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ARYLTETRALIN LIGNANS, PHENOLIC ACID DERIVATIVES, AND AN ALKYL GLYCOSIDE FROM RHIZOMES OF *DYSOSMA TONKINENSE*

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Summary

A phytochemical study on the rhizomes of *Dysosma tonkinense* resulted in the isolation of eight compounds (**1**–**8**). Their structures were elucidated through 1D- and 2D-NMR spectroscopy, mass spectrometry (MS), and comparison with reported spectroscopic data. The isolated compounds were identified as *α*-peltatin (**1**), podophyllotoxin (**2**), picropodophyllotoxin (**3**), *α*-peltatin 2-*O*-*β*-D-glucopyranoside (**4**), ethyl 4-hydroxybenzoate (**5**), vanillic acid (**6**), a salt form of protocatechuic acid (**7**), and ethyl-*O*-*β*-D-glucopyranoside (**8**). This is the first report of compounds (**3**–**7**) from *D. tonkinense*, and notably, compound **4** is the first record from the genus *Dysosma*.

Keywords: *Dysosma tonkinense; Lignans; Phenolic acid derivatives; Sugar.*

1. Introduction

The genus *Dysosma* Woodson (Berberidaceae) comprises approximately 7-10 species, mainly distributed in China and northern Vietnam [1],[2]. It is taxonomically distinct from the related genus *Podophyllum* L., represented by *P. peltatum* in the Americas [3]. Most *Dysosma* species found in China are considered endemic, including *D. versipellis*, *D. pleiantha*, *D. aurantiocaulis*, *D. delavayi*, *D. majoensis*, *D. tsayuensis*, and *D. villosa* [1],[4],[5]. Species of this genus have been identified as rich sources of bioactive metabolites, particularly lignans (*β*-apopicropodophyllin, podophyllotoxin, picropodophyllin, podophyllotoxone, diphyllin,…) [6] and flavonoids (astragalin, kaempferid-3-*O*-*β*-D-glucopyranoside, isoquercitrin, quercetin-4'-*O*-*β*-D-glucopyranoside,…) [7]. In addition, other constituents such as anthranoids [8], phenolic acids [6], sterols [9], glucosides [10], and terpenoids [6] have also been reported in *Dysosma* species. Furthermore, extracts and isolated compounds from this genus exhibited diverse biological activities, such as anticancer [11], antioxidant [12], and antibacterial [6] properties, as well as inhibitory effects on acetylcholinesterase [13] and tyrosinase [13].

Dysosma tonkinense (Gagnep.) M. Hiroe, known in Vietnam as Bat giac lien, is a traditional medicinal plant. Its taxonomic position has been uncertain because it shares many similarities with *Podophyllum versipelle* and *Dysosma difformis*, and has sometimes been treated as the same species. In the study by Pham Ngoc Khanh et al., a combination of morphological observations and ITS sequence analysis was used to clarify its status. The results showed that *D. tonkinense* can be distinguished by its thick rhizomes, peltate leaves with 4-8 lobes, dull-red petals, and ellipsoid fruits that turn green-yellow when ripe and contain 20-25 arillate seeds. Overall, this species should be recognized as distinct and is more closely related to *D. difformis* than to *D. versipellis* [14]. Although the chemical constituents and biological activities of *D. tonkinense* have not yet been extensively investigated, its close phylogenetic relationship with other *Dysosma* species suggests considerable potential for pharmacological applications, particularly for the treatment of cancer and disorders associated with oxidative stress and inflammation. This species has long been used in traditional medicine to treat various conditions, including tuberculosis, cough, asthma, hematemesis, pharyngitis, stomachache, scrofula, boils, wound abscesses, snake bites, swelling, and ulcers [15],[16].

This study presents the isolation and structural elucidation of aryltetralin lignans, phenolic acid derivatives, and an alkyl glycoside from the rhizomes of *D. tonkinense* to support future phytochemical and pharmacological studies.

