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**GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY**

**PHAM THANH BINH**

# STUDY ON THE CHEMICAL CONSTITUENTS AND EVALUATION OF CYTOTOXIC ACTIVITY OF TWO VIETNAMESE SEA SLUG,

## *Aplysia dactylomela* AND *Aplysia oculifera*

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# INTRODUCTION

The incidence of cancer is increasing over time. Cancer is a general term for a group of diseases that can affect any part of the body. Other commonly used terms are malignant tumor and neoplasm. Cancer is the rapid formation of abnormal cells, which grow beyond their normal boundaries and then invade other parts and organs of the body (metastasis). Metastasis is also the main cause of death from cancer.

According to the World Health Organization (WHO) report on cancer, there were approximately 9.7 million deaths and approximately 20 million new cases in 2020 [1]. Cancer is a malignant tumor characterized by local tissue invasion and/or distant metastasis. In the early stages of cancer development, surgery or surgery combined with adjuvant radiotherapy can cure in most cases. When the cancer has progressed and invaded other tissues, systemic chemotherapy (oral or intravenous) is added to surgery and radiotherapy to eliminate isolated cancer cells and/or cancer cell subgroups that have not been removed or destroyed. As a general rule (there are always exceptions), the more advanced the cancer at the time of diagnosis, the more intense the adjuvant or multi-chemotherapy will be applied. Therefore, finding specific drugs as well as supportive drugs for cancer treatment and chemopreventive agents is extremely necessary and urgent.

Humanity is urgently researching and searching to discover natural and synthetic compounds with good activity and for the prevention and treatment of cancer. Of the 39.500 marine natural compounds that have been identified and published (as of 2023) [2],

only 15 pharmaceutical products derived from sponges, sea squirts, microorganisms, fish and mollusks have been approved by the FDA [3]. Natural compounds account for a large proportion of the current pharmacopoeia, with approximately 70% of currently used antibiotics and anticancer drugs derived from natural sources, such as plants and bacteria [4]. While plant-derived drugs have a documented history of more than 5,000 years and bacterial-derived drugs have been used for nearly a century, active ingredients from marine sources have only recently been studied as new drugs [4]. To date, there have been 15 marine-derived pharmaceuticals clinically approved by the FDA (US Food and Drug Administration), mainly for cancer treatment and funded by the NCI (US National Cancer Institute) since the 1960s [3].

About 39.560 bioactive compounds derived from marine animals such as sponges, soft corals, echinoderms, sea slugs and other marine organisms have been published [5]. Of these, the phylum molluscs has many diverse and rich species and is the largest group of marine animals, accounting for about 23% of the total number of named marine organisms. In tropical regions, including Vietnam, this phylum has more than 90 thousand existing species, including species such as mussels, clams, snails, mussels, clams, squid, octopus, etc. Along with economic value, the rich diversity has shown the great potential for exploitation of molluscs, especially sea slugs (sea rabbits). From some species of sea slugs studied, many valuable active ingredients have been found, including some active ingredients that have been applied in clinical trials and as medicines to treat diseases. Such as the compound dolastatin to produce the drug Brentuximab vedotin (Adcetris®) to treat liver cancer, the compound Jorumycin

synthesized into a drug (Zalypsis®) is in phase II clinical trial to treat epithelial cancer, multiple myeloma, uterine cancer, the compound kahalalide F is synthesized into elisidepsin (PM02734), this compound is in phase II clinical trial to treat breast cancer and leukemia.

Vietnam has a coastline of over 3,000 km, more than 3,000 large and small islands, including two archipelagos Hoang Sa and Truong Sa, with an exclusive economic zone of about 1 million km2, 3 times larger than the mainland area according to the United Nations Convention on the Law of the Sea 1982 (UNCLOS). This is an important premise for Vietnam to develop a multi-sector, multi-purpose economy, promoting domestic and international maritime trade. During the research process, the graduate student surveyed mollusks, specifically the sea slug Aplysia dactylomela, and found some substances with strong activity that are toxic to cancer cells. However, in our country, there have not been many studies on chemical composition and biological activity, there have only been a few studies in recent years. Therefore, to continue researching on mollusks, specifically sea slugs, to discover biologically active compounds that can be applied in cancer treatment, I chose the title **"Study on the chemical constituents and evaluation of cytotoxic activity of two Vietnamese sea slugs, *Aplysia dactylomela* and *Aplysia oculifera*"**. The thesis includes the following objectives and contents:

- Objectives of the thesis:

1. Isolation and determination of chemical structures of compounds from two sea slugs *Aplysia dactylomela* and *Aplysia oculifera*.

2. Evaluate the cytotoxic effects of isolated compounds on some human cancer cell lines to search for biologically active substances, providing a scientific basis for further biomedical and pharmaceutical research.

- Contents of the thesis:

1. Isolation and structural determination of compounds isolated from the sea slug *Aplysia dactylomela* collected on Phu Quy island, Lam Dong.

2. Isolation and structural determination of compounds isolated from the sea slug *Aplysia dactylomela* collected on Ly Son island, Quang Ngai.

3. Isolation and structural determination of compounds isolated from the sea slug *Aplysia oculifera* collected in Lang Co, Hue.

4. Evaluation of the cytotoxic effects on human cancer cells from [Three cancer cell lines: liver cancer (Hep-G2), lung cancer (A549) and breast cancer (MCF-7)] with compounds isolated from two sea slug samples *Aplysia dactylomela*.

5. Evaluation of the cytotoxic effects on human cancer cells from [Three cancer cell lines: liver cancer (Hep-G2), cervical cancer (Hela) and breast cancer (MCF-7) with compounds isolated from *Aplysia oculifera*.

