# MINISTRY OF EDUCATION VIETNAM ACADEMY OF AND TRAINING SCIENCE AND TECHNOLOGY

### GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY



## **TRAN VAN HIEN**

### STUDY ON THE CHEMICAL CONSTITUENTS AND SOME BIOLOGICAL ACTIVITIES OF Moringa oleifera

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#### INTRODUCTION

Vietnam belongs to a typical tropical monsoon region with extremely diverse and abundant flora and fauna. The 8th National Conference on Ecology and Biological Resources, coorganized by the Institute of Ecology and Biological Resources and the Graduate Academy of Science and Technology, identified approximately 51,400 species in Vietnam, including about 7,500 species/strains of microorganisms, 20,000 plant species, 10,900 animal species, 2,000 freshwater invertebrates and fish species, and 11,000 marine species. This represents an invaluable natural resource for scientists to explore and discover compounds that meet human needs, such as medicines for humans, livestock, and crops.

Historically, the use of plants for providing nutrients and treating illnesses was based on accumulated experience over many generations. While traditional medicine formulations have been developed from these practices, there remains a lack of scientific studies to substantiate the chemical composition and biological activities of the natural compounds found in these traditional remedies.

In recent years, advancements in science and technology, particularly in the extraction, isolation, and structural determination of natural compounds, have allowed scientists to discover, isolate, identify, and test numerous natural compounds. These findings have clarified the mechanisms of action of many traditional remedies and led to the discovery of new bioactive compounds with valuable therapeutic properties for human health. The **Moringaceae family** consists of a single genus, *Moringa*, which includes 22 species distributed widely in tropical and subtropical regions. Studies on several *Moringa* species have revealed numerous interesting bioactive compounds, such as those with antioxidant, anti-inflammatory, antibacterial, antifungal, anticancer, and antidiabetic activities.

Today, despite scientific advancements and improved living standards, many diseases are becoming increasingly prevalent and severe, threatening human health and quality of life. In particular, environmental pollution and the overuse of antibiotics have made inflammatory diseases more complex and difficult to treat. As older generations of anti-inflammatory drugs become less effective, the search for new natural antiinflammatory agents has become critical. Isolating and extracting anti-inflammatory compounds from medicinal plants used in traditional remedies, with the goal of discovering highly active natural compounds for human and veterinary medicine, is a research focus of significant scientific interest.

Research on several *Moringa* species has identified many valuable bioactive compounds, such as those with antioxidant, anti-inflammatory, antibacterial, antifungal, anticancer, and antidiabetic activities. Among these, *Moringa oleifera* has received significant attention not only for its nutritional value but also for its biological activities and potential applications in medicine and pharmaceuticals.

The Thesis, titled "Study on the chemical constituents and some biological activities of *Moringa oleifera*", focuses on investigating the chemical composition of the leaves and roots of *Moringa oleifera*. It also examines the antiinflammatory activity (through NO production inhibition) and antibacterial activity against certain *Vibrio* strains that cause diseases in aquaculture. The study aims to discover new compounds with strong bioactivity for potential development into treatments for inflammatory diseases in humans and aquaculture.

## **Objectives of the Thesis**

To study the chemical composition of *Moringa oleifera Lam* in order to identify new chemical structures and explore the biological activities of isolated compounds, providing a scientific basis for further research.

## **Thesis Content**

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1. Isolation of compounds from the leaves, stems, and roots of *Moringa oleifera Lam* collected in Hoanh Bo District, Quang Ninh Province, using column chromatography.

2. Determination of the chemical structures of the isolated compounds using spectroscopic methods such as IR, MS, 1D-NMR, and 2D-NMR.

3. Evaluation of cytotoxic, anti-inflammatory, hypoglycemic, antioxidant, and antimicrobial activities of the extracts and isolated compounds.

#### **CHAPTER I. OVERVIEW**

## 1.1. Scientific Classification, morphological characteristics, and distribution 1.1.1. Scientific classification

Moringa oleifera belongs to the following classification:

- Kingdom (Regnum): Plantae
- Order (Ordo): Brassicales
- Family (Familia): Moringaceae
- Genus (Genus): Moringa
- Species (Species): Moringa oleifera

*Moringa oleifera*, commonly known as the drumstick tree, is the most prevalent woody plant in the *Moringa* genus. Native to South Asia, it also grows wild and is cultivated in various parts of the world due to its high economic value.

