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Original Article

Quantitative and qualitative analyses of cytotoxic triterpenoids in the rhizomes of *Anemone raddeana* using HPLC and HPLC-ESI-Q/TOF-MS

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ABSTRACT

Anemone raddeana Regel, a Traditional Chinese Medicine, has been demonstrated to possess cytotoxicity and anti-inflammatory activities. The purpose of this study is to establish analytical methods to identify and quantify the major active constituents in *Anemone raddeana*. A high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (HPLC-ESI-Q/TOF-MS) was used to identify the components in the title plant material. To quantify the major components, a HPLC-UV method was developed and validated. The results showed that **37** compounds were identified based on the MS data and retention times. The contents of eight main bioactive compounds were determined by HPLC simultaneously. These methods could be used to effectively evaluate the quality of *A. raddeana* and provide a valuable reference for further study. In addition, the cytotoxicity activity of the different fractions of *A. raddeana* was determined. Hederacolchiside A1 (**f**) showed promising activity against ten human cancer cell lines with IC_{50} values from 0.29 to 3.48 μM.

1. Introduction

Anemone raddeana Regel is a well-known Traditional Chinese Medicine (TCM) recorded in the Chinese Pharmacopoeia (2015 edition) for the treatment of rheumatism and pain [1]. This herbal is the major components in a few TCM formulas such as Huo-Luo-Wan and Zai-Zao-Wan [2]. Pharmacological and clinical results showed that the total triterpenoid saponins prepared from *A. raddeana* had cytotoxic and anti-inflammation activities [3]. Furthermore, chemical analysis demonstrated that triterpenoids were the major constituents in *A. raddeana* [4]. Some of the pure compounds have been tested using cell line or animal models. For example, raddeanoside A displayed significant cytotoxicity by inhibiting VEGFR2 signaling [5]. In the previous studies, we found that some triterpenoid isolated from *A. raddeana* exhibited promising effects on superoxide generation in human neutrophil, which was associated with anti-inflammation activity [6–9].

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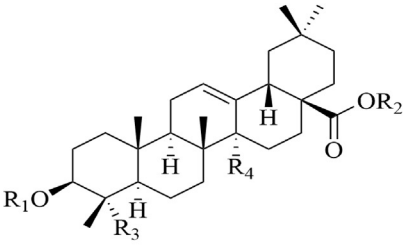

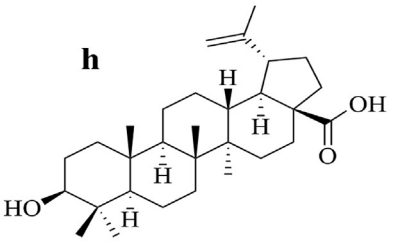

Compound	R_1	R_2	R_3	R_4
a	rha^{2}(glc^{4})ara	rha^{4}glc^{6}glc	CH_3	CH_2OH
b	rha^{2}(glc^{4})ara	rha^{4}glc^{6}glc	CH_3	CH_3
c	glc^{4}ara	rha^{4}glc^{6}glc	CH_3	CH_3
d	rha^{2}ara	rha^{4}glc^{6}glc	CH_3	CH_3
e	rha^{2}(glc^{4})ara	H	CH_3	CH_2OH
f	rha^{2}(glc^{4})ara	H	CH_3	CH_3
g	rha^{2}ara	H	CH_3	CH_3

Fig. 1 – Chemical structures of the eight compounds.

Table 1 – Samples information of *A. raddeana*.

Sample No.	Growth location	Sample No.	Growth location
1	Zuojia, Jilin	11	Yabuli, Heilongjiang
2	Huadian,Jilin	12	Heilongjiang
3	Tiangang,Jilin	13	Heilongjiang
4	Jingyu, Jilin	14	Qingyuan, Liaoning
5	Tonghua, Jilin	15	Benxi, Liaoning
6	Meihekou, Jilin	16	Fengcheng, Liaoning
7	Jilinshi, Jilin	17	Qianshan, Liaoning
8	Wuchang, Heilongjiang	18	Kuandian, Liaoning
9	Acheng, Heilongjiang	19	Aiyang, Liaoning
10	Yimianpo, Heilongjiang		

TCM plays an important role in public health due to its effectiveness [10]. However, quality control is challenge for TCM. A few methods, such as HPLC-UV, LC-MS/MS, and LC-NMR, have been developed and tested for the quality control of TCM [11–14]. For *A. raddeana*, a few analytic methods using HPLC have been reported. However, most of them only measured raddeanin A or raddeanin D [15–17]. The concern is that other components may also be active. To globally control the quality of the title plant material, we developed a HPLC-ESI-Q/TOF-MS method to identify the constituents in *A. raddeana*. In addition, we developed and validated an HPLC method to quantify eight bioactive triterpenoids. To provide scientific evidence to justify the traditional usage, we tested the activity of the major components in *A. raddeana* against several cancer cell lines.

