MINISTRY OF EDUCATION AND TRAINING
VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

ACADEMY OF SCIENCE AND TECHNOLOGY

PHAM THI HAI YEN

STUDY ON ANTI-INFLAMMATORY AND ANTI-CANCER ACTIVITIES OF SOME COMPOUNDS ISOLATED FROM TWO SPECIES OF *P. ANGULATA* AND *P. MINIMA*, SOLANACEAE FAMILY

Specialization: Human and Animal Physiology
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ABSTRACT OF DOCTORAL THESIS IN BIOLOGY

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The thesis will be defended in front of the Academy-level doctoral thesis grading committee, meeting at the Academy of Science and Technology - Vietnam Academy of Science and Technology.

At ,on day month year 2023 .

The thesis can be found more at:

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FOREWORD

Cancer is a disease with a high mortality rate and is becoming a major burden in countries around the world, especially in poor and developing countries. According to statistics from the International Organization for Research on Cancer (Globocan), in 2020 there will be 19.3 million new cancer cases and 10 million cancer deaths worldwide, in which Asia accounts for the highest rate of new cases, accounting for 49.6% of new cancer patients globally.

In Vietnam, cancer is also a disease that is increasing rapidly and fast. According to statistics of the International Organization for Research on Cancer (Globocan), in 2020 there were about 182,563 new cancer cases in Vietnam, nearly 122,690 deaths and more than 353,826 people were living with cancer. Besides, according to many reports, inflammation and chronic inflammation are considered as one of the factors that stimulate the development of cancerous tumors. Scientists have documented the presence of various types of inflammatory cells along with an increase in inflammatory cytokines in the tumor microenvironment. Therefore, it is very necessary and urgent to find new medicine types/drugs for anti-tumor, anti- inflammation as well as treatment or prevention support. Although in modern medicine there have been developments in the synthesis of drugs to treat cancer, inflammatory diseases, up to present, no drug has been found to be completely effective and safe.

Plants and products (active ingredients) of plant origin are still considered effective and appropriate in treating and controlling cancer. It is due to natural compounds with low toxicity, well tolerated in the organism, capable of killing tumor cells, and protecting healthy cells. Studies show that plant-extracted secondary metabolites inhibit cancer cells through anti-DNA damage, activate apoptosis-inducing enzymes through inhibition of signaling pathways such as: RAS-ERK pathway, c-Met signaling pathway, PI3K/Akt signaling pathway, mitochondrial pathway etc.. Therefore, nowadays, most of the research works looking for new anti-cancer or anti-inflammatory drugs are directed at plants and natural compounds derived from plant origin.

Vietnam is a country with extremely diverse and rich medicinal plant resources, distributed throughout the territory. According to statistics in 2016, Vietnam has about 5117 species and under higher plants used as medicine in folk. This shows the great potential of Vietnamese medicinal herbs that can be used in screening studies to find rare and precious pharmacologically active substances, in which there are new anti-tumor and anti-inflammatory agents with high efficiency, little or no side effects. Among the species that have been discovered, Physalis angulata and Physalis minima of the Physalis genus are species widely used in traditional medicine for the treatment of diseases including inflammatory diseases and cancer. In recent years, in Vietnam, there have also been a number of scientific studies on isolation and determination of chemical structures of bioactive compounds of P. angulata and P. minima species. Learning about the chemical composition and anti-tumor and anti-inflammatory activities of these two species will supplement a source of scientific basis for use in the process of supporting and treating these diseases.

Based on the above reasons, we carried out the research project **"*Study on anti-inflammatory and anti-cancer activities of some compounds isolated from two species of P. angulata and P. minima, Solanaceae family*"**. The results of the study of the project will contribute to the evaluation of the potential anti-cancer and anti-inflammatory activities of the extracts, detection of pure compounds with supportive and therapeutic effects on inflammatory diseases and cancer isolated from *P. angulata* and *P. minima* species distributed in Vietnam. The results of the project are the scientific basis, contributing to explain the anti-tumor and anti-inflammatory activities of folk remedies, improving the use value of these plants.

Topic objective of the thesis

1. Isolating and determinating chemical structure of some compounds from 02 species of *P. angulata* and *P. minima* of the Physalis genus in Vietnam;

2. Detecting compounds with potential anti-inflammatory and anti-cancer activities from these two plant species as a basis for further pharmacological studies.

Research subjects

The research subjects of the project are two species of the *Physalis* genus: *P. angulata* collected in Thai Binh province and *P. minima* collected in Thua Thien Hue province.

Thesis contents include

1. Screening for anti-inflammatory and anticancer activities from extracts of two species *P. angulata* and *P. minima* of Physalis genus in Vietnam.

2. Determinating chemical structures of compounds isolated from potential extracts of two species *P. angulata* and *P. minima* of the Physalis genus in Vietnam.

3. Evaluating anti-inflammatory-directed NO inhibitory activity of isolated compounds.

4. Evaluating the antitumor activity of the isolated compounds.

CHAPTER 1. OVERVIEW

1.1. Some information about inflammation and cancer

1.2. An overview of the Physalis genus

1.2.1. Botanical characteristics of the Physalis genus

The Physalis genus belongs to the Solanaceae family, Solanales order, dicotyledonous plants (Magnoliopsida), flowering plant (Magnoliophyta). In Vietnam, the Physalis genus includes 5 species, namely *Physalis angulata, Physalis alkekengi, Physalis peruviana, Physalis cordata Mill, and Physalis minima.*

1.2.2. Chemical composition and biological activity of the Physalis genus

In terms of chemical composition, the main class of substances of the Physalis genus are withanolides, then labdane diterpenes, sucrose esters, flavonoids, ceramides and some other substances. Species of the

Physalis genus have been shown to have anti-cancer, anti-inflammatory, analgesic, antipyretic, anti-diabetic, antibacterial, anti-tuberculosis, anti-malarial and immunomodulatory effects.

1.2.3. Introduction about P.angulata

1.2.4. Introduction about P. minima

CHAPTER 2. RESEARCH SUBJECTS AND METHOD

2.1. Research subjects and materials

2.1.1. Samples of P.angulata: Collected in Tien Hai district and Dong Hung district, Thai Binh province in August 2015.

2.1.2. Samples of P. minima: Collected in Huong Hoa commune, Nam Dong district, Thua Thien Hue province in September 2018.

2.1.3. Research materials: Cell lines: RAW 264.7 lines; Human cancer cell lines: SK-LU-1 (lung cancer), A549 (lung cancer), HeLa (cervical cancer), PANC-1 (pancreatic cancer), HepG2 (hepatocellular carcinoma) and MCF7 (breast cancer).

