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内生真菌交织枝顶孢次生代谢产物及其生物活性

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摘　要:从番茄内生真菌交织枝顶孢(*Acremonium implicatum*)中分离到5个次生代谢产物,经理化特性和波谱数据分析鉴定为对羟基苯甲醛(**1**)、对羟基苯甲酸(**2**)、吲哚-3-甲醛(**3**)、5′-*O*-乙酰胸苷(**4**)和酵母甾醇(**5**)。化合物**1**和**2**表现出强的抗细菌、抗真菌、抗氧化和抗线虫活性。化合物**3**对秀丽隐杆线虫和南方根结线虫表现出一定的抑制活性,半抑制浓度(IC_{50})分别为82.3 μg/mL和93.4 μg/mL。结果表明对羟基苯甲醛(**1**)、对羟基苯甲酸(**2**)、吲哚-3-甲醛(**3**)是交织枝顶孢中的主要活性成分,为深入研究交织枝顶孢对线虫的生防机制以及该真菌的开发与应用提供了依据。上述化合物均为首次从交织枝顶孢中分离得到。

关键词:番茄;内生真菌;交织枝顶孢;次生代谢产物;抗菌活性;抗氧化活性;抗线虫活性

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Secondary Metabolites of Endophytic Fungus *Acremonium implicatum* and Their Biological Activities

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Abstract: Five secondary metabolites were isolated from the ethyl acetate extract of the endophytic fungus *Acremonium implicatum* from tomato root galls infected with *Meloidogyne incognita*. They were identified as 4-hydroxy benzaldehyde (**1**), 4-hydroxy benzoic acid (**2**), indole-3-carboxaldehyde (**3**), 5′-*O*-acetylthymidine (**4**) and cerevisterol (**5**) by physicochemical properties and spectrometric methods. Compounds **1** and **2** showed strong antibacterial, antifungal, antioxidant and antinematodal activities. Compound **3** exhibited moderate antinematodal activity on *Caenorhabditis elegans* and *M. incognita* with the median inhibitory concentration (IC_{50}) values of 82.3 μg/mL and 93.4 μg/mL, respectively. The results indicated that 4-hydroxy benzaldehyde (**1**), 4-hydroxy benzoic acid (**2**), and indole-3-carboxaldehyde (3) could be the main bioactive components in the endophytic fungus *A. implicatum*. The results will be helpful for clarifying biocontrol mechanisms of *A. implicatum* on the root-knot nematode as well as for development and utilization of this fungus. All the compounds were isolated from *A. implicatum* for the first time.

Key words: *Lycopersicon esculentum*; endophytic fungus; *Acremonium implicatum*; secondary metabolites; antimicrobial activity; antioxidant activity; antinematodal activity

Introduction

Plant endophytic fungi are microorganisms that live within plant tissues without causing symptoms of disease[1]. Endophytic fungi are rich of valuable bioactive metabolites with antioxidant, anti-viral, insecticidal, anti-tumor, antimicrobial and antinematodal activities to display their potential applications in agriculture, medicine and food industry[2,3].

Acremonium implicatum was reported to show its antinematodal activity[4,5]. It was isolated as an endophytic fungus from tomato (*Lycopersicon esculentum*) root galls infected with *Meloidogyne incognita*, an important root-knot nematode causing great economic losses in vegetable production. This fungus suppressed egg

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hatching, killed second-stage juveniles (J2s), and also promoted the growth of tomato[4]. *A. implicatum* was also isolated as an opportunistic fungus from the eggs and females of the root-knot nematode, and was revealed that this fungus produced chitinase and parasitized different life stages of *M. incognita*, including eggs, J2s, and females[5].

To the best of our knowledge, there was no report about the secondary metabolites from *A. implicatum* and their biological activities. This study aimed to investigate the secondary metabolites from the endophytic fungus *A. implicatum* isolated from tomato root galls infected with *M. incognita* as well as to evaluate their antimicrobial, antioxidant and antinematodal activities in order to provide the support data for developing biologically active metabolites and biocontrol agents as well as for clarifying its biocontrol mechanisms on the root-knot nematode.

