UNIVERSITY OF DANANG
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STUDY ON CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF *ARALIA ARMATA* SPECIES OF GENUS *ARALIA*, ARALIACEAE FAMILY IN VIETNAM

Major: **Organic chemistry**
Major code: **9 44 01 14 (62 44 01 14)**

SUMMARY OF DOCTORAL THESIS

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This thesis was completed at:
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The thesis can be found in:
The library of Nation;
The library of University of Science and Education, The University of Danang.

INTRODUCTION

1. Preface

Aralia armata species belongs to the genus *Aralia*, family Araliaceae is one of the most common plants in Vietnam, widely distributed from the mountains to the midlands, sometimes in the plains, growing wild everywhere from the North to the Midlands. This plant is known as a folk medicine used for various medicinal purposes.

Up to now, the publications on *A. armata* species are minimal. In particular, there has not been any research on the chemical composition and biological activities of *A. armata* species in Vietnam. Therefore, the topic "Study on extraction, isolation, structure determination and biological activities of several compounds from *Aralia armata* species (Araliaceae) in Vietnam" will contribute to creating a scientific database on the chemical composition and biological activities of *A. armata* and answer the therapeutic effects of this species in our country. The success of the project not only creates a scientific database but also has the potential to create safe products from nature.

2. Aims of the thesis

- Isolation and structural determination of several chemical compounds from leaves and roots of *A. armata* (Araliaceae) in Vietnam;
- Evaluation of biological activities of several compounds isolated from leaves and roots of *A. armata*.

3. Contents of the thesis

- Isolation and determination of chemical structures of several compounds from leaves and roots of *A. armata* in Vietnam;
- Evaluation of cytotoxic activity on cancer (HT29 cell lines - Human colon cancer cell, A549 - Human lung carcinoma cell, A2058 - Human melanoma cell) and HEK-293A normal cells - Human embryonic kidney cell), molluscicidal activity on the golden apple snail (*P. canaliculata*) of several compounds isolated from the leaves and roots of *A. armata* plant;
- Acute toxicity test in mice and brine shrimp (*Artemia* sp.) for selected fractions of leaves and roots of *A. armata* plant.

4. New contributions of the thesis

From leaves and roots of *Aralia armata*, isolated:

- 05 new compounds: araliaarmoside, aramatoside A, aramatoside B, aramatoside C, aramatoside D
- 08 compounds were isolated for the first time from *A. armata* species: 3-*O*-*β*-D-glucuronopyranosyl-23-hydroxyoleanolic acid-28-*O*-*β*-D-glucopyranoside, 3-*O*-[*α*-L-arabinopyranosyl-(1→3)-*β*-D-

glucuronopyranosylhederagenin 28-*O*-*β*-D-glucopyranosyl ester, Oleanolic acid-[28-*O*-*β*-D-glucopyranosyl]-3-*O*-[*β*-D-galactopyranosyl (1→3)]-[*β*-D-glucopyranosyl (1→2)]-*β*-D-glucuronopyranoside, 3-*O*-{*β*-D-glucopyranosyl-(1→3)-[*β*-D-galactopyranosyl-(1→2)]-*β*-D-galactopyranosyl}-oleanolic acid, araliasaponin XVI, pseudogisenoside RT1 methyl ester, linalool 3-*O*-*β*-D-xylopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside, linalool 3-*O*-*α*-L-arabinopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside.

- According to the literature review at the time of the study, the study results on cytotoxic activity on three human cancer cell lines (HT29, A2058, A549) and normal cell lines (HEK- 293A) of 04/12 compounds isolated from the leaves of *A. armata* were published for the first time.
- For the first time, the molluscicidal activity on the golden apple snail (*Pomacea canaliculata*) of 13/13 compounds isolated from the roots of *A. armata* was studied.

5. The layout of the thesis

The thesis consists of 145 pages containing 30 Tables and 73 Figures. Preface 04 pages, conclusions and recommendations 02 pages, publications 01 page, references 14 pages. Contents of the thesis are divided into 04 chapters:

Chapter 1. Overview, 19 pages.

Chapter 2. Materials and methods, 07 pages.

Chapter 3. Experimental section, 15 pages.

Chapter 4. Results and discussion, 83 pages.

CHAPTER 1. OVERVIEW

This part mentioned domestic and international research on the following issues:

1.1. Overview of the genus *Aralia*

1.2. Introduction to *Aralia armata* species

CHAPTER 2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant material

Leaf samples of *A. armata* were collected in Vinh Phuc province in December 2017. Root samples of *A. armata* were collected in Danang in January 2021. Plant samples identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. Specimens of leaves and roots with codes NCCT-P71 and NCCT-P71R, respectively, are kept at the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology.

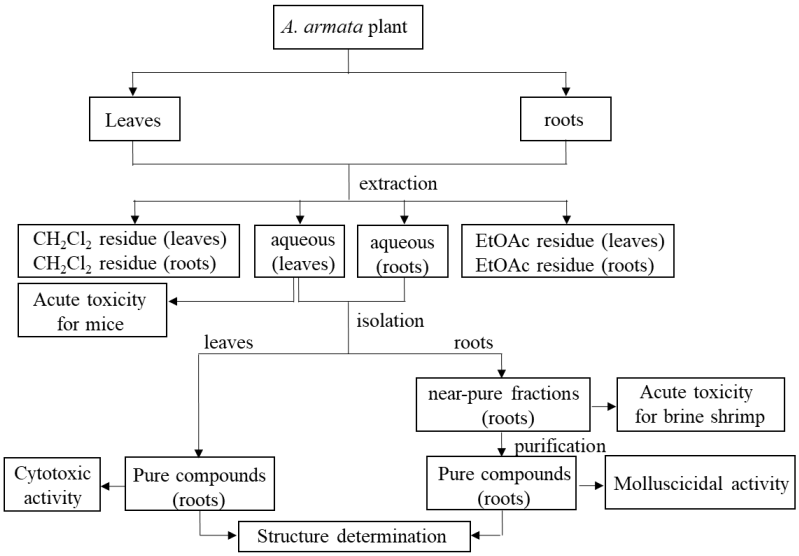
2.1.2. Other material

Swiss white mice, golden apple snail eggs, brine shrimp, cancer cell lines (HT29, A549, A2058), normal cell (HEK-293A).

2.2. Chemicals, tools and equipment

Brief presentation of chemicals, tools and equipment used in the thesis.

2.3. Research content



The content of the study is summarized in **Figure 2.2**.

Figure 2.2. The summary diagram of the research content.

2.4. Research methods

2.4.1. Plant extraction method

Plant materials were extracted using the solid-liquid extraction method and liquid-liquid extraction methods.

2.4.2. Method for isolation of compounds

Chromatographic methods include thin-layer chromatography (TLC) and column chromatography (CC).

2.4.3. Method for determination of the chemical structure of compounds

The general method used for the determination of the chemical structure of the compounds is a combination of physical parameters; modern spectroscopic methods such as high-resolution mass spectrometry (HR-ESI-MS), infrared spectroscopy (FT-IR), nuclear magnetic resonance spectroscopy (1D, 2D-NMR); and comparing with references.

2.4.4. Method for evaluation of the cytotoxic activity of compounds

The cytotoxic activity of the isolated compounds was determined by the MTS and SRB methods.

2.4.5. Method for evaluation of molluscicidal activity

The molluscicidal activity was carried out according to the method of Ding et al. (2018).

2.4.6. Method of acute toxicity test for mice

The acute toxicity test method on mice was conducted according to the method of Do Trung Dam (2014).

2.4.7. Acute toxicity test method for brine shrimp

The acute toxicity test method for brine shrimp was performed according to the method of Cong et al. (2021).

CHAPTER 3. EXPERIMENTAL

3.1. Sample processing and preparation of fractional extracts

Figure 3.1 illustrates the process of producing the fractional extracts from the leaves and roots of *A. armata*.

Through the extraction process, 240 g MeOH residue, CH_2Cl_2 residue (35 g), EtOAc (26 g) residue and an aqueous fraction were obtained in the leaf part. Similarly, 135 g of MeOH residue, 37 g of CH_2Cl_2 residue, 33 g of EtOAc residue and an aqueous fraction were obtained in the root part.

3.2. Isolation of compounds

The scheme of isolation of compounds from aqueous fractions of leaves and roots of *A. armata* is shown in **Figure 3.2** and **Figure 3.3**.

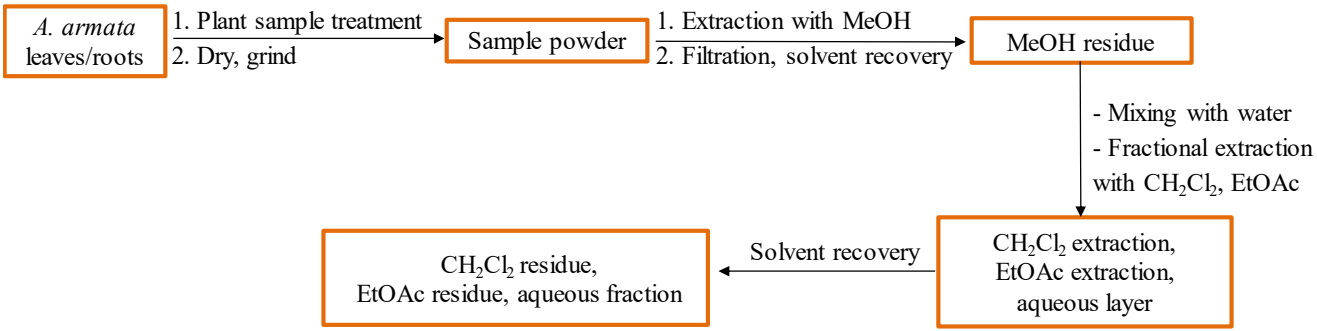


Figure 3.1. Schematic diagram of leaf and root extracts of *A. armata*.

