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ORIGINAL RESEARCH PAPER

Simultaneous qualitative and quantitative analysis for evaluating constituents of *Atractylodis macrocephalae* rhizome by UPLC-QTOF-MS

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ABSTRACT

Atractylodis macrocephalae rhizome (AMR) belongs to medicine food homology. Its' clinical application of invigorating the spleen-stomach of AMR was applied to various diseases. In this research, a UPLC-QTOF-MS method was developed for qualitative and quantitative analysis of AMR, simultaneously. A Waters Acquity BEH C_{18} column (2.1 mm × 100 mm, 1.7 µm particle size) was used for separation of AMR multi-components. The column was eluted with a mobile phase of 0.1% formic acid-water and 0.1% formic acid-acetonitrile. Electron spray ionization with positive-ion mode and external standard method was utilized for quantifying the nine analytes in AMR. Constituents of AMR were scanned by UPLC-QTOF-MS and then identified by mass fragments and chromatographic information compared with the published literature and reference standards. Under positive mode, a total of 61 chemical compositions including 16 terpenoids, 8 polyacetylenes, 6 aromatics, 5 flavonoids, 5 coumarins, 5 organic acids, 4 amino acids, 3 fatty acids, 3 aliphatics, 2 steroids, and 2 alkenes, a nucleoside and an aldehyde were identified. Simultaneously, the contents of three amino acids (L-tyrosine, L-phenylalanine, and L-tryptophan), three sesquiterpenoids (atractylenolide III, atractylenolide II, and atractylenolide I), a flavonoid (rutin), an organic acid (ferulic acid), and a pentacyclic triterpenoid (oleanolic acid) were determined in seventeen AMR batches. Amino acids and triterpenoid were quantified for the first time in AMR. The UPLC-QTOF-MS method developed in this article was reliable, practical, and useful for qualitative and quantitative evaluation of AMR multi-components.

KEYWORDS

Atractylodis macrocephalae rhizome, qualitative analysis, quantitative analysis, UPLC-QTOF-MS

INTRODUCTION

There is a kind of substance with innocuity, nutrients, therapeutic for the sick, and pleasure for the hunger, simultaneously. The substance is defined as "medicine and food homology", which was put forward in the ancient *Huang Di Nei Jing Su Wen* [1]. They are developed as health-care products broadly circulated in the market, such as ginseng, *Polygonatum sibiricum,* and *Angelica sinensis*. *Atractylodis macrocephalae* rhizome (AMR), as a traditional Chinese medicine (TCM), belongs to the genus Atractylodes (family Asteraceae), and is known as medicine food homology species. AMR is an indispensable herb appeared in more than 122 kinds of health-care products, 912 kinds of Chinese medicine preparation, and

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4,333 kinds of herbal prescriptions for treating chronic diseases in line with the database [2]. Zhejiang and Anhui provinces of China were authentic producing areas of AMR [3]. AMR have a diversity of pharmacological effects of anti-tumor activity, enhancing immunostimulatory, improving gastrointestinal function, anti-inflammatory activity, anti-Alzheimer's disease, anti-aging, anti-oxidative and neuro-protective activities [4, 5]. Phytochemical investigation has shown that AMR contains polysaccharides, sesquiterpenoids, alkynes, amino acids, pyrazines, phenolic acids, and acyl sugar compounds [6]. Among them, the sesquiterpene-type lactones were acknowledged as principal bioactive compounds. Former literatures intensively focused on the quantitative determination and biological activities of atractylenolide I, atractylenolide II, and atractylenolide [7–9]. However, the therapeutic effects of AMR should be comprehensively revealed based on its multiple constituents.

AMR is rich in amino acids supporting human nutrition to maintain good health and prevent diseases. Tyrosine, phenylalanine, aspartic acid, tryptophan, glutamic acid, and alanine contribute to health benefits. Amino acids play important roles in the fundamental building blocks supporting life [10], preventing intestinal dysfunction [11], supporting immune function [12], and so on. Tryptophan is a precursor of serotonin that was synthesized by brain neurons [13]. Phenylalanine is a necessary amino acid for human absolutely. Yet, phenylalanine can be transformed into tyrosine, which is the precursor of epinephrine, norepinephrine, thyroxine, and neurotransmitters dopamine. From the perspective of the elementary theory of TCM, AMR possess the function of strengthening the spleen, dispel dampness for diuresis, and miscarriage prevention. The research suggested the requirement for phenylalanine during early and late gestation in healthy pregnant women [14]. Tyrosine was a versatile amino acid and proved participating in structural conformation transitions of proteins [15]. Even, it was shown that phenylalanine and tyrosine were linked with a raised risk of diabetes [16]. Chronic dietary phenylalanine, tryptophan, and tyrosine depletion brought about consequences that behavioral alterations in mice were present [14]. However, there are very rare attention on the amino acids of AMR. In addition, flavonoids and triterpenoids in AMR were also suffering a lack of attention. It is worth noting that measurement of amino acid, flavonoid, and triterpenoid in AMR is important precondition that illustrates health protection of AMR.

Previously, it has reported that gas chromatograph-mass spectrometer (GC-MS) analysis for volatile oil [17], liquid chromatography coupled with mass spectrometry (LC-MS) for multi-component characterization [18], and high performance liquid chromatography-diode array detection (HPLC-DAD) combined with chemometrics [19] have been established for composition assessment of AMR. Synchronous full-scan MS^1 and MS^2 capabilities of quadrupole time-of-flight mass spectrometry (QTOF-MS/MS) make both of qualitative and quantitative analyses accomplished, simultaneously [20]. To make a supplement for multi-ingredient excavation and quality control of AMR, with the intense separation ability, excellent resolution, sensitivity, and structural characterization capabilities, the strategy of UPLC-QTOF-MS was established to capture and profile the chemical components of AMR as much as possible, while we determined the contents of the representative amino acid, sesquiterpene, triterpene, and flavonoid.

EXPERIMENTAL

Materials and reagents

Twelve standards of L-tyrosine (**2**), L-phenylalanine (**6**), L-tryptophan (**9**), rutin (**14**), ferulic acid (**18**), luteolin (**21**), baicalein (**26**), wogonin (**27**), atractylenolide III (**34**), atractylenolideII (**37**), atractylenolide I (**43**), and oleanolic acid (**53**) with 98% purity were obtained from Weikeqi Biological Technology Co., Ltd. (Sichuan, China). HPLC-grade acetonitrile and methanol were procured from Merck (Darmstadt, Germany). Formic acid for LC-MS analysis was supplied by Fluka (Steinheim, Germany). Distilled water was fetched from an Aquapro water purification system (Aquapro, Chongqing, China).

Sample preparation

Stock standard solution of compounds **2**, **6**, **9**, **14**, **18**, **34**, **37**, **43**, and **53** were prepared in pure methanol at 3.28, 21.27, 16.2, 3.8, 6.8, 201.3, 25.55, 25.7, and 13.15 ug/mL, respectively. Proper concentration levels ($n = 6$) for determining calibration curves were obtained by diluting mixed stock solutions. The commercial AMRs from Anhui (S1–S4), Gansu (S5–S6), Henan (S7–S8), Sichuan (S9–S11), Shanxi (S12–S13), Yunnan (S14), and Zhejiang (S15–S17) were collected from different medicinal stores (Table S1). 0.5 g herb powder of seventeen AMR batches ground into 80 mesh were weighted, and added into 20 mL pure methanol, shaken, stood still for 20 min, and weighted again. After vortexed for 30 min, cooled down, weighed, and made up for weight loss with methanol, the supernatant was passed through a 0.22 μm microporous membrane before UPLC-QTOF-MS/MS analysis.

