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STUDY ON CHEMICAL CONSTITUENTS, CYTOTOXIC AND ANTI-INFLAMMATORY ACTIVITIES OF TWO SPECIES *Barringtonia acutangula* AND *Barringtonia racemosa* OF THE *Barringtonia* GENUS, Lecythidaceae FAMILY

Major: Organic chemistry

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SUMMARY OF CHEMISTRY DOTORAL THESIS

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INTRODUCTION

The use of medicinal plants is always associated with the history of the existence and development of human society. Natural compounds have many advantages such as diverse chemical structures and biological activities, easy absorption and metabolism in the body as well as low toxicity, and easy decomposition without affecting the environment. Therefore, compounds of natural origin are interested in research by domestic and foreign scientists to develop into pharmaceuticals for human treatment. Vietnam is blessed with nature and a vibrant flora with over 12,000 species of higher vascular plants, of which an estimated 5,000 species are used in traditional medicine. In addition to the richness of the species composition, the source of Vietnamese medicinal herbs is also of great value in that they are widely used in the community to treat many different diseases.

The genus *Barringtonia* has been studied by scientists around the world and has shown many valuable activities such as cytotoxicity [1-3], α-glucosidase enzyme inhibition [4], antimiccrobial effect [5-9] and anti-inflammatory activity by inhibiting PGE2, TNF-α, iNOS, and COX-2 and activating nuclear factor NF-kB [58]. However, in Vietnam, there are very few scientific publications on the chemical constituents and biological activities of this genus. Currently, there is only a preliminary study on *Barringtonia acutangula* with isolation of three flavan-3-ol compounds [10]. From the above practice, the author chose her thesis topic with the title "Study on chemical constituents, cytotoxic and anti-inflammatory activities of two species *Barringtonia acutangula* and *Barringtonia racemosa* of the *Barringtonia* genus, Lecythidaceae family" in order to study the isolation of compounds with cytotoxic and anti-inflammatory activities from *B. acutangula* and *B. racemosa* collected in Vietnam.

The objectives of the thesis

- Determine the chemical constituents of *B. acutangula* and *B. racemosa* growing in Vietnam.
- Evaluation of the cytotoxic and anti-inflammatory activities of isolated compounds from two studied species to search for active ingredients

that serve as a scientific basis for further studies to create care products for community healthcare.

The main contents of the thesis

- Isolation of compounds from *B. acutangula* and *B. racemosa* collected in Vietnam by the chromatographic methods.
- Determine the chemical structure of the isolated compounds by spectroscopic methods.
- Evaluation of the cytotoxic and anti-inflammatory activities of the isolated compounds.

CHAPTER 1: OVERVIEW

Includes an overview of national and international studies on the chemical composition and biological activity of the genus *Barringtonia* and the two species of *B. acutangula* and *B. racemosa.*

1.1. General overview of the genus *Barringtonia*

1.1.1. Taxonomic features

Barringtonia Forst is a plant genus in the family Lecythidaceae, with about 45 species worldwide, usually woody or shrub, distributed in tropical regions. In Vietnam, there are 14 species, description was shown in the thesis, especially 2 species *B. acutangula* and *B. racemosa.*

1.1.2. Parts and uses in traditional medicine

The bark of *B. acutangula* is used to treat abdominal pain, diarrhea, and fever with a dose of 8-16g, a decoction for drinking. The young fruit, pressed to get juice to cure eczema, or crushed and soaked with alcohol to cure toothache (do not swallow the juice). In India, crushed fruit and seeds are applied to the chest to treat colds, and to the abdomen to treat colic and flatulence [11].

B. racemosa root is used to treat measles. The fruit is used to treat coughs and asthma. Crushed kernels, mixed with flour and oil, are used to treat dysentery and diarrhea. The seeds are used to treat stomachaches and eye diseases. The seeds and pods are also used to treat worms. In Malaysia, the leaves of *B. racemosa* are used in the treatment of hypertension and as an analgesic [14].

1.2. A review of the study on the chemical composition of the genus *Barringtonia*

1.2.1. Domestic researches

In Vietnam, there are 14 species of *Barringtonia*, but only few studies on chemical composition of *Barringtonia* species in our country was published. Initial research results on the chemical composition of the bark of *B. acutangula* have isolated 03 flavan-3-ol compounds: (+)-epigallocatechin (1), (+)-gallocatechin 4′-*O*-methyl ether (2), and (+)-gallocatechin 4′-*O*-methyl ether 5-*O*-β-D-glucopyranoside (3) [10].

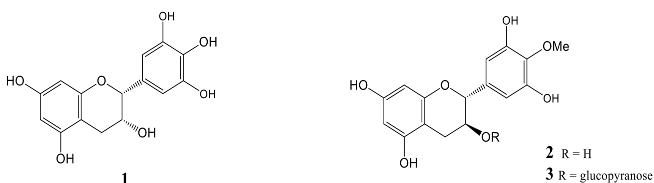


Figure 1.1. Structure of flavan-3-ol isolated from B. acutangula in Vietnam

In 2022, Nguyen Pham Tuan et al. [17] using the method of Yadav et al (2014) have identified a number of compounds present in the extract of *B. acutangular* leaves as Table 1.1.

Compounds	Experiment	Phenomena
Alkaloid	1mL Alkaloid + some drop TT Mayer	White precipitate
(Dragendorff)	1mL Alkaloid + some drop TT Dragendorff	Red-orange precipitate
Flavonoid Saponin	1mL Flavonoid Saponin + some drop $FeCl_3$	Red-brown precipitate
(Foam)	3mL Flavonoid Saponin + 6mL H_2O→ boil	Foam appears
Steroid (Salkowski)	1mL Steroid + 2mL $CHCl_3$ + 2mL H_2SO_4	A red-brown ring appears between the 2 layers
Tannin or phenol (Braymer)	0,5mL Steroid + 10mL H_2O + 2-3 drop $FeCl_3$ 0,1%	Dark blue precipitate
Terpenoid	2mL Tannin or phenol + 2mL $(CH_3CO)_2O$ + 2-3 drops of condensed H_2SO_4	Appears deep red

1.2.2. Research situation in the world

The genus *Barringtonia* has been studied by scientists around the world for a long time. The first studies from 1898 until now from the genus *Barringtonia* have published 113 compounds belonging to the classes of saponins, terpennoids, alkaloids, lignans, flavonoids, flavanones and steroids. In which, the substances were isolated and structure elucidated mainly from *B. acutangula* and *B. racemosa* species.

a. Saponins compounds

In 1994, three new monouzoidic glucuronide saponin compounds, barringtosides A-C (**4-6**) were reported from the species *B. Acutangula* [21]. In 2002, two saponins from the seeds of *B. asiatica* species were isolated and structurally determined [22]. Nine new saponins, acutangulosides A-F (**9-14**) and acutanguloside D-F methyl ester (**15-17**) were further published from *B. acutangula* in 2005 [23].

