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RESEARCH ON CHEMICAL CONSTITUENTS AND ANTICANCER ACTIVITIES OF TWO SPECIES *DIANELLA ENSIFOLIA* AND *ELAEOCARPUS HAINANENSIS*

Major: Organic chemistry
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SUMMARY OF CHEMISTRY DOCTORAL THESIS

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PREFACE

1. The urgency of the thesis

Vietnam has an extremely rich and diverse flora and fauna due to the hot and humid tropical monsoon climate. Secondary metabolites are diverse in chemical structures, biological activities, and mechanisms of action.

Medicinal plants have been used to treat various disease since ancient times. About 50% of the anticancer drugs were isolated compounds or semi-synthesized from nature such as taxol, vinca alkaloid, camptothecin, podophyllotoxin, and their semi-synthetic derivatives, which were important anticancer drugs used in cancer treatment.

Dianella genus consists about 30 species, but only 9 out of them have been studied for their chemical compositions. Among these, *Dianella ensifolia* is the most studied species. Extracts and some purified compounds of this species have been investigated for biological activities to show many important effects such as anti-inflammatory, antiviral, antibacterial, anticancer, antioxidant and tyrosinase/melanogenesis inhibition.

Elaeocarpus hainanensis belonging to the genus *Elaeocarpus*, family Elaeocarpaceae, has been studied for its chemical constituents and biological activities. According to literatures published up to 2022, 16 terpenoids have been isolated from this species with cucurbitane triterpenes as were predominant subgroup. Notably, the cucurbitane triterpenes have been extensively studied for their biological effects due to their interesting antitumor activities both in terms of their inhibitory ability and mechanism of action.

With the aim of finding compounds with multi-effect and multi-mechanical anticancer abilities from Vietnamese medicinal plants, the author chose the topic of the thesis "**Research on chemical constituents and anticancer activities of two species *Dianella ensifolia* and *Elaeocarpus hainanensis***''.

2. Research aims of the thesis

➢ To study the chemical constituents of two species, including: Xuong quat (*Dianella ensifolia*) and Com hai nam (*Elaeocarpus hainanensis*).

➢ To evaluate the cytotoxic and anticancer activities of compounds isolated from two studied species; The potential active compounds are selected for further study on the effect at molecular level.

3. Research contents of the thesis

➢ To isolate compounds from two species: *Dianella ensifolia* and *Elaeocarpus hainanensis* by using chromatography methods;

➢ To determine the chemical structures of isolated compounds by using spectroscopic methods;

➢ To evaluate cytotoxic activities of isolated compounds. The potential active compounds are selected for further study on the effect at molecular level. The obtained results set up the scientific basis and recommendations for research and application of selected species in the prevention and treatment of cancer.

CHAPTER 1. OVERVIEW

The overview summarized the domestic and foreign studies on the following: botanical features, traditional medicinal uses,

chemical constituents and biological activities *Dianella* genus, *Dianella ensifolia* species; and *Elaeocarpus* genus, *Elaeocarpus hainanensis* species.

Regarding the biological activities of compounds isolated from two investigated species, the thesis summarized the biological effects of cucurbitane triterpenes, which showed strong inhibitory activity on cancer cells, induced apoptosis in cancer cells, induced synergistic effect with some chemotherapeutic agents, and increased the effectiveness of cancer treatment.

CHAPTER 2. RESEARCH OBJECTS AND METHODS

2.1. Plant samples

In the thesis, the materials used for study were two plant samples: *Dianella ensifolia* and *Elaeocarpus hainanensis* collected in Vietnam.

2.2. Research methods

2.2.1. Isolation methods

Using column chromatographic method (CC) in combination with thin layer chromatography (TLC) or high-performance liquid chromatography (HPLC) in some necessary cases.

2.2.2. Methods of structure determination

The general method used to determine the chemical structures of compounds was the combination of physical parameters with modern spectroscopic methods, including:

Specific rotation ($[\alpha]_D$)

Infrared spectrum (IR)

High-resolution electrospray ionization-mass spectroscopy (HR-ESI-MS)

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

NMR

Two-dimensional nuclear magnetic resonance: ^{1}H-^{1}H COSY, NOESY, HSQC, HMBC.

2.2.3. Methods for biological evaluation

2.2.3.1. Methods for cytotoxic activity evaluation

2.2.3.2. Methods for analysis of cell death, cell cycle progression and inducing apoptosis.

2.2.3.3. Methods for detection of gene expression by using RT-PCR

CHAPTER 3. EXPERIMENTS

3.1. ***Dianella ensifolia***

3.1.1 Extraction distribution of compound groups

3.1.2. Extraction and isolation of compounds

3.1.3. Physical parameters and spectroscopic data

3.2. ***Elaeocarpus hainanensis***

3.1.1 Extraction distribution of compound groups

3.2.1. Extraction and isolation of compounds

3.2.2. Physical parameters and spectroscopic data

3.3. Anti cancer activitive assays

3.3.1. Isolated compounds from D. Ensifolia

3.3.2. Isolated compounds from E. hainanensis

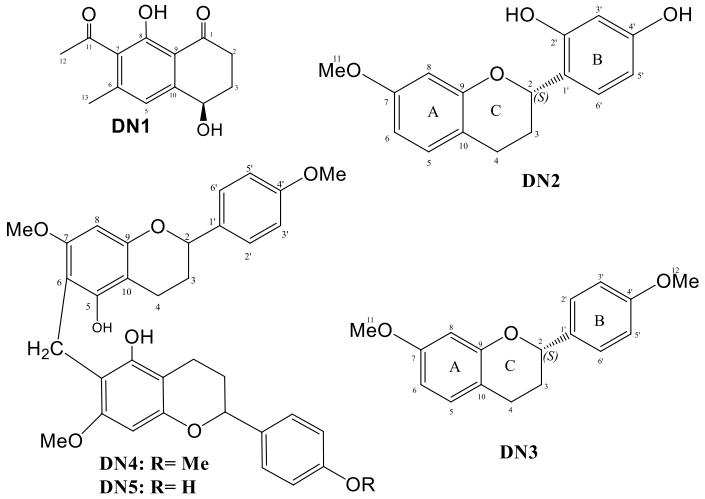
CHAPTER 4. RESULTS AND DISCUSSION

4.1. Results of study on chemical constituents

4.1.1. Determining the structure of compounds isolated from ***Dianella ensifolia***

10 compounds were isolated and structurally elucidated from *D. ensifolia,* including 4 new phenolic compounds: 7-acetyl-4R,8-dihydroxy-6-methyl-1-tetralone **(DN1**), (2*S*)-2′,4′-dihydroxy-7-methoxyflavan (**DN2**), diaensi-biflavan (5-hydroxy-7,4'-dimethoxy-(6,6"-methylen)-biflavan) (**DN4**), and diaensi-biflavan A (5-hydroxy-7,4'-dimethoxy-6,6"-methylen-(5,4'-dihydroxy-7-methoxy)-flavan

(**DN5*)***; 1 compound isolated for the first time as a natural product (2*S*)-7,4′-dimethoxy flavan (**DN3**) and 5 known compounds (**DN6–DN10**): methyl *β*-orcinolcarboxylate (**DN6**), dianellose (**DN7**), amentoflavone (**DN8**), sitosterone (**DN9**), and *β*-sitosterol (**DN10**).



