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STUDY ON CHEMICAL CONSTITUENTS, CYTOTOXIC AND ANTI-INFLAMMATORY ACTIVITIES OF TWO CRINOIDS *CAPILLASTER MULTIRADIATUS* (LINNAEUS, 1758) VÀ *COMANTHUS DELICATA* (AH CLARK, 1909) IN VIETNAMESE SEAS

Major: Biochemistry

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SUMMARY OF BIOLOGY DOTORAL THESIS

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The thesis can be found in:
- The Library of Graduate University of Science and Technology, Vietnam Academy of Science and Technology.
- National Library of Vietnam.
- Institute of Biotechnology

INTRODUCTION

The oceans cover more than 70% of the Earth's total surface area with up to 90% of the planet's habitable space. Many countries around the world have exploited biologically active substances from marine organisms in order to research and find drugs to treat serious diseases, especially some common diseases such as cancer and inflammation. The two diseases are on the rise every year, claiming the lives of millions of people around the world each year. Up to now, there have been many active ingredients derived from marine organisms that have been developed into drugs and licensed for circulation, such as Ara-C, Trabectedin for cancer treatment; Ara-A for Herpes, Ziconotide as a pain reliever… There are also many active ingredients that are currently under clinical research and will soon be available on the market. To get this result, research institutes around the world have researched, searched and screened for the biological activity of millions of compounds from marine species, and spent many millions of dollars and time on research, preclinical and clinical studies.

Echinoderms including crinoids distributed quite commonly in many seas around the world, has been and is being interested by many scientists because this object is still quite new. About 25 species of crinoid belonging to 16 genera from the class lily were studied out of a total of 190 accepted species and genera. Vietnam is located in the Pacific Ocean region with over 3,260 km of coastline and large bays and islands, where there is a rich source of marine

species. There are about 60 crinoid species in Vietnam, but so far there has not been a scientific study on the chemical constituents and biological activity of crinoid.

Therefore, the topic: ***"Study on chemical constituents, cytotoxic and anti-inflammatory activities of two crinoids Capillaster multiradiatus (Linnaeus, 1758) and Comanthus delicata (AH Clark, 1909) in Vietnamese seas"*** was selected to proceed.

The aim of the thesis:

- Determine the chemical constituents of two crinoid species *Capillaster multiradiatus* and *Comanthus delicata* collected in Vietnamese seas.
- Searching for compounds with cytotoxic and anti-inflammatory activities in the studied species for application in pharmacological research.

Thesis content includes:

1. Isolation of compounds from two crinoid species *Capillaster multiradiatus* and *Comanthus delicata* in Vietnamese seas by chromatographic methods
2. Determination of chemical structures of isolated compounds from two crinoid species *Capillaster multiradiatus* and *Comanthus delicata* in Vietnamese seas
3. Evaluation of the cytotoxic activity of isolated compounds
4. Evaluation of anti-inflammatory activity of isolated compounds

CHAPTER 1. OVERVIEW

Includes an introductory overview of natural compounds, anti-cancer and anti-inflammatory activities, common characteristics of sea lilies; Typical compounds from sea lilies and their biological activities.

1.1. Overview of natural compounds

1.1.1. Classification of natural compounds

1.1.2. Marine natural compounds and drugs derived from marine

1.2. General introduction to cancer

1.2.1. Cancer and cancer treatments

1.2.2. The link between apoptosis and cancer

1.3. Introduction to inflammatory disease

1.3.1. Stages of the inflammatory process

1.3.2. Factors involved in the inflammatory process

1.3.3. Inhibition of iNOS and COX2 expression in anti-inflammatory activity studies

1.4. General introduction about crinoid

1.4.1. Body structure

1.4.2. Reproduction and development

1.4.3. Bioactivity of typical compounds from crinoids

A review of published studies may suggest that crinoid contain many valuable biologically active compounds. The main chemical constituents of crinoids are anthraquinones and napthopyrones with potential anticancer and anti-inflammatory activities. The species of crinoid *Capillaster multiradiatus* has been studied by scientists around the world on the chemical constituents as well as biological activities of some compounds, however, in Vietnam so far no researches about this species have been published. The crinoid *Comanthus delicata*, according to reference materials, both in Vietnam and abroad, have no scientific publications on chemical constituents as well as biological activity. Therefore, the study of chemical constituents and biological activity of these two species will

contribute to the scientific basis for further research and application orientation.

CHAPTER 2. SUBJECTS AND METHODS

2.1. Subjects

2.1.1. The crinoid Capillaster multiradiatus (Linnaeus, 1758)

Samples of *Capillaster multiradiatus* (Linnaeus, 1758) were collected at Son Tra, Đa Nang, Vietnam (8/2016)

Figure 2.1. The crinoid *Capillaster multiradiatus* (Linnaeus, 1758)

2.1.2. The crinoid Comanthus delicata (AH Clark, 1909)

Figure 2.2. The crinoid *Comanthus delicata* (AH Clark, 1909)

Samples of *Comanthus delicata* (AH Clark, 1909) were collected at Van Phong, Khanh Hoa, Vietnam (7/2020).

The scientific names of the above species are indentified by Prof. Dr. Do Cong Thung, Institute of Marine Environment and Resources, VAST.

2.2. Methods

2.2.1. Process of processing and creating extracts for crinoid samples

The collected crinoid samples were washed with water to remove sand and debris, then blotted dry with blotting paper. The sample portion as a specimen is fixed in 96% alcohol and kept in a plastic bottle with a tight-fitting stopper. The rest is freeze-dried using a freeze-drying device.

