MINISTRY OF EDUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

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TRAN THI HOAI VAN

STUDY CHEMICAL COMPOSITION AND BIOACTIVITIES OF SOME VIETNAMESE PLANT SPECIES AGAINST *VIBRIO PARAHAEMOLYTICUS* AND THEIR CYTOTOXICITY ACTIVITIES

Speciality: Chemistry of Natural Products

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SUMMARY OF DOCTORAL THESIS IN CHEMISTRY

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Reviewer 1:

Reviewer 2:

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at …… pm., ….. …., 2023 .

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The thesis can be found at:

- Library of Graduate University of Science and Technology
- National Library of Vietnam

INTRODUCTION

1. The necessity of the dissertation subject

Since 2010 until now, acute hepatopancreatic necrosis disease (AHPND) with the causative agent of *Vibrio parahaemolyticus* carrying a plasmid containing the virulent gene causing acute hepatopancreatic necrosis, has caused great economic losses to the industrial shrimp farming industry in our country.The searching for plant species with good effects on disease prevention and treatment in aquaculture in general and shrimp farming in particular is currently a development trend of the world. On the basis of screening for activity against *Vibrio parahaemolyticus* combined with experience that has been used in folklore, many herbal species have been identified that exhibit good activity and have many potential advantages for drug development.

The computer-aided drug research model is increasingly popular and has made many important contributions to drug discovery. This is a reliable and effective technique in future new drug development. From the above reasons, we carried out the topic "**Study chemical composition and bioactivities of some Vietnamese plant species against *Vibrio parahaemolyticus* and their cytotoxicity activities**".

2. The objectives of the thesis

Study on the chemical composition orienting resistance to Vibrio parahaemolyticus causing acute hepatopancreatic necrosis disease in cultured shrimp and anti-tumor activity of some Vietnamese plant species including: *Aralia armata*, *Pouzolzia zeylanica*, *Croton tonkinensis*, *Urena lobata*, *Polygonum chinense*, *Ricinus communis* and *Solanum xanthocarpum*.

Study on bioactivity towards preparing preparations against AHPND caused by Vibrio parahaemolyticus in shrimp and study on the cytotoxicity against A549 cell line of isolated diterpenoid ent-kaurane.

In silico screening of a database of compounds from research plants to rapidly predict potential compounds acting on specific protein targets for use in the treatment of *Vibrio parahaemolyticus* and antitumor. Study the mechanism of action and determine the structure-activity correlation of potential compounds.

3. The main content of the thesis

- Isolation and structure elucidation of compounds isolated from 07 researched plant species.
- Evaluation of the activity against *V. parahaemolyticus in vitro* of crude extracts and some isolated compounds.
- Study mechanism of action of some bioactive compounds on proline metabolism of *V. parahaemolyticus* bacteria.
- Evaluation of the activity against *V. parahaemolyticus in vivo* of the crude extracts of the studied plants.
- Conduct molecular docking studies of ent-kaurane diterpenoid compounds directed to inhibit the PI3K signaling pathway.

4. Structure of the thesis

The thesis consists of 126 typed pages with 17 tables, 50 pictures. The specific distribution is as follows: Introduction 02 pages, literature overview 26 pages, materials and methods 09 pages, experimental 18 pages, results and discussion 58 pages, conclusions and recommendations 02 pages, publications related to the thesis 01 page, references 10 pages.

MAIN CONTENT OF THE THESIS

CHAPTER 1. LITERATURE OVERVIEW

The literature review is a collection of national and international studies on the following issues: 1. Introduction to 7 researched plant species; 2. Research situation on antibacterial and cytotoxicity activities; 3. Research on drugs of plant origin against pathogenic Vibrio strains in aquaculture; 4. Virtual screening searching for potential compounds with antibacterial and cytotoxic activity.

CHAPTER 2. MATERIALS AND METHODS

2.1. Plant materials and isolation and structure elucidation methods

2.1.1. Plant materials

The research object of the thesis is 07 plant samples: stem and leaves of *Pouzolzia zeylanica*, leaf of *Ricinus communis*, stem of *Urena lobata*, branches and leaves of *Croton tonkinensis*, branches and leaves of *Polygonum chinense*, stem and leaves of *Aralia armata* and fruit of *Solanum xanthocarpum* in Vietnam.

2.1.2. Extraction methods

Plant samples were treated according to the method in chemistry, production of methanol extract residue, then water was added and the extract redistributed with the solvents n-hexane, chloroform, ethyl acetate and water, respectively.

2.1.3. Isolation methods

Combination of different chromatographic methods such as: Thin layer Chromatography (TLC), normal phase column chromatography (CC) on silica gel (Merck), reverse phase column chromatography YMC RP 18 (Merck), diaion HP-20, sephadex LH-20 (Merck) and high performance liquid chromatography (HPLC) for the separation of compounds.

2.1.4. Structure elucidation methods

Using modern spectroscopic methods while combining analysis and reference: specific rotation [α]; mass spectrometry (MS); high resolution mass spectrometry (HR-ESI-MS); nuclear magnetic resonance (NMR): ^{1}H-NMR, ^{13}C-NMR và DEPT, HSQC, HMBC, COSY, NOESY, ROESY; circular dichroism (CD).

2.2. Biological activity testing method

The antibacterial activity of V. parahaemolyticus was tested *in vitro* according to Kirby-Bauer's agar antibiogram and Chaweepack's agar diffusion (2015) and *in vivo* testing was carried out at Research Institute for Aquaculture No.1 and No.2.

The cytotoxic activity assay was performed using MTT [3- [4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide] on liver (Hep-G2), and lung cancer cell lines (A549) at Institute of Natural Compound Chemistry, VAST.

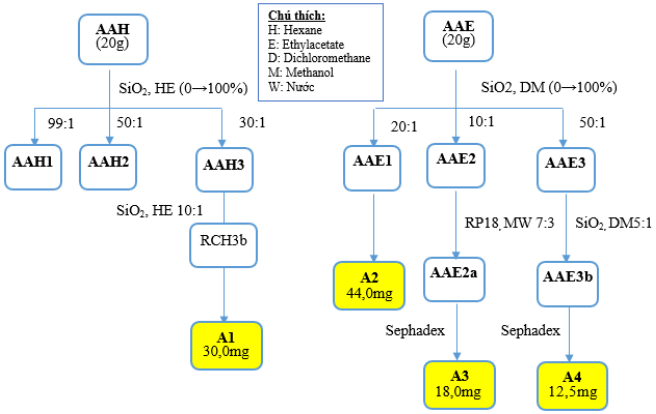
2.3. Molecular docking studies

Molecular docking simulations using AutoDock 4.2.6 and Molegro Virtual Docker software to investigate the mechanism of action on proline metabolism of V.parahaemolyticus and predict potential compounds that inhibit the PI3K signaling pathway in the treatment of lung cancer at Institute of Natural Compound Chemistry, VAST.