UPLC-QTOF-MS/MS conditions

The UPLC separation was conducted using a Waters Acquity BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm, Waters Corporation) on the Shimadzu LC-30AD system (Shimadzu, Japan). The column was eluted with mobile phase of 0.1% formic acid-water (A) and 0.1% formic acid-acetonitrile (B), which was conducted as follows: 90% A (0–2 min), 79%–75% A (2–4 min), 75%–55% A (4–6 min), 55%–45% A (6–14 min), 45%–43% A (14–17 min), 43%–42% A (17–19 min), 42%–30% A (19–20 min), 30–20% A (20–30 min), 20%–5% A (30–31 min), and 5% A (31–33 min). The flow rate, column temperature, and injection volume were set at 0.3 mL min^{-1}, 40 °C, and 5 μL, respectively.

QTOF-MS/MS analysis was performed using a Triple TOF^{TM} 5600^{+} mass spectrometer (AB Sciex, Foster City,

CA). The mass acquisition conditions were as follows: Ion source, DuoSpray ion source; polarity, positive mode; ion source gas 1, 55 psi; ion source gas 2, 55 psi; curtain gas, 35 psi; temperature, 550 °C; Ion Spray Voltage Floating, 4500 V; declustering potential, 90 V; collision energy, 5 V. For the information dependent acquisition criteria, the eight most intense fragmentation ions of each target were chosen to conduct a product ion scan when they exceeded 100 cps counts. MS and MS/MS scan range were 100–1550 and 50–1000 with a 250 ms accumulation time, respectively. Dynamic background subtraction was turned on during the full scans. Calibration delivery system was applied for precursor and product ion calibration at every 4 h. Data acquisition and processing were executed by Analyst®TF 1.7 and PeakView® 2.0 software, respectively.

RESULTS AND DISCUSSION

Optimization of UPLC-QTOF-MS method

The pivotal UPLC-QTOF-MS parameters such as mobile phase composition (0.1% formic acid-water/0.1% formic acid-methanol, 0.1%-formic acid water/0.1%-formic acid acetonitrile), analytical columns Waters Acquity BEH C_{18} (2.1 mm × 100 mm, 1.7 μm) and Waters Cortecs UPLC C_{18} (2.1 mm × 100 mm, 1.6), MS acquisition modes (negative or positive), CE values (25V/35V/45V) were inspected for obtaining an advantageous acquisition method. Taking separation performance, chromatographic peak shape, response intensity, and fragment ions into consideration, a Waters Acquity BEH C_{18} (2.1 mm × 100 mm, 1.7 μm) column at 0.1%-formic acid-water/0.1%-formic acid-acetonitrile with the optimized gradient elution was applied for the UPLC analysis. The CE value of 35V was recommended for qualitative analysis. Both negative and positive modes were adopted for acquiring chromatograms and MS data facilitating mutual verification. However, extract ion chromatograms (EICs) of active compounds **37** and **43** in AMR were unable to be detectable in ESI^- mode, while the EICs of the two compounds were extracted perfectly in ESI^+ mode (Fig. S1). Therefore, a quantitative analysis of nine compounds was detected in positive acquisition mode.

Qualitative analysis of AMR

Before characterization of chemical ingredients in AMR, a lot of beforehand work is necessary. A database of AMR components covering names, formulas, original literatures, CAS number, and compound structures was established for identification. For QTOF-MS/MS was acclaimed as a high-resolution technique, in the first round, molecular weights with the error (ppm >5) were excluded based on the full scan mass spectra. In the second round, the final identified component corresponding to single or multiple EIC was found out according to characteristic fragments and retention time (Rt) existing in published literature or standards. Additionally, the cracking law conforming to chemical structure was employed for inferring target compound, which particularly suits the circumstance of no contrast reference. As a result, the total ion chromatogram of AMR in positive mode was displayed in Fig. 1A. And the chromatograms of the quantified nine analytes were seen in Fig. 1B. A total of 61 chemical compositions (16 terpenoids, 8 polyacetylenes, 6 aromatics, 5 flavonoids, 5 coumarins, 5 organic acids, 4 amino acids, 3 fatty acids, 3 aliphatics, 2 steroids, and 2 alkenes, a nucleoside and an aldehyde) were tentatively identified, and their Rt, formula, ppm, fragmentation, mass value, as well as references were described in Table 1. The chemical structures of 61 identified compositions are shown in Fig. S2. Fragmentation pathways and MS^2 spectrum of atractylenolide VI, (8R,9R)-8,9-dihydroxylatractylodinol-9-O-β-D-glucopyranoside, scoparone, and proline were displayed in Fig. S3.

Identification of terpenoids. Sixteen compounds (**8, 23, 28, 30, 31, 34, 36, 37, 38, 40, 42, 43, 44, 53, 54,** and **60**) were identified as terpenoids, which have no irregular parent nucleus structure. These compounds were assigned as sesquiterpenoids (**23, 30, 31, 34, 37, 40, 42, 43, 44, 54,** and **60**), diterpenes (**8, 28, 36,** and **38**), and pentacyclic triterpene (**53**). Compound **60** exhibited a *m/z* 203.1791 $[M+H]^+$ at a Rt of 31.04 min. By comparing Rt and the characteristic ions at *m/z* 173.0990 $[M+H-2CH_3]^+$, 147.1182$[M+H-C_3H_5-CH_3]^+$ with literature data [21]. Compound **60** was tentatively identified as atractylenolide VI. Compound **44** was inferred as selina-4(14), 7(11)-dien-8-one by consulting literature in the same way [22]. Compound **23** (ppm −1.3) with an adduct ion at *m/z* 237.1846 $[M+H]^+$ produced ions at *m/z* 201.1597 $[M+H-2H_2O]^+$, 173.1307$[M+H-2H_2O-2CH_2]^+$, and 161.1351$[M+H-H_2O-C_3H_6O]^+$. Compound **23** with the core skeleton similar to compounds **60** and **44** was identified as eudesm-4(15),7-diene-9α,11-diol or its isomers. Compounds **34, 37**, **43**, and **53** were confirmed as atractylenolide III, atractylenolideII, atractylenolide I, and oleanolic acid by comparison to standards. Atractylenolide III, atractylenolide II, and atractylenolide I possess multiple activities. Atractylenolide I and atractylenolide II have noticeable anti-tumor activities [9, 23]. Atractylenolide I and atractylenolide III have excellent anti-inflammatory and neuroprotective activities [24]. Oleanolic acid exerted beneficial bio-active effects including anti-viral, antibacterial, anticarcinogenic, anti-atherosclerotic, anti-diabetes, etc [25]. Mass spectral and chromatographic information, and reference literatures for identification of compounds **30**, **31**, **36 38**, **40**, **42**, and **54** were listed in Table 1.