Materials and Methods

General

Silica gel (60-80 and 200-300 mesh, Qingdao Marine Chemical Company, China) was used for column chromatography (CC). Thin layer chromatography (TLC) plates (Qingdao Marine Chemical Company, China) were coated with 0.5 mm layer of silica gel (GF_{254}, 300-400 mesh). Melting points (m. p.) were determined on an XT4-100B microscopic melting point apparatus (Tianjin Tianguang Optical Instruments Company, China) and were uncorrected. NMR spectra were recorded on a Bruker ARX-400 (^1H at 400 MHz and ^{13}C at 100 MHz). HR-ESI-MS spectra were recorded on a Bruker Apex IV FTMS mass spectrometer. A microplate spectrophotometer (PowerWave HT, BioTek Instruments, USA) was employed to measure the light absorption value. Carbendazim, β-carotene and streptomycin sulfate were purchased from Sigma-Aldrich (USA). 3-(4,5-Dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide (MTT) was purchased from Amresco (USA). Salicylic acid (SA), ascorbic acid (AA), butylated hydroxytoluene (BHT) and Tween-40 were from Beijing Chemical Company. All other chemicals and reagents were of analytical grade.

Endophytic fungus

The endophytic fungus *Acremonium implicatum* was isolated from the root galls of tomato (*Lycopersicon esculentum*) and showed its antinematodal activity on *Meloidogyne incognita*[4]. It was stored on PDA (potato 200 g/L, dextrose 20 g/L and agar 20 g/L) slants at 4 ℃.

Preparation of the crude extract

The fungus *A. implicatum* was cultured on PDA medium in Petri dishes at 25 ℃ for 7 days. For seed culture, several plugs of agar medium with mycelia were inoculated in each 250-mL Erlenmeyer flask containing 100 mL of potato dextrose broth (PDB) medium, and incubated on a rotary shaker at 150 rpm and 25 ℃ for 5 days. For fermentation culture, 10 mL of seed culture broth was inoculated in each 1000-mL Erlenmeyer flask containing 300 mL PDB medium. After incubation on a rotary shaker at 150 rpm and 25 ℃ for 8 days, a total of 100 L fermentation broth was harvested. Then the mycelia were separated from the culture broth by filtration. The filtrate was concentrated under vacuum at 50 ℃ to give the brown residue which was further suspended in water (1 L) and fractionated successively with petroleum ether (3 × 1 L) and ethyl acetate (3 × 1 L) for three times to give their corresponding fractions. The dry mycelia (200 g) were soaked in ethyl acetate (EtOAc) at room temperature for three times at an interval of 7 days (3 × 4 L). The above EtOAc extracted and fractionated solutions were combined and concentrated under vacuum at 40 ℃ to give EtOAc crude extract (70 g).

Isolation and purification of the metabolites

Firstly, the EtOAc extract was subjected to silica gel (200 – 300 mesh) column chromatography (CC) eluted with CH_2Cl_2-MeOH (30∶1, v/v) to obtain nine fractions (FA-FI) monitored by TLC. Fraction FC (3.45 g) was selected for further fractionation by middle-pressure liquid chromatography (MPLC) eluted with a step gradient of CH_2Cl_2-MeOH (from 100∶0 to 0∶100, v/v) to give 6 sub-fractions. Sub-fraction FC-3 was purified by semi-preparative HPLC eluted with MeOH-H_2O (30∶70, v /v) at a flow rate of 3.0 mL/min, detection wavelength of 266 nm, injection volume

of 2.0 mL for each time to afford compound **4** (4.0 mg). Sub-fraction FC-5 was crystallized to afford compound **5**(5.5 mg).

Similarly, fraction FE (7.72 g) was subjected to silica gel CC eluted with a step gradient of CH_2Cl_2-MeOH (from 100∶0 to 0∶100, v/v) to give nine sub-fractions. Sub-fraction FE-6 (3.78 g) was further separated by MPLC eluted with a step gradient of petroleum ether-acetone (from 50∶0 to 50∶20, v/v) followed with a step gradient of CH_2Cl_2-MeOH (from 20∶1 to 0∶20, v/v), and purified by semi-preparative HPLC eluted with MeOH-H_2O (25∶75, v/v) to afford compound **2** (7.5 mg). Fraction FG (10.1 g) was subjected to silica gel CC eluted with a step gradient of CH_2Cl_2-MeOH (from 100∶0 to 0∶100, v/v) to give 13 sub-fractions. Sub-fraction FG-4 was further separated by MPLC and semi-preparative HPLC to afford compounds **1** (45.0 mg) and **3**(4.0 mg).