CHAPTER 3. EXPERIMENTAL

3.1. Stem and leaves of *Aralia armata*

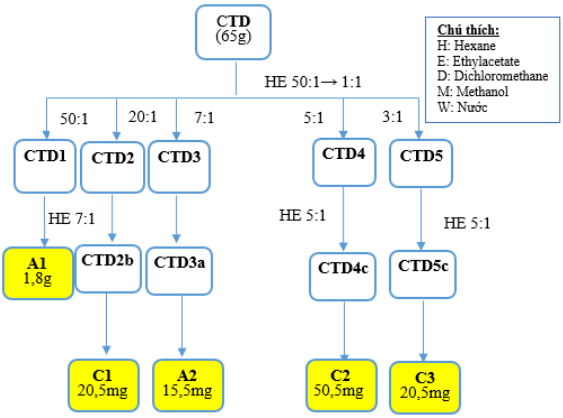
The process of isolating compounds from n-hexane (AAH) and ethyl acetate (AAE) extracts of the stem and leaves of *Aralia armata* are presented in Scheme 3.1.



Scheme 3.1. Isolation process from *n*-hexane and ethyl acetate extracts of *Aralia armata*

3.2. Branches and leaves of *Croton tonkinensis*

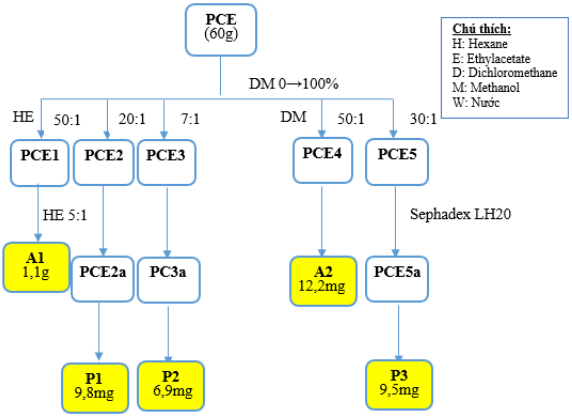
The process of isolating compounds from dichloromethane (CTD) extracts of *Croton tonkinensis* are presented in Scheme 3.2.



Scheme 3.2. Isolation process from dichloromethane extracts of *Croton tonkinensis*

3.3. Branches and leaves of *Polygonum chinense*

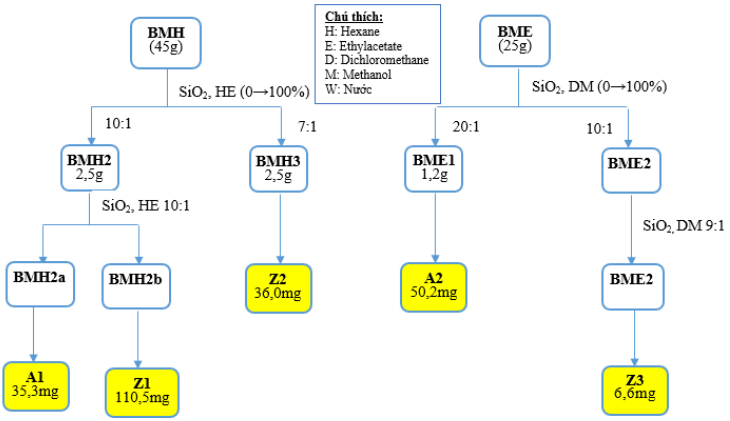
The process of isolating compounds from ethyl acetate (PCE) extracts of *Polygonum chinense* are presented in Scheme *3.3.*



Scheme 3.3. Isolation process from ethyl acetate extracts of *Polygonum chinense*

3.4. Stem and leaves of *Pouzolzia zeylanica*

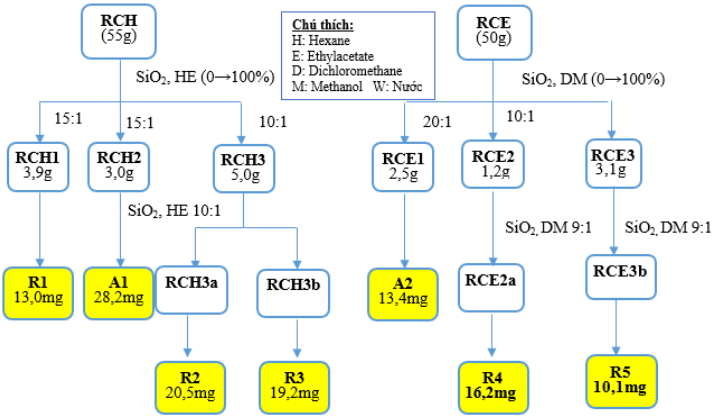
The process of isolating compounds from *n*-hexane (BMH) and ethyl acetate (BME) extracts of *Pouzolzia zeylanica* are presented in Scheme *3.4.*



Scheme 3.4. Isolation process from *n*-hexane and ethyl acetate extracts of *Pouzolzia zeylanica*

3.5. Leaf of *Ricinus communis*

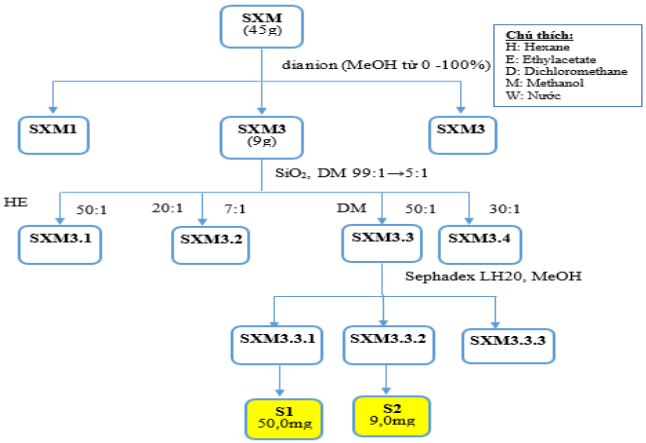
The process of isolating compounds from *n*-hexane (RCH) and ethyl acetate (RCE) extracts of *Ricinus communis* are presented in Scheme *3.5.*



