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Chemical characterization of *Dioscorea cirrhosa* by UHPLC-Q-Exactive Orbitrap Mass Spectrometry and assessment of their antioxidant activities

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Dioscorea cirrhosa Lout.(*D. cirrhosa)* has been traditionally used as a dye and folk medicine. The tuber is the pharmaceutical part of this plant, which has the effects of antioxidant, anti-tumor, anti-radiation, and anti-pressure. Previous studies on the tuber of *D. cirrhosa* have been detailed; however, its peels are often discarded as waste and fewer studies. Consequently, it is necessary to conduct a systematic and comprehensive research on the chemical constituents and pharmacological activities of *D. cirrhosa* peels . In this study, UHPLC-Q-Exactive Orbitrap MS was used for the first time to qualitatively analyze *D. cirrhosa* peels, and combined it with *in vitro* antioxidant activities. Eventually, A total of 196 compounds, including 118 flavonoids, 38 organic acids, 37 phenols and other components ,etc, were identified based on the chromatographic retention time, bibliography data, MS^2 information, neutral loss fragment (NLFs), and diagnostic fragment ion (DFIs) . Among of these, 176 compounds were reported for the first time in peel. *D. cirrhosa* present decent potent ABTS and DPPH scavenging abilities and Copper ion reducting ability. This new discovery elucidates the pharmacodynamic material basis and further proves its remarkable antioxidant activity.

Keywords: UHPLC-Q-Exactive Orbitrap mass spectrometry; Chemical constituent; *Dioscorea cirrhosa*; antioxidant activity

Dioscorea cirrhosa Lout. (*D. cirrhosa*) , named as "Hongyaozi" and "Honghaier" in China, is a perennial winding sturdy vine of the genus Dioscorea of Dioscoreacease, It is widely distributed in Guangdong, Guangxi, Fujian, Jiangxi, Hunan and other southern low-altitude mountains[1]. According to literature records, it is rich in tannin and polyphenols, polysaccharides, amino acids, unsaturated fatty acids and volatile oils[2]. The medicinal part is tuber, which is mostly used in folk for hemostasis, uterine contraction, antibacterial, etc.[3-5], and is also used in the food industry such as brewing wine[6]. However, the current use of tubers is mainly in the production of fragrant gauze in the dyeing and weaving industry [7], and peels are often discarded as a physical waste, mainly because their chemical composition and efficacy have not been reported yet.

Previously, a detailed investigation on its tubers have indicated that the plant contains a variety of different compounds with different activities, etc, including antioxidant, anti-tumor, anti-radiation, and antihypertensive ,etc.[8-11]. Recently, only a few reports [12]about *D. cirrhosa* peels have been reported; therefore, it is necessary to elucidate its chemical composition and pharmacological activities.

UHPLC-MS/MS is a powerful analytical technique that has been widely used in the detection and identification of in *vitro* chemical components in traditional Chinese medicine. UHPLC-Q-Orbitrap-MS has the characteristics of high resolution, high sensitivity and high separation efficiency, which is conducive to the identification of chemical components of traditional Chinese medicine[39-41].This study was aim to establish a rapid and efficient method based on UHPLC-Q-Exactive Orbitrap mass spectrometry to identify the unknown chemical constituents of *D. cirrhosa* peels and investigate their antioxidant activities, which will lay a foundation for future development and provide a guidance for the search for natural sources of antioxidants.

Materials and methods

Chemicals and reagents

The detail information of reference standards is listed in Table S1. LC-MS grade

of formic acid was obtained from Fisher Scientific (New Jersey, USA). Methanol and acetonitrile were of chromatographic grade and provided by Merck KGaA (New Jersey, USA). Deionized water was further purified using a Milli-Q system (Millipore, Milford, MA, USA). All other reagents were of analytical grade.

Catechin, bovine serum albumin and 2,2'-alnitrogen-double-3-ethyl benzothiazole Lin-6-sulfonic acid (ABTS) were purchased from Yuanye Biotechnology Co.(Shanghai). Potassium persulfate ($K_2S_2O_8$,AR), ascorbic acid (Vc, AR) and ammonium acetate were purchased from Sinopharm Chemical Reagent Co., Ltd. 1,1-Diphenyl-2-picrohydrazine Free Radical (DPPH) was purchased from Shanghai Development Co., Ltd. anhydrous cupric sulfate was purchased from Tianjin Guangfu; Neocuproine was purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. Sodium hydroxide purchased from Nanjing Chemical Reagent Co., Ltd. Other chemicals and solvents used in this study were of analytical grade.

Plant materials and extraction procedure

The powder of *D. cirrhosa* peels was purchased from Kunming ZhiFen Biotechnology Co., LTD. The voucher specimens were deposited at the School of Pharmaceutical Sciences, Hunan University of Medicine.

One gram of the *D. cirrhosa* peels was accurately weighed, and extracted by 10mL methanol with ultrosonic machine for 30min at 30 ℃ and 40 kHz. Then, the extracting solution was centrifuged at 5000 rpm, 15min and 4℃ to collect supernatant. Then the supernatant was dried by rotary steaming at 45℃, and lyophilized in the LGJ-10C vacuum freeze dryer (Sihuan Furuikeyi Technology Development Co., Ltd.) to gain powder of (EDP), which were stored at −20 ℃ before further experiments.

Total tannin, condensed tannin, flavonoidsand protein content assay

The content of total tannin was determined by Phosphomolybdic Acid Casein Colorimetric method[13]. The Vanillin Sulfate method[14]was used to determine condensed tannin content. The Aluminum Nitrate method was used[15]to determine

flavonoids content. The total protein content was determined by Coomassie Brilliant Blue G-250[16].