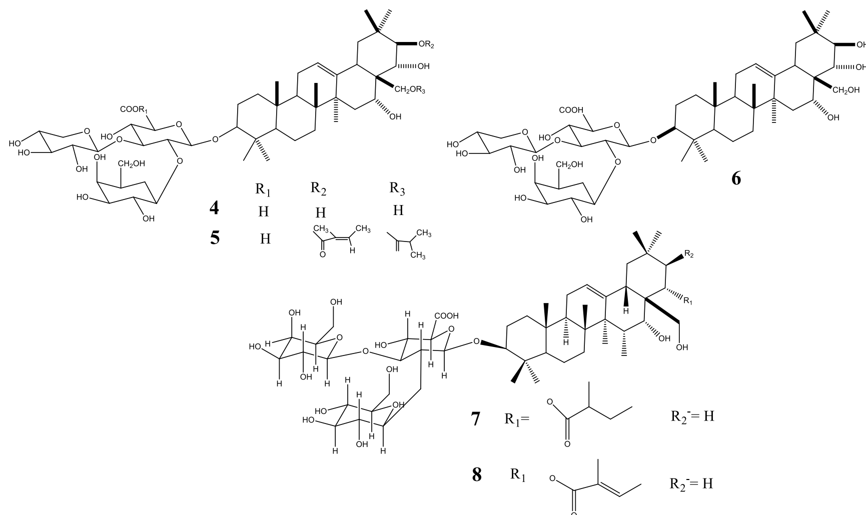


Figure 1.2. Structures of saponins isolated from *B. acutangula* and *B. asiatica*

b. Flavonoid compounds

In 2006, dihydromyricetin (**18**) was reported from leaves of *B. racemosa* [24]. Also from the leaves of *B. Racemosa,* a flavanone, a flavone and two flavonols, naringenin (**19**), luteolin (**20**), kaempferol (**21**) and

quercetin 3-O-rutinoside (**22**) and with gallic acid (**23**) and ferulic acid (**24**) were isolated and structurally determined [2, 25, 26].

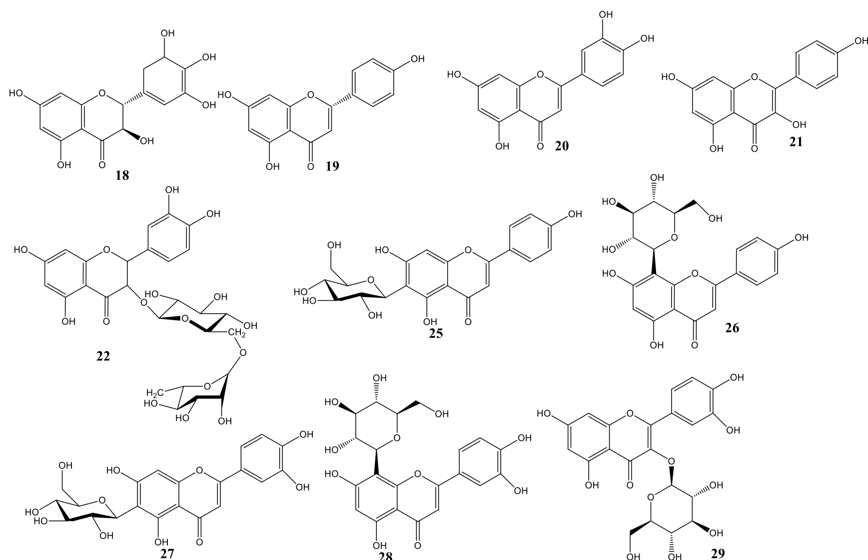


Figure 1.3. Structures of flavonoid compounds isolated from genus *Barringtonia*

c. Terpenoids and other compounds

In 1942, from the seeds of *B. acutungula* collected in Dacca (Bangladesh) recorded the presence of saponins in the form of white powder [28]. In 1957, from the fruit of *B. racemosa* species Yau-Tang Lin and colleagues isolated two triterpenoid sapogenin compounds R2-barringenol (**33**) that could resemble barringtogenol (2:3:23:28-tetra-hydroxyolean-12) -ene) and R1-Barrigenol: $C_{30}H_{50}O_7$ (**32**) [29]. In 1967, from the ^{1}H-NMR spectrum, the structure of the compound R1-barrigenol (**32**) was determined to be 3β,15α,16α,22α,28β-pentahydroxyolean-12-ene, and the compound R2-barringenol (**33**) had the same structure as camelliagenin A, similar to the previous inference that 3β,16α,22α,28β-tetrahydroxyolean-12-ene or 15-deoxy-R1-barrigenol [30, 31].

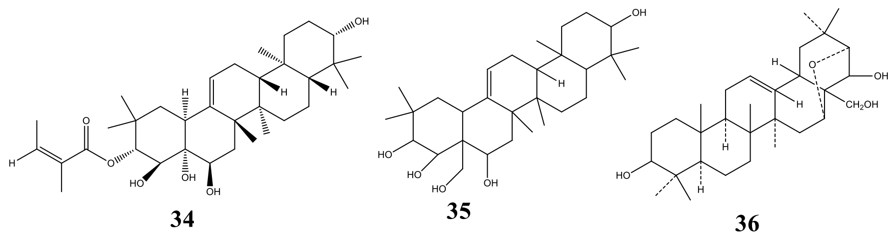


Figure 1.6. Structures of new triterpenoids isolated from *B. racemosa*

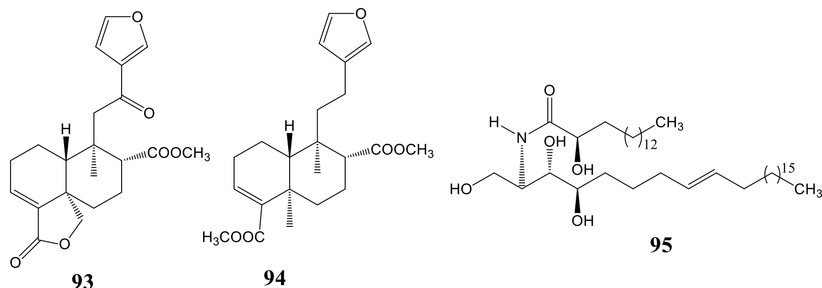


Figure 1.9. Structures of 2 diterpenoids and ceramide isolated from *B. racemosa*

1.3. Studies on the biological activity of the genus *Barringtonia*

1.3.1. Studies towards cytotoxicity

According to folk experience, the seeds of *B. racemosa* species are used in Kerala (India) to prevent and treat cancer but have not been fully reported. Recently, there have been several studies reporting on the anticancer activity of this genus. A study by Murakami et al. in 2000 showed that the leaf extract of *B. racemosa* species has the ability to inhibit 12-O hexadecanoylphorbol-13-acetate, a tumor promoter that causes the activation of Epstein-Barr virus [49]. This herpes virus is known to produce viral proteins which can then lead to malignancy by affecting transcription factors [49].