Structural identification

4-Hydroxy benzaldehyde (**1**) was obtained as white amorphous powder; m. p. 95-97 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.76 (2H, d, J = 4.7 Hz, H-2,6), 6.92 (2H, d, J = 4.7 Hz, H-3,5), 10.59 (1H, s, OH-4), 9.79 (1H, s, H-7); ^{13}C NMR (DMSO-d_6, 150 MHz) δ (ppm): 128.5 (C-1), 132.1 (C-2,6), 115.9 (C-3,5), 163.3 (C-4), 191.0 (C-7). Those data were consistent with literature[6].

4-Hydroxy benzoic acid (**2**) was obtained as white amorphous powder; m. p. 212-215 ℃. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 6.81 (2H, d, J = 4.7 Hz, H-3,5), 7.78 (2H, d, J = 4.7 Hz, H-2,6); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 121.5 (C-1), 131.5 (C-2,6), 115.1 (C-3,5), 161.6 (C-4), 167.3 (C-7). Those data were consistent with literature[7].

Indole-3-carboxaldehyde (**3**) was obtained as yellow amorphous powder; m. p. 198-199 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.28 (1H, s, H-2), 8.09 (1H, d, J = 5.1 Hz, H-5), 7.24 (2H, m, H-6, 7), 7.51 (1H, d, J = 5.4 Hz, H-8), 9.93 (1H, s, CHO-10); ^{13}C NMR (DMSO-d_6, 150 MHz) δ (ppm): 138.5 (C-2), 124.1 (C-3), 123.5 (C-4), 122.1 (C-5), 118.2 (C-6), 120.8 (C-7), 112.4 (C-8), 137.1 (C-9), 185.0 (CHO-10). The structure was confirmed by comparison with literature[8].

5′-*O*-Acetylthymidine (**4**) was obtained as yellow amorphous powder; m. p. 150 ℃; The molecular formula C_{12} H_{16} N_2O_6 was assigned by HR-ESI-MS m/z 307.09014 [M + Na]$^+$ (calcd. 307.09006); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.44 (1H, s, H-6), 1.79 (3H, s, H-7), 6.17 (1H, t, J = 6.9 Hz, H-1′), 2.18 (2H, m, H-2′), 3.89 (1H, d, J = 5.3 Hz, H-3′), 4.19 (3H, m, H-4′, 5′), 2.05 (3H, s, H-7′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 152.3 (C-2), 120.4 (C-3), 166.2 (C-4), 111.6 (C-5), 137.4 (C-6), 12.4 (C-7), 86.5 (C-1′), 40.5 (C-2′), 72.2 (C-3′), 85.7 (C-4′), 65.0 (C-5′), 172.2 (C-6′), 20.6 (C-7′). Those data were consistent with literature[9].

Cerevisterol (**5**) was obtained as white needle crystal; m. p. 243-246 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 5.13-5.26 (1H, m, H-7), 0.54 (3H, s, H-18), 0.99 (3H, s, H-19), 0.90 (3H, d, J = 5.1 Hz, H-21), 0.81 (3H, d, J = 5.4 Hz, H-26), 0.80 (3H, d, J = 6.5 Hz, H-27), 0.89 (3H, d, J = 6.8 Hz, H-28); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 33.0 (C-1), 30.9 (C-2), 67.7 (C-3), 39.5 (C-4), 76.0 (C-5), 73.7 (C-6), 117.6 (C-7), 144.0 (C-8), 43.5 (C-9), 37.2 (C-10), 22.1 (C-11), 39.2 (C-12), 43.8 (C-13), 54.8 (C-14), 22.9 (C-15), 27.9 (C-16), 56.0 (C-17), 12.3 (C-18), 18.8 (C-19), 40.4 (C-20), 21.1 (C-21), 135.4 (C-22), 132.2 (C-23), 42.8 (C-24), 33.1 (C-25), 20.0 (C-26), 19.7 (C-27), 17.6 (C-28). Those data were consistent with literature[10].