UPLC-Q-Exactive Orbitrap mass spectrometry analyses

Standard solutions and sample preparation

All standard solutions were precisely weighed and dissolved was precisely weighed and dissolved in methanol in(1 mg/mL). The solutions of 41 standards was mixed and successively diluted in a appropriate concertration of 10 μg/mL in methanol, then stored in 4 ℃.

The approximately weighed portion of 1g EDP was dissolved in 10 mL methanol and centrifuged at 12000 r/min for 10 min. and then re-dissolved with 1 mL methanol. Next, centrifuge at 12000 rpm again for 10 minutes. The volume of 2μL was injected into UHPLC-MS for analysis.

Instrumentation and UHPLC-MS/MS conditions

UHPLC analyses were performed using an Ultimate 3000 (Dionex, Sunnyvale, CA, USA) system consisting of an autosampler, a vacuum degasser, and a binary pump, a column compartment, controlled by Xcalibur 4.2 software (Thermo Fisher Scientific, California, USA). The chromatography was performed on a Thermo Scientific Hypersil GOLD aQ (100 mm ×2.1mm, 1.9 μm) column with the column temperature at 40℃ and the sample at 10℃. The mobile phase is water consisting of 0.1% formic acid (A) and acetonitrile (B). The gradient procedure is :0 min, 5% B; 2 minutes, 10% B; 5 minutes, 10% B; 10 min, 15% B; 12 min, 30% B; 20 minutes, 40% B; 25 minutes, 95% B; 26 minutes, 5% B. The flow rate was set at 0.30 mL/min.

The UHPLC system was equipped with a Q-Exactive Orbitrap MS (Thermo Fisher Scientific, Bremen, Germany), which uses electrospray ionization (ESI) in the scan range *m/z* 100-1000, Mass spectrometry was performed in positive and negative ionization mode with a resolution of 35,000. The gas source conditions are sheath gas 30, auxiliary gas 10, spray voltage (-)-ESI is 3.0kV, (+) -ESI is 3.5kV, capillary temperature is 320℃, auxiliary gas heater temperature is 350℃. The MS^1 spectrum was obtained by full mass spectrometry mode with a resolution of 35,000, and the MS^2 spectrum was obtained by triggered ddMS^2 or parallel reaction monitoring (PRM)

mode. The normalized collision energy (NEC) is set at 30% and the automatic gain control (AGC) target is $5.0 \times e^5$.

Data processing and analysis

All the data acquisition and analysis were made by Xcalibur™ version 4.1 and Compound Discovery (CD) 3.0 (Thermo Fisher scientific, California, USA). In this research, LC-MS original data was imported into Compound Discovery with Traditional Chinese Medicine (TCM) workflow templates. First, MS and MS^2 data were compared with data from mzCloud, mzvault, and Orbitrap Traditional Chinese Medicine Library (OTCML) databases to identify compounds in EDP. The optimized parameters of TCM workflow template were set as follows: MS and MS^2 with mass tolerances of 5 and 10 ppm; the maximum element count:C-60 H-60 O-60 N-10; the min peak intensity: 1,000,000; S/N threshold: 10; isotopic peaks: used. Then, The Xcalibur 4.2 was used for further chemical characterization of retention time, fragmentation ions.

In *vitro* antioxidant activity

ABTS radical scavenging aasay

The ABTS radical scavenging activity of EDP was determined by the previous method[17]. Briefly, 7 mmol/L ABTS solution was mixed with 2.45 mmol/L potassium persulfate solution in equal volume, incubated away from light for 16 h, and diluted with phosphate buffer (0.2 mol/L) to obtain a light absorption value of 0.7 0± 0.02 at 734nm wavelength. 195 μL ABTS working liquid and 5 μL EDP test liquid with different concentrations (25, 50, 100, 200, 400 μg/mL) were placed in a 96-well plate for 30min, and absorbance values were measured at 734nm. Ascorbic acid (Vc) was used as a positive control. The radical scavenging activity was calculated by the following equation:

$$\text{ABTS radicalscavengingactivity}(100\%)=\left(1-\frac{A_1-A_2}{A_0}\right)\times 100\%$$

Where A0 stands for the absorbance of the control (distilled water without sample), A2 is the absorbance of the mixture with ABTS replaced by anhydrous ethanol, and A1 stands for the absorbance of the sample.

DPPH radical scavenging assay

The DPPH radical scavenging activity of EKL was determined by the previous

method [18]. Firstly, 100ul DPPH solution (0.2 mmol /L) and 100ul EDP solution with different concentrations (2.5, 5, 7.5, 10, 25 μg/mL) were placed in a 96-well plate, shaken well, and subjected to a light-free reaction at 37℃ for 30 min. The absorbance was determined at 517 nm. Ascorbic acid (Vc) was used as a positive control. The radical scavenging activity was calculated by the following equation：

$$\mathrm{DPPHradicalscavengingactivity}(100\%)=\left(1-\frac{A_1-A_2}{A_0}\right)\times 100\%$$

Where B1 stands for the absorbance of the mixture with the sample replaced by anhydrous ethanol, and B2 is the absorbance of control with DPPH replaced by distilled water. A2 is the absorbance of the mixture with DPPH replaced by anhydrous ethanol, and A1 stands for the absorbance of the sample.

Copper ion reducting assay

The copper ion reduction capacity of EDP was determined by CUPRAC method [19-20]. Firstly, EDP solution 20 μL, 0.01 mmol/L copper sulfate solution 60 μL, 7.5 mmol/L new cuprous reagent 60 μL and 1 mmol/L ammonium acetate buffer 60μL were added into the pores of the 96-well plate. After mixing, the absorbance A was measured at 450 nm, and distilled water was used as blank. The copper ion reducing ability was calculated by the following equation：

$$\mathrm{Copperionreductingactivity}(100\%)=\left(1-\frac{A_1-A_2}{A_0}\right)\times 100\%$$

A is the absorbance of the experimental group, A0 is the absorbance of the blank control group, and Amax is the maximum absorbance. Where A0 stands for the absorbance of the control, A stands for the absorbance of the sample, and Amax is the maximum absorbance.

