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STUDY ON CHEMICAL CONSTITUENTS AND EVALUATION OF THE CYTOTOXIC ACTIVITY OF *AMESIODENDRON CHINENSE* (SAPINDACEAE) AND *BACCAUREA SYLVESTRIS* (PHYLLANTHACEAE) SPECIES

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SUMMARY OF CHEMISTRY DOCTORAL THESIS

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INTRODUCTION

1. The urgency of the thesis

Along with the development of science and technology, people today have to face to many challenges, in which the biggest problems are environmental pollution and many dangerous epidemics and diseases. In which, cancer has been concerned specialy. According to the World Health Organization (WHO), the number of people diseased cancer in the world is increasing and the number of deaths is increasing every year. WHO's data is estimated to account for 9.6 million deaths in 2018 in the global. Vietnam has about 165 000 new cancer cases and about 115000 cancer deaths each year. New methods treated cancer using modern equipment and new cancer drugs has been continued to make to served needs of people. However, synthesized drugs often have extra effects. So the search and discovery of active anti-cancer compounds from plants is always an urgent requirement in order to create new valuable cancer drugs with standards as safe, effect, few extra effects. Vietnam is a country located in the tropical monsoon climate zone, so flora in here contains many various of kinds. It is estimated that there are nearly 13.000 species of higher plants, of which more than 4000 species are used as medicine. The Sapindaceae and the Phyllanthaceae are two large families with many species of different genera exhibiting many valuable and diverse biological activities, in which many active ingredients have been used for treating incurable diseases. The expurgatorial process of extracts from Vietnamese flora through the France-Vietnamese cooperation project (Research on phytochemistry of Vietnamese vegetation) has discovered many species with valuable biological activities. Among these extracts, ethyl acetate

extract from leaves of *Amesiodendron chinense* (Merr.) Hu specie belonging to Bo Hon family (Sapindaceae) inhibited 100% of SW13 adrenal cancer cells at a concentration of 1 µg/mL, and *Baccaurea sylvestris* Lour specie of the Diep Ha Chau family (Phyllanthaceae), inhibited 11.3% of the TB carcinoma cell line at the concentration of 1 µg/mL and 100% of the SW13 cancer cells at the concentration of 5 µg/mL. Two species *A. chinense* and *B. sylvestris* have never been studied in Vietnam and in the world, so it is necessary for the study of chemical constituents as well as biological activity, especially anticancer activity of the plant. The purpose of this thing is to search and discover bioactive compounds from plants with high anti-cancer activity. Based on the urgency and results of expurgatorial about bioactive compounds from Vietnamese plants, we chose the thesis as: **"Study on chemical constituents and evaluation of the cytotoxic activity of *Amesiodendron chinense* (Sapindaceae) and *Baccaurea sylvestris* (Phyllanthaceae) species".**

2. Aims of thesis

- Study on chemical constituents of as *Amesiodendron chinense* (Sapindaceae) and *Baccaurea sylvestris* (Phyllanthaceae) species.

- Evaluation of the cytotoxic activity of isolated compounds.

The main contents of the thesis:

- Collecting and identifying plant samples of *A. chinense* and *B. sylvestris* species.

- Extraction and isolation of chemical constituents of *A. chinense* and *B. sylvestris* species.

- Determinating structures of isolated compounds by modern spectroscopy methods.

- Test cytotoxic activity of extracts and some isolated compounds on 4 cancer cell lines KB, Hep-G2, LU, MCF-7.

Chapter 1. OVERVIEW

The overview included 28 pages display contents:

Introduction to Sapindaceae family and genus *Amensiodendron* includes taxonomy, distribution and botanical characteristics of genus *Amensiodendron*, genus *Baccaurea.* Applying in traditional medicine of some species of Sapindaceae family, and chemical constituents of some species of Sapindaceae family and some species of *Baccaurea* genus as well as their biological activities. The survey results showed of *Amensiodendron chinense* and *Baccaurea sylvestris* species have never been studied in the world and in Vietnam.

Chapter 2: RESEARCH METHODS AND EXPERIMENTS

Chapter 2 included 19 pages presenting research objects, chemicals, equipments, methods of isolation and determination of the structure of compounds as well as a diagram of the isolation of compounds from *A. chinense* and *B. sylvestris* species. In addition, the cytotoxic activity test method as well as the spectral datas and physical parameters of the isolated compounds are also presented in this chapter.

Chapter 3: RESULTS AND DISCUSSION

Chapter 3 included 73 pages discussing the structures of the isolated compounds, as well as the results of testing the cytotoxic activity of some isolated compounds, of some extracts from *A. chinense* and *B. sylvestris* species.

3.1. Structures of compounds isolated from *A. chinense* and *B. sylvestris* species

From leaf and flower parts of *A. chinense* specie collected in Da Nang, 18 compounds from **AC1**-**AC19** were isolated, in which **AC1**-**AC3** are three new substances.

