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BRIEF COMMUNICATION

Chromones with lipoprotein oxidation inhibitory activity from an endophytic fungus *Alternaria brassicae* JS959 derived from *Vitex rotundifolia*

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Abstract

Chemical investigation of the ethyl acetate extract of an endophytic fungus, *Alternaria brassicae* JS959 derived from a halophyte, *Vitex rotundifolia*, led to the isolation of a new chromone, (2′*S*)-2-(2-acetoxypropyl)-7-hydroxy-5-methylchromone (**1**), along with sixteen known compounds: a chromone (**2**), twelve benzopyranones (**3**−**14**) and three perylenequinones (**15**−**17**). The chemical structures of the isolated compounds were identified by extensive spectroscopic data analysis including 1D, 2D NMR, HRESIMS, and optical rotation. Of these compounds, **1** and **2** showed inhibitory activity on Cu^{2+}–induced low density lipoprotein (LDL) and high density lipoprotein (HDL) oxidation in human blood plasma. The results suggest that metabolites of endophytic microbes could provide the basis for developing treatments for heart disease.

Endophytes are microorganisms that live inside plant tissues for all or part of their lifespan, and have a symbiotic relationship with the host plant [1]. It has been reported that the secondary metabolites produced by endophytes possess rich chemical diversity and exert various biological functions such as antibiotic, anticancer, antibacterial, antiviral, plant growth, and regulatory activities [2]. Therefore, the substances derived from endophytes have been recognized as new sources of novel bioactive natural product leads for drug discovery, agriculture, and industry [1].

In our ongoing effort to research the novel bioactive secondary metabolites from endophytic fungi, the fungal strain *Alternaria brassicae* (JS959) was obtained from a halophytic plant *Vitex rotundifolia* (Verbenaceae). *A. brassicae* has been found to produce a variety of secondary metabolites, including phytotoxic cyclic peptides (destruxin B, homodestruxin B, and desmethyldestruxin B) [3], a phytotoxic dibenzo-α-pyranone (alternariol monomethyl ether) [4], and a sesquiterpene (abscisic acid) with plant growth regulatory properties [5]. In this study, the chemical investigation of the EtOAc extract of *A.brassicae* (JS959) endophytic fungus derived from *V. rotundifolia* yielded seventeen compounds (**1–17**) including a new chromone derivative (**1**) (Fig. 1).

Human plasma low density lipoprotein (LDL) and high density lipoprotein (HDL) are known as primary blood carriers for cholesterol transportation. The modification of those lipoproteins by oxidation is associated with the initiation and progression of atherosclerosis, hypertension, and coronary heart disease [6]. Therefore, finding LDL and/or HDL oxidation inhibitors could be a promising strategy for the prevention of heart disease mentioned above. Moreover, several compounds originating from natural sources such as guggulsterone and 1,8-cineole have been reported to inhibit lipoprotein oxidation [7, 8]. Herein, we evaluated the LDL and HDL inhibitory effects of isolated chromone-type compounds (**1** and **2**) from *A. brassicae* (JS959) derived from *V. rotundifolia* on cupric-ion–mediated oxidation of lipoprotein in human blood plasma.

Supplementary information The online version of this article (https://doi.org/10.1038/s41429-019-0198-4) contains supplementary material, which is available to authorized users.