The dried crinoid samples were then cut into small pieces, extracted 3 times with methanol on the ultrasonic device. Extract each sample by evaporating under reduced pressure to obtain a methanol extract. The MeOH extract of the study sample was mixed with distilled water and then extracted by a liquid-liquid distribution with poorly soluble organic solvents and gradually increased polarity from hexane, chloroform, and ethyl acetate.

2.2.2. Methods for isolation of compounds

2.2.2.1. Isolation of compounds from Capillaster multiradiatus

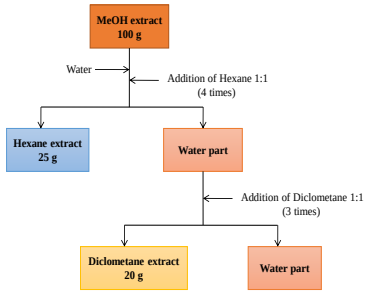


Figure 2.3. Class extraction diagram *Capillaster multiradiatus*

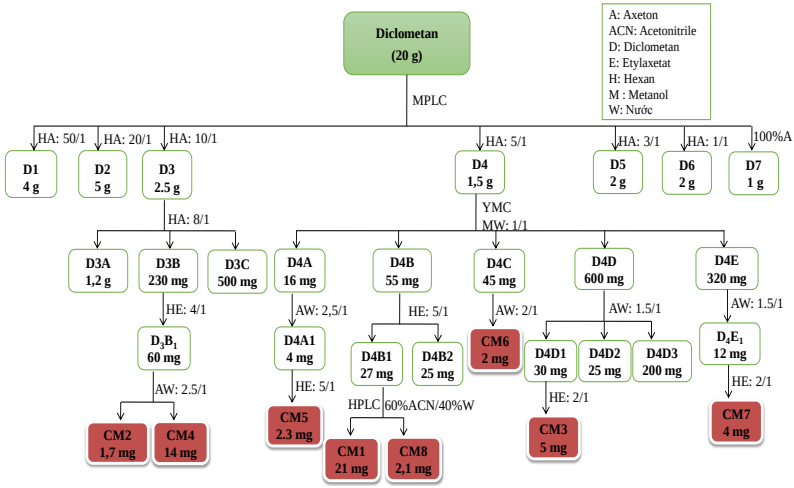


Figure 2.4. Isolation of compounds from the crinoid *Capillaster multiradiatus*

2.2.1.2. Isolation of compounds from the crinoid Comanthus delicata

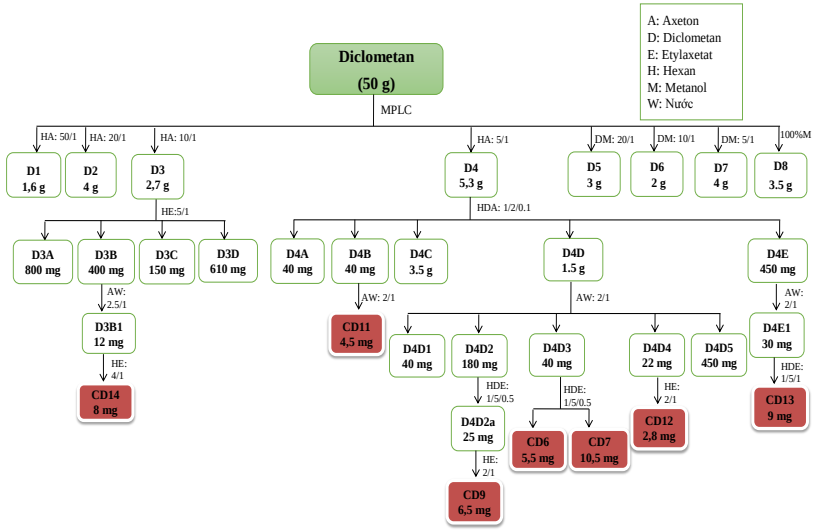


Figure 2.5. Isolation of compounds from fraction D3, D4 of the crinoid *Comanthus delicata*

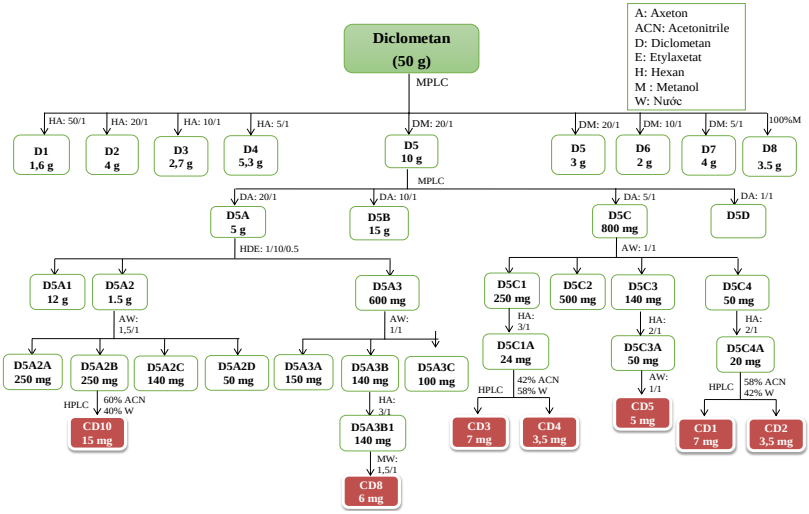


Figure 2.6. Isolation of compounds from fraction D5 of the crinoid *Comanthus delicata*