**1.1.2.** Morphological characteristics and distribution *Moringa oleifera* is a medium-sized tree. At maturity, it can grow to a height of tens of meters. Within one year, if not pruned, it can reach 5–6 meters in height with a trunk diameter of 10 cm. The tree reaches full maturity after 3–4 years. The trunk is smooth and thornless. The compound leaves are 30–60 cm long, pinnately shaped, and dusty green. The leaflets are 12–20 mm long, ovate, and arranged oppositely in 6–9 pairs.

The tree flowers from January to February. The cream-colored flowers, similar in shape to pea flowers, grow in panicles from leaf axils, are covered with fine hairs, and are rich in nectar. The fruit is a hanging pod, 25–40 cm long and 2 cm wide, with three ribs and slight bulges where seeds are located. The seeds are black, round, and triangular.

#### 1.2. Global research on Moringa oleifera

## 1.2.1. Studies on the chemical composition of the Moringa genus

The *Moringa* genus, the only genus in the Moringaceae family, comprises 13 species, including *Moringa arborea*, *Moringa borziana*, *Moringa concanensis*, *Moringa drouhardii*, *Moringa hildebrandtii*, *Moringa longituba*, *Moringa ovalifolia*, *Moringa peregrina*, *Moringa pygmaea*, *Moringa rivae*, *Moringa ruspoliana*, *Moringa stenopetala*, and the most widely known species, *Moringa oleifera* [3].

Among these 13 species, the most studied are *Moringa oleifera*, *Moringa stenopetala*, *Moringa concanensis*, and *Moringa peregrina*. Previous research has identified the following groups of bioactive compounds in the *Moringa* genus:

- 1.2.1.1. Flavonoids
- 1.2.1.2. Glucosinolate and isothiocyanate compounds
- 1.2.1.3. Phenolic acids
- 1.2.1.4. Terpenoids
- 1.2.1.5. Steroids
- 1.2.1.6. Alkaloids
- 1.2.1.7. Others

#### 1.2.2. Studies on the biological activities of Moringa oleifera

*Moringa oleifera* is a medicinal plant with significant value in traditional medicine. Pharmacological studies have demonstrated its potential to alleviate pain, reduce inflammation, lower fever, combat cancer, act as an antioxidant, improve cognitive function, protect the liver and stomach, prevent ulcers, support cardiovascular health, combat obesity, control epilepsy and asthma, treat diabetes, prevent kidney stones, act as a diuretic, provide local anesthesia, alleviate allergies, combat parasites, promote wound healing, fight bacteria, modulate the immune system, and treat diarrhea.

This summary offers a comprehensive overview of the chemical and pharmacological activities, as well as the traditional and therapeutic applications of this plant. *Moringa oleifera* has extensive medicinal and therapeutic potential for addressing a wide range of pathological conditions.

Modern studies have revealed the following biological activities of *Moringa oleifera*:

- 1.2.1. Analgesic, anti-inflammatory, and antipyretic activities
- 1.2.2. Neuroprotective activities
- 1.2.3. Anticancer activities
- 1.2.4. Effects on the reproductive system
- *1.2.5. Hepatoprotective activities*
- 1.2.6. Gastroprotective and anti-ulcer activities
- 1.2.7. Cardiovascular effects
- 1.2.8. Anti-obesity activities
- 1.2.9. Anti-asthmatic activities
- 1.2.10. Hematological effects
- 1.2.11. Antidiabetic activities
- 1.2.12. Diuretic and anti-kidney stone activities
- 1.2.13. Anti-allergic activities
- 1.2.14. Anthelmintic activities
- 1.2.15. Wound healing activities
- 1.2.16. Antibacterial activities
- 1.2.17. Immune-modulating activities
- 1.2.18. Anti-diarrheal activities
- 1.2.19. Other activities

#### 1.3. Domestic research on Moringa oleifera

Research conducted by Vietnamese scientists has demonstrated numerous valuable properties of *Moringa oleifera*, including cytotoxicity against cancer cell lines (e.g., A375, A2058), enzyme inhibition, and antibacterial effects.

