Isolation and characterization of ardicrenin from *Ardisia crenata* Sims

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ABSTRACT

A new, effective and economical method to extract ardicrenin from *Ardisia crenata* Sims collected in the Wolong natural reserve, Sichuan, China, is established. *Ardisia crenata* Sims powder is counter-current extracted with 80% methanol reflux, decompressively enriched and centrifuged to defat. Supernatant is applied to macroporous resin column (AB-8) with 80% methanol, ardicrenin is isolated by silica gel chromatography with dichlormethane-aceto-acetate-methanol (4:1.5:1) washing, and recrystallized in methanol. The final product which proved to be ardicrenin by analytic procedure including Furier transform infrared (FTIR) and ultraviolet spectrum (UV), mass spectroscopy (MS), nuclear magnetic resonance (NMR) and high performance liquid chromatography (HPLC) is white amorphous powder with yield of 1.59 ± 0.02%.

Keywords: *Ardisia crenata* Sims; ardicrenin; isolation; determination

The genus *Ardisia*, a Myrsinaceae family, consists of more than 200 species growing in the warm climates of tropical and subtropical regions on the earth (Bailey 1925). *Ardisia crenata* Sims, distributed along the Yangzi River in China with names of coral bush, coralberry, hen's eyes and spiceberry, is small evergreen shrub and most commonly with non-variegated foliage and red berries (Lee 1998). Genus *Ardisia* is widely used as the traditional medicine to cure diseases, e.g. pulmonary tuberculosis, hepatitis, chronic bronchitis and irregular menstruation (Jiangsu New Medical College 1986). Therefore, many constituents were extracted from genus *Ardisia* and were characterized, i.e. cyclic depsipeptide (Fujioka et al. 1988), peptide (Gibbs et al. 2004), alkenylphenol (Horgen et al. 1997) and triterpenoid saponins (de Tommasi et al. 1993, Koike et al. 1999, Huang et al. 2000, Zheng et al. 2004, Chang et al. 2007). Recent study has shown that compounds extracted from *Ardisia* genus are health-promoting (Kobayashi et al. 2005); biological peptide, Fr900359, discovered by Fujioka (Fujioka et al. 1988) has been proved to inhibit platelet aggregation; ardipusilioside isolated by Zheng and his coworkers have anti-tumor activities (Jia et al. 1994a, Zheng et al. 2008) discovers that ardisicrenoside C, D have inhibitory activity on cAMP phosphodiesterase, Dat (2007) found that dimeric lactone has HIV inhibitory activity and ardisiacrispin A, B characterized by Jansakul and Piacente can promote utero-contraction (Jansakul et at. 1987, Piacente 1996) and have pro-apoptotic and microtubule-disassembly effects on human hepatoma Bel-7402 cells (Li et al. 2008). Usually, the isolated saponins have similar structures; it is an aglycon of different glyco-units combined with different oleanane derivate. However, reported articles underline the discovery and effect of the extracted compounds, but an effective and economical isolation method of ardicrenin which is a potential pharmaceutical still does not exist. Our research put emphasis on large scale of isolation of one single saponin ardicrenin of high purity which fits for industrialized production.

Table 1. Selection of extraction methods for ardicrenin (n = 3)

Test No.	Extraction method	Sample amount (g)	Content (mg/g)
1	water, reflux	10.000	20.13
2	95% ethanol, reflux	10.000	24.06
3	80% ethanol, reflux	10.000	25.97
4	60% ethanol, reflux	10.000	23.91
5	methanol, reflux	10.000	25.09
6	80% methanol, reflux	10.000	26.72
7	60% methanol, reflux	10.000	24.35

MATERIALS AND METHOD

Analytic procedure. HPLC is measured on Shimadzu SPD-20A. UV spectrum is collected on a Perkin Elmer Lambda 25 UV/Vis spectrometer. IR spectrum is recorded with a Thermo Nicolet Avatar 360 infrared spectrometer. ^{13}C and ^{1}H NMR spectra are acquired on Bruker Avance 600 and chemical shift is in ppm (δ) with tetramethylsilane (TMS) as an internal reference at 150 MHz and 500 MHz, respectively. High-resolution mass spectrum of ardicrenin (Figure 1) with no derivation is obtained using a JEOL LCmate magnetic sector instrument in the FAB mode with a glycerol matrix.