Chemical structures of 5 new compounds isolated from *D. ensifolia*

❖ *Compound diaensi-biflavan (5-hydroxy-7,4'-dimethoxy-(6,6''-methylen)-biflavan) (**DN4**)*

DN4: white powder, $[\alpha]_D^{25}$ = +29 ($CHCl_3$, *c* = 0,1), pseudo-molecular ion peak at *m/z* 585.2477 $[M + H]^+$ (calcd. for $C_{35}H_{37}O_8$, 585.2488) in the HR-ESI-MS indicated the molecular formula of $C_{35}H_{36}O_8$. In addition to the typical signals indicating a flavan structure ($C_{17}H_{18}O_4$), a methylene group (δ_H 3.80, δ_C 16.2) was also evident in the ^{1}H- and ^{13}C-NMR spectra. These data suggested that **DN4** includes two identical flavan units, connected through a methylene group. The ^{13}C-NMR spectra of each unit showed the signals of aromatic carbons of 2 aromatic rings A and B ranging

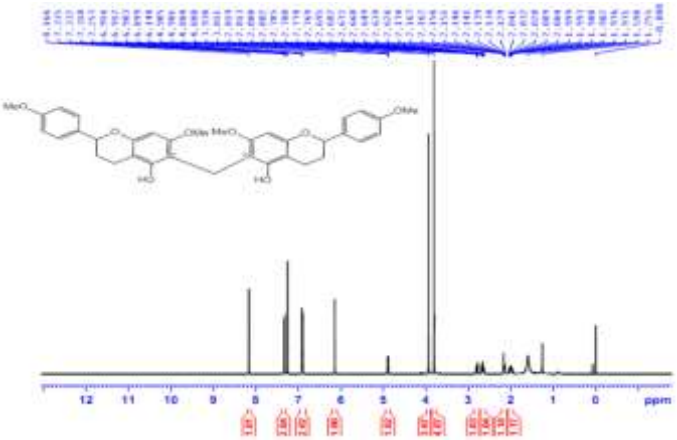
from δ_C 92.0 to 159.3. The ring C of each unit indicated the presence of one oxymethine at δ_H 4.89 (H-2, dd, 2.0, 10.0; δ_C 77.7) and two methylene at δ_H 2.15 và 1.99 (H_2-3, m; δ_C 29.5), and at δ_H 2.79 and 2.66 (H_2-4, m; δ_C 19.8). Two doublet protons at δ_H 7.33 (H-2′/H-6′, dd, 2.0; 8.0) and 6.91 (H-3′/H-5′, dd, 2.0; 8.0) characterized a benzene ring with submission at C-1' and C-4' positions. The presence of a hydroxy group (δ_H 8.17) and two methoxy groups on each unit was indicated by signals at δ_H 3.81 (δ_C 56.1) and δ_H 3.94 (δ_C 55.3).

The structure of each unit was determined to be 5-hydroxy-7,4'-dimethoxy flavan based on HMBC correlations between H-8 (δ_H 6.14) and C-6 (δ_C 106.0), C-7 (δ_C 155.1), C-9 (δ_C 154.5), C-10 (δ_C 104.2); between OH-8 (δ_H 8.17) and C-5 (δ_C 154.1), C-6 (δ_C 106.0), C-10 (δ_C 104.2); between H-4 (δ_H 2.66) and C-3 (δ_C 29.5), C-5 (δ_C 154.1), C-10 (δ_C 104.2); and between H-2 (δ_H 4.89) and C-4 (δ_C 19.8), C-10 (δ_C 133.8), C-2', C-6′ (δ_C 127.4). The connection position of the two flavan units at C-6 through a methylene group was deduced based on HMBC correlations among signals between CH_2 (δ_H 3.80) and C-5 (δ_C 154.1), C-6 (δ_C 106.0) and C-7 (δ_C 155.1). Accordingly, the structure of **DN4** was determined as 5-hydroxy-7,4′-dimethoxy- (6,6″-methylen)-biflavan (named diaensi-biflavan), a new compound.

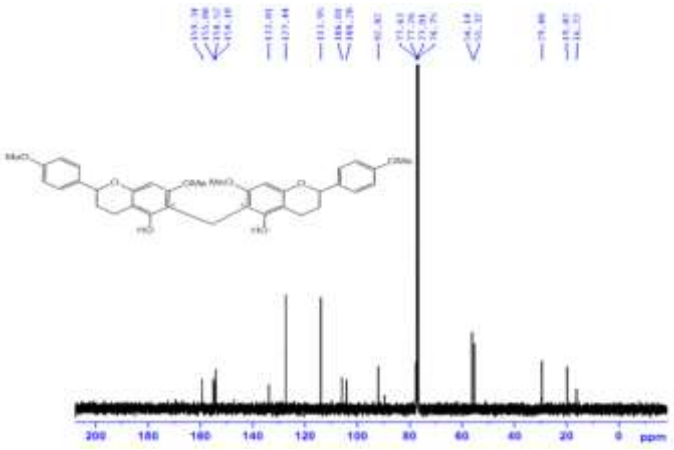
Table: NMR spectroscopic data of compound **DN4**

C	**δ_C[a]**	**δ_H[b]**
2	77.6 CH	4.89, dd (2.0, 10.0) 1H
3	29.5 CH_2	2.15, m, 1H; 1.99, m, 1H
4	19.8 CH_2	2.79, m, 1H; 2.66, m, 1H

