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GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

NGUYEN THI THU HIEN

STUDY ON CHEMICAL CONSTITUENTS AND CYTOTOXIC ACTIVITY OF *ARALIA DASYPHYLLA* AND *ARALIA HIEPIANA* SPECIES IN WESTERN HIGHLANDS OF VIETNAM

Major: Chemistry of natural compounds
Code: 9 44 01 17

SUMMARY OF CHEMICAL DOCTORAL THESIS

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Adviser 1: Dr. Nguyen Huu Toan Phan
Adviser 2: Prof. Dr. Nguyen Manh Cuong

1st Reviewer: Assoc. Prof. Dr. Nguyen Xuan Nhiem
2nd Reviewer: Assoc. Prof. Dr. Pham Thi Nguyet Hang
3rd Reviewer: Assoc. Prof. Dr. Nguyen Quang Tung

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Thesis can be found in - The library of the Graduate University of Science and Technology, Vietnam Academy of Science and Technology.
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INTRODUCTION

1. The urgency of the thesis

The genus *Aralia* (Araliaceae) was found in Asia, and North America. The dried leaves, roots and stem barks of several *Aralia* species have been used in the traditional medicines to treat diabetes, hepatitis, stomach ulcer, and other diseases. Previous chemical studies of *Aralia* species have reported the isolation of triterpenoid saponins, diterpenoids, phenolics, and acetylenic lipids. *Aralia* plants exhibited antibacterial, anti-inflammatory, antioxidant, cytotoxic... However, there are few researches on the chemical components and biological activities of *Aralia* species growing in Vietnam. In the screening of biologically active plant in Lam Dong province project, we found that *Aralia dasyphylla* Miq. and *Aralia hiepiana* J. Wen & Lowry of Araliaceae family were distributed in Lam Dong, Vietnam have biological activities to againt cancer cells and antibacterial. *A. hiepiana* species is a newly discovered in 2002. Up to now, there have not been any studies on the chemical composition and biological activity of this species. From above reasons, thesis title was chosen to be "**Study on chemical constituents and cytotoxic activity of *Aralia dasyphylla* and *Aralia hiepiana* species in western highlands of Vietnam**".

2. The objectives of the thesis

Study on chemical constituents of *Aralia dasyphylla* Miq. and *Aralia hiepiana* J.Wen & Lowry species.

Evaluation of biological activities of isolated compounds to find potential compounds.

3. The main contents of the thesis

1. Isolation of compounds from the leaves of *Aralia dasyphylla* Miq. and *Aralia hiepiana* J.Wen & Lowry.

2. Determination of chemical structures of the isolated compounds.

3. Evaluation on the cytotoxic activity inhibiting of the isolated compounds.

The layout of the thesis:

The thesis consists of 150 pages with 38 tables, 74 pictures and 155 references. The thesis includes 4 chapters: Introduction (1 pages), Chapter 1: Overview (37 pages); Chapter 2: Materials and research methods (9 pages); Chapter 3: Experimental (17 pages);, Chapter 4: Results and discussion (75 pages); Conclusion (1 page); Recommendations (1 page); Articles related to the thesis (1 page); References (10 pages); Appendix (112 pages).

CHAPTER 1: OVERVIEW

Overview of all researches related to my studies of the chemical constituents and biological activities of *Aralia* genus, Araliaceae.

1.1. Introduction to *Aralia genus*

1.1.1. Plant characteristics of Aralia genus

Aralia genus belongs to the Araliaceae family, consisting of 79 accepted species of deciduous or evergreen trees, shrubs, and rhizomatous herbaceous perennials, distributed in the Asia and America.

1.1.2. The review of Aralia genus in traditional medicine

1.1.3. The review of Aralia chemical constituents

In recent years, there have been many studies on chemical constituents and biological activities of *Aralia* species. According to published papers in the liturature, the chemical constituents of the *Aralia* genus include main classes: Saponins (Compound **1-172**), triterpenes (**173-180**), diterpenes (**181-223**), sterols (**224-228**), phenolics (**229-266**), polyacetylens (**267-276**), alkaloids (**277-282**), glycolipids (**283-284**), polysaccharides (**285-291**).

Especially, triterpene saponins with olean-12-en skeleton are quite common compounds in the species of *Aralia*.

1.1.4. The review of Aralia biological activities

Studies showed that *Aralia* extract and compounds possessed a wide range of biological activities such as: Anti-inflammatory, cytotoxicity, many members of genus *Aralia* have been investigated for their potential usefulness in treating diabetes mellitus, antiproliferative, antibacterial.

1.2. Introduction about two plant were researched

1.2.1. Introduction about plant characteristics of Aralia dasyphylla Miq.: This section introduces scientific names, synonyms, Vietnamese names, botanical characteristics, distribution and previous research of *A. dasyphylla*. Currently, there are only two studies on the chemical composition of the roots of this species, and there are no studies on the leaves.

1.2.1. Introduction about plant characteristics of Aralia hiepiana J.Wen & Lowry: This section introduces the scientific name, Vietnamese name, botanical characteristics, distribution of *A. hiepiana.* There have not been any studies on the chemical composition of this species in Vietnam and in the world.

CHAPTER 2: PLANT MATERIALS AND METHODS

2.1. Plant materials

The samples of the plant *A. dasyphylla* Miq. and *A. hiepiana* J.Wen & Lowry were collected in Lam Dong province and identified by Dr. Nong Van Duy from the Tay Nguyen Institute for Scientific Research, VAST.

2.2. Methods

2.2.1. Isolation methods

This section presents methods for isolating pure compounds: thin-layer chromatography and column chromatography.

2.2.2. Methods for determination of chemical struture of compounds

This section showed the general methods to determine the chemical structure of the compounds are combination of physical parameters and modern spectroscopic methods including: Mass spectrometry and high-resolution mass spectrometry (HR-ESI-MS), magnetic resonance spectrum (1D, 2D-NMR), ultraviolet-visible (UV), infrared (IR).

2.5. Methods for evaluation of biological activities

This section presents chemicals and equipment for the *in vitro* biological activity test method and *in silico* in hGLUT1 protein.

CHAPTER 3: EXPERIMENTALS

3.1. Extraction of *A. dasyphylla*

This section presents the process of making methanol extracts and partitioned extract from *A. dasyphylla*.

3.2. Isolation compounds from *A. dasyphylla*

This section presents in detail the isolated procedure of 21 compounds from *A. dasyphylla*.

