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**INSTITUTE OF MARINE BIOCHEMISTRY**

**TRAN THI HONG HANH**

# STUDY ON CHEMICAL COMPOSITIONS AND BIOLOGICAL ACTIVITIES OF THE STARFISH *ASTERINA BATHERI* Goto, 1914 AND *ASTROPECTEN POLYACANTHUS* Müller & Troschel, 1842

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**SUMMARY OF CHEMICAL DOCTORAL THESIS**

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Advisors: Prof. Acad. Chau Van Minh
Dr. Nguyen Hoai Nam
Institute of Marine Biochemistry

1st Reviewer:

2nd Reviewer:

3rd Reviewer:

The dissertation will be defended at Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, No.18 Hoang Quoc Viet, Cau Giay District, Ha Noi City.

at hour date month 2015

Thesis can be found in the library of Institute of Marine Biochemistry

# I. INTRODUCTION THESIS

## 1. Preamble

The ocean covering approximately 70% of the Earth's surface and it occupy over 90% volume of living area of the earth and most life activities are related to marine life. Thus the marine environment where many things are still secret contains the largest biological diversity of species.

From the early 90s of last century, the marine scientists had the same concerns which are discoveries of the diversity of biological resources in the ocean. But the researches at that time are incomplete, scattered and unsystematic. There are many debates between the scientists because some species are counted multiple times, even dozens of times; so the statistics become inaccurate. The question of the diversity of species, where marine creatures live and the complex relationships among marine species lead to a new and urgent requirements of a global systematic study

Vietnam with over 3260km of coastline running from north to south and thousands of islands in coastal areas. Geographical conditions bring Viet Nam many advantages, a large quantity of natural resources and the diversity of marine ecosystems abundant in both reserves and species. In Vietnam, biological resources have been attracted the attention of scientists for about 30 recent years. The scientists have isolated many compounds from marine species. The compounds shows biological activity high and valuable, such as cytotoxic activity, antioxidant activity, anti-inflammatory activity and antibacterial activity…

Starfish is a marine species which are common in the waters of Vietnam. They are nutritious foods which help people to improve the health. In addition, recent studies showed that products from starfish also work to prevent and treat disease. In Vietnam, the study of the chemical composition and biological activity of the starfish are very limited. The further studies on this abundant resource will create the scientific basis for the applications in practice.

On the basis, we proceed to implement the thesis "**Study on chemical compositions and biological activities of the starfish *Asterina batheri* Goto, 1914 and *Astropecten polyacanthus* Müller & Troschel, 1842"**.

**2. Subjects and contents of the thesis**

Subjects of dissertation research is the two starfish *Asterina batheri* and *Astropecten polyacanthus.*

The main contents of the thesis are:

1. Extract and isolate the compounds from the two starfish *Asterina batheri* and *Astropecten polyacanthus* of Vietnam.

2. Determine the structures of the isolated compounds.

3. Evaluate the biological activity of isolated compounds to guide the subsequent applied research.

**3. New contributions of the thesis**

**1.** Eight new compounds were isolated from two starfish of Vietnam: *Astropecten polyacanthus* and *Asterina batheri*, they are: Astebatherioside A (**AB1**), Astebatherioside B (**AB2**), Astebatherioside C (**AB3**), Astebatherioside D (**AB4**), Astropectenol A (**ASP1**), Astropectenol B (**ASP2**), Astropectenol C (**ASP3**), Astropectenol D (**ASP4**).

**2.** The results of *in vitro* cytotoxicity tests of the diclometane fraction and 7 compounds isolated from *A. polyacanthus* were first time found: The dichloromethane fraction and 5 compounds showed significant inhibitory effects on 3 cell lines with $IC_{50}$ ranging from 2.70 ÷ 82.95 μM. Specially, compound **ASP7** and $CH_2Cl_2$ fraction exhibit potent cytotoxic effects against HL-60 human leukemia cells with the $IC_{50}$ of 2.70 μM and 8.29 μg/mL, respectively, comparing to the position control, mitoxantrone ($IC_{50}$=6.80 μM).

**3.** The first time, investigation for the possible mechanism underlying the induction of apoptosis showed that $CH_2Cl_2$ fraction or compound ASP7 induced apoptosis via alteration of expression of apoptosis-related protein in HL-60 cells.

**4.** The first time, the inhibitory effect of 12 compounds isolated from two starfish on pro-inflammatory cytokine IL-12 p40, IL-6 and tumor necrosis factor α (TNF-α) production in lipopolysaccharide (LPS)-stimulated bone marrow-derived dendritic cells (BMDCs). Among those tested, compounds ASP1, ASP5, ASP7 showed potent inhibitory effects on the production of all three pro-inflammatory cytokines with $IC_{50}$ values ranging from 1.82 ÷ 7.00 μM.

**4. The layout of the thesis**

The thesis consists of 115 pages with 18 tables, 98 pictures, 95 references. The layout of the thesis: Introduction (2 pages), Chapter 1: Overview (28 pages), Chapter 2: Objects and methods (6 pages), Chapter 3: Experimentals (7 pages), Chapter 4: Results and discussion (61 pages), Conclusions and recommendations (1 page), Publications (1 page), and References (7 pages).

## II. CONTENTS OF THE THESIS

**PREAMBLE:** mention scientific sense, practicality, object, objectives and tasks of the dissertation research.

## CHAPTER 1: OVERVIEW

1.1. Introduction to class Asteroidea

1.2. The study of starfish in the world

1.3. The study of starfish in Vietnam

## CHAPTER 2: OBJECTS AND METHODS

### 2.1. Biological materials

Samples of two starfishs *A. polyacanthus* (phylum Echinodermata, class Asteroidea, order Paxillosida, family Astropectinidae) and *A. batheri* (order Valvatida, family Oreasteridae) were collected at Cat Ba, Hai Phong, Vietnam

### 2.2. Isolated methods

Combine chromatographic methods include thin-layer chromatography (TLC), column chromatography (CC), medium pressure liquid chromatography (MPLC) and high pressure liquid chromatography (HPLC).

### 2.3. Method of determining the chemical structure of compounds

General method to determine the chemical structure of the compound is a combination of physical parameters and modern spectroscopic methods include: the optical rotation ($[\alpha]_D$), mass spectrometry (ESI-MS) and high-resolution mass spectrometry (FT-ICR-MS), magnetic resonance spectrum (1D, 2D-NMR).

### 2.4. Cytotoxic activity assay

Cytotoxic activity was determined by the method of MTT.

### 2.5. Anti-inflammatory effects

The effects of compounds on the production of pro-inflammatory cytokines IL-12, IL-6 and TNF- α in lipopolysaccharide-stimulated (LPS) bone marrow-derived dendritic cells (BMDCs)

## CHAPTER 3: EXPERIMENTALS

### 3.1. Extraction

This section presents the process of making methanol extracts, partitioned extract and isolated compounds from two samples starfish: *A. batheri* and *A. polyacanthus*.