Ten triterpenes, three steroids and one vitamin E derivative from *B. maunwongyathiae* were evaluated for their antitumor potential based on inhibition of TPA-induced ornithine decarboxylase expression, COX-1 and COX-2 activities, and phorbol-induced NF-κB luciferase expression, as well as antioxidant response factor activation mediated by luciferase

expression. Among these compounds, taraxerol (**45**), 3-(E)-coumaroyl taraxerol (**56**) and α-tocopherylquinone (**68**) show promising chemopreventive potential. Compound α-tocopherylquinone (**68**) inhibited TPA-induced ornithine decarboxylase activity with an IC value of 5.9 μM and enhanced ARE expression with an EC_{50} of 5.2 μM [41].

1.4.2. Anti-inflammatory studies

The fruit of *B. racemosa* species is commonly used in Indian medicine to treat pain and inflammation through inhibition of PGE2, TNF-α, iNOS, COX-2 and activation of nuclear factor NF-kB [36].

Chandra Mohan. S et al studied the anti-inflammatory and anti-arthritic activity of the leaves of *B. acutangula*. The ethanol extract of *B. acutangula* was investigated for its anti-inflammatory activity *in vitro* by human red blood cell membrane stabilization (HRBC) and *in vitro* anti-arthritic activity by bovine serum protein denaturation and method for denaturing egg albumin. The activity of the ethanol extract of *B. acutangula* was compared with that of the standard anti-inflammatory drug Diclofenec. The authors found that *B. acutangula* extract at concentrations of 10, 20, 30, 40 and 50 μg/mL showed 29.95, 43.97, 47.63, 48.66 and 49.69% protection. Protect HRBC in the corresponding hypotonic solution (IC_{50} = 43.71), while standard diclofenac at 20, 40, 60, 80 and 100 μg/mL showed 56.28, 60.14, 67.49, 72.78 and 78.69% (IC_{50} = 0.592). In the egg albumin denaturation method, *B. acutangula* extract at concentrations of 10, 20, 30, 40 and 50 μg/mL showed inhibition of 37.57, 44.16, 60.57, 66.24 and 70, respectively. 98% for egg albumin denaturation (IC_{50} = 23.36); meanwhile, standard diclofenac 20,40, 60, 80 and 100 μg/mL showed 47.76, 57.71, 63.89, 75.87 and 84.81% inhibition of egg albumin denaturation (IC_{50} = 25)). From this study, it was found that the ethanol extract of *B. acutangula* had a stronger inhibitory effect on egg albumin denaturation than the anti-inflammatory drug diclofenac. It can be concluded that *B. acutangula* has good anti-inflammatory and rheumatic activities *in vitro* [62].

1.4.3. Studies in other directions

In addition, there are research directions for inhibiting bacteria, cardiovascular disease, antioxidant, and type 2 diabetes.

CHAPTER 2: RESEARCH SUBJECTS AND METHODS

2.1. Research subjects

The research objects of the thesis are two species *B. acutangula* and *B. racemosa* growing in Vietnam.

2.1.1. Barringtonia acutangula species

Figure 2.1. Photograph of *Barringtonia acutangula* (L.) Gaertn.

Number: **PL 01**

Location: Loc Tri commune, Phu Loc district, Thua Thien Hue province

Date: 17/08/2016

Collector: Nguyen The Cuong et al.

Determination: Dr. Nguyen The Cuong

2.1.2. Barringtonia racemosa *species*

Figure 2.2. Photograph of *Barringtonia racemosa* (L.) Spreng

Number: **PL BH 01**

Location: Bien Hoa, Đong Nai

Date: 25/08/2016

Collector: Nguyen The Cuong et al.

Determination: Dr. Nguyen The Cuong

2.2. Research Methods

2.2.1. Methods for isolating compounds

Using the methods of fractional extraction, thin plate chromatography, column chromatography, medium pressure liquid chromatography (MPLC), high performance liquid chromatography (HPLC).

2.2.2. Structure determination method

The general methods to determine the chemical structure of compounds is a combination of physical parameters with modern spectroscopic methods including:

HR-ESI-MS high-resolution electrospray ionisation mass spectrometry.

One-dimensional nuclear magnetic resonance spectra: ^{1}H-NMR, ^{13}C-NMR, 1D TOCSY

Two-dimensional nuclear magnetic resonance spectra: ^{1}H-^{1}H COSY, NOESY, HSQC, HMQC, HMBC, 2D TOCSY.

2.2.3. Method to determine biological activity

2.2.3.1. Evaluation of anti-inflammatory activity

Compounds were evaluated for their anti-inflammatory activity based on the inhibition of nitric oxide (NO) production on RAW264.7 cell line according to Dirsch's method.

2.2.3.2. Method to evaluate cytotoxic activity

2.2.3.2.1. In vitro cell culture method

- The cancer cell lines were cultured as monolayers in DMEM culture medium with an accompanying composition of 2 mM L-glutamine, 10 mM HEPES, and 1.0 mM sodium pyruvate, in addition to the addition of 10% fetal bovine serum – FBS (GIBCO).

- Cells were cultured after 3-5 days with the ratio (1:3) and cultured in a CO_2 incubator at 37°C, 5% CO_2.

2.2.3.2.2. Bioassay for cytotoxicity

The *in vitro* cytotoxicity test method has been confirmed by the US National Cancer Institute (NCI) as a standard cytotoxicity test to screen and detect substances capable of inhibiting growth or kill cancer cells under *in vitro* conditions. This test was performed according to Monks's method [42]. MCF-7: Human breast carcinoma and LNCaP: Human prostate carcinoma cell lines were provided by Prof. Dr. J. M. Pezzuto, University of Long-Island, US and Prof. Jeanette Maier, University of Milan, Italy.

CHAPTER 3: EXPERIENCE

3.1. Isolation of compounds

3.1.1. Isolation of compounds from B. acutangula

The leaves of *B. acutangula* were cleaned, dried at the temparatures below 50ºC, and powdered. The dried powder (3.0 kg) of *B. acutangula* leaves was extracted under ultrasonic condition (3x5L, 3h each), filtered and concentrated in rotary vapor to obain 240 g MeOH residue. This residue was suspended in water and partioned in turn with *n*-hexane and dichloromethane to obtain *n*-hexane (20.0 g), CH_2Cl_2 (16,5 g) extracts, water layer and indissolvable part.