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade methanol and acetonitrile were purchased from Yuwang Group Co., Ltd. (Shandong, China). The standard compounds, 3-O-α-L-rhamnopyranosyl (1 → 2)[β-D-glucopyranosyl (1 → 4)]-α-L-arabinopyranosyl-27-hydroxy-oleanolic acid 28-O-α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranosyl (1 → 6)-β-D- glucopyranoside (**a**), hederacholichiside E (**b**), raddeanoside R_{19} (**c**), hederacholichiside B (**d**), raddeanoside R_{20} (**e**), hederacolchiside A1 (**f**), eleutheroside K (**g**), and betulinic acid (**h**) were purified from the rhizome of *A. raddeana*. The purity of

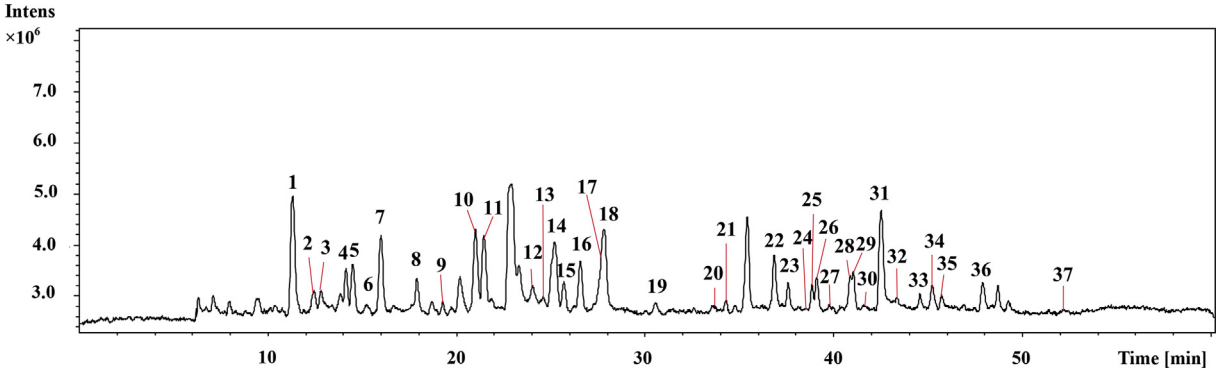

Fig. 2 – Representative total ion chromatograms of *A. raddeana* obtained by HPLC-ESI-Q/TOF-MS in negative scan mode.

Table 2 – The compounds identified by HPLC-ESI-Q/TOF-MS.