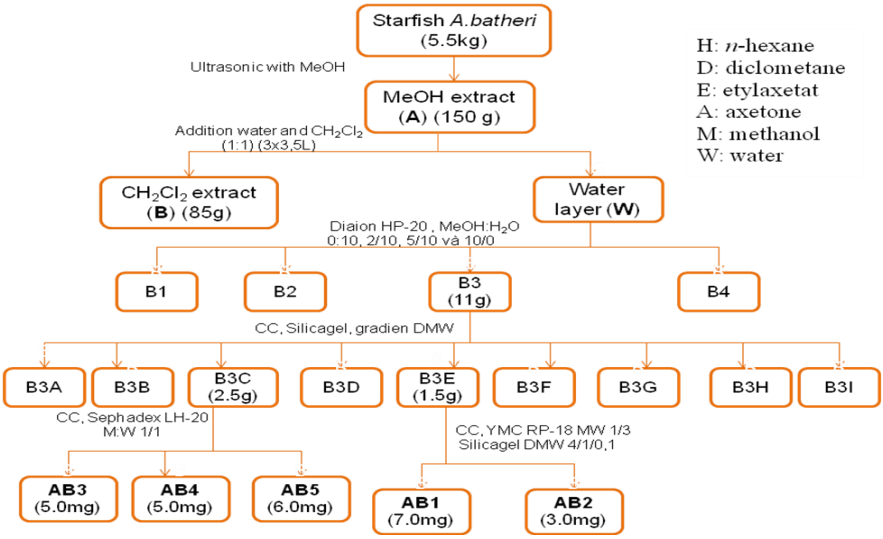



Figure 3.1a: Isolation compounds from *A. batheri*

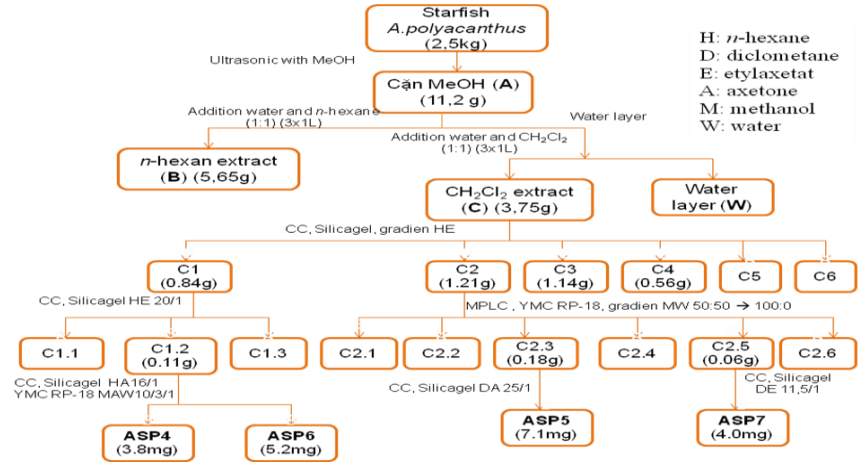



Figure 3.1b: Isolation compounds from C1, C2 extracts of *A. polyacanthus*

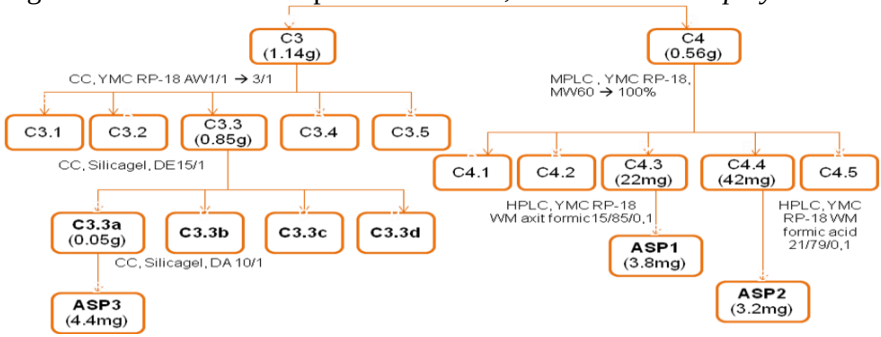



Figure 3.1c: Isolation compounds from C3, C4 extracts of *A. polyacanthus*

## 3.2. Physical and spectroscopic data

### 3.2.1. Compound AB1: Astebatherioside A (new)

White power, $[\alpha]_D^{25}$ + 5,0 (*c*, 0,25, MeOH);

FT-ICR-MS *m/z* 842,32887 $[M + H]^+$ (calcd for $C_{35}H_{56}O_{22}N$, 842,32940).

$^1$H-NMR and $^{13}$C-NMR data (see Table 4.2.2 and discussion).

### 3.2.2. Compound AB2: Astebatherioside B (new)

White power, $[\alpha]_D^{25}$ – 2,3 (*c*, 0,25, MeOH);

FT-ICR-MS *m/z* 850,29569 $[M + Na]^+$ (calcd for $C_{34}H_{53}NO_{22}Na$, 850,29570)

**$^1$H-NMR** (DMSO-$d_6$, 500 MHz) $\delta_H$ (ppm) **Aglycon**: 5,91 (br s, H-4), 6,85 (br s, H-5), 2,34 (s, H-2′), 11,15 (s, NH), **Sugar**: **Qui I**: 4,99 (d, *J* = 6,5 Hz, H-1″), 3,67 (H-2″), 3,68 (H-3″), 3,20 (H-4″), 3,64 (H-5″), 1,28 (d, *J* = 6,0 Hz, H-6″).

**Qui II**: 4,61 (d, *J* = 8,0 Hz, H-1‴), 2.96 (dd, *J* = 8,0, 9,0 Hz, H-2‴), 3,11 (H-3‴), 2,75 (t, *J* = 9,0 Hz, H-4‴), 3,10 (H-5‴), 1,05 (d, *J* = 6,0 Hz, H-6‴). **Ara(p):** 4,57 (d, *J* = 4,0 Hz, H-1⁗), 3,64 (H-2⁗), 3,66 (H-3⁗), 3,72 (br s, H-4⁗), 3,42 (dd, *J* = 4,0, 12,0 Hz, H-5⁗). **Fuc:** 4,31 (d, *J* = 6,5 Hz, H-1⁗), 3,41 (H-2⁗), 3,42 (H-3⁗), 3,55 (br s, H-4⁗), 3,58 (H-5⁗), 1,12 (d, *J* = 6,0 Hz, H-6⁗). **Ara(f)**: 5,03 (br s, H-1‴‴), 3,90 (br s, H-2‴‴), 3,65 (H-3‴‴), 3,85 (H-4‴‴), 3,44 and 3,51 (H-5‴‴)

**$^{13}$C-NMR** (DMSO-*$d_6$*, 125 MHz) $\delta_C$ (ppm) **Aglycon**: 118,1 (C-2), 150,3 (C-3), 97,1 (C-4), 123,1 (C-5), 185,2 (C-1′), 27,7 (C-2′).**Sugar: Qui I**: 99,3 (C-1″), 80,0 (C-2″), 74,5 (C-3″), 82,5 (C-4″), 70,5 (C-5″), 17,7 (C-6″). **Qui II**: 103,2 (C-1‴), 74,6 (C-2‴), 76,1 (C-3‴), 75,2 (C-4‴), 71,7 (C-5‴), 17,6 (C-6‴). **Ara(p)**: 100,5 (C-1⁗), 79,4 (C-2⁗), 71,0 (C-3⁗), 65,8 (C-4⁗), 63,3 (C-5⁗). **Fuc**: 104,7 (C-1‴‴), 70,2 (C-2‴‴), 79,3 (C-3‴‴), 70,6 (C-4‴‴), 69,9 (C-5‴‴), 16,3 (C-6‴‴). **Ara(f)**: 109,0 (C-1‴‴), 81,3 (C-2‴‴), 77,6 (C-3‴‴), 85,0 (C-4‴‴), 61,6 (C-5‴‴).

### 3.2.3. Compound AB3: Astebatherioside C (new)

White power, $[\alpha]_D^{25}$ + 18,9 (*c*, 0,25, MeOH);

FT-ICR-MS *m/z* 418.17310 $[M + H]^+$ (calcd for $C_{18}H_{28}NO_{10}$, 418,17132).

**$^1$H-NMR** (DMSO-*$d_6$*, 500 MHz) $\delta_H$ (ppm) **Aglycon**: 5,92 (t, *J* = 3,0 Hz, H-4), 6,85 (t, *J* = 3,0 Hz, H-5), 2,32 (s, H-2′), 11,14 (s, NH), **Sugar**: **Qui I**: 4,96 (d, *J* = 7,5 Hz, H-1″), 3,55 (dd, *J* = 7,5, 9,0 Hz, H-2″), 3,50 (H-3″), 2,98 (H-4″), 3,49 (H-5″), 1,17 (d, *J* = 6,5 Hz, H-6″). **Qui II**: 4,52 (d, *J* = 8,0 Hz, H-1‴), 3,00 (H-2‴), 3,08 (H-3‴), 2,75 (t, *J* = 9,0 Hz, H-4‴), 3,07 (H-5‴), 0,99 (d, *J* = 6,5 Hz, H-6‴).

**$^{13}$C-NMR** (DMSO-*$d_6$*, 125 MHz) $\delta_C$ (ppm) **Aglycon**: 118,1 (C-2), 150,6 (C-3), 97,1 (C-4), 123,4 (C-5), 185,3 (C-1′), 27,8 (C-2′). **Sugar: Qui I**: 99,5 (C-1″), 81,5 (C-2″), 76,3 (C-3″), 74,6 (C-4″), 71,7 (C-5″), 17,7 (C-6″). **Qui II**: 103,8 (C-1‴), 74,8 (C-2‴), 76,1 (C-3‴), 75,2 (C-4‴), 71,9 (C-5‴), 17,7 (C-6‴).

### 3.2.4. Compound AB4: Astebatherioside D (new)

White power, $[\alpha]_D^{25}$ – 1.0 (*c*, 0.25, MeOH);

FT-ICR-MS *m/z* 733,25355 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_{19}Na$, 733,25310).

**$^1$H-NMR** (DMSO-*$d_6$*, 500 MHz) $\delta_H$ (ppm) **Aglycon**: 6,71 (d, *J* = 2,0 Hz, H-4), 7,82 (d, *J* = 2,0 Hz, H-5), 2,34 (s, H-2′). **Sugar**: **Qui I**: 5,18 (d, *J* = 7,5 Hz, H-1″), 3,73 (H-2″), 3,69 (H-3″), 3,21 (t, *J* = 9,0 Hz, H-4″), 3,72 (H-5″), 1,34 (d, *J* = 6,5 Hz, H-6″). **Qui II**: 4,58 (d, *J* = 8,0 Hz, H-1‴), 2.95 (dd, *J* = 8,0, 8,5 Hz, H-2‴),

3,10 (H-3‴), 2,75 (t, *J* = 8,5 Hz, H-4‴), 3,08 (H-5‴), 1,01 (d, *J* = 6,5 Hz, H-6‴). **Fuc I:** 4,42 (d, *J* = 7,0 Hz, H-1⁗), 3,56 (H-2⁗), 3,55 (H-3⁗), 3,48 (br s, H-4⁗), 3,70 (dd, *J* = 4,0, 12,0 Hz, H-5⁗), 1,15 (d, *J* = 6,0 Hz, H-6⁗). **Fuc II:** 4,31 (d, *J* = 7,5 Hz, H-1′′′′′), 3,32 (H-2′′′′′), 3,32 (H-3′′′′′), 3,40 (br s, H-4′′′′′), 3,55 (H-5′′′′′), 1,13 (d, *J* = 6,0 Hz, H-6′′′′′).