Identification of polyacetylenes. Polyacetylenes are a class of vigorous compounds consisting of carbon-carbon triple bond that are abundant in natural medicine. Diverse biological functions including immune regulation, tumor suppression, anti-depressant, and neuroprotection have attracted extensive attention [26]. Compounds **11**, **15**, **25**, **32**, **35**, **39**, **49**, and **50** were judged as polyacetylenes. Compound **11** (Rt 2.3 min) displayed an $[M+H]^+$ precursor ion peak at *m/z* 395.1320 (error ppm −4.2). The formula

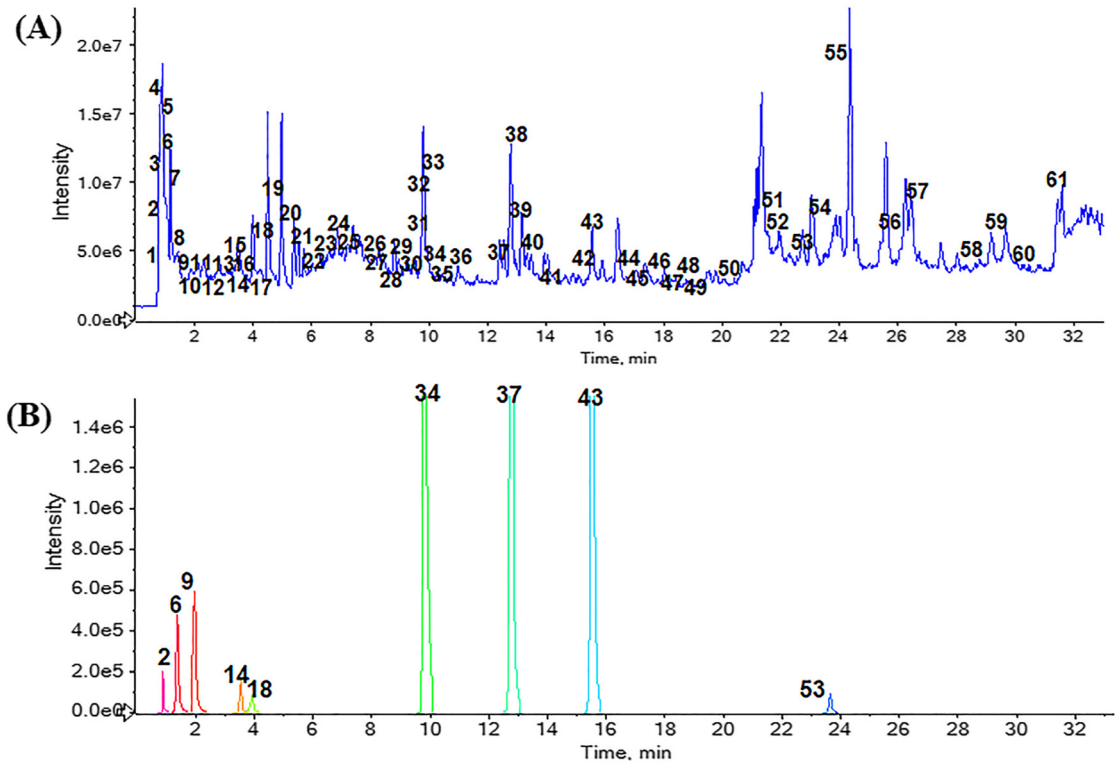

Fig. 1. The ion chromatograms of ARM (A) and reference standards (B) by UPLC-QTOF-MS/MS analysis. (peak **2**: L-tyrosine; **6**: L-phenylalanine; **9**: L-tryptophan; **14**: rutin; **18**: ferulic acid; **34**: atractylenolide III; **37**: atractylenolide II; **43**: atractylenolide I; **53**: oleanolic acid)

was calculated as $C_{19}H_{22}O_9$. For the characteristic ion at *m/z* 232.0632 corresponds to the loss of glucopyranoside, compound **11** was conducted as (8R,9R)-8,9-dihydroxylatractylodinol-9-O-β-D-glucopyranoside. Compound **50** present a precursor ion $[M+H]^+$ at *m/z* 303.1587 (error ppm −1.2, $C_{18}H_{22}O_4$). The mass spectrum showed the primary fragment ion was *m/z* 243.1329 by losing of terminal methyl ester. By comparing Rt of literature [27, 28], the compound **50** was considered as (6E,12E)-tetradecadiene-8,10-diyne-1,3-diol diacetate. Other identified polyacetylenes were introduced in Table 1, correspondingly.

Identification of flavonoids and coumarins. Flavonoids have a wide variety of bioactivities including antioxidation, cardio-protective, anti-inflammatory, anticancer, and other properties [29, 30]. Flavonoids possessed a characteristic "C6–C3–C6" skeleton, easy to break glycosidic bonds and Diels-Alder (RDA) reaction generating major fragment ions [29]. Compounds **14, 16, 21, 26,** and **27** belong to flavonoids, divided into flavone aglycones (**14** and **16**) and glycosyl flavonoids (**21, 26,** and **27**). Compound **14** was ascribed to rutin compared with reference standard, which yielded an $[M+H]^+$ ion at *m/z* 611.1602 and produced fragments ions $[M+H-C_6H_{10}O_4]^+$ at *m/z* 465.1037, $[M+H-C_6H_{10}O_4-C_6H_{10}O_5]^+$ at *m/z* 303.0537. As a result, compounds **16**, **21**, **26,** and **27** were identified as puerarin, luteolin, baicalein, and wogonin based on the differentiated data in Table 1.

Coumarins were part of benzopyrone family. Natural coumarins have extensive pharmacological activities such as antifungal, antiviral, Alzheimer's disease inhibition, etc. [31]. The ordinary fragmentation pathway was observed losing neutral molecules carbon monoxide, carbon dioxide, methyl, H_2O, and sugar, etc. [30]. For example, compound **19** gave an $[M+H]^+$ ion at *m/z* 207.0553 and generated fragments ions at *m/z* 191.0322$[M+H-CH_4]^+$, 151.0726 $[M+H-C_3H_4O]^+$, and 145.0237$[M+H-C_2H_6O_2]^+$ (seen in Table 1). Thus, compound **19** was identified as scoparone according to previous literatures [32]. Compounds **10**, **17**, **20**, and **24** were recognized as scopoletin-D-xylopyranosyl-(1→6)-D-glucopyranoside, scopoletin, 4-methylumbelliferone, and umbelliferone in comparison with standard or literatures, respectively.

Identification of organic acids, amino acids, fatty acids, steroids, and the others. Five compounds (**3**, **4**, **12**, **13**, and **18**) were identified as organic acids in the positive mode. Through the characteristic ions at *m/z* 175.0864 $[M+H-H_2O]^+$, 147.0943$[M+H-H_2CO_2]^+$, 129.0172 $[M+H-H_2O-H_2CO_2]^+$, and precursor ion at *m/z* 193.0345 $[M+H]^+$, compound **3** was predicted as citric acid. Compound **4** had deprotonated molecular at *m/z* 175.0235 $[M+H]^+$ at 1.16 min. The loss of carboxyl (HCO_2) generated a major fragment ion at *m/z* 130.0994. Compound **4** was identified as aconitic acid. Fragmentary ions at *m/z* 145, 149 and 117 can result from compounds **12**, **13** and **18,** because they have a common mono-acyl chlorogenic acid.