Scheme 3.5. Isolation process from *n*-hexane and ethyl acetate extracts of *Pouzolzia zeylanica*

3.6. Fruit of *Solanum xanthocarpum*

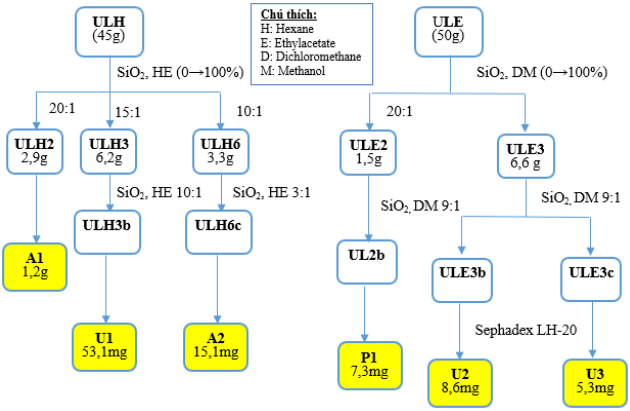
The process of isolating compounds from methanol (SXM) extracts of *Solanum xanthocarpum* are presented in Scheme *3.6.*



Scheme 3.6. Isolation process from methanol extracts of *Solanum xanthocarpum*

3.7. Stem of *Urena lobata*

The process of isolating compounds from *n*-hexane (ULH) and ethyl acetate (ULE) extracts of *Urena lobata* are presented in Scheme *3.7.*



Scheme 3.7. Isolation process from *n*-hexane and ethyl acetate extracts of *Urena lobata*

3.8. Molecular docking studies

Perform molecular docking studies to investigate mechanism of action on PDH target: The crystal structure of proline dehydrogenase was obtained from the Protein Data Bank database. The flavonoid and

diterpenoid compounds used in this study were constructed with three-dimensional structures. The known PDH inhibitor naucleidinal was used as the standard. The simulations were performed using AutoDock 4.2.6 software.

Perform molecular docking studies to investigate mechanism of action on PI3K signaling pathway: The crystal structures of proteins in the PI3K signaling pathway including AKT, mToR, COX-2, MDM2 and PDK1 were obtained from the Protein Data Bank database. For ranking, the MolDock Score [GRID] scoring system of the MVD software was used. Oxaliplatin was used as a reference standard.

3.9. Antibacterial and cytotoxic activities of crude extracts and isolated compounds

In vitro antibacterial activity testing method: according to Kirby-Bauer's agar-disc preparation method and Chaweepack's agar diffusion method (2015). Efficacy is assessed based on the sterile ring.

In vivo antibacterial activity testing method: according to the experimental method using raw extracts mixed into feed for shrimp and for aquatic environment.

Determination of LC50 value of Croton tonkinensis extracts: performed based on the method of APHA (2005). Record daily mortality of shrimp and monitor for 96 hours to determine the LC_{50} value (lethal concentration 50%) using a computer program (Stephan & Rodgers, 1985).

Cytotoxic activity assay by MTT method: human lung cancer cell line A549 was obtained from the Center for Nutrition and Pharmaceutical Materials, Myongji University, Korea. The cytotoxic activity was performed by the MTT method. IC_{50} inhibitory concentration was calculated using Graphpad Prism software (USA).

Vibrio parahaemolyticus *in vitro* and *in vivo* antibacterial activities of extracts and pure compounds were tested in Research Institute for Aquaculture No.1 and No.2.

The cytotoxic assay on lung cancer cell line (A549) was tested at the Institute of Natural Products Chemistry, VAST.

CHAPTER 4. RESULTS AND DISCUSSION

A. ANTIBACTERIAL ACTIVITY AGAINST *VIBRIO PARAHAEMOLYTICUS*

4.1. Results of antibacterial activity against *V. parahaemolyticus* of crude extracts *in vitro*

Total ethanol extracts of 13 samples: *Polygonum chinense*-**PC.M**, *Ricinus communis*-**RC.M**, *Solanum xanthocarpum*-**SX.M**, *Urena lobata*-**UL.M**, *Aralia armata*-**AA.M**, *Pouzolzia zeylanica*-**PZ.M** and *Croton tonkinensis*-**CT.M**, *Sida rhambifolia*-**SR.M**, *Desmodium heterophyllum*-**DM.M**, *Biidens pilosal*-**BP.M**, *Curcuma longa*-**CL.M**, *Chenopodia abrosioides*-**CA.M** and *Euphorbia tithymaloides*-**ET.M** were tested for antibacterial activities against V. parahaemolyticus causing AHPND. The test results are shown in Table 4.1.

Table 4.1. Antibacterial results against *V. parahaemolyticus* of crude extracts

Sample	Concentration	d (mm)	Method	Conclusion
AA.M	1,2 mg/ 2% ethanol	25 ± 0,3	Chaweepack (2015)	Sensitive *****
CT.M	1,2 mg/ 2% ethanol	18 ± 0,3	Chaweepack (2015)	Sensitive *****
PZ.M	1,2 mg/ 2% ethanol	17 ± 0,3	Chaweepack (2015)	Sensitive *****
UL.M	1,2 mg/ 2% ethanol	15 ± 0,3	Chaweepack (2015)	Sensitive *****
DM.M	1,2 mg/ 2% ethanol	7 ± 0,0	Chaweepack (2015)	Resistance *
ET.M	1,2 mg/ 2% ethanol	7 ± 0,1	Chaweepack (2015)	Resistance *
PC.M	200 µg 66,7 µg 40 µg 22,2 µg	20,6 ± 0,41 19,8 ± 0,45 15,3 ± 0,16 0	W. Kirby and A. Bauer (1961)	Sensitive ** Sensitive ** Moderate** No **
RC.M	200 µg 66,7 µg 40 µg 22,2 µg	15,3 ± 0.55 14,3 ± 0.22 11,0 ± 0.37 0	W. Kirby and A. Bauer (1961)	Moderate ** Moderate ** Resistance ** No **
SX.M	200 µg 66,7 µg	14,4 ± .24 14,2 ± 0,37	W. Kirby and A. Bauer (1961)	Moderate ** Moderate **