Results

Total content of tanin, flavonoides, and protein in EDP

EDP was mainly composed of total tannin (29%), condensed tannin (25%), Flavonoids (19%) and protein (16%). According to previous studies, tannins in tuberculus are mainly catechins condensed tannins, which have good free radical scavenging effect. Flavonoids are polyphenol compounds, which have

anti-inflammatory and antioxidant effects, cardiovascular protection, regulation of glucose and lipid metabolism. Thus, we speculate that condensed tannins and flavonoids play an important role in its antioxidant of the EDP .

Identification of chemical composition

A total of 194 compounds were firstly identified from peels of *D. cirrhosa,* according to retention time, molecular ions, and MS^2 spectra by comparing with standards, literature data, and inhouse database (OTCML), which are mainly classified into four groups: flavonoids, organic acids, tannins, anthocyanin, and others. Retention time, molecular ions, error, MS/MS fragment data, and identified compounds are listed in Table 1.

Organic Acids

As shown in Table 1, a total of 38 organic acids were identified. Compond 48, eluted at 4.74 min, showed a molecular ion $[M-H]^-$ at *m/z* 353.0880($C_{16}H_{18}O_9$), and fragment ions at *m/z* 191.0554, 135.0440, 173.0448, 179.0342 and 161.0236. The MS/MS profiles were the same compared with the reference standard; therefore, it was identified as chlorogenic acid (trans 5-CQA).

Componds 54, 76, 81 were eluted at 5, 6.26 and 6.56 min, showed a molecular ion $[M-H]^-$ at *m/z* 179.0349($C_9H_8O_4$), and fragment ions at *m/z* 179.0342, The MS/MS profiles were the same compared with the reference standard; therefore, they were identified as caffeic acid. Compounds 25 and 40 were eluted at 3.72, and 4.40 min, showed a molecular ion $[M-H]^-$ *m/z* 341.0878 ($C_{15}H_{18}O_9$), and fragment ions at *m/z* 135.0441, 153.0184 and 179.0341, which indicated they contained the caffeic acid part. With a fragment iron at *m/z* 179.0341 resulted from the neutral loss of 162 Da, corresponding with the loss of one hexose moiety; hence, they were considered caffeic acid-hexoside.

Compounds 11 and 80 were eluted at 2.15, 6.51 min, respectively, showing the same molecular ion $[M-H]^-$ *m/z* 167.0349 ($C_8H_8O_4$), as well as fragment ions at *m/z* 123.044, 108.021, and 152.010, This indicates they were an isomer of vanillic acid [21]. Compounds 17, 19, 36, 66 and 68 were detected at 2.52, 3.14, 4.25, 5.59, and 5.82 min, with the same molecular ion $[M-H]^-$ *m/z* 329.0878 ($C_{14}H_{18}O_9$) . All of those compounds yield the fragment ions *m/z* 123.0437, 108.0203, 152.0103, which indicated they contained vanillic acid part. With a fragment iron at *m/z* 167.0338 resulted from the neutral loss of 162 Da, corresponding with the loss of one hexose

moiety; therefore, they were considered to be vanillic acid-o-glucoside. Similarly, compound 50 was eluted at 4.76 min with molecular ion position *m/z* 475.1457 ($C_{20}H_{28}O_{13}$), with a fragment iron at *m/z* 167.0339 resulted from the neutral loss of 308 Da, corresponding with the loss of one rutinose moiety; therefore, compound 50 were considered as vanillic acid-o-rutinoside[21].

Compounds 20 and 59 were eluted at 3.16 ,and 5.33 min with a quasi-molecular ions at *m/z* 197.0455($C_9H_{10}O_5$) and fragment ions at *m/z* 153.0184, 182.0214 and 166.9977, indicating it was syringic acid isomer[22].

Compound 63 produced a molecular ion at $[M-H]^-$ *m/z* 353.0878 ($C_{16}H_{18}O_9$), and was observed at 5.48 min with the same MS1 data and similar MS2 data as chlorogenic acid (trans 5-CQA), so compound 63 was identified as chlorogenic acid (trans 5-CQA). Compound 66 was identified as cis-5-CQA based on retention time, MS_2 data and published papers [23-25].

Compond 92, eluted at 9.40min, showed a molecular ion $[M-H]^-$ at *m/z* 193.0497($C_{10}H_{10}O_4$), and fragment ions at *m/z* 178.0266, 134.0364, 158.8460, 149.0600 and 137.0235. The MS/MS profiles were the same compared with the mzValut, massbank database; therefore, it was identified as ferulic acid. Compounds 42, 49, 57, 62, 75, and 83 were detected at 4.50, 4.74, 5.23, 5.41, 6.25, and 6.98 min with fragment ions $[M-H]^-$ at *m/z* 193.049, 178.026, 134.036, which corresponded to ferulic acid part. With a fragment iron at *m/z* 355.1034 resulted from the neutral loss of 162 Da, corresponding with the loss of one glucose moiety; therefore, they were considered as glucosides of ferulic acid, and according to the published paper [21], they were identified as isomers of ferulic acid-o-glucoside.

The identification of organic acids was observed at 1.13 and 6.17 min for compounds 3 and 74, respectively, with the quasi-molecular ions $[M-H]^-$ at *m/z* 191.0197 ($C_6H_8O_7$) and 163.0401 ($C_9H_8O_3$), according to comparing the mzValut, massbank database, which had the same MS^1 data and similar MS^2 data for citric acid and p-coumaric acid. However, their retention time were inconsistent with citric acid and p-coumaric acid. Therefore, compounds 3 and 74 were inferred as isomers of citric acid isomer and p-coumaric acid isomer.