**$^{13}$C-NMR** (DMSO-$d_6$, 125 MHz) $\delta_C$ (ppm) **Aglycon**: 136,9(C-2), 152,2 (C-3), 104,3 (C-4), 147,0 (C-5), 183,2 (C-1′), 27,3 (C-2′).**Sugar: Qui I**: 99,3 (C-1′′), 79,6 (C-2′′), 74,2 (C-3′′), 84,6 (C-4′′), 70,5 (C-5′′), 17,7 (C-6′′). **Qui II**: 103,0 (C-1′′′), 74,0 (C-2′′′), 76,1 (C-3′′′), 75,2 (C-4′′′), 71,6 (C-5′′′), 17,6 (C-6′′′). **Fuc I**: 101,3 (C-1⁗), 73,4 (C-2⁗), 80,4 (C-3⁗), 70,2 (C-4⁗), 70,3 (C-5⁗), 16,2 (C-6⁗). **Fuc II**: 105,2 (C-1′′′′′), 71,9 (C-2′′′′′), 73,2 (C-3′′′′′), 70,9 (C-4′′′′′), 70,3 (C-5′′′′′), 16,4 (C-6′′′′′).

### 3.2.5. Compound AB5: 3-[*O*-*β*-D-fucopyranosyl-(1→3)-*β*-D-fucopyranosyl-(1→4)-[*β*-D-quinovopyranosyl-(1→2)]-*β*-D-quinovopyranosyl]-2-acetyl-pyrrole

White power, $[\alpha]_D^{25}$ + 2 (*c*, 0.25, MeOH);

**$^{1}$H-NMR** (DMSO-$d_6$, 500 MHz) $\delta_H$ (ppm) **Aglycon**: 5,90 (br s, H-4), 6,85 (br s, H-5), 2,34 (s, H-2′), 11,15 (s, NH). **Sugar**: **Qui I**: 4,99 (d, *J* = 6,5 Hz, H-1′′), 3,70 (H-2′′), 3,71 (H-3′′), 3,20 (t, *J* = 9,0 Hz, H-4′′), 3,65 (H-5′′), 1,33 (d, *J* = 5,5 Hz, H-6′′). **Qui II**: 4,61 (d, *J* = 7,5 Hz, H-1‴), 2.95 (dd, *J* = 7,5, 9,0 Hz, H-2‴), 3,11 (H-3‴), 2,75 (t, *J* = 9,0 Hz, H-4‴), 3,10 (H-5‴), 1,05 (d, *J* = 5,5 Hz, H-6‴). **Fuc I:** 4,42 (d, *J* = 7,0 Hz, H-1⁗), 3,57 (H-2⁗), 3,56 (H-3⁗), 3,48 (br s, H-4⁗), 3,70 (dd, *J* = 4,0, 12,0 Hz, H-5⁗), 1,15 (d, *J* = 6,5 Hz, H-6⁗). **Fuc II:** 4,31 (d, *J* = 7,5 Hz, H-1′′′′′), 3,34 (H-2′′′′′), 3,33 (H-3′′′′′), 3,39 (br s, H-4′′′′′), 3,53 (H-5′′′′′), 1,13 (d, *J* = 6,5 Hz, H-6′′′′′).

**$^{13}$C-NMR** (DMSO-$d_6$, 125 MHz) $\delta_C$ (ppm) **Aglycon**: 118,2 (C-2), 150,3 (C-3), 97,1 (C-4), 123,2 (C-5), 185,2 (C-1′), 27,7 (C-2′). **Sugar**: **Qui I**: 99,3 (C-1′′), 79,7 (C-2′′), 78,9 (C-3′′), 84,8 (C-4′′), 70,3 (C-5′′), 17,7 (C-6′′). **Qui II**: 102,9 (C-1‴), 74,6 (C-2‴), 76,1 (C-3‴), 75,2 (C-4‴), 71,6 (C-5‴), 17,6 (C-6‴). **Fuc I**: 101,3 (C-1⁗), 73,4 (C-2⁗), 80,5 (C-3⁗), 70,3 (C-4⁗), 70,3 (C-5⁗), 16,2 (C-6⁗). **Fuc II**: 105,3 (C-1′′′′′), 71,9 (C-2′′′′′), 73,2 (C-3′′′′′), 70,9 (C-4′′′′′), 70,3 (C-5′′′′′), 16,4 (C-6′′′′′).

### 3.2.6. Compound ASP1: Astropectenol A (new)

White power; $[\alpha]_D^{25}$ + 2,6 (*c*, 0,25, MeOH);

FT-ICR-MS *m/z* 441,33458 $[M+Na]^+$ (calcd for $C_{27}H_{46}O_3Na$, 441,33447);

$^1$H-NMR **and** $^{13}$C-NMR **(see table 4.2.3 and discussion)**

### 3.2.7. Compound ASP2: Astropectenol B (new)

White powder; $[\alpha]_D^{25}$ –3,4 (*c*, 0,25, MeOH);

FT-ICR-MS *m/z* 455,31369 $[M+Na]^+$ (calcd for $C_{27}H_{44}O_4Na$, 455,31373);

**$^1$H-NMR** (DMSO-$d_6$, 500 MHz) $\delta_H$ (ppm): 1,05 (m, $H_a$-1)/1,60 (m, $H_b$-1), 1,18 (m, $H_a$-2)/1,65 (m, $H_b$-2), 3,37 (m, H-3), 1,17 (m, $H_a$-4)/1,53 (m, $H_b$-4), 1,32 (m, H-5), 1,15 (m, $H_a$-6)/1,44 (m, $H_b$-6), 4,35 (dd, *J* = 5,5, 11,0 Hz, H-7), 1,62 (m, H-9), 1,22 (m, $H_a$-11)/1,57 (m, $H_b$-11), 1,06 (m, $H_a$-12)/1,82 (m, $H_b$-12), 1,60 (m, $H_a$-16)/1,85 (m, $H_b$-16), 1,17 (m, H-17), 1,04 (s, H-18), 0,65 (s, H-19), 1,44 (m, H-20), 0,87 (d, *J* = 6,5 Hz, H-21), 0,95 (m, $H_a$-22)/1,25 (m, $H_b$-22), 1,14 (m, $H_a$-23)/1,25 (m, $H_b$-23), 1,10 (m, H-24), 1,50 (m, H-25), 0,84 (d, *J* = 6,5 Hz, H-26), 0,82 (d, *J* = 6,5 Hz, $H_b$-27), 4,54 (s, 3-OH), 6,35 (15-OH).

**$^{13}$C-NMR** (DMSO-$d_6$, 125 MHz) $\delta_C$ (ppm): 36,0 (C-1), 31,1 (C-2), 69,0 (C-3), 37,7 (C-4), 40,0 (C-5), 30,9 (C-6), 76,0 (C-7), 126,3 (C-8), 45,2 (C-9), 36,6 (C-10), 19,3 (C-11), 38,2 (C-12), 42,0 (C-13), 139,3 (C-14), 101,6 (C-15), 40,2 (C-16), 55,6 (C-17), 17,8 (C-18), 12,5 (C-19), 34,7 (C-20), 18,4 (C-21), 34,9 (C-22), 23,0 (C-23), 38,8 (C-24), 27,3 (C-25), 22,3 (C-26), 22,6 (C-27).

### 3.2.8. Compound ASP3: Astropectenol C (new)

White power, $[\alpha]_D^{25}$ + 4,2 (*c*, 0,25, MeOH);

FT-ICR-MS *m/z* 415,32125 $[M+H]^+$ (calcd for $C_{27}H_{43}O_3$, 415,32122);

**$^1$H-NMR** ($CDCl_3$, 500 MHz) $\delta_H$ (ppm): 1,55 (m, $H_a$-1)/1,73 (m, $H_b$-1), 1,48 (m, $H_a$-2)/1,90 (m, $H_b$-2), 3,62 (m, H-3), 1,19 (m, $H_a$-4)/1,68 (m, $H_b$-4), 1,73 (m, H-5), 1,96 (m, $H_a$-6)/2,44 (dd, *J* = 7,0, 18,0 Hz, $H_b$-6), 1,98(m, H-11), 1,28 (m, $H_a$-12)/2,32 (m, $H_b$-12), 6,23 (dd, *J* = 1,5, 3,5 Hz, H-15), 2,10 (ddd, *J* = 1,5, 7,5, 10,0 Hz, $H_a$-16)/2,36 (m, $H_b$-16), 1,45 (m, H-17), 0,77 (s, H-18), 0,97 (s, H-19), 1,55 (m, H-20), 0,90 (d, *J* = 6,5 Hz, H-21), 1,03 (m, $H_a$-22)/1,33 (m, $H_b$-22), 1,10 (m, $H_a$-23)/1,31 (m, $H_b$-23), 1,05 (m, $H_a$-24), 1,10 (m, $H_b$-24), 1,48 (m, H-25), 0,84 (d, *J* = 6,5 Hz, H-26), 0,82 (d, *J* = 6,5 Hz, $H_b$-27).

**$^{13}$C-NMR** ($CDCl_3$, 125 MHz) $\delta_C$ (ppm): 33,0 (C-1), 31,5 (C-2), 70,3 (C-3), 37,6 (C-4), 33,3 (C-5), 41,9 (C-6), 205,4 (C-7), 62,2 (C-8), 73,3 (C-9), 37,0 (C-10), 20,9 (C-11), 33,2 (C-12), 45,0 (C-13), 139,5 (C-14), 134,8 (C-15), 36,8 (C-16), 56,5 (C-17), 16,3 (C-18), 16,0 (C-19), 34,5 (C-20), 19,5 (C-21), 36,5 (C-22), 24,1 (C-23), 39,9 (C-24), 28,5 (C-25), 22,0 (C-26), 23,3 (C-27).

