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Antibacterial and antioxidant metabolites from the insect-associated fungus *Aspergillus fumigatus*

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Abstract: The research on bioactive secondary metabolites from *Aspergillus fumigatus* afforded six compounds, which were identified by mass spectrometer (MS) and nuclear magnetic resonance (NMR) spectroscopic analysis as cyclopyazonic acid (**1**), trypacidin A (**2**), asterric acid (**3**), methyl asterrate (**4**), demethylcitreoviranol (**5**), as well as (5-hydroxy-2-oxo-2*H*-pyran-4-yl) methyl acetate (**6**). Cyclopyazonic acid (**1**) was found to have potent antibacterial effects, especially against *Bacillus licheniformis* with minimal inhibitory concentration (MIC) value of 3.7μg/mL. Its antibacterial effects were possibly related to the olefinic acid group in the structure. Phenyl ether derivatives **3** and **4**, and trypacidin A (**2**) also exhibited antimicrobial effects. In addition, compound **6** showed significant antioxidant effects with half maximal effective concentration (EC_{50}) value of 10.2μM in the ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) assay, which was better than the positive control.

Keywords: Insect-associated fungus, *Aspergillus fumigatus*, antibacterial activity, antioxidant.

INTRODUCTION

Drug resistant bacteria, spreading worldwide and increasing progressively, have imposed threat on human health and clinical treatment for a long time (Poirel *et al*, 2010). This kind of microbe can cause serious skin, bloodstream and respiratory infections. (Magiorakos *et al*, 2012). Recent researches indicate that fungal secondary metabolites could be one of the potential resources of antimicrobial drugs. Various types of fungal metabolites such as dimeric tetrahydroxanthones (Ola *et al*, 2014), alkaloids (Wagenaar *et al*, 2010), phenyl ethers (Liu *et al*, 2016), and terpenes (Ratnaweera *et al*, 2014) have significant inhibitions on the growth of human pathogenic bacteria.

Insect-associated fungi are one kind of special microorganisms that inhabit the host insects. They could induce immune reactions and provide nutrition and antibiotics for the host growth (Baumann, 2014). In our ongoing search for potential antimicrobial compounds (Li *et al*, 2015), the EtOAc extract from an insect-associated fungus *Aspergillus fumigatus*, showed obvious antibacterial activity against *Staphylococcus aureus* ATCC 25923 [MIC (minimal inhibitory concentration) = 195.0 μg/mL]. Further chemical study of this extract afforded the six compound (fig. 1), cyclopyazonic acid (**1**) (Yokota *et al*, 1981), trypacidin A (**2**) (Pinheiro *et al*, 2013), asterric acid (**3**) (Hargreaves *et al*, 2002), methyl asterrate (**4**) (Hargreaves *et al*, 2002), demethylcitreoviranol (**5**) (Shizuri *et al*, 1988), as well as (5-hydroxy-2-oxo-2*H*-pyran-4-yl) methyl acetate (**6**) (Lin *et al*, 2008). In the antibacterial assays, compound **1**-**4** exhibited potent effects against four strains of G^+ and two strains of G^- bacteria. Cyclopyazonic acid (**1**) was found to have powerful antibacterial effects, especially against *Bacillus licheniformis* with MIC value of 3.7μg/mL. In addition, compound **6** showed significant antioxidant effects with half maximal effective concentration (EC_{50}) value of 10.2 μM in the ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) assay, which was even better than the positive control. The isolation, structure elucidation, and bioactive studies of these compounds are described here.

MATERIALS AND METHODS

General experimental procedures

Using tetramethyl silane (TMS) as the internal standard, nuclear magnetic resonance (NMR) measurements were performed on the Bruker AVIII-500 NMR instrument (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR). An Agilent 6520B quadrupole-time-of-flight (Q-TOF) mass equipment (Agilent Technologies, USA) was applied for the electrospray ionization mass spectrometer (ESIMS) spectrums. Semi-preparative high performance liquid chromatography (HPLC) was achieved on a Waters 1525 HPLC instrument (detector, Waters 2998, USA; column, Zorbax XDB-C_{18}, 250×10mm, 5μm, Agilent Technologies, USA). Column chromatographies (CCs) were used for the compound isolation and the packing materials were chosen as microporous resin (MCI) (Mitsubishi, Japan), Sephadex LH-20 (Pharmacia, Sweden), and silica gel (Qingdao Marine Chemical Co. Ltd., China). The OD (optical density) values were recorded on a microplate reader (Tecan, Switzerland).

