ARALIA ARMATA ROOTS: EXTRACTION, ISOLATION AND MOLLUSCICIDAL ACTIVITY AGAINST GOLDEN APPLE SNAILS, *POMACEA CANALICULATA*

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Abstract - *Aralia armata* is one of the common herb species in mountainous areas, especially in the northern mountainous areas of Vietnam, which has potential in pharmaceutical and agricultural industries. Herein, we report that an oleanolic acid saponin named pseudogisenoside RT1 methyl ester (**A**) was isolated from the roots of the species *Aralia armata*. The fractionated extracts of dichloromethane, ethyl acetate, water and compound **A** were investigated for their molluscicidal effects, specifically on *Pomacea canaliculata*. The tests showed that the extracts and compound **A** from *A. armata* roots had good activity against *P. canaliculata* snail. In particular, compound **A** had a Lethal concentration of 50 (LC_{50}) value of 16.443 µg/mL.

Key words - Araliaceae; *Aralia armata;* oleanolic acid saponin; triterpene glycoside; molluscicidal activity

1. Introduction

The *Aralia armata* (Wall.) Seem. (Araliaceae) which is a common herb in the Northern mountainous region in Vietnam used in traditional medicine to cure hepatitis, arthritis, stomachache, malaria, and snake-rolling [1].

The golden apple snail (*Pomacea canaliculata* Lamarck. (Ampullariidae)), native to Southern America [2], is a species of snail that has a negative impact on the harvest of agricultural products, especially rice [3-5]. Besides, it is an intermediate host for the human eosinophilic meningitis *Angiostrongylus cantonensis* [6-8], the intestinal trematode *Echinostom ilocanum* (Garrison) (Echinostomatidae) [9-11].

The previous studies on the chemical constituents of *A. armata* have reported that the oleane-type triterpene glycosides are the main component of the leaves [12] and roots [13]. We also have reported fourteen oleane-type triterpene glycosides isolated from the leaves of this plant, and some of them exhibited cytotoxic activity against some cancer cell lines in the previous papers [14-16]. However, no chemical and bioactive works have been done on the roots of Vietnamese *A. armata*.

In this study, we report the isolations, the chemical structure elucidation of oleanolic acid saponin from the roots of this plant, and evaluate the molluscicidal activity of extracts and the compound isolated from this plant against *P. canaliculata.*

2. Materials and methods

2.1. Plant materials

The roots of *Aralia armata* were collected in Hoa Vang District, Danang City, Vietnam (16°00′55″N 108°07′8″E) in January 2021 and identified by botanist Nguyen The Cuong at the Institute of Ecology and Biological Resources, VAST. A voucher specimen (coded: NCCT-P71B) was deposited at the Institute of Marine Biochemistry, VAST.

2.2. General experimental procedures

NMR spectra of the isolated compound have been resolved at 500 MHz on a Bruker WM500 spectrometer. NMR data were processed using MestReNova (version 9.0.1). HPLC was carried out with the Agilent 1200 HPLC system. Column chromatography was performed using the silica gel (Kieselgel 60, 230-400 mesh, Merck) or RP-18 resins (30-50 µm, Fuji Silysia Chemical Ltd.), and thin-layer chromatography using a pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck) [17, 18].

2.3. Extraction and isolation

The roots of *A. armata* (5.0 kg) were cut into small pieces, dried, powdered, then ultrasonically extracted with MeOH three times (12 L of MeOH each in 60 minutes). After filtration, the solvent was removed *in vacuo* to give 450 g of methanol extract. This was suspended in water and successively partitioned with dichloromethane and ethyl acetate (EtOAc) to give organic soluble fractions and a water layer. The water layer was chromatographed on a diaion (HP-20) column, washed with water to remove salts and oligosaccharides. Next, the obtained mixture was stepwise eluted by methanol/water (25%, 50%, 75%, and 100% volume of methanol) to give four fractions AA1-AA4. Fraction AA4 (15.0 g) was chromatographed on a silica gel column with dichloromethane/methanol solvent (1/0-0/1, v/v) to give four fractions AA4A-AA4D. Fraction AA4A (3.5 g) was chromatographed on a reverse-phase C18 column with methanol/water solvent (3/3, v/v) to give 04 fractions AA4A1-AA4A4. Fraction AA4A3 (890 mg) was further chromatographed on HPLC (J'sphere H-80 column, 250mm length x 20mm ID with a mixed solvent of 20% acetonitrile in water, a flow rate of 2 mL/min) to give compound **A** (8.8 mg) (Figure 1).