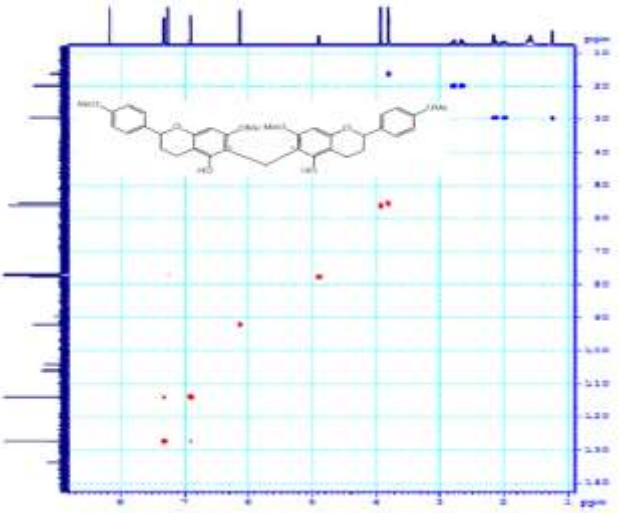
5	154.1 C	-
6	106.0 C	-
7	155.1 C	-
8	92.0 CH	6.14, s, 1H
9	154.5 C	-
10	104.2 C	-
1'	133.8 C	-
2'	127.4 CH (x2)	7.33, dd (2.0, 8.0) 2H
3'	113.9 CH (x2)	6.91, dd (2.0, 8.0) 2H
4'	159.3 C	-
5'	113.9 CH (x2)	6.91, dd (2.0, 8.0) 2H
6'	127.4 CH (x2)	7.33, dd (2.0, 8.0) 2H
5-OH	-	8.17, s, 1H
7-OMe	55.3 CH_3	3.94, s, 3H
4'-OMe	56.1 CH_3	3.81, s, 3H
CH_2	16.2 CH_2	3.80, s, 2H



^{1}H-NMR (500 MHz, $CDCl_3$) spectrum of compound **DN4**



^{13}C-NMR (125 MHz, $CDCl_3$) spectrum of compound **DN4**



HSQC spectrum of compound **DN4**

By analyzing the spectroscopic data similar to that of **DN4**, the structures of all isolated compounds were determined as listed in the following table:

Isolated compounds from *D. ensifolia*

№	Compounds	Name of compounds	Notes
1	**DN1**	7-Acetyl-4R, 8-dihydroxy-6-methyl-1-tetralone	**New compound**
2	**DN2**	(2*S*)-2′,4′-Dihydroxy-7-methoxyflavan	**New compound**
3	**DN3**	(2*S*)-7,4′-Dimethoxy flavan	Isolated for the first time as a natural product
4	**DN4**	Diaensi-biflavan (5-hydroxy-7,4′-dimethoxy-(6,6″-methylen)-biflavan)	**New compound**
5	**DN5**	Diaensi-biflavan A (5-hydroxy-7,4'-dimethoxy-6,6"-methylen-(5,4'-dihydroxy-7-methoxy)-flavan)	**New compound**
6	**DN6**	Methyl*β*-orcinolcarboxylate	
7	**DN7**	Dianellose	
8	**DN8**	Amentoflavone	Isolated for the first time from *Dianella* genus
9	**DN9**	Sitosterone	
10	**DN10**	β-sitosterol	

4.1.2. Determining the structure of compounds isolated from *Elaeocarpus hainanensis* Oliv.

15 compounds were isolated and structurally elucidated from *E. hainanensis*, including: 13 triterpenes, which cover 8 cucurbitacins (**EH1-EH8**), 5 oleanens (**EH9-EH13**); 1 furfural terpenoid (**EH14**); and 1 megastigmane (**EH15**). Among these, 3 compounds **EH8, EH9**, and **EH13** were new**.**

❖ *New compound elaeohainensin A (**EH8**)*

Molecular formula of **EH8** was assigned as $C_{30}H_{46}O_6$ on the basis of the pseudo-molecular ion peak at *m/z* 503.3358 $[M + H]^+$ (cald. for $C_{30}H_{47}O_6$, 503.3367) and 537.2989 $[M + Cl]^-$ (cald. for $C_{30}H_{46}O_6Cl$, 537.2988) in the positive and negative HR-ESI-MS, respectively.

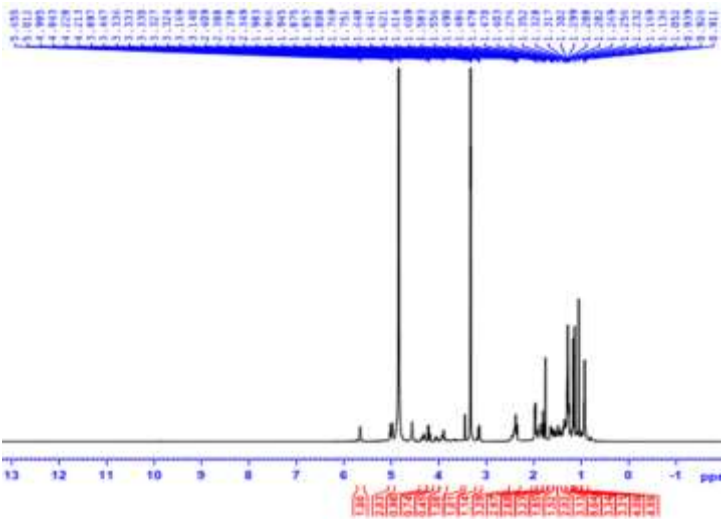
The ^{13}C NMR spectrum of **EH8** revealed the presence of 30 carbons, which were classified as eight quaternary carbons, eight methines, seven methylenes and seven methyl groups (8 x C, 8 x CH, 7 x CH_2, 7x CH_3), including five oxygen-bearing carbons, a trisubstituted double bond, a disubstituted terminal double bond, and a carbonyl group (δ_C 216.6). The ^1H- and ^{13}C-NMR spectra revealed the presence of seven singlet methyl groups at δ_H 0.93-1.75, a doublet olefinic methine at δ_H 5.66 (br d, J = 6.0 Hz, H-6)/δ_C 120.4, and four oxygenated methines at δ_H 3.45 (brs, H-3)/δ_C76.9, δ_H 4.32 (ddd, J = 10.5, 10.5, 3.5 Hz, H-16)/δ_C 78.0, δ_H 3.92 (m, H-23)/ δ_C 76.2, and δ_H 4.22 (d, J = 7.5 Hz, H-24)/ δ_C 93.2. Most notably, the typical signals of an isopropylidene moiety were recognized in the NMR spectra at δ_H 5.02 (H-26a, d, 4.5) and 5.00 (H-26b, d, 4.5)/δ_C 116.2, 1.75 (CH_3-27, s)/δ_C 18.0 và 143.7 ppm (C-25). Therefore, the structure of **EH8** was suggested as a tetracyclic triterpene with one carbonyl group, one double bond in the ring and one terminal double bond, which was specific to the cucurbitacin group (one double bond at -C5=C6-). In addition, the characteristic NMR spectral data of **EH8** were similar to those of **EH6**, except for the significant resonance changes in the side chain, with a stronger field shift of C-24 from δ_C 126.5 to 93.2 ppm. Moreover, ^{13}C NMR data of **EH8** were almost similar to those of elaeocarpucins A and B [53], with the only significant downfield shift difference at δ_C 93.2 ($\Delta\delta_C$ > 25 ppm), which