Peak	t_R (min)	Formula M	[M−H]⁻		Fragmentation	Structure[a]				Reference
			Measured mass	Calcd mass		R_1	R_2	R_3	R_4	
1	11.371	$C_{65}H_{106}O_{31}$	1381.6645	1381.6645	911.5011 [M-H-rha-glc-glc]	rha1 → 2 [glc (1 → 4)]ara	rha1 → 4glc1 → 6glc	CH_2OH	CH_3	[23,24]
2	12.509	$C_{59}H_{96}O_{27}$	1235.6066	1235.6138	765.4435 [M-H-rha-glc-glc]	glc1 → 4ara	rha1 → 4glc1 → 6glc	CH_2OH	CH_3	[23]
3	12.86	$C_{65}H_{106}O_{31}$	1381.6645	1381.665	911.4989 [M-H-rha-glc-glc]	rha1 → 2 [glc (1 → 4)]ara	rha1 → 4glc1 → 6glc	CH_3	CH_2OH	[25]
4	14.200	$C_{65}H_{106}O_{31}$	1381.6645	1381.6698	911.4986 [M-H-rha-glc-glc]	rha1 → 2glc1 → 2ara	rha1 → 4glc1 → 6glc	CH_3	CH_2OH	[20]
5	14.568	$C_{59}H_{96}O_{26}$	1219.6117	1219.6134	749.4490 [M-H-rha-glc-glc]	rha1 → 2ara	rha1 → 4glc1 → 6glc	CH_2OH	CH_3	[24,26]
6	15.455	$C_{53}H_{86}O_{22}$	1073.5538	1073.5523	603.3893 [M-H-rha-glc-glc]	ara	rha1 → 4glc1 → 6glc	CH_2OH	CH_3	[23,24]
7	16.041	$C_{59}H_{96}O_{27}$	1235.6066	1235.6083	765.4467 [M-H-rha-glc-glc]	glc1 → 2ara	rha1 → 4glc1 → 6glc	CH_2OH	CH_3	[27]
8	17.933	$C_{59}H_{96}O_{27}$	1235.6066	1235.6141	–	glc1 → 4ara	rha1 → 4glc1 → 6glc	CH_3	CH_2OH	[25]
9	19.322	$C_{59}H_{96}O_{26}$	1219.6117	1219.6166	749.4540 [M-H-rha-glc-glc]	rha1 → 2ara	rha1 → 4glc1 → 6glc	CH_3	CH_2OH	[21]
10	21.013	$C_{65}H_{106}O_{31}$	1381.6645	1381.6715	911.5004 [M-H-rha-glc-glc]	rha1 → 2glc1 → 2ara	rha1 → 4glc1 → 6glc	CH_3	CH_2OH	[25]
11	21.465	$C_{70}H_{114}O_{34}$	1497.7119	1497.7165	1027.549 [M-H-rha-glc-glc]	ara1 → 3rha1 → 2 [glc (1 → 4)]ara	rha1 → 4glc1 → 6glc	CH_3	CH_3	[25]
12	24.027	$C_{65}H_{106}O_{30}$	1365.6696	1365.6686	895.5039 [M-H-rha-glc-glc]	rha1 → 2glc1 → 2ara	rha1 → 4glc1 → 6glc	CH_3	CH_3	[25,26]
13	24.579	$C_{65}H_{106}O_{30}$	1365.6696	1365.6678	–	rha1 → 2 [glc (1 → 4)]ara	rha1 → 4glc1 → 6glc	CH_3	CH_3	[28]
14	25.266	$C_{59}H_{96}O_{26}$	1219.6117	1219.6166	749.4502 [M-H-rha-glc-glc]	glc1 → 4ara	rha1 → 4glc1 → 6glc	CH_3	CH_3	[19]
15	25.701	$C_{59}H_{96}O_{26}$	1219.6117	1219.6155	749.4504 [M-H-rha-glc-glc]	glc1 → 2ara	rha1 → 4glc1 → 6glc	CH_3	CH_3	[29]
16	26.572	$C_{64}H_{104}O_{29}$	1335.6591	1335.6655	865.4963 [M-H-rha-glc-glc]	ara1 → 3rha1 → 2ara	rha1 → 4glc1 → 6glc	CH_3	CH_3	[25]
17	27.559	$C_{65}H_{106}O_{31}$	1381.6645	1381.6715	911.5011 [M-H-rha-glc-glc]	glc1 → 2 [glc (1 → 4)]ara	rha1 → 4glc1 → 6glc	CH_3	CH_3	[25]
18	27.76	$C_{59}H_{96}O_{25}$	1203.6168	1203.627	733.4583 [M-H-rha-glc-glc]	rha1 → 2ara	rha1 → 4glc1 → 6glc	CH_3	CH_3	[25,30]
19	30.573	$C_{53}H_{86}O_{21}$	1057.5589	1057.5684	587.4005 [M-H-rha-glc-glc]	ara	rha1 → 4glc1 → 6glc	CH_3	CH_3	[23,26]
20	33.603	$C_{47}H_{76}O_{18}$	927.4959	927.5085	–	glc1 → 2 [glc (1 → 4)]ara	H	CH_2OH	CH_3	[27]
21	34.205	$C_{52}H_{84}O_{21}$	1043.5432	1043.5538	–	ara1 → 3rha1 → 2 [glc (1 → 4)]ara	H	CH_2OH	CH_3	[26]
22	36.767	$C_{47}H_{76}O_{17}$	911.5010	911.5055	–	rha1 → 2 [glc (1 → 4)]ara	H	CH_2OH	CH_3	[21,31]
23	37.503	$C_{47}H_{76}O_{17}$	911.5010	911.5126	–	rha1 → 2 [glc (1 → 4)]ara	H	CH_3	CH_2OH	[25]
24	38.457	$C_{48}H_{78}O_{17}$	925.5166	925.5286	455.3650 [M-H-rha-glc-glc]	H	rha1 → 4glc1 → 6glc	CH_3	CH_3	[32]
25	38.809	$C_{41}H_{66}O_{12}$	749.4482	749.4586	–	rha1 → 2ara	H	CH_2OH	CH_3	[24]
26	39.077	$C_{41}H_{66}O_{13}$	765.4431	765.4547	603.3992 [M-H-glc]	glc1 → 4ara	H	CH_2OH	CH_3	[23,24]
27	39.646	$C_{36}H_{56}O_9$	631.3852	631.3931	455.2545 [M-H-glcA]	glcA	H	CH_3	CH_3	[33]
28	40.784	$C_{53}H_{86}O_{21}$	1057.5589	1057.5677	–	glc1 → 2ara	rha1 → 4glc	CH_3	CH_3	[25,34]
29	40.868	$C_{47}H_{76}O_{17}$	911.5010	911.5102	455.2526 [M-H-glc-glc-ara]	glc1 → 2 [glc (1 → 4)]ara	H	CH_3	CH_3	[28]
30	41.571	$C_{41}H_{66}O_{12}$	749.4482	749.4543	–	rha1 → 2ara	H	CH_3	CH_2OH	[22,25]
31	42.459	$C_{47}H_{76}O_{16}$	895.5061	895.5156	–	rha1 → 2 [glc (1 → 4)]ara	H	CH_3	CH_3	[22,24]
32	43.296	$C_{35}H_{55}O_8$	603.3891	603.3922	471.3511 [M-H-ara]	ara	H	CH_2OH	CH_3	[26]
33	44.518	$C_{47}H_{76}O_{16}$	895.5061	895.5102	–	rha1 → 2glc1 → 2ara	H	CH_3	CH_3	[35]
34	45.187	$C_{41}H_{66}O_{12}$	749.4482	749.4516	587.3973 [M-H-glc]	glc1 → 2ara	H	CH_3	CH_3	[25,35]
35	45.639	$C_{46}H_{74}O_{15}$	865.4955	865.4979	–	ara1 → 3rha1 → 2ara	H	CH_3	CH_3	[18]
36	47.849	$C_{41}H_{66}O_{11}$	733.4521	733.4602	587.4005 [M-H-rha]	rha1 → 2ara	H	CH_3	CH_3	[22,26]
37	52.152	$C_{35}H_{56}O_7$	587.3953	587.3992	–	ara	H	CH_3	CH_3	[25]