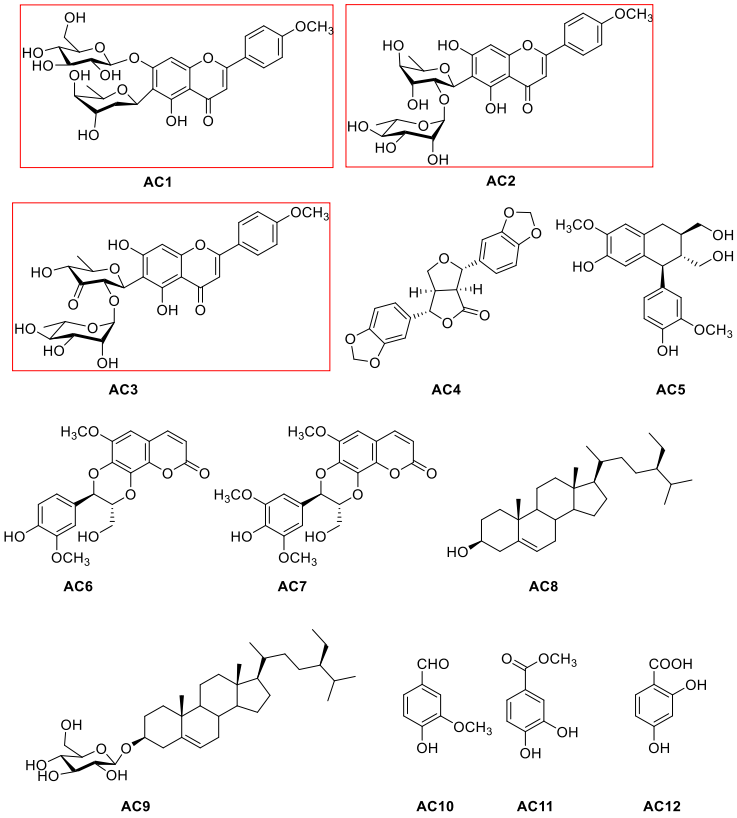


Figure 3.1. Structures of isolated compounds from leave of *A. chinense* specie

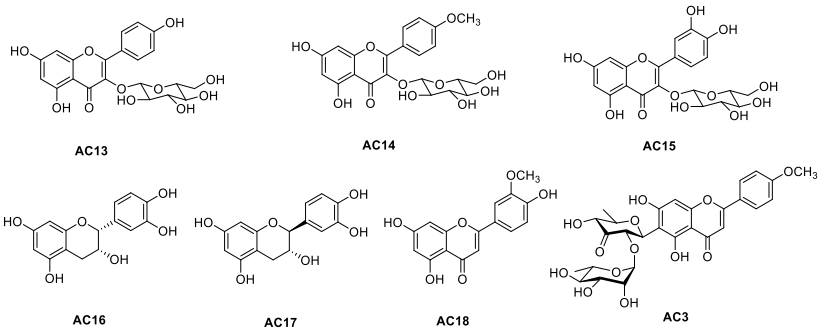


Figure 3.2. Structures of isolated compounds from flower of *A. chinense* specie

3.1.1. Determinating structure of some isolated compounds from leave of A. chinense specie

*3.1.1.1. Compound **AC1**: amesiflavone A*

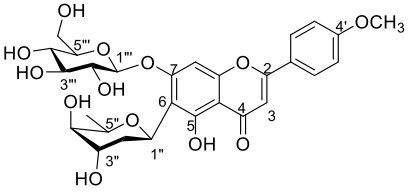


Figure 3.3. Structure of compound **AC1**

Compound **AC1** was obtained as a yellow amorphous powder. $[\alpha]_D^{25}$ = -76 (c 0.1, MeOH). The HR-ESI-MS analysis revealed the molecular formula to be $C_{28}H_{32}O_{13}$ with a cluster ion peak at m/z 577.1910 $[M+H]^+$ (Calcd. for $[C_{28}H_{33}O_{13}]^+$, 577.1916). NMR spectrum of **AC1** showed specific signals for flavonoid glycoside. The ^{1}H-NMR spectrum of **AC1** revealed signals of four aromatic protons of B ring at δ_H 8.00 (2H, d, J = 9.0 Hz, H-2′ and H-6′) and 7.10 (2H, d, J = 9.0 Hz, H-3′ and H-5′), two singlet signals of protons including 1 proton of A ring at δ_H 7.06 (s, H-8) and 1 proton

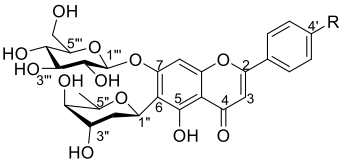
of C rings at δ_H 6.74 (s, H-3) assigned to a flavone aglycone. The ^{13}C-NMR and DEPT spectra of this aglycone moiety gave the corresponding signal of 15 flavone carbon skeleton consisting of a carbonyl group (C-4); 6 carbon methine sp^2; and 8 non-protonated quaternary carbons (sp^2) including 5 oxygenated quaternary carbon (sp^2), and 3 quaternary carbons (sp^2) (see table 3.1). There was also a signal of a methoxy group at δ_H 3.90 (4′-OCH_3)/δ_C 56.1(4′-OCH_3). The above ^{1}H, ^{13}C-NMR spectral datas suggested the presence of an apigenin moeity with an additional substituent at the A ring at the C-6 position (δ_C 114.3) because the usual chemical shift in flavone of 6-CH is around δ_C 99.0. The signals of the two monosaccharide moieties were assigned to one of the rare natural boivinopyranose and the other to glucopyranose. The boivinopyranosyl unit was indicated as the 2,6-dideoxyhexopyranosyl, which was deoxygenated at the 2″ and 6″ positions. The ^{1}H-NMR spectrum of **AC1** showed proton signals consisting of 1 proton anomer at δ_H 5.54 (dd, J = 3.0; 12.5 Hz, H-1″), the large $^3J_{H\text{-}1''/H\text{-}2''}$ = 12.5 Hz showed that H-1″ was in the *axial* position and monosaccharide unit had *β*- linkage; 2 non-equivalent geminal protons of 1 methylene group at δ_H 3.08 (ddd, J = 3.0; 12.5; 14.0, H_{ax}-2″) and 1.47 (dd, J = 3.0; 14.0 Hz, H_{eq}-2″); 3 protons of 3 oxymethine groups at δ_H 4.12 (q, J = 6.5, H-5″), 4.06 (br d, J = 3.0, H-3″), and 3.39 (br d, J = 3.5, H-4″); a methyl group at δ_H 1.24 (d, J = 6.5, 3H-6″). The small $^3J_{H\text{-}3''/H\text{-}4''}$ so they had *equatorial* bonds and *axially* oriented 3″-OH and 4″-OH groups. Thus, the 2,6-dideoxyhexopyranosyl unit was identified and the orientation of the bonds suggested the structure of the *β*-boivinopyranosyl unit. The ^{13}C-NMR, DEPT and HSQC spectra showed that the corresponding carbon signals of the units. The *β*-boivinopyranosyl unit showed the

signal of 6 carbons at δ_C 72.3 (C-5″), 71.1 (C-4″), 69.5 (C-3″), 67.1 (C-1″), 31.3 (C-2″), and 17.5 (C-6″). The chemical shift of the anomer carbon at δ_C 67.1 (C-1″) indicated the monosaccharide unit was directly linked (C-C) to the carbon of the aglycone to form a *C*-glycoside or *C*-*β*-boivinopyranosyl. The remaining glucopyranosyl unit displayed a signal of 1 proton anomer/carbon anomer at δ_H 4.96 (d, *J* = 7.5 Hz, H-1‴)/103.7 (C-1‴); an oxymethylene group at δ_H 4.04 (dd, *J* = 6.5; 12.0), and 3.78 (dd, *J* = 2.0; 12,0)/62,7 (C-6‴); 4 oxymethine groups at δ_H 3.64 (dd, *J* = 7.5; 9.5, H-2‴)/75.0 (C-2‴), 3.62 (m)/78.7 (C-5‴), 3.55 (dd, *J* = 9.0; 9.5, H-3‴)/77.1 (C-3‴), and 3.43 (dd, *J* = 9.0; 9.5, H-4‴)/71.6 (C-4‴). The large *J* between the protons H-1‴/H-2‴, H-2‴/ H-3‴, H-3‴/ H-4‴ indicated that these protons were oriented *axial*, the *equatorial* oriented 2‴-OH, 3‴-OH, 4‴-OH groups showed the structure of the glucopyranose as *O*-*β*-glucopyranosyl unit. The COSY spectrum displayed correlations between H-2′/H-3′ and H-5′/H-6′ of B ring, between H-1″/H_{2ax}-2″, H_{2eq}-2″/H-3″, H-3″/H-4″, H-4″/H-5″ and H-5″/H-6″ of the *β*-boivinopyranosyl unit, and between H-1‴/H-2‴, H-2‴/H-3‴, H-3‴/H-4‴, H-4‴/H-5‴ and H-5‴/H_2-6‴ of the *β*-glucopyranosyl unit. The HMBC spectrum showed that correlations between between H-8 (δ_H 7.06) with C-7 (δ_C 164.8)/C-9 (δ_C 158.5)/C-6 (δ_C 114.3)/C-10 (δ_C 107.0) and H-3 (δ_H 6.74) and C-4 (δ_C 184.2)/C-2 (δ_C 166.3)/C-1′ (δ_C 124.3)/C-10 (δ_C 107.0) confirmed the positions of two protons at C-3, C-8 and the structure of the A and C rings; between H-3′ and H-5′ (δ_H 7.10) with C-1′/C-4′/C-2′/C-3′/C-5′ and between H-2′ and H-6′ (δ_H 8.00) with C-2/C-4′ (δ_C 164.6)/C-2′/C-6′, and between the methoxy group (δ_H 3.90) with C-4′ (δ_C 164.6) proven the structure of the B ring and the position of the methoxy

