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SECONDARY ANTI-FUNGI METABOLITES FROM THE ENDOPHYTIC FUNGUS *Fusarium* sp. IN *Eucommia ulmoides*

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Endophytic fungi that inhabit normal tissues of the host plants without causing apparent pathogenic symptoms have been demonstrated to be rich sources of bioactive secondary metabolites [1, 2]. Endophytic fungi show high potential as sources of novel antiviral, anticancer, antioxidant, and insecticidal compounds [3]. In an ongoing effort to screen biologically active secondary metabolites, we have isolated four compounds from an endophytic fungus *Fusarium* sp. among the medicinal plants (*Eucommia ulmoides*). In this paper, we report the isolation, structure elucidation, and antimicrobial activity *in vitro* of these compounds.

The endophytic fungus *Fusarium* sp. was grown on potato dextrose agar (PDA) plate at 28°C for 5 days. The inoculum was prepared by introducing the periphery of the PDA plate of the endophytic fungus into 500 mL Erlenmeyer flasks containing 200 mL of liquid Czapek medium, followed by shaking (130 rpm) continuously for 5 days at 28°C. The follow-up fermentation was accomplished by adding the inoculum (30 mL) into 1000 mL Erlenmeyer flasks containing 400 mL of the same medium, and then shaking for 15 days in the same condition. The culture (40 L) was filtered to remove mycelia and concentrated to 3.5 L below 60°C, then extracted five times with ethyl acetate.

The ethyl acetate extract (13.9 g) was subjected to chromatography on silica gel eluting successively with chloroform–methanol gradient (1:0, 100:1, 50:1, 20:1, 10:1, 5:1, 2:1, 1:1, 0:1) to give nine fractions (I–IX). Repeated chromatography of fraction II on silica gel with a gradient of chloroform in petroleum ether and Sephadex LH-20 with chloroform–methanol (1:1) afforded two diketopiperazines (DKPs), compound **1** (fumitremorgin B, 55 mg) and compound **2** (fumitremorgin C, 22 mg). Fraction I was repeatedly subjected to column chromatography on silica gel with a gradient of chloroform in petroleum ether and Sephadex LH-20 with chloroform–methanol (1:1) to afford two steroids, compound **3** (ergosterol, 42 mg) and compound **4** (cerevisterol, 18 mg). The structures of these compounds were confirmed using a combination of spectral analyses and by comparison with reported spectral data in the literature. Moreover, compounds **1** and **2** were isolated for the first time from *Fusarium* sp., and were isolated for the first time from the endophytic fungus of *Eucommia ulmoides*.

Fumitremorgin B (1). Colorless needle crystalline sold, mp 210–212°C. HR-ESI-MS *m*/*z* 502.2302 $[M + Na]^+$, calcd for $C_{27}H_{33}N_3O_5Na$ 502.2318. ^1H NMR and ^{13}C NMR [4, 5].

Fumitremorgin C (2). Colorless needle crystalline solid, mp 126–127°C. HR-ESI-MS *m*/*z* 402.1791 $[M + Na]^+$, calcd for $C_{22}H_{25}N_3O_3Na$ 402.1794. ^1H NMR and ^{13}C NMR [6].

Ergosterol (3). Colorless needle crystalline solid, mp 155–157°C. ^1H NMR and ^{13}C NMR [7].

Cerevisterol (4). Colorless needle crystalline solid, mp 240–242°C. ^1H NMR and ^{13}C NMR [8].

The minimal inhibition concentration (MIC) of compounds **1–3** against five kinds of bacteria and ten kinds of fungi were determined. It was found that compounds **1** and **2** had good antimicrobial activity. Compound **3** could inhibited *Staphylococcus aureus*, *Streptococcus lactis*, and *Bacillus subtilis*. Moreover, compound **1** showed stronger bioactivity against *Streptococcus lactis* with an MIC value of 3.13 μg/mL; by comparison, the MIC value of streptomycin sulfate was 6.25 μg/mL (Table 1).

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TABLE 1. Results of Anti-Fungus Test of Compounds **1–3**

Test Microbes	MIC, μg/mL			
	1	**2**	**3**	Standard
				Streptomycin sulfate
Streptococcus lactis	3.13	12.5	12.5	6.25
Staphylococcus aureus	12.5	12.5	50	3.13
Bacillus subtilis	100	25	100	6.25
Escherichia coli	–	–	–	3.13
Pseudomonas aeruginosa	–	–	–	3.13
				Ketoconazole
Alternaria alternata	50	25	–	6.25
Phytophthora capsici	12.5	12.5	–	6.25
Colletotricum gloeosporioides	–	–	–	50
Fusarium graminearum	6.25	6.25	–	3.13
F. oxysporum f. sp. *niveum*	12.5	25	–	6.25
F. oxysporumf. sp. *fragariae*	12.5	25	–	12.5
Alternaria brassicae	12.5	12.5	–	12.5
Valsa mali	12.5	12.5	–	12.5
Botrytis cinerea	–	–	–	50
Penicillium glaucum	–	–	–	50
Candida albicans	50	25	–	3.13

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