Prev. Nutr. Food Sci. 2022;27(2):223-233
https://doi.org/10.3746/pnf.2022.27.2.223
ISSN 2287-8602

Antioxidant Activities of Bioactive Compounds Isolated from *Rheum emodi* Wall (Himalayan Rhubarb) Based on LC-DAD-ESI/MS and Preparative LC/MS System

Sang Koo Park

Food Safety Management Division, Seoul Regional Korea Food and Drug Administration, Seoul 07978, Korea

ABSTRACT: Natural compounds are a good substitute for synthetic antioxidants. Attempts have been made to characterize the antioxidant capacity of natural resources (e.g., medicinal plants). Thus, the *Rheum emodi* Wall was evaluated using liquid chromatography with diode array detection and electrospray ionization-mass spectrometry. Three antioxidant compounds (i.e., myricitrin, myricetin-3-galloyl rhamnoside, and myricetin) were isolated, identified, and used to screen the antioxidant capacity of the new compounds. 2,2-Diphenyl-1-picrylhydrazyl, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and superoxide dismutase assay results are presented in the half-maximal inhibitory concentration values ranging 1.50～28.46, 102.01～137.55, and 4.06～15.74 μg/mL, respectively. Myricetin had the highest antioxidant activity among the other compounds. A significantly positive correlation was noted between the ethyl acetate fraction and the antioxidant compound. In a partial least squares-discriminant analysis model, identified antioxidant compounds were shown to play a role in the structure of the compound and its contents based on the antioxidant activity. The study suggests that myricetin from *R. emodi* possesses the most potent antioxidant activity, and thus is the most efficient in extracting antioxidant contents.

Keywords: antioxidant activity, DPPH, Himalayan rhubarb, LC-DAD-ESI/MS, *Rheum emodi* Wall

INTRODUCTION

Rheum emodi Wall. ex Meissn. (Polygonaceae) is a stout herb found in the temperate and subtropical Himalayas, from Kashmir to Sikkim, at elevations ranging from 2,800 to 3,800 m. For almost 2,000 years, *R. emodi* rhizomes have been used to treat piles, bleeding, gastroenteritis, and other inflammatory disorders in traditional Chinese and Tibetan medicine. Extracts of *R. emodi* rhizomes have antioxidant activities, and these therapeutic qualities may be due to phenolic chemicals (Peigen et al., 1984; Rajkumar et al., 2011). *R. emodi* possesses many phytoconstituents, e.g., anthraquinones (rhein, chrysophanol, and emodin), anthrones, stilbenes (piceatannol and resveratrol), flavonoids, lignans, and phenols (Wani et al., 2009), and many of which were discovered using liquid chromatography (LC)-mass spectrometry (MS) analysis. These compounds can potentially improve the bioavailability of antibacterial and antifungal medicines. Methanolic extracts of several *R. emodi* plants were studied for their phytochemical profile, including phenolic content, flavonoid content, and antioxidant activity (Rolta et al., 2018; Rolta et al., 2020).

The majority of modern illnesses transition from acute to chronic metabolic diseases. These are hypothesized to be involved in oxidative stress, free radicals, and reactive oxygen species (ROS). These are generally produced due to environmental stresses, e.g., such as ultraviolet (UV) radiation, drought, chilling, and salinity. Antioxidants reduce the level of ROS or free radicals in the body to prevent, delay, or eliminate oxidative stress (Cross et al., 1987; Frei, 1994; Sözmen et al., 1994; Halliwell, 2007; Burgos-Morón et al., 2019).

Synthetic antioxidants (e.g., butylated hydroxyl toluene, hydroxyanisole, tertbutyl hydroquinone, and propyl gallate) have been widely used because of their low cost, excellent stability, and efficiency. However, toxicity associated with synthetic antioxidants exists (Bandonienė et al., 2002). Consequently, a significant effort to utilize natural medicinal products to screen for more potent antioxidant agents and identify free radical prevention processes was noted. Oriental medicinal plants have piqued

Received 29 March 2022; Revised 8 May 2022; Accepted 9 May 2022; Published online 30 June 2022

Correspondence to Sang Koo Park, E-mail: rokmc3588@korea.kr

© 2022 The Korean Society of Food Science and Nutrition.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

the interest of researchers as a potential source of novel drugs due to their diverse species and long-standing usage as traditional remedies in Asia (Ahmed et al., 2018; Blando et al., 2019; Dienaitė et al., 2019).

In this study, the chemical patterns of all compounds were analyzed and quantified from the chemical library based on the results of the isolation and identification. LC with diode array detection (DAD) and electrospray ionization (ESI)/MS was used to discover the structures of individual antioxidant compounds in *R. emodi* extract. The antioxidant compounds were subjected to a rigorous single-MS fragmentation analysis of preparative LC-MS purification. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), superoxide dismutase (SOD) activity, and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity were among the techniques used to evaluate the antioxidant activity of antioxidant compounds. Furthermore, it was measured and assessed to determine their correlation with antioxidant activities of bioactive compounds isolated from *R. emodi* Wall.

MATERIALS AND METHODS

Materials

R. emodi was discovered in Langtang, Nepal, at an elevation of 3,500 m. A botanist recognized the plant using established sources and authenticated it. Fig. 1 shows that the samples were prepared following the method (Park et al., 2021). The plant materials were air-dried, and 800 g of the sample were powdered and extracted with 70% ethanol under reflux (3×2.5 L, 2 h each time), then filtered through Whatman No. 2 filter paper. The solvent of the combined extract was evaporated under reduced pressure using a rotary vacuum-evaporator at 50°C and the remaining water was removed by freeze-dry. The vacuumed crude extract of EtOH extract was successively extracted with *n*-hexane (room temperature, 2×1 L), dichloromethane (2×1 L), ethyl acetate (2×1 L), *n*-butane (2×1 L) and water. The solvent fractions were stored below −18°C for further analysis.

