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ORIGINAL ARTICLE

Two New Phenolic Glycosides from the Aerial Part of *Dryopteris erythrosora*

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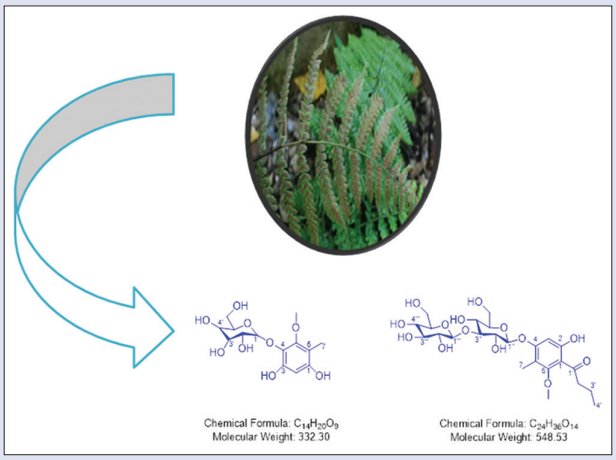
ABSTRACT

Background: *Dryopteris erythrosora* (D.C. Eaton) Kuntze is a species of fern in the family of *Dryopteridaceae,* which is distributed throughout East Asia. The genus *Dryopteris* has been used as traditional medicine, especially to treat hepatitis and protect liver. However, only few studies of chemical constituents of *D. erythrosora* have been conducted so far. **Objective:** In this study, we investigated the phytochemical constituents of *D. erythrosora*. **Materials and Methods:** The 80% methanol extract of the aerial part of *D. erythrosora* was used for the isolation of phenolic compounds. The isolated compounds were elucidated by various spectroscopic methods including nuclear magnetic resonance and mass spectrometry. **Results:** The present phytochemical investigation on the aerial part of *D. erythrosora* led to the isolation of two new phenolic glycosides, 1 and 2, as well as nine known flavonoids including two flavones (3 and 4) and seven flavonols (5-11). **Conclusion:** In this study, two new phenolic glycosides together with nine known flavonoids were isolated from the aerial part of *D. erythrosora*. Among them, compounds 4, 8, and 11 were isolated for the first time in *Dryopteridaceae* family from the present investigation. These results helped us to enrich our understanding of the chemical constituents of *D. erythrosora* and to identify compounds 1 and 2 which could be potential chemotaxonomic markers for the species.

Key words: *Dryopteridaceae, Dryopteris erythrosora,* phenolic glycoside

SUMMARY

- The genus *Dryopteris* has been used as traditional medicine, especially to treat hepatitis and protect liver
- Two new phenolic glycosides were isolated from *D. erythrosora*
- Nine known flavonoids (3-11) were isolated from *D. erythrosora*
- Compounds 4, 8, and 11 were isolated for the first time in *Dryopteridaceae* family.



Abbreviations used: HPLC: High-performance liquid chromatography; Q-TOF LC/MS: Quadrupole-time-of-flight liquid chromatography/mass spectrometry; NMR: Nuclear magnetic resonance; TMS: Tetramethylsilane

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INTRODUCTION

Dryopteris erythrosora (D.C. Eaton) Kuntze is a species of fern in the family of *Dryopteridaceae,* which is distributed throughout East Asia.[1] *Dryopteris* genus is a well-known traditional medicine and extensively used to treat hepatitis and protect liver.[2] The major identified constituents in *Dryopteris* genus are phenols, flavonoids, and terpenoids.[3-5] In a previous study, the chemical constituents of 18 *Dryopteris* genus were investigated and compared.[6] However, only few studies of chemical constituents of *D. erythrosora* have been conducted so far.[7]

MATERIALS AND METHODS

General procedures

All organic solvents, such as hexane, chloroform (CHCl3), ethyl acetate (EtOAc), methanol (MeOH), and n-butanol (n-BuOH) used for extraction and column chromatography were of analytical grade and purchased from Duksan Chemical (Anseong, Korea). ^{1}H nuclear magnetic resonance (NMR) (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on an Agilent 400-MR NMR spectrometer (Agilent Technologies, Santa Clara, CA), and tetramethylsilane was used as an internal standard. Data processing was carried out with the MestReNova 6.0.2 program. HRESIMS spectra were obtained using an Agilent 6550 iFunnel quadrupole-time of flight (Q-TOF) liquid chromatography/mass spectrometry (LC/MS) system (Agilent Technologies, Santa Clara, CA). Preparative high-performance LC (HPLC) was carried out using an Agilent 1260 HPLC system. Column chromatography was performed

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on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany) and YMC RP-18 resins (Fuji Silysia Chemical, Aichi, Japan).