Plant collection. *Ardisia crenata* Sims plants used in the following experiments were collected in Dujiangyan under the direction of Tongpei YI research worker in Sichuan Agriculture University (SCAU). Voucher specimens were retained form the SCAU.

Extraction and isolation. 100 g plant powder of *Ardisia crenata* Sims is counter-current extracted with 800, 600 and 400 ml 80% methanol for 3, 2 and 1 h, respectively. When decompressively enriched, merge it filtrate at 70°C. Crude extract is incubated in 90°C water bath for 20 min with stirring, standing for 12 h and defatted with centrifugation. Supernatant is applied to macroporous resin column (AB-8) with water and 90% methanol washing. Then the decompressively dehydrated washing is admixed with 6 g silica gel (MIX). MIX is isolated by silica gel (ODS) chromatography (Huang et al. 2003) with dichlormethane-acetoacetate-methanol (4:1.5:1). Collected ardicrenin solution qualified by TLC analysis combined foam test with developing agent of dichlormethane-acetoacetate-methanol (4:2:1) and color developing agent of 10% sulphuric acid methanol solution. Then ardicrenin is crystallized, washed with ether and recrystallized in methanol. Recrystal is vacuum dried at 70°C and final product is white amorphous powder and applied to IR, UV/Vis spectrometer and GC-MS and NMR. The purity of amorphous powder is tested through RP-HPLC method compared to standard sample (Zhen et al. 2007). The yield (1.59 ± 0.02%) is calculated gravitationally. All experiments are repeated at least for three times.

RESULT AND DISCUSSION

In order to improve the extracting yield which was strongly affected by the lipids, deffating is necessary and it is important to seek a safe, efficient, and economical method. According to the previous study, ligarine is used in defatting procedure (Jia et al. 1994b). Ligarine extraction and centrifugation are attempted to defat. In the procedure, it is showed that centrifugation is more suitable and no further problems such as emulsification, demixing and unsafety occur in contrast to ligarine extraction. Furthermore, centrifugation is more fitted for industrialized production. Consequently, centrifugation is used for ardicrenin extraction.