confirmed the assignment of hydroperoxide group at C-24. This was confirmed by the base peak in the positive ion HR-ESI-MS spectrum at *m/z* 471.3479 $[M+H-O_2]^+$ (calcd. for $C_{30}H_{47}O_4^+$, 471.3469), originating from a molecular ion after loss of two oxygen atoms (O_2) and characteristic for the presence of a hydroperoxide group in the parent compound. Furthermore, key HMBC correlations from H-24 (δ_H 4.22) and H-27 (δ_H 1.75) to C-26 (δ_C 116.2), as well as the terminal olefinic methylene protons of H_2-26 to C-24 (δ_C 93.2), C-25 (δ_C 143.7), and C-27 (δ_C 18.0) confirmed the structure of the side chain moiety. Therefore, the relative configuration at all asymmetric carbons of **EH8,** except for C-24, were determined as 16*α*,23*α*-epoxy-3*β*,20*β*-dihydroxy-10*α*H,23*β*H. In the above-mentioned data, the structure of **EH8** was elucidated as 16*α*,23*α*-epoxy-3*β*,20*β*-dihydroxy-24*α*-hydroperoxy-10*αH*,23*βH*-cucurbit-5,25-dien-11-one, a new natural cucurbitacin, and named elahainencin A.

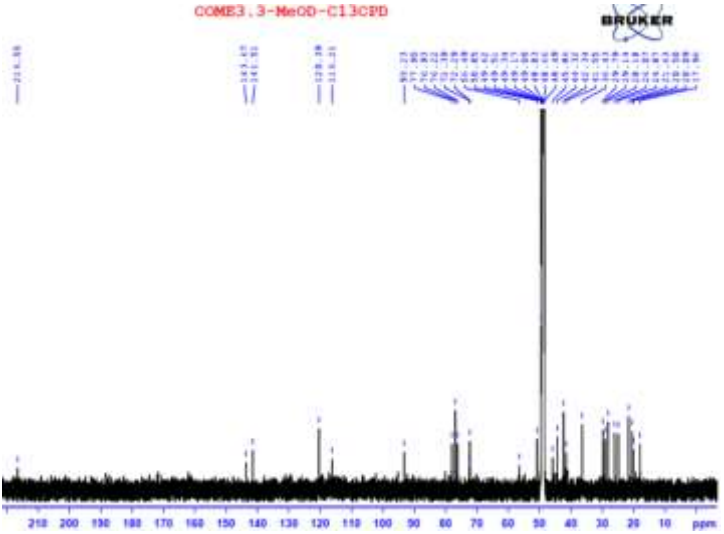
NMR spectroscopic data of compound **EH8**

C	δ_C[a]	δ_H[b]	**HMBC correlations (H→C)**
1	21.6	*α*: 1.62, dd (8.5, 7.5) *β*: 1.33, m	-
2	29.8	*α*: 1.88, m *β*: 1.61, dd (8.5, 7.5)	C-4, C-1
3	76.9	3.45, br s	C-5, C-1
4	42.3	-	
5	141.5	-	
6	120.4	5.66, br d (6.0)	C-8, C-4, C-10
7	24.9	*α*: 1.92, m *β*: 2.46, m	-
8	44.3	1.94, m	-

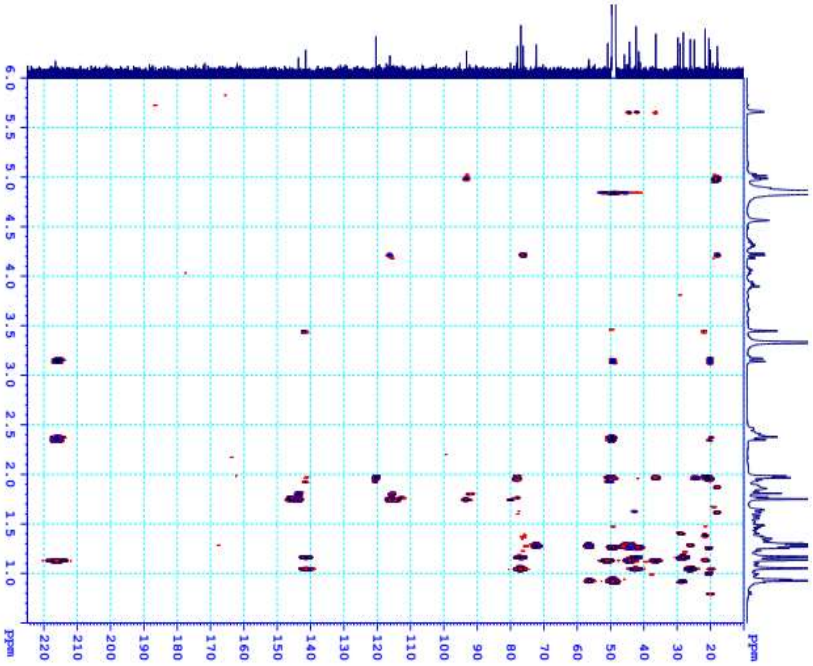
9	49.0 [b]	-	-
10	36.4	2.37, br d (9.5)	-
11	216.6	-	-
12	49.6 [b]	α: 3.15, d (14.5) β: 2.36, d (14.5)	C-11, C-13, C-18
13	49.2[b]	-	-
14	50.9	-	-
15	41.6	α: 1.48, m β: 1.86, m	-
16	78.0	4.32, ddd (3.5, 10.5, 10.5)	-
17	56.5	1.97, br d (8.5)	C-14, C-16
18	20.1	0.93, s	C-14
19	20.5	1.14, s	-
20	72.4	-	-
21	29.1	1.30, s	C-20, C-17, C-13
22	45.9	α: 1.34, m β: 1.36, m	C-20
23	76.2	3.92, m	-
24	93.2	4.22, d (7.5)	C-26, C-23, C-27
25	143.7	-	-
26	116.2	5.02, d (5.2) 5.00, d (5.2)	C-24, C-27
27	18.0	1.75, s	C-24, C-25, C-26
28	28.2	1.17, s	C-3, C-4, C-29
29	26.1	1.05, s	C-3, C-4, C-28
30	26.1	1.27, s	C-14, C-15