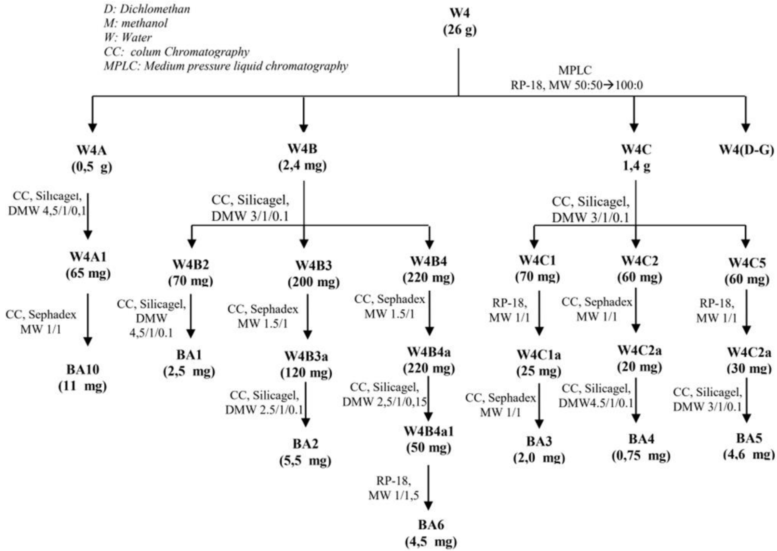


Figure 32. Diagram of isolation of compounds from fraction W4 of B. acutangula

The water fraction was separated on a Diaion column with gradient methanol/water to obtain 4 fractions from W1→W4. The W4 fraction was separated on reverse phase MPLC with methanol:water (1:1) solvent system to obtain 7 fractions W4A→W4G. Further separations led to isolation of 7 compounds **BA1**, **BA2**, **BA3**, **BA4**, **BA5**, **BA6**, **BA10** according to the diagram of Figure 3.2.

The ethyl residue (16.5 g) was subjected to normal phase MPLC using mobile phase of the dichloromethane:methanol gradient from 100% dichloromethane → 100% methanol to obtain 8 fractions E1 → E8. The E6 fraction (1.7 g) was separated on MPLC using a reversed-phase column with a methanol:water (1:1) solvent system to obtain 8 fractions E6A→E6H. The E6B fraction (50 mg) was further separated on a normal phase CC with dichloromethane:methanol:water solvent system (4.5:1:0.1), and then on Sephadex CC using methanol:water system (1:1) to obtain compounds **BA7** (2.0 mg), **BA8** (1.5 mg) and **BA9** (1.3 mg).

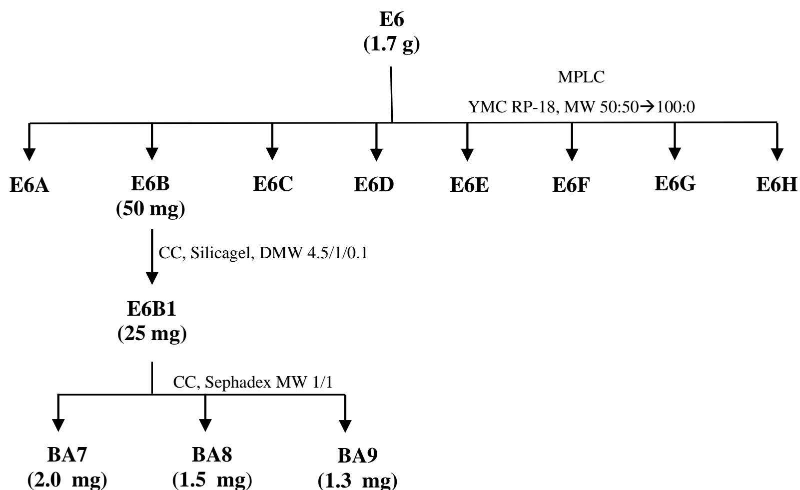


Figure 3.1.1.c. Diagram of isolation of compounds from ethyl residue of *B. acutangula*

3.1.2. Isolation of compounds from B. racemosa

The leaves of *B. racemosa* were cleaned, dried at the temparatures below 50ºC, and powdered (5.5 kg). The dried powder (3.0 kg) of *B. acutangula* leaves was extracted under ultrasonic condition (3x5L, 3h each), filtered and concentrated in rotary vapor to obain 190 g MeOH residue. This residue was suspended in water and partioned in turn with *n*-hexane and ethyl acetate to obtain *n*-hexane (13.5 g), EtOAc (15.0 g) extracts, water layer and indissolvable part.

The water part was filtered out of insoluble residue before being passed through the Diaion HP-20 column and firstly eluted with water to remove the sugar and inorganic salts. Then elute with a gradient solvent system with a solvent system of 100% water methanol:water (25:75) methanol:water (50:50) methanol:water (25:75) 100% methanol to obtain 5 fractions W1→W5. Further chromatographic separations led to isolation of 5 compounds **BR1**, **BR2**, **BR3**, **BR4**, **BR6** according to the diagram of Figure 3.5.

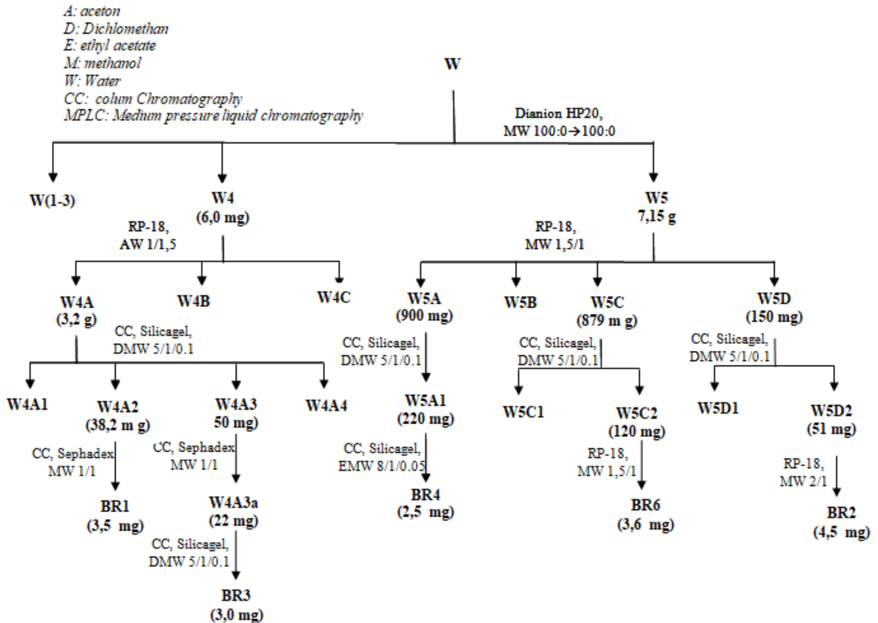


Figure 3.5. Diagram of isolation of compounds from aqueous of B. racemosa

Ethyl acetate residue (E, 15 g) was separated into 6 fractions, E1-E6, using MPLC with normal-phase silica gel column and mobile phase gradient of CH_2Cl_2/MeOH from 50/1 to 1/1 (v/v). The E3 fraction (3.48 g) was further separated on column chromatography with reverse-phase silica gel using the MeOH/H_2O elution system (1/1, 2/1, 3/1 and 10/1) obtained 5 small fractions, E3A-E3E. The E3C fraction (0.9 g) was further separated into three smaller fractions, E3C1-E3C3, using a chromatographic column with normal phase silica gel adsorbent and EtOAc/MeOH elution system (17/1), v/v). The E3C3 fraction (22 mg) was purified by column chromatography with reverse phase silica gel adsorbent and MeOH/H_2O elution system (1.5/1), then purified on a Shephadex LH-20 CC column. using mobile phase MeOH/H_2O (1.5/1) obtained compound **BR5** (5 mg). The E2 fraction (2.1 g) was separated into three fractions, E2A-E2C, using reversed-phase silica gel-filled column chromatography and the mobile phase of MeOH/H_2O (3/1). The E2A fraction (1.6 g) was further separated into two smaller fractions E2A1 and E2A2 using a column packed with

normal-phase silica gel and an elution system of CH_2Cl_2/MeOH (20/1). Compounds **BR7** (6.0 mg) and **BR8** (4.8 mg) were purified from the E2A1 fraction (0.8 g) on RP-18 CC with acetone/H_2O (2/1), then further purification was carried out on silica gel CC using an elution system of CH_2Cl_2/MeOH (20/1).