Table 1. Characterisation of the chemical constituents of AMR by UPLC-QTOF-MS method

No.	Compounds	Rt (min)	Theoretical mass	Detected mass	ppm	Detected mode	Formula	Fragments	Type	Reference
1	Proline	0.88	115.0633	116.0711	4.5	$[M+H]^+$	$C_5H_9NO_2$	70.0735,53.0483	amino acids	[33]
2	L-tyrosine	0.90	181.0739	182.0805	−3.6	$[M+H]^+$	$C_9H_{11}NO_3$	147.0444,136.0759,123.0448,119.0501, 107.0508,103.0562,95.0508,77.0412	amino acids	*
3	citric acid	1.13	192.0270	193.0345	1.0	$[M+H]^+$	$C_6H_8O_7$	193.0952,175.0864,147.0943,139.0018 129.0172,111.0090,93.0331,83.0494,68.9989	organic acids	[37]
4	aconitic acid	1.16	174.0164	175.0235	−1.0	$[M+H]^+$	$C_6H_6O_6$	175.1191,130.0994,116.0747, 104.0508	organic acids	[37]
5	Uridine	1.18	244.0695	245.0774	2.3	$[M+H]^+$	$C_9H_{12}N_2O_6$	245.1161,217.0954,142.0891,113.0371	nucleoside	[38]
6	L-phenylalanine	1.41	165.0790	166.0856	−4.0	$[M+H]^+$	$C_9H_{11}NO_2$	120.0821,119.0749,118.0673,103.0564 102.0492	amino acids	*
7	5-hydroxymethyl furaldehyde	1.56	126.0317	127.0390	0.4	$[M+H]^+$	$C_6H_6O_3$	109.0324	aldehyde	[39]
8	atractyloside A	1.87	448.2309	449.2379	−0.3	$[M+H]^+$	$C_{21}H_{36}O_{10}$	287.0572,269.1752,251.1682,233.1590 223.1689 215.1457,187.1565,147.1209	terpenoids	–
9	L-tryptophan	2.05	204.0899	205.0969	−3.7	$[M+H]^+$	$C_{11}H_{12}N_2O_2$	143.0738,142.0662,132.0825,130.0662 128.0514,	amino acids	*
10	scopoletin-D-xylopyranosyl -(1→6)-D-glucopyranoside	2.27	486.1373	487.1448	0.4	$[M+H]^+$	$C_{21}H_{26}O_{13}$	355.0976,193.0539,163.0440,133.0678 87.0647	coumarins	–
11	(8R,9R)-8,9-dihydroxylatractylodinol-9-O-β-D-glucopyranoside	2.30	394.1264	395.1320	−4.2	$[M+H]^+$	$C_{19}H_{22}O_9$	232.0632,215.0721,198.1827,164.0443 114.1107	polyacetylenes	–
12	chlorogenic acid	2.31	354.0951	355.1013	−4.5	$[M+H]^+$	$C_{16}H_{18}O_9$	163.0392,145.0296,135.0460,117.0358 107.0523	organic acids	[40]
13	5-O-feruloylquinic acid	3.23	368.1107	369.1176	−1.1	$[M+H]^+$	$C_{17}H_{20}O_9$	177.0546,149.0628,145.0313,117.0387	organic acids	[33]
14	Rutin	3.51	610.1534	611.1602	−0.4	$[M+H]^+$	$C_{27}H_{30}O_{16}$	465.1037,303.0537,129.0578,71.0537	flavonoids	*
15	1-(2-Furyl)-(1E,7E)-nonadiene-3,5-diyne-9-yl 4-methylbenzoate or isomers	3.61	314.1307	315.1368	−3.5	$[M+H]^+$	$C_{22}H_{18}O_2$	283.1007,247.0852,235.0827,222.0770 211.0851 206.0838,193.0768,167.0604	polyacetylenes	–
16	Puerarin	3.61	432.1057	433.1127	−0.5	$[M+H]^+$	$C_{21}H_{20}O_{10}$	415.0701,397.0648,379.0570,361.0456, 337.0506,323.0739,309.0626,283.0486, 255.0580,165.0197,149.0250,121.0339	flavonoids	[41]
17	scopoletin	3.88	192.0423	193.0493	−1.1	$[M+H]^+$	$C_{10}H_8O_4$	193.0507,178.0266,165.0574,161.0246, 150.0326,137.0609,133.0298,122.0381, 107.0507,105.0360	coumarins	[42]
18	ferulic acid	3.93	194.0579	195.0652	0.2	$[M+H]^+$	$C_{10}H_{10}O_4$	152.0364,149.0598,145.330,134.0391, 117.0352,106.0433	organic acid	*
19	scoparone	5.46	206.0579	207.0553	−4.9	$[M+H]^+$	$C_{11}H_{10}O_4$	191.0322,163.0394,151.0760,146.0394, 135.0483,133.0324,117.0365,107.0551 105.0400	coumarins	[32]
20	4-methylumbelliferone	5.83	176.0473	177.0543	−2	$[M+H]^+$	$C_{10}H_8O_3$	149.0636,145.0293,134.0372,117.0361, 115.0533,106.0435,105.0357	coumarins	[43]

(continued)

Table 1. Continued

No.	Compounds	Rt (min)	Theoretical mass	Detected mass	ppm	Detected mode	Formula	Fragments	Type	Reference
21	luteolin	6.20	286.0477	287.0547	−1.0	$[M+H]^+$	$C_{15}H_{10}O_6$	287.0457,269.0372,241.0458,153.0196, 139.0584,135.0468,	flavonoids	[42]*
22	2-[(2′E)-3′,7′-dimethyl-2′,6′-octadienyl] -4-methoxy6-methylphenol	6.35	272.2140	273.2208	−1.7	$[M+H]^+$	$C_{19}H_{28}O$	220.1760,182.0983,165.0724,160.1258,	aromatics	–
23	eudesm-4(15),7-diene-9α,11-diol or isomers	6.47	236.1776	237.1846	−1.3	$[M+H]^+$	$C_{15}H_{24}O_2$	201.1597,173.1307,161.1351,145.1041 119.0902,107.0936	terpenoids	–
24	umbelliferone	6.99	162.0317	163.0387	−1.6	$[M+H]^+$	$C_9H_6O_3$	135.0465,133.0284,105.0349,103.0554	coumarin	[40]
25	(4E,6E,12E)-tetradeca-4,6,12-trien-8,10-diyne-1,3,14-triol	7.01	232.1099	233.1166	−2.6	$[M+H]^+$	$C_{14}H_{16}O_3$	215.1072,187.1096,175.1111,169.1042 159.1181,153.0705,141.0718,131.0877 129.0725,119.0885,115.0578,105.0747,	polyacetylenes	[37]
26	baicalein	7.32	270.0528	271.0589	−4.3	$[M+H]^+$	$C_{15}H_{10}O_5$	271.0560, 253.0458,225.0520, 179.0477 169.0115, 151.0026, 123.0077, 95.0141	flavonoids	*
27	wogonin	8.05	284.0685	285.0752	−2.0	$[M+H]^+$	$C_{16}H_{12}O_5$	270.0443,253.0438,242.0519,168.0051 140.0119	flavonoids	*
28	2-methoxy-4-methyl-1-(1-methylethyl)benzene	8.29	164.1201	165.1275	0.5	$[M+H]^+$	$C_{11}H_{16}O$	165.0689,163.0529,119.0826,109.0649, 107.0872,105.0707	terpenoids	–
29	ethyl 3-(4-Hydroxyphenyl) acrylate or isomers	8.36	192.0786	193.0857	−1.1	$[M+H]^+$	$C_{11}H_{12}O_3$	161.0597,135.0497,133.0670,131.0500, 118.0442,115.0571,105.0731,103.0578	aromatics	–
30	β-elemene or isomers	9.16	204.1878	205.1950	−0.3	$[M+H]^+$	$C_{15}H_{24}$	149.1343,121.1009,119.0887,107.0865,	terpenoids	–
31	4α,7α-epoxyguaiane-10α,11-diol or isomers	9.31	254.1882	255.1955	0.3	$[M+H]^+$	$C_{15}H_{26}O_3$	158.0252,141.0051,133.1038,128.0633, 117.0704,107.0893	terpenoids	–
32	(4E,6E,12E)-1-acetoxy-3-(2-methylbutyryloxy) −4,6,12-trien-8,10-diyn-14-ol or isomers	9.53	358.1780	359.1856	0.8	$[M+H]^+$	$C_{21}H_{26}O_5$	359.1732,341.1554,331.1816,313.1741, 311.1558,295.1587,271.1628,243.1348, 225.1588,217.1617, 211.1128,105.0704	polyacetylenes	–
33	Safrole	9.74	162.0681	163.0754	0.4	$[M+H]^+$	$C_{10}H_{10}O_2$	163.0764,135.0829,117.0731,115.0559, 107.0509,103.0564	aromatics	[44]
34	atractylenolide III	9.80	248.1412	249.1481	−1.6	$[M+H]^+$	$C_{15}H_{20}O_3$	231.1394,213.1286,203.1434,198.1056, 189.0929,175.0764,163.0761,155.0870, 142.0788,129.0709,117.0710,105.0708	terpenoids	*
35	(6E,12E)-1-acetoxytetradeca −6,12-dien-8,10-diyn-3-ol or isomers	10.02	260.1412	261.1487	0.7	$[M+H]^+$	$C_{16}H_{20}O_3$	261.1412,243.1405,219.1771,201.1634, 187.0770,173.1337,159.1172,145.1035, 131.0875,115.0575	polyacetylenes	–
36	5-Isopropyl-2-methyl-2,4-cyclohexadien-1-one	11.10	150.1045	151.1116	−0.7	$[M+H]^+$	$C_{10}H_{14}O$	117.0711,115.0555,109.0644,105.0705, 103.0531	terpenoids	–
37	atractylenolide II	12.66	232 .1463	233.1533	−1.5	$[M+H]^+$	$C_{15}H_{20}O_2$	233.1493,215.1396,197.1312,187.1462, 167.0861,159.0830,145.1020,141.0722, 117.0734,115.0579,105.0741	terpenoids	*