Antimicrobial activity assay

Two Gram-negative (*Pseudomonas lachrymans* ATCC 11921 and *Xanthomonas vesicatoria* ATCC 11633) and two Gram-positive (*Bacillus subtilis* ATCC 11562 and *Staphylococcus haemolyticus* ATCC 29970) bacteria were selected for antibacterial activity assay. They were grown in liquid LB medium (yeast extract 5 g/L, peptone 10 g /L, NaCl 5 g/L, pH 7.0) overnight at 28 ℃, and the diluted bacterial suspension (1×10^6 CFU/mL) was ready for detection. A modified broth dilution-colorimetric assay by using the chromogenic reagent 3-(4, 5-dimethylthiazol-2-yl) -2, 5-diphenyl tet-

razolium bromide (MTT) was employed to detect the antibacterial activity of the compounds according to our previous report[11]. Rice blast fungus, *Magnaporthe oryzae* (strain P131) was cultured on the oatmeal-tomato agar medium (oatmeal 30 g/L, tomato juice 150 mL/L, and agar 20 g/L) at 25 ℃. The spores were prepared from 7-day-old cultures of *M. oryzae*. A modified spore germination assay was employed to detect the antifungal activity of the compounds according to our previous report[12]. The compound-ethanol solution was mixed with an equivalent volume of fungal spore suspension containing 2×10^6 spores per mL. Of the mixture, 50 mL was then placed on a separate concave glass slide. The final compound concentrations ranged from 2.5 to 50 μg/mL containing 10% (v/v) ethanol. The negative control was 10% ethanol, and the positive control was carbendazim, with different concentrations ranging from 0.5 to 50 μg/mL.

Antioxidant activity assay

Both hydroxyl radical scavenging and β-carotene/linoleic acid bleaching assays were employed to determine antioxidant activity of the samples by a microplate spectrophotometric method according to our previous reports[13]. All tests were carried out in triplicate. Both AA and BHT were used as the positive controls.

Antinematodal activity assay

The nematode *Caenorhabditis elegans*, which was kindly supplied by Dr. Chonglin Yang of the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, was inoculated on the nematode growth medium (NGM) that was cultured previously with *Escherichia coli* OP_{50} according to our previous report[14]. Southern root-knot nematode *Meloidogyne incognita* was kindly supplied by Dr. Heng Jian of the Department of Plant Pathology, China Agricultural University. It was cultured *in vivo* in the roots of *Ipomoea aquatica* Forsk in the greenhouse. The egg masses were isolated and hatched to get second-stage juveniles (J2s) for the assay. The antinematodal activity of the compounds was tested according to our previous report[15]. 5% acetone-water solution was used as the negative control. Avermectin B1, which was kindly provided by Dr. Shankui Yuan at the Institute for the Control of Agrochemicals, Chinese Ministry of Agriculture, was used as the positive control with the purity of 97.2%.

Results and Discussion

Elucidation of the metabolites

Five compounds (**1-5**) were isolated from the EtOAc fraction or extract of *A. implicatum*. After comparing their physicochemical and spectrometric data with those reported in literatures, they were known compounds and confirmed as 4-hydroxy benzaldehyde (**1**)[6], 4-hydroxy benzoic acid (**2**)[7], indole-3-carboxaldehyde (**3**)[8], 5′-*O*-acetylthymidine (**4**)[9], and cerevisterol (**5**)[10], respectively. The chemical structures were shown in Fig. 1. These compounds were isolated from the endophytic fungus *A. implicatum* for the first time.

4-Hydroxy benzaldehyde (**1**) and 4-hydroxy benzoic acid (**2**), belonging to phenols, have been isolated from many plant and fungal species such as 4-hydroxy benzaldehyde (**1**) from fungus *Aspergillus nishimurae*[16] and plant *Litchi chinensis*[6] as well as 4-hydroxy benzoic acid (**2**) from fungus *Penicillium* sp. HS-5[17] and plant *Halostachys caspica*[7].