CHAPTER 4: RESULTS AND DISCUSSION

4.1. Structure elucidation of compounds from *B. acutangula*

4.1.1. Compound BA1: barringoside A (new)

Compound **BA1** was obtained a a pale yellow solid with the molecular fomular of $C_{42}H_{46}O_{22}$, determining by HR-ESI-MS with a quasi-moleular ion peak at *m/z* 925.23789 $[M+Na]^+$.

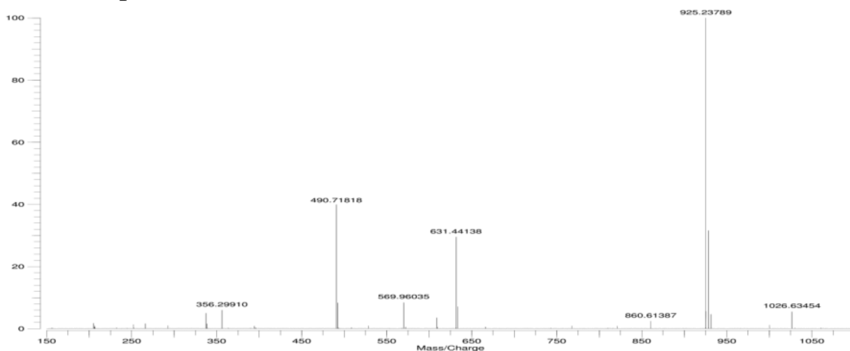


Figure 4.1.1.a. HR-ESI-MS spectrum of **BA1**

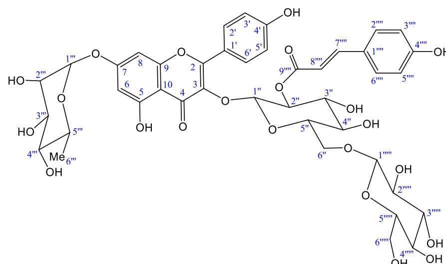


Figure 4.1.1.b. Structure of **BA1**

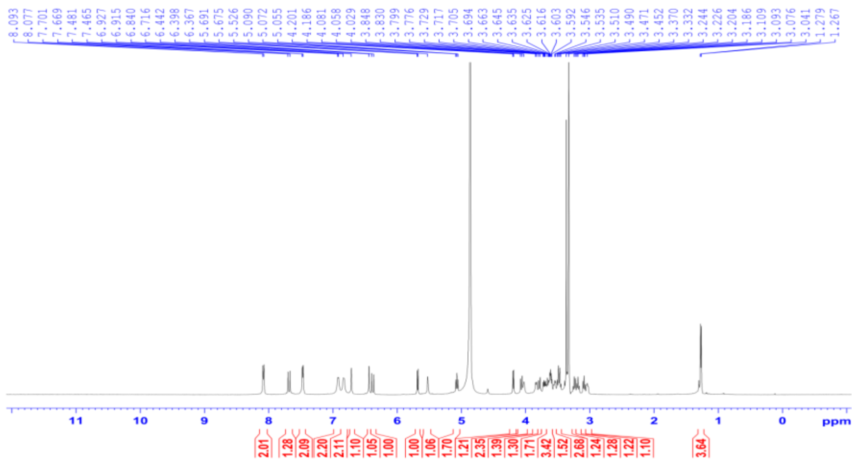


Figure 4.1.1.c. ^{1}H-NMR spectrum of **BA1**

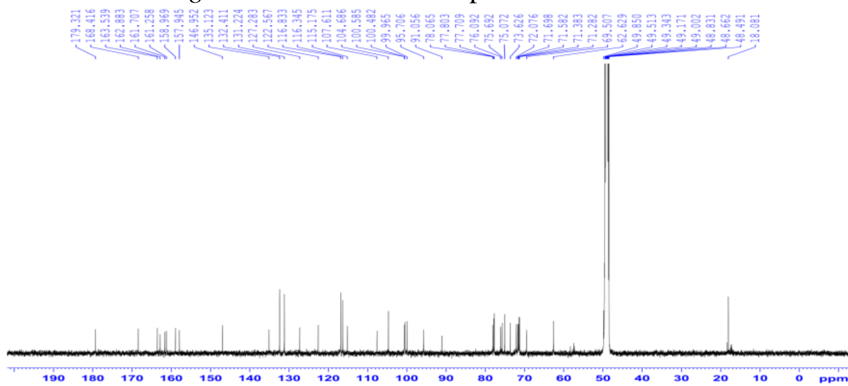


Figure 4.1.1.d. ^{13}C-NMR spectrum of **BA1**

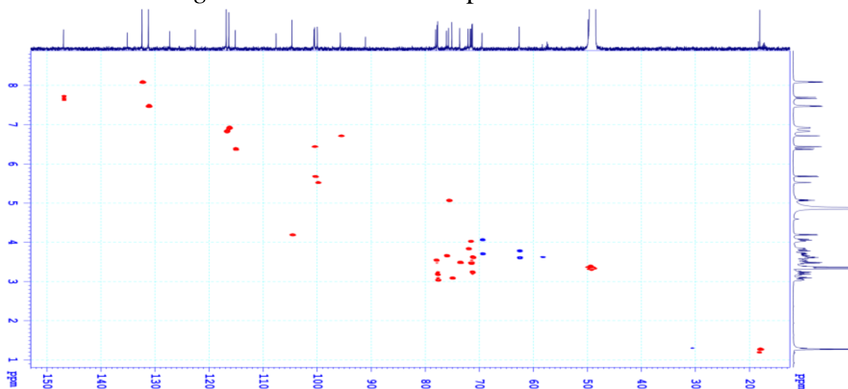


Figure 4.1.1.e. HSQC spectrum of **BA1**

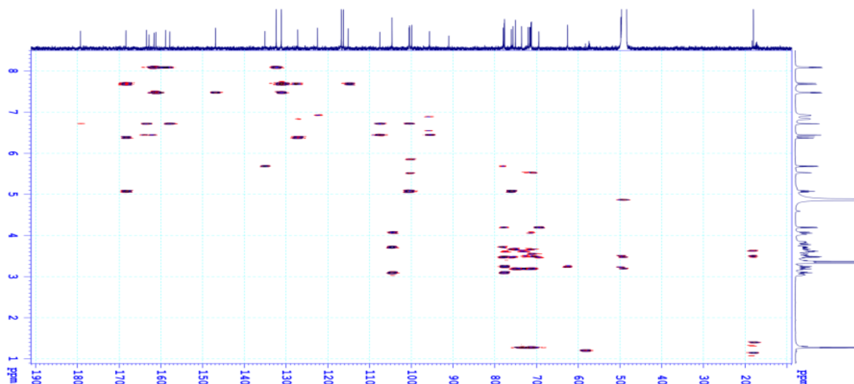


Figure 4.1.1.f. HMBC spectrum of **BA1**

Table 4.1.1. NMR spectral data of **BA1** and relate compounds

C	[a]δ_C	[b]δ_C	δ_C [c,d]	δ_H [c,e] mult. (J = Hz)	**HMBC** (H → C)
Aglycon					
2		159.3	158.9	-	
3		135.6	135.1	-	
4		179.6	179.3	-	
5		163.3	162.8	-	
6		100.7	100.5	6.44 s	5, 7, 8, 10
7		163.6	163.5	-	
8		95.9	95.7	6.71 s	4, 6, 7, 9, 10
9		158.0	157.9	-	
10		107.5	107.6	-	
1′		122.5	122.5	-	
2′		132.5	132.4	8.08 d (8.0)	2, 4′, 6′
3′		116.3	116.3	6.92 d (8.0)	
4′		161.8	161.7	-	
5′		116.3	116.3	6.92 d (8.0)	
6′		132.5	132.4	8.08 d (8.0)	
Glc1					
1″	**101.0**	**103.5**	**100.5**	5.68 d (8.0)	3
2″	75.8	75.8	75.6	5.07 dd (8.0, 9.0)	9′′′′
3″	**76.3**	**77.9**	**76.0**	3.66 t (9.0)	
4″	71.0	71.4	71.5	3.47 t (9.0)	
5″	77.6	77.9	78.1	3.55 m	
6″	68.6	69.5	69.5	3.71 dd (6.0, 12.0)	

C	[a]δ_C	[b]δ_C	δ_C [c,d]	δ_H [c,e] mult. (J = Hz)	HMBC (H → C)
				4.07 br d (12.0)	
Rha					
1‴		100.0	99.9	5.52 s	7
2‴		71.6	71.7	4.03 br s	
3‴		72.0	72.0	3.80 br d (9.0)	
4‴		73.5	73.6	3.49 t (9.0)	
5‴		71.3	71.2	3.62[f]	
6‴		18.2	18.0	1.27 d (6.0)	4‴, 5‴
p-coumaric acid					
1⁗	127.2		127.2	-	
2⁗	131.2		131.2	7.47 d (8.0)	4⁗, 7⁗
3⁗	116.8		116.8	6.83 d (8.0)	
4⁗	161.1		161.2	-	
5⁗	116.8		116.8	6.83 d (8.0)	
6⁗	131.2		131.2	7.47 d (8.0)	
7⁗	146.9		146.9	7.68 d (16.0)	
8⁗	115.2		115.1	6.39 d (16.0)	1⁗, 7⁗, 9⁗
9⁗	168.5		168.4	-	
Glc2					
1⁗′		104.6	104.6	4.19 d (7.5)	6″
2⁗′		75.1	75.0	3.09 dd (7.5, 9.0)	
3⁗′		77.8	77.8	3.18 t (9.0)	
