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RESEARCH ARTICLE

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Metabolite Chemical Composition of the *Bletilla striata* (Thunb.) Reichb. f. Endophyte *Penicillium oxalicum*

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ABSTRACT

Penicillium oxalicum strain can be isolated from the *Bletilla striata* (Thunb.) Reichb. f. tubers. Its solid-state fermentation products are concentrated by percolation extraction. Separation and purification have been conducted to the ethyl acetate extracts by preparative HPLC. Based on the use of spectrometry, we have determined 17 known compounds, 12,13-dihydroxy-fumitremorgin C (**1**), pseurotin A (**2**), tyrosol (**3**), cyclo-(*L*-Pro-*L*-Val) (**4**), *cis*-4-hydroxy-8-O-methylmellein (**5**), uracil (**6**), cyclo-(*L*-Pro-*L*-Ala) (**7**), 1,2,3,4-tetrahydro-4-hydroxy-4-quinolin carboxylic acid (**8**), cyclo-(Gly-*L*-Pro) (**9**), 2′-deoxyuridine (**10**), 1-(*β*-D-ribofuranosyl)thymine (**11**), cyclo-(*L*-Val-Gly) (**12**), 2′-deoxythymidine (**13**), cyclo-(Gly-*D*-Phe) (**14**), cyclo-*L*-(4-hydroxy-prolinyl)-*D*-leucine (**15**), cyclo-(*L*)-4-hydroxy-Pro-(*L*)-Phe (**16**), uridine (**17**). Here, we report compounds **1**–**3**, **5**, **7**–**8**, **11**–**12**, **14**–**17** are first found and isolated from this endophyte.

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1. Introduction

The Orchidaceae family *Bletilla striata* (Thunb.) Reichb. f. has a long history of being used as a medicinal herb due to its effects for treating traumatic bleeding, hematemesis, hemoptysis, sores and ulcers, turgescence, chapped skin, etc [1]. The compounds found in *Bletilla striata* mainly include bibenzyls, phenanthrenes, anthraquinone, glycosides, anthocyanidins, steroids, phenols, triterpenes, flavonoids, etc [2–7]. Modern pharmacological research has demonstrated these compounds contribute to the pharmacological activities of *Bletilla striata*, such as antibiosis, anti-neoplasm, anti-fibrosis, anti-oxidation, anti-ulcer, hemostasis, and promoting wound healing, etc [8–13].

An endophyte is an endosymbiont that lives within a plant without causing apparent disease. Endophytic species are very diverse, including endophytic fungi and endophytic bacteria [14–15]. Secondary metabolites of some endophytic species are proven to regulate the plant's immune system, increase plant growth and consequently enhance the plant's tolerance against biological and non-biological threats [16–20]. Studies have shown endophytes of medicinal plants produce new bioactive compounds rather than mere secondary metabolites that are same as or similar to the active ingredients of the host plant. Qi, etc. isolated four new butenolides (Terrusnolides A-D) from an endophytic *Aspergillus* from *Tripterygium wilfordii*.

These butenolides were tested in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. These four analogs showed good inhibition against interleukin-1*β* (IL-1*β*), tumor necrosis factor-α (TNF-α), and nitric oxide (NO) in LPS-induced macrophages [21].

Including endophytic bacteria and endophytic fungi, *Bletilla striata* endophytes are very various. The endophytic bacteria mainly consist of *Sphingomonas*, *Pedobacter*, *Halomonas*, *Agrobacterium*, *Kaistobacter*, *Shewanella*, *Pseudomonas,* and *Brevundimonas,* among which the first four genera are the dominant [22]. On the other hand, the endophytic fungi evenly include *Trichoderma*, *Fusarium*, *Pestalotiopsis*, *Nemania*, *Nectria*, *Xylaria*, *Purpureocillium*, *Colletotrichum*, *Nigrospora*, *Biscogniauxia*, *Humicola*, *Neurospora*, *Phomopsis*, *Phytopythium*, *Mucor,* and *Umbelopsis*, among which the first three genera are the dominant [23].

The pharmacological activities of *Bletilla striata* endophytes have been widely studied. Wang's group isolated a *Bletilla striata* endophytic bacterium strain that turned to be *Pseudomonas* which has inhibition against *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus luteus*, *Pectobacterium carolovorum* at

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different levels [24]. From the fresh root of *Bletilla striata*, Dong's team isolated ten endophytic fungus strains later identified as six different strains [25]. Their antioxidant activities on exopolysaccharides (EPS) were respectively, determined. *Fusarium* has the most potent exopolysaccharides total reducing power and ABTS free radical scavenging activity; *Chaetomium aureum* and *Fusarium solani* show the best DPPH free radical scavenging activity; *Fusarium solani* also gives the highest potency on hydroxyl radical scavenging.