(continued)

Table 1. Continued

No.	Compounds	Rt (min)	Theoretical mass	Detected mass	ppm	Detected mode	Formula	Fragments	Type	Reference
38	azulene	13.06	128.0626	129.0698	−0.6	$[M+H]^+$	$C_{10}H_8$	128.0632,117.0701,115.0577,113.0462, 103.0537, 102.0487	terpenoids	[37]
39	(4E,6E,12E)-tetradecatriene−8,10-diyne-1,3-diyl diacetate	13.44	300.1362	301.1426	−2.7	$[M+H]^+$	$C_{18}H_{20}O_4$	199.1113,178.0808,165.0705,152.0629, 141.0694, 128.0633,115.0557,105.0706	polyacetylenes	[37]
40	furanodiene	13.78	216.1514	217.1582	−2.3	$[M+H]^+$	$C_{15}H_{20}O$	157.1003,143.0871,119.0879,105.0727	terpenoids	[45]
41	amylcinnamyl alcohol or isomers	14.74	204.1514	205.1588	0.6	$[M+H]^+$	$C_{14}H_{20}O$	187.1524,161.1366,149.0260,141.9626, 128.9546,119.0892,100.9363,97.9725, 81.0740,55.9393	alkenes	–
42	curcumene or isomers	15.62	202.1722	203.1791	−1.6	$[M+H]^+$	$C_{15}H_{22}$	147.1176,133.1014,119.0875,105.0729	terpenoids	[46]
43	atractylenolide I	15.62	230.1307	231.1376	−1.7	$[M+H]^+$	$C_{15}H_{18}O_2$	231.1393,215.1089,201.0933,188.0854, 185.1326,175.0779,165.0710,155.0857, 142.0780,129.0700,115.0546,105.0709	terpenoids	*
44	selina-4(14),7(11)-dien-8-one	17.32	218.1671	219.1740	−1.7	$[M+H]^+$	$C_{15}H_{22}O$	177.1255,141.0732,131.0856,119.0868, 107.0863, 105.0708	terpenoids	[22]
45	(E,E,E)-2,4,6-octatriene	17.36	108.0939	109.1011	−0.3	$[M+H]^+$	C_8H_{12}	100.9589	alkenes	–
46	(1S,4S)-Bicyclo[2.2.1]hept-5-en-2-one	18.12	108.0575	109.0652	4	$[M+H]^+$	C_7H_8O	109.0839,100.9582	alkenes	–
47	diisobutyl phthalate or isomers	18.26	278.1518	279.1591	0.2	$[M+H]^+$	$C_{16}H_{22}O_4$	201.0448,149.0245,121.0306,	aromatics	[47]
48	diethyl phthalate	18.31	222.0892	223.0965	−0.1	$[M+H]^+$	$C_{12}H_{14}O_4$	223.1675,207.0263, 191.0000, 149.0245,121.0316	aromatics	[48]
49	atractylodin	19.85	182.0732	183.0806	0.6	$[M+H]^+$	$C_{13}H_{10}O$	152.06017,141.0767,139.0542,128.0632 115.0564	polyacetylenes	[37]
50	(6E,12E)-tetradecadiene-8,10-diyne-1,3-diol diacetate	20.38	302.1518	303.1587	−1.2	$[M+H]^+$	$C_{18}H_{22}O_4$	243.1329,172.8635,135.0464	polyacetylenes	[28]
51	methyl linolenate	22.25	292.2402	293.2470	−1.7	$[M+H]^+$	$C_{19}H_{32}O_2$	293.2021,145.0908,121.1054,109.1036, 107.0918,105.0757	aliphatics	[49]
52	Sitosterol	22.31	414.3862	415.3934	4.2	$[M+H]^+$	$C_{29}H_{50}O$	397.2353,369.2470,341.2659,313.3311,	steroids	[50]
53	oleanolic acid	23.63	456.3604	457.3667	−2	$[M+H]^+$	$C_{30}H_{48}O_3$	411.3532,333.1758,315.2569,297.2513, 269.2217, 31.2073,217.1558,203.1818, 189.1646,163.1486,149.1339,135.1182, 119.0892,107.0880	terpenoids	*
54	atractyline	23.94	216.1514	217.1584	−1.5	$[M+H]^+$	$C_{15}H_{20}O$	199.1529,161.1019,147.1244,143.0913, 133.1072,105.0777,95.0565,77.0465, 67.0629	terpenoids	[51]*
55	stigmasterol	25.22	412.3705	413.3798	3.6	$[M+H]^+$	$C_{29}H_{48}O$	395.3149,383.2175,365.1762,294.1277, 273.1184,243.1100,229.0963,215.0814, 202.0743,165.0706,141.0720,115.0577	steroids	[52]
56	monoolein	26.125	356.2927	357.3000	0.1	$[M+H]^+$	$C_{21}H_{40}O_4$	357.2905,310.1592,247.2414,149.1308 135.1218,107.0918	aliphatics	[37]

(continued)