Flavonoids

A total of 57 flavonoids were characterized in extracts of *D. cirrhosa* peels ,

including flavonols, flavones, flavanols and dihydrochalcones.

Identification of flavonols

Compond 168, eluted at 13.72 min, showed a molecular ion $[M-H]^-$ at *m/z* 317.0305 ($C_{15}H_{10}O_8$), and fragment ions at *m/z* 317.0304, 178.0027, 151.0027, 137.0234, 180.9118 and 112.9845. According to comparing the reference standard, it was identified as myricetin. Compounds 77 and 121 generated a molecular ion $[M-H]^-$ at *m/z* 317.0303 ($C_{15}H_{10}O_8$) and a fragment ion at *m/z* 151.0029, 178.9981 and 137.0234 . Therefore, they were identified as myricetin isomer.

Compond 179, eluted at 14.81 min, showed a molecular ion $[M-H]^-$ at *m/z* 301.0353 ($C_{15}H_{10}O_7$), and fragment ions at *m/z* 301.0356, 151.0027, 178.9979 and 121.0284. The MS/MS profiles were the same compared with the reference standard; therefore, it was identified as quercetin. Compounds 69, 98, 100 and 107, showed a molecular ion $[M-H]^-$ at *m/z* 625.141 ($C_{27}H_{30}O_{17}$), and the fragment ions at *m/z* 301.0348, 151.0025 and 463.0868. Indicating they contain quercetin component. Resulting from the neutral loss at 162 Da, corresponding with the loss of one sophorose moiety; therefore, they were identified as quercetin-o-sophoroside.

Compond 189, eluted at 16.15 min, showed a molecular ion $[M-H]^-$ at *m/z* 269.0458 ($C_{15}H_{10}O_5$), and fragment ions at *m/z* 269.0457, 225.0553, 151.0026, and 117.0332. According to comparing the reference standard, it was identified as apigenin. Compound 70 exhibited a molecular ion $[M-H]^-$ at *m/z* 269.0455 ($C_{15}H_{10}O_5$) and a fragment ion at *m/z* 117.0334 and 151.0027. Hence, it was identified as apigenin isomer.

Compond 190, eluted at 16.23min, showed a molecular ion $[M-H]^-$ at *m/z* 269.0455 ($C_{15}H_{10}O_5$), and fragment ions at *m/z* 285.0406 and 151.0026. According to comparing the reference standard, it was identified as kaempferol. Compounds 129, 151, 155, 167 and 174 were eluted at 12.55, 13.29, 13,51, 13.71 and 13.93 min, possessed a similar quasi-molecular ion $[M-H]^-$ at *m/z* 447.0392 ($C_{21}H_{20}O_{11}$) and a fragment ion at *m/z* 285.0401. which indicated they contained kaempferol part. Resulting from the neutral loss of 162 Da, corresponding with the loss of one hexose moiety. So, they were determined to be kaempferol-o-hexoside.

Compond 192, eluted at 16.61 min, showed a molecular ion $[M-H]^-$ at *m/z* 315.0515 ($C_{16}H_{12}O_7$), and fragment ions at *m/z* 300.0278 and 3150514. According to the comparing the reference standard, it was identified as isorhamnetin. Compounds 102, 172 and 181 produced a quasi-molecular ion $[M-H]^-$ at *m/z* 315.0510 ($C_{16}H_{12}O_6$) and the fragment ion at *m/z* 300.0277. Therefore, they were identified as isorhamnetin isomer. Compounds 104 and 113 eluted at 10.59, 11.62min, possessed a similar quasi-molecular ion $[M-H]^-$ at *m/z* 477.1038 ($C_{22}H_{22}O_{12}$) and a fragment ion at *m/z* 315.0513, Indicating they had an isorhamnetin part. Resulting from the neutral loss at 162 Da, corresponding with the loss of a glucose moiety, hence, they were identified as isorhamnein-o-glucoside. Similarly, compounds 108, 120, 146, 157 exhibited a quasi-molecular ion $[M-H]^-$ at *m/z* 623.1617 ($C_{28}H_{32}O_{16}$), indicating the loss of 308 Da, corresponding with the loss of one rutinose moiety. Hence, it was considered asisorhamnein-o-rutin. Compound 187 showed a quasi-molecular ion $[M-H]^-$ at *m/z* 461.1089 ($C_{22}H_{22}O_{11}$), and a fragment ion $[M-H]^-$ in *m/z* 315.0506, resulting from the neutral loss of 146 Da, corresponding with the loss of one rhamnose moiety. Therefore, it was identified as isorhamnein-o-rhamnoside.

Compound 191 displayed a quasi-molecular ion $[M-H]^-$ at *m/z* 299.0561 ($C_{16}H_{12}O_6$) and a fragment ion at *m/z* 285.0358, which indicated the loss of methyl. It was identified as methyl kaempferol, according to the literature [26].

Compounds 126 and 159, eluted at 12.47 and 13.6 min, produced an quasi-molecular ion -$[M]^+$ at *m/z* 331.08232, and a fragment ion at *m/z* 301.0356 and 151.0027. According to the database, Their MS/MS profiles were nearly the same compared with isoquercitrin; however, none of them could be identified as isoquercitrin because their retention time was incongruent with the standard. Hence, they were regarded as isoquercitrin isomer.

Identification of flavonoids.

Compond 178, eluted at 14.22 min, showed a molecular ion $[M-H]^-$ at *m/z* 287.0566($C_{15}H_{12}O_6$), and fragment ions at *m/z* 151.0027, and 135.0441. The MS/MS profiles were the same compared with the standard; therefore, it was identified as eriodictyol. Compound 106 displayed a molecular ion $[M-H]^-$ at *m/z* 287.0561

($C_{15}H_{12}O_6$), with a fragment ion at *m/z* 151.0027 and 135.0441. Hence, compound 106 was identified as eriodictyol isomer.