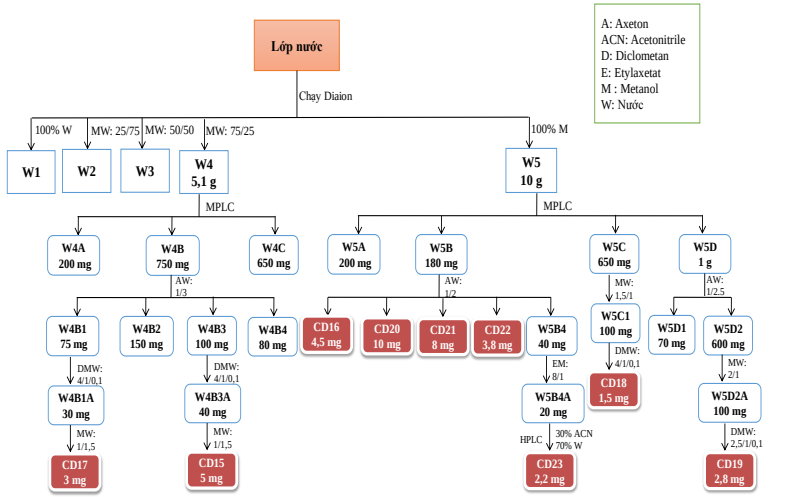


Figure 2.7. Isolation of compounds from fraction W of the crinoid *Comanthus delicata*

2.2.3. Methods for determination of chemical structures of compounds

Using a combination of modern spectroscopy methods such as high-resolution mass spectroscopy, nuclear magnetic resonance spectroscopy, circular dichroism...

2.3. Methods to assess biological activity

2.3.1. Methods to evaluate the cytotoxic activity of cancer cells

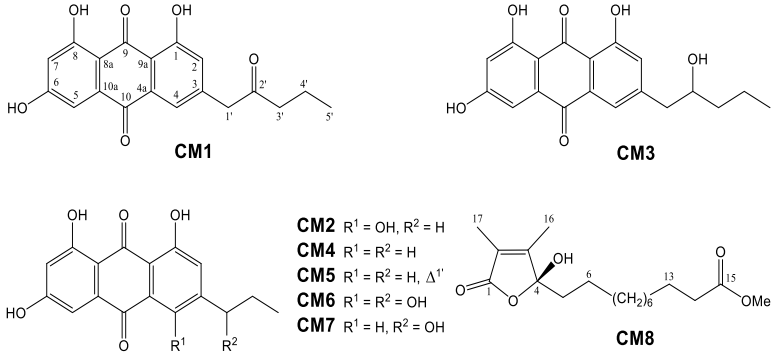
2.3.2. Methods to evaluate the anti-inflammatory activity

CHAPTER 3: RESULTS

3.1. Results of isolation and determination of chemical structures of compounds

3.1.1. Isolation and determination of chemical structures of compounds from the crinoid Capillaster multiradiatus

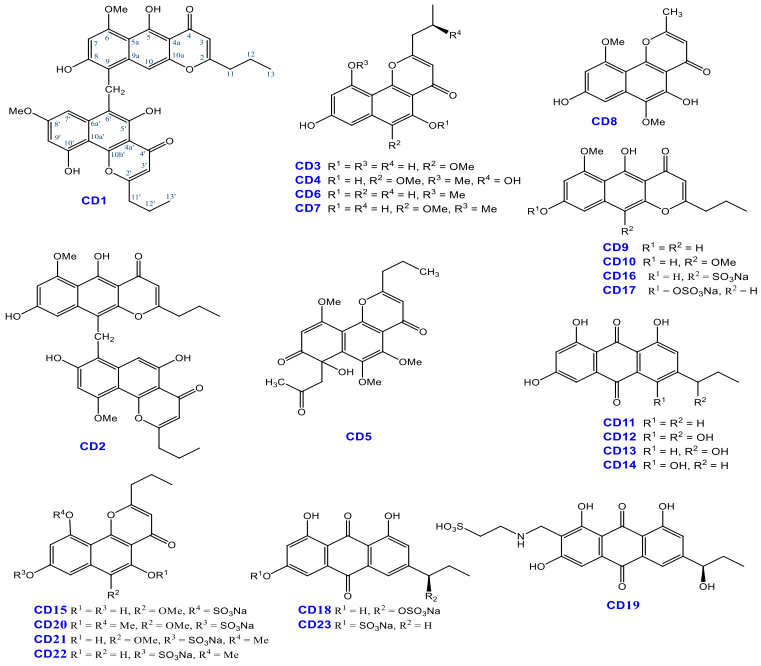
Using combined chromatographic methods, 08 compounds were isolated and structurally determined from the crionid *Capillaster multiradiatus* as shown in Figure 3.1.



Hình 3.1. Chemical structure of isolated compounds from the crinoid *Capillaster multiradiatus*

3.1.2. Isolation and determination of chemical structure of compounds from the crinoid Comanthus delicata

Using combined chromatographic methods, 08 compounds were isolated and structurally elucidated from the crionid *Comanthus delicata* as shown in Figure 3.2.