2.1.4. Chemicals used in the study

2.2. Research Methods

2.2.1. Methods for producing methanol extracts and fractions of P. angulata and P. minima. samples

After collection, plant samples are removed, washed, dried at room temperature, dried at 50-60^{0}C, then ground into a dry powder. This dry powder is extracted with methanol with the help of ultrasonic extraction equipment. The extract is collected, filtered through filter paper and then distilled to recover the solvent by a rotary distillation device under reduced pressure obtaining methanol extract containing most of the compounds present in the sample. The methanol extract is dissolved in water and extracted with a liquid-liquid distribution with diclomethane and ethyl acetate solvents of increasing polarity respectively, to extract plant samples from *P. angulata* and *P. minima* species. Rotary distillation removes the solvent under reduced pressure to obtain the corresponding extracts.

Table 2.1. Extracts from *P. angulata* and *P. minima* species

Segmentation	***Mark of for fractional extracts***	
	P. angulata	***P. minima***
Total (MeOH) extract	PA	PM
Dichlomethane	PAD	PMD
Ethyl acetate	PAE	PME
Water extract	PAW	PMW

2.2.2. Method of isolation of compounds from P. angulate

2.2.2.1. Production of methanol extracts and fractional extracts from P.angulata species

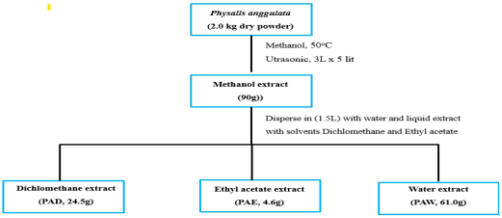


Figure 2.1. Diagram of the preparation of extracts from *P. angulata*

2.2.2.2. Extraction and isolation of compounds from PAD extract of P. angulata

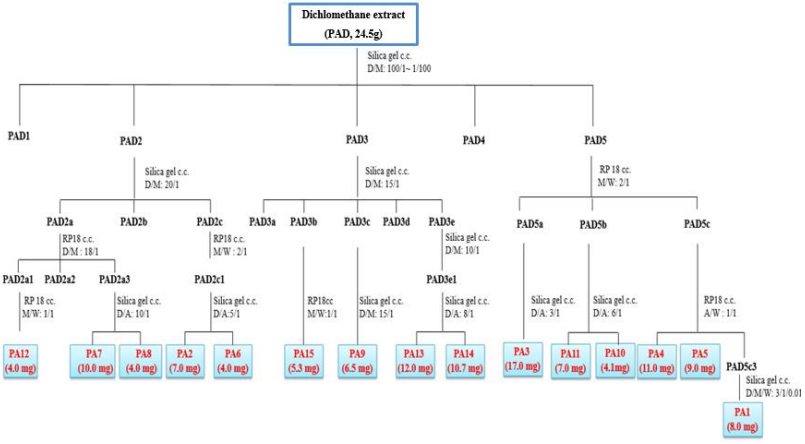


Figure 2.2. Diagram of isolation of compounds from PAD extract of P. angulata

2.2.3. Method of isolation of compounds from P. minima species

2.2.3.1. Production of methanol extracts and fractional extracts from P. minima

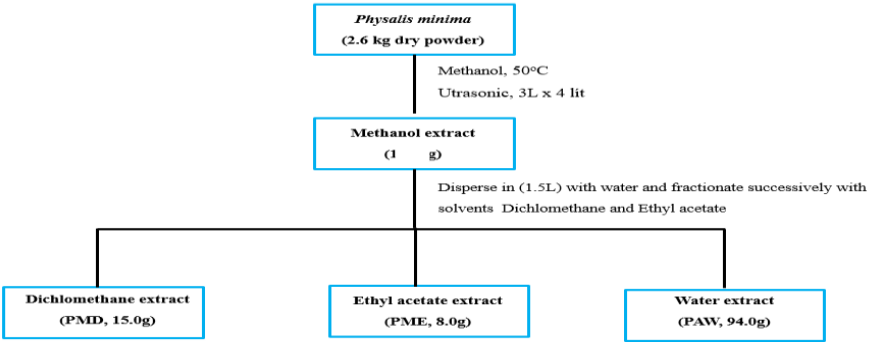


Figure 2.3. Diagram of the preparation of extracts from *P. minima* species

2.2.3.2. Extraction and isolation of compounds from PMD and PME extracts of P. minima

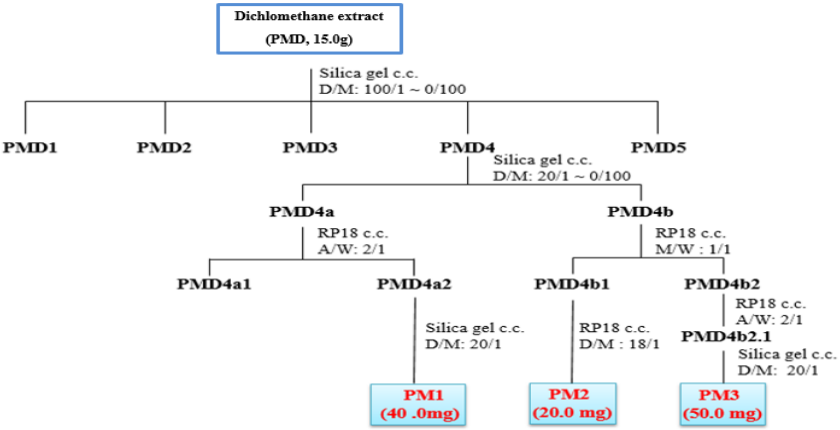


Figure 2.4. Diagram of isolation of compounds from PMD extract of P. minima

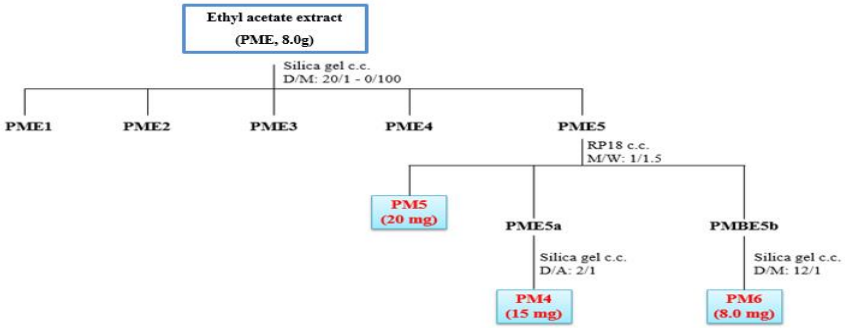


Figure 2.5. Diagram of isolation of compounds from PME extract of P. minima

2.3. Methods to determine the chemical structure of compounds

2.3.1. Chromatographic method for the isolation of compounds from plant samples

2.3.2. Nuclear resonance spectroscopy method