Compond 187, eluted at 15.37min, showed a molecular ion $[M-H]^-$ at *m/z* 271.0615($C_{15}H_{12}O_5$), and fragment ions at *m/z* 151.0027, 119.0491, 93.0333, 177.0186 and 107.0127. The MS/MS profiles were the same compared with the reference standard; therefore, it was identified as naringenin. Compounds 112 and 147, eluted at 11.48 and 13.12 min, exhibited a quasi- molecular ion $[M-H]^-$ at *m/z* 433.1140 ($C_{21}H_{22}O_{10}$), with a fragment ion at *m/z* 271.0608 and 151.0024, which indicated they contained naringin part, resulting from the neutral loss at *m/z* 162 Da, corresponding with the loss of one glucose moiety; therefore, they were identified as naringenin-O-glucoside.

Compound 182 generated a molecular ion $[M-H]^-$ at *m/z* 271.0611 ($C_{15}H_{12}O_5$) and a fragment ion at *m/z* 151.0027. Therefore, compound was determined to be naringenin isomer.

Compounds 64 and 71, eluted at 5.52 and 5.86 min, displayed a similar molecular ion $[M-H]^-$ at *m/z* 449.1089 ($C_{21}H_{22}O_{11}$) , with a fragment ion at *m/z* 287.0563. According to the comparing the database, which were corresponded to malvidin part. Resulting from the neutral loss at *m/z* 162 Da, corresponding with the loss of one glicose moiety, therefore, they were identified as holophenol-o-glucoside.

Identification of flavanols

Compound 58 showed a molecular $[M-H]^-$ ion at *m/z* 319.0459 ($C_{15}H_{12}O_8$) and a fragment ion at *m/z* 151.0027. According to the mzValut, massbank database, hence, compound 58 was identified as dihydromyricetin.

Compound 99 showed a molecular ion $[M-H]^-$ at *m/z* 303.0510 ($C_{15}H_{12}O_7$) and a fragment ion at *m/z* 153.0184 and 125.0233. According to the mzValut, Massbank database, therefore, compound 99 was identified as taxifolin. Compounds 22, 35 and 89 eluted at 3.42, 4.22 and 7.95 min, produced a qausi-molecular ion $[M-H]^-$ at *m/z* 465.1038 ($C_{21}H_{22}O_{12}$), and a fragment ion at *m/z* 125.0233 and 303.0512. Indiciating they had a taxifolin component. Resulting from the neutral loss of 162 Da, corresponding with the loss of one hexose moiety they were identified as taxifolin-

hexoside.

Identification of dihydrochalcone

Compond 161, eluted at 13.61 min, showed a molecular ion $[M-H]^-$ at *m/z* 435.1298($C_{21}H_{24}O_{10}$), and fragment ions at *m/z* 273.0771, and 167.0342. The MS/MS profiles were the same compared with the reference standard; therefore, it was identified as phlorizin. Compound 93 generated a molecular ion $[M-H]^-$ at *m/z* 435.1296($C_{21}H_{24}O_{10}$) and a fragment ion at *m/z* 273.0770. Hence, compound 93 was identified as phlorizin isomer.

Compond 188, eluted at 15.59 min, showed a molecular ion $[M-H]^-$ at *m/z* 273.0772($C_{15}H_{14}O_5$), and fragment ions at m/z 123.0438, 119.0489, 167.0338 and 125.0231. The MS/MS profiles were the same compared with the reference standard; therefore, it was identified as phloretin. Compound 5, eluted at 5.09 min, showed a quasi-molecular ion $[M-H]^-$ at *m/z* 597.1824 （$C_{27}H_{34}O_{15}$), and a fragment ions at m/z 273.0765 and 435.1292, Indicating it had a phloretin component. Resulting from the neutral loss of 162 Da, corresponding with the loss of a glucose moiety, so it was considered as phloretin-di-O-glucoside.

Tannins

Compond 37, eluted at 4.27 min, showed a molecular ion $[M-H]^-$ at *m/z* 289.0720 ($C_{15}H_{14}O_6$), and fragment ions at *m/z* 289.0720, 245.0819, 203.0708, 205.0502 and 187.0396. The MS/MS profiles were the same compared with the reference standard; therefore, it was identified as chlorogenic acid catechin.

Compound 4 was eluted at 1.19 min with a quasi-molecular ions $[M-H]^-$ at *m/z* 187.0248 ($C_7H_8O_6$) and fragment ions at *m/z* 125.0233. According to literature [27], it was identified as gallic acid hydrate.

Compounds 5, 8, and 12, eluted at 1.34, 1.59, and 2.19 min, showed a molecular ion $[M-H]^-$ at *m/z* 331.0670 ($C_{13}H_{16}O_{10}$) , and the fragment ions at *m/z* 125.0233 and 169.0134. Indicating This they had a gallic acid component. Resulting from the neutral loss of 162 Da, corresponding to a loss of a glucose moiety, they were identified as gallic glucose.

Compound 6 was eluted at 1.38 min, exhibited a molecular ion $[M-H]^-$ at *m/z*

493.1198 ($C_{19}H_{26}O_{15}$). The main fragment ion at *m/z* 331.0673 lost 162 Da, due to the neutral loss of glucose and other fragment ions at *m/z* 169.0135, 125.0234, indicating the gallic acid portion. It was preliminarily identified as galaloylsucrose.

Compounds 8 and 12, eluted at 1.95 and 2.46 min, displayed a similar quasi-molecular ion $[M-H]^-$ at *m/z* 345.0827($C_{13}H_{16}O_{10}$), it also showed a similar loss to gallyl glucose, with an additional loss of the methyl component (14 Da). Based on the major fragment ions at *m/z* 183.0292 ([M-h-hexose]-), 139.0391 ([M-h-hexose -CO2]-), these compounds were identified as the methylgallic glucose isomer.