group 4′-OCH_3; between H-1″ (δ_H 5.54) and C-7 (δ_C 164.8) C-5 (δ_C 160.1)/C-6 (δ_C 114.3) and between H-1‴ (δ_H 4.96) with C-7 (δ_C 164.8) indicated that the *β*-boivinopyranosyl and the *β*-glucopyranosyl units linked to C-6, C-7 of the A ring. The *β*-boivinopyranosyl unit bond to C-6 was also confirmed when compared the chemical shift of C-6 (δ_C 114.3) of **AC1** to the case like the A-ring of flavone has 2 substituents, a 7-*O*-glycoside substituent and the other was *C*-glycoside at C-6 or C-8, the chemical shift values of 8-*C*-glycoside at δ_C 107.7 and 6-*C*-glycoside at δ_C 112.9. In addition, the HMBC correlations between H_{ax}-2″ with C-1″ and H_{eq}-2″ with C-3″, between H-5″ and C-1″/C-6″/C-4″/C-3″, between H-6″ and C-4″/C-5″ further confirmed the position of the 2″-CH_2 group in the structure of 6-*C*-*β*-boivinopyranosyl unit. Assumptions about the configuration of the monosaccharide units are affirmed more clearly on the NOESY spectrum. The results of NOESY spectrum of the 6-*C*-*β*-boivinopyranosyl unit showed that NOESY correlation between H-1″ (δ_H 5.54) and H-5″ (δ_H 4.12) without NOESY correlation between H-1″ (δ_H 5.54) with H-3″ (δ_H 4.06) indicated the *equatorial* orientation of H-3″ and further confirmed the structure of the boivinopyranose unit. The NOESY correlations of *O*-*β*-D-glucopyranosyl unit between H-1‴/H-3‴/H-5″ confirmed the *axial* orientation of the protons H-1″/H- 3‴/H-5‴. The NOESY correlation between H-1‴ (4.96) and H-8 (7.06) further confirmed the bond position of the 7-*O*-*β*-glucopyranosyl moiety to the C-7 of the aglycone. Combining the 1D, 2D-NMR spectral analysis of the compound **AC1** and comparing the spectral datas of this compound with the reference datas (table 3.1) showed the spectral datas of **AC1** similar to apigenin 6-*C*-*β*-D-boivinopyranosyl-

7-*O*-*β*-D-glucopyranoside (**AC1′**) except for the addition of a 4′-OCH_3 methoxy group in the structure of **AC1**. Thus, the structure of **AC1** was identified as acacetin-6-*C*-*β*-D-boivinopyranosyl-7-*O*-*β*-D-glucopyranoside which was a new compound and named amesiflavone A.

Table 3.1. ^{1}H-, ^{13}C-NMR spectra of compound **AC1** and reference compound



AC1: R = OCH_3

AC1': R = OH (Apigenin-6-*C*-*β*-boivinopyranosyl-7-*O*-*β*-glucopyranoside)

No	**AC1**		Reference compound	
C	δ_C[a]	δ_H[b](mult., *J* in Hz)	δ_C[c]	δ_H[d](mult., *J* in Hz)
2	166.3	-	164.1	-
3	104.8	6.74 (s)	103.2	6.88 (s)
4	184.2	-	182.0	-
5	160.1	-	157.8	-
6	114.3	-	112.9	-
7	164.8	-	163.0	-
8	96.3	7.06 (s)	94.7	6.96 (s)
9	158.5	-	156.1	-
10	107.0	-	105.0	-
1′	124.3	-	120.8	-
2′, 6′	129.5	8.00 (d, 9.0)	128.4	7.95 (d, 8,8)
3′, 5′	115.7	7.10 (d, 9.0)	115.9	6.94 (d, 8,8)
4′	164.6	-	161.0	-
4′-OMe	56.1	3.90 (s)	-	-
	Boi			**Boi**
1″	67.1	5.54 (dd, 3.0; 12.5)	64.6	5.31 (dd, 2.3; 12)
2″	31.3	3.08 (ddd, 3.0; 12.5;	30.0	3.90 (dt, 2.7;12), *ax*

		14.0), *ax*		1.25 (br d, 13), *eq*
		l.47 (dd, 3.0; 14.0), *eq*		
3″	69.5	4.06 (m)	67.1	3.86 (d, 2.7)
4″	71.1	3.39 (br d, 3.5)	69.8	3.19 (d, 3.7)
5″	72.3	4.12 (q, 6.5)	70.0	3.89 (q, 6.3)
6″	17.5	1,24 (d, 6.5)	17.1	1.05 (d, 6.6)
	Glc			**Glc**
1‴	103.7	4.96 (d, 7.5)	102.0	4.86 (d, 7.6)
2‴	75.0	3.64 (dd, 7.5; 9.5)	73.6	3.34 (m)
3‴	77.1	3.55 (dd, 9.0; 9.5)	77.3	3.40 (m)
4‴	71.6	3.43 (dd, 9.0; 9.5)	69.2	3.20 (m)
5‴	78.7	3.62 (m)	75.1	3.29 (m)
6‴	62.7	4.04 (dd, 6.5; 12.0)	60.1	3.80 (dd, 5.3; 10.9)
		3.78 (dd, 2.0; 12.0)		3.52 (dd, 6.0; 10.9)

[a,b] recored in CD_3OD; [c,d] recored in DMSO-d_6; [a,c]125 MHz, [a,d]500 MHz; Boi: boivinopyranosyl; Glc: glucopyranosyl.