Indole-3-carboxaldehyde (**3**) has also been isolated from a variety of species such as the fungus *Aspergillus versicolor*[18], actinomycete *Jishengella endophytica*[19], and plant *Isodon excisoides*[8].

5′-*O*-Acetylthymidine (**4**) has been isolated from the Formosan soft coral *Cladiella australis*[20] and the actinomycete *Streptomyces* sp.[9].

Cerevisterol (**5**), a sterol, was also named as (3β,5α,6β,22E)-ergosta-7,22-diene-3,5,6-triol. It has been isolated from many fungal species such as *Fusarium* sp.[21] and *Penicillium sclerotiorum*[22]. It was also

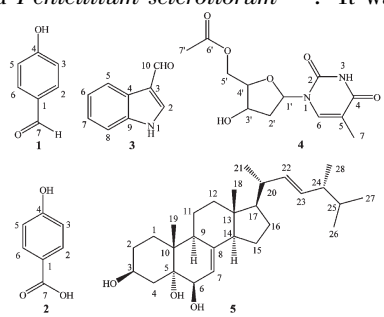

Fig. 1 **Chemical structures of the isolated compounds (1-5)**

isolated from the endophytic fungi *Aspergillus awamori* from *Acrostichum speciosum*[10], and *Chaetomium* sp. from *Panax ginseng*[23].

Biological activities of the metabolites

Both 4-hydroxy benzaldehyde (**1**) and 4-hydroxy benzoic acid (**2**) showed antibacterial activity on Gram-negative bacteria. The median inhibitory concentration (IC_{50}) values for 4-hydroxy benzaldehyde (**1**) on *Pseudomonas lachrymans* and *Xanthomonas vesicatoria* were 13.7 μg/mL and 14.1 μg/mL, respectively, and those for 4-hydroxy benzoic acid (**2**) on *P. lachrymans* and *X. vesicatoria* were 12.5 μg/mL and 13.4 μg/mL, respectively. Both 4-hydroxy benzaldehyde (**1**) and 4-hydroxy benzoic acid (**2**) also showed inhibitory activity on spore germination of *Magnaporthe oryzae* with the IC_{50} values of 17.3 μg/mL and 21.5 μg/mL, respectively.

Both 4-hydroxy benzaldehyde (**1**) and 4-hydroxy benzoic acid (**2**) showed strong hydroxyl radical scavenging activity with the IC_{50} values of 13.6 μg/mL and 19.7 μg/mL, respectively, and also showed moderate inhibitory activity on β-carotene/linoleic acid oxidation with IC_{50} values of 50.9 μg/mL and 62.1 μg/mL, respectively. Cerevisterol (**5**) showed weak inhibitory activity on β-carotene/linoleic acid oxidation with IC_{50} value of 81.7 μg/mL.

Among the compounds, 4-hydroxy benzaldehyde (**1**), 4-hydroxy benzoic acid (**2**) and indole-3-carboxaldehyde (**3**) exhibited antinematodal activity on *Caenorhabditis elegans* with IC_{50} values of 50.6 μg/mL, 60.1 μg/mL and 82.3 μg/mL, respectively, and on *Meloidogyne incognita* with IC_{50} values of 54.5 μg/mL, 64.5 μg/mL and 93.4 μg/mL, respectively. Both 5′-*O*-acetylthymidine (**4**) and cerevisterol (5) had no antinematodal activity at the maximum concentration of 2.0 mg/mL.

4-Hydroxy benzaldehyde (**1**) was previously reported to have moderate antioxidant activity by chelating capacity on ferrous ion[24]. 4-Hydroxy benzoic acid (**2**) was also reported to have antimicrobial and antioxidant activities[7]. Indole-3-carboxaldehyde (**3**) was previously reported to have nematicidal activity against *Caenorhabditis elegans* and *Meloidogyne incognita*[25]. Some indoles including indole, indole-3-carboxaldehyde (**3**), and indole-3-acetic acid (IAA) in their combination were sufficiently toxic to *Caenorhabditis elegans*[26]. Indole-3-carboxaldehyde (**3**) also showed its antivirus activity on the influenza A virus subtype H1N1[19]. The antimicrobial, antioxidant and antinematodal activities of the isolated compounds in the present study were basically accord with the previous reports[7,19,24-26]. It indicated that the phenols (**1** and **2**) and indole-3-carboxaldehyde (**3**) could be the main bioactive components in the endophytic fungus *A. implicatum*.