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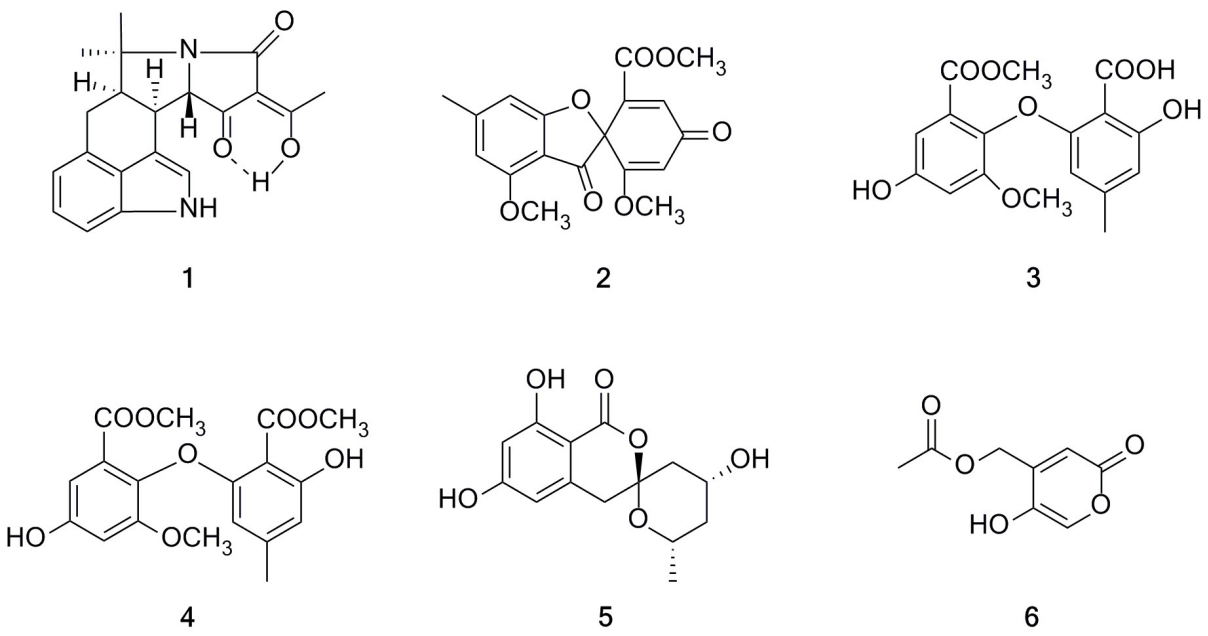

Fig. 1: structures of compounds **1**-**6**. **1**, cyclopyazonic acid; **2**, trypacidin A; **3**, asterric acid; **4**, methyl asterrate; **5**, demethylcitreoviranol; **6**, (5-hydroxy-2-oxo-2*H*-pyran-4-yl) methyl acetate.

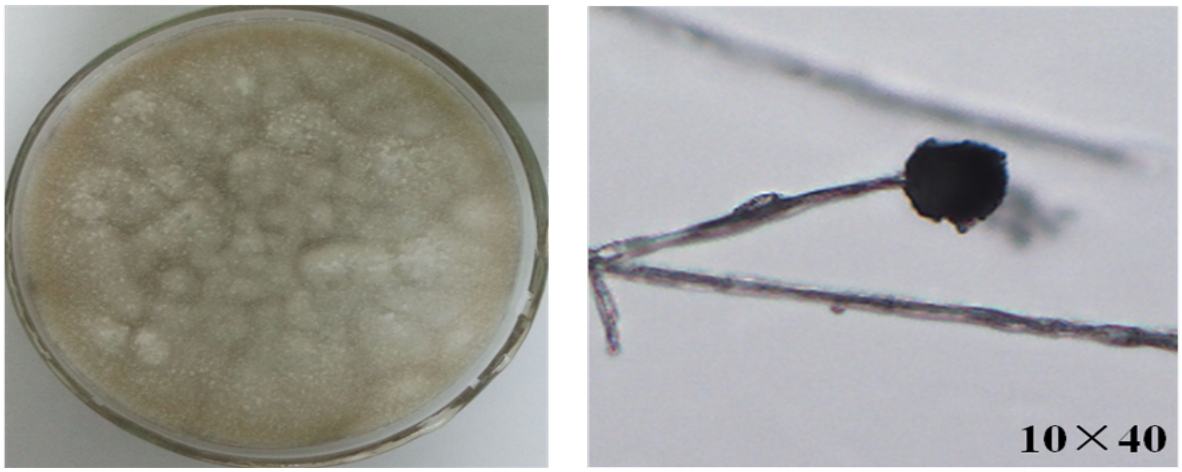

Fig. 2: morphological and microscopic characteristics of *A. fumigatus*.