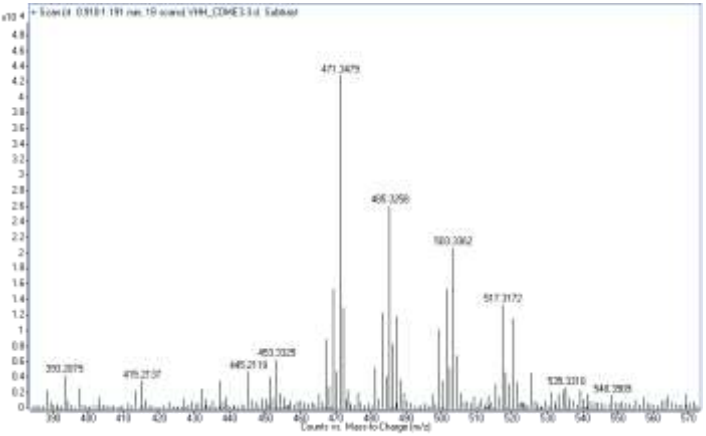
^{1}H NMR (500 MHz, CD_3OD) spectrum of compound **EH8**



^{13}C NMR (125 MHz, CD_3OD) spectrum of compound **EH8**



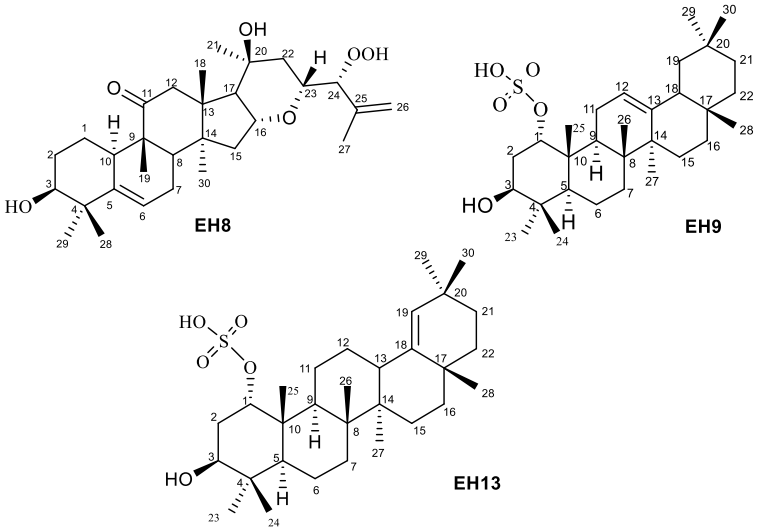
HMBC spectrum of compound **EH8**



HR-ESI-MS (positive) spectrum of compound **EH8**

Isolated compounds from *E. hainanensis*

№	Compounds	Name of compounds	Notes
1	**EH1**	Cucurbitacin D	
2	**EH2**	Cucurbitacin I	
3	**EH3**	3-*epi*-isocucurbitacin D	
4	**EH4**	Cucurbitacin F	
5	**EH5**	Cucurbitacin H	
6	**EH6**	16*α*, 23α-Epoxy-3*β*, 20R-dihydroxy-10*α*H, 23*β*H-cucurbit-5,24-dien-11-one	
7	**EH7**	16*α*, 23*α*-Epoxy-cucurbit-3*β*, 20*β*-dihydroxy-10*α*H, 23*β*H-5,24-dien-11-one 3-O-*β*-D-glucopyranoside	
8	**EH8**	Elaeohainensin A (16*α*,23*α*-epoxy-3*β*,20*β*-dihydroxy-24*α*-hydroperoxy-10*αH*,23*βH*-cucurbit-5,25-dien-11-one)	**New compound**
9	**EH9**	1*α*,3*β*-Dihydroxy-olean-12-ene 1- sulphate	**New compound**
10	**EH10**	1*α*-Hydroxy-olean-12-en-3*β*-O-*α*-L-arabinopyranoside	
11	**EH11**	1*α*-Hydroxy-olean-11-oxo-12-en-3-O-*α*-L-arabinopyranoside	
12	**EH12**	1*α*-Hydroxy-olean-12-en-3-O-*β*-D-xylopyranoside	
13	**EH13**	1*α*,3*β*-Dihydroxy-olean-18-ene 1- sulphate	**New compound**
14	**EH14**	5-(Hydroxymethyl)-2-furancarboxaldehyde	
15	**EH15**	Blumenol A	



Chemical structure of new compounds isolated from *E. hainanensis*

4.2. The results for anticancer activit

4.2.1. The anticancer activity of the isolated compounds from D. ensifolia

DN1, DN2, DN3, DN4 isolated from *D. ensifolia* were investigated the cancer cell proliferation inhibition on four cancer cell lines of A549 (human lung carcinoma), Hep3B (human hepatocellular carcinoma), Hela (human cervix carcinoma), MCF7 (human breast carcinoma).

All of them at the concentration of 100 µM weakly inhibited four cancer cell lines except the compound **DN1.**

4.2.2. The anticancer activity of the isolated compounds from E. hainanensis

❖ The cytotoxic activity

The results of cytotoxic activity of the compounds isolated from *E. hainanensis*

Test	50% inhibitory concentration (IC_{50} µM)

