MINISTRY OF EDUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY SCIENCE AND TECHNOLOGY

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RESEARCH ON THE CHEMICAL COMPOSITION AND *IN-VITRO* NITRIC OXIDE PRODUCTION INHIBITORY ACTIVITY OF TWO SPECIES *Cryptolepis buchananii* VÀ *Ailanthus triphysa*

SUMMARY OF DOCTORAL DISSERTATION IN MATERIALS SCIENCE

Major: Organic Chemistry

Code: 9 44 01 14

Hà Nội - 2026

The thesis was completed at: Graduate University Science and Technology - Vietnam Academy of Science and Technology

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Referee 2:

The dissertation will be defended before the Academy-level Doctoral Dissertation Evaluation Council, convened at the Academy of Science and Technology – Vietnam Academy of Science and Technology at .... /...../2026

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INTRODUCTION

Rationale of the dissertation

The diverse natural conditions and characteristic climates across different regions have endowed Vietnam with a rich plant ecosystem. In addition, Vietnam is one of the countries with a long-standing tradition of traditional medicine, using a wide variety of herbs for disease treatment and health promotion. According to scientists, Vietnam has about 12,000 species of vascular plants, of which more than 5,000 species are used as medicinal herbs and remedies. The role of medicinal plant resources is increasingly recognized due to their great potential for research and the development of therapeutic drugs. Research focusing on the discovery of biologically active chemical compounds from traditional medicinal plants is currently an area of great interest to scientists. These studies aim to identify chemical constituents and discover active compounds that possess medicinal properties and contribute to health improvement.

Cryptolepis buchananii belongs to the family Apocynaceae. Worldwide, *Cryptolepis buchananii* is mainly distributed in the tropical regions of Asia. In Vietnam, this species grows in low mountainous areas near the northern border and extends to the midlands and plains of southern provinces, as well as on large islands.

Ailanthus triphysa (family Simaroubaceae) is mainly distributed in India, Sri Lanka, China, Malaysia, Myanmar, Thailand, the Philippines, Indonesia, and Vietnam. In Vietnam, this species grows wild in forests in Phu Tho, Thanh Hoa, Nghe An, and Ha Tinh provinces.

These two species have been used in traditional medicine in China, India, Vietnam, Indonesia, Thailand, and other countries. They are known for their uses in treating skin diseases, fever, influenza, cough, diarrhea, and other ailments. Worldwide, studies have also demonstrated their cytotoxic, insecticidal, anti-inflammatory, antimicrobial, and other notable biological activities. Screening studies aimed at discovering anti-inflammatory compounds from plants in Vietnam showed that the methanol extracts of *Ailanthus triphysa* and *Cryptolepis buchananii* inhibited nitric oxide (NO) production effectively, with inhibition rates of 78% and 69%, respectively, at a concentration of 100 µg/mL.

However, there are still no detailed studies in Vietnam on the chemical composition and biological activities of these two species. Therefore, these species were selected for investigation under the research topic: **"Research on the chemical composition and in vitro nitric oxide production inhibitory activity of two species, *Cryptolepis buchananii* and *Ailanthus triphysa*."** The results of this study will contribute to clarifying the chemical composition and NO production inhibitory activity of *Cryptolepis buchananii* and *Ailanthus triphysa*, thereby providing a scientific basis for further applied research to support healthcare and practical applications.

Objectives of the dissertation:

- To identify the major chemical components of the two species *Cryptolepis buchananii* and *Ailanthus triphysa*.

- To evaluate the inhibitory activity on NO production in RAW 264.7 cells of the compounds isolated from the two species *Cryptolepis buchananii* and *Ailanthus triphysa*.

Scope of the dissertation includes:

1. Isolate compounds from the two species *Cryptolepis buchananii* and *Ailanthus triphysa* using chromatographic methods.

2. Structural elucidation of the isolated compounds using modern spectroscopic methods such as HR-ESI-MS, NMR, UV, and IR.

3. To evaluate the inhibitory activity on NO production in RAW 264.7 cells of the compounds isolated from the two species *Cryptolepis buchananii* and *Ailanthus triphysa*.

CHAPTER 1. LITERATURE REVIEW

1.1. Introduction to the genus *Cryptolepis*

1.1.1. Botanical characteristics of the genus *Cryptolepis*

The genus *Cryptolepis* R.Br. belongs to the family Apocynaceae, order Gentianales, subclass Magnoliidae, class Equisetopsida, and phylum Streptophyta.

1.1.2. Research status on the chemical composition of the genus *Cryptolepis*

Fifty-four compounds have been isolated from species of the genus *Cryptolepis*, including alkaloids (22 compounds), steroids (11 compounds), lignans (5 compounds), flavonoids (4 compounds), and other compounds (12 compounds).

1.1.3. Research status on the biological activity of the genus *Cryptolepis*

Studies have shown that some species of the genus *Cryptolepis* exhibit cytotoxic, anti-inflammatory, antibacterial, antifungal, and other biological activities.

1.1.4. Introduction to the species *Cryptolepis buchananii* R.Br. ex Roem. & Schult.

Twenty-nine compounds were isolated from the species *C. buchananii*, including four alkaloids, eight steroid compounds, five

lignan compounds, and 12 other compounds. The biological activities of *C. buchananii* include cytotoxic, anti-inflammatory, antibacterial, antifungal, and antioxidant effects.

1.2. Introduction to the genus *Ailanthus*

1.2.1. Botanical characteristics of the genus *Ailanthus*

The genus *Ailanthus* Desf. belongs to the family Simaroubaceae, order Sapindales, subclass Magnoliidae, class Equisetopsida, and phylum Streptophyta.

1.2.2. Research status on the chemical composition of the genus *Ailanthus*

Twenty-two compounds have been isolated from species of the genus *Ailanthus*, including alkaloids, terpenoids, quassinoids, steroids, flavonoids, benzopyranoids, and several other compounds.

1.2.3. Research status on the biological activity of the genus *Ailanthus*

Studies have shown that some species of the genus *Ailanthus* exhibit anti-inflammatory, antibacterial, antifungal, antioxidant, and cytotoxic activities.

1.2.4. Introduction to the species *Ailanthus triphysa* (Dennst.) Alston.

Seventy compounds were isolated from the species *A. triphysa*, including 10 alkaloids, 40 terpenoids, 4 quassinoids, 3 steroids, 2 flavonoids, 5 benzopyranoids, and 6 other compounds. Some of the biological activities of *A. triphysa* include cytotoxic, antibacterial, antifungal, and antioxidant effects.