DPPH radical scavenging assay

The influence of solvent fractions on the DPPH free radical was measured using the Brand-Williams et al. (1995) technique as reported by Yoo et al. (2008). Four milliliters of the fractions were mixed with 1 mL of DPPH, then homogenized and left to stand in the dark for 30 min. The DPPH level in each well was determined using a spectrophotometer to measure the absorbance at 520 nm, with ascorbic acid as the standard.

ABTS radical scavenging assay

The ABTS radical scavenging activity was used to deter-

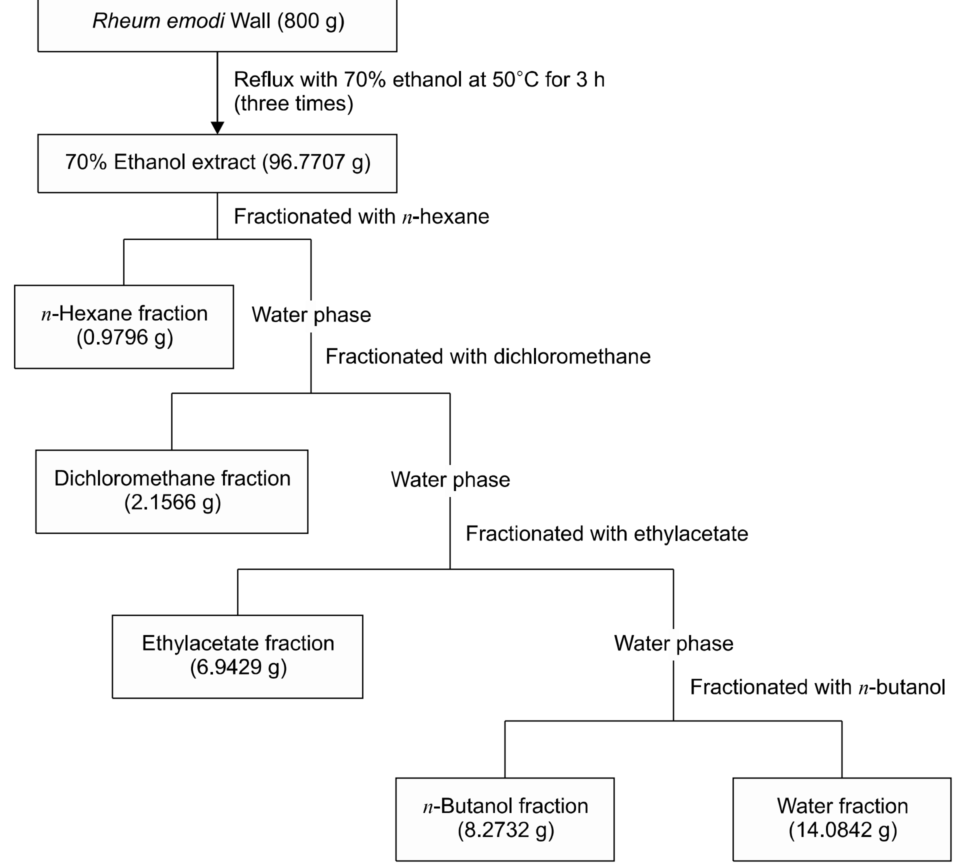

Fig. 1. Extraction and fractionation of *Rheum emodi*.

mine the total antioxidant activity of the samples (Re et al., 1999) as described by Park et al. (2021). The $ABTS^+$ was produced by reacting 7 mM ABTS in H_2O with 2.45 mM potassium persulfate ($K_2S_2O_8$) and stored in a dark at room temperature for 12∼16 h. It was then diluted with 99.5% ethanol to obtain an absorbance of 0.70±0.02 at 734 nm. The percentage inhibition at 734 nm for 190 μL of ABTS reagent mixed with 10 μL of the sample was evaluated after 30 min. Ascorbic acid was utilized as a reference. The replicates of this experiment were performed.

SOD assay

SOD assay was measured using a SOD kit (Dojindo Molecular Technologies Inc., Kumamoto, Japan) following the manufacturer's instructions. Moreover, 20 μL of double-distilled water was added to 20 μL for each sample solution. In addition, 200 μL of WST working solution was added to the mixture in each well. Each well received 20 mL of working enzyme solution and adequately mixed. The plate was then incubated at 37°C for 20 min. A microplate reader was used to measure absorbance at 450 nm, and ascorbic acid was employed as a reference. All of the tests were conducted in triplicate.

Qualitative analysis of LC-MS library for *R. emodi*

Based on various literature sources, an LC-MS library of 10 different stilbenoids, 16 different anthraquinones, and three different flavonols of antioxidant compounds from *R. emodi* was developed and used to efficiently determine individual components.

Quantitative and qualitative analyses of antioxidant compounds by LC-DAD-ESI/MS

The compounds present in *R. emodi* fractions were isolated following the method (Park et al., 2021) using Micromass ZQ MS (Waters Corp., Milford, MA, USA) and an Alliance e2695 high-performance liquid chromatography (HPLC) system (Waters Corp.) equipped with a 2998 photodiode array detector (PDA). In addition, the reversed-phase column YMC PACK ODS-AM (4.6×250 nm I.D., 5 m, YMC Co., Ltd., Kyoto, Japan) was used. The analysis was conducted at a flow rate of 1 mL/min in the detection wavelength range of 190∼600 nm (a representative wavelength of 254,350 nm) with a column heater set to 30°C. Trifluoroacetic acid in water (0.1%, phase A) and acetonitrile (phase B) were utilized as mobile phases. The pretreatment sample was evaluated using the following gradient conditions: a gradient of 10∼30% phase B over 25 min, 30% phase B for 5 min, a gradient of 30∼10% phase B for 3 min, and a final wash with 10% phase B for 7 min. The MS analysis was performed with an ESI source in positive ionization mode. The MS parameters were adjusted at 30 V cone voltage, 120°C source temperature, 350°C desolvation temperature, and 500 L/h desolvation N_2 gas flow. In full scan mode, the molecular weight range was 100∼1,200 m/z.