### 3.2.9. Compound ASP4: Astropectenol D (new)

White power, $[\alpha]_D^{25}$ + 1,8 (*c*, 0,25, MeOH);

FT-ICR-MS *m/z* 425,33996 $[M+Na]^+$ (calcd for $C_{27}H_{46}O_2Na$, 425,33955);

**$^1$H-NMR** ($CDCl_3$, 500 MHz) $\delta_H$ (ppm): 1,43 (m, $H_a$-1)/1,76 (m, $H_b$-1), 1,37 (m, $H_a$-2)/1,83 (m, $H_b$-2), 3,57 (m, H-3), 1,25 (m, $H_a$-4)/1,78 (m, $H_b$-4), 1,78 (m, H-5), 1,23 (m, $H_a$-6)/1,67 (m, $H_b$-6), 5,25 (dt, *J* = 2,0, 5,0 Hz, H-7), 1,55 (m, $H_a$-11)/1,81 (m, $H_b$-11), 1,53 (m, $H_a$-12)/1,84 (m, $H_b$-12), 2,29 (m, H-14), 1,35 (m, $H_a$-15)/1,51 (m, $H_b$-15), 1,25 (m, H-16), 1,27 (m, H-17), 0,53 (s, H-18), 0,88 (s, H-19), 1,52 (m, H-20), 0,91 (d, *J* = 6,5 Hz, H-21), 0,98 (m, $H_a$-22)/1,32 (m, $H_b$-22), 1,11 (m, $H_a$-23)/1,31 (m, $H_b$-23), 1,10 (m, H-24), 1,51 (m, H-25), 0,85 (d, *J* = 6,5 Hz, H-26), 0,83 (d, *J* = 6,5 Hz, $H_b$-27).

**$^{13}$C-NMR** ($CDCl_3$, 125 MHz) $\delta_C$ (ppm): 30,0 (C-1), 32,0 (C-2), 71,1 (C-3), 38,5 (C-4), 33,7 (C-5), 30,4 (C-6), 121,9 (C-7), 141,1 (C-8), 74,4 (C-9), 39,4 (C-10), 27,7 (C-11), 36,3 (C-12), 44,2 (C-13), 51,6 (C-14), 23,6 (C-15), 28,5 (C-16), 56,5 (C-17), 11,6 (C-18), 15,6 (C-19), 36,7 (C-20), 19,3 (C-21), 36,6 (C-22), 24,4 (C-23), 40,0 (C-24), 28,5 (C-25), 23,1 (C-26), 23,3 (C-27).

### 3.2.10. CompoundASP5: 5*α*-cholest-7-ene-3*β*,6*α*-diol

Whiter power, $[\alpha]_D^{25}$ + 45 (*c*, 0.25, MeOH);

**$^1$H-NMR (**$CDCl_3$, 500 MHz) $\delta_H$ (ppm): 1,09 (m, $H_a$-1)/1,21 (m, $H_b$-1), 1,75 (m, $H_a$-2)/1,37 (m, $H_b$-2), 3,53 (m, H-3), 1,22 (m, $H_a$-4)/2,20 (m, $H_b$-4), 1,65 (m, H-5), 3,75 (br d, *J* = 7,0 Hz, H-6), 5,13 (br s, H-7), 1,21 (m, H-9), 1,56 (m, $H_a$-11)/1,42 (m, $H_b$-11), 1,07 (m, $H_a$-12)/2,01 (m, $H_b$-12), 1,79 (m, H-14), 1,11 (m, $H_a$-15)/1,31 (m, $H_b$-15), 1,49 (m, $H_a$-16)/1,25 (m, $H_b$-16), 1,18 (m, H-17), 0,51 (s, H-18), 0,79 (s, H-19), 1,31 (m, H-20), 0,89 (d, *J* = 7,0 Hz, H-21), 1,30 (m, $H_a$-22)/0,96 (m, $H_b$-22), 1,54 (m, $H_a$-23)/1,36 (m, $H_b$-23), 1,10 (m, $H_a$-24)/1,73 (m, $H_b$-24), 1,86 (m, H-25), 0,84 (d, *J* = 7,0 Hz, H-26), 0,83 (d, *J* = 7,0 Hz, $H_b$-27).

**$^{13}$C-NMR** ($CDCl_3$, 125 MHz) $\delta_C$ (ppm): 39,7 (C-1), 31,2 (C-2), 70,9 (C-3), 34,1 (C-4), 48,9 (C-5), 70,1 (C-6), 122,4 (C-7), 141,5 (C-8), 49,5 (C-9), 35,5 (C-10), 21,7 (C-11), 39,8 (C-12), 43,9 (C-13), 55,1 (C-14), 24,2 (C-15), 28,3 (C-16), 56,5 (C-17), 12,3 (C-18), 14,3 (C-19), 36,5 (C-20), 19,2 (C-21), 36,4 (C-22), 23,2 (C-23), 37,5 (C-24), 28,3 (C-25), 22,9 (C-26), 23,2 (C-27).

### 3.2.11. Compound ASP6: 5*α*-cholest-8(14)-ene-3*β*,7*α*-diol

White power, $[\alpha]_D^{25}$ – 85 (*c*, 0.25, MeOH);

**$^{1}$H-NMR** ($CDCl_3$, 500 MHz) $\delta_H$ (ppm): 1,60 (m, $H_a$-1)/1,63 (m, $H_b$-1), 1,30 (m, $H_a$-2)/1,67 (m, $H_b$-2), 3,57 (m, H-3), 1,18 (m, $H_a$-4)/1,54 (m, $H_b$-4), 1,69 (m, H-5), 1,34 (m, $H_a$-6)/1,46 (m, $H_b$-6), 4,45 (br s, H-7), 2,02 (m, H-9), 1,41 (m, $H_a$-11)/1,58 (m, $H_b$-11), 1,09 (m, $H_a$-12)/1,58 (m, $H_b$-12), 1,31 (m, $H_a$-15)/2,27 (m, $H_b$-15), 1,34 (m, $H_a$-16)/1,81 (m, $H_b$-16), 1,11 (m, H-17), 0,79 (s, H-18), 0,60 (s, H-19), 1,41 (m, H-20), 0,88 (d, *J* = 6,0 Hz, H-21), 1,03 (m, $H_a$-22)/1,10 (m, $H_b$-22), 1,08 (m, $H_a$-23)/1,31 (m, $H_b$-23), 1,07 (m, $H_a$-24)/1,08 (m, $H_b$-24), 1,47 (m, H-25), 0,80 (d, *J* = 7,0 Hz, H-26), 0,82 (d, *J* = 7,0 Hz, $H_b$-27).

**$^{13}$C-NMR** ($CDCl_3$, 125 MHz) $\delta_C$ (ppm): 36,8 (C-1), 31,8 (C-2), 71,4 (C-3), 38,3 (C-4), 37,7 (C-5), 36,1 (C-6), 67,2 (C-7), 128,9 (C-8), 44,7 (C-9), 37,3 (C-10), 19,9 (C-11), 37,3 (C-12), 43,5 (C-13), 148,7 (C-14), 25,6 (C-15), 27,4 (C-16), 57,0 (C-17), 18,5 (C-18), 12,5 (C-19), 34,9 (C-20), 19,6 (C-21), 36,5 (C-22), 24,3 (C-23), 40,1 (C-24), 28,5 (C-25), 23,3 (C-26), 23,1 (C-27).

### 3.2.12. Compound ASP7: 5*α*-cholest-7,9(11)-diene-3*β*-ol

White power, $[\alpha]_D^{25}$ + 15 (*c*, 0.25, MeOH);

**$^{1}$H-NMR** ($CDCl_3$, 500 MHz) $\delta_H$ (ppm): 1,94 (m, $H_a$-1)/1,34 (m, $H_b$-1), 1,47 (m, $H_a$-2)/1,87 (m, $H_b$-2), 3,58 (m, H-3), 1,29 (m, $H_a$-4)/1,71 (m, $H_b$-4), 1,40 (m, H-5), 1,88 (m, $H_a$-6)/1,49 (m, $H_b$-6), 5,35 (br s, H-7), 5,44 (d, *J* = 6,5 Hz, H-11), 2,28 (m, $H_a$-12)/2,09 (m, $H_b$-12), 2,14 (m, H-14), 1,11 (m, $H_a$-15)/1,33 (m, $H_b$-15), 1,49 (m, $H_a$-16)/1,34 (m, $H_b$-16), 1,23 (m, H-17), 0,49 (s, H-18), 0,88 (s, H-19), 1,33 (m, H-20), 0,90 (d, *J* = 7,0 Hz, H-21), 1,37 (m, $H_a$-22)/0,99 (m, $H_b$-22), 1,73 (m, $H_a$-23)/1,36 (m, $H_b$-23), 1,12 (m, $H_a$-24)/1,08 (m, $H_b$-24), 1,50 (m, H-25), 0,84 (d, *J* = 7,0 Hz, H-26), 0,83 (d, *J* = 7,0 Hz, $H_b$-27).

**$^{13}$C-NMR** ($CDCl_3$, 125 MHz) $\delta_C$ (ppm): 35,3 (C-1), 32,2 (C-2), 71,6 (C-3), 38,4 (C-4), 39,8 (C-5), 29,9 (C-6), 120,9 (C-7), 136,9 (C-8), 144,5 (C-9), 36,3 (C-10), 119,2 (C-11), 42,9 (C-12), 42,9 (C-13), 52,2 (C-14), 24,5 (C-15), 29,0 (C-16),

56,9 (C-17), 11,8 (C-18), 20,1 (C-19), 36,7 (C-20), 19,0 (C-21), 36,7 (C-22), 23,8 (C-23), 40,1 (C-24), 28,6 (C-25), 22,4 (C-26), 23,2 (C-27).

