isocoumarin derivatives from the endophytic fungus *Xylomelasma* sp. Samif07, and their antibacterial and antioxidant activities, *Natural Product Research,* 1-5. **9.** Zhao J. T., Ma D. H., Luo M., Wang W., Zhao C., Zu Y. G., Fu Y., Wink M. (2014), *In vitro* antioxidant activities and antioxidant enzyme activities in HepG2 cells and main active compounds of endophytic fungus from pigeon pea [*Cajanus cajan* (L.) Millsp.], *Food Research International,* 56, 243-251. **10.** Zhao J., Mou Y., Shan T., Li Y., Zhou L., Wang M., Wang J. (2010), Antimicrobial metabolites from the endophytic fungus *Pichia guilliermondii* isolated from *Paris polyphylla* var. *yunnanensis*, *Molecules,* 15(11), 7961-7970. **11.** Zhan J. X., Burns A. M., Liu M. P. X., Faeth S. H, Gunatilaka A. A. L. (2007), Search for cell motility and angiogenesis inhibitors with potential anticancer activity: Beauvericin and other constituents of two endophytic strains of *Fusarium oxysporum*, *Journal of Natural Products,* 70(2), 227-232. **12.** Li H., Xiao J., Gao Y. Q., Tang J. J., Zhang A. L., Gao J. M. (2014), Chaetoglobosins from *Chaetomium globosum*, an endophytic fungus in *Ginkgo biloba*, and their phytotoxic and cytotoxic activities, *Journal of Agricultural and Food Chemistry,* 62(17), 3734-3741. **13.** Sanger F., Coulson A. R. (1975), A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase, *Journal of Molecular Biology*, 94, 441-448. **14.** Sanger F., Nicklen S., Coulson A. R. (1977), DNA sequencing with chain-terminating inhibitors, *PNAS*, 74, 5463-5467. **15.** Li X., Wu Q., Xie Y., Ding Y., Du W. W., Sdiri M., Yang B. B. (2015), Ergosterol purified from medicinal mushroom *Amauroderma* rude inhibits cancer growth *in vitro* and *in vivo* by up-regulating multiple tumor suppressors, *Oncotarget,* 6(19), 17832-17846. **16.** Nozawa K., Nakajima S., Kawai K. (1988), Isolation and structures of indoloditerpenes, possible biosynthetic Intermediates to the tremorgenic mycotoxin, paxilline, from *Emericella striata*, *Journal of the Chemical Society Perkin Transaction I*, 1988, 2607-2610. **17.** Sarah C. M. F., Alistair L. W., Christopher O. M. (1996), Isolation of paspaline B, an indole-diterpenoid from *Penicillium paxilli*, *Phytochemistry*, 41(1), 327-332. **18.** Silvio U., Christo J. B., Trude V., Elin R., Christopher O. M. (2009), Indole-diterpenes and ergot alkaloids in *Cynodon dactylon* (Bermuda grass) Infected with *Claviceps cynodontis* from an outbreak of tremors in Cattle, *Journal of Agricultural and Food Chemistry*, 57, 11112-11119. **19.** Guo S., Feng B., Zhu R., Ma J., Wang W., (2011), Preparative isolation of three anthraquinones from *Rumex japonicus* by high-speed counter-current chromatography, *Molecules*, 16, 1201-1210.

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PHENOLIC COMPOUNDS FROM *CURCULIGO ORCHIOIDES*

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Summary

Phenolic Compounds from *Curculigo orchioides*

Six compounds were isolated from ethyl acetate and *n*-butanol fractions from the rhizome of *Curculigo orchioides* Gaertn. (Hypoxidaceae). Their chemical structures were identified as curculigoside A (**1**), curculigoside B (**2**), orcinol *β*-D-glucoside (**3**), orcinol gentiobioside (**4**), glucosyringic acid (**5**) and vanillic acid 4-*O*-*β*-D-glucoside (**6**) by NMR, MS spectroscopic data and comparison with the reported literature. Compound **6** was isolated for the first time from this plant.