# Chapter 1. OVERVIEW

## 1.1. Introduction to the sea slug genus *Aplysia*

There are about 40 species of *Aplysia* sea slugs in the world, belonging to the family Aplysiidae, order Anaspidea, class Gastropoda, phylum Molluscs. *Aplysia* sea slugs (Gastropoda: Opisthobranchia) have a short lifespan, fast growth rate and high egg production [3]. Fast-growing juveniles can grow up to 13,000% in a two-week period and individual sea slugs can lay more than 108 eggs during their lifetime [3]. Sea slugs also show great variation in growth rate and maximum body size in different populations of the same species living in different locations or times or under different environmental conditions [4, 5]. These data suggest that *Aplysia* sp. have high potential productivity and live in different environmental conditions.

Sea slugs are widely distributed across continents, in tropical and subtropical regions [7]. In Vietnam, sea slugs are distributed in the seas of Thanh Hoa, Quang Binh, Quang Ngai, Nha Trang... There have been many studies in the world on ecology as well as chemical composition and biological activity. Some compounds from sea slugs have good activities and are used as anti-cancer drugs. Their main class of substances is terpenoids, in addition to some classes of substances such as alkaloids, sterols, macrolides...

Table 1.1. List of sea slugs of the genus *Aplysia*

| No | Genus | No | Genus | No | Genus |
|---|---|---|---|---|---|
| 1 | *A. angasi* | 15 | *A. gigantea* | 28 | *A. perfata* |
| 2 | *A. brasiliana* | 16 | *A. gracilis* | 29 | *A. rehderi* |
| 3 | *A. californica* | 17 | *A. inca* | 30 | *A. reticulata* |
| 4 | *A.* cedrosensis | 18 | *A. juliana* | 31 | *A. reticulopoda* |
| 5 | *A. cervina* | 19 | *A. keraudreni* | 32 | *A. robertsi* |
| 6 | *A. corrigera* | 20 | *A. kurodai* | 33 | *A. rudmani* |
| 7 | *A. cronullae* | 21 | *A. maculata* | 34 | *A. sagamiana* |
| 8 | *A. dactylomela* | 22 | *A. morio* | 35 | *A. sowerbyi* |
| 9 | *A. denisoni* | 23 | *A. nigra* | 36 | *A. sydneyensis* |
| 10 | *A. depilans* | 24 | *A. oculifera* | 37 | *A. tanzannensis* |
| 11 | *A. dura* | 25 | *A. parvula* | 38 | *A. vaccaria* |
| 12 | *A. euchlora* | 26 | *A. pulmonica* | 39 | *A. willcoxi* |
| 13 | *A. extraordinaria* | 27 | *A. punctata* | 40 | *A. winneba* |
| 14 | *A. fasciata* | | | | |

# Chpater 2. RESEARCH OBJECTS AND METHODS

## 2.1. Research subjects

### *2.1.1. The sea slug Aplysia dactylomela*

The sea slug *Aplysia dactylomela* Rang, 1828 was collected in May 2021 on Phu Quy Island, Lam Dong with a fresh weight of 600g by Prof. Do Cong Thung, Institute of Marine Resources and Environment, Vietnam Academy of Science and Technology, identified by morphological characteristics. The specimen was soaked in alcohol and kept at the Biological Actives Department, Institute of Marine Biochemistry, Vietnam Academy of Science and Technology.

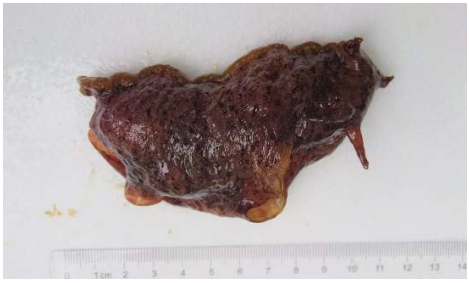


Fig. 2.1. *A. dactylomela*

### *2.1.2. The sea slug Aplysia dactylomela*

The sea slug *Aplysia dactylomela* Rang, 1828 was collected in Ly Son, Quang Ngai in June 2023 with a fresh sample weight of 1.25 kg by MSc. Nguyen Chi Mai, Department of Biological Resources, using molecular biology methods. The specimen was soaked in alcohol and kept at the Department of Biological Activities, Institute of Marine Biochemistry, Vietnam Academy of Science and Technology.

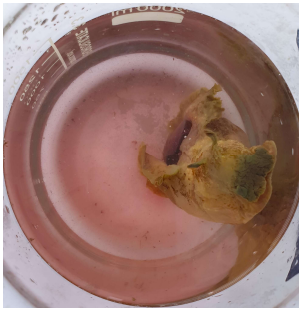
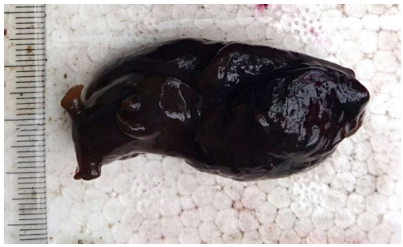


Fig. 2.1. *A. dactylomela*

### *2.1.3. The sea slug Aplysia oculifera*

The sea slug *Aplysia oculifera* was collected in June 2023 in Lang Co, Hue with a fresh weight of 9.0 kg by MSc. Nguyen Chi Mai, Department of Biological Resources, Institute of Marine Biochemistry, using molecular biology methods. The specimen was soaked in alcohol and kept at the Department of Marine Biology Research, Institute of Chemistry, Vietnam Academy of Science and Technology.