Earlier, our group has isolated *Penicillium oxalicum* from fresh tubers of *Bletilla striata* for the first time. Studies have shown the plant disease prevention and plant growth increasement of *Penicillium oxalicum*. For instance, A. De Cal, etc. reported that *Penicillium oxalicum* alleviates tomato (*Lycopersicon esculentum*) disease caused by *Fusarium oxysporum* f. sp. *lycopersici* [26]; Aili.Tureke, etc. have demonstrated the Po-41 strain of *Penicillium oxalicum* promotes the growth of wheat seedlings [27]. However, researches on *Bletilla striata* endophytes remain still relatively little. To find new scaffolds and potential pharmacologically active compounds, we applied solid-state fermentation for culturing *Penicillium oxalicum* and identified 17 compounds, 12 of them are the first time to be isolated from this fungus.

2. Materials and methods

2.1. General

Nuclear magnetic resonance spectra were recorded on Bruker Avance 600 and Avance 700 spectrometers with TMS as internal standard. Compounds were purified by (NP7000, Jiangsu Hanbon Science&.Technology Co., Ltd., Jiangsu, China) Preparative High Performance Liquid Chromatography (PHPLC) with (YMC-Pack ODS-A, YMC Co., Ltd., Kyoto, Japan) semi-preparative column (250 × 10 mm) and (ChromCore 120 C18, NanoChrom Co., Ltd., Suzhou, China) preparative column (21.2 × 250 mm, 5 μm). All used solvents were commercially available and of analytical grade or higher.

2.2. Source of the strain

The *Penicillium oxalicum* was isolated from fresh tubers of the *Bletilla striata* in the medicinal botanical garden of Chengdu University of Traditional Chinese Medicine. And it is preserved in 4 °C, Laboratory of Biochemistry & Pharmaceutical, School of Pharmacy, Chengdu University of Traditional Chinese Medicine.

2.3. Medium recipe

Potato Dextrose Agar (PDA) medium: 200 g potatoes, 20 g glucose, 20 g agar, 1000 mL distilled water; Potato Dextrose Broth (PDB) medium: 200 g potatoes, 20 g glucose, 1000 mL distilled water; Brown Rice Solid-state (BRS) medium: 40 g brown rice, 2 g peptone, and 25 mL distilled water.

2.4. Fermentation of Penicillium oxalicum

The strain of *Penicillium oxalicum* was taken out and put flat in a constant temperature and humidity incubator for revival. The fungus culture was inoculated on the sterilized PDA medium and incubated at 30 °C, 50% humidity for 3 days until even mycelia had grown. The activated fungus colonies were inoculated into the liquid PDB medium and incubated at 30 °C and 120 rpm vibration for 3 days. And the obtained liquid culture was inoculated into 770 erlenmeyers of BRS medium (2 mL for each) and left to ferment at room temperature for 40 days.

2.5. Extraction and isolation of chemical components of Penicillium oxalicum

After the fermentation was complete, the solid fermentation product of *Penicillium oxalicum* was percolated with 75% ethanol followed by concentration. The diacolation crude was diffused with ultrapure water and extracted with ethyl acetate (equal volumes of ultrapure water × 3 times). The extracts were combined and the solvent was removed under vacuum. 1.94 kg of extract paste was given.

The extracted paste was pre-purified with silica column chromatography and eluted with petroleum ether and acetone (v/v, 100:0 to 1:1). All the collected fractions were monitored by TLC and fractions with the same component were combined. After this, 30 fractions (Fr.1 to Fr.30) were given.

Fr.13 was eluted by semi-preparative column (250 × 10 mm) using a MeOH-H_2O (v/v, 55:45; 3 mL/min) solvent system and gave three subfractions (Fr.13-1 to Fr.13-3). Fr.13-1 was purified by semi-preparative column (250 × 10 mm) using a MeOH-H_2O (v/v, 53:47; 3 mL/min) eluent system and afforded 2.62 mg of compound **1**. Fr.13-3 was re-purified by semi-preparative column (250 × 10 mm) using a MeOH-H_2O (v/v, 40:60; 3 mL/min) eluent system and obtained 3.61 mg of compound **2**.

Fr.17 was eluted by preparative column (21.2 × 250 mm, 5 μm) using a MeOH-H_2O (v/v, 10:90 to 100:0; 10 mL/min) gradient eluent system and gave 11 subfractions (Fr.17-1 to Fr.17-11). Fr.17-7 was eluted by semi-preparative column (250 × 10 mm) using a MeOH-H_2O (v/v, 25:75; 3 mL/min) solvent system and gave five secondary

subfractions (Fr.17-7-1 to Fr.17-7-5). Fr.17-7-1 was eluted by semi-preparative column (250 × 10 mm) using a MeOH-H_2O (v/v, 10:90; 3 mL/min) eluent system and afforded 6.20 mg of compound **3** and 1.68 mg of compound **4**. Fr.17-7-4 was re-purified by semi-preparative column (250 × 10 mm) using a MeOH-H_2O (v/v, 13:87; 3 mL/min) eluent system and obtained 4.58 mg of compound **5**.

