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CHEMICAL CONSTITUENTS OF THE HEXANE FRACTION OF THE LICHEN *Parmotrema dilatatum* AND THEIR α-GLUCOSIDASE INHIBITION ACTIVITY

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Lichen metabolites have a unique skeleton and have tremendous biological activity. The phenolic compounds from lichen are responsible for their bioactivity [1]. Antibacterial, enzyme inhibitory, and antiviral activities are shown by the depsidone skeleton, while cytotoxicity against several cancer cell lines are displayed by depsides and diphenyl ethers [2]. Monoaromatic compounds such as methyl haematommate, methyl-β-orcinolcarboxylate, and 2-hydroxy-4-methoxy-3,6-dimethylbenzoic acid have antioxidant, antibacterial, and antifungal activity and are potential anticancer agents [3–8]. Vietnam is considered as a new source for novel lichen metabolite investigation. The *Parmotrema* genus, widely distributed in Vietnam, such as *P. sancti-angelii*, *P. prasorediosum*, and *P. tsavoense,* contained many new compounds with remarkable biological activity [9–11]. For the sake of research efficiency on lichen constituents in Vietnam, *P. dilatatum* was selected for its bioactive constituents. Generally, *P. dilatatum* (Vainio.) Hale is dispersed in South Vietnam at high attitude. There is inadequate research on this species, with atranorin and salazinic acid reported as its major compounds [12]. The biological activities of this species are rarely reported as well, and only protocetraric acid isolated from this lichen has been reported to induce the release of nitric oxide in *Thioglycollate*-elicited peritoneal cells [13]. In this paper, the isolation of nine compounds from the *n*-hexane fraction of lichen *P. dilatatum* is described. NMR and MS were used to determine the chemical structures of lichen compounds, which were further compared with the data described in the literature. Compounds **1**, **2**, **7**, and **9** were isolated from this lichen for the first time according to the information collected. The α-glucosidase inhibition activity of compounds from the *n*-hexane fraction was evaluated.

The α-glucosidase inhibition activity of the compounds isolated from the lichen was determined. Compounds **1** and **4** showed excellent α-glucosidase inhibition activity with IC_{50} 77.9 ± 0.82 and 48.7 ± 1.59 μM, respectively. Compound **4,** which possesses an aldehyde group at position C-3, showed better activity compared to **1,** which contains an α,β-unsaturated ketone substituent. The methyl ester substituent in **4** also increases the activity greatly compared to **7** bearing the aldehyde group at C-3 but lacking the methyl ester substituent at C-1. Compounds **1** and **4** displayed promising activity compared to acarbose as positive control with IC_{50} 93.6 ± 0.49 μM. It was suggested that phenolic compounds are active against α-glucosidase and α-amylase and thus will be valuable for the development of type 2 diabetes drugs [14].

Lichen Material. Lichen thalli were obtained from rocks in Lam Dong Province, Vietnam in August–September, 2015. The species was identified by Dr. Wetchasart Polyiam, Lichen Research Unit, Department of Biology, Faculty of Science, Ramkhamhaeng University. A voucher specimen (No. UP-002) was deposited in the Herbarium of the Department of Organic Chemistry, University of Education, Ho Chi Minh City, Vietnam.

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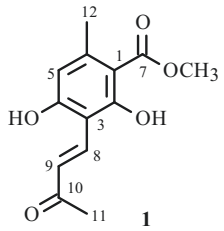

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Extraction and Isolation. The lichen thalli were air-dried and ground. Then 703 g of ground lichen was macerated in acetone (3 × 10 L) at ambient temperature. The solution was filtered and concentrated *in vacuo* to afford an acetone extract (112.5 g). The acetone crude extract was subjected to normal-phase silica gel quick column chromatography and eluted consecutively with *n*-hexane, dichloromethane, ethyl acetate, acetone, and methanol to afford five fractions, PH (3.02 g), PC (6.17 g), PEA (44.53 g), PA (31.71 g), and PM (4.91 g), respectively. Fraction PH (3.02 g) was subjected to normal-phase silica gel column chromatography (CC) and eluted with solvent system *n*-hexane–ethyl acetate (8.0:2.0) to obtain five fractions, MT1 (1.57 g), MT2 (0.37 g), MT3 (0.32 g), MT4 (0.19 mg), and MT5 (0.55 g). Fraction MT1 was further subjected to normal-phase CC and isocratically eluted with *n*-hexane–ethyl acetate–acetone–acetic acid (8.0:2.0:0.4:0.1) to obtain four compounds, **2** (11 mg), **3** (21 mg), **7** (13 mg), and **8** (210 mg). Furthermore, Fr. MT2 was chromatographed using a similar solvent system as previously described above to obtain a new compound **1** (5.0 mg). Fraction MT5 was then purified by CC to furnish four compounds, **4** (67 mg), **5** (25 mg), **6** (3 mg), and **9** (7 mg).