Preparative LC/MS system

The preparative LC/MS system included a Micromass ZQ MS (Waters Corp.) and a fractionation system of 2,767 samples manager (Waters Corp.) with 2,998 PDA. The analysis protocol was similar to isolating and identifying antioxidant compounds by LC-DAD-ESI/MS. Furthermore, the fraction settings were adjusted to 78 maximum fractions and tubes per injection, 60 s for the solvent front delay, 10 s for the collector delay, and 60 s for the maximum fraction width. The ethyl acetate fraction from *R. emodi* was used at 200 μL per injection at a concentration of 50,000 ppm. The collected fractions were examined straight from collection tubes without any further liquid handling. The peak purity of collected fractions relative to the principal component was evaluated using relative peak areas in mass chromatograms.

Statistical analysis

SPSS was used to analyze the data, which is reported as mean±standard deviation (version 12.0 for Windows XP, SPSS Inc., Chicago, IL, USA). One- and two-way analyses of variance and Duncan's multiple comparisons were used to test for any significant differences between the means; the mean values of antioxidant activity between two extracts or two treatments were assessed using an independent-sample *t*-test. Pearson's correlation coefficient was used to calculate bivariate correlations. A $P<0.05$ was considered significant. The chemical structures of compounds were drawn using the ChemDraw Ultra 8.0 program (PerkinElmer Inc., Waltham, MA, USA).

Multivariate statistical analysis (partial least squares-discriminant analysis)

The data matrix was made by normalizing and arranging all the qualitative and quantitative information, which was used for multivariate statistical analysis as $\log_{10}$-transformed data. Partial least squares-discriminant analysis (PLS-DA) models were run using SIMCA-P 11.0 software (Umetrics, Umeå, Sweden).

RESULTS

Diversity of *R. emodi* chemical library

Library information was used to investigate the antioxidant compounds and identify new compounds from *R. emodi*. Table 1 shows the database of information on approximately 10 different stilbenoids, 16 different anthraquinones, and three flavonols, respectively, built by a stilbenoid and anthraquinone library, which includes chemical names, molecular formulas, molecular weights, MS

Table 1. The chemical library of stilbenoids, anthraquinones, and flavonols from *Rheum emodi* based on literature sources

No.	Compound name	Molecular formula	Molecular weight	Fragment ions pattern	Chemical structure	Plant resources	State	References
				Stilbenoids				
1	Piceatannol	$C1_4H_{12}O_4$	244	$243(M-H)^-$		*R. emodi*, rhubarb, rhaponticum	Confirmed	Matsuda et al., 2001; Püssa et al., 2009; Chai et al., 2012
2	Piceatannol glycoside	$C_{20}H_{22}O_9$	406	$405(M-H)^-$, $243(M-H-glc)^-$		*R. emodi*, rhubarb, rhaponticum	Confirmed	Matsuda et al., 2001; Püssa et al., 2009; Chai et al., 2012
3	Desoxyrhapontigenin	$C_{15}H_{14}O_3$	242	$241(M-H)^-$		*R. emodi*, rhubarb rhaponticum	Confirmed	Matsuda et al., 2001; Babu et al., 2004; Püssa et al., 2009
4	Rhapontigenin	$C_{15}H_{14}O_4$	258	$257(M-H)^-$		*R. emodi*, rhubarb, rhaponticum	Confirmed	Matsuda et al., 2001; Babu et al., 2004; Püssa et al., 2009
5	Desoxyrhaponticin	$C_{21}H_{24}O_8$	404			*R. emodi*, rhubarb	Confirmed	Matsuda et al., 2001; Babu et al., 2004
6	Rhaponticin	$C_{21}H_{24}O_9$	420	$419(M-H)^-$, $257(M-H-glc)^-$		Rhubarb	Confirmed	Matsuda et al., 2001; Püssa et al., 2009
7	Isorhaponticin	$C_{21}H_{24}O_9$	420	$419(M-H)^-$, $257(M-H-glc)^-$		Rhubarb	Confirmed	Matsuda et al., 2001; Püssa et al., 2009
8	Isorhapontigenin	$C_{15}H_{14}O_4$	258	$257(M-H)^-$		*R. emodi*, rhubarb	Confirmed	Matsuda et al., 2001; Babu et al., 2004; Püssa et al., 2009
9	Rhaponticin 2″-*O*-gallate	$C_{28}H_{28}O_{13}$	572	$571(M-H)^-$, $419(M-H-glc)^-$, $257(M-H-glc-gal)^-$		Rhubarb	Confirmed	Matsuda et al., 2001; Püssa et al., 2009

Table 1. Continued

No.	Compound name	Molecular formula	Molecular weight	Fragment ions pattern	Chemical structure	Plant resources	State	References
10	Rhaponticin 6″-*O*-gallate	$C_{28}H_{28}O_{13}$	572	$571(M\text{-}H)^-$, $419(M\text{-}H\text{-}glc)^-$, $257(M\text{-}H\text{-}glc\text{-}gal)^-$		Rhubarb	Confirmed	Matsuda et al., 2001; Püssa et al., 2009
					Anthraquinones			
1	Emodin	$C_{15}H_{10}O_5$	270	$269(M\text{-}H)^-$		*R. emodi*, *Rhamnus alpines*, rhubarb	Confirmed	Matsuda et al., 2001; Singhet al., 2005; Verma et al., 2005; Püssa et al., 2009; Genovese et al., 2010; Singh et al., 2012
2	Physcion	$C_{16}H_{12}O_5$	284			*R. emodi*, *R. alpines*, rhubarb	Confirmed	Matsuda et al., 2001; Singhet al., 2005; Verma et al., 2005; Genovese et al., 2010; Singh et al., 2012
3	Chrysophanol	$C_{15}H_{10}O_4$	254			*R. emodi*, *R. alpines*, rhubarb	Confirmed	Matsuda et al., 2001; Singhet al., 2005; Verma et al., 2005; Genovese et al., 2010; Singh et al., 2012
4	Aloe-emodin	$C_{15}H_{10}O_5$	270			*R. alpines*, rhubarb	Confirmed	Genovese et al., 2010; Singh et al., 2012
5	Rhein	$C_{15}H_8O_6$	284			*R. emodi*, *R. alpines*, rhubarb	Confirmed	Matsuda et al., 2001; Singh et al., 2005; Verma et al., 2005; Genovese et al., 2010; Singh et al., 2012
6	Emodin 8-*O*-Glc	$C_{21}H_{20}O_{10}$	432	$431(M\text{-}H)^-$, $269(M\text{-}H\text{-}glc)^-$		*R. emodi*, rhubarb	Confirmed	Arun et al., 2005b; Püssa et al., 2009 Singh et al., 2012
7	Chrysophanol 8-*O*-Glc	$C_{21}H_{20}O_9$	416	$415(M\text{-}H)^-$, $253(M\text{-}H\text{-}glc)^-$		*R. emodi*, rhubarb	Confirmed	Matsuda et al., 2001; Singh et al., 2005; Verma et al., 2005; Püssa et al., 2009