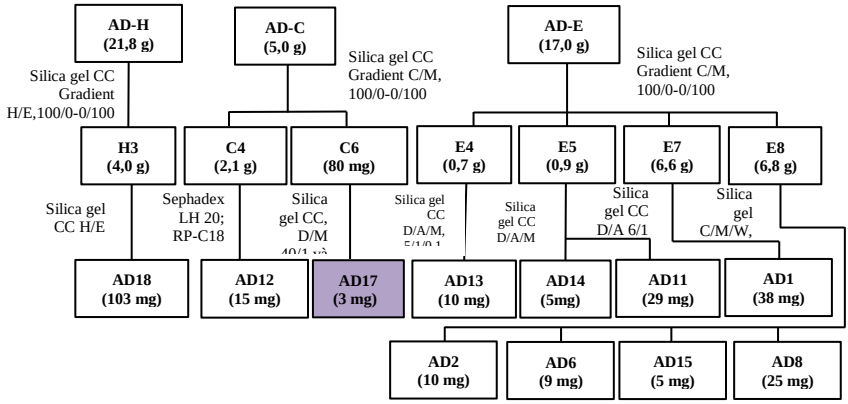


Figure 3.2. Schematic diagram of compounds isolated from *n*-hexane, chloroform and ethyl acetate fractions of *A. dasyphylla*

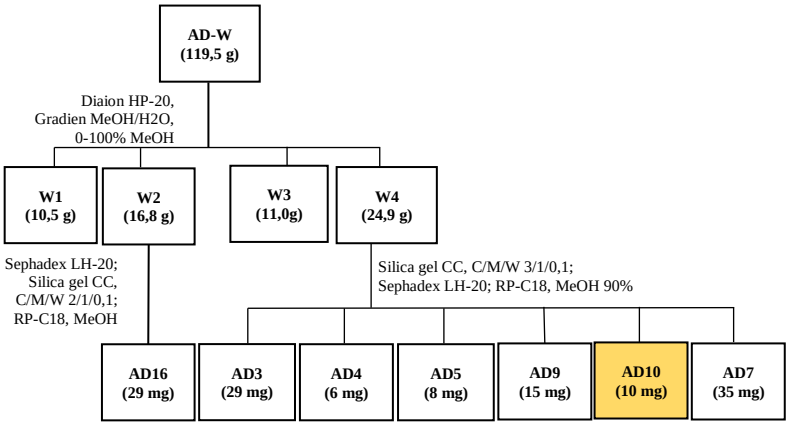


Figure 3.3. Schematic diagram of compounds isolated from water fractions of *A. dasyphylla*

3.3. Physical properties and spectroscopic data of the isolated compounds from *A. dasyphylla*

3.3.1. Compound AD1: Acid ursolic

3.3.2. Compound AD2: 3-O-β-D-glucopyranosyl (1→3)-α-L-arabinopyranosyl ursolic acid

3.3.3. Compound AD3: Matesaponin 1

3.3.4. Compound AD4a: 3-O-α-L-arabinopyranosyl oleanolic acid

3.3.5. Compound AD4b: 3-O-α-L-arabinopyranosyl ursanolic acid

3.3.6. Compound AD5a: Oleanolic acid 28-O-β-D-glucopyranosyl ester

3.3.7. Compound AD5b: Ursolic acid 28-O-β-D-glucopyranosyl ester

3.3.8. Compound AD6a: Elatoside F

3.3.9. Compound AD6b: Araliasaponin VIII

3.3.10. Compound AD7: Elatoside E

3.3.11. Compound AD8: Acutoside A

3.3.12. Compound AD9: Oleanderolide

3.3.13. Compound AD10: 3-O-β-D-glucopyranosyl (1→3)-α-L-arabinopyranosyl 12α-hydroxyolean 28,13-olide (New compound)

White powder. IR (ν_{max}) cm^{-1}: 3394, 2927, 1775, 1078. ESI-MS:

m/z 767.4 $[M+H]^+$, *m/z* 765.7 $[M-H]^-$, M = 766. Molecular formula: $C_{41}H_{66}O_{13}$.

^{1}H NMR (CD_3OD, 500 MHz) δ ppm: 3.18 (1H, dd, *J* = 8.0, 8.0 Hz, H-3), 4.17 (1H, br s, H-12), 1.03 (3H. s, H-23), 0.84 (3H, s, H-24), 0.89 (3H, s, H-25), 0.91 (3H, s, H-26), 1.40 (3H, s, H-27), 1.00 (3H, s, H-29), 0.91 (3H, s, H-30), 4.26 (1H, d, *J* = 7.5 Hz, H-1'), 4.53 (1H, d, *J* = 7.5 Hz, H-1").

^{13}C NMR (CD_3OD, 125 MHz) δ ppm: 39.0 (C-1), 26.1 (C-2), 89.3 (C-3), 39.5 (C-4), 56.0 (C-5), 17.8 (C-6), 34.8 (C-7), 42.7 (C-8), 45.2 (C-9), 36.6 (C-10), 29.6 (C-11), 65.4 (C-12), 92.0 (C-13), 43.4 (C-14), 29.2 (C-15), 21.6 (C-16), 45.6 (C-17), 52.3 (C-18), 40.1 (C-19), 31.9 (C-20), 34.2 (C-21), 27.8 (C-22), 28.1 (C-23), 16.4 (C-24), 16.8 (C-25), 19.0 (C-26), 20.3 (C-27), 179.3 (C-28), 33.3 (C-29), 23.7 (C-30), 105.7 (C-1'), 71.1 (C-2'), 83.0 (C-3'), 68.2 (C-4'), 65.7 (C-5'), 104.3 (C-1"), 74.0 (C-2"), 76.4 (C-3"), 70.0 (C-4"), 76.3 (C-5"), 61.9 (C-6").

3.3.14. Compound AD11: Kaempferol

3.3.15. Compound AD12: Hispidulin

3.3.16. Compound AD13: Eupafolin

3.3.17. Compound AD14: Kaempferol-7-O-α-L-rhamnoside

3.3.18. Compound AD15 Kaempferitrin

3.3.19. Compound AD16: Kaempferol 3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside

3.3.20. Compound AD17: 4-O-Methyl burseneolignan (New compound)

White powder. HR-TOF-MS *m/z* 475.1964 $[M+Na]^+$ (for formular $C_{23}H_{32}O_9Na$ is 475,1944). M = 452. Molecular formula: $C_{23}H_{32}O_9$.

^{1}H NMR (CD_3OD, 500 MHz) δ ppm: 6.78 (2H, s, H-2, H-6), 5.03 (1H, d, *J* = 6.0 Hz, H-7), 4.11 (1H, m, H-8), 3.40 (1H, dd, *J* =

4.0; 12.0 Hz, Ha-9), 3.81 (1H, dd, *J* = 4.8; 12.0 Hz, Hb-9), 6.55 (2H, s, H-2', H-6'), 2.65 (2H, t, *J* = 7.8 Hz, H-7'), 1.84 (2H, m, H-8'), 3.59 (2H, t, *J* = 6.5, H-9'), 3.86 (6H, s, 3,5-OCH_3), 3.76 (3H, s, 4-OCH_3), 3.84 (6H, s, 3',5'-OCH_3). ^{13}C NMR (CD_3OD, 125 MHz) δ ppm: 138.4 (C-1), 105.3 (C-2, C-6), 154.2 (C-3, C-5), 138.4 (C-4), 74.4 (C-7), 88.4 (C-8), 61.9 (C-9), 140.3 (C-1'), 106.8 (C-2', C-6'), 154.1 (C-3', C-5'), 135.3 (C-4'), 33.4 (C-7'), 35.4 (C-8'), 62.1 (C-9'), 56.61 (3,5-OCH_3), 61.1 (4-OCH_3), 56.6 (3',5'-OCH_3).