Table 1: The MIC values of compounds **1**-**6** (μg/mL)[a]

Compounds	Sa	Bs	Bl	Ba	Ec	Pa
1	5.5 ± 0.9	8.7 ± 2.0	3.7 ± 1.3	7.6 ± 1.7	13.8 ± 1.4	18.5 ± 2.1
2	64.7 ± 2.4	35.2 ± 3.6	38.4 ± 3.1	45.5 ± 4.0	25.2 ± 2.5	46.5 ± 4.0
3	23.6 ± 4.2	55.0 ± 7.2	18.9 ± 2.1	27.6 ± 4.2	> 100.0	> 100.0
4	68.8 ± 6.5	> 100.0	56.5 ± 3.6	> 100.0	> 100.0	> 100.0
5	> 100.0	> 100.0	> 100.0	> 100.0	> 100.0	> 100.0
6	> 100.0	> 100.0	> 100.0	> 100.0	> 100.0	> 100.0
Penicillin[b]	0.8 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1		
Streptomycin[b]					0.7 ± 0.2	1.0 ± 0.2

[a]Data are expressed as mean of three measurements of triplicate tests ± SD, compounds were inactive when MICs > 100.0 μg/mL, Sa (*S. aureus* ATCC 25923), Bs (*B. subtilis* ATCC 6633), Bl (*B. licheniformis*), Ba (*B. altitudinis*), Ec (*E.coli* ATCC 25922), Pa (*P. aeruginosa* ATCC 27853). [b] Positive control.

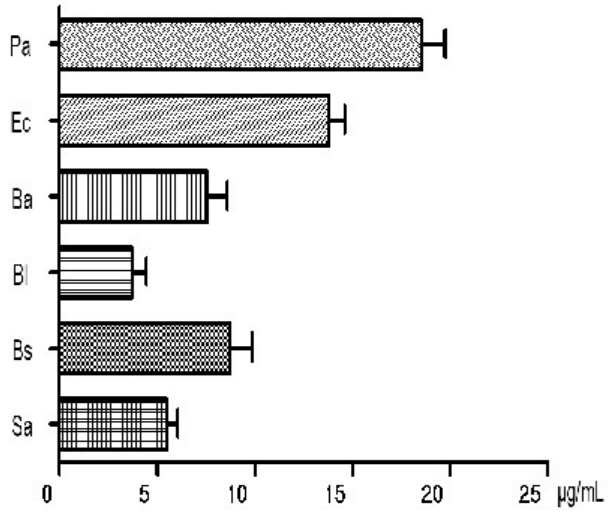

Fig. 3: antibacterial effects of cyclopyazonic acid (**1**). Data are expressed as mean of three measurements of triplicate tests ± SD. Sa (*S. aureus* ATCC 25923), Bs (*B. subtilis* ATCC 6633), Bl (*B. licheniformis*), Ba (*B. altitudinis*), Ec (*E.coli* ATCC 25922), Pa (*P. aeruginosa* ATCC 27853).

Table 2: EC_{50} values of compounds **1**-**6** (μM)[a]

Compounds	ABTS	DPPH
1	> 200.0	> 200.0
2	> 200.0	> 200.0
3	145.5 ± 5.9	128.7 ± 6.1
4	164.7 ± 7.4	105.2 ± 3.6
5	23.2 ± 1.8	45.0 ± 2.4
6	10.2 ± 1.0	41.1 ± 2.9
Trolox[b]	18.5 ± 0.5	25.9 ± 0.7

[a]Data are expressed as mean of three measurements of triplicate tests ± SD, compounds were inactive when EC_{50} values > 200.0 μM. [b]Positive control.

