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ORIGINAL ARTICLE

A strategy for antioxidant quality evaluation of *Aster yunnanensis* based on fingerprint-activity relationship modeling and chemometric analysis

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Abstract In this study, a fingerprint-activity relationship between ultrahigh performance liquid chromatography (UHPLC) fingerprints and antioxidant activity was established to evaluate the quality of *Aster yunnanensis* Franch.(AYF) from different collecting spots. First, the fingerprint of AYF was established by UHPLC, and the similarity analysis was analyzed based on twenty-one common peaks. Then the chemical constituents from AYF were analyzed and identified by UHPLC-quadrupole time-of-flight tandem mass spectrometry (QTOF-MS/MS). Next, the antioxidant activity of twelve batches of AYF was assessed in vitro. Subsequently, eleven chemical markers were screened out by fingerprints and antioxidant activity utilizing grey relational analysis (GRA) and partial least squares (PLS). Finally, the contents of eleven chemical markers in twelve batches of AYF were detected by UHPLC, and the antioxidant quality of AYF was evaluated using chemometric analysis, such as principal components analysis (PCA) and technique for order preference by similarity to ideal solution (TOPSIS). The results showed that the antioxidant efficacy was associated with the total content of eleven compounds of AYF. Moreover, this method to discover quality markers was reasonable by fingerprint-activity relationship combination with chemometric analysis. The present study will certify quality markers associated with therapeutic effects, and provide a powerful strategy for evaluating the resource quality of AYF.

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1. Introduction

Aster yunnanensis Franch.(AYF), a perennial herb of Asteraceae(Ma, et al.,2021), was mainly distributed in Gansu, Qinghai, Yunnan and Tibet of China at an altitude of 2500 to 4000 m. AYF is a well-known food and herbal medicine, which has various bioactivities and can be used to treat various diseases, such as flu, fever, food poisoning, ringworm, blast fever, spasm and so on(Li, et al.,2021).Phytochemical studies have shown that AYF contained many kinds of bioactive constituents, such as phenylpropanoids, flavonoids, organic acids, alkaloids, saponins, glycosides and so on. Modern pharmacological studies have also shown that flavonoids(Papadopoulos, et al.,2021), organic acids(Shang, et al.,2012), alkaloids(Trinh, et al.,2020), saponins(César, et al.,2018), and glycosides(Yu, et al.,2018) have good antioxidant activity. However, there are no reports about the antioxidant activity and bioactive components of AYF in the previous study. In addition, there is no research on the quality control of AYF. Usually, single or several index components can be employed to control the quality of medicinal herbs. However, it is unscientific to control the quality of medicinal herbs based on only one or a few index components. Furthermore, it is widely believed that Chinese medicines exert therapeutic efficacies holistically by a 'multi-component, multi-targeted, and multi-pathway' mode(Chen, et al.,2019). As described above, AYF is composed of a variety of chemical constituents, and its efficacy is not limited to a single bioactive component. Therefore, it is necessary to establish an effective and scientific evaluation method for controlling the quality of AYF.

The fingerprint technique was demonstrated to be an effective method in evaluating the chemical consistency of traditional Chinese medicine (TCM), which also has been well received by the World Health Organization (WHO), China Food and Drug Administration (CFDA), the United States Food and Drug Administration (FDA) and European Medicines Agency (EMEA)(Chen, et al.,2019). Chromatographic fingerprinting is widely used to evaluate the quality of various medicinal herbs because of its high efficiency and low cost (Chen, et al.,2020; Dilaram, et al.,2021). However, the chromatographic fingerprint is unclear to identify the unknown bioactive components. In addition, although it can explain the similarities and differences between different batches of samples, it is still some disadvantages to evaluating the quality. To solve this problem, UHPLC-QTOF-MS/MS, a fast, strong separation ability and high mass accuracy analysis technology, can be employed to identify chemical components(Chen, et al.,2018; Xu, et al.,2018). Nevertheless, it remains unclear the relationship between the bioactive activity and chemical constituents. In recent years, fingerprint-activity relationship modeling and chemometric analysis have been used to solve these problems and discover potential bioactive components(Chen, et al.,2019; Wu, et al.,2020; Deng, et al.,2021; Han, et al.,2022).It was also meaningful to explore quality control that can reflect the intrinsic quality of medicinal herbs.

Therefore, this study was conducted to discover the potential bioactive markers related to the most potent antioxidant capacity by using fingerprint-activity relationship modeling, and to evaluate the quality of AYF by merging chemometric analysis, including grey relational analysis (GCA), partial least squares (PLS), principal components analysis (PCA) and technique for order preference by similarity to ideal solution (TOPSIS). Firstly, the chromatography fingerprints were established by UHPLC, and UHPLC-Q-TOF-MS was used to identify the chemical components of AYF qualitatively. Secondly, three models, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS)radical scavenging assay and ferric-reducing antioxidant power (FRAP) assay were used to determine the antioxidant capacity of AYF. Thirdly, GCA and PLS were used to build the relationship between chromatographic fingerprints and antioxidant activity of AYF to screen out major potential antioxidant markers. Finally, the content of eleven markers was determined by UHPLC, and the quality of AYF was evaluated based on PCA and TOPSIS.

2. Materials and methods

2.1. Reagents and materials

Formic acid (HPLC grade), acetonitrile and methanol were obtained from Merck (Darmstadt, Germany).Ultrapure water was provided by Watsons Food and Beverage Company (Guangzhou, China).Apigenin (Lot NO.,20080706) and kaempferol (Lot NO.,20042613) were provided by Sichuan Weiqi Biotechnology Co. Ltd (Chengdu, China).Chlorogenic acid (Lot NO.,327979), isoquercetin (Lot NO.,15070211), isochlorogenic acid B (Lot NO.,14534613), isochlorogenic acid A (Lot NO.,2450535) and isochlorogenic acid C (Lot NO.,57378720) were provided by Chengdu Maide Biotechnology Co. Ltd (Chengdu, China).Neochlorogenic acid (Lot NO.,20042105), cryptochlorogenic acid (Lot NO.,20080301) and Rutin (Lot NO.,15040524) were purchased from Chengdu Kangbang Biotechnology Co. Ltd (Chengdu, China). Quercitrin (Lot NO.,100081200907) was purchased from the National Institute for the control of Pharmaceutical and Biological Products (Beijing, China).The purity of eleven standards was more than 98% determined by HPLC.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH, Lot NO.,2010289) and 2,20-azinobis (3-ethylbenzothia-zo-line-6-sulphonicacid) diammonium salt (ABTS, Lot NO.,20112801) were purchased from Chengdu Kangbang Biotechnology Co. Ltd (Chengdu, China). Ascorbic acid (Vc, Lot NO., B21293) and 2,4,6-tri(2-pyridinyl)-1,3,5-triazine (TPTZ, Lot NO., S30632) were purchased from Shanghai Yuanye Biotechnolog-y Co. Ltd (Shanghai, China). All other chemicals were of analytical grade.

Twelve batches of AYF samples were collected from different locations and authenticated by Professor Zhifeng Zhang (Institute of Qinghai-Tibetan Plateau, Southwest Minzu University). The information of samples is shown in Table 1. The fresh samples were dried in the sun till complete dryness. The samples were preserved in the herbarium of Southwest Minzu University (Chengdu, China).

2.2. UHPLC fingerprint

2.2.1. Sample preparation

The dried AYF samples were grilled to powder and passed through a 0.30 mm sample sieve. Subsequently, the sample powder (0.5 g) was ultrasonicated with 10 mL of 80% methanol for 30 min at room temperature. The solution was filtered through a 0.22 μm microfiltration membrane before injection of UHPLC analysis and UHPLC-Q-TOF-MS/MS analysis.

2.2.2. Chromatographic conditions

Sample analysis was performed on the Waters UHPLC®–CLASS system (Waters, Milford, MA, USA) with the separation conducted by an ACQUITY UHPLC®HSS C18 column (2.1 × 100 mm, 1.8 μm). The solvent system consisted of 0.1% aqueous formic acid (A) and acetonitrile (B)with the following gradient elution program: 0–10 min, 5–18% B; 10–12 min, 18–19% B; 12–17 min, 19–20% B; 17–19 min, 19–20% B; 19–21 min, 20–22% B; 21–29 min, 22–60% B; 29–32 min, 60–95% B; 32–37 min, 96% B. The flow rate was at 0.2 mL/min. The column was kept at 35 °C, and the injection volume

Table 1 Sample information and similarity of *Aster yunnanensis* from different collecting spots.