2. Materials and methods

2.1. Plant material

The rhizomes of *Dysosma tonkinense* (Gagnep.) M. Hiroe were collected in March 2024 from the Sa Pa Medicinal Plants Research

Center, Lao Cai Province, Vietnam. The samples were taxonomically authenticated by MSc. Nguyen Quynh Nga and MSc. Phan Van Truong at the Center of Medicinal Material Resources, NIMM. A voucher specimen (MT.03.05) has been deposited at the Department of Analytical Chemistry and Standardization, NIMM (Code: MT.03.05).

2.2. General experimental procedures

NMR analyses were carried out on Bruker 500 and 600 MHz spectrometers (Karlsruhe, Germany) with TMS as an internal standard. ESI-MS data were obtained on an LC-MS/MS system equipped with a DAD detector (Shimadzu, Japan). Column chromatography was performed on *silica gel* (Merck, 0.040–0.063 mm), RP-18 (Merck, 30-50 µm), and MCI gel CHP20P (Mitsubishi Chemical, 75–150 µm, Japan). Thin-layer chromatography was conducted on pre-coated *silica gel* 60 F_{254} (Merck, 0.25 mm) and *silica gel* 60 RP-18 $F_{254}S$ (Merck, 0.25 mm) plates. Compounds were visualized under UV light and by spraying with 10% sulfuric acid in ethanol followed by heating.

2.3. Extraction and isolation

Dried rhizomes of *D. tonkinense* (4.0 kg) were extracted three times with 70% EtOH at room temperature, each for 3 days. After filtration, the combined extracts were evaporated under reduced pressure to yield a brown residue (596.55 g), of which 500 g was suspended in hot water and sequentially partitioned with *n*-hexane, EtOAc, and BuOH (1:1, v/v), affording *n*-hexane (16.9 g), EtOAc (117.74 g), BuOH (83.41 g), and aqueous (249.6 g) extracts.

The EtOAc extract (110.0 g) was subjected to *silica gel* CC, eluting with 100% DCM, a DCM–MeOH gradient (50:1 → 1:1, v/v), and 100% MeOH, to afford 18 fractions (E1-E18). Fraction E6 (5.04 g) was further separated by Sephadex LH-20 CC (MeOH–H_2O 2.5:1 and 5:1, v/v, and 100% MeOH) into 18 subfractions (E6.1-E6.18). Subfraction E6.3 (0.51 g) was purified by *silica gel* CC (DCM–acetone 15:1 → 10:1, v/v) to yield compound **2** (314.4 mg). Subfraction E6.4 (1.67 g) was fractionated on MCI gel with acetone–H_2O (1:1, v/v), yielding compound **6** (139.6 mg) and five smaller subfractions (E6.4.2–E6.4.6). Compound **1** (131 mg) was obtained from E6.4.3 (301.1 mg) via *silica gel* CC (DCM–acetone 8:1, v/v). Using similar procedures, compounds **3** (11.5 mg) and **5** (8.2 mg) were isolated from E6.4.5 (225.7 mg). Fraction E15 (15 g) was separated by *silica gel* CC (DCM–MeOH 20:1 → 1:1, v/v, and 100% MeOH) into 10 subfractions (E15.1–E15.10). Subfraction E15.8 (1.5 g) was separated by Sephadex LH-20 (MeOH–H_2O 4:1, v/v) to give seven smaller subfractions (E15.8.1–E15.8.7). E15.8.1 (150 mg) was sequentially purified by RP-18 CC (MeOH–H_2O 1:1, v/v) and MCI gel CC (MeOH–H_2O 1:1, v/v) to afford compound **8** (5 mg). E15.8.2 (211 mg) was purified using MCI gel (MeOH–H_2O 2:1, v/v) followed by *silica gel* CC (DCM–MeOH 8:1, v/v) to yield compound **4** (6.7 mg). Compound **7** (8 mg) was obtained from E15.8.3 (350 mg) by MCI CC using a MeOH–H_2O (1.5:1, v/v) elution system.

Compound 1: White powder; ESI-MS: *m/z* = 399.35 $[M–H]^–$; ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1 & 2.

