Research article

α-Glucosidase inhibitory activity of compounds isolated from the twig and leaf extracts of *Desmos dumosus*

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ABSTRACT

Four new compounds, (+)-(2S)-desmosdumosone (**1**), (+)-(2R)-7,8-dimethoxy-5-hydroxyflavanone (**7**), (+)-(2R)-7-methoxychamanetin (**9**), and (+)-(1′R,2′R)-phebalosin (**18**), and 25 known compounds were isolated from the twig and leaf extracts of *Desmos dumosus*. Compounds (±)-**7** and (±)-**9** were isolated as racemates and their enantiomers were separated by chiral HPLC. Their structures were elucidated by spectroscopic methods as well as comparisons made from the literature. The absolute configuration of (+)-(1′R,2′R)-**18** was established by X-ray diffraction analysis using Cu Kα radiation and electronic circular dichroism (ECD) spectroscopy. In contrast, the absolute configuration of compounds (+)-(2S)-**1**, (+)-(2R)-**7**, and (+)-(2R)-**9** were identified by comparing their ECD spectra and specific rotations with those of reported known compounds. Compounds **9**, **11**, **13**, **14**, **22**, **25**, and **28** showed α-glucosidase inhibitory activities with IC_{50} values ranging from 5.3-52.7 μM, much better than that of standard control (acarbose, IC_{50} value 83.5 μM). Compound **13** was the most active with an IC_{50} value of 5.3 μM.

1. Introduction

Diabetes mellitus (DM) accompanied by an increased blood glucose level is a metabolic disorder in which the pancreatic beta cells generate insufficient insulin [1]. Multiple complications can arise, including both macro and microvascular dysfunctions [2], where complete treatment with insulin and oral hypoglycemic agents without side effects is difficult [3]. In the recent literature, many phenolic compounds isolated from Annonaceae plants have been recommended to treat diabetes mellitus alone or in combination with hypoglycemic agents [4, 5].

Desmos dumosus (Roxb.) Saff. is a shrub belonging to the Annonaceae family and is distributed throughout Southeast Asia and Northern Australia [6]. The roots and leaves of this plant have been used in traditional medicines to treat malaria, inflammation, rheumatoid arthritis, roseola infantum rash, and dysentery in Chinese folk medicine [7, 8, 9, 10]. Previous phytochemical investigations of *Desmos* genus

resulted in the isolation and identification of several types of compounds including, polyoxygenated flavonoids [7, 8, 9, 10, 11], cinnamylphenol [9, 12], artefact [11], aristolactam [13], aporphine [11, 13, 14], berberine [13, 14], orientalinone [11, 13], canadine [11, 13], isoquinoline [13, 14], morphinandienone alkaloids [14], benzoate ester [6], *C*-benzylated chalcone [10], sterol [15], hybrid flavan-chalcone [16], and oxepinone [17]. Some of these isolated compounds displayed a wide range of biological activities, including anti-HIV [12], anti-plasmodial [13], cytotoxicity [9, 13], antioxidant, aromatase and lipoxygenase inhibition [16], anti-fungal [18], and α-glucosidase [17], and tyrosine kinase inhibitory [19] activities. In this study, we report herein the isolation, structure elucidation, resolution, and absolute configuration assignments of three new flavonoids (**1**, **7**, and **9**) and one coumarin (**18**) together with 25 known compounds (**2–29**) from the twig and leaf extracts of *D. dumosus*, which were collected from Doi Tung, Chiang Rai

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Province, Thailand. The crude extracts and some of isolated compounds were evaluated their α-glucosidase inhibitory activities.

2. Materials and methods

2.1. General experimental procedures

Melting points were measured on a Buchi melting point B-540 visual thermometer. The optical rotations were measured with a Bellingham and Stanley APD440 polarimeter. The UV spectra were recorded with a Perkin Elmer UV-Vis spectrophotometer. Infrared (IR) spectra were recorded using a Perkin Elmer Frontier Optica FT-IR spectrometer. Electronic circular dichroism spectra were recorded on a JASCO J-815 spectrometer. The NMR spectra recorded using 400 MHz Bruker FT-NMR Ultra Shield and 600 MHz Bruker AV-600 spectrophotometers. The HRESITOFMS were obtained on a Bruker microTOF mass spectrometer and Bruker-Hewlett-Packeard 1100 Esquire-LC system mass spectrometer. The HREIMS were recorded using Kratos MS-50 mass spectrometer. Chiral HPLC was performed on a CHIRALPAKIA 15 column of 10–250 mm and attached to Waters 2487 Dual λ absorbance detector.

2.2. Plant material

The twigs and leaves of *D. dumosus* were collected from Doi Tung, Chiang Rai Province, Thailand in February 2016 (GPS coordinates: 20°17′30″N 99°48′30″E). The plant was identified by Mr. Matin Van de Bult (Doi Tung Development Project, Chiang Rai, Thailand). The specimen (MFU-NPR0110) was deposited at the Natural Products Research Laboratory, School of Science, Mae Fah Luang University.

2.3. Extraction and isolation

The fresh twigs of *D. dumosus* were dried at temperature (ca 27–35 °C, in summer season) for a week. Air-dried twigs of *D. dumosus* were extracted with EtOAc (20 L) for three days at room temperature. The crude extract (92.72 g) was subjected to QCC over silica gel using a gradient of hexanes and EtOAc (100% hexanes to 100% EtOAc) to afford 12 fractions (TF1-TF12). Fraction TF5 (7.70 g) was further fractionated by CC over Sephadex-LH 20 (100% MeOH) to provide nine subfractions (TF5A-TF5I). Subfraction TF5E (1.45 g) was further separated by CC (1:4, *v/v* EtOAc/hexanes) to give compound **5** (35.5 mg) together with six subfractions (TF5E1-TF5E6). Compounds **4** (67.5 mg) and **6** (21.5 mg) were obtained from subfraction TF5E2 (421 mg) by CC (1:4, *v/v* EtOAc/hexanes), whereas compounds **1** (4.5 mg) and **13** (5.0 mg) were isolated from subfraction TF5E5 (125.6 mg) by CC (1:4, *v/v* EtOAc/hexanes). Subfraction TF5C (402.3 mg) was further separated by CC (1:4, *v/v* EtOAc/hexanes) to provide compounds **14** (13.5 mg) and **16** (3.9 mg). Fraction TF2 (12.83 g) was separated by CC (1:19, *v/v* EtOAc/hexanes) to give 11 subfractions (TF2A-TF2L). Fraction TF9 (2.42 g) was purified by CC (1:4, *v/v* EtOAc/hexanes) to afford 12 subfractions (TF9A-TF9L) and compound **25** (99.8 mg) was isolated from subfraction TF9F (108.4 mg) by CC (1:9, *v/v* EtOAc/CH₂Cl₂). Subfraction TF9J (339.8 mg) was separated by CC over Sephadex-LH 20 (100% MeOH) to provide four subfractions (TF9J1-TF9J4). Compound **19** (107.6 mg) was isolated from subfraction TF9J2 (301.7 mg) by CC (1:19, *v/v* EtOAc/hexanes). Subfraction TF9I (271.7 mg) was purified by CC over Sephadex LH-20 (100% MeOH) to afford four subfractions (TF9I1-TF9I4). Compound **20** (26.6 mg) was obtained from subfraction TF9I2 (31.9 mg) by CC (1:4, *v/v* EtOAc/hexanes), whereas compound **21** (5.0 mg) was isolated from TF9I4 (9.0 mg) by PTLC (1:10, *v/v* EtOAc/hexanes). Fraction TF7 (5.11 g) was further fractionated by CC over Sephadex LH-20 (100% MeOH) to give five subfractions (TF7A-TF7E). Subfraction TF7D (356.2 mg) was separated by CC (1:50, *v/v* EtOAc/CH₂Cl₂) to give nine subfractions (TF7D1-TF7D9). Compound **22** (1.3 mg) together with eight subfractions (TF7D8A-TF7D8H) were isolated from subfraction TF7D8 (128.1 mg) by CC (1:50, *v/v* EtOAc/CH₂Cl₂). Compounds **24** (7.3 mg) and **26** (5.4 mg)

