MINISTRY OF EDUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

TRAN THI BICH HA

STUDY ON THE CHEMICAL CONSTITUENTS, BIOLOGICAL ACTIVITIES, AND QUANTIFICATION OF LIMONOIDS ISOLATED FROM *Xylocarpus moluccensis and Xylocarpus granatum*

SUMMARY OF DISSERTATION ON SCIENCES OF MATTER

Major: Analytical chemistry

Code: 9 44 01 18

Hanoi – 2026

The dissertation is completed at: Graduate University of Science and Technology, Vietnam Academy Science and Technology

Supervisor:

1. Supervisor 1: **Dr. Nguyen Van Thanh**, Institute of Chemistry, Vietnam Academy of Science and Technology
2. Supervisor 2: **Prof., PhD. Tran Viet Hung**, Institute of Drug Quality Control - Ho Chi Minh City

Referee 1:

Referee 2:

Referee 1:

The dissertation is examined by Examination Board of Graduate University of Science and Technology, Vietnam Academy of Science and Technology at h00 am, date / /2026.

The dissertation can be found at:

1. Graduate University of Science and Technology Library
2. National Library of Vietnam

INTRODUCTION

Vietnamese mangrove forests are among the most unique and valuable ecosystems, formed at the interface between rivers and the sea. They are not only resource-rich ecological zones but also play an essential role in environmental protection. With their ability to stabilize soil, prevent erosion, absorb CO_2, and mitigate the adverse impacts of climate change, mangrove forests are increasingly regarded as a "green shield" protecting coastal areas. Mangroves depend on tidal cycles and brackish water conditions for their survival and development. The plant species within these ecosystems not only contribute to biodiversity and provide food resources for human life but also serve as valuable sources of raw materials for medicinal applications.

Among them, species of the genus *Xylocarpus*—particularly *Xylocarpus granatum* and *Xylocarpus moluccensis*—are considered promising candidates in the medical field. Given the significant therapeutic properties of these two species, the research potential for the genus *Xylocarpus* is immense. Therefore, we have selected the topic: "Study on the chemical constituents and biological activities of the mangrove plants *Xylocarpus granatu*m and *Xylocarpus moluccensis*."

The low concentration of analytes in medicinal materials, coupled with complex sample matrices and the limited detection capabilities of HPLC-PDA, poses significant challenges for identifying trace constituents. Consequently, HPLC-UV methods lack the necessary sensitivity and specificity for trace analysis in these matrices, necessitating a more sensitive and specialized approach.

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) has emerged as a sophisticated analytical method, offering high sensitivity and precision for analyzing natural constituents in plants. Data from

MS/MS analysis provides crucial information for structural identification and confirmation, as well as proposing fragmentation pathways. Furthermore, MS/MS spectra facilitate both the qualitative and quantitative analysis of individual compounds within plant materials.

Objectives of the thesis:

- Isolation and determination of chemical structures of compounds from *Xylocarpus granatum (XG)* and *Xylocarpus moluccensis (XM)*.
- Investigating antimicrobial and cytotoxic activities against selected cancer cell lines.
- Develop and validate a qualitative and quantitative analytical procedure for unique, characteristic limonoids from *XM* using the LC-MS/MS method.

NEW CONTRIBUTIONS OF THE THESIS:

- Isolation and structural determination of **21 compounds** isolated from *Xylocarpus granatum* and *Xylocarpus moluccensis*, including **7 new compounds** has not been found in nature (internationally published) and **14 known compounds**:

✓**04 new compounds** isolated from *XM*: **XM01** (Xylomoluccenoid A), **XM02** (Xylomoluccenoid B), **XM03** (Xylomoluccenoid C), and **XM04** (Xylomoluccenoid D).

✓**03 new compounds** isolated from *XG*: **XG01** (Xylocagranoside A), **XG02** (Xylocagranoside B), and **XG03** (Xylocargranin A).