Fr.23 was eluted by preparative column (21.2 × 250 mm, 5 μm) using a MeOH-H_2O (v/v, 10:90 to 100:0; 10 mL/min) gradient eluent system and gave five subfractions (Fr.23-1 to Fr.23-5). Fr.23-1 was re-purified by semi-preparative column (250 × 10 mm) using a MeOH-H_2O (v/v, 5:95; 3 mL/min) eluent system and obtained 1.66 mg of compound **6**. Fr.23-2 was re-purified by semi-preparative column (250 × 10 mm) using a MeOH-H_2O (v/v, 8:92; 3 mL/min) eluent system and obtained 9.42 mg of compound **7**. Fr.23-3 was re-purified by semi-preparative column (250 × 10 mm) using a MeOH-H_2O (v/v, 13:87; 3 mL/min) eluent system and obtained 1.01 mg of compound **8**.

Fr.26 was eluted by preparative column (21.2 × 250 mm, 5 μm) using a MeOH-H_2O (v/v, 10:90 to 100:0; 10 mL/min) gradient eluent system and gave eight subfractions (Fr.26-1 to Fr.26-8). Fr.26-2 was re-eluted by semi-preparative column (250 × 10 mm) using a MeOH-H_2O (v/v, 5:95; 3 mL/min) eluent system and, respectively, afforded 31.51 mg of compound **9**, 4.50 mg of compound **10** and 1.56 mg of compound **11**. Fr.26-3 was re-eluted by semi-preparative column (250 × 10 mm) using a MeOH-H_2O (v/v, 5:95; 3 mL/min) eluent system and obtained 1.93 mg of compound **12** and 16.16 mg of compound **13**. Fr.26-4 was re-eluted by semi-preparative column (250 × 10 mm) using a MeOH-H_2O (v/v, 13:87; 3 mL/min) eluent system and obtained 1.26 mg of compound **14** and 3.54 mg of compound **15**. Fr.26-6 was re-purified by semi-preparative column (250 × 10 mm) using a MeOH-H_2O (v/v, 28:72; 3 mL/min) eluent system and obtained 23.21 mg of compound **16**.

Fr.27 was re-purified by semi-preparative column (250 × 10 mm) using a MeOH-H_2O (v/v, 5:95; 3 mL/min) eluent system and afforded 177.14 mg of compound **17**.

2.6. Identification of chemical components of Penicillium oxalicum

All the structures of the above 17 compounds have been identified by ^{1}H and ^{13}C NMR. We compared the determined NMR spectrums with that of reported candidate compounds and confirmed the structure of each compound.

3. Results

A total of 17 compounds were isolated and identified from the metabolites of *Penicillium oxalicum*, and the chemical structures of all these compounds are given in Figure 1. The determined NMR spectrum data are shown below:

Compound **1** (Supplementary Figures S1 and S2): Light yellow powder. ESI-MS *m/z*: 412 $[M+H]^+$. ^{1}H-NMR (700 MHz, CD_3OD) δ: 7.74 (1H, d, J = 8.7 Hz, H-16), 6.87 (1H, d, J = 2.2 Hz, H-19), 6.68 (1H, dd, J = 8.7, 2.3 Hz, H-17), 5.94 (1H, dd, J = 9.6, 0.7 Hz, H-3), 5.69 (1H, d, J = 1.1 Hz, H-13), 4.82 − 4.78 (1H, m, H-21), 4.51 (1H, dd, J = 9.5, 7.2 Hz, H-6), 3.81 (3H, s, CH_3O-18), 3.64-3.58 (2H, m, H-9), 2.45 (1H, dt, J = 10.5, 6.1 Hz, H-7a), 2.11-2.07 (1H, m, H-7b), 2.05 (1H, dd, J = 15.6, 10.8 Hz, H-8a), 2.02-2.01 (3H, m, H-24), 2.00-1.96 (1H, m, H-8b), 1.67 (3H, d, J = 0.7 Hz, H-23); ^{13}C-NMR (175 MHz, CD_3OD) δ: 173.2 (C-11), 168.1 (C-5), 157.6 (C-18), 139.5 (C-20), 135.6 (C-22), 131.2 (C-2), 125.0 (C-21), 122.2 (C-16), 121.9 (C-15), 110.0 (C-17), 106.6 (C-14), 95.6 (C-19), 85.0 (C-12), 69.7 (C-13), 60.2 (C-6), 55.9 (CH_3O-18), 51.4 (C-3), 46.5 (C-9), 30.2 (C-7), 25.9 (C-23), 23.5 (C-8), and 18.4 (C-24). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as 12,13-dihydroxy-fumitremorgin C [28].

Compound **2** (Supplementary Figures S3 and S4): White needle crystals. ESI-MS *m/z*: 454 $[M+Na]^+$. ^{1}H-NMR (700 MHz, CD_3OD) δ: 8.39-8.34 (2H, m, H-19, H-23), 7.69-7.63 (1H, m, H-21), 7.53-7.50 (2H, m, H-20, H-22), 5.63 (1H, dt, J = 11.7, 7.4 Hz, H-13), 5.50-5.46 (1H, m, H-12), 4.70-4.67 (1H, m, H-10), 4.55 (1H, s, H-9), 4.51 (1H, d, J = 6.6 Hz, H-11), 3.35 (3H, s, OMe-8), 2.21-2.10 (2H, m, H-14), 1.77 (3H, s, H-16), 0.99 (3H, t, J = 7.5 Hz, H-15); ^{13}C-NMR (175 MHz, CD_3OD) δ: 199.2 (C-4), 197.1 (C-17), 188.7 (C-6), 169.2 (C-2), 137.3 (C-13), 135.1 (C-21), 134.9 (C-18), 131.7 (C-19, 23), 129.5 (C-20, 22), 128.8 (C-12), 114.4 (C-3), 93.9 (C-5), 93.6 (C-8), 76.3 (C-9), 72.9 (C-10), 69.6 (C-11), 52.5 (OMe-8), 22.3 (C-14), 14.5 (C-15), and 5.8 (C-16). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as pseurotin A [29].

