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A simple and rapid method to identify and quantitatively analyze triterpenoid saponins in *Ardisia crenata* using ultrafast liquid chromatography coupled with electrospray ionization quadrupole mass spectrometry

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ABSTRACT

Ardisia plant species have been used in traditional medicines, and their bioactive constituents of 13,28-epoxy triterpenoid saponins have excellent biological activities for new drug development. In this study, a fast and simple method based on ultrafast liquid chromatography coupled to electrospray ionization mass spectrometry (UFLC–MS) was developed to simultaneously identify and quantitatively analyze triterpenoid saponins in *Ardisia crenata* extracts. In total, 22 triterpenoid saponins, including two new compounds, were identified from *A. crenata*. The method exhibited good linearity, precision and recovery for the quantitative analysis of eight marker saponins. A relative quantitative method was also developed using one major saponin (ardisiacrispin B) as the standard to break through the choke-point of the lack of standards in phytochemical analysis. The method was successfully applied to quantitatively analyze saponins in commercially available plant samples. This study describes the first systematic analysis of 13,28-epoxy-oleanane-type triterpenoid saponins in the genus *Ardisia* using LC-ESI–MS. The results can provide the chemical support for further biological studies, phytochemotaxonomical studies and quality control of triterpenoid saponins in medicinal plants of the genus *Ardisia*.

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1. Introduction

Triterpenoid saponins, which are structurally defined as the glycosides of triterpenes, are among the most widely distributed plant component types with a large diversity of chemical structures and biological activities [1,2]. The triterpenoid saponins with a 13,28-epoxy moiety in their sapogenin structure are distributed only in certain plant genera of the families of Myrsinaceae, Primulaceae, Aceraceae, Icacinaceae and Apiaceae, but numerous compounds of this group of triterpenoid saponins are reported to have excellent biological activities [3]. For example, saikosaponins from *Bupleurum chinense* (Apiaceae) have significant anti-inflammatory and hepatoprotective effects [4,5], maesabalides from *Maesa balansae* (Myrsinaceae) have potent and specific *in vitro* and *in vivo* antileishmanial activity [6], and sakuraso-saponins from *Primula sieboldii* (Primulaceae) have strong antifungal activities against *Candida albicans* [7].

Ardisia, which is the largest genus in the family Myrsinaceae, contains approximately 500 species. The plants that belong to the genus *Ardisia* are evergreen shrubs or trees, which are found throughout subtropical and tropical regions, and various species are used in traditional medicines. The plants that belong to the genus *Ardisia* are also important sources of 13,28-epoxy triterpenoid saponins [8,9]. Until now, approximately 50 triterpenoid saponins have been isolated from 10 *Ardisia* species, and these *Ardisia* saponins are reported to have biological activities such as cAMP phosphodiesterase inhibitory activity [10], a prostaglandin E2-like effect [11], and cytotoxicity against human cancer cells [12]. In addition, we recently reported that 13,28-epoxy triterpenoid saponins from *Ardisia japonica* selectively inhibited the proliferation of liver cancer cells without affecting normal liver cells [13]. The structural features and diverse biological

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Table 1
UFLC-ESI–MS analysis for triterpenoid saponins in *Ardisia*.