Hình 2. 2. Sên biển *A. oculifera*

## 2.3. Isolation of compounds

### *2.3.1. Isolation of compounds from A. dactylomela in Phu Quy, Lam Dong*

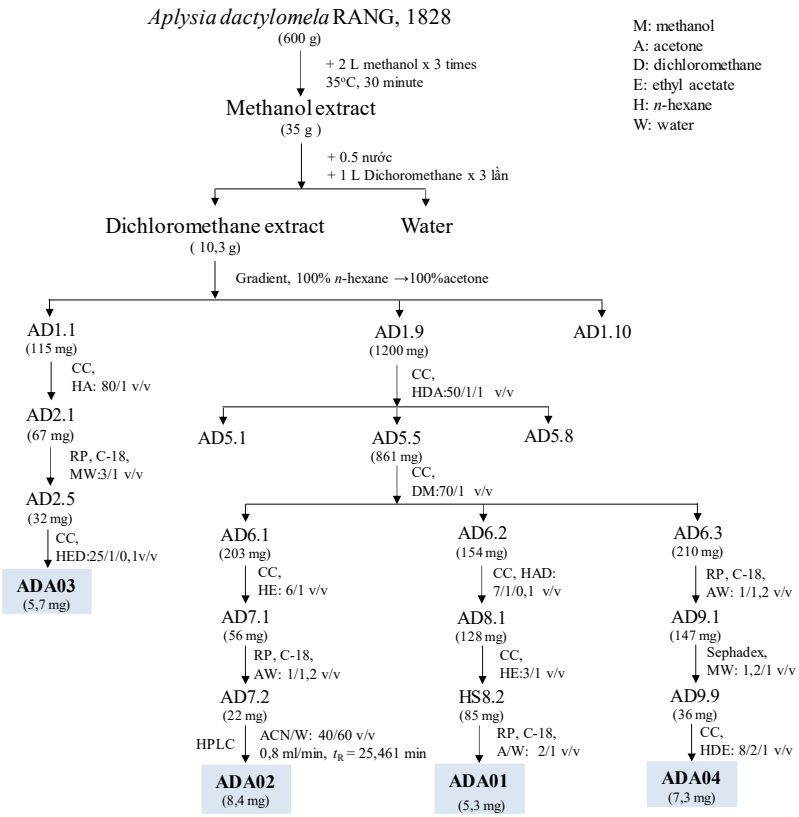



Fig. 2.3. Diagram of isolation of compounds from *A. dactylomela*

### *2.3.2. Isolation of compounds from A. dactylomela in Ly Son, Quang Ngai*

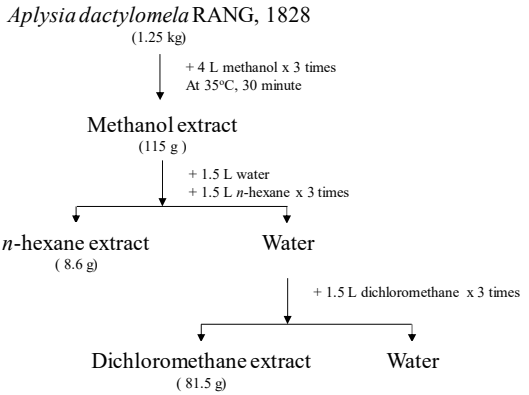



Fig. 2.4. Diagram of isolation of compounds form *A. dactylomela* in Quang Ngai

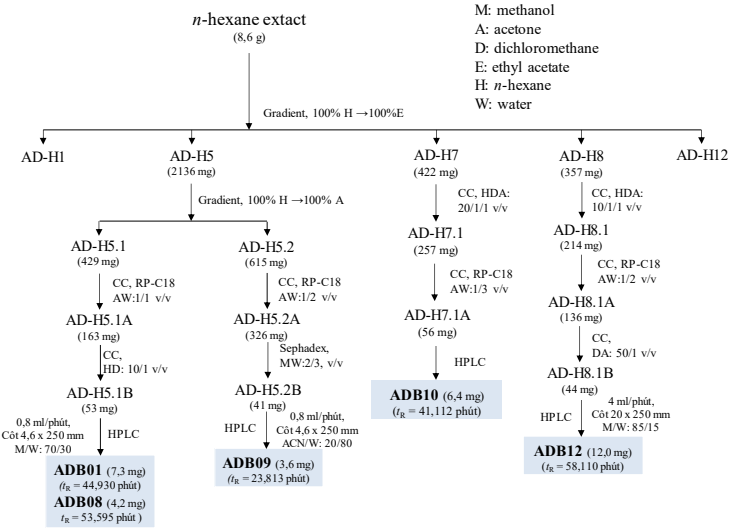



Fig. 2.5. Diagram of isolation of compounds from *n*-hexane of *A. dactylomela*

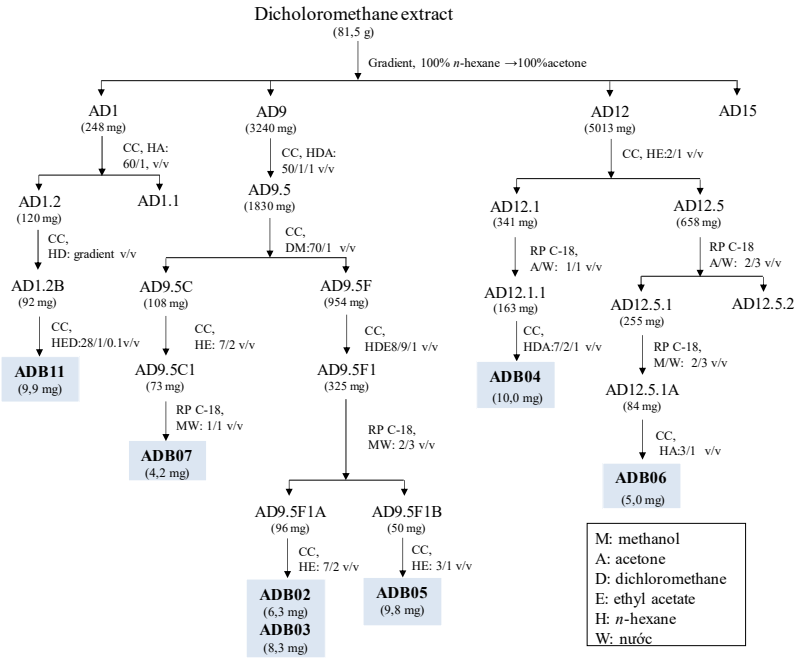



Fig. 2. 6. Diagram of isolation of compounds from dichloromethane extract of *A. dactylomela*