Keywords: *Curculigo orchioides, Curculigoside, Glucosyringic acid, Orcinol, phenolic compounds, Vanillic acid.*

1. Introduction

Curculigo orchioides Gaertn. is a perennial herb belonging to the family Hypoxidaceae. The rhizomes of *Curculigo orchioides* have been used in traditional medicine of Asian countries including Vietnam, for the treatment of impotence, rheumatism, hepatitis, and heart diseases… [1],[2]. This medicinal plant has shown a wide range of pharmacological activities such as antioxidant, anti-inflammatory, immunostimulant, hepatoprotective, anticancer, and antidiabetic activities [1],[3]. Previous phytochemical investigations of *C. orchioides* have reported phenolic glycosides, cycloartane saponins, flavonoids and carbohydrates [1],[3],[4],[5]. In Vietnam, *C. orchioides* collected in Tay Nguyen has been found to have orcinol glucoside, curculigoside, orcinol-1-*O*-(6′-*O*-acetyl)-*α*-D-glucopyranoside [6]. This study focused on the chemical constituents of *C. orchioides* in Vietnam for further evaluation of its pharmacological properties.

2. Material and method

2.1. Plant material

The rhizomes of *C. orchioides* were collected in Lao Cai province in March 2021. The sample was authenticated by Ph.D Tran Thi Van Anh (Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City). In additionally, DNA analysis of sample showed that the chloroplast gene trnH-psbA và rps16 had 100% query cover in BLAST match of data of *C. orchioides* in gene bank (NC-053892.1). Voucher specimen of the

plant (SC-LC0321) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh city, Vietnam. Plant material was air-dried and ground to powder before further processing.

2.2. General experimental procedures

The NMR spectra were recorded by a Bruker Avance Neo 600 spectrometer (Bruker, MA, USA) using tetramethylsilane as an internal standard. The high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was obtained on a UPLC-Xevo QTof system (Water, USA). Column chromatography was performed with *silica gel* (40-63 µm, Merck) and Sephadex LH-20 (GE Healthcare Life). Thin-layer chromatography (TLC) was carried out on *silica gel* 60 F_{254} plates (Merck). Compounds were visualized by UV lamp and spraying with vanillin-sulfuric acid reagent followed by heating to 110°C.

2.3. Extraction and isolation

The powder of *C. orchioides* dried rhizomes (11 kg) was percolated with 96% EtOH. The extract was evaporated *in vacuo* to obtain the concentrated extract which was suspended to 1L of H_2O and successively extracted with increasing polarity solvents. The organic solvent was removed *in vacuo* to obtain 4 fractions including petroleum ether fraction (63.0 g), chloroform fraction (28.9 g), ethyl acetate fraction (92.4 g), and *n*-butanol fraction (85.9 g).

The ethyl acetate fraction (49.26 g) was subjected to a *silica gel* column chromatography using gradient solvent system of $CHCl_3$-MeOH (100:0, 98:2; 96:4, 94:6; 92:8; 90:10…50:50 v/v) to obtain 17 fractions (C.1-C.17). Fraction C.11 (2.267 g) was separated by *silica gel* column eluting with CH_2Cl_2-MeOH (9:1, v/v) to obtain 10 subfractions (C.11.1 - C.11.10). Subfraction C.11.6 (93.7 mg) was purified through a Sephadex LH-20 column with the mobile phase $CHCl_3$ - MeOH (9:1, v/v) to obtain compound **1** (80.0 mg). Compound **2** (66.3 mg) was obtained by filtration of the precipitate from the fraction C.11.8. Fraction C.13 had the precipitate when evaporating solvent. The precipitate was filtrated to yield compound **3** (397.9 mg).

The *n*-butanol fraction (50.0 g) was chromatographed on a *silica gel* column eluting with gradient mobile phase $CHCl_3$-MeOH (8:2, 7:3, 6:4, 5:5 v/v) to yield 15 fractions (D.1 - D.15). Fraction D.7 (7.92 g) was subjected to a *silica gel* column using $CHCl_3$ - MeOH (8:2, 7:3, 6:4, 5:5, v/v) as eluent to obtain 12 subfractions (D.7.1 - D.7.12). Subfraction D.7.11 (512.6 mg) was separated by a Sephadex LH-20 column using MeOH as mobile phase to get 3 subfractions (D.7.11.1 -D.7.11.3). Subfraction D.7.11.2 (263.3 mg) was purified by a Sephadex LH-20 column with MeOH-H_2O (7:3, v/v) to yield compounds **5** (4 mg) and **6** (43.0 mg).