2.3.3. Mass spectroscopy method

2.3.4. Method of measuring polar rotation [α]D

2.4. Methods to assess biological activity

2.4.1. In vitro cell culture method

2.4.2. Methods to evaluate the cytotoxic activity of cancer cells

2.4.3. Method to determine the ability to inhibit NO production of of cells using RAW 264.7 macrophage cells

2.4.4. Western Blot Method

2.4.5. Methods to evaluate the effects of apoptosis induction

2.5. Statistical analysis method

CHAPTER 3. RESEARCH RESULTS AND DISCUSSION

3.1. Screening results for inhibitory activity of NO production in RAW 264.7 cells and cytotoxic activity of *P. angulata* and *P. minina* species

3.1.1. Screening results of the inhibitory activity of NO production in RAW 264.7 cells of the extracts isolated from P.angulata and P.minina species

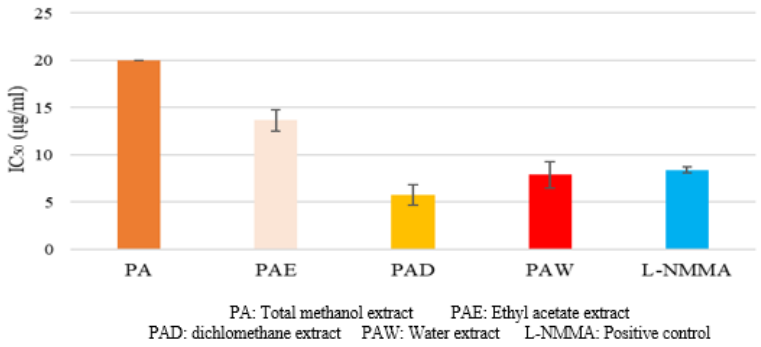


Figure 3.1. Inhibitory ability of NO production of extracts isolated from P. angulata species through IC_{50} *value* (µg/mL)

PA, PAD, PMW and PAE extracts isolated from *P. angulata* species are evaluated for their ability to inhibit NO production at the concentration of 20 µg/mL and the results show that PAD, PMW and PAE extracts are able to inhibit >50% of NO production in RAW264.7 cells. These extracts are further tested at different concentrations to determine the IC_{50} value. The results show that PAD extract have the strongest inhibitory effect on NO production compared with PAE and PAW extracts.

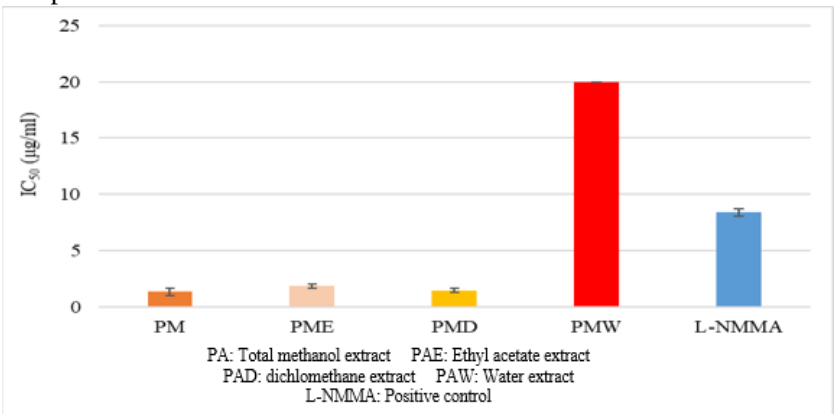
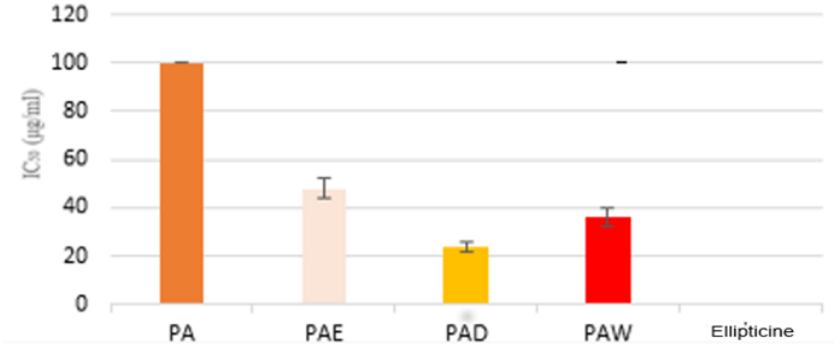


Figure 3.2. Inhibitory ability of NO production of extracts isolated from P. minima species through IC_{50} *value (µg/mL)*

For *P. minima* species, by comparing the IC_{50} values of the extracts, it can be seen that the inhibitory activity level of NO production

increases in order: PMW <PME <PMD<PM, corresponding to decreasing IC_{50} value.

3.1.2. Screening results for cytotoxic activity of extracts from two species P. angulata and P. minima

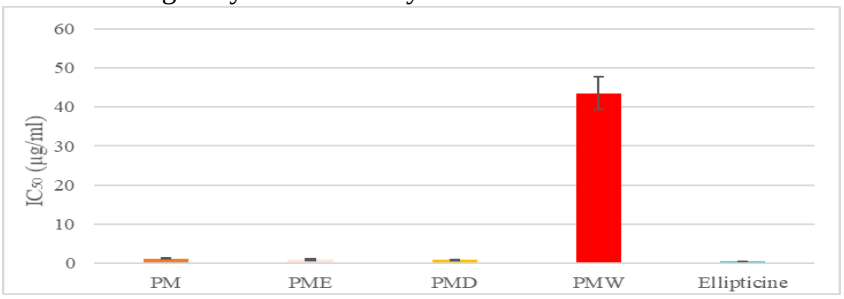


PA: Total methanol extract PAE: Ethyl acetate extract

PAD: dichlomethane extract PAW: Water extract Ellipticine: Positive control

Figure 3.3. IC_{50} value in the A549 line cancer cytotoxic activity test of extracts isolated from *P. angulata* species

Thus, in *P. angulata* species, the PA extract does not show cytotoxic activity against all three tested cell lines and the PAD extract shows the strongest cytotoxic activity.