3.3.18. Compound AD18: β-sistosterol

3.4. Extraction of *A. hiepiana*

This section presents the process of making methanol extracts and partitioned extract from *A. hiepiana*.

3.5. Isolation compounds from *A. hiepiana*

This section presents in detail the isolated procedure of 21 compounds from *A. hiepiana*

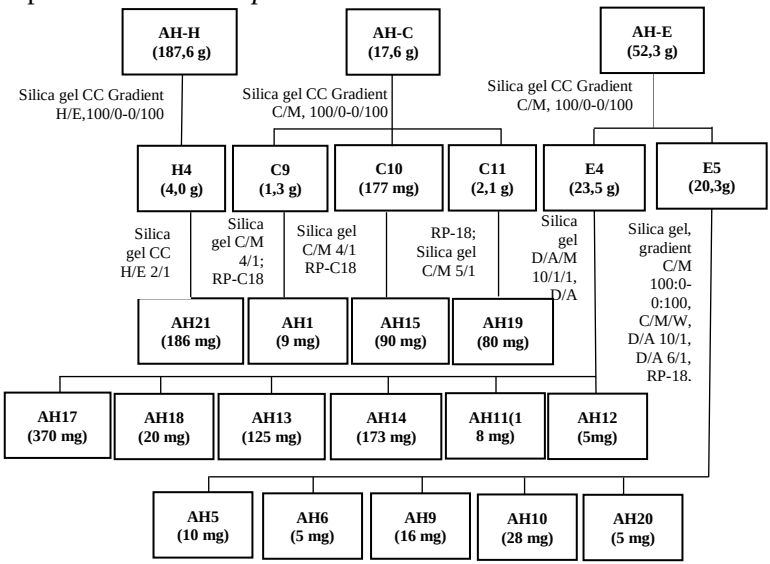


Figure 3.5. Schematic diagram of compounds isolated from *n*-hexane, chloroform and ethyl acetate fractions of *A. hiepiana*

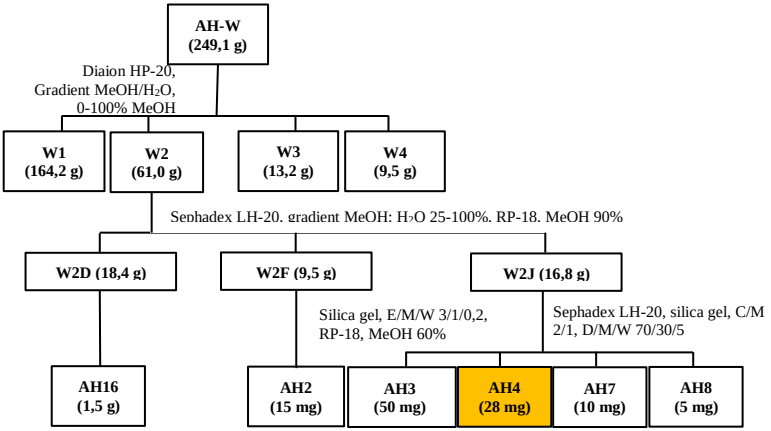


Figure 3.6. Schematic diagram of compounds isolated from water fractions of *A. hiepiana*

3.6. Physical properties and spectroscopic data of the isolated compounds from *A. hiepiana*

3.6.1. Compound AH1: 3-O-(α-L-arabinopyranosyl)-β-D-glucopyranosyl olean-12-en oic acid

3.6.2. Compound AH2: Araliasaponin IV

3.6.3. Compound AH3: Congmujingnoside B

3.6.4. Compound AH4: 3-O-([β-D-xylopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyl) oleanolic acid 28-O-β-D-glucopyranosyl ester (New compound)

White powder, HR-ESI-MS $[M+Na]^+$ *m/z* 1229.5934, (1229.5926 for $C_{58}H_{94}O_{26}Na$). Molecular formula $C_{58}H_{94}O_{26}$.

^1H NMR (500 MHz, pyridine-d_5) δ ppm: 3.20 (1H, dd, *J* = 4.0, 11.0 Hz, H-3), 5.37 (1H, t, H-12), 3.14 (1H, dd, *J* = 3.5, 13.5 Hz, H-18), 1.21 (3H, s, H-23), 1.03 (3H, s, H-24), 0.84 (3H, s, H-25), 1.04 (3H, s, H-26), 1.21 (3H, s, H-27), 0.84 (3H, s, H-29), 0.84 (3H, s, H-30), 4.72 (1H, d, *J* = 7.0 Hz, H-1'), 5.32 (1H, d, *J* = 7.5 Hz, H-1"),

5.21 (1H, d, *J* = 7.0 Hz, H-1'''), 4.96 (1H, d, *J* = 8.0 Hz, H-1''''), 6.18 (1H, d, *J* = 8.0 Hz, H-1''''').

^{13}C NMR (125 MHz, pyridine-d_5): 38.7 (C-1), 26.6 (C-2), 89.1 (C-3), 39.6 (C-4), 55.8 (C-5), 18.4 (C-6), 32.4 (C-7), 39.8 (C-8), 48.0 (C-9), 36.9 (C-10), 23.3 (C-11), 122.7 (C-12), 144.0 (C-13), 42.0 (C-14), 28.2 (C-15), 23.7 (C-16), 46.9 (C-17), 41.6 (C-18), 46.2 (C-19), 30.6 (C-20), 33.9 (C-21), 33.0 (C-22), 27.7 (C-23), 16.4 (C-24), 15.5 (C-25), 17.4 (C-26), 25.9 (C-27), 176.5 (C-28), 33.0 (C-29), 23.5 (C-30), 105.4 (C-1'), 77.2 (C-2'), 83.5 (C-3'), 68.7 (C-4'), 77.7 (C-5'), 62.3 (C-6'), 104.9 (C-1''), 74.0 (C-2''), 78.6 (C-3''), 71.3 (C-4''), 66.9 (C-5''), 104.9 (C-1'''), 74.9 (C-2'''), 78.3 (C-3'''), 75.7 (C-4'''), 70.6 (C-5'''), 69.1 (C-6'''), 104.9 (C-1''''), 78.3 (C-2''''), 78.2 (C-3''''), 71.1 (C-4''''), 65.9 (C-5''''), 95.5 (C-1'''''), 73.6 (C-2'''''), 75.0 (C-3'''''), 71.3 (C-4'''''), 78.0 (C-5'''''), 62.4 (C-6''''').