CHAPTER 2: RESEARCH SUBJECTS AND METHODS

2.1. Research Subjects

2.1.1. Species C. buchananii

Leaf and fruit samples of *Cryptolepis buchananii* were collected in Dakrong, Quang Tri province in April 2023 and examined by Assoc.

Prof. Dr. Ninh Khac Ban, Institute of Chemistry, Vietnam Academy of Science and Technology. The specimen (NCCT-P144) is stored at the Institute of Chemistry, Vietnam Academy of Science and Technology.

2.1.2. Species A. triphysa

Leaf samples of *Ailanthus triphysa* (Dennst.) Alston were collected in Me Linh, Vinh Phuc (now Phu Tho province) in April 2024 and identified by Dr. Nguyen The Cuong, Institute of Biology, Vietnam Academy of Science and Technology. The specimen (NCCT-P155) is kept at the Institute of Chemistry, Vietnam Academy of Science and Technology.

2.2. Research Methods

2.2.1. Compound Isolation Methods

Using fractionation extraction methods, thin-layer chromatography, column chromatography, high-performance liquid chromatography (HPLC)

2.2.2. Structure Determination Methods

The general method for determining the chemical structure of compounds involves combining the determination of physical parameters with modern spectroscopic techniques, including HR-ESI-MS, IR, ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY, NOESY, HSQC, HMQC, and HMBC.

2.2.3. Methods for Evaluating the Activity of Inhibiting NO Production

Evaluation of the in vitro nitric oxide (NO) inhibition ability of the analyzed samples in LPS-activated RAW 264.7 cells using the Griess reaction.

CHAPTER 3: EXPERIMENTS AND RESULTS

3.1. Isolation of compounds

3.1.1. Isolation from the leaves of C. buchananii

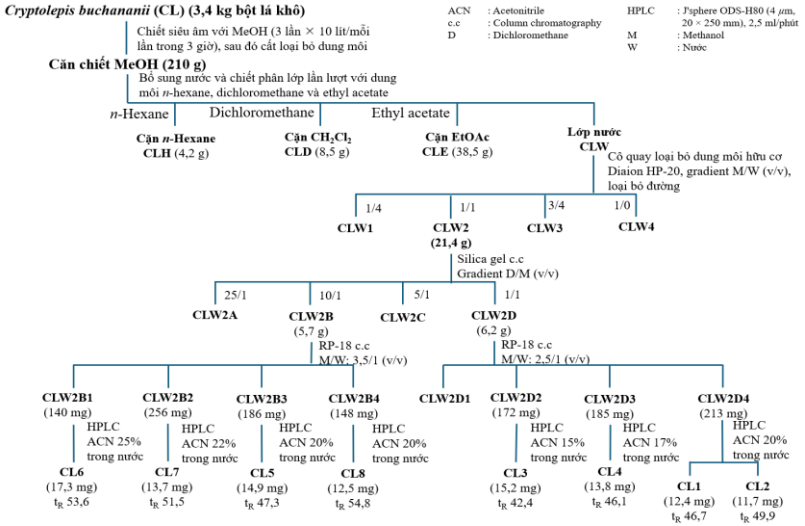

Figure 3.1. Isolation scheme of compounds from the leaves of *C. buchananii*

3.1.2. Isolation from the fruit of C. buchananii

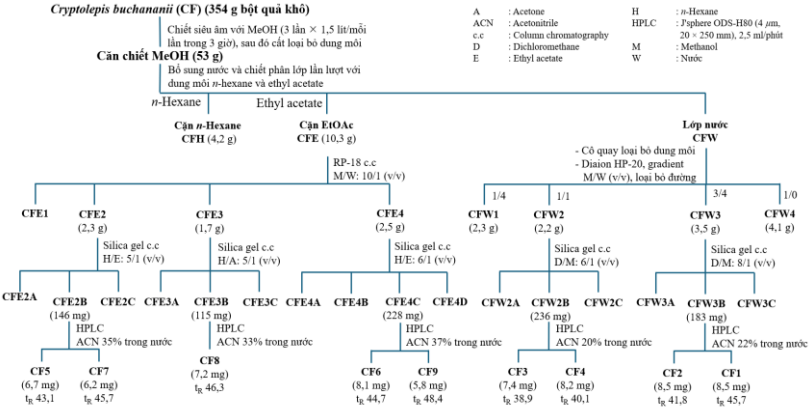

Figure 3.2. Isolation scheme of compounds from the fruit of *C. buchananii.*

3.1.3. Isolation from the leaves of A. triphysa

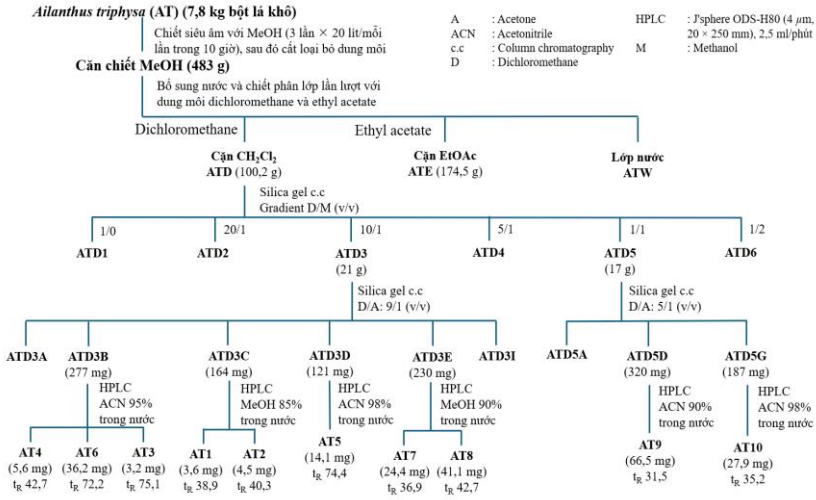

Figure 3.3. Isolation scheme of compounds from the leaves of *A. triphysa*

3.2. Evaluation of the inhibitory activity on nitric oxide production of compounds isolated from *C. buchananii* and *A. triphysa*

Table 3.1. IC_{50} values for NO production inhibition of **CL1-CL8** and **CF1-CF9**