Table 1. Continued 2

No.	Compound name	Molecular formula	Molecular weight	Fragment ions pattern	Chemical structure	Plant resources	State	References
8	Chrysophanol 1-*O*-Glc	$C_{21}H_{20}O_9$	416	415(M-H)$^-$, 253(M-H-glc)$^-$		Rhubarb	Confirmed	Matsuda et al., 2001
9	Aloe-emodin 8-*O*-Glc	$C_{21}H_{20}O_{10}$	432	431(M-H)$^-$, 269(M-H-glc)$^-$		Rhubarb	Confirmed	Püssa et al., 2009; Singh et al., 2012
10	Aloe-emodin 1-*O*-Glc	$C_{21}H_{20}O_{10}$	432	431(M-H)$^-$, 269(M-H-glc)$^-$		Rhubarb	Confirmed	Matsuda et al., 2001; Püssa et al., 2009
11	Chrysophanol 8-*O*-(6´-galloyl)-Glc	$C_{28}H_{24}O_{13}$	568	567(M-H)$^-$, 415(M-H-gal)$^-$, 253(M-H-gal-glc)$^-$		Rhubarb	Confirmed	Matsuda et al., 2001
12	Revandchinone-1	$C_{34}H_{46}O_6$	550			*R. emodi*	Confirmed	Babu et al., 2003
13	Revandchinone-2	$C_{43}H_{66}O_5$	662			*R. emodi*	Confirmed	Babu et al., 2003
14	Revandchinone-3	$C_{37}H_{54}O_5$	578			*R. emodi*	Confirmed	Babu et al., 2003
15	Revandchinone-4	$C_{33}H_{48}O_7$	556			*R. emodi*	Confirmed	Babu et al., 2003
16	Torachrysone 8-*O*-Glc	$C_{20}H_{24}O_9$	408			*R. emodi*, rhubarb	Confirmed	Matsuda et al., 2001

Table 1. Continued 3

No.	Compound name	Molecular formula	Molecular weight	Fragment ions pattern	Chemical structure	Plant resources	State	References
					Flavonols			
1	Myricetin	$C_{15}H_{10}O_8$	318	$319(M+H)^+$		*R. emodi*	Confirmed	Püssa et al., 2009
2	Myricetin 3-rhamnoside	$C_{21}H_{20}O_{12}$	464	$465\ (M+H)^+$, 463 $(M-H)^-$, 319 $(M+H-rham)^+$		*R. emodi*	Confirmed	Taamalli et al., 2014
3	Myricetin 3-galloyl rhamnoside	$C_{28}H_{24}O_{16}$	616	$617\ (M+H)^+$, 465 $(M+H-gal)^+$, 319 $(M+H-rham-gal)^+$, 299 $(rham+gal+H)^+$		*R. emodi*	Confirmed	New compounds in *R. emodi*

glc, glycoside (glucose); gal, galloyl (gallic acid).

fragment ion patterns, UV spectra, chemical structures, plant resources, and references from *R. emodi* (Matsuda et al., 2001; Babu et al., 2003; Babu et al., 2004; Singh et al., 2005; Verma et al., 2005; Püssa et al., 2009; Genovese et al., 2010; Chai et al., 2012; Singh et al., 2012; Taamalli et al., 2014). The structures of individual stilbenoids and anthraquinone isolated from *R. emodi*, rhubarb, and *Rheum rhaponicum* were identified based on the fragmentation patterns of rhamnoside (m/z 146), gallic acid (m/z 152), and glucoside (m/z 162). Stilbenoids with a basic piceatannol or piceatannol glycoside structure composed of primary agricones (i.e., desoxyrhapontigenin, rhapontigenin, isorhapontigen) were identified. In rhaponticin 2”-*O*-gallate and rhaponticin 6”-*O*-gallate, gallic acids were acylated with rhaponticin at positions 2” and 6”, respectively.

Anthraquinone was found to have a basic structure of emodin, physcion, chrysophanol, and so on. In chrysophanol 8-*O*-(6’-galloyl)-glc, gallic acid was acylated with chrysophanol as aglycone. The flavonols had a basic myricetin structure. Gallic acid was acylated with myricetin as aglycone in myricetin 3-galloylrhamnoside.

Identification of antioxidant compounds from *R. emodi*

According to Park et al. (2021), the polyphenol contents and antioxidant activities of the ethyl acetate fraction were the greatest. It was chosen as an extraction solvent for further investigation into the identification of antioxidant compounds using HPLC with gradient elution. Preparative HPLC separation was performed using an ethyl acetate fraction of *R. emodi* to identify the compounds (Fig. 2A). A chemical library comprising of the three components with their molecular formulas and weights, chemical names and structures, MS fragment ion patterns, UV spectra, and references was created from published *R. emodi* data. The mass fragmentation patterns of myricetin (m/z 318), rhamnoside (m/z 146), and gallic acid (m/z 152) were used to identify the structures of individual components from *R. emodi*. According to the LC-DAD-ESI/MS spectra, three components were recovered from the ethyl acetate fraction of *R. emodi* based on their retention time and detection wavelength of 350 nm.