Fungal material

The living insects of *Coridius chinensis* were obtained from the western area of Zhengzhou, Henan, China at the end of May 2017, which were immediately disinfected by 75% ethanol and 1% NaClO. Its gut was ground and the tissue fluid was spread on potato dextrose agar (PDA). Afterwards, the title strain was isolated. By the detailed comparison of the morphological and microscopic characteristics with those in the literature (fig. 2), the fungus was identified as *A. fumigatus* (Chen *et al*, 2013). The fungal identification was unambiguously confirmed by the 18S rDNA sequences and ITS (internal transcribed spacer) analysis (100% identical to the *A. fumigatus* isolate No. JN246062.1).

At 28°C for a week, *A. fumigatus* was activated on the PDA. The seed cultures were prepared in four 500mL Erlenmeyer flasks, which were inoculated with 8 pieces of the fungal agar and cultivated at the conditation of 28°C, 120 rpm for a week. For the solid fermentation, 20 Erlenmeyer flasks (500mL) were prepared and 80g of sterilized rice and 125mL of sterile water were added to each flask. Then 20mL of seed culture was transferred into each flask, which was fermented at room temperature for around a month.

Extraction and isolation

The rice cultures were extracted with EtOAc three times. The organic solvent was combined and evaporated under reduced pressure to afford a crude extract (18.2g). It was interesting to note that the crude extract could significantly inhibit the growth of *S. aureus* ATCC 25923 (MIC = 195.0μg/mL). Through a gradient elution of petroleum ether-EtOAc from 20:1 to 1:2, silica gel CC was performed, giving fractions A-G. The combined bioactive parts C-F (2.5g) were purified by MCI CC to obtain 8 subfractions M1-M8 (MeOH-H_2O, 10% to 100%). After being purified by Sephadex LH-20 CC (CH_2Cl_2−MeOH, 1:1), subfraction M7 was further fractionated on semi-preparative HPLC using MeOH−H_2O (75:25, 0.1% HCOOH), giving compounds **2** (28.5mg, t_R 25.8 min), **3** (9.3mg, t_R 30.7min), and **4** (8.1mg, t_R 33.6 min). Compound **1** (30.8mg, t_R 17.2min) were isolated from M6 by semi-preparative HPLC (65:35, 0.1% HCOOH). And compounds **6** (19.2mg, t_R 31.4min, 30% MeOH + 0.1% HCOOH) and **5** (2.5mg, t_R 33.1min, 30% MeOH + 0.1% HCOOH) were obtained from M4.

Antimicrobial assay

According to the broth microdilution method (Li *et al*, 2015), the bacterial inhibition effects of the compounds were tested in 96-well plates using four strains of G^+ (*B. subtilis* ATCC 6633, *B. licheniformis*, *B. altitudinis*, *S. aureus* ATCC 25923) and two strains of G^- bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853). The compounds were firstly prepared in DMSO and were subsequently diluted with MH (Mueller-Hinton) liquid broth using 2-fold method in the range of 1-100μg/mL. Penicillin G and streptomycin were chosen as the positive control for G^+ and G^- bacteria and were diluted with MH broth in the range of 0.1-10μg/mL. Bacterial suspension solutions were prepared at the concentration of $1.0*10^4$-$1.0*10^6$ CFU/mL. Then 100μL of compound solutions together with equal volume of bacterial suspensions were transferred to the test wells. Blank control wells (200μL of MH broth) and growth control wells (100μL of MH broth and 100μL of bacterial suspensions) were also prepared. After being incubated at 37 °C for one day, the 96-well plates were measured for the OD values at 530 nm. The inhibition ratio of each test well was calculated as inhibition (100%)=$[(OD_{growth}-OD_{blank})-(OD_{test}-OD_{blank})]/(OD_{growth}-D_{blank})\times100\%$. The compound concentrations that could inhibit 50% growth of the bacteria were regarded as the MIC values.

Antioxidant assay

The ABTS and DPPH (2,2-diphenyl-1-picrylhydrazyl)

radical scavenging assays (Li *et al*, 2016) were applied for the antioxidant determination of **1**-**6** (trolox as the positive control). For the ABTS assay, the ABTS and oxidant solutions were mixed equally, which were reacted at 28°C in the dark place for around 16h. The final absorbance 0.7 ± 0.05 (734 nm) of the mixture was required. Compound test solutions (10mM) were prepared, which were diluted with 80% ethanol to 0.005-1.00mM. Subsequently in the 96-well microtiter plates, 200μL of dilute ABTS solution were added. Then 20μL of test solutions were transferred and the plates were kept avoid light for 6 min before the absorbance measurement at 734 nm. For DPPH assays, 0.15mM DPPH solutions were prepared at first. Then 100μL of DPPH solutions and equal volume sample solutions were pipetted and reacted avoid light for half an hour. The measurement wavelength was chosen at 517 nm. The test concentrations that yielded 50% decrease of the absorbance were denoted as EC_{50} values.