PM: Total methanol extract PME: Ethyl acetate extract

PMD: dichlomethane extract PMW: Water extract Ellipticine: Positive control

Figure 3.4. IC_{50} value in the A549 line cancer cytotoxic activity test of extracts isolated from P. minima species

The results in Figure 3.4 show that the cytotoxic activity of PMD and PME extracts show cancer cell cytotoxic activity on the tested lines is stronger than that of PMW and total PM extracts.

3.2. *Results of chemical structure determination of compounds isolated from P. angulata and P. minina species*

3.2.1. Results of chemical structure determination of compounds isolated from *P. angulata*

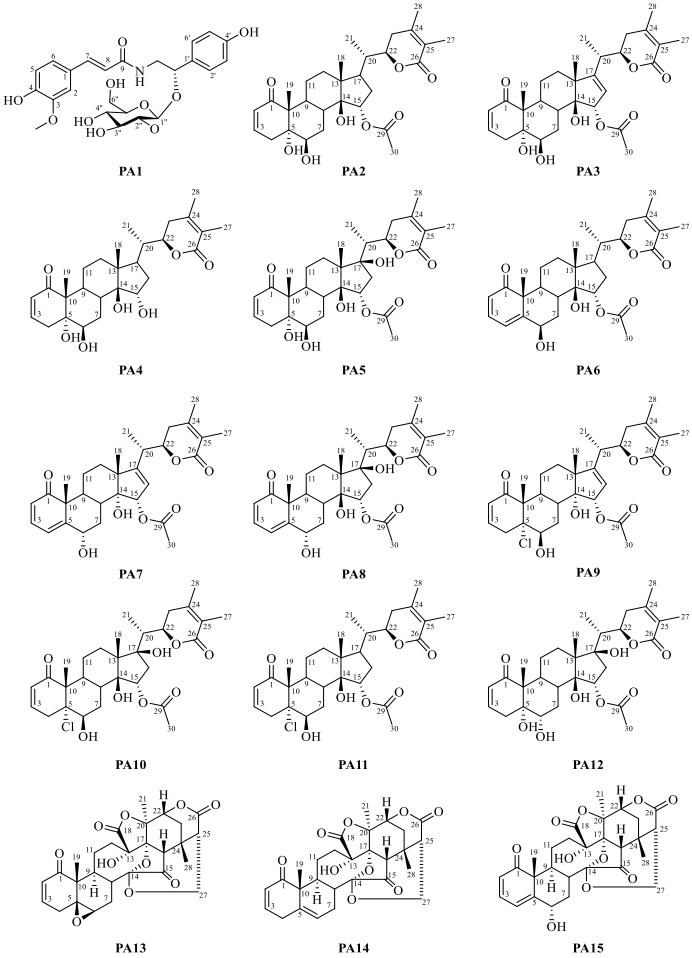


Figure 3.11. Chemical structures of compounds isolated from *P. angulata*

4.2.2. Determination of chemical structures of compounds isolated from P. minima

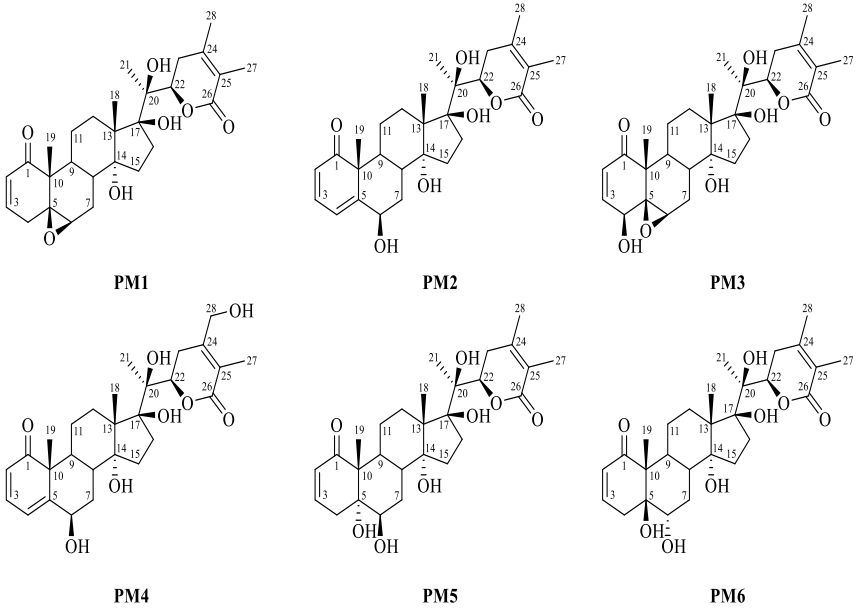


Figure 3.12. Chemical structures of compounds isolated from P.minima

3.3. Results of evaluating anti-inflammatory and cytotoxic oriented NO production inhibitory activities of compounds isolated from *P. angulata* species

3.3.1. Results of evaluating anti-inflammatory-directed NO production inhibitory activities of compounds isolated from *P. angulate* species

Table 3.4. Results of evaluating the inhibitory activity of NO production in RAW 264.7 cells of the compound isolated from *P. angulata*

No.	Compound name	Mark	IC_{50} (μM)
1	physalucoside A	PA1	**2,69 ± 0.17**
2	physagulin *J*	PA2	*
3	Withaminimine	PA3	**69,6±4.5**

4	Physagulin N	PA4	*****
5	physagulin K	PA5	*****
6	physagulin P	PA6	*****
7	physagulin L	PA7	**> 100**
8	physagulin M	PA8	**> 100**
9	physagulin B	PA9	**0,24 ± 0.09**
10	Physagulide Q	PA10	**0,57 ± 0.18**
11	(20S, 22R)-15α-acetoxy-5α-chloro-6β,14β-dihydroxy-1-oxowitha-2,24-dienonide	PA11	**0,68 ± 0.02**
12	physagulin Q	PA12	*****
13	physalin F	PA13	**1,06 ± 0,68**
14	physalin B	PA14	**0,28 ± 0,10**
15	physalin G	PA15	**3,74 ± 0,29**
L-NMMA		**7,84 ± 0,87**	

*Notes: * causing cell death of RAW264.7 test.*

Fifteen compounds isolated from *P. angulata* were investigated for their inhibitory activity on NO production in RAW 264.7 cells. Test results show that compounds **PA2, PA4, PA5, PA6** and **PA12** are highly toxic, causing tested cell death at the studied concentration, so it did not show obvious inhibitory activity on NO production. Meanwhile, compounds **PA7** and **PA8** did not inhibit NO production with IC_{50} value > 100 µM. Compounds **PA9, PA10, PA11, PA13, PA14** strongly inhibited NO production in RAW 264.7 cells with IC_{50} value from 0.24 ± 0.09 to 1.06 ± 0.68 µM compared to the positive control L-NMMA (IC_{50} = 7.84 ± 0.87 µM). Showing weaker inhibitory activity on NO production includes compounds **PA1, PA3** and **PA15** with IC_{50} value of 2.69 ± 0.17, respectively; 69.6 ± 4.5; 12.5 ± 1.70, and 3.74 ± 0.29 µM. This result

shows that compounds **PA9, PA10, PA11, PA13, PA14** have strong inhibitory activity on NO production generated by LPS in RAW 264.7 cells when caused inflammation.