The crude extract of *A. implicatum* was reported to show strong antinematodal activity on *M. incognita*[4]. We only isolated a few compounds (**1-3**) with moderate antinematodal activity in this study, which indicated that other antinematodal compounds with very low content in the fungus have not been isolated. It is also possible that these compounds have their synergistically antinematodal activity that needs to be further studied. Some extracellular enzymes such as chitinase from *A. implicatum* have been reported to suppress *M. incognita*[5,27]. Therefore, the action mechanism of the endophytic fungus *A. implicatum* on nematode needs to be studied in detail.

Conclusion

In this study, five secondary metabolites (**1-5**) were firstly isolated from the endophytic fungus *A. implicatum*, and were identified as 4-hydroxy benzaldehyde (**1**), 4-hydroxy benzoic acid (**2**), indole-3-carboxaldehyde (**3**), 5′-*O*-acetylthymidine (**4**), and cerevisterol (**5**). Among them, both 4-hydroxy benzaldehyde (**1**), 4-hydroxy benzoic acid (**2**) showed strong antimicrobial, antioxidant and antinematodal activities. Indole-3-carboxaldehyde (**3**) only exhibited moderate antinematodal activity on *Caenorhabditis elegans* and *Meloidogyne incognita*. Cerevisterol (**5**) only showed weak inhibitory activity on β-carotene/linoleic acid oxidation. The results indicated that two phenols (**1** and **2**) along with indole-3-carboxaldehyde (**3**) could be the main bioactive components against *M. incognita* in the endophytic fungus *A. implicatum*. They could be used as the potential antimicrobial, antioxidant and antinematodal agents. The results could provide additional data

for studying the biocontrol mechanisms of *A. implicatum on the root-knot nematode, as well as for development and utilization of A. implicatum* as the biocontrol agent and bioactive component producer.

References

1 Porras-Alfaro A, Bayman P. Hidden fungi, emergent properties: endophytes and microbiomes. *Annu Rev Phytopathol*, 2011, 49: 291-315.

2 Gutierrez RMP, Gonzalez AMN, Ramirez AM. Compounds derived from endophytes: a review of phytochemistry and pharmacology. *Curr Med Chem*, 2012, 19: 2992-3030.

3 Gimenez C, Cabrera R, Reina M, *et al*. Fungal endophytes and their role in plant protection. *Curr Org Chem*, 2007, 11: 707-720.

4 Tian X, Yao Y, Chen G, *et al*. Suppression of *Meloidogyne incognita* by the endophytic fungus *Acremonium implicatum* from tomato root galls. *Int J Pest Manage*, 2014, 60: 239-245.

5 Lin S, Wu X, Cao J, *et al*. Biocontrol potential of chitinase-producing nematophagous fungus *Acremonium implicatum* against *Meloidogyne incognita*. *Acta Phytopathol Sin*, 2013, 43: 509-517.

6 Guo J, Chen J, Lin L, *et al*. Five chemical constituents of the ethyl acetate fraction from ethanol extract of semen litchi. *J Med Plants Res*, 2012, 6: 168-170.

7 Liu H, Wang K, Zhang J, *et al*. Secondary metabolites from *Halostachys caspica* and their antimicrobial and antioxidant activities. *Rec Nat Prod*, 2012, 6: 57-61.

8 Li H, Jiao K, Zhang P, *et al*. Chemical constituents from *Isodon excisoides*. *Chin Tradit Herb Drugs*, 2014, 45: 154-160.

9 Wang T, Jiang Y, Jin R, *et al*. Studies on secondary metabolites of *Streptomces* sp. isolated from *Elephas maximus* feces. *Nat Prod Res Dev* (天然产物研究与开发), 2014, 26: 509-512.