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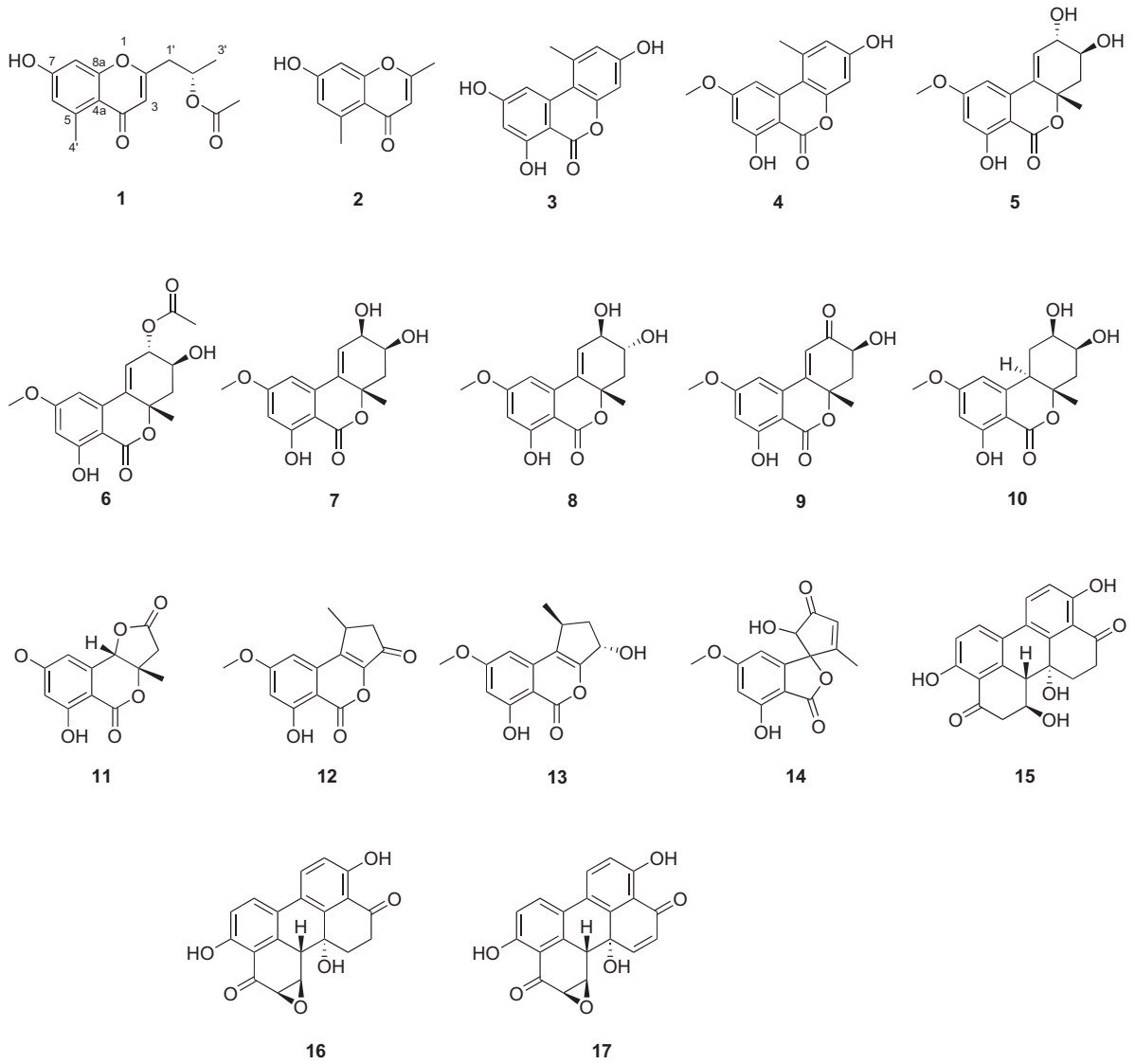

Fig. 1 Chemical structures of isolated compounds **1–17**

Compound **1** was obtained as an amorphous white powder and its molecular formula, $C_{15}H_{16}O_5$, was established by the positive mode HRESIMS ion peak at *m/z* 277.1071 $[M+H]^+$ (calcd for $C_{15}H_{17}O_5$, 277.1076), indicating eight degrees of unsaturation. The ^{1}H NMR spectrum of **1** exhibited signals for three methyls [δ_H 1.33 (3H, d, $J = 6.3$Hz), 1.98 (3H, s), and 2.69 (3H, s)], a methylene [δ_H 2.82 (1H, dd, $J = 14.6$, 7.7Hz) and 2.85 (1H, dd, $J = 14.6$, 5.0Hz)], an oxygenated methine [δ_H 5.29 (1H, m)], an olefinic proton [δ_H 6.01 (1H, s)] and two meta-coupled aromatic protons [δ_H 6.60 (1H, d, $J = 2.3$Hz) and 6.61 (1H, d, $J = 2.3$Hz)] (Table 1). Its ^{13}C NMR spectrum showed 15 carbon signals due to three methyls (δ_C 20.9, 21.8, and 23.9), a methylene (δ_C 41.7), four methines (δ_C 70.2, 102.6, 113.3, and 119.6), and seven quaternary carbons (δ_C 116.1, 144.4, 162.4, 165.4, 166.4, 172.9, and 182.6). The HMBC correlation signals from H-3