Table 1. Continued

No.	Compounds	Rt (min)	Theoretical mass	Detected mass	ppm	Detected mode	Formula	Fragments	Type	Reference
57	linoleic acid	26.27	280.2402	281.2475	−0.1	$[M+H]^+$	$C_{18}H_{32}O_2$	263.2292,245.2210,219.2089,203.1790, 189.1640,175.1473,165.1255,149.1339, 147.1179,133.1031,119.0881,105.0733	fatty acids	[53]
58	palmitic acid	29.04	256.2402	257.2471	−1.4	$[M+H]^+$	$C_{16}H_{32}O_2$	257.1879,178.0783,165.0697,128.0630, 115.0553	fatty acids	[53]
59	oleic acid	29.72	282.2559	283.2623	−3.1	$[M+H]^+$	$C_{18}H_{34}O_2$	263.2288,245.2234,219.2126,203.1795 189.1614,175.1468,161.1329,149.1342 147.1178,135.1181,133.1032, 119.0874, 105.0728	fatty acids	[53]
60	atractylenolide VI	31.04	202.1722	203.1791	−1.6	$[M+H]^+$	$C_{15}H_{22}$	173.0990,147.1182,133.1031,119.0880 105.0729	terpenoids	[21]
61	methyl octadeca-9,12-dienoate	32.31	294.2559	295.2628	−1.4	$[M+H]^+$	$C_{19}H_{34}O_2$	295.1587,280.1339,237.1231,226.0852, 199.0727,187.0738,159.0804,142.0815, 141.0720,128.0639,115.0562,119.0867	aliphatics	[54]

*Compounds **2**, **6**, **9**, **14**, **18**, **21**, **26**, **27**, **34**, **37**, **43** and **53** were identified by comparison with reference standards.

Table 2. Linear equation, correlation coefficient, linear range, LODs, LOQs, precision, repeatability, stability, and recovery of nine reference standards

NO.	Linear equation	Correlation coefficient	Linear range (μg mL^{-1})	LODs (ng mL^{-1})	LOQs (ng mL^{-1})	Precision RSD (%)		Repeatability RSD (%)	Stability RSD (%)	Recovery (%)	
						Intra-day	Inter-day			Mean	RSD
2	$y = 2.42684e^5x+19475.34990$	0.9994	0.10–3.28	12.81	51.25	1.02	1.43	2.71	1.45	98.92	2.51
6	$y = 2.81161e^5x+5.21415e4$	0.9999	0.66–21.27	10.39	83.09	1.99	2.16	2.43	3.02	103.57	1.75
9	$y = 4.90522e^5x+17522.03931$	0.9997	0.51–16.20	31.64	126.56	1.46	1.31	3.30	3.54	102.32	4.66
14	$y = 7.76104e^5x + -4218.35468$	0.9992	0.01–0.48	1.86	7.42	2.81	4.51	2.75	2.65	95.61	0.41
18	$y = 2.01936e^5x + -4679.18156$	0.9996	0.11–3.40	13.28	106.25	0.92	1.20	2.15	2.00	96.47	3.87
34	$y = 6.88885e^5x+1.64143e^6$	0.9996	1.57–50.31	3.07	12.28	0.79	2.12	3.10	1.16	98.89	1.45
37	$y = 3.01940e^6x+2.53101e^6$	0.9990	0.40–12.78	3.12	12.48	1.54	1.71	3.15	2.86	99.10	3.36
43	$y = 2.25477e^6x+1.91026e^6$	0.9994	0.80–25.70	6.27	25.10	1.25	1.60	3.01	2.51	99.63	2.87
53	$y = 6.20298e^4x+3.72172e^4$	0.9993	0.21–6.58	25.68	102.73	0.71	1.01	1.56	1.02	97.82	1.13

Compounds **12**, **13**, and **18** were recognized as chlorogenic acid, 5-O-feruloylquinic acid, and ferulic acid. Compounds **1**, **2**, **6**, and **9** were classified as amino acids. A parent ion at *m/z* 116.0711$[M+H]^+$ was observed with Rt of 0.88 min, with the predicted formula $C_5H_9NO_2$. The fragmentation ion at *m/z* 70.0735 $[M+H-H_2CO_2]^+$ was similar to that in documents [33] and human metabolome database. Compound **1** was attributed to proline. Compounds **57**, **58**, and **59** belong to fatty acids, while compounds **52** and **55** are steroids. By consulting the reference substances with the same MS^2 spectra and Rt (seen in Table 1), compounds **2**, **6**, **9**, **52**, **55**, **57**, **58**, and **59** were identified as L-tyrosine, L-phenylalanine, L-tryptophan, sitosterol, stigmasterol, linoleic acid, palmitic acid, and oleic acid, respectively.

There are thirteen additional chemicals including **5** (alkaloid), **7** (aldehyde), **22** (aromatics), **29** (aromatics), **33** (aromatics), **41** (alkenes), **45** (alkenes), **46** (alkenes), **47** (aromatics), **48** (aromatics), **51** (aliphatic), **56** (aliphatic), and **61** (aliphatic). On basis of the data found in literature or regular MS splitting decomposition law, compounds **5**, **7**, **22**, **29**, **33**, **41**, **45**, **46**, **47**, **48**, **51**, **56**, and **61** were assigned to uridine, 5-hydroxymethyl furaldehyde, 2-[(2′E)-3′,7′-dimethyl-2′,6′-octadienyl]-4-methoxy6-methylphenol, ethyl 3-(4-Hydroxyphenyl) acrylate or isomers, safrole, amylcinnamyl alcohol or isomers, (E,E,E)-2,4,6-octatriene, (1S,4S)-Bicyclo[2.2.1]hept-5-en-2-one, diisobutyl phthalate or isomers, diethyl phthalate, atractylodin, methyl linolenate, monoolein, and methyl octadeca-9,12-dienoate, respectively.

Quantitative analysis

Method validation. Six different concentrations of mixed standard solutions were applied for constructing standard curves by plotting the peak area (y) versus the concentration (x). Table 2 shows the linear equation with good correlation coefficient (0.9990–0.9999) over a wide linear range of the nine standards. The limit of detection (LOD) and the limit of quantification (LOQ) of analytes were calculated as signal-to-noise of 3:1 and 10:1, respectively. The LODs and LOQs of nine analytes were in the range of 1.86–31.64 ng mL^{-1} and 7.42–126.56 ng mL^{-1}, respectively. The intra- and inter-day precision of the developed method were analyzed by repeated injection for six times in one day or over three consecutive days, respectively. The relative standard deviations (RSDs) of intra- and inter-day precisions of nine targets were 0.71%–2.81% and 1.01%–4.51%, respectively. The repeatability was examined by continuously injecting the same sample for six times. The same sample was analyzed at 0, 4, 8, 12, 24 and 48 h to examine stability. The RSDs of repeatability and stability performed on S7 were ranged from 1.56%–3.30% and 1.02%–3.54%, respectively. A certain quantity of the nine analytes at low, medium, and high concentrations were added into a 0.25 g powder of S7. The recovery was analyzed by comparing the detected amounts of reference compounds with the spiked amount (Tables S2). The average recoveries ($n = 3$) of the nine analytes were in the range of 95.61%–103.57% with the RSD <4.66. The results indicated that the analytical methods are appropriate for determination of three amino acids, three sesquiterpenoids, a flavonoid, an organic acid, and a pentacyclic triterpenoid, simultaneously.