Saponins	Structure		R_t[a] (min)	Formula	MW	Positive ion (*m/z*)			Negative ion (*m/z*)	
	Aglycone	Sugar				$[M+Na]^+$	$[Aglycone-H_2O+H]^+$	Others	$[M-H]^-$	$[M+HCOOH-H]^-$
1[b,d]	I	S4x	4.46	$C_{58}H_{94}O_{28}$	1239.35	n.d.[e]	471.35	453.10, 435.25	1237.80	1283.65
2[b,d]	I	S4r	4.62	$C_{59}H_{96}O_{28}$	1253.38	n.d.[e]	471.15	453.10, 435.20	1252.30	1298.25
3[b,d]	III	S4x	6.62	$C_{52}H_{84}O_{23}$	1077.21	1100.10	471.20	453.15, 435.10	1075.65	1121.30
4[c]	VI	S4x	7.05	$C_{52}H_{84}O_{22}$	1061.20	1084.35	455.15	437.20, 419.15	1060.10	1105.95
5[b,d]	VI	S4r	7.23	$C_{53}H_{86}O_{22}$	1075.24	1097.90	455.10	437.05, 419.20	1073.75	1120.30
6	VII	S4x	7.98	$C_{52}H_{84}O_{23}$	1077.21	1099.55	471.30	453.21, 435.10	1076.00	1122.00
7[b,d]	VII	S4r	8.28	$C_{53}H_{86}O_{23}$	1091.24	1113.80	471.10	453.10, 435.20	1090.00	1136.00
8[b,d]	VIII	S4x	8.39	$C_{52}H_{86}O_{22}$	1063.23	1085.50	457.25	439.10, 421.15	1061.50	1107.80
9[b,d]	VIII	S4r	8.73	$C_{53}H_{88}O_{22}$	1077.25	1101.10	457.20	439.15, 421.10	1076.10	1121.65
10[c]	IX	S4x	9.35	$C_{52}H_{82}O_{23}$	1075.19	1097.80	469.00	453.15	1073.30	1139.55
11[b,d]	IX	S4r	9.53	$C_{53}H_{84}O_{23}$	1089.22	1111.05	469.20	453.15	1087.20	1133.10
12[b,d]	X	S4x	11.73	$C_{52}H_{84}O_{22}$	1061.21	1083.85	455.15	437.10, 419.25	1060.10	1106.45
13[b,d]	X	S4r	12.04	$C_{54}H_{88}O_{21}$	1075.24	1097.95	455.15	437.20, 419.10	1073.60	1119.60
14	X	S3g	12.46	$C_{47}H_{76}O_{18}$	929.00	951.15	455.15	437.35, 419.20	927.20	973.30
15	XII	S4x	12.83	$C_{52}H_{82}O_{22}$	1059.19	1081.70	453.30	435.10, 417.35	1057.85	1104.30
16[b,d]	XII	S4r	13.14	$C_{53}H_{84}O_{22}$	1072.55	1096.10	453.20	435.00, 417.10	1071.75	1117.90
17[b,d]	X	S3x	14.49	$C_{46}H_{74}O_{17}$	899.10	921.55	455.15	437.20, 419.25	898.10	944.30
18[b,d]	X	S3r	14.56	$C_{47}H_{76}O_{17}$	913.10	936.15	455.10	437.30, 419.15	912.10	957.65
19[b,d]	X	S2g1	15.47	$C_{41}H_{66}O_{13}$	766.45	789.10	455.20	437.20, 407.05	765.30	811.20
20[b,d]	X	S2g2	15.58	$C_{41}H_{66}O_{13}$	766.45	789.20	455.30	437.30, 407.05	765.50	811.45
21	XII	S3x	16.60	$C_{46}H_{72}O_{17}$	897.10	921.75	453.35	435.15, 417.20	896.45	942.10
22[b,d]	XII	S3r	16.76	$C_{46}H_{72}O_{17}$	911.20	934.20	453.15	435.2, 417.35	910.10	956.55
23[d]	II	S4r	6.27	$C_{53}H_{88}O_{22}$	1077.25	1099.75	457.15	439.15, 421.10	1076.35	1122.20
24[d]	III	S4r	6.65	$C_{53}H_{86}O_{23}$	1091.24	1114.10	471.10	453.10, 435.20	1090.20	1136.10
25[d]	IV	S4r	6.97	$C_{53}H_{86}O_{23}$	1091.21	1113.85	471.15	453.15, 435.15	1090.15	1136.15
26[d]	V	S4r	7.35	$C_{52}H_{86}O_{22}$	1063.24	1086.15	425.10	407.15	1062.25	1108.10
27[d]	X	S5x	11.39	$C_{58}H_{94}O_{27}$	1223.35	1245.65	455.15	437.20, 419.15	1222.40	1266.65
28[d]	XI	S7x	12.53	$C_{70}H_{114}O_{37}$	1547.60	1570.10	455.20	437.20	1545.95	1591.50
29[d]	XIII	S7x	18.19	$C_{70}H_{116}O_{36}$	1533.62	1555.95	441.15	423.10, 405.15	1532.35	1578.75
30[d]	XIII	S4r	18.23	$C_{53}H_{88}O_{21}$	1061.25	1084.10	441.10	423.10, 405.10	1060.25	1106.10
31[d]	XIII	S6x	19.10	$C_{64}H_{106}O_{31}$	1371.55	1393.85	441.25	423.15, 405.15	1370.45	1416.35
32[d]	XIII	S3g	19.98	$C_{47}H_{78}O_{18}$	915.10	938.20	441.20	423.10, 405.20	914.10	960.15
33[d]	XIII	S3x	20.81	$C_{46}H_{76}O_{16}$	885.12	907.55	441.30	423.20, 405.15	883.65	929.75
34[d]	XIII	S3r	20.95	$C_{47}H_{78}O_{16}$	899.13	921.70	441.15	423.10, 405.20	898.10	944.05