	40 µg 22,2 µg	10,4 ± 0,24 0		Resistance ** Resistance **
SR.M	200 µg 66,7 µg 40 µg 22,2 µg	0 0 0 0	W. Kirby and A. Bauer (1961)	No **
CL.M	200 µg 66,7 µg 40 µg 22,2 µg	11,2 ± 0,24 10,3 ± 0,14 8,9 ± 0,11 0	W. Kirby and A. Bauer (1961)	Resistance ** Resistance ** Resistance ** Resistance **
CA.M	200 µg 66,7 µg 40 µg 22,2 µg	0 0 0 0	W. Kirby and A. Bauer (1961)	No **
BP.M	200 µg 66,7 µg 40 µg 22,2 µg	0 0 0 0	W. Kirby and A. Bauer (1961)	No **

Notes: * Diameter of bacterial growth inhibition zone: Resistance: ≤ 9 mm; Moderate: ≥ 10-13 mm; Sensitive: ≥ 14 mm. ** Sterile ring diameter: Resistance: ≤ 11 mm, Moderate: 12-15 mm, Sensitive: ≥ 16 mm

Table 4.1 shows: 07 total ethanol extracts of *Polygonum chinense*-**PC.M**, *Ricinus communis*-**RC.M**, *Solanum xanthocarpum*-**SX.M**, *Urena lobata*-**UL.M**, *Aralia armata*-**AA.M**, *Pouzolzia zeylanica*-**PZ.M** and *Croton tonkinensis*-**CT.M** exhibited better antibacterial activity than the rest, thus, they are selected for further investigation.

Among the samples tested by antibacterial method on agar plate according to W. Kirby and A. Bauer (1961), Polygonum chinense extract has the highest ability to kill V. parahaemolyticus causing AHPND. At the concentration of 66,7-200µg, the test sample had no difference in bactericidal effect with Doxycycline (30µg).

Among the samples tested by the antibacterial method on agar plates according to Chaweepack (2015), *Aralia armata* has the most

sensitivity, followed by *Croton tonkinensis* extract, which inhibits growth of *V. parahaemolyticus* with an average inhibition ring diameter of 25 and 18 mm, respectively.

Based on the above results:

The total extract of the 07 samples mentioned above continues to be studied further (testing on shrimp, study chemical composition to determine which main classes of compounds in the tested samples have inhibitory effect on *V. parahaemolyticus*).

Antibiogram results (Table 4.1) showed that the high concentration of extract used increased threefold from từ 66,7µg to 200µg, but the bactericidal effect did not have a significant difference. Therefore, when testing on shrimp, a low concentration of 66,7µg was selected for application to increase economic efficiency (reducing the cost of extracting herbs, but the bactericidal effect was still significant, respectively, at high concentration (200µg).

Although the total extract of *A. armata* was able to inhibit the growth of bacteria V. parahaemolyticus which is higher than that of *C. tonkinensis*, however, the species *A. armata* is less common, depending on the season, it is very difficult to collect samples, affecting large-scale experimental trials. In contrast, the species *C. tonkinensis* is widely used in folklore, grows wild and is grown as medicine in Ba Vi, Ninh Binh, Hoa Binh, etc. This is an easy source of raw materials. On the other hand, the antibacterial activity against V. parahaemolyticus of ent-kauran isolated from *C. tonkinensis* showed much higher activity than isolated triterpenoid compounds from *A. armata*. Therefore, the selection of *C. tonkinensis* for further studies on shrimp is reasonable.

Total extracts of *P. chinense* and *R. communis* were tested on shrimp. *R. communis* is preferred for testing for the following reasons: *S. xanthocarpum* and *R. communis* have similar efficacy in the results of antibiotic preparation. However, table 4.1 shows that the sterile

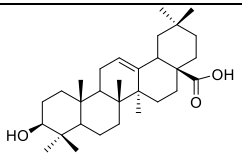
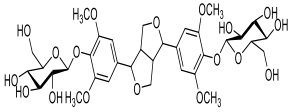
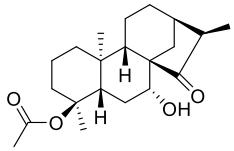
diameter of *R. communis* is greater than 0,9; 0,1 and 0,6mm compared with *S. xanthocarpum* at concentrations of 200, 66,7 and 40µg, respectively. In addition, in Vietnam in aquaculture, *R. communis* is one of 6 herbs recommended to be used to treat diseases of farmed fish with high results (soaked 15-20kg of leaves/8-10m^3 fish cage).

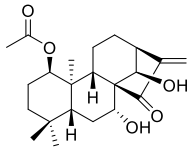
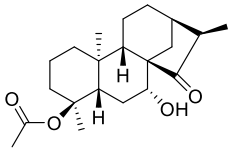
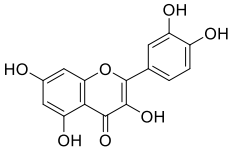
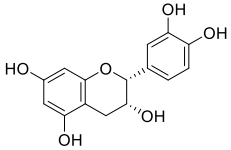
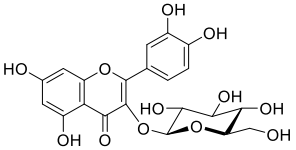
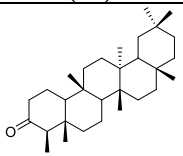
In aquaculture, *R. communis* has been determined to be effective against bacteria and fungi, with a broad bactericidal spectrum against Gr (+) and Gr (-) (V. parahaemolyticus) bacteria when the diameter of the sterile ring reaches 18,0 mm (*Staphylococcus aureus*) and 22,3 mm (*Bacillus subtilis*) with concentrations of 100 µg/paper roll, respectively.