- **XM10** (Xyloccensin E) was isolated for the first time from *XM*.
- Two compounds, **XM06** (1H-Dibenzo[b,e][1,4]dioxepin-11-one, 3,8-dihydroxy-4-(methoxymethyl)-1,6-dimethyl) and **XM08** (Swietmanin H), were reported for the first time from the genus *Xylocarpus* (specifically from *XM*).

- **Biological activity** indicated that two novel compounds, **XM01** (Xylomoluccenoid A) and **XM02** (Xylomoluccenoid B), along with **Xylomolin S** from *XM*, exhibited inhibitory effects on the growth of **HeLa, MCF-7, and HepG2** cancer cell lines (IC_{50} 26.77 μM - 54.19 μM). Additionally, **Odoratone** and **Xylogranatin C** from *XG* showed cytotoxic activity against **A549, MCF-7, and HepG2** lines (IC_{50} 27.54 μM - 46.99 μM).
- The high-resolution-mass spectrometry (HRMS) and nuclear magnetic resonance (NMR) were used to determine the structure of isolated compounds meanwhile the quantitative NMR (qNMR) with ethylene carbonate as the internal standard was used to confirm their purity. These pure compounds were used as standards for the development of the LC-MS/MS method.
- Moluccensin Y, 3,30-Diacetylphragmalin, and Xyloccensin E were determined using the qNMR method.
- In this study, a new rapid LC-MS/MS method was developed to determine Moluccensin Y, 3,30-Diacetylphragmalin, and Xyloccensin E with high sensitivity and specificity. This method then was validated according to ICH guidelines and applied to qualitative and quantitative analysis of the above compounds from the leaves of Vietnamese *Xylocarpus moluccensis* collected in the Ca Mau mangrove forests.

Significance of the Study:

This research provides comprehensive scientific data on the chemical constituents and biological activities of these species, serving as a foundation for future studies, pharmaceutical development, and quality control by regulatory authorities and manufacturers. Beyond its scientific value, the study holds practical significance by contributing several compounds with triterpenoid scaffolds and other structures identified as

having potent pharmacological potential, supporting the advancement of the domestic pharmaceutical industry.

THESIS STRUCTURE

The thesis consists of 138 pages, organized into 3 chapters, featuring 37 tables, 73 figures and schemes, and 141 references. The appendix includes 113 supplemental figures of spectra and LC-MS/MS analytical data.

The specific breakdown of the thesis is as follows:

- ✓ Introduction: 3 pages.
- ✓ Chapter I - Literature Review: 31 pages.
- ✓ Chapter II - Materials and Methods: 17 pages.
- ✓ Chapter III - Results and Discussion: 85 pages.
- ✓ Conclusions and Recommendations: 3 pages.
- ✓ References: 7 pages.
- ✓ List of Publications: 1 page.
- ✓ Appendices: 84 pages.

THESIS CONTENT

CHAPTER 1. OVERVIEW

Introduction to the genus *Xylocarpus* and the two species: *Xylocarpus moluccensis* and *Xylocarpus granatum*.

Overview of the chemical constituents and biological activities of the genus *Xylocarpus*: To date, more than **444 compounds** have been isolated and structurally identified from the three species within this genus: *Xylocarpus granatum*, *Xylocarpus moluccensis*, and *Xylocarpus rumphii*. The chemical profile of the genus *Xylocarpus* is summarized in **Table 1.1**.

Table 1.1. Chemical Constituents Identified in *Xylocarpus* Species**.**

No	Component /Class	Compound group	No.of compounds	No.of compounds *XG*	No.of compounds *XM*	No.of compounds *XR*
1	Triterpenoid					
		Obacunol limonoid	1	1	-	-
		Andirobin limonoid	7	2	4	1
		Mexicanolide limonoid	**253**	**144**	**95**	**19**
		Phragmalin limonoid	**70**	**37**	**32**	**1**
		Protolimonoid	33	29	9	-
		Other limonoid	44	20	29	-
2	Steroid		9	9	3	-
3	Alkanoid		6	6	-	-
4	Phenolic		6	3	3	-
5	Monoterpen		1	-	1	-
6	Flavanol		1	1	-	-
7	Others		13	11	2	-
Total :			**444**	**263**	**178**	**21**
Note: These compounds were mainly isolated from fruits, seeds, leaves, bark, and roots.						