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
CL1	26.72 ± 0.99	**CF1**	37.57 ± 1.88
CL2	52.24 ± 3.09	**CF2**	26.04 ± 1.45
CL3	58.54 ± 3.28	**CF3**	20.55 ± 0.35
CL4	49.19 ± 2.28	**CF4**	25.37 ± 1.36
CL5	56.86 ± 3.56	**CF5**	8.91 ± 0.36
CL6	52.53 ± 2.73	**CF6**	22.69 ± 1.58
CL7	38.15 ± 2.39	**CF7**	18.78 ± 0.96

CL8	18.79 ± 0.95	**CF8**	19.21 ± 0.83
Dexamethasone[a]	14.05 ± 1.17	**CF9**	22.01 ± 0.72

[a] *Dexamethasone was used as a positive control*

Table 3.2. IC_{50} values for NO production inhibition of **AT1-AT10**

Compound	IC_{50} (µM)	Compound	IC_{50} (µM)
AT1	8.12 ± 0.41	**AT6**	24.71 ± 1.38
AT2	22.64 ± 0.73	**AT7**	63.04 ± 1.85
AT3	34.34 ± 1.80	**AT8**	65.41 ± 2.67
AT4	31.54 ± 1.21	**AT9**	54.42 ± 3.14
AT5	48.84 ± 2.63	**AT10**	55.14 ± 2.52
Dexamethasone[a]	14.05 ± 1.17		

[a] *Dexamethasone was used as a positive control*

The test of inhibitory activity on nitric oxide production of compounds isolated from the two species *C. buchananii* and *A. triphysa* on LPS-stimulated RAW 264.7 cell line was carried out according to the method described in section 2.2.3. The concentration of NO in the experimental medium was determined by the Griess reaction.

CHAPTER 4: DISCUSSION OF RESULTS

4.1. Chemical composition of *C. buchananii* and *A. triphysa*

4.1.1. Determination of the chemical structure of compounds isolated from C. buchananii

4.1.1.1. Compound **CL1***: Cryptobuchanoside A (new compound*)

Compound **CL1** was isolated as a colorless amorphous powder. In the infrared (IR) spectrum (Figure I.1.1 – appendix section) of compound **CL1**, peaks characteristic of hydroxy, carbonyl, C=C double bond, and ether functional groups were observed at ν_{max} 3401,

1736, 1639, 1075 cm^{-1}, respectively. Analysis of the high-resolution electrospray ionization mass spectrum (HR-ESI-MS) of compound **CL1** showed a pseudo-molecular ion peak at *m/z* 1155.5401 $[M+^{35}Cl]^-$ (theoretical calculation for the ion $[C_{54}H_{88}O_{24}{}^{35}Cl]^-$: 1155.5359), determined the molecular formula of this compound as $C_{54}H_{88}O_{24}$ (M = 1120) with 11 degrees of unsaturation.

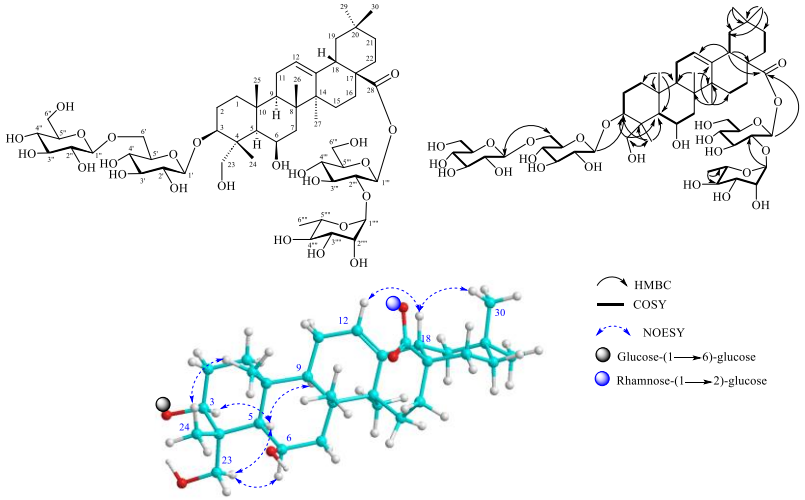

*Figure 4.1. Chemical structure, HMBC, COSY, and NOESY interactions of **CL1***

Analysis of the ^1H NMR and HSQC spectra of compound **CL1** revealed signals such as: six methyl groups at δ_H 0.90 (3H, s, H-29), 0.95 (3H, s, H-30), 1.07 (3H, s, H-26), 1.08 (3H, s, H-24), 1.13 (3H, s, H-27), and 1.34 (3H, s, H-25); one olefinic proton at δ_H 5.30 (1H, t, J = 3.6 Hz, H-12); three methine protons at δ_H 1.23 (1H, br s, H-5), 1.65 (1H, m, H-9), and 2.85 (1H, dd, J = 13.2, 4.2 Hz, H-18); two oxymethine protons at δ_H 3.58 (1H, dd, J = 13.2, 4.2 Hz, H-3) and 4.44 (1H, br s, H-6); one oxymethylene group at δ_H 3.71 (1H, d, J = 12.0 Hz, Ha-23) and 3.47 (1H, d, J = 12.0 Hz, Hb-23); and other

saturated protons resonating in the region δ_H 0.90-2.03 ppm. Additionally, four anomeric protons were identified at δ_H 4.34 (1H, d, J = 7.8 Hz, H-1″), 4.40 (1H, d, J = 7.8 Hz, H-1′), 5.42 (1H, d, J = 7.8 Hz, H-1‴), and δ_H 5.46 (1H, d, J = 1.2 Hz, H-1⁗) (Table 4.1).

