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ORIGINAL ARTICLE

Comparative chemical diversity and antioxidant activities of three species of *Akebia* herbal medicines

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Antioxidant activity

Abstract *Akebia* stem has long been used extensively as a rare Chinese herbal medicine. The three most significant *Akebia* medicinal species are *Akebia quinata* (Thunb.) Decne. (*A. quinata*), *Akebia trifoliata* (Thunb.) Koidz. (*A. trifoliata*), and *Akebia trifoliata* (Thunb.) Koidz. var. Australis (Diels) Rehd. (*A. trifoliata. var*). They have significant therapeutic effects and are widely used in the pharmaceutical and cosmetics industries. Only a few studies compared their chemical differences and antioxidant activities. To better demonstrate each species' characteristics and antioxidant properties, the ultra-performance liquid chromatography coupled with quadrupole Orbitrap mass spectrometry (UPLC-Q-Orbitrap/MS)-based metabolomics was applied to investigate the chemome diversity of three *Akebia* species. Their antioxidant activities were evaluated by DPPH and ABTS assays. In total, 65 different metabolites were identified, including 5 phenolic acids, 2 phenylpropanoids, 4 lignan glycosides, and 54 triterpenoid saponins. The different aglycone types of triterpenoid saponins were found to be the component differences between the three *Akebia* species. The chemical composition of *A. trifoliata* and *A. trifoliata. var* is similar. The 2-(3,4-dihydroxyphenyl)-ethyl-O-β-D-glucopyranoside has been found only in *A. quinata*. In contrast, the triterpenoid saponins akemisaponin B, akemisaponin D, oleanolic-acid-3-O-arabinopyranosyl-28-O-glucopyranosyl-glucopyranosyl-rhamnopyranosyl-arabinopyranosyl, akemisaponin C and saponin P_{j1} have been found *A. trifoliata* and *A. trifoliata. var*. As a result, these six compounds can be considered marker compounds that distinguish three *Akebia* species. The antioxidant activities results indicated that

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the antioxidants of three *Akebia* species were the same in different antioxidative test systems. *A. trifoliata* (IC_{50}: 2.28–6.97 mg·mL^{-1}) and *A. trifoliata. var* (IC_{50}: 2.09–6.87 mg·mL^{-1}) showed 2–3 times higher antioxidant activity than *A. quinata* (IC_{50}: 5.56–11.21 mg·mL^{-1}). This study reveals the antioxidant activity differences of three *Akebia* species, laying a foundation for further development and utilization. This type of study can lead to the identification of a compound that, with further work and more extensive studies, has the potential to be used as a biomarker, in this case to distinguish different medicinal species.

1. Introduction

The Lardizabalaceae family includes 9 genera, *Akebia, Archakebia, Boquila, Decaisnea, Holboellia, Lardizabala, Sargentodoxa, Sinofranchetia,* and *Stauntonia*, representing 35 plant species (The Plant List, 2020). *Akebia* is the most populous genus of the Lardizabalaceae family. They are found in Asia, specifically in China, Japan, and Korea (Maciąg et al., 2021). *Akebia quinata* (Thunb.) Decne. (*A. quinata*), *Akebia trifoliata* (Thunb.) Koidz. (*A. trifoliata*) and *Akebia trifoliata* (Thunb.) Koidz. var. Australis (Diels) Rehd. (*A. trifoliata. var*), have been described as mutong in the pharmacopoeia of China and also named moku-tsu in the pharmacopoeia of Japan (Ma et al., 2019). Because of their medicinal and high nutritional values, they are the most economically important three *Akebia* species. They have long been used as a rare herbal medicine. Biological activity studies of the three *Akebia* species have confirmed diuretic, hepatoregenerative, neuroprotective, analgesic, anti-inflammatory, anti-obesity, antibacterial and anticancer (liver and stomach) activities (Jiang et al., 2020; Bian et al., 2021; Lee et al., 2017; Maciąg et al., 2021; Peng et al., 2020; Song et al., 2018; Sung et al., 2015). Furthermore, as natural plants, *A. quinata and A. trifoliata* extracts have been shown to have antioxidant activity and free radical scavenging capability (Jiang et al., 2021; Jung et al., 2004; Luo et al., 2021). The *A. trifoliata* and *A. trifoliata. var* is widely cultivated and considered a new medicinal and edible homologous resource type (Niu et al., 2021). The strong antioxidant activity makes Akebia stems widely used in cosmetics, health care products, and food. Studies have proven *A. quinata* as a crude drug material for treating obesity in traditional Korean medicine and also as an ingredient of a traditional Korean weight-loss tea used as a folk remedy (Du et al., 2012; Jeon et al., 2014; Lee et al., 2014; Maciąg et al., 2021; Shin et al., 2015). As a result, three *Akebia* species have recently received much attention. However, there have been few studies on the comparative antioxidant activities of the three *Akebia* species.

The content of bioactive principles is strongly related to the complex chemical composition in Traditional Chinese Medicine (TCM) varies with the species, geographic origin, environmental conditions, harvesting, and processing of the plant (Liu et al., 2018; Liu et al., 2020; Sun et al., 2020; Wang et al., 2020). At present, the chemical composition of *Akebia* has been studied through classical targeted analyses, especially with traditional Chinese medicine theory (Ling et al., 2015). Several studies showed that the activities of *Akebia* are mainly related to four classes of compounds: triterpenoid saponins, triterpenoids, phenolic acids, and phenylpropanoids, especially triterpenoid saponins (Chen et al., 2019; Gao and Wang, 2006; Iwanaga et al., 2012; Jin et al., 2014; Jin et al., 2014b; Lu et al., 2014; Wang et al., 2014; Xu et al., 2016; Yoshihiro et al., 2007). Previous research has shown that 38 triterpenoid saponins were separated from a 70 % ethanol extract of *A. trifoliata. var* stems and identified using spectroscopic analysis (Gao and Wang, 2006). 30 triterpenoid saponins, 10 triterpenoids, 7 phenylpropanoids, 4 phenolic acids, and 10 other compounds have been identified in the stem of *A. quinata* and A. *trifoliata* (Liu et al., 2020). Gao and colleagues showed that the biological activity of *Akebia* is related to the structure of triterpenoid saponins (Gao and Wang, 2006). *Akebia* contains a variety of triterpenoid saponins belonging to different aglycone types, including oleanolic acid, noroleanolic acid, hederagenin, norhederagenin, arjunolic acid, norajunolic acid, and gypsogenin. The oleanolic acid aglycone is a characteristic *Akebia* chemical constituent (Maciąg et al., 2021). In addition, the sugar chain constituents have been reported, mainly including glucopyranose, arabinopyranose, rhamnopyranosyl, and xylopyranosyl (Ma et al., 2019). As the differences in the chemical composition of *Akebia* are directly related to its quality and its correct clinical use, so it is necessary to establish a rapid and effective analytical method to ensure the effectiveness of *Akebia*. Several studies have performed qualitative and quantitative analyses of three *Akebia* species based on HPLC or LC-MS (Liu et al., 2020; Wei et al., 2015; Zhan et al., 2016; Zhang et al., 2021; Zhang et al., 2014). However, these studies generally only focus on a limited number of compounds. Few reports have focused on the systematic characterization of the differences in the chemical constituents between the three *Akebia* species.

One emerging analytical method of systematic characterization is untargeted metabolomics with unbiased detection and extensive coverage. This relatively recent discipline is becoming increasingly popular, particularly within areas of research such as disease diagnostics, toxicology, and environmental research, because of its holistic property conforming to the "Multi-component & Multi-target" feature of TCM (Danuta et al., 2018; He and Zhou, 2021; Zhou et al., 2021). It has also been used for TCM authentication studies, the easily confusing species, different geographic origins, developmental stages, and the discrimination of sulfur-fumigation and non-sulfur-fumigation TCM materials. For example, by untargeted metabolomics, Wang et al. facilitated a better understanding of flavonoid metabolites between *Citrus reticulata* and four other *Citrus* peels (Wang et al., 2019). Fu et al. analyzed the diversity among *Echinacea* species by non-target metabolomics (Fu et al., 2021). Li et al. investigated the metabolite differences of *Lonicerae japonicae flos* through a non-targeted metabolomics approach (Li et al., 2022). Following these examples, untargeted metabolomic studies provide information on thousands of compounds found in the samples, discovering compounds that show a significant trend across different sample groups. Following further research and larger studies, identifying just one of these compounds could serve as a marker to aid in distinguishing the three *Akebia* species.

This study aims to show how an untargeted metabolomics technique using UPLC-Q-Orbitrap/MS can be applied to a distinguishing study to reveal differences in the chemical composition of three *Akebia* species. This type of study can lead to the identification of a compound that has the potential to be used as a biomarker, in this case, to distinguish different medicinal species. Furthermore, the antioxidant activities assay provides evidence for *Akebia's* functional antioxidant, laying the groundwork for its further development and utilization.