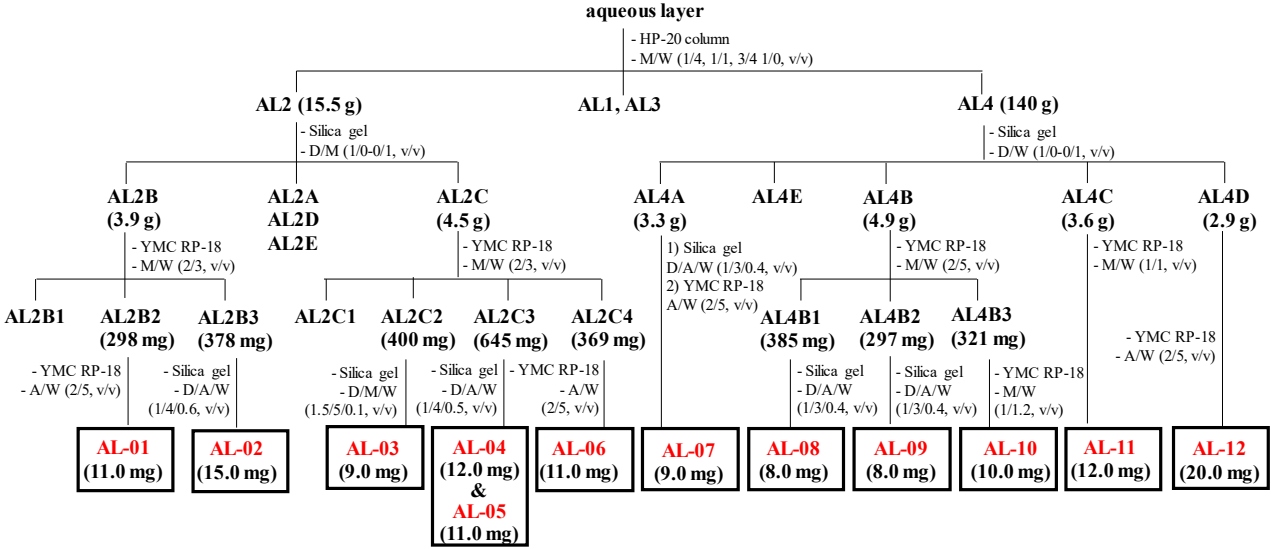


Figure 3.2. Scheme of aqueous fraction isolation of *A. armata* leaves.

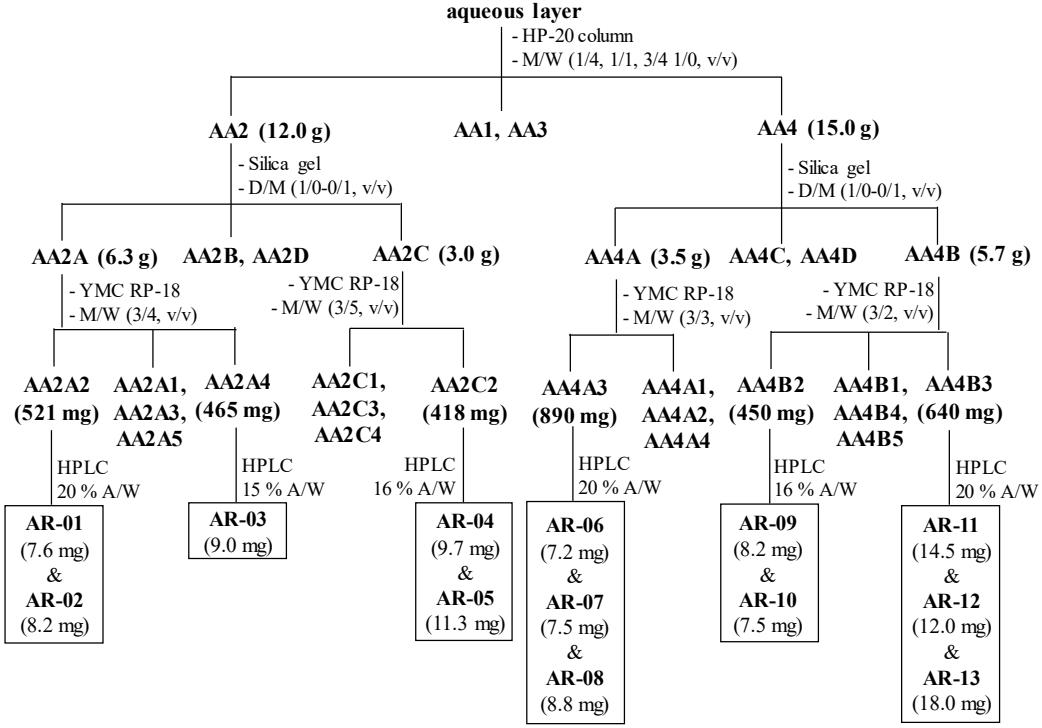


Figure 3.3. Scheme of aqueous fraction isolation of *A. armata* roots.

3.3. Physical and spectroscopic data

3.3.1. Compound AL-01: 3-O-β-D-glucuronopyranosyl-23-hydroxyoleanolic acid-28-O-β-D-glucopyranoside

Amorphous, white powder; Molecular formula: $C_{42}H_{66}O_{15}$; Molecular mass: 810.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,82 (3H, s, H-26); 0,88 (3H, s, H-24); 0,93 (3H, s, H-30); 0,95 (3H, s, H-29); 0,97 (3H, s, H-25); 1,08 (2H, s, H-23); 1,17 (3H, s, H-27); 3,69 (1H, m, H-3); 3,70 and 3,84 (2H, m, H-6″); 4,46 (1H, d, *J* = 8,0 Hz, H-1′); 5,27 (1H, br s, H-12); 5,40 (1H, d, *J* = 8,0 Hz, H-1″).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 13,7 (C-24); 16,5 (C-25); 17,7 (C-26); 23,9 (C-29); 26,3 (C-27); 33,4 (C-30); 62,4 (C-6″); 64,6 (C-23); 82,2 (C-3); 95,7 (C-1″); 104,9 (C-1′); 123,7 (C-12); 144,9 (C-13); 177,0 (C-6'); 178,2 (C-28).

3.3.2. Compound AL-02: 3-O-[α-L-arabinopyranosyl-(1→3)-β-D-glucuronopyranosylhederagenin 28-O-β-D- glucopyranosyl ester

Amorphous, white powder; Molecular formula: $C_{47}H_{74}O_{19}$; Molecular mass: 942.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,71 (3H, s, H-24); 0,82 (3H, s, H-26); 0,93 (3H, s, H-30); 0,95 (3H, s, H-29); 0,99 (3H, s, H-25); 1,18 (3H, s, H-27); 3,27 and 3,64 (2H, m, H-23); 3,62 and 3,96 (2H, m, H-5″); 3,69 (1H, m, H-3); 3,70 and 3,83 (2H, m, H-6‴); 3,83 (1H, m, H-4″); 4,50 (1H, d, *J* = 8,0 Hz, H-1′); 4,59 (1H, d, *J* = 7,0 Hz, H-1″); 5,27 (1H, br s, H-12); 5,40 (2H, d, *J* = 8,0 Hz, H-1‴).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 13,3 (C-24); 16,5 (C-25); 17,7 (C-26); 23,9 (C-29); 26,3 (C-27); 33,2 (C-30); 62,4 (C-6‴); 64,9 (C-23); 67,4 (C-5″); 69,7 (C-4″); 82,2 (C-3); 95,7 (C-1‴); 104,7 (C-1′); 105,2 (C-1″); 123,7 (C-12); 144,9 (C-13); 176,6 (C-6′); 178,1 (C-28).

3.3.3. Compound AL-03: chikusetsusaponin IVa methyl ester

Amorphous, white powder; Molecular formula: $C_{43}H_{68}O_{14}$; Molecular mass: 808.

HR-ESI-MS *m/z*: 843,4320 $[M+^{35}Cl]^-$, 845,4324 $[M+^{37}Cl]^-$

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,81 (3H, s, H-26); 0,86 (3H, s, H-24); 0,93 (3H, s, H-29); 0,95 (3H, s, H-30); 0,97 (3H, s, H-25); 1,08 (3H, s, H-23); 1,18 (3H, s, H-27); 3,16 (1H, dd, *J* = 5,0 Hz, *J* = 12,0 Hz, H-3); 3,69 (1H, dd, *J* = 11,5 Hz, *J* = 5,5 Hz, H-6′); 3,79 (3H, s, OCH_3); 3,82 (1H, dd, *J* = 11,5 Hz, J = 2,5 Hz, H-6′); 4,40 (1H, d, J = 8,0 Hz, H-1″); 5,27 (1H, br s, H-12); 5,40 (1H, d, J = 7,5, H-1′).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 15,9 (C-25); 16,9 (C-24); 17,7 (C-26); 23,9 (C-30); 26,2 (C-27); 28,4 (C-23); 33,4 (C-29); 52,7 (OCH_3); 62,4

(C-6′); 91,1 (C-3); 95,7 (C-1′); 107,0 (C-1″); 123,8 (C-12); 144,8 (C-13); 171,4 (C-6″); 177,0 (C-28).

3.3.4. Compound AL-04: oleanolic acid-[28-O-β-D-glucopyranosyl]-3-O-{[6‴-O-β-D-glucopyranosyl-(3″-O-β-D-glucopyranosyl)]-(4″-O-α-L-arabinofuranosyl)-β-D-glucuronopyranoside (araliaarmoside)

Amorphous, white powder; pole rotation $[\alpha]_D^{25}$: +13,0° (*c* 0,1, MeOH); Molecular formula: $C_{59}H_{94}O_{28}$; Molecular mass: 1250; HR-ESI-MS *m/z*: 1249,5860 $[M-H]^-$.