was isolated from subfraction TF7D8E (57.2 mg) by CC (1:4, *v/v* EtOAc/hexanes). Subfraction TF7D4 (52.3 mg) was purified by CC (3:10, *v/v* EtOAc/hexanes) to afford compound **23** (3.0 mg). Finally, fraction TF8 (499.7 mg) was separated by CC (1:5, *v/v* EtOAc/hexanes) to provide 17 subfractions (TF8A-TF8Q). Subfraction TF8O (69.3 mg) was purified by CC (2:8, *v/v* acetone/CH₂Cl₂) to yield a mixture of **2** and **3** (8.1 mg) and a mixture of **11** and **12** (15.5 mg). The mixture of compounds **11** and **12** (15.5 mg) was purified using preparative HPLC to provide two peaks eluting at 32.67 min (11, 0.6 mg) and 36.22 min (12, 0.6 mg), which was eluted with 8:2, *v/v* ACN-H₂O, 2.5 mL/min (Figures 1 and 2).

The fresh leaves of *D. dumosus* were dried at temperature (ca 27–35 °C, in summer season) for a week. Air-dried leaves of *D. dumosus* (LF, 336.7 g) were extracted with EtOAc (20 L) for three days at room temperature. The EtOAc extract (35.74 g) was subjected to QCC over silica gel and eluted with gradient of hexanes and EtOAc (100% hexanes to 100% EtOAc) to yield ten fractions (LF1-LF10). Fraction LF6 (6.73 g) was separated by RP CC (7:3, *v/v* MeOH/H₂O) to provide 14 subfractions (LF6A-LF6N). Fraction LF6B (691.8 mg) was further purified by CC (1:4, *v/v* EtOAc/hexanes) to afford compounds **29** (3.3 mg), **17** (7.3 mg), **18** (50.1 mg), and **28** (5.2 mg). Compound **7** (4.3 mg) was purified from subfraction LF6C (444.1 mg) by CC (1:4, *v/v* acetone/hexanes). Subfraction LF6G4 (301.7 mg) was further isolated by CC (1:8, *v/v* EtOAc/CH₂Cl₂) to provide **27** (1.2 mg) and **10** (4.7 mg). Compounds **8** (2.3 mg) and **9** (11.9 mg) were purified from subfraction LF6F (156.7 mg) by CC (1:4, *v/v* acetone/hexanes). Fraction LF7 (3.74 g) was separated by CC over Sephadex-LH20 (100% MeOH) to give six subfractions (LF7A-LF7F). Subfraction LF7D (652.7 mg) was further separated by CC over Sephadex LH-20 (100% MeOH) to afford six subfractions (LF7D1-LF7D6), and compound **15** (7.3 mg) was purified by RP (1:1, *v/v* MeOH/H₂O) from subfraction LF7D3 (223.0 mg) (Figure 3).

2.3.1. (+)-(2S)-Desmosdumosone (1)

Yellow viscous oil, $[\alpha]_D^{25}$ + 16 (*c* 1.5, MeOH), UV (MeOH) λ_{max} (log ε) 231 (2.07), 282 (2.04) and 341 (1.73) nm; IR (neat) v_{max} 3364, 2923, 2853, 1615, 1580, 1449, 1354, 1121, 1045, 878 and 768 cm⁻¹; ECD (7.49 × 10⁻³ M, MeOH) λ_{max} (Δε) 227 (+0.92), 242 (−0.45), 282 (−0.46), 297 (+0.26), 331 (+0.08) nm. ¹H and ¹³C NMR data, see Table 1; HRESITOFMS *m/z* 299.0913 [M − H]⁺ (calcd for C₁₇H₁₅O₅, 299.0919).

2.3.2. (±)-7,8-Dimethoxy-5-hydroxyflavanone (7)

Colorless crystals, mp 158–159 °C, UV (MeOH) λ_{max} (log ε) 215 (0.16), 290 (0.04), 345 (0.60) nm; IR (neat) v_{max} 3414, 1626, 1452, 1323, 1221 and 826 cm⁻¹.

2.3.3. Resolution of compound 7 and ECD spectroscopic data of (−)-(2S)-7 and (+)-(2R)-7

Resolution of compound (±)-**7** (4.3 mg) was performed by semi-preparative HPLC on a chiral column (CHIRALCEL OD-H, 4.6 × 250 mm, flow rate 1 mL/min, *n*-hexane/MeOH/iPrOH, 70:20:10 *v/v*).

Compound (−)-(2S)-**7** (t_R 7 min) [(1.3 mg), $[\alpha]_D^{23}$ −23 (*c* 0.1, CHCl₃)]; ECD (1.0 × 10⁻³ M, MeOH) λ_{max} (Δε) 225 (+1.27), 287 (−1.15), 331 (+0.60) nm.

Compound (+)-(2R)-**7** (t_R 7.5 min) [(0.3 mg), $[\alpha]_D^{23}$ + 31 (*c* 0.1, CHCl₃)]; ECD (1.0 × 10⁻³ M, MeOH) λ_{max} (Δε) 225 (−1.19), 287 (+1.26), 331 (−0.54) nm]; ¹H and ¹³C NMR data, see Table 2; HRESI-TOFMS *m/z* 323.0898 [M + Na]⁺ (calcd for C₁₇H₁₆O₅Na, 323.0895).