Compound 1 was identified using literature and mass spectra of the samples as myricitrin (myricetin 3-*O*-rhamnoside) at 17.80 min through comparison of the retention times and fragmentation at m/z 465 with a molecular formula $C_{21}H_{20}O_{12}$ (Fig. 2B). Compound 2 was identified as myricetin 3-galloylrhamnoside at 23.82 min and fragmented at m/z 617 with a molecular formula of $C_{28}H_{24}O_{16}$ (Fig. 2C). Remarkably, this compound was found in this plant for the first time. Compound 3 was identified as myricetin (Fig. 2D) at 24.37 min and fragmented at 319 m/z with a molecular formula of $C_{15}H_{10}O_8$.

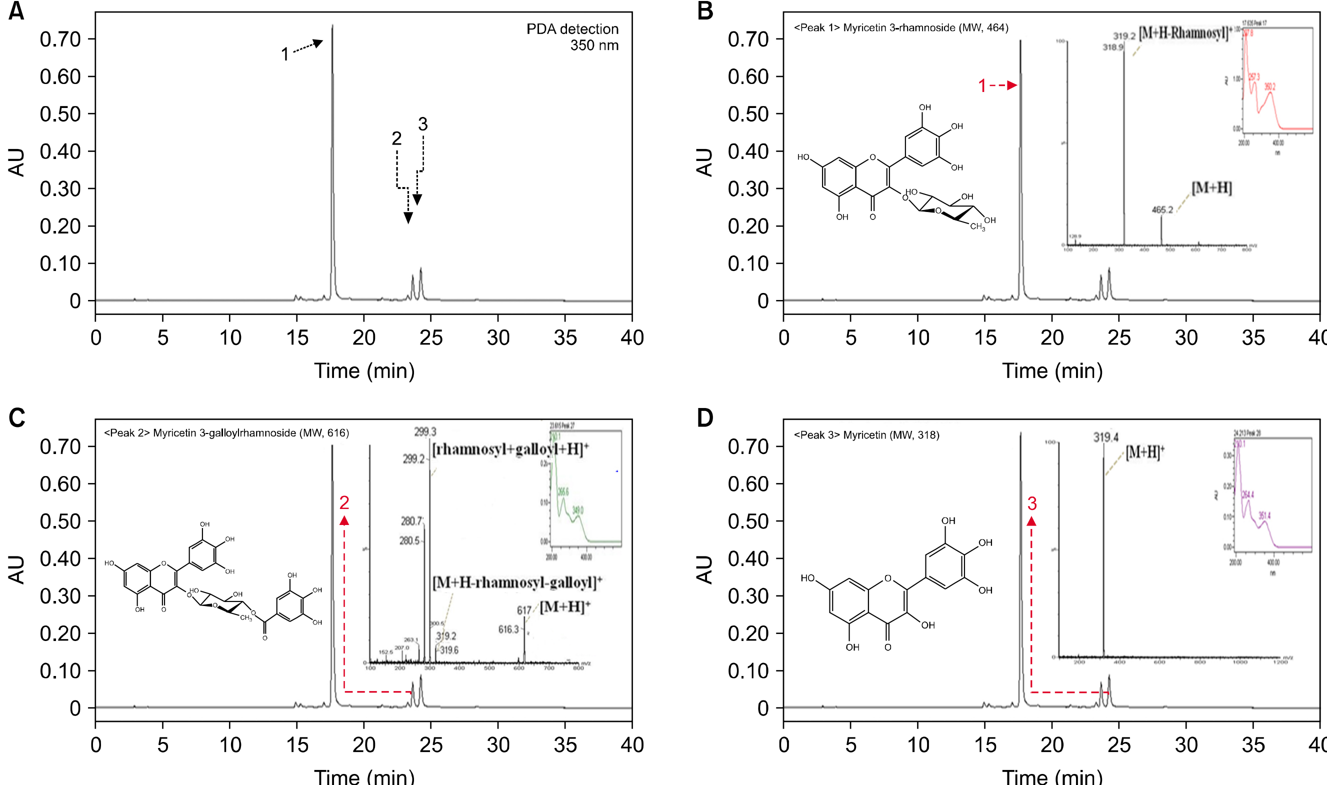

Fig. 2. (A) Liquid chromatography (LC) chromatogram (350 nm) of ethyl acetate fraction from *Rheum emodi*. LC, mass spectrometry, ultraviolet spectrum, and chemical structures of myricitrin (B), myricetin 3-galloyl rhamnoside (C), and myricetin (D). PDA, photodiode array detector; MW, molecular weight.

Antioxidant activity of the analysis of the compounds from *R. emodi*

Several approaches were used to screen the antioxidant activity capabilities of the *R. emodi* antioxidant compounds. The fractions' DPPH, $ABTS^{+}$ radical scavenging activity, and SOD activity, as well as the positive control, L-ascorbic acid, were assessed and converted into the half-maximal inhibitory concentration (IC_{50}) values (Table 2). DPPH radical scavenging activity of the compounds ranged from 1.50 to 28.46 μg/mL, and the IC_{50} value of positive control was 64.24 μg/mL. The IC_{50} values of myricetin (1.50 μg/mL), myricetin 3-galloylrhamnoside (26.66 μg/mL), and myricitrin (28.46 μg/mL) components were much lower than L-ascorbic acid. The components' levels were much lower than L-ascorbic acid.

The compounds' ABTS radical scavenging activity varied from 102.01 to 137.55 μg/mL, and the positive control's IC_{50} value was 91.55 μg/mL. The IC_{50} values of the myricetin (102.01 μg/mL), myricetin 3-galloylrhamnoside (120.32 μg/mL), and myricitrin (137.55 μg/mL) compounds were higher than the positive control.

SOD radical scavenging activity of the compounds varied from 4.06 to 15.74 μg/mL, and the positive control's IC_{50} value was 74.43 μg/mL. Myricetin (4.06 μg/mL), myricetin 3-galloylrhamnoside (8.18 μg/mL), and myricitrin (15.74 μg/mL) had considerably lower IC_{50} values than the positive control.