### 3.3. Biological activity

#### 3.3.1. Cytotoxic activity assay

These assay were carried at College of Pharmacy, Chungnam National University, Korea.

#### 3.3.2. Anti-inflammatory effects

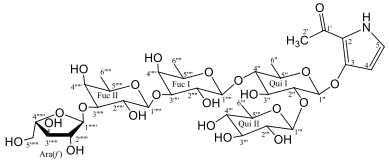
These assay were carried at College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea.

## CHAPTER 4. RESULTS AND DISCUSSIONS

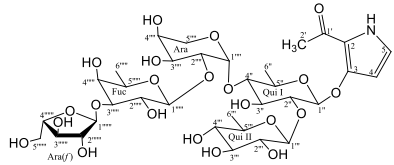
### 4.1. Isolated procedure

Summary isolation of 12 compounds from *A. batheri* and *A. polyacanthus* is shown in Figure 4.1.1 and 4.1.2.

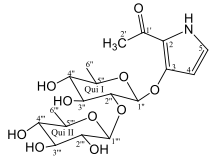
Figure 4.1.1. Structure of compounds were isolated from *A. batheri*



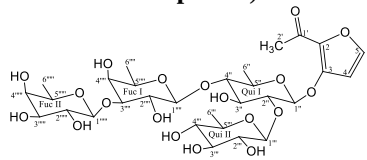
**AB1: Astebatherioside A (new compound)**



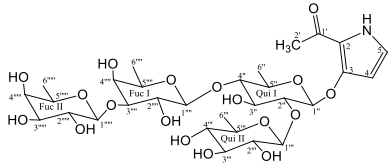
**AB2: Astebatherioside B (new compound)**



**AB3: Astebatherioside C (new compound)**

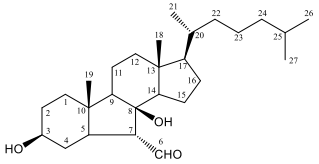


**AB4: Astebatherioside D (new compound)**

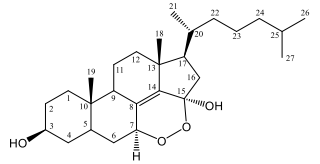


**AB5: 3-[O-$\beta$-D-fucopyranosyl-(1→3)-$\beta$-D-fucopyranosyl-(1→4)-[$\beta$-D-quinovopyranosyl-(1→2)]-$\beta$-D-quinovopyranosyl]-2-acetyl-pyrrole**

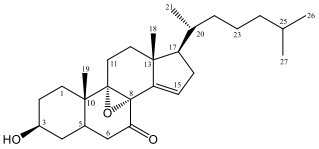
Figure 4.1.2. Structure of compounds were isolated from *A. polyacanthus*



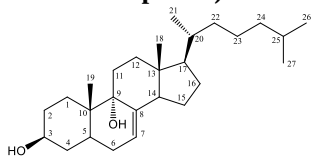
**ASP1: Astropectenol A (new compound)**



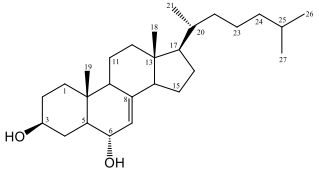
**ASP2: Astropectenol B (new compound)**



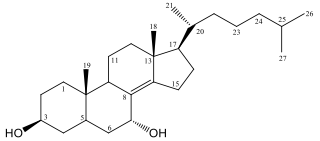
**ASP3: Astroptectenol C (new compound)**



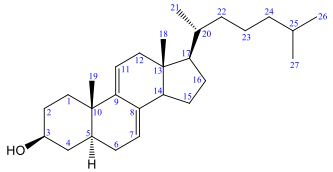
**ASP4: Astroptectenol D (new compound)**



**ASP5: 5*α*-cholest-7-ene-3*β*,6*α*-diol**



**ASP6: -cholest-8(14)-ene-3*β*,7*α*-diol**



**ASP7: 5*α*-cholest-7,9(11)-diene-3*β*-ol 5*α***

## 4.2. Chemical structure of compounds

This section presents the detailed results of spectral analysis and structure determination of 12 compounds isolated from *A. batheri* and *A. polyacanthus* including 8 new compounds and 4 known compounds.

Below details the method for determining the structure of the two new compounds **AB1** and **ASP1.**

- **Compound AB1 (Astebatherioside A)**

Astebatherioside A was obtained as a white powder. Its molecular formula was defined as $C_{35}H_{55}O_{22}N$ by a pseudo-molecular ion peak at m/z842.32887 $[M+H]^+$

(calcd for $C_{35}H_{56}O_{22}N$, 842.32940) in the Fourier transform ion cyclotron resonance mass spectrum (FTICRMS). The $^{1}H$ NMR exhibited typical signals corresponding to one acetoxyl methyl [$\delta_H$ 2.34 (3H, s, H-2')], one amine [$\delta_H$ 11.15 (1H, s, NH)], and two olefinic [$\delta_H$ 5.90 (H-4) and 6.85 (H-5); each 1H, br s] protons suggesting the presence of a pyrrole aglycon with an acetyl substitute. This was confirmed by carbon signals of the aglycon at $\delta_C$ 118.2 (C, C-2), 150.3 (C, C-3), 97.2 (CH, C-4),123.2 (CH, C-5), 185.2 (C, C-1'), and 27.7 ($CH_3$, C-2') and by correlation spectroscopy (COSY) and heteronuclear multiple-bond correlation spectroscopy (HMBC, see Figure 2). Moreover, five anomeric carbon signals were observed at $\delta_C$ 99.3 (C-1''), 102.9 (C-1'''), 101.3(C-1''''), 105.1 (C-1'''''), and 109.0 (C-1''''''), which correlated with anomeric protons at $\delta_H$ 4.98 (1H, d, *J* = 7.0 Hz, H-1''), 4.61 (1H, d, *J* = 7.5 Hz, H-1'''), 4.41 (1H, d, *J* = 6.5 Hz, H-1''''), 4.38 (1H, d, *J* = 6.5 Hz, H-1'''''), and 5.02 (1H, br s, H-1''''''), respectively, in heteronuclear single quantum coherence spectroscopy (HSQC), indicating the presence of five sugar moieties. In the $^{1}H$ NMR spectrum, four secondary methyl groups (each d, *J* = 6.0 Hz) were observed at $\delta_H$ 1.33 (3H, H-6''), 1.05 (3H, H-6'''), and 1.14 (6H, H-6'''' and H-6''''') suggesting the presence of four quinovose and/or fucose moieties. The $^{1}H$ and $^{13}C$ NMR data of AB1 were identical to those of 3-[*O*-*β*-D-fucopyranosyl-(1→3)-*β*-D-fucopyranosyl-(1→4)-[*β*-D-quinovopyranosyl-(1→2)]-*β*-D-quinovopyranosyl]-2-acetyl-pyrrole (Zhang *et al.*, 2006), except for additional signals of a sugar moiety. The $^{13}C$ NMR data of the additional sugar at $\delta_C$ 109.0 (CH, C-1''''''), 81.3 (CH, C-2''''''), 77.5 (CH, C-3''''''), 85.2 (CH, C-4''''''), and 61.7 ($CH_2$, C-5''''''), and the small coupling constant (J ~ 0 Hz) of the anomeric proton H-1'''''' at $\delta_H$ 5.02 indicated an arabinofuranosyl moiety and a-glycosidic linkage.

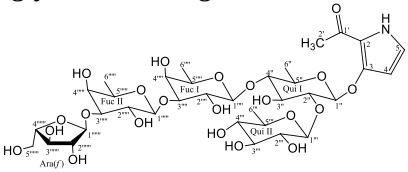
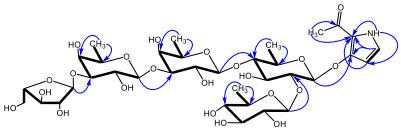