Analysis of the ^{13}C NMR and HSQC spectra of compound **CL1** revealed the presence of resonances for 54 carbons. Among them, 30 carbon signals belong to the olean-triterpene aglycone framework [69], and the remaining 24 carbon signals were assigned to 4 sugar molecules. In the aglycone portion, a double bond at δ_C 124.0 (C-12) and 144.1 (C-13), one carbonyl group at δ_C 178.2 (C-28), two oxymethine groups at δ_C 83.3 (C-3) and 68.7 (C-6), and a oxygenated methylene group at δC 64.7 (C-23) were identified. The above NMR spectral data suggested that the aglycone structure is uncargenin C [69, 70], with an olean-triterpene framework containing a C-12/C-13 double bond, a carboxylic acid group at C-28, and three hydroxy groups at C-3, C-6, and C-23. The exact positions of the substituents were confirmed by the aforementioned HMBC combined with HSQC spectra. HMBC interaction from H_2-23 (δ_H 3.71/3.47) to C-3 (δ_C 83.3)/C-4 (δ_C 44.8)/C-5 (δ_C 48.9)/C-24 (δ_C 15.0) and from H_3-24 (δ_H 1.08) to C-3 (δ_C 83.3)/C-4 (δ_C 44.8)/C-5 (δ_C 48.9)/C-23 (δ_C 64.7) suggests that the carbon at C-3 and C-23 must be connected to oxygen atom. The HMBC interaction from H_3-27 (δ_H 1.13) to C-8 (δ_C 39.9)/C-13 (δ_C 144.1)/C-14 (δ_C 43.6)/C-15 (δ_C 29.5), and from H-18 (δ_H 2.85) to C-28 (δ_C 178.2) confirmed a Δ^{12} double bond and a 28-oic acid (Figure 4.1). The 6-OH group was demonstrated by COSY interaction between H-5 (δ_H 1.23) / H-6 (δ_H 4.44), as well as HMBC interaction between H-5 and C-6 (δ_C 68.7). The stereochemistry of compound **CL1** is elucidated based on coupling constant analysis

from the ^{1}H-NMR spectrum and NOESY interactions. The large coupling constant ($^{3}J_{2,3}$ = 13.2 Hz) between H-2 and H-3 confirmed that proton H-3 is oriented *alpha/axial*. Proton H-6 (δ_H 4.44) appeared as a broad singlet with nearly zero coupling constant ($^{3}J_{5,6}$ ~ 0 Hz), indicating that proton H-6 occupies the *alpha/equatorial* position. In addition, the above stereochemical orientation was further confirmed by the NOESY interactions between H-3 (δ_H 3.58) and H-5 (δ_H 1.23), between H_2-23 (δ_H 3.71 and 3.47) and H-3 (δ_H 3.58), and between H-5 and H-6. In the sugar part of compound **CL1**, four hexose units were identified, specifically: 18 carbon signals belonging to three glucose groups at δ_C (105.5, 75.7, 78.0, 71.4, 77.7 and 69.7), δ_C (104.7, 75.2, 77.7, 71.6, 78.0 and 62.7) and δ_C (95.1, 77.1, 79.4, 71.1, 78.4 and 62.7); and 6 carbon signals belonging to one rhamnose unit at δ_C (101.5, 72.3, 72.2, 73.7, 70.3 and 18.2). The linkages of the sugar groups were determined through interactions observed in the HSQC, COSY, and HMBC spectra (Figure 4.1). The HMBC correlations from Glu H-1″ (δH 4.34) to C-6′ (δC 69.7), from Glu H-1′ (δH 4.40) to C-3 (δC 83.3), from Glu H-1‴ (δH 5.42) to C-28 (δC 178.2), and from Rha H-1⁗ (δH 5.46) to C-2‴ (δC 77.1) established the sugar units as glucopyranosyl-(1→6)-glucopyranoside and rhamnopyranosyl-(1→2)-glucopyranoside linked to C-3 and C-28, respectively, via glycosidic and ester bonds. In addition, the ^{1}H NMR spectra of the sugar moieties also showed large coupling constants (J = 7.8 Hz) for the three anomeric protons Glu H-1′ (δ_H 4.40), Glu H-1″ (δ_H 4.34), and Glu H-1‴ (δ_H 5.42), indicating β-glycosidic linkages, while the small coupling constant (J = 1.2 Hz) of the anomeric proton H-1⁗ at (δ_H 5.46) of the rhamnose indicated an α-glycosidic linkage. Compound **CL1** was hydrolyzed in

Table 4.1. NMR spectral data of **CL1**

C	δ_C[a,b]	δ_H[a,c] (multi., *J* = Hz)	C	δ_C[a,b]	δ_H[a,c] (multi., *J* = Hz)
1	41.7	0.90*/1.57 (dd. 12.6. 1.8)	30	24.3	0.95 (s)
2	26.4	1.82 (m)/1.93 (m)	3-*O*-Glucopyranosyl		
3	83.3	3.58 (dd. 13.2. 4.2)	1′	105.5	4.40 (d. 7.8)
4	44.8	-	2′	75.7	3.20 (dd. 9.0. 7.8)
5	48.9	1.23 (br s)	3′	78.0	3.26*
6	68.7	4.44 (br s)	4′	71.4	3.31*
7	41.1	1.61*/1.72 (br d. 13.8)	5′	77.7	3.51 (ddd. 9.0. 5.4. 1.8)
8	39.9	-	6′	69.7	4.09 (dd. 12.0. 1.8)
					3.73 (dd. 12.0. 5.4)
9	49.1	1.65 (m)	6′-*O*-Glucopyranosyl		
10	37.3	-	1″	104.7	4.34 (d. 7.8)
11	24.5	1.94*/2.03*	2″	75.2	3.23*
12	124.0	5.30 (t. 3.6)	3″	77.7	3.26*
13	144.1	-	4″	71.6	3.31*
14	43.6	-	5″	78.0	3.25*
15	29.5	1.22*/1.68 (m)	6″	62.7	3.67*/3.84*
16	23.9	1.68 (m)/2.04 (m)	28-*O*-Glucopyranosyl		
17	49.7	-	1‴	95.1	5.42 (d. 7.8)
18	42.8	2.85 (dd. 13.2. 4.2)	2‴	77.1	3.63 (dd. 9.0. 7.8)
19	47.3	1.73 (dd. 13.2. 13.2)/1.17*	3‴	79.4	3.57 (t. 9.0)
20	31.6	-	4‴	71.1	3.42 (t. 9.0)
21	35.0	1.22*/1.39 (m)	5‴	78.4	3.36 (m)
22	33.2	1.58 (m)/1.75 (m)	6‴	62.7	3.67*/3.84*
23	64.7	3.71 (d. 12.0)/3.47 (d. 12.0)	2‴-*O*-Rhamnopyranosyl		
24	15.0	1.08 (s)	1⁗	101.5	5.46 (d. 1.2)
25	18.0	1.34 (s)	2⁗	72.3	3.93 (dd. 3.0. 1.2)

26	19.0	1.07 (s)	3‴′	72.2	3.67 (dd. 9.0. 3.0)
27	26.2	1.13 (s)	4‴′	73.7	3.42 (t. 9.0)
28	178.2	-	5‴′	70.3	3.77 (m)
29	33.5	0.90 (s)	6‴′	18.2	1.26 (d. 6.0)

*[a] CD_3OD, [b] 150 MHz, [c] 600 MHz, * Overlapped signals.*

an acidic medium [63, 64], yielding glucose and rhamnose sugars using thin-layer chromatography and comparing them with the corresponding standard sugars. Then, the glucose and rhamnose obtained were subjected to optical rotation measurement and identified as D-glucose and L-rhamnose, after comparing their specific rotations, which corresponded to the published values (section 2.2.2.6). From the above analyses, compound **CL1** was identified as 3-O-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyluncargenin C 28-O-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester. Upon investigation, it was found that **CL1** is a new compound and was named cryptobuchanoside A.