Calcd for $C_{59}H_{93}O_{28}$: M = 1249,5853.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,82 (3H, s, H-26); 0,85 (3H, s, H-24); 0,93 (3H, s, H-30); 0,96 (3H, s, H-25 và H-29); 1,05 (3H, s, H-23); 1,17 (3H, s, H-27); 3,15 (1H, dd, *J* = 4,0 Hz, *J* = 12,0 Hz, H-3); 3,64 and 3,69 (2H, dd, *J* = 12,0 Hz, *J* = 4,0 Hz, H-5‴′); 3,70 and 3,82 (2H, m, H-6′); 3,70 and 3,83 (2H, m, H-6‴′); 3,78 and 4,14 (2H, d, *J* = 12,0 Hz, H-6‴); 4,36 (1H, d, *J* = 7,5 Hz, H-1″); 4,37 (1H, d, *J* = 7,5 Hz, H-1‴′); 4,87 (1H, d, *J* = 8,0 Hz, H-1‴); 5,20 (1H, br s, H-1‴′); 5,27 (1H, br s, H-12); 5,37 (1H, d, *J* = 8,0 Hz, H-1′).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 16,0 (C-25); 17,0 (C-24); 17,8 (C-26); 24,0 (C-29); 26,2 (C-27); 28,5 (C-23); 33,4 (C-30); 62,2 (C-6′); 62,7 (C-6‴′); 63,3 (C-5‴′); 69,5 (C-6‴); 90,7 (C-3); 95,7 (C-1′); 104,4 (C-1‴); 104,6 (C-1‴′); 106,3 (C-1″); 108,3 (C-1‴′); 123,8 (C-12); 144,8 (C-13); 176,5 (C-6″); 178,1 (C-28).

3.3.5. Compound AL-05: oleanolic acid-[28-O-β-D-glucopyranosyl]-3-O-[β-D-galactopyranosyl (1→3)]-[β-D-glucopyranosyl (1→2)]-β-D-glucuronopyranoside

Amorphous, white powder; pole rotation $[\alpha]_D^{25}$: +15,0° (*c* 0,1, MeOH), Molecular formula: $C_{54}H_{86}O_{24}$; Molecular mass: 1118; HR-ESI-MS *m/z*: 1153,5208 $[M + {}^{35}Cl]^-$, 1155,5154 $[M + {}^{37}Cl]^-$; Calcd for $C_{54}H_{86}O_{24}{}^{35}Cl$: M = 1153,5198, $C_{54}H_{86}O_{24}{}^{37}Cl$: M = 1155,5168.

^{1}H NMR (500 MHz, Py-d_5) δ (ppm): 0,82 (3H, s, H-26); 0,88 (3H, s, H-24); 0,93 (3H, s, H-30); 0,95 (3H, s, H-29); 0,97 (3H, s, H-25); 1,08 (3H, s, H-23); 1,17 (3H, s, H-27); 3,22 (1H, m, H-3); 3,57 and 3,84 (2H, m, H-6‴); 3,70 và 3,83 (2H, m, H-6′); 3,70 và 3,84 (2H, m, H-6‴′); 3,82 (1H, dd, *J* = 3,5 Hz, *J* = 3,0 Hz, H-4‴′); 4,49 (1H, d, *J* = 7,5 Hz, H-1″); 4,72 (1H, d, *J* = 8,0 Hz, H-1‴′); 4,99 (1H, d, *J* = 8,0 Hz, H-1‴); 5,27 (1H, br s, H-12); 5,40 (1H, d, *J* = 8,0 Hz, H-1′).

^{13}C NMR (125 MHz, Py-d_5) δ (ppm): 16,0 (C-25); 16,8 (C-24); 17,7 (C-26); 23,8 (C-30); 26,2 (C-27); 28,3 (C-23); 33,4 (C-29); 62,4 (C-6‴′); 62,5 (C-6′); 63,6 (C-6‴); 70,6 (C-4‴′); 91,9 (C-3); 95,7 (C-1′); 103,2 (C-1‴); 104,4 (C-1‴′); 105,6 (C-1″); 123,8 (C-12); 144,7 (C-13); 178,1 (C-28).

3.3.6. Compound AL-06: chikusetsusaponin IV

Amorphous, white powder; pole rotation $[\alpha]_D^{25}$: +13,0° (*c* 0,1, MeOH); Molecular formula: $C_{47}H_{74}O_{18}$; Molecular mass: 926; HR-ESI-MS *m/z*: 961,4598 $[M+^{35}Cl]^-$, 963,4597 $[M+^{37}Cl]^-$; Calcd for $C_{47}H_{74}O_{18}{}^{35}Cl$: M = 961,4564, $C_{47}H_{74}O_{18}{}^{37}Cl$: M = 963,4534.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,81 (3H, s, H-26); 0,86 (3H, s, H-24); 0,93 (3H, s, H-29); 0,95 (3H, s, H-30); 0,97 (3H, s, H-25); 1,08 (3H, s, H-23); 1,18 (3H, s, H-27); 3,16 (1H, dd, *J* = 4,0 Hz, *J* = 11,5 Hz, H-3); 3,65 và 3,71 (2H, dd, *J* = 12,0 Hz, *J* = 4,5 Hz, H-5‴); 3,71 (1H, dd, *J* = 11,5 Hz, *J* = 5,5 Hz, H-6′); 3,83 (1H, dd, *J* = 11,5 Hz, *J* = 2,5 Hz, H-6′); 4,35 (1H, d, 7,5, H-1″); 5,27 (1H, br s, H-12); 5,09 (1H, br s, H-1‴); 5,40 (1H, d, *J* = 7,5 Hz, H-1′).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 15,9 (C-25); 17,0 (C-24); 17,7 (C-26); 23,9 (C-30); 26,4 (C-27); 28,5 (C-23); 33,6 (C-29); 62,5 (C-6′); 63,2 (C-5‴); 90,7 (C-3); 95,7 (C-1′); 106,8 (C-1″); 109,5 (C-1‴); 123,9 (C-12); 144,8 (C-13); 172,2 (C-6″); 177,0 (C-28).

3.3.7. Compound AL-07: oleanolic acid 28-O-β-D-glucopyranosyl ester

Amorphous, white powder; Molecular formula: $C_{36}H_{58}O_8$; Molecular mass: 618.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,77 (3H, s, H-24); 0,83 (3H, s, H-26); 0,93 (3H, s, H-30); 0,95 (3H, s, H-29); 0,97 (3H, s, H-25); 0,99 (3H, s, H-23); 1,18 (3H, s, H-27); 3,16 (1H, dd, *J* = 4,0 Hz, *J* = 11,0 Hz, H-3); 3,68 và 3,81 (2H, m, H-6′); 5,27 (1H, br s, H-12); 5,40 (1H, d, *J* = 8,0 Hz, H-1′).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 15,9 (C-25); 16,3 (C-24); 17,7 (C-26); 23,9 (C-29); 26,3 (C-27); 28,7 (C-23); 33,4 (C-30); 62,4 (C-6′); 79,7 (C-3); 95,7 (C-1′); 123,8 (C-12); 144,8 (C-13); 178,1 (C-28).

3.3.8. Compound AL-08: narcissiflorine

Amorphous, white powder; Molecular formula: $C_{41}H_{64}O_{13}$; Molecular mass: 764; HR-ESI-MS *m/z*: 763,4230 $[M–H]^-$, 799,4034 $[M+^{35}Cl]^-$

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,84 (3H, s, H-26); 0,86 (3H, s, H-24); 0,93 (3H, s, H-29); 0,95 (3H, s, H-25); 0,96 (3H, s, H-30); 1,07 (3H, s, H-23); 1,18 (3H, s, H-27); 3,17 (1H, dd, *J* = 4,2 Hz, *J* = 11,4 Hz, H-3); 3,64 (1H, dd, *J* = 12,0 Hz, *J* = 5,0 Hz, H-5″); 3,74 (1H, *J* = 12,0 Hz, *J* = 1,8 Hz, H-5″); 4,36 (1H, d, *J* = 7,8 Hz, H-1′); 5,10 (1H, br s, H-1″); 5,26 (1H, br t, *J* = 3,0 Hz, H-12).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 16,0 (C-25); 17,0 (C-24); 17,8 (C-26); 24,0 (C-30); 26,4 (C-27); 28,5 (C-23); 33,6 (C-29); 63,2 (C-5″); 90,7 (C-3); 106,8 (C-1′); 109,5 (C-1″); 123,6 (C-12); 145,3 (C-13); 176,0 (C-6′); 182,0 (C-28).

3.3.9. Compound AL-09: 3β,23α-dihydroxyolean-12-ene-28-oic acid 3-O-[4′-O-α-L-arabinofuranosyl-(3′-O-β-D-glucopyranosyl)]-β-D-glucuronopyranoside (aramatoside B)

Amorphous, white powder; Molecular formula: $C_{47}H_{74}O_{19}$; Molecular mass: 942; HR-ESI-MS *m/z*: 941,4768 $[M - H]^-$, 977,4536 $[M + {}^{35}Cl]^-$; Calcd for $C_{47}H_{73}O_{19}$: M = 941,4746, $C_{47}H_{74}O_{19}{}^{35}Cl$: M = 977,4513.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,71 (3H, s, H-24); 0,88 (3H, s, H-26); 0,90 (3H, s, H-29); 0,97 (3H, s, H-30); 0,99 (3H, s, H-25); 1,17 (3H, s, H-27); 3,28 and 3,62 (2H, m, H-23); 3,64 (1H, m, H-3); 3,64 and 3,70 (2H, dd, *J* = 4,0 Hz, *J* = 12,0 Hz, H-5‴); 3,71 and 3,84 (2H, br d, *J* = 12,0 Hz, H-6′); 4,47 (1H, d, *J* = 8,0 Hz, H-1′); 4,84 (1H, d, *J* = 8,0 Hz, H-1′); 5,20 (1H, br s, H-1‴); 5,23 (1H, br s, H-12).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 13,4 (C-24); 16,5 (C-25); 18,3 (C-26); 24,3 (C-30); 26,5 (C-27); 33,9 (C-29); 62,2 (C-6″); 63,3 (C-5‴); 65,0 (C-23); 83,1 (C-3); 104,6 (C-1″); 105,0 (C-1′); 108,3 (C-1‴); 122,6 (C-12); 146,6 (C-13); 176,6 (C-6′); 181,0 (C-28).