Compound **3** (Supplementary Figures S5 and S6): Light yellow powder. ESI-MS *m/z*: 137 $[M-H]^-$. ^{1}H-NMR (700 MHz, CD_3OD) δ: 7.03 (2H, d, J = 8.5 Hz, H-3, 5), 6.70 (2H, d, J = 8.5 Hz, H-2, 6), 3.69 (2H, t, J = 7.2 Hz, H-2′), 2.72 (2H, t, J = 7.2 Hz, H-1′); ^{13}C-NMR (175 MHz, CD_3OD) δ: 156.8 (C-1), 131.0 (C-4), 130.9 (C-3, 5), 116.1 (C-2, 6), 64.6 (C-2′), and 39.4 (C-1′). The above data are in general agreement

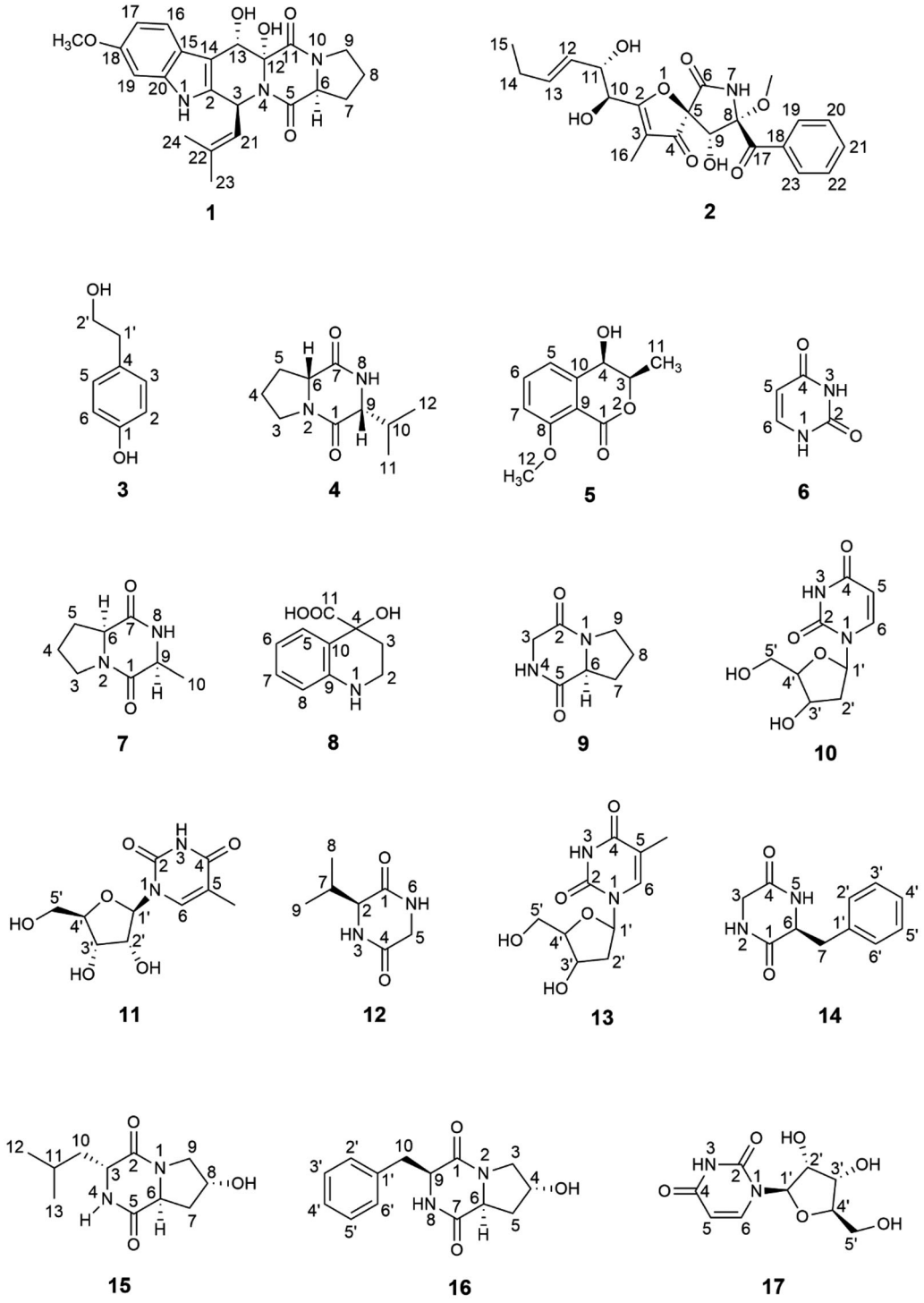

Figure 1. Chemical structures of compounds **1**–**17** that are isolated from the metabolites of fermented *Penicillium oxalicum*.

with the NMR data reported in the literature, so the compound was identified as tyrosol [30].