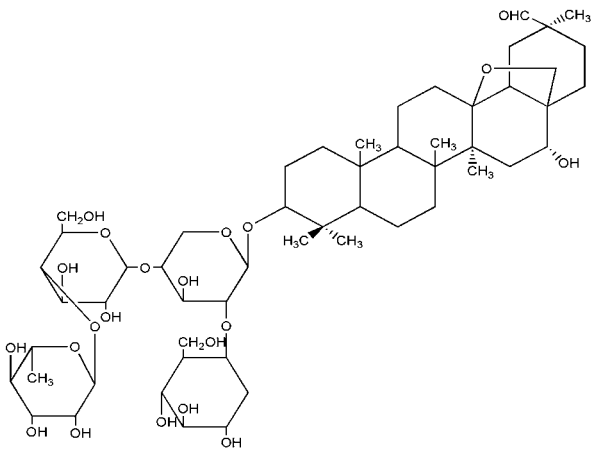

Figure 1. Structure of ardicrenin

Table 2. ^{13}C NMR spectra data for ardicrenin

Aglycone	Δ	DEPT	Sugars	δ	DEPT
1	39.2	CH_2	arabinose (A)	104.8	CH
2	26.2	CH_2	A-1	80.6	CH
3	89.4	CH	A-2	74.2	CH
4	39.3	C	A-3	74.7	CH
5	55.6	CH	A-4	62.8	CH_2
6	17.7	CH_2	A-5		
7	33.2	CH_2	glucose (G) (terminal)		
8	43.3	C	G-1	104.5	CH
9	50.4	CH	G-2	76.4	CH
10	36.7	C	G-3	77.7	CH
11	18.8	CH_2	G-4	71.3	CH
12	31.3	CH_2	G-5	78.8	CH
13	86.5	C	G-6	62.2	CH_2
14	47.6	C	glucose (G') (inner)		
15	45.2	CH_2	G'-1	103.5	CH
16	212.9	CH	G'-2	71.3	CH
17	55.6	C	G'-3	79.5	CH
18	55.3	CH	G'-4	78.3	CH
19		CH_2	G'-4	78.7	CH
20	50.4	C	G'-6	62.3	CH_2
21	29.4	CH_2	rhamnose (R)		
22	33.7	CH_2	R-1	101.6	CH
23	28.0	Me	R-2	72.1	CH
24	16.4	Me	R-3	72.7	CH
25	16.1	Me	R-4	74.9	CH
26	18.4	Me	R-5	69.4	CH
27	21.1	Me	R-6	18.6	Me
28	74.7	CH_2			
29	23.8	Me			
30	206.5	CH			

When MIX is applied to silica gel chromatography, a better washing solution is necessary to determine. Two mixed liquors of chloroform-methanol-water (Jia et al. 1994b) and dichlormethane-dichlormethane-methanol with different ratio in volume are tested. Since ardicrenin is dissolved easily in polarity solution, it shows that dichlormethane-acetoacetate-methanol of a ratio 4:1.5:1 has the best isolating result. This is mainly due to the dichlormethane-acetoacetate-methanol solution which has a larger polarity and as a result ardicrenin is much more easily dissolved in dichlormethane-acetoacetate-methanol solution. Contrast to Huang's work (Huang et al. 2003) with the yield is at 0.1 mg/g degree, a high extracting level at 20 mg/g degree is obtained.

Ardisia crenata Sims are extracted with methanol (Wang et al. 1992) and ethanol (Liu et al. 2007) of different concentrations with reflux or not. Compared to the reported article, the yield of

Table 3. ^{1}H NMR spectra data for ardicrenin

Aglycone	δ	Sugars	δ
1	0.76, 1.58	arabinose (A)	
2	1.80, 2.00	A-1	4.90
3	3.15	A-2	4.54
4	–	A-3	4.31
5	0.62	A-4	4.57
6	1.43	A-5	4.27
7	0.97		4.43
8	–	glucose (G) (Terminal) (Terminal)	
9	1.08	G-1	5.35
10	–	G-2	4.07
11	1.67	G-3	4.32
12	1.52	G-4	4.28
13	–	G-5	4.31
14	–	G-6	4.27
15	2.82		4.53
16	4.14	glucose (G') (inner)	
17	–	G'-1	4.00
18	1.88	G'-2	3.88
19	2.16	G'-3	4.08
20	–	G'-4	4.18
21	1.93	G'-4	4.23
22	0.96	G'-6	4.51
23	1.18		
24	1.03	rhamnose (R)	
25	0.76	R-1	6.43
26	1.22	R-2	4.77
27	1.10	R-3	4.69
28	3.88, 4.32	R-4	4.58
29	0.89	R-5	5.05
30	9.52	R-6	1.86

the saponin extracted from *Ardisia crenata* are usually at a low level of 0.1 mg/g (Zheng et al. 2008), the result shows that ardicrenin can be sufficiently extracted by countercurrent extraction with reflux, which reaches top extracting with 80% methanol at 26.72 mg/g (Table 1). It indicates that ardicrenin is more easily dissolved in methanol solution than in water or ethanol solution, while 80% is the optical concentration.