3.6.5. Compound AH5: Quercetin

3.6.6. Compound AH6: Apigenin 7-O-β-D-glucopyranoside

3.6.7. Compound AH7: Quercetin-3-O-β-D-glucopyranoside-7-O-α-L-rhampyranoside

3.6.8. Compound AH8: Rutin

3.6.9. Compound AH9: Methyl 3,4-dihydroxybenzoate

3.6.10. Compound AH10: Methyl caffeate

3.6.11. Compound AH11: Acid caffeic

3.6.12. Compound AH12: 2-Hydroxy-4-methoxybenzoic acid

3.6.13. Compound AH13: Methyl-α-L-rhamnopyranoside

3.6.14. Compound AH14: Methyl-α-D-glucopyranoside

3.6.15. Compound AH15: Kaempferitrin

3.6.16. Compound AH16: Matesaponin 1

3.6.17. Compound AH17: Acid ursolic

3.6.18. Compound AH18: Kaempferol

CHAPTER 4. RESULTS AND DISCUSSIONS

4.1. The result of isolation from *A. dasyphylla*

From the MeOH extract of *A. dasyphylla* has led to isolated 21 compounds:

- 01 New triterpene saponin: AD10

- 01 New neolignan: AD17

- 12 known triterpenes: **AD3**, **AD2**, **AD6a**, **AD6b**, **AD8**, **AD1**, **AD9**, **AD4a**, **AD4b**, **AD5a**, **AD5b**, and **AD7**

- 06 known flavonoids: **AD12**, **AD13**, **AD14**, **AD11**, and **AD10**

- 01 known sterol: **AD18**

4.2. Determined the structures of the isolated compound from *A. dasyphylla*

4.2.10. 3-O-(β-D-glucopyranosyl (1→3)-α-L-arabinopyranosyl) 12α-hydroxyolean-28,13-olide (New compound)

Compound **AD10** was isolated as a white powder. The ^{1}H NMR spectrum of **AD10** exhibited signals of 7 methyls at δ_H 1.03 (s, H-23), 0.84 (s, H-24), 0.91 (s, H-25), 1.20 (s, H-26), 1.40 (s, H-27), 1.00 (s, H-29), 0.91 (s, H-30), 01 methine hydroxyl at δ_H 4.17 (t, J = 2.0 Hz, H-12). Additionally, signals of 02 anomer protons of 02 unit of sugar moiety were observed at δ_H 4.26 (d, J = 7.5 Hz, H-1') and δ_H 4.53 (d, J = 7.5 Hz, H-1").

The analysis of ^{13}CNMR, DEPT and HSQC datas of **AD10** revealed the presence of 41 carbons, including 7 methyls, 11 methylenes, 15 methines, 4 oxygenated tertiary, 6 quaternary carbons, 8 tertiary carbons.

The HMBC correlations of anomer protons δ_H 4.26 (H-1', Ara) with C-3 (δ_C 89.3) and the correlations of anomer protons at δ_H 4,53 (H-1", Glc) with carbon at C-3' (δ_C 83.0, Ara), determined the linkage of Ara with C-3 of aglycone and Glc with C-3' of Ara.

Consequently, the structure of **AD10** was determined and named 3-*O*-(*β*-D-glucopyranosyl-(1→3)-*α*-L-arabinopyranosyl)-12*α*-hydroxyolean-28,13-olide.

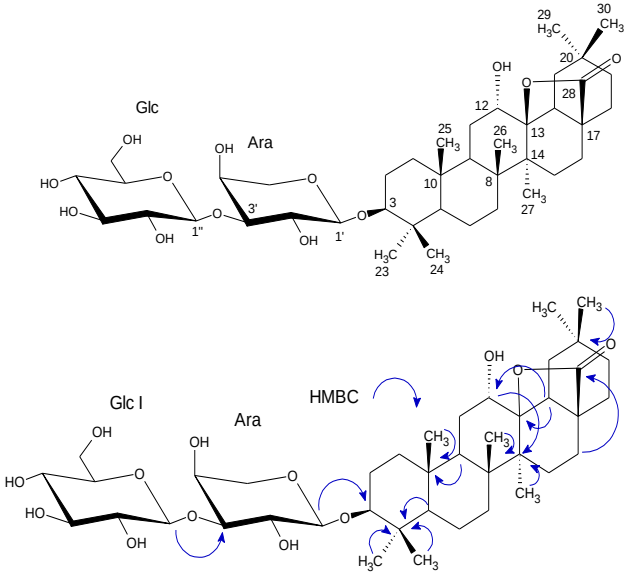
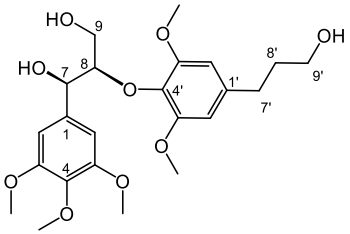
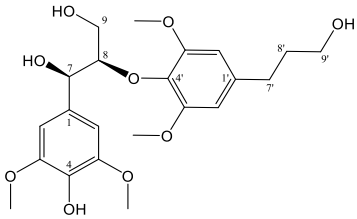


Figure 4.10. Structures and the key HMBC correlations of compound **AD10**.

4.2.17. 4-O-Methyl burseneolignan (New compound)



AD17



Burseneolignan

Figure 4.23. Structure of compound **AD17** and burseneolignan

Compound **AD17** was obtained as a white powder with a molecular of $C_{23}H_{32}O_9$ base on the positive HR-TOF-MS data $[M+Na]^+$ *m/z* 475.1964 (calcd. 475.1944 for $C_{23}H_{32}O_9Na$). The ^1H NMR spectrum of **AD17** showed an additional 1,3,4,5-tetrasubstituted aromatic ring with two equivalent aromatic protons at δ_H 6.78 (2H, s, H-2 and H-6) and 6.55 (2H, s, H-2' and H-6'), five methoxyl groups at δ_H 3.86 (6H, 3, 5-OMe), 3.76 (3H, s, 4-OMe) and 3.84 (6H, s, 3', 5'-OMe), two oxymethines at δ_H 5.03 (1H, d, *J* = 6.0 Hz, H-7) and 4.11 (1H, m, H-8), two oxymethylenes at δ_H 3.40 (1H, dd, *J* = 4.0, 12.0 Hz, Ha-9/3,81 (1H, dd, *J* = 4.8, 12.0 Hz, Hb-9) and 3.59 (2H, t, *J* = 6.5 Hz, H-9') and two methylenes at δ_H 2.65 (2H, t, *J* = 7.8 Hz, H-7') and 1.84 (2H, m, H-8'). The ^{13}C NMR and DEPT spectral data showed the presence of 23 carbons, including 12 carbons of two tetrasubstituted aromatic rings, five methoxyl carbons [δ_C 56.6 (3, 5-OMe), 61.1 (4-OMe) and 56.6 (3', 5'-OMe)], two oxymethines carbons [δ_C 74.4 (C-7) and 88.4 (C-8)], two oxymethylencarbons [δ_C 61.9 (C-9) and 62.1 (C-9')] and two methylen [δ_C 33.4 (C-7') and 35.4 (C-8')]. A comparison of the ^1H and ^{13}C NMR spectroscopic data of **AD17** to those of burseneolignan [2] indicated that the structure of **AD17** very similar with burseneolignan [4], except for the addition of a methoxy at δ_C 61.1.