Compound 2: White powder; ESI-MS: *m/z* = 415.05 $[M+H]^+$; ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1 & 2.

Compound 3: White solid; ESI-MS: *m/z* = 415.25 **$[M+H]^+$**; ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1 & 2.

Compound 4: White solid; White powder; ESI-MS: *m/z* = 561.20 $[M–H]^–$; ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1 & 2.

Compound 5: Pale yellow solid; ESI-MS: *m/z* = 165.05 $[M–H]^–$; ^{1}H-NMR (500 MHz, CD_3OD): δ_H 7.84 (2H, d, *J* = 8.5 Hz, H-2, H-6), 7.77 (2H, d, *J* = 8.5 Hz, H-3, H-5), 4.30 (2H, dd, *J* = 7.0, 14.5 Hz, H-2′), 1.37 (3H, t, *J* = 7.0 Hz, H-1′); ^{13}C-NMR (125 MHz, CD_3OD) δ_C (ppm): 132.7 (C-2, C-6), 117.1 (C-3, C-5), 14.7 (C-1′), 61.5 (C-2′).

Compound 6: Pale yellow solid; ESI-MS: *m/z* = 167.05 $[M–H]^–$; ^{1}H-NMR (600 MHz, CD_3OD): δ_H 7.56 (2H, m, H-2, H-6), 6.83 (1H, d, *J* = 7.8 Hz, H-5), 3.89 (3H, s, 3-OCH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 123.8 (C-1), 113.9 (C-2), 148.6 (C-3), 152.4 (C-4), 115.8 (C-5), 125.2 (C-6), 170.5 (C-7), 56.4 (3-OCH_3).

Compound 7: Pale yellow solid; ^{1}H-NMR (600 MHz, CD_3OD): δ_H 7.49 (1H, s, H-2), 7.44 (1H, d, *J* = 8.4 Hz, H-6), 6.78 (1H, d, *J* = 8.4 Hz, H-5); ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 127.4 (C-1), 115.4 (C-2), 145.6 (C-3), 150.1 (C-4), 117.9 (C-5), 123.5 (C-6), 174.4 (C-7).

Acidification of compound 7: Compound **7** (4 mg) was dissolved in 0.1 N aqueous HCl (5 mL) and stirred at 50°C for 30 min. The reaction mixture was then partitioned with EtOAc, and the organic layer was concentrated in vacuo to afford **7a** (3.5 mg). ^{1}H-NMR (600 MHz, CD_3OD): δ_H

7.49 (1H, s, H-2), 7.45 (2H, m, H-2, H-6), 6.86 (1H, d, J = 7.8 Hz, H-5); ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 123.3 (C-1), 115.9 (C-2), 145.7 (C-3), 151.2 (C-4), 117.8 (C-5), 124.0 (C-6), 170.9 (C-7).

Compound 8: Pale yellow solid; ESI-MS: m/z = 207.05 $[M-H]^-$; ^{1}H-NMR (500 MHz, CD_3OD) δ_H (ppm): 4.28 (1H, d, J = 7.8 Hz, H-1), 3,18 (1H, dd, J = 7.8, 9.0 Hz, H-2), 3.36 (1H, m, H-3), 3.30 (2H, m, H-4, H-5), 3.63 (1H, dd, J = 5.4, 12.0 Hz, H-6a), 3.87 (1H, dd, J = 2.4, 12.0 Hz, H-6b), 1.25 (3H, t, J = 7.2, H-1′), 3.97 (1H, m, H-2′b), 3.68 (1H, dd, J = 7.2, 9.6 Hz, H-2′a); ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 104.1 (C-1), 75.1 (C-2), 78.1 (C-3), 71.7 (C-4), 77.9 (C-5), 62.8 (C-6), 15.4 (C-1′), 66.2 (C-2′).

3. Results and discussion

Eight compounds (**1**–**8**) were isolated from the 70% EtOH extract of *D. tonkinense* rhizomes through combined chromatographic techniques, and their structures were elucidated by spectroscopic analysis in comparison with reported data (**Fig. 1**).