Sample ID	Collecting locations	Altitude /m	Collecting time	Longitude and latitude	Similarity
S1	Dege, Sichuan	3910	2020.7.30	E99°31′45″, N31°46′00″	0.984
S2	Ruoergai, Sichuan	3471	2020.7.26	E102°36′24″, N33°56′43″	0.979
S3	Baiyu, Sichuan	3676	2020.7.13	E99°18′58″, N31°04′57″	0.974
S4	Shiqu, Sichuan	4018	2020.7.30	E98°18′33″, N33°03′04″	0.985
S5	Ruoergai, Sichuan	3463	2020.7.26	E102°35′30″, N33°11′25″	0.990
S6	Shiqu, Sichuan	3977	2020.7.30	E98°38′46″, N32°22′00″	0.980
S7	Nangqian, Qinhai	4065	2020.7.29	E95°31′47″, N32°19′51″	0.965
S8	Yushu, Qinhai	3980	2020.7.28	E96°55′22″, N32°51′57″	0.951
S9	Xiangcheng, Sichuan	3919	2020.7.10	E99°45′34″, N28°57′10″	0.665
S10	Ruoergai, Sichuan	3436	2020.7.26	E102°28′09, N33°36′29″	0.941
S11	Zaduo, Qinhai	4034	2020.7.28	E95°28′54″, N32°51′12″	0.961
S12	Kangding, Sichuan	3258	2020.7.31	E101°50′07″, N30°16′46″	0.717

was 1 μL each time. The detection wavelength was set as 266 nm.

2.2.3. Analysis of UHPLC fingerprints

UHPLC fingerprinting was validated for precision, stability, and repeatability. The precision test was performed by injecting six times the same sample solution. The stability test was analyzed by replicate injecting of the same sample solution at0, 2, 6, 8, 12, and 24 h, respectively. The repeatability test was performed by injections six times working solutions of the same sample. The relative standard deviation (RSD) from Relative retention time (RRT) and relative peak area (RPA) of the common peak were used to evaluate precision, stability and repeatability, respectively. The RRT and RPA of common peak were calculated using peak 11 (sochlorogenic acid B) as the reference peak. Furthermore, the original chromatographic data were imported into the Similarity Evaluation System for Chromatographic Fingerprint of traditional Chinese medicine (version 2012, Chinese Pharmacopoeia Commission), and UHPLC fingerprints of AYF samples were established.

2.3. UHPLC-Q-TOF-MS/MS conditions

Sample analysis was performed on the Agilent 6530 Accurate-Mass Q-TOF-LC-MS/MS system (Agilent, USA) with the separation conducted by a Thermo Hypersil GOLD C_{18} Column (100 mm × 2.1 mm, 1.9 μm). The solvent system was consisted of 0.1% aqueous formic acid (A) and acetonitrile (B)with the following gradient elution program: 0–3 min, 5–20% B; 3–4 min, 20–22% B; 4–10 min, 22–32% B; 10–12 min, 32–60% B; 12–14 min, 60–80% B; 14–16 min, 80–90% B; 16–17 min, 90–95% B. The flow rate was at 0.4 mL/min. The column was kept at 35 °C, and the injection volume was 3 μL each time.

Mass spectrometry was achieved by Agilent 6530 Accurate-Mass Q-TOF-LC-MS/MS Mass technology equipped with an electrospray ionization (ESI) source. Both positive and negative ionization modes were applied to acquire by scanning range from 50 to 1000 Da with scanning time of 0.2 s and 17 min detection period. The MS parameters were set as follows: The capillary voltage was set at 4.0 kV (positive mode) and 3.2 kV (negative mode); the source and desolation temperatures were 100 and 350 ℃, respectively; the drying gas flow rate was 6.8 L/min. Finally, Processing and analysis of the data were carried out using Agilent HPLC-ESI-Q-TOF-MS MassHunter A.01.00 software (Agilent, USA).

2.4. Determination of antioxidant capacity

2.4.1. Sample preparation

The12 batches of dried AYF samples were grilled to powder and passed through a 0.30 mm sieve. Subsequently, the sample powder (15 g) was added to 250 mL conical flash and sonicated with 150 mL of 80% methanol twice, 30 min each time. The solution was filtered and the solvent was reduced by pressure distillation. The extract was diluted with methanol to five different concentrations of sample solutions (0.0125,0.025,0.05,0.10,0.25, 0.5 mg/mL).

2.4.2. DPPH assay

The DPPH activity of twelve batches of AYF was assessed according to the procedure mentioned by Liu et al.(2017) with a small modification. For DPPH assay, 100 μL different concentrations of sample extract or positive control was mixed with the 100 μL of the DPPH solution (0.0799 mg/mL) in 96-well plates. Then, the mixed solutions were kept and reacted in the dark place for 30 min. Finally, the absorbance of the mixed solutions was recorded at 517 nm with a multifunctional microplate reader and calculated DPPH radical scavenging activity as Eq (1):

$$\text{Scavenging activity} = \left(1 - \frac{\text{Ad} - \text{Ax}}{\text{Ac}}\right) \times 100\% \quad (1)$$

Ascorbic acid was used as the positive control. Where A_d is the absorbance of 100 μL sample extract or Ascorbic acid with 100 μL the DPPH solution; A_x is 100 μL sample extract with 100 μL the methanol; A_c is 100 μL methanol with 100 μL the DPPH solution. Using regression analysis of data to estimate the IC_{50} values.

2.4.3. ABTS assay

The ABTS activity of the AYF was determined as described by Chen et al.(2020) with a few modifications. For ABTS assay, The ABTS aqueous solution (7 mmol/L) mixed with the $K_2S_2O_8$ (2.45 mmol/L), and protected from light at

room temperature for 12 to 16 h. The configured $ABTS^+$ solution was diluted with anhydrous ethanol and the absorbance of 0.70 ± 0.02 was measured at 734 nm for $ABTS^+$ analysis. Then, 25 μL of different concentrations of sample solution or positive control was mixed with175 μL of $ABTS^+$ solution in 96-well plates. After 30 min of reaction in the darkness, the absorbance was measured at the wavelength of 734 nm and calculated DPPH radicals cavenging activity as Eq (2):

$$\text{Scavenging activity} = \left(1 - \frac{\text{Ad} - \text{Ax}}{\text{Ac}}\right) \times 100\% \quad (2)$$

Ascorbic acid was used as the positive control. Where A_d is the absorbance of 25 μL sample solution or Ascorbic acid with 175 μL the $ABTS^+$ solution; A_x is 25 μL sample solution with 175 μL the methanol; A_c is 25 μL methanol with 175 μL the $ABTS^+$ solution. Using regression analysis of data to estimate the IC_{50} values.

2.4.4. FRAP assay

The FRAP assay was tested as reported by Xu et al.(2020) with some modifications. The working stock solutions (Fe^{3+}–TPTZ solution) were prepared by mixing 10 parts of 0.3 mol/L acetate buffer (3.1 g $C_2H_3NaO_2{\cdot}3H_2O$ and 16 mL $C_2H_4O_2$, pH 3.5), with 1 part of 20 mmol/L $FeCl_3$ solution and with 1 part of 20 mmol/L TPTZ solution in 40 mmol/L hydrochloric acids (HCl), then stored at 37 ℃ until further use. For analysis, 100 μL different concentrations of sample solution or the positive control were mixed with 100 μL Fe^{3+}–TPTZ working stock solution in 96-wellplate, left in dark for 10 min at room temperature. Then, the absorbance of the mixed solution was recorded at the wavelength of 593 nm. The standard curve was set up by $FeSO_4$ with the concentrations of 2.02,4.04,6.06,8.08,10.10,12.12,16.16 and 20.20 μ mol/L. The positive control was ascorbic acid, and solutions without samples were set as blank control. The results of the FRAP were presented as mmol Fe^{2+} /g of samples.

All those antioxidant tests (DPPH, ABTS, FRAP) were determined on Thermo Fisher Scientific Oy Ratastie 2FI-01620 (Vantaa, Finland) equipment. All the tests were repeated three times.

2.5. Fingerprint-activity relationship modeling

2.5.1. Grey relational analysis

GRA was performed by using the Data Processing System (DPS 9.50) to analyze the correlation degree between the peak area of the twenty-one common peaks and their antioxidant capacity. The grey relational grade was obtained with a distinguishing coefficient of 0.5,and the contribution of fingerprint peaks to pharmacological effects could be evaluated.

2.5.2. Partial least square analysis

In the PLS model, the areas of common peaks were taken as the independent variables (X), and the antioxidant activity levels by different assays were set as the dependent variables (Y). PLS models were built to reveal the relation between the fingerprint peaks and their antioxidant capacity. PLS analysis was performed by SIMCA 14.1 software (Umetrics AB, Umea, Sweden).

2.6. Determination of 11 constituents of AYF

2.6.1. Chromatographic conditions, preparation of sample solutions and standard solutions

The content analysis of sample solution preparation and chromatographic conditions were the same as the Section "2.2.1" and "2.2.2", respectively. A mixed standard stock solution of eleven standards was prepared with methanol, and their concentrations were as follows: Neochlorogenic acid, 0.348 mg/mL; chlorogenic acid,0.389 mg/mL; cryptochlorogenic acid, 0.359 mg/mL; rutin, 0.364 mg/mL; isoquercetin, 0.351 mg/mL; sochlorogenic acid B, 0.355 mg/mL; isochlorogenic acid A, 0.357 mg/mL; isochlorogenic acid C, 0.378 mg/mL; quercetin, 0.513 mg/mL; apigenin 0.350 mg/mL; kaempferol 0.345 mg/mL. The stock solutions were further diluted and stored in a refrigerator at 4℃ until further UHPLC analysis.