10 Gao H, Hong K, Zhang X, *et al*. New steryl esters of fatty acids from the mangrove fungus *Aspergillus awamori*. *Helv Chim Acta*, 2007, 90: 1165-1178.

11 Wang J, Liu H, Zhao J, *et al*. Antimicrobial and antioxidant activities of the root bark essential oil of *Periploca sepium* and its main component 2-hydroxy-4-methoxybenzaldehyde. *Molecules*, 2010, 15: 5807-5817.

12 Liu H, Wang J, Zhao J, *et al*. Isoquinoline alkaloids from *Macleaya cordata* active against plant microbial pathogens. *Nat Prod Commun*, 2009, 4: 1557-1560.

13 Wang J, Zhao J, Liu H, *et al*. Chemical analysis and biological activity of the essential oils of two valerianaceous species from China *Nardostachys chinensis* and *Valeriana officinalis*. *Molecules*, 2010, 15: 6411-6422.

14 Wang K, Luo C, Liu H, *et al*. Nematicidal activity of the alkaloids from *Macleaya cordata* against certain nematodes. *Afr J Agric Res*, 2012, 7: 5925-5929.

15 Luo C, Yu R, Liu H, *et al*. Phenolic compounds from *Caragana leucophloea* and their biological activities. *Nat Prod Res Dev* (天然产物研究与开发), 2015, 27: 386-392.

16 Hosoe T, Mori N, Kamano K, *et al*. A new antifungal yellow pigment from *Aspergillus nishimurae*. *J Antibiot*, 2011, 64: 211-212.

17 Ying YM, Shan W G, Liu WH, *et al*. Studies on the metabolites of a fungal endophyte *Penicillium* sp. HS-5 from *Huperzia serrata*. *Asian J Chem*, 2013, 25: 1208-1210.

18 Liu XH, Miao F P, Li XD, *et al*. A new sesquiterpene from an endophytic *Aspergillus versicolor* strain. *Nat Prod Commun*, 2012, 7: 819-820.

19 Wang P, Kong F, Wei J, *et al*. Alkaloids from the mangrove-derived actinomycete *Jishengella endophytica* 161111. *Mar Drugs*, 2014, 12: 477-490.

20 Ahmed AF, Wu MH, Wu YC, *et al*. Metabolites with cytotoxic activity from the Formosan soft coral *Cladiella australis*. *J Chin Chem Soc*, 2006, 53: 489-494.

21 Wang Y, Kuang Y, Yang S, *et al*. Chemical constituents produced by *Fusarium* sp. S13 isolated from *Pleioblastus amarus*. *Nat Prod Res Dev* (天然产物研究与开发), 2014, 26: 1597-1601.

22 Tao M, Li L, Chen Y, *et al*. Chemical constituents of marine fungus *Penicillium sclerotiorum* FS50. *Nat Prod Res Dev* (天然产物研究与开发), 2014, 26: 1593-1596.

23 Liang W, Wang D, Cheng H, *et al*. Chemical constituents from endophytic fungus *Chaetomium* sp. RSQMK-9 isolated from *Panax ginseng*. *Nat Prod Res Dev* (天然产物研究与开发), 2014, 26: 1202-1206.

24 Li Y, Han J, Yang X, *et al*. Bioactive composition of the solid culture of the edible mushroom *Pleurotus citrinopileatus* on rice and the antioxidant effect evaluation. *Mycosystema*, 2013, 32: 876-886.

25 Chen MJ, Wang X, Zhang KQ, *et al*. Chemical constituents from the bacterium *Wautersiella falsenii* YMF 3.00141. *Appl Biochem Biotechnol*, 2015, 51: 402-405.

26 Bommarius B, Anyanful A, Izrayelit Y, *et al*. A family of indoles regulate virulence and Shiga toxin production in pathogenic *E. coli*. *PLoS One*, 2013, 8: e54456.

27 Yang J, Tian B, Liang L, *et al*. Extracellular enzymes and the pathogenesis of nematophagous fungi. *Appl Microbiol Biotechnol*, 2007, 75: 21-31.