2.3.4. (±)-7-Methoxychamanetin (9)

Colorless viscous oil, UV (MeOH) λ_{max} (log ε) 216 (0.03), 289 (0.37), 346 (1.21) nm; IR (neat) v_{max} 3452, 2937, 1636, 1497, 1313, 1100 and 1024 cm⁻¹.
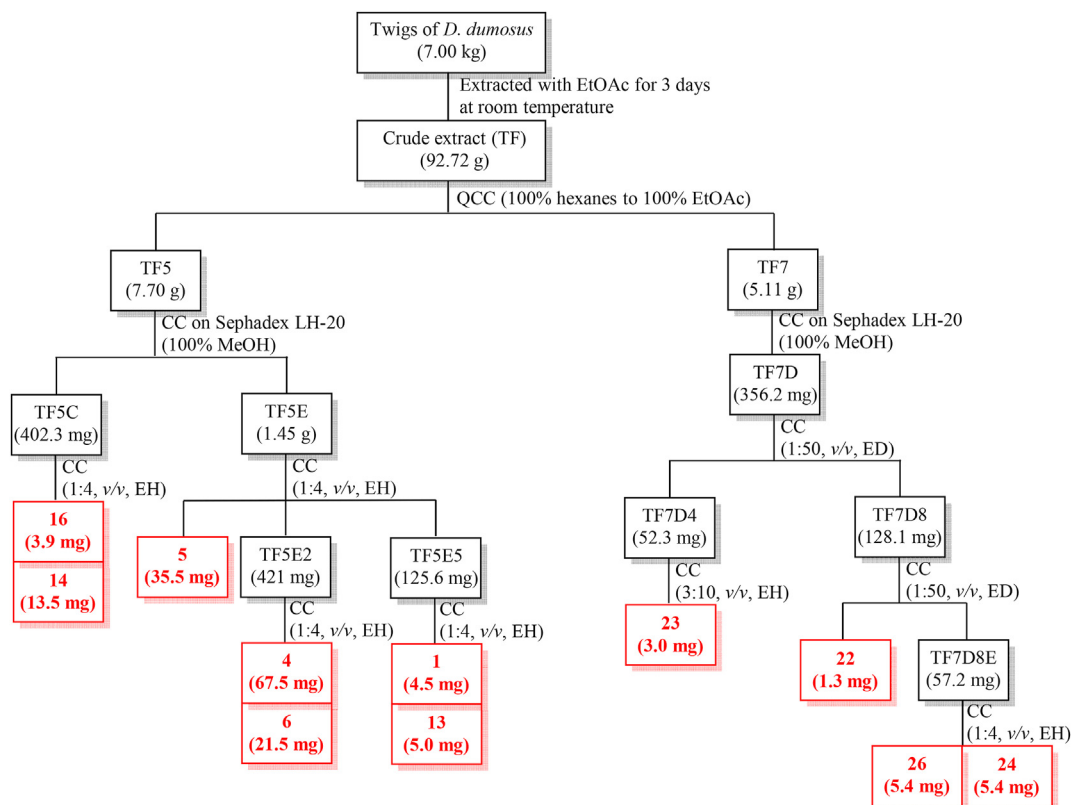
Figure 1. Isolation and purification diagram of *D. dumosus* twigs (Fr. TF5 and TF7).
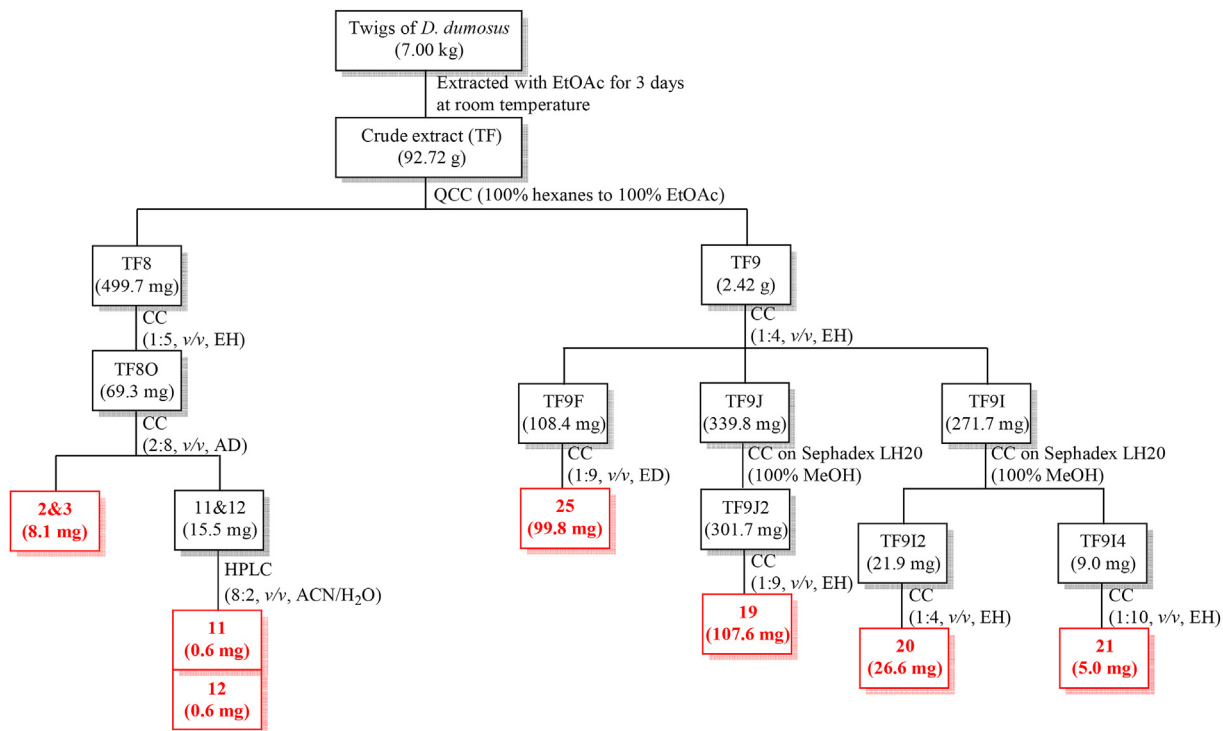

Figure 2. Isolation and purification diagram of *D. dumosus* twigs (Fr. TF8 and TF9).
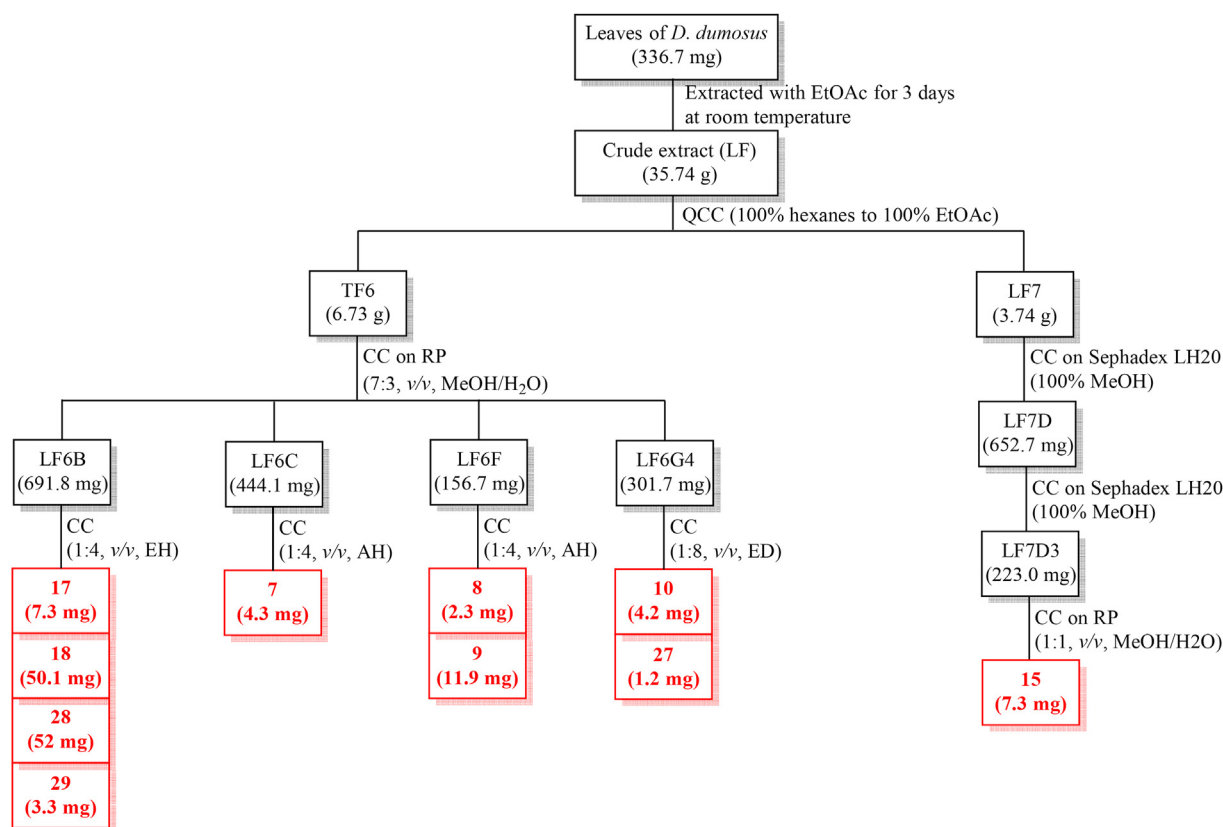
Figure 3. Isolation and purification diagram of *D. dumosus* leaves.