Quantitative determination of nine components in AMR. As obviously displayed in Fig. 2 and Table S3, atractylenolide III (150.30–303.20 μg g^{-1}), atractylenolide II (119.60–210.80 μg g^{-1}) and atractylenolide I (72.84–185.76 μg g^{-1}) were the major bioactive components, which was consistent with previous reports [34]. Aractylenolide III, atractylenolide II, and atractylenolide I were frequently considered as key chemicals for AMR quality control. Few reports concerning quantification of amino acids in AMRs were published. As shown in Table S3, L-tyrosine, L-phenylalanine, and L-tryptophan (14.60–40.00 μg g^{-1}, 117.00–245.60 μg g^{-1}, 27.00–211.60 μg g^{-1}, respectively) were detected in seventeen AMR batches. L-phenylalanine and L-tryptophan were observed abundant in AMR samples. The level of tryptophan in the body is both tightly relevant to depression pathophysiology [35]. The exogenous amino acid could be supplied from the diet of AMR for disease prevention. In addition, the amount of oleanolic acid (2.44–144.36 μg g^{-1}) was reported for the first

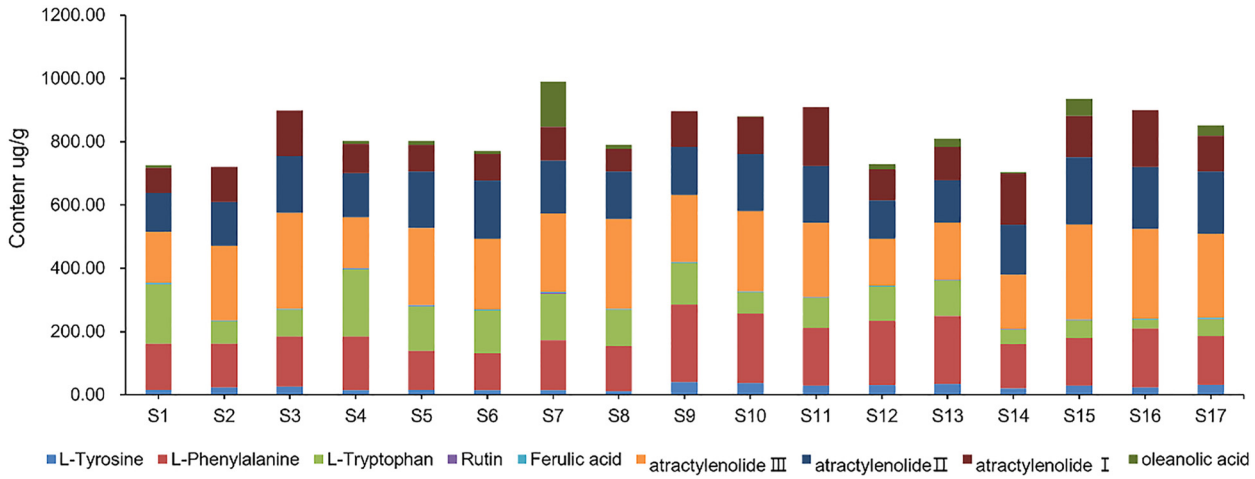

Fig. 2. Composition profiles of the quantified nine components in AMR extracts

time. As a note, the amounts of oleanolic acid in S2, S3, S9, S11, and S16 were unable to be calculated for their content was not up to LOQ standard. Rutin, as a familiar flavonoid, had low content (0.4–3.10 μg g^{-1}) in seventeen AMR batches (seen in Table S3). An assessment of the amount of ferulic acid had also been observed at 1.46–3.60 μg g^{-1}. Ferulic acid has multiple functions such as anti-oxidant and anti-inflammatory [36]. The detailed composition profiles of the nine quantified components in seventeen batches of AMR were presented in Fig. 2.

CONCLUSION

In this research, a UPLC-QTOF-MS method was developed for qualitative and quantitative analysis of AMR, simultaneously. The results highlighted nutritional composition such as amino acids, which significantly complement for the chemical profiling of AMR. A total of 61 chemical compositions including 16 terpenoids, 8 polyacetylenes, 6 aromatics, 5 flavonoids, 5 coumarins, 5 organic acids, 4 amino acids, 3 fatty acids, 3 aliphatics, 2 steroids, and 2 alkenes, a nucleoside and an aldehyde were identified. Simultaneously, the contents of three amino acids, three sesquiterpenoids, a flavonoid, an organic acid, and a pentacyclic triterpenoid were determined in seventeen AMR batches. Amino acids and triterpenoid were quantified for the first time in AMR. The established UPLC-QTOF-MS method in this article was practical, useful, and reliable for qualitative and quantitative evaluation of AMR multi-components.

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SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1556/1326.2023.01151.

