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Cao Thanh Hải

**STUDY ON CHEMICAL CONSTITUENTS AND
BIOACTIVITIES OF *ATRACTYLODES MACROCEPHALA*
KOIDZ . (ASTERACEAE)**

**SUMMARY OF DISSERTATION ON SCIENCES OF
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Supervisors:

1. Supervisor 1: *Prof. Dr. Nguyễn Tiến Đạt*
Center for high technology research and development
2. Supervisor 2: *Prof. Dr. Phạm Thế Chính*
Thai Nguyen University of sciences

Phản biện 1:

Phản biện 2:

Phản biện 3:

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INTRODUCTION

The urgency of the thesis

Today, as society is increasingly developing, the trend of using natural medicinal sources in general, especially natural compounds derived from plants for prevention and treatment of diseases is becoming a top concern not only in the pharmaceutical industry in our country but also in countries worldwide.

Atractylodes macrocephala Koidz. is one of eight species of the *Atractylodes* genus of the Asteraceae family. This is a perennial herb best known in East Asia, especially in China. Currently, *Astragalus* is grown quite commonly in some northern mountainous provinces in Vietnam. Phytochemical evaluation has shown that *Eucalyptus* contains compounds with interesting biological activities that have potential for drug research and development. However, only a few of these compounds have been tested for biological activity and their respective structures have not been fully determined. Additionally, the quality control of *Atractylodes macrocephala* Koidz. has not been thoroughly researched and there is a lack of critical pharmacological assessment of its relationship to the traditional medicinal uses of *Atractylodes macrocephala*. To contribute to enriching the scientific database on the chemical composition and biological activity of *Eucalyptus* species grown in Vietnam, we propose the topic “Study on chemical constituents and bioactivities of *Atractylodes macrocephala* Koidz. (Asteraceae). This research result will be one of the contributions to the chemical treasure of natural compounds.

The objectives of the thesis :

Research on the chemical composition and evaluation of some *in vivo* biological activities of extracts and isolated compounds of *Atractylodes macrocephala* Koidz. of the Asteraceae family collected in Vietnam. Compare some changes between *Atractylodes macrocephala* samples and *Atractylodes macrocephala* samples processed according to traditional medicine methods using the HPLC-DAD method.

The main contents of the thesis:

- Isolation and structural determination of compounds from *Atractylodes macrocephala* Koidz.
- Evaluation of biological activity of extracts and compounds isolated from *Atractylodes macrocephala* Koidz.
- Applying metabolomics method to evaluate the changes in some chemical components in *Atractylodes macrocephala* Koidz. processed by traditional medicine methods

CHAPTER 1. OVERVIEW**1.1. Overview of *Atractylodes macrocephala* Koidz.****1.1.1. Botany of *Atractylodes* species**

Atractylodes has the scientific name *Atractylodes macrocephala* Koidz. belongs to the genus *Atractylodes*, order Asterales, family Asteraceae, class Eudicots, phylum Angiospermatophyta. The tree originates from China. Currently, *Atractylodes macrocephala* Koidz. is grown in many mountainous provinces in the North of our country.

Plant description: the plant prefers cool, dry climates and grows rapidly at temperatures of 22-28°C. The stem is straight, solitary or branched at the top, the lower part is woody, average height is about 30-80 cm. The leaves grow alternately, the lower part of the stem is long, the upper part is short, the base of the leaf is wide. The leaf blades are often deeply divided into 3 lobes and have serrated teeth. Flowers grow and cluster in clusters at the top of the stem. The corolla is reddish purple in color and the pistil is a long thread extending outward. The corolla is tubular, the upper part is purple red, the lower part is white. *Atractylodes macrocephala* fruit is oblong, flat and gray in color. The root acts as the main medicinal ingredient and is used as medicine.

1.1.2. Application of *Atractylodes* species in traditional medicine

In addition to regulating the digestive system, immune system, and urinary system, it also has many pharmacological effects such as

anti-cancer, anti-inflammatory, anti-aging, antioxidant, osteoporosis treatment, neuroprotection and immune regulation. The roots are used in more than 835 preparations and are an integral part of more than 4340 classical prescriptions for the treatment of chronic diseases in traditional Chinese medicine. It is also known as “Baizhu” in Chinese, recorded in the Shennong medicinal herb in the Eastern Han Dynasty, and later described in other traditional Chinese medicine books. Besides, *Atractylodes* is also a part of the traditional Chinese medicine Fangji Huangqi Tang (FHT) for the treatment of chronic glomerulonephritis, cardiac edema and rheumatoid arthritis that has been clinically tested in China.

In Japan, *Atractylodes macrocephala* is used as a diuretic and to treat some ulcers and gastric mucosal bleeding. Today, Japanese people use *Atractylodes macrocephala* to enhance digestion, diuretic, treat body pain, cough, excessive phlegm, nausea, wet dreams, and dysentery.

In traditional Korean medicine, *Atractylodes* is often used clinically to treat gastrointestinal diseases, abdominal pain, diarrhea, edema, obesity and excessive sweating. It is one of the main ingredients of traditional Korean medicines such as Soamsan and Boyangwhanotang, which have long been used for cancer prevention and chronic disease prevention.

In Vietnam, *Atractylodes* is one of the herbs in traditional herbal medicine used to treat acute and chronic gastritis in oral form, treat rheumatism, measles, itching, diarrhea, and long-term dysentery not cured. *Atractylodes macrocephala* combined with other herbs is effective in treating some liver diseases and helping to ease pregnancy.

1.2. The status of study on *Atractylodes* species

1.2.1. The status of study on chemical composition of *Atractylodes* species

There have been 79 compounds isolated from the *Atractylodes macrocephala* root with an extremely rich chemical composition with many classes of substances such as benzoquinone, coumarin,

flavonoids, flavonoid glycosides, phenylpropanoids, polyacetylene, polysaccharides, sesquiterpenoids, steroids, triterpenoids and many other compounds other substances. Among them, sesquiterpenoids, polyacetylene and polysaccharides are the main biologically active components of *Atractylodes macrocephala*.