4.1.1.18. Summary of compounds isolated from the species C. buchananii

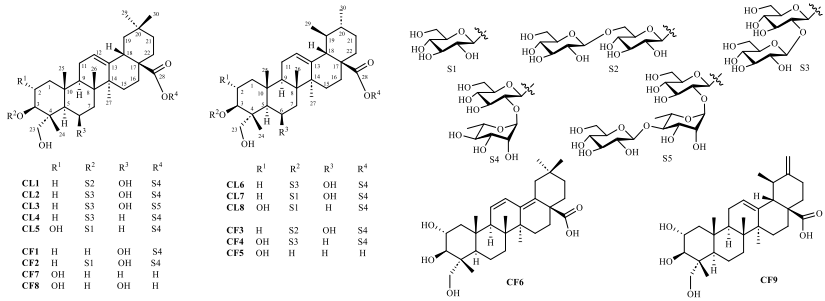

Figure 4.2. Summary of compounds isolated from *C. buchananii*

Eight compounds (**CL1-CL8**) were isolated from the leaves: among them, seven new compounds were named cryptobuchanoside

A-G (**CL1**-**CL7**), and one known compound is named 3-O-*β*-D-glucopyranosylasiatic acid 28-O-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**CL8**).

Nine compounds (**CF1**-**CF9**) were isolated from the fruit part of the species: Among them, 4 new compounds were named: uncargenin C 28-O-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**CF1**), 3-O-*β*-D-glucopyranosyluncargenin C 28-O-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**CF2**), 3-O-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-6*β*,23-dihydroxyursolic acid 28-O-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**CF3**), 3-O-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosylasiatic acid 28-O-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**CF4**); and 5 known compounds named: asiatic acid (**CF5**), 2*α*,3*β*,23-trihydroxyoleana-11,13(18)-dien-28-oic acid (**CF6**), arjunolic acid (**CF7**), 6*β*-hydroxyarjunolic acid (**CF8**) and actinidic acid (**CF9**).

4.1.2. Determination of the chemical structures of compounds isolated from the species A. triphysa

4.1.2.1. Compound AT1: Ailantriphysa A (new compound).

Compound **AT1** was isolated as a colorless solid. In the infrared (IR) spectrum (Figure II.1.1 – appendix section) of **AT1**, characteristic wavelengths for the hydroxy (OH), carbonyl (C=O), olefinic double bond (C=C), and ether (C-O-C) functional groups were observed at 3309, 1730, 1469, and 1062 cm^{-1}, respectively. The high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) analysis of **AT1** showed a pseudo-molecular ion peak at *m/z* 503.3745 $[M-H]^-$ (theoretical calculation for the ion $[C_{31}H_{51}O_5]^-$: 503.3742), determined its molecular formula to be $C_{31}H_{52}O_5$ (M = 504), with 6 degrees of unsaturation.

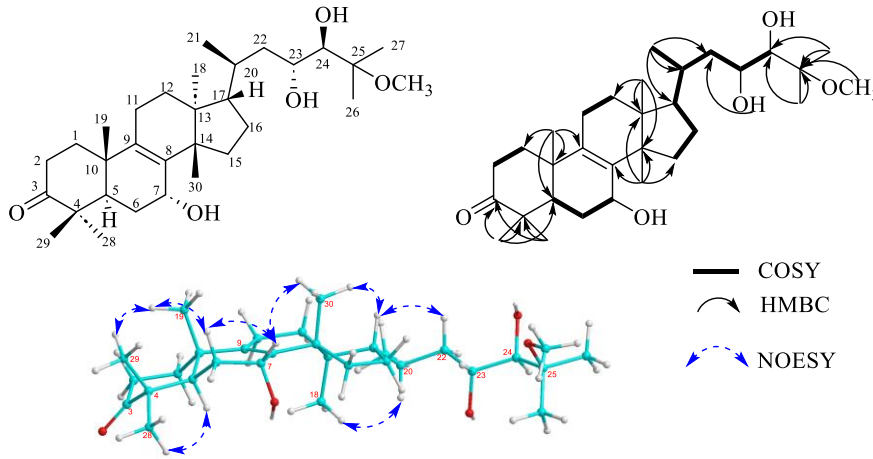

Figure 4.3. Chemical structure, HMBC, COSY, and NOESY interactions of **AT1**

Analysis of the ^{1}H NMR spectrum and the HSQC spectrum of compound **AT1** showed the presence of signals for: seven tertiary methyl groups at δ_H 0.75 (3H, s, H-18), 0.91 (3H, s, H-30), 1.00 (3H, s, H-19), 1.09 (3H, s, H-29), 1.16 (3H, s, H-28), 1.23 (3H, s, H-26), and 1.30 (3H, s, H-27); one secondary methyl group at δ_H 0.97 (3H, d, J = 6.0 Hz, H-21); three methine protons at δ_H 1.38-1.42 (1H, m, H-20), 1.52 (1H, d, J = 9.0 Hz, H-17), and 2.19 (1H, overlapped signal, H-5); three oxymethine protons at δ_H 3.13 (1H, d, J = 9.6 Hz, H-24), 4.07-4.09 (1H, m, H-23), and 4.40 (1H, br s, H-7); one methoxy group at δ_H 3.25 (3H, s, O-CH3); proton signals of two hydroxy groups at δ_H 2.44 (1H, d, J = 9.6 Hz, 24-OH) and 3.49 (1H, d, J = 2.4 Hz, 23-OH); and other saturated protons resonating in the δ_H 1.25-2.5 ppm region (Table 4.19). Analysing of the ^{13}C NMR spectrum of **AT1** showed signals corresponding to 31 carbons. Among them, one carbon belongs to a methoxy group (δ_C 49.3) and 30 carbons belong to the tirucallane