The methoxy group appearing at C-4 is determined by the long-range correlations between the methoxy proton at δ_H 3.76 to C-4 (δ_C 138.4). The other HMBC correlations agreed with the structure of **AD17**. Thus, **AD17** was determined to be 1R*-(4-methoxy-3,5-dimethoxy-phenyl)-2R*-[4-(3-hydroxy-propyl)-2,6-dimethoxy-phenoxy]- propane-1,3-diol.

4.1.2. Biological activities results of *A. dasyphylla*

The biological activity test results of MeOH extract from *A. dasyphylla* leaves showed the cytotoxic activity on two cancer cell lines Hep-G2 and RD with IC_{50} values of 7.81 and 9.1 µg/mL respectively. Bioactivity testing of isolated compounds showed that three compounds **AD2**, **AD4** and **AD7** had cytotoxic activity on both HepG2 and LU-1 cell lines with IC_{50} values of (5.36, 2.85 µM), (7.21, 4.56 µM), (3.24, 2.55 µM), compound **AD1** had cytotoxic activity against LU-1 cell line with IC_{50} value of 7.04 µM.

Table 4.17. Cell survial value (%) of isolated compound from *A. dasyphylla*

No	Sample	Concentration (µg/mL)	Cell lines/ CS (%)		
			HepG2	LU-1	RD
	DMSO	-	100	100	100
	Positve (+)	5	**1,25±0,3**	**1,87±0,2**	**0**
1	**AD1**	5	68,42±0,96	**29,61±0,15**	66,79±1,51
2	**AD2**	5	**37,2±2,30**	**15,12±0,60**	70,0±2,19
3	**AD3**	5	98,28±0,95	78,70±1,15	98,42±1,47
4	**AD4**	5	**45,98±1,45**	**25,11±1,54**	72,81±1,56
5	**AD5**	5	59,88±1,80	65,52±2,53	64,52±1,34
6	**AD6**	5	97,01±0,90	76,47±2,00	95,57±1,90
7	**AD7**	5	**0**	**18,51±1,20**	67,13±2,17
8	**AD8**	5	98,04±1,66	78,2±3,06	98,77±2,00
9	**AD9**	5	91,72±1,23	74,89±1,82	97,54±2,32
10	**AD10**	5	96,25±2,41	81,54±2,84	97,53±1,74
11	**AD14**	5	79,30±0,70	75,87±2,80	90,40±1,67
12	**AD15**	5	99,04±0,57	84,89±1,77	99,87±0,21
13	**AD17**	5	92,37 ±1,30	99,32±020	92,63 ±0,10
14	**Total Saponin**	20	93,65±1,64	79,67±0,14	91,99±0,99

Positive control (+): Ellipticine

Table 4.18. IC_{50} value of bioactive compounds

No	Sample	Cell lines/ IC_{50} (μM)		
		HepG2	LU-1	RD
	Positive (+)	**1.22**	**1.30**	**1.78**
1	**AD1**	-	**7.04**	-
2	**AD2**	**5.36**	**2.85**	-
3	**AD4**	**7.21**	**4.56**	-
4	**AD7**	**3.24**	**2.55**	-

Positive control (+): Ellipticine

The results of *in silico* bioactivity evaluation on hGLUT1 sugar transporter for saponins isolated from *A. dasyphylla* showed that most of the compounds have medicinal potential with their binding ability and propertie, ADMET properties, among which the compounds elatoside E (**AD7**), 3-*O*-*α*-L-arabinopyranosyl oleanolic acid (**AD4a**), and oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester (**AD5a**) are potential compounds that need further investigation for their potential applications as cytotoxic.

4.2. The result of the isolation of *A. hiepiana*

From the MeOH extract of the leaves of *A. hiepiana* has led to isolated 21 compounds, including:

- **01 new saponin: AH4**
- 05 known triterpenes: **AH1**, **AH2**, **AH3**, **AH16**, **AH17**
- 08 known flavonoids : **AH5**, **AH6**, **AH7**, **AH8**, **AH15**, **AH18**, **AH19**, **AH20**
- 04 known aromatic ring compounds : **AH9**, **AH10**, **AH11**, **AH12**
- 03 anothers: **AH13**, **AH14**, **AH21**

4.2.1 Determination the chemical structure of isolated compounds

4.2.1.4. Compound AH4: 3-O-([β-D-xylopyranosyl-(1→2)]-[β-D-glucopyranosy-(1→6)-β-D-glucopyranosyl-(1→3)]-β-D-

arabinopyranosyl) oleanolic acid 28-O-β-D-glucopyranosyl ester (New compound)

Compound **AH4** was obtained as a white amorphous powder and its molecular formula, $C_{58}H_{94}O_{26}$, was determined by HR-ESI-MS with a quasi-molecular ion peak at *m/z* 1229.5926 $[M+Na]^+$. The seven tertiary methyl groups [δ_H 1.21 (s, H-23), 1.03 (s, H-24), 0.84 (s, H-25), 1.04 (s, H-26), 1.21 (s, H-27), 0.84 (s, H-29), 0.84 (s, H-30)] and one trisubstituted olefinic proton δ_H 5.38 (t, J = 3.5 Hz, H-12), were observed in the ^{1}H NMR spectra as well as the information of the ^{13}C NMR spectra (seven sp^3 carbons at δ_C 27.7 (C-23), 16.4 (C-24), 15.5 (C-25), 17.4 (C-26), 25.9 (C-27), 33.0 (C-29) and 23.5 (C-30), two sp^2 olefinic carbons at δ_C 122.7 and 144.0 analyzed with DEPT and HSQC) showed that the compound was an oleanane-type triterpene saponin.