Compound **4** (Supplementary Figures S7 and S8): Light yellow oil. ESI-MS *m/z*: 195 $[M-H]^-$. ^{1}H-NMR (700 MHz, CD_3OD) δ: 4.21 (1H, t, $J=7.6$ Hz, H-9), 4.05 (1H, t, $J=2.0$ Hz, H-6), 3.61-3.55 (1H, m, H-3a), 3.54-3.49 (1H, m, H-3b), 2.53-2.47 (1H, m, H-10), 2.36-2.31 (1H, m, H-5a), 2.06-2.01 (1H, m, H-5b), 1.99-1.91 (2H, m, H-4), 1.10 (3H, d, $J=7.2$ Hz, H-11), 0.95 (3H, d, $J=6.9$ Hz, H-12); ^{13}C-NMR (175 MHz, CD_3OD) δ: 172.6 (C-1), 167.6 (C-7), 61.5 (C-6), 60.0 (C-9), 46.2 (C-3), 29.9 (C-10), 29.5 (C-5), 23.3 (C-4), 18.8 (C-11), and 16.7 (C-12). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as cyclo-(*L*-Pro-*L*-Val) [31].

Compound **5** (Supplementary Figures S9–S13): White solid. ESI-MS *m/z*: 231 $[M+Na]^+$. ^{1}H-NMR (700 MHz, CD_3OD) δ: 7.65 (1H, dd, $J=8.4$, 7.6 Hz, H-6), 7.20 (1H, d, $J=8.5$ Hz, H-5), 7.08 (1H, d, $J=7.4$ Hz, H-7), 4.60 (1H, qd, $J=6.5$, 1.9 Hz, H-3), 4.56 (1H, d, $J=1.7$ Hz, H-4), 3.92 (3H, s, H-12), 1.48 (3H, d, $J=6.6$ Hz, H-11); ^{13}C-NMR (175 MHz, CD_3OD) δ: 164.8 (C-1), 162.2 (C-8), 145.1 (C-10),

136.7 (C-6), 120.8 (C-7), 113.9 (C-5), 113.1 (C-9), 78.2 (C-3), 68.5 (C-4), 56.5 (C-12), and 16.2 (C-11). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as *cis*-4-hydroxy-8-O-methylmellein [32].

Compound **6** (Supplementary Figures S14 and S15): White powder. ESI-MS *m/z*: 113 $[M+H]^+$. ^{1}H-NMR (700 MHz, D_2O) δ: 7.53 (1H, d, $J=7.7$ Hz, H-6), 5.80 (1H, d, $J=7.7$ Hz, H-5); ^{13}C-NMR (175 MHz, D_2O) δ: 167.4 (C-4), 153.0 (C-2), 143.4 (C-6), and 101.0 (C-5). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as uracil [33].

Compound **7** (Supplementary Figures S16 and S17): White needle crystals. ESI-MS *m/z*: 169 $[M+H]^+$. ^{1}H-NMR (700 MHz, CD_3OD) δ: 4.27-4.23 (1H, m, H-9), 4.20-4.16 (1H, m, H-6), 3.53-3.49 (2H, m, H-3), 2.32-2.27 (1H, m, H-5a), 2.04-1.97 (2H, m, H-5b, 4a), 1.96-1.88 (1H, m, H-4b), 1.37 (3H, d, $J=6.9$ Hz, H-10); ^{13}C-NMR (175 MHz, CD_3OD) δ: 172.6 (C-1), 169.0 (C-7), 60.5 (C-6), 52.1 (C-9), 46.4 (C-3), 29.2 (C-5), 23.6 (C-4), and 15.7 (C-10). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as cyclo-(*L*-Pro-*L*-Ala) [34].

Compound **8** (Supplementary Figures S18 and S19): Light yellow powder. ESI-MS *m/z*: 194 $[M+H]^+$. ^{1}H-NMR (700 MHz, CD_3OD) δ: 7.33-7.31 (1H, m, H-5), 7.23 (1H, td, $J=7.7$, 1.2 Hz, H-7), 7.04 (1H, td, $J=7.6$, 0.9 Hz, H-6), 6.86 (1H, d, $J=7.7$ Hz, H-8), 3.49 (2H, t, $J=7.2$ Hz, H-2), 2.20-2.16 (1H, m, H-3a), 2.13-2.09 (1H, m, H-3b); ^{13}C-NMR (175 MHz, CD_3OD) δ: 182.0 (C-11), 142.7 (C-9),132.7 (C-10), 130.6 (C-7), 125.1 (C-5), 123.7 (C-6), 111.3 (C-8), 76.4 (C-4), 58.4 (C-2), and 41.2 (C-3). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as 1,2,3,4-tetrahydro-4-hydroxy-4-quinolin carboxylic acid [35].