3.3.2. Results of evaluating iNOS and COX-2 enzyme inducing activities of compounds PA13 and PA14 isolated from P. angulata

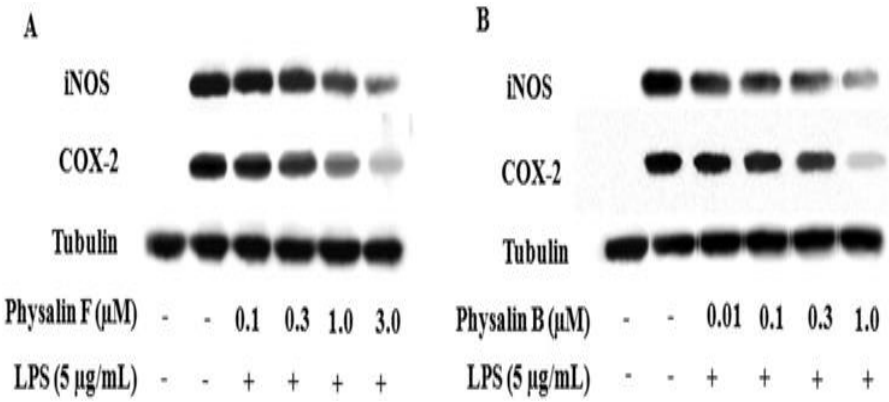


Figure 3.18. Inhibitory effect of iNOS, COX-2 protein expression of compounds PA13(A) and PA14(B) in LPS-inflammation induced RAW 264.7 cells

The data in Figure 3.18 show that iNOS and COX-2 proteins are barely detectable in RAW 264.7 cells when not stimulated with LPS. However, after RAW 264.7 cells are stimulated inflammation with LPS, protein expression levels of iNOS and COX-2 are markedly increased. On the other hand, when increasing the concentration of **PA13** from 0.1 μM to 3.0 μM and **PA14** from 0.01 μM to 1.0 μM, the protein concentration of two enzymes iNOS and COX-2 in the cell is significantly reduced. On the other hand, during incubation with LPS (1 μg/ml) the expression of tubulin protein does not change. This suggests that both compounds **PA13** and **PA14** can downregulate iNOS and COX-2 expression caused by LPS at the translational level.

3.3.3. Results of evaluating the inhibitory activity of some cancer cell lines of compounds isolated from P. angulata species

Table 3.5. Results of evaluating cytotoxic activity of 15 compounds isolated from PAD extract of *P. angulata* species

No.	Compound name	Mark	IC_{50} (µM)		
			A-549	**HeLa**	**PANC-1**
1	physalucoside A	PA1	**> 100**	**> 100**	**> 100**
2	physagulin *J*	PA2	**8,27 ± 0,97**	**> 100**	**> 100**
3	Withaminimine	PA3	**11,8 ± 2,06**	**> 100**	**34,06 ± 2,08**
4	physagulin N	PA4	**> 100**	**> 100**	**> 100**
5	physagulin K	PA5	**> 100**	**> 100**	**> 100**
6	physagulin P	PA6	**13,47 ± 2,73**	**> 100**	**20,23 ± 1,38**
7	physagulin L	PA7	**21.54 ± 1.32**	**> 100**	**6.30 ± 1,19**
8	physagulin M	PA8	**17,47 ± 2,37**	**> 100**	**3,18 ± 0,12**
9	physagulin B	PA9	**> 100**	**> 100**	**> 100**
10	physagulide Q	PA10	**> 100**	**> 100**	**> 100**
11	(20S, 22R)-15α-acetoxy-5α-chloro-6β,14β-dihydroxy-1-oxowitha-2,24-dienonide	PA11	**1,03 ± 0,09**	**29,89 ± 1,15**	**11,53 ± 0,36**
12	physagulin Q	PA12	**> 100**	**> 100**	**> 100**
13	physalin F	PA13	**0,68 ± 0,05**	**0,23 ± 0,03**	**> 100**
14	physalin B	PA14	**0,95 ± 0,04**	**13,84 ±1,27**	**12,77 ±1,07**
15	physalin G	PA15	**6,88 ± 2,41**	**> 100**	**> 100**
	Etoposide		**2,68 ± 0,89**	**3,29 ± 0,05**	**0,08 ± 0,11**

The above results show that compounds **PA1, PA4, PA5, PA9, PA10, PA12** do not show cytotoxic activity on all 3 tested cancer cell lines. Compounds **PA2, PA3, PA6, PA7, PA8, PA11, PA13, PA14** and **PA15** have cytotoxic effects against the A549 cell line. The remaining compounds show almost no cytotoxic effect on this cell line (IC_{50} > 100

μM). For the HeLa cancer cell line, compounds **PA11, PA13** and **PA14** have strong cytotoxic effects. The remaining compounds are considered almost no cytotoxic effect on HeLa line at the value IC_{50} > 100 μM. In addition, compounds **PA3, PA6, PA7, PA8, PA11, PA14** show significant cytotoxic activity against PANC-1 cell line. The evaluation results of cytotoxic activity show that compounds **PA2, PA3, PA6-PA8, PA11, PA13, PA14, PA15** isolated from *P. angulata* species are potential compounds and need to be further studied on the mechanism to guide clinical application.

When analyzing the spectrum, it is shown that compounds **PA7** and **PA3** have similar structure, except that the appearance of an extra double bond at the C4-C5 position in **PA7** led to cytotoxic activity on the A549 and PANC-1 cancer cell lines of **PA7** and **PA3** differentially.

Compound **PA8** shows cytotoxic activity against A549, PANC-1 cell lines and no HeLa cell cytotoxic activity.

Compound **PA11** shows cytotoxic activity on tested cancer lines because at there is a Cl atom position 5, which increases the cytotoxic activity of **PA11** compound.