1.2.3. Some domestic research on *Atractylodes macrocephala* species

In Vietnam, so far there have been only a few research projects on essential oils and pharmacological effects of *Atractylodes macrocephala* extracts. There is no published research on the process of quantitative analysis of the chemical composition of *Atractylodes macrocephala* roots.

1.3. Some studies on the chemical composition and bioactivities of *Atractylodes macrocephala* roots processed according to traditional medicine methods.

Research projects on *Atractylodes macrocephala* roots processed by traditional medicine methods mainly focus on studying the changes in substance content between *Atractylodes macrocephala* and *Atractylodes macrocephala* after processing by traditional medicine methods transmitted like stars with bran honey, stars with loess. Studies show that after treatment, the properties and uses of *Atractylodes macrocephala* increase and the toxicity and side effects may decrease.

CHAPTER 2. RESEARCH SUBJECTS AND METHODS

2.1. Subjects

Samples of *Atractylodes macrocephala* Koidz. were collected in Quyet Tien commune, Quan Ba district, Ha Giang province at the end of May 2021. The scientific name was determined by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. Ha Giang 06 specimen is kept at the Center for high technology research and development.

2.2. Methods

2.2.1. Processing method of *Atractylodes macrocephala* root samples according to traditional medicine

Traditional medicine processing methods are carried out according to the Guidelines of the Ministry of Health of Vietnam on traditional medicine processing methods (2017).

2.2.2. Methods for isolation

Compound isolation method: Combination of chromatography methods including thin layer chromatography (TLC), column chromatography (CC)

2.2.3. Methods for determining the chemical structure

Using modern spectroscopic methods including: mass spectrometry (ESI-MS), high resolution mass spectrometry (HR-ESI-MS), 1-dimensional and 2-dimensional nuclear magnetic resonance (NMR) spectroscopy, polar rotation ($[\alpha]_D$)

2.2.4. Method to evaluate biological activity

- DPPH free radical scavenging activity was conducted according to the method of Williams et al.
- Anti-inflammatory activity was evaluated through the inhibition of NO production on RAW264.7 cells stimulated by LPS
- Cancer cytotoxic activity was determined by the MTT method

2.2.5. Analytical and quantitative methods

The content of compounds was determined by high performance liquid chromatography (HPLC). Data collection was handled by Microsoft Excel 2016 (Microsoft Corporation, USA). Standardized and multivariate statistics were conducted in R statistical software (R Foundation for Statistical Computing, Vienna, Austria).

2.2.6. Qualitative analysis method

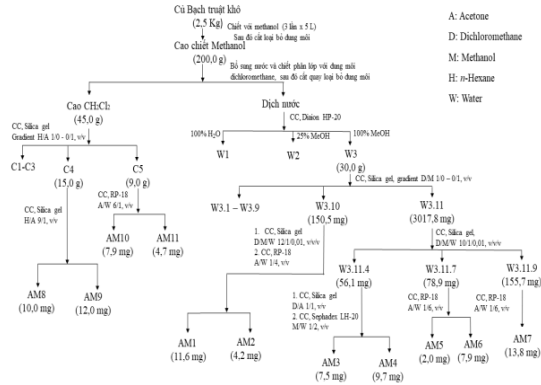
The compounds isolated from *Atractylodes macrocephala* roots was determined by HPLC method on HPLC-DAD Agilent 1100 instrument.

CHAPTER 3: EXPERIMENT AND RESULTS

3.1. Isolation of compounds

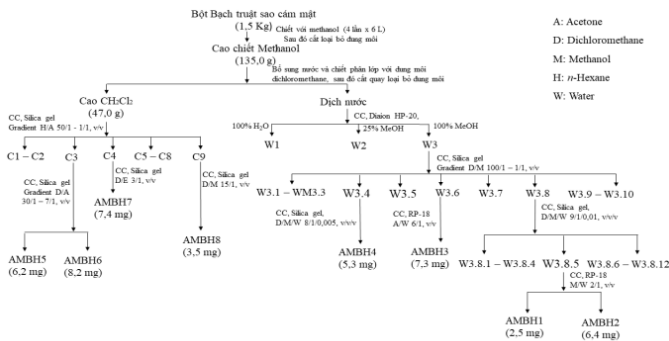
3.1.1. Isolation of compounds from *Atractylodes macrocephala*

From *Atractylodes macrocephala* species, 11 compounds were isolated using chromatographic methods according to the following diagram:



3.1.2. Isolation of compounds from *Atractylodes macrocephala* bran honey

From *Atractylodes macrocephala* species bran honey, 08 compounds were isolated using chromatographic methods according to the following diagram:



3.2. Physical and spectroscopic data of compounds

13 compounds isolated from *Atractylodes macrocephala* species are presented with physical parameters such as existing forms,

color, specific rotation, high resolution mass spectrometry (HR-ESI-MS), molecular formula, molecular mass (M)

3.3. Activity of the isolated compound

3.3.1. Results of DPPH free radical scavenging activity

The results of DPPH free radical scavenging activity test are shown in the following two tables:

Table 3.1. Test results of DPPH free radical scavenging activity of compounds isolated from Atractylodes macrocephala roots and Atractylodes macrocephala bran molasses

STT	Tên mẫu	IC ₅₀ (μM)	STT	Tên mẫu	IC ₅₀ (μM)
1	AM1	>100,00	8	AM8	>100
2	AM2	>100,00	9	AM9	>100
3	AM3	>100,00	10	AM10	>100
4	AM4	32,63 ± 2,78	11	AM11	17,64 ± 3,08
5	AM5	85,17 ± 2,56	12	AMBH1	74,41 ± 2,13
6	AM6	45,54 ± 3,82	13	AMBH5	56,76 ± 2,05
7	AM7	>100,00	ĐC	Ascorbic acid *	55,64 ± 4,37