### *2.3.3. Isolation of compounds from A. oculifera in Lan Co, Hue*

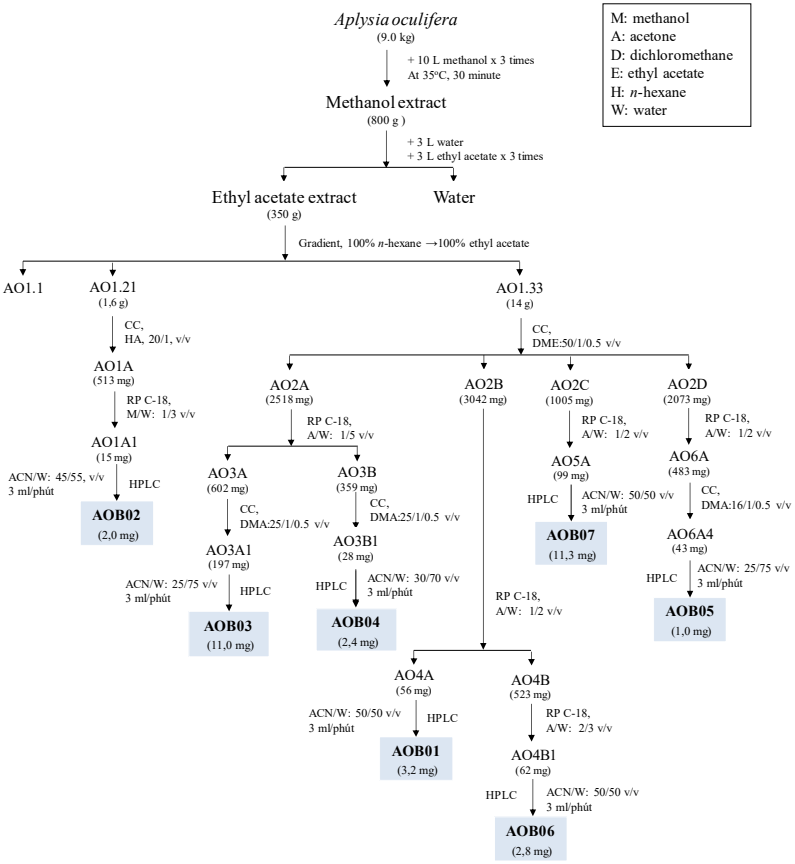



Fig. 2.7. Diagram of isolation of compounds from *A. oculifera*

# Chương 3. DISCUSS RESULTS

## 3.1. Determine the structure of the isolated compounds

### *3.1.1. Determination of the structures of compounds isolated from A. dactylomela collected on Phu Quy island, Binh Thuan*

3.1.1.1. Compound **ADA1**: Dactylomelanin C (new)

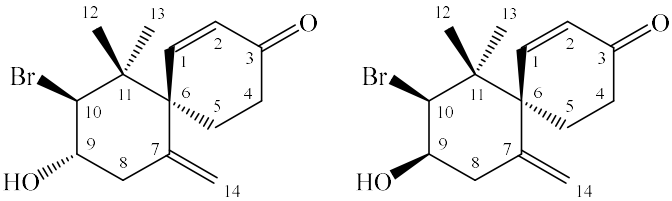



Fig. 3.1. Structure of **ADA1**

Dactylomelanin C (**ADA1**) was obtained as a white amorphous powder. Its HR-ESI-MS spectrum exhibited the isotopic ion peaks at m/z 299.0657 and 301.0624 $[M + H]^+$ with a ratio of 1:1, suggesting a molecular formula of $C_{14}H_{19}BrO_2$ (calcd. for $C_{14}H_{20}BrO_2^+$, 299.0641 and 301.0621). The $^{13}C$ NMR spectrum of **ADA1** showed signals for 14 carbons, which were categorized by the assistance of the HSQC experiment as four non-protonated carbons, four methines, four methylenes, and two methyls. Among these, one ketone group ($\delta_C$ 198.8), four olefinic carbons ($\delta_C$ 152.3, 142.9, 131.4, and 117.0), and two oxygenated or halogenated methines ($\delta_C$ 73.2 and 71.2) were detected. Furthermore, the number of $sp^2$ carbons accounted for three of the total five degrees of unsaturation deduced from the molecular formula, indicating the existence of a bicyclic system in **ADA1**. The $^1H$ NMR spectrum revealed the presence of a 1,2-disubstituted double