2. Materials and methods

2.1. Reagents and materials

Formic acid in MS grade was acquired from Sigma-Aldrich (St. Louis, MO, USA). Chromatographic grade acetonitrile

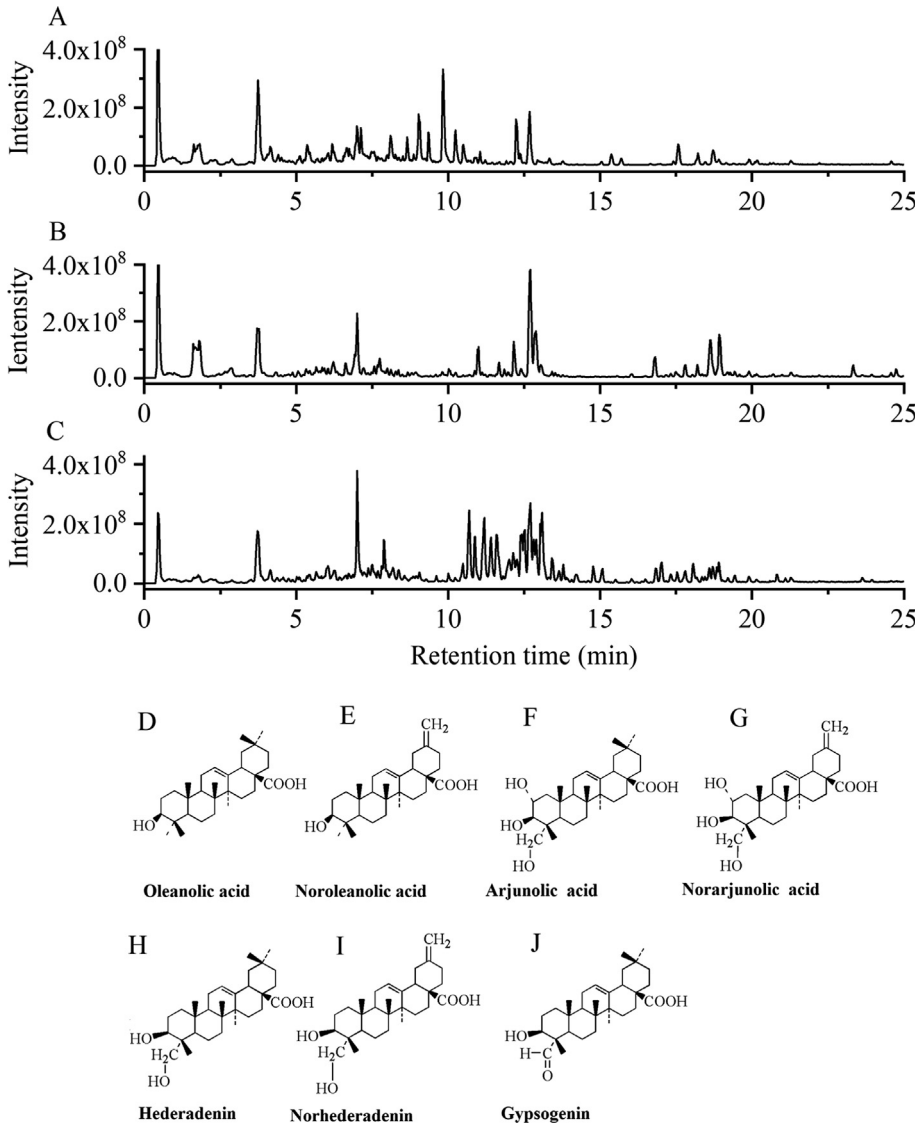

Fig. 1 The representative base peak intensity (BPI) chromatograms and aglycones of triterpene saponins characteristic of three medicinal *Akebia* species. A: *A. trifoliata. var*, B: *A. trifoliata*, and C: *A. quinata*.

and methanol were obtained from Fisher Scientific (Pittsburgh, PA, USA). Distilled water was produced via a Millipore water purification system (Millipore, Billerica, MA, USA). All other chemicals were analytical grade. As standards, calceolarioside B (111910–201604) and chlorogenic acid (110753–202018) were purchased from NIFDC (National Institute of Food and Drug Control, Changchun, Jilin, China). DPPH (R27137) and ABTS (R24146) were obtained from Shanghai Yuanye Biotechnology Co. ltd. (Shanghai, China).

In total, 27 batches of *Akebia* herbal materials, including 9 batches *Akebia quinata* (Thunb.) Decne, 9 batches *Akebia trifoliata* (Thunb.) Koidz and 9 batches *Akebia trifoliata* (Thunb.) Koidz. var. Australis (Diels) Rehd were collected from the primary origin of *Akebia* in China and identified by Prof. Jiyu Gong of Changchun University of Chinese Medicine, School of Pharmaceutical Sciences. The information on samples is shown in Table S1.

2.2. Sample solution preparation

In total, 27 batches of *Akebia* samples solution were prepared. An aliquot of 0.5 g fine powder of each sample was properly weighed and ultrasonically extracted with 25 mL of 70 % (v/v) ethanol/water for 25 min at room temperature. The mixture was filtered using a 0.22 μm membrane filter. The filtrate was transferred into a sample vial for UPLC-Q-Orbitrap/MS analysis.

Besides, 27 batches of *Akebia* samples dry ointment extracts were prepared. First, *Akebia* stem (50 g) was ground into a fine powder, soaked in 1000 mL of 70 % ethanol, and extracted in a reflux condenser at 70 °C for 3 h. Next, the extract was filtered through a testing 150 μm sieve, evaporated on a rotary evaporator, concentrated by lyophilization, and then stored at −20 °C. Then, 1 g extract of each sample was weighed correctly and ultrasonically extracted with 20 mL of 70 % (v/v)

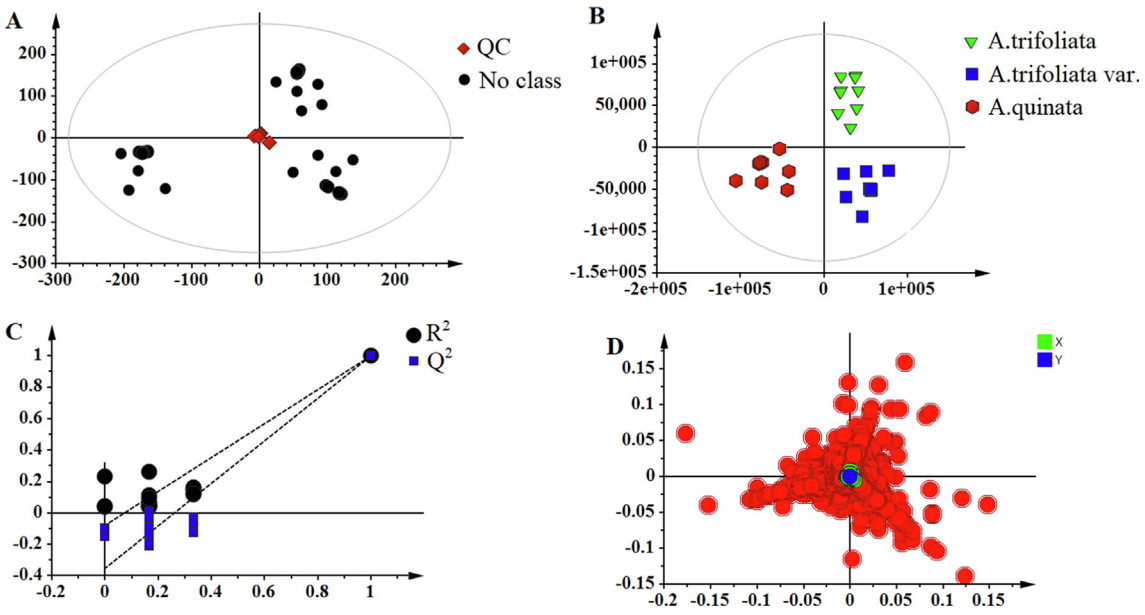

Fig. 2 PCA sore plot (A) of all samples, and PLS-DA score plot (B) of three *Akebia* species and the corresponding permutation test (C) and loading plot (D).

ethanol/water for 20 min at room temperature. Then, the mixture was filtered and diluted according to DPPH and ABTS test systems.

2.3. UPLC-Q-Orbitrap/MS analysis

Chromatographic separation was performed on an Ultimate 3000 ultra-performance liquid chromatography system (Thermo, San Jose, CA, USA) coupled with the Supelco C18 column (3.0 × 50 mm, 2.7 μm; Sigma-Aldrich). The column was maintained at 30 °C. The mobile phases A and B were acetonitrile and water with 0.1 % formic acid, respectively. The separation of experimental samples was programmed with the following gradient elution: 95 % B (0–5 min), 95–60 % B (5–10 min), 60–35 % B (10–20 min), 35–5 % B (20–25 min), 5–95 % B (25–30 min), and maintained at 95 % for 5 min. The injection volume was 5 μL, and the flow rate was 0.4 mL/min.

Mass spectrometric detection was carried out on a Q-Orbitrap-MS/MS (Thermo, San Jose, CA, USA) equipped with an electrospray ionization source under the negative ion mode. The ion source parameters were set to 40 Arb for sheath gas flow, 10 Arb for aux gas flow, and 1 Arb for sweep gas flow. The S-Lens RF was 55 %. The capillary voltage was set to − 3.5 kV with a capillary temperature of 350 °C. Full MS data were acquired at the centroid mode from m/z 150 to 1500 Da with a resolution of 70,000 with the automatic gain control (AGC) target of 1×10^6, and a maximum injection time (IT) of 100 ms. In addition, the tandem mass spectrum was obtained in Full-MS/ddMS2 mode using the following settings: 17,000 for resolution, 1×10^5 for automatic gain control (AGC) for the target, 50 ms for maximum IT, 5 for Loop count, 5 for TopN, 4.0 m/z for Isolation window and 30, 40, 55 for stepped NCE.

2.4. Antioxidant activities

2.4.1. DPPH assay

All *Akebia* samples were tested for DPPH radical scavenging activity according to the manufacturer's protocol with minor modifications. In brief, an aliquot (20 μL) extract from each sample was added to 80 μL of 0.105 $\mu M \cdot mL^{-1}$ DPPH solution to initiate the reaction, and 70 % ethanol was used as the blank solution. After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm. Each measurement was performed in triplicate.