Table 3.2. Test results of DPPH free radical scavenging activity of extracts from Atractylodes macrocephala roots processed by traditional methods

Tên mẫu	IC ₅₀ (μg/mL)
MeOH extract of RA	>150
MeOH extract of AL	115,74 ± 3,67
MeOH extract of BH	>150
MeOH extract of SO	101,43 ± 2,44
Ascorbic acid *	15,47 ± 1,78

* positive control

3.3.2. Results of NO production inhibitory activity

Anti-inflammatory activity was evaluated through the ability to inhibit NO production on the RAW264.7 cell line shown in the following two tables:

Table 3.3. Results of testing the NO production inhibitory activity of compounds isolated from Atractylodes macrocephala and Atractylodes macrocephala bran honey

Tên chất	IC ₅₀ (μM) [#]	Tên chất	IC ₅₀ (μM) [#]
AM1	43,52 ± 4,08	AM8	78,48 ± 6,47
AM2	60,13 ± 5,78	AM9	48,94 ± 3,41
AM3	32,74 ± 3,22	AM10	86,14 ± 5,83
AM4	46,38 ± 4,47	AM11	42,71 ± 4,35
AM5	52,12 ± 4,85	AMBH1	>100
AM6	76,18 ± 5,75	AMBH5	>100
AM7	51,96 ± 5,81	Cardamonin *	2,14 ± 0,14

Table 3.4. Results of testing the NO production inhibition activity of extract samples from Atractylodes macrocephala roots processed by traditional methods

Tên mẫu	IC ₅₀ (μg/mL) [#]
MeOH extract of RA	> 500
MeOH extract of AL	377,16 ± 26,8
MeOH extract of BH	> 500
MeOH extract of SO	394,9 ± 44,3
* Cardamonin	0,78 ± 0,11

**positive control*

% cell survival was above 85% at concentrations of 25 μM and 100 μM for all samples

3.3.3. Results of cancer cell cytotoxic activity

Results of evaluating in vitro cytotoxicity on two human cancer cell lines including lung cancer (A549) and leukemia (K562) are shown in the following table:

Table 3.5. Results of testing the cytotoxic activity of compounds isolated from *Atractylodes macrocephala* roots and *Atractylodes macrocephala* roots bran honey

Tên chất	IC ₅₀ (μM)		Tên chất	IC ₅₀ (μM)	
	A549	A549		A549	K562
AM1	67,6 ± 8,42	77,4 ± 5,59	AM8	28,56 ± 1,27	51,76 ± 3,45
AM2	54,3 ± 4,31	>100	AM9	>100	78,5 ± 4,29
AM3	62,5 ± 7,91	>100	AM10	>100	>100
AM4	>100	>100	AM11	>100	>100
AM5	>100	>100	AMBH1	>100	>100
AM6	>100	>100	AMBH5	>100	>100
AM7	>100	84,7 ± 6,13	Camptothecin*	0,76 ± 0,09	0,98 ± 0,11

Table 3.6. Results of cytotoxic activity of extracts from *Atractylodes macrocephala* roots processed by traditional methods

STT	Tên mẫu	IC ₅₀ (μg/mL)	
		A549	K562
1	MeOH extract of RA	78,97 ± 6,45	>100
2	MeOH extract of AL	64,57 ± 5,75	80,21 ± 7,16
3	MeOH extract of BH	>100	>100
4	MeOH extract of SO	77,45 ± 9,18	>100
	Camptothecin *	0,26 ± 0,03	0,34 ± 0,04

* positive control

Atractylodes macrocephala (RA), *Atractylodes macrocephala* soaked in alcohol (AL), *Atractylodes macrocephala* bran honey (BH), *Atractylodes macrocephala* loess (SO)

CHAPTER 4: DISCUSSIONS

4.1. Determine the structure of the isolated compounds

4.1.1. Compounds isolated from *Atractylodes macrocephala* root samples

Compound **AM1**: eudesma-4(15),7-diene-3 α ,9 β ,11-triol

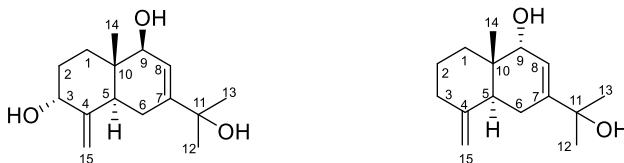


Figure 4.1. Chemical structure of compound **AM1** and reference compound

Compound **AM1** was isolated as a colorless solid. From the HR-ESI-MS high-resolution mass spectrum of compound **AM1**, a pseudomolecular ion peak was obtained at m/z 251.1645 $[M-H]^-$ (theoretical calculation for the formula $[C_{15}H_{23}O_3]$, 251,1647), combined with ^{13}C NMR spectral data allows determining the molecular formula of compound **AM1** as $C_{15}H_{24}O_3$. The 1H NMR spectrum of compound **AM1** appears two exomethylene signals at δ_H 5,08 (1H, t, $J = 1,5$ Hz, Ha-15) and δ_H 4,80 (1H, t, $J = 1,5$ Hz, Hb-15) and an olefinic proton at δ_H 5,55 (1H, d, $J = 1,5$ Hz, H-8), the characteristic signal of the eudesmane framework. In addition, the 1H NMR spectrum of compound **AM1** also shows two oxymethine protons at δ_H 4,28 (1H, br s, H-3) and δ_H 4,04 (1H, d, $J = 2,0$ Hz, H-9) along with three methyl groups at δ_H 0,69 (3H, s, H-14), 1,34 (3H, s, H-13), 1,35 (3H, s, H-12).