Hình 3.2. Chemical structures of isolated compounds from the crinoid *Comanthus delicata*

3.2. Evaluation of cytotoxic activity

3.2.1. Evaluation of cytotoxic activity of isolated compounds from Capillaster multiradiatus

The results showed that only **CM2** and **CM5** exhibited cytotoxic activity on all 05 tested cancer cell lines. The remaining compounds showed no activity at the tested concentrations.

Table 3.1. Evaluation of cytotoxic activity of isolated compounds from *Capillaster multiradiatus*

Compounds	IC_{50} (µM)				
	KB	SK-Mel-2	HepG2	LNCaP	MCF7
CM1	-	-	-	-	-
CM2	49.13±2.52	53.68±2.51	79.86±6.67	73.97±4.71	65.75±3.02
CM3	-	-	-	-	-
CM4	-	-	-	-	-
CM5	86.38±5.02	86.65±4.86	98.68±6.48	86.30±5.16	90.87±3.61
CM6	-	-	-	-	-
CM7	-	-	-	-	-
CM8	-	-	-	-	-
Ellipticine*	1.62±0.24	1.38±0.08	1.54±0.08	1.38±0.08	1.67±0.12

*"*****":Positive control; "-": No activity*

3.2.2. Evaluation of cytotoxic activity of isolated compounds from Comanthus delicata

Results in Table 3.2 shows that two compounds **CD1** and **CD4** exhibit cytotoxic activity on all 05 tested cancer cell lines; compounds **CD2**, **CD7**, **CD8**, **CD10** showed cytotoxic activity on 2 tested cancer cell lines, SK-Mel-2 and MCF7; compounds **CD19**, **CD20**, **CD21**, **CD22** showed cytotoxic activity on 2 cancer cell lines SK-Mel-2 and LNCaP; compounds **CD3**, **CD5**, **CD6**, **CD9**, **CD15**, **CD16**, **CD17**, **CD19** only showed selective cytotoxic activity on SK-Mel-2 cancer cell line; the remaining two compounds **CD18** and **CD23** did not show activity against all five cancer cell lines tested.

Table 3.2. Evaluation of cytotoxic activity of compounds isolated from *Comanthus delicata*

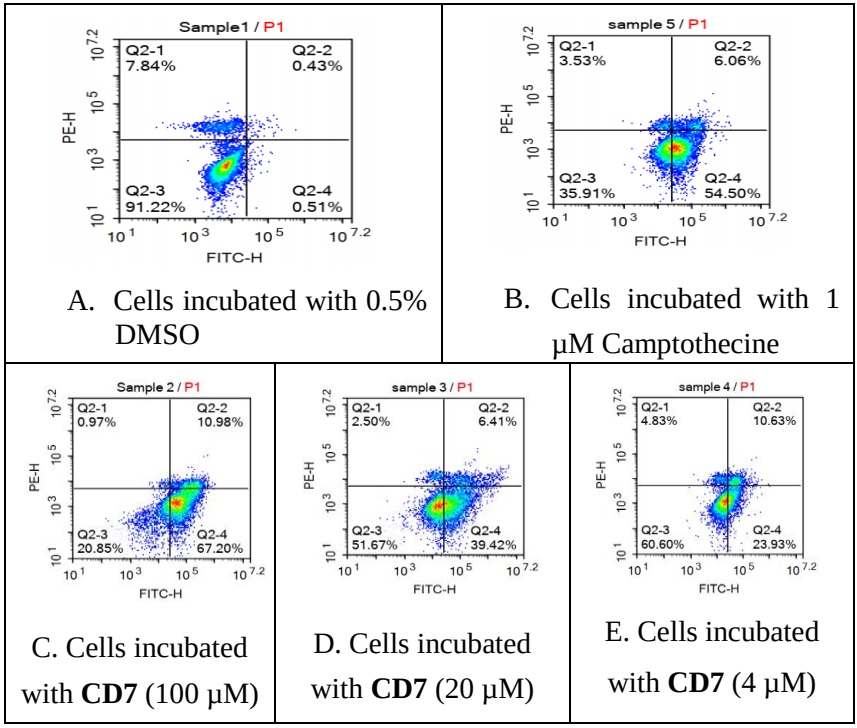
Compounds	IC_{50} (µM)				
	KB	**SK-Mel-2**	**HepG2**	**LNCaP**	**MCF7**
CD1	52.20±6.01	44.45±2.86	55.53±5.16	54.53±1.82	64.28±2.03
CD2	-	11.99±0.69	-	-	14.90±2.25
CD3	-	47.03±2.66	-	-	-
CD4	80.12±3.54	8.51±0.98	79.03±2.29	63.57±2.69	39.98±4.47
CD5	-	38.15±4.24	-	-	-
CD6	-	12.03±0.54	-	-	-
CD7	-	11.68±0.88	-	-	25.76±3.85
CD8	-	10.49±1.25	-	-	84.49±7.24
CD9	-	36.01±2.08	-	-	-
CD10	-	31.46±3.36	-	-	60.26±2.81
CD15	-	76.92±5.85	-	-	-
CD16	-	74.53±7.27	-	-	-
CD17	-	64.78±1.97	-	-	-
CD18	-	-	-	-	-
CD19	-	49.96±1.74	-	-	-
CD20	-	61.98±1.45	-	20.29±2.43	-
CD21	-	70.05±4.62	-	66.16±1.26	-
CD22	-	68.86±4.73	-	54.64±4.14	-
CD23	-	-	-	-	-
Ellipticine*	1.46±0.12	1.22±0.12	1.63±0.16	1.67±0.16	1.42±0.16

*"*****":Positive control; "-": No activity*

Compounds **CD11** (same as **CM4**), **CD12** (same as **CM6**), **CD13** (same as **CM7**), **CD14** (same as **CM2**) isolated from the crinoid *Capillaster multiradiatus* species have been evaluated and presented in section 3.2.1.