triterpene framework, suggesting a typical class of compounds isolated from the genus *Ailanthus* [47, 79, 80]. The carbon signals assigned to the tirucallane triterpene framework included a ketone at δ_C 217.7 (C-3); a tetrasubstituted double bond (four substituents attached to the two carbons of the double bond other than hydrogen) at δ_C 131.3 (C-8) and 143.8 (C-9); three oxymethine groups at δ_C 79.3 (C-7), 68.8 (C-23), and 76.6 (C-24); a quaternary carbon linked to a methoxy group at δ_C 79.8 (C-25); and eight methyl groups at δ_C 15.8 (C-18), 18.7 (C-19), 19.3 (C-21), 21.2 (C-29), 21.5 (C-27), 21.6 (C-26), 27.1 (C-28), and 25.7 (C-30), which were identified similarly to previous reports [81, 82]. The ketone groups at C-3, the double bond at C-8/C-9, the methoxy group at C-25, and three hydroxy groups at C-7, C-23, and C-24 were further identified by interactions in the HSQC, ^{1}H-^{1}H COSY, and HMBC spectra. The NMR spectral data of the four rings (A, B, C, and D) of compound **AT1** (Table 4.19) are similar to those of dymacrin K [82], allowing the identification of ketone groups at position C-3, the double bond at position C-8/C-9, and the hydroxy group attached to carbon C-7. On the HMBC spectrum of compound **AT1**, interactions from H_3-28 (δ_H 1.16)/H_3-29 (δ_H 1.09) to C-3 (δ_C 217.7)/C-4 (δ_C 46.6)/C-5 (δ_C 44.3), from H_3-18 (δ_H 0.75) to C-12 (δ_C 30.8)/C-13 (δ_C 44.1)/C-14 (δ_C 49.9), from H_3-19 (δ_H 1.00) to C-1 (δ_C 34.8)/C-5 (δ_C 44.3)/C-9 (δ_C 143.8)/C-10 (δ_C 38.0), from H_3-21 (δ_H 0.97) to C-17 (δ_C 51.1)/C-20 (δ_C 34.2)/C-22 (δ_C 40.2), from H_3-30 (δ_H 0.91) to C-8 (δ_C 131.3)/C-13 (δ_C 44.1)/C-14 (δ_C 49.9)/C-15 (δ_C 29.6), from H_3-26 (δ_H 1.23)/H_3-27 (δ_H 1.30) to C-24 (δ_C 76.6)/C-25 (δ_C 79.8), from the methoxy protons -OCH_3 (δ_H 3.25) to C-25 (δ_C 79.8), along with the ^{1}H-^{1}H COSY interactions of H-5 (δ_H 2.19)/H_2-6 (δ_H 1.43-1.47 and 2.20-2.24)/H-7 (δ_H 4.40) and H-23 (δ_H 4.07-4.09)/H-24

(δ_H 3.13) were observed, confirming a tirucallane triterpene compound. In addition, the H-7 proton appeared as a broad singlet (at δ_H 4.40) with a coupling constant close to zero ($^3J_{6,7} \sim 0$ Hz),

Table 4.2. NMR spectral data of AT1

C	$\boldsymbol{\delta_C}^{a,b}$	$\boldsymbol{\delta_H}^{a,c}$ (multi., *J* = Hz)	**C**	$\boldsymbol{\delta_C}^{a,b}$	$\boldsymbol{\delta_H}^{a,c}$ (multi., *J* = Hz)
1	34.8	1.66-1.70 (m)	18	15.8	0.75 (s)
		1.94-1.96 (m)			
2	34.3	2.48-2.55 (m)	19	18.7	1.00 (s)
3	217.7	-	20	34.2	1.38-1.42(m)
4	46.6	-	21	19.3	0.97 (d. 6.0)
5	44.3	2.19*	22	40.2	1.20-1.24 (m)
					1.79-1.82 (m)
6	24.3	1.43-1.47 (m)	23	68.8	4.07-4.09 (m)
		2.20-2.24 (m)			
7	79.3	4.40 (br s)	24	76.6	3.13 (d. 9.6)
8	131.3	-	25	79.8	-
9	143.8	-	26	21.6	1.23 (s)
10	38.0	-	27	21.5	1.30 (s)
11	22.1	2.08-2.10 (m)	28	27.1	1.16 (s)
12	30.8	1.72-1.78 (m)	29	21.2	1.09 (s)
13	44.1	-	30	25.7	0.91 (s)
14	49.9	-	OCH_3	49.3	3.25 (s)
15	29.6	1.28-1.30 (m)	23-OH		3.49 (d. 2.4)
		1.99-2.01 (m)			
16	28.4	1.37-1.39 (m)	24-OH		2.65 (d. 9.6)
		2.00-2.04 (m)			
17	51.1	1.52 (t. 9.0)			

*[a] $CDCl_3$, [b] 150 MHz, [c] 600 MHz, * Overlapped signals*

suggesting that this proton is oriented *beta/equatorial* [82]. This assertion is further confirmed by NOESY interactions of H-7 (δ_H 4.40) and H_3-30 (δ_H 0.91). Furthermore, the NOESY spectrum also showed interactions from H_3-29 (δ_H 1.09) to H_3-19 (δ_H 1.00), from H-5 (δ_H 2.19) to H_3-28 (δ_H 1.16), and from H-17 (δ_H 1.52) to H_3-30 (δ_H 0.91), determined protons H-5, H_3-18, and H_3-28 were α-orientation, while protons H-7, H-17, H_3-19, H_3-29, and H_3-30 were β-orientation. The NMR spectral data of C-23 (δ_C 68.8) and C-24 (δ_C 76.6) of **AT1** completely matched the data of hispidol A 25-methyl ether with positions [23α-OH (at $\delta_{C\text{-}23}$ 68.21), 24β-OH (at $\delta_{C\text{-}24}$ 76.65)] and hispidol B 25-methyl ether with positions [23α-OH ($\delta_{C\text{-}23}$ 68.13), 24β-OH ($\delta_{C\text{-}24}$ 76.75)] [79]. In addition, the proton of the 24-OH group appeared as a doublet (J = 9.6 Hz) at δ_H 2.65 due to the coupling interaction of H-24 (at δ_H 3.13, d, J = 9.6 Hz). Therefore, the $J_{23/24}$ coupling constant value is considered to be zero. This evidence indicated that C-23/C-24 had a *syn-gauche (erythro)* configuration [83], similar to the configuration of piscidinol A ($^3J_{23/24}$ = 0 Hz) and different from 24-*epi*-piscidinol A (23α-OH, 24α-OH) which has a coupling constant value of $^3J_{23/24}$ = 8.0 Hz [84]. From the above evidence, compound **AT1** was determined as 25-methoxy-7α,23α,24β-trihydroxytirucallane-8-en-3-one, a new compound named ailantriphysa A.