#### **CHAPTER II . EXPERIMENTS AND RESULTS**

#### 2.1. Research materials

The leaves and roots of *Moringa oleifera* were collected in Hoanh Bo, Quang Ninh, in July 2019 and were identified by Dr. Nguyen The Cuong from the Institute of Ecology and Biological Resources.

#### 2.2. Research methods

#### 2.2.1. Extraction method

The collected plant samples were cleaned, air-dried, ground into fine powder, and extracted at room temperature with methanol. The extracts were combined and evaporated under reduced pressure to obtain methanol residue. This residue was dissolved in water and subjected to liquid-liquid extraction with chloroform, yielding a chloroform fraction (CHCl3) and an aqueous fraction.

#### 2.2.2. Compound isolation methods

2.2.2.1. Thin-layer chromatography (TLC)

2.2.2.2. Column chromatography

2.2.2.3. High-performance liquid chromatography (HPLC)

2.2.3. Structural determination methods

2.2.3.1. Melting point (mp)

2.2.3.2. Optical rotation ( $[\alpha]D$ )

2.2.3.3. Infrared spectroscopy (IR)

2.2.3.4. Mass spectrometry (MS)

2.2.3.5. *High-resolution electrospray ionization mass spectrometry (HR-ESI-MS)* 

2.2.3.6. Nuclear magnetic resonance (NMR) spectroscopy

2.2.4. Biological activity evaluation methods

2.2.4.1. Nitric oxide (NO) production inhibition assay

2.2.4.2. Antimicrobial activity assay

#### 2.3. Compound isolation

2.3.1. Isolation of compounds from the roots of Moringa oleifera



Figure 2.2. Extraction and fractionation diagram of Moringa oleifera roots.



Figure 2.3. Isolation diagram of compounds from Moringa oleifera roots.



Figure 2.4. Additional isolation diagram of compounds from Moringa oleifera roots.

#### 2.3.2. Isolation of compounds from Moringa leaves



Figure 2.5. Extraction diagram of Moringa oleifera leaves



Figure 2.6. Isolation diagram of compounds from the ethyl acetate fraction of Moringa oleifera leaves.



Figure 2.7. Isolation diagram of compounds from the aqueous fraction of Moringa oleifera leaves.

## 2.3.3. Physical parameters and spectral data of isolated compounds

The isolated compounds' physical parameters and spectral data are presented in tables 3.2 to 3.30.

#### CHAPTER III. RESULTS AND DISCUSSION

From the leaves and roots of *Moringa oleifera*, 30 compounds have been isolated. Among them, 18 compounds were found in the roots, and 16 compounds were found in the leaves.

Substance name	Structure	Root	Leaf
Lasiodipline D	$\begin{array}{c} 14 \\ 13 \\ 14 \\ 15 \\ 11 \\ 11 \\ 10 \\ 11 \\ 11 \\ 10 \\ 11 \\ 10 \\ 11 \\ 10 \\$	х	
7- epi -Lasiodipline D	$\begin{array}{c} 13 \\ 13 \\ 11 \\ 11 \\ 11 \\ 11 \\ 11 \\ 11 $	х	
Lasiodipline C	S <i>III</i> 0 6 NH	х	
Retusin (5-hydroxy- 3,3',4',7- tetramethoxyflavone )		x	

*Table 3.31. Summary of isolated compounds from the roots and leaves of Moringa oleifera* 

5-hydroxy-3,4',7- trimethoxyflavone		X	
5-hydroxy-7- methoxyflavone	0 7 8 9 0 2 U 3 6 0 4 0 0 10 0 3 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 1	х	
2-phenylethyl $\beta$ -D-glucopyranoside	O-Glu	х	
Phenylethyl $\beta$ - primeveroside	O-Glu(1-6)Xyl	х	
Benzyl $\beta$ -D-glucopyranoside	O-Glu	x	
2-( Aminomethyl )phenyl $\beta$ -D-glucopyranoside	GleO , SleO , A	х	
3,4-dihydroxybenzoic acid	HO <sup>4</sup> OH	x	
Vanillic acid		x	x

Ferulic acid		x	x
Salicifoliol	8 0 6 0 1 5 HO 0 3	x	
Marumoside B	Gle(1 - 3) Rhao	х	
Isolariciresinol	ОН НО ОН ОН	x	x
Pinoresinol		он Х	x
Kojic acid	НО ОН	x	
Niazirin	Rha-O N		х