Compound 15 was detected at 2.52 min, exhibited a quasi-molecular ion $[M-H]^-$ at *m/z* 153.0193 ($C_7H_6O_4$) and a fragment ion at *m/z* 109.0283, According to the literature[28], it was identified as protocatechuic acid.

Compound 73, eluted at 6.02 min, compared with standard catechins and non-catechins, the same molecular ions $[M-H]^-$ are shown at *m/z* 289.0717 ($C_{15}H_{14}O_6$) and their fragment ions at *m/z* 245.0818, 203.0708, 205.0502, thus consistent with epicatechin[29-30].

Compounds 24, and 46, eluted at 3.60 and 4.68min, showed a molecular ion $[M-H]^-$ at *m/z* 451.1245 ($C_{21}H_{24}O_{11}$), and the fragment ions at m/z 289.0719 and 245.0818. Indicating This they had a catechin or epicatechin component. Resulting from the neutral loss of 162 Da, corresponding to a loss of a glucose moiety. Therefore, they were identified as catechin-glucoside and epicatechin-glucoside.

Compounds 9, 43, 52, 90 and 125 were eluted at 1.90, 4.55, 4.90, 8.07 and 12.40 min, showed the same molecular ion $[M-H]^-$ at *m/z* 865.1985 ($C_{45}H_{38}O_{18}$), resulting from their fragment ions *m/z* 125.0233, 289.0721, 407.0774, 425.0896, indicating they were catechin polymers. Therefore, they were identified as (epicatechin -(epicatechin) -(epicatechin) [31].

Compounds 14, 86, 95,103 and 137 eluted at 2.47, 7.76, 9.66, 10.57 and 12.85 min, possessed a similar molecular ion $[M-H]^-$ at *m/z* 849.2036($C_{45}H_{38}O_{17}$), resulting from the *m/z* 125.0233, 289.0721, 407.0774, 425.0896 of their fragment ions, it can be inferred they were polymers of epicatechin, and the *m/z* 271.0615 of the fragment ions indicated the epicatechin part. Therefore, they were identified as

epicafcatechin-epicatechin-epicatechin [32].

Compounds 26, 34, 56, 61 and 88 were eluted at 3.80, 4.19, 5.17, 5.38 and 7.81 min, generated the same molecular ion $[M-H]^-$ at *m/z* 609.1249（$C_{30}H_{26}O_{14}$）and their fragment ions at *m/z* 125.0232、305.0667. According to literature[33], it was identified as prodelphinium B type.

Compounds 27, 39 and 45 eluted at 3.85, 4.30 and 4.66 min, displayed a similar molecular ion $[M-H]^-$ at *m/z* 739.1879($C_{36}H_{36}O_{17}$), with a fragment ion at *m/z* 289.0719 and 125.0233, indicating they had catechins or epicatechin parts. They were identified as proanthocyanidins B-O-glucopyranoside.

Compounds 30, 38 and 47 eluted at 4.00, 4.28 and 4.72 min, displayed a similar molecular ion $[M-H]^-$ at *m/z* 457.0776 ($C_{22}H_{18}O_{11}$), with a fragment ion at *m/z* 169.0134 and 289.0724, indicating they had gallic acid or (epicatechin) components. Hence, they were considered as gallic catechin gallate (GCG) and epigallocatechin gallate (EGCG) [31].

Compound 33, 51 and 65 eluted at 4.08, 4.87 and 5.56 min, showed a molecular ion $[M-H]^-$ at *m/z* 577.1351 ($C_{30}H_{26}O_{12}$), and the fragment ions at *m/z* 289.0719, 407.0773, 125.0233. Indicating they had (superficial) catechin parts. Therefore, they were identified as proanthocyanidin B type.

Compounds 32, 41 and 44 eluted at 4.04, 4.46 and 4.63min, showed a same molecular ion $[M-H]^-$ at *m/z* 305.0666 ($C_{15}H_{14}O_7$), and their fragment ions at *m/z* 125.0233 correspond to (-) -galligallocatechin and (-) -epigallocatechin[31].

Derivatives of Compounds 53, 78, 85 and 111, eluted at 4.04, 4.46 and 4.63min, showed a quasi-molecular ion $[M-H]^-$ at *m/z* 561.1402($C_{30}H_{26}O_{11}$), and fragment ions at *m/z* 271.0614, indiciting it had the epicatechin part, as well as other fragment ions at *m/z* 289.0719, 407.0775, 125.0233, indiciting they had the (epicatechin) part. Therefore, they are identified as epicatechin - (4-6) - epicatechin [34].

Compounds 67, 91 and 94 eluted at 5.70, 9.24 and 9.58 min, showed a molecular ion $[M-H]^-$ at *m/z* 441.0827 ($C_{22}H_{18}O_{10}$), and the fragment ions at *m/z* 125.0233, corresponding to catechin gallate and epicatechin gallate [35].

Anthocyanins

Anthocyanins were the most common in tubularins, such as: pelargonidin (*m/z* 271.0600), centaurin (*m/z* 287.0550), paeonidin (*m/z* 301.0706), delphinidin (*m/z* 303.0499), petunia (*m/z* 303.0499). *m/z* 317.0666), malvacin aglytin (*m/z* 331.0823) and its sugar derivatives.

Identification of pelargonidin derivatives

Compound 72 showed a quasi-molecular ion $[M-H]^-$ at *m/z* 271.0600 ($C_{15}H_{11}O_5$) and a fragment ion at *m/z* 215.0702, 149.0234 and 243.0651. Therefore, it was identified as pelargonidin[36,37].