3.2.3. Study on the mechanism of cytotoxicity of CD7

*3.2.3.1. Evaluation of the ability of **CD7** to induce apoptosis on SK-Mel-2 cell line*



A. Cells incubated with 0.5% DMSO

B. Cells incubated with 1 µM Camptothecine

C. Cells incubated with **CD7** (100 µM)

D. Cells incubated with **CD7** (20 µM)

E. Cells incubated with **CD7** (4 µM)

Figure 3.3. The effect of **CD7** on SK-Mel-2 cell apotosis through Annexin V-FITC and PI Kit (x-axis represents FITC- Annexin V staining level, y-axis represents PI staining level in Log units)

Table 3.3. The rate of apoptosis cell types under the influence of **CD7** on SK-Mel-2 cell line

Compounds	The rate of survival cells (%)	The rate of early apoptosis cells (%)	The rate of late apoptosis cells (%)	The rate of necrotic cells (%)
Control	91.22	0.51	0.43	7.84
CD7 (100 µM)	20.85	67.20	10.98	0.97
CD7 (20 µM)	51.67	39.42	6.41	2.50
CD7 (4 µM)	60.60	23.93	10.63	4.83
Camptothecine* (1µM)	35.91	54.50	6.06	3.53

*"**":Positive control*

The results presented in Figure 3.3 and Table 3.3 show that the percentage of early and late apoptosis cells changed under the influence of compound **CD7**.

3.2.3.2. *Evaluation of the ability to stimulate caspase-3 production of* ***CD7***

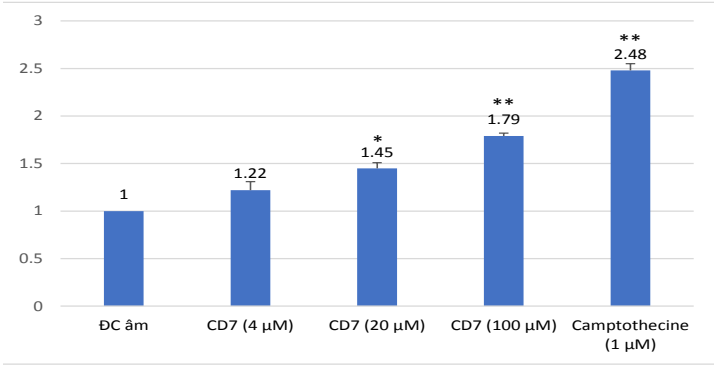


Figure 3.4. Capability to stimulate caspase-3 in SK-Mel-2 cells under the influence of **CD7** (*P<0.05; **P<0.01 compared with negative control)

The results showed that compound **CD7** has the ability to induce SK-Mel-2 cancer cells to produce caspase-3.

3.3. Results of evaluation of NO production inhibitory activity

3.3.1. Results of evaluating the inhibitory activity of NO production compounds from Capillaster multiradiatus

Table 3.4. Results of evaluating the inhibitory activity on NO production of compounds from *Capillaster multiradiatus*

Compounds	IC_{50} (µM)
CM1	**5.89 ± 0.11**
CM2	12.02 ± 0.45
CM3	20.89 ± 0.76
CM4	13.18 ± 0.15
CM5	19.05 ± 0.32
CM6	-
CM7	16.98 ± 0.98
CM8	-
Cardamonin*	2.59 ± 0.18

*"*****":Positive control; "-": No activity*

The inhibitory activity of NO production of 08 isolated compounds from *Capillaster multiradiatus* is shown in Table 3.4. The results showed that compounds **CM1**, **CM2**, **CM3**, **CM4**, **CM5** and **CM7** exhibited inhibitory activity on NO production. Compounds **CM6** and **CM8** showed no activity.

3.3.2. Results of evaluation of NO production inhibitory activity of compounds from Comanthus delicata

The results in Table 3.5 showed that **CD3**, **CD4**, **CD6**, **CD7**, **CD8** and **CD23** exhibited inhibitory activity on NO production. The remaining compounds showed no activity.

Compounds **CD11** (same as **CM4**), **CD12** (same as **CM6**), **CD13** (same as **CM7**), **CD14** (same as **CM2**) isolated from *Capillaster multiradiatus* have been evaluated and presented in section 3.3.1.

Table 3.5. Results of evaluating NO production inhibitory activities of compounds from *Comanthus delicata*

Compounds	**IC_{50} (μM)**
CD1	-
CD2	-
CD3	63.55±4.08
CD4	36.18±2.98
CD5	-
CD6	24.98±1.13
CD7	55.44±2.77
CD8	31.25±3.09
CD9	-
CD10	-
CD15	-
CD16	63.55±4.08
CD19	36.18±2.98
CD20	-
CD21	-
CD22	55.44±2.77
CD23	31.25±3.09
L-NMMA*	2.59 ± 0.18