bond [$\delta_H$ 6.87 (dd, $J$ = 10.2, 1.8 Hz) and 6.14 (d, $J$ = 10.2 Hz)], a 1,1-disubstituted double bond [$\delta_H$ 5.18 (br s) and 4.81 (t, $J$ = 1.8 Hz)], and two singlet methyls [$\delta_H$ 1.20 and 1.13]. Additionally, two deshielded methine protons resonating at $\delta_H$ 4.38 (d, $J$ = 10.5 Hz) and $\delta_H$ 3.90 (ddd, $J$ = 10.5, 10.5, 6.0 Hz) could be assigned to a brominated methine and an oxymethine group, respectively (Davyt et al., 2001). Analysis of the COSY and HMBC data revealed that the 2D structure of 1 (Fig. 2) was identical to that of ma'ilione, a nor-chamigrane isolated from the red alga *Laurencia cartilaginea* (Francisco et al., 1998; Juagdan et al., 1997). Specifically, the COSY cross-peaks of H-1/H-2 and $H_2$-4/H2-5, in combination with the HMBC correlations from $H_2$-5 to C-1, C-3, and C-6, and from H-2 to C-4 confirmed the structure of a cyclohexenone ring. The structure of a cyclohexane ring as well as a spiro[5.5] chamigrane skeleton were established by the COSY correlations between $H_2$-8/H-9/H-10 and HMBC correlations from $H_3$-12 to C-10, C-11, and C-6, from $H_2$-14 to C-8 and C-6, from $H_2$-8 to C-7, and from $H_2$-5 to C-7. The $^1H$–$^1H$ coupling constants and NOESY spectrum were used to assign the relative configuration of **ADA1**. The large coupling constants of 10.5 Hz between H-10 and H-9, and between H-9 and H-8b indicated the axial orientation for these protons. This was further confirmed by the observed NOESY correlations between H-10/$H_2$-5, $H_2$-5/H-8b, and H-9/ $H_3$-12. The obvious NOESY correlations between $H_3$-12/H-1, H-1/H-14b, and $H_2$-5/$H_3$-13 verified the spiro[5.5] chamigrane structure with a 6*S** configuration. Among the bromochamigrane sesquiterpenes isolated from *Aplysia* species and the marine red alga of the genus *Laurencia*, most contain a bromine atom at C-10, but some were reported as unusual 9-bromochamigranes (Gonzalez ´ et al., 1983; Hu et al., 2020;

Li et al., 2013; Wessels et al., 2000). Accordingly, in order to elucidate the position of the bromine atom in **ADA1**, $^{1}H$ and $^{13}C$ NMR chemical shift calculations for the two possible isomers **ADA1**-1a-(6*S**,9*S**-OH,10*S**-Br) and 1b-(6*S**,9*S**-Br,10*S**-OH) were performed using a gauge independent atomic orbital (GIAO) method at the PCM-CHCl3/mPW1PW91/6–31+G(d,p) level. The experimental and calculated NMR data were then compared by analyses of the linear correlation coefficient ($R^2$), the corrected mean absolute error (CMAE), the root mean square deviation (RMSD), and DP4+ probability (Zanardi and Sarotti, 2021). The results showed that the calculated $^{1}H$ and $^{13}C$ NMR chemical shifts of C-9 and C-10 of isomer 1a matched the experimental values much better than those of **ADA1-**b. Furthermore, a comparison of the $R^2$, CMAE, and RMSD of the two isomers revealed that both $^{1}H$ and $^{13}C$ NMR data of **ADA1-**a were also more accurate than those of **ADA1-**b. Finally, the DP4+ calculation predicted that 1a was the correct isomer with 100% probability. To determine the absolute configuration of **ADA1**, the isomer **ADA1**-(6S,9S,10S) and its enantiomer, **ADA1′**-(6R,9R,10R), were subjected to ECD calculation using the time-dependent density functional theory (TDDFT) at the PCM-MeOH/CAM-B3LYP/cc-PVTZ level. The results indicated that the calculated ECD curve of **ADA1**-(6*S*,9*S*,10*S*) was in good agreement with the experimental data. Therefore, the absolute configuration of dactylomelanin C (**ADA1**) was assigned as 6*S*,9*S*,10*S*.

Table 3.1. $^{1}H$, $^{13}C$-NMR data of **ADA1**

| **Position** | $*\delta_C$[a,d] | $\delta_C$[a,b] | $\delta_H$[a,c] **(mult., *J* in Hz)** |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 1 | 152,9 | 152,3 | 6,87 (1H, dd, 10,2, 1,8) |
| 2 | 131,1 | 131,4 | 6,14 (1H, d, 10,2) |
| 3 | 198,7 | 198,8 | - |
| 4 | 34,2 | 34,0 | 2,35 (2H, m) |
| 5 | 26,3 | 26,3 | 2,21 (2H, m) |
| 6 | 51,8 | 51,5 | - |
| 7 | 141,7 | 142,9 | - |
| 8 | 38,4 | 39,2 | 2,80 (1H, dd, 13,8, 6,0)<br>2,44 (1H, dddd, 13,8, 10,5, 2,4, 1,8) |
| 9 | 71,7 | 71,2 | 3,90 (1H, ddd, 10,5, 10,5, 6,0) |
| 10 | 68,8 | 73,2 | 4,38 (1H, d, 10,5) |
| 11 | 42,7 | 42,5 | - |
| 12 | 21,4 | 19,2 | 1,20 (3H, s) |
| 13 | 26,9 | 26,3 | 1,13 (3H, s) |
| 14 | 117,9 | 117,0 | 5,18 (1H, br s)<br>4,81 (1H, br t, 1,8) |

[*] *Reference of ma'ilione [6],* [a] *recorded in* $CDCl_3$,, [b] *150 MHz,* [c] *600 MHz,* [d] *50 MHz*

### *3.1.2. Compounds isolated from A. dactylomela*

From *A. dactylomela*, 16 compounds were isolated and their structures were determined: dactylomelanin C **(ADA1)**, dactylomelanin D **(ADA2)**, dactylomelanin E **(ADA3)**, (2*S*,3*R*,7*S*,7*R*)-2-chloro-3,7-epoxychamigrane-9-one **(ADA4)**, elatol **(ADB1)**, aplydactylonin I **(ADB2)**, aplydactylonin K **(ADB3)**,

aplydactylonin G **(ADB4)**, aplydactylonin H **(ADB5)**, aplydactylonin F **(ADB6)**, aplydactylonin E **(ADB7)**, [3(15)*E*,4*Z*,6*S*,9*S*,10*R*]-10,15-dibromochamigra-3(15),4,7(14)-trien-9-ol **(ADB8)**, pacifidiene **(ADB9)**, 11-hydroxy-8-oxo-*β*-cyperon **(ADB10)**, aplydactylonin D **(ADB11)**, thyrsiferol **ADB12)**.