[a] The basic skeleton is the same as depicted in Fig. 1 for compounds **a**–**g**.

these standards was all above 98.0% by HPLC analysis. Their structures (Fig. 1) were elucidated by spectroscopic analysis (1D, 2D NMR) [4,18–22]. Other chemicals were analytical grade.

2.2. Plants materials

Nineteen batches of *A. raddeana* (Sample 1–19) were collected from different regions in northeast of China in May 2014. These samples were authenticated by Professor Lu, (Department of Pharmacognosy, Shenyang Pharmaceutical University). The information was listed in Table 1.

2.3. Preparation of sample solutions

2.3.1. Preparation of sample solutions for quantification

Nineteen batches of *A. raddeana* were grounded into powder. The powder (5.0 g) of each batch was extracted by reflux with 75% ethanol (3 × 50 mL, each 1 h). The combined extract was filtered and the solvent was removed using rotary vaporization under vacuum. The residue was dissolved in water and loaded to the HPD400 macroporous resin column (10.0 g), which was eluted with water till the eluate showed negative response to Molish reaction, followed by 70% ethanol till void of saponin. The saponin containing fractions were evaporated to remove ethanol, followed by in vacuo-drying at 40 °C to obtain the total saponin. An accurately weighed total saponin was dissolved in methanol and filtrated through 0.45 μm micropore membrane for analysis.

2.3.2. Preparation of sample solutions for cytotoxic activity assay

Air-dried and powdered *A. raddeana* was refluxed with 75% ethanol. The dry extract was suspended in water and then successively partitioned with petroleum ether (PE), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and n-butanol (BuOH). Remove solvent under vacuum to afford PE, CH_2Cl_2, EtOAc, and BuOH fractions. These fractions were dissolved in DMSO to afford stock solutions used in MTT assay. Doxorubicin was used as the positive control.

2.4. Preparation of standard solutions

A standard solution of initial concentration was prepared by accurately weighing each standard sample (compound **a**–**h**) and dissolved with methanol, which was stored in a 10 mL volumetric flask. Then the standard solution was diluted to a series of different concentrations. Each standard working solution was filtered by 0.45 μm micropore membrane.

2.5. HPLC-ESI-Q/TOF-MS analysis

The experiment was performed on quadrupole time-of-flight tandem mass spectrometer coupled with an electrospray ionization source in the negative ion mode from *m/z* 50 to 1500 (Bruker Co., Karlsruhe, Germany). The instrumental parameters for the mass spectrometric analysis were set as follows: end plate offset, −500 V; capillary voltage, 3800 V; nebulizer gas pressure, 1.2 bar; temperature 180 °C and the flow rate was 8.0 L/min. All data were acquired and processed using Bruker Daltonics DataAnalysis 3.4. Software (Bruker Co., Germany).

2.6. HPLC analysis

2.6.1. HPLC conditions

HPLC analysis was operated on a Lab Alliance-Series III (SSI, USA). A C_{18} analytical column (250 mm × 4.6 mm, 5 μm, Alltech Associates Co., USA) was used. The separation was

Table 3 – Results of calibration curves, LOD and LOQ.

Compound	Calibration curve	R^2	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
a	$y = 1.006 \times 10^6x + 371\,381$	0.9990	34–270	1.05	4.06
b	$y = 1.174 \times 10^6x + 4753$	0.9991	86–688	0.33	0.79
c	$y = 6.916 \times 10^5x + 92951$	0.9997	42–338	1.21	3.86
d	$y = 1.088 \times 10^6x + 1528223$	0.9998	27–213	0.48	1.28
e	$y = 1.155 \times 10^6x + 67640$	0.9999	34–270	0.52	1.37
f	$y = 1.880 \times 10^6x + 195490$	0.9997	44–354	0.24	0.80
g	$y = 1.458 \times 10^6x + 261874$	0.9990	46–371	0.21	0.69
h	$y = 4.394 \times 10^5x + 367245$	0.9995	27–216	0.41	1.62

Table 4 – Precision, Stability, Reproducibility and Recovery ($n = 6$) in quantitation.