Ardicrenin is obtained as white amorphous powder, with a strong adsorption at 3417/cm attributed to hydroxyl group, a weak adsorption at 886/cm attributed to epoxy group and a strong adsorption at 1046/cm attributed to cyclohexane backbone in IR spectrum (Figure 2). The ion at *m/z* 1073.8 of the mass spectrum (Figure 3) corresponds to the molecular formula $C_{53}H_{86}O_{22}$. The ^{13}C NMR data (Table 2) indicate 53 carbon

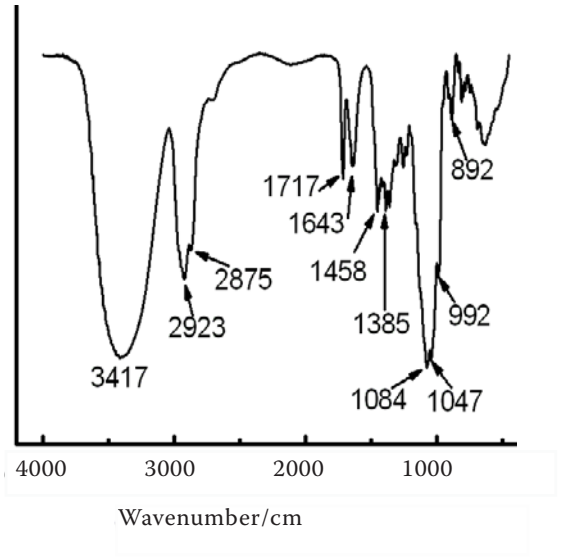

Figure 2. FTIR spectrum of ardicrenin

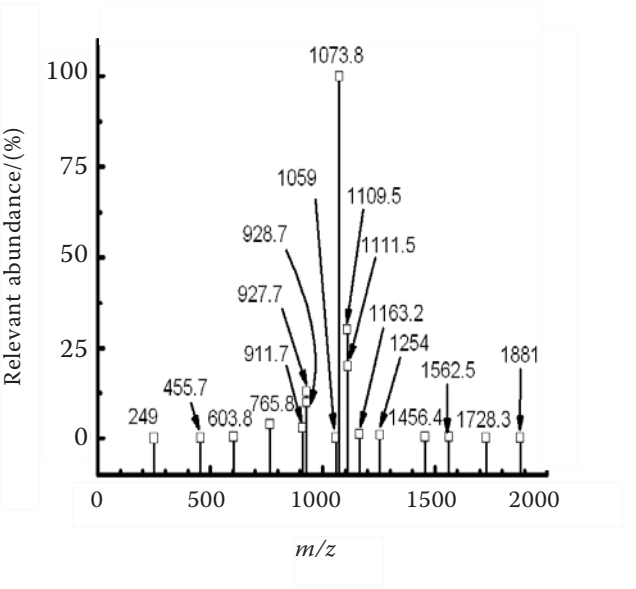

Figure 3. Mass spectrum of ardicrenin

signals, 30 of which are assigned to aglycone part, while the other 23 are assigned to carbonhydrate (arabinose, glucose and rhamnose) moiety. The ^{1}H (Table 3) and ^{13}C NMR spectrum data determine the structure (Figure 1) of ardicrenin as 3-O-[α-L-rhamnopyranosy(1-4)-β-D-glucopyranosyl(1-4)][β-D-glucopyranosyl(1-2)]α-L-arabinopyranosyl cyclamiretin A, which is according to Wang (1992) and Jia's (1994) works.

Method that effectively and economically extracts ardicrenin from *Ardisia crenta* Sims is found to be suitable for industrialized production. The final product is white powder and its purity and yield are 98% and 1.6%, respectively.

REFERENCE

Bailey L.H. (1925): The Standard Cyclopedia of Horticulture. McMillan Co., London.

Chang X.L., Li W., Jia Z.H., Satou T., Fushiya S., Koike K. (2007): Biologically active triterpenoid saponins from *Ardisia japonica*. Journal of Natural Products, *70*: 179–187.

Dat N.T., Bae K.H., Wamiru A., McMahon J.B., Le Grice S.F.J., Bona M., Beutler J.A., Kim Y.H. (2007): A dimeric lactone from *Ardisia japonica* with inhibitory activity for HIV-1 and HIV-2 ribonuclease H. Journal of Natural Products, *70*: 839–841.

De Tommasi N., Piacente S., de Simone F., Pizza C., Zhou Z.L. (1993): Characterization of three new triterpenoid saponins from *Ardisia japonica*. Journal of Natural Products, *56*: 1669–1675.