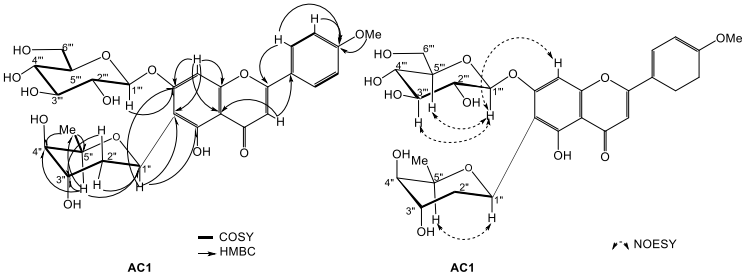


Figure 3.16. The key COSY, HMBC and NOESY correlations in **AC1** compound

*3.1.1.2. Compound **AC2**: amesiflavone B*

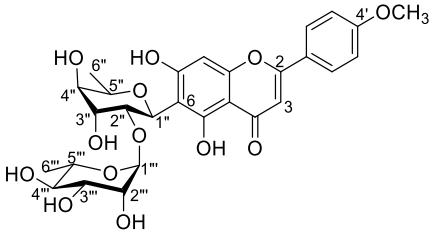


Figure 3.17. Structure of compound **AC2**

Compound **AC2** was obtained as a yellow amorphous powder, $[\alpha]_D^{25}$ = -43.0 (c 0.1, MeOH) and its molecular formula was determined to be $C_{28}H_{32}O_{13}$ by HR-ESI-MS at m/z 577.1915 $[M+H]^+$ (Calcd. for $[C_{28}H_{33}O_{13}]^+$, 577.1916). Similar to compound **AC1**, the NMR spectrum of **AC2** also showed the signals of flavone glycoside acacetin-skeleton, except for the difference in the two monosaccharide moieties. The ^{1}H-NMR spectrum of **AC2** displayed the signals of the protons of the apigenin substituted part at C-6 including two douplet signals of four para-substituted benzene ring protons at δ_H 7.93 (2H, d, *J* = 7.5 Hz, H-2′ and H-6′) and 7.09 (2H, d, *J* = 7.5 Hz, H-3′ and H-5′) of the B ring; two singlets at δ_H 6.63 (1H, s, H-3) and 6.54 (1H, s, H-8) of the C ring and A ring; a methoxy group at δ_H 3.91 (3H, s, 4′-OCH_3). The ^{13}C-NMR, DEPT and HSQC spectra of **AC2** showed 15 carbons respectively signals of 3 rings A, B and C of the flavone acacetin skeleton including 1 carbonyl group, 6 carbon methine sp^2; 8 non-protonated carbons sp^2 (see table 3.2). There was also a carbon of the methoxy group at δ_C 56.1 (4′-OCH_3). The above ^{1}H-, ^{13}C-NMR spectral analysis suggested that the presence of an acacetin part with an additional substituent at C-6 (δ_C 110.1) of the A ring. Beside the characteristic signals of aglycone acacetin protons, the NMR spectrum revealed signals of two monosaccharide moieties with two proton anomer/carbon anomer signals at δ_H 5.41 (1H, d, *J* = 9.8 Hz, H-1′′)/δ_C 70,3 (C-1′′) and 4.82 (1H, br s, H-1′′′)/δ_C 97.5 (C-1′′′). The large $^3J_{H\text{-}1''/H\text{-}2''}$ = 9.8 Hz showed that H-1′′ was *axially* oriention and the it had *β*-bonding linkage; conversely, the small $^3J_{H\text{-}1'''/H\text{-}2'''}$ (br s) indicated H-1′′′ was in the *equatorial* oriention and the second monosaccharide unit had *α* bonding linkage. Otherwise, the chemical shift of the anomer carbon

at δ_C 70.3 (C-1″) has shown that this carbon was directly bonded to the carbon of the aglycone part forming a 6-*C*-*β*-glycoside bond. The first monosaccharide unit was identified as 6-deoxyhexopyranosyl unit which was deoxygenated at the C-6″ position. NMR spectrum of the deoxyhexopyranosyl unit showed that the remaining proton signals of 4 oxymethine groups at δ_H 4.45 (1H, br d, *J* = 9.8 Hz, H-2″)/δ_C 70.8 (C-2″), δ_H 4.26 (1H, m, H-3″)/δ_C 67.7 (C-3″), δ_H 4.22 (1H, m, H-5″)/δ_C 72.3 (C-5″), and δ_H 3.69 (1H, br d, *J* = 3.5 Hz, H-4″)/δ_C 73.0 (C-4″); a methyl group at δ_H 1.29 (3H, d, *J* = 6.5 Hz, H-6″)/δ_C 17.8 (C-6″). The small $^3J_{H\text{-}3''/H\text{-}4''}$ indicated that these protons were *equatorially* orientation and the 3″-OH and 4″-OH hydroxy groups were *axially* orientation. The 6-*C*-*β*-glycoside linkage and the orientation of the bonds in the first monosaccharide unit suggested that this was the 6-deoxygulopyranose and that the first monosaccharide was 6-*C*-*β*-deoxygulopyranosyl. The second monosaccharide unit, identified as the rhamnopyranosyl unit, gave the remaining proton signals of four oxymethine groups at δ_H 4.22 (1H, m, H-5‴)/δ_C 72.3 (C-5‴), δ_H 3.85 (1H, br s, H-2‴)/δ_C 72.4 (C-2′) ″), δ_H 3.40 (1H, dd, *J* = 3.0, 9.5 Hz, H-3‴)/δ_C 72.2 (C-3‴), and δ_H 3.14 (1H, dd, *J* = 3.0; 9.5 Hz, H-4‴)/δ_C 73.0 (C-4‴); a methyl group at δ_H 0.87 (3H, d, *J* = 6.5 Hz, H-6‴)/δ_C 16.6 (C-6‴). The large $^3J_{H\text{-}3'''/H\text{-}4'''}$ (*J* = 9.5 Hz) showed that these two protons are *axially* orientation and the hydroxy groups 3‴-OH and 4‴ -OH was oriented *equatorial*. The *O*-*α*-glycoside linkage and the orientation of the bonds in the second monosaccharide unit indicated that it was *O*-*α*-L-rhamnopyranosyl. The COSY spectrum of **AC2** showed between protons including between H-2′/H-3′ and H-5′/H-6′ of the B ring, between H-1″/H_2-2″, H_2-2″/H-3″, H-3″/H-4″, H-4″/H-5″ and H-5″/