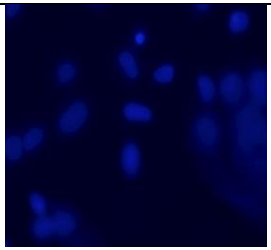
The appearance of epoxy structure of 5β,6β-epoxy, double bond at the position C-5, C-6 and substitution of hydroxy group at C-6 instead of epoxy connection 5β,6β of **PA13** has great influence on cytotoxic activity. This compound shows strong activity against two cancer cell lines A549 and Hela. While compound **PA14** has moderate activity on all 3 tested cancer cell lines and compound **PA15** only shows cytotoxic activity on cancer cell line A549.

3.3.4. Results of evaluating apoptosis induction by compound PA6 on lung cancer cell line (A549)

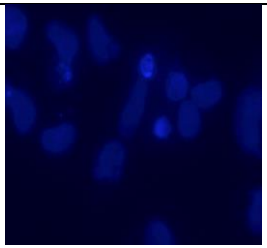
3.3.4.1. Determination of apoptosis induction by staining cell nucleus with Hoechst 33342

Table 3.6. Percentage of cells with nuclear condensation or fractions under the active ingredient of **PA6**

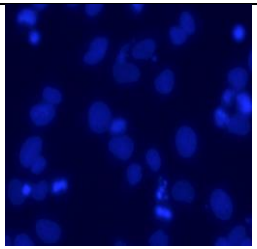
% cells with nuclear morphology undergo apoptosis				
PA6 (5 µg/mL)	**PA6** (10 µg/mL)	**PA6** (15 µg/mL)	**Camptothecin** (0,5 µM)	**Control** (-)
4,89±0,91	7,36±1,76	16,01±2,26	23,21±3,24	3,79±1,03



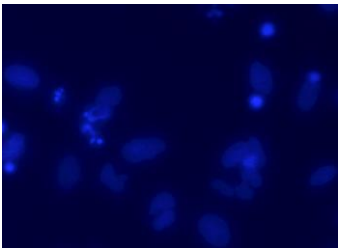
Negative control



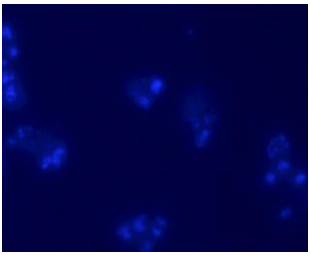
PA6 (5 µg/mL)



PA6 (10 µg/mL)



PA6 (15 µg/mL)



Camptothecin (0.5 µg/mL)

Figure 3.19. Image of A549 cells stained with Hoechst 33342 under the influence of **PA6** at different concentrations

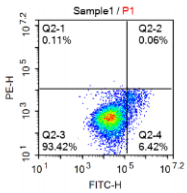
When increasing the concentration of **PA6** reagent from 5 µg/mL to 15 µg/mL, the number of apoptotic cells increases from 4.89% to 16.01% (Table 3.11). This means that the ability to induce nucleation of compound **PA6** is dependent on the concentration. In the camptothecin-positive control, the cell with fraction and concentration in the nucleus is very obvious. While negative control cells have bright, round and uniform staining nucleus. Thus, in this test, compound **PA6** shows the ability to induce apotosis and change the morphology of the tested cancer cells.

*3.3.4.2. Determination of the ability of **PA6** to induce apoptosis by Flowcytometry method*

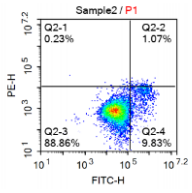
Compound **PA6** at concentration 5 M, 10 M and 15 M shows the ability to induce early and late apoptosis in A549 cells after 24h of incubation.

Table 3.7. The rate of apoptosis cells under the effect of **PA6**

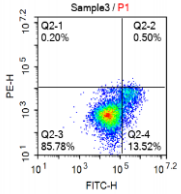
Sample for experiment	% living cells (Q2-3)	% of early apoptosis cells (Q2-4)	% of cells with late apoptosis (Q2-2)	% necrotic cells (Q2-1)
ĐC	93,42	6,42	0,11	0,06
PA6 (5 µg/mL)	88,86	9,83	1,07	0,23
PA6 (10 µg/mL)	85,78	13,52	0,50	0,20
PA6 (15 µg/mL)	80,17	15,92	3,26	0,65
Camptothecin (0,5 µM)	80.61	18,67	0,30	0,41



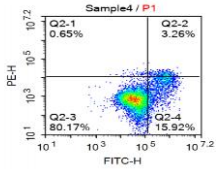
Cells incubated with 0.5% DMSO for 48h



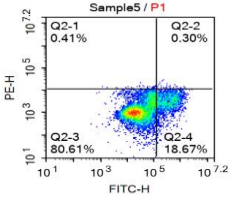
Cells incubated with PA7 sample (5 g/mL) for 48h



Cells incubated with PA6 sample (10 g/mL) for 48h



Cells incubated with PA6 sample (15 g/mL) for 48h



Cells incubated with Camptothecin (0.5 M) for 48h

Figure 3.20. The effect of **PA6** at different concentrations on A549 cell apoptosis process after 48h of incubation using Annexin V/cell dead apoptosis Kit

Research results show that compound **PA6** significantly increases the rate of apoptosis cell and is concentration-dependent. These results showed the ability of **PA6** to induce and activate apoptosis, especially in the early stages of apoptosis in A549 cells (15.92%) and also cause a cytotoxic effect in the form of necrosis, although not significant (0.65%).

3.3.4.3. Study on apoptosis induction activity through caspase 3 enzyme

Table 3.8. Effects of **PA6** on caspase 3 production ability in A549 cells (The rate of caspase-3 induction upon PA6 treatment on lung cancer cell line (A549) reaches 1.30 times (5 µM), 2.00 times (10 M), 2.59 times (15 M).); camptothecin reaches 3.23 times; **P<0.01; ***P<0.001)

Samples	Number of times of caspase 3 stimulation compared with negative control	Deviation
PA6 15 µM	2,59 **	0,26
PA6 10 µM	2,00 ***	0,06
PA6 5 µM	1,30	0,22
Camptothecin (0,5 µM)	3,23***	0,06
Negative control	1,00	0,06

Experimental results show that compound **PA6** is able to induce A549 cancer cell apoptosis by caspase-3 production with an increasing stimulation times of 2.59 times, 2.00 times and 1.30 times respectively compared with the negative control at concentration 15, 10 and 5 M, respectively. Camptothecine positive control with caspase-3 activity increases 3.23 times compared to negative control and stable activity in the test (P<0.01). This indicates that the active ingredient **PA6** activated the

caspase pathway to induce the activity of apoptosis process in A549 cells and subsequently resulted in inhibition of proliferation.