* *XG: Xylocarpus granatum; XM: Xylocarpus moluccensis; XR: Xylocarpus rumphii*

A summary of Quantitative Nuclear Magnetic Resonance (qNMR) spectroscopy [115]. Circular Dichroism (CD) spectroscopy [116]. Mass Spectrometry (MS) method [117], [118].

CHAPTER 2. RESEARCH OBJECTS AND METHODS

2.1. Materials

Leaf samples of *Xylocarpus granatum*: Collected in 2021 from the mangroves of Bai Tu Long Bay, Quang Ninh, Vietnam.

Leaf samples of *Xylocarpus moluccensis*: Collected in 2019 and 2022 from the mangroves of Ngoc Hien, Ca Mau, Vietnam.

2.2. Research Methods

- This section provides a brief description of the equipment and chemicals used in the experimental part of the thesis. Structural elucidation was performed using spectroscopic methods (**^{1}H and ^{13}C NMR, HSQC, HMBC, COSY, and NOESY**), combined with **ECD** and **HR-MS** data.
- A summary of the **qNMR** quantitative method used to determine the purity of three compounds from *XM*: Moluccensin Y, 3,30-Diacetylphragmalin**,** and Xyloccensin E.
- A summary of **Circular Dichroism (CD) spectroscopy** used to determine the absolute configuration of the novel compounds.
- A summary of the **Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)** analytical method.
- A summary of sample preparation procedures.
- A summary of the investigation of ionization and fragmentation conditions, as well as the development and validation of the LC-MS/MS analytical procedure for Moluccensin Y, 3,30-Diacetylphragmalin, and Xyloccensin E.

2.2.1. Isolation of Compounds

2.2.1.1. Isolation of compounds from Xylocarpus moluccensis

This section outlines the isolation procedure for compounds from *XM* leaves. After collection, cleaning, drying, and grinding, 6.7 kg of dry powder was obtained. This was extracted with Methanol (30 L, ×3 times) to

yield 1.1 kg of MeOH crude extract. The crude extract was suspended in water and partitioned sequentially with solvents of increasing polarity: *n*-Hexane and Ethyl acetate, yielding the respective extracts and an aqueous residue. Concentration of these fractions yielded the *n*-Hexane extract (154.5 g) and the ethyl acetate extract (115.3 g). Based on literature reviews indicating that most triterpenoids are found in the Ethyl acetate fraction, this extract was selected for the isolation of triterpenoid compounds. Using column chromatography, combined chromatographic techniques, and appropriate solvent systems, 10 pure compounds were isolated.

2.2.1.2. Isolation of compounds from Xylocarpus granatum

This section describes the isolation procedure from *XG* leaves. The dried powder (2.6 kg) was extracted with Ethanol (10 L, ×3 times) for 48 hours, then filtered and concentrated under reduced pressure at 50°C using a rotary evaporator. This process was repeated twice more to obtain 410.5 g of Ethanol crude extract. The extract was dissolved in warm water and partitioned with *n*-Hexane, Dichloromethane, and *n*-Butanol. The fractions were concentrated at 50°C under reduced pressure, yielding 82 g of *n*-Butanol extract. Following recent studies identifying relevant compounds in the *n*-Butanol fraction, this extract was selected for further processing. Through column chromatography and combined methods, 11 pure compounds were isolated.

2.2.2. Biological Activity Assays

2.2.2.1. Antimicrobial Activity

Microbial Strains: The strains used for activity testing were obtained from Microbiologics (USA):

✓ **Gram-positive (Gr+) bacteria:** *Enterococcus faecalis* ATCC 29212; *Staphylococcus aureus* ATCC 25923; *Staphylococcus aureus* ATCC 14579.