H-6″ of the *C-β*-6-deoxygulopyranoside unit; and between H-1‴/H-2‴, H-2‴/H-3‴, H-3‴/H-4‴, H-4‴/H-5‴ and H-5‴/H2-6‴ of the O-*α*-*L*-rhamnopyranosyl unit. The HMBC spectrum showed that correlations between between H-3 (δ_H 6.63) and C-10 /C-1′/C-2 /C-4 and H-8 (δ_H 6.54) and C-6/C-7/C-9/C-10 confirmed the positions of two protons at C-3, C-8 and the structure of the C and A rings; between H-2′ and H-6′ with C-2/C-4′/C-2′/C-6′ and between H-3′ and H-5′ with C-1′/C-4′ /C-3′/C-5′, and between the methoxy group (δ_H 3.90) and C-4′ demonstrated the structure of the B ring and the position of the methoxy group at C-4′; between H-1″ (δ_H 5.41) and C-5/C-6/C-7 indicated that the *β*-6-deoxygulopyranoside unit bonded to C-6 of the A ring; and between H-1‴(δ_H 4.82) and C-2″(δ_C 70.8) showed that the O-*α*-*L*-rhamnopyranosyl unit bonded to C-2″ of the 6-*C-β*-6-deoxygulopyranosyl unit. The 6-*C-β*-6-deoxygulopyranosyl unit gives NOESY correlations between H-5″ (δ_H 4.22) and H-1″ (δ_H 5.41), between H-3″/H-4″ without the NOESY correlations between H-1″ and H-3″/H-4″ further confirmed the *axial* orientation of H-1″ and H-5″, the *equatorial* orientation of H-3″/H-4″ and *axial* orientation of the 3″-OH, 4″-OH groups. Combining the above 1D and 2D-NMR spectral analysis and comparison with the literature allowed to determine that the first monosaccharide unit was *β*-6-deoxygulopyranosyl. The NOESY correlations in second *α*-L-rhamnopyranosyl unit between H-1‴/H-2‴ and H-1‴ with H-2‴/H-3″ further confirmed *equatorial* orientation of H-2‴ and the connection of the *α*-L-rhamnopyranosyl sugar unit to the C-2″ of *β*-6-deoxygulopyranosyl; between H-3‴/H-5‴ further confirms the *axial* co-orientation of the H-3‴/H-5‴ protons. Combining the above 1D and 2D-NMR spectral analysis allowed to confirm that the

second monosaccharide unit was the *α*-L-rhamnopyranosyl and bonded to the C-2″ of the first monosaccharide unit. Analysis of the spectrum datas for compound **AC2** showed that it was also a flavone *C*-glycoside compound with the aglycone acacetin. The two monosaccharides bonded to each other and bonded to the aglycone at C-6 of the A ring by a C-C bond. The first monosaccharide unit that directly bonded C-C to acacetin was the *β*-6-deoxygulopyranose unit, which was rare in nature, and the second monosaccharide unit was the *α*-L-rhamnopyranose, which bonded to the C-2″ of the first unit. Consequently, the **AC2** compound identified as acacetin-6-*C*-(2″-*α*-L-rhamnopyranosyl)-*β*-6-deoxygulopyranoside was a new compound named amesiflavone B. ^{1}H-, ^{13}C-NMR spectral datas of compound **AC2** was shown in table 3.2.

Table 3.1. ^{1}H-, ^{13}C-NMR datas of compound **AC2**

No C	**AC2**			
	δ_C[a]	δ_H[b](mult., *J* in Hz)	HMBC[c] (H→C)	NOESY (H→C)
2	165.9	-	3, 2′,6′	
3	104.7	6.63 (s)		
4	184.3	-	3	
5	161.1	-	1″	
6	110.1	-	8	
7	164.9	-	8, 1‴	
8	96.0	6.54 (s)	1‴	
9	158.9	-	8	
10	105.5	-	8	
1′	124.7	-	3, 3′, 5′	
2′, 6′	129.3	7.93 (d, 7.5)	2′, 6′	2′, 6′
3′, 5′	115.7	7.09 (d, 7.5)	3′,5′	3′, 5′
4′	164.4	-	2′, 6′, 3′, 5′, 4′-OMe	
4′-	56.1	3.91 (s)		

OMe				
	Gul			
1″	70.3	5.41 (d, 9.8)		5″
2″	70.8	4.45 (br d, 9.8)	1‴, 1″, 4″	3″
3″	67.7	4.26 (m)	4″	4″, 2″
4″	73.0	3,69 (br d, 3.5)		3″, 6″
5″	72.3	4,22 (m)	3″	1″, 6″
6″	17.8	1.29 (d, 6.5)	5″	5″
	Rha			
1‴	97.5	4.82 (br s)		2‴,3″,2″
2‴	72.4	3.85 (br s)	1‴	1‴, 3‴
3‴	72.2	3.40 (dd, 3.0; 9.5)	1‴, 4‴	5‴, 4‴
4‴	73.3	3.14 (dd, 9.0; 9.5)	2‴, 3‴	6‴
5‴	69.8	2.41 (m)	1‴, 4‴	5‴
6‴	16.6	0.87 (d, 6.5)	4‴	4‴, 5‴

[a,b] recored in CD_3OD; [c,d] recored in DMSO-d_6 ; [a]125 MHz, [b]500 MHz; [c]90 MHz, [d]360 MHz; Gul: 6-deoxygulopyranosyl; Rha: rhamnopyranosyl.