Fraction D.12 (6.12 g) was chromatographed by a *silica gel* column, eluting with $CHCl_3$-MeOH - H_2O (80:20:10, 70:30:10, 60:40:10, v/v/v) to give 8 fractions (D.12.1 -D.12.8). Subfraction D.12.5 (436.9 mg) was purified through a Sephadex LH-20 column using MeOH-H_2O (7:3, v/v) as eluent to obtain compound **4** (25 mg).

Curculigoside A (1): pale yellow gum; HR-ESI-MS *m/z* 489.13783 $[M+Na]^+$ (calcd. for $C_{22}H_{26}O_{11}Na^+$, 489.13728); ^{1}H-NMR (600 MHz, CD_3OD): δ_H (*ppm*) 7.35 (1H, t, J = 8.5 Hz, H-4′), 7.08 (1H, d, J = 8.8 Hz, H-3), 6.92 (1H, d, J = 3.0 Hz, H-6), 6.70 (1H, dd, J = 8.8, 3.0 Hz, H-4), 6.67 (2H, d, J = 8.5 Hz, H-3′/5′), 5.46 (H, d, J = 13.3 Hz, H-7a), 5.40 (1H, d, J = 13.3 Hz, H-7b), 4.76 (1H, d, J = 7.5 Hz, H-1″), 3.86 (1H, J = 12.0, 2.2 Hz, H-6a″), 3.81 (6H, s, -OCH_3), 3.69 (1H, J = 12.0, 5.4 Hz, H-6b″), 3.40 -3.47 (4H, m, H-2″/ H-3″/H-4″/ H-5″); ^{13}C-NMR (150 MHz, CD_3OD) δ_C (*ppm*): 128.8 (C-1), 149.6 (C-2), 118.8 (C-3), 116.2 (C-4), 154.0 (C-5), 116.4 (C-6), 63.2 (C-7), 114.3 (C-1′), 158.2 (C-2′/C-6′), 105.2 (C-3′/C-5′), 132.6 (C-6′), 168.5 (C-7′), 56.5 (2′/6′-OCH_3), 104.3 (C-1″), 75.0 (C-2″); 78.1 (C-3″), 71.4 (C-4″), 78,1 (C-5″), 62.6 (C-6″).

Curculigoside B (2): yellow amorphous powder, HR-ESI-MS *m/z* 475.12178 $[M+Na]^+$ (calcd. for $C_{21}H_{24}O_{11}Na^+$, 475.12163); ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H (*ppm*) 7.20 (1H, t, J = 8.3 Hz, H-4′), 6.98 (1H, d, J = 8.8 Hz, H-3), 6.85 (1H, d, J = 3.0 Hz, H-6), 6.64 (1H, dd, J = 8.8, 3.0 Hz, H-4), 6.52 (1H, d, J = 4.9 Hz, H-3′), 6.51 (1H, d, J = 4.9 Hz, H-5′), 5.33 (1H, d, J = 14.0 Hz, H-7a), 5.29 (1H, d, J = 14.0 Hz, H-7b), 4.60 (1H, J = 4,7 Hz, H-1″), 3.76 (3H, s, 2′-OCH_3), 3.69 (1H, dd, J = 11.8, 2.1 Hz, H-6″), 3.47 (1H, dd, J = 11.8, 5.8 Hz, H-6″), 3.15 -3.25 (4H, m, H-2″/ H-3″/H-4″/H-5″). ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C (*ppm*): 126.8 (C-1), 147.4 (C-2), 117.1 (C-3), 114.6 (C-4), 152.3 (C-5), 114.3 (C-6), 61.1 (C-7), 110.9 (C-1′), 156.6 (C-2′), 102.0 (C-3′), 131.1 (C-4′), 108.5 (C-5′), 157.3 (C-6′), 166.1 (C-7′), 55.6 (-OCH_3), 102.5 (C-1″), 73.3 (C-2″), 76.5 (C-3″), 69.8 (C-4″), 76.9 (C-5″), 60.8 (C-6″).

