

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

Guijiae Yoo, SeonJu Park, HeeJung Yang¹, Xuan Nhiem Nguyen², Nanyoung Kim, Jun Hyung Park, Seung Hyun Kim

College of Pharmacy, Yonsei Institute of Pharmaceutical Science, Yonsei University, Incheon 406-840, Korea, ¹Research center natural medicine research Team, Richwood Pharmaceutical company, Ltd., Seoul, 08826, Republic of Korea, ²Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Caujgay, Hanoi, Vietnam

Submitted: 06-07-2016

Revised: 01-09-2016

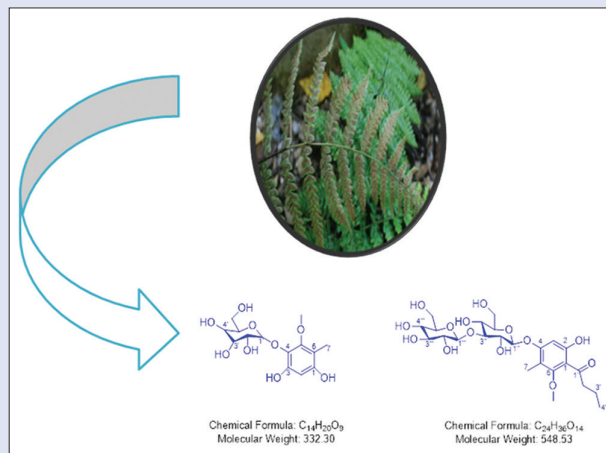
Published: 13-11-2017

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

Correspondence:

Prof. Seung Hyun Kim,
College of Pharmacy, Yonsei University,
162-1, Songdo-dong, Yeonsu-gu,
Incheon 406-840, Korea.
E-mail: kimsh11@yonsei.ac.kr
DOI: 10.4103/pm.pm_326_16

Access this article online

Website: www.phcog.com

Quick Response Code:



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). ¹H nuclear magnetic resonance (NMR) (400 MHz) and ¹³C 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

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Yoo G, Park S, Yang H, Nguyen XN, Kim N, Park JH, et al. Two new phenolic glycosides from the aerial part of *Dryopteris erythrosora*. *Phcog Mag* 2017;13:673-6.

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 H₂O and then partitioned successively with CH₂Cl₂ (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 CHCl₃–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–H₂O (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 CHCl₃–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 a silica gel column eluted with CHCl₃–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 C₁₄H₂₀O₉ by the HR-ESI-MS [M + H]⁺ ion at m/z 333.1107 (calcd for C₁₄H₂₁O₉, 333.1186). The ¹H-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 ¹³C-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, ¹³C 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 C₂₄H₃₆O₁₄ by the HR-ESI-MS [M + H]⁺ ion at m/z 549.2105 (calcd for C₂₄H₃₇O₁₄, 549.2137). The ¹H-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 ¹³C-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)

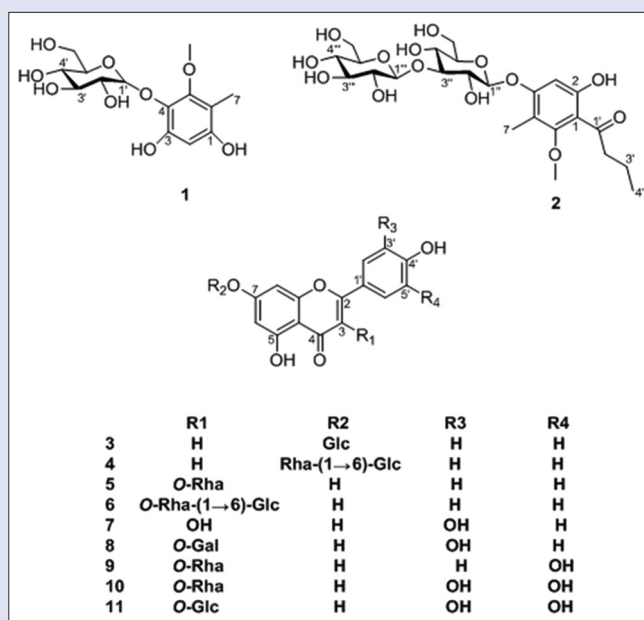


Figure 1: Chemical structures of compounds 1–11

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

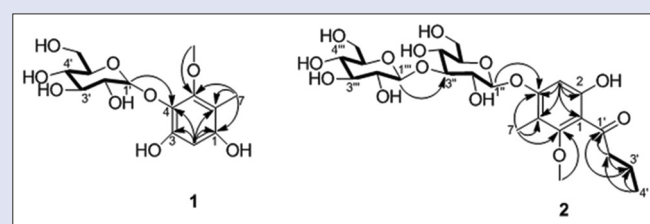
Position	1		Pos.	2	
	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (J in Hz)		$\delta_C^{a,b}$	$\delta_H^{a,c}$ (J in Hz)
1	154.02	-	1	112.03	-
2	100.11	6.18 (s)	2	163.37	4.92 (s)
3	149.63	-	3	99.82	6.42 (s)
4	132.82	-	4	162.56	-
5	153.09	-	5	113.37	-
6	110.59	-	6	161.87	-
7	8.8	2.02 (s)	7	9.49	2.12 (s)
5-OMe	61.80	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)	2'''	76.24	3.76-3.77*
		3.70 (m)	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)