Compound **1** was isolated as a white powder. The ESI-MS spectrum of **1** displayed a deprotonated molecular ion peak at m/z 399.35 $[M-H]^-$, consistent with the molecular formula $C_{21}H_{20}O_8$. The ^{1}H-NMR spectrum indicated six protons of two methoxy groups at δ_H 3.79 (6H, s); two geminally coupled protons of a methylenedioxy group at δ_H 5.93 (1H, d, J = 1.2 Hz) and 5.95 (1H, d, J = 1.2 Hz); three aromatic protons at δ_H 6.23 (1H, s) and 6.37 (2H, s); and aliphatic protons in the range δ_H 2.49-4.46, including two methylene and three methine groups, suggesting that **1** possesses the structure of an aryltetralin-type lignan. The ^{13}C-NMR spectrum combined with HSQC revealed signals for 21 carbons, among which the signal at δ_C 175.2 indicated the presence of a lactone ring. The coupling constants helped determine the relative stereochemistry of H-7′, H-8′, and H-8. The $J_{8,8'}$ value distinguishes *cis* (≈ 9 Hz) from *trans* (≈ 14 Hz) isomers [17]. In our data, $J_{8,8'}$ was 13.8 Hz (in CD_3OD) for compound **1**, confirming a *trans* configuration. Likewise, the relatively large $J_{7',8'}$ (4.8 Hz, in CD_3OD) indicates a *cis* relationship between H-7′ (equatorial) and H-8′ (axial). Based on these spectroscopic data and comparison with the published literature [18],[19], **1** was elucidated as *α*-peltatin.

Compound **2** was also isolated as a white powder, and its ESI-MS spectrum displayed a protonated molecular ion peak at m/z 415.05 $[M+H]^+$, corresponding to the molecular formula $C_{22}H_{22}O_8$. The NMR data of **2** were similar to those of **1**, except that the aliphatic carbon signals at δ_C 26.9 and 32.2 were shifted downfield to δ_C 72.8 (C-7) and δ_C 40.8 (C-8), suggesting hydroxylation at C-7. A further aromatic proton singlet at δ_H 7.12 indicated the absence of a hydroxyl group at C-2 on the aromatic ring. Furthermore, an additional methoxy group at δ_H 3.81, δ_C 60.8, downfield relative to the methoxy groups at C-3′ and C-5′, suggested attachment to C-4′, which was confirmed by the HMBC correlation between δ_H 3.81 and δ_C 137.3 (C-4′). The relative configurations at H-7′, H-8′, and H-8 of **2** were determined to be similar to those of **1**. As reported by Jullian-Pawlicki N. et al. [20], a large $J_{7,8}$ value is characteristic of diaxial proton coupling in the 7*α*-hydroxyl configuration. In **2**, the observed large coupling constant (10.2 Hz) confirms a diaxial relationship between H-7 and H-8. Based on these analyses, **2** was identified as podophyllotoxin [21].

Compound **3** was obtained as a white solid. Its ESI-MS spectrum displayed a protonated molecular ion peak at m/z 415.25 $[M+H]^+$, in accordance with the molecular formula $C_{22}H_{22}O_8$. The NMR data of **3** were similar to those of **2**, except for significant chemical shift differences at C-7 (δ_C 69.4, $\Delta\delta_C$ -3.4 ppm), C-8 (δ_C 42.6, $\Delta\delta_C$ +1.8 ppm), C-1′ (δ_C 139.3, $\Delta\delta_C$ +3.8 ppm), C-2′/C-6′ (δ_C 105.7, $\Delta\delta_C$ -2.8 ppm), and C-9′ (δ_C 177.8, $\Delta\delta_C$ +3.3 ppm), suggesting a configurational change at C-8′. The $J_{8,8'}$ value of 9.0 Hz confirmed a *cis* configuration between H-8 and H-8′ in **3**. Likewise, the relatively large $J_{7',8'}$ (5.4 Hz) indicated a *cis* relationship between H-7′ (equatorial) and H-8′ (axial). In addition, the small $J_{7,8}$ value (2.4 Hz) was consistent with an axial-equatorial relationship between H-7 and H-8 in the 7*α*-hydroxyl configuration [20]. Based on these spectroscopic data and comparison with reported values [17], compound **3** was identified as picropodophyllotoxin. Based on these spectroscopic data and comparison with reported values [21], **3** was determined as picropodophyllotoxin.