Orcinol *β-D*-glucoside (3): white amorphous powder, HR-ESI-MS *m/z* 309.09566 $[M+Na]^+$ (calcd. for $C_{13}H_{18}O_7Na^+$, 309.09502); ^{1}H-NMR

(600 MHz, CD_3OD): δ_H (*ppm*) 6.44 (1H, brs, H-6), 6.39 (1H, t, J = 2.2 Hz, H-2), 6.31 (1H, brs, H-4), 4.86 (1H, d, J = 7.5 Hz, H-1′), 3.91 (1H, dd, J = 12.0; 2,1Hz, H-6′), 3.73 (1H, dd, J = 12.0, 5.0 Hz, H-6′), 3.41-3.45 (1H, m, H2′/H3′/H4′/H5′), 2.23 (3H, s, H-7); ^{13}C-NMR (150 MHz, CD_3OD) 160.1(C-1), 102.2 (C-2), 159.2 (C-3), 111.2 (C-4), 141.2 (C-5), 109.7 (C-6), 21.6 (C-7), 102.2 (C-1′), 74.9 (C-2′), 78.1 (C-3′), 71.4 (C-4′), 78.1 (C-5′), 62.5 (C-6′).

Orcinol gentiobioside (anacardoside) (4): white amorphous powder, HR-ESI-MS *m/z* 447.15003 $[M-H]^-$ (calcd. for $C_{19}H_{27}O_{12}^-$, 447.15025), ^{1}H-NMR (600 MHz, CD_3OD): δ_H(*ppm*) 6.44 (1H, br s, H-2), 6.41 (1H, br s, H-6), 6.29 (1H, s, H-4), 4.83 (1H, d, J = 7.4 Hz, H-1′), 4.41 (1H, d, J = 7.8 Hz, H-1″), 4.16 (dd, J = 11.8, 2.0 Hz, H-6′), 3.81 (1H, m, H-6″), 3.84 (1H, m, H-6′), 3.66 (1H, m, H-6″), 3.65 (1H, m, H-3′) 3.47 - 3.20 (7H, m, H2′-H5′, H2″-H5″); ^{13}C-NMR (150 MHz, CD_3OD) 160.0 (C-1), 102.0 (C-2), 159.2 (C-3), 111.2 (C-4), 141.3 (C-5), 109.9 (C-6), 21.7 (C-7), 102.1 (C-1′), 74.9 (C-2′), 77.5 (C-3′), 71.4 (C-4′), 77.9 (C-5′), 69.6 (C-6′), 104.6 (C-1″), 75.2 (C-2″), 77.8 (C-3″), 71.6 (C-4″), 77.9 (C-5″), 62.7 (C-6″).

Glucosyringic acid (5): pale yellow solid, HR-ESI-MS *m/z* 359.09847 $[M-H]^-$ (calcd. for $C_{15}H_{19}O_{10}^-$, 359.09782) ; ^{1}H-NMR (600 MHz, CD_3OD) 7.35 (2H, s, H-3/H-5), 4.94 (1H, d, J = 7.6 Hz, H-1′), 3.49 (1H, ddd, J = 7.6, 6.7, 2.7 Hz, H-2′), 3.22 (1H, ddt, J = 7.5, 5.2, 2.5 Hz, H-3′), 3.87 (6H, s, -OCH_3), 3.77 (1H, dd, J = 12.0, 2.5 Hz, H-6a), 3.67 (dd, J = 12.0, 5.0 Hz, H′6b), 3.44-3.40 (1H, m, H-4′, H-5′). ^{13}C-NMR (150 MHz, CD_3OD) 174.4 (-COOH), 135.4 (C-1), 108.4 (C-2/C-6), 153.4 (C-3/C-5), 138.0 (C-4), 56.9 (-OCH_3), 105.2 (C-1′), 75.7 (C-2′), 78.3 (C-3′), 71.3 (C-4′), 77.8 (C-5′), 62.5 (C-6′)