Methyl (*E*)-2,4-dihydroxy-6-methyl-3-(3-oxobut-1-en-1-yl)benzoate (1), white amorphous powder. UV (MeOH, λ_{max}, nm) (log ε): 195 (4.6), 210 (4.7), 615 (3.3). IR (KBr, ν_{max}, cm^{-1}): 3437, 2922, 1654, 1617, 1384. ^{1}H NMR (500 MHz, acetone-d_6, δ, ppm, J/Hz): 12.76 (1H, br, 2-OH), 7.83 (1H, d, J = 16.4, H-8), 7.11 (1H, d, J = 16.4, H-9), 6.35 (1H, s, H-5), 3.16 (3H, s, 7-OCH_3), 2.36 (3H, s, H-12), 2.15 (3H, s, H-11). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 197.9 (C-10), 172.2 (C-7), 165.0 (C-2), 161.0 (C-4), 141.4 (C-6), 133.5 (C-8), 129.3 (C-9), 111.6 (C-5), 107.5 (C-3), 103.3 (C-1), 51.8 (7-OCH_3), 23.6 (C-12), 14.3 (C-11). HR-ESI-MS *m/z* 249.0754 $[M - H]^-$ (calcd for $C_{13}H_{13}O_5$, 249.0763).

Hopane-16-*β*,22-diol (2). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.89 (1H, d, J = 8.1, 16-OH), 3.81 (1H, s, 22-OH), 3.72 (1H, ddd, J = 10.5, 4.5, 2.5, H-16), 2.10 (1H, dd, J = 19.5, 8.5, H-21), 1.93 (1H, dd, J = 10.0, 4.5, H-17), 1.62 (1H, m, H-15a), 1.32 (1H, m, H-15b), 1.58 (1H, m, H-12a), 1.36 (1H, m, H-12b), 1.54 (1H, m, H-11a), 1.38 (1H, m, H-11b), 1.49 (1H, m, H-20a), 1.42 (1H, m, H-20b), 1.49 (1H, m, H-2a), 1.32 (1H, m, H-2b), 1.49 (1H, m, H-19a), 1.01 (1H, m, H-19b), 1.49 (1H, m, H-1a), 0.75 (1H, m, H-1b), 1.47 (1H, m, H-9), 1.35 (1H, m, H-13), 1.12 (3H, s, H-30), 1.07 (3H, s, H-29), 1.03 (3H, s, H-27), 0.97 (3H, s, H-26), 0.94 (3H, s, H-28), 0.92 (3H, s, H-25), 0.81 (3H, s, H-23), 0.78 (1H, m, H-5), 0.71 (3H, s, H-24). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 71.6 (C-22), 66.6 (C-16), 60.0 (C-17), 53.8 (C-5), 50.4 (C-21), 49.3 (C-9), 48.9 (C-13), 44.7 (C-18), 43.6 (C-15), 42.1 (C-3), 41.5 (C-19), 40.9 (C-8), 38.6 (C-10), 39.9 (C-1), 39.0 (C-14), 36.6 (C-23), 33.9 (C-7), 33.3 (C-4), 30.8 (C-30), 28.9 (C-29), 26.1 (C-20), 23.6 (C-12), 21.9 (C-24), 21.3 (C-6), 20.6 (C-11), 18.1 (C-2), 18.0 (C-27), 16.9 (C-25), 16.8 (C-26), 15.9 (C-28) [15, 16].

Methyl orsellinate (3), colorless crystals. Spectral data agreed with those published [17].

Methyl haematommate (4), colorless needles. Spectral data agreed with those published [18].

Methyl *β*-orcinol carboxylate (5), colorless needles. Spectral data agreed with those published [19].

2-Hydroxy-4-methoxy-3,6-dimethylbenzoic acid (6), white amorphous powder. Spectral data agreed with those published [20].

Atranol (7), white amorphous powder. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 10.21 (1H, s, CHO-7), 6.14 (2H, s, H-1, 5), and 2.20 (3H, s, CH_3-8) [21].

Atranorin (8), white needles. Spectral data agreed with those published [22].

Lecanorin (9), amorphous solid. Spectral data agreed with those published [21].

***α*-Glucosidase Inhibitory Assay**. The *α*-glucosidase inhibition activity was determined according to [23]; 0.1 U/mL of *α*-glucosidase and 1 mM *p*-nitrophenyl-*α*-D-glucopyranoside as substrate were dissolved in 0.1 mM pH 6.9 phosphate buffer. Then 10 μL of inhibitors dissolved in DMSO was added into a 96-well microplate followed by 40 μL of enzyme. The mixture was preincubated at 37°C for 10 min. Then 50 μL of substrate was added into the reaction mixture. The enzymatic reaction was carried out at 37°C for 20 min and stopped by adding 1 M Na_2CO_3 (100 μL). Enzymatic activity was

quantified by measuring the absorbance at 405 nm (Allsheng microplate reader AMR-100). All samples were analyzed in triplicate at different concentrations to obtain the IC_{50} value of each inhibitor, and the mean values were retained. The inhibition percentage (%) was calculated by the following equation:

$$\text{Inhibition (\%)} = [(A_{\text{sample}}/A_{\text{control}})/A_{\text{control}}] \times 100,$$

where A_{control} is the absorbance of the reaction mixture without inhibitor and A_{sample} is the absorbance of the reaction mixture with inhibitors. Acarbose® was used as standard control.

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