2.4.2. ABTS assay

The ABTS radical scavenging activity of each sample was determined using the manufacturer's protocol with slight modifications. First, the same volume of 7.4 mM ABTS solution and 2.60 mM $K_2S_2O_8$ were mixed and kept at room temperature for 12 h under dark conditions to prepare the stock solution. Before use, the ABTS working solution was obtained by adding 70 % ethanol to the stock solution until the absorbance reached 0.70 at 734 nm. Then, 10 μL of extract of each sample solution was mixed with the 90 μL of ABTS working solution and was kept at ambient temperature for 10 min. Finally, the absorbance at 734 nm against the blank sample (70 % ethanol) was measured and recorded. All measurements were done in triplicate.

Table 1 Compounds identified from three *Akebia* species by UPLC-Q-Orbitrap/MS.

Compound	RT (min)	Identity	Formula	Detected m/z	Adducts	Mass Error (ppm)	MS/MS Fragment ions	Type	Ref
1	2.08	5-caffeoylquinic acid	C16H18O9	353.0875	$[M-H]^-$	2.27	270.1496, 191.0557, 179.0344, 161.0238	Phenolic acid	(Liu et al., 2020)
2	3.76	3-caffeoylquinic acid	C16H18O9	353.0873	$[M-H]^-$	1.70	278.0404, 191.0557, 173.0449, 161.0236	Phenolic acid	(Liu et al., 2020)
3	4.15	4-caffeoylquinic acid	C16H18O9	353.0874	$[M-H]^-$	1.98	278.9987, 191.0557, 179.0346, 173.0452, 161.0233	Phenolic acid	(Liu et al., 2020)
4	4.28	syringin	C17H24O9	371.1336	$[M-H]^-$	0.00	353.0781, 315.1113, 211.0453, 173.0449	Phenylpropanoid	(Liu et al., 2020)
5	5.10	akeqintoside B	C26H32O11	519.1859	$[M-H]^-$	−0.39	475.1239, 438.8632, 415.1505, 373.1384, 341.0656	Lignan glycoside	(Iwanaga et al., 2012)
6	5.53	2-(3,4-dihydroxyphenyl)-ethyl-O-β-D-glucopyranoside	C14H20O8	315.1086	$[M-H]^-$	3.81	258.0984, 247.3074, 195.0291, 179.0556	Lignan glycoside	(Maciąg et al., 2021; Yen et al., 2014)
7	6.26	quinatoside A	C34H52O7	571.3620	$[M-H]^-$	−1.58	537.1602, 455.3164, 395.0985, 359.1502, 323.0773	Norhederadenin	(Gao and Wang, 2006)
8	7.01	calceolarioside B	C23H26O11	477.1391	$[M-H]^-$	0.00	414.9501, 315.1091, 281.0674, 251.0554, 221.0458	Phenylpropanoid	(Maciąg et al., 2021; Yen et al., 2014)
9	7.37	1,3-Dicaffeoylquinic acid	C25H24O12	515.1180	$[M-H]^-$	−0.78	477.1339, 353.0856, 335.0762, 315.1083, 281.0667, 225.0764	Phenolic acid	(Liu et al., 2020)
10	7.86	3,5-Dicaffeoylquinic acid	C25H24O12	515.1179	$[M-H]^-$	−0.97	477.1379, 353.0883, 315.1089, 265.0729, 223.0603	Phenolic acid	(Liu et al., 2020)
11	7.87	aradecoside D	C59H96O27	1281.6073	$[M + HCOO]^-$	−2.89	1249.5806, 941.4784, 897.4841, 765.4432, 669.2201, 589.3745, 455.3524	Oleanolic acid	(Gao and Wang, 2006)
12	8.63	akemisaponin B	C52H82O23	1119.5188	$[M + HCOO]^-$	−2.59	1046.7434, 973.4631, 865.4536, 781.4393, 649.3872, 535.1987, 471.3122	Norarjunolic acid	(Iwanaga et al., 2012)
13	8.81	akeqintoside C	C25H30O13	537.1641	$[M-H]^-$	7.07	485.2163, 351.1101, 273.5634, 207.0655, 175.0392	Lignan glycoside	(Gao and Wang, 2006)
14	9.27	dipsacoside B	C53H86O22	1119.5583	$[M + HCOO]^-$	0.18	1073.5428, 945.5416, 749.4471, 683.2810, 637.4318, 471.3472	Hederadenin	(Jiang et al., 2008; Liu et al., 2018)
15	9.60	akeboside Stj	C65H106O30	1365.6607	$[M-H]^-$	−5.71	1251.6073, 1087.5337, 957.5073, 781.4371, 649.3947, 569.3472, 455.3524	Oleanolic acid	(Gao and Wang, 2006)
16	9.96	akemisaponin C	C53H86O23	1135.5505	$[M + HCOO]^-$	−2.20	997.4979, 913.4703, 701.3907, 601.1979, 487.3424, 471.3116, 469.1591	Arjunolic acid	(Iwanaga et al., 2012)
17	9.99	saponin PJ1	C53H86O23	1089.5479	$[M-H]^-$	0.28	957.5103, 811.2541, 649.3956, 487.3425	Arjunolic acid	(Maciąg et al., 2021;

(*continued on next page*)

Table 1 (*continued*)

Compound	RT (min)	Identity	Formula	Detected m/z	Adducts	Mass Error (ppm)	MS/MS Fragment ions	Type	Ref
									Yen et al., 2014)
18	10.42	saponin F	C53H86O23	1089.5543	$[M-H]^-$	7.62	1064.4519, 651.3111, 541.9780, 528.9768, 471.3471	Hederadenin	(Maciąg et al., 2021; Yen et al., 2014)
19	10.88	asiaticoside	C48H78O19	1003.5083	$[M + HCOO]^-$	−2.49	927.4586, 765.4420, 651.3297, 585.3799, 487.3435	Arjunolic acid	(Gao and Wang, 2006)
20	11.42	akemisaponin D	C58H92O27	1265.5722	$[M + HCOO]^-$	−5.93	1219.6104, 1103.5272, 941.4739, 893.4537, 749.4479, 603.3901, 455.3165	Norhederadenin	(Iwanaga et al., 2012)
21	11.75	Gypsogenin-3-O-β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-28-O-β-D-glucopyranosyl-(1 → 6)-β–D-glucopyranoside	C52H82O22	1057.5234	$[M-H]^-$	1.89	1103.5329, 955.4643, 733.4183, 601.1994, 469.3289	Gypsogenin	(Ma et al., 2019)
22	11.88	saponin PJ3	C59H96O25	1203.6098	$[M-H]^-$	−4.82	1057.5098, 939.4621, 881.4887, 733.4522, 587.3971, 469.1553, 455.3511	Oleanolic acid	(Maciąg et al., 2021; Yen et al., 2014)
23	12.59	Oleanolic acid-3-O-arabinopyranosyl-28-O-glucopyranosyl-glucopyranosyl-rhamnopyranosyl-arabinopyranosyl	C58H94O25	1189.5972	$[M-H]^-$	−2.44	1023.6034, 895.5052, 719.4357, 601.1978, 587.3971, 469.1593, 455.3534	Oleanolic acid	(Ma et al., 2019)
24	12.65	saponin H3	C65H106O31	1381.6559	$[M-H]^-$	−5.43	1281.6471, 1103.5633, 911.4983, 749.4584, 651.3703, 603.4025, 471.3472	Hederadenin	(Maciąg et al., 2021; Yen et al., 2014)
25	12.75	arjunolic acid-3-O-arabinopyranosyl-28-O-glucopyranosyl-glucopyranosyl-rhamnopyranosyl-arabinopyranosyl	C58H93O27	1220.5737	$[M-H]^-$	−6.80	1103.5208, 997.5363, 895.4691, 833.4669, 619.3912, 601.1978, 469.1594	Arjunolic acid	(Ma et al., 2019)
26	12.98	akeboside Sth	C59H96O26	1265.6248	$[M + HCOO]^-$	6.95	1219.6103, 1103.5271, 749.4501, 603.3958, 471.3466	Hederadenin	(Gao and Wang, 2006)
27	13.39	akebiaoside K	C53H84O23	1087.5288	$[M-H]^-$	−2.85	1041.4906, 925.4789, 865.4933, 763.4215, 601.1992, 485.3255, 469.1550	Noroleanolic acid	(Gao and Wang, 2006)
28	13.43	saponin G	C65H106O31	1381.6588	$[M-H]^-$	−3.33	1219.5756, 1107.5917, 925.4725, 785.4073, 733.4513, 701.4271, 587.3948, 471.3475	Hederadenin	(Maciąg et al., 2021; Yen et al., 2014)
29	13.65	saponin PH	C47H74O19	987.5175	$[M + HCOO]^-$	38.48	987.4832, 795.4154, 471.3122, 469.1589,323.0854	Norarjunolic acid	(Maciąg et al., 2021; Yen et al.,

Table 1 (*continued*)