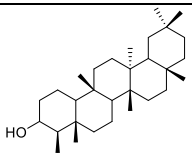
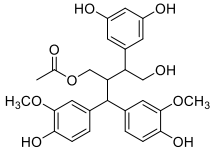
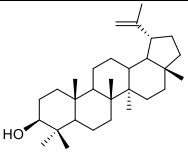
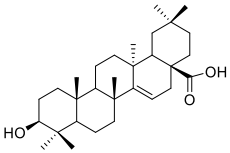
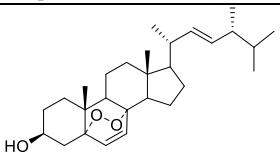
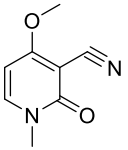
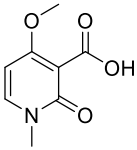
4.2. Main chemical composition of studied plants

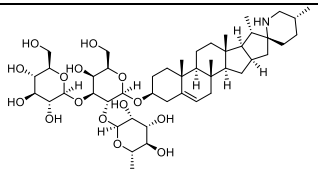
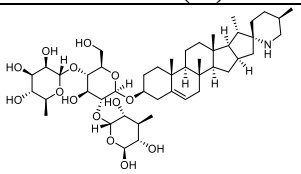
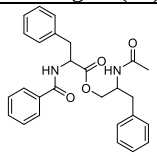
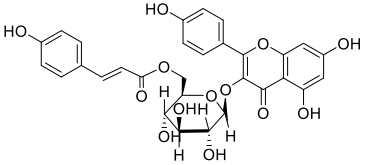
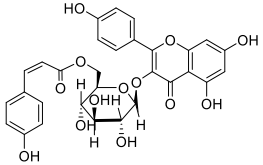
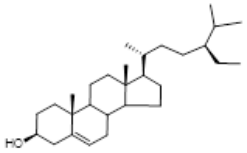
The results of isolation and structural elucidation of compounds in 7 studied species are summarized in Table 4.9.

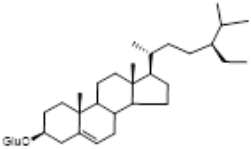
Table 4.9. Compounds isolated from 7 studied species

No.	Compounds	Structure class	Species	Weigh (mg)	Novelty
1	 oleanolic acid (**A3**)	Triterpenoit	*A. armata*	18,0	
2	 liriodendrin (**A4**)	Lignan	*A. armata*	12,5	L
3	 *ent*-18-axetoxy-7β-hydroxy-kauran-15-on (**C1**)	Diterpenoid	*C. tonkinensis*	20,5	

4	 *ent*-1α-axetoxy-7β,14α-dihydroxykaur-16-en-15-on (**C2**)	Diterpenoid	*C. tonkinensis*	50,5	
5	 *ent*-18-axetoxy-7β-hydroxykaur-16-en-15-on (**C3**)	Diterpenoid	*C. tonkinensis*	20,3	
6	 quercetin (**P1**)	Flavonoid	*P. chinense* *U. lobata*	9,8 7,3	
7	 (-)-epicatechine (**P2**)	Flavonoid	*P. chinense*	6,9	L
8	 quercetin-3-O-β-D-glucopyranoside (**P3**)	Flavonoid	*P. chinense*	9,5	L
9	 friedelan-3-one (**Z1**)	Triterpenoid	*P. zeylanica*	110,5	L

10	 3β-friedelanol (**Z2**)	Triterpenoid	*P. zeylanica*	36,0	L
11	 pouzolignan F (**Z3**)	Lignan	*P. zeylanica*	6,6	H
12	 lupeol (**R1**)	Triterpenoid	*R. communis*	13,0	
13	 epialeuritolic acid (**R2**)	Triterpenoid	*R. communis*	20,5	L
14	 ergosterol peroxide (**R3**)	Steroid	*R. communis*	19,2	L
15	 ricinine (**R4**)	Alkaloid	*R. communis*	16,2	
16	 3-carboxy-4-methoxy-N-methyl-2-pyridone (**R5**)	Alkaloid	*R. communis*	10,1	H

17	 solasonine (**S1**)	Alkaloid	*S. xanthocarpum*	50,0	
18	 solamargine (**S2**)	Alkaloid	*S. xanthocarpum*	9,0	
19	 α-acetylamino-phenylpropyl α-benzoylamino-phenylpropanoate (**U1**)	Alkaloid	*U. lobata*	53,1	H
20	 *trans*-tiliroside (**U2**)	Flavonoid	*U. lobata*	8,6	H
21	 *cis*-tiliroside (**U3**)	Flavonoid	*U. lobata*	5,3	H
22	 β-sitosterol (**A1**)	Steroid (Phytosterol)	*A. armata* *C. tonkinensis* *P. chinense* *P. zeylanica* *R. communis* *U. lobata*	30,0 1800 1100 35,3 28,1 1200	

23	 Daucosterol (**A2**)	Steroid (Phytosterol)	*A. armata* *C. tonkinensis* *P. chinense* *P. zeylanica* *R. communis* *U. lobata*	44,0 15,5 9,5 50,2 13,4 15,1	

L: First isolated from species H: First isolated from genus

4.3. Antibacterial activities against *V. parahaemolyticus* of isolated compounds

Isolated compounds from 7 studied species continued to be tested *in vitro* against *V. parahaemolyticus* causing AHPND. Preparation of the strain *V. parahaemolyticus* and the test method was performed as described in section 3.9.

Tested results of compounds: oleanolic acid, liriodendrin (*Aralia armata*), 3-friedelanone, 3*β*-friedelanol, pouzolignan F (*Pouzolzia zeylanica*), lupeol, epialeuritolic acid, ergosterol peroxide, ricinine (*Ricinus communis*) and solasonine và solamagine (*Solanum xanthocarpum*) against *V. parahaemolyticus* bacteria at 4 concentrations of 50, 100, 200 and 300/pie by the method of antibiotic disc diffusion on agar plate according to W. Kirby and A. Bauer (1961) were not effective against V. parahaemolyticus causing AHPND.

Antibacterial results of compounds: *ent*-18-axetoxy-7*β*-hydroxykauran-15-on (**C1**, content 0,012%), *ent*-1*α*-axetoxy-7*β*,14*α*-dihydroxykaur-16-en-15-on (**C2**, content 0,80%) and *ent*-18-axetoxy-7*β*-hydroxykaur-16-en-15-on (**C3**, content 0,52%) (*Croton tonkinensis*) and quercetin-3-O-*β*-D-glucopyranoside (**P3**, content 1,11%) by diffusion method on agar plates is shown in Table 4.10.