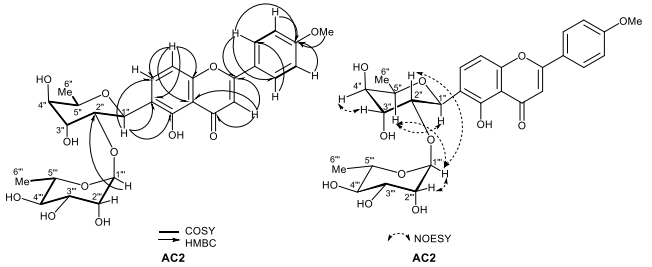


Figure 3.29. The key COSY, HMBC and NOSEY correlations in **AC2** compound

*3.1.1.3. Compound **AC3**: amesiflavone C*

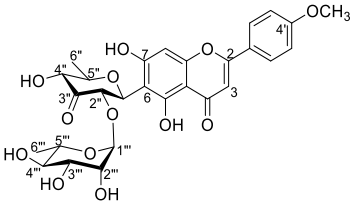


Figure 3.30. Structure of compound **AC3**

Compound **AC3** was obtained as a yellow amorphous powder, $[\alpha]_D^{25}$ = -25.0 (c 0.1, MeOH) and its molecular formula was determined to be $C_{28}H_{30}O_{13}$ by HR-ESI-MS at m/z 575.1761 $[M+H]^+$ (Calcd. for $[C_{28}H_{31}O_{13}]^+$, 575.1759). Similar to compounds **AC1** and **AC2**, the ^{1}H-, ^{13}C-NMR spectra of compound **AC3** also displayed the signals of a flavone glycoside compound with proton signals of the aglycone that is substituted at C-6 including signals of 4 protons of a para-substituted benzene ring (B ring) at δ_H 8.02 (2H, d, J = 9.0 Hz, H-2′ and H-6′) and 7.12 (2H, d, J = 9.0 Hz, H-3′ and H-5′); 1 proton of the C ring at δ_H 6.80 (1H, s, H-3); 1 proton of the A ring at δ_H 6.56 (1H, br s, H-8); 3 protons of the methoxy group at δ_H 3.87 (3H, s). The ^{13}C-NMR, DEPT and HSQC spectra of **AC3** showed that 15 carbons respectively of 3 rings A, B and C of the flavone skeleton including 1 carbonyl, 6 carbon methine sp^2; 8 quaternary carbons (sp^2), of which 5 oxygenated carbons (sp^2) and 3 quaternary carbons sp^2 (see table 3.3); and a carbon of the methoxy group at δ_C 55.3 (4′-OCH_3). Unlike **AC1** and **AC2**, the ^{1}H, ^{13}C-NMR signals of **AC3** were unexpected when measured at room temperature. Some signals do not split, but give broaden signals, such as the signal of H-1″ when measured at room temperature (30 ^{o}C) give a singlet signal at δ_H 4.86 but when measured at 60 ^{o}C gave a doublet signal $J = {}^3J_{H\text{-}1''/H\text{-}}$

$_{2''}$ = 10.0 Hz. This phenomenon was related to the restriction of rotation around the C (sp^3) - C (sp^2) axis of the *C*-glucosyl bond at C-6 by the 5-OH and 7-OH groups of the flavones. This thing had also shown that the monosaccharide unit was bonded to C-6 but not to C-8 at the A-ring of the flavone. Beside the characteristic proton signals of the aglycone moiety, the NMR spectra revealed the signals of the two monosaccharide moieties with two proton anomer/carbon anomer signals at δ_H 4.88 (1H, d, J = 10.0 Hz, H-1″)/δ_C 73.0 (C-1″) and 4.69 (1H, s, H-1‴)/δ_C 99.0 (C-1‴). The large value of $^3J_{H\text{-}1''/H\text{-}2''}$ = 10.0 Hz showed that H-1″ was *axially* oriented and the first monosaccharide unit had *β*-bonding linkage; conversely, the small value of $^3J_{H\text{-}1'''/H\text{-}2'''}$ (s) showed that H-1‴ was in the *equatorial* orientation and the second monosaccharide unit had an *α*-bond. On the other hand, the chemical shift of the anomer carbon at δ_C 73.0 (C-1″) of the monosaccharide unit suggested that this carbon was directly bonded to the carbon of the aglycone unit. The first monosaccharide unit was identified as *β*-6-deoxy-ribo-hexos-3-ulopyranosyl because it was deoxygenated at the C-6″ position and was ketonization at C-3″. The NMR spectrum of the *β*-6-deoxy-ribo-hexos-3-ulopyranosyl unit showed that the remaining signals of a ketone group at 205.7 (C-3″); 3 oxymethine groups at δ_H 5.29 (1H, br s, H-2″)/δ_C 75.4 (C-2″), δ_H 3.92 (1H, m, H-4″)/δ_C 77.2 (C-4″), and δ_H 3.42 (1H, m, H-5″)/δ_C 77.8 (C-5″); a methyl group at δ_H 1,29 (3H, d, J = 6.5 Hz, H-6″)/δ_C 17.8 (C-6″). In the presence of a ketone group at the C-3″ position, the deoxidation at C-6″ in the first monosaccharide unit as *β*-6-deoxy-ribo-hexos-3-ulopyranose. The second monosaccharide unit was identified as the L-rhamnopyranosyl, gave the remaining signals of 4 oxymethine groups at δ_H 3.74 (1H, br s, H-2‴)/δ_C 69 ,9 (C-2‴),

δ_H 3.09 (1H, m, H-3‴)/δ_C 70.2 (C-3‴), δ_H 3.01 (1H, dd, *J* = 9.0, 9.5 Hz, H-4‴)/δ_C 71.1 (C-4‴), and δ_H 2.45 (1H, m, H-5‴)/ δ_C 68.5 (C-5‴); a methyl group at δ_H 0.74 (3H, br s, H-6‴)/δ_C 17.1 (C-6‴). The large *J* between H-4‴/H-3‴/ and H-4‴/H-5‴ (*J* = 9.0 and 9.0 Hz) proved that 3 protons were *axially* oriented and the hydroxy groups 3‴-OH and 4‴-OH and 6‴-CH_3 were *equatorial* oriented. The orientation of the bonds in the monosaccharide unit suggested that this was an *α*-L-rhamnopyranose unit. The COSY spectrum showed that correlations between H-2′/H-3′ and H-5′/H-6′ of the C ring, between H-1″/H2-2″, H-4‴/H-5″ and H-5″/H-6″ of the *β*-6-deoxy-ribo-hexos-3-ulopyranosyl unit; and between H-1‴/H-2‴, H-2‴/H-3‴, H-3‴/H-4‴, H-4‴/H-5‴ and H-5‴/H_2-6‴ of the *α*-L-rhamnopyranosyl unit. The HMBC correlations between H-3 (δ_H 6.80) and C-10 /C-1′/C-2 /C-4 and H-8 (δ_H 6.56) and C-6/C-7/C-9/C-10 confirmed the positions of two protons at C-3, C-8 and the structure of the C and A rings; between H-2′ and H-6′ with C-2/C-4′/C-2′/C-6′ and between H-3′ and H-5′ with C-1′/C-4′ /C-3′/C-5′, and between the methoxy group (δ_H 3.87) and C-4′ demonstrated the structure of the B ring and the position of the methoxy group at C-4′; between H-1″ (δ_H 4.88) and C-5/C-6/C-7 showed that the *β*-6-deoxy-ribo-hexos-3-ulopyranosyl bonded to C-6 of the A ring. In addition, the HMBC correlations between H-1″ and C-5″/C-3″ (205.7) demonstrated the ketones group at C-3″ in this monosaccharide unit. The correlations between H-1‴ (δ_H 4.69) with C-2″ (δ_C 75.4) indicated that the *α*-L-rhamnopyranosyl unit linked to C-2″ of the *β*-6-deoxy-ribo-hexos-3-ulopyranosyl unit. The configuration of the monosaccharide units was further confirmed on the NOESY spectrum. The NOESY correlations between H-2″/H-4″ without the