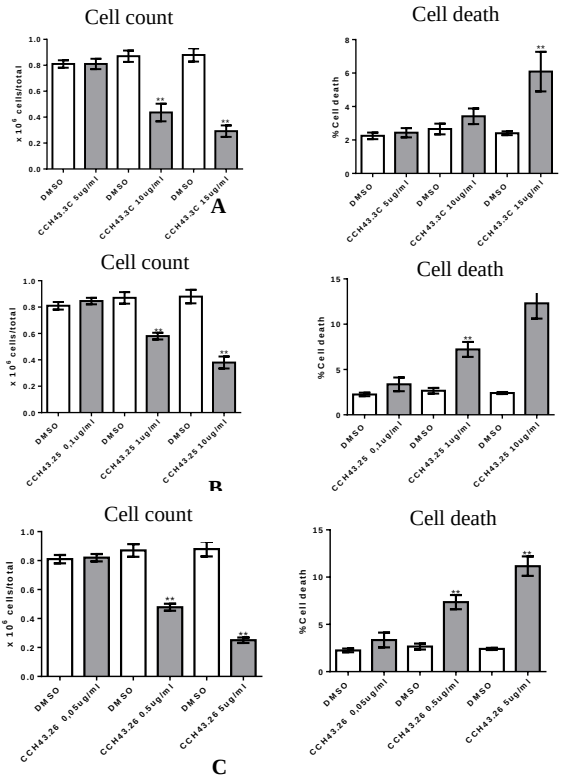
compound	A549	T24	Huh-7	8505	SNU-1
EH6	83.49 ± 4.11	104.49 ± 4.40	**56.19 ± 3.34**	80.19 ± 6.17	75.96 ± 3,57
EH7	108.53 ± 3.72	**116.22 ± 4.22**	111.17 ± 8.23	101.68 ± 3.80	95.06 ± 8.62
EH8	100.60 ± 3.13	101.71 ± 5.28	63.82 ± 5.46	114.12 ± 7.55	98.92 ± 4.54
Ellipticine	1.87 ± 0.20	2.11 ± 0.16	1.58 ± 0.16	1.54 ± 0.12	1.58 ± 0.12

* **EH6:** 16*α*,23*α*-epoxy-3*β*,20*R*-dihydroxy-10*α*H,23*β*H-cucurbit-5,24-dien-11-one ; **EH7:** 16*α*,23*α*-epoxy-3*β*,20*R*-dihydroxy-10*α*H,23*β*H-cucurbit-5,24-dien-11-one 3-O-*β*-D-glucopyranoside; **EH8:** 16*α*,23*α*-epoxycucurbitacin-24-hydroperoxy

The cytotoxicity of compounds **EH6, EH7, EH8** on human cancer cell lines (A549, T24, Huh-7, 8505, SNU-1) was accessed using SRB asay. As showed in the above table, all of them showed cytotoxic activity on all of tested cancer cell lines with IC_{50} values about 56.19 ± 3.34 to 116.22 ± 4.22 µM. These cucurbitacins showed the cytotoxic activity less than the other cucurbitacins, this result is also suitable for the results of the previous studies.

❖ The cytotoxic activity on cancer cell line of OCI-AML3

The results in below figure showed that the compounds of **EH10**, **EH2a (mixture EH1 và EH2) , and EH3** exhibited the cytotoxic activity on OCI-AML3 of 15, 1, 0,5 µg/mL treatment, respectively. Cucurbitacin compounds exhibited the cytotoxicity on OCI-AML3 more strongly than the triterpenoid oleans isolated from *E. hainanensis.*



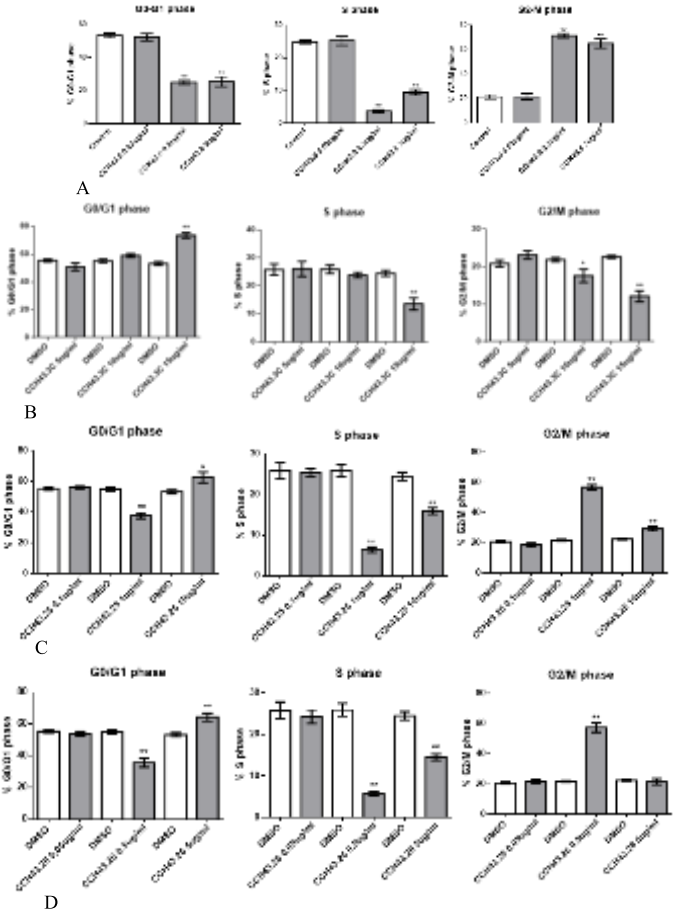
*A: **EH10 (**5; 10; 15 µg/mL **)**, B: **EH2a (**0.1; 1; 10 µg/mL**)**, C: **EH3** (0.05; 0.5; 5 µg/mL).*

The results of cytotoxicity activity on OCI-AML3 of some isolated compounds from *E. hainanensis.*

❖ Analysing of cell cycle on OCI-AML3 of the isolated compounds from *E. Hainanensis*

The results in the below figure showed that the triterpenoid compounds isolated from *E. hainanensis* as **EH10** (1α-hydroxy-olean-12-en-3-O-β-L arabinopyranoside), **EH1 (**cucurbitacin D**), EH2a (**Mixture of cucurbitacin D and cucurbitacin I (**EH2**)**)**, **EH3** (3-*epi*-cucurbitacin D) inhibited the OCI-AML3 growth with the mechanism was effection on cell cycle arrest, increasing the number of cells in G_0/M and G_2/M phases. Among them the **EH1**, **EH2a, EH3**

compounds displayed very good active.



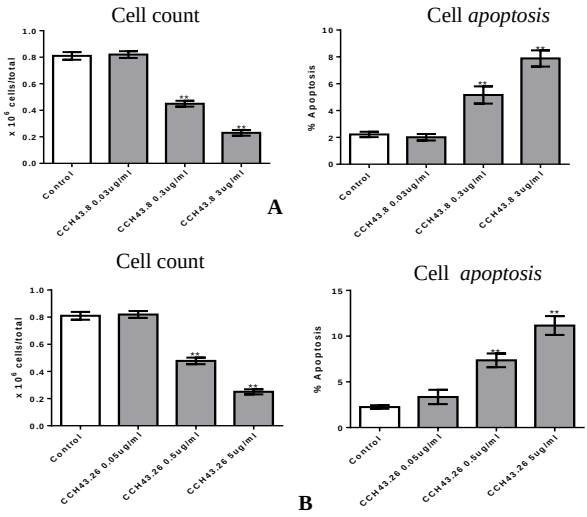
*A: **EH1 (**0.03; 0.3; 3 µg/mL**)**, B: **EH10 (**5; 10; 15 µg/mL**)**, C: **EH2a (**0.1; 1; 10 µg/mL**)**, D: **EH3 (**0.05; 0.5; 5 µg/mL**)***

The results of cell cycle analysing on OCI-AML3 of **EH1, EH10, EH2a, EH3** isolated from *E. Hainanensis*

❖ The *apoptosis* inducing on OCI-AML3 cells

The results in the above figure show that, both of the **EH1** and **EH3** compounds induce *apoptosis* on OCI-AML3 of the low

concentration treatments (< 0,5 µg/ml).