The ^{13}C NMR spectrum and HSQC spectrum of **AM1** have the appearance of 15 carbon signals, including 4 carbons not bonded to hydrogen (at δ_C 152,1, 145,5, 40,4 and 72,9), three groups methyl (at δ_C 29,1, 29,0 and 11,0), four methylene groups (at δ_C 32,4, 30,6, 25,4 and 110,8), four methine groups (at δ_C 74,0, 40,1, 123,3 and 79,2). The

NMR spectrum data of compound **AMI** shows similarities with the data of compound eudesma-4(15),7-diene-9 α ,11-diol (Table 4.1), a sesquiterpene compound with the eudesmane frame form isolated from *Atractylodes lancea* in previous studies

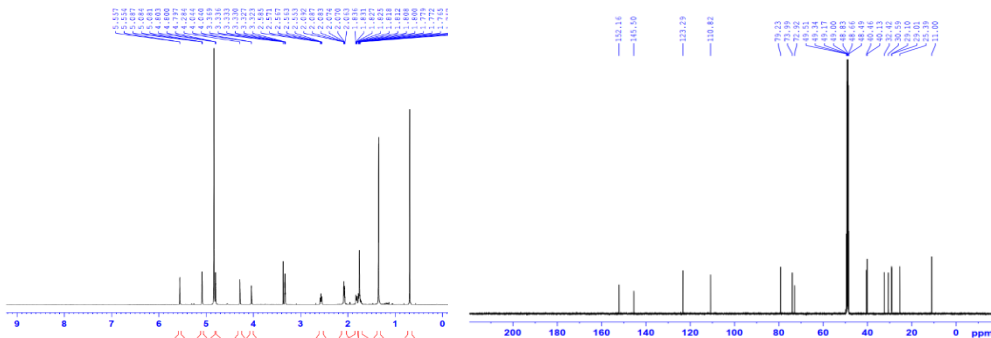


Figure 4.2. ^1H NMR and ^{13}C NMR spectrum of compound **AMI**

Table 4.1. NMR spectrum of compound **AMI** and reference compound

C	# δ_{C}	$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}^{\text{a,c}}$ (multi., $J = \text{Hz}$)
1	78,9	32,4	1,78 m
2	31,5	30,6	1,83 m/1,77 m
3	34,2	74,0	4,28 s
4	148,7	152,1	-
5	41,9	40,1	2,56 dd (9,0, 2,0)
6	28,8	25,4	2,08 m
7	75,6	145,5	-
8	26,3	123,3	5,55 d (1,5)
9	32,1	79,2	4,04 d (2,0)
10	39,8	40,4	-
11	75,5	72,9	-
12	24,8	29,1	1,35 s
13	24,8	29,0	1,34 s
14	9,1	11,0	0,69 s
15	106,7	110,8	5,08 (t, 1,5, H _a -15) 4,80 (t, 1,5, H _b -15)

^aCD₃OD, ^b125 MHz, ^c500 MHz, # δ_{C} : data of 4(15)-eudesmene-1 β ,7,11-triol in CDCl₃, 100 MHz.

The ^1H - ^1H COSY spectrum of compound **AM1** shows the interaction of protons attached to two adjacent carbons of H-1 (δ_{H} 1,78)/ H-2 α (δ_{H} 1,83), H-2 β (δ_{H} 1, 77)/ H-3 (δ_{H} 4,28), H-5 (δ_{H} 2,56)/ H-6 (δ_{H} 2,08) and H-8 (δ_{H} 5,55)/ H-9 (δ_{H} 4,04). From the analysis of the above spectroscopic data, it was confirmed that this compound is a eudesmane-type sesquiterpene. HMBC spectrum of compound **AM1** shows interactions of H-15 α (δ_{H} 5,08) and H-15 β (δ_{H} 4,80) with C-3 (δ_{C} 74,0)/C-4 (δ_{C} 152,1) /C-5 (δ_{C} 40,1), and of H-12 (δ_{H} 1,35) together with H-13 (δ_{H} 1,34) with C-7 (δ_{C} 145,5), C-11 (δ_{C} 72,9) demonstrated the binding of exomethylene (CH₂-15) and propan-2-ol-2-yl groups at C -4 and C-7 respectively. Besides, the interaction of H-14 (δ_{H} 0,69) with C-1 (δ_{C} 32,4)/C-5 (δ_{C} 40,1)/C-9 (δ_{C} 79,2) proves that the methyl (CH₃-14) bonded at C-10. NMR spectrum data of compound **AM1** has one more hydroxyl group (at position C-3) than the published compound eudesm-4(15),7-diene-9 α ,11-diol.

The very small J constant value at H-3 (br s) characterizes the equatorial-axial and equatorial-equatorial proton interactions of H-2 with H-3. Besides, the constant J of H-5 (dd, $J = 9,0, 2,0$ Hz) proves the interaction of protons at the *axial-axial* and *axial-equatorial* positions of H-5 and H-6. In addition, on the NOESY spectrum, an interaction between H-5 (δ_{H} 2,56) with H-9 (δ_{H} 4,04) appears but no interaction between H-5 and H-14 appears, showing that the trans configuration of eudesmane frame. The resonance signal of H-14 at (δ_{H} 0,69) shows that the methyl group of C-14 has a β configuration similar to eudesmane sesquiterpenes compounds from species of the genus *Atractylodes*. From the analysis of the above spectral data and comparison with reference materials of the similar published compound 4(15)-eudesmene-1 β ,7,11-triol, it can be determined that compound **AM1** is a new substance, named eudesma-4(15),7-diene-3 α ,9 β ,11-triol.

From *Atractylodes macrocephala*, 11 compounds have been isolated, including 2 new compounds: **AM1** (eudesma-4(15),7-diene-3 α ,9 β ,11-triol) and **AM2** (eudesma-4(15).),7-diene-1 β ,3 α ,9 β ,11-

tetraol) has the same eudesmane frame. The known compounds **AM3-AM11** are compounds belonging to different framework groups.