Compound **9** (Supplementary Figures S20 and S21): White crystals. ESI-MS *m/z*: 155 $[M+H]^+$. ^{1}H-NMR (700 MHz, CD_3OD) δ: 4.28-4.24 (1H, m, H-6), 4.16-4.11 (1H, m, H-3a), 3.77 (1H, d, $J=16.8$ Hz, H-3b), 3.62-3.52 (2H, m, H-9), 2.37-2.32 (1H, m, H-7a), 2.08-2.03 (1H, m, H-7b), 2.03-1.93 (2H, m, H-8); ^{13}C-NMR (175 MHz, CD_3OD) δ: 172.0 (C-5), 166.4 (C-2), 59.8 (C-6), 47.0 (C-3), 46.3 (C-9), 29.4 (C-7), and 23.3 (C-8). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as cyclo-(Gly-*L*-Pro) [36].

Compound **10** (Supplementary Figures S22 and S23): White crystals. ESI-MS *m/z*: 229 $[M+H]^+$. ^{1}H-NMR (700 MHz, CD_3OD) δ: 7.99 (1H, d, $J=8.1$ Hz, H-6), 6.28 (1H, t, $J=6.8$ Hz, H-1′), 5.70 (1H, d, $J=8.1$ Hz, H-5), 4.41-4.38 (1H, m, H-4′), 3.93 (1H, q, $J=3.4$ Hz, H-3′), 3.79 (1H, dd, $J=12.0$, 3.3 Hz, H-5'a), 3.73 (1H, dd, $J=12.1$, 3.8 Hz, H-5'b), 2.31-2.28 (1H, m, H-2'a), 2.23-2.19 (1H, m, H-2'b); ^{13}C-NMR (175 MHz, CD_3OD) δ: 166.2 (C-4), 152.2 (C-2), 142.5 (C-6), 102.6 (C-5), 89.0 (C-1′), 86.6 (C-4′), 72.3 (C-3′), 62.8 (C-5′), and 41.4 (C-2′). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as 2′-deoxyuridine [37].

Compound **11** (Supplementary Figures S24 and S25): Light yellow oil. ESI-MS *m/z*: 259 $[M+H]^+$. ^{1}H-NMR (700 MHz, CD_3OD) δ: 7.86 (1H, d, $J=1.2$ Hz, H-6), 5.91 (1H, d, $J=4.7$ Hz, H-1′), 4.20 − 4.16 (2H, m, H-2′, 3′), 4.00 (1H, dt, $J=4.2$, 2.9 Hz, H-4′), 3.86 (1H, dd, $J=12.2$, 2.7 Hz, H-5'a), 3.75 (1H, dd, $J=12.2$, 3.0 Hz, H-5'b), 1.89 (3H, d, $J=1.2$ Hz, 5-Me); ^{13}C-NMR (175 MHz, CD_3OD) δ: 166.4 (C-4), 152.7 (C-2), 138.4 (C-6), 111.5 (C-5), 90.4 (C-1′), 86.3 (C-4′), 75.5 (C-2′), 71.3 (C-3′), 62.3 (C-5′), and 12.4 (5-Me). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as 1-(*β*-D-ribofuranosyl)thymine [38].

Compound **12** (Supplementary Figures S26 and S27): White powder. ESI-MS *m/z*: 157 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 3.99 (1H, d, $J=18.1$ Hz, H-5a), 3.82 (1H, d, $J=18.1$ Hz, H-5b), 3.73 (1H, d, $J=4.0$ Hz, H-2), 2.28-2.21 (1H, m, H-7), 1.03 (3H, d, $J=7.0$ Hz, H-8), 0.96 (3H, d, $J=6.8$ Hz, H-9); ^{13}C-NMR (150 MHz, CD_3OD) δ: 170.3 (C-1), 168.9 (C-4), 61.7 (C-2), 45.2 (C-5), 34.4 (C-7), 19.0 (C-8), and 17.2 (C-9). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as cyclo-(*L*-Val-Gly) [39].

Compound **13** (Supplementary Figures S28 and S29): White crystals. ESI-MS *m/z*: 243 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.81 (1H, s, H-6), 6.28 (1H, t, $J=6.8$ Hz, H-1′), 4.41-4.38 (1H, m, H-3′), 3.90 (1H, q, $J=3.3$ Hz, H-4′), 3.79 (1H, dd, $J=12.0$, 3.2 Hz, H-5'a), 3.72 (1H, dd, $J=12.0$, 3.6 Hz, H-5'b), 2.24-2.18 (2H, m, H-2′), 1.88 (3H, s, 5-CH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ: 166.4 (C-4), 152.4 (C-2), 138.2 (C-6), 111.5 (C-5), 88.8 (C-4′), 86.2 (C-1′), 72.2 (C-3′), 62.8 (C-5′), 41.2 (C-2′), and 12.5 (5-CH_3). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as 2′-deoxythymidine [40].