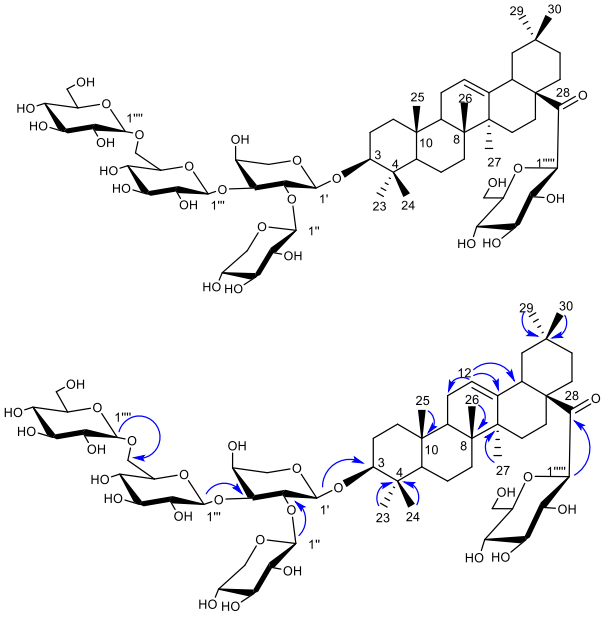


Figure 4.35. The structure and important HMBC corelations of compound **AH4**

The HSQC spectrum of compound **AH4** showed that it contained five sugar units. Their anomeric protons at δ_H 4.72 (d, *J* = 8.0 Hz, H-1', Ara), 5.33 (d, *J* = 7.0 Hz, H-1", Xyl-I), 5.21 (d, *J* = 8.0 Hz, H-1'", Glc I), 4.96 (d, *J* = 8.0 Hz, H-1"", Glc II), và 6.18 (d, *J* = 8.0 Hz, H-1""", Glc III) were correlated with carbons signals at δ_C 105.4 (C-1', Ara), 104.9 (C-1", Xyl), 104.9 (C-1'", Glc I), 104.9 (C-1"", Glc II), và δ_C 95.5 (C-1""", Glc III), respectively.

The spin-system for sugar moieties were assigned based on spectroscopic evidence obtained by 1H-1H COSY, HMBC, and ROESY experiments. The sugar sequences of the sugar chains as well as the glycoside sites were subsequently determined by the HMBC spectrum. In the HMBC spectrum of **AH4**, the correlations could be achieved between the anomeric proton of Ara at δ_H 4.72 (H-1', Ara) and C-3 of aglycone at δ_C 89.3, the anomeric proton of xylose at δ_H 5.33 (H-1", Xyl) and the C-2′ of arabinose at δ_C 77.2, the anomeric proton of glucose-I at δ_H 5.21 (H-1"", Glc I) and the C-3′ of arabinose at δ_C 83.5, the anomeric proton of glucose-II at δ_H 4.96 (H-1"", Glc II) and the C-6′ of glucose-I at δ_C 69.1, and the anomeric proton of glucose-III at δ_H 6.18 (H-1""", Glc III) and C-28 of aglycone at δ_C 176.5. The assignment for all carbon signals was achieved by 2D NMR. Based on this evidence and comparison with the previous literature, the structure of **AH4** was established as 3-*O*-([*β*-D-xylopyranosyl-(1→2)]-[*β*-D-glucopyranoside-(1→6)-*β*-D-glucopyranosyl-(1→3)]-*α*-L-arabinopyranosyl) oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester.

4.2.2. Results of biological activity testing of *A. hiepiana*

The activity test results showed that compound **AH1** exhibited cytotoxic activity against HeLa line with IC_{50} value of 13.19 µM.

Table 4.28. The cytotoxic activity of isolated compounds from *A. hiepiana*

No	Sample	Concentration (μg/mL)	Cell lines/ CS (%)		
			HepG2	LU-1	HeLa
	DMSO	-	100	100	
	Positive (+)	5	**1.25±0.3**	**1.87±0.2**	
1	**AH1**	10	53.65±0.27	61.30±1.18	**45.85±1.71**
2	**AH2**	10	99.45±0.43	99.53±0.27	99.63±0.24
3	**AH3**	10	98.22±1.10	97.96±1.38	99.57±0.16
4	**AH4**	10	99.71±0.16	98.32±0.76	98.90±0.61
5	**AH15**	5	98.75±0.68	99.69±0.3	

Table 4.29. The IC_{50} value of bioactive compound

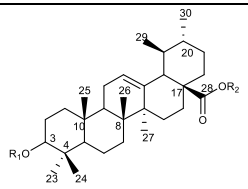
TT	Sample	IC_{50} (μM)/ HeLa
	Positive (+)	0.85
1	**AH1**	**13.19**

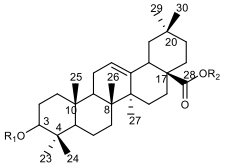
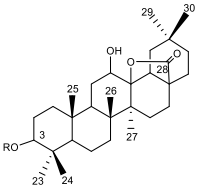
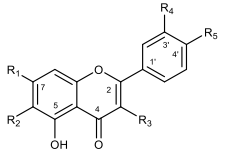
Positive (+): Ellipticine

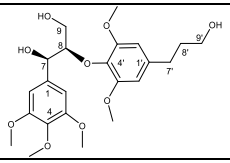
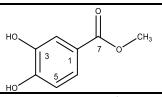
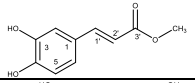
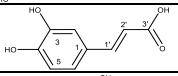
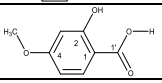
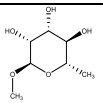
4.3. Summary of research results

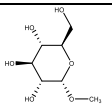
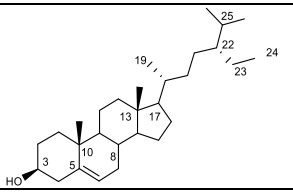
From the leaves of two species *A. dasyphylla* and *A. hiepinana*, **35** compounds have been isolated, mainly belonging to the classes of triterpenoids, flavonoids, lignans, phenolics and sterols.

Table 4.30. The results of isolating compounds

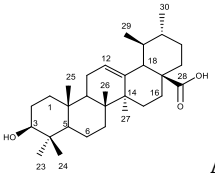
Compound	Name	Structure
Triterpenes		
AD1=AH17	Acid ursolic	 **AD1:** R_1= H, R_2 = H **AD2:** R_1= Glc (1→3) Ara, R_2 = H **AD3:** R_1= Glc (1→3) Ara, R_2 = Glc **AD4b**: R_1= Ara, R_2 = H **AD5b**: R_1= H, R_2 = Glc
AD2	3-*O*-*β*-D-glucopyranosyl (1→3 *O*-*α*-L-arabinopyranosyl) ursanolic acid	
AD3=AH16	Matesaponin 1	
AD4b	3-*O*-*α*-L-arabinopyranosyl ursolic acid	
AD5b	Ursolic acid 28-*O*-*β*-D-glucopyranosyl ester	
AD6b	Araliasaponin VIII	