4.1.2. Determining the structure of compounds isolated from samples of *Atractylodes macrocephala* bran honey

Compound **AMBH1**: methyl (4*R**, 5*S**)-2,5-dihydroxy-3,5-dimethyl-4-oxocyclopent-2-ene-1-carboxylate



AMBH1 (4*S*,5*S*)-2,4,5-trihydroxy-3-methoxy-4-methoxycarbonyl-5-methyl-2-cyclopenten-1-one

Figure 4.3. Chemical structure of compound AMBH1 and reference compound

Compound **AMBH1** is obtained in oily, yellow form. The molecular formula $C_9H_{12}O_5$ was determined based on HR-ESI-MS high-resolution mass spectrometry data with a pseudomolecular ion fragment at m/z 223,0578 $[M+Na]^+$, corresponding to the calculated mass number, theoretical 223,0582 of the formula $C_9H_{12}O_5Na$.

On the 1H NMR spectrum of **AMBH1**, signals of two methyl groups appear at δ_H 1,39 (3H, s, 5- CH_3) and δ_H 1,92 (3H, br s, 3- CH_3), a methoxy group with δ_H at 3,65 (3H, s, 4'- OCH_3) and a signal of a methine proton at δ_H 3,56 (1H, s, H-4) and an OH group signal at δ_H 4,48 (1H, s, 5-OH). Combining the ^{13}C NMR spectrum and HSQC spectrum of **AMBH1** shows the presence of signals of 9 carbon atoms, including the appearance of a carbonyl group δ_C at 201,1 (C-1), a carboxyl group δ_C at 170,9 (C-4'), two methyl carbon atoms at δ_C 13,0 (3- CH_3), 25,8 (5- CH_3), one methine signal at δ_C 59,3 (C-4), one signal of methoxy carbon δ_C at 51,9 (4'- OCH_3), two olefine carbon atoms at δ_C 138,5 (C-2) and 149,5 (C-3).

Table 4.2. NMR spectrum data of compound **AMBH1**
and reference compounds

C	[#] δ _C ^{a,b}	δ _C ^{a,b}	δ _H ^{a,c} multi. (J = Hz)
1	197,95	201,1	-
2	132,96	138,5	-
3	157,68	149,5	-
4	81,97	-	3,56, s
5	78,15	-	-
3-CH ₃	-	13,0	1,92, d (1,2)
5-CH ₃	22,2	25,7	1,38 s
4'	171,13	170,9	-
4'-OCH ₃	52,06	51,9	3,65 s

^aAcetone-d₆, ^b150 MHz, ^c600 MHz, [#]δ_C: data of (4*S*,5*S*)-2,4,5-trihydroxy-3-methoxy-4-methoxycarbonyl-5-methyl-2-cyclopenten-1-one in (CD₃)₂SO

From *Atractylodes macrocephala* bran honey, the structures of 8 compounds have been determined, including 1 new compound **AMBH1** (methyl (4*R**, 5*S**)-2,5-dihydroxy-3,5-dimethyl-4-oxocyclopent-2-ene-1-carboxylate). **AMBH2** - **AMBH8** which 6 compounds are similar to compounds isolated from *Atractylodes macrocephala* root and another compound is **AMBH5**

4.2. Evaluation of biological activity of isolated compounds

4.2.1. DPPH free radical scavenging activity

Compounds **AM4-AM6**, **AM11**, **AMBH1**, **AMBH5** show DPPH antioxidant activity with IC₅₀ values in the range of 17,64 – 85,17 μM, in which compound **AM11** shows the best activity with IC₅₀ values, equal to 17,64 ± 3,08 μM compared to the positive control. The remaining compounds show weak to no activity. Methanol extract samples of *Atractylodes macrocephala* processed by traditional medicine methods were tested for either weak activity (**AL** and **SO**) or no activity (**RA** and **BH**).

4.2.2. NO production inhibitory activity

After the compounds were tested at different concentrations to determine the IC₅₀ value. The results showed that all compounds showed weak ability to inhibit NO production on RAW264.7 cells

with IC₅₀ values in the range of 32,74 – 86,14 µM, only two compounds **AMBH1** and **AMBH5** did not show any effect. this activity. The total extract samples showed weak activity (**AL** and **SO**) and no activity (**RA** and **BH**).

4.2.3. Cancer cell toxicity

Compounds **AM1-AM3**, **AM8-AM10** all show moderate cytotoxic activity against lung cancer lines with IC₅₀ values in the range of 28,56 – 67,6 µM and demonstrate the ability to cause toxicity. Weak on leukemia lines with IC₅₀ value in the range of 51,76 – 84,7 µM. MeOH extract samples of *Atractylodes macrocephala* processed by traditional medicine methods all showed weak to inactive toxicity on both tested cancer cell lines.

4.3. Analyze and quantify the composition of *Atractylodes macrocephala* processed according to traditional medicine methods

4.3.1. Optimize analytical conditions

Analytical results on the HPLC-DAD system with optimized analytical conditions are presented on a chromatogram suitable for simultaneous analysis of 6 reference substances: **AM4**, **AM5**, **AM8**, **AM9**, **AM10**, **AM11**.

4.3.2. Set up standard curve, limit of detection (LOD) and limit of quantification (LOQ)

The values are presented in the table below:

Table 4.3. Standard curve equation, limit of detection (LOD) and limit of quantification (LOQ) for both reference substances

Reference substance	Equation standard	Correlation coefficient R ²	LOD (µg/mL)	LOQ (µg/mL)
AM4	y = 6,4907x – 13,9640	0,9996	0,2442	0,7399
AM5	y = 5,8912x – 5,8315	0,9997	0,3270	0,9909
AM8	y = 30,5416x – 30,7566	0,9995	0,0628	0,1902
AM9	y = 27,8722x – 30,7026	0,9997	0,1541	0,4671
AM10	y = 12,1393x – 9,5048	0,9996	0,5355	1,6227
AM11	y = 1,1595x – 1,3971	0,9990	0,8570	2,5969

All methods have correlation coefficients $R^2 > 0,999$ and LOD (range from 0,1 to 0,9 $\mu\text{g/mL}$) and LOQ (range from 0,2 to 2,6 $\mu\text{g/mL}$) values are very low. low, ensuring linearity and sensitivity for quantification.