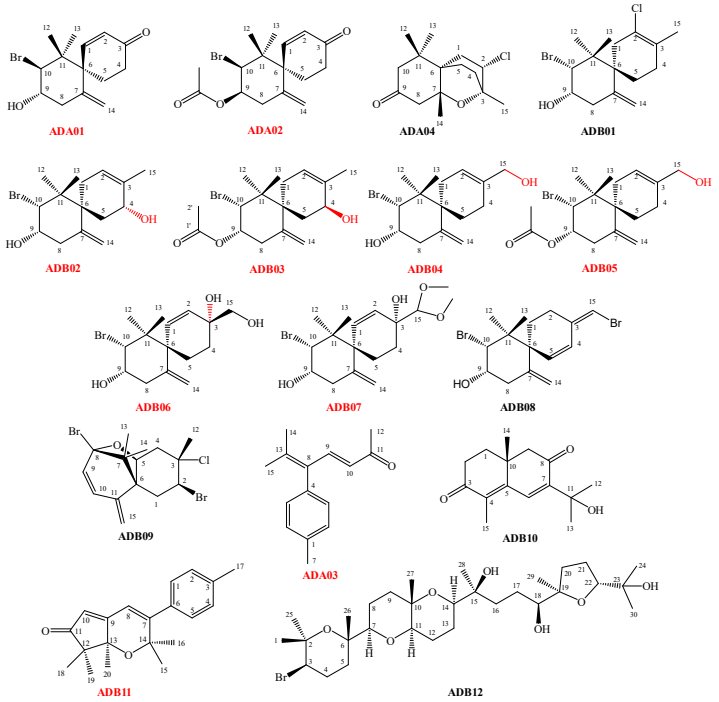



Fig. 3.2. Structure of compounds from *A. dactylomela*

From the structural analysis of compounds from *A. dactylomela*, the main compounds belonging to the class of substances are sesquiterpene, diterpene and triterpene. These compounds show high similarity in chemical composition that has been published by researchers for *A. dactylomela*.

### *3.1.3. Determination of the structures of compounds isolated from A. oculifera*

### *3.1.4. Compounds isolated from A. oculifera*

From *A. oculifera* 07 compound were isolated and their structures were determined: oculiferanin A **(AOB1)**, oculiferanin B **(AOB2)**, oculiferanin C **(AOB3)**, oculiferanin D **(AOB4)**, oculiferanin E **(AOB5)**, oculiferanin F **(AOB6)**, oculiferanin **G (AOB7)**. The above compounds are all new compounds and are also the first class of substances isolated from *A. oculifera*.

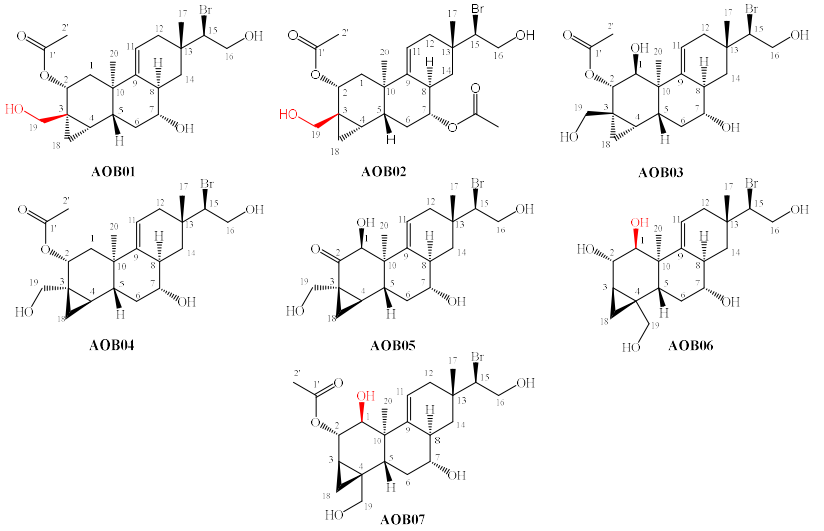



Fig. 3.3. Structure of compounds **AOB1** - **AOB7** from *A. oculifera*

## 3.2. Cytotoxic activity of some isolated compounds

### *3.2.1. Cytotoxic activity of compounds isolated from A. dactylomela*

The compounds **ADA1 - ADA4** and **ADB1 - ADB12** isolated from *A. dactylomela* were evaluated for in vitro cytotoxic activity on three human cancer cell lines: lung cancer (A549), breast cancer (MCF-7) and liver cancer (HepG2) by MTT method. The results are shown in Table 3.26.