Compound	RT (min)	Identity	Formula	Detected m/z	Adducts	Mass Error (ppm)	MS/MS Fragment ions	Type	Ref
									2014)
30	13.9	Arjunolic acid-3-O-arabinopyranosyl-rhamnopyranosyl-28-O-glucopyranosyl-glucopyranosyl	C53H86O23	1089.5487	$[M-H]^-$	1.01	1135.5538, 765.4464, 619.3847, 471.3116, 469.1594	Arjunolic acid	(Ma et al., 2019)
31	14.76	hederagenin-3-O-arabinopyranosyl-rhamnopyranosyl-28-O-glucopyranosyl-rhamnopyranosyl	C53H86O21	1051.5184	$[M-H]^-$	−5,710.61	1103.5289, 749.4449, 603.2031, 471.3472	Hederadenin	(Ma et al., 2019)
32	15.05	akemisaponin F	C52H80O23	1071.5221	$[M-H]^-$	20.07	1117.5111, 601.1976, 469.2952	Noroleanolic acid	(Iwanaga et al., 2012; Jin et al., 2014)
33	15.36	30-norarjunolic acid	C29H44O5	471.3099	$[M-H]^-$	−1.27	943.6324, 471.3122, 323.1008, 195.1037	Norarjunolic acid	(Gao and Wang, 2006)
34	15.51	akeqintoside A	C25H32O11	507.1860	$[M-H]^-$	−0.20	471.3121, 313.0723, 293.2125, 275.1922	Lignan glycoside	(Iwanaga et al., 2012)
35	15.79	saponin PJ2	C53H86O22	1073.553	$[M-H]^-$	0.28	1119.5632, 927.4979, 603.3961, 471.3475	Hederadenin	(Maciąg et al., 2021; Yen et al., 2014)
36	16.34	hederagenin-28-O-glucopyranosyl-glucopyranosyl-rhamnopyranosyl	C48H78O18	941.5077	$[M-H]^-$	−2.87	471.3475, 323.1014	Hederadenin	(Ma et al., 2019)
37	16.44	hederagenin-3-O-β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-28-O-β-D-glucopyranoide	C46H74O17	897.4799	$[M-H]^-$	−4.79	735.4333, 603.3907, 471.3467	Hederadenin	(Ma et al., 2019)
38	16.73	saponin E	C52H84O22	1053.5331	$[M-H]^-$	−5,666.53	897.9884, 735.43405, 603.39156, 471.3472	Hederadenin	(Maciąg et al., 2021; Yen et al., 2014)
39	17.01	trifoside A	C46H72O18	911.4607	$[M-H]^-$	−2.96	865.4600, 777.4048, 733.4250, 585.3850, 529.1929, 471.3470, 455.3164	Norhederadenin	(Gao and Wang, 2006)
40	17.50	saponin D	C47H76O18	927.4922	$[M-H]^-$	−2.70	603.3914, 471.3475	Hederadenin	(Maciąg et al., 2021; Yen et al., 2014)
41	17.58	arjunolic acid	C30H48O5	487.3417	$[M-H]^-$	−0.21	471.3116, 469.1594, 455.3165, 323.0864	Arjunolic acid	(Maciąg et al., 2021; Yen et al., 2014)
42	17.73	saponin PG	C46H74O16	881.4910	$[M-H]^-$	1.93	749.4484, 603.3908, 471.3472	Hederadenin	(Maciąg et al., 2021; Yen et al.,

(*continued on next page*)

Table 1 (*continued*)

Compound	RT (min)	Identity	Formula	Detected m/z	Adducts	Mass Error (ppm)	MS/MS Fragment ions	Type	Ref
									2014)
43	17.89	hederagenin-28-O-glucopyranosyl-glucopyranosyl-rhamnopyranosyl-arabinopyranosyl	C53H86O22	1073.5488	$[M-H]^-$	−3.63	1027.5428, 939.4994, 779.4211, 717.4209, 667.3887, 601.1998, 471.3479	Hederadenin	(Ma et al., 2019)
44	18.01	saponin PD	C41H66O12	795.4506	$[M + HCOO]^-$	−2.39	779.4297, 749.4468, 677.4963, 603.3895, 471.3492	Hederadenin	(Maciąg et al., 2021; Yen et al., 2014)
45	18.05	saponin PE	C41H66O12	749.4466	$[M-H]^-$	−0.53	749.4466, 587.3956, 455.3523	Oleanolic acid	(Maciąg et al., 2021; Yen et al., 2014)
46	18.36	akeboside Stc	C41H66O12	749.4397	$[M-H]^-$	−9.74	651.4957, 595.7773, 471.3456	Hederadenin	(Gao and Wang, 2006)
47	18.41	quinatoside B	C40H62O13	749.4057	$[M-H]^-$	−6.54	651.4952, 613,8746, 455.3165, 423.3266, 314.4899	Norhederadenin	(Gao and Wang, 2006)
48	18.42	gypsogenin-3-O-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside	C41H64O12	747.4297	$[M-H]^-$	−2.27	677.4975, 651.5189, 603.3768, 515.0312, 487.3383, 469.3289	Gypsogenin	(Ma et al., 2019)
49	18.50	akeboside Stb	C35H56O8	603.3845	$[M-H]^-$	−7.62	527.2416, 471.3472, 441.2963	Hederadenin	(Gao and Wang, 2006)
50	18.52	saponin A	C35H56O8	603.3895	$[M-H]^-$	0.66	1207.7914, 649.3957, 471.3473	Hederadenin	(Maciąg et al., 2021; Yen et al., 2014)
51	18.54	2α,23-dihydroxy-3β-sulfoxylean-12-en-28-oic acid-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl	C36H58O10	649.3932	$[M-H]^-$	−2.16	603.3878, 487.3427, 471.3116, 469.1594	Arjunolic acid	(Ma et al., 2019)
52	18.76	norhederagenin-3-O-arabinopyranosyl-rhamnopyranosyl-glucopyranosyl-28-oic acid	C47H76O16	895.4995	$[M-H]^-$	−6.03	733.4113, 587.4077, 455.3154	Norhederadenin	(Ma et al., 2019)
53	18.85	trifoside C	C46H72O17	895.4661	$[M-H]^-$	−2.68	733.4543, 651.5479, 587.3938, 501.3192, 455.3153	Norhederadenin	(Gao and Wang, 2006)
54	19.17	CP3	C46H74O15	865.4920	$[M-H]^-$	−2.66	733.4533, 587.3971, 501.3192, 455.3522	Oleanolic acid	(Ma et al., 2019)
55	19.20	trifoside B	C45H70O16	865.4521	$[M-H]^-$	−6.82	733.4504, 651.5241, 587.3935, 551.3763, 455.3135	Norhederadenin	(Gao and Wang, 2006)
56	19.34	oleanolic acid-3-O-arabinopyranosyl-glucopyranosyl-glucopyranosyl-28-oic acid	C47H76O17	911.4976	$[M-H]^-$	−2.41	651.4787, 588.8561, 603.3897, 455.3537	Oleanolic acid	(Ma et al., 2019)
57	19.58	2α,3β-dihydroxy-23-oxo-olean-12-en-28-oic acid	C30H46O5	485.3252	$[M-H]^-$	−1.85	455.3165, 414.9921, 339.2006	Noroleanolic acid	(Ma et al., 2019)
58	19.71	saponin C	C41H66O13	765.4414	$[M-H]^-$	−0.65	651.4847, 603.3915, 471.3472	Hederadenin	(Maciąg et al., 2021)

Table 1 (*continued*)

Compound	RT (min)	Identity	Formula	Detected m/z	Adducts	Mass Error (ppm)	MS/MS Fragment ions	Type	Ref
59	19.85	gypsogenin- 3-O-β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranoside	C40H62O12	733.4461	$[M-H]^-$	41.38	779.4216, 601.3729, 469.3246	Gypsogenin	(Ma et al., 2019)
60	19.91	saponin B	C40H64O12	733.4167	$[M-H]^-$	1.36	651.5366, 545.2958, 504.3094, 471.3476	Hederadenin	(Maciąg et al., 2021; Yen et al., 2014)
61	21.07	hederagenin	C30H48O4	471.3452	$[M-H]^-$	−3.39	471.3472, 425.3421, 217.1206, 162.8501	Hederadenin	(Maciąg et al., 2021)
62	22.42	quinatoside C	C40H64O11	719.4259	$[M-H]^-$	−14.59	587.3971, 455.3167	Norhederadenin	(Gao and Wang, 2006)
63	24.62	quinatoside D	C39H60O11	703.4047	$[M-H]^-$	−0.57	677.4915, 651.2538, 542.0229, 453.3168	Norhederadenin	(Gao and Wang, 2006)
64	22.67	gypsogenin	C30H46O4	469.3324	$[M-H]^-$	2.56	439.1219, 409.2361, 339.1991, 313.0732, 279.2335	Gypsogenin	(Lee et al., 2017; Maciąg et al., 2021)
65	24.01	saponin PB	C41H66O11	733.4553	$[M-H]^-$	4.36	587.3971, 455.3524	Oleanolic acid	(Maciąg et al., 2021; Yen et al., 2014)

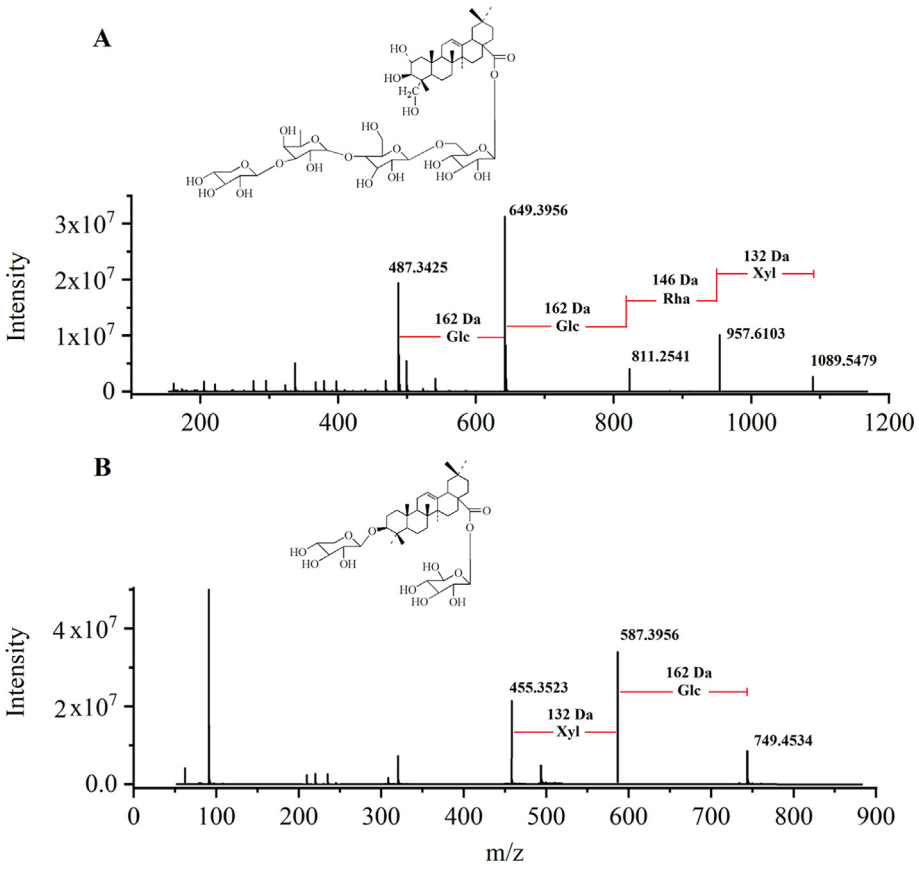

Fig. 3 The fragmentation pathways of saponin P_{J1} (A) and saponin P_E (B) in negative ion mode.