4.3.3. Precision and accuracy

The precision (CV%) during the day and over 3 consecutive days ranged from 0,6 to 7,8%. The highest precision for compound **AM11** at a concentration of 5 $\mu\text{g/mL}$ was 4,6% and 7,8%, respectively. The accuracy of the distributions is between 96,0% and 104,8% with less than 6,0%. From the results table, it can be seen that the developed quantitative method has precision and accuracy within the FDA's allowable limits, ensuring correct assessment of the content of reference substances in test samples.

Table 4.4. Intra-day and inter-day precision and accuracy of reference substances

Name	Concentration ($\mu\text{g/mL}$)	Precision (n=6)		Accuracy (n=6)	
		(CV%)		(%Recovery \pm error number)	
		Intra-day	Inter-day	Intra-day	Inter-day
AM4	5	3,9	3,6	100,4 \pm 1,6	101,0 \pm 2,6
	40	2,0	3,5	99,7 \pm 0,6	99,6 \pm 1,2
	100	0,8	1,1	99,5 \pm 0,6	99,5 \pm 0,6
AM5	5	2,7	4,2	104,8 \pm 5,4	101,7 \pm 4,8
	40	2,0	1,9	98,5 \pm 3,1	100,3 \pm 3,1
	100	0,4	2,0	98,8 \pm 2,1	98,5 \pm 2,1
AM8	5	2,0	4,4	99,4 \pm 2,3	98,7 \pm 4,5
	40	1,6	2,3	99,1 \pm 1,4	99,6 \pm 3,3
	100	0,6	0,8	98,5 \pm 1,1	99,0 \pm 1,5
AM9	5	2,5	3,0	98,2 \pm 2,6	97,9 \pm 3,2
	40	2,5	2,3	98,1 \pm 1,7	97,5 \pm 2,9
	100	1,1	1,2	98,9 \pm 1,2	99,2 \pm 1,5
AM10	5	3,1	3,3	97,9 \pm 4,1	99,4 \pm 3,5
	40	2,2	1,9	98,6 \pm 1,7	97,0 \pm 3,4
	100	1,0	0,8	98,6 \pm 0,9	99,5 \pm 1,2

Name	Concentration (µg/mL)	Precision (n=6)		Accuracy (n=6)	
		(CV%)		(%Recovery ± error number)	
		Intra-day	Inter-day	Intra-day	Inter-day
AM11	5	4,6	4,9	96,0 ± 6,0	97,7 ± 5,7
	40	3,3	3,4	97,4 ± 1,9	98,4 ± 2,0
	100	3,6	3,2	98,2 ± 1,7	99,6 ± 2,2

4.3.4. Quantitative results and multivariate statistics

The content of **AM4**, **AM5** and **AM11** fluctuates between 7,8 µg/g and 88,6 µg/g, while the content of sesquiterpenes (**AM8**, **AM9**, **AM10**) fluctuates in a larger value range, from 80,7 µg/g to 588,5 µg/g.

Table 4.5. Content of 6 reference substances in *Atractylodes macrocephala* samples

Processing method	Name	AM5	AM11	AM4	AM10	AM9	AM8
AM	RA 1	8,5 ± 0,7	28,0 ± 2,8	16,3 ± 1,8	184,9 ± 18,1	118,7 ± 11,2	97,5 ± 11,6
	RA 2	11,0 ± 1,1	43,1 ± 3,0	18,6 ± 2,2	196,5 ± 15,1	132,6 ± 15,6	110,3 ± 11,8
	RA 3	8,3 ± 0,6	42,6 ± 3,5	12,6 ± 1,3	220,5 ± 26,5	139,1 ± 15,5	121,8 ± 13,5
	RA 4	11,6 ± 1,3	31,8 ± 3,6	14,9 ± 1,6	211,4 ± 25,1	167,3 ± 13,2	126,5 ± 9,9
	RA 5	7,8 ± 0,9	23,3 ± 2,5	13,2 ± 1,6	156,9 ± 18,1	112,3 ± 12,7	80,7 ± 7,2
	RA 6	10,3 ± 1,3	38,6 ± 3,9	24,7 ± 3,0	163,9 ± 13,8	128,5 ± 13,8	97,4 ± 9,0
AM soaked in alcohol	AL 1	35,0 ± 3,9	54,9 ± 6,1	20,5 ± 1,9	297,0 ± 22,2	240,2 ± 20,8	197,8 ± 14,9
	AL 2	62,7 ± 6,2	69,2 ± 7,3	36,4 ± 3,7	312,4 ± 22,2	221,1 ± 21,8	209,0 ± 19,3
	AL 3	53,6 ± 4,9	61,6 ± 5,5	32,8 ± 2,7	262,4 ± 23,6	149,2 ± 14,1	204,4 ± 25,4
	AL 4	40,6 ±	53,2 ±	25,9 ±	313,5 ±	191,5 ±	341,8 ±