3.3.10. Compound AL-10: stipuleanoside R1

Amorphous, white powder; Molecular formula: $C_{47}H_{74}O_{18}$; Molecular mass: 926; HR-ESI-MS *m/z*: 925,4696 $[M-H]^-$, 961,4506 $[M+{}^{35}Cl]^-$; Calcd for $C_{47}H_{73}O_{18}$: M = 925,4797), $C_{47}H_{74}O_{18}{}^{35}Cl$: M = 961,4564.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,83 (3H, s, H-26); 0,85 (3H, s, H-24); 0,93 (3H, s, H-29); 0,96 (6H, s, H-25 and H-30); 1,06 (3H, s, H-23); 1,18 (3H, s, H-27); 3,16 (1H, dd, *J* = 4,5 Hz, *J* = 12,0 Hz, H-3); 3,66 (2H, m, H-6″); 3,71; 3,84 (2H, m, H-5‴); 4,37 (1H, d, *J* = 7,5 Hz, H-1′); 4,84 (1H, d, *J* = 7,5 Hz, H-1″); 5,20 (1H, br s, H-1‴); 5,25 (1H, br s, H-12).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 15,9 (C-25); 17,0 (C-24); 17,7 (C-26); 24,0 (C-30); 26,4 (C-27); 28,5 (C-23); 33,6 (C-29); 62,2 (C-5‴); 63,3 (C-6″); 90,8 (C-3); 104,4 (C-1″); 106,4 (C-1′); 108,3 (C-1‴); 123,6 (C-12); 145,2 (C-13); 176,8 (C-6′); 178,0 (C-28).

3.3.11. Compound AL-11: 3-O-β-D-glucuronopyranosyl-oleanolic acid-28-O-β-D-glucopyranoside (chikusetsusaponin IVa)

Amorphous, white powder; Molecular formula: $C_{42}H_{66}O_{14}$; Molecular mass: 794; HR-ESI-MS *m/z*: 793,4349 $[M-H]^-$, 829,4121 $[M+{}^{35}Cl]^-$; Calcd for $C_{47}H_{73}O_{18}$: M = 925,4797, $C_{47}H_{74}O_{18}{}^{35}Cl$: M = 961,4564.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,82 (3H, s, H-26); 0,87 (3H, s, H-24); 0,93 (3H, s, H-29); 0,96 (6H, s, H-25 and H-30); 1,07 (3H, s, H-23); 1,18 (3H, s, H-27); 3,22 (1H, dd, 4,0, 11,5, H-3); 3,70/3,83 (2H, m, H-6″); 4,35 (1H, d, *J* = 7,5 Hz, H-1′); 5,27 (1H, br s, H-12); 5,40 (1H, d, *J* = 7,5 Hz, H-1″).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 16,04 (C-25); 17,00 (C-24); 17,75 (C-26); 23,95 (C-30); 26,28 (C-27); 28,53 (C-23); 33,47 (C-29); 62,45 (C-6″); 90,72 (C-3); 95,93 (C-1″); 106,69 (C-1′); 123,87 (C-12); 144,80 (C-13); 177,0 (C-6′); 178,12 (C-28).

3.3.12. Compound AL-12: 3β,22α-dihydroxyolean-12-ene-28-oic acid 3-O-[4'-O-α-L-arabinofuranosyl-(3'-O-β-D-glucopyranosyl)]-β-D-glucuronopyranoside (aramatoside A)

Amorphous, white powder; pole rotation $[\alpha]_D^{25}$: +15,0° (*c* 0,1, MeOH); Molecular formula: $C_{47}H_{74}O_{19}$; Molecular mass: 942; HR-ESI-MS *m/z*: 941,4767 $[M - H]^-$, 977,4533 $[M + Cl]^-$; Calcd for $C_{47}H_{73}O_{19}$: M = 941,4746, $C_{47}H_{74}O_{19}{}^{35}Cl$: M = 977,4513.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,85 (1H, s, H-24); 0,86 (1H, s, H-26); 0,96 (6H, s, H-25 và H-29); 1,01 (1H, s, H-30); 1,06 (1H, s, H-23); 1,19 (1H, s, H-27); 3,14 (1H, dd, *J* = 4,5 Hz, *J* = 12,0 Hz, H-3); 3,65 and 3,70 (2H, dd, *J* = 5,5 Hz, *J* = 11,5 Hz, H-5‴); 3,86 (1H, m, H-22); 4,37 (1H, d, *J* = 8,0 Hz, H-1′); 4,88 (1H, d, *J* = 8,0 Hz, H-1″); 5,20 (1H, br s, H-1‴); 5,26 (1H, br s, H-12).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 15,9 (C-25); 17,0 (C-24); 17,9 (C-26); 25,2 (C-30); 26,8 (C-27); 28,5 (C-23); 33,6 (C-29); 62,2 (C-6″); 63,3 (C-5‴); 72,5 (C-22); 90,9 (C-3); 104,3 (C-1″); 106,3 (C-1′); 108,3 (C-1‴); 123,7 (C-12); 144,6 (C-13); 176,5 (C-6′); 181,0 (C-28).

3.3.13. Compound AR-01: 3-O-β-D-glucuronopyranosylhederagenin 28-O-β-D-glucopyranosyl ester

Amorphous, white powder; Molecular formula: $C_{47}H_{74}O_{18}$; Molecular mass: 926; HR-ESI-MS *m/z*: 961,4507 $[M+{}^{35}Cl]^-$, 925,4698 $[M - H]^-$; Calcd for $C_{47}H_{74}O_{18}{}^{35}Cl$: M = 961,4564, $C_{47}H_{73}O_{18}$: M = 925,4797.

^{1}H-NMR (500 MHz, Py-d_5) δ (ppm): 0,82 (1H, s, H-26); 0,86 (1H, s, H-24); 0,93 (1H, s, H-29); 0,96 (1H, s, H-30); 0,97 (1H, s, H-25); 1,07 (1H, s, H-23); 1,18 (1H, s, H-27); 3,17 (1H, dd, *J* = 11,5 Hz, *J* = 4,5 Hz, H-3); 3,65 (1H, *J* = 12,0 Hz, *J* = 5,0 Hz, H-5‴); 3,71 (1H, *J* = 12,0 Hz, *J* = 5,0 Hz, H-6′); 4,35 (1H, d, 8,0, H-1″); 5,09 (1H, br s, H-1‴); 5,27 (1H, br t, 3,0, H-12); 5,40 (1H, d, *J* = 8,5 Hz, H-1′).

^{13}C-NMR (125 MHz, Py-d_5) δ (ppm): 15,9 (C-25); 17,0 (C-24); 17,8 (C-26); 24,0 (C-30); 26,4 (C-27); 28,5 (C-23); 33,5 (C-29); 62,4 (C-6′); 63,2 (C-5‴); 90,7 (C-3); 95,7 (C-1′); 106,8 (C-1″); 109,5 (C-1‴); 123,9 (C-12); 144,8 (C-13); 176,0 (C-6″); 178,1 (C-28).

3.3.14. Compound AR-02: narcissiflorine

Amorphous, white powder; Molecular formula: $C_{41}H_{64}O_{13}$; Molecular mass: 764.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,83 (3H, s, H-26); 0,86 (3H, s, H-24); 0,92 (3H, s, H-29); 0,96 (6H, s, H-25 and H-30); 1,06 (3H, s, H-23); 1,18 (3H, s, H-27); 3,17 (1H, dd, 4,5; 12,5, H-3); 3,65 and 3,74 (2H, m, H-5″); 4,37 (1H, d, *J* = 8,0 Hz, H-1′); 5,10 (1H, br s, H-1″); 5,25 (1H, br s, H-12).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 15,93 (C-25); 16,97 (C-24); 17,73 (C-26); 23,97 (C-30); 26,38 (C-27); 28,49 (C-23); 33,83 (C-29); 63,19 (C-5″); 90,87 (C-3); 109,24 (C-1″);123,67 (C-12); 145,16 (C-13); 106,84 (C-1′); 176,9 (C-6′); 181,85 (C-28).

3.3.15. Compound AR-03: stipuleanoside R1

Amorphous, white powder; Molecular formula: $C_{47}H_{74}O_{18}$; Molecular mass: 926.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,83 (3H, s, H-26); 0,85 (3H, s, H-24); 0,93 (3H, s, H-29); 0,96 (6H, s, H-25 and H-30); 1,06 (3H, s, H-23); 1,18 (3H, s, H-27); 3,16 (1H, dd, *J* = 4,5 Hz, *J* = 12,0 Hz, H-3); 3,66 (2H, m, H-6″); 3,71 and 3,84 (2H, m, H-5‴); 4,37 (1H, d, 7,5, H-1′); 4,84 (1H, d, 7,5, H-1″); 5,20 (1H, br s, H-1‴); 5,25 (1H, br s, H-12).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 15,93 (C-25); 16,98 (C-24); 17,76 (C-26); 23,99 (C-30); 26,39 (C-27); 28,50 (C-23); 33,57 (C-29); 62,23 (C-5‴); 63,29 (C-6″); 90,79 (C-3); 104,42 (C-1″); 106,36 (C-1′); 108,24 (C-1‴); 123,60 (C-12); 145,23 (C-13); 176,80 (C-6′).