2.6.2. Validation of the method for quantitative analysis

To verify the applicability of UHPLC analysis methods, linearity, precision, repeatability, stability and recovery were conducted respectively. The linearity of calibration curves was examined by drawing the peak area and the corresponding different concentrations of each standard. Then, the regression equation, correlation coefficient and linear range were obtained through the curves. The detection limit (LOD) and quantification limit (LOQ) were evaluated at the signal-to-noise ratio (S/N) of about 3 and 10, respectively. The precision test was achieved by continuously injecting six times mixed standard solution. The repeatability was determined by intra-day and inter-day tests. The intra-day test was measured by detecting the mixed standard solution in six duplicates on the same day, and the inter-day test was measured on three successive days. The stability was analyzed by the same sample at 0, 2, 4, 8, 12, and 24 h, respectively. The recovery test was obtained by adding the corresponding constituents at 80%, 100% and 120% to the AYF sample. Three replicates on each level were determined. The precision, repeatability, stability and recovery of the method were measured by determining the RSD of RRT and the RPA of compounds.

2.7. Chemometric analysis

Principal components analysis (PCA), an unsupervised chemometric pattern recognition method, was employed to classify the AYF samples based on the content of 11 constituents using SIMCA-P 14.1. Technique for order preference by similarity to ideal solution (TOPSIS) is an approach of ordering a limited amount of assessment objectives according to their proximity to an idealized goal, which was used to evaluate the relative merits of the sample.

3. Results

3.1. UHPLC fingerprints analysis of AYF samples

3.1.1. Optimization of UHPLC conditions

In order to get the best separation and higher analytical efficiency, a series of experimental factors were optimized, including column type, mobile phase, flow rate, detection wavelength and column temperature. As a result, a satisfactory chromato-

graphic separation of AYF samples was obtained by ACQUITY UHPLC®HSS C18 column (2.1 × 100 mm, 1.8 μm). The solvent system of 0.1% aqueous formic acid and acetonitrile presented better separation and more peak shapes than other systems. The column temperature and flow rate for the best separation were obtained at 35 °C and 0.2 mL/min, respectively. The chromatographic conditions were present in Section 2. As shown in Fig. 1, the major components of AYF were separated well within36min.

3.1.2. Methodology validation

In method validation, peak 11 (P11) with large peak area and moderate retention time was selected as the reference peak and the RSD of average RRT and RPA of other 20 common peaks were calculated to analyze the precision, stability and repeatability. The RSD of RRT and RPA in precision, stability and repeatability tests were all less than 3.2 %. It indicated that the method was accurate and reliable, which could apply to establish UHPLC fingerprint for analyzing samples.

3.1.3. Similarity analysis of the fingerprints

To establish the fingerprint of AYF,12 batches of samples that came from different origins in China were analyzed by UHPLC and presented in Fig. 1. Twenty-one peaks(P1-P21) were selected as common peaks of different samples.

The similarity among the sample was evaluated by using the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (version2012). The similarity values of different samples were in the range of0.665–0.990 and were listed in Table 1. The similarities of the two batches from Xiangcheng Sichuan Province (S9) and Kangding Sichuan Province (S12) were 0.665 and 0.717, respectively. However, the similarities of the other 10 batches (Sichuan and Qinghai origins) were all higher than0.94. The similarity value of different samples may be caused by geographic location, sunlight condition, climate and growing environment. It indicated that chemical characteristics of the AYF samples were similar to a larger extent, but the relative content was different. Also, it suggested that the fingerprint analysis for AYF could be employed to evaluate the quality and authenticity of Chinese herbal medicines.

3.2. Identification of chemical constituents in AYF by UHPLC-Q-TOF-MS/MS

The method of UHPLC-Q-TOF-MS/MS was employed to identify the chemical constituents of twelve batches of AYF samples for further systematic evaluation of their quality. The total ion currents (TIC) of the extracts of AYF are displayed in Fig 2. A total of forty-six compounds were detected and thirty-eight compounds were identified, including thirteen flavonoids, ten terpenoids, seven organic acids, three fatty acid and five other compounds. The peaks for compounds 1, 2, 4, 9, 11, 16, 18, 20,23, 25 and 26 were identified by comparing the retention time, UV absorption and reference standards. The detailed information on mass data, retention time, and chemical formula for these compounds was listed in Table 2.

3.2.1. Identification of flavonoids

The fragmentation pathways of flavonoids follow by the Retro-Diels-Alder (RDA), then the loss of methyl group, carbonyl group, H_2O, CO_2 and other ions(Chen, et al.,2018). In this study, there were a total of thirteen flavonoids or their derivatives were identified in AYF extracts.

Compound 9 showed an $[M-H]^-$ ion at m/z 609 with the molecular formula $C_{20}H_{34}O_{21}$, which further produced a fragment ion at m/z 463 $[M-H-146]^-$ and 301 $[M-H-146-162]^-$ corresponding to the successive loss of the rhamnosyl and glucosyl units. Thus, compared with the reference standard, MS/MS fragmentation data and literature, it was definitely identified as rutin. The fragmentation pathway of compound 9 was shown in Figure 3A. By using a similar approach, compound 11was unambiguously identified as isoquercetin by comparison with the reference standard(Zhao,2014). Compound 8, 13, 14, 17, 19 and 32 were tentatively identified as hyodeoxycholate-6-O-glucuronide, kaempferol-3-rutinoside

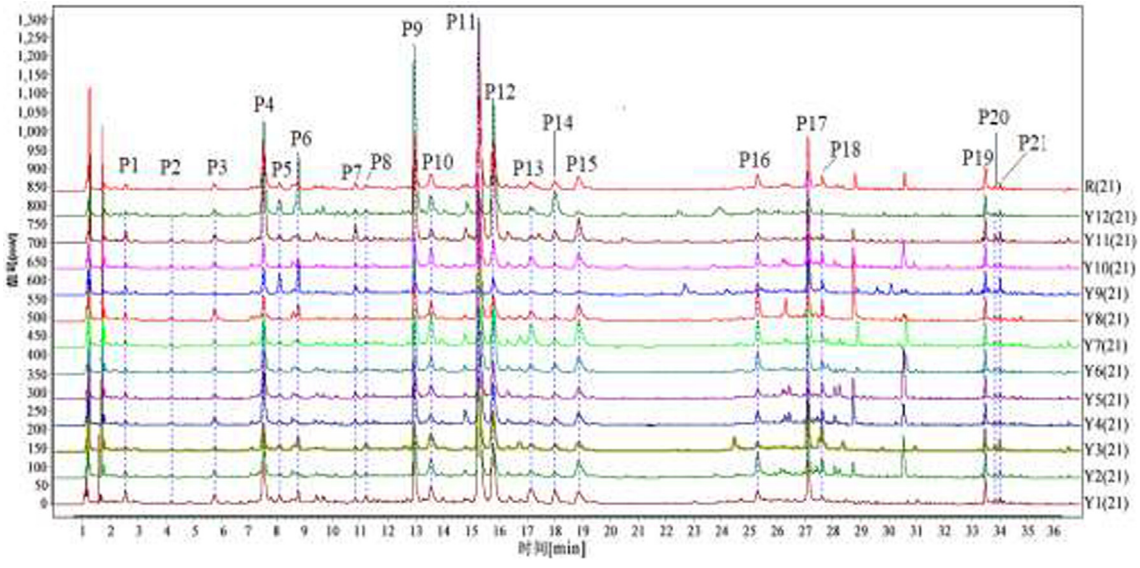

Fig. 1 UHPLC fingerprints of 12 batches of AYF (Y1-Y12) samples and Twenty-one common peaks (P1–P21).

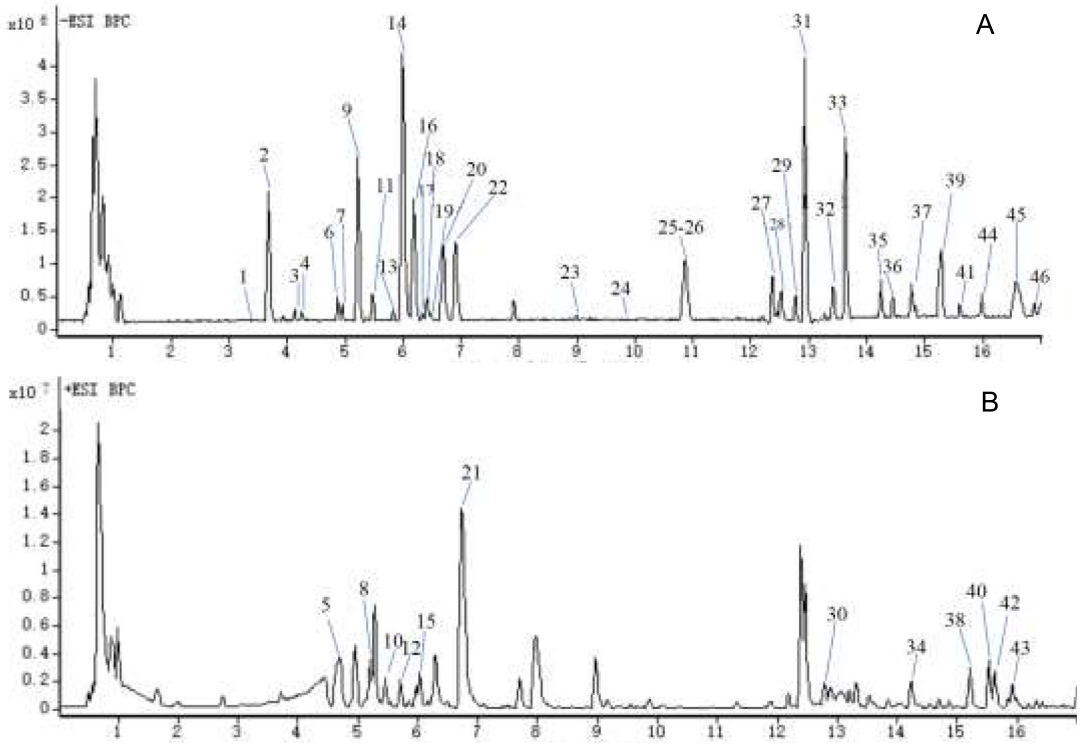

Fig. 2 The TIC of the extract of AYF in negative (A) and positive (B) ion mode.