Compound 162, eluted at 13.63min, showed a molecular ion $[M-H]^-$ at *m/z* 433.1129 ($C_{21}H_{21}O_{10}$), and the fragment ions at *m/z* 271.0600, indiciting it had pelargonidin part. Resulting from the neutral loss of 162 Da, corresponding to a loss of a hexose moiety, it was identified as pelargonidin -o-hexoside.

Identification of cyanidin derivatives

Compond 82, eluted at 6.82 min, showed a molecular ion $[M-H]^-$ at *m/z* 287.0549 ($C_{15}H_{11}O_6$), and fragment ions at *m/z* 287.0549. According to comparing the reference standard, it was identified as cyanidin.

Compounds 142, 152, 156, 169 and 175, eluted at 12.99, 13.31, 13.53, 13.72 and 13.94 min, showed a molecular ion $[M-H]^-$ at *m/z* 449.1078 ($C_{21}H_{21}O_{11}$), and the fragment ions at *m/z* 287.0548, indiciting they had cyanidin parts. Resulting from the neutral loss of 162 Da, corresponding to a loss of a hexose moiety, Hence, it was identified as cyanidin-o-hexoside.

Identification of peonidin derivatives

Compound 164 produced a quasi-molecular ion $[M-H]^-$ at *m/z* 301.0706 ($C_{16}H_{13}O_6$), and the fragment ion at *m/z* 286.0471, according to literature [37,38]; It was identified as peonidin isomer.

Compounds 96, 97, 123 and 171, eluted at 9.67, 9.80, 12.26, 13.86 min, showed a molecular ion $[M-H]^-$ at *m/z* 463.1234 ($C_{22}H_{23}O_{11}$), and the fragment ions at *m/z* 301.0706, indiciting they had peonidin parts. Resulting from the neutral loss of 162 Da, corresponding to a loss of a glucose moiety, it was identified as

peonidin-o-glucoside.

Compounds 166, eluted at 13.86 min, showed a molecular ion $[M-H]^-$ at *m/z* 609.1813 ($C_{28}H_{33}O_{15}$), and the fragment ions at *m/z* 301.0705 and 463.1228, indiciting they had peonidin parts. Resulting from the neutral loss of 308 Da, 146Da, and 162 Da, corresponding with the loss of one rutinose moiety, it was identified as peonidin o-rutin.

Identification of delphinidin derivatives.

Compounds 110, 117, 130 and 134 were eluted at 11.43, 11.82, 12.58 and 12.75 min, showed a molecular ion $[M-H]^-$ at *m/z* 611.1606 ($C_{27}H_{31}O_{16}$), and the fragment ions at *m/z* 303.0498 and 449.1066, indiciting they had delphinidin components. Resulting from the neutral loss of 308 Da, 162 Da and 146 Da, corresponding with the loss of one rutinose moiety. Hence, they were identified as delphinidin-o-rutinoside.

According to references [36-38], compounds 124, 165 and 180 were detected at 12.32, 13.66 and 14.82 min, produced a molecular ion $[M-H]^-$ at *m/z* 303.0499 ($C_{15}H_{11}O_7$), and the fragment ion at *m/z* 257.0443, 229.0497 and 153.0182, Therefore, it was identified as an isomer of delphinidin.

Compounds 127, 132 and 163 were detected at 12.49, 12.70 and 13.65 min, produced a molecular ion $[M-H]^-$ at *m/z* 465.1027 ($C_{21}H_{21}O_{12}$), and the fragment ion at *m/z* 303.0497, indiciting they had a delphinidin component. Resulting from the neutral loss 162 Da, corresponding with the loss of one hexose moiety, they were identified as delphinidin-o-hexoside.

Identification of petunidin derivatives

Compounds 105, 114, 119 and 138 were eluted at 10.60, 11.64, 11.88 and 12.87 min, showed a molecular ion $[M-H]^-$ at *m/z* 479.1184 ($C_{22}H_{23}O_{12}$), and the fragment ions at *m/z* 317.0655, indiciting they had a petunidin component. Resulting from the neutral loss of 162 Da, corresponding with the loss of one hexose moiety, they were identified as petunidin-o-hexoside.

Compounds 109, 122, and 158 were eluted at 11.40, 12.01, and 13.55 min, showed a molecular ion $[M-H]^-$ at *m/z*, and the fragment ions at *m/z* 317.0655, indiciting they had a petunidin component. Resulting from the neutral loss of 308 Da,

corresponding with the loss of one rutinose moiety, they were identified as petunidin-o-rutinoside.

Compounds 118, 140 and 173 were eluted at 11.85, 12.91, and 13.91 min, according to the determination of reference[33], the molecular ion $[M-H]^-$ is *m/z* 317.0655 ($C_{16}H_{13}O_7$), and the fragment ion is *m/z* 302.0419, hence, it is identified as petunidin isomer.

Compounds 128, and 135 were eluted at 12.53 and 12.79 min, showed a molecular ion $[M-H]^-$ at *m/z* 449.1078 ($C_{21}H_{21}O_{11}$), and the fragment ions at *m/z* 317.0653, indiciting they had a petunidin component. Resulting from the neutral loss of 132 Da, corresponding with the loss of one glycose moiety, they were identified as petunidin-o-arabic glycoside.

Compound 144, eluted at 13.06 min, showed a molecular ion $[M-H]^-$ at *m/z* 463.1234 ($C_{22}H_{23}O_{11}$), and the fragment ions at *m/z* 317.0654, indiciting they had a petunidin component. Resulting from the neutral loss of 146 Da (463.1234-317.0654), corresponding with the loss of one rhamnose moiety, they were identified as petunidin-o-rhamnoside.