2.5. Data processing and multivariate statistical analysis

After data acquisition, the raw data of *Akebia* samples were processed by Sieve software (version 2.1, Thermo, San Jose, CA, USA) to perform the peak extraction, alignment, and normalization. The main parameters for the raw data preprocessing were as follows: retention time range (0–25 min), mass range (100–1500 Da), mass tolerance (5 mDa), intensity threshold (2000 counts), mass window (0.15 Da), and retention time window (0.10 min). Then a data set containing the sample code, peak retention time (RT)_*m*/*z* value, and peak intensity was constructed for the statistical analysis. Multivariate statistical analysis was performed using Simca 14.1 software (Umetrics, Malmö, Sweden) to find the marker compounds contributing to the difference among three *Akebia* species. The principal component analysis (PCA) model was established first to perform pattern recognition and obtain an overview of sample classification, followed by the partial least-squares discriminant analysis (PLS-DA) model to obtain the most significant separation among groups. A permutation test was performed to provide reference distributions of the R^2 and Q^2 values that could show the fitness of the established PLS-DA model. The variable importance for the projection (VIP) value was used to screen the marker compounds. Then the ANOVA was used to further assess the significant differences of these compounds among groups using SPSS 21.0 (Chicago, IL, USA). The compounds with VIP $>$ 1 and $P < 0.05$ were considered significant and were selected as marker compounds. GraphPad Prism 8.0 software (GraphPad Prism Software, San Diego, CA) was used for IC_{50} analysis and mapping.

3. Results

*3.1. Metabolomic profiles of three medicinal **Akebia** species based on UPLC-Q-Orbitrap/MS*

The metabolites of three *Akebia* species were profiled with the UPLC-Q-Orbitrap/MS system. The typical total ion current chromatograms of the three *Akebia* species were analyzed in negative ion mode, as shown in Fig. 1. Several peak differences can be observed in the samples, indicating that the chemical composition significantly differs among the three *Akebia* species. An unsupervised PCA was conducted to obtain a clear overview of the different metabolic profiles among *Akebia* samples. The PCA score plot in Fig. 2**A** shows that the QC sample was tightly clustered in the center of all samples, reflecting the stability and reliability of the metabolomics analysis. The samples were separated into three groups in the PCA score plot, suggesting the chemical composition diversity in three *Akebia* species. To investigate the chemome diversity of three *Akebia* species, the supervised PLS-DA model produced analogous results to examine the metabolites with the most significant. As a result, complete separation among the *A. quinata, A. trifoliata* and *A. trifoliata. var* groups was accomplished

in the score plot (Fig. 2**B**). Furthermore, R^2X, R^2Y, and Q^2 were calculated as 0.956, 0.975, and 0.984 respectively. The permutation test (n = 200) was performed to further validate the constructed model, and the permutated R^2 and Q^2 observed in Fig. 2**C** were lower than the original values, which indicates that no overfitting was observed established model.

3.2. Metabolomic profiles selection and identification

Next, to further evaluate metabolomic profiles in three *Akebia* species, the loading plot and VIP value were used to select the marker compounds. The features with a VIP value > 1 were highlighted as marker candidates and marked in Fig. 2**D**. A total of 235 ions were found displaying VIP > 1. To screen the different major components, in the current work, the ANOVA was performed to evaluate the change significance of these features, and the compounds with the statistically significant difference ($P < 0.05$) were finally selected as marker compounds. A total of 65 different abundant metabolites, including 5 phenolic acids, 2 phenylpropanoids, 4 lignan glycosides, and 54 triterpenoid saponins, were identified in negative ion mode and were listed in Table 1. The compound annotation was conducted by searching PubMed and Chinese National Knowledge Infrastructure (CNKI) databases. We compared the MS and MS/MS information of detected compounds using the database, literature records, and standard references.

The detailed annotation procedures were demonstrated by taking the ion of saponin P_{J1} at RT 9.99 min_m/z 1089.5479 as an example. The tandem MS spectrum is shown in Fig. 3**A**. The ions at m/z 957.5103, 811.2541, 649.3956 and 487.3425 were generated by $[M\text{-Xyl-H}]^-$, $[M\text{-Xyl-Rha-H}]^-$, $[M\text{-Xyl-Rha-Glc-H}]^-$, $[M\text{-Xyl-Rha-2(Glc)-H}]^-$, respectively. According to the ion of m/z 487.3425 $[M-H]^-$ corresponding to arjunolic and comparing with the literature, this ion at RT

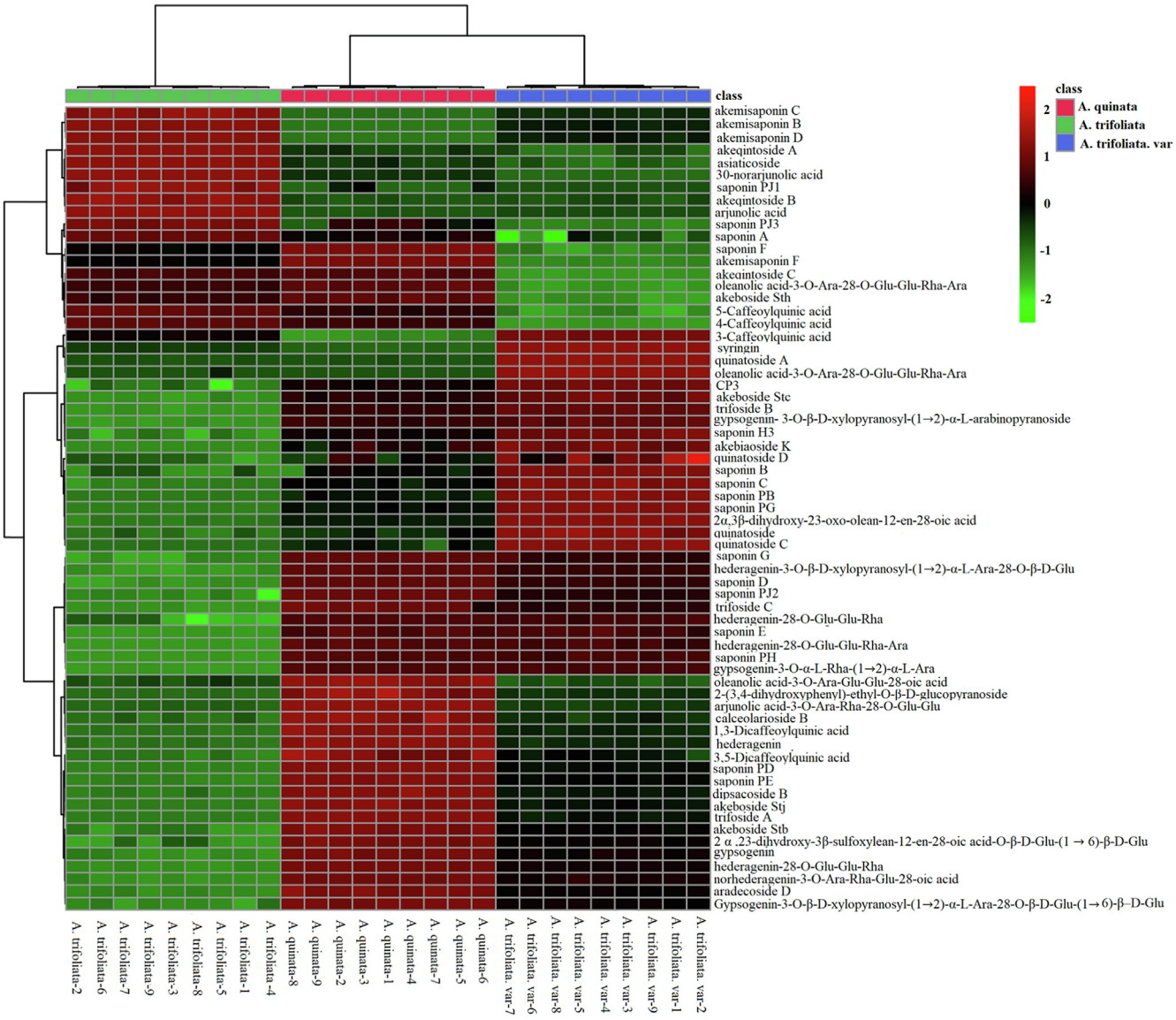

Fig. 4 Heat map visualizing the changes in the intensities of marker compounds.

9.99 min_m/z 1089.5479 was collectively assigned to saponin P_{J1}.