(Zhao,2014), isorhamnetin-3-O-neohespeidoside(Li, et al., 2021), Isorhamnetin-3-O-glucoside(Li, et al.,2021), 11-O-Syringylbergenin and echinocystic acid 3-O-glucoside (Zhao,2014), respectively; while combined with their molecular formula and MS data reported in the literature.

Compound 23showedan $[M-H]^-$ ion at *m*/*z* 301and product ions at 235 and 151, which was identified as quercetin by comparing with the reference standards(Wang, et al.,2021). By using a similar approach, compound 25 and 26 were unambiguously identified as apigenin and kaempferol, respectively, by comparing with corresponding reference standards(Li, et al.,2021). Compound 40 and 43 were tentatively identified as isopropylapiosyl glucoside and 4′-methylxanthohumol respectively, according to the literature data.

3.2.2. Identification of organic acids

As apparent from Table.3, a total of seven compounds were assigned to organic acids. Compound 1, 2 and 4 produced the same molecular ion[M-H]$^-$ at *m*/*z*353 and fragment ions at *m*/*z* 191, which suggested that they were isomers with the same formula $C_{16}H_{18}O_9$. Besides, compound 1 further yielded characteristic fragment ion at *m*/*z* 127, whereas compound 2 and 4 generated characteristic fragment ion at *m*/*z*135 and 161, respectively, thus, they were unambiguously identified as neochlorogenic acid, chlorogenic acid and cryptochlorogenic acid, respectively, by comparing with reference standards (Zhao,2014).

As for compound 7, it was tentatively identified as butanedioic acid, considering it generated the ion $[M-H]^-$ at *m*/*z* 329, and further produced fragment ions at *m*/*z* 248 and 179.

Compounds 16, 18 and 20 shared the same molecular ion $[M-H]^-$ at *m*/*z* 515 and product ions at 353, 191, and 179, indicating that they were structural isomers. they were unambiguously identified as 3,4-dicaffeoylquinic acid,3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid, respectively, by comparing retention time, polarity and reference standards(Zhao,2014). The fragmentation pathway of representative compound (3,5-dicaffeoylquinic acid) is shown in Figure 3B.

3.2.3. Identification of terpenoids

In this work, A total of ten terpenoids or their derivatives were detected, and they were tentatively identified as orcinol gentiobioside (3)(Chen, et al.,2019), verbasoside (6), diterpene glycoside (28)(Zhao,2014), momordicoside E (31), excisanin B (35), 2,3,24-Trihydroxyole-an-12-en-28-oic acid (36)(Sun, et al.,2018), hederagenin (39), sterebin D (41)(Zhao,2014), glycyrrhetinic acid (44) and 23-Hydroxybetulinic acid (45)(Li, et al.,2021), respectively, through comparison with the MS data combined with the literature data.

3.2.4. Identification of fatty acid and other compounds

For compound 15, it displayed an ion $[M + H]^+$ at *m*/*z* 647, which further generated fragment ion at *m*/*z* 501, 331. Compound 15 was tentatively identified as Sucrose citrate succinate by comparing it with the MS data reported in literature. Similarly, according to the reported literature and MS data, compounds 37and 46 were tentatively assigned as 13-keto-9Z,11E-octadecadienoic acid and 16-Hydroxyhexadecanoic acid, respectively.

Table 2 The identified chemical components of AYF by UHPLC-Q-TOF-MS/MS.

NO.	t_R/min	Mass ([M−H]$^-$, *m*/*z*)	Error (ppm)	Fragment Ions (*m*/*z*)	Formula	Identification
1	3.504	353.0865	−10.04	191.0552,127.0387	$C_{16}H_{18}O_9$	Neochlorogenic acid
2	3.682	353.0873	−8.57	286.8643, 218.8703, 135.0435, 191.0547	$C_{16}H_{18}O_9$	Chlorogenic acid
3	4.133	447.1510	−9.58	401.1444,269.1926, 161.0439	$C_{19}H_{28}O_{12}$	Orcinol Gentiobioside
4	4.249	353.0900	−8.89	286.8637, 191.0547,161.0237	$C_{16}H_{18}O_9$	Cryptochlorogenic acid
5	4.697	481.2514[M + H]$^+$	1.55	413.0754	$C_{29}H_{36}O_6$	Vedelianin
6	4.867	461.1665	−8.12	415.1598, 325.9165, 259.9290,149.0423	$C_{20}H_{30}O_{12}$	Verbasoside
7	4.950	329.1231	−7.53	248.9580,179.0563	$C_{15}H_{22}O_8$	Butanedioic acid
8	5.197	569.3055[M + H]$^+$	0.36	435.3255, 281.5880,170.0965	$C_{30}H_{48}O_{10}$	Hyodeoxycholate-6-O-glucuronide
9	5.217	609.1449	0.99	463.0894, 301.0337, 300.0253, 178.9999, 151.0023	$C_{20}H_{34}O_{21}$	Rutin
10	5.448	634.3201[M + H]$^+$	0.93	428.3852, 318.1577, 277.0415,173.0006	$C_{30}H_{50}O_{14}$	unknown
11	5.484	463.0877	−8.63	301.0343,271.0239, 151.0040	$C_{21}H_{20}O_{12}$	Isoquercetin
12	5.715	678.3099[M + H]$^+$	−3.27	546.3193, 341.1407,137.0574,	$C_{31}H_{50}O_{16}$	unknown
13	5.834	593.1493	−7.56	459.1102, 327.0475, 285.0383,151.0027,	$C_{27}H_{30}O_{15}$	Kaempferol-3-rutinoside
14	5.984	623.1603	−8.45	463.0934, 357.0609, 315.0504,151.0022	$C_{28}H_{32}O_{16}$	Isorhamnetin-3-O-neohespeidoside
15	6.031	647.1870[M + H]$^+$	3.09	501.1181, 331.1144	$C_{22}H_{30}O_{22}$	Sucrose citrate succinate
16	6.185	515.1171	−8.25	447.0927, 353.0864, 242.9452,191.0541	$C_{25}H_{24}O_{12}$	3,4-Dicaffeoylquinic acid
17	6.335	477.1038	−6.28	411.4614, 314.0421,151.0007	$C_{22}H_{22}O_{12}$	Isorhamnetin-3-O-glucoside
18	6.418	515.1185	−6.95	431.0958,353.0862,310.9243,242.9434,191.0539,135.0448	$C_{25}H_{24}O_{12}$	3,5-Dicaffeoylquinic acid
19	6.485	507.1142	−7.19	461.9059, 345.0603, 238.9370,130.0396	$C_{23}H_{24}O_{13}$	11-O-Syringylbergenin
20	6.702	515.1262	−7.21	353.0860, 255.0674, 173.0438,135.0444	$C_{25}H_{24}O_{12}$	4,5-Dicaffeoylquinic acid
21	6.732	701.4338[M + H]$^+$	0.45	701.4338	$C_{38}H_{68}O_{11}$	unknown
22	6.902	625.1177	−2.92	463.0876, 301.0339,178.9967	$C_{48}H_{18}O_2$	unknown
23	8.987	301.0347	−2.95	235.9628, 189.9557, 178.9976,151.0030	$C_{15}H_{10}O_7$	Quercetin
24	9.869	447.228	−6.89	447.228	$C_{18}H_{38}O_{12}$	unknown
25	10.856	269.0438	−7.72	225.0544,151.0017	$C_{15}H_{10}O_5$	apigenin
26	10.860	285.0450	−5.40	162.0093,151.0026	$C_{15}H_{10}O_6$	Kaempferol
27	12.458	389.0868	−7.32	345.1702,231.1385	$C_{19}H_{18}O_9$	Bicyclol
28	12.524	329.2322	−7.22	171.1018	$C_{18}H_{34}O_5$	Diterpene glycoside
29	12.775	389.1599	7.39	345.1705, 263.1261,129.9765	$C_{14}H_{30}O_{12}$	Dimethylene-D-Sorbitol
30	12.788	365.2584[M + H]$^+$	−9.74	194.0924	$C_{21}H_{32}O_5$	Dihydrocortisol
31	12.925	695.4003	−8.08	649.3984, 503.3368, 409.3158,207.0489	$C_{37}H_{60}O_{12}$	Momordicoside E
32	13.425	647.3790	−6.32	571.3642, 407.3331, 308.8903, 242.9377,175.0210	$C_{36}H_{56}O_{10}$	Echinocystic acid 3-O-glucoside
33	13.626	343.1545	−8.21	299.1640,217.1201	$C_{20}H_{24}O_5$	Nectandrin B
34	14.240	437.2057[M + H]$^+$	−5.33	303.1294,169.0438	$C_{19}H_{32}O_{11}$	unknown
35	14.243	391.2125	−6.45	331.1892, 287.2014,190.9240	$C_{22}H_{32}O_6$	Excisanin B
36	14.443	487.3426	−6.37	437.2292, 329.1390,175.9586	$C_{30}H_{48}O_5$	2,3,24-Trihydroxyolean-12-en-28-oic acid
37	14.777	295.2264[M + H]$^+$	−6.77	195.1373	$C_{18}H_{30}O_3$	13-keto-9Z,11E-octadecadienoic acid
38	15.208	343.1444[M + H]$^+$	5.57	255.0934	$C_{13}H_{26}O_{10}$	unknown
39	15.294	471.3474	−7.74	407.3333,248.9521	$C_{30}H_{48}O_4$	Hederagenin
40	15.525	357.1740[M + H]$^+$	7.47	255.1030,185.0596	$C_{14}H_{26}O_{10}$	Isopropyl apiosylglucoside
41	15.594	293.2106	−6.69	249.2207,185.1174	$C_{18}H_{30}O_3$	Sterebin D
42	15.641	357.1859[M + H]$^+$	−3.23	255.1121	$C_{14}H_{28}O_{10}$	unknown
43	15.925	369.1611[M + H]$^+$	5.26	267.0975,133.0917	$C_{22}H_{24}O_5$	4′-methylxanthohumol
44	15.978	469.3306	−6.29	407.3317, 270.2515,137.0957	$C_{30}H_{46}O_4$	Glycyrrhetinic acid
45	16.579	471.3467	−6.9	428.2415, 336.9307, 270.9398, 211.1362,144.9204	$C_{30}H_{48}O_4$	23-Hydroxybetulinic acid
46	16.896	271.2269	−6.62	225.2132,144.9214	$C_{16}H_{32}O_3$	16-Hydroxyhexadecanoic acid