Compound	Precision (RSD, %)		Stability	Repeatability	Recovery	
	Intra-day	Inter-day	(RSD, %)	(RSD, %)	Average (%)	RSD (%)
a	1.12	2.59	1.45	1.45	98.3 ± 1.78	1.81
b	1.64	1.68	2.45	2.20	101.03 ± 2.92	2.89
c	1.02	2.05	1.10	1.10	95.92 ± 3.41	3.55
d	2.22	2.20	1.60	0.88	94.55 ± 2.95	3.12
e	1.81	3.18	1.81	1.81	96.67 ± 3.25	3.36
f	2.49	2.74	1.80	1.88	99.13 ± 2.57	2.59
g	1.07	1.21	0.70	2.74	103.57 ± 2.45	1.90
h	1.02	1.97	2.05	2.44	96.77 ± 2.91	3.01

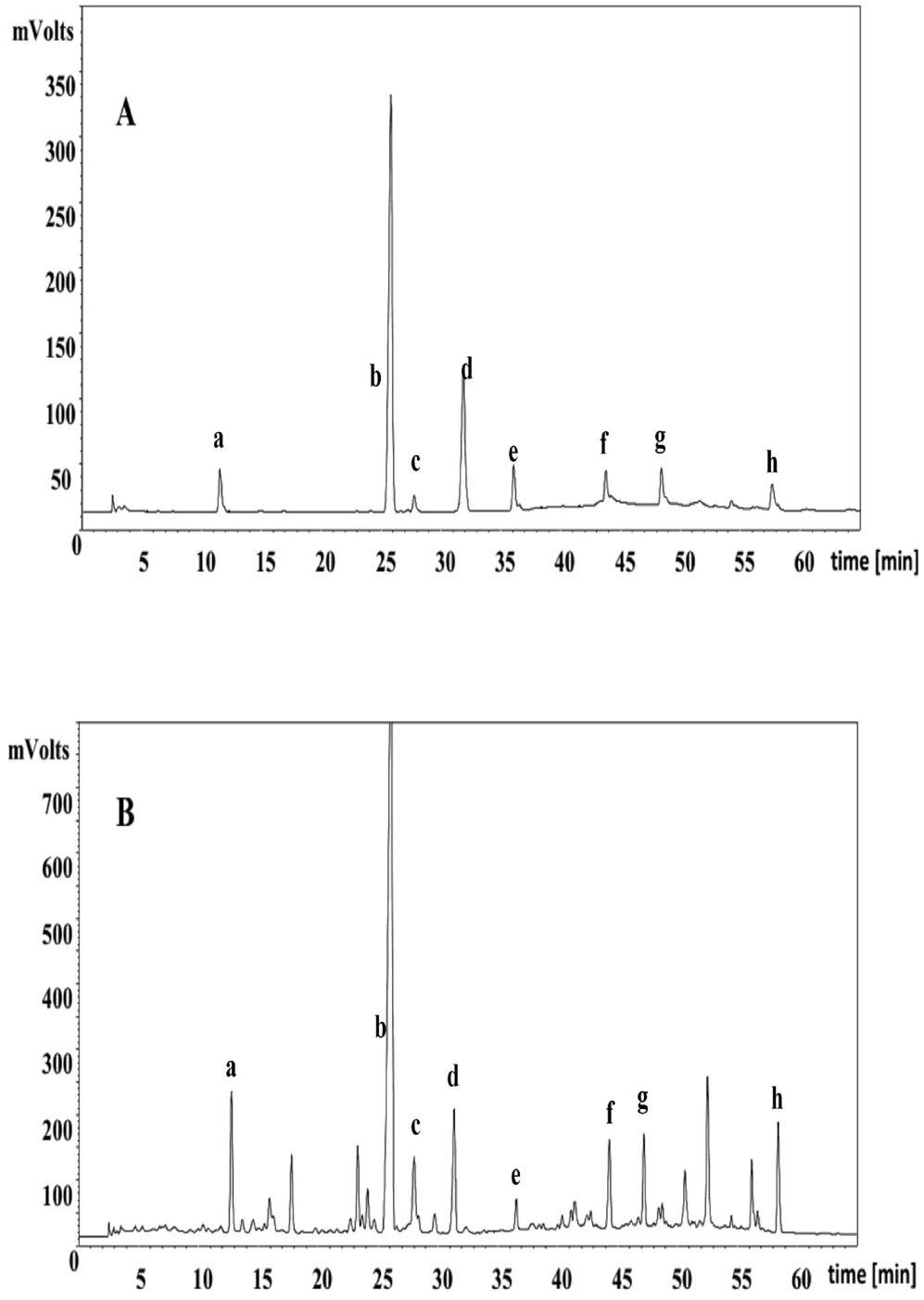

Fig. 3 – HPLC chromatogram of standard chemicals (A) and sample extract (B).

achieved by a linear gradient elution program of the two combined eluents: A (acetonitrile) and B [0.1% phosphoric acid (v/v) in water]. The detailed gradient elution was as follows: 0–28 min, 23%–36% A; 28–42 min, 36%–56% A; 42–52 min, 56%–90% A. The flow rate of the mobile phase was 1.0 mL/min. The wavelength was set at 203 nm. The column temperature was 30 °C. The injection volume was 10 μL.