Table 1 The ^{1}H and ^{13}C NMR spectral data of **1** in CD_3OD (800 MHz)

Position	**1**	
	δ_H (J in Hz)	δ_C
1		
2		166.4
3	6.01, s	113.3
4		182.6
4a		116.1
5		144.4
6	6.61, d (2.3)	119.6
7		162.4
8	6.60, d (2.3)	102.6
8a		165.4
1′	2.82 (dd, 14.6, 7.7) 2.85 (dd, 14.6, 5.0)	41.7
2′	5.29, m	70.2
3′	1.33, d (6.3)	20.9
4′	2.69, s	23.9
$OCOCH_3$	1.98, s	21.8
		172.9

(δ_H 6.01) to C-4 (δ_C 182.6), C-2 (δ_C 166.4), C-4a (δ_C 116.1), and C-1′ (δ_C 41.7), from H-8 (δ_H 6.60) to C-8a (δ_C 165.4) and C-7 (δ_C 162.4) from H-6 (δ_H 6.61) to C-8 (δ_C 102.6) and C-4a (δ_C 116.1), and from H_3-4′ (δ_H 2.69) to C-6 (δ_C 119.6), C-5 (δ_C 144.4), and C-4a (δ_C 116.1) suggested the presence of a chromone skeleton in **1** (Fig. 2). The UV maxima for **1** were 227, 244, and 251 nm, supporting that this compound had a chromone skeleton. The additional HMBC correlations from H-1′ (δ_H 2.82) to C-3 (δ_C 113.3), C-2, C-2′ (δ_C 70.2), and C-3′ (δ_C 20.9), from H-3′ (δ_H 1.33) to C-2′, from H-2′ (δ_H 5.29) to the carbonyl carbon of acetyl group (δ_C 172.9) indicated the connection of an isopropyl acetate group to the chromone backbone. The absolute configuration at C-2′, bearing a secondary alcohol group, was assigned to be *S* by the measurement of optical rotation ($[\alpha]_D^{25} = +24.7$ (*c* 0.3, CH_3OH)) [9]. Based on these results, compound **1** was assigned as (2′*S*)-2-(2-acetoxypropyl)-7-hydroxy-5-methyl-chromone.

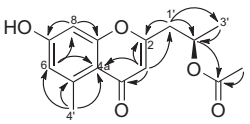

Fig. 2 Key HMBC correlation signals of compound **1**

The sixteen known compounds were identified as 7-hydroxy-2,5-dimethyl-4*H*-chromen-4-one (**2**) [10], alternariol (**3**) [11], alternariol 5-*O*-methyl ether (**4**) [11], altenuene (**5**) [11], altenuene-5′-acetoxy ester (**6**) [11], 5′-*epi*-altenuene (**7**) [12], isoaltenuene (**8**) [12], dehydroaltenuene B (**9**) [12], dihydroaltenuene A (**10**) [12], 6-hydroxy-8-methoxy-3a-methyl-3a,9b-dihydro-3*H*-furo[3,2-c]isochromene-2,5-dione (**11**) [12], 1-deoxyrubralactone (**12**) [13], phialophoriol (**13**) [14], talaroflavone (**14**) [10], altertoxin I (**15**) [15], altertoxin II (**16**) [15], and stemphyltoxin III (**17**) [16] by comparing their spectroscopic data values with those of previously reported literature (Fig. 1). All the isolates except for **4** and **16** are reported for the first time from this endophytic fungus.