Table 1. NMR spectroscopic data of compound (+)-**1** (600 MHz in CDCl₃).

Position	δ_C	δ_H mult. (*J* in Hz)
2	101.3	
3	48.4	3.09, s
4	194.8	
4a	104.9	
5	159.2	
6	103.0	
7	160.9	
8	102.4	
8a	154.2	
6-CH₃	8.0	2.11, s
8-CH₃	7.0	2.16, s
1′	142.3	
2′	125.1	7.73, d (7.0)
3′	128.9	7.49, t (7.3)
4′	129.3	7.47, d (7.1)
5′	128.9	7.49, t (7.3)
6′	125.1	7.73, d (7.0)
5-OH		12.19, s

2.3.5. Resolution of compound **9** and ECD spectroscopic data of (−)-(2S)-**9** and (+)-(2R)-**9**

Resolution of compound (±)-**9** (1.3 mg) was performed by semi-preparative HPLC on a chiral column (CHIRALCEL OD-H, 4.6 × 250 mm, flow rate 1 mL/min, *n*-hexane/iPrOH, 90:10 *v/v*).

Compound (−)-(2S)-**9** (t_R 7 min) [(0.5 mg), $[\alpha]_D^{23}$ −71 (*c* 0.1, acetone)]; ECD (3.12 × 10⁻³ M, MeOH) λ_{max} (Δε) 220 (+0.89), 287 (−0.90), 335 (+0.45) nm].

Compound (+)-(2R)-**9** (t_R 13 min) [(0.3 mg), $[\alpha]_D^{23}$ + 56.7 (*c* 0.1, acetone)]; ECD (3.12 × 10⁻³ M, MeOH) λ_{max} (Δε) 220 (−0.86), 287 (+0.85), 335 (−0.34) nm]; ¹H and ¹³C NMR data, see Table 2; HRESI-TOFMS *m/z* 399.1211 [M + Na]⁺ (calcd for C₂₃H₂₀O₅Na, 399.1208).

2.3.6. (+)-(1′R, 2′R)-Phebalosin (**18**)

Colorless crystals, $[\alpha]_D^{23}$ + 18 (*c* 0.5, CHCl₃), mp 121–122 °C, UV (MeOH) λ_{max} (log ε) 322 (0.97), and 211 (1.12) nm; IR (neat) υ_{max} 1726, 1605, 1496, 1252, and 835 cm⁻¹; ECD (4.12 × 10⁻³ M, MeOH) λ_{max} (Δε) 213 (+2.01), 250 (−0.72), 270 (+0.68), 293 (−0.89) and 335 (+1.74) nm. ¹H and ¹³C NMR data, see Table 3; HREIMS *m/z* 258.08961 [M]⁺ (calcd for 258.08921, C₁₅H₁₄O₄).

2.4. X-ray crystallographic analysis of compounds (±)-7 and (+)-18

Single colorless crystals of (±)-**7** (0.36 × 0.23 × 0.05 mm³) and (+)-**18** (0.40 × 0.06 × 0.04 mm³) were selected and mounted on a mylar loop in oil on a Bruker APEX II area detector diffractometer. The structure was solved with the XT [20] structure solution program using the Intrinsic Phasing solution method and using Olex2 [21] as the graphical interface. The model was refined with version 2018/3 of XL [22] using Least Squares minimization.

X-Ray Crystallographic Data for (±)-7,8-dimethoxy-5-hydroxy-flavanone: C₁₇H₁₆O₅, *M* = 300.30, crystal size = 0.40 × 0.06 × 0.04 mm³, monoclinic, space group *P2₁/c*, *a* = 5.16560 (10) Å, *b* = 25.7165 (7) Å, *c* = 10.6823 (3) Å, α = 90°, β = 90.631° (2), γ = 90°, *V* = 1418.96 (6) Å³, *T* = 90 (2) K, *Z* = 4, *Dcalc* = 1.406 g/cm³. The x-ray diffraction analysis using Cu Kα radiation values was = 0.862 cm⁻¹, 13976 reflections measured, 2483 unique (R_int = 0.0398) which were used in all

Table 2. NMR spectroscopic data of compounds (+)-**7** (500 MHz in CDCl$_3$) and (+)-**9** (600 MHz in CDCl$_3$).

Position	(+)-7		(+)-9	
	δ_C	δ_H mult. (J in Hz)	δ_C	δ_H mult. (J in Hz)
2	79.2	5.48, dd (12.3, 3.2)	79.5	5.52, dd (13.2, 2.9)
3	43.5	3.08, dd (17.2, 12.3); 2.90, dd (12.3, 3.2)	42.9	3.20, dd (17.2, 13.2); 2.89, dd (17.2, 2.9)
4	196.1		195.7	
4a	102.6		102.8	
5	159.9		162.7	
6	93.1	6.12, s	92.7	6.22, s
6a			22.8	3.86, d (6.3)
7	161.6		164.0	
8	129.8		106.8	
8a	153.7		158.3	
1′	138.6		137.4	
2′, 5′	126.0	7.48, d (7.2)	126.0	7.57, m
3′, 6′	128.9	7.43, t (7.3)	128.6	
4′	128.8	7.38, t (7.3)	128.8	
1″			124.9	
2″			153.9	
3″			119.5	6.80, t (8.0)
4″			127.5	7.13, m
5″			115.6	6.86, d (8.0)
6″			131.3	7.29, dd (8.0, 1.2)
5-OH		11.97, s		12.18, s
7-OMe	56.3	3.90, s	55.9	4.02, s
8-OMe	61.5	3.79, s		

Table 3. NMR spectroscopic data of compound (+)-**18** (400 MHz in CDCl$_3$).