NOESY correlations between H-1″ and H-2″/H-4″ indicated H-2″ /H-4″ proton were on the same plane on the opposite side as the H-1″/H-5″ protons of the pyranose monosaccharide plane and these protons were *axially* oriented as shown in the figure. Combining the above 1D and 2D-NMR spectral analysis allowed to confirm that the first monosaccharide unit was *β*-6-deoxy-ribo-hexos-3-ulopyranosyl and the second monosaccharide unit was the *α*-L-rhamnopyranosyl unit. The *α*-L-rhamnopyranosyl unit for the NOESY correlations between H-1‴/H-2‴/H-2″/H-3″ showed the *equatorial* orientation of H-2‴ and the *α*-L-rhamnopyranosyl unit into the C-2″ portion of the β-6-deoxy-ribo-hexos-3-ulopyranosyl unit; between H-3‴/H-5‴/H-6‴ showed the *axial* orientation of the H-3‴/H-5‴ protons. Analysis of ^{1}H-, ^{13}C-NMR spectral datas of compound **AC3** presented in table 3.3 almost similarly to cassiaoccidentalin A except there was an additional methoxy group at C-4′ in the molecule of **AC3**. Consequently, **AC3** was identified as acacetin-6-*C*-(2″-*α*-L-rhamnopyranose)-*β*-6-deoxy-ribo-hexos-3-ulopyranosyl. It was the new compound named **amesiflavone C**. ^{1}H, ^{13}C-NMR spectral datas of **AC3** and the reference compound cassiaoccidentalin A were presented in table 3.3.

Table 3.3. ^{1}H-, ^{13}C-NMR datas recorded in DMSO-d_6 of **AC3** and cassiaoccidentalin A

No	**AC3**		Cassiaoccidentalin A	
C	δ_C[a]	δ_H[b] (mult., *J* in Hz)	δ_C[c]	δ_H[d] (mult., *J* in Hz)
2	163.0	-	163.9	-
3	103.3	6.80 (s)	103.3	6.74 (s)
4	181.6	-	182.4	-
5	161.5	-	161.1	-
6	107.4	-	107.8	-
7	161.8	-	162.3	-

8	93.3	6.56 (br s)	93.5	6.53 (s)
9	156.5	-	156.9	-
10	103.3	-	103.1	-
1′	122.6	-	121.4	-
2′, 6′	128.0	8.02 (d, 9.0)	128.8	7.88 (d, 9.0)
3′, 5′	114.4	7.12 (d, 9.0)	116.3	6.92 (d, 9.0)
4′	162.2	-	161.3	-
4′-OMe	55.3	3.87 (s)	-	-
	Rib			
1″	73.0	4.88 d, 10.0)	73.6	4.84 (d, 10.0)
2″	75.4	5.29 (br s)	75.8	5.27 (d, 10.0)
3″	205.7	-	206.2	-
4″	77.2	3.92 (m)	78.2	3.88 (d, 10.0)
5″	77.8	3.42 (m)	78.4	3.37 (m)
6″	18.7	1.33 (d, 6.0)	19.2	1.29 (d, 5.5)
	Rha			
1‴	99.0	4.69 (s)	99.5	4.64 (br s)
2‴	69.9	3.74 (br s)	70.4	3.69 (m)
3‴	70.2	3.09 (m)	70.3	3.02 (m)
4‴	71.1	3.01 (like t, 9.0)	71.4	2.95 (d, 9.5)
5‴	68.5	2.45 (m)	69.1	2.34-2.41 (m)
6‴	17.1	0.74 (br s)	17.6	0.78 (m)

[a,b] recorded in 60°C ; [c,d] recorded in 40°C; [a,c]125 MHz, [b,d]500 MHz;

Rha: rhamnopyranosyl; Rib: 6-Deoxy-ribo-hexos-3-ulose

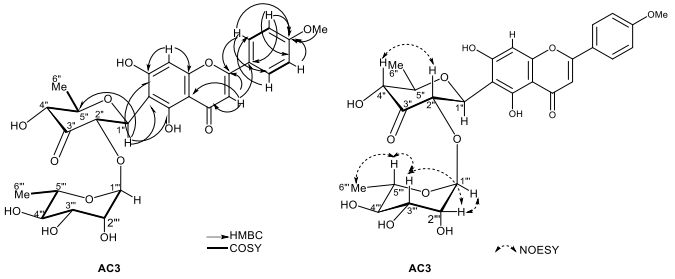


Figure 3.44. The key COSY, HMBC and NOSEY correlations in **AC3** compound

3.1.2. Determinating structure of some isolated compounds from flower of A. chinense specie

3.2. Determinating structure of some isolated compounds from leave of *B. sylvestris specie*

From leaves of *B. sylvestris* collected in Gia Lai province, 7 compounds were isolated, **BS1**-**BS7**.

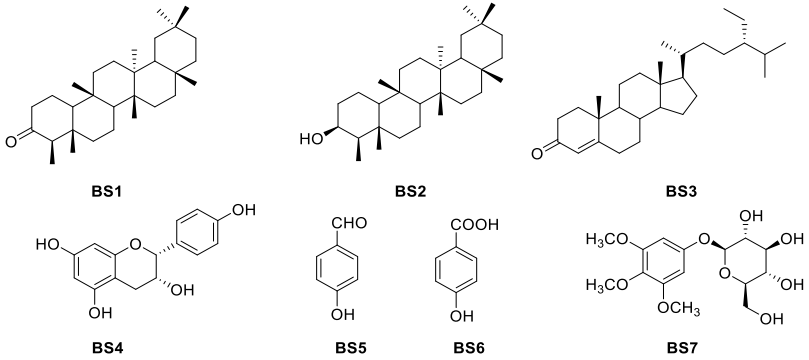


Figure 3.78. Structures of isolated compounds from leaves of *B. sylvestris* specie

3.3. Biological activities of extracts and isolated compounds from *A. chinense* and *B. sylvestris* species

3.3.1. Test results of biological activities of some extracts from A. chinense and species B. sylvestris species

The results of toxicity testing of the extracts showed that only AE extract, which was the leaf ethyl acetate extract of specie *A. chinense* species, was selectively active on cancer cell lines with IC_{50} = 20.55 µg/ml. All remaining extracts showed weak or no activity against the tested cancer cell lines KB, HepG2, Lu and MCF7.

3.3.2. Test results of cytotoxic activity of some selective compounds from A. chinense specie

Compounds were isolated from the ethyl acetate fraction from the leaf part of *A. chinense* specie including **AC1**, **AC2**, **AC3**, **AC4**, **AC5**, **AC6** and **AC7**, were evaluated for their cytotoxic potency by the MTT assay to cancer cell lines KB, SK-LU-1, MCF7, HepG2 and SW480 using Ellipticine as a positive control. The results showed that compounds **AC3** and **AC5** did not show cytotoxic activity, the remaining compounds **AC1, AC2, AC4, AC6 and AC7** had weak inhibitory activity on 5 tested cell lines in IC_{50} assay 71.0-146.0 μM. Fractional extracts exhibited cytotoxicity with IC_{50} values ranging from 20.55 to 153.9 μM. However, the potency of the isolated compounds **AC1**, **AC2**, **AC4**, **AC6** and **AC7** indicated by the IC_{50} value was much higher than that of the fractional extracts. This observation supported that the isolated compounds were less cytotoxic in the single test than the fractional extracts. The results suggested that compounds from the leaf extracts of *A. chinense* species may have synergistic effects in cytotoxicity assays.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

This is the first time in Vietnam and in the world, chemical constituent and biological activities of *Amesiodendron chinense* and *Baccaurea sylvestris* species have been studied.