Partial least square discriminant analysis

PLS-DA has recently been utilized to quickly discriminate or discover variations in a wide range of food, pharmaceutical, and agricultural products. PLS-DA is more ad-

Table 2. Radical scavenging and antioxidant compounds activity of *Rheum emodi*

Fraction	IC_{50} (μg/mL)
DPPH radical scavenging	
Myricitrin	28.46 ± 2.82^{b}
Myricetin 3-galloylrhamnoside	26.66 ± 0.73^{ab}
Myricetin	1.50 ± 0.31^{a}
Ascorbic acid	64.24 ± 0.43^{c}
$ABTS^{+}$ radical scavenging	
Myricitrin	137.55 ± 4.72^{d}
Myricetin 3-galloylrhamnoside	120.32 ± 1.29^{c}
Myricetin	102.01 ± 3.01^{b}
Ascorbic acid	91.55 ± 3.69^{a}
SOD activity	
Myricitrin	15.74 ± 2.12^{c}
Myricetin 3-galloylrhamnoside	8.18 ± 1.04^{b}
Myricetin	4.06 ± 0.52^{a}
Ascorbic acid	73.43 ± 3.27^{d}

Values are presented as mean±standard deviation. Values within a column followed by different letters (a-d) are significantly different (P<0.05).
Ascorbic acid was used as a positive control.
IC_{50}, the half-maximal inhibitory concentration; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); SOD, superoxide dismutase.

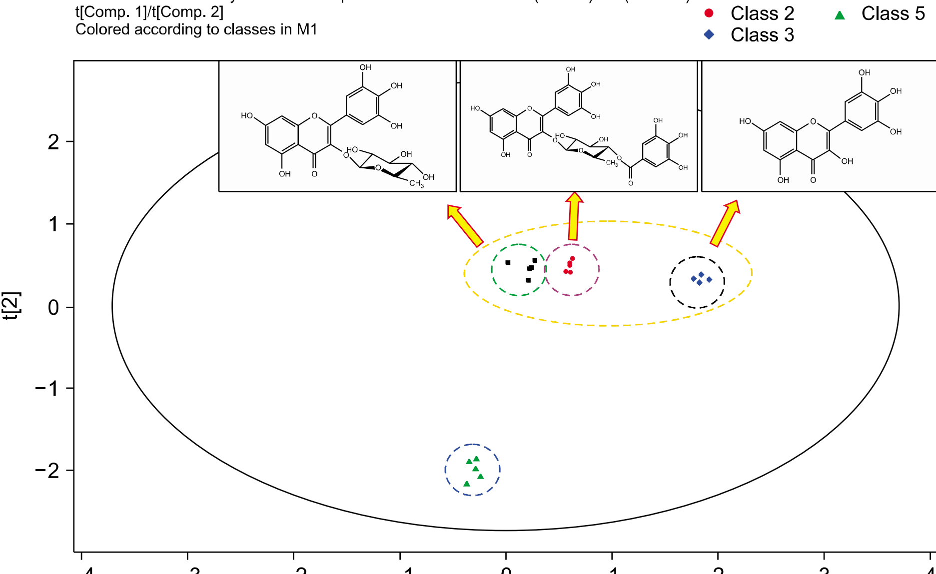

Fig. 3. Score plotting chart of principal components 1 and 2 of the partial least squares-discriminant analysis (PLS-DA) results obtained from the data set by compound profiling of the antioxidant compounds. This chart shows classification by the origin of all samples. Yellow circle indicates myricitrin, myricetin-3-galloylrhamnoside, and myrictin; blue circle indicates L-ascorbic acid.

vantageous in distinguishing the characteristics of predefined groups (Pérez-Enciso et al., 2003) than PCA. The individual score showed its pattern, change, and cluster formation that contained antioxidant activities of compounds from *R. emodi*. Fig. 3 shows that the correlation between antioxidant activities and purified compounds (myricitrin, myricetin 3-galloylrhamnoside, myricetin, and L-ascorbic acid) were expressed through PLS-DA score plotting. They were classified into two groups: myricetin aglycones and the L-ascorbic acid. The yellow cluster in compounds (myricitrin, myricetin 3-galloylrhamnoside, and myricetin) was located on the center-right side. The blue in L-ascorbic acid was located at the bottom. Thus, antioxidant activities are related to the compound's structure and contents, meaning that compounds belonging to the myricetin group cultivar had higher antioxidant activity than the other compounds belonging to L-ascorbic acid cultivars. In addition, the myricetin 3-galloylrhamnoside showed that the gallate acylation on the glycoside moiety on a flavonoid had higher antioxidant activities than myricitrin; however, the potency did not exceed the activity of the corresponding aglycon. Galloylation to the mother molecule increased antioxidant activity. The activity of the galloyl compounds was greater than the corresponding aglycones in their assay using an erythrocyte membrane ghost (Okamura et al., 1993).

Correlation of ethyl acetate fraction and antioxidant activity of antioxidant compounds from *R. emodi*

Table 3 shows the correlation of ethyl acetate fraction and antioxidant activity of antioxidant compounds from *R. emodi*. A highly significant correlation was noted between the ethyl acetate fraction and the antioxidant compounds determined by myricetin 3-galloylrhamnoside (R^2=0.998, P<0.01), myricitrin (R^2=0.992, P<0.01), and myricetin (R^2=0.972, P<0.01). These results suggest that the highly significant correlations obtained in this study support the hypothesis that the ethyl acetate fraction significantly contributes to the flavonol compounds from *R. emodi*. The antioxidant activity of *R. emodi* was

Table 3. Correlation between the total ethyl acetate fraction and the antioxidant activity of antioxidant compounds from *Rheum emodi*

Variable	Fraction	Antioxidant activity		
	Ethyl acetate	Myricitrin	Myricetin 3-galloylrhamnoside	Myricetin
Fraction				
Ethyl acetate	1			
Antioxidant activity				
Myricitrin	0.992**	1		
Myricetin 3-galloylrhamnoside	0.998**	0.997**	1	
Myricetin	0.972**	0.991**	0.984**	1

The values represent the correlation coefficient (r).
**P<0.01.

confirmed by comparing DPPH and ABTS radical scavenging activity in the nonenzymatic system with SOD activity in the enzymatic system. Thus, antioxidant compounds may effectively exploit *R. emodi* in the pharmaceutical industry.