Vanillic acid 4-*O*-*β*-D-glucoside (6): pale yellow solid, HR-ESI-MS *m/z* 329.08850 $[M-H]^-$ (calcd. for $C_{14}H_{17}O_9^-$, 329.08726), ^{1}H-NMR (600 MHz, CD_3OD) 7.62, (1H, m, H-2), 7.61 (1H, m, H-6), 7.17 (1H, d, J = 8.8 Hz, H-5), 5.00 (1H, d, J = 7.5 Hz, H-1′), 3.89 (3H, s, -OCH_3) 3.87 (1H, m, H-6′a), 3.70 (1H, J =12.2, 5.0 Hz, H-6b), 3.50-3.38 (4H, m, H2′-H5′). ^{13}C-NMR (150 MHz, CD_3OD) 171.8 (-COOH) 128.9 (C-1), 114.5 (C-2), 150.1 (C-3), 151.2 (C-4), 116.4 (C-5), 124.1 (C-6), 56.6 (-OCH_3), 102.1(C-1′), 74.9 (C-2′), 77.8 (C-3′), 71.3 (C-4′), 78.2 (C-5′) 62.4 (C-6′).

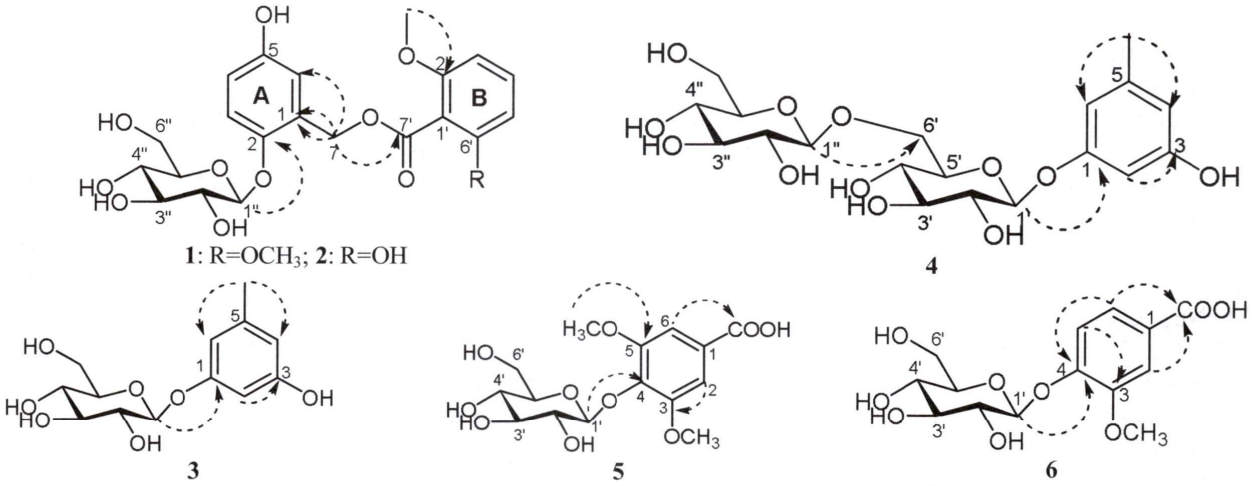

Fig. 1. Chemical structures of the compounds **1- 6** and their key HMBC correlations

3. Result and discussion

Compound **1** was isolated as a pale-yellow gum. The HR-ESI-MS spectrum exhibited a quasi-molecular peak at *m/z* 489.13783 $[M+Na]^+$ corresponding to the molecular formula $C_{22}H_{26}O_{11}$. The ^{1}H-NMR spectrum of **1** showed proton signals for two methoxy groups δ_H 3.81 (6H, s), one methylene δ_H 5.46 (H, d, J = 13.3 Hz, H-7a), 5.40 (1H, d, J = 13.3 Hz, H-7b), one anomeric proton δ_H 4.76 (1H, d, J = 7.5 Hz, H-1") due to a *β*-glucosyl moiety together with two aromatic rings: ring A with an ABX system at δ_H 7.08 (1H, d, J = 8.8 Hz), 6.70 (1H, dd, J = 8.8, 3.0 Hz) and δ_H 6.92 (1H, d, J = 3.0 Hz) and ring B with protons at δ_H 6.67 (2H, d, J = 8.5 Hz) and 7.35 (1H, t, J = 8.5 Hz). The ^{13}C-NMR spectrum of compound **1** showed 19 signals including three signal of symmetric carbons (δ_C 158.2, 105.2, and 56.2) which suggested that compound **1** have two aromatic rings, one carboxyl carbon (δ_C 168.5), one methylene (δ_C 63.2), two methoxys (δ_C 56.5) as well as one set of *β*- glucopyranosyl