Identification of malvidin derivatives

Compounds 154, and 166 were eluted at 13.17 and 13.37 min, showed a molecular ion $[M-H]^-$ at *m/z* ($C_{23}H_{25}O_{12}$), and the fragment ions at *m/z* 331.0810, indiciting they had malvidin parts. Resulting from the neutral loss of 162 Da (493.1340-331.0810), corresponding with the loss of one hexose moiety, they were identified as malvidin-o-hexoside.

Compounds 145, and 150 were eluted at 13.06 and 13.23 min, showed a molecular ion $[M-H]^-$ at *m/z* 639.1919($C_{29}H_{35}O_{16}$), and the fragment ions at *m/z* 331.0810, indiciting they had malvidin parts. Resulting from the neutral loss of 308 Da (639.1919-331.0810), corresponding with the loss of one rutinose moiety, they were identified as malvidin-o-rutinoside.

Compound 184 and 185, eluted at 15.10 and 15.19 min, generated a molecular ion $[M-H]^-$ at *m/z* 331.0823($C_{17}H_{15}O_7$). According to references [37,38], hence, it was identified as malvidin isomer.

In *vitro* antioxidant activity of EDP

ABTS radical scavenging ability

The ABTS radical scavenging abilities of EDP are shown in Figure 1. The tests of Vc and EDP showed a concentration-dependent scavenging activity against ABTS within the tested concentration range (25-400 μg/mL).The IC_{50} value of EDP was 83.66 μg/mL. The results demonstrated that EDP exhibited a significant ABTS radical scavenging ability.

DPPH radical scavenging ability

The DPPH radical scavenging abilities of EDP are shown in Figure 2. In the experimental range, the scavenging rate of EDP and Vc on DPPH radical increased gradually with the increase of concentration. When the EDP concentration reaches 2.5 μg/mL, the DPPH clearance rate can reach 94.18 ± 0.01% and the IC_{50} value is 4.320 μg/mL. The IC50 value of Vc is 5.534 μg/mL, which exhibited that EDP has strong DPPH radical scavenging ability.

Copper ion reduction ability

The copper ion reduction abilities of EDP are shown in Figure 3. The reducing ability of EDP to copper ions is related to concentration. The IC_{50} value of EDP and Vc is 205.2 μg/mL and 186.2 μg /mL, respectively, indicating that EDP has a good reducing ability to copper ions.

Conclusion

The chemical composition and antioxidant properties of EDP were investigated in this study. A total of 194 chemical components were efficiently characterized, including 38 organic acids, 59 flavonoids, 37 tannins, 59 anthocyanins and 3 other components. 176 components were identified for the first time, which indicated that flavonoids and anthocyanins were the main compounds in the D. cirrhosa peels. EDP has strong ABTS scavenging ability, DPPH scavenging ability and copper ion reduction ability. The results of this study open up a new pharmacological prospect and provide a pharmacodynamic basis for the further study of the extracts of Dioscorea cirrhosa

peels, which have the potential to be used as a functional food or pharmaceutical in food, medicine, cosmetics and other industries.

Data availability

Data is available upon reasonable request. For inquiries, please contact the corresponding author.

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Declarations

Competing interests

The authors declare no competing interests.

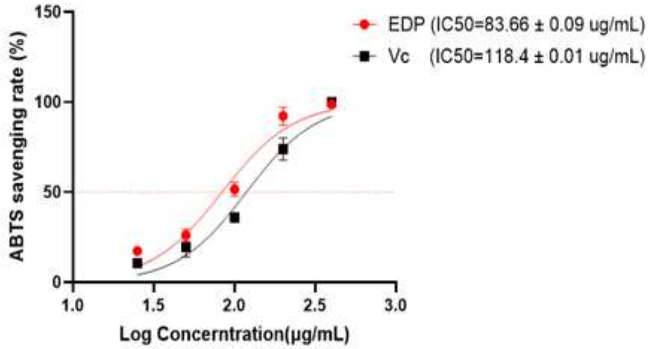

Figure 1. ABTS radical scavenging aasay

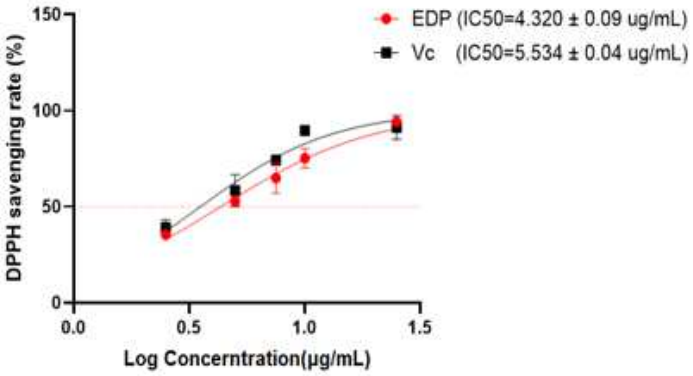

Figure 2. DPPH radical scavenging assay

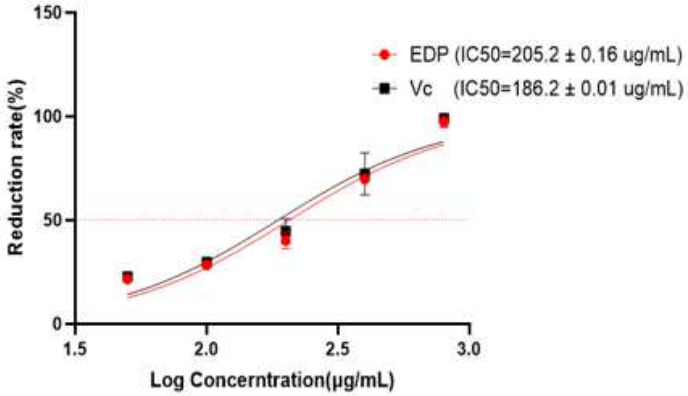

Figure 3. Copper ion reducting assay

Supplementary Files

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- Table.docx