Niazirinin	Ac-4Rha-O N	х
keampferol 3- glucopyranoside (astragalin)	HO OH O-Glu	x
6" -O- Acetylastragalin	HO O-Glu-6-Ac	x
Isoquercetin	HO OH OH OH OH	x
6 -O - acetyl tamarixin	HO O-Glu-6-Ac	x
Pinoresinol-4 - <i>O-β</i> -D- glucoside	Gleo y z	x
Lariciresinol 9 - <i>O</i> -β -D- glucopyranoside	HO y y y g g hO y hO y hO y hO y hO y hO y hO y hO y hO y hO y hO y hO y hO y hO y hO hO hO hO hO hO hO hO hO hO	X

(+)- isolarisiresinol 3a - $O$ - $\beta$ -D-glucopyranoside	HO s t t t t t t t t t t t t t		x
L-Tryptophan	4 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0		х
9-hydroxymegastigma- 4,7-dien-3-one 9 - $O$ - $\beta$ - D-glucopyranoside	$0 \xrightarrow{12} 4 \xrightarrow{11} 5 \xrightarrow{10} 9 $		x
Benzyl $\beta$ - primeveroside	O-Glu(1-6)Xyl		х
Total		18	16

#### 3.2. Biological activities of the isolated compounds

This section evaluates the biological activities of the compounds isolated from the leaves and roots of *Moringa oleifera*, focusing on two primary activities: nitric oxide (NO) inhibition activity related to anti-inflammatory effects and antimicrobial activity against aquaculture pathogens.

#### **3.2.1.** Nitric oxide (NO) inhibition activity related to antiinflammatory effects

Nitric oxide (NO) plays a role in regulating various physiological processes in mammals, but excessive NO production can lead to inflammatory diseases, cancer, and diabetes. NO production depends on the activity of nitric oxide synthase (NOS) enzymes, particularly inducible NOS (iNOS). Normally, iNOS is absent in non-activated cells, but under pathological conditions, iNOS is induced and produces large amounts of NO in response to inflammatory signals such as cytokines, TNF- $\alpha$ , and lipopolysaccharide (LPS). The NO inhibition activity assay was performed over 24 hours using the Griess method, as described in the experimental section.

The results, shown in *Table 3.32*, indicate that the methanol extracts from the leaves and roots of *Moringa oleifera* exhibit significant NO inhibition activity at a concentration of 100  $\mu$ g/mL. The ethyl acetate fraction demonstrated stronger inhibition compared to the n-hexane and aqueous fractions.

No.	Sample code	Sample name	IC50 (µg/mL) NO inhibition	% cell viability
1	MOL-M	Crude methanol extract of leaves	$70.4\pm5.8$	>95%
2	MOL-H	n-hexane fraction of leaves	>100	>95%
3	MOL-E	Ethyl acetate fraction of leaves	26.5 ± 2.1	>95%
4	MOL-W	Aqueous fraction of leaves	80.9 ± 6.3	>95%
5	MOR-M	Crude methanol extract of roots	$59.5\pm4.0$	>95%
6	MOR-H	n-hexane fraction of roots	>100	>95%
7	MOR-E	Ethyl acetate fraction of roots	35.6 ± 2.7	>95%

Table 3.32. NO inhibition activity of crude extracts and fractions from Moringa oleifera

No.	Sample code	Sample name	IC50 (µg/mL) NO inhibition	% cell viability	
8	MOR-W	Aqueous fraction of roots	>100	>95%	
9	Control	L-NMMA	$11.6 \pm 0.3$	>95%	

The results of the NO inhibition activity of isolated compounds are shown in **Table 3.32**. Among the 30 compounds isolated, 1/30 demonstrated NO inhibition activity with IC50 values ranging from 38.7 to 81.5  $\mu$ M, compared to the positive control L-NMMA. The phenolic acid 3,4-dihydroxybenzoic acid (MO-11) exhibited the strongest NO inhibition activity (IC50 = 46.3  $\mu$ M), while the flavonoid group showed moderate effects. Two nitrile-containing compounds, niazirin (MO-19) and niazirinin (MO-20), exhibited weak inhibition. However, niazirinin (MO-20), an acetyl derivative of niazirin (MO-19), showed stronger activity, suggesting that acetyl substitution could enhance NO inhibition in this group of compounds.