[a] R_t, retention time.
[b] Structurally confirmed by comparison with reference compounds.
[c] New compounds.
[d] Reference.
[e] n.d., not detectable.

activities of *Ardisia* saponins make them attractive targets for new drug discovery. However, no comprehensive profile of the saponins in *Ardisia* species is available, and analytical studies were not reported. Therefore, a simple and rapid analytical method to identify and quantify triterpenoid saponins in the genus *Ardisia* should be developed.

HPLC-coupled MS detection is a powerful tool to both identify and quantitatively analyze triterpenoid saponins because it has a much better detection sensitivity and produces better structural information than classical analytical methods such as TLC, HPLC-UV, and HPLC-ELSD [14,15]. Ultrafast liquid chromatography (UFLC) results in short analysis time and increased peak resolution, capacity, and sensitivity using columns that contain particles with a diameter of <2 μm and fluidic systems that operate at higher pressures [16]. Therefore, to improve the sensitivity, selectivity, applicability and operability and decrease the solvent consumption and time, the fast and simple UFLC–MS method was selected for the qualitative and quantitative analysis of saponins in *Ardisia* species.

Ardisia crenata is a low-growing evergreen shrub widely dispersed in eastern Asia. Its roots are used as a traditional Chinese medicine (TCM) to treat respiratory tract infections, menstrual disorders, tonsillitis, toothaches, trauma and arthralgia, and it is listed in the Chinese Pharmacopeia. Triterpenoid saponins have been isolated as the major constituents from *A. crenata* [17], but no quantitative data are available. Therefore, we selected *A. crenata* as a model plant to develop a method with UFLC-ESI–MS to rapidly separate, identify and accurately determine triterpenoid saponins.

In this study, we report in detail the establishment of a UFLC–MS-based method that can identify 22 triterpenoid saponins in one chromatographic run, and we demonstrate its applicability to quantitatively analyze triterpenoid saponins in *A. crenata*.

2. Experimental

2.1. Chemicals and materials

Acetonitrile, methanol, ethanol, formic acid and trifluoroacetic acid were of HPLC grade and purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Pure water for the UFLC analysis was purified using a Milli-Q water purification system (Millipore, MA, USA) and used for all solutions and dilutions. Other reagents and chemicals were of analytical grade.

In this study, 28 triterpenoid saponin, which were isolated from *A. crenata* and *A. japonica* in our laboratory, were used as the standards to investigate their chromatographic and mass spectrometric behaviors (Table 1, Figs. 1 and 2) [12,17,18]. The purity of each compound was determined to be higher than 98% by normalizing the peak area detected using the HPLC analysis and their ^1H NMR spectra.

Five batches of *A. crenata* (samples 1–5) were collected from Yunnan, Zhejiang, Yunnan, Hunan, or Guangxi provinces, P.R. China.

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