		4,2	6,4	3,2	27,6	20,1	35,1
	AL 5	36,0 ± 4,1	87,2 ± 8,7	30,0 ± 2,7	261,2 ± 26,9	251,5 ± 27,9	298,3 ± 21,9
	AL 6	55,9 ± 4,1	59,5 ± 5,8	37,0 ± 2,9	230,5 ± 25,7	190,4 ± 17,7	185,4 ± 16,7
	AL 7	41,2 ± 4,7	55,8 ± 6,4	20,4 ± 1,7	262,0 ± 32,1	274,8 ± 26,8	248,3 ± 18,8
	AL 8	34,1 ± 4,1	66,7 ± 5,0	34,1 ± 3,8	311,7 ± 33,3	227,0 ± 26,0	198,8 ± 20,7
	AL 9	40,7 ± 4,7	37,6 ± 3,8	39,4 ± 3,1	319,2 ± 24,9	191,9 ± 17,8	209,7 ± 19,0
AM loess	SO 1	35,5 ± 3,5	80,5 ± 6,1	39,3 ± 3,5	489,7 ± 35,1	274,5 ± 26,0	185,4 ± 20,0
	SO 2	41,8 ± 4,2	70,7 ± 7,1	35,1 ± 2,6	495,7 ± 49,4	246,0 ± 21,3	150,4 ± 15,6
	SO 3	40,4 ± 4,0	74,5 ± 8,0	36,7 ± 3,0	545,6 ± 45,1	220,4 ± 26,2	121,4 ± 14,5
	SO 4	42,1 ± 5,1	88,7 ± 7,1	41,7 ± 3,2	395,1 ± 45,9	202,6 ± 23,4	149,3 ± 16,3
	SO 5	33,4 ± 2,6	79,5 ± 7,6	29,9 ± 2,2	388,5 ± 32,6	218,2 ± 22,1	124,4 ± 14,5
	SO 6	47,5 ± 3,4	58,5 ± 5,2	24,3 ± 1,8	588,5 ± 60,7	253,7 ± 22,1	182,1 ± 18,2
	SO 7	37,5 ± 3,2	80,5 ± 8,7	37,6 ± 2,8	369,0 ± 39,2	272,9 ± 27,4	219,2 ± 18,8
AM bran honey	BH 1	30,5 ± 2,8	31,6 ± 3,2	31,6 ± 3,1	361,9 ± 45,0	184,3 ± 19,6	184,3 ± 18,4
	BH 2	27,0 ± 3,1	38,8 ± 4,4	35,8 ± 3,9	351,8 ± 41,0	167,4 ± 17,4	150,0 ± 13,4
	BH 3	32,7 ± 3,3	29,7 ± 2,8	29,7 ± 2,4	303,8 ± 37,2	142,7 ± 16,8	135,7 ± 10,1
	BH 4	23,6 ± 2,5	39,5 ± 2,9	39,5 ± 3,3	244,4 ± 23,2	155,1 ± 13,2	143,3 ± 10,4
	BH 5	25,1 ± 2,2	28,6 ± 2,4	28,6 ± 2,9	358,3 ± 32,4	123,6 ± 14,9	119,2 ± 11,5
	BH 6	17,5 ± 1,6	25,2 ± 2,1	25,2 ± 2,9	325,2 ± 25,4	179,5 ± 21,9	170,6 ± 15,2
	BH 7	25,3 ± 2,0	25,0 ± 2,9	25,0 ± 2,4	268,1 ± 29,5	128,3 ± 16,0	110,9 ± 8,7

	BH 8	21,0 ± 2,5	38,1 ± 4,8	38,1 ± 3,4	365,4 ± 33,0	196,5 ± 21,0	165,1 ± 16,8
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The HCA tree diagram shows that *Atractylodes macrocephala* can be divided into four separate groups corresponding to four processing processes. The only exception is sample **AL9**, which instead of being in the **AL** group, is found in the **BH** sample group.

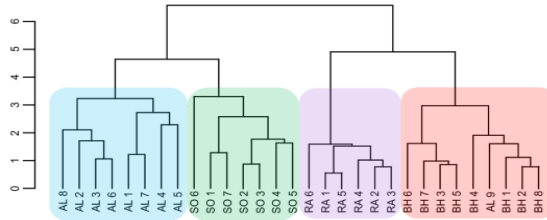


Figure 4.5. Tree diagram of *Atractylodes macrocephala* based on quantified metabolite content.

From figure 4.6, it shows that the total percentage of variance explained on PC1 and PC2 is nearly 80%, so these two PCs can be used to represent and analyze the entire data.

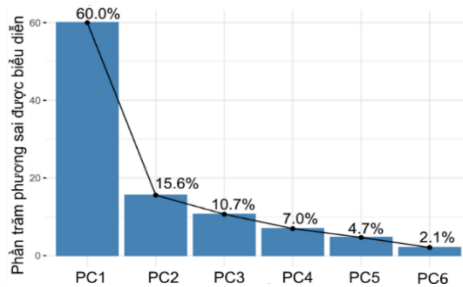


Figure 4.6. Variance drop plot of principal components

From figure 4.7 below, it shows that the **BH** group is located in quadrant IV, opposite the position of quadrant II, of the **AL** group and the representative vector is **AM8** (F). Therefore, **AM8** (F) is considered simultaneously as a positive indicator and a negative indicator of the **AL** and **BH** sample groups.



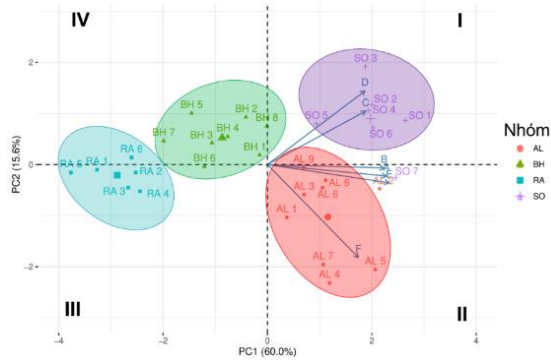


Figure 4.7. Double PCA plot of *Atractylodes macrocephala* with reference substances

The chart figure 4.8 shows that **AM4**, **AM5**, **AM11** can be used as markers to distinguish samples of *Atractylodes macrocephala* (**RA**), loess (**SO**), *Atractylodes macrocephala* soaked in alcohol (**AL**), bran honey (**BH**)