 Table 3.32. NO inhibition activity of isolated compounds from

 Moringa oleifera

#	Code	Name	IC <sub>50</sub> (µM)	% cell viability
1	MO-01	Lasiodipline D	93.6±2.08	>95%
2	MO-02	7-epi-Lasiodipline D	94.2±3.37	>95%
3	MO-03	Lasiodipline C	>100	>95%
4	MO-04	Retusine	> 100	>95%
5	MO-05	5-hydroxy-3.4'.7- trimethoxyflavone	97.5±3.2	>95%
6	MO-06	5-hydroxy-7- methoxyflavone	76.8±5.3	>95%
7	MO-07	2-phenylethyl $\beta$ -D-glucopyranoside	>100	>95%

8	MO-08	Phenylethyl $\beta$ - primeveroside	>100	>95%
9	MO-09	Benzyl $\beta$ -D-glucopyranoside	>100	>95%
10	MO-10	2-(Aminomethyl)phenyl $\beta$ -D-glucopyranoside	>100	>95%
11	MO-11	3.4-dihydroxybenzoic acid	46.3±1.1	>95%
12	MO-12	Vanillic acid	>100	>95%
13	MO-13	Ferulic acid	>100	>95%
14	MO-14	Salicifoliol	>100	>95%
15	MO-15	Marumoside B	>100	>95%
16	MO-16	Isolariciresinol	>100	>95%
17	MO-17	Pinoresinol	>100	>95%
18	MO-18	Kojic acid	>100	>95%
19	MO-19	Niazirin	83.8±2.3	>95%
20	MO-20	Niazirinin	63.4±5.6	>95%
21	MO-21	keampferol 3- glucopyranoside	68.4±2.5	>95%
22	MO-22	6"-O-Acetylastragalin	56.9±2.1	>95%
23	MO-23	Isoquercetin	72.1±3.2	>95%
24	MO-24	6- <i>O</i> -acetyl tamarixin	81.5±1.4	>95%
25	MO-25	Pinoresinol-4- <i>O</i> -β-D- glucoside	>100	>95%
26	MO-26	Lariciresinol 9- <i>O-β</i> -D- glucopyranoside	>100	>95%
27	MO-27	(+)-isolarisiresinol 3a- $O$ - $\beta$ -D-glucopyranoside	>100	>95%
28	MO-28	L-Tryptophan	>100	>95%
29	MO-29	9-hydroxymegastigma- 4.7-dien-3-one 9- <i>O</i> -β-D- glucopyranoside	>100	>95%
30	MO-30	Benzyl $\beta$ -primeveroside	>100	>95%

31	Đối chứng	L-NMMA	46.6±0.78	>95%
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#### 3.2.2. Antimicrobial activity

The antimicrobial activity of extracts and some pure compounds isolated from the leaves and roots of *Moringa oleifera* was evaluated against pathogenic *Vibrio* strains that affect aquaculture. The results showed that most extracts, except for the n-hexane root fraction, exhibited activity against *Vibrio parahaemolyticus*. The crude methanol extract and the ethyl acetate root fraction exhibited the strongest activity, with MIC =  $32 \ \mu g/mL$ , while the leaf extracts showed weaker activity with MIC =  $64 \ \mu g/mL$ . Among the tested pure compounds, methyl gallate, isolated from the roots, exhibited broad-spectrum activity against four *Vibrio* strains: *V. parahaemolyticus*, *V. harveyi*, *V. vulnificus*, and *V. cholera*.

Table 3.33. Antimicrobial activity (MIC,  $\mu g/mL$ ) against aquaculture pathogens

#	Code	Name	Vp	Vh	Vv	Vc	Va
1	MOL-M	Crude methanol extract of leaves	64	-	-	-	I
2	MOL-H	n-hexane fraction of leaves	64	-	-	-	I
3	MOL-E	Ethyl acetate fraction of leaves	128	-	128	256	-
4	MOL-W	Aqueous fraction of leaves	128	-	-	-	I
5	MOR-M	Crude methanol extract of roots	32	-	256	-	256
6	MOR-H	n-hexane fraction of roots	-	-	-	-	-