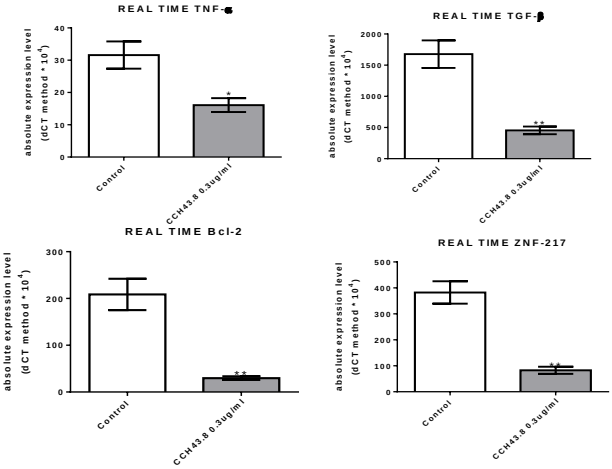


*A: **EH1 (0.03; 0.3; 3** µg/mL) B: **EH3 (0.05; 0.5; 5** µg/mL)*

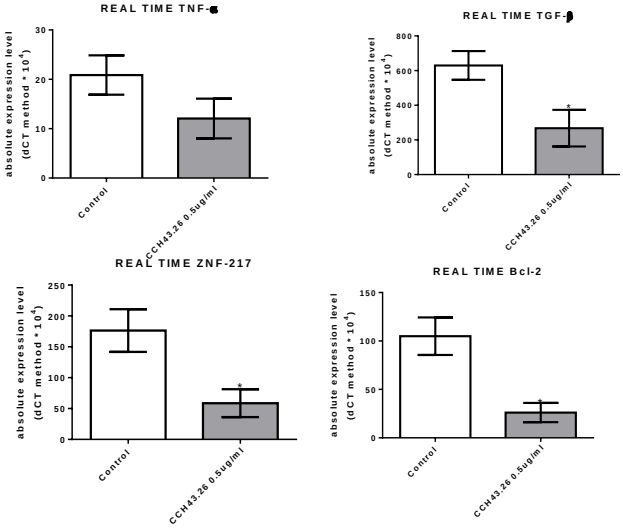
The results of *apoptosis* inducing on OCI-AML3 of **EH1** and **EH3**

❖ Researching on the expressions of genes relate to cancer stimulation of cucurbitacin

0.3 µg/ml of **EH1 and** 0.5 µg/ml of **EH3** were treated with OCI-AML3 cells. Using RT-PCR methods to evaluate the expression of genes as TNF-α, Bcl-2, TGF-β, ZNF-217 (The overexpression of these gene induce promotion, invasive and metastatic cancer). In above figure showed the results of downregulation all of genes of **EH1** and **EH3.**



The effection of **EH1** (0.3 µg/mL) to gene expression (TNF-α, Bcl-2, TGF-β, ZNF-217) of OCI-AML3.



The effection of **EH3** (0.5 µg/mL) to gene expression (TNF-α, Bcl-2, TGF-β, ZNF-217) of OCI-AML3

CONCLUSION

1. Results of study on chemical constituents

❖ 10 compounds were isolated and structurally elucidated from *D. ensifolia*, including:

4 new phenolic compounds: 7-acetyl-4R,8-dihydroxy-6-methyl-1-tetralone **(DN1),** **(**2*S*)-2',4'-dihydroxy-7-methoxyflavan **(DN2),** diaensi-biflavan **(DN4)**, and diaensi-biflavan A **(DN5)**.

1 compound isolated for the first time as a nature product: (2*S*)-7,4'-dimethoxyflavan (**DN3**).

6 known compounds: methyl*β*-orcinolcarboxylate (**DN6**), dianellose (**DN7**), amentoflavone (**DN8**), sitosterone (**DN9**), and *β*-sitosterol (**DN10**).

❖ 15 compounds were isolated and structurally elucidated from *E. hainanensis*. Among these, cucurbitacins and oleanane triterpenes are the main compounds, including:

3 new compounds, including 1 cucurbitacin elaeohainencin A (**EH8**); and 2 oleanane triterpene sulfates 1*α*,3*β*-dihydroxy-olean-12-ene 1- sulfate (**EH9**), and 1*α*,3*β*-dihydroxy-olean-18-ene 1-sulfate (**EH13**).

12 known compounds, including 7 cucurbitacins: cucurbitacin D (**EH1**), cucurbitacin I **(EH2),** 3-*epi*-isocucurbitacin D **(EH3),** cucurbitacin F **(EH4),** cucurbitacin H (**EH5**), 16*α*,23*α*-epoxy-3*β*,20*R*-dihydroxy-10*α*H,23*β*H-cucurbit-5,24-dien-11-one **(EH6)**, and 16*α*, 23*α*-epoxy-cucurbit-3*β*, 20*β*-dihydroxy-10*α*H,23*β*H-5,24-dien-11-one 3-O-*β*-D-glucopyranoside **(EH7)**; and 3 oleanane triterpenes: 1*α*-hydroxy-olean-12-en-3-O-*α*-L-arabinopyranoside **(EH10),** 1*α*-hydroxy-olean-11-oxo-12-en-3-O-*α*-L-arabinopyranoside (**EH11**), and 1*α*-hydroxy-olean-12-en-3-O-*β*-D-xylopyranoside **(EH12)**; 1 furfurane 5-(hydroxymethyl)-2-furancarboxaldehyde **(EH14)**; and 1 megastigmane blumenol A **(EH15).**

2. The results for anticancer activity

❖ ***The anticancer activity of the compounds isolated from D. ensifolia***

➢ For the first time, four isolated compounds from *D.*

ensifolia were tested cytotoxic activity on human cancer cell lines as A549, Hela, HepG3 and MCF7. Three flavans (**DN2, DN3,** and **DN4**) displayed the inhibitory activity of these cancer cell lines but it was weak, while **DN1** is not flavan did not exhibit cytotoxic activity.