*"*****":Positive control; "-": No activity*

3.3.3. Results of evaluating the inhibition of CM1 on iNOS and COX-2 expression

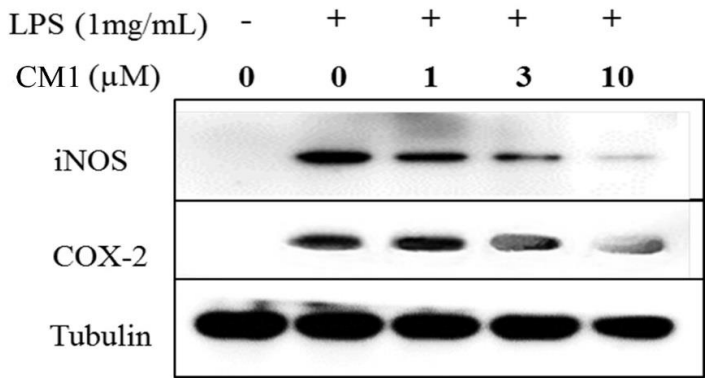


Figure 3.5. Effects of **CM1** at concentrations of 1, 3 and 10μM on the expression of protein iNOS, COX-2 in LPS-stimulated RAW264.7 cell line

The results of the inhibition of iNOS and COX-2 expression in RAW264.7 cells are shown in Figure 3.5. The results showed that **CM1** had an inhibitory effect on the expression of iNOS and COX-2 in RAW264.7 cells stimulated with LPS.

CHAPTER 4: DISCUSSIONS

4.1. Determination of chemical structures of compounds

4.1.1. Determination of chemical structures of compounds from the crinoid Capillaster multiradiatus (Linnaeus, 1758)

From the crinoid *Capillaster multiradiatus*, 08 compounds were isolated. In which, there are 03 new compounds named: capillasterquinone A (**CM1**), capillasterquinone B (**CM2**),

capillasterolide (**CM8**) and 05 known compounds: 3-(2′-hydroxy-n-pentyl)-1, 6,8-trihydroxy-9,10-anthraquinone (**CM3**), 3-propyl-1,6,8-trihydroxy-9,10-anthraquinone (**CM4**), 3-(trans-prop-1′-enyl)-1 ,6,8-trihydroxy-9,10-anthra-quinone (**CM5**), 3-(1′-hydroxypropyl)-1,4,6,8-tetrahydroxy-9,10-anthraquinone (**CM6**), 3-(1'-hydroxypropyl)-1,6,8-trihydroxy-9,10-anthraquinone (**CM7**).

4.1.2. Determination of chemical structures of compounds from the crinoid Comanthus delicata (AH Clark, 1909)

From the crinoid *Comanthus delicata*, 23 compounds were isolated. Of which, 10 new compounds are delicapyrons A-H (**CD1-CD5** and **CD15-CD17**), delicaquinone A (**CD18**) and delicaquinon B (**CD19**); The 13 known compounds are comaparvin (**CD6**), 6-methoxycomaparvin (**CD7**), 5,8-dihydroxy-6,10-dimethoxy-2-methyl-4H-naphtho[1,2-b]pyran-4-one (**CD8**), 5,8-dihydroxy-6-methoxy-2-propyl-4H-naphtho[2,3-b]pyran-4-one (**CD9**), 5,8-dihydroxy-6,10-dimethoxy-2 -propyl-4H-naphtho[2,3-b]pyran-4-one (**CD10**), **CD11** coincides with **CM4**, **CD12** coincides with **CM6**, **CD13** coincides with **CM7**, **CD14** coincides with **CM2** , 6-methoxycomaparvin-5-methylether-8-O-sodium sulfate (**CD20**), 6-methoxycomaparvin-8-O-sodium sulfate (**CD21**), comaparvin-8-O-sodium sulfate (**CD22**), and 3-propyl-1,6,8-trihydroxy-9,10-anthraquinone-6-O-sodium sulfate (**CD23**).

4.1.3. Summary and comment on the results of determining the chemical structures of compounds

From the crinoid *Capillaster multiradiatus*, 07 of 08 isolated compounds were anthraquinone derivatives, including 2 new ones, capillasterquinone A (**CM1**), capillasterquinone B (**CM2**) and a new butenolide derivative, capillasterolide (**CM8**) (Figure 3.1).

International studies on this crinoid species have reported the presence of naphthopyrone and pyrano[2,3-f]chromene derivatives. However, this is the first time report of anthraquinone and butenolide derivatives from the crinoid *Capillaster multiradiatus*.

From the crinoid *Comanthus delicata*, among 23 isolated compounds (Figure 3.2), there are 2 new bisnaphthopyrone derivatives, delicapyrons A (**CD1**) and B (**CD2**); 8 napthopyrone derivatives including 3 new ones: delicapyrons C-E (**CD3-CD5**); 6 naphthopyrone sulfate derivatives, including 03 new ones, delicapyrons F-H (**CD15-CD17**) and 4 anthraquinone derivatives and 3 anthraquinone sulfate derivatives, including 2 new ones, delicaquinones A (**CD18**) and B (**CD19**). The two compounds **CD1** and **CD2** have a novel bisnaphthopyrone skeleton containing an angular naphthopyrone unit and a linear naphthopyrone unit linked by a CH_2 group. According to the reference search, this is the first time this structure has been discovered and published. This is also the first studies on the chemical constituents and biological activity of the crinoid *Comanthus delicata* have been conducted. The main chemical constituents from this species are naphthopyrone and anthraquinone derivatives, showing the agreement with the published researches on crinoids in the world.