Table 4.10. Inhibitory effect of compounds on growth of *Vibrio parahaemolyticus* (VP) cultured in ISB medium after 24 h

Compounds	**Concentration**	**Density (CFU/ml)**	**Bacterial inhibition rate after 24 hours**
Absolute ethanol + VP +		$1,37 \times 10^8$	

ISB			
P3	0,1%	2,4 x 10^7	82,5%
C1		4,0 x 10^7	70,8%
C2		7,6 x 10^7	44,5%
C3		6,8 x 10^7	50,4%

Table 4.10 show that the inhibition rate of V. parahaemolyticus in the treatment containing compound **P3** was 82,5%, next, the treatment containing compound **C1** accounted for 70,8% at a concentration of 0,1%. In the treatments containing compounds **C3** and **C2**, the inhibition rate of *V. parahaemolyticus* was 50,4% and 44,5%, respectively. This shows that **P3** and **C1** at a concentration of 0,1% have the ability to inhibit the growth of *V. parahaemolyticus*. Therefore, they are used for AHPND prevention experiments in the laboratory at a dose of 0,1% (1g/kg feed).

4.4. Molecular docking studies to investigate mechanism of action of bioactive compounds on proline metabolism of *V. parahaemolyticus*

Proline dehydrogenase (PDH) is a regulatory enzyme that plays an important role in protein self-organization in bacteria. Mechanism of action of compounds *ent*-1α-axetoxy-7β,14α-dihydroxykaur-16-en-15-on (**C2**), *ent-1*8-axetoxy-7*β*-hydroxy-kauran-15-on (**C1**), *ent*-18-axetoxy-7*β*-hydroxykaur-16-en-15-on (**C3**) and quercetin-3-O-*β*-D-glucopyranoside (**P3**) will be analyzed using molecular docking method between the compounds and the target enzyme PDH.

Table 4.11. Dock score and residues participated in forming interaction with PDH

Compounds	**ΔG (kcal/mol)**	**No. of H-bond**	**Residues participated in H-bond**
C2	-9.91	2	Gly64; Asp281
C1	-10.85	2	Gly64; Gln102
C3	-10.34	3	Gly64; Asp281; Arg289
P3	-11.59	8	Asp61; Leu62; Gly64; Leu98; Leu100; Gln102 ;

			Arg288 ; Arg289
Naucleidinal	-7.82	2	Gly64; Arg289

The docking results of potential compounds within the active site of proline dehydrogenase enzyme are presented in Table 4.11. According to the ranking criteria of Autodock 4.2.6, the more negative value of docking energy, the better binding affinity of the compound towards targeted receptor. Considering the above criteria, all four compounds showed better binding affinity at the active region of PDH than standard ligand (naucleidinal), in which compound P3 showed the highest binding affinity (-11.59 kcal/mol), compound C2 has the lowest binding affinity (-9.91 kcal/mol).

These initial data show a high correlation between calculation and experiment as shown by the correlation coefficient R^2 = 0.8014. This suggests that the computational model used in this study has potential applications in predicting compounds capable of inhibiting *V. parahaemolyticus*. In general, the compounds all formed bonds with important amino acids within the active region of the PDH enzyme, which suggests a reasonable explanation for the mechanism underlying the antimicrobial activity of the studied compounds. .

4.5. Antibacterial results against *V. parahaemolyticus* of crude extracts *in vivo*

4.5.1. Extracts of Polygonum chinense and Ricinus communis were added to the aqueous medium

Test results of adding total ethanol extract of *Polygonum chinense* (**PC.M**) and *Ricinus communis* (**RC.M**) to the water environment: **PC.M** extract at a concentration of 30g/m^3 was mixed with AHPND-infected shrimp culture water at 2 time points (initiation of pathogen appearance and repeated after 24 hours) which improved the survival rate of shrimp (60%) compared with the positive control. **PC.M** extract (25g/m^3) and **RC.M** extract (35g/m^3; 40g/m^3) did not completely kill bacteria in water, so 100% mortality after 6 days of experiment.

4.5.2. Extracts of Croton tonkinensis, Pouzolzia zeylanica and Urena lobata were added to the aqueous medium

Test results of adding total ethanol extract of *Croton tonkinensis* (**CT.M**), *Aralia armata* (**AA.M**), *Pouzolzia zeylanica* (**PZ.M**) and *Urena lobata* (**UL.M**) to the water environment before infected for 1 hour and soaked for the second time after 24 hours of infection with a dose of 20 ppm: The average survival rate of shrimp after 7 days of infection was highest recorded at 71,7 ± 2,9% in the treatment with **CT.M**, followed by soaking in **AA.M** at 61,7 ± 2,9%, **PZ.M** immersion is 35% ± 10,0% and the lowest **UL.M** immersion is 11,7 ± 7,6%. Meanwhile, the mean survival rate in the positive control treatment was 10 ± 5,0% and the negative control shrimp still survived 100% after the end of the experiment.

The mortality rate of shrimp was obtained, LC_{50} values determined at 48, 72 and 96 hours were 93,02ppm, 81,25ppm and 81,25ppm. Meanwhile, at 24 h, the LC_{50} value could not be determined due to low mortality.

4.5.3. Antibacterial effect against V. Parahaemolyticus when mixed with shrimp feed

4.5.3.1. Total extract of Polygonum chinense (PC.M) mixed with shrimp feed

For the formula of mixing **PC.M** extract into the feed: shrimp were fed with 2 concentrations of 25 and 30g/100kg of shrimp continuously for 6 days, then increase the bacterial concentration by adding bacteria to the culture water at a density of 10^5-10^6 cfu/mL. The results showed that by feeding at a concentration of 25g/100kg of shrimp, the mortality rate was 27%, while the batch fed with 30g/100kg of shrimp had a mortality rate of 60%. High mortality in the batch fed with 30g/100kg of shrimp was observed on the 3rd day after the treatment. Thus, through this experimental model, **PC.M** extract is effective in preventing AHPND with a high survival rate of >60% compared to the positive control group.