Compound **14** (Supplementary Figures S30 and S31): White powder. ESI-MS *m/z*: 205 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.31-7.28 (3H, m, H-2′, 4′, 6′), 7.23-7.19 (2H, m, H-3′, 5′), 4.22 (1H, t,

$J = 4.4$ Hz, H-6), 3.41 (1H, dd, $J = 17.7$, 0.7 Hz, H-3a), 3.23 (1H, dd, $J = 13.7$, 4.1 Hz, H-7a), 2.99 (1H, dd, $J = 13.7$, 4.7 Hz, H-7b), 2.63 (1H, dd, $J = 17.7$, 1.0 Hz, H-3b); ^{13}C-NMR (150 MHz, CD_3OD) δ: 170.0 (C-1), 168.7 (C-4), 136.4 (C-1′), 131.5 (C-2′, 6′), 129.6 (C-3′, 5′), 128.5 (C-4′), 57.5 (C-6), 44.6 (C-3), and 40.9 (C-7). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as cyclo-(Gly-*D*-Phe) [41].

Compound **15** (Supplementary Figures S32 and S33): Light yellow crystals. ESI-MS *m/z:* 227 $[M + H]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 4.43-4.39 (1H, m, H-8), 4.37-4.33 (1H, m, H-6), 3.86 (1H, dd, $J = 9.7$, 5.4 Hz, H-3), 3.66 (1H, dd, $J = 12.1$, 3.6 Hz, H-9a), 3.44 (1H, dd, $J = 12.1$, 5.5 Hz, H-9b), 2.47 (1H, ddd, $J = 14.2$, 8.8, 5.5 Hz, H-7a), 2.25-2.20 (1H, m, H-7b), 1.81-1.73 (1H, m, H-11), 1.68 (1H, ddd, $J = 13.7$, 9.7, 5.5 Hz, H-10a), 1.57 (1H, ddd, $J = 13.8$, 8.5, 5.5 Hz, H-10b), 0.99 (3H, d, $J = 6.6$ Hz, H-13), 0.96 (3H, d, $J = 6.6$ Hz, H-12); ^{13}C-NMR (150 MHz, CD_3OD) δ: 171.2 (C-5), 169.4 (C-2), 68.8 (C-8), 57.5 (C-6), 56.9 (C-3), 54.3 (C-9), 43.4 (C-10), 37.7 (C-7), 25.6 (C-11), 23.3 (C-13), and 21.9 (C-12). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as cyclo-*L*-(4-hydroxyprolinyl)-*D*-leucine [42].

Compound **16** (Supplementary Figures S34 and S35): Light yellow oil. ESI-MS *m/z:* 261 $[M + H]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.30-7.20 (5H, m, H-2′, 3′, 4′, 5′, 6′), 4.47 (1H, s, H-9), 4.36 (1H, dd, $J = 11.6$, 5.8 Hz, H-6), 4.27 (1H, t, $J = 4.6$ Hz, H-4), 3.69 (1H, dd, $J = 13.0$, 4.5 Hz, H-3b), 3.31-3.29 (1H, m, H-3a), 3.18 (1H, dd, $J = 14.4$, 4.7 Hz, H-10b), 3.14 (1H, dd, $J = 14.3$, 5.2 Hz, H-10a), 2.06 (1H, dd, $J = 12.9$, 5.9 Hz, H-5a), 1.37 (1H, td, $J = 11.9$, 3.5 Hz, H-5b); ^{13}C-NMR (150 MHz, CD_3OD) δ: 171.3 (C-7), 167.1 (C-1), 137.4 (C-1′), 131.0 (C-2′, 6′), 129.5 (C-3′, 5′), 128.1 (C-4′), 68.6 (C-4), 58.4 (C-6), 57.6 (C-9), 55.3 (C-3), 38.9 (C-5), and 38.0 (C-10). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as cyclo-(*L*)-4-hydroxy-Pro-(*L*)-Phe [43].

Compound **17** (Supplementary Figures S36 and S37): White powder. ESI-MS *m/z*: 267 $[M + Na]^+$. ^{1}H-NMR (700 MHz, D_2O) δ: 7.89 (1H, d, $J = 8.1$ Hz, H-6), 5.90 (1H, d, $J = 4.5$ Hz, H-1′), 5.89 (1H, d, $J = 8.1$ Hz, H-5), 4.34 (1H, t, $J = 4.9$ Hz, H-2′), 4.24 (1H, t, $J = 5.4$ Hz, H-3′), 4.16-4.12 (1H, m, H-4′), 3.93 (1H, dd, $J = 12.8$, 2.8 Hz, H-5'a), 3.83 (1H, dd, $J = 12.8$, 4.2 Hz, H-5'b); ^{13}C-NMR (175 MHz, D_2O) δ: 165.9 (C-4), 151.5 (C-2), 141.8 (C-6), 102.2 (C-5), 89.3 (C-1′), 84.2 (C-4′), 73.8 (C-2′), 69.4 (C-3′), and 60.7 (C-5′). The above data are in general agreement with the NMR data reported in the literature, so the compound was identified as uridine [44].

4. Discussion

In this research, we fermented *Bletilla striata* endophyte *Penicillium oxalicum* and collected its metabolites by percolation extraction. With silica column chromatography and preparative HPLC, 17 compounds were isolated and purified.