To analyze, saponin P_E was used as an example of bidesmosidic saponin. The precursor ion at RT 18.05 min_m/z 749.4534 $[M-H]^-$ was discovered, and the formula was calculated to be $C_{41}H_{66}O_{12}$. The tandem MS spectrum was shown in Fig. 3**B** by the diagnostic ion at m/z 455.3523 [aglycone-H]$^-$, saponin P_E was attributed to the oleanolic type. The fragment ion at m/z 587.3956 $[M-Glc-H]^-$ was produced by the complete loss of 162 Da from the precursor ion. First, the signal was powered, which result indicated that the sugar chain of Glc was attached at C-28. Then, by a Xyl (132 Da) in turn, the fragment ions at m/z 455.3523 were produced, which indicated the branched sugar chain unit was attached at C-3. Compared with the literature, this ion at RT 18.05 min_m/z 749.4534 was assigned to saponin P_E.

3.3. Characteristics of three Akebia species

A heat map based on the mean values of the 65 different abundant metabolites provided a comprehensive overview of the differences in the metabolite contents among three *Akebia* species, as shown in Fig. 4**.** Accordingly, significant differences in the contents of phenolic acids, phenylpropanoids, lignan glycosides, and triterpenoid saponins were observed among three *Akebia* species. We compared characteristic metabolite ion intensity between samples to detect significantly changed metabolites according to the three *Akebia* species. The compounds with VIP > 1 and $P < 0.05$ were retained as different accumulated metabolites.

3.3.1. Phenolic acids

In this study, 5 different phenolic acids were identified by UPLC-Orbitrap-MS in all samples, including 5-caffeoylquinic acid, 4-caffeoylquinic acid, 3-caffeoylquinic acid, 1,3-Dicaffeoylquinic acid, and 3,5-Dicaffeoylquinic acid. Among the detected the three *Akebia* species, phenolic acids were most abundant in *A. trifoliata* (Fig. 5**A**). 5-caffeoylquinic acid, 3-caffeoylquinic acid, and 4-caffeoylquinic acid are the most abundant characteristic metabolites in *A. trifoliata* (Fig. 5**B-D**). Furthermore, 4-caffeoylquinic acid was the most abundant, with significantly higher levels in *A. trifoliata* than in the other two species. In addition, we observed a significant decrease of 1,3-Dicaffeoylquinic acid and 3,5-Dicaffeoylquinic acid ion intensity in *A. trifoliata. var* and *A. trifoliata* (Fig. 5**E-F**).

3.3.2. Phenylpropanoids

The results showed phenylpropanoids as common components of three *Akebia* species. High concentrations of phenyl-

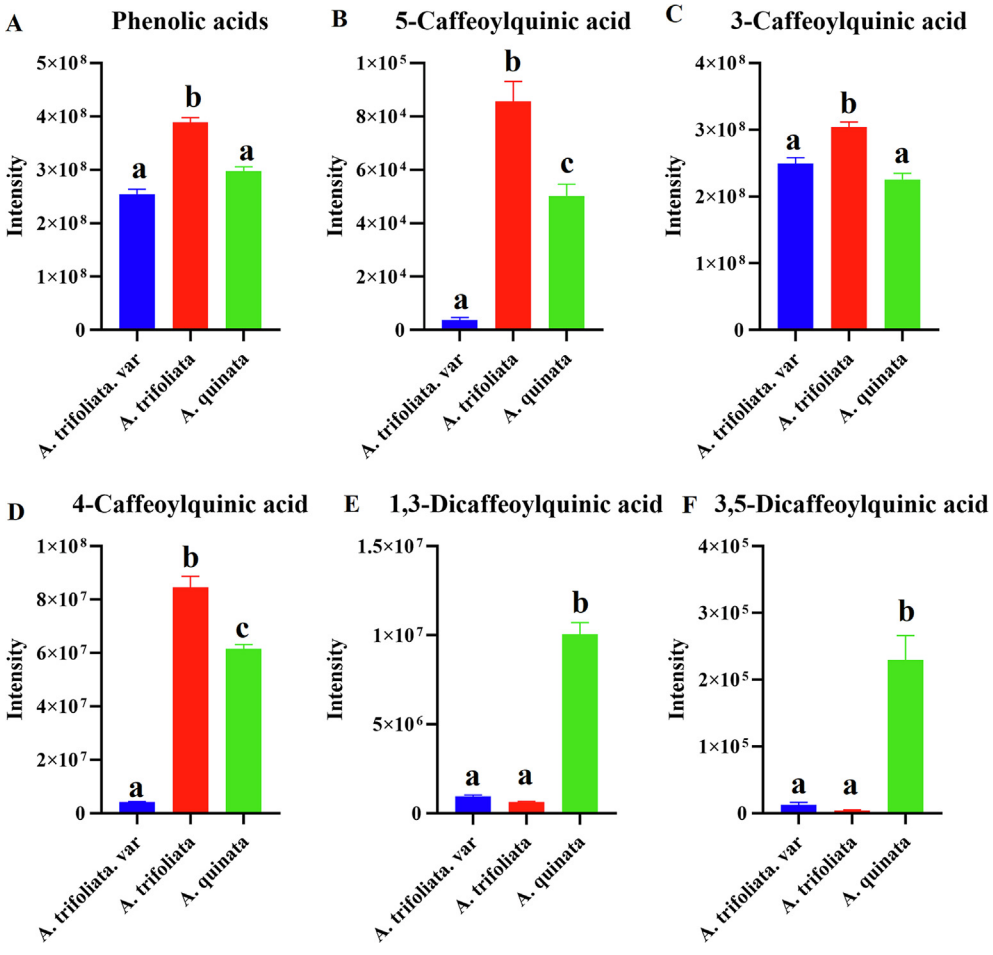

Fig. 5 Comparison of the total ion intensity of phenolic acids of three *Akebia* species (a, b, and c, $p < 0.05$).

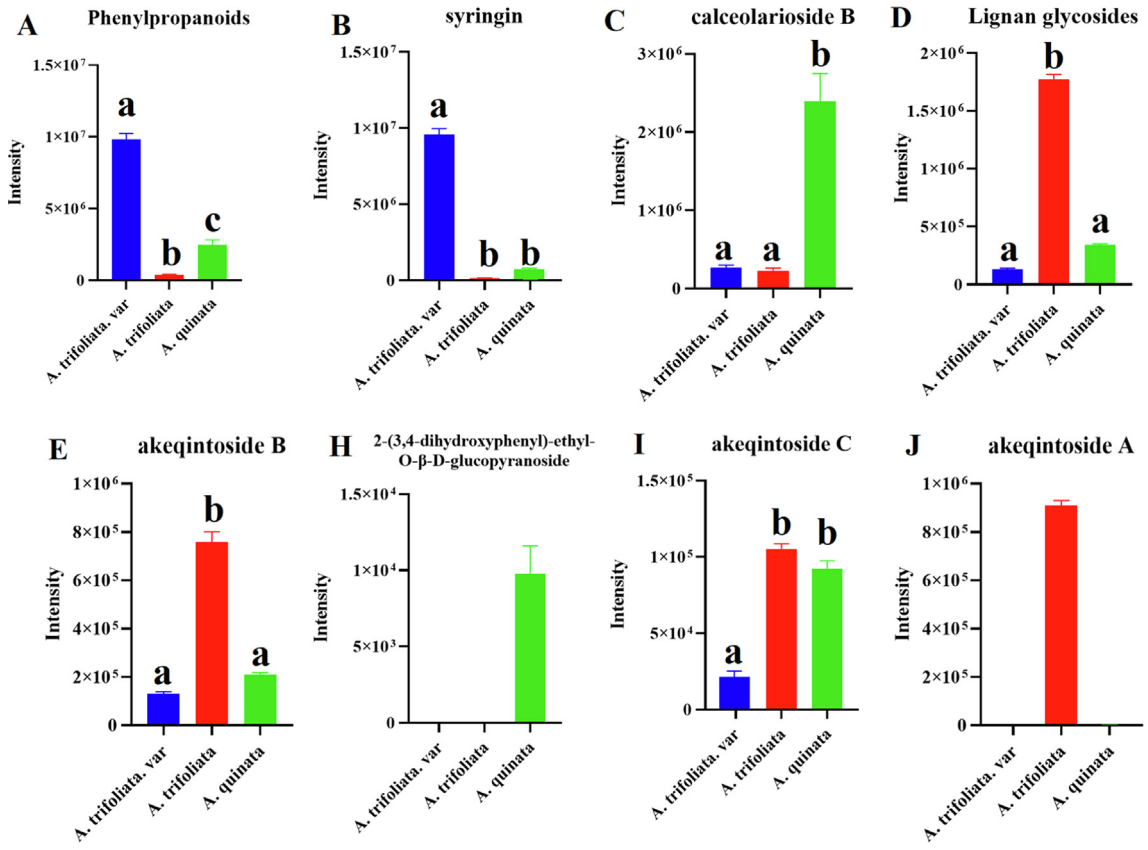

Fig. 6 Comparison of the total ion intensity of phenylpropanoid and lignan glycosides of three *Akebia* species (a, b, and c, $p < 0.05$).

propanoids were confirmed in *A. trifoliata.var* (Fig. 6**A**). The results indicated that syringin was more abundant in *A. trifoliata.var* (Fig. 6**B**). Furthermore, the compound character of the three *Akebia* species, whose concentration is used to standardize that medicinal material according to pharmacopoeial requirements, is calceolarioside B, which was highly concentrated in *A. quinata* (Fig. 6**C**).

3.3.3. Lignan glycosides

Lignan glycosides are essential compounds in *Akebia*. Comparative studies of the lignan glycosides of three *Akebia* species have shown differences in the distributions of individual metabolites, which were slightly increased in *A. trifoliata* (Fig. 6**D-J**). The result revealed that the metabolite 2-(3,4-dihy droxyphenyl)-ethyl-O-β-D-glucop-Yranoside could be used as a marker to distinguish *A. quinata* from the other two species (Fig. 6**H**). Furthermore, the metabolite akeqintoside A was also demonstrated as a marker in *A. trifoliata* (Fig. 6**J**).