REFERENCES

1. Gong, X.; Ji, M.; Xu, J.; Zhang, C.; Li, M. *Crit. Rev. Food* **2020**, *60*, 2303–26.
2. Zhan, C.; Wang, H.; Wang, Y. *J. Pharmaceut Biomed.* **2022**, *219*, 114899.
3. Hu, L.; Chen, X.; Yang, J.; Guo, L. *Rapid Commun. Mass Sp.* **2019**, *33*, 1703–10.
4. Yang, L.; Yu, H.; Hou, A.; Man, W.; Wang, S.; Zhang, J.; Wang, X.; Zheng, S.; Jiang, H.; Kuang, H. *Front. Pharmacol.* **2021**, *12*, 727154.
5. Zhu, B.; Zhang, Q. L.; Hua, J. W.; Cheng, W. L.; Qin, L. P. *J. Ethnopharmacol.* **2018**, *226*, 143–67.
6. Zhang, W. J.; Zhao, Z. Y.; Chang, L. K.; Cao, Y.; Wang, S.; Kang, C. Z.; Wang, H. Y.; Zhou, L.; Huang, L. Q.; Guo, L. P. *J. Ethnopharmacol.* **2021**, *266*, 113415.
7. Wang, K.; Huang, W.; Sang, X.; Wu, X.; Shan, Q.; Tang, D.; Xu, X.; Cao, G. *Phytomedicine* **2020**, *68*, 153191.
8. Zhou, Y.; Huang, S.; Wu, F.; Zheng, Q.; Zhang, F.; Luo, Y.; Jian, X. *Neurosci. Lett.* **2021**, *759*, 136050.
9. Zhang, Y.; Liu, Y.; Wang, J.; Jiang, Z.; Zhang, L.; Cui, Y.; Zhao, D.; Wang, Y. *Immunopharm. Immunot.* **2022**, *44*, 227–37.
10. Yu, Z.; Yang, Z. *Crit. Rev. Food* **2020**, *60*, 844–58.
11. Kong, X.; Wu, G.; Yin, Y. *Front. Biosci.* **2011**, *3*, 372–84.
12. Kelly, B.; Pearce, E. L. *Cell Metab* **2020**, *32*, 154–75.
13. Luo, C.; Xu, X.; Wei, X.; Feng, W.; Huang, H.; Liu, H.; Xu, R.; Lin, J.; Han, L.; Zhang, D. *Pharmacol. Res.* **2019**, *148*, 104409.
14. Ennis, M. A.; Rasmussen, B. F.; Lim, K.; Ball, R. O.; Pencharz, P. B.; Courtney, M. G.; Elango, R. *Am. J. Clin. Nutr.* **2020**, *111*, 351–9.
15. Lee, J.; Ju, M.; Cho, O. H.; Kim, Y.; Nam, K. T. *Adv. Sci.* **2019**, *6*, 1801255.
16. Le, D. G.; Solon-Biet, S. M.; Cogger, V. C.; Ribeiro, D. R.; Cabo, R. D.; Raubenheimer, D.; Cooney, G.; Simpson, S. J. *Arr.* **2020**, *64*, 101198.
17. Wu, Y. X.; Lu, W. W.; Geng, Y. C.; Yu, C. H.; Sun, H. J.; Kim, Y. J.; Zhang, G.; Kim, T. *Chem. Biodivers.* **2020**, *17*, e2000268.
18. Qian, Y.; Li, W.; Wang, H.; Hu, W.; Wang, H.; Zhao, D.; Hu, Y.; Li, X.; Gao, X.; Yang, W. *Arab. J. Chem.* **2021**, *14*, 102957.
19. Tong, G. Y.; Wu, H. L.; Wang, T.; Chang, Y. Y.; Chen, Y.; Yang, J.; Fu, H. Y.; Yang, X. L.; Li, X. F.; Yu, R. Q. *J. Chromatogr. A.* **2022**, *1674*, 463121.
20. Zhu, M.; Wei, P.; Peng, Q.; Qin, S.; Zhou, Y.; Zhang, R.; Zhu, C.; Zhang, L. *Phytochem. Anal.* **2019**, *30*, 164–81.
21. Shan, G. S.; Zhang, L. X.; Zhao, Q. M.; Xiao, H. B.; Zhuo, R. J.; Xu, G.; Jiang, H.; You, X. M.; Jia, T. Z. *J. Pharm. Biomed. Anal.* **2014**, *98*, 74–84.
22. Lei, R. *Chin. Tradit. Herbal Drugs* **2017**, *48*, 3511–6.
23. Long, F.; Lin, H.; Zhang, X.; Zhang, J.; Xiao, H.; Wang, T. *Front. Pharmacol.* **2020**, *11*, 598939.
24. Ruqiao, L.; Yueli, C.; Xuelan, Z.; Huifen, L.; Xin, Z.; Danjie, Z.; Le, S.; Yanxue, Z. *Pharmazie* **2020**, *75*, 42–55.
25. Castellano, J. M.; Ramos-Romero, S.; Perona, J. S. *Nutrients* **2022**, *14*, 623.
26. Xie, Q.; Wang, C. *Phytochemistry* **2022**, *201*, 113288.
27. Kawamura, A.; Iacovidou, M.; Takaoka, A.; Soll, C. E.; Blumenstein, M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6879–82.
28. Chae, H. S.; Kim, S. Y.; Pel, P.; Huh, J.; Joo, S. W.; Lim, Y. Y.; Park, S. J.; Lim, J. L.; Chin, Y. W. *Molecules* **2020**, *25*, 3064.
29. Liang, C.; Yao, Y.; Ding, H.; Li, X.; Li, Y.; Cai, T. *Phytochem. Anal.* **2022**, *33*, 943–60.
30. Xiang, Z.; Chen, Y.; Qiu, J. *Phytochem. Anal.* **2022**, *33*, 851–68.
31. Sharifi-Rad, J.; Cruz-Martins, N.; López-Jornet, P.; Lopez, E. F.; Harun, N.; Yeskaliyeva, B.; Beyatli, A.; Sytar, O.; Shaheen, S.; Sharopov, F. *Oxid. Med. Cell Longev.* **2021**, *2021*, 6492346.
32. Gao, Q. Q.; Yan, C. P.; Xu, Z. S.; Wu, Y.; Weng, Z. B.; Zhao, G. H.; Zhang, L. J.; He, J. Y.; Cai, B. C.; Chen, Z. P. *Biomed. Chromatogr.* **2016**, *30*, 528–35.
33. Reçber, T.; Nemutlu, E.; Beksac, K.; Aksoy, S.; Kır, S. *Microchemical J.* **2020**, *159*, 105559.
34. Zhan, C.; Wang, H.; Wang, Y. *J. Pharm. Biomed. Anal.* **2022**, *219*, 114899.

35. Wang, T.; Song, Y.; Ai, Z.; Liu, Y.; Li, H.; Xu, W.; Chen, L.; Zhu, G.; Yang, M.; Su, D. *Phytomedicine* **2023**, 154852.
36. Li, D.; Rui, Y. X.; Guo, S. D.; Luan, F.; Liu, R.; Zeng, N. *Lsci* **2021**, *284*, 119921.
37. Zhang, Y.; Bo, C.; Fan, Y.; An, R.; Chen, L.; Zhang, Y.; Jia, Y.; Wang, X. *Biomed. Chromatogr.* **2019**, *33*, e4443.
38. Huang, L. P.; Huang, L. Y.; Li, X. Z.; Yu, X. L. *Chin. J. Hosp. Pharm.* **2015**, *35*, 678–82.
39. Gao, X.; Li, Y.; Meng, M.; Wang, P.; Feng, Y.; Jia, J.; Qin, X. *J. Pharm. Biomed. Anal.* **2020**, *187*, 113293.
40. Xu, L.; Xu, Z.; Strashnov, I.; Liao, X. *Metabolomics* **2020**, *16*, 1–10.
41. Jung, H. R.; Kim, S. J.; Ham, S. H.; Cho, J. H.; Lee, Y. B.; Cho, H. Y. *J. Chromatogr. B.* **2014**, *971*, 64–71.
42. Shi, F.; Pan, H.; Lu, Y.; Ding, L. *J. Sep. Sci.* **2018**, *41*, 3830–9.
43. Zheng, Z.; Hu, H.; Zeng, L.; Yang, H.; Yang, T.; Wang, D.; Zhang, C.; Deng, Y.; Zhang, M.; Guo, D. *Phytochem. Anal.* **2022**, *33*, 72–82.
44. Taipabu, M. I.; Sohilait, H. J.; Viswanathan, K.; Wu, W.; Fransina, E. G.; Naqvi, S. R. *Biomass. Convers. Bior.* **2022**, 1–18.
45. Qin, Y.; Fei, C.; Zhang, W.; Su, L.; Ji, D.; Bian, Z.; Wang, M.; Li, Y.; Mao, C.; Zhao, X. *Chem. Biodivers.* **2022**, *19*, e202200361.
46. Ahn, C.; Obendorf, S. K. *Fiber. Polym.* **2006**, *7*, 158–63.
47. Feas, C. P.; Barciela-Alonso, M.; Bermejo-Barrera, P. *J. Chromatogr. B.* **2011**, *879*, 231–5.
48. Thummar, K.; Vasoya, R.; Dama, B.; Ladolkar, H.; Raval, M.; Sheth, N. *Anal. Chem. Lett.* **2020**, *10*, 93–103.
49. Zhou, T.; Guo, W.; Ren, S.; Li, Y.; Wu, J.; Yang, B. *Carbohyd. Res.* **2021**, *510*, 108462.
50. Kaur, J.; Dhiman, V.; Bhadada, S.; Katare, O.; Ghoshal, G. *Food Chem. Adv.* **2022**, *1*, 100084.
51. Huang, X. F.; Ouyang, H.; Li, J. M.; Lu, Y. M.; Li, W.; Gong, Q. F. *Chin. J. Exp. Tradit. Med. Form.* **2017**, *23*, 27–33.
52. Rafi, M.; Karomah, A. H.; Septaningsih, D. A.; Rahminiwati, M.; Putri, S. P.; Iswantini, D. *Arab. J. Chem.* **2022**, *15*, 104232.
53. Liang, Y.; Yan, G. Y.; Wu, J. L.; Zong, X.; Liu, Z.; Zhou, H.; Liu, L.; Li, N. *Phytochem. Anal.* **2018**, *29*, 398–405.
54. Aboshi, T.; Shimizu, N.; Nakajima, Y.; Honda, Y.; Kuwahara, Y.; Amano, H.; Mori, N. *Insect Biochem. Mol.* **2013**, *43*, 991–6.