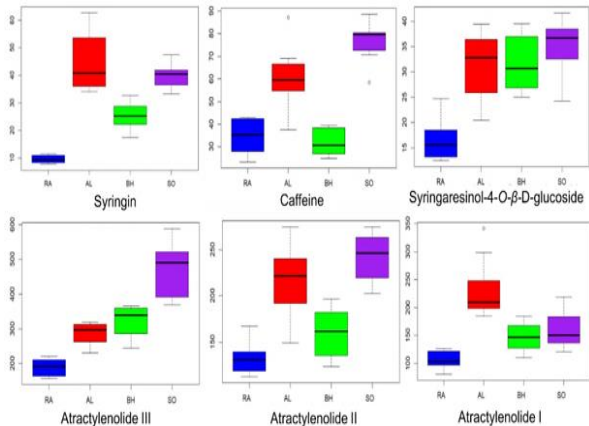


Figure 4.8. Box plot of reference substance content in *Atractylodes macrocephala* sample groups

CONCLUSIONS

Research on chemical composition

- From *Atractylodes macrocephala* root, 11 compounds (**AM1-AM11**) have been isolated and their structures determined. Among them, two new compounds **AM1** (eudesma-4(15),7-diene-3 α ,9 β ,11-triol), **AM2** (eudesma-4(15),7-diene-1 β ,3 α ,9 β ,11-tetraol) and 9 known compounds (**AM3-AM11**).

- From *Atractylodes macrocephala* root processed according to traditional medicine methods, bran honey has been isolated and identified 8 compounds. Among them, two other compounds compared to *Atractylodes macrocephala* root are **AMBH1** (methyl (4R*, 5S*)-2,5-dihydroxy-3,5-dimethyl-4-oxocyclopent-2-ene-1-carboxylate) (new substance), **AMBH5** (methyl (E)-4-[5-(hydroxymethyl) furan-2-yl]but-3-en-2-one) and 6 compounds similar to those isolated from the Bach sample. avulsion (**AM4, AM5, AM8-AM11**)

Research results on biological activity

- DPPH free radical scavenging activity: **AM11** shows the strongest radical scavenging activity with an IC₅₀ value of 17,64 ± 3,08 μ M. The remaining compounds showed moderate antioxidant activity or no activity.

- Anti-inflammatory activity was evaluated through the ability to inhibit NO production in RAW264.7 macrophages stimulated by LPS: all compounds had weak activity with IC₅₀ values in the range of 32,74 - 86,14 μ M. Particularly, two compounds **AMBH1** and **AMBH5** do not show this activity.

- Cancer cytotoxic activity on lung cancer cell line (A549) and leukemia cell line (K562): **AM8** exhibits moderate cytotoxic activity with IC₅₀ value = 28,56 ± 1,27 μ M on A549 cell line. The remaining compounds showed weak to no activity on these two cancer cell lines.

In all three of the above activity testing methods, MeOH extract samples of *Atractylodes macrocephala* root processed by traditional medicine methods all showed weak to no activity.

Research results on analysis and quantification of compounds in *Atractylodes macrocephala* processed according to traditional medicine

- Developed a method to simultaneously quantitatively analyze 6 active ingredients in *Atractylodes macrocephala* using HPLC-DAD technique.
- Distinguished and identified a number of indicators for *Atractylodes macrocephala* processed according to traditional medicine methods: **AM4, AM5, AM11** can be used as markers to distinguish *Atractylodes macrocephala* (**RA**), *Atractylodes macrocephala* soaked in alcohol (**AL**), *Atractylodes macrocephala* bran honey (**BH**), *Atractylodes macrocephala* loess (**SO**)

NEW CONTRIBUTIONS OF THE THESIS

- Isolate and identify 2 new compounds from *Atractylodes macrocephala*: **AM1** (eudesma-4(15),7-diene-3 α ,9 β ,11-triol) and **AM2** (eudesma-4(15),7- diene-1 β ,3 α ,9 β ,11-tetraol)
- Isolate and identify a new compound from *Atractylodes macrocephala* bran honey according to traditional medicine method: **AMBH1** (methyl (4*R**, 5*S**)-2,5-dihydroxy-3,5-dimethyl-4-oxocyclopent-2-ene-1-carboxylate)
- This is the first study on the transformation of the compounds syringaresinol-4'-O- β -D-glucoside (**AM4**), syringin (**AM5**), atractylenolide I (**AM8**), atractylenolide II (**AM9**), atractylenolide III (**AM10**), caffeine (**AM11**) due to commonly used medicinal processing methods (honey bran, wine soaking, ocher) are combined with multivariate statistical analysis to distinguish and identify chemical components. learn characteristics of sample groups.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. Cao Thanh Hai, Nguyen Thi Luyen, Do Hoang Giang, Bui Quang Minh, Nguyen Quang Trung, Pham The Chinh, Dang Viet Hau, Nguyen Tien Dat, *Atractylodes macrocephala* rhizomes contain anti-inflammatory sesquiterpenes, Chemical and Pharmaceutical Bulletin, 2023, c22-00779.

<https://doi.org/10.1248/cpb.c22-00779>

2. Hai Thanh Cao, Uyen Thu Nguyen, Giang Hoang Do, Minh Thi Thu Nguyen, Duong Thuy Hoang, Le Thi Nhat Bui, Minh Ngoc Truong, Dat Tien Nguyen, *Quantitative HPLC-based Metabolomics Approach for the Discrimination of Processed Rhizomes of Atractylodes macrocephala*, Current Analytical Chemistry 20.1 (2024): 41-51.

<https://doi.org/10.2174/0115734110283469231204061131>

3. Cao Thanh Hai, Nguyen Thi Luyen, Pham The Chinh, Ngo Thi Thuy Ngan, Nguyen Tuong Vy, Nguyen Tien Dat, Dang Viet Hau, *Phenolic glycoside constituents from the rhizomes of Atractylodes macrocephala* Koidz, Journal of Medicinal Materials, 27(2), 86-90, 3-2022.