3.3.4. Triterpenoid saponins

Most triterpene saponins were identified from three *Akebia* species by UPLC-Q-Orbitrap/MS. The investigation revealed that *Akebia* contains a variety of triterpenoid saponins from various aglycone types, and the composition of triterpenoid saponins differed between the three *Akebia* species. These aglycones were classified into seven groups including oleanolic acid, noroleanolic acid, arjunolic acid, norarjunolic acid, hederagenin, norhederagenin and gypsogenin (Fig. 1**D-J**). *A. quinata* was the richest in oleanolic acid, hederadenin, norhederadenin, and gypsogenin (Fig. 7**A-D**). *A. trifoliata* was rich in arjunolic acid and norarjunolic acid (Fig. 7**E-F**). The noroleanolic acid was significantly more abundant in *A. trifoliata. var* than other two species (Fig. 7**H**).

Comparative studies of the triterpenoid saponins composition of three *Akebia* species, the species *A. trifoliata* and *A. trifoliata var,* were characterized by the presence of five triterpene saponins: akemisaponin B, akemisaponin D, oleanolic acid-3-O-arabinopyranosyl-28-O-glucopyranosyl-glucopyranosyl-rha mnopyranosyl-arabinopyranosyl, akemisaponin C, and saponin P_{j1} can be treated as markers distinguishing the *A. quinata* (Fig. 8). Finally, the aglycones composition of *A. trifoliata* and *A. trifoliata. var* is more similar to *A. quinata.*

3.4. Antioxidant activities of three Akebia species

Before the assay, the dry ointment yield of extracts was obtained. The antioxidant inhibitory concentration $(IC)_{50}$ values of 27 extracts were evaluated by DPPH and ABTS assays. These results are listed in Table 2. *A. trifoliata* and *A. trifoliata. var* showed 2–3 times higher antioxidant activity than *A. quinata*. DPPH free radical scavenging IC_{50} values of *A. quinata* varied from 8.99 to 11.21 mg·mL^{-1}. *A. trifoliata* and *A. trifoliata. var* showed a considerable and stable ability to scavenge the DPPH free radical (5.45–6.97 mg·mL^{-1} and 5.36–6.87 mg ·mL^{-1}, respectively), are shown in Fig. 9**A.** ABTS free radical scavenging IC_{50} values of *A. quinata* varied from 5.56 to 7.54 mg·mL^{-1}. The IC_{50} values of *A. trifoliata* and *A. trifoliata. var* were 2.28–3.22 mg·mL^{-1} and 2.09–3.22 mg·mL^{-1},

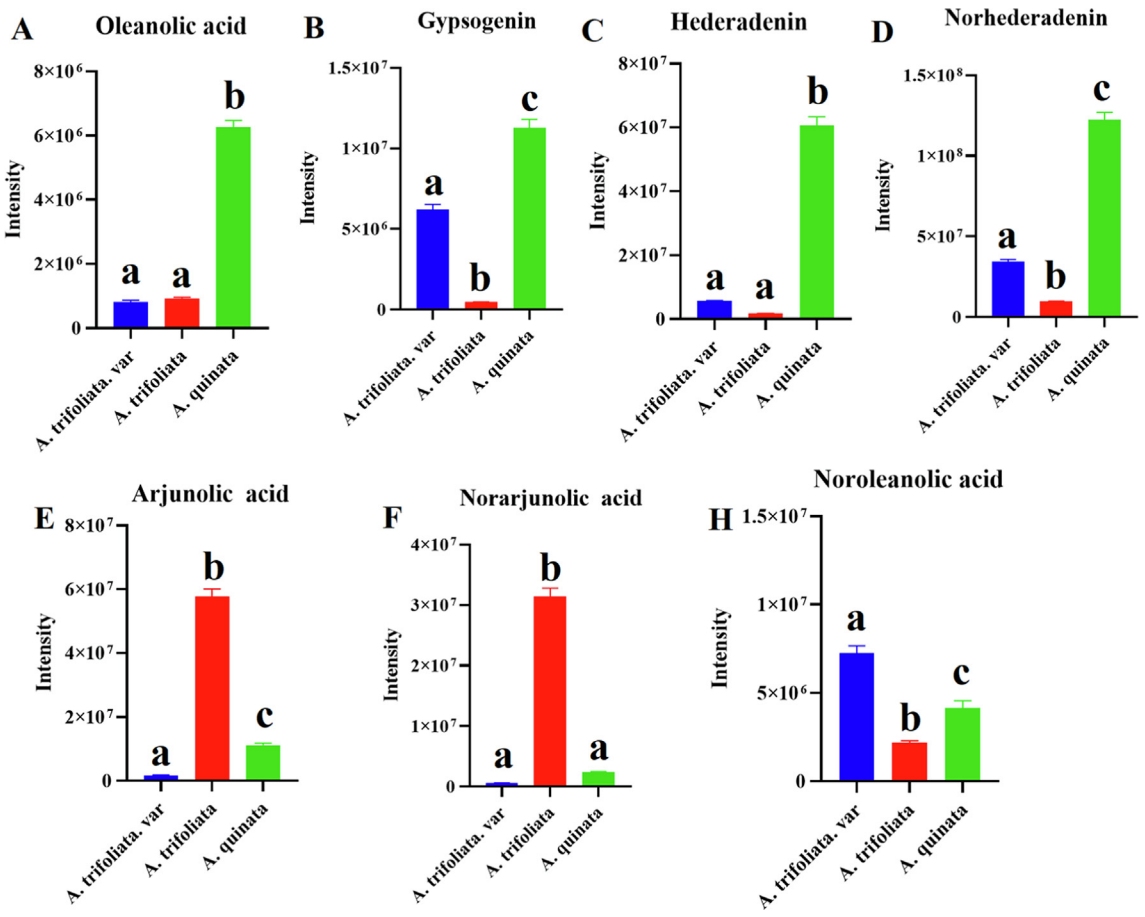

Fig. 7 Comparison of the total ion intensity of triterpenoid saponins of three *Akebia* species (a, b, and c, $p < 0.05$).

respectively (Fig. 9**B**). The antioxidant activities results indicated that the antioxidants of three *Akebia* species were the same in different antioxidative test systems and the antioxidant activities of *A. trifoliata* and *A. trifoliata. var* was stronger than *A. quinata*.

4. Discussion

In this study, an untargeted UPLC-Q-Orbitrap/MS approach combined with multivariate analysis was used to investigate the metabolite profile of three *Akebia* species. In total, 65 different metabolites were identified in *A. quinata*, *A. trifoliata* and *A. trifoliata. var*, including 5 phenolic acids, 2 phenylpropanoids, 4 lignan glycosides, and 54 triterpenoid saponins. Among these, the contents of phenolic acids and lignan glycosides were significantly higher in *A. trifoliata* than in *A. quinata* and *A. trifoliata. var*. The results showed that the phenylethanoids are common components of three *Akebia* species, and high concentrations of phenylpropanoids were confirmed in *A. trifoliata.var*. The composition of triterpenoid saponins is different. Seven aglycones groups, including oleanolic acid, noroleanolic acid, arjunolic acid, norarjunolic acid, hederagenin, norhederagenin and gypsogenin were investigated in three *Akebia* species. The aglycones composition of *A. trifoliata* and *A. trifoliata. var* is more similar to *A. quinata*. Scientific research has proven significant differences in the composition of these compounds in the stems of different species, not only between *A. quinata* and *A. trifoliata* but also in the *A. trifoliata. var* (Maciąg et al., 2021; Yen et al., 2014). Acidic aglycones are characteristic of the species *A. trifoliata*. In contrast, noroleanane derivatives have been isolated only from *A. quinata*. 2α-hydroxy groups are derivatives of arjunolic acid and norarjunolic (Jiang et al., 2008; Liu et al., 2018).

In addition, the phenolic glycoside 2-(3,4-dihydroxyphenyl)-ethyl-O-β-D-*gluco*-pyranoside has been found only in *A. quinata*, whereas the triterpenoid saponins akemisaponin B, akemisaponin D, oleanolic acid-3-O-arabinopyranosyl-28-O-*gluco*-pyranosyl-glucopyranosyl-rhamnopyranosyl-arabinopyranosyl, akemisaponin C and saponin P_{j1} have been found in *A. trifoliata* and *A. trifoliata. var*. Accordingly, these six compounds can be regarded as marker compounds distinguishing three *Akebia* species.