Of the 16 compounds tested for activity, 9 compounds **ADA1 - ADA3**, **ADB1**, **ADB3**, **ADB5**, **ADB8** and **ADB11** showed cytotoxic activity in all 3 cancer lines. Of which, compound elatol (**ADB1**) showed the strongest cytotoxic activity with $IC_{50}$ values of 6.22, 5.07 and 6.55 μM corresponding to 3 cell lines HepG2, A549 and MCF-7. This result is also consistent with previous studies. Compound elatol (**ADB1**) has cytotoxic effect due to its ability to arrest cells in G1 and post-G1 phase, leading to programmed cell death (apoptosis) [107]. Compound **ADB1** also exhibited cytotoxic activity against the Vero cell line, showing an $IC_{50}$ value of 25.0 μg/mL. In addition to its cytotoxic activity, **ADB1** has several other reported activities. For example, it is used as a parasiticide against blood-sucking bugs such as *Trypanasoma cruzi* and *Leishmania amazonensis* [124], as an antitumor [124], and as a larvicidal agent against the dengue mosquito *Aedes aegypti* [125]. Compound **ADB1** exhibited antibacterial activity against all 13 bacterial strains tested. It showed a minimum inhibitory concentration (MIC) value of 5.5 mg/plate against *Clostridium cellobioparum*, *Proteus mirabilis* và *Flavobacterium helmiphilum* và 15–30 mg/đĩa đối với các loài vi khuẩn *Chromobacterium violaceum*,

*Clostridium fallax*, *Clostridium novyi*, *Clostridium sordellii*, *Escherichia coli*, *Enterobacter aerogenes*, *Shigella flexneri*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, và *Vibrio vulnificus*. [8].

Three compounds dactylomelanin C **(ADA1)**, dactylomelanin E **(ADA3)**, aplydactylonin K **(ADB3)** exhibited activity with $IC_{50}$ values in the range 19,91 - 42,66 µM for three tested cell lines. Four compounds dactylomelanin D **(ADA2)**, aplydactylonin H **(ADB5)**, [3(15)*E*,4*Z*,6*S*,9*S*,10*R*]-10,15-dibromochamigra-3(15),4,7(14)-trien-9-ol **(ADB8)**, aplydactylonin D **(ADB11)** showed weaker activity with $IC_{50}$ values in the range 34,51 – 68,71 µM for three tested cell lines. The remaining compounds did not show activity at the concentrations studied.

### *3.2.2. Cytotoxic activity of compounds isolated from A. oculifera*

The purified compounds **AOB1** to **AOB4** isolated from the sea slug A. oculifera were tested for in vitro cytotoxic activity on three human cancer cell lines: liver cancer HepG2, cervical cancer (Hela) and breast cancer (MCF-7) by MTT method. The results are shown in

The results of the cytotoxic activity test of the four compounds showed that: Compound oculiferanin A (**AOB1**) showed effects on two cancer cell lines HepG2 and Hela with $IC_{50}$ values of 21.38 and 23.44 µM, respectively. Compound oculiferanin C (**AOB3**) also showed inhibition on two cancer cell lines with $IC_{50}$ values of 34.67 and 36.73 µM, respectively, like **AOB1** but weaker. Compound oculiferanin B (**AOB2**) showed inhibition on all three cancer cell lines HepG2, Hela and MCF-7 with $IC_{50}$ values of 34.94, 41.91 and 35.89 µM, respectively. Compounds oculiferanin D (**AOB4**) and

oculiferanin G (**AOB7**) with $IC_{50}$ values of 54.95 and 31.99 μM, respectively, on the same cancer cell line HepG2. Compounds oculiferanin E (**AOB5)** and oculiferanin F (**AOB6**) showed no activity on all three tested cancer cell lines.

## CONCLUSIONS AND RECOMMENDATIOS

❖ CONCLUSIONS

By combining chromatographic methods and modern spectroscopic methods, and comparing with the spectral data of compounds published in reference documents, the structures of 23 pure compounds from two sea slugs *A. dactylomela* and *A. oculifera* were isolated and determined. The isolated compounds were tested for their cytotoxic activity against cancer cells, specifically as follows:

- Chemical composition:

1. From *A. dactylomela* collected in Phu Quy island, Binh Thuan isolated and determined the structure of 04 clean compounds: dactylomelanin C (**ADA1**), dactylomelanin D (**ADA2**), dactylomelanin E (**ADA3**), (2S,3R,7S,7R)-2-chloro-3,7-epoxychamigrane-9-one (**ADA4**). The above compounds belong to the sesquiterpene class, including three new compounds and one known compound.

2. From *A. dactylomela* collected in Son Tinh, Quang Ngai isolated and determined the structure of 12 clean compounds: elatol (**ADB1**), aplydactylonin I (**ADB2**), aplydactylonin K (**ADB3**), aplydactylonin G (**ADB4**), aplydactylonin H (**ADB5**), aplydactylonin F (**ADB6**), aplydactylonin E (**ADB7**), [3(15)E,4Z,6S,9S,10R]-10,15-

dibromochamigra-3(15),4,7(14)-trien-9-ol (**ADB8**), pacifidiene (**ADB9**), 11-hydroxy-8-oxo-β-cyperon (**ADB10**), aplydactylonin D (**ADB11**), thyrsiferol (**ADB12**). The above compounds belong to the terpenoid class, including eleven sesquiterpene compounds, one diterpene compound and one triterpene compound. Of the twelve pure compounds isolated, seven were new and five were known compounds.

3. From *A. oculifera* collected in Lang Co, Hue isolated and determined the structure of 07 clean compounds: oculiferanin A (**AOB1**), oculiferanin B (**AOB2**), oculiferanin C (**AOB3**), oculiferanin D (**AOB4**), oculiferanin E (**AOB5**), oculiferanin F (**AOB6**), oculiferanin G (**AOB7**). The above compounds all belong to the diterpene class and are new compounds, of which five compounds have a frame rearranged from the parguerane frame and two compounds have a parguerane frame.