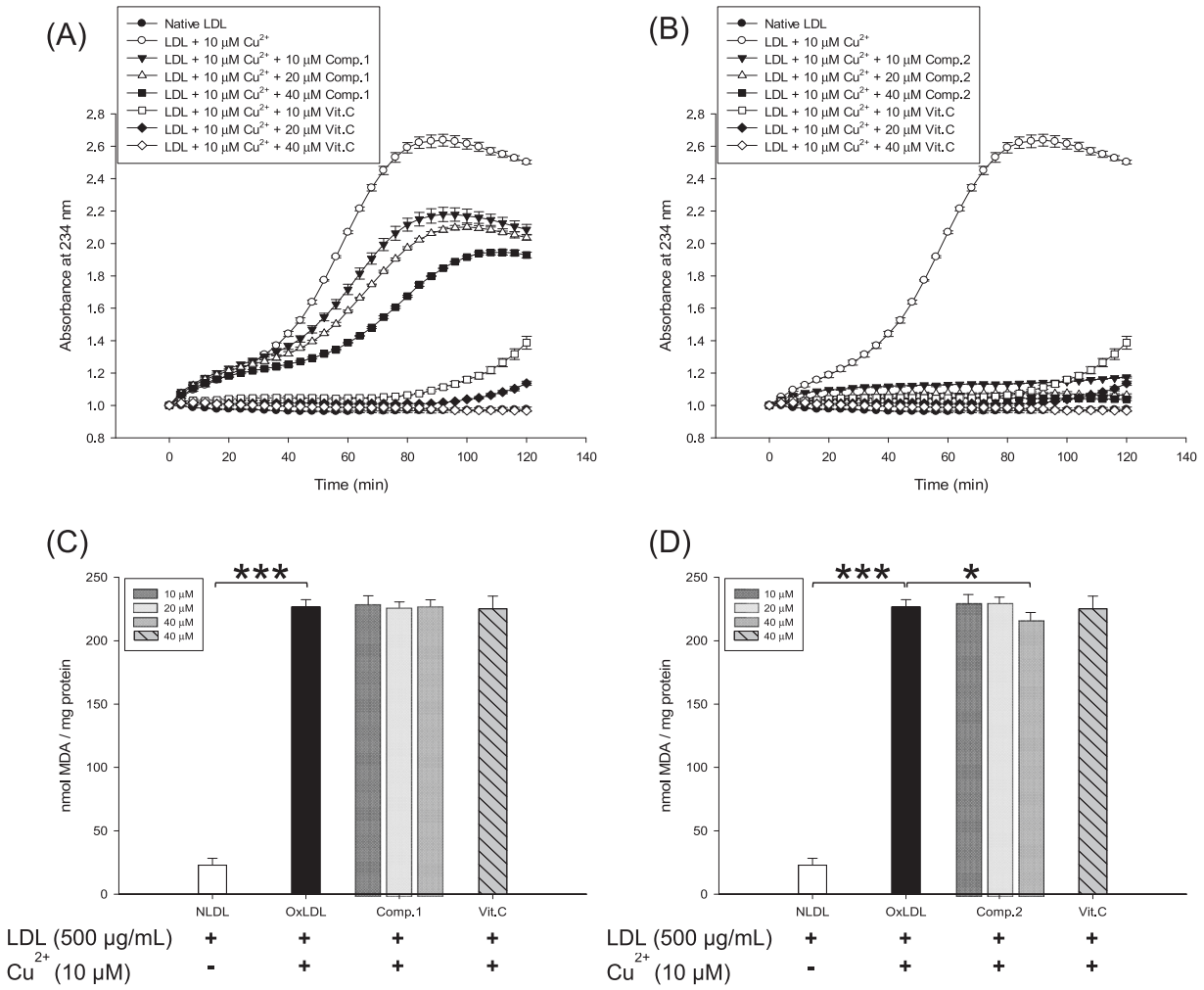

Fig. 3 Effect of compounds **1** and **2** on Cu^{2+}-induced LDL oxidation. **a** The effect of compound **1** on conjugated diene levels by doses. **b** The effect of compound **2** on conjugated diene levels by doses. **c** The effect of compound **1** on TBARS production. **d** The effect of compound **2** on TBARS production. Data are expressed as mean ± S. D. of three independent experiments. $*p < 0.05$, $***p < 0.001$