moiety. In the HMBC spectrum, methylene proton (δ_H 5.46 (H, d, J = 13.3 Hz, H-7a), 5.40 (1H, d, J = 13.3 Hz, H-7b) correlated with three aromatic carbons (δ_C 116.4; 128.8 and 149.6) and carboxyl carbon (δ_C 168.5). Additionally, the anomeric proton δ_H 4.76 (1H, d, J = 7.5 Hz) had the correlation with oxygenated carbon δ_C 149.6 that revealed ring A linked with β-glucopyranosyl moiety through hydroxyl group at C-2. Ring B had the symmetrical structure with two methoxy groups at C-2′/C6′ indicated by the HMBC correlation of δ_H 3.81 to aromatic carbon δ_C 158.2. Ring B connected with ring A through an ester bond. Analysis of the NMR spectroscopic data and comparison with those in literature [7] led to identify compound **1** as curculigoside A (5-hydroxy-2-O-β-glucopyranosylbenzyl 2,6-dimethoxybenzoate).

Compound **2** was isolated as a yellow amorphous powder. The HR-ESI-MS spectrum exhibited a quasi-molecular peak *m/z* 475.12178 $[M+Na]^+$ corresponding to the molecular formular $C_{21}H_{24}O_{11}$. The ^{1}H-NMR and ^{13}C-NMR spectra of **2** were similar to those of compound **1** except a methoxy group at δ_H 3.76 (3H, s); δ_C 55.6 in ring B. In HMBC, the correlation of methoxy proton at δ_H 3.76 with carbon at 155.6 indicated methoxy group at C-2′. Furthermore, by comparison with the published spectra data [7], compound **2** was determined as curculigoside B (5-hydroxy-2-O-β- glucopyranosylbenzyl 2-methoxy-6-hydroxybenzoate).

Compound **3** was isolated as a white amorphous powder. The HR-ESI-MS spectrum exhibited a quasi-molecular peak *m/z* 309.09566 $[M+Na]^+$ corresponding to the molecular formula $C_{13}H_{18}O_7$. The ^{1}H-NMR spectrum of compound **3** showed three aromatic protons at δ_H 6.31 (1H, br s); 6,39 (1H, t, J = 2,2 Hz) and 6,44 (1H, brs), one methyl group at δ_H 2,23 (3H, s), one anomeric proton δ_H 4,86 (1H, *d*, J = 7,5 Hz) with other six carbinol protons of a β-glucopyranosyl group. The ^{13}C NMR showed 13 carbon signals including six aromatic carbons, one methyl carbon at δ_C 21.6 and six carbons of a glucosyl moiety. In HMBC spectrum, the anomeric proton δ_H 4.86 (1H, d, J = 7.5 Hz) had correlation with oxygenated carbon δ_C 160.1 revealed the sugar linked with aromatic ring at C-1. Additionally, the correlation of methyl proton δ_H 2,23 (3H, s) with δ_C 141.2, 111.22, 109.7 and the correlation of δ_H 6.39 (1H, t, J =2.2 Hz) with δ_C 160.1 and 159.2 revealed aromatic ring had substituted methyl group at C-5 and hydroxy group at C-3. Therefore, compound **3** was identified as orcinol O-β-*D*-glucopyranoside based on the comparison of the NMR data with reported values [8].

Compound **4** was isolated as a white amorphous powder. The HR-ESI-MS spectrum exhibited a quasi-molecular peak at *m/z* 447.15003 [M-H]- corresponding to the molecular formula $C_{19}H_{27}O_{12}$. ^{1}H-NMR and ^{13}C-NMR spectrum of **4** were similar to those of compound **3** except compound **4** had signals of two sugar moieties. There were two anomeric protons δ_H 4.41 (1H, d, J = 7.8 Hz), δ_H 4.83 (1H, d, J = 7.4 Hz) together with two anomeric carbons δ_C 102.1 and 104.6. In HMBC spectrum, the anomeric proton δ_H 4.83 and δ_H 4.41 had the correlation with δ_C 160.0 (C-1) and δ_C 69.6 (C-6′), respectively. Hence, the chemical structure of compound **4** was identified as orcinol *O*-gentiobioside (anacardoside) [9].