3.4. Results of evaluating anti-inflammatory and cytotoxic oriented NO production inhibitory activities of compounds isolated from P. minima species

3.4.1. Results of evaluating NO inhibitory activities of compounds isolated from P. minima species

The anti-inflammatory-directed NO production inhibitory activity of compounds isolated from P. minima species is evaluated by using the RAW 264.7 cell model stimulated by LPS. The results of evaluating the anti-inflammatory-directed NO production inhibitory activity of the isolated compounds are presented in Table 3.9 below:

Table 3.9. Results of evaluating inhibitory activity on NO production in RAW 264.7 cells of compounds isolated from *P. minima* species

No.	Compound name	Mark	IC_{50} (μM)
1	withanolide E	PM1	0,15 ± 0,02
2	withaperuvin C	PM2	> 100
3	4β-hydroxywithanolide E	PM3	NA
4	28-hydroxywithaperuvin C	PM4	> 100
5	physaperuvin G	PM5	70,25 ± 2,43
6	4-deoxywithaperuvin	PM6	> 100
	L-NMMA		**7,84 ± 0,87**

NA: not determined due to RAW264.7 tested cell death.

The results of evaluating the ability to inhibit NO production according to the concentration of 06 compounds isolated from *P. minima* species show, exhibit that the most active compound is **PM1** with IC_{50} value as 0.15 ± 0. 02 M, several times stronger than the positive control L-NMMA (IC_{50} = 7.84 ± 0.87 μM). Compound **PM5** shows weak activity

with an IC_{50} of 70.25 ± 2.43 µM. The remaining compounds are considered inactive due to IC_{50} > 100 µM (**PM2, PM4**) or causing tested cell death (**PM3**).

Compound PM1 shows the effect of NO production due to the presence of a 5β,6β-epoxy group in the B ring that increases its anti-inflammatory activity. Compounds PM2, PM4, PM6 have no inhibitory effect on NO production. Compound PM3 shows high toxicity, causing tested cell death. Compound **PM5** has selective inhibitory effect on NO production on different cancer cell lines.

3.4.2. Results of evaluating the inhibitory activity of some cancer cell lines of compounds isolated from P. minima species

Six compounds (**PM1-PM6**) isolated from **P. minima** species are evaluated for their cytotoxic activity against liver cancer cell lines (HepG2), lung cancer (SK-LU-1) and breast cancer (MCF7) according to the in vitro cytotoxic activity tesing method. The results of the evaluation of cytotoxic activity of the compounds are presented in the table below:

Table 3.10. Results of evaluating cytotoxic activity of 6 compounds isolated from PMD and PME fractions of *P. minima* species

No.	Compound name	Mark	IC_{50} (µM)		
			HepG2	SK-LU-1	MCF7
1	withanolide E	PM1	**0,051 ± 0,004**	**0,056 ± 0,003**	**0,059 ± 0,006**
2	withaperuvin C	PM2	**19,50 ± 1.75**	**14.65 ± 0,82**	**11,74 ± 1,01**
3	4*β*-hydroxywithanolide E	PM3	**0,80 ± 0,05**	**0,86 ± 0,09**	**0,83 ± 0,13**
4	28-hydroxywithaperuvin C	PM4	**>100**	**>100**	**>100**
5	physaperuvin G	PM5	**>100**	**>100**	**>100**
6	4-deoxywithaperuvin	PM6	**64,44 ± 3,93**	**56,22 ± 6,22**	**65,33 ± 4,06**
Etoposide			**2,68 ± 0,89**	**3,29 ± 0,05**	**0,38 ± 0,02**

Evaluation results show that compounds **PM1** and **PM3** exhibit strong cytotoxic activity against liver cancer (HepG2), lung cancer (SK-LU-1) and breast cancer (MCF7) cells. Compounds **PM2** and **PM6**

exhibited weaker activity. Two compounds **PM4** and **PM5** with IC_{50} value > 100 μM are considered to have no cytotoxic activity against the tested cancer cell lines. The results of cytotoxic activity show that 2 steroid compounds belonging to the withanolde framework (**PM1, PM3**) isolated **from P. minima** species are potential compounds and need to be further studied on the mechanism to guide clinical application.

According to the results of structural analysis, compound **PM1** has the presence of 5β,6β-epoxy group at ring B. This is the reason for the compound **PM1** causing toxicity to HepG2, SK-LU-1, MCF7.

^{1}H and ^{13}C-NMR spectral data of **PM2** is quite similar to compound **PM1** except for the signals in the A and B ring regions by the presence of the double bond at C-4/C-5 and the loss of the epoxy connection at C-5/C-6 at **PM2.** The substitution of substituents affected the cytotoxic activity on 3 tested cancer cell lines HepG2, SK-LU-1, MCF7 of compound **PM2** which is weaker than **PM1**.

According to NMR spectrum analysis, the chemical structure of **PM3** is similar to that of **PM1** except for the presence of more than 1 hydroxy group at C-4 in **PM3**. Substituent substitution in the structure explains the cytotoxic activity on 3 cancer cell lines HepG2, SK-LU-1, MCF-7 of compound **PM3** which is weaker than compound **PM1**.

Compounds **PM4** and **PM5** do not show activity on 3 tested cancer cell lines.

The NMR spectral data of PM6 is quite similar to that of compound **PM1**, except for the positions from C-4 to C-8, which reduces the cytotoxic activity. Therefore, according to the study results, we found that the cytotoxic activity of HepG2, SK-LU-1 and MCF-7 of **PM6** is lower than that of **PM1**.

CONCLUSIONS AND RECOMMENDATIONS

1. Screened for anti-inflammatory and anti-cancer activities from extracts of two species *P. angulata* and *P. minima* of the Physalis genus in Vietnam.

- For *P. angulata* species, the PAD extract has the strongest inhibitory activity on NO production and cytotoxic activity.

- For *P. minima* species, PMD and PME extracts have stronger inhibitory activity on NO production and cytotoxic activity than PMW extracts and total PM extracts.

2. Isolated and determined the chemical structures of compounds isolated from potential extracts of two species of *P. angulata* and *P. minima* of the Physalis genus in Vietnam.

- From the sample of *P. angulata* species, the chemical structures of 15 new compounds have been isolated and determined, including 03 new compounds, which were recorded for the first time as: physalucoside A (**PA1**), physagulin P **(PA6),** physagulin Q **(PA12)**.

- From the sample of *P. minima* species, the chemical structure of 06 compounds has been isolated and determined, of which 01 compound was isolated for the first time from the Physalis genus as 4-deoxywithaperuvin **(PM6)** and 05 compounds were isolated for the first time from *P.minima* species including: withanolide E (**PM1**), withaperuvin C (**PM2**), 4*β*-hydroxywithanolide E (**PM3**), 28-hydroxywithaperuvin C (**PM4**), physaperuvin G (**PM5**).