Plant materials

The whole plants of *D. erythrosora* were collected at Jiri Mountain, Sancheong-gun, Gyeongsangnam-do province, South Korea, in July 2013, and authenticated by Dr. Jong Hee Park, a professor emeritus of Pusan National University. A voucher specimen (YIPS-DE-141220) was deposited at the Herbarium of College of Pharmacy, Yonsei Institute of Pharmaceutical Sciences, Yonsei University, Incheon, Korea.

Extraction and isolation

The dried and powdered aerial part of *D. erythrosora* (270 g) was extracted with 80% MeOH four times using ultrasound for 3 days at room temperature. After removal of the solvent under reduced pressure *in vacuo*, the resulted MeOH extract (37.6 g, yield: 13.9%) was suspended in H_2O and then partitioned successively with CH_2Cl_2 (3.68 g), EtOAc (13.41 g), and *n*-BuOH (18.6 g) fractions. The EtOAc fraction was chromatographed on a silica gel column and eluted with a gradient of $CHCl_3$– MeOH (40:1 → 1:1, v/v) to obtain eight subfractions as follows: Fr. E1 (1.58 g), Fr. E2 (1.21 g), Fr. E3 (1.03 g), Fr. E4 (0.67 g), Fr. E5 (4.56 g), Fr. E6 (1.65 g), Fr. E7 (2.34 g), and Fr. E8 (0.42 g). The Fr. E5 fraction was chromatographed on ODS silica gel column eluted with MeOH– H_2O (3:7, v/v) to yield 1 (5.1 mg), 2 (4.8 mg), and 7 (6.0 mg). The Fr. E6 fraction was chromatographed on HPLC using J'sphere ODS H-80 (250 mm × 20 mm, 4 μm, 8 nm) column eluted with 25% aqueous acetonitrile at a flow rate of 3 mL/min to yield 5 (6.4 mg) and 6 (7.5 mg). The Fr. E7 fraction was chromatographed on silica gel column eluted with CHCl3– MeOH (5:1, v/v) to yield 3 (2.3 mg) and 4 (2.8 mg). The *n*-BuOH fraction was chromatographed on a silica gel column and eluted with a gradient of $CHCl_3$– MeOH (20:1 → 1:1, v/v) to obtain six subfractions as follows: Fr. B1 (1.35 g), Fr. B2 (1.54 g), Fr. B3 (2.04 g), Fr. B4 (1.87 g), Fr. B5 (5.78 g), and Fr. B6 (3.54 g). The Fr. B5 fraction was chromatographed on an silica gel column eluted with $CHCl_3$– MeOH (5:1, v/v) to yield 8 (2.7 mg) and 9 (4.1 mg). The Fr. B6 fraction was chromatographed on HPLC using J'sphere ODS H-80 (250 mm × 20 mm, 4 μm, 8 nm) column eluted with 30% aqueous acetonitrile at a flow rate of 3 mL/min to yield 10 (3.1 mg) and 11 (2.5 mg). The isolated compounds were elucidated by ESI-Q-TOF-MS and several NMR techniques including 1D and 2D NMR spectroscopic methods and by comparison of their data with those reported previously in the related literatures.

RESULTS AND DISCUSSION

Two new phenolic glycosides (1 and 2), together with nine known compounds (3–11), were isolated from the aerial part of *D. erythrosora*. By comparing their spectroscopic methods with those reported in the literature, the isolated compounds were identified as apigenin-7-*O*-glucopyranoside and apigenin-7-*O*-rutinoside (3 and 4),[8] keampferol-3-*O*-rhamnoside (5),[9] kaempferol-3-*O*-rutinoside (6),[10] quercetin (7),[11] quercetin-3-*O*-galactoside (8),[12] quercetin-3-*O*-rhamnoside (9),[13] myricetin-3-*O*-rhamnoside (10),[14] and myricetin-3-*O*-glucopyranoside (11)[15] [Figure 1].

The structures of two new compounds 1 and 2 were elucidated on the basis of spectroscopic analysis [Figure 2] and comparison with literature data as described below.