Figure 4.2.1: Structure of AB1



Hình4.2.2: Key HMBC and COSY correlations of AB1

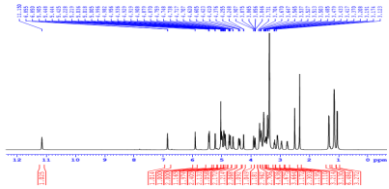


Figure 4.2.4: $^{1}H$ NMR spectrum of AB1

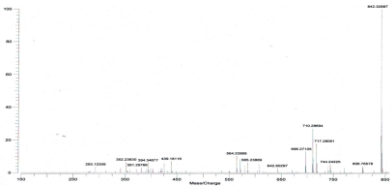


Figure 4.2.3: FT-ICR-MS spectrum of AB1

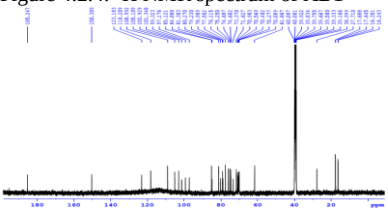


Figure 4.2.5: $^{13}C$ NMR spectrum of AB1

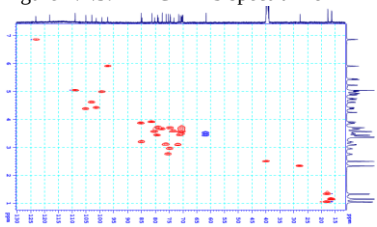


Figure 4.2.6: HSQC spectrum of AB1

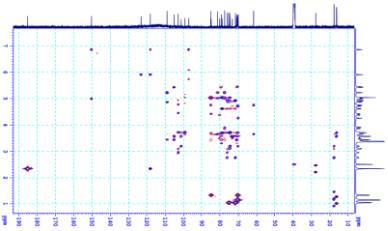


Figure 4.2.7: HMBC spectrum of AB1

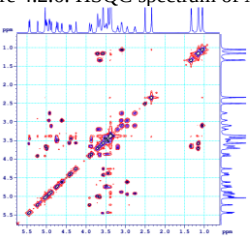


Figure 4.2.8: COSY spectrum of AB1

Detailed analyses of correlations in the $^{1}H$–$^{1}H$ COSY spectrum allowed the assignment of proton positions for all of the sugar moieties. These data combined with the HMBC correlations between H-1" ($\delta_H$ 4.98) and C-3 ($\delta_C$ 150.3), H-1'" ($\delta_H$ 4.61) and C-2" ($\delta_C$ 78.9), H-1"" ($\delta_H$ 4.41) and C-4" ($\delta_C$ 84.9), and H-1""" ($\delta_H$ 4.38) and C-3"" ($\delta_C$ 80.3) indicated that the two quinovose and two fucose moieties were attached at the same positions as those in5. The carbon signal C-3"" of AB1 at $\delta_C$ 79.2 was strongly shifted downfield relative to that of 5at $\delta_C$ 73.1 (C-3"") suggesting the attached position of the arabinofuranose moiety at this carbon pyrrole (Zhang *et al.*, 2006). This was further confirmed by an HMBC correlation between H-1"""($\delta_H$ 5.02) and C-3"" ($\delta_C$ 79.2). Consequently, the structure of AB1was elucidated as 3-[O-*α*-L-arabinofuranosyl-(1→3)-*β*-D-fucopyranosyl-(1→3)-*β*-D-fucopyranosyl-(1→4)-[*β*-D-quinovopyranosyl-(1→2)]-*β*-D-quinovopyranosyl]-2-acetyl-pyrrole, which was named astebatherioside A.

*Table 4.2.2. NMR spectroscopic data of AB1*

| C | [a]$\delta_C$ | $\delta_C$[b.c] | $\delta_H$[b.d], mult. (*J in* Hz) | HMBC |
|---|---|---|---|---|
| *Aglycon* | | | | |
| 2 | 118.0 | 118.2 | - | |
| 3 | 150.3 | 150.3 | - | |
| 4 | 97.1 | 97.2 | 5.90 br s | 2, 5 |
| 5 | 123.1 | 123.2 | 6.85 br s | 2, 3, 4 |
| 1' | 185.2 | 185.2 | - | |
| 2' | 27.9 | 27.7 | 2.34 s | 2, 1' |
| NH | | | 11.15 s | 2, 3, 4 |
| *Qui I* | | | | |
| 1" | 99.3 | 99.3 | 4.98 d (7.0) | 3 |
| 2" | 74.6 | 78.9 | 3.70 | |
| 3" | 78.8 | 74.6 | 3.70 | |
| 4" | 85.0 | 84.9 | 3.20 t (9.0) | |
| 5" | 70.2 | 70.3 | 3.65 | |
| 6" | 17.7 | 17.7 | 1.33 d (6.0) | 4", 5" |
| *Qui II* | | | | |
| 1"' | 102.9 | 102.9 | 4.61 d (7.5) | 2" |
| 2"' | 74.6 | 74.7 | 2.95 dd (7.5. 9.0) | |
| 3"' | 76.0 | 76.1 | 3.11 | |
| 4"' | 75.1 | 75.2 | 2.76 t (9.0) | |
| 5"' | 71.6 | 71.6 | 3.10 | |
| 6"' | 17.7 | 17.6 | 1.05 d (6.0) | 4"', 5"' |
| *Fuc I* | | | | |
| 1"" | 101.4 | 101.3 | 4.41 d (6.5) | 4" |
| 2"" | 73.3 | 73.4 | 3.56 | |
| 3"" | 80.5 | 80.3 | 3.56 | |
| 4"" | 70.3 | 70.4 | 3.65 | |
| 5"" | 70.3 | 70.3 | 3.70 | |
| 6"" | 16.3 | 16.2 | 1.14 d (6.0) | 4"", 5"" |
| *Fuc II* | | | | |
| 1""' | 105.4 | 105.1 | 4.38 d (6.5) | 3"" |
| 2""' | 71.9 | 71.0 | 3.43 | |
| 3""' | **73.1** | **79.2** | 3.43 | |
| 4""' | 70.9 | 70.6 | 3.48 | |
| 5""' | 70.2 | 70.1 | 3.56 | |
| 6""' | 16.5 | 16.3 | 1.14 d (6.0) | 4""', 5""' |
| Ara(f) | | | | |
| 1""'' | | 109.0 | 5.02 br s | 3""' |
| 2""'' | | 81.3 | 3.91 br s | |
| 3""'' | | 77.5 | 3.65 | |
| 4""'' | | 85.2 | 3.86 dd (5.0. 9.0) | |
| 5""'' | | 61.7 | 3.43/3.51 | |

[a]$\delta_C$ of 3-[*O*-*β*-D-fucopyranosyl-(1→3)-*β*-D-fucopyranosyl-(1→4)-[*β*-D-quinovopyranosyl-(1→2)]-*β*-D-quinovopyranosyl]-2-acetyl-pyrrole(Zhang *et al*., 2006), [b]recorded in DMSO-$d_6$, [c]125 MHz, [d]500 MHz

- **Compound ASP1 (astropectenol A)**

Astropectenol A (ASP1) was isolated as a white powder with the molecular formula of $C_{27}H_{46}O_3$, identified by a pseudomolecular ion peak at m/z 441.33458 $[M+Na]^+$ in the Fourier transform ion cyclotron resonance mass spectrum (FT-ICRMS). The $^1$H- and $^{13}$C-NMR features were typical for a steroidal compound. The presence of an oxymethine and an aldehyde groups was confirmed by proton signals at $\delta_H$ 3.70(1H, m, H-3) and 9.81 (1H, d, $J$ = 3.0 Hz, H-6), respectively. Moreover, two tertiary methyl [$\delta_H$ 0.84 (H-18) and 0.73 (H-19), each 3H, s] and three secondary methyl [$\delta_H$ 0.87 (H-21), 0.84 (H-26), and 0.82 (H-27), each 3H, d, $J$ = 6.5 Hz] groups were observed on $^1$H-NMR spectrum suggesting for the presence of a cholesterol-like side chain.