Compound **4** was isolated as a white solid. Its ESI-MS spectrum revealed a deprotonated molecular ion peak at m/z 561.20 $[M-H]^-$, which corresponds to the molecular formula $C_{27}H_{30}O_{13}$. The NMR spectrum of **4** was similar to that of **1** except for the presence of a *β*-glucose moiety, characterized by an anomeric proton at δ_H 5.29 (1H, d, J = 7.8 Hz), and hydroxymethine and hydroxymethylene protons in the range δ_H 3.35-3.79,

corresponding to six carbons (δ_C 103.0, 78.2 (x 2C), 75.4, 71.4, and 62.5). The sugar was attached at C-2, as indicated by HMBC correlations between H-1′ (δ_H 5.29), H-7 (δ_H 3.35), and C-2 (δ_C 139.0). The relative configurations at H-7, H-7′, H-8, and H-8′ in **4** were the same as in **1**. The spectral data matched those previously reported for *α*-peltatin 2-*O*-*β*-D-glucopyranoside [22].

Table 1. ^{1}H-NMR data of compounds **1**–**4**

No.	1		2		3	4
	δ_H [a,b]	δ_H [d,b]	δ_H [a,b]	δ_H [d,b]	δ_H [a,b]	δ_H [d,b]
2	-	-	7.12 (s)	7.14 (s)	7.04 (s)	-
5	6.23 (s)	6.12 (s)	6.50 (s)	6.46 (s)	6.38 (s)	6.29 (s)
7	2.49 (dd, 10.2, 16.2) 3.19 (dd, 4.8, 10.2)	2.47 (dd, 12.0, 16.2) 3.20 (dd, 5.4, 16.2)	4.75 (m)	4.75 (d, 10.2)	4.49 (d, 2.4)	2.55 (dd, 11.4, 16.8) 3.35 (m)
8	2.69 (m)	2.69 (m)	2.76 (m)	2.76 (m)	2.73 (m)	2.64 (m)
9	3.94 (dd, 8.4, 10.2) 4.46 (dd, 6.6, 8.4)	4.00 (dd, 8.4, 10.8) 4.48 (t, 8.4)	4.08 (t, 9.5) 4.58 (m)	4.14 (dd, 8.4, 10.2) 4.58 (m)	4.42 (dd, 6.0, 9.6) 4.50 (m)	3.97 (dd, 8.4, 10.2) 4.46 (t, 8.4)
2′, 6′	6.37 (s)	6.38 (s)	6.38 (s)	6.46 (s)	6.45 (s)	6.37 (s)
7′	4.59 (d, 3.6)	4.53 (d, 4.8)	4.58 (m)	4.58 (m)	4.11 (d, 5.4)	4.52 (d, 4.8)
8′	2.69 (m)	2.77 (dd, 4.8, 13.8)	2.82 (dd, 4.2, 14.4)	3.05 (dd, 4.8, 14.4)	3.22 (dd, 5.4, 9.0)	2.77 (dd, 4.8, 13.8)
OCH_2O	5.95 (d, 1.2) 5.93 (d, 1.2)	5.89 (d, 1.2) 5.88 (d, 1.2)	5.96 (d, 1.2) 5.98 (d, 1.2)	5.94 (d, 1.8) 5.95 (d, 1.2)	5.96 (d, 1.2) 5.98 (d, 1.2)	5.89 (d, 1.2) 5.91 (d, 1.2)
3′,5′-OCH_3	3.79 (s)	3.73 (s)	3.75 (s)	3.74 (s)	3.75 (s)	3.72 (s)
4′-OCH_3	-		3.81 (s)	3.74 (s)	3.81 (s)	-
1″						5.29 (d, 7.8)
2″-5″						3.35 – 3.47 (m)
6″						3.69 (dd, 5.4, 12.0) 3.79 (dd, 3.0, 12.0)