4⁗′		71.3	71.3	3.24 t (9.0)	
5⁗′		77.7	77.7	3.04 m	
6⁗′		62.5	62.6	3.61[f]/3.79 br d (11.5)	

[a]δ_C of quercetin 3-*O*-[2″-*O*-(*E*)-*p*-coumaroyl][*β*-D-glucopyranosyl(1→3)-*α*-L-rhamnopyranosyl(1→6)]-*β*-D-glucoside [76], [b]δ_C of kaempferol 3-*O*-*β*-[*β*-D-glucopyranosyl(1→6)D-glucopyranoside]-7-*O*-*α*-L-rhamnopyranoside [77], [c]recorded in CD_3OD, [d]125 MHz, [e]500 MHz, [f]overlapped signals.

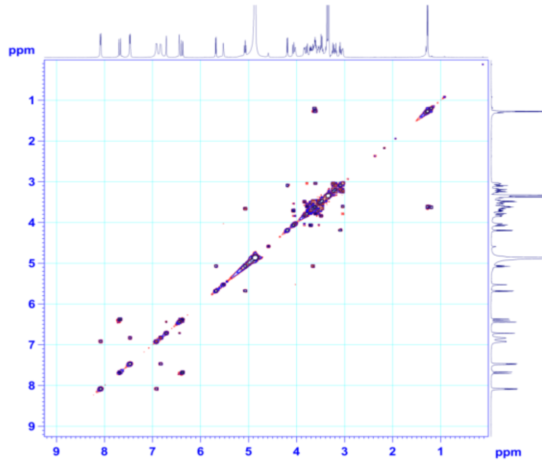


Figure 4.1.1.g. COSY spectrum of **BA1**

The NMR data of **BA1** indicated a flavonoid triglycoside with three anomeric protons at δ_H 5.68 (1H, d, J = 8.0 Hz, H-1″), 5.52 (1H, br s, H-1‴) và 4.19 (1H, d, J = 7.5 Hz, H-1′′′′′), had HSQC correlations with relevant anomeric carbons at δ_C 100.5 (C-1″), 100.0 (C-1‴) and 104.7 (C-1′′′′′) confirming three sugar units. Detailed analysis of the HSQC correlations led to assignment of carbon signals with their corresponding protons. From the HSQC results, and COSY connectivities of H-1″ (δ_H 6.58)/H-2″ (δ_H 5.07)/H-3″ (δ_H 3.66)/H-4″ (δ_H 3.47)/H-5″ (δ_H 3.55)/H-6″ (δ_H 3.71 và 4.07). H-1‴ (δ_H 5.52)/H-2‴ (δ_H 4.03)/H-3‴ (δ_H 3.80)/H-4‴ (δ_H 3.49)/H-5‴ (δ_H 3.62)/H-6‴ (δ_H 1.27) và H-1′′′′′ (δ_H 4.19)/H-2′′′′′ (δ_H 3.09)/H-3′′′′′ (δ_H 3.18)/H-4′′′′′ (δ_H 3.24)/H-5′′′′′ (δ_H 3.04)/H-6′′′′′ (δ_H 3.61 và 3.79), led to assignment of the ^{1}H-NMR and ^{13}C-NMR for all three sugar moieties as shown in Table 4.1.1. In addition, the presence of two *meta* coupled aromatic protons [δ_H 6,44 (H-6) and 6,71 (H-8), each 1H, br s] and four *ortho* coupled aromatic protons [δ_H 8.08 (H-2′ and H-6′) and 6.92 (H-3′ and H-5′), each 2H, d, J = 8.0 Hz] indicated a kaempferol skeleton. The ^{1}H and ^{13}C NMR spectral data of **BA1** were similar to those of kaempferol 3-*O*-β-[β-D-glucopyranosyl(1→6)D-glucopyranoside]-7-*O*-α-L-

rhamnopyranoside [77], except for additional presence of a *trans-p*-coumaroyl moiety at δ_C 127.3 (C-1′′′′), 131.2 (C-2′′′′ và C-6′′′′), 116.8 (C-3′′′′ và C-5′′′′), 161.3 (C-4′′′′), 146.9 (C-7′′′′), 115.2 (C-8′′′′) and 168.4 (C-9′′′′)/δ_H 7.47 (2H, d, J = 8.0 Hz, H-2′′′′ and H-6′′′′), 6.83 (2H, d, J = 8.0 Hz, H-3′′′′ and H-5′′′′), 7.68 (1H, d, J = 16.0 Hz, H-7′′′′) and 6.39 (1H, d, J = 16.0 Hz, H-8′′′′).