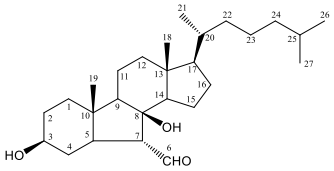



Figure 4.2.9: Chemical structure of AB1

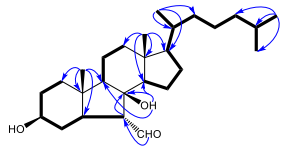



Figure 4.2.10: Key HMBC and COSY correlations of ASP1

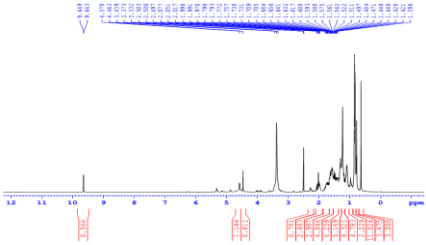


Figure 4.2.12: $^1$H NMR spectrum of ASP1

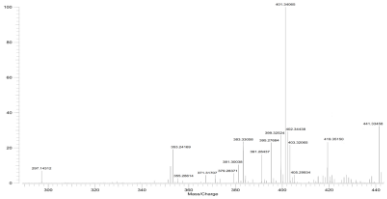


Figure 4.2.11: FT-ICR-MS spectrum of ASP1

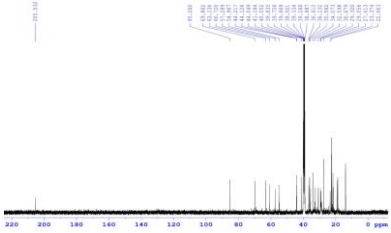


Figure 4.2.13: $^{13}$C NMR spectrum of ASP1

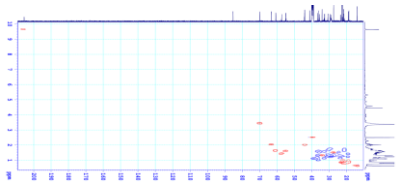


Figure 4.2.14: HSQC spectrum of ASP1

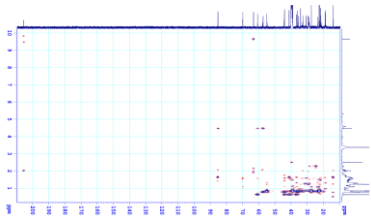


Figure 4.2.15: HMBC spectrum of ASP1

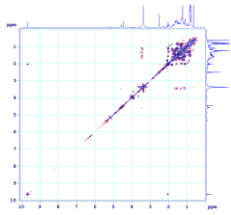


Figure 4.2.16: COSY spectrum of ASP1

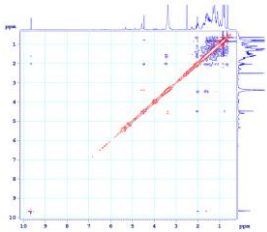


Figure 4.2.17: ROESY spectrum of ASP1

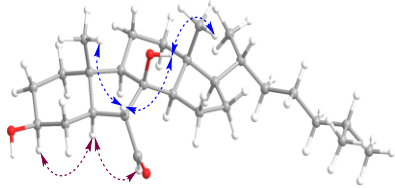


Figure 4.2.18 Key ROESY correlations of ASP1

The $^{13}$C-NMR spectrum (in $CDCl_3$) revealed 27 signals including one oxymethine [$\delta_C$ 71.8 (CH, C-3)], one aldehyde [$\delta_C$ 206.3 (CH, C-6)], and one oxygenated quaternary [$\delta_C$ 87.4 (C, C-8)] carbons. All carbons were assigned to relevant protons by an heteronuclear single quantum coherence (HSQC) experiment (Table 4.2.3). The $^1$H-and $^{13}$C-NMR data of ASP1 were similar to those of 8*β*-hydroxy-*B*-norconicasta-6*α*-aldehyde (Zhang *et al.*, 2010) suggesting for the placement of the aldehyde group at C-6 and hydroxy groups at C-3 and C-8. To confirm structure of ASP1, the 1D- and 2D-NMR spectra were recorded again in DMSO-$d_6$. The $^1$H–$^1$H correlation spectroscopy (COSY) experiment (in DMSO-$d_6$) allowed to assign the proton-proton correlations of $H_2$-1/$H_2$-2/H-3/$H_2$-4/$H_2$-5/H-7/H-6, H-9/$H_2$-11/$H_2$-12, H-14/$H_2$-15/$H_2$-16/H-17/H-20/$H_3$-21, H-20/$H_2$-22/$H_2$-23/$H_2$-24/H-25/$H_3$-26, and H-25/$H_3$
-27. These data and the heteronuclear multiple bond connectivity (HMBC) correlations (in DMSO-$d_6$) between H-18 ($\delta_H$ 0.77) and C-12 ($\delta_C$ 35.6)/C-13 ($\delta_C$ 44.1)/C-14 ($\delta_C$ 57.3)/C-17 ($\delta_C$ 55.0), H-19 ($\delta_H$ 0.63) and C-1 ($\delta_C$ 36.6)/C-5 ($\delta_C$ 44.0)/C-9 ($\delta_C$ 60.7)/C-10 ($\delta_C$ 41.2), H-7 ($\delta_H$ 2.02)/C-8 ($\delta_C$ 85.2), and 8-OH ($\delta_H$ 4.46) and C-8 ($\delta_C$ 85.2)/C-9 ($\delta_C$ 60.7)/C-14 ($\delta_C$ 57.3), clearly confirmed the planar structure of ASP1(Fig. 4.2.9).

Table 4.2.3. NMR spectrum data of **ASP1**

| **C** | **[a]$\delta_C$** | **$\delta_C$ [b.c]** | **$\delta_C$ [c.e]** | **$\delta_H$ [d.e]** mult. ($J$ = Hz) | **HMBC** (H → C) |
|---|---|---|---|---|---|
| 1 | | 37.3 | 36.6 | 1.20 m/1.59 m | |
| 2 | | 31.3 | 30.7 | 1.20 m/1.65 m | |
| 3 | | 71.8 | 69.8 | 3.43 m | |
| 4 | | 33.0 | 32.3 | 1.12 m/1.54 m | |
| 5 | 51.9 | 46.0 | 44.0 | 2.00 m | |
| 6 | 205.0 | 206.3 | 205.5 | 9.64 d (3.0) | 7 |
| 7 | 60.1 | 63.6 | 63.2 | 2.02 dd (11.0. 3.0) | 8 |
| 8 | 87.2 | 87.4 | 85.2 | - | |
| 9 | 62.9 | 63.3 | 60.7 | 1.62 m | |
| 10 | 44.6 | 42.0 | 41.2 | - | |
| 11 | 19.7 | 19.7 | 18.9 | 1.22 m/1.40 m | |
| 12 | 36.4 | 37.0 | 35.6 | 1.50 m/1.57 m | |
| 13 | 44.8 | 45.2 | 44.1 | - | |
| 14 | 58.1 | 58.2 | 57.3 | 1.45 m | |
| 15 | 22.0 | 22.7 | 21.5 | 1.52 m/1.58 m | |
| 16 | 29.6 | 30.1 | 29.3 | 1.20 m/1.75 m | |
| 17 | 56.9 | 57.3 | 55.0 | 1.60 m | |
| 18 | 22.0 | 22.5 | 21.7 | 0.77 s | 12, 13, 14, 17 |
| 19 | 14.9 | 14.6 | 13.9 | 0.63 s | 1, 5, 9, 10 |
| 20 | 35.0 | 35.3 | 34.1 | 1.32 m | |
| 21 | 18.6 | 19.1 | 18.6 | 0.86 d (6.5) | 17, 20, 22 |
| 22 | | 36.9 | 36.1 | 0.98 m/1.31 m | |
| 23 | | 24.5 | 23.4 | 1.15 m/1.30 m | |
| 24 | | 39.9 | 39.7 | 1.10 m | |
| 25 | | 28.5 | 27.4 | 1.50 m | |
| 26 | | 23.0 | 22.3 | 0.84 d (6.5) | 24, 25, 27 |
| 27 | | 23.3 | 22.6 | 0.82 d (6.5) | 24, 25, 26 |
| 8-OH | | | - | 4.46 s | 8, 9, 14 |

[a]$\delta_C$ of 8$\beta$-hydroxy-*B*-norconicasta-6$\alpha$-aldehyde (Zhang *et al.*, 2010), [b]recorded in $CDCl_3$, [c]125 MHz, [d]500 MHz, [e]recorded in DMSO-$d_6$.

The $^{13}$C-NMR chemical shift of C-3 (in $CDCl_3$) at $\delta_C$ 71.8 was indicative for the $\beta$-orientation of the hydroxyl group at C-3 (Miyaoka *et al.*, 1997). The relative configurations at C-7 and C-8 of ASP1was suggested to be the same as those of 8$\beta$-hydroxy-*B*-norconicasta-6$\alpha$-aldehyde (Zhang *et al.*, 2010) by the agreement of $^1$H- and $^{13}$C-NMR data between the two compounds and further confirmed by rotating-frame Overhauser spectroscopy (ROESY) in DMSO-$d_6$. The ROESY correlations (Fig. 3) between H-19 ($\delta_H$ 0.63) and H-7 ($\delta_H$ 2.02) and 8-OH ($\delta_H$ 4.46)

and H-7 ($\delta_H$ 2.02)/H-18 ($\delta_H$ 0.77) suggested the same *β*-orientation of H-7 and 8-OH. In addition, proton H-5 ($\delta_H$ 2.00) had correlations with H-3 ($\delta_H$ 3.43) and H-6 ($\delta_H$ 9.64) indicating that H-3, H-5,and H-6 were all *α*-orientation. Thus, the structure 3*β*,8*β*-dihydroxy-*B*-norcholest-7*α*-carboxaldehyde of astropectenol A (ASP1) was elucidated.

### 4.3. Biological activity

#### 4.3.1. Cytotoxic activity

The cytotoxic effects of compounds (1–7) and the $CH_2Cl_2$ fraction of *A. polyacanthus* were tested for cytotoxic effects against HL-60, PC-3, and SNU-C5 cancer cells using MTT assay for 72 h (see Table 4.3.1). On the HL-60 cells, compound ASP7 and the $CH_2Cl_2$ fraction showed potent activity ($IC_{50}$=2.70 µM and 8.29 µg/mL); whereas ASP1, ASP3-ASP5 were moderate active ($IC_{50}$: 27.85–39.12 µM) and ASP2 and ASP6 were inactive ($IC_{50}$>100 µM); comparing to the positive control, mitoxantrone ($IC_{50}$=6.80 µM). Due to the potent cytotoxic activity, we next investigated whether the inhibitory effects of the $CH_2Cl_2$ fraction and compound 7on HL-60 cells might arise from the induction of apoptosis

Table 4.3.1. Effects of compounds on the growth of human cancer cell lines

| **Compound** | **$IC_{50}$ values on the cell lines (µM)** | | |
|---|---|---|---|
| | HL-60 (leukemia cells) | PC-3 (prostate cancer cells) | SNU-C5 (colorectal cancer cells) |
| **ASP1** | 27.91 ± 1.37 | 31.29 ± 0.75 | 45.49 ± 7.40 |
| **ASP2** | (-) | (-) | (-) |
| **ASP3** | 39.12 ± 3.11 | 64.10 ± 0.41 | (-) |
| **ASP4** | 27.85 ± 1.70 | 41.19 ± 2.64 | 74.45 ± 3.15 |
| **ASP5** | 28.99 ± 1.19 | 82.95 ± 5.02 | (-) |
| **ASP6** | (-) | (-) | (-) |
| **ASP7** | **2.70 ± 0.05** | 27.28 ± 1.57 | (-) |
| **ASP-C[b]** | **8.29 ± 0.20** | 25.42 ± 0.84 | (-) |
| **Mitoxantrone[a]** | 6.80 ± 0.90 | 5.17 ± 0.34 | 17.96 ± 4.40 |

[a] positive control, [b]diclometane extract (µg/ml)

### 4.3.2. The inhibitory effects of the $CH_2Cl_2$ fraction and compound ASP7 on HL-60 cells might arise from the induction of apoptosis

Apoptotic characteristics were examined after the HL-60 cells were treated with $CH_2Cl_2$ fraction (1, 10 μg/mL) and compound ASP7(1, 10 μM) for 24 h. Flow cytometric analysis showed that the percentage of sub-G1 hypodiploid cells exposed to $CH_2Cl_2$ fraction (10 μg/mL) and compound 7(10 μM) increased to 17.21 and 43.42%, respectively. These results indicated that the $CH_2Cl_2$ fraction and compound ASP7 induced the apoptosis of HL-60 cells, which was supported by the increase in the number of apoptotic bodies found in the sample-treated cells stained with Hoechst 33342.