Several previous researches have reported the inhibitory activities of natural simple phenolic compounds on lipoprotein oxidation. For example, some flavonoids such as isorhamnetin and hesperidin inhibited the oxidation of HDL by $CuSO_4$ [17]. An isopentylated coumarin, osthole, alleviated the oxidized LDL-induced vascular injury in human umbilical vein endothelial cells by suppression of transforming growth factor-β1/Smad pathway [18]. Another coumarin, bergapten, also showed the protective activity against LDL oxidation in a hamster model [19].

Among the isolated compounds, alternariol derivatives (**3–10**) and altertoxin derivatives (**14–16**) were known for their toxic properties, thus, we investigated the inhibitory effects of the chromone–type compounds (**1** and **2**) on Cu^{2+}–induced lipoprotein oxidation in human blood plasma. Compound **1** was found to have a new structure and **2** had previously been isolated only once before, and reported to have antifungal activity against *Candida albicans* SC 5314 [10].

The extent of LDL and HDL oxidation was measured by the formation and accumulation of conjugated diene (CD) and thiobarbituric acid reactive substance (TBARS). Oxidation of LDL by $CuSO_4$ forms approximately a two-fold higher amount of CD compared to native LDL [20]. Both chromones, **1** and **2**, significantly reduced the CD formation and increased the lag time on cupric cation–induced LDL and HDL oxidation in human plasma (Figs. 3a, b and 4a, b). Both compounds inhibited the formation of CD in a dose-dependent manner in LDL and HDL (Figs. 3a, b and 4a, b). Notably, compound **2** with a methyl group at position 3 in the chromone skeleton had more potent CD inhibitory activity than compound **1** with its isopropyl acetate group. Compound **2** showed stronger inhibitory activity against