Position	δ_C	δ_H mult. (J in Hz)
2	160.3	
3	113.4	6.29, d (9.5)
4	143.4	7.65, d (9.5)
4a	112.8	
5	128.8	7.43, d (8.7)
6	107.6	6.89, d (8.7)
7	162.0	
8	112.6	
8a	153.9	
1′	51.8	4.00, d (2.4)
2′	60.7	3.93, d (2.4)
3′	141.3	
4′	113.5	5.31, brs; 5.09, brs
5′	17.4	1.88, s
7-OMe	56.4	3.98, s

calculations. The final wR_2 was 0.0886 (all data), R_1 was 0.0330 (I > 2(I)) and R_1 was 0.0412 (all data). The crystallographic data of (±)-**7** (Figure 7) have been deposited in the Cambridge Crystallographic Data Centre as CCDC 2006685 and data can be obtained free of charge from the via http://www.ccdc.cam.ac.uk/data_request/cif.

X-Ray Crystallographic Data for (+)-phebalosin: $C_{16}H_{16}Cl_2O_4$, $M = 343.19$, crystal size = 0.36 × 0.23 × 0.05 mm^3, orthorhombic, space group $P2_12_12_1$, $a = 4.04710 (10)$ Å, $b = 16.5976 (5)$ Å, $c = 23.3280 (8)$ Å, $\alpha = 90^o$, $\beta = 90^o$, $\gamma = 90^o$, $V = 1566.99 (8)$ Å^3, $T = 90 (2)$ K, $Z = 4$, $Dcalc = 1.455$ g/cm^3. The x-ray diffraction analysis using Cu Kα radiation values

was 3.866 cm^{-1}, 11560 reflections measured, 2762 unique ($R_{int} = 0.0389$) which were used in all calculations. The final wR_2 was 0.1267 (all data), R_1 was 0.0525 (I > 2(I)) and R_1 was 0.0534 (all data). The absolute configurations of compound (+)-**18** was assigned as *1′R2′R* (Figure 8) with a Flack parameter was 0.072 (8). The crystallographic data of (+)-**18** have been deposited in the Cambridge Crystallographic Data Centre as CCDC 2006686 and data can be obtained free of charge from the via http://www.ccdc.cam.ac.uk/data_request/cif.

2.5. α-Glucosidase inhibitory activity

A colorimetric α-glucosidase assay was performed according to the previously described method [4, 5]. Briefly, the samples were dissolved in DMSO and further diluted with KH$_2$PO$_4$ buffer (c 100 mM, pH 6.8) to make the final concentration of 10% DMSO. The substrate, *p*-nitrophenyl α-D-glucoside (Sigma, St. Louis, USA; cat. no. N1377), was prepared in KH$_2$PO$_4$ buffer (c 1.5 mM). The mixture of 100 μL of enzyme solution (c 0.35 U/mL) and 50 μL of the isolated compounds was preincubated at 37 °C for 10 min. The enzymatic reaction was started by adding 100 μL of a substrate (c 1.5 mM), and the mixture was further incubated at 37 °C for 20 min. The enzyme activity was terminated by adding Na$_2$CO$_3$ (c 1 M, 1 mL), and the absorption at 405 nm was measured immediately. Acarbose was used as a positive control with an IC$_{50}$ value of 83.5 μM.

3. Results and discussion

3.1. Isolation and structure elucidation

The isolation and elucidation of EtOAc extracts of twigs and leaves from *D. dumosus* was purified by repeated chromatographic techniques to obtain fourteen flavonoids, a chalcone, two benzyl benzoates, two coumarins, eight aristolactam alkaloids, a *seco*-cyclohexenes, and a cyclohexenes compounds (Figure 4) were identified to be 2,5,7-trihydroxyl-8-formyl-6-methylflavanone (or desmal) (**2**) [19,23] 2,5,7-trihydroxyl-6-formyl-8-methylflavanone (**3**) [23] 5,7-dihydroxy-8-formyl-6-methylflavanone (or desmosal) (**4**) [7], 5,7-dihydroxy-8-formyl-6-methylflavanone (**5**) [24], demethoxymetteucinol (**6**) [24,25], 7,8-dimethoxy-5-hydroxy-flavanone (**7**) [45], 5-*O*-methylchamanetin (or chamanetin 5-Me ether) (**8**) [26], 7-methoxychamanetin (or 7-*O*-methylchamanetin) (**9**) [26,48, 49], cryptochrysin (**10**) [27], 5,7-dihydroxy-6-methyl-8-formylflavone (**11**) [24], 5,7-dihydroxy-8-methyl-6-formylflavone (or isounonal) (**12**) [24], matteuorien (**13**) [25], desmosdumotin B (or dasytrichon) (**14**) [28], desmosdumotin C (**15**) [29], benzyl benzoate (**16**) [30], 8-methoxycoumarin (**17**) [31], aristolactam BII (**19**) [32-36], 3,5-dihydroxy-2,4-dimethoxyaristolactam (**20**) [33], aristolactam BIII (**21**) [32], 10-amino-3,6-dihydroxy-2,4-dimethoxyphenanthrene-1-carboxylic acid lactam (**22**) [34], goniopedalin (**23**) [34], stigmalactam (**24**) [34], piperolactam D (**25**) [35], aristolactam I (**26**) [36], flexuvarin B (**27**) [37], cherrevenol A (**28**) [38], and benzoic acid (**29**) [39]. These structures were confirmed by comparisons of their spectroscopic and chemical properties with previously reported data.