- Biological activity:

The cytotoxic activity of 23 pure compounds from *A. dactylomela* and *A. oculifera* was tested. The compounds isolated from *A. dactylomela* were tested for cytotoxicity on three cancer lines HepG2, A549 and MCF-7, showing that nine compounds showed activity. Of which, compound elatol (**ADB1**) showed the strongest cytotoxic activity with $IC_{50}$ values of 6.22, 5.07 and 6.55 μM against three cell lines HepG2, A549 and MCF-7, respectively. Three compounds dactylomelanin C (**ADA1**), dactylomelanin E (**ADA3**), aplydactylonin K (**ADB3**) showed activity with $IC_{50}$ values ranging from 19.91 to 42.66 μM. Five compounds dactylomelanin D (**ADA2**), aplydactylonin H (**ADB5**), [3(15)E,4Z,6S,9S,10R]-10,15-

dibromochamigra-3(15),4,7(14)-trien-9-ol (**ADB8**), aplydactylonin D (**ADB11**) showed weaker activity with $IC_{50}$ values ranging from 34.51 - 85.31 μM. Of the compounds isolated from *A. oculifera* tested on the cancer cell lines HepG2, Hela and MCF-7, two compounds **AOB1** and **AOB3** showed activity against both the cancer cell lines HepG2 and Hela with $IC_{50}$ values ranging from 21.38 - 36.73 μM. Compound **AOB4** showed weak activity against the HepG2 cancer cell line with an $IC_{50}$ value of 54.95 μM. Compound oculiferanin B (**AOB2**) showed inhibition on all three cancer cell lines HepG2, Hela and MCF-7 with $IC_{50}$ values of 34.94, 41.91 and 35.89 μM, respectively. Compounds oculiferanin D (**AOB4**) and oculiferanin G (**AOB7**) showed $IC_{50}$ values of 54.95 and 31.99 μM, respectively, on the same HepG2 cancer cell line. Compounds oculiferanin E (**AOB5**) and oculiferanin F (**AOB6**) showed no activity against all three tested cancer cell lines.

## ❖ RECOMMENDATIONS

From two species of sea slugs of the genus *Aplysia*: *A. dactylomela* and *A. oculifera*, many new compounds have been isolated. Further studies are needed to further study the chemical composition and discover new compounds with good activity.

The new compounds isolated from the two species *A. dactylomela* and *A. oculifera* need to be further tested for some other activities, such as anti-inflammatory activity, antibacterial activity, enzyme inhibition... to detect better activities, serving for further in-depth studies later.

## NEW CONTRIBUTIONS OF THE THESIS

1. From *A. dactylomela* collected in Phu Quy Island, Binh Thuan and Son Tinh, Quang Ngai, 18 clean substances were isolated, including 10 new compounds: dactylomelanin C **(ADA1)**, dactylomelanin D **(ADA2)**, dactylomelanin E **(ADA3),** aplydactylonin I **(ADB2)**, aplydactylonin K **(ADB3)**, aplydactylonin G **(ADB4)**, aplydactylonin H **(ADB5)**, aplydactylonin F **(ADB6)**, aplydactylonin E **(ADB7)**, [aplydactylonin D **(ADB11)**.

2. From *A. oculifera* collected in Lang Co, Hue isolated and determined the structure of 07 compounds.: oculiferanin A **(AOB1)**, oculiferanin B **(AOB2)**, oculiferanin C **(AOB3)**, oculiferanin D **(AOB4)**, oculiferanin E **(AOB5)**, oculiferanin F **(AOB6)**, oculiferanin **G (AOB7)**. The above compounds all belong to the diterpene class and are new compounds, including five compounds with a frame rearranged from the parguerane frame and two compounds with a parguerane frame.

3. The new compounds (17 substances) isolated from two sea slugs *A. dactylomela* and *A. oculifera* were tested for biological activity with four cancer cell lines (HepG2, A549, MCF-7 and Hela) showing that three compounds dactylomelanin C (**ADA1**), dactylomelanin E (**ADA3**), aplydactylonin M (**ADB3**) showed activity with $IC_{50}$ values ranging from 19.91 - 42.66 μM. Compound oculiferanin A (**AOB1**) showed effect on two cancer cell lines HepG2 and Hela with $IC_{50}$ values of 21.38 and 23.44 μM, respectively.

## LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **Pham Thanh Binh**, Duong Thu Trang, Vu Thanh Trung, Kieu Thi Phuong Linh, Nguyen Viet Phong, Nguyen Phuong Thao, Nguyen Xuan Cuong, Do Cong Thung, Nguyen Hoai Nam, Nguyen Van Thanh, 2023, New nor-chamigrane and bisabolane sesquiterpenoids from the sea hare *Aplysia dactylomela*, *Phytochemistry Letters*, 53, pp. 92-97.

2. **Pham Thanh Binh**, Duong Thu Trang, Kieu Thi Phuong Linh, Nguyen Phuong Thao, Nguyen Chi Mai, Tran My Linh, Dang Vu Luong, Nguyen Hoai Nam, Nguyen Van Thanh, 2025, A novel diterpene and six new sesquiterpene from the sea hare *Aplysia dactylomela*. *Organic & Biomolecular Chemistry*, 23, pp. 8040-8052.

3. **Pham Thanh Binh**, Duong Thu Trang, Vu Thanh Trung, Kieu Thi Phuong Linh, Nguyen Phuong Thao, Nguyen Chi Mai, Tran My Linh, Nguyen Van Thanh, 2024, Cytotoxic constituents from the sea hare *Aplysia dactylomela, Vietnam Journal of Marine Science and Technology*. (Reviewing).