DISCUSSION

In this study, the antioxidant properties of antioxidant compounds from *R. emodi* were assessed by LC-DAD-ESI/MS and LC-MS library, as well as DPPH, SOD activity, and ABTS radical scavenging activity. Consequently, three *R. emodi* compounds were identified: myricitrin, myricetin 3-galloylrhamnoside, and myricetin. *R. emodi* contains various anthraquinone and stilbene which are the most common components of *R. emodi*. Anthraquinones include rhein, chrysophanol, aloe-emodin, emodin, physcion, chrysophanein, and emodin glycoside. Piceatannol, resveratrol, and their glycosides make up stilbene (Malik et al., 2010). Recent research suggests and supports the potential use of myricitrin, rather than rhamnosides and quercetin, to reduce malondialdehyde, H_2O_2-induced oxidative damage, and increase antioxidant enzyme activity in ROS-induced cell dysfunction (Zhang et al., 2017). Despite these various antioxidant properties, in this work, myricitrin showed a lower antioxidant effect than myricetin. Based on its high iron-chelating capability, antioxidant, and free radical scavenging activities, recent research show and support the potential use of myricetin in processes of intrinsic resistance to cancer, diabetes, and cardiovascular protection (Mira et al., 2002; Ma et al., 2012; Yao et al., 2014; Park et al., 2021). In this study, myricetin had the lowest IC_{50} value among the others, even substantially lower than the control by DPPH radical scavenging and SOD activity, when the antioxidant activity of the discovered compounds was compared. These findings show that myricetin may be effective in exploiting *R. emodi* in the pharmaceutical industry to benefit from a medicinal herb.

In particular, the antioxidant activity of myricetin 3-galloylramnoside was lower than myricitrin in DPPH and SOD activity. Gallate acylation on the glycoside molecule importantly functions to increase antioxidant activity compared to the equivalent glycoside. Many flavonols occur in the plant body as glycosides at the 3-*O*-position, and such glycosylation reduces antioxidant activity (Yao et al., 2014). Furthermore, a significantly positive correlation was observed between the ethyl acetate fraction and the antioxidant compound. These results show that the ethyl acetate fraction contributes considerably to the flavonol compound from *R. emodi*. Park et al. (2021) suggested that antioxidant activity be validated by comparing DPPH, ABTS radical scavenging activity, and SOD activity. Among the five fractions tested, ethyl acetate had the lowest IC_{50} value, followed by *n*-hexane, *n*-butanol, dichloromethane, and water. Thus, the ethyl acetate fraction effectively scavenged free radicals in *R. emodi*.

In the PLS-DA model, they were classified into two different groups (i.e., myricetin aglycones and L-ascorbic acid). It will be used to differentiate their function into clusters created according to their antioxidant activity properties. Variation influences cluster formation or is regarded as crucial compound information. These results clearly demonstrate the role of the PLS-DA model based on the chemical structure of antioxidant compounds and the content of antioxidants. Collectively, these results suggest that the causal antioxidant compounds were identified by HPLC analysis using ethyl acetate fraction of *R. emodi* extract. They were evaluated using LC-DAD-ESI/MS and LC-MS library were identified, i.e., myricitrin, myricetin 3-galloylrhamnoside, and myricetin.

In the correlation coefficient, a significantly positive correlation was observed between ethyl acetate fraction and antioxidant compound. According to the results, the ethyl acetate fraction significantly contributes to the flavonol molecules from *R. emodi*. These compounds can act as antioxidant bioavailability enhancers and could be valuable in the pharmaceutical industry products.

FUNDING

None.

AUTHOR DISCLOSURE STATEMENT

The author declares no conflict of interest.

REFERENCES

Ahmed QU, Alhassan AM, Khatib A, Shah SAA, Hasan MM, Sarian MN. Antiradical and xanthine oxidase inhibitory activity evaluations of *Averrhoa bilimbi* L. leaves and tentative identification of bioactive constituents through LC-QTOF-MS/MS and molecular docking approach. Antioxidants. 2018. 7:137. https://doi.org/10.3390/antiox7100137

Babu KS, Srinivas PV, Praveen B, Kishore KS, Murty US, Rao JM. Antimicrobial constituents from the rhizomes of *Rheum emodi*. Phytochemistry. 2003. 62:203-207.

Babu KS, Tiwari AK, Srinivas PV, Ali AZ, China Raju B, Rao JM. Yeast and mammalian α-glucosidase inhibitory constituents from Himalayan rhubarb *Rheum emodi* Wall.ex Meisson. Bioorg Med Chem Lett. 2004. 14:3841-3845.

Bandonienė D, Venskutonis PR, Gruzdienė D, Murkovic M. Antioxidative activity of sage (*Salvia officinalis* L.), savory (*Satureja hortensis* L.) and borage (*Borago officinalis* L.) extracts in rapeseed oil. Eur J Lipid Sci Technol. 2002. 104:286-292.