Compound **5** was isolated as a pale-yellow solid. The HR-ESI-MS spectrum exhibited a quasi-molecular peak at *m/z* 359.09847 [M-H]$^-$ corresponding to the molecular formula $C_{15}H_{20}O_{10}$. The ^{1}H-NMR spectrum of compound **5** showed a signal of an aromatic proton δ_H 7.35 (2H, s), an anomeric proton δ_H 4.94 (1H, d, J = 7.6 Hz) and other six carbinol protons of a β-glucopyranosyl group. The ^{13}C-NMR showed 12 signals including six aromatic carbons, a carbonyl carbon δ_C 174.4, two methoxy groups δ_C 56.9 (2C), and six carbons of a sugar moiety. In HMBC spectrum, the proton δ_H 7.35 correlated with carbon δ_C 174.4, 153.4, 108.4, and the anomeric proton δ_H 4.94 had the correlation with δ_C 138.0 suggested the structure of a benzoic acid derivative substituted with two methoxy groups at C-3/C-5 and a glucosyl group at C-4. Compound **5** was identified as glucosyringic acid, which was confirmed by comparison with published data [10].

Compound **6** was isolated as a pale-yellow solid. The HR-ESI-MS spectrum exhibited a quasi-molecular peak at *m/z* 329.08850 [M-H]$^-$ corresponding to the molecular formula $C_{14}H_{18}O_{10}$. The ^{1}H-NMR spectrum of compound **6** showed three protons δ_H 7.17 (1H, d, J = 8.8 Hz), 7.62 (1H, m), 7.61 (1H, m), an anomeric proton δ_H 5.00 (1H, d, J = 7.5 Hz) and other six carbinol protons of a β-glucopyranosyl group. The ^{13}C-NMR spectrum of compound **6** showed 14 signals including 6 aromatic carbons in which two oxygenated carbons (δ_C 151.1 and 150.1), a carbonyl carbon δ_C 171.8, a methoxy group δ_C 56.6, and a β-glucopyranosyl moiety. In HMBC spectrum, the correlation of proton δ_H 7.17 with δ_C 128.9 and 150.1

and the cross-peak of δ_H 7.61 with δ_C 114.5 and 151.2 revealed the oxygenated substituents on C-3 and C-4 of benzoic acid. Additionally, the cross-peak of methyl proton δ_H 3.89 and anomeric proton δ_H 5.00 (1H, J=7.5 Hz) with δ_C 150.1 and δ_C 151.2, respectively revealed the methoxy group at C-3 and the *O*-glucosyl at C-4. Thus, compound **6** was identified as vanillic acid 4-*O*-*β*-D-glucopyranoside [11].

4. Conclusion

A phytochemical investigation of *C. orchioides* collected in Vietnam led to the isolation of six phenolic compounds. They were identified as curculigoside A (**1**), curculigoside B (**2**), orcinol *O*-*β*-D-glucopyranoside (**3**), orcinol *O*-gentiobioside (**4**), glucosyringic acid (**5**) and vanillic acid 4-*O*-*β*-D-glucopyranoside (**6**). Compound **6** was found in *C. orchioides* for the first time.