3.3.16. Compound AR-04: 23-hydroxyoleanolic acid-[28-O-β-D-glucopyranosyl]-3-O-[2″-O-β-D-glucopyranosyl-(3″-O-β-D-glucopyranosyl)]-β-D-galactopyranoside (aramatoside C)

Amorphous, white powder; pole rotation $[\alpha]_D^{25}$: +51,0° (c 0,1, MeOH); Molecular formula: $C_{54}H_{88}O_{24}$; Molecular mass: 1120; HR-ESI-MS *m/z*: 1155,5341 $[M+^{35}Cl]^-$, 1157,5289 $[M+^{37}Cl]^-$; Calcd for $C_{54}H_{88}O_{24}{}^{35}Cl$: M = 1155,5354, $C_{54}H_{86}O_{24}{}^{37}Cl$: M = 1157,5325.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,76 (3H, s, H-24); 0,83 (3H, s, H-26); 0,93 (3H, s, H-29); 0,95 (3H, s, H-30); 1,00 (3H, s, H-25); 1,19 (3H, s, H-27); 3,57 and 3,83 (2H, m, H-6″); 3,67 (1H, dd, 13,5, 5,4, H-3); 3,70 and 3,84 (2H, m, H-6′); 3,78 and 3,28 (2H, d, *J* = 12,0 Hz, H-23); 4,14 (1H, br d, *J* = 3,0 Hz, H-4″); 4,53 (1H, d, *J* = 7,5 Hz, H-1″); 4,63 (1H, d, *J* = 7,5 Hz, H-1‴); 4,89 (1H, d, *J* = 7,5 Hz, H-1‴); 5,27 (1H, t, *J* = 3 Hz, *J* = 5 Hz, H-12); 5,40 (1H, d, *J* = 8,0 Hz, H-1′).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 13,2 (C-24); 16,4 (C-25); 24,0 (C-30); 26,4 (C-27); 33,5 (C-29); 62,4 (C-6‴); 62,5 (C-6′); 63,5 (C-6″); 64,8 (C-23); 70,0 (C-4″); 84,9 (C-3); 95,7 (C-1′); 103,5 (C-1‴); 104,8 (C-1″); 105,3 (C-1‴); 123,8 (C-12); 144,9 (C-13); 178,1 (C-28).

3.3.17. Compound AR-05: oleanolic acid-[28-O-β-D-glucopyranosyl]-3-O-[2''-O-β-D-glucopyranosyl-(3''-O-β-D-glucopyranosyl)]-β-D-galactopyranoside (aramatoside D)

Amorphous, white powder; pole rotation $[\alpha]_D^{25}$: +45,0° (*c* 0,1, MeOH); Molecular formula: $C_{54}H_{88}O_{23}$; Molecular mass: 1104; HR-ESI-MS *m/z*: 1139,5402 $[M + {}^{35}Cl]^-$, 1141,5414 $[M + {}^{37}Cl]^-$; Calcd for $C_{54}H_{88}O_{23}{}^{35}Cl$: M = 1139,5405, $C_{54}H_{88}O_{23}{}^{37}Cl$: M = 1141,5375.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,82 (3H, s, H-26); 0,86 (3H, s, H-24); 0,93 (3H, s, H-29); 0,95 (3H, s, H-30); 0,98 (3H, s, H-25); 1,08 (3H, s, H-23); 1,17 (3H, s, H-27); 3,14 (1H, dd, *J* = 13,5 Hz, *J* = 5,0 Hz, H-3); 3,70 and 3,84 (2H, m, H-6′); 3,70 and 3,79 (2H, m, H-6‴); 3,72 and 3,84 (2H, m, H-6″); 4,13 (1H, br d, *J* = 3,0 Hz, H-4″); 4,42 (1H, d, *J* = 7,5 Hz, H-1″); 4,62 (1H, d, *J* = 7,5 Hz, H-1⁗); 4,74 (1H, d, *J* = 7,5 Hz, H-1‴); 5,27 (1H, t, *J* = 3 Hz, *J* = 5 Hz, H-12); 5,40 (1H, d, *J* = 8,0 Hz, H-1′).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 16,0 (C-25); 16,6 (C-24); 17,8 (C-26); 24,0 (C-30); 26,3 (C-27); 28,3 (C-23); 33,5 (C-29); 62,3 (C-6‴); 62,4 (C-6′); 62,5 (C-6″); 70,0 (C-4″); 91,5 (C-3); 95,7 (C-1′); 104,7 (C-1‴); 105,3 (C-1⁗); 105,9 (C-1″); 123,9 (C-12); 144,8 (C-13); 178,1 (C-28).

3.3.18. Compound AR-06: 3-O-{β-D-glucopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-galactopyranosyl}-oleanolic acid

Amorphous, white powder; Molecular formula: $C_{48}H_{78}O_{18}$; Molecular mass: 942; HR-ESI-MS *m/z*: 943 $[M+H]^+$; Calcd for $C_{48}H_{79}O_{18}$: M = 943.5266.

^{1}H-NMR (500 MHz, Py-d_5) δ (ppm): 0,72 (3H, s, H-25); 0,88 (3H, s, H-26); 0,90 (3H, s, H-29); 0,94 (3H, s, H-30); 1,05 (3H, s, H-24); 1,23 (3H, s, H-27); 1,25 (3H, s, H-23); 3,20 (1H, dd, *J* = 13,5 Hz, *J* = 5,4 Hz, H-3); 4,15 and 4,31 (2H, m, H-6′); 4,74 (1H, d, *J* = 7,8 Hz, H-1′); 5,18 (1H, d, *J* = 7,8 Hz, H-1‴); 5,33 (1H, d, *J* = 7,8 Hz, H-1″); 5,40 (1H, br s, H-12).

^{13}C-NMR (125 MHz, Py-d_5) δ (ppm): 15,2 (C-25); 16,5 (C-24); 17,2 (C-26); 23,5 (C-30); 26,0 (C-27); 27,9 (C-23); 33,1 (C-29); 61,4 (C-6″); 61,8 (C-6′); 62,1 (C-6‴); 69,1 (C-4′); 89,4 (C-3); 104,4 (C-1″); 104,8 (C-1‴); 105,1 (C-1′); 122,4 (C-12); 144,6 (C-13); 180,1 (C-28).

3.3.19. Compound AR-07: 3-O-{β-D-glucopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-galactopyranosyl}- oleanolic acid-28-O-β-D-glucopyranosy ester (araliasaponin XVI)

Amorphous, white powder; Molecular formula: $C_{54}H_{88}O_{23}$; Molecular mass: 1104; HR-ESI-MS *m/z*: 1105 $[M+H]^{+;}$ Calcd for $C_{54}H_{89}O_{23}$: M = 1105,6795.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,82 (3H, s, H-26); 0,89 (3H, s, H-24); 0,93 (3H, s, H-29); 0,95 (3H, s, H-30); 0,97 (3H, s, H-25); 1,11 (3H, s, H-23); 1,18 (3H, s, H-27); 3,18 (1H, dd, *J* = 13,5 Hz, *J* = 5,4 Hz, H-3); 4,46 (1H, d, *J* = 8,0 Hz, H-1′); 4,64 (1H, d, *J* = 7,5 Hz, H-1‴); 4,78 (1H, d, *J* = 7,5 Hz, H-1″); 5,27 (1H, br s, H-12); 5,41 (1H, d, *J* = 8,0 Hz, H-1⁗).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 15,2 (C-25); 16,5 (C-24); 17,2 (C-26); 23,5 (C-30); 26,0 (C-27); 27,9 (C-23); 33,1 (C-29); 91,9 (C-3); 95,7 (C-1⁗); 104,4 (C-1″); 105,2 (C-1‴); 105,9 (C-1′); 122,4 (C-12); 144,6 (C-13); 178,0 (C-28).

3.3.20. Compound AR-08: pseudogisenoside RT1 methyl ester

Amorphous, white powder; Molecular formula: $C_{48}H_{76}O_{18}$; Molecular mass: 940.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,80 (1H, s, H-25); 0,83 (1H, s, H-30); 0,86 (1H, s, H-29); 1,01 (1H, s, H-24); 1,03 (1H, s, H-26); 1,20 (2H, s, H-23 and H-27); 3,32 (1H, dd, *J* = 13,5 Hz, *J* = 5,4 Hz, H-3); 3,69 (3H, s, OCH_3); 4,90 (1H, d, 8,0, H-1′); 5,20 (1H, d, *J* = 7,5 Hz, H-1″); 5,37 (1H, br s, H-12); 6,30 (1H, d, *J* = 8,0 Hz, H-1‴).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 15,4 (C-25); 16,2 (C-24); 17,3 (C-26); 23,5 (C-30); 26,0 (C-27); 27,7 (C-23); 33,0 (C-29); 52,0 (OCH_3); 89,3 (C-3); 95,6 (C-1‴); 105,0 (C-1′); 106,5 (C-1″); 122,7 (C-12); 144,0 (C-13); 176,5 (C-28).

3.3.21. Compound AR-09: linalool 3-O-β-D-xylopyranosyl-(1→6)-O-β-D-glucopyranoside

Amorphous, white powder; Molecular formula: $C_{21}H_{36}O_{10}$; Molecular mass: 448; HR-ESI-MS *m/z*: 483,2012 $[M + {}^{35}Cl]^-$; Calcd for $C_{21}H_{36}O_{10}Cl$: M = 483,1997.