Compound **1** was obtained from the twig extract as a yellow viscous oil. Its molecular formula was established as $C_{17}H_{15}O_5$, based on HRESITOFMS, which showed an ion peak at *m/z* 299.0913 [M − H]$^-$ (calcd for 299.0919). The UV spectrum displayed characteristics of flavanone at λ_{max} 231, 282, and 341 nm [40, 41], while the IR spectrum showed bands for hydroxy (3364 cm^{-1}) and conjugated carbonyl (1615 cm^{-1}) functionalities. The ^{1}H and ^{13}C NMR data of compound **1** (Table 1) displayed resonances for hydrogen-bonded hydroxy proton [δ_H 12.19 (1H, s, OH-5)], a set of monosubstituted aromatic protons [δ_H 7.73 (2H, t, $J = 7.0$ Hz, H-2′/6′)/δ_C 125.1, 7.47 (2H, d, $J = 7.0$ Hz, H-4′)/δ_C 129.3, and δ_H 7.49 (2H, t, $J = 7.3$ Hz, H-3′/5′)/δ_C 128.9], methylene protons [δ_H 3.09 (2H, s, CH$_2$-3)/δ_C 48.4], and two methyl groups [δ_H 2.11 (3H, s, CH$_3$-6)/δ_C 8.0 and δ_H 2.16 (3H, s, CH$_3$-8)/δ_C 7.0]. The locations of the three hydroxy groups (C-2, C-5, and C-7) were assigned from the HMBC correlations shown in Figure 5. The unaccustomed lower field chemical
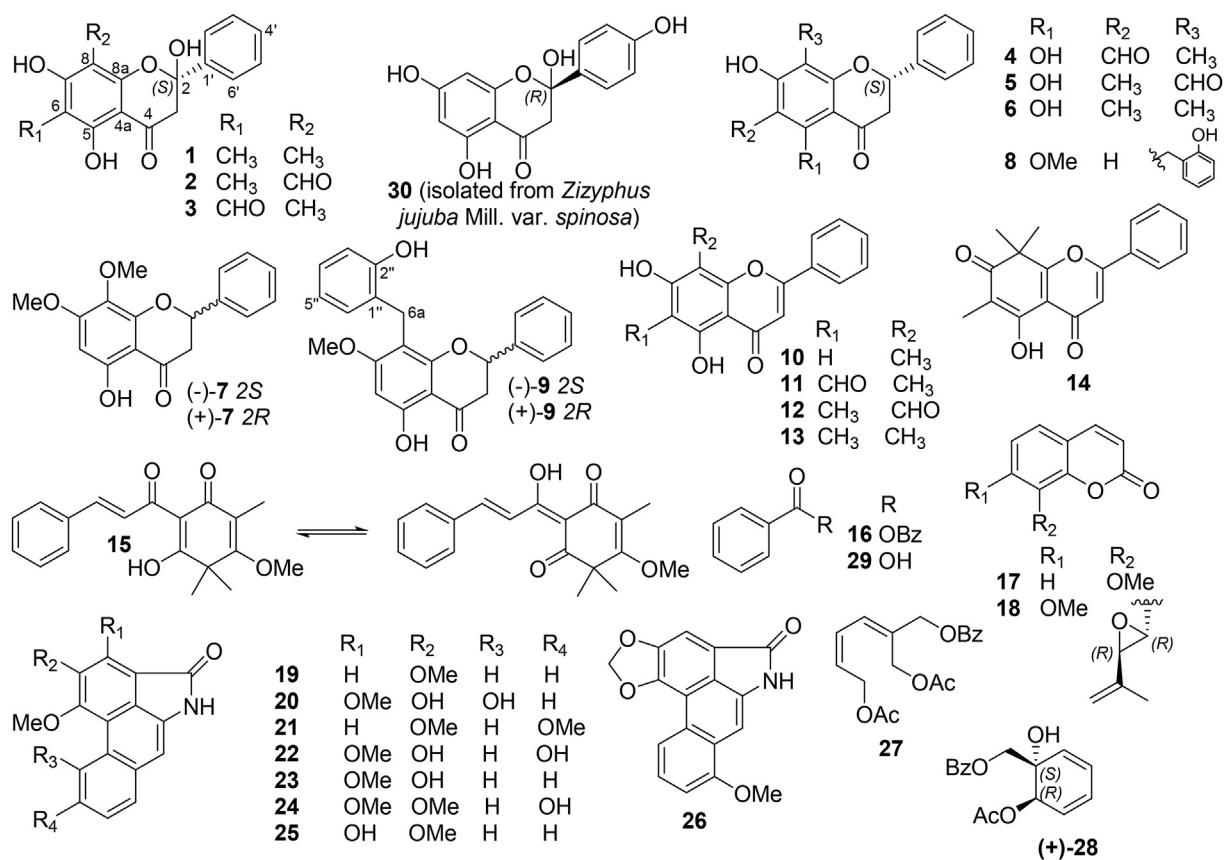
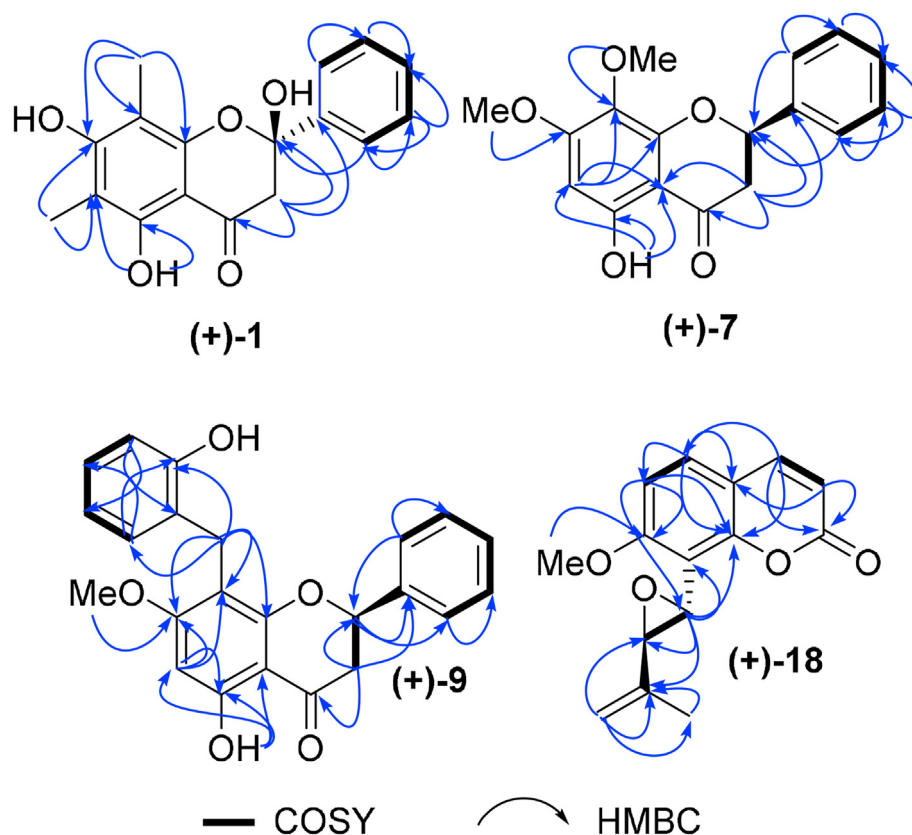**Figure 4.** Compounds isolated from twig and leaf extracts of *D. dumosus*.

— COSY ⌒ HMBC

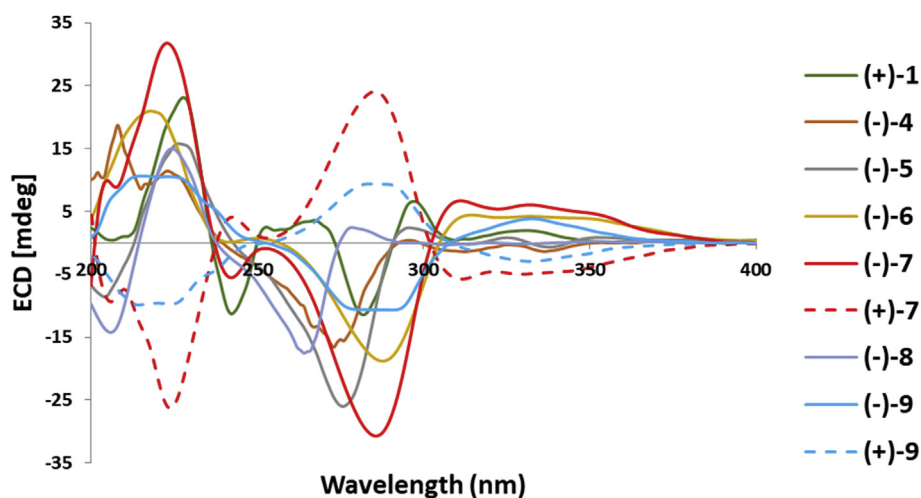**Figure 5.** COSY and selected HMBC correlations of compounds (+)-**1**, (+)-**7**, (+)-**9**, and (+)-**18**.