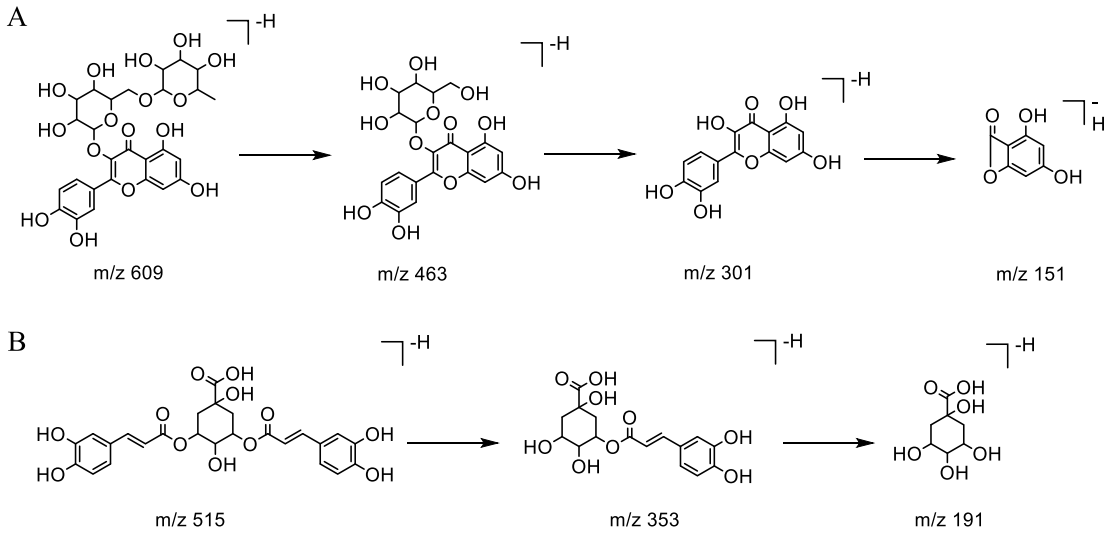

Fig. 3 The hypothesized fragmentation pathway of compound 9(A) and 18(B).

Table 3 Antioxidant activity of 12 batches of AYF.

Sample ID	DPPH	ABTS	FRAP
	IC_{50} (μg/mL)	IC_{50} (μg/mL)	(mmol Fe^{2+}/g)
Ascorbic acid	4.78 ± 0.05	4.46 ± 0.02	13.72 ± 2.16
S1	69.14 ± 2.07	74.25 ± 4.29	1.59 ± 0.07
S2	97.22 ± 1.98	84.24 ± 0.76	1.26 ± 0.02
S3	124.13 ± 3.02	98.53 ± 1.39	1.25 ± 0.04
S4	136.46 ± 2.88	101.82 ± 2.87	1.31 ± 0.03
S5	139.86 ± 3.02	98.94 ± 3.37	1.21 ± 0.06
S6	112.93 ± 2.98	93.58 ± 1.22	1.24 ± 0.02
S7	82.26 ± 2.10	86.58 ± 2.15	1.32 ± 0.03
S8	182.92 ± 8.45	129.07 ± 4.88	0.92 ± 0.04
S9	151.79 ± 5.07	129.77 ± 2.57	1.08 ± 0.06
S10	156.38 ± 7.62	118.00 ± 3.97	1.03 ± 0.04
S11	59.16 ± 1.87	63.89 ± 1.92	1.69 ± 0.02
S12	65.72 ± 2.17	79.75 ± 0.88	1.58 ± 0.04

Table 4 Correlations and Grade of GRA.

Peaks	r_i		
	DPPH	ABTS	FRAP
P1	0.6848	0.5547	0.4859
P2	0.8654	0.8991	0.8764
P3	0.6854	0.5851	0.6827
P4	0.5832	0.616	0.5549
P5	0.8426	0.8234	0.8103
P6	0.7960	0.7637	0.7782
P7	0.6405	0.7451	0.7333
P8	0.6705	0.691	0.6476
P9	0.8203	0.8517	0.8237
P10	0.7853	0.8424	0.8441
P11	0.588	0.6447	0.7186
P12	0.8425	0.8273	0.7755
P13	0.6862	0.5848	0.5141
P14	0.7088	0.5899	0.4700
P15	0.8225	0.7675	0.7102
P16	0.6964	0.658	0.8413
P17	0.6625	0.6799	0.8069
P18	0.6401	0.6184	0.6004
P19	0.5542	0.5669	0.6634
P20	0.5816	0.6365	0.5642
P21	0.7615	0.7882	0.7864

Compound 5 generated an $[M + H]^+$ ion at m/z481 with formula C29H36O6. It further showed the fragment ion at 413. It was tentatively assigned as vedelianin.

Compounds 27 and 29 showed the same molecular ion $[M-H]^-$ at *m/z* 389 with the same formula $C_{14}H_{30}O_{12}$. In addition, compounds27 and 29 generated the characteristic fragment at *m/z* 231 and 129, respectively. Thus, they were tentatively identified as bicyclol and dimethylene-D-sorbitol respectively.

Compound 30 produced an $[M + H]^+$ ion at m/z365 with formula C21H32O5. It further showed the fragment ion at 194, which was tentatively assigned as dihydrocortisol.

Compound 33 showed an $[M-H]^-$ion at *m/z* 343 and further produced fragment ion at *m/z* 299, 217. It was tentatively assigned as nectandrin B.

3.3. Antioxidant activities of AYF

The antioxidant activities of AYF were determined by using DPPH, ABTS and FRAP assays. DPPH and ABTS free radical scavenging assay are the most common approach to evaluate the antioxidant activity because of its good stability and repeatability(Xu, et al.,2020).FRAP is also a common method to measure antioxidant activity. These three types of methods are frequently used to evaluate the antioxidant activity because methods for the evaluation of antioxidant capacity are simple, rapid, low cost and good repeatability(Apak, et al.,2007). Therefore, these three kinds of antioxidant tests were conformed to determine the antioxidant capacity of AYF, and the results are listed in Table 3. As shown in Table 3, the IC_{50} values of DPPH, ABTS, and FRAP assays were in the range 59.16 ± 1.87–182.92 ± 8.45 μg/mL, 63.89 ± 1.92–129 .77 ± 2.57 μg/mL, 0.92 ± 0.04–1.69 ± 0.02 mmol Fe^{2+}/g,