STATISTICAL ANALYSIS

For the antimicrobial and antioxidant assays, all the tests were performed in triplicate and ± SD values were calculated based on the three individual results. The data were analyzed by one-way analysis of variance (ANOVA) using GraphPad Prism 5 (GraphPad Software, USA) with $p < 0.05$ defined as statistical significance.

RESULTS

Fungal identification

The fungus *A. fumigatus* grew on PDA as a white cottony colony and orange color on the reverse side (fig. 1). Conidiophore stipes could be observed with a lot of egg-like spores on the top of the stipes. The fungal strain was unambiguously identified as *A. fumigatus* by the 18S rDNA sequences and ITS analysis (100% identical to the *A. fumigatus* isolate No. JN246062.1).

Structure elucidation

Cyclopyazonic acid (**1**): white solid; ESIMS *m/z* 337 $[M+H]^+$, $C_{20}H_{20}N_2O_3$; ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 8.16 (1H, br s, H-1-NH), 7.11-7.21 (3H, m, H-2, H-15, H-16), 6.91 (1H, d, *J*=6.9 Hz, H-14), 4.06 (1H, d, *J* = 11.0 Hz, H-5), 3.66 (1H, dd, *J*=11.0, 5.8 Hz, H-4), 3.05 (2H, m, H-12), 2.63 (1H, dt, *J*=6.9 Hz, H-11), 2.45 (3H, s, H-20), 1.68 (3H, s, H-21), 1.63 (3H, s, H-22), 1.00 (3H, s, H-22); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 195.1 (C-6), 184.9 (C-19), 175.3 (C-8), 133.5 (C-18), 128.7 (C-13), 125.9 (C-17), 123.1 (C-15), 120.8 (C-2), 116.5 (C-14), 110.1 (C-3), 108.7 (C-16), 105.6 (C-7), 71.9 (C-5), 63.4 (C-10), 53.1 (C-11), 36.1 (C-4), 26.6 (C-12), 26.4 (C-21), 24.4 (C-22), 19.7 (C-20).

Trypacidin A (**2**): white needle-like crystal; ESIMS *m/z* 345 $[M+H]^+$, $C_{18}H_{16}O_7$; ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.10 (1H, br s, H-2′), 6.55 (1H, br s, H-7), 6.37 (1H, s, H-5), 5.77 (1H, s, H-4′), 3.95 (3H, s, OCH_3-9), 3.69 (3H, s, OCH_3-8′), 3.66 (3H, s, OCH_3-6′), 2.44 (3H, s, CH_3-8); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 190.4 (C-3), 185.6 (C-3′), 174.4 (C-7a), 169.5 (C-5′), 163.5 (C-7′), 158.4 (C-4), 152.0 (C-6), 138.3 (C-1′), 137.1 (C-2′), 108.4 (C-3a), 105.6 (C-7), 105.4 (C-5), 104.0 (C-4′), 84.0 (C-2), 56.7 (C-6′), 56.1 (C-9), 52.7 (C-8′), 23.1 (C-8).

Asterric acid (**3**): yellow powder; ESI-MS *m/z* 349 $[M+H]^+$, $C_{17}H_{16}O_8$; ^{1}H-NMR (CD_3OD, 500 MHz) δ_H 6.92 (1H, s, H-3), 6.80 (1H, s, H-5), 6.41 (1H, br s, H-6′), 5.76 (1H, br s, H-4′), 3.71 (3H, s, OCH_3-7), 3.76 (3H, s, OCH_3-9), 2.13 (3H, s, CH_3-7′); ^{13}C-NMR (CD_3OD, 125 MHz) δ_C 167.2 (C-8′), 164.3 (C-8), 160.9 (C-1′), 157.1 (C-3′), 155.1 (C-4), 146.3 (C-6), 135.8 (C-5′), 126.5 (C-1), 112.2 (C-2), 109.3 (C-6′), 106.6 (C-3), 106.3 (C-5), 106.1 (C-2′), 103.4 (C-4′), 56.7 (C-7), 52.8 (C-9), 22.0 (C-7′).