Figure 6. ECD spectra (MeOH) of compounds (+)-**1**, (−)-**4**, (−)-**5**, (−)-**6**, (−)-**7**, (+)-**7**, (−)-**8**, (−)-**9**, and (+)-**9**.
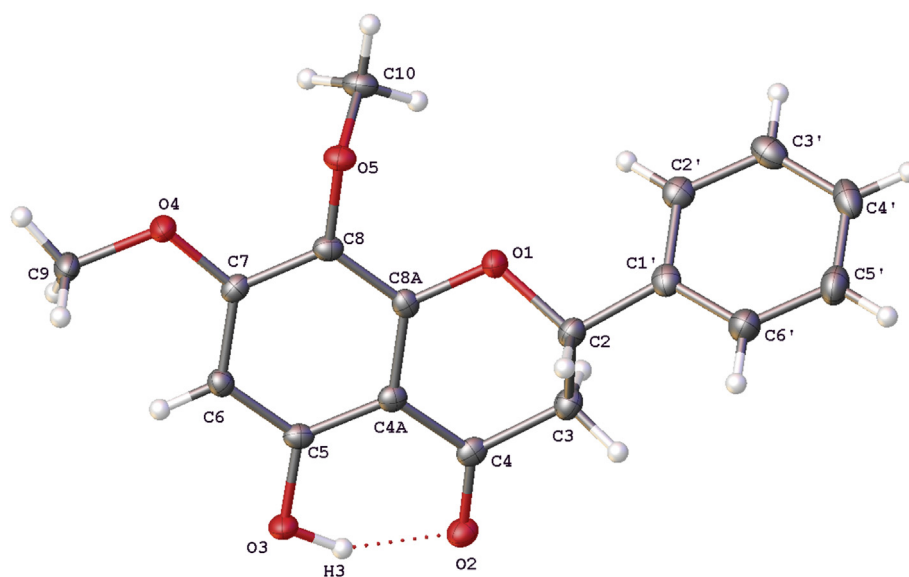
Figure 7. X-ray crystallographic data of (±)-7,8-dimethoxy-5-hydroxyflavanone (±)-**7**.

shift of the C-2 sp^3 carbon (δ_C 101.3) indicated a hemiketal carbon of the flavanone core skeleton [42]. The full assignments of all ^{1}H and ^{13}C NMR resonances, as well as HMBC correlations, were shown in Table 1. It should be noted that almost all of the naturally occurring absolute C-2 flavanone configurations containing the α-orientation 2-aryl substituent unit have been defined as (2*S*) [43, 44]. They will display a negative Cotton effect of the acetophenone chromophore transitions at 270–290 nm (π→π*) and a positive Cotton effect at 320–330 nm (n→π*) in ECD spectra [43, 44]. The ECD spectrum of **1** (Figure 6) showed positive Cotton effects at 227, 297, and 331 nm and negative Cotton effects at 242 and 282 nm similar to that of compound (−)-**7** suggesting the 2*S* absolute configuration of **1**. Besides, the ECD spectrum and the specific rotation ($[\alpha]_D^{23}$ + 16 (*c* 1.5, MeOH)) of **1** were opposite to that of (−)-(*R*)-2-hydroxynaringenin (**30**), previously isolated from *Zizyphus jujuba* Mill. var. spinosa [44], which further supported the identification of the 2*S* absolute configuration of **1**. Therefore, compound **1** was identified as (+)-(2*S*)-desmosdumosone.

The racemic mixture of (±)-7,8-dimethoxy-5-hydroxyflavanone (**7**), was first isolated from the root extracts of *Andrographis paniculata* in 1983 by Gupta and co-workers [45]. Then, in 1987, the *S*-configuration of

(−)-**7** was identified and isolated from *Andrographis paniculate* root extracts [46]. In our study, we isolated the racemic mixture of compound **7** as a colorless crystal. Its structure was confirmed by a single X-ray diffraction analysis using Cu Kα radiation (Figure 7, CCDC 2006685). Compound (±)-**7** was resolved by semipreparative chiral HPLC to give compounds (−)-**7** (t_R 7 min), $[\alpha]_D^{23}$ −23 (*c* 0.1, CHCl₃)] and (+)-**7** (t_R 7.5 min), $[\alpha]_D^{23}$ + 31 (*c* 0.1, CHCl₃) in a ratio of *ca* 1:0.9 (Figure S28). The (2*R*) absolute configuration of (+)-**7** was established by the comparison of its specific rotation and ECD spectrum with those of (−)-(2*S*)-7, 8-dimethoxy-5-hydroxyflavanone [46]. The ECD spectrum (Figure 6) of (2*R*)-**7** [225 (−1.19), 287 (+1.26), 331 (−0.54) nm] was the mirror image of that of (2*S*)-**7** [225 (+1.27), 287 (−1.15), 331 (+0.60) nm] and its specific rotation was also opposite sign {$[\alpha]_D^{23}$ + 31.2 (*c* 0.1, CHCl₃) for (2*R*)-**7** and $[\alpha]_D^{23}$ −23 (*c* 0.1, CHCl₃)] for (2*S*)-**7**}. Therefore, the new compound (+)-**7** was identified as (+)-(2*R*)-7,8-dimethoxy-5-hydroxyflavanone. Detailed assignments of the protons and carbons as well as selected HMBC correlations are shown in Table 2 and Figure 5, respectively.