[a] $CDCl_3$, [b] 600 MHz, [d] CD_3OD

Table 2. ^{13}C-NMR data of compounds **1**–**4**

No.	1		α-Peltatin [18]	2	Podophyllotoxin [21]	3	Picropodophyllotoxin [21]	4	α-Peltatin glucoside [22]
	δ_C [a,c]	δ_C [d,c]	δ_C [d,e]	δ_C [a,c]	δ_C [a,c]	δ_C [a,e]	δ_C [a,c]	δ_C [d,c]	δ_C [d,c]
1	118.2	120.4	120.4	133.3	133.1	132.1	131.9	123.7	123.7
2	134.0	135.6	135.3	106.4	106.3	105.4	105.4	139.0	140.0
3	136.8	139.3	139.4	147.8	147.9	147.4	147.5	136.3	136.3
4	147.2	148.7	148.6	147.7	147.8	147.0	147.1	149.5	149.5
5	103.5	103.2	103.2	109.8	109.9	109.2	109.3	106.3	106.3
6	132.0	133.1	133.1	131.2	131.3	130.6	130.6	133.1	133.1
7	26.9	27.8	27.8	**72.8**	73.0	**69.4**	69.6	28.5	28.5
8	32.2	33.9	33.8	**40.8**	40.9	**42.6**	42.7	33.9	33.9
9	72.4	74.0	73.9	71.4	71.3	69.8	69.8	74.0	74.0
1′	131.8	133.4	133.4	**135.5**	135.3	**139.3**	139.3	133.4	133.4
2′, 6′	108.0	109.4	109.2	**108.5**	108.5	**105.7**	105.5	109.6	109.6
3′, 5′	146.4	148.5	148.4	152.6	152.7	153.6	153.7	148.6	148.6
4′	132.9	134.5	134.4	137.3	137.5	137.1	137.4	135.7	135.7
7′	43.7	45.0	44.9	44.1	44.1	44.0	44.1	45.0	45.0
8′	47.5	48.6	48.3	45.4	45.4	45.4	45.4	48.3	48.3
9′	175.2	178.1	178.1	**174.5**	174.2	**177.8**	177.6	178.0	178.0
OCH_2O	101.6	102.3	102.3	101.5	101.5	101.5	101.2	102.6	102.6
3′,5′-OCH_3	56.5	56.7	56.7	56.3	56.4	56.3	56.3	56.9	56.9
4′-OCH_3				60.8	60.8	60.8	60.9		
1″								103.0	103.0
2″								75.4	75.4
3″								78.2	78.2
4″								71.4	71.4
5″								78.2	78.2
6″								62.5	62.5

[a] $CDCl_3$, [c] 150 MHz, [d] CD_3OD, [e] 125 MHz, [f] 100 MHz.

Compound **5** was isolated as a pale yellow solid. Its ESI-MS spectrum exhibited a deprotonated molecular ion peak at *m/z* 165.05 $[M-H]^-$, consistent with the molecular formula $C_9H_{10}O_3$. The ^{1}H-NMR spectrum of **5** showed an AA′BB′ system with two proton pairs at δ_H 7.84 (2H, d, J = 8.5 Hz) and 7.77 (2H, d, J = 8.5 Hz), corresponding to two carbon signals at δ_C 132.7 and 117.1. A methyl triplet [δ_H 1.37 (3H, t, J = 7.0 Hz)] and oxymethylene signals [δ_H 4.30 (2H, dd, J = 7.0, 14.5 Hz), δ_C 61.5] indicated the presence of an ethyl group. Signals of non-protonated carbons were not observed, which is likely due to the low sample amount of **5**. Based on these spectral data and comparison with the reported literature [23], **5** was confirmed as ethyl 4-hydroxybenzoate.