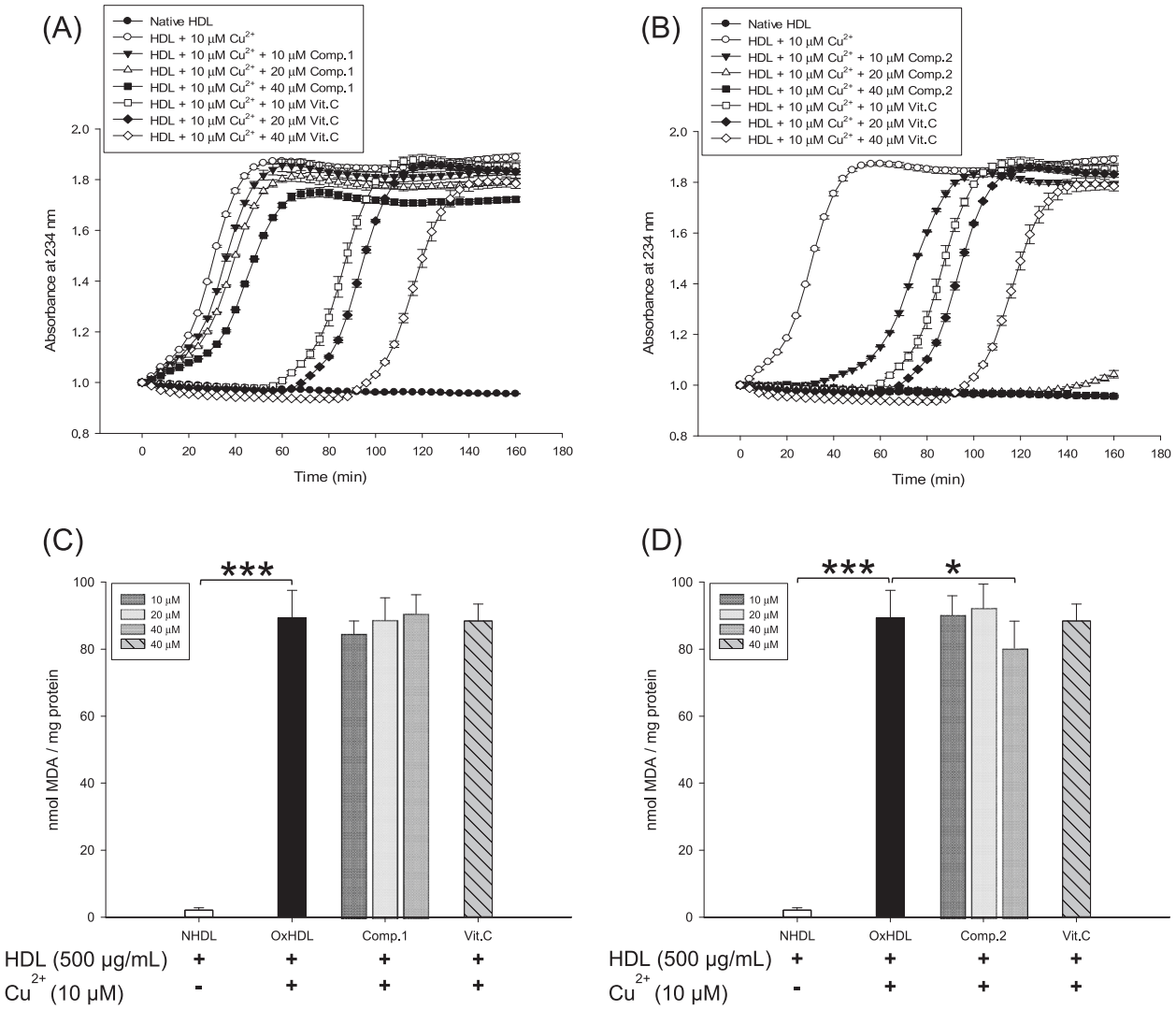

Fig. 4 Effect of compounds **1** and **2** on Cu^{2+}-induced HDL oxidation. **a** The effect of compound **1** on conjugated diene levels by doses. **b** The effect of compound **2** on conjugated diene levels by doses. **c** The effect of compound **1** on TBARS production. **d** The effect of compound **2** on TBARS production. Data are expressed as mean ± S. D. of three independent experiments. *$p < 0.05$, ***$p < 0.001$

CD formation than vitamin C, a positive control (Figs. 3b and 4b). Malondialdehyde (MDA) is known to be generated by lipid peroxidation of lipoprotein and also used as a marker of oxidative stress. We evaluated the effect of **1** and **2** on MDA level during Cu^{2+}-induced LDL and HDL oxidation by TBARS assay (Figs. 3c, d and 4c, d). MDA levels of oxidized LDL and oxidized HDL were shown significantly higher than those of native lipoproteins. However, when compounds **1** and **2** were simultaneously incubated with Cu^{2+}-treated lipoproteins, the MDA levels were strongly decreased. As with the inhibition of CD production, compound **2** showed a significant inhibitory effect on MDA production at a 40 μM concentration while compound **1** did not show the activity.

To summarize, the chemical investigation of the ethyl acetate extract of the fungal endophyte *A. brassicae* grown on solid rice media resulted in the isolation of seventeen compounds (**1–17**) including a new chromone derivative, (2′*S*)-2-(2-acetoxypropyl)-7-hydroxy-5-methyl-chromone (**1**). Two chromones, **1** and **2**, exhibited inhibitory effects on the lipoprotein oxidation by reducing CD and MDA formation in Cu^{2+}-induced human blood plasma. These findings provide further information about chemical characteristics of the endophytic fugus. *A. brassicae* and the potential use of chromones as lipoprotein oxidation inhibitory agents.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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