Blando F, Russo R, Negro C, De Bellis L, Frassinetti S. Antimicro-

bial and antibiofilm activity against *Staphylococcus aureus* of *Opuntia ficus-indica* (L.) Mill. cladode polyphenolic extracts. Antioxidants. 2019. 8:117. https://doi.org/10.3390/antiox8050117
Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT—Food Sci Technol. 1995. 28:25-30.
Burgos-Morón E, Abad-Jiménez Z, Marañón AM, Iannantuoni F, Escribano-López I, López-Domènech S, et al. Relationship between oxidative stress, ER stress, and inflammation in type 2 diabetes: the battle continues. J Clin Med. 2019. 8:1385. https://doi.org/10.3390/jcm8091385
Chai YY, Wang F, Li YL, Liu K, Xu H. Antioxidant activities of stilbenoids from *Rheum emodi* Wall. Evid Based Complement Alternat Med. 2012. 2012:603678. https://doi.org/10.1155/2012/603678
Cross CE, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, et al. Oxygen radicals and human disease. Ann Intern Med. 1987. 107:526-545.
Dienaitė L, Pukalskienė M, Pukalskas A, Pereira CV, Matias AA, Venskutonis PR. Isolation of strong antioxidants from *Paeonia officinalis* roots and leaves and evaluation of their bioactivities. Antioxidants. 2019. 8:249. https://doi.org/10.3390/antiox8080249
Frei B. Natural antioxidants in human health and disease. Academic Press, San Diego, CA, USA. 1994. p 25-55.
Genovese S, Tammaro F, Menghini L, Carlucci G, Epifano F, Locatelli M. Comparison of three different extraction methods and HPLC determination of the anthraquinones aloe-emodine, emodine, rheine, chrysophanol and physcione in the bark of *Rhamnus alpinus* L. (*Rhamnaceae*). Phytochem Anal. 2010. 21:261-267.
Halliwell B. Biochemistry of oxidative stress. Biochem Soc Trans. 2007. 35:1147-1150.
Malik S, Sharma N, Sharma UK, Singh NP, Bhushan S, Sharma M, et al. Qualitative and quantitative analysis of anthraquinone derivatives in rhizomes of tissue culture-raised *Rheum emodi* Wall. plants. J Plant Physiol. 2010. 167:749-756.
Matsuda H, Morikawa T, Toguchida I, Park JY, Harima S, Yoshikawa M. Antioxidant constituents from rhubarb: structural requirements of stilbenes for the activity and structures of two new anthraquinone glucosides. Bioorg Med Chem. 2001. 9:41-50.
Ma Z, Liu T. Myricetin facilitates potassium currents and inhibits neuronal activity of PVN neurons. Neurochem Res. 2012. 37:1450-1456.
Mira L, Fernandez MT, Santos M, Rocha R, Florêncio MH, Jennings KR. Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. Free Radic Res. 2002. 36:1199-1208.
Okamura H, Mimura A, Yakou Y, Niwano M, Takahara Y. Antioxidant activity of tannins and flavonoids in *Eucalyptus rostrata*. Phytochemistry. 1993. 33:557-561.
Park SK, Lee YK. Antioxidant activity in *Rheum emodi* Wall (Himalayan rhubarb). Molecules. 2021. 26:2555. https://doi.org/10.3390/molecules26092555
Peigen X, Liyi H, Liwei W. Ethnopharmacologic study of Chinese rhubarb. J Ethnopharmacol. 1984. 10:275-293.
Pérez-Enciso M, Tenenhaus M. Prediction of clinical outcome with microarray data: a partial least squares discriminant analysis (PLS-DA) approach. Hum Genet. 2003. 112:581-592.
Püssa T, Raudsepp P, Kuzina K, Raal A. Polyphenolic composition of roots and petioles of *Rheum rhaponticum* L. Phytochem Anal. 2009. 20:98-103.
Rajkumar V, Guha G, Ashok Kumar R. Antioxidant and anti-cancer potentials of *Rheum emodi* rhizome extracts. Evid Based Complement Alternat Med. 2011. 2011:697986. https://doi.org/10.1093/ecam/neq048
Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999. 26:1231-1237.
Rolta R, Kumar V, Sourirajan A, Upadhyay NK, Dev K. Bioassay guided fractionation of rhizome extract of *Rheum emodi* Wall as bio-availability enhancer of antibiotics against bacterial and fungal pathogens. J Ethnopharmacol. 2020. 257:112867. https://doi.org/10.1016/j.jep.2020.112867
Rolta R, Sharma A, Kumar V, Sourirajan A, Baumler DJ, Dev K. Methanolic extracts of the rhizome of *R. emodi* act as bioenhancer of antibiotics against bacteria and fungi and antioxidant potential. Med Plant Res. 2018. 8:74-85.
Singh NP, Gupta AP, Sinha AK, Ahuja PS. High-performance thin layer chromatography method for quantitative determination of four major anthraquinone derivatives in *Rheum emodi*. J Chromatogr A. 2005. 1077:202-206.
Singh P, Negi JS, Rawat MSM, Pant GJ. HPLC separation of anthraquinones from rhubarbs. Int J Med Aromat Plants. 2012. 2:531-535.
Sözmen EY, Tanyalçin T, Onat T, Kutay F, Erlaçin S. Ethanol induced oxidative stress and membrane injury in rat erythrocytes. Eur J Clin Chem Clin Biochem. 1994. 32:741-744.
Taamalli A, Iswaldi I, Arráez-Román D, Segura-Carretero A, Fernández-Gutiérrez A, Zarrouk M. UPLC-QTOF/MS for a rapid characterisation of phenolic compounds from leaves of *Myrtus communis* L. Phytochem Anal. 2014. 25:89-96.
Verma SC, Singh NP, Sinha AK. Determination and locational variations in the quantity of hydroxyanthraquinones and their glycosides in rhizomes of *Rheum emodi* using high-performance liquid chromatography. J Chromatogr A. 2005. 1097:59-65.
Wani PA, Nawchoo IA, Wafai BA. The role of phenotypic plasticity, phenology, breeding behaviour and pollination systems in conservation of *Rheum emodi* Wall. ex Meisn. (*Polygonaceae*)—A threatened medicinal herb of North West Himalaya. Int J Plant Reprod Biol. 2009. 1:179-189.
Yao Y, Lin G, Xie Y, Ma P, Li G, Meng Q, et al. Preformulation studies of myricetin: A natural antioxidant flavonoid. Pharmazie. 2014. 69:19-26.
Yoo KM, Lee CH, Lee H, Moon BK, Lee CY. Relative antioxidant and cytoprotective activities of common herbs. Food Chem. 2008. 106:929-936.
Zhang B, Shen Q, Chen Y, Pan R, Kuang S, Liu G, et al. Myricitrin alleviates oxidative stress-induced inflammation and apoptosis and protects mice against diabetic cardiomyopathy. Sci Rep. 2017. 7:44239. https://doi.org/10.1038/srep44239