4.1.2.11. Summary of compounds isolated from the species A. triphysa

Ten compounds (**AT1**-**AT10**) including four new compounds (**AT1**-**AT4**) are named ailantriphysa A-D (**AT1**-**AT4**) and six known compounds 24*S*,25-dihydroxytirucall-7-en-3-one (**AT5**), phellochin (**AT6**), hispidol B 25-methyl ether (**AT7**), meliasenin G (**AT8**), 21-*O*-

methyltoosendanpentol (**AT9**) and agladupol A (**AT10**) had been isolated.

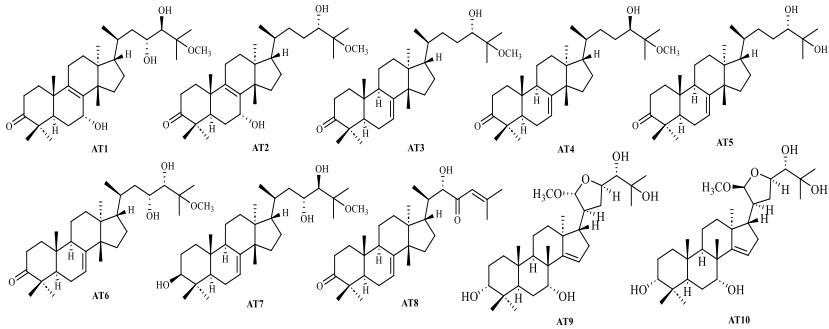

Figure 4.4. Summary of compounds isolated from *A. triphysa*

4.2. Evaluation of the inhibitory activity on NO production of two species *C. buchananii* and *A. triphysa*

4.2.1. NO inhibitory activity of compounds isolated from C. buchananii

The activity test results of 18 compounds isolated from *C. buchananii* showed that compounds **CL1-CL8** and **CF1-CF9** (at a concentration of 100 μM) were considered non-cytotoxic at a statistically significant level (cell viability from 92.79% to 100.99%). Thus, the results of evaluating NO production inhibition were not affected or caused by the cytotoxicity of the tested compounds. At a concentration of 100 μM, compounds **CL1-CL8** and **CF1-CF9** inhibited NO production with inhibition percentages greater than 50% (ranging from 81.09% to 99.74%). Therefore, these compounds were further evaluated for their NO production inhibitory effects in a concentration-dependent manner to calculate the IC_{50} values. The results showed that the compounds **CL1-CL8** inhibited NO production with IC_{50} values of 26.72, 52.24, 58.54, 49.19, 56.86, 52.53, 38.15, and 18.79 μM, respectively, while the compounds **CF1-CF9** inhibited NO

production with IC_{50} values of 37.57, 26.04, 20.55, 25.37, 8.91, 22.69, 18.78, 19.21, and 22.01 μM, compared to the positive control dexamethasone, which had an IC_{50} value of 14.05 μM.

From the results of NO production inhibition and the structures of the tested compounds, it can be seen that compound **CF5** exhibited the best inhibitory activity with an IC_{50} value of 8.91 μM. Compounds **CL1**, **CL8**, **CF2-CF4**, and **CF6-CF9** showed IC_{50} values ranging from 18.78 to 26.72 μM, indicating moderate activity compared to the positive control dexamethasone. The remaining compounds showed weak effects with IC_{50} values ranging from 37.57 to 58.54 μM. These results suggest that pentacyclic triterpenes containing 3*β*,23-dihydroxy-12-en-28-oic acid and their glycosyl derivatives at C-3 and C-28 may play an important role in the NO production inhibitory effect of *C. buchananii*. These findings indicate that saponin components may play a significant role in the NO production inhibitory activity of *C. buchananii*.

4.2.2. **NO inhibitory activity of compounds isolated from *A. triphysa***

The activity test results of 10 compounds isolated from the species *A. triphysa* showed that the compounds **AT1-AT10** (at a concentration of 100 μM) were considered not to cause statistically significant cell death (% cell viability from 82.21% to 94.52%), so the results evaluating the ability to inhibit NO production were not affected by or caused by the cytotoxic effects of the tested compounds. Also at a concentration of 100 μM, the compounds **AT1-AT10** inhibited NO production with inhibition percentages greater than 50% (ranging from 67.72% to 94.24%). Therefore, these compounds were further studied for dose-dependent inhibition of NO

production to calculate IC_{50} values. The results showed that the compounds **AT1**-**AT10** inhibited NO production with IC_{50} values of 8.12, 22.64, 34.34, 31.54, 48.84, 24.71, 63.04, 65.41, 54.42, and 55.14 μM, respectively, compared to the positive control dexamethasone, which had an IC_{50} value of 14.21 μM.

From the results of NO production inhibition and the structures of the tested compounds, it can be seen that compound **AT1** has the best inhibitory activity with an IC_{50} value of 8.12 μM. Compounds **AT2**-**AT4** and **AT6** exhibit moderate activity with IC_{50} values ranging from 22.64 to 34.34 μM. The remaining compounds show weak activity with IC_{50} values ranging from 48.84 to 65.41 μM. These results indicate that tirucallane triterpenes and their ketone and hydroxy derivatives at C-3, C-7, C-23, and C-24 may play an important role in the NO production inhibitory effect of the species *A. triphysa*. Regarding the relationship between structure and NO inhibitory activity, preliminary research results suggest that among the **AT1**-**AT6** group, when the double bond shifts from the C-8/C-9 position to C-7/C-8, the activity may decrease, and the loss of the 23-OH group may also reduce activity. For the remaining compounds, the relationship between structure and biological activity has not yet shown any defined indication.

CONCLUSION

By using chromatography methods and modern spectroscopic methods, the chemical structures of 27 compounds from the two species *C. buchananii* and *A. triphysa* have been isolated and identified, including 15 new compounds and 12 known compounds.