		AD6b: R_1= [Xyl (1→2), Glc (1→3)] Ara, R_2 = Glc
AD4a	3-*O*-*α*-L-arabinopyranosyl oleanolic acid	
AD5a	Oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester	
AD6a	Elatoside F	
AD7	Elatoside E	
AD8	Acutside A	
AH1	3-*O*-*β*-D-glucopyranosyl -(1→3)-*O*-*α*-L-arabinopyranosyl olean-12-en-28-oic acid	
AH2	Araliasaponin IV	
AH3	Congmujingnoside B	
AH4 (New compound)	3-*O*-([*β*-D-xylopyranosyl-(1→2)]-[*β*-D-glucopyranosyl (1→6)-*β*-D-glucopyranosyl-(1→3)]-*α*-L-arabinopyranosyl) oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester	**AD4a**: R_1 = Ara, R_2 = H **AD5a**: R_1 = H, R_2 = Glc **AD6a**: R_1 = [Xyl (1→2), Glc (1→3)] Ara, R_2 = Glc **AD7:** R_1 = [Xyl (1→2), Glc (1→3)] Ara, R_2 = H **AD8:** R_1 = Glc (1→2) Glc, R_2 = H **AH1:** R_1= Glc (1→3) Ara, R_2 = Glc **AH2**: R_1= [(Xyl (1→2)), (Glc (1→3)] Glc, R_2 = Glc **AH3:** R_1= [Glc (1→6) Glc (1→3), Xyl (1→2)] Glc, R_2 = Glc **AH4:** R_1 = [Xyl (1→2), Glc-(1→6) Glc (1→3)] Ara, R_2 = Glc
AD9 (Isolated for the first time from *Aralia* genus)	Oleanderolide	
AD10 (New compound)	3-*O*-(*β*-D-glucopyranosyl (1→3)-*α*-L-arabinopyranosyl) 12*α*-hydroxyolean-28o,13-olide	**AD9**: R=H **AD10**: R= Glc (1→3) Ara
Flavonoids		
AD11=AH18	Kaempferol	
AD12 (Isolated for the first time from *Aralia* genus)	Hispidulin	
AD13 (Isolated for the first time from *Aralia* genus)	Eupafolin	**AD11**: R_1= OH, R_2= H, R_3= OH, R_4= H, R_5= OH **AD12**:R_1= OH, R_2= OCH_3, R_3= H, R_4= H, R_5= OH

AD14=AH20	Kaempferol-7-*O*-*α*-L-rhamnopyranoside	**AD13**: R_1= OH, R_2= OCH_3, R_3= H, R_4= OH, R_5= OH **AD14**: R_1= O-Rha, R_2= H, R_3= OH, R_4= H, R_5= OH **AD15**: R_1= O-Rha, R_2= H, R_3= O-Rha, R_4= H, R_5= OH **AD16**: R_1= O-Rha, R_2= H, R_3= O-Glc, R_4= H, R_5= OH **AH5**: R_1= OH, R_2= H, R_3= OH, R_4= OH, R_5= OH **AH6**: R_1= O-Glc, R_2= H, R_3= H, R_4= H, R_5= OH **AH7**: R_1= O-Rha, R_2= H, R_3= O-Glc, R_4= OH, R_5= OH **AH8:** R_1= O-Rha, R_2= H, R_3= O- Glc (6→1)Rha, R_4= OH, R_5= OH
AD15=AH15	Kaempferitrin	
AD16=AH19 (Isolated for the first time from *Aralia* genus)	Kaempferol 3-*O*-*β*-D-glucopyranosyl-7-*O*-*α*-L-rhamnopyranoside	
AH5	Quercetin	
AH6 (Isolated for the first time from *Aralia* genus)	Apigenin 7-*O*-*β*-glucoside	
AH7	Quercetin-3-*O*-*β*-D-glucopyranosyl-7-*O*-*α*-L-rhampyranoside	
AH8 (Isolated for the first time from *Aralia* genus)	Rutin	
Lignan		
AD17 (New compound)	4-*O*-Methyl burseneolignan	
Aromatic ring compounds		
AH9	Methyl 3,4-dihydroxybenzoate	
AH10	Methyl caffeate	
AH11	Acid caffeic	
AH12	2-Hydroxy-4-methoxybenzoic acid	
Glycosides		
AH13 (Isolated for the first time from *Aralia* genus)	Methyl *α*-L-rhamnopyranoside	

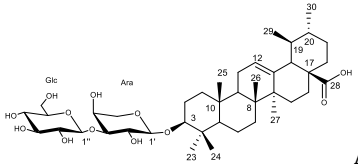
AH14 (Isolated for the first time from *Aralia* genus)	Methyl α-D-glucopyranoside	
Steroid		
AD18=AH21	β-sistosterol	

The MeOH extract of the leaves of A. dasyphylla showed cytotoxic activity against two cancer cell lines HepG2 and RD with IC_{50} values of 7.81 and 9.1 µg/mL, respectively. The n-hexane, chloroform and aqueous extracts also exhibited cytotoxic activity against two cancer lines HepG2 and RD with IC_{50} values in the range of 32-37 µg/mL.



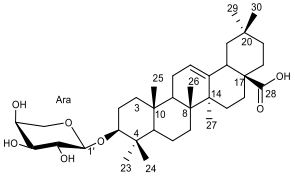
AD1

AD1 showed cytotoxic activity against LU-1 cell line with IC_{50} value of 7.04 µM.

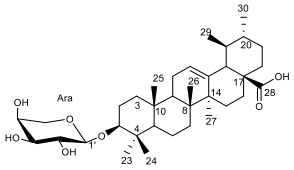


AD2

AD2 showed the cytotoxic activity on both HepG2 and LU-1 cell lines with IC_{50} value of 5.36, 2.85 µM, respectively. This is the compound with the second most negative binding energy value (-11.54 kcal/mol) compared to the 5RE standard.

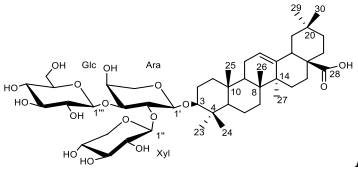


AD4a



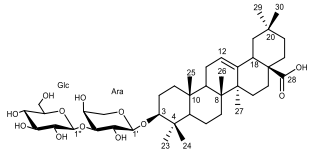
AD4b

The mixture of AD4a and AD4b exhibited cytotoxic activity on both HepG2 and LU-1 cell lines with IC_{50} value is 7.21, 4.56 µM, respectively.



AD7

AD7 showed cytotoxic activity on both HepG2 and LU-1 cell lines with IC_{50} value of 3.24, 2.55 µM, respectively. This is the compound with the most negative binding free energy value (-11.61 kcal/mol) compared to the 5RE standard.



AH1

AH1 showed cytotoxic activity on HeLa cell lines with IC_{50} value of 13.19 µM.