Compound **6** was obtained as a pale-yellow solid. Its ESI-MS spectrum displayed a deprotonated molecular ion peak at *m/z* 167.05 $[M-H]^-$, which corresponds to the molecular formula $C_8H_8O_4$. The ^{1}H-NMR spectrum of **6** showed three aromatic protons at δ_H 7.56 (2H, m, H-2, H-6) and 6.83 (1H, d, J = 7.8 Hz, H-5) of an ABX system, and a methoxy singlet at δ_H 3.89 (3H, s). The ^{13}C-NMR spectrum of **6** revealed eight carbons, including a carboxylic carbonyl (δ_C 170.5), two oxygenated aromatic carbons (δ_C 148.6 and 152.4), one quaternary carbon (δ_C 123.8), three methine carbons (δ_C 113.9, 115.8, and 125.2), and one methoxy carbon (δ_C 56.4). HMBC correlations between protons H-2 (δ_H 7.56), H-5 (δ_H 6.83), and the methoxy proton (δ_H 3.89) with C-3 (δ_C 148.6) confirmed the methoxy position. By comparing its spectral data with reported compounds [24], **6** was identified as vanillic acid.

Compound **7** was isolated as a pale yellow solid. Its spectral data closely resembled those of **6**, except for the absence of a methoxy group. Comparison of the ^{1}H- and ^{13}C-NMR data of **7** with those of protocatechuic acid [25] showed similar spectra, except for chemical shift differences at C-1 (δ_C 127.4, $\Delta\delta_C$ +3.7 ppm), C-7 (δ_C 174.4, $\Delta\delta_C$ +3.8 ppm), suggesting that **7** was obtained as a carboxylate salt. Although the ESI-MS data did not reveal the counter cation, the presence of a carboxylate ion was confirmed by converting **7** to its free acid form (**7a**) with 0.1 N HCl. The ^{1}H- and ^{13}C-NMR data of **7a** exactly matched those reported for protocatechuic acid [25]. Thus, compound **7** was established as a salt form of protocatechuic acid.

Compound **8**, obtained as a pale-yellow solid, exhibited a protonated molecular ion peak at *m/z* 207.05 $[M-H]^-$ in its ESI-MS spectrum, in agreement with the molecular formula $C_8H_{16}O_6$. The ^{1}H-NMR spectrum revealed five hydroxymethine protons (δ_H 3.18–4.28**)**, including an anomeric proton at δ_H 4.28 (1H, d, J = 7.8 Hz, H-1) along with a methyl signal at δ_H 1.25. The ^{13}C-NMR spectrum was in agreement, showing five oxymethine carbons (δ_C 104.1, 77.9, 78.1, 75.1, and 71.7), two oxymethylene carbons (δ_C 62.8 and 66.2), and a methyl carbon (δ_C 15.4). The ethyl group was characterized by a methyl triplet at δ_H 1.25 (3H, t, J = 7.2 Hz) and oxymethylene signals at δ_H 3.79 (1H, m) and 3.68 (1H, dd, J = 7.2, 9.6 Hz), δ_C 66.2. On the basis of these spectroscopic data and comparison with reported data [26], **8** was assigned as ethyl-*O*-*β*-D-glucopyranoside.

Currently, no studies have investigated the chemical constituents or biological activities of *D. tonkinense*. In Vietnam, some studies, such as those by Man Thanh Long (2018) [27] and Nguyen Thi Dung (2018) [28], have been conducted on *D. difformis*, which was considered a synonym of *P. tonkinense* in that publication. These studies reported the isolation of seven compounds, namely podophyllotoxin, deoxypodophyllotoxin, kaempferol, quercetin, *α*-peltatin, rutin, and nicotiflorin. Extracts and isolated compounds from this species were also evaluated for their DPPH radical scavenging activity and cytotoxicity against cancer cell lines. In addition, Cao Thanh Mai (2014) described the morphological features of the examined specimens and proposed that they might belong to one of three species: *D. pleiantha* (Hance) Woodson (*P. pleianthum* Hance), *D. majoensis* (Gagnepain) M. Hiroe (*P. majoense* Gagnepain), or *D. difformis* (Hemsley & E. H. Wilson) T. H. Wang ex T. S. Ying (*P. tonkinense* Gagnepain). A preliminary phytochemical screening revealed the presence

of flavonoids, saponins, coumarins, cardiac glycosides, and reducing sugars in the rhizomes, with flavonoids being the most abundant [29]. However, according to Pham Ngoc Khanh (2020), *D. differmis* is a distinct species (or a sister species) of *D. tonkinense* [14].