4.2. Evaluation of the cytotoxic activity of the isolated compounds

4.2.1. The cytotoxic activity of the isolated compounds

With 14 isolated naphthopyrones, 08 derivatives including **CD3**, **CD4**, **CD5**, **CD6**, **CD7**, **CD8**, **CD9**, **CD10** all showed strong and moderate activity on skin cancer cell line SK-Mel-2, while 06 sulfate derivatives including **CD15**, **CD16**, **CD17**, **CD20**, **CD21**, **CD22** showed weak cytotoxic activity (Table 3.2). This suggests that

the presence of a sulfate group in the naphthopyrone structure may reduce the cytotoxic activity of these compounds.

Previous work demonstrated that compound **CD7** at a concentration of 300 μM completely inhibited TNF-α induced by NF-κB (an inducible transcription factor with important roles in cancer development and inflammation) by inhibiting the activity of the kinase enzyme IKKβ. In addition, **CD7** also has the ability to inhibit the ABCG2-mediated transporter, a breast cancer-fighting protein implicated in chemotherapy drug resistance. From the crinoid *Comanthus delicata*, we have isolated compound **CD7** with big amount (10.5 mg). Based on published studies on the antitumor activity, compound **CD7** was further investigated for its ability to induce apoptosis in SK-Mel-2 cancer cell line.

4.2.2. Evaluation ability of CD7 to induce apoptosis on cancer cell line SK-Mel-2

4.2.2.1. Determination of the ability to induce apoptosis by Annexin V-FITC kit

The results in Figure 3.3 and Table 3.3 show that: In the negative control sample, the number of alive cells was high up to 91.22% and only 0.94% of the cells showed apoptosis signs (Figure 3.3A). Under the influence of the positive control Camptothecine, the living cell population decreased to only 35.91%, the total number of apoptotic cells was 60.56% (Figure 3.3B). Compound **CD7** with all the tested concentrations strongly increases the percentage of cells in the early stage of apoptosis, late apoptosis was also increased relative to the control. In the control cells, the percentage of early apoptosis cells was only 0.51%, however, this cell population increased to 67.20% under the influence of **CD7** at 100 μM. When reducing

reagent concentrations to 20 and 4 µM, the percentage of early apoptosis cells was 39.42% and 23.93%, respectively. The total % of apoptosis cells is proportional to the concentrations of compound **CD7**, treatment at 4 µM concentration was 34.56%, increased to 45.83% when using 20 µM concentration and up to 78.18% when the concentration of **CD7** is 100 µM.

Thus, compound **CD7** clearly showed the ability to induce cell death through apoptosis, so it was selected to evaluate the ability to activate caspsae-3.

*4.2.2.2. Evaluation of the ability to activate caspase-3 of **CD7***

The results in Figure 3.2 show that, at concentrations of 100 and 20 µM, compound **CD7** clearly showed the ability to induce caspase-3 at a statistically significant level ($P<0.01$ and $P<0.05$). The number of times of stimulation of caspase-3 production increased by 1.45 times compared with the negative control when using **CD7** at 20 µM and increased by 1.79 times compared with negative control when using **CD7** at 100 µM. At the concentration of 4 µM, **CD7** increased caspase-3 activity by 1.22 times compared to the negative control but not at a statistically significant level ($P>0.05$). The positive control camptothecin (1 µM) exhibited stable activity with a 2.48-fold increase in caspase-3 activity compared to the negative control ($P<0.05$).

Thus, **CD7** exhibited cytotoxic effect on SK-Mel-2 skin cancer cells through apoptosis and caspase-3 induction. The thesis's research is the first report on the ability of **CD7** to activate caspase-3 in human skin cancer cell line SK-Mel-2. This contributes to confirm that compound **CD7** is a potential compound for cancer treatment-oriented studies.

4.3. Evaluation of the anti-inflammatory activity of the isolated compounds

4.3.1. Evaluation of the ability to inhibit NO production

According to previous studies, compounds **CM4** and **CM7** have been shown to significantly inhibit the accumulation of pro-inflammatory iNOS protein in LPS-stimulated RAW264.7 macrophage cells. In this study, it is also appropriate since these two compounds are initially evaluated to have good activity by significantly inhibiting the production of NO, a pro-inflammatory mediator.

Compounds **CD20**, **CD21**, **CD22** were isolated containing sulfate group at position C-8 and did not show activity while the corresponding compounds with OH group at position C-8 as 6-methoxycomaparvin 5-methyl ether, 6-methoxycomaparvin and comaparvin have potential anti-inflammatory activity. These results suggest that the substitution of the sulfate group at the C-8 position in angular naphthopyrone may affect the anti-inflammatory activity of these compounds.

The new compound **CM1** is structurally similar to 3-propyl-1,6,8-trihydroxy-9,10-anthraquinone, which has been shown to exhibit potential anti-inflammatory activity through inhibition of iNOS expression in RAW264.7 macrophage cells stimulated by LPS. With a large obtained amount and strong activity, **CM1** was selected for further evaluation of the effect of inhibiting the expression of pro-inflammatory proteins iNOS and COX-2 in RAW264.7 macrophages.

4.3.2. Evaluation of the inhibition of iNOS and COX-2 expression by CM1

The effect of compound **CM1** on the expression of two proteins iNOS and COX-2 involved in inflammation was evaluated by Western Blot method. RAW264.7 cells were stimulated by LPS and treated with **CM1** at experimental concentrations of 1, 3 and 10 µM. Western Blot results showed that **CM1** decreased the expression markers of iNOS and COX-2 with increasing concentration. Meanwhile, the expression level of tubulin remained unchanged (Figure 3.5).