1. From the species *C. buchananii*, 17 compounds **CL1-CL8** and **CF1-CF9** were isolated and their structures determined: including 11 new compounds named as: cryptobuchanoside A-G (**CL1-CL7**), uncargenin C 28-O-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**CF1**), 3-O-*β*-D-glucopyranosyluncargenin C 28-O-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**CF2**), 3-O-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-6*β*,23-dihydroxyursolic acid 28-O-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**CF3**), 3-O-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosylasiatic acid 28-O-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**CF4**); and 6 known compounds including: 3-O-*β*-D-glucopyranosylasiatic acid 28-O-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**CL8**), asiatic acid (**CF5**), 2*α*,3*β*,23-trihydroxyoleana-11,13(18)-dien-28-oic acid (**CF6**), arjunolic acid (**CF7**), 6*β*-hydroxyarjunolic acid (**CF8**), and actinidic acid (**CF9**).

2. From the species *A. triphysa*, 10 compounds **AT1-AT10** were isolated and their structures identified: including 4 new compounds named ailantriphysa A-D (**AT1-AT4**) and 6 known compounds: 24*S*,25-dihydroxytirucall-7-en-3-one (**AT5**), phellochin (**AT6**), hispidol B 25-methyl ether (**AT7**), meliasenin G (**AT8**), 21-O-methyltoosendanpentol (**AT9**), and agladupol A (**AT10**).

3. Research results on biological activity:

- Compounds **CL1-CL8** and **CF1-CF9** were evaluated for their inhibitory activity on NO production in RAW 264.7

macrophages. The results showed that these compounds inhibited NO production with IC_{50} values ranging from 8.91 to 58.54 μM. Among them, compound **CF5** exhibited the best inhibitory activity with an IC_{50} value of 8.91 μM. Compounds **CL1**, **CL8**, **CF2-CF4**, and **CF6-CF9** had IC_{50} values from 18.78 to 26.72 μM, showing moderate activity compared to the positive control dexamethasone. The remaining compounds showed weak activity with IC_{50} values ranging from 37.57 to 58.54 μM.

- Compounds **AT1-AT10** were evaluated for their inhibitory activity on NO production in RAW 264.7 macrophages. The results showed that these compounds inhibited NO production with IC_{50} values ranging from 8.12 to 65.41 μM. Among them, compound **AT1** exhibited the best inhibitory activity with an IC_{50} value of 8.12 μM. Compounds **AT2-AT4** and **AT6** showed moderate activity with IC_{50} values ranging from 22.64 to 34.34 μM. The remaining compounds showed weak activity with IC_{50} values ranging from 48.84 to 65.41 μM.

RECOMMENDATIONS

Two compounds asiatic acid (**CF5**) and ailantriphysa A (**AT1**) exhibited inhibitory activity on NO production in LPS-stimulated RAW264.7 cells with IC_{50} values of 8.91 μM and 8.12 μM, respectively, which are stronger than the positive control dexamethasone (14.05 μM). These results suggested that further studies to determine the mechanism of inflammatory activity would be taken. The isolated pentacyclic triterpene glycoside and tirucallane triterpenoid compounds have unique chemical structures. Thus, research on screening additional activities could be expanded to guide future applied studies.

NOVEL CONTRIBUTIONS OF THE DISSERTATION

This is the first report of the chemical constituents and nitric oxide inhibitory activity on RAW 264.7 cells of *C. buchananii* and *A. triphysa* growing in Viet Nam.

From *C. buchananii*, 11 new compounds, named cryptobuchanoside A-G, (**CL1**-**CL7**), uncargenin C 28-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**CF1**), 3-*O*-*β*-D-glucopyranosyluncargenin C 28-*O*-*α*-L-rhamnopyranosyl-(1→2)- *β*-D-glucopyranosyl ester (**CF2**), 3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-6*β*,23-dihydroxyursolic acid 28-O-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**CF3**), 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosylasiatic acid 28-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**CF4**) have been isolated. These compounds showed NO inhibitory activity with IC_{50} values ranging from 20,55 to 58,54 μM.

From *A. triphysa*, four new compounds named ailantriphysa A-D (**AT1**-**AT4**) have been isolated. These compounds showed strong NO inhibitory activity. Of these, **AT1** showed the best inhibition with IC_{50} = 8.12 μM.

LIST OF PUBLISHED PAPERS RELATED TO THE DISSERTATION

1. **Nguyen Duc Duy**, Ngo Anh Bảng, Pham Hai Yen, Do Thi Trang, Bui Thi Nha Trang, Nguyen Thi Kim Thuy, Nguyen Thi Cuc, Nguyen Xuan Nhiem, Phan Van Kiem, Ninh Khac Ban, Bui Huu Tai, Four new pentacyclic triterpene glycosides isolated from the fruits of *Cryptolepis buchananii* R.Br. ex Roem. & Schult and their inhibition of NO production in LPS-activated RAW 264.7 Cells, *Chem. Biodiversity*, 2023, 20(12), e202301683.
2. Ngo Anh Bang, **Nguyen Duc Duy**, Bui Huu Tai, Nguyen Thi Kim Thuy, Pham Hai Yen, Duong Thi Dung, Nguyen Huy Hoang, Nguyen Xuan Nhiem, Ninh Khac Ban, Phan Van Kiem, Cryptobuchanosides A-G: seven previously undescribed triterpene glycosides from *Cryptolepis buchananii* R.Br. ex Roem. and Schult. with nitric oxide production inhibition activity, *J. Nat. Med*., 2024, 78(3), 741-752.
3. **Nguyen Duc Duy**, Duong Thi Dung, Do Thi Trang, Ngo Anh Bảng, Pham Hai Yen, Nguyen Xuan Nhiem, Nguyen Thi Cuc, Phan Thi Thanh Huong, Nguyen Viet Dung, Nguyen Huy Hoang, Duong Thi Hai Yen, Nguyen Thi Kim Thuy, Nguyen The Cuong, Bui Huu Tai, Phan Van Kiem, Four new tirucallane triterpenoids from the leaves of *Ailanthus triphysa* with anti-inflammatory activities, *Chem. Biodiversity*, 2025, 22(3), e202402584.
4. **Nguyen Duc Duy**, Bui Huu Tai, Nguyen Thi Kim Thuy, Nguyen Thi Hanh, Quach Thi Thanh Van, Quan Cam Thuy, Bui Thi Phuong Thao, Phan Van Kiem, *Ailanthus triphysa*: a review of phytochemistry and pharmacology, *Vietnam Journal of Science and Technology*, 2025, 63(6), 1050-1073