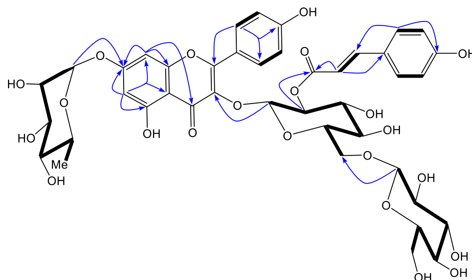
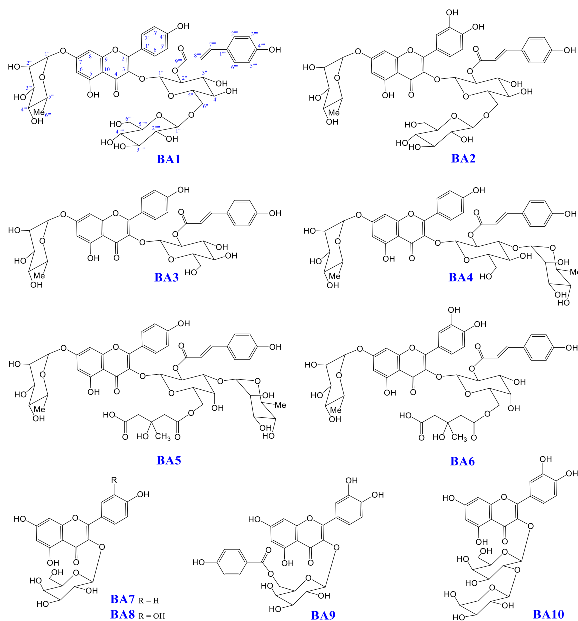


Figure 4.1.1.h. Key HMBC and COSY correlations of **BA1**

Detailed analysis of COSY and HMBC correlations (Figure 4.1.1.h) clearly confirmed the structure of **BA1**. Attached positions of the first glucose at C-3, rhamnose at C-7 and second glucose at C-6″ were determined by HMBC cross-peaks of H-1″ (δ_H 5.68) with C-3 (δ_C 135.1), H-1‴ (δ_H 5.52) with C-7 (δ_C 163.5) and H-1′′′′′ (δ_H 4.19) with C-6″ (δ_C 69.5). Moreover, ^{13}C NMR data of the first glucose of **BA1** (Table III.1) were similar to those of quercetin 3-*O*-[2″-*O*-(*E*)-*p*-coumaroyl][*β*-D-glucopyranosyl(1→3)-*α*-L-rhamnopyranosyl-(1→6)]-*β*-D-glucopyranoside [76] in combination with the strong low field shift of H-2″ at δ_H 5.07 led to determine the esterification of the *trans-p*-coumaroyl moiety at C-2″. This was further supported by HMBC cross-peak (Figure II.1.h) of H-2″ (δ_H 5.07) with C-9′′′′ (δ_C 168.4). Thus, the structure of **BA1** was elucidated as kaempferol 3-*O*-[2″-*O*-(*E*)-*p*-coumaroyl][*β*-D-glucopyranosyl (1→6)*β*-D-glucopyranoside]-7-*O*-*α*-L-rhamnopyran-oside, một hợp, a new compound and named barringoside A.

4.1.2. Compounds from *B. acutangula*

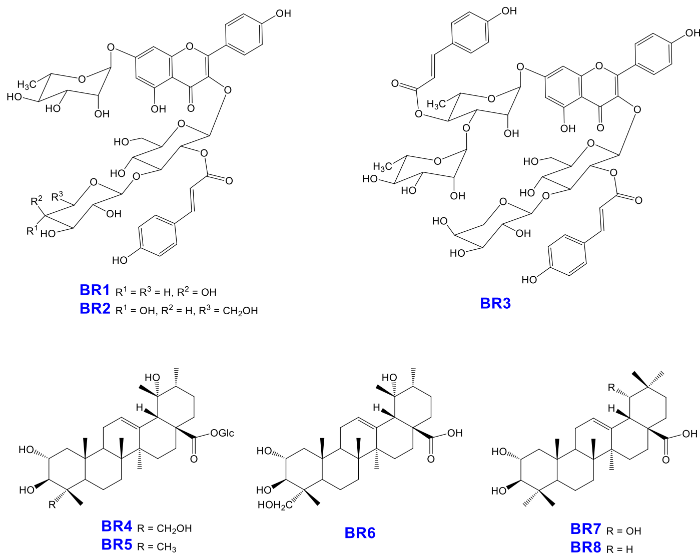


Structures of isolated compounds from *B. acutangula*

Using various spectroscopic experiments, the author elucidated structures of all 10 compounds from *B. acutangula* including: 6 new acylated flavonoid glycosides namely barringosides A-F (**BA1**-**BA6**). The known compounds is ditermined as kaempferol 3-*O*-*β*-D-galactopyranoside (**BA7**), quercetin-3-*O*-*β*-D-galactopyranoside (**BA8**), quercetin 3-*O*-*β*-D-(6-*p*-hydroxybenzoyl)galacto-pyranoside (**BA9**) and quercetin 3-*O*-*α*-L-arabinopyranosyl-(1→2)-*β*-D-galacto-pyranoside (**BA10**).

4.2. Structure elucidation of compounds from *B. racemosa*

Using various spectroscopic experiments, the author elucidated structures of all 8 compounds from *B. racemosa* including: 3 new acylated flavonoid glycosides namely barringosides G-I (**BR1**-**BR3**) and 5 known triterpenoids as niga-ichigoside F1 (**BR4**), rosamultin (**BR5**), 23-hydroxytormentic acid (**BR6**), arjunic acid (**BR7**) và maslinic acid (**BR8**).



Structures of isolated compounds from B. racemosa

4.3. Results of bioactivity testing of isolated compounds

4.3.1. Anti-inflammatory activity

The results of anti-inflammatory evaluation showed that, among 18 studied compounds, only 2 compounds, **BA9** and **BR3** showed anti-inflammatory activity through inhibition of NO production in RAW264.7 cells stimulated by LPS with the corresponding IC_{50} values of 20,00±1,68 and 52,48±1,04 μM.