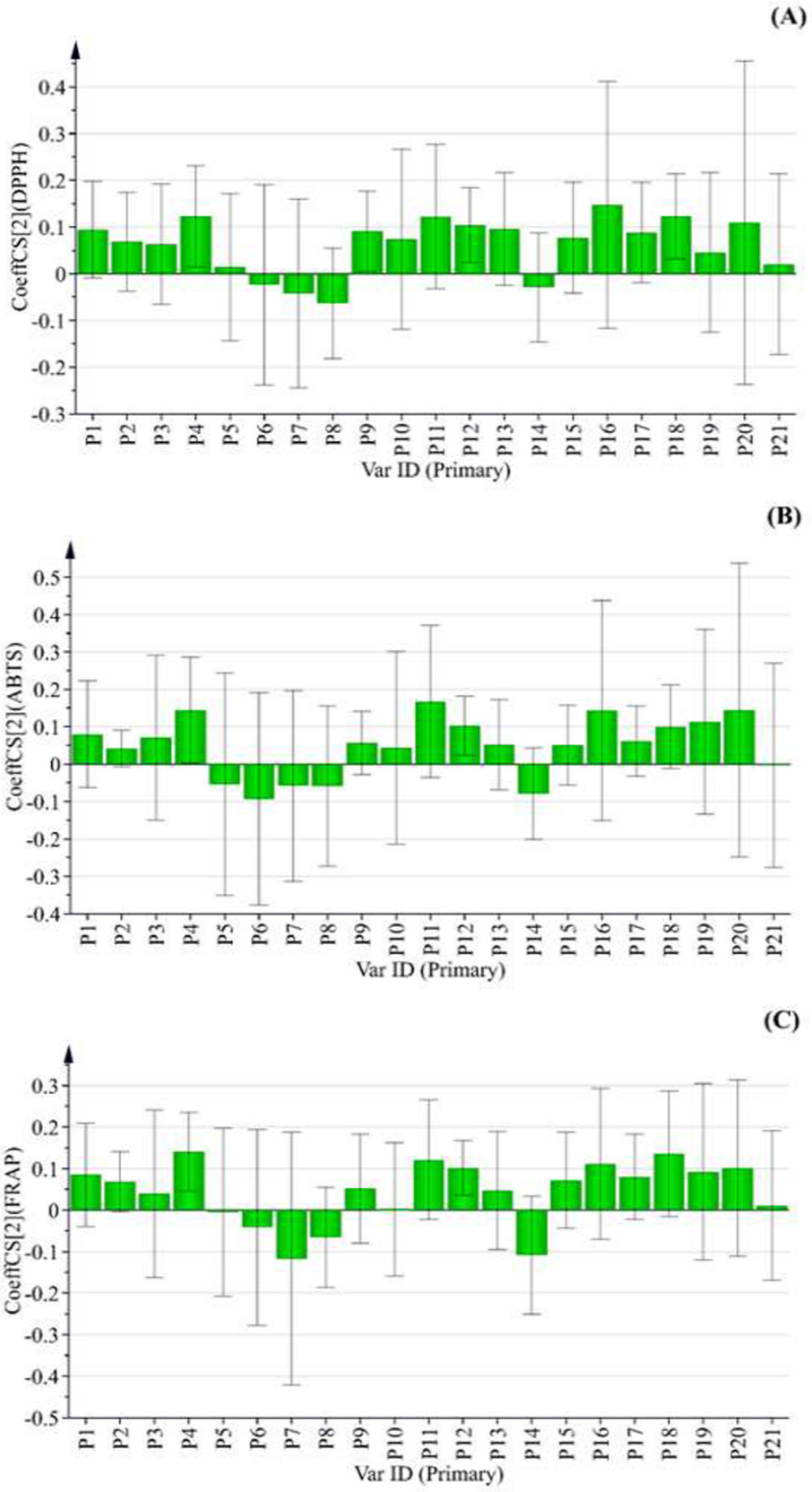

Fig. 4 Regression coefficients of 21 common peak of AYF in the PLS models of DPPH (A), ABTS (B) and FRAP (C) assay, respectively.

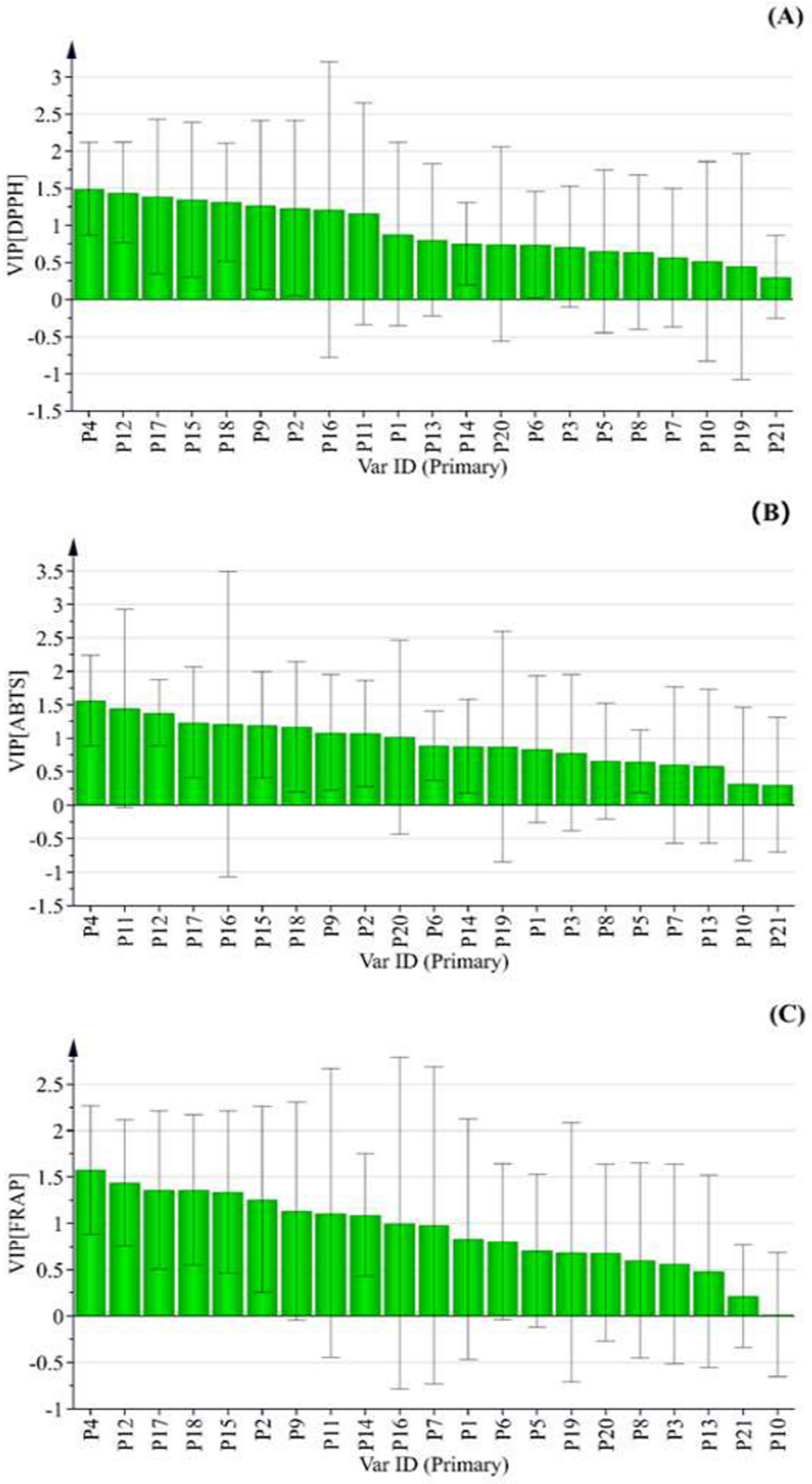

Fig. 5 The VIP values of 21 commonpeaks of AYF in thePLS models of DPPH (A), ABTS (B) and FRAP (C) assay, respectively.

respectively, indicating that the extracts of AYF showed strong antioxidant activity. Moreover, different batches of sample showed different levels of antioxidant capacity in tests. The batch of S11, S12, and S1 showed the higher radical scavenging activity for DPPH, ABTS, and FRAP assays. It was indicating that the antioxidant capacity of AYF might be related to geographic location, chemical compound and content.

3.4. Fingerprint-activity relationship analysis

In order to find out efficacy markers, chemometric analysis, including GRA and PLS, were employed to analyze the correlation between common characteristic peaks and antioxidant ability.

3.4.1. Gray relational analysis

GRA was used to analyze the spectrum-efficacy relationship between antioxidant activity and peak area values of 21 common peaks. As shown in Table 4, the relational degree (r_i) was in the range of 0.4700–0.8991. The r_i value of the peak was greater than 0.6000, which indicated that it was related to antioxidant activity. The r_i value was greater than 0.8000, indicating that it had a high correlation to antioxidant activity (Liu, et al.,2020). As a result, P2, P5, P9, P12 and P15 showed high relations to DPPH; P2, P5, P6, P9, P10 and P12 exhibited high relations to ABTS; P2, P5, P9, P10, P16 and P17 displayed high relations to FRAP. Therefore, eight peaks (P2, P5, P9, P10, P12, P15, P16 and P17) were significantly potential active compounds correlated with antioxidant capacity.