Methyl asterrate (**4**): white powder; ESI-MS *m/z* 363 $[M+H]^+$, $C_{18}H_{18}O_8$; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 11.55 (1H, s, 3′-OH), 6.97 (1H, br s, H-3), 6.71 (1H, br s, H-5), 6.42 (1H, s, H-6′), 5.75 (1H, s, H-4′), 3.94 (3H, s, OCH_3-7), 3.75 (3H, s, OCH_3-9), 3.69 (3H, s, OCH_3-9′), 2.13 (3H, s, CH_3-7′); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ_C 171.8 (C-8′), 165.9 (C-8), 163.2 (C-1′), 160.0 (C-3′), 154.1 (C-4), 153.3 (C-6), 146.2 (C-5′), 136.9 (C-1), 126.0 (C-2), 111.2 (C-6′), 108.6 (C-3), 106.0 (C-5), 105.1 (C-4′), 101.0 (C-2′), 56.6 (C-7), 52.5 (C-9), 52.4 (C-9′), 22.1 (C-7′).

Demethylcitreoviranol (**5**): white powder; ESIMS *m/z* 281 $[M+H]^+$, $C_{14}H_{16}O_6$; ^{1}H NMR (CD_3OD, 500 MHz) δ_H 6.20 (1H, br s, H-5), 6.18 (1H, br s, H-7), 4.19 (1H, m, H-9), 4.01 (1H, m, H-11), 3.19 (1H, d, *J* = 16.0 Hz, H-4a), 2.96 (1H, d, *J* = 16.0 Hz, H-4b), 2.35 (1H, m, H-10a), 2.02 (1H, m, H-10b), 1.49 (1H, dd, *J* = 12.8, 12.5 Hz, H-12a), 1.20 (1H, dd, *J* = 18.8, 11.7 Hz, H-12b), 1.11 (3H, d, *J* = 6.3 Hz, CH_3-13); ^{13}C-NMR (CD_3OD, 125 MHz) δ_C 170.2 (C-1), 166.7 (C-8), 165.7 (C-6), 141.7 (C-4a), 108.9 (C-5), 106.4 (C-3), 102.2 (C-7), 101.1 (C-8a), 69.0 (C-9), 64.3 (C-11), 43.7 (C-10), 42.9 (C-12), 39.0 (C-4), 21.9 (C-13).

(5-Hydroxy-2-oxo-2*H*-pyran-4-yl) methyl acetate (**6**): white powder; ESI-MS *m/z* 185 $[M+H]^+$, $C_8H_8O_5$; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 7.83 (1H, s, H-6), 6.50 (1H, s, H-3), 4.41 (2H, s, H-7), 2.14 (3H, s, H-10); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ_C 174.1 (C-3), 169.8 (C-9), 162.7 (C-5), 145.9 (C-4), 138.4 (C-6), 111.3 (C-3), 61.3 (C-7), 20.5 (C-10).

Antibacterial activities

As shown in table 1, compound **1** was the most active metabolite against four strains of G^+ and two strains of G^- bacteria (fig. 3), possessing the best effect against *B. licheniformis* (MIC = 3.7μg/mL). Trypacidin A (**2**) had moderate activities (MIC = 25.2 to 64.7μg/mL). In comparison with its derivatives **4**, asterric acid (**3**) showed better effects with MIC values from 18.9 to 55.0μg/mL.

However, no inhibition effects have been found for **5** and **6** on the tested bacteria.

Antioxidant activities

Pyran derivative **6** was revealed as the best antioxidant with EC_{50} value of 10.2μM (table 2) in the ABTS assay, which was better that the positive control trolox. Compound **5** also exhibited potent effects with EC_{50} values of 23.2 and 45.0μM in the ABTS and DPPH assays, while **3** and **4** showed moderate activities.

DISCUSSION

Insect-associated fungi are a kind of special and valuable microbial resources, and they could induce immune reactions and provide nutrition and antibiotics for the host growth (Baumann, 2014; Hu *et al*, 2015; Li *et al*, 2015). The secondary metabolites **1**-**6** were purified by silica gel CC, MCI CC, and semi-preparative HPLC. Their structures were determined by careful comparison of the ^{1}H NMR, ^{13}C NMR and MS data with those in the literatures (Hargreaves *et al*, 2002; Lin *et al*, 2008; Pinheiro *et al*, 2013; Shizuri *et al*, 1988; Yokota *et al*, 1981).