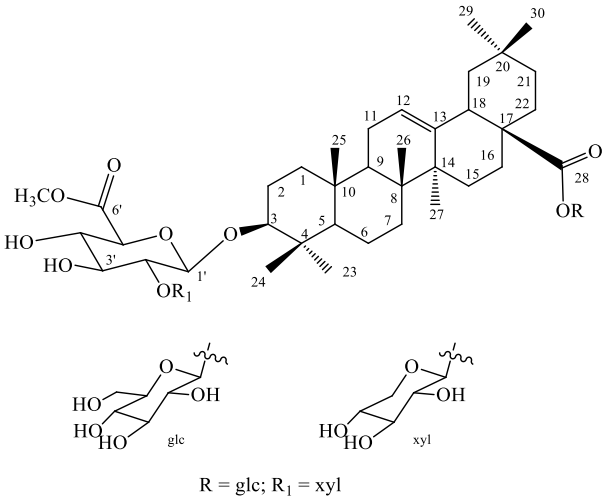

Figure 1. *Saponin isolated from Aralia armata*

2.4. Molluscicidal assay

Eggs of *Pomacea canaliculata* were collected from a rice field at Dien Ban District, Quang Nam province (15°55′58″N 108°11′46″E). Snail eggs were incubated in the laboratory at 25 ± 2°C, 70 ± 5 % humidity. Newly hatched snails were fed with *Ipomoea aquatica* leaves. After 07 days, the snails were 1.0-3.0 mm in size and used for experiments. The snail was identified by Dr. Nguyen Huy Hung at the Center for Advanced Chemistry, Institute of Research and Development, Duy Tan University.

The molluscicidal activity of extraction and the pure compound was performed according to the protocol of Ding et al. [19]. The samples were prepared by completely dissolving in DMSO (1% stock solution). The samples were placed in 1000 mL beakers containing 150 mL of distilled water, shook slightly to dissolve the samples in the water, and added 20 snails to each beaker. Tests were performed with sample concentrations of 1.5, 3.0, 6.0, 12.5, 25, 50, 100, 200 µg/mL, with four replicated experiments at each concentration. The positive and negative controls used in this trial were tea saponin and DMSO, respectively. The experiments were conducted at a temperature of 26 ± 2°C with a photoperiod of 12/12 h (light/dark). After 24 h, the snails under test were transferred to a beaker containing 150 mL of distilled water for recovery. After the next 24 h, snails that did not recover (immobilized, did not adhere to the wall of the cup and did not respond to retraction into the shell when lightly pushed by a blunt object) were presumed dead. The number of dead/living snails was recorded.

The lethal concentration 50 (LC_{50}) value of the test was calculated through log-probit analysis [20] using SPSS25 with 95% confidence limits.

3. Results and discussion

3.1. Isolated compound structure

One oleanolic acid saponin was isolated from the methanol extract of the roots of *A. armata* by various chromatography methods, including HPLC. The NMR spectra of this compound suggested that it had the same aglycone oleanane-12-ene-28-oic acid identified from the C-12 (CH)/C-13 (C) double bond signals, C-28 acid or ester, an oxygenated methine group at C-3, and all seven methyl groups appeared as singlets. The identification of this compound was first based on comparing its NMR data with the data of suggested compounds previously reported [21-25] and further confirmed by HSQC, HMBC spectra. The NMR assignments are shown in Table 1. Compound **A** was identified to be pseudogisenoside RT1 methyl ester [21]. The NMR data of this compound was identical to those reported.

Table 1. *Molluscicidal activity against Pomacea canaliculata of the fractional extracts and compound **A***

Sample	LC_{50} (µg/mL) 95% confidence interval
Dichloromethane fraction	48.984 (33.962 – 75.925)
EtOAc fraction	40.698 (34.020 – 49.226)
Water fraction	22.571 (15.985 – 31.713)
Compound **A**	16.443 (10.834 – 25.854)
Positive control (tea saponin)	9.225 (6.069 – 13.882)

3.2. Molluscicidal activity

The fractional extracts were tested for molluscicidal activity against *Pomacea canaliculata.* After testing, it was found that the fractional extracts from the roots of *A.armata* species had good molluscicidal activity. The LC_{50} values of dichloromethane, EtOAc fractional extracts, and water layer were 48.984 µg/mL, 40.698 µg/mL, and 22.571 µg/mL, respectively. Herein, the molluscicidal activity of the water layer was about two times more effective than that of the other fractional extracts. Therefore, we further isolated and tested the activity of the compound from the water layer. Compound **A** obtained from the isolation of the water layer had an outstanding activity with an LC_{50} value of 16.443 µg/mL, which was no big difference from the positive control of 9.225 µg/mL. 100% of snails were alive during the experiment with negative control (DMSO). The results of the molluscicidal activity of the fractions, compound **A**, and the positive control (tea saponin) [26] were presented in Table 2.