^{1}H-NMR (500 MHz, Py-d_5) δ (ppm): 1,48 (3H, s, H-10); 1,52 (3H, s, H-8); 1,60 (3H, s, H-9); 5,17 (1H, dd, *J* = 16,0 Hz, *J* = 1,0 Hz, H-1); 4,83 (1H, d, *J* = 7,5 Hz, H-1′); 4,91 (1H, d, *J* = 7,5 Hz, H-1″); 5,16 (1H, m, H-6); 5,18 (1H, dd, *J* = 10,0 Hz, *J* = 1,0 Hz, H-1); 6,38 (1H, dd, *J* = 16,0 Hz, *J* = 10,0 Hz, H-2).

^{13}C-NMR (125 MHz, Py-d_5) δ (ppm): 23,8 (C-10); 25,5 (C-9); 99,0 (C-1′); 105,3 (C-1″); 114,1 (C-1); 125,3 (C-6); 130,9 (C-7); 144,0 (C-2).

3.3.22. Compound AR-10: linalool 3-O-α-L-arabinopyranosyl-(1→6)-O-β-D-glucopyranoside

Amorphous, white powder; Molecular formula: $C_{21}H_{36}O_{10}$; Molecular mass: 448; HR-ESI-MS *m/z*: 483,2018 $[M + {}^{35}Cl]^-$; Calcd for $C_{21}H_{36}O_{10}Cl$: M= 483,1997.

^{1}H-NMR (500 MHz, Py-d_5) δ (ppm): 1,47 (3H, s, H-10); 4,80 (1H, d, *J* = 7,5 Hz, H-1′); 1,50 (3H, s, H-8); 1,59 (3H, s, H-9); 4,87 (1H, d, 7,0, H-1″), 5,15

(1H, m, H-6); 5,19 (1H, dd, *J* = 16,0 Hz, *J* = 1,0 Hz, H-1); 5,21 (1H, dd, *J* = 10,0 Hz, *J* = 1,0 Hz, H-1).

^{13}C-NMR (125 MHz, Py-d_5) δ (ppm): 23,0 (C-10); 25,5 (C-9); 98,9 (C-1′); 104,5 (C-1″); 114,2 (C-1); 125,2 (C-6); 130,9 (C-7); 143,8 (C-2).

3.3.23. Compound AR-11: 3-O-β-D-glucuronopyranosyl-oleanolic acid-28-O-β-D-glucopyranoside (chikusetsusaponin IVa)

Amorphous, white powder; Molecular formula: $C_{42}H_{66}O_{14}$; Molecular mass: 794.

^{1}H-NMR (500 MHz, Py-d_5) δ (ppm): 0,82 (3H, s, H-25); 0,89 (3H, s, H-30); 0,92 (3H, s, H-29); 0,95 (3H, s, H-24); 1,07 (3H, s, H-26); 1,26 (3H, s, H-27); 1,27 (3H, s, H-23); 3,32 (1H, dd, *J* = 13,5 Hz, *J* = 5,4 Hz, H-3); 4,81 (1H, d, *J* = 7,0 Hz, H-1′); 5,41 (1H, br s, H-12); 6,29 (1H, d, *J* = 8,0 Hz, H-1″).

^{13}C-NMR (125 MHz, Py-d_5) δ (ppm): 15,5 (C-25); 17,0 (C-24); 17,5 (C-26); 23,1 (C-30); 26,1 (C-27); 28,3 (C-23); 33,1 (C-29); 62,2 (C-6″); 89,2 (C-3); 95,7 (C-1″); 106,5 (C-1′); 122,9 (C-12); 144,1 (C-13); 176,5 (C-28); 176,6 (C-6′).

3.3.24. Compound AR-12: 3-O-β-D-glucuronopyranosyl-23-hydroxyoleanolic acid-28-O-β-D-glucopyranoside

Amorphous, white powder; Molecular formula: $C_{42}H_{66}O_{15}$; Molecular mass: 810; HR-ESI-MS *m/z*: 809,4308 $[M-H]^+$, 845,4018 $[M+^{35}Cl]^-$; Calcd for $C_{42}H_{65}O_{15}$: M = 809,4323, $C_{42}H_{66}O_{15}{}^{35}Cl$: M = 845,4090.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 0,88 (1H, s, H-24); 0,93 (1H, s, H-30); 0,95 (1H, s, H-29); 0,97 (1H, s, H-25); 1,17 (1H, s, H-27); 3,27 and 3,65 (2H, d, *J* = 12,0 Hz, H-23); 3,69 (1H, m, H-3); 4,46 (1H, d, *J* = 8,0 Hz, H-1″); 5,27 (1H, br s, H-12); 5,40 (1H, d, *J* = 8,0 Hz, H-1′).

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 13,7 (C-24); 16,5 (C-25); 17,7 (C-26); 23,9 (C-29); 26,3 (C-27); 33,4 (C-30); 62,4 (C-6′); 64,6 (C-23); 82,2 (C-3); 95,7 (C-1′); 123,7 (C-12); 144,9 (C-13); 104,9 (C-1″); 177,0 (C-6″); 178,2 (C-28).

3.3.25. Compound AR-13: chikusetsusaponin IVa methyl ester

Amorphous, white powder; Molecular formula: $C_{43}H_{68}O_{14}$; Molecular mass: 808

^{1}H-NMR (500 MHz, Py-d_5) δ (ppm): 0,79 (3H, s, H-25); 0,84 (3H, s, H-30); 0,87 (3H, s, H-24); 0,93 (3H, s, H-29); 1,04 (3H, s, H-26), 1,22 (3H, s, H-27); 1,25 (3H, s, H-23); 3,32 (1H, dd, *J* = 13,5 Hz, *J* = 5,4 Hz, H-3); 3,70 (OCH_3); 4,94 (1H, d, *J* = 8,0 Hz, H-1″); 5,38 (1H, br s, H-12); 6,24 (1H, d, *J* = 8,0 Hz, H-1′).

^{13}C-NMR (125 MHz, Py-d_5) δ (ppm): 15,4 (C-25); 16,8 (C-24); 17,3 (C-26); 23,5 (C-30); 26,0 (C-27); 28,1 (C-23); 33,0 (C-29); 52,0 (OCH_3); 61,9 (C-6′);

89,1 (C-3); 95,6 (C-1′); 107,1 (C-1″); 122,7 (C-12); 144,0 (C-13); 170,7 (C-6″); 176,5 (C-28).

3.4. Biological activities of *A. armata* plant

3.4.1. Biological activities of A. armata leaves

a. Cytotoxic activity

Compounds isolated from *A. armata* leaves were first tested for their cytotoxic effect on cancer cell line HT29 at a concentration of 30 μM by the MTS method to screen and remove compounds with low or no activity.

Active compounds were further tested on cancer cell lines HT29 (human colon cancer), A549 (human lung cancer), A2058 (human melanoma) and HEK-293A (human embryonic kidney cells - normal cells) by SRB method. Samples were sent and performed at the Institute of Biotechnology - Vietnam Academy of Science and Technology, the Institute of Pharmaceutical Sciences - Yonsei University, Korea.

b. Acute toxicity to mice

Acute mice toxicity of the aqueous fraction from the leaves of *A. armata* was performed according to the method described in section 2.4.6. The experiments were conducted at the Department of Pharmacy, Duy Tan University.

3.4.2. Biological activities of A. armata roots

a. Molluscicidal activity for golden apple snail

The molluscicidal activity of pure compounds and the fractions closest to the pure compounds (AA2A2, AA2A4, AA2C2, AA4A3, AA4B2, AA4B3) and were performed according to the method in section 2.4.5. The experiments were conducted at the Research and Development Institute, Duy Tan University.

b. Acute toxicity to brine shrimp

Acute toxicity to brine shrimp of the fractions closest to the pure compounds (AA2A2, AA2A4, AA2C2, AA4A3, AA4B2, AA4B3) was performed according to the method described in section 2.4.7. The experiments were conducted at the Research and Development Institute, Duy Tan University.

CHAPTER 4. RESULTS AND DISCUSSIONS

4.1. Chemical structure of compounds

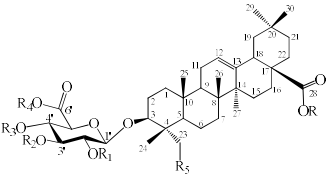
This section presents the detailed results of the spectral analysis and structure determination of 25 isolated compounds from *A. armata* leaves and roots, including 05 new compounds (first published chemical structure), 08 compounds isolated for the first time from *A. armata*, 06 compounds found in both leaves and roots of this plant.

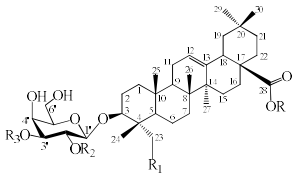
The chemical structure and names of 25 compounds from *A. armata* are shown in **Table 4.21** and **Figure 4.57**.