Cyclopyazonic acid (**1**), a toxin to silkworm, was first reported as the secondary metabolite of *A. flavus* and *A. oryzae*. Interestingly, its structure was revealed as a kind of alkaloid with an olefinic acid group, which afforded acid and alkaline abilities simultaneously. In this study, the antimicrobial effects of cyclopyazonic acid (**1**) were determined against four strains of G^+ and two strains of G^- bacteria, giving more inhibitions on the G^+ bacteria *B. subtilis*, *B. licheniformis*, *B. altitudinis*, *S. aureus* (fig. 3). The result was in accordance with those in the literature against *Staphylococcus albus* (MIC = 10.0 μg/mL) and *E. coli* (inhibition zone of 20mm) (Reddy and Reddy, 1995; Asiri *et al*, 2015). A lot of tetrahydroxanthone and anthraquinone compounds such as tetracycline (Chopra and Roberts, 2001), neosartorin (Ola *et al*, 2014), and lentulins A-H (Li *et al*, 2016) showed significant antibacterial activities, and all of them possessed olefinic acid group in their structures when considering the structure-function relationships. Thus, the antibacterial effects of cyclopyazonic acid might be associated with the olefinic acid group.

Compounds **3** and **4** were revealed as phenyl ether derivatives, which were known as a class of potent antibiotics (Hu *et al*, 2015; Chen *et al*, 2013). The stronger antibacterial activities were found for some brominated diphenyl ethers (Liu *et al*, 2016). It has been reported that asterric acid (**3**) could inhibit the growth of *B. subtilis* (Zhang *et al*, 2009). In this research, compound **3** showed moderate effects against four strains of G^+ bacteria but had no inhibition on the growth of *E. coli* and *P. aeruginosa* (table 1). In comparison with the activities of **3**, its esterification derivative **4** exhibited weak effects. These results suggested the carboxyl group could enhance their antibacterial activities, which were also in accordance with those in the literatures (Hu *et al*, 2015; Liu *et al*, 2016).

Compounds **2**, **5**, and **6** were revealed as furanone and pyranone compounds and they also possessed phenolic groups in the structures. A lot of phenolic compounds such as kojic acid and flavonoids are reported to exhibit significant radical scavenging effects, which are associated with the phenolic hydroxy groups (Hu *et al*, 2015; Li *et al*, 2016). In this study, compounds **3**-**6** were found to have antioxidant activities and the small molecule compound **6** exhibited most potent effects. It seems that small molecule compound could react with the free radicals more fast and efficiently. In addition, due to the lack of phenolic hydroxy groups, compound **1** and **2** were inactive in the ABTS and DPPH radical scavenging assays.

CONCLUSION

Six bioactive secondary metabolites were isolated from *A. fumigatus*. Cyclopyazonic acid (**1**) was found to have potent antibacterial effects, especially against *B. licheniformis* with MIC value of 3.7μg/mL. Its antibacterial effects were possibly related to the olefinic acid group in the structure. Phenyl ether derivatives (**3** and **4**) exhibited moderate antibacterial activities and phenolic compound **6** showed significant antioxidant effects that was better than the positive control.

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REFERENCES

Asiri IAM, Badr JM and Youssef DTA (2015). Penicillivinacine, antimigratory diketopiperazine alkaloid from the marine-derived fungus *Penicillium vinaceum*. *Phytochem. Lett.*, **13**(1): 53-58.

Baumann P (2005). Biology bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annu. Rev. Microbiol.*, **59**(1): 155-189.

Chen M, Shao CL, Fu XM, Xu RF, Zheng JJ, Zhao DL, She ZG and Wang CY (2013). Bioactive indole alkaloids and phenyl ether derivatives from a marine-derived *Aspergillus* sp. Fungus. *J. Nat. Prod.*, **76**(4): 547-553.

Chopra I and Roberts M (2001). Tetracycline antibiotics:

mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol. Mol. Biol. Rev.*, **65**(2): 232-260.

Hargreaves J, Park JO, Ghisalberti EL, Sivasithamparam K, Skelton BW and White AH (2002). New chlorinated diphenyl ethers from an *Aspergillus* species. *J. Nat. Prod.*, **65**(1): 7-10.

