant, anti-inflammatory, immunostimulant, hepatoprotective, anticancer, and antidiabetic activities [1],[3]. Previous phytochemical investigations of *C. orchioides* have reported phenolic glycosides, cycloartane saponins, flavonoids and carbohydrates [1],[3],[4],[5]. In Vietnam, *C. orchioides* collected in Tay Nguyen has been found to have orcinol glucoside, curculigoside, orcinol-1-*O*-(6′-*O*-acetyl)-*α*-D-glucopyranoside [6]. This study focused on the chemical constituents of *C. orchioides* in Vietnam for further evaluation of its pharmacological properties.

2. Material and method

2.1. Plant material

The rhizomes of *C. orchioides* were collected in Lao Cai province in March 2021. The sample was authenticated by Ph.D Tran Thi Van Anh (Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City). In additionally, DNA analysis of sample showed that the chloroplast gene trnH-psbA và rps16 had 100% query cover in BLAST match of data of *C. orchioides* in gene bank (NC-053892.1). Voucher specimen of the