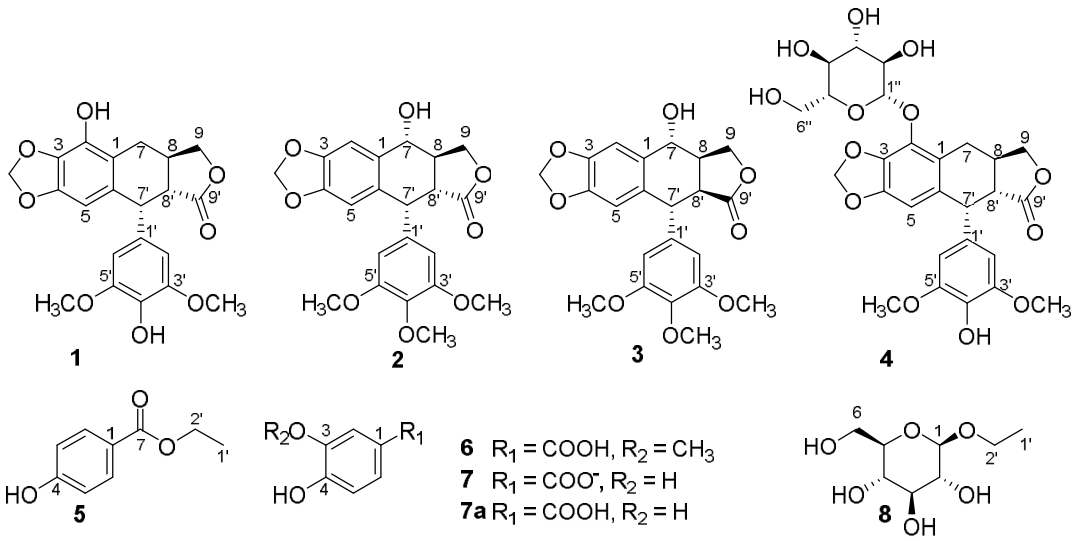

Fig. 1. Chemical structure of isolated compounds (**1** – **7**) from *D. tonkinense*

In the present study, lignans, phenolic acid derivatives, and an alkyl glycoside were isolated and structurally elucidated from *D. tonkinense* rhizomes. These findings are consistent with previous reports on the genus *Dysosma*, which is known to be rich in lignans (podophyllins) [6], especially podophyllotoxin, a compound with diverse biological activities such as antitumor, antiviral, anti-inflammatory, and immunosuppressive effects,... Nevertheless, because of its strong toxicity, podophyllotoxin is mainly used as a precursor for the semisynthesis of less toxic and more effective derivatives such as etoposide and teniposide [30]. Besides lignans, other classes of compounds, including phenolic acids, sugars, and their derivatives, have also been reported in the genus *Dysosma*. For instance, 4-hydroxybenzoic acid [31] and sucrose [32] have been identified in *D. versipellis* [31], while ethyl *β*-D-glucoside has been found in *D. differmis* [10].

4. Conclusion

Four lignans (**1**–**4**), three phenolic acid derivatives (**5**–**7**), and an alkyl glycoside (**8**) were isolated and structurally identified from the rhizomes of *D. tonkinense*. Among them, compounds **3**–**7** are reported from this species for the first time, and *α*-peltatin 2-*O*-glucopyranoside (**4**) is additionally reported as the first record from the genus *Dysosma*. These findings expand the chemical profile of *D. tonkinense* and provide a valuable basis for further studies on its potential biological activities.

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