2.6.2. *HPLC method validation*

The developed method was validated for its linearity, LODs (limit of detection), LOQs (limit of quantification), precision, repeatability, and sample stability. All calibration curves were plotted based on linear regression analysis of the integrated peak areas (y) versus concentrations (x) of identified constituents in the standard solutions to obtain a linear equation $y = ax + b$. The standard solution containing eight reference compounds was diluted to six different concentrations with methanol for HPLC injection. The experiment was performed in triplicate.

The LODs and LOQs were determined at signal-to-noise ratios (S/N) of 3 and 10, respectively.

Intra-day and inter-day variations were utilized to evaluate the precision. The intra-day variation was determined by successive analysis of the same sample solution six times within one day and inter-day variation was determined on three consecutive days. For testing stability, the same sample solution was analyzed after prepared for 0 h, 2 h, 4 h, 8 h, 12 h, and 24 h. To confirm the repeatability, six different working solutions from the same sample were injected. The recovery was determined by spiking the extracts with exact amount of each reference compound. Eight triterpenoids were spiked into the samples (the same as the known amounts), and then extracted, processed and quantified in accordance with the established method. The precision, repeatability and the stability were analyzed and variations expressed by RSD. The percent recovery rates for the analytes were presented as mean ± SD.

2.7. *Cell culture*

All of the cell lines, including Lung cancer (A549), human hepatocarcinoma (Hep-G2), human breast adenocarcinoma cell lines (MCF-7), human pancreatic cancer (CFPAC-1), human hepatocarcinoma (Hep 3B), human colon cancer (HT-29), human oral epidermoid carcinoma cells (KB), human esophageal cancer cell line (Eca-109), Lung cancer (SPC-A-1), gastric cancer cell line (SGC-7901), bladder cancer cell (5637), acute myeloid leukemia cells (HL-60), and Human glioma cells (U251), were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and maintained at 37 °C with 5% CO_2.

2.8. *Viability assay*

The cells were treated with the test compounds at different concentrations for 72 h. Then 10 μL MTT solution was added and the mixture was incubated for another 4 h at 37 °C. After the removal of the culture medium, 100 μL dimethyl sulfoxide (DMSO) was added. The absorbance was measured at 570 nm on Varioskan Flash Microplate Reader (Thermo Scientific, USA).

2.9. *Statistical analysis*

All values were represented by mean ± SD. The IC_{50} values were calculated by the SPSS 19.0 software.

3. Results and discussion

3.1. *Identification of main chemical constituents of* A. raddeana

To identify the main chemical constituents in A. *raddeana*, a HPLC-ESI-Q/TOF-MS method was used in negative scan mode. A representative chromatogram was shown in Fig. 2. Based on

Table 5 – Contents of the eight components in different batches of A. *raddeana* samples (mg/g, n = 3).