*4.5.3.2. Total extract of Croton tonkinensis (**CT.M**) mixed with shrimp feed*

The results of the prevention experiment with *Croton tonkinensis* extract mixed into the feed and fed to the shrimp for 7 days and 12 days: The survival rate of shrimp in the positive control treatment (shrimp fed the feed not mixed with the extract) was 15%±5% and the mean survival rate of shrimp in the negative control was 100%. Meanwhile, treatments for disease prevention by extracts at concentrations of 1%; 2% and 4% had a survival rate of 43,3%±5,8%; 63,3%±7,6% and 71,7%±2,9%. This can be suggested that *Croton tonkinensis* extract is effective in preventing acute hepatopancreatic necrosis disease in whiteleg shrimp with both 2% and 4% doses mixed into the feed with an average survival rate of over 60%. The results of histopathological analysis of shrimp samples for prevention of AHPND showed that the hepatopancreas of shrimp fed with 2% and 4% mixed *Croton tonkinensis* extract had normal structure.

The average mortality of experimental shrimp at the time points of 24, 48, 72, and 96 hours were tested. Shrimp were fed daily with high concentrations of gentian extract mixed into the feed including 0, 25, 30, 35, 40 and 45%. Experimental results showed that no shrimp died in the control treatment (0%) during the 96 hours of the experiment. In all treatments, shrimp died after 24 h, with an average mortality from 1,67%±2,89% to 5%±0%. The average mortality rate of shrimp after 48 hours at the highest concentration of extract (45%) was 15%±5%. At 72 and 96 hours, the average mortality rate of shrimp at the highest concentration of the experiment (45%) was similar (21,67%±2,89%). Due to the results of shrimp mortality <22%, the LC_{50} value at key time points could not be determined. Shrimp hepatopancreas has a normal structure.

B. DOCKING STUDIES AND CYTOTOXICITY ACTIVITIES

4.6. Molecular docking studies of ent-kaurane diterpenoid compounds directed to inhibit the PI3K signaling pathway

The objective of this study was to conduct molecular docking to identify diterpenoid compounds capable of inhibiting the PI3K signaling pathway, and then to study their cytotoxic activity on lung cancer cell line (A549).

Based on compounds isolated from *Croton tonkinensis*, performed docking simulation for 7 ent-kaurane diterpenoid compounds including: ent-18-axetoxy-7β-hydroxykaur-16-en-15-one (**1**); ent-1α-axetoxy-7β,14α-dihydroxykaur-16-en-15-one (**2**); ent-18-axetoxy-7β-hydroxykaur-15-one (**3**); ent-7β,14α-dihydroxykaur-16-en-15-one (**4**); ent-18α-axetoxy-7α,14β-dihydroxykaur-16-en-15-one (**5**); ent-1α,14α-diacetoxy-7β-hydroxykaur-16-en-15-one (**6**) and ent-1α,7β-diacetoxy-14α-hydroxykaur-16-en-15-one (**7**) directed inhibition on the main proteins of the PI3K signaling pathway including: AKT (PDB ID: 1UNQ), mToR (PDB ID: 4JSV), COX-2 (PDB ID: 3NT1), MDM2 (PDB ID: 4JRG), PDK1 (PDB ID: 5LOV).

The results show that compound **6** has a highest dock score with mToR of -124.5 kcal/mol, followed by **1** docked with COX-2 at a dock score of -110.2 kcal/mol. In terms of hydrogen bonding, **6** does not form hydrogen bonds with mToR, so the interaction with mToR is not stable even though the dock score is higher than the others. Compound **1** forms hydrogen bonds with Phe210, Gln289 and Tyr385 through the O17 and H1 positions. The binding affinity results show that **7** and **1** have the strongest binding affinity for mToR of -16.1 kJ/mol and -15.8 kJ/mol, respectively. In general, compound **7** had the strongest binding affinity for AKT, mToR, MDM2 and PDK1, The compound with the best affinity for COX-2 is **6**, compound **2** also exhibits high dock energy with AKT, MToR and MDM2, ranked after compounds **6** and **7**. Therefore, **1, 2**, **6** and **7** are predicted to have the potential to inhibit the PI3K signaling pathway.

4.7. Cytotoxicity of ent-kaurane diterpenoid from *Croton tonkinensis* against lung cancer cell line A549

Cytotoxicity assays were performed using MTT method: Cells were incubated with the studied compounds for 48 h at different concentrations. IC_{50} values of compounds **7**, **6**, **1** and **2** were 11,17 ± 0,8; 12,87 ± 0,3; 18,55 ± 1.3 and 20,07 ± 0.8 µM, which higher than oxaliplatin (22,12 ± 1,1 µM). The remaining compounds showed moderate and weak activity. These preliminary results show the potential of the studied compounds to inhibit lung cancer cell proliferation in which it shows high correlation to the results of molecular docking studies.

CONCLUSION

The thesis has obtained the following main results:

1. Research on chemistry:

From 07 studied species, isolated and determined the structure of 33 compounds (of which 10 were duplicated, the remaining 23 compounds) mainly belonging to the classes of diterpenoids, triterpenoids, steroids and polyphenols.

2. Research on bioactivity towards preparing preparations against acute hepatopancreatic necrosis disease caused by *Vibrio parahaemolyticus* in shrimp:

- Preliminary survey results on *Vibrio parahaemolyticus* inhibition of crude extract of 13 plant showed that: *Urena lobata*, *Pouzolzia zeylanica*, *Croton tonkinensis* and *Aralia armata* (average diameter of inhibition ring is 15 mm, 17 mm, 18 mm, and 25 mm, respectively, according to the method of Chaweepack-2015); *Ricinus communis* and *Polygonum chinense* (diameter of sterile ring is 10,4 - 15,3 mm and 15,3 – 20,6 mm corresponding to the concentration of 40-200µg/circle according to the method of W. Kirby and A. Bauer - 1961) exhibited the best activity and was selected for the study *of V. parahaemolyticus*

in shrimp (*in vivo*) supplemented with water and feed. The results of *in vivo* activity test showed that the extract of *Croton tonkinensis* (**CT.M**) at a dose of 15 ppm and *Polygonum chinense* (**PC.M)** at a concentration of 30g/m^3 added to AHPND-infected shrimp culture water *in vivo* gave the best antibacterial results, improving shrimp survival rate by more than 60%. *C.tonkinensis* extract (at dose of 2% (20g/kg feed) and 4% (40g/kg feed)) and *P.chinense* (at dose 25-30g/100kg shrimp) added to shrimp feed effectively raising the survival rate of shrimp to over 70,4%.