^aMeasured in methanol-d₄, ^b100 MHz, ^c400 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'' \rightarrow 3''')-glucopyranoside sugar moiety at C-4. Based on the evidence mentioned above, compound 2 was established as 4-O- β -D-glucopyranosyl-(1'' \rightarrow 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 *Dryothyrium boryanum*,^[17] kaempferol-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

This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science, and Technology (NRF-2011-0025129).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Zhang M, Cao J, Dai X, Chen X, Wang Q. Flavonoid contents and free radical scavenging activity of extracts from leaves, stems, rachis and roots of *Dryopteris erythrosora*. Iran J Pharm Res 2012;11:991-7.
- Cao J, Xia X, Chen X, Xiao J, Wang Q. Characterization of flavonoids from *Dryopteris erythrosora* and evaluation of their antioxidant, anticancer and acetylcholinesterase inhibition activities. Food Chem Toxicol 2013;51:242-50.
- Gao Z, Li R, Wang B, Lu Y. Progress in chemical constituents of genus *Dryopteris*. China J Chin Mater Med 2003;9:50-5.
- Chang X, Li W, Koike K, Wu L, Nikaido T. Phenolic constituents from the rhizomes of *Dryopteris crassirhizoma*. Chem Pharm Bull (Tokyo) 2006;54:748-50.
- Zhao DD, Zhao QS, Liu L, Chen ZQ, Zeng WM, Lei H, *et al.* Compounds from *Dryopteris fragrans* (L.) Schott with cytotoxic activity. Molecules 2014;19:3345-55.
- Hiraoka A. Flavonoid patterns in Athyriaceae and *Dryopteridaceae*. Biochem Syst Ecol 1978;6:171-5.
- Chang Y, Hu J, Jiang S, Qiang S. Study on the distribution and the total flavonoids content of medical pteridophytes in Nanjing Zijin Mountain. J Northeast Agric Univ 2005;36:320-3.
- Baris O, Karadayi M, Yanmis D, Guvenalp Z, Bal T, Gulluce M. Isolation of 3 flavonoids from *Mentha longifolia* (L.) Hudson subsp. longifolia and determination of their genotoxic potentials by using the *E. coli*WP2 test system. J Food Sci 2011;76:T212-7.
- Kim SK, Kim HJ, Choi SE, Park KH, Choi HK, Lee MW. Anti-oxidative and inhibitory activities on nitric oxide (NO) and prostaglandin E2 (COX-2) production of flavonoids from seeds of *Prunus tomentosa* Thunberg. Arch Pharm Res 2008;31:424-8.
- Song N, Xu W, Guan H, Liu X, Wang Y, Nie X. Several flavonoids from *Capsella bursa-pastoris*

- (L.) Medic. Asian J Tradit Med 2007;2:218-22.
11. Dhasan PB, Jegadeesan M, Kavimani S. Cucurbitacins isolated from the fruits of *Momordica cymbalaria* Hook f. Pharmacogn Mag 2008;4:96.
 12. Wei Y, Ito Y. Isolation of hyperoside and luteolin-glucoside from *Agrimonia pilosa* ledeb using stepwise elution by high-speed countercurrent chromatography. J Liq Chromatogr Relat Technol 2007;30:1465-73.
 13. Hema K, Sukumar D. Isolation and phytochemical studies of quercetin and quercetin 3-O-rhamnoside. Int J Pharm Bio Sci 2013;4:519-24.
 14. Gedara SR, Galala AA. New cytotoxic spirostane saponin and biflavonoid glycoside from the leaves of *Acacia saligna* (Labill.) H.L. Wendl. Nat Prod Res 2014;28:324-9.
 15. Manguro LOA, Ugi I, Lemen P. Further flavonol glycosides of *Embelia schimperi* leaves. B CHEM SOC ETHIOPIA. 2004;18:51-7.
 16. Zuo L, Wang HQ, Chen RY. Chemical constituents in roots of *Dryopteris championii*. Zhong Cao Yao 2005;2:177-9.
 17. Cao J, Xia X, Dai X, Xiao J, Wang Q, Andrae-Marobela K, *et al.* Flavonoids profiles, antioxidant, acetylcholinesterase inhibition activities of extract from *Dryoathyrium boryanum* (Willd.) Ching. Food Chem Toxicol 2013;55:121-8.
 18. Min BS, Tomiyama M, Ma CM, Nakamura N, Hattori M. Kaempferol acetylramnosides from the rhizome of *Dryopteris crassirhizoma* and their inhibitory effects on three different activities of human immunodeficiency virus-1 reverse transcriptase. Chem Pharm Bull (Tokyo) 2001;49:546-50.
 19. Imperato F. Kaempferol 3-O-(acetylrutinoside), a new flavonoid and two new fern constituents, quercetin 3-O-(acetylglucoside) and 3-O-(acetylrutinoside) from *Dryopteris villarii*. Am Fern J 2006;96:93-6.
 20. Li B, Zhu JF, Zou ZJ, Yin YQ, Shen ZB. Studies on the chemical constituents of *Dryopteris fragrans*. Zhong Yao Cai 2009;32:1232-3.