7	MOR-E	Ethyl acetate fraction of roots	32	-	-	-	-
8	MOR- W	Aqueous fraction of roots	128	-	256	-	-
9	MO-11	3,4-dihydroxybenzoic acid	256	-	-	128	-
10	MO-20	Niazirinin	128	256	-	-	-
11	MO-19	Niazirin	256	-	-	-	I
12	MO-18	Kojic acid	128	256	-	-	-
	Control	Kanamycin	128	64	64	32	32

#### Notes:

- *Vibrio* strains include:
  - Vp: V. parahaemolyticus
  - Vh: V. harveyi
  - Vv: V. vulnificus
  - Vc: V. cholera
  - Va: V. alginolyticus

#### Conclusions

#### 1. Chemical composition

From the roots of Moringa oleifera, 18 compounds were isolated and identified, including: lasiodipline D (MO-01), 7-epilasiodipline D (MO-02), lasiodipline C (MO-03), retusine (MO-04), 5-hvdroxy-3.4'.7-trimethoxyflavone 5-hvdroxv-7-(MO-05). methoxyflavone (MO-06), 2-phenylethyl β-D-glucopyranoside (MOβ-D-primeveroside 07). phenethyl (MO-08), benzyl β-Dglucopyranoside 2-(aminomethyl)phenyl (MO-09), β-Dglucopyranoside (MO-10), 3,4-dihydroxybenzoic acid (MO-11), vanillic acid (MO-12), ferulic acid (MO-13), salicifoliol (MO-14), marumoside B (MO-15), isolariciresinol (MO-16), pinoresinol (MO-17), and kojic acid (MO-18). Among these, MO-02 is a novel compound published for the first time.

From the leaves of Moringa oleifera, 16 compounds were isolated and identified, including: niazirin (MO-19), niazirinin (MO-20), kaempferol 3-glucopyranoside (MO-21), 6"-O-acetylastragalin (MO-22), isoquercetin (MO-23), 6-O-acetyl tamarixin (MO-24), pinoresinol-4-O-β-D-glucoside (MO-25), lariciresinol 9-O-β-D-(MO-26), (+)-isolariciresinol glucopyranoside 3a-O-β-Dglucopyranoside (MO-27), L-tryptophan (MO-28), 9hydroxymegastigma-4,7-dien-3-one 9-O-B-D-glucopyranoside (MO-29), and benzyl  $\beta$ -D-primeveroside (MO-30).

Among these compounds, only four (vanillic acid, ferulic acid, isolariciresinol, and pinoresinol) were found in both roots and leaves.

#### 2. Biological activities

#### 2.1. Nitric oxide (NO) inhibition activity related to inflammation

The ethyl acetate extract of *Moringa oleifera* leaves (MOL-E) exhibited significant activity at both tested concentrations (30  $\mu$ M and 100  $\mu$ M). The n-hexane extracts from leaves (MOL-H), roots (MOR-H), and the aqueous extract from roots (MOR-W) showed weak activity at both concentrations.

Pure compounds MO-01, MO-09, MO-10, and MO-19 demonstrated activity with IC50 values of 63.4, 68.4, 56.9, and 46.3  $\mu$ M, respectively. Other compounds showed weak activity.

#### 2.2. Antimicrobial activity against test microorganisms

Two extracts, the methanol crude extract (MOR-M) and the ethyl acetate fraction (MOR-E) from *Moringa oleifera* roots, demonstrated strong antibacterial activity against *Vibrio parahaemolyticus* (Vp) with a MIC value of 32 µg/mL.

Two extracts, the methanol crude extract (MOL-M) and the n-hexane fraction (MOL-H) from *Moringa oleifera* leaves, showed moderate activity against *Vibrio parahaemolyticus* with a MIC value of  $64 \mu g/mL$ .

Against other *Vibrio* strains, such as *Vibrio harveyi*, *Vibrio vulnificus*, *Vibrio cholera*, and *Vibrio alginolyticus*, neither the extracts nor the isolated compounds demonstrated significant activity.

#### Recommendations

From the leaves and roots of *Moringa oleifera*, 30 pure compounds (MO-01 to MO-30) were isolated, including one novel compound (MO-02). As the isolated compounds have not undergone extensive biological testing, further studies are recommended to explore additional biological activities, such as cytotoxicity against cancer cell lines and antimicrobial activity against other bacterial strains. This would help expand the understanding of their potential applications.