Thus, compound **CM1** strongly inhibits NO production through inhibiting the expression of iNOS and COX-2 proteins. Therefore, **CM1** is a potential anti-inflammatory compound. This is also the first published study on the anti-inflammatory activity of compound **CM1** to date.

CONCLUSIONS AND RECOMMENDATION

CONCLUSIONS

This is the first study in Vietnam on the chemical constituents and biological activities of two crinoid species *Capillaster multiradiatus* and *Comanthus delicata* collected in Vietnamese seas.

1. **Research on chemical constituents**

Isolated and determined the structure of 31 compounds from two species of the crinoid *Capillaster multiradiatus* and *Comanthus delicata*. Specifically:

From the crinoid *Capillaster multiradiatus*, 08 compounds have been isolated and structurally elucidated, of which 3 compounds

are new and named capillasterquinone A (**CM1**), capillasterquinone B (**CM2**), and Capillasterolide (**CM8**).

From the crinoid *Comanthus delicata*, 23 compounds have been isolated and structurally determined, of which 10 compounds are new and named delicapyrons A-H (**CD1**-**CD5** and **CD15**-**CD17**) and delicaquinones A (**CD18**) and B (**CD19**).

2. Research on biological activity

+ The cytotoxic activity of the isolated compounds was evaluated on 05 human cancer cell lines as KB, SK-Mel-2, HepG2, LNCaP and MCF7. The results showed that: compound **CD2** exhibited strong activity against skin cancer cell line SK-Mel-2 and breast cancer cell line MCF7 with IC_{50} values of 11.99 ± 0.69 and 14.90 ± 2,25 µM. Compounds **CD4**, **CD6**, **CD7**, **CD8** exhibited strong activity against skin cancer cell line SK-Mel-2 with IC_{50} values of 8.51 ± 0.98, 12.03 ± 0.54, 11.68 ± 0.88, 10.49 ± 1.25 µM, respectively.

+ It has been identified that compound **CD7** clearly exhibits the ability to induce SK-Mel-2 cell death through apoptosis and caspase-3 induction.

+ Evaluated the inhibitory effects on NO production in RAW264.7 cell line of the isolated compounds. The results showed that compound **CM1** showed the strongest activity with IC_{50} value of 5.89 ± 0.11 µM.

+ It has been identified that compound **CM1** at a concentration of 10 µM strongly inhibits NO production through inhibiting the expression of iNOS and COX-proteins in RAW264.7 cell line.

RECOMMENDATION

Compound **CD7** is a potential compound in cancer research and compound **CM1** has a potential anti-inflammatory effect, so further *in vivo* researches on the mechanism of action of these two compounds should be carried out.

Compound **CD2** exhibited strong inhibitory activity against the growth of skin cancer cell line SK-Mel-2 and breast cancer cell line MCF7 while compounds **CD4**, **CD6**, **CD8** inhibited only SK-Mel-2 cell lines. Therefore, it is necessary to further study the mechanism of cytotoxicity on cancer cell lines to clarify the mechanism of action of these compounds.

NEW FINDING OF THE THESIS

1. The completed isolation of 13 new compounds from the two crinoids *Capillaster multiradiatus* and *Comanthus delicata* collected in Vietnamese seas, evaluation of cytotoxic and anti-inflammatory activities of these 13 new compounds.
2. Compound 6-methoxycomaparvin (**CD7**) exhibited cytotoxic effect through apoptosis and caspase-3 induction in SK-Mel-2 cancer cells; Capillasterquinone A (**CM1**) exhibited anti-inflammatory activity through reduction of the expression of iNOS and COX-2 proteins in macrophage RAW264.7 cells.

LIST OF PUBLISHED ARTICLES

1. **Le Thi Vien**, Tran Thi Hong Hanh, Phan Thi Thanh Huong, Nguyen Hai Dang, Nguyen Van Thanh, Nguyen Xuan Cuong, Nguyen Hoai Nam, Do Cong Thung, Phan Van Kiem, and Chau Van Minh. Anthraquinone and butenolide constituents from the crinoid *Capillaster multiradiatus*. **Chemical and Pharmaceutical Bulletin**, 2018, 66, 1023–1026.
2. **Le Thi Vien**, Tran Thi Hong Hanh, Tran Hong Quang, Do Thi Thao, Do Cong Thung, Nguyen Xuan Cuong, Nguyen Hoai Nam, Pham Van Cuong, Phan Van Kiem, Chau Van Minh. Naphthopyrone and anthraquinone derivatives from *Comanthus delicata*. **Phytochemistry Letters**, 2021, 46, 129–135.
3. **Le Thi Vien**, Tran Thi Hong Hanh, Tran Hong Quang, Do Cong Thung, Nguyen Xuan Cuong, Nguyen Hoai Nam, Pham Van Cuong, Phan Van Kiem, Chau Van Minh. Sulfated naphthopyrones and anthraquinones from the Vietnamese crinoid *Comanthus delicata*. **Chemical and Pharmaceutical Bulletin**, 2022, 70(5), 408-412.
4. Chau Van Minh, Nguyen Hoai Nam, Phan Van Kiem, Nguyen Xuan Cuong, **Le Thi Vien**, Tran Thi Hong Hanh, Phan Thi Thanh Huong, Nguyen Hai Dang, Nguyen Van Thanh, Do Cong Thung. Anthraquinone compound with anti-inflammatory activity and isolation method of this compound from the crinoid *Capillaster multiradiatus*. **Patent**, 2021, number 29299.