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References

1. Nie Y., Dong X., He Y., Yuan T., Ting H. T., Rahman K., Qin L., Zhang Q. (2013), Medicinal plants of genus Curculigo: Traditional uses and a phytochemical and ethnopharmacological review, *Journal of Ethnopharmacology*, 147(3), 547-563. **2.** Do Huy Bich (2006), Medicinal plants and animals in Vietnam, *Science and Technics Publishing House, Hanoi*, 2, 693-694. **3.** Chauhan N. S., Sharma V., Thakur M., Dixit V. K. (2010), Curculigo orchioides: the black gold with numerous health benefits, *Zhong Xi Yi Jie He Xue Bao* (*Journal of Chinese Integrative Medicine*), 8(7): 613-23. **4.** Xu R. S., Li X. Y., Xu J. P. X. (1992), Four new cycloartane saponins from *Curculigo orchioides*, *Planta Medica*, 58(2), 208-210. **5.** Wang X., Zhang M., Zhang D., Wang X., Cao H., Zhang Q., Yan C. (2019), Structural elucidation and anti-osteoporosis activities of polysaccharides obtained from *Curculigo orchioides, Carbohydrate Polymers*, 203: 292-301. **6.** Nguyen Bich Ngoc, Nguyen Manh Tuyen, Phuong Thien Thuong, Vu Van Tuan, Nguyen Van Khiem, Vu Duy Hong (2015), Three phenolic glycosides from the rizhome of *Curculigo orchioides* Gaertn. collected in Tay Nguyen, *Journal of Pharmaceutical Science*, 55(7), 54-57. **7.** Peng J., Jiang Y., Fan G., Chen B., Zhang Q., Chai Y., Wu Y. (2006), Optimization suitable conditions for preparative isolation and separation of curculigoside and curculigoside B from *Curculigo orchioides* by high-speed counter-current chromatography, *Separation Purification Technology*, 52(1), 22-28. **8.** Lee S. Y., Kim M. R., Choi H. S., Moon H. I., Chung J. H., Lee D. G., Woo E. R. (2009), The effect of curculigoside on the expression of matrix metalloproteinase-1 in cultured human skin fibroblasts, *Archives Pharmacal Research*, 32(10), 1433-1439. **9.** Gil R. R., Lin L. Z., Cordell G. A., Kumar M. R., Ramesh M., Reddy B. M., Mohan G. K., Narasimha A. V., Rao A. (1995), Anacardoside from the seeds of *Semecarpus anacardium*l, *Phytochemistry*, 39(2), 405-407. **10.** Niu C., Zhang Z., Yang L., Zhai Y. Y., Li S. N., Hao S. Y., Chen X. Y., Wang J. H. and Wang Z. H. (2020), Chemical constituents of *Curculigo orchioides*, *Chemistry of Natural Compounds*, 56(5), 818-819. **11.** Sakushima A., Coşkun M., Maoka T. (1995), Hydroxybenzoic acids from *Boreava orientalis*, *Phytochemistry*, 40(1), 257-261.

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QUANTITATIVE DETERMINATION OF DAPHNORETIN IN AERIAL PARTS OF *WIKSTROEMIA INDICA* L. (THYMELAEACEAE) BY HPLC-DAD

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Summary

Quantitative Determination of Daphnoretin in Aerial Parts of *Wikstroemia indica* L. (Thymelaeaceae) by HPLC-DAD

A simple and sensitive HPLC method was proposed for the quantification of daphnoretin in aerial parts of *Wikstroemia indica* (L.) (*W. indica*). The chromatographic separations were obtained by an Agilent C_{18} column (250 x 4.6 mm, 5 µm particle size) using isocratic elution with acetonitrile and water (40/60, v/v), at a flow rate of 0.6 mL/min, with an injection volume of 10 µL and detection wavelength of 345 nm. The calibration curve displayed a good linear relationship (linearity ranging from 14.88 - 238 µg/mL and R^2=0.9999). The method was validated for system suitability (RSD of peak area = 0.21%; RSD of t_R = 0.15%); precision (RSD < 3.0%) and accuracy (recovery: 95.60-102.92%). The validation results displayed good accuracy, repeatability, linearity, and selectivity according to the ICH guideline. The percentage content of daphnoretin in aerial part samples of *W. indica* ranged from 0.010% to 0.207%. The obtained results provided useful information for quality assurance and standardization of *W. indica* in Vietnam.

Keywords: *Wikstroemia indica* (L.), *Aerial parts, HPLC-DAD, Daphnoretin.*

1. Introduction

Wikstroemia indica (WI) is a wild plant belonging to the Thymelaeaceae family. It is distributed in several countries, including Australia, China, India, Indonesia, Malaysia, Papua New Guinea, Philippines, Taiwan,