Figure 4.52. Summary of biological activity results

CONCLUSIONS AND RECOMMENDATIONS

1. CONCLUSIONS

This is the first publication in Vietnam as well as in the world on the chemical composition and cytotoxic activity of the leaves of *A. dasyphylla* Miq. distributed in Lac Duong district, Lam Dong province.

This is the first publication in Vietnam as well as in the world on the chemical composition and cytotoxic activity of the leaves of *A. hiepiana* J. Wen & Lowry.

From the leaves of two studied species, **35** compounds were isolated and identified and the cytotoxic activity of the extracts and some selected compounds were evaluated, namely:

1.1. Chemical constituent

From leaves plant *A. dasyphylla* 21 compounds were isolated including 2 new compounds namely: 3-*O*-(*β*-D-glucopyranosyl (1→3)-*α*-L-arabinopyranosyl) 12*α*-hydroxyoleanolic-28,13-olide (**AD10**), 4-*O*-Methyl burseneolignan (**AD17**) and 19 known compounds: acid ursolic (**AD1**), 3-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*α*-L-arabinopyranosyl ursanolic acid (**AD2**), matesaponin 1 (**AD3**), elatoside E (**AD7**), acutoside A (**AD8**), oleanderolide (**AD9**), kaempferol (**AD11**), hispidulin (**AD12**), eupafolin (**AD13**), kaempferol-7-*O*-*α*-L-rhamnopyranoside (**AD14**), kaempferitrin (**AD15**), kaempferol 3-*O*-*β*-D-glucopyranosyl-7-*O*-*α*-L-rhamnopyranoside (**AD16**), *β*-sistosterol (**AD18**), elatoside F (**AD6a**), araliasaponin VIII (**AD6b**), 3-*O*-*α*-L-arabinopyranosyl oleanolic acid (**AD4a**), 3-*O*-*α*-L-arabinopyranosyl ursolic acid (**AD4b**), oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester (**AD5a**), ursolic acid 28-*O*-*β*-D-glucopyranosyl ester (**AD5b**).

From leaves plant *A. hiepiana* 21 compounds were isolated including 1 new compounds namely: 3-*O*-(*[β*-D-xylopyranosyl-(1→2)]-[*β*-D-glucopyranosyl (1→6)-*β*-D-glucopyranosyl-(1→3)]-*α*-L-arabinopyranosyl) oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester (**AH4**), and 20 known compounds: 3- *O*-*β*-D-glucopyranosyl -(1→3)-*O*-*α*-L-arabinopyranosyl olean-12-en-28-oic acid (**AH1**), araliasaponin IV (**AH2**), congmujingnoside B (**AH3**), quercetin (**AH5**), apigenin 7-*O*-*β*-glucoside (**AH6**), quercetin-3-*O*-*β*-D-glucopyranosyl-7-*O*-*α*-L-rhampyranoside (**AH7**), rutin (**AH8**), methyl 3,4-dihydroxybenzoate (**AH9**), methyl caffeate (**AH10**), acid caffeic (**AH11**), 2-hydroxy-4-methoxybenzoic acid (**AH12**), methyl *α*-L-rhamnopyranoside (**AH13**), methyl *α*-D-glucopyranoside (**AH14**), kaempferitrin (**AH15**), matesaponin 1 (**AH16**), acid ursolic (**AH17**), kaempferol (**AH18**), kaempferol 3-*O*-*β*-D-glucopyranosyl-7-*O*-*α*-L-rhamnopyranoside (**AH19**), kaempferol-7-*O*-*α*-L-rhamnopyranoside (**AH20**), *β*-sistosterol (**AH21**).

1.2. Biological activity

+ Results of *in vitro* cytotoxicity assessment

The MeOH extract of the leaves of *A. dasyphylla* showed cytotoxic activity against two cancer cell lines HepG2 and RD with IC_{50} values of 7.81 and 9.1 μg/mL, respectively. Bioactivity testing of isolated compounds showed that three compounds **AD2**, **AD4** and **AD7** exhibited cytotoxic activity against both HepG2 and LU-1 cell lines with IC_{50} values of **AD2** (5.36, 2.85 μM, respectively), **AD4** (7.21, 4.56 μM, respectively), **AD7** (3.24, 2.55 μM, respectively) and compound **AD1** exhibited cytotoxic activity against the LU-1 cell line with values IC_{50} is 7.04 μM. Compound **AH1** exhibited cytotoxic activity against the HeLa cell line with an IC_{50} value of 13.19 μM.

+ Results of bioactivity evaluation *in silico*

The docking results on hGLUT1 sugar transporter for saponins isolated from *A. dasyphylla* showed most of the binding capacity and good ADMET properties, among which compounds **AD2, AD5a, AD7** are potential compounds and need to be further studied for their applicability as drugs and functional foods in cancer prevention and treatment through inhibition of GLUT1 protein.

2. RECOMMENDATIONS

- Research on chemical composition and biological activities of other species of the genus *Aralia* in Vietnam.

- Continue to further investigate the cytotoxic activity on the inhibition of glucose transport in vitro on the GLUT1 protein.

NEW FINDINGS OF THE THESIS

1. The thesis provides the first results on the chemical composition of the leaves of *A. dasyphylla* Miq. From the leaves of *A. dasyphylla* collected in Lac Duong district, Lam Dong province, 21 compounds have been isolated and identified, including 2 new compounds and 19 known compounds.

2. The thesis provides the first results on the chemical composition of the leaves of *A. hiepiana* J.Wen & Lowry. From the leaves of *A. hiepiana* collected in Da Lat, 21 compounds were isolated, including 01 new compound and 20 known compounds.

3. The thesis provides the first results on cytotoxic activity *in vitro* and evaluation results of *in silico* biological activity on GLUT1 protein of compounds isolated from leaves of two species *A. dasyphylla* and *A. hiepiana.*

PUBLICATIONS WITHIN THE SCOPE OF THESIS

1. **Nguyen Thi Thu Hien**, Nguyen Huu Huong Duyen, Nguyen Thi Dieu Thuan, Tran Thi Ngoc Hanh, Pham Van Huyen, Hoang Thi Ngoc Anh, Nguyen Xuan Ha, Pham Ngoc Khanh, Nguyen Manh Cuong, Nguyen Huu Toan Phan, *In vitro* and *in silico* cytotoxic activities of triterpenoids from the leaves of *Aralia dasyphylla* Miq. and the assessment of their ADMET properties, *Journal of Biomolecular Structure and Dynamics*, **2022, SCI, Q2.**

2. **Nguyen Thi Thu Hien**, Nguyen Thi Dieu Thuan, Pham Van Huyen, Truong Thi Anh Hong, Nguyen Huu Toan Phan. Flavonoids from the leaves of *Aralia dasyphylla* Miq. (Araliaceae), *Vietnam Journal of Chemistry* 56(6), **2018**, 695-699.

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