In recent studies, scientists have demonstrated molluscicidal activity against *P. canaliculata* of extracts, essential oils, and compounds isolated from different plant species. For example, the MeOH extract from the leaves of *Ipomoea batatas* species had an LC_{50} value of 1000 µg/mL (48 h) [27], the *n*-butanol extract from the fruit of *Ilex paraguariensis* species had an LC_{50} value of 24.75 µg/mL (24 h) [28] for molluscicidal activity against the golden apple snail (*P. canaliculata*). Essential oil from the leaves of *Lantana camara* species was also active against the snail *P. canaliculata* with an LC_{50} value of 23.63 µg/mL [29]. Several compounds isolated from the bark of *Eucalyptus exserta* F. Muell. also exhibits molluscicidal activity, in which the most active compound was yangambin with an IC_{50} value of 23.70 µg/mL against golden apple snail (*P. canaliculata*) [30].

The above results show that the molluscicidal activity of extracts and compound **A** from *A. armata* roots are great.

Table 2. *^{1}H- and ^{13}C-NMR data for compound **A** (in Py-d_5) and reference compound*

C	Compound A		
	[#,d]δ_C	[a,b]δ_C	[a,c]δ_H (mult., *J* in Hz)
1	39.5	38.6	0.78 (m)/ 1.36 (m)
2	27.0	26.4	1.79 (m)/ 2.03 (m)
3	91.2	89.3	3.32 (dd, 13.5, 5.4)
4	40.2	39.4	-
5	57.1	55.7	0.75 (m)
6	19.3	18.3	1.25 (m)/ 1.44 (m)
7	33.9	33.0	1.28 (m)/ 1.39 (m)
8	40.7	39.7	-
9	49.1	47.9	1.54 (m)
10	37.8	36.8	-
11	24.5	23.6	1.81 (m)/ 1.83 (m)
12	123.7	122.7	5.37 (br s)
13	144.8	144.0	-
14	42.9	42.0	-
15	28.8	28.1	1.12 (m)/ 2.26 (m)
16	23.9	23.2	1.92 (m)/ 2.04 (m)
17	48.0	46.9	-
18	42.5	41.6	3.12 (dd, 11.5, 4.5)
19	47.2	46.1	1.20 (m)/ 1.73 (m)
20	31.5	30.6	-
21	34.8	33.8	1.12 (m)/ 1.31 (m)
22	33.1	32.4	1.50 (m)/ 1.57 (m)
23	28.2	27.7	1.20 (s)
24	16.5	16.2	1.01 (s)
25	16.0	15.4	0.80 (s)
26	17.7	17.3	1.03 (s)
27	26.4	26.0	1.20 (s)
28	178.0	176.5	-
29	33.5	33.0	0.86 (s)
30	23.9	23.5	0.83 (s)
3-O-GluA			
1′	105.5	105.0	4.90 (d, 8.0)
2′	82.7	82.9	4.12 (dd, 9.0, 8.0)
3′	77.3	76.3	4.27 (dd, 9.0, 9.0)
4′	72.9	72.5	4.35 (dd, 9.0, 9.0)
5′	77.4	76.6	4.45 (d, 9.0)
6′	171.2	170.3	-
OCH_3	52.8	52.0	3.69 (s)
2′-O-xyl			
1″	106.2	106.5	5.20 (d, 7.5)
2″	76.2	76.1	4.03 (dd, 9.0, 7.5)
3″	77.7	77.8	4.09 (dd, 9.0, 9.0)
4″	71.1	70.	4.24 (m)
5″	67.1	67.2	4.30 (m)/3.62 (m)
28-O-glc			
1‴	95.6	95.6	6.30 (d, 8.0)
2‴	73.9	73.8	4.14 (dd, 9.0, 8.0)
3‴	78.2	78.5	4.22 (dd, 9.0, 9.0)
4‴	71.1	70.8	4.16 (dd, 9.0, 9.0)
5‴	78.6	79.0	3.97 (m)
6‴	62.4	62.0	4.30 (dd, 12.0, 5.0) 4.38 (dd, 12.0, 2.0)

Measured in [a)]Py-d_5, [b)]125 MHz, [c)]500 MHz, [d)]CD_3OD. [#δ]C of pseudogisenoside RT1 methyl ester [21]. The data were assigned from the HSQC and HMBC spectra.

4. Conclusions

In summary, from this study, several conclusions can be concluded. The extracts from *A. armata* roots are toxic to the *Pomacea canaliculata* snail. By which the water-extracted fraction had the highest activity. In particular, pseudogisenoside RT1 methyl ester - an oleanolic acid saponin isolated from the water layer shows great results compared to previously published results.

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