#### **NEW CONTRIBUTIONS OF THE THESIS**

#### 1. Chemical composition

Eighteen compounds were isolated and identified from Moringa oleifera roots, including lasiodipline D (MO-01), 7-epi-lasiodipline D (MO-02), lasiodipline C (MO-03), retusine (MO-04), 5-hydroxy-3.4'.7-trimethoxyflavone (MO-05). 5-hydroxy-7-methoxyflavone (MO-06), 2-phenylethyl β-D-glucopyranoside (MO-07), phenethyl β-D-primeveroside (MO-08), benzyl β-D-glucopyranoside (MO-09), 2-(aminomethyl)phenyl β-D-glucopyranoside (MO-10), 3.4dihydroxybenzoic acid (MO-11), vanillic acid (MO-12), ferulic acid (MO-13). salicifoliol (MO-14). marumoside В (MO-15). isolariciresinol (MO-16), pinoresinol (MO-17), and kojic acid (MO-18). Among these, MO-02 is a novel compound published for the first time.

Sixteen compounds were isolated and identified from *Moringa* oleifera leaves, including niazirin (MO-19), niazirinin (MO-20), kaempferol 3-glucopyranoside (MO-21), 6"-O-acetylastragalin (MO-(MO-23), 22), isoquercetin 6-O-acetyl tamarixin (MO-24), pinoresinol-4-O-β-D-glucoside (MO-25), lariciresinol 9-O-β-Dglucopyranoside (MO-26), (+)-isolariciresinol 3a-O-β-Dglucopyranoside (MO-27), L-tryptophan (MO-28). 9hydroxymegastigma-4,7-dien-3-one 9-O-B-D-glucopyranoside (MO-29), and benzyl  $\beta$ -D-primeveroside (MO-30).

Among the identified compounds, four (vanillic acid, ferulic acid, isolariciresinol, and pinoresinol) were found in both roots and leaves.

#### 2. Biological effects

For the first time, the antimicrobial activity of extracts and compounds from *Moringa oleifera* against aquatic pathogenic *Vibrio* strains (*V. harveyi*, *V. vulnificus*, *V. cholera*, *V. alginolyticus*) was tested. Two extracts, the methanol crude extract (MOR-M) and the ethyl acetate fraction (MOR-E) from roots, showed strong antibacterial activity against *V. parahaemolyticus* (MIC =  $32 \mu g/mL$ ).

Methanol crude extract (MOL-M) and n-hexane fraction (MOL-H) from leaves demonstrated moderate activity against *V*. *parahaemolyticus* (MIC =  $64 \mu g/mL$ ).

#### LIST OF PUBLICATIONS RELATED TO THE THESIS

1. **Tran Van Hien**, Nguyen Quang Trung, Nguyen Tien Dat. Nitrogen containing compounds from *Moringa oleifera* leaves. Vietnam J. Chem., 2020, 58(6E12), 246-248.

2. **Trần Văn Hiện**, Nguyễn Thị Hồng Anh, Nguyễn Thị Luyến, Nguyễn Thị Thu Minh, Đỗ Hoàng Giang, Nguyễn Tiến Đạt. Chiết xuất, phân lập và tinh chế niazirin từ lá chùm ngây (*Moringa oleifera*) để thiết lập chất chuẩn. Tạp chí phân tích Hóa, Lý và Sinh học, 2023, 29(4), 46-50.

3. Do Hoang Giang, **Tran Van Hien**, Nguyen Thi Thu Minh, Nguyen Thu Uyen, Hoang Thuy Duong, Bui Thi Nhat Le, Mai Thi Thu Ha, Nguyen Tien Dat. Optimization of the Extraction Process for Bioactive Compounds from the Root Barks of *Moringa oleifera*. Natural Product Sciences, 2023, 29(4), 281-286. (SCOPUS, Citescore 2,4).

4. **Hien Tran Van**, Do Hoang Giang, Nguyen Thi Hong Anh, Luyen Nguyen Thi, Quynh Le Thi Phuong, Hang Nguyen Thi Thuy, Thuy Nguyen Thi Thu, Dat Nguyen Tien. New sulfureous diketopiperazine from roots of *Moringa oleifera*. Records of Natural Products, 2024, 18(4), 463-467 (SCIE, IF 1,9)