1. Chemical constituent

From *A. chinense* specie collected in Da Nang, 18 compounds were isolated:

- 12 compounds isolated from leaves including 3 new flavone *C*-glucoside compounds: amesiflavone A (**AC1**), amesiflavone B

(**AC2**) and amesiflavone C (**AC3**); The four lignan compounds were (+)-aptosimon (**AC4**), (+)-isolariciresinol (**AC5**), (-)-cleomiscosin A (**AC6**), (-)-cleomiscosin C (**AC7**); 2 steroid compounds were *β*-sitosterol (**AC8**) and daucosterol (**AC9**); and three phenolic compounds were 4-hydroxy-3-methoxybenzaldehyde (**AC10**), protocatechuic acid methyl ester (**AC11**), and protocatechuic acid (**AC12**).

- 6 flavonoids isolated from flowers, including 3 flavonols astragalin (**AC13**), kaempferide 3-*O*-*β*-D-glucopyranoside (**AC14**), and quercetin 3-*O*-*β*-D-glucoside (**AC15**); 2 flavanol were (-)-epi-catechin (**AC16**) and (-)-catechin (**AC17**); 1 flavone compound was chrysoeriol (**AC18**); and a new compound was isolated that coincided with the one isolated from the leaves, amesiflavone C (**AC3**).

From the leaves of *B. sylvestris*, 7 compounds were isolated, including 2 triterpenoid compounds: friedelin (**BS1**) and 3*β*-friedelanol (**BS2**); another steroid was stigmast-4-en-3-one (**BS3**); one flavanol was (-)-epiafzelechin (**BS4**); and three phenolic compounds were 4-hydroxybenzaldehyde (**BS5**), 4-hydroxybenzoic acid (**BS6**) and 3,4,5-trimethoxyphenyl-*β*-D-glucopyranoside (**BS7**).

2. *In vitro* cancer cytotoxic activity of extracts and compounds isolated from *A. chinense* and *B. sylvestris* species

- Tested the cytotoxic activity of some extracts with 4 cell lines KB, LU, MCF7 and HepG2, the results showed that the leaf ethyl acetate extract of *A. chinense* specie had strong activity against KB cells. with IC_{50} = 20.55 µg/ml. All the remaining extracts showed weak or no activity against the 4 tested cancer cell lines. *In vitro*

cancer cytotoxicity of extracts and compounds isolated from *A. chinense* and *B. sylvestris* species.

- Selecting 7 compounds **AC1**, **AC2**, **AC3**, **AC4**, **AC5**, **AC6** and **AC7** for cytotoxicity assessment with cell lines KB, LU, MCF7, HepG2 and SW480. Results showed compounds from *A. chinense* has weak cytotoxic activity in the IC_{50} range = 71.0–146.0 μM or non-cytotoxic activity.

NEW FINDINGS OF THE THESIS

For the first time, *A. chinense* (Merr.) Hu belongs to the Sapindaceae family and *B. sylvestris* Lour belongs to the Phyllanthaceae family have been studied detailly for their chemical constituent and biological activity. 25 compounds were isolated from 2 species, of which 3 compounds were new (**AC1**, **AC2**, **AC3**) and 15 compounds were first isolated from the genus *Amensiodendron* (**AC1**-**AC18**), (**AC19** coincides with **AC3**); the rest seven compounds were isolated for the first time from *B. sylvestris* Lour (**BS1**-**BS7**).

RECOMMENDATIONS

Research results on two species of *A. chinense* (Merr.) Hu and *B. sylvestris* Lour showed that *A. chinense* and *B. sylvestris* had rich chemical compositions such as *C*-glucoside compounds, lignans and lignans, triterpenes, phenolic derivatives, which were classes of substances with many valuable biological activities. Among them, flavonoid compounds from flowers of *A. chinense* species were valuable compounds that have many applications in pharmaceuticals. However, it is necessary to continue to study much more about their biological activities in order to make fundament for the development of public health care products.

PUBLICATIONS WITHIN THE SCOPE OF THESIS

1. **Ho Van Ban**, Trinh Thi Thanh Van, Vu Van Chien, Nguyen Thi Hue, Pham Thi Hang, Nguyen Le Tuan, Nguyen Xuan Nhiem, Pham Van Cuong, Nguyen Quoc Vuong, *Lignans from leaves of Amesiodendron Chinense and their cytotoxic activity*, Vietnam Journal of Science and Technology, 2020, 58(4), 442-449. Doi:10.15625/2525-2518/58/4/14877.
2. **Ho Van Ban**, Trinh Thi Thanh Van, Vu Van Chien, Nguyen Thi Hue, Pham Thi Hang, Pham Van Cuong, Nguyen Le Tuan, Nguyen Quoc Vuong, *Flavonoids from flowers of Amesiodendron Chinense*, Vietnam Journal of Science and Technology, 2020, 58(6), 676-684. doi:10.15625/2525-2518/58/6/15127.
3. **Ho Van Ban**, Trinh Thi Thanh Van, Vu Van Chien, Nguyen Thi Hue, Pham Thi Hang, Nguyen Le Tuan, Marc Litaudon, Chau Van Minh, Pham Van Cuong, Nguyen Quoc Vuong, Nguyen Xuan Nhiem, *Flavone C-glycosides from the leaves of Amesiodendron chinense*, Phytochemistry Letters, 2020, 40 105-108. https://doi.org/10.1016/j.phytol.2020.09.017.
4. **Ho Van Ban**, Vu Van Chien, Nguyen Thi Hue, Pham Thi Hang, Nguyen Le Tuan, Hoang Nu Thuy Lien, Nguyen Quoc Vuong, *Phenolic compounds from leaves of Amensiodendron chinense (Sapindaceae)*, Hue University Journal of Science: Natural Science, 2021, Vol. 130, No. 1B, 53–57. DOI: 10.26459/hueunijns.v130i1B.6169.
5. **Ho Van Ban**, Vu Van Chien, Nguyen Thi Hue, Pham Thi Hang, Phạm Van Cuong, Nguyen Quoc Vuong, Nguyen Le Tuan, *Anti-inflammatory activity and phytochemistry of the leaf extracts of Baccaurea sylvestris Lour* was submitted on Vietnam Journal of Science and Technology.