- *In vitro* biological activity test results showed that compounds quercetin-3-O-*β*-D-glucopyranoside, *ent*-18-axetoxy-7*β*-hydroxykaur-16-en-15-on, *ent*-18*α*-axetoxy-7*α*,14*β*-dihydroxykaur-16-en-15-on and *ent*-1*α*-axetoxy-7*β*,14*α*-dihydroxykaur-16-en-15-on at a concentration of 0,1% have the ability to inhibit the growth of *Vibrio parahaemolyticus* of 82,5; 70,8; 50,4; 44,5 and 35,5%, respectively.

- Initially investigate mechanism of action of *ent*-1α-axetoxy-7β,14α-dihydroxykaur-16-en-15-on (**C2**), *ent-1*8-axetoxy-7*β*-hydroxy-kauran-15-on (**C1**), *ent*-18-axetoxy-7*β*-hydroxykaur-16-en-15-on (**C3**) and quercetin-3-O-*β*-D-glucopyranoside (**P3**) on the proline metabolism of *Vibrio parahaemolyticus*. The docking results showed that the binding affinity of studied compounds at the active site of the PDH protein was better than that of the naucleidinal, in which compound **P3** showed the highest binding affinity (-11.59 kcal/mol), compound **C2** has the lowest binding affinity (-9.91 kcal/mol).

3. Research on cytotoxic activities:

- Investigated cytotoxicity against human lung cancer cell line A549 of ent-kaurane diterpenoids isolated from *C.tonkinensis* including: *ent*-1α,7*β*-diacetoxy-14α-hydroxykaur-16-en-15-one (**7**); *ent*-1α,14α-diacetoxy-7*β*-hydroxykaur-16-en-15-one (**6**); *ent*-18α-axetoxy-7α,14*β*-dihydroxykaur-16-en-15-one (**5**); *ent*-7*β*,14α-

dihydroxykaur-16-en-15-one (**4**); *ent*-18-axetoxy-7*β*-hydroxykaur-16-en-15-one (**C3**); *ent*-1α-axetoxy-7*β*,14α-dihydroxykaur-16-en-15-one (**C2**) and *ent*-18-axetoxy-7*β*-hydroxykaur-15-one (**C1**). Obtained results indicated that potential compounds were **7**, **6**, **C3** and **C2** with IC_{50} value of 11,17 ± 0,8; 12,87 ± 0,3; 18,55 ± 1.3 và 20,07 ± 0.8 µM, respectively, which are higher than oxaliplatin (22,12 ± 1,1 µM).

- Docking simulation of 7 ent-kaurane diterpenoid compounds directed to inhibit PI3K signaling pathway in lung cancer cells also indicated 4 compounds: *ent*-18-axetoxy-7*β*-hydroxykaur-16-en-15-one (**C3**), *ent*-1α-axetoxy-7*β*,14α-dihydroxykaur-16-en-15-one (**C2**), *ent*-1α,14α-diacetoxy-7*β*-hydroxykaur-16-en-15-one (**6**) and *ent*-1α,7*β*-diacetoxy-14α-hydroxykaur-16-en-15-one (**7**) exhibited inhibitory potential for proteins when compared with oxaliplatin.

RECOMMENDATIONS

The research results in the thesis show that *Croton tonkinensis* and *Polygonum chinense* are two species with interesting chemical compositions and biological activities. Therefore, in the future, we will continue to evaluate the effectiveness of AHPND prevention of *Croton tonkinensis* and *Polygonum chinense* extracts to create products. The results of the study on the cytotoxic activity on the lung cancer cell line A549 showed that the ent-kaurane diterpenoid compounds have the potential to be considered for further studies in cancer drugs development.

PUBLICATIONS RELATED TO THE THESIS

❖ **International articles (ISI/Scopus):**

1. Pham Thi Hong Minh, **Tran Thi Hoai Van**, et al. Identification of *ent*-kaurane diterpenoid compounds as potential inhibitors of the PI3K pathway in nonsmall cell lung cancer through molecular docking simulations, *Natural Product Communications*, (2021), 16(9): 1–8.

❖ **National articles:**

2. **Trần Thị Hoài Vân**, Đỗ Tiến Lâm và cộng sự. Đóng góp vào kết quả nghiên cứu thành phần hóa học cây Bọ mắm *Pouzolzia zeylanica* (L.) Benn). *Tạp chí Hóa học*, (2015), 53 (6e 1,2), p. 149 - 153.

3. Đỗ Tiến Lâm, Vũ Thị Thu Lê, **Trần Thị Hoài Vân** và cộng sự. Các kết quả nghiên cứu ban đầu về thành phần hóa học của thân cây Đơn châu chấu (*Aralia armata*) ở Thái nguyên. *Tạp chí Khoa học và Công nghệ*, Trường ĐH Thái Nguyên, KHTN-KT, **(**2016), 150 (05), p. 9-14. ISSN: 1859-2171.

4. **Tran Thi Hoai Van**, Luan Thi Thu và cộng sự. Contribution to results of the chemical constituents of *Ricinus communis***.** *Tạp chí Khoa học và Công nghệ*, (2016), 54 (2C), p. 523-529. ISSN: 1859-2171.

5. Trương Thị Mỹ Hạnh, Phạm Thị Yến, Phạm Thị Huyền, Huỳnh Thị Mỹ Lệ, Phạm Thị Hồng Minh, Đỗ Tiến Lâm, **Trần Thị Hoài Vân** và cộng sự. Tác dụng diệt khuẩn của dịch chiết thân lá Thồm lồm (*Polygonum chinenses* L,) đối với vi khuẩn gây bệnh hoại tử gan tụy cấp ở tôm nuôi nước lợ. *Tạp chí Khoa học và Công nghệ Việt Nam*, (2017), 17 (6), p. 20-24. ISSN: 1859-4794.

6. Pham Minh Quan**, Tran Thi Hoai Van**, Do Tien Lam et al. Study on the chemical composition of *Urena lobata* (L.) growing in Vietnam. *Tạp chí Vietnam Journal of Science and Technology*, (2019), 57 (2), p.162-169.

7. **Tran Thi Hoai Van**, Pham Thi Hong Minh, Pham Quoc Long, et al. Effect of some phyto-flavonoids and terpenoid on proline metabolism of *Vibrio parahaemolyticus*: inhibitory mechanism and interaction with molecular docking simulation. *Vietnam Journal of Science and Technology*, (2020), 58 (6A) p. 189-198.