3. The inhibitory activity on NO production of compounds isolated from *P. angulata* and *P. minima* species was evaluated.

- Compounds physagulin B **(PA9)**, physagulide Q (**PA10)**, (20*S*, 22*R*)-15*α*-acetoxy-5*α*-chloro-6*β*,14*β*-dihydroxy-1-oxowitha-2,24-dienonide (**PA11)**, physalin F (**PA13)**, physalin B (**PA14)** have a very strong inhibitory effect on NO production in RAW 264.7 cells. Two compounds, physalin F (PA13), and physalin B (PA14) show clear anti-inflammatory activity when reducing the expression of iNOS and COX-2 proteins.

- The withanolide compound E (**PM1**) showed the strongest inhibitory activity on NO production with IC_{50} = 0,15 ± 0,02 μM. The remaining compounds have not shown the activity to be studied.

4. The cell cytotoxic activity of compounds isolated from *P. angulata* and *P. minima* species was evaluated. The results are as the followings:

- Compound (20S,22R)-15α-acetoxy-5α-chloro-6β,14β-dihydroxy-1-oxowitha-2,24-dienonide (PA11), physalin F (PA13), physalin B (PA14) showed activity Highly toxic to lung cancer cell lines (A549) (IC_{50} from 0,68 to 1,03 μM). Compound PA13 had the strongest cytotoxic effect on cervical cancer cell line (HeLa) with IC50 = 0.23 ± 0.03 M. Compounds Withaminimine **(PA3)**, physagulin P **(PA6),** physagulin L **(PA7),** physagulin M **(PA8)**, (20*S*,22*R*)-15α-acetoxy-5*α*-chloro-6*β*,14β-dihydroxy-1-oxowitha-2,24-dienonide (**PA11)**, physalin B **(PA14**) showed significant cytotoxic activity against pancreatic cancer cell line (PANC-1) with IC50 from 3.18 to 34.06 M. The remaining compounds did not show activity at the studied concentrations.

- The new compound physagulin P (**PA6**) showed apotosis-inducing activity in lung cancer cell line (A549) through its ability to induce nuclear concentration/fractions, activate caspase-3, increased percentage of cells with early apoptosis, late apoptosis and necrosis.

- Two compounds withanolide E (**PM1**) and 4β-hydroxywithanolide E (**PM3**) showed very strong cytotoxic activity against liver cancer cell line (HepG2), lung cancer (SK-LU-1) and breast cancer (MCF7) with IC_{50} values from 0.051 to 0.86 μM. The remaining compounds did not show activity at the studied concentrations

RECOMMENDATION

From the obtained research results of compounds isolated from two plant species *P. angulata* and *P. minima*, we recommend:

- The compound Physagulin P (**PA6**) is a potential new compound in the research and development of drugs for treating lung cancer (A549) and pancreatic cancer (PANC-1). Therefore, further studies are needed to

understand the mechanism of action at the molecular level of these two compounds to guide clinical applications.

- The compound Withanolide E (PM1) shows potential anti-inflammatory activity by strongly inhibiting NO production and need to be further evaluated for anti-inflammatory effects through the ability to inhibit pro-inflammatory cytokines (TNF-α, IL-6), inflammatory cytokines (PGE-2) or COX-2 enzyme etc. for affirmation

NEW CONTRIBUTIONS OF THE THESIS

1. For the first time, the three new compounds, which are physalucoside A (**PA1**), physagulin P (**PA6),** physagulin Q (**PA12**) from *P. angulata*, are isolated and elucidated. From the species *P. minima* distributed in Vietnam, the compound, 4-deoxywithaperuvin (**PM6**) that was first isolated from the genus Physalis, was obtained.

2. For the first time, the cytotoxicity against six cancer cell lines and NO productive inhibition in RAW 264.7 cells of the three new compounds, which are physalucoside A (**PA1**), physagulin P (**PA6),** physagulin Q (**PA12**) from *P. Angulata*, has been evaluated.

3. For the first time, the anti-inflammatory capacity of the two compounds, Physalin F (**PA13**) and physalin B (**PA14**) isolated from *P. angulata*, have been identified with their promising iNOS and COX-2 inhibitory activity.

4. For the first time, a new compound, physagulin P (**PA6**), has been identified and documented to induce apoptosis in A549 lung cancer cells.

LIST OF SCIENTIFIC PUBLICATIONS OF THE AUTHOR RELATED TO THE THESIS

1. Hoang Le Tuan Anh, Do Thi Thao, Duong Thi Dung, Phan Van Kiem, Tran Hong Quang, Pham Thi Hai Yen, Do Thanh Tuan, Pham Viet Cuong, Le Canh Viet Cuong, Tran Manh Hung (2018), Phytochemical constituents and cytotoxic activity of *Physalis angulata* L growing in Vietnam, *Phytochemistry Letters* 27: 193-196.

2. Le Canh Viet Cuong, Le Ba Vinh, **Pham Thi Hai Yen**, Le Thi Lien, Pham Thi Thuy Hoai, Ton That Huu Dat, Do Thi Thao, Bach Long Giang, Ho Kim Young, Hoang Le Tuan Anh (2019), Identification of potential cytotoxic inhibitors from *Physalis minima*, *Natural Product Research*, 35(12): 2082-2085. .

3. **Pham Hai Yen**, Le Canh Viet Cuong, Ton That Huu Dat, Dinh Thi Quy Thuy, Dang Thi Ngoc Hoa, Nguyen Thi Cuc, Duong Thi Hai Yen, Do Thi Thao, Hoang Le Tuan Anh (2019). Whithanolides from the whole plant of *Physalis angulata* and their anti-inflammatory activities, *Journal of Chemistry*, 57(3):334-338.

4. Hoang Le Tuan Anh, Le Ba Vinh, Do Thi Thao, Phan Van Kiem, Pham Thi Hai Yen, Bach Long Giang, Tran Manh Hung, Tran Thi Phuong Anh Ho Kim Young (2020), Bioactive compounds from *Physalis angulata* and their anti-inflamatory and cytotoxic activities, *Natural Products Research* 23(8): 809-818.

5. **Pham Thi Hai Yen**, Nguyen Thi Nga, Trieu Ha Phuong, Nguyen Thi Cuc, Do Thi Phuong, Hoang Le Tuan Anh, Do Thi Thao (2022). Determination of apoptotic inductive activities of physalin P from *Physalis angulata* plant in Vietnam, *Journal of Biology* 20(1):81-87