To investigate the possible mechanism underlying the induction of apoptosis, we confirmed that apoptosis-related proteins expression such as Bcl-2, Bax, caspase-3, caspase-9 and poly(ADP-ribose) polymerase (PARP) in HL-60 cells. When treated with $CH_2Cl_2$ fraction (1, 10 μg/mL) and compound 7(1, 10 μM), we could observe the alteration of expression of apoptosis-related proteins such as increase of Bax level, decrease of Bcl-2 level, cleavage of caspase-9, cleavage of caspase-3 and cleavage of PARP in dose dependent manner . These results indicated that $CH_2Cl_2$ fraction and compound ASP7 induced apoptosis *via* alteration of expression of apoptosis-related proteins in HL-60 cells.

Activation ERK 1/2 pathway contribute stabilization of C-myc, which is one of oncoprotein.Thus, we examined expression of ERK 1/2 and C-myc in HL-60 cells. The treatment of $CH_2Cl_2$ fraction or compound ASP7decreased the phospho-ERK1/2 and C-myc level in the treatment condition that could induce apoptosis of HL-60 cells. These results suggested that $CH_2Cl_2$ fraction and compound 7 induced apoptosis via the down-regulation of ERK 1/2 pathway and C-myc in HL-60 cells.

### 4.3.3. Anti-inflammatory effects

In the present study, we describe the inhibitory effect of compounds isolated from the starfish *A. polyacanthus* and *A. batheri* on LPS-induced expression of the pro-inflammatory cytokines IL-12 p40, IL-6, and TNF-α in bone marrow-derived dendritic cells.

Of those tested, compound ASP4 showed potent inhibition on IL-12 p40 production, compound ASP1, ASP7 showed potent inhibition on IL-12 p40 and IL-6 production, compound ASP5 showed potent inhibition on IL-12 p40 and TNF-α production (see Table 4.3.3). Other compounds showed moderate inhibition or did not show significant inhibition compared to the positive control.

Figure 4.3.3. Anti-inflammatory effects of compounds on LPS-stimulated BMDCs

| Compounds | $IC_{50}$ (μM) | | |
|---|---|---|---|
| | **IL-12 p40** | **IL-6** | **TNF-α** |
| **ASP1** | **3.96 ± 0.12** | **4.07 ± 0.13** | (-) |
| **ASP2** | 34.86 ± 1.31 | (-) | (-) |
| **ASP3** | 6.55 ± 0.18 | (-) | 22.80 ± 0.21 |
| **ASP4** | **5.06 ± 0.16** | 16.73 ± 0.25 | (-) |
| **ASP5** | **1.82 ± 0.11** | 5.76 ± 0.14 | **4.94 ± 0.12** |
| **ASP6** | 79.05 ± 2.05 | (-) | (-) |
| **ASP7** | **3.90 ± 0.14** | **2.61 ± 0.10** | 7.00 ± 0.16 |
| **ASP-C**[b] | **1.27 ± 0.11** | 8.82 ± 0018 | 11.48 ± 0.16 |
| **ASP-M**[b] | 11.47 ± 0.16 | 20.28 ± 0.22 | 36.99 ± 0.24 |
| **AB2** | 36.4 ± 0.25 | (-) | (-) |
| **AB3** | 31.1 ± 0.18 | (-) | (-) |
| **AB4** | 22.8 ± 0.15 | (-) | (-) |
| **SB203580** [a] | 5.00 ± 0.16 | 3.50 ± 0.12 | 7.20 ± 0.13 |

[a] positive control, [b](μg/mL)

## CONCLUSIONS

### 1. Chemical investigation

From two starfish *A.polyacanthus* and *A. batheri*, 12 compound were isolated including eight new AB1-AB4, and ASP1-ASP4. The structure of them was elucidated by spectroscopic and chemical methods.

- Astebatherioside A **(AB1)**
- Astebatherioside B **(AB2)**
- Astebatherioside C **(AB3)**
- Astebatherioside D **(AB4)**
- 3-[*O*-*β*-D-fucopyranosyl-(1→3)-*β*-D-fucopyranosyl-(1→4)-[*β*-D-quinovopyranosyl-(1→2)]-*β*-D-quinovopyranosyl]-2-acetyl-pyrrole **(AB5)**
- Astropectenol A **(ASP1)**
- Astropectenol B **(ASP2)**
- Astropectenol C **(ASP3)**
- Astropectenol D **(ASP4)**
- 5*α*-cholest-7-ene-3*β*,6*α*-diol **(ASP5)**
- 5*α*-cholest-8(14)-ene-3*β*,7*α*-diol **(ASP6)**
- 5*α*-cholest-7,9(11)-diene-3*β*-ol **(ASP7)**

### 2. Biological activity

- The results of *in vitro* cytotoxicity tests of the dichloromethane fraction and 7 compounds isolated from *A. polyacanthus* were found: The diclometane fraction and 5 compounds showed significant inhibitory effects on 3 cell lines with $IC_{50}$ ranging from 2.70 ÷ 82.95 μM. Specially, compound **ASP7** and $CH_2Cl_2$ fraction exhibit poten cytotoxic effects against HL-60 human leukemia cells with the $IC_{50}$ of 2.70 μM and 8.29 μg/mL, respectively, comparing to the position control, mitoxantrone ($IC_{50}$ = 6.80 μM).
- Investigation for the possible mechanism underlying the induction of apoptosis showed that $CH_2Cl_2$ fraction or compound ASP7 induced apoptosis via alteration of expression of apoptosis-related protein in HL-60 cells.
- The inhibitory effect of 12 compounds isolated from two starfishs on pro-inflammatory cytokine IL-12 p40, IL-6 and tumor necrosis factor α (TNF-α)

production in lipopolysaccharide (LPS)-stimulated bone marrow-devived dendritic cells (BMDCs). Among those tested, compounds ASP1, ASP5, ASP7 showed potent inhibitory effects on the production of all three pro-inflammatory cytokines with $IC_{50}$ values ranging from 1.82 ÷ 7.00 μM.

**RECOMMENDATIONS**

This is the first study of chemical compositions and biological activities of two starfish *A. batheri* and *A.polyacanthus* of Vietnam. Based on these obtained results show that :

- The starfish *A. polyacanthus* likely develop into materials to prevent and treatment inflammatory and cancer disease.
- The starfish *A. batheri* should be further study of the chemical composition.

**Publications**

1. Nguyen Phuong Thao, Nguyen Xuan Cuong, Bui Thi Thuy Luyen, Nguyen Hoai Nam, Pham Van Cuong, Nguyen Van Thanh, Nguyen Xuan Nhiem, **Tran Thi Hong Hanh**, Eun-Ji Kim, Hee-Kyoung Kang, Phan Van Kiem, Chau Van Minh, and Young Ho Kim. Steroidal constituents from the starfish *Astropecthen polyacanthus* and their anticancer effects. Chem. Pharm. Bull. 61(10) 1044-1051 (2013).
2. Nguyen Phuong Thao, Nguyen Xuan Cuong, Bui Thi Thuy Luyen, Tran Hong Quang, **Tran Thi Hong Hanh**, Sohyun Kim, Young-Sang Koh, Nguyen Hoai Nam, Phan Van Kiem, Chau Van Minh, and Young Ho Kim. Anti-inflammatory components of the starfish *Astropecthen polyacanthus*. Mar. Drugs 2013, 11, 2917-2926.
3. Nguyen Phuong Thao, Le Duc Dat, Ninh Thi Ngoc, Vu Anh Tu, **Tran Thi Hong Hanh**, Phan Thi Thanh Huong, Nguyen Xuan Nhiem, Bui Huu Tai, Nguyen Xuan Cuong, Nguyen Hoai Nam, Pham Van Cuong, Seo Young Yang, Sohyun Kim, Doobyeong Chae, Young-Sang Koh, , Phan Van Kiem, Chau Van Minh, and Young Ho Kim. Pyrrole and furan oligoglycosides from the starfish *Asterina batheri* and their inhibitory effect on the production of pro-inflammatory cytokine in lipopolysaccharide-stimulated bone marrow-derived dendritic cell. Bioorganic & Medicinal Chemistry Letters 23 (2013) 1823-1827.
4. **Trần Thị Hồng Hạnh**, Nguyễn Phương Thảo, Lê Đức Đạt, Ninh Thị Ngọc, Phan Thị Thanh Hương, Vũ Anh Tú, Châu Ngọc Điệp, Nguyễn Tiến Đạt, Nguyễn Xuân Cường, Nguyễn Hoài Nam, Phan Văn Kiệm, Young Ho Kim, Châu Văn Minh. Các hợp chất steroid phân lập từ loài sao biển *Astropecthen polyacanthus*. Tạp chí Hóa học, Tập 51 (6ABC) 10-13, 2013.

5. **Tran Thi Hong Hanh**, Ninh Thi Ngoc, Le Đuc Đat, Phan Thi Thanh Huong, Nguyen Phuong Thao, Nguyen Tien Đat, Do Thi Thao, Nguyen Xuan Cuong, Nguyen Hoai Nam, Đo Cong Thung, Phan Van Kiem, Chau Van Minh. An anti-imflammatory pyrrole oligoglycoside from the starfish *Asterina batheri* living in Vietnamese seas. Journal of medicinal materials Vol.19, No.5, 279-283, 2014.