3.4.2. Partial least square analysis

PLS analysis was further employed to build the correlation between characteristic peak and antioxidant activity, so as to find out the potential antioxidant compounds of AYF (Zhang, et al.,2018; Zhang, et al.,2019).The lower theIC_{50} score, the stronger free radical scavenging capacity is for antioxidant tests. In the PLS model, the peak areas of common peaks were set as independent variable (X), the inverse of IC_{50} values was defined as depended variable (Y). In the PLS model, the value of the regression coefficient was positive, meaning that the correlation between the peak areas of common peaks and antioxidant capacity also was positive. As shown in Figure 4A, P1, P2, P3, P4, P5, P9, P10, P12, P13, P15, P16, P17, P18, P19, P20 and P21 were positive correlated with DPPH free radical scavenging capacity. P1, P2, P3, P4, P9, P10, P12, P13, P15, P16, P17, P18, P19, P20 and P21 showed positive relations to ABTS and FRAP assay in Figure 4B-C. It indicates that these selected peaks contributed to the main antioxidant activity of AYF. However, the variable importance in projection (VIP) values were usually used to reflect the importance of the independent variable (X) to the dependent variable (Y). The VIP value is larger and the peak is more important. Generally, VIP value was greater than 1, indicating that the independent variable of peak was meaningful(Cao, et al.,2018; Jiang, et al.,2018; Zhang, et al.,2018). As shown in Figure 5(A–C), P4, P12, P17, P15, P18, P9, P2, P16 and P11 were the main important peak for the DPPH assay; P4, P11, P12, P17, P16, P15, P18, P9 and P2 the ABTS assay; P4, P12, P17, P18, P15, P2, P9, P11, P14 and P16 for the FRAP assay. Therefore, combining regression coefficient with VIP value, P4, P12, P17, P15, P18, P9, P2, P16 and P11had an obvious effect on DPPH free radical scavenging capacity; P4, P11, P12, P17, P16, P15, P18, P9 and P2 the ABTS assay; P4, P12, P17, P18, P15, P2, P9, P11 and P16 for the FRAP assay. The PLS result indicated that nine peaks (P2, P4, P9, P11, P12, P15, P16, P17and P18) were selected to be correlated with better antioxidant capacity in AYF.

3.4.3. Identification of efficacy-related markers

Based on GRA and PLS analysis, eleven markers (P2, P4, P5, P9, P10, P11, P12, P15, P16, P17and P18) were selected and regarded as potential markers to be related to antioxidant effect. Eleven of these compounds were identified as neochlorogenic acid (P2), chlorogenic acid (P4), cryptochlorogenic acid (P5), rutin (P9), isoquercitrin (P10), isochlorogenic acid B (P11), isochlorogenic acid A (P12), isochlorogenic acid C (P15), quercetin (P16), apigenin (P17), kaempferol (P18), respectively, by comparison with the characteristic fragment ions and retention time of corresponding standards. It was reported that neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, rutin, isoquercitrin, isochlorogenic acid B, isochlorogenic acid A, isochlorogenic acid C, quercetin, apigenin and kaempferol presented a much stronger antioxidant activity(Singh, et al.,2014; Ekiert, et al.,2020).Six of the eleven markers were organic acids, and the other markers were flavonoids, so it was supposed that these six organic acids and five flavonoids were the main material basis for antioxidant efficacy and play an oxidative inhibitory role in AYF. In conclusion, these compounds may be beneficial to distinguish from

Table 5 Results of linear regression, LOQs and LODs for eleven compounds in AYF.

Compounds	Regression equation	Linearity range (μg/mL)	r	LOQ (μg/mL)	LOD (μg/mL)
Neochlorogenic acid	y = 31909321 × - 27,460	0.5 ~ 87	0.9996	7.074×10^{-2}	1.192×10^{-2}
Chlorogenic acid	y = 33985913 × + 94527	13 ~ 389	0.9991	9.772×10^{-2}	2.892×10^{-2}
Cryptochlorogenic acid	y = 27139161 × - 35,120	2.4 ~ 179.7	0.9998	6.007×10^{-2}	2.087×10^{-2}
Rutin	y = 18984830 × + 66186	12.1 ~ 364	0.9993	8.693×10^{-2}	0.812×10^{-2}
Isoquercetin	y = 30307965 × - 11,600	1.5 ~ 175.3	0.9999	5.604×10^{-2}	1.004×10^{-2}
Isochlorogenic acid B	y = 34247909 × - 117,686	2.4 ~ 177.7	0.9998	7.977×10^{-2}	1.134×10^{-2}
Isochlorogenic acid A	y = 45513167 × + 25180	11.9 ~ 357	0.9993	6.924×10^{-2}	0.929×10^{-2}
Isochlorogenic acid C	y = 39776147x − 134,832	6.3 ~ 189	0.9993	7.017×10^{-2}	0.985×10^{-2}
Quercetin	y = 61932766 × - *8964*	0.2 ~ 42.8	0.9999	4.085×10^{-2}	0.745×10^{-2}
Apigenin	y = 36052453 × + 9360	0.8 ~ 58.4	0.9995	5.099×10^{-2}	1.142×10^{-2}
Kaempferol	y = 38572923 × + 11084	0.8 ~ 57.4	0.9995	4.126×10^{-2}	1.197×10^{-2}

Table 6 Content(mg/g) of 11 components in AYF (n = 3).

Sample ID	Neochlorogenic acid	Chlorogenic acid	Cryptochlorogenic acid	Rutin	Isoquercetin	Isochlorogenic acid B	Isochlorogenic acid A	Isochlorogenic acid C	Quercetin	Apigenin	Kaempferol	total
S1	0.0934 ± 0.0010	3.2322 ± 0.0020	0.4402 ± 0.0037	1.0578 ± 0.0012	0.3059 ± 0.0041	1.6846 ± 0.0010	2.7497 ± 0.0013	1.0333 ± 0.0015	0.1169 ± 0.0013	0.4255 ± 0.0024	0.1055 ± 0.0013	11.2449
S2	0.0557 ± 0.0012	1.5820 ± 0.0321	0.1911 ± 0.0053	0.9566 ± 0.0026	0.4158 ± 0.0013	0.9668 ± 0.0029	1.4560 ± 0.0026	0.4119 ± 0.0013	0.2733 ± 0.0024	0.5096 ± 0.0025	0.2022 ± 0.0009	7.0208
S3	0.0835 ± 0.0022	1.0425 ± 0.0142	0.3569 ± 0.0081	0.4516 ± 0.0024	0.3490 ± 0.0016	0.8428 ± 0.0034	1.7241 ± 0.0017	0.4350 ± 0.0018	0.1241 ± 0.0007	0.4506 ± 0.0010	0.2522 ± 0.0008	6.1122
S4	0.0693 ± 0.0013	2.2420 ± 0.0223	0.1971 ± 0.0047	0.8834 ± 0.0031	0.2872 ± 0.0026	1.2200 ± 0.0039	1.8200 ± 0.0034	0.5403 ± 0.0026	0.1867 ± 0.0011	0.3268 ± 0.0005	0.1299 ± 0.0004	7.9027
S5	0.0607 ± 0.0012	2.1475 ± 0.0461	0.2546 ± 0.0015	0.8590 ± 0.0016	0.2669 ± 0.0207	1.0234 ± 0.0048	1.7830 ± 0.0037	0.4257 ± 0.0031	0.1609 ± 0.0010	0.2986 ± 0.0013	0.1865 ± 0.0011	7.4667
S6	0.0814 ± 0.0007	2.3116 ± 0.0036	0.2282 ± 0.0022	1.0123 ± 0.029	0.3235 ± 0.0011	1.6317 ± 0.0020	2.8198 ± 0.0022	0.7071 ± 0.0031	0.2262 ± 0.0011	0.3340 ± 0.0008	0.0976 ± 0.0004	9.7734
S7	0.0614 ± 0.0004	1.3417 ± 0.0052	0.2002 ± 0.0014	1.8139 ± 0.0001	0.6842 ± 0.0032	1.3912 ± 0.0019	1.9302 ± 0.0022	0.5458 ± 0.0015	0.3069 ± 0.0014	0.7454 ± 0.0012	0.0792 ± 0.0012	9.1003
S8	0.0426 ± 0.0010	1.0827 ± 0.0407	0.1768 ± 0.0053	0.9571 ± 0.0021	0.4170 ± 0.0017	1.3675 ± 0.0044	1.1671 ± 0.0040	0.3730 ± 0.0019	0.3651 ± 0.0007	0.7851 ± 0.0032	0.1976 ± 0.0011	6.9315
S9	0.0660 ± 0.0014	1.0460 ± 0.0080	1.0576 ± 0.0172	0.4370 ± 0.0016	0.1445 ± 0.0013	0.3067 ± 0.0015	0.7427 ± 0.0020	0.3372 ± 0.0016	0.1315 ± 0.0011	0.4233 ± 0.0022	0.1806 ± 0.0016	4.8731
S10	0.0497 ± 0.0023	1.0744 ± 0.0283	0.1345 ± 0.0028	0.6406 ± 0.0030	0.4157 ± 0.0018	1.0052 ± 0.0026	1.2002 ± 0.0028	0.3665 ± 0.0019	0.2013 ± 0.0016	0.8451 ± 0.0010	0.1821 ± 0.0010	6.1154
S11	0.1044 ± 0.0021	4.7134 ± 0.0367	0.4853 ± 0.0033	1.7104 ± 0.0017	0.3109 ± 0.0018	2.3287 ± 0.0017	4.0948 ± 0.0013	0.8306 ± 0.0009	0.1523 ± 0.0015	0.2973 ± 0.0006	0.1061 ± 0.0017	15.1342
S12	0.2070 ± 0.0025	4.3406 ± 0.0502	1.0673 ± 0.0127	3.2792 ± 0.0024	0.4107 ± 0.0023	1.1243 ± 0.0025	5.6985 ± 0.0076	1.8067 ± 0.0013	0.1289 ± 0.0022	0.2339 ± 0.0025	0.0985 ± 0.0032	18.3956

different AYF samples. Moreover, they can be employed to analyze the quality of AYF.