❖ ***The anticancer activity of the compounds isolated from E. hainanensis***

➢ The cucurbitacin derivations (**EH6, EH7, EH8**) were tested cytotoxic activity on five humun cancer cell lines A549, T24, Huh, 8505, SNU-1 at the first time. The results show that all of test compounds exhibited on average of cytotoxic activity with IC_{50} values about 56.19 to 116.22 µM.

➢ **EH10**, **EH2a (mixture EH1 and EH2), EH3** exhibited the cytotoxicity activity on OCI-AML3 of 15, 1, 0.5 µg/mL, respectively. This is the first time these compounds were tested cytotoxic activity on OCI-AML3 cell line.

➢ At the high concentration of **EH10**, **EH2, EH3 (**15; 10; 5; µg/mL, respectively) were induced G_0/M arrest in OCI-AML3 cell line. While at the low concentration of **EH1, EH2a, EH3** (0.3; 1; 0.5 µg/mL respectively) were induced G_2/M arrest in OCI-AML3 cell line.

➢ The **EH1** and **EH3** compounds induced *apoptosis* on OCI-AML3 at the low concentration (< 0.5 µg/mL).

➢ At the concentration of 0.3 µg/mL (**EH1) and** 0.5 µg/mL (**EH3)** downregulated the expression of OCI-AML3 genes as TNF-α, Bcl-2, TGF-β, ZNF-217.

RECOMMENDATION

The results of this study showed that two compounds cucurbitacin D (**EH1**) and 3-*epi*-cucurbitacin D (**EH3**) had cytotoxic activities, induced apotosis, and produced significant effect on down-expression of cancer genes for the acute myeloid leukemia (OCI-AML3) cell line. These cucurbitacins were predicted to have a great potent and further research need to be done on its pharmacological activities in order to research and develop materials to produce anticancer products for the community.

NEW CONTRIBUTIONS OF THE THESIS

1. For the first time *D. ensifolia* was studied on chemical constituents. From this species, 4 new compounds were isolated and structurally elucidated, including 7-acetyl-4*R*,8-dihydroxy-6-methyl-1-tetralone **(DN1), (**2*S*)-2',4'-dihydroxy-7-methoxyflavan **(DN2),** diaensi-biflavan **(DN4),** and diaensi-biflavan A **(DN5**). Compound (2*S*)-7,4'-dimethoxyflavan (**DN3**) was isolated for the first time as natural product.
2. New compound elaeohainencin A (**EH8**) was the first representative of cucurbitacin with hydroperoxide group in structure; and two compounds 1α,3β-dihydroxy-olean-12-ene 1-sulfate (**EH9**), and 1α,3β-dihydroxy-olean-18-ene 1-sulfate (**EH13**) isolated from *E. hainanensis* were new oleanane triterpenes with substitution of the sulfate group in structure.
3. For the first time the cytotoxicity of compounds as cucurbitacin D (**EH1**) and **EH2a** (mixture of cucurbitacin D và –I (**EH2**)) và 3-*epi*-cucurbitacin D (**EH3**) against acute myeloid leukemia cell line (OCI-AML3) was tested, all of them exhibited strongly cytotixic activity.
4. For the first time the cell cycle analysis on OCI-AML3 of triterpenoid compounds (**EH1**, **EH10, EH2a, EH3**) and the apoptosis inducing on OCI-AML3 of **EH1 (**cucurbitacin D**), EH3 (**3-*epi*-cucurbitacin D**)** were evaluated.
5. This is also the first time that **EH1** and **EH3** were evaluated the effection of the decreasing of gene expression as TNF-α, Bcl-2, TGF-β, ZNF-217 on OCI-AML3. The overexpression of these genes promote cancer growth.

LIST OF PUBLISHED ARTICLES

1. **Ba Thi Cham**, Nguyen Thi Thuy Linh, Nguyen Thi Hoang Anh, Trinh Thi Thuy, Nguyen Hai Dang, Domenico V. Delfino, Le Thi Hong Nhung, Cytotoxic activity of new phenolic from *Dianella ensifolia* (L.) DC., Journal of science and Technology (Hanoi university of industry), 2021, 57, 123-125.
2. Le Thi Hong Nhung, Nguyen Thi Thuy Linh, **Ba Thi Cham**, Trinh Thi Thuy, Nguyen Thanh Tam, Dao Duc Thien, Pham Thi Mai Huong, Vu Minh Tan, Bui Huu Tai, Nguyen Thi Hoang Anh, New phenolics from *Dianella ensifolia*, Natural Product Research, 2021, 35(18), 3063–3070, doi.org/10.1080/14786419.2019.1689499.
3. **Ba Thi Cham**, Vu Dinh Hoang, Nguyen Thi Thuy Linh, Bui Huu Tai, Domenico V Delfino & Trinh Thi Thuy, Noncytotoxic 16,23-epoxycucurbitacin-type triterpenoids from *Elaeocarpus hainanensis*, Natural Product Research, online 10/2022, doi.org/10.1080/14786419.2022.2137507.
4. **Ba Thi Cham,** Le Thi Hong Nhung, Nguyen Thi Thuy Linh, Vu Đinh Hoang, Trinh Thi Thuy, Study on isolation and structural determination of some aromatic and flavonoid compounds from *Dianella ensifolia*, Journal of Science and Technology (Da Nang university), 2023, 21 (3), 93-96.
5. **Ba Thi Cham,** Vu Dinh Hoang, Nguyen Thi Thuy Linh, Nguyen Thi Thu Hoa, Nguyen Thi Hoang Anh, Bui Huu Tai, Le Thi Hong Nhung, Domenico V Delfino, Trinh Thi Thuy, Oleanane-type triterpenoid sulfates, two new α-glucosidase inhibitors from *Elaeocarpus hainanensis*. Natural Product Research, Natural Product Research, online 24/8/2023, doi.org/10.1080/14786419.2023.2250900.
6. Nguyen Thi Thuy Linh, **Ba Thi Cham**, Le Thi Hong Nhung, Bui Huu Tai, Vu Dinh Hoang, Tran Duc Quan, Nguyen Thi Hoang Anh, Nguyen Huu Truc, Trinh Thi Thuy. A new biflavan from *Dianella ensifolia* and its cytotoxic activity. Submitted to Natural Product Communication, 05/2023.