Table 4.57. Compounds isolated from male *A. armata.*

Symbol	Compound name	Symbol	Compound name
AL-01 and **AR-12**	3-*O*-*β*-D-glucuronopyranosyl-23-hydroxyoleanolic acid-28-*O*-*β*-D-glucopyranoside	**AL-11** and **AR-11**	3-*O*-*β*-D-glucuronopyranosyl-oleanolic acid-28-*O*-*β*-D-glucopyranoside (chikusetsusaponin IVa)
AL-02	3-*O*-[*α*-L-arabinopyranosyl-(1→3)-*β*-D-glucuronopyranosylhederagenin 28-*O*-*β*-D-glucopyranosyl ester	**AL-12**	3*β*,22*α*-dihydroxyolean-12-ene-28-oic acid 3-*O*-[4′-*O*-*α*-L-arabinofuranosyl-(3′-*O*-*β*-D-glucopyranosyl)]-*β*-D-glucuronopyranoside (aramatoside A)
AL-03 and **AR-13**	chikusetsusaponin IVa methyl ester	**AR-04**	23-hydroxyoleanolic acid-[28-*O*-*β*-D-glucopyranosyl]-3-*O*-[2″-*O*-*β*-D-glucopyranosyl-(3″-*O*-*β*-D-glucopyranosyl)]-*β*-D-galactopyranoside (aramatoside C)
AL-04	oleanolic acid-[28-*O*-*β*-D-glucopyranosyl]-3-*O*-{[6‴-*O*-*β*-D-glucopyranosyl-(3″-*O*-*β*-D-glucopyranosyl)]-(4″-*O*-*α*-L-arabinofuranosyl)-*β*-D-glucuronopyranoside (araliaarmoside)	**AR-05**	oleanolic acid-[28-*O*-*β*-D-glucopyranosyl]-3-*O*-[2″-*O*-*β*-D-glucopyranosyl-(3″-*O*-*β*-D-glucopyranosyl)]-*β*-D-galactopyranoside (aramatoside D)
AL-05	oleanolic acid-[28-*O*-*β*-D-glucopyranosyl]-3-*O*-[*β*-D-galactopyranosyl (1→3)]-[*β*-D-glucopyranosyl (1→2)]-*β*-D-glucuronopyranoside	**AR-06**	3-*O*-{*β*-D-glucopyranosyl-(1→3)-[*β*-D-galactopyranosyl-(1→2)]-*β*-D-galactopyranosyl}-oleanolic acid
AL-06 and **AR-01**	chikusetsusaponin IV	**AR-07**	3-*O*-{*β*-D-glucopyranosyl-(1→3)-[*β*-D-galactopyranosyl-(1→2)]-*β*-D-galactopyranosyl}-oleanolic acid-28-*O*-*β*-D-glucopyranosy ester (araliasaponin XVI)

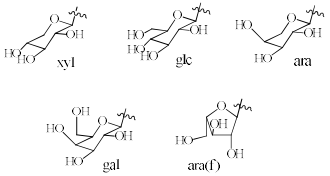
AL-07	oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester	**AR-08**	pseudogisenoside RT1 methyl ester
AL-08 and **AR-02**	narcissiflorine	**AR-09**	linalool 3-*O*-*β*-D-xylopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside
AL-09	3*β*,23*α*-dihydroxyolean-12-ene-28-oic acid 3-*O*-[4′-*O*-*α*-L-arabinofuranosyl-(3′-*O*-*β*-D-glucopyranosyl)]-*β*-D-glucuronopyranoside (aramatoside B)	**AR-10**	linalool 3-*O*-*α*-L-arabinopyranosyl-(1→6)-*O*-*β*-D- glucopyranoside
AL-10 and **AR-03**	stipuleanoside R1		

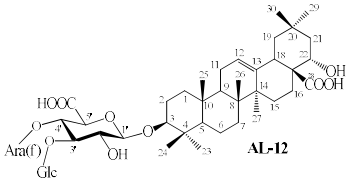


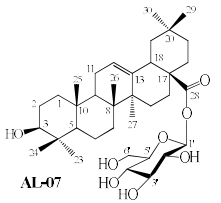


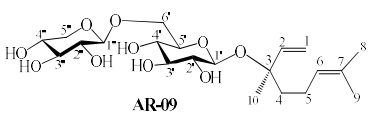
AR-12/AL-01 R = glc; $R_1 = R_2 = R_3 = R_4 = H$; $R_5 = OH$
AL-02 R = glc; $R_1 = R_3 = R_4 = H$; R_2 = ara ; $R_5 = OH$
AL-04 R = glc; $R_1 = R_4 = R_5 = H$; R_3 = ara(f); R_2 = glc 1→6 glc
AL-05 R = R_1 = glc; R_2 = gal; $R_3 = R_4 = R_5 = H$
AL-09 $R = R_4 = H$; R_2 = glc; R_3 = ara(f); $R_5 = OH$
AL-06/AR-01 R = glc; $R_1 = R_2 = R_4 = H$; R_3 = ara(f); $R_5 = H$
AL-08/AR-02 $R = R_1 = R_2 = R_4 = R_5 = H$; R_3 = ara
AL-10/AR-03 $R = R_1 = R_4 = R_5 = H$; R_2 = glc; R_3 = ara(f)
AR-08 R = glc; R_1 = xyl; $R_2 = R_3 = R_5 = H$, $R_4 = CH_3$
AL-11/AR-11 R = glc, $R_1 = R_2 = R_3 = R_4 = R_5 = H$
AL-03/AR-13 R = glc, $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = CH_3$

AR-04 $R = R_2 = R_3$ = glc, $R_1 = OH$
AR-05 $R = R_2 = R_3$ = glc, $R_1 = H$
AR-06 $R = R_1 = H$, R_2 = gal, R_3 = glc
AR-07 $R = R_3$ = glc, $R_1 = H$, R_2 = gal









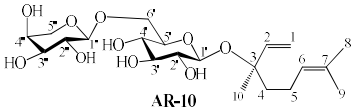


Figure 4.57. Chemical structure of compounds isolated from *A. armata.*

4.2. The results of the evaluation of biological activities of *Aralia armata* leaves

4.2.1. Cytotoxic activity of pure compounds isolated from Aralia armata leaves

04/12 pure compounds from *A. armata* leaves (**AL-07**, **AL-08**, **AL-10**, **AL-11**) exhibit cytotoxic activity against three human cancer cell lines (HT29, A2058, A549) well, the IC_{50} values range from 2,01 ± 0,17 µM to 18,8 ± 1,17 µM. Besides, compounds **AL-07**, **AL-10** and **AL-11** are safe for normal cells (HEK-293A). It is worth mentioning that compound **AL-08**, with strong cytotoxic activity, can be toxic to normal cells (IC_{50} 42,05 ± 1,77 µM); however, compound **AL-08** is still evaluated as safe because the 50% inhibitory concentration for normal cells is much greater than that of cancer cells as well as the positive control (ellipticine) (**Table 4.23**).

Table 4.23. Cytotoxic activity of several compounds from *A. armata* leaves.

No.	Compound	IC_{50} (µM)			
		HT29	**A2058**	**A549**	**HEK-293A**
1	**AL-07**	6,51 ± 1,64	9,41 ± 1,72	6,51 ± 1,6	>100
2	**AL-08**	2,02 ± 1,65	4,26 ± 0,50	2,01 ± 0,17	42,05 ± 1,77
3	**AL-10**	13,9 ± 1,16	18,8 ± 1,17	12,5 ± 0,9	>100
4	**AL-11**	11,9 ± 3,79	4,61 ± 1,24	12,0 ± 2,79	>100
5	Positive control	10,3 ± 1,32 (*)	1,27 ± 0,56 (*)	9,89 ± 0,19 (*)	0,45 ± 0,03 (#)

(*): Irinotecan hydrochloride, (#): Ellipticine.

4.2.2. Acute toxicity for mice of aqueous fraction

The results of the acute toxicity test for the aqueous fraction from the leaves of *A. armata* (**Table 4.24**) show that the mean lethal dose (LD_{50}) of the extract is determined to be 149,256 g/kg, nearly 30 times higher than the toxicity level of According to the Organization for Economic Cooperation and Development (5000 mg/kg), which proves the high safety of the aqueous extract of the leaves of *A. armata* to mice.

Table 4.24. Acute toxicity in mice to the aqueous fraction from *A. armata* leaves

Group	Number of mice for testing (individual)	Dosage (g/kg)	Volume for drinking (mL)	Death/survival rate (individual/individual)
1	10	25	0,6 mL x 03 times	0/10
2	10	50	0,6 mL x 03 times	0/10
3	10	100	0,6 mL x 03 times	3/7

4	10	150	0,6 mL x 03 times	5/5
5	10	200	0,6 mL x 03 times	7/3
6	10	250	0,6 mL x 03 times	8/2
Control	10	Distilled water	0,6 mL x 03 times	0/10

4.3. Results of evaluating the biological activity of the *Aralia armata* roots

4.3.1. Molluscicidal activity of the compounds isolated from Aralia armata roots

The molluscicidal activity of the compounds from the aqueous fraction of *A. armata* roots were tested and compared with the positive control, which is saponins. The results demonstrate that the compounds belonging to the roots of *A. armata* indicate really effective molluscicidal activity; the LC_{50} values of the compounds are in the range of 7,90 – 17,50 µg/mL. Notably, the molluscicidal activity of several compounds (**AR-01**, **AR-04**, **AR-05**, **AR-10**, **AR-11**, **AR-12**, **AR-13**) is higher than saponins - a compound with strong molluscicidal ability. In addition, the isolated fractions closest to the pure compounds (AA2A2, AA2A4, AA2C2, AA4A3, AA4B2, AA4B3) also show significant molluscicidal activity. This is proven by the LC_{50} values of most isolated fractions from 11,54 – 17,88 µg/mL – close to that of the positive control (LC_{50} 11,02 µg/mL) (**Table 4.25**).

Table 4.25. Test results of molluscicidal activity of extracts, isolated fractions and pure compounds from the roots of *A. armata*.