3.5. Quantitative analysis of eleven components in AYF

3.5.1. Method validation for quantitative analysis

The method was validated before the simultaneous determination of eleven components. As shown in Table 3, the results of linear regression for eleven compounds exhibited good linearity (r greater than 0.9990) for every reference standard among a certain concentration range. The results of LOQs and LODs were listed in Table 5. It meant that LOQs and LODs were used to simultaneously determine eleven components. As for the precision test, the RSDs of intra-day and inter-day peak areas of the eleven reference standards were all less than 2.5%, indicating that the instrument was in good condition. The RSDs of the repeatability test was less than 2.0%, suggesting that the method had good repeatability. The RSDs of the stability test in 24 h was less than 3.2%, indicating that the sample solution was stable for 24 h at room temperature. Moreover, the average recoveries were in the range of 98.2% to 102.9% and the RSDs of recovery were all less than 2.4%. The results suggested that the method was feasible and could be applicable for quantitative analysis.

3.5.2. Quantitative analysis of sample

The quantitative results of samples are shown in Table 6. the contents of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, rutin, isoquercitrin, isochlorogenic acid B, isochlorogenic acid A, isochlorogenic acid C, quercetin, apigenin, kaempferoland total of eleven compounds in twelve batches of AYF varied from 0.0426 to 0.2070 mg/g, 1.0425 to 4.3406 mg/g, 0.1768 to 1.0673 mg/g,0.4370 to 3.2792 mg/g,0.1445 to 0.6842 mg/g,0.3067 to 2.3287 mg/g,0.7427 to 5.6985 mg/g,0.3372 to 1.8067 mg/g,0.1169 to 0.3069 mg/g,0.2339 to 0.8451 mg/g, 0.0792 to 0.2522 mg/g and 4.8731 to 18.3956 mg/g, respectively. The contents of each component were observably different in sample obtained from different areas. For example, the content of isochlorogenic acid A in AYF from Xiangcheng county of Sichuan (S9), was 0.7427 mg/g, while it was 5.6985 mg/g in AYF from Kangding city of Sichuan (S12). However, the similarities of S9 and S12 were 0.665 and 0.717, respectively, indicating that the quality of the sample was not only dependent on a single component but a multi-component interaction. The batch of S11, S12 and S1 had higher total content of eleven compounds, while the batch of S9, S10 and S3 had lower total content. It was indicated that the antioxidant capacity might be related to the total content of eleven compounds. Thus, it is necessary to use multicomponent and comprehensive evaluation methods to evaluate the antioxidant quality of samples.

3.6. Multivariate statistical analysis

3.6.1. PCA of samples

PCA was used to classify different batches of AYF samples based on the content of eleven constituents. The content of eleven constituents from twelve batches of samples was led into the SIMCA-P software, and the score scatters plot of PCA was obtained. To predict discrimination in the model, the parameters R2 and Q2 were calculated to predict and evaluate

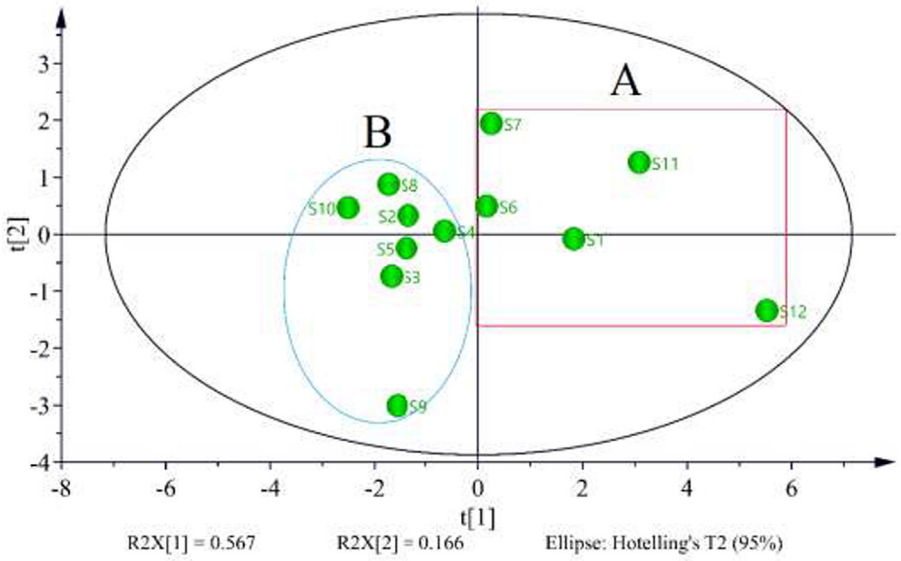

Fig. 6 The score plot of PCA for 12 batch samples.

discrimination in the model. As a result, the R^2X and Q^2 were 0.941and 0.506, respectively, indicating that the model was used to distinguish the samples. As shown in Fig. 6, the score scatters plot of PCA indicated that all samples were divided into two groups. In detail, the batches of S1, S6, S7, S11, and S12 were gathered into group A with the total content of eleven constituents exceeding 9 mg/g (Table 6) and distributed in the right of t [1], while the batches of S2, S3, S4, S5, S8, S9 and S10 were gathered into group B and distributed in the left of t [1]. However, a comprehensive evaluation is necessary to determine which batch is of better quality.

3.6.2. TOPSIS analysis

TOPSIS analysis is a multi-index decision analysis method, which has been widely used in the comprehensive quality evaluation of traditional Chinese medicine. As shown in Table 7, the chemical comprehensive evaluation index *Ci* values were in the range of [0, 1]. The value of *Ci* is closer to 1, which the comprehensive quality of the sample is better(Zhao, et al.,2020). Thus, the antioxidant efficacy batches of S12, S11, S1, S7 and S6 were ranked in the top five, while the batches of S9, S8, S4, S2, S5, S3 and S10 were ranked from 6th to 12th. It was suggested that TOPSIS analysis could be used to explain which group gave a better quality in the PCA analysis. It also was found that the rank of comprehensive quality evaluation of the sample agreed with the antioxidant activity.

4. Conclusion

In this work, fingerprint-activity relationship modeling was employed to review a quality evaluation of AYF based on chemical fingerprints and efficacy. Firstly, UHPLC fingerprints were established to evaluate the similarity of twelve batches of sample based on the twenty-one common characteristic, and the similarity of twelve batches of AYF from different origins was in the range of0.665 to 0.990. Then, the identification of characteristic chemical components in AYF was carried out by UHPLC-Q-TOF-MS/MS, and forty-six compounds were detected and thirty-eight compounds were identified. Secondly, the protective effect of AYF against antioxidant activities was evaluated by DPPH, ABTS, and FRAP assays. Thirdly, fingerprint-activity relationship modeling between the spectrum and the antioxidant efficacy was preform by GRA and PLS to screen out bioactive markers without expensive and tedious phytochemical separation. Finally, the content

Table 7 Results of TOPSIS analysis of AYF samples.

Sample ID	D_i^+	D_i^-	C_i	Ranking of C_i
S1	1.0587	0.6551	0.4271	3
S2	1.3016	0.3930	0.2373	9
S3	1.3425	0.3173	0.1957	11
S4	1.2049	0.4295	0.2624	8
S5	1.2625	0.3789	0.2365	10
S6	1.0947	0.5977	0.3665	5
S7	1.1256	0.7570	0.3934	4
S8	1.3783	0.5050	0.2733	7
S9	1.4868	0.5553	0.2966	6
S10	1.3834	0.2956	0.1771	12
S11	0.8345	0.9536	0.5440	2
S12	0.4981	1.3975	0.7645	1

of markers in AYF was simultaneously determined by UHPLC. Then, PCA and TOPSIS were used to assess the antioxidant efficacy of AYF. As a result, there were obvious differences in the contents of the eleven markers among the twelve batches of AYF. The results of antioxidant efficacy showed that the batches of S12, S11, and S1 had high content of eleven markers and strong antioxidant activity, which was supposed to be of good quality. However, the batch of S10, S3, and S5 had low content of eleven markers and weak antioxidant activity, which was thought to be of poor quality. It was reasonable that eleven compounds could be selected and considered as markers for quality control of AYF. Therefore, this strategy could provide a useful method for the discovery of bioactive markers and quality evaluation of Chinese herbal medicine in the future.

Author contributions

This study was conceived by Zhifeng Zhang. Jianguang Zhang conducted the analysis of UHPLC-QTOF-MS and draft the manuscript. Junjun Wang and Yue Wang performed the antioxidant experiment. Li Li evaluated the quantitative analysis. Wenfang Jin conducted the statistical analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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