Hu SS, Jiang N, Wang XL, Chen CJ, Fan JY, Wurin G, Ge HM, Tan RX and Jiao RH (2015). Prenylated diphenyl ethers from the mantis-associated fungus *Aspergillus versicolor* GH-2. *Tetrahedron Lett.*, **56**(25): 3894-3897.

Li TX, Yang MH, Wang XB, Wang Y and Kong LY (2015). Synergistic antifungal meroterpenes and dioxolanone derivatives from the endophytic fungus *Guignardia* sp. *J. Nat. Prod.*, **78**(11): 2511-2520.

Li TX, Wang XB, Luo J, Yang MH and Kong LY (2016). Antioxidant sordariol dimers from *Sordaria macrospora* and the absolute configuration determinations of their two simultaneous linear 1,2-diols. *Tetrahedron Lett*., **57**(25): 2754-2757.

Li TX, Yang MH, Wang Y, Wang XB, Luo J, Luo JG and Kong LY (2016). Unusual dimeric tetrahydroxanthone derivatives from *Aspergillus lentulus* and the determination of their axial chiralities. *Sci. Rep.*, **6**(1): 38958-38968.

Lin A, Lu X, Fang Y, Zhu T, Gu Q and Zhu W (2008). Two new 5-hydroxy-2-pyrone derivatives isolated from a marine-derived fungus *Aspergillus flavus*. *J. Antibiot.*, **61**(4): 245-249.

Liu H, Lohith K, Rosario M, Pulliam TH, O'Connor RD, Bell LJ and Bewley CA (2016). Polybrominated diphenyl ethers: structure determination and trends in antibacterial activity. *J. Nat. Prod.*, **79**(7): 1872-1786.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G and Olsson-Liljequist B (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect*, **18**(3): 268-281.

Ola ARB, Debbab A, Aly AH, Mandi A, Zerfass I, Hamacher A, Kassack MU, Brotz-Oesterhelt H, Kurtan T and Proksch P (2014). Absolute configuration and antibiotic activity of neosartorin from the endophytic fungus *Aspergillus fumigatiaffinis*. *Tetrahedron Lett*., **55**(5): 1020-1023.

Pinheiro EA, Carvalho JM, Santos DC, Feitosa AO, Marinho PS, Guilhon GM, Santos LS, Souza AL and Marinho AM (2013). Chemical constituents of *Aspergillus* sp EJC08 isolated as endophyte from *Bauhinia guianensis* and their antimicrobial activity. *An. Acad. Bras. Cienc*., **85**(4): 1247-1252.

Poirel L, Lagrutta E, Taylor P, Pham J and Nordmann P (2010). Emergence of metallo-*β*-lactamase NDM-1-producing multidrug-resistant *Escherichia coli* in Australia. *Antimicrob. Agents Chemother.*, **54**(11): 4914-4916.

Ratnaweera PB, Williams DE, Silva EDD, Wijesundera RLC, Dalisay DS and Andersen RJ (2014). Helvolic acid, an antibacterial nortriterpenoid from a fungal endophyte, *Xylaria* sp. of orchid *Anoectochilus setaceus* endemic to Sri Lanka. *Mycology*, **5**(1): 23-28.

Reddy VK and Reddy S M (1995). Bioassay of cyclopiazonic acid by *Staphylococcus albus*. *Natl. Acad. Sci. Lett.*, **18**(1): 59-60.

Shizuri Y, Shigemori H, Sato R, Yamamura S, Kawai K and Furukawa H (1988). Four new metabolites produced by *Penicillium citreo-viride* B. on addition of NaBr. *Chem. Lett.*, (8):1419-1422.

Wagenaar MM, Gibson DM and Clardy J (2010). Akanthomycin, a new antibiotic pyridone from the entomopathogenic fungus *Akanthomyces gracilis*. *Org. Lett.*, **33**(5): 671-673.

Yokota T, Sakurai A, Iriuchijima S and Takahashi N (1981). Isolation and ^{13}C NMR study of cyclopyazonic acid, a toxic alkaloid produced by muscardine fungi *Aspergillus flavus* and *A. oryzae*. *Agric. Biol. Chem.*, **45**(1): 53-56.

Zhang JW, Yang CP, Ji ZQ, Wei SP and Wu WJ (2009). Isolation and identification of the antibacterial composition from the fermentation broth of endophytes in *Celastrus angulatus* (Strain Hd3). *Chin. J. Pest. Sci.*, **11**(2): 225-229.