Compound 1 was obtained as a pale brown amorphous powder and its molecular formula was determined as $C_{14}H_{20}O_9$ by the HR-ESI-MS [M + H] + ion at m/z 333.1107 (calcd for $C_{14}H_{21}O_9$, 333.1186). The ^{1}H-NMR spectra of 1 revealed an olefinic proton at δ_H 6.18, a methyl at δ_H 2.02, and a methoxy signal at δ_H 3.82. The β-linkage of the glucopyranosyl moiety was deduced from the coupling constant (J = 7.2 Hz) of the anomeric proton signal at δ_H 4.64 [Table 1]. The ^{13}C-NMR and DEPT spectra of 1 revealed 14 carbon signals, including five quaternary carbons (δ_C 110.59, 132.82, 149.63, 153.09, and 154.02), six methines (δ_C 71.03, 75.33, 77.84, 78.41, 100.11, and 107.24), one methylene (δ_C 62.25), one methyl (δ_C 8.81), and one methoxy (δ_C 61.80). Among the exhibited carbon signals, ^{13}C chemical shifts of C-1' (δ_C 107.24), C-2' (δ_C 78.41), C-3' (δ_C 77.84), C-4' (δ_C 71.03), C-5' (δ_C 75.33), and C-6' (δ_C 62.25) suggested the presence of β-glucopyranosyl sugar moiety. The NMR data of 1 were similar to those of pseudo-aspidinol B[16] except for the replacement of β-glucopyranosyl sugar moiety instead of a butanone. The HMBC correlations between H-1' (δ_H 4.64) and C-4 (δ_C 132.82) suggested the presence of *O*-β-glucopyranosyl sugar moiety at C-4. The HMBC correlations from H-OMe (δ_H 3.82) to C-5 (δ_C 153.09) suggested that the methoxy is attached at C-5. The HMBC correlations from H-7 (δ_H 2.02) to C-1, C-5, and C-6 (δ_C 154.02, 153.09, and 110.59, respectively) confirmed that methyl was located at C-6. Based on the evidence mentioned above, compound 1 was established as 1-1,3-dihydroxy-5-methoxyphenyl-4-*O*-*β*-D-glucopyranoside.

Compound 2 was also obtained as a pale brown amorphous powder and its molecular formula was determined as $C_{24}H_{36}O_{14}$, by the HR-ESI-MS [M + H] + ion at m/z 549.2105 (calcd for $C_{24}H_{37}O_{14}$, 549.2137). The ^{1}H-NMR spectra of 2 revealed an olefinic proton (δ_H 6.42), two methylene protons (δ_H 1.67 and 3.01), two methyl (δ_H 0.94 and 2.12), and a methoxy signal (δ_H 3.70). The β-linkage of the sugar moiety was deduced from the coupling constant (J = 7.7, 7.5 Hz) of the anomeric proton signal at δ_H 4.68 and 5.15, respectively [Table 1]. The ^{13}C-NMR and DEPT spectra of 2 revealed 24 carbon signals, including six quaternary carbons (δ_C 112.02, 113.37, 161.87, 162.56, 163.37, and 207.98), eleven methines (δ_C 70.91, 71.21, 76.24, 77.81, 78.00, 78.10, 78.18, 82.85, 99.72, 99.82, and 105.37), four methylenes (δ_C 19.32, 46.19, 62.28, and 62.31), two methyls (δ_C 9.49 and 14.32), and one methoxy (δ_C 62.54). The backbone of 2 was deduced from the HMBC correlations from methyl (δ_H 2.12) to C-4 (δ_C 162.56), C-5 (δ_C 113.37), and C-6 (δ_C 161.87), and from olefinic proton (δ_H 6.42)

