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

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**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-sulfonyl 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
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-
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 H2O with 2.45 mM potassium persulfate (K2S2O8) 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 desolation temperature, and 500 L/h desolvation N2 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 log10 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
| No. | Compound name | Molecular formula | Molecular weight | Fragment ions pattern | Chemical structure | Plant resources | State | References |
|-----|---------------|-------------------|------------------|----------------------|-------------------|----------------|-------|------------|
| 1   | Piceatannol   | C_{14}H_{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_{38}H_{36}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 |
| No. | Compound name          | Molecular formula | Molecular weight | Fragment ions pattern                      | Chemical structure | Plant resources | State       | References                                  |
|-----|-----------------------|------------------|------------------|--------------------------------------------|--------------------|----------------|------------|--------------------------------------------|
| 1   | Emodin                | C_{6}H_{10}O_{5}  | 270              | 269(M-H)^{+}                               | R. emodi, Rhamnus  | Confirmed      | Matsuda et al., 2001: Singh et al., 2005: | Singh et al., 2005: Verma et al., 2005: Püssa et al., 2009 |
| 2   | Physcion              | C_{6}H_{12}O_{5}  | 284              |                                            | R. emodi, R. alpines, rhubarb | Confirmed      | Matsuda et al., 2001: Singh et al., 2005: | Singh et al., 2005: Verma et al., 2005: Genovese et al., 2010: Singh et al., 2012 |
| 3   | Chrysophanol          | C_{6}H_{10}O_{4}  | 254              |                                            | R. emodi, R. alpines, rhubarb | Confirmed      | Matsuda et al., 2001: Singh et al., 2005: | Singh et al., 2005: Verma et al., 2005: Genovese et al., 2010: Singh et al., 2012 |
| 4   | Aloe-ewoodin          | C_{6}H_{10}O_{5}  | 270              |                                            | R. alpines, rhubarb | Confirmed      | Genovese et al., 2010: Singh et al., 2012 |                                             |
| 5   | Rhein                 | C_{13}H_{14}O_{6} | 284              |                                            | R. emodi, R. alpines, rhubarb | Confirmed      | Matsuda et al., 2001: Singh et al., 2005: | Singh et al., 2005: Verma et al., 2005: Genovese et al., 2010: Singh et al., 2012 |
| 6   | Emodin 8-O-Glc        | C_{8}H_{10}O_{10} | 432              | 431(M-H)^{+}, 269(M-H-glc)^{+}             | R. emodi, rhubarb  | Confirmed      | Arun et al., 2005b: Püssa et al., 2009    | Singh et al., 2012 |
| 7   | Chrysophanol 8-O-Glc  | C_{7}H_{12}O_{9}  | 416              | 415(M-H)^{+}, 253(M-H-glc)^{+}             | R. emodi, rhubarb  | Confirmed      | Matsuda et al., 2001: Singh et al., 2005: | Singh et al., 2005: Verma et al., 2005: Püssa et al., 2009 |

**Anthraquinones**
| No. | Compound name                  | Molecular formula | Molecular weight | Fragment ions pattern                                      | Chemical structure | Plant resources | State      | References                     |
|-----|--------------------------------|-------------------|------------------|------------------------------------------------------------|--------------------|----------------|------------|--------------------------------|
| 8   | Chrysophanol 1-\(\beta\)-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-\(\beta\)-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-\(\beta\)-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-\(\beta\)-(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_{34}H_{46}O_{6} | 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-\(\beta\)-Glc | C_{20}H_{24}O_{9} | 408              |                                                           | R. emodi, rhubarb | Confirmed      | Matsuda et al., 2001 |
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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}.

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**Table 1. Continued 3**

| No. | Compound name | Molecular formula | Molecular weight | Fragment ions pattern | Chemical structure | Plant resources | State | References |
|-----|---------------|-------------------|------------------|----------------------|--------------------|----------------|-------|------------|
| 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)^+          |                    | R. emodi       | Confirmed | Taamalli et al., 2014 |
| 3   | Myricetin 3-galloylrhamnoside | C_{28}H_{24}O_{16} | 616              | 617 (M+H)^+          |                    | R. emodi       | Confirmed | New compounds in *R. emodi* |

glc, glycoside (glucose); gal, galloyl (gallic acid).
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 (IC50) values (Table 2). DPPH radical scavenging activity of the compounds ranged from 1.50 to 28.46 μg/mL, and the IC50 value of positive control was 64.24 μg/mL. The IC50 values of myricetin (1.50 μg/mL), myricetin 3-galloyl rhamnoside (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 IC50 value was 91.55 μg/mL. The IC50 values of the myricetin (102.01 μg/mL), myricetin 3-galloyl rhamnoside (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 IC50 value was 74.43 μg/mL. Myricetin (4.06 μg/mL), myricetin 3-galloyl rhamnoside (8.18 μg/mL), and myricitrin (15.74 μg/mL) had considerably lower IC50 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 3. Correlation of ethyl acetate fraction and antioxidant activity of antioxidant compounds from *R. emodi*

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

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

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-galloylhrhamnoside ($R^2=0.998$, $P<0.01$), 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 advantageous 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-galloylhrhamnoside, 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-galloylhrhamnoside, 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-galloylhrhamnoside 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).
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-galloylramnoside, and myricetin. *R. emodi* contains various anthraquinone and stilbene which are the most common components of *R. emodi*. Anthraquinones include rhein, chrysophanol, aloem-emin, emodin, physcion, chrysophanein, and emodin glycoside. Piceatannol, resveratrol, and their glycosides make up stilbene physcion, chrysophanein, and emodin glycoside. Piceatannol was lower than myricitrin in DPPH and ABTS radical scavenging activity, when the antioxidant compound. These results show that myricetin may be effective in exploiting *R. emodi* in the pharmaceutical industry to benefit from a medicinal herb.

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.

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**AUTHOR DISCLOSURE STATEMENT**

The author declares no conflict of interest.

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