Traditionally, the three *Akebia* species have been used only based on TCM. TCM states that the therapeutic effects of *Akebia* raw material include diuretic, anti-inflammatory, analgesic, cardiostimulatory, antibacterial, and antioxidant activities (Bian et al., 2021; Lee et al., 2017; Maciąg et al., 2021; Peng et al., 2020; Song et al., 2018; Sung et al., 2015; Wang et al., 2015). However, *Akebia* has important cosmetological properties, including antiaging, antioxidant, and moisturizing properties. Modern scientific research confirms the utility of *Akebia* extracts in cosmetics (Du et al., 2012; Jeon et al.,

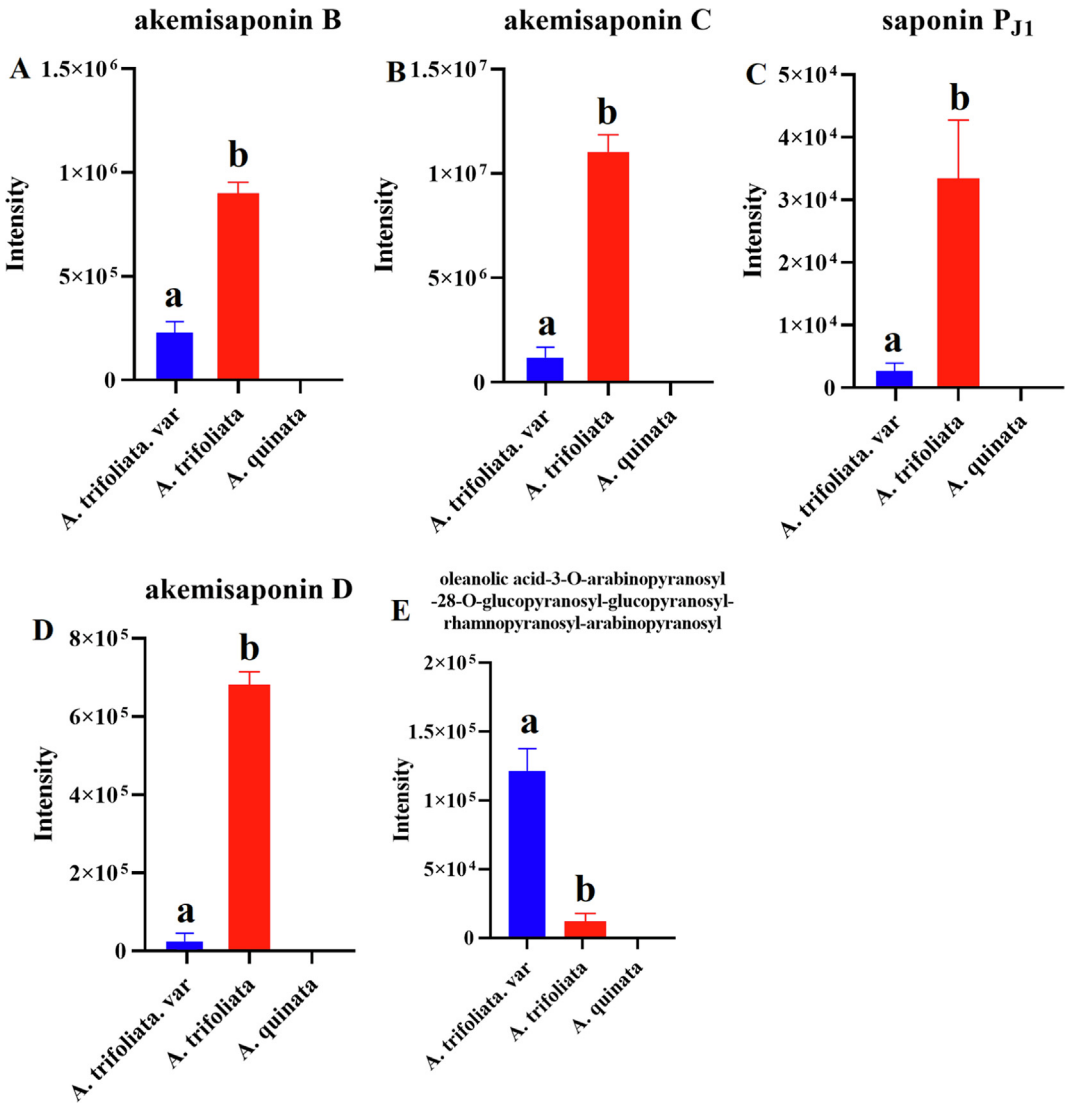

Fig. 8 The characteristic triterpenoid saponins composition of three *Akebia* species (a and b, $p < 0.05$).

2014; Lee et al., 2014; Maciąg et al., 2021; Shin et al., 2015). Recently, cosmetics companies have become particularly interested in the valuable antioxidant biological properties of *Akebia*, introducing stem extracts into the production of various preparations. *Akebia* species are described in the CosIng database. According to the CosIng database, the forms in which they are approved for cosmetic use and their potential effects have been summarized. The companies that produce cosmetics from *A. quinata* are mainly Korean, Japanese, Italian and American, including Kleladarm, Pola, Neogen, Beauty of Joseon, Hello Products, Missha, Sum37°, the Face Shop, Kose, Rosette, Sioris, Bonajour, etc. These companies use *A. quinata* fruit extract and *A. quinata* stem extract in their products. In addition, the companies Decorte and Muji produce cosmetics containing *A. trifoliata* stem extract. (Du et al., 2012; Jeon et al., 2014; Lee et al., 2014; Maciąg et al., 2021; Shin et al., 2015). These applications are closely related to antioxidant effects.

DPPH and ABTS tests were used to evaluate antioxidant activity in order to better demonstrate the antioxidant properties of each species. The free radical scavenging activity in the DPPH and ABTS tests for the *A. quinata* extract at IC_{50} values range 8.99–11.21 mg·mL^{-1} and 5.56–7.54 mg·mL^{-1}, respectively. In addition, *A. trifoliata* and *A. trifoliata. var* exhibited outstanding DPPH scavenging activity with IC_{50} ranging from 5.45 to 6.97 mg·mL^{-1} and 5.36–6.87 mg·mL^{-1}, respectively. Meanwhile, as observed with the ABTS assay, lower IC_{50} (2.28–3.22 mg·mL^{-1} and 2.09–3.22 mg·mL^{-1}, respectively) values were obtained from *A. trifoliata* and *A. trifoliata. var*. The antioxidant activities of *A. trifoliata* and *A. trifoliata. var* was stronger than *A. quinata*.

5. Conclusion

This study reveals the antioxidant activity differences between three *Akebia* species. Furthermore, it provides a reliable identification of a high number of metabolites, contributing to the characterization of the chemical composition of three *Akebia* species. Moreover, it lays a foundation for its further development or utilization. Furthermore, the current findings suggested that metabolomics is a powerful tool for distinguishing between easily confused species.

Table 2 The antioxidant inhibitory concentration (IC) 50 values of 27 dry ointment extracts (n = 3).

Sample No.	species	DPPH/(IC50, mg·mL − 1)	ABTS/(IC50, mg·mL − 1)	Sample No.	species	DPPH/(IC50, mg·mL − 1)	ABTS/(IC50, mg·mL − 1)	Sample No.	species	DPPH/(IC50, mg·mL − 1)	ABTS/(IC50, mg·mL − 1)
S1	*A. trifoliata. var*	6.24 ± 0.04	2.11 ± 0.05	S10	*A. trifoliata*	6.23 ± 0.02	3.11 ± 0.09	S19	*A. quinata*	9.56 ± 0.21	6.12 ± 0.02
S2		6.05 ± 0.08	2.09 ± 0.04	S11		6.19 ± 0.01	2.93 ± 0.16	S20		11.21 ± 0.04	6.22 ± 0.01
S3		6.97 ± 0.24	2.22 ± 0.01	S12		6.87 ± 0.01	2.56 ± 0.08	S21		11.03 ± 0.13	5.56 ± 0.14
S4		5.87 ± 0.16	3.02 ± 0.23	S13		5.43 ± 0.22	2.67 ± 0.08	S22		9.12 ± 0.24	6.25 ± 0.21
S5		5.99 ± 0.04	2.95 ± 0.08	S14		6.65 ± 0.07	2.87 ± 0.01	S23		8.99 ± 0.11	6.44 ± 0.08
S6		6.67 ± 0.01	3.11 ± 0.06	S15		5.36 ± 0.04	2.28 ± 0.01	S24		9.47 ± 0.08	7.58 ± 0.04
S7		6.74 ± 0.21	3.06 ± 0.06	S16		5.76 ± 0.35	3.22 ± 0.22	S25		9.23 ± 0.01	6.26 ± 0.19
S8		6.45 ± 0.35	2.33 ± 0.04	S17		6.76 ± 0.24	3.13 ± 0.20	S26		9.26 ± 0.06	6.01 ± 0.05
S9		5.45 ± 0.27	3.22 ± 0.13	S18		5.87 ± 0.02	2.29 ± 0.02	S27		10.21 ± 0.06	6.22 ± 0.01

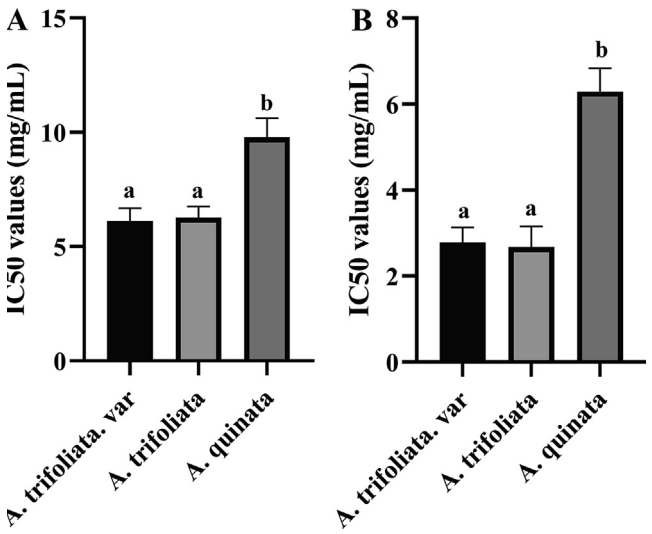

Fig. 9 The IC_{50} results of DPPH (A) and ABTS (B) antioxidant assay (a and b, $p < 0.05$).

CRediT authorship contribution statement

Xiaokang Liu: Conceptualization, Methodology, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing. **Kangyu Wang:** Methodology, Investigation, Data curation. **Guangzhi Cai:** Resources, Project administration. **Huitao Li:** Methodology, Investigation, Resources. **Yunlong Guo:** Conceptualization, Writing – review & editing, Project administration. **Jiyu Gong:** Conceptualization, Writing – review & editing, Project administration, Funding acquisition.

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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Appendix A. Supplementary material

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