Gibbs B.F., Zougman A., Masse R., Mulligan C. (2004): Production and characterization of bioactive peptides from soy hydrolysate and soy-fermented food. Food Research International, *37*: 123–131.

Fujioka M., Koda S., Morimoto Y. (1988): Structure of Fr900359, a cyclic depsipeptide from *Ardisia crenata* Sims. Journal of Organic Chemistry, *53*: 2820–2825.

Horgen F.D., Guinaudeau H., Pezzuto J.M., Soejarto D.D., Farnsworth N.R., Agcaoili F., de los Reyes G, Edrada R.A. (1997): Isolation and structure elucidation of ardisenone a new, cytotoxic alkenylphenol from *Ardisia iwahigensis*. Journal of Natural Products, *60*: 533–535.

Huang J., Ogihara Y., Zhang H., Shimizu N., Takeda T. (2000): Triterpenoid saponins form *Ardisia mamillata*. Photochemistry, *54*: 817–822.

Huang J., Zhang H., Shimizu N., Takeda T. (2003): Ardisimamillosides G and H, two new triterpenoid saponins from *Ardisia mamillata*. Chemical and Pharmaceutical Bulletin, *51*: 875–877.

Jansakul C., Baumann H., Kenne L., Samuelsson G. (1987): Ardisiacrispin A and B, two utero-contracting saponins from *Ardisia crispa*. Planta Medica, *53*: 405–409.

Jia Z.H., Koike K., Nikaido T., Ni M. (1994a): Triterpenoid saponins from *Ardisia crenata* and their inhibitory activity on cAMP phosphodiesterase. Chemical and Pharmaceutical Bulletin, *42*: 2309–2312.

Jia Z.H., Koike K., Ohmoto T. (1994b): Triterpenoid saponins from *Ardisia crenata*. Phytochemistry, *37*: 1389–1396.

Jiangsu New Medical College (1986): The Chinese Medicine Dictionary. Shanghai Science and Technology Publishing House, Shanghai.

Kobayashi H., de Mejia E. (2005): The genus *Ardisia*: a novel source of health-promoting compounds and

phytopharmaceuticals. Journal of Ethnopharmacology, *96*: 347–354.

Koike K., Jia Z.H., Ohura S., Mochida S., Nikaido T. (1999): Minor triterpenoid saponin from *Ardisia crenata*. Chemical and Pharmaceutical Bulletin, *47*: 434–435.

Lee A.K. (1998): Ecological studies on *Ardisia* native to Korea and the significance as a potential indoor horticultural crop. [Ph.D. Dissertation.] Dankook University, Cheonan.

Li M., Wei S.Y., Xu B., Guo W., Liu D.L., Cui J.R., Yao X.S. (2008): Pro-apoptotic and microtubule-disassembly effects of ardisiacrispin (A plus B), triterpenoid saponins from *Ardisia crenata* on human hepatoma Bel-7402 cells. Journal of Asian Natural Products Research, *10*: 729–736.

Liu D.L., Wang N.L., Zhang X. (2007): Two new triterpenoid saponins from *Ardisia crenata*. Journal of Asian Natural Products Research, *9*: 119–127.

Piacente S. (1996): Constituents of *Ardisia japonica* and their *in vitro* anti-HIV activity. Journal of Natural Products, *59*: 565–569.

Wang M.T., Guan X.T., Han X.W., Hong S.H. (1992): A new triterpenoid saponin from *Ardisia crenata*. Planta Medica, *58*: 205–207.

Zhen H., Pu S.R., Ma M.D. (2007): Determination of ardicrenin in *Ardisia crenata* using RP-HPLC. Chinese Journal of Chromatography, *25*: 944–945.

Zheng Y., Deng Y., Wu F.E. (2004): Ardisinones A-E, novel diarylundecanones from *Ardisia arborescens*. Journal of Natural Products, *67*: 1617–1619.

Zheng Z.F., Xu J.F., Feng Z.M., Zhang P.C. (2008): Cytotoxic triterpenoid saponins from the roots of *Ardisia crenata*. Journal of Asian Natural Products Research, *10*: 833–839.

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