Samples.NO.	compound a	compound b	compound c	compound d	compound e	compound f	compound g	compound h
1	3.48 ± 0.080	10.65 ± 0.269	1.25 ± 0.030	2.16 ± 0.042	0.61 ± 0.008	1.66 ± 0.037	–	0.18 ± 0.004
2	3.06 ± 0.080	10.73 ± 0.178	1.96 ± 0.036	2.19 ± 0.039	0.58 ± 0.007	2.18 ± 0.039	0.46 ± 0.012	–
3	3.59 ± 0.090	12.25 ± 0.239	1.98 ± 0.041	1.86 ± 0.022	0.81 ± 0.015	2.16 ± 0.029	0.35 ± 0.004	0.17 ± 0.005
4	3.63 ± 0.100	12.13 ± 0.232	1.40 ± 0.036	0.68 ± 0.009	0.42 ± 0.006	1.16 ± 0.023	0.19 ± 0.004	–
5	4.17 ± 0.060	12.58 ± 0.308	1.92 ± 0.021	1.59 ± 0.016	0.32 ± 0.008	0.74 ± 0.014	0.22 ± 0.006	0.10 ± 0.001
6	4.21 ± 0.090	11.71 ± 0.253	1.27 ± 0.014	1.87 ± 0.028	0.82 ± 0.018	2.33 ± 0.046	0.52 ± 0.009	–
7	2.95 ± 0.050	10.88 ± 0.185	1.46 ± 0.038	1.99 ± 0.044	0.49 ± 0.006	1.50 ± 0.017	0.36 ± 0.006	0.10 ± 0.001
8	3.06 ± 0.060	10.73 ± 0.145	1.96 ± 0.030	2.19 ± 0.037	0.58 ± 0.016	2.18 ± 0.054	0.46 ± 0.011	–
9	2.39 ± 0.040	8.70 ± 0.114	1.20 ± 0.020	3.41 ± 0.080	0.64 ± 0.010	2.45 ± 0.026	0.62 ± 0.017	0.31 ± 0.003
10	3.93 ± 0.070	9.01 ± 0.105	1.64 ± 0.028	2.13 ± 0.052	0.78 ± 0.020	1.92 ± 0.020	0.47 ± 0.013	0.10 ± 0.002
11	3.60 ± 0.070	10.38 ± 0.274	2.78 ± 0.064	2.14 ± 0.037	0.48 ± 0.006	1.58 ± 0.037	0.25 ± 0.004	0.10 ± 0.001
12	3.93 ± 0.100	9.29 ± 0.252	2.09 ± 0.058	4.32 ± 0.055	0.72 ± 0.011	2.41 ± 0.045	0.88 ± 0.020	0.13 ± 0.002
13	1.96 ± 0.030	12.14 ± 0.243	1.25 ± 0.032	1.88 ± 0.040	0.86 ± 0.015	2.87 ± 0.068	0.42 ± 0.008	0.18 ± 0.002
14	2.66 ± 0.050	8.20 ± 0.216	2.39 ± 0.057	1.39 ± 0.024	0.73 ± 0.010	2.58 ± 0.050	0.27 ± 0.006	0.27 ± 0.004
15	2.94 ± 0.070	11.65 ± 0.308	0.48 ± 0.009	2.18 ± 0.047	0.69 ± 0.016	2.29 ± 0.062	0.56 ± 0.010	0.60 ± 0.011
16	2.73 ± 0.030	10.20 ± 0.141	1.11 ± 0.026	5.94 ± 0.163	0.68 ± 0.009	1.86 ± 0.042	0.84 ± 0.016	0.53 ± 0.006
17	4.06 ± 0.110	14.12 ± 0.387	2.85 ± 0.048	2.03 ± 0.049	0.55 ± 0.011	1.55 ± 0.018	0.16 ± 0.004	–
18	3.31 ± 0.050	13.93 ± 0.299	1.61 ± 0.042	1.83 ± 0.027	0.46 ± 0.012	1.30 ± 0.028	0.14 ± 0.003	0.17 ± 0.002
19	3.69 ± 0.050	12.25 ± 0.272	1.70 ± 0.026	2.09 ± 0.037	0.66 ± 0.016	1.81 ± 0.034	0.26 ± 0.006	0.27 ± 0.005