The racemic compound **9** (7-methoxychamanetin or 7-*O*-methylchamanetin) is a *C*-benzylated flavanone and was first isolated from the
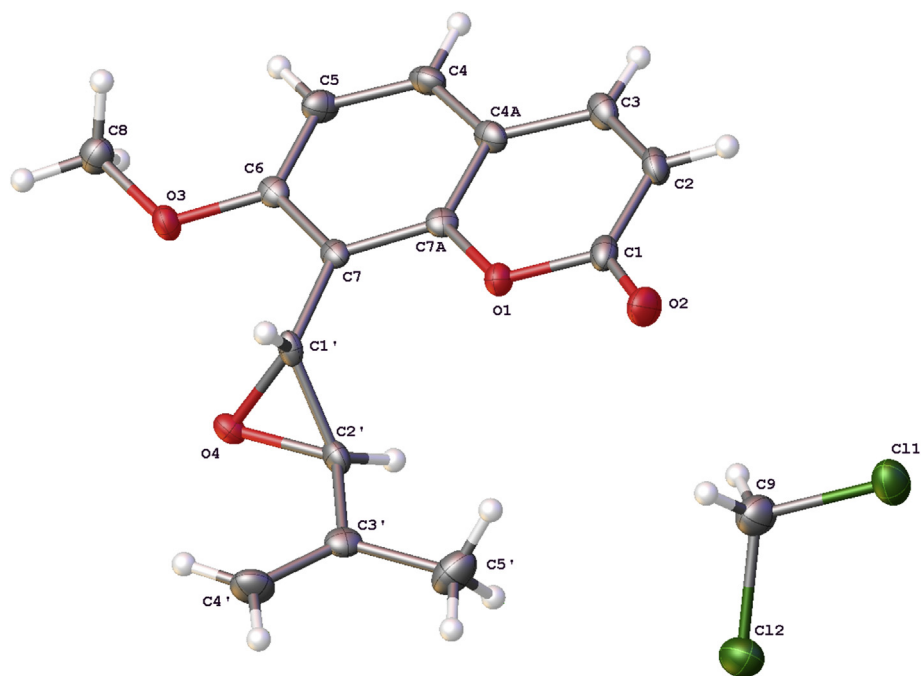
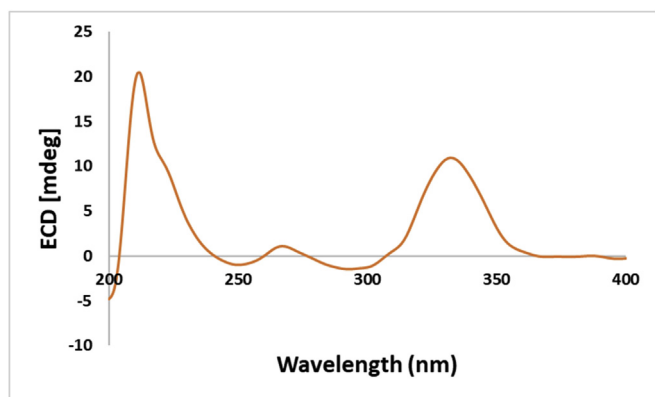**Figure 8.** X-ray crystallographic data for (+)-(1′R, 2′R)-phebalosin (**18**).

Figure 9. ECD spectra (MeOH) of compounds (+)-**18**.

Phebalosin was first identified and isolated from *Phebalium tuberculosum* by Chow and co-workers in 1966 [49]. In 1989, Muhamad and co-workers isolated (−)-phebalosin [[α]$_D^{20}$ −36 (c 0.5, CHCl$_3$)] from *Merrillia caloxylon* [50]. In this study, (+)-phebalosin (**18**) was isolated and its absolute configuration was identified from its specific rotation, ECD spectrum, and X-ray diffraction analysis. The NMR spectroscopic data (Table 3) were similar with (−)-phebalosin. The (1′R, 2′R) absolute configuration of (+)-phebalosin was established from X-ray crystallographic data analysis (Figure 8, CCDC 2006686) with the Flack parameter was showed to 0.072 (8). The ECD spectrum [213 (+2.01), 250 (−0.72), 270 (+0.68), 293 (−0.89), and 335 (+1.74) nm] is shown in Figure 9. Thus, compounds **18** was identified as (+)-(1′R, 2′R)-phebalosin as an enantiomer of (−)-phebalosin. Detailed assignments of the protons and carbons as well as selected HMBC correlations are shown in Table 3 and Figure 5, respectively.

3.2. α-Glucosidase inhibitory activity of isolated compounds

Some isolated compounds (**4**, **7**, **9–16**, **19**, **22**, **25**, and **28**) were evaluated for their α-glucosidase activities (Table 4). Compounds **9**, **11**, **13**, **14**, **22**, **25**, and **28** showed α-glucosidase inhibitory activities with IC$_{50}$ values ranging from 5.3-52.7 μM, which were much better than that of standard control (acarbose, IC$_{50}$ value 83.5 μM), and compound **13** was the most active with the IC$_{50}$ value of 5.3 μM. Compounds **7**, **15**, and **19** exhibited weak α-glucosidase inhibitory activities with IC$_{50}$ values of 120.9, 154.4, and 198.6 μM, respectively. Two remaining compounds (**10** and **12**) were shown to the inactive with α-glucosidase inhibitory activity at 200 μg/mL.

4. Conclusions

The phytochemical investigations of *D. dumosus* collected from Doi Tung, Chiang Rai Province, Thailand led to the isolation and identification of four new compounds together with 25 known compounds. Most of flavanone previously isolated from Nature were reported to have the 2*S* configuration or were isolated as racemic mixtures [17, 51, 52, 53, 54]. In this study, (+)-(2*R*)-7,8-dimethoxy-5-hydroxyflavanone and

root extract of *Xylopia africana* (Benth.) Oliver [47]. In 1976, William and co-workers [48] synthesized (−)-(2*S*)-7-methoxychamanetin, which had a specific rotation of [α]$_D^{25}$ −107.5 (c 1.7, acetone) and its EDC spectrum displayed positive Cotton effects at 332 (+860), 311 (+1290), and 212 (+3020) nm and negative Cotton effects at 287 (−4320) and 235 (−1330). In this study, we resolved compound **9** by semi-preparative chiral HPLC to provide compounds (−)-**9** (t_R 7 min), [α]$_D^{23}$ −71 (c 0.1, CHCl$_3$)] and (+)-**9** (t_R 13 min), [α]$_D^{23}$ + 57 (c 0.1, CHCl$_3$) in a ratio of ca 1:0.9 (Figure S28). The ECD spectrum (Figure 6) of (+)-**9** [220 (−0.86), 287 (+0.85), 335 (−0.34) nm] was the mirror image of that of (−)-(2*S*)-7-methoxychamanetin ((−)-**9**) [48] indicating the absolute configuration of (+)-**9** was opposite to that of 7-methoxychamanetin Thus, compound (+)-**9** was assigned as (+)-(2*R*)-7-methoxychamanetin. Full assignments of the protons and carbons as well as selected HMBC correlations are shown in Table 2 and Figure 5, respectively.

Table 4. α-Glucosidase inhibitory activity of crude extract and isolated compounds from *D. dumosus*.

Compounds	α-Glucosidase inhibitory activity (IC$_{50}$, μM)
4	85.1
7	120.9
9	15.4
10	inactive
11	36.0
12	inactive
13	5.3
14	32.7
15	154.4
19	198.6
22	11.2
25	10.5
28	52.7
Acarbose	83.5

(+)-(2*R*)-7-methoxychamanetin were obtained by the resolution of compounds (±)-**7** and (±)-**9**, respectively, using semi-preparative chiral HPLC. The absolute configuration of all new compounds was established by comparing their ECD spectra with those of relevant reported compounds, except for compound (+)-(1′*R*,2′*R*)-**18** which was determined using X-ray diffraction analysis (Cu K*α* radiation). Compound **13** showed the most active α-glucosidase inhibitory activity (IC$_{50}$ 5.3 μM), much better than standard control (acarbose, IC$_{50}$ value 83.5 μM). This compound may have the potential for further development as an anti-diabetic agent.

Declarations

Author contribution statement

Virayu Suthiphasilp: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Tharakorn Maneerat: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Raymond J. Andersen, Stephen G. Pynee: Analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; Wrote the paper.

Brian O. Patrick: Performed the experiments; Analyzed and interpreted the data.

Surat Laphookhieo: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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