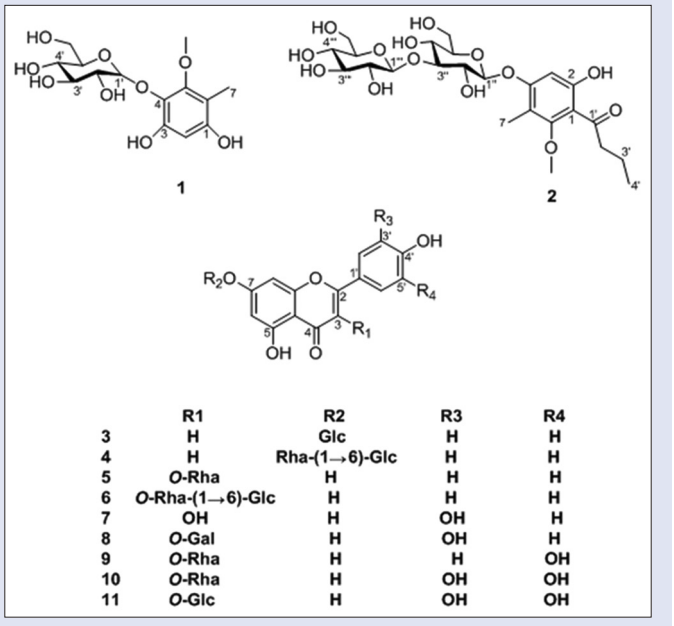

Figure 1: Chemical structures of compounds 1–11

Table 1: Nuclear magnetic resonance spectroscopic data for compounds 1 and 2

Position	1		Pos.	2	
	δ_C [a,b]	δ_H [a,c] (J in Hz)		δ_C [a,b]	δ_H [a,c] (J in Hz)
1	154.02	-	1	112.03	-
2	100.11	6.18 (s)	2	163.37	4.92 (s)
3	149.63	-	3	99.82	6.42 (s)
4	132.82	-	4	162.56	-
5	153.09	-	5	113.37	-
6	110.59	-	6	161.87	-
7	8.8	2.02 (s)	7	9.49	2.12 (s)
5-OMe	61.80	3.82 (s)	6-OMe	62.54	3.70 (s)
1'	107.24	4.64 (d, 7.2)	1'	207.98	-
2'	78.41	3.43-3.47*	2'	46.19	3.01 (t, 7.2)
3'	77.84	3.43-3.47*	3'	19.32	1.67 (m)
4'	71.03	3.37-3.38*	4'	14.32	0.94 (t, 7.4)
5'	75.33	3.37-3.38*	1"	99.72	5.15 (d, 7.8)
6'	62.25	3.87 (dd, 2.2, 12.2) 3.70 (m)	2"	76.24	3.76-3.77*
			3"	82.85	3.11-3.13*
			4"	70.91	3.64-3.66 (m)
			5"	78.18	3.75-3.77 (m)
			6"	62.28	3.54-3.56 (m) 3.79-3.81*
			1"'	105.37	4.68 (d, 7.8)
			2"'	77.81	3.76-3.80 (m)
			3"'	78.10	3.49-3.58 (m)
			4"'	71.21	3.40-3.49 (m)
			5"'	78.00	3.73-3.75 (m)
			6"'	62.31	3.56-3.59* 3.79 (m)

[a]Measured in methanol-d4, [b]100 MHz, [c]400 MHz, *Overlapped signal. Assignments were done by HSQC, HMBC, and COSY experiments

to C-1 (δ_C 112.03), C-2 (δ_C 163.37), C-4 (δ_C 162.56), and C-5 (δ_C 113.37). The NMR data of 2 were similar to those of methylphlorbutyrophenon[5] except for the sugar moiety and a methoxy group. The presence of a methoxy group was confirmed by the HMBC correlation between H-OMe (δ_H 3.70) and C-6 (δ_C 161.87) suggesting that the methoxy is located at C-6. The HMBC correlations from H-7 (δ_H 2.12) to C-4, C-5, and C-6 (δ_C 162.56, 113.37, and 161.87, respectively) confirmed that methyl was located at C-5. The HMBC correlations from H-1' (δ_H 5.15) to C-4 (δ_C 162.56) and from H-1" (δ_H 4.68) to C-3" (δ_C 82.85) suggested the presence of glucopyranosyl-(1"'→3")-glucopyranoside sugar moiety at C-4. Based on the evidence mentioned above, compound 2 was established as 4-*O*-*β*-D-glucopyranosyl-(1"'→3")-glucopyranosyl-2-hydroxy-6-methoxy-5-methylphenyl-1-butanone.

CONCLUSION

The present phytochemical investigation on the aerial part of *D. erythrosora* led to the isolation of two new phenolic glycosides, 1 and 2, as well as nine known flavonoids including two flavones (3 and 4) and seven flavonols (5-11). These results were in a good agreement with other

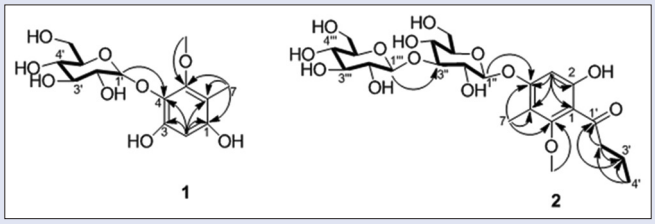

Figure 2: The key HMBC correlations of compounds 1 and 2

chemical composition reports of the *Dryopteris* genus such as apigenin-7-*O*-glucoside (3) from *Dryoathyrium boryanum*,[17] keampferol-3-*O*-rhamnoside (5) from *D. crassirhizoma*,[18] kaempferol-3-*O*-rutinoside (6) from *Dryopteris villarii*,[19] and quercetin (7) from *Dracaena fragrans*.[20] Two 3-*O*-rhamnoside flavonols, quercetin-3-*O*-rhamnoside (9), and myricetin-3-*O*-rhamnoside (10) were previously isolated from *D. erythrosora*.[2] These results led to the conclusion that apigenin-7-*O*-rutinoside (4), quercetin-3-*O*-galactoside (8), and myricetin-3-*O*-glucopyranoside (11) were isolated for the first time in *Dryopteris* genus as well as in *Dryopteridaceae* family from the present investigation. This phytochemical investigation helped us to enrich our understanding of the chemical constituents of *D. erythrosora* and to identify that compounds 1 and 2 could be potential chemotaxonomic markers for the species.

Acknowledgement

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Nil.

Conflicts of interest

There are no conflicts of interest.

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