Table 4.3.1. **The results of the evaluation of the inhibitory effect on NO production of 18 compounds**

No.	**Symbol**	**IC_{50} (μM) Value**	**No.**	**Symbol**	**IC_{50} (μM) Value**
1	**BA1**	>100	11	**BR1**	>100
2	**BA2**	>100	12	**BR2**	>100
3	**BA3**	>100	**13**	**BR3**	**52.48 ± 1.04**
4	**BA4**	>100	14	**BR4**	>100

5	**BA5**	>100	15	**BR5**	>100
6	**BA6**	>100	16	**BR6**	>100
7	**BA7**	>100	17	**BR7**	>100
8	**BA8**	>100	18	**BR8**	>100
9	**BA9**	**20.00±1.68**	19	**Cardamonin***	2,2±0,27
10	**BA10**	>100			

**positive control*

Thus, **BA9** and **BR3** can exhibit anti-inflammatory activity. Compound **BA9** exhibits inhibitory activity on NO production with an IC_{50} value of 20.00±1.68 µM, which can be selected to conduct further studies towards evaluating the mechanism of action. This is also the first evaluation of the inhibitory effect on NO production of this compound.

4.3.2. Cytotoxic activity

18 isolated compounds were evaluated for cytotoxic activity on 02 human cancer cell lines, LNCaP (prostate) and MCF-7 (breast). The obtained results showed that: **BA8**, **BR6** and **BR7** had activity with IC_{50} values from 29.98 - 84.99 µM on two tested cancer cell lines. The remaining samples showed less activity at the highest studied concentrations of 100 M.

Table 4.3.2. The results of the cytotoxic effects on cancer cells

Cell line	**IC_{50} (µM) value of compound**			
	BA8	**BR6**	**BR7**	**Ellipticine***
LNCaP	41.76±4.86	29.98±2.40	58.58±5.08	1.91±0.08
MCF-7	54.11±5.67	37.11±2.07	84.99±7.37	1.99±0.12

**positive control*

CONCLUSION

1. Research on chemical composition

Using a combination of chromatographic and spectral experiments led to isolation and structure elucidation of 10 compounds from *Barringtonia acutangula* and 08 compounds from *Barringtonia racemosa*.

+ From *Barringtonia acutagula*: 10 flavonoid glycosides including 6 new compounds named barringosides A-F (**BA1-BA6**) and four known compounds as kaempferol 3-*O*-*β*-D-galactopyranoside (**BA7**), quercetin-3-*O*-*β*-D-galactopyranoside (**BA8**), quercetin 3-*O*-*β*-D-(6-*p*-hydroxybenzoyl)galacto-pyranoside (**BA9**) and quercetin 3-*O*-*α*-L-arabinopyranosyl-(1→2)-*β*-D-galacto-pyranoside (**BA10**).

+ From *Barringtonia racemosa*: 3 new flavonoid glycosides named barringoside G-I (**BR1**-**BR3**) and 05 known compounds as niga-ichigoside F1 (**BR4**), rosamultin (**BR5**), 23-hydroxytormentic acid (**BR6**), arjunic acid (**BR7**) and maslinic acid (**BR8**).

The acylated flavonoid glycosides were firstly discovered from *Baringtonia* species.

2. Research on biological activity

The anti-inflammatory activity of all isolates was evaluated through inhibition of NO production in LPS-stimulated RAW264.7 cells. The results showed that two compounds, quercetin 3-O-β-D-(6-p-hydroxybenzoyl)galacto-pyranoside (**BA9**) and barringoside I (**BR3**), exhibited anti-inflammatory activity through inhibition of NO production in RAW264.7 cells stimulated with LPS with IC_{50} values of 20.00±1.68 and 52.48±1.04 µM, respectively.

The cytotoxic activity of the isolated compounds was evaluated on 02 human cancer cell lines, LNCaP (prostate) and MCF-7 (breast). The obtained results showed that only compounds quercetin-3-O-*β*-D-galactopyranoside (**BA8**), 23-hydroxytormentic acid (**BR6**) and arjunic acid (**BR7**) showed activity on two tested cancer cell lines with IC_{50} values from 29.98 - 84.99 µM.

SUGGESTIONS

The compound quercetin 3-O-β-D-(6-p-hydroxybenzoyl)galacto-pyranoside (**BA9**) exhibited significant anti-inflammatory activity through inhibition of NO production in RAW264.7 cells stimulated with LPS with IC_{50} value of 20.00±1.68 µM, so it is necessary to conduct further studies to determine the mechanism of action. The acylated flavonoid glycosides have

unique chemical structures, so it is necessary to expand the study to other types of activities to guide the applied studies.

NEWTH OF THE THESIS

1. Completed solation and structure determination 06 new acylated flavonoid glycosides namely barringosides A-F (**BA1**-**BA6**) from *Baringtonia acutangula*, and 03 new acylated flavonoid glycosides namely barringosides G-I (**BR1**-**BR3**) from *B. racemosa*.

2. Acylated flavonoid glycosides was isolated and reported for the first time from *Barringtonia* species.

3. For the first time, the compound quercetin 3-O-*β*-D-(6-*p*-hydroxybenzoyl)galacto-pyranoside (**BA9**) was found to exhibit significant anti-inflammatory activity through inhibition of NO production in RAW264.7 was stimulated by LPS with an IC_{50} value of 20.00±1.68 μM.

LIST OF PUBLISHED ARTICLES

1. Le Thi Vien, **Quach Thi Thanh Van**, Tran Thi Hong Hanh, Phan Thi thanh Huong,Nguyen Thi Kim Thuy, Nguyen The Cuong, Nguyen Hai Dang, Nguyen Van Thanh,Nguyen Xuan Cuong, Nguyen Hoai Nam, Phan Van Kiem, Chau Van Minh.*Flavonoid glycosides from Barringtonia acutangula*, Bioorganic & Medicinal Chemistry Letters, **2017**, 27, 3776–3781.

2. **Quach Thi Thanh Van**, Le Thi Vien, Tran Thi Hong Hanh,Phan Thi Thanh Huong, Nguyen The Cuong, Nguyen Phuong Thao,Nguyen Huy Thuan, Nguyen Hai Dang, Nguyen Van Thanh,Nguyen Xuan Cuong, Nguyen Hoai Nam, Phan Van Kiem andChau Van Minh.*Acylated flavonoid glycosides from Barringtonia racemosa*, Natural Product Research, **2020**, 34(9), 1276–1281.

3. **Quach Thi Thanh Van**, Le Thi Vien, Tran Thi Hong Hanh, Phan Thi Thanh Huong,Nguyen Van Thanh, Nguyen Xuan Cuong, Nguyen Hoai Nam, Chau Van Minh. *Structural elucidation of four flavonoid glycosidesfrom Barringtonia acutangula*, Vietnam Journal of Chemistry, **2018**, 56(2), 187-190.

4. **Quach Thi Thanh Van**, Le Thi Vien, Tran Thi Hong Hanh, Phan Thi Thanh Huong,Nguyen Van Thanh, Nguyen Xuan Cuong, Nguyen Hoai Nam, Chau Van Minh. *Triterpenoid derivatives from Barringtonia racemosa*, Vietnam Journal of Chemistry, **2019**, 57(1), 96-100.