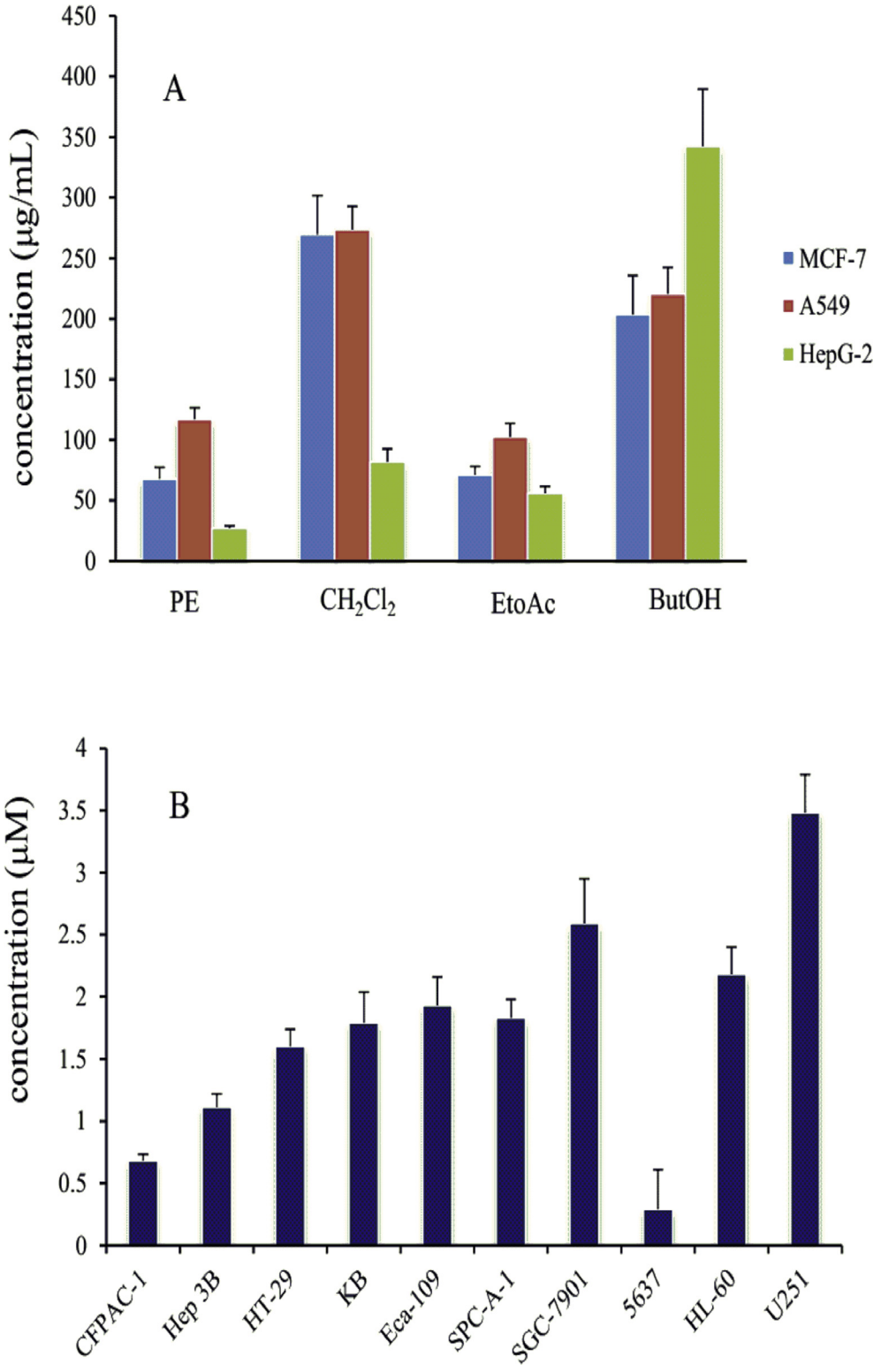

Fig. 4 – Cytotoxic activity of different part of extract (A) and hederacolchiside A1 (B).

the retention times, molecular formula, and the MS/MS data, **37** compounds were identified (Table 2).

3.2. *Optimization of HPLC method*

The RP-HPLC condition for analyzing these triterpenoid glycosides was optimized. Acetonitrile and 0.1% phosphoric acid system as eluent using a C_{18} reversed-phase column and detection at 203 nm showed the best separation and peak shape.

3.3. *Calibration and validation*

The method was validated in terms of linearity, LODs, LOQs, precision, stability, repeatability and recovery test [36]. All calibration curves showed good linear regression ($R^2 > 0.999$). The LODs and the LOQs for the analytes were less than 1.21 and 4.06 μg/mL (Table 3). The RSD values of precision, stability, and repeatability were less than 2.74%. The average recoveries of the analytes were 94.55%–103.57% and RSD values were less than 3.55% (Table 4). Therefore, the HPLC method was precise, accurate and sensitive enough for simultaneously quantitating eight compounds (**a**–**h**) in the extracts of *A. raddeana*.

3.4. *Quality evaluation of the eight compounds*

The proposed method was applied to simultaneously determine the eight compounds in 19 batches of *A. raddeana* samples. A representative HPLC chromatogram was shown in Fig. 3A. The content of eight analytes in 19 samples were listed in Table 5 and that of hederacholichiside B (**d**) (0.68–5.94 mg/g) showed the most remarkable difference and that of hederacholichiside E (**b**) (8.20–14.12 mg/g) had the highest amount. Based on this result, the content of each compound varied to a great extent among different sources. The relationship between the bioactivity and the content of these major glycosides should be further studied.

3.5. *MTT cytotoxicity assay*

The results of different fraction of *A. raddeana* were shown in Fig. 4A. Petroleum ether and ethyl acetate fraction showed the potential cytotoxicity. The anti-proliferation activity of hederacolchiside A1 (**f**) against ten cancer cells lines was evaluated. As compound **f** exhibited a strong cytotoxicity with IC_{50} values from 0.29 to 3.48 μM (Fig. 4B) and was isolated from the ethyl acetate fraction, this fraction contained the cytotoxic ingredients of *A. raddeana*.

4. Conclusions

Thirty seven compounds were identified from *A. raddeana* primarily by HPLC-ESI-Q/TOF-MS for the first time. Eight triterpenoid glycosides were simultaneously quantified by HPLC for the quality control. The cytotoxic activity of the extract of *A. raddeana* was tested. The result indicated that the ethyl acetate soluble fraction was the most potent. Further separation from this fraction yielded hederacolchiside A1, a triterpenoid glycoside, which showed good inhibitory activity against ten human cancer cells lines, providing scientific evidence for the potential of *A. raddeana* as an anti-cancer traditional medicine.

Conflicts of interest statement

The authors declare that there is no conflict of interest.

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