Sample	**LC_{50} (µg/mL)**	**Sample**	**LC_{50} (µg/mL)**
AR-01	**7,90 (6,83-9,11)**	**AR-11**	**7,59 (6,64-8,67)**
AR-02	17,50 (15,72-19,61)	**AR-12**	**8,73 (7,57-10,06)**
AR-03	17,33 (15,34-19,71)	**AR-13**	**7,61 (6,70-8,64)**
AR-04	**9,83 (8,20-11,75)**	AA2A2	14,40 (12,46-16,56)
AR-05	**9,40 (7,89-11,17)**	AA2A4	13,02 (11,34-14,90)
AR-06	16,17 (14,02-18,69)	AA2C2	11,64 (10,08-13,38)
AR-07	15,03 (13,11-17,22)	AA4A3	17,88 (15,36-20,50)
AR-08	16,44 (10,83-25,85)	AA4B2	12,45 (10,75-14,34)
AR-09	15,59 (13,87-17,64)	AA4B3	11,54 (9,89-13,39)
AR-10	**10,30 (8,96-11,82)**	saponin	11,02 (9,35 - 12,79)

4.3.2. Acute toxicity for brine shrimp of near-pure fractions

To evaluate the toxicity of compounds isolated from *A. armata* roots to aquatic environment, acute toxicity test on brine shrimp (*Artemia* sp.) was conducted. The LC_{50} values (µg/mL) of the near-pure fractions (AA2A2, AA2A4, AA2C2, AA4A3, AA4B2, AA4B3) for brine shrimp are 148,55 – 193,22 µg/mL after 24 hours of testing. Comparing the molluscicidal

activity of these fractions to golden apple snail (LC_{50} 11.54 - 17.88 µg/mL), the toxicity to brine shrimp is much lower (10 - 15 times) under the same experimental conditions (**Table 4.26**). Morever, at a concentration of 30 µg/mL, or at concentrations higher than the lethal concentration of 50% of golden apple snails, there was no mortality of brine shrimp. Thus, it can be seen that the compounds from the roots of *A. armata* are non-poisonous to brine shrimp at the lethal concentration of 50% of the golden apple snail.

Table 4.26. Results of acute toxicity test for brine shrimp.

Fraction	LC_{50} (µg/mL)	SI*
AA2A2	148,55 (138,53-158,39)	10,32
AA4A3	178,36 (168,00-188,82)	9,98
AA2A4	193,22 (171,02-221,94)	14,84
AA4B2	155,41 (143,89-167,07)	12,48
AA4B3	164,48 (150,52-178,11)	14,25
AA2C2	162,11 (152,10-172,08)	13,93

**SI (selectivity index) = LC_{50} (Artemia)/LC_{50} (P. canaliculata)*

CONCLUSIONS

1. Chemical composition

From the leaves and roots of *Aralia armata*, **25** compounds are isolated and determined chemical structure. According to the literature review at the time of the study, among the isolated compounds, there were **05 new compounds**, **08 compounds isolated for the first time from *A. armata* species**, **06 compounds presenting in both leaves and roots of this plant**, specifically as follows:

From the leaves of *A. armata*, 12 compounds are isolated (**AL-01** – **AL-12**), including **03 new compounds** named araliaarmoside (**AL-04**), aramatoside B (**AL-09**), aramatoside A (**AL-12**) and **09 compounds known** as 3-*O*-*β*-D-glucuronopyranosyl-23-hydroxyoleanolic acid-28-*O*-*β*-D-glucopyranoside (**AL-01**), 3-*O*-[*α*-L-arabinopyranosyl-(1→3)-*β*-D-glucuronopyranosylhederagenin 28-*O*-*β*-D-glucopyranosyl ester (**AL-02**), chikusetsusaponin IVa methyl ester (**AL-03**), oleanolic acid-[28-*O*-*β*-D-glucopyranosyl]-3-*O*-*[β*-D-galactopyranosyl (1→3)]-[*β*-D-glucopyranosyl (1→2)]-*β*-D-glucuronopyranoside (**AL-05**), chikusetsusaponin IV (**AL-06**), oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester (**AL-07**), narcissiflorine (**AL-08**), stipuleanoside R1 (**AL-10**), chikusetsusaponin IVa) (**AL-11**).

From the roots of *A. armata*, 13 compounds are isolated (**AR-01** – **AR-13**), including **02 new compounds** named aramatoside C (**AR-04**), aramatoside D (**AR-05**) and **11 compounds known** as chikusetsusaponin IV (**AR-01**), narcissiflorine (**AR-02**), stipuleanoside R1 (**AR-03**), 3-*O*-{*β*-D-glucopyranosyl-(1→3)-[*β*-D-galactopyranosyl-(1→2)]-*β*-D-

galactopyranosyl}-oleanolic acid (**AR-06**), araliasaponin XVI (**AR-07**), pseudogisenoside RT1 methyl ester (**AR-08**), linalool 3-*O*-*β*-D-xylopyranosyl-(1→6)-*O*-*β*-D- glucopyranoside (**AR-09**), linalool 3-*O*-*α*-L-arabinopyranosyl-(1→6)-*O*-*β*-D- glucopyranoside (**AR-10**), chikusetsusaponin IVa (**AR-11**), 3-*O*-*β*-D-glucuronopyranosyl-23-hydroxyoleanolic acid-28-*O*-*β*-D-glucopyranoside (**AR-12**), chikusetsusaponin IVa methyl ester (**AR-13**).

Among the compounds mentioned above, 08 compounds are isolated for the first time from *A. armata* species, including **AL-01**/**AR-12**, **AL-02**, **AL-05**, **AR-06**, **AR-07**, **AR-08**, **AR-09**, **AR-10**; 06 compounds presenting in both leaf and root parts of this plant are **AL-01**/**AR-12**, **AL-03**/**AR-13**, **AL-06**/**AR-01**, **AL-08**/**AR-02**, **AL-10**/**AR-03**, **AL-11**/**AR-11**.

2. Biological activities

The cytotoxic activity of 04/12 compounds isolated from *Aralia armata* leaves is evaluated *in vitro*. For three human cancer cell lines (HT29, A2058, A549), the compounds have IC_{50} values ranging from 2,01 ± 0,17 µM to 18,8 ± 1,17 µM. For the normal cell line HEK-293A, compound **AL-08** has an IC_{50} value at 55,04 ± 2,32 µM; compounds **AL-07**, **AL-10**, **AL-11** have IC_{50} value >100 µM.

Acute mice toxicity of the aqueous fraction of the leaves of *A. armata* is evaluated. The mean lethal dose (LD_{50}) for white mice is 149,256 g/kg.

The molluscicidal activity of compounds isolated from *A. armata* roots is evaluated on the golden apple snail. The LC_{50} values of the compounds range from 7,90 to 17,50 µg/mL.

Acute brine shrimp toxicity of the near-pure fractions (AA2A2, AA2A4, AA2C2, AA4A3, AA4B2, AA4B3) from the roots of *A. armata* is evaluated, the LC_{50} values of the fractions range from 148,55 to 193,22 g/mL.

RECOMMENDATIONS

From the results on the isolation and biological activities of Aralia armata leaves and roots in Vietnam, we recommend:

- Continue to investigate the chemical composition of other extracts from the leaves and roots of Aralia armata to search for new compounds in this plant.
- Continue testing other activities for isolated compounds.

PUBLICATIONS WITHIN THE SCOPE OF THESIS

[1] **Nguyen Thi Hong Chuong,** Do Thi Thuy Van, Giang Thi Kim Liên, Pham Hai Yen, Dan Thuy Hang, Nguyen Xuan Nhiem, Bui Huu Tai, Phan Van Kiem (2021), "Aramatosides C and D, Two Previously Undescribed Triterpene Glycosides Isolated from The Roots of *Aralia armata*", *Natural Product Communications*, *16*(7), 1934578X211033686. Link: https://journals.sagepub.com/doi/pdf/10.1177/1934578X211033686

[2] **Thi Hong Chuong Nguyen**, Giang Thi Kim Lien, Pham Hai Yen, Thanh-Tam Ho, Do Thi Thuy Van, Phan Van Kiem, Nguyen Huy Hung, Ping-Chung Kuo, William N. Setzer (2022), "Molluscicidal activity of compounds from the roots of *Aralia armata* against the golden apple snail (Pomacea canaliculata)", *Natural Product Communications*, 17(12), 1–12. Link: https://journals.sagepub.com/doi/full/10.1177/1934578X221144573

[3] Pham Hai Yen, **Nguyen Thi Hong Chuong**, Giang Thi Kim Lien, Nguyen Thi Cuc, Nguyen Xuan Nhiem, Nguyen Thi Viet Thanh, Bui Huu Tai, Yohan Seo, Wan Namkung, SeonJu Park, Seung Hyun Kim, Chau Van Minh, Pham Van Kiem (2020), "Oleanane-type triterpene saponins from *Aralia armata* leaves and their cytotoxic activity", *Natural Product Research*, 36(1), 142-149. Link: https://www.tandfonline.com/doi/abs/10.1080/14786419.2020.1768090

[4] Pham Hai Yen, Nguyen Thi Cuc, Phan Thi Thanh Huong, Nguyen Xuan Nhiem, **Nguyen Thi Hong Chuong**, Giang Thi Kim Lien, Bui Huu Tai, Nguyen Van Tuyen, Chau Van Minh, Phan Van Kiem (2020), "Araliaarmoside: A New Triterpene Glycoside Isolated From the Leaves of *Aralia armata*". *Natural Product Communications*, *15*(9), 1934578X20953300. Link: https://journals.sagepub.com/doi/pdf/10.1177/1934578X20953300

[5] **Nguyen Thi Hong Chuong**, Phan Nguyen Phuong Thao, Nguyen Huy Hung, Giang Thi Kim Lien, Pham Hai Yen, Do Thi Thuy Van (2021). *Aralia armata* roots: extraction, isolation and molluscicidal activity against golden apple snails, *Pomacea canaliculata*, *The University of Danang - Journal of science and technology*, 19, 21-24. Link: https://jst-ud.vn/jst-ud/article/view/7640