Among them, the biological activities of some compounds have been studied extensively (Table 1). Compound **2** has been reported to be antimicrobic and antineoplastic due to its activity on anti-*Klebsiella pneumoniae* and anti-*Bacillus subtilis* and some inhibition against human alveolar A549 cells

Table 1. List of biological activities of compounds **1–17**.

Compound No.	Chemical name	Molecular formula	Biological activity	References
1	12,13-dihydroxy-fumitremorgin C	$C_{22}H_{25}N_3O_5$	–	–
2	pseurotin A	$C_{22}H_{25}NO_8$	Antibiosis; antitumor	[45]
3	tyrosol	$C_8H_{10}O_2$	Anti-allergic inflammation; antibiosis; antioxidation; antitumor; immune enhancement	[46–49]
4	cyclo-(*L*-Pro-*L*-Val)	$C_{10}H_{16}N_2O_2$	–	–
5	*cis*-4-hydroxy-8-O-methylmellein	$C_{11}H_{12}O_4$	–	–
6	uracil	$C_4H_4N_2O_2$	Protection of hepatocyte injury *in vitro*; neuroprotective effect	[50,51]
7	cyclo-(*L*-Pro-*L*-Ala)	$C_8H_{12}N_2O_2$	–	–
8	1,2,3,4-tetrahydro-4-hydroxy-4-quinolin carboxylic acid	$C_{10}H_{11}NO_3$	–	–
9	cyclo-(Gly-*L*-Pro)	$C_7H_{10}N_2O_2$	Antitumor	[52]
10	2′-deoxyuridine	$C_9H_{12}N_2O_5$	–	–
11	1-(*β*-D-ribofuranosyl)thymine	$C_{10}H_{14}N_2O_6$	–	–
12	cyclo-(*L*-Val-Gly)	$C_7H_{12}N_2O_2$	–	–
13	2′-deoxythymidine	$C_{10}H_{14}N_2O_5$	Antioxidation	[53]
14	cyclo-(Gly-*D*-Phe)	$C_{11}H_{12}N_2O_2$	Inhibition against VEGFR2-CD	[54]
15	cyclo-*L*-(4-hydroxyprolinyl)-*D*-leucine	$C_{11}H_{18}N_2O_3$	–	–
16	cyclo-(*L*)-4-hydroxy-Pro-(*L*)-Phe	$C_{14}H_{16}N_2O_3$	Antibiosis	[43]
17	uridine	$C_9H_{12}N_2O_6$	–	–

[45]. Compound **3** has anti-allergic inflammatory effects by inhibiting the degranulation of mast cells and the expression of inflammatory cytokines [46]. Besides, compound **3** has shown various kinds of activities, e.g. antibiosis, antioxidation, antitumor, and immune enhancement [47–49]. Liver cells can be protected from acetaminophen (APAP) overdose caused damage by compound **6** at a concentration of 10 μM [50]. Another research has shown compound **6** has an obvious neuroprotective effect on 100 μM 6-hydroxydopamine (6-OHDA)-induced apoptosis of pheochromocytoma cells (PC12) [51]. The antineoplastic activity of compound **9** has been proven based on its inhibition against a series of cancer cell lines, such as HL-6, A-549, SMMC-7721, MCF-7 and SW-480 [52]. Compound **13** also shows some antioxidant activity [53]. Compound **14** has some inhibition against vascular endothelial growth factor receptor-2 tyrosine kinase (VEGFR2-CD) [54]. At a concentration of 2.9 mM, compound **16** can restrain the growth of *Staphylococcus aureus* and *Micrococcus luteus* [43].

Penicillium is a genus of ascomycetous fungi that widely distributed in nature. The antibiotic, antineoplastic and antioxidant activities of its secondary metabolites make it a significant source of lead compounds in drug discovery. Many of these metabolites (**4**, **7**, **9**, **12**, **14**, **15**, and **16**) in this research are cyclodipeptides. Studies on cyclodipeptides have shown their biological activities in antioxidation, antitumor, antibiosis as well as cardiovascular and neural protection [55–60]. Other reported *Penicillium* metabolites include butyrolactones, anthraquinones, and hexenones, etc. and these scaffolds also show biological activities in certain aspects [61–64].

By isolating and identifying metabolite chemical compositions of the *Bletilla striata* endophyte *Penicillium oxalicum*, combined with reported compounds' biological activities, our study partially revealed the medicinal properties of *Bletilla striata* at the molecular level.

5. Conclusions

We fermented *Penicillium oxalicum* which was earlier isolated from the *Bletilla striata* tubers. The metabolites of *Penicillium oxalicum* were collected, isolated, purified and identified. 17 known compounds were found in this study. Compounds **1**–**3**, **5**, **7**–**8**, **11**–**12**, **14**–**17** are first found and isolated from *Bletilla striata* endophyte *Penicillium oxalicum*. We expect this study inspires the possibilities for future wider development and utilization of medicinal plant endophytes, as well as for *Bletilla striata* researches.

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Author contributions

Ran Liu designed and wrote the manuscript. Jing Gao and Min Luo organized the figures. Xuehua Han participated in the experiment. Dale Guo and Guangzhi Wang reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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