✓ **Gram-negative (Gr-) bacteria:** *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853; *Salmonella enterica* ATCC 13076.

✓ **Yeast:** *Candida albicans* ATCC 10231.

2.2.2.2. Cytotoxic Activity

The cancer cell lines were sourced from the **ATCC** (American Type Culture Collection): **MCF-7:** Human breast adenocarcinoma cell line; **HepG2:** Human hepatocellular carcinoma cell line; **HeLa:** Human cervical cancer cell line; **SK-LU-1:** Human lung carcinoma cell line; **A549:** Human lung carcinoma cell line.

2.2.3. Analytical Method Validation

According to **AOAC [128]** and **ICH [129]** guidelines, the parameters required for the validation of qualitative and quantitative procedures for active pharmaceutical ingredients and/or metabolites in herbal matrices include: System Suitability; Specificity/Selectivity; Recovery (Extraction efficiency); Calibration Curve and Linearity Range; Accuracy and Precision; Limit of Detection (LOD); Limit of Quantitation (LOQ); Sample Stability.

CHAPTER 3. DISCUSS RESULTS

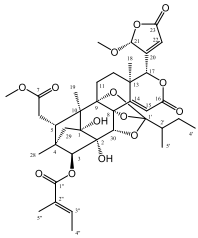
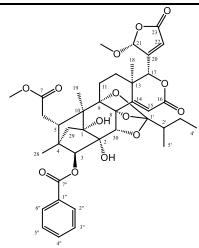
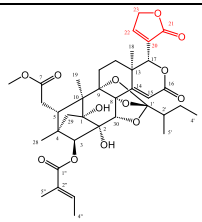
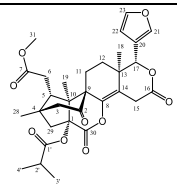
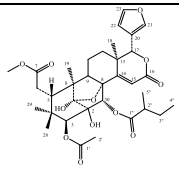
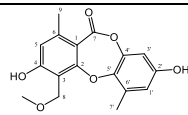
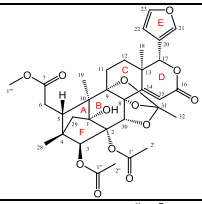
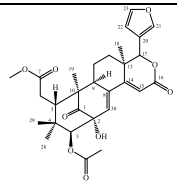
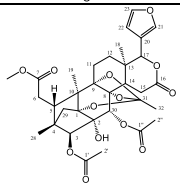
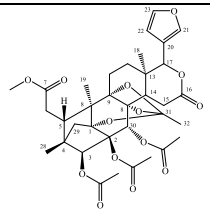
3.1. Structural Elucidation of Compounds from *Xylocarpus moluccensis*

Previous studies have demonstrated that *Xylocarpus moluccensis* is a prominent subject in experimental research, revealing that the genus *Xylocarpus* primarily contains **triterpenoid** as its major constituents. In this study, the compounds isolated from *XM* collected in the Ca Mau mangroves (Vietnam) are consistent with prior findings, belonging to the triterpenoid group—a characteristic chemical marker of this genus.

Among the **10 isolated compounds**:

❖ **04 are novel compounds** that have not been identified in any other genus besides *Xylocarpus*, nor in any other family besides Meliaceae.

❖ **06 are known compounds**, all of which possess **triterpenoid scaffolds** similar to those previously reported in the literature.

Code	Chemical structure	Code	Chemical structure
XM01: New Xylomoluccenoid A Formula $C_{38}H_{48}O_{14}$, M 728.3044		**XM02: New Xylomoluccenoid B** Formula $C_{40}H_{46}O_{14}$ M 750.2888	
XM03: New Xylomoluccenoid C Formula $C_{37}H_{46}O_{13}$ M 698.2938		**XM04: New Xylomoluccenoid D** Formula $C_{31}H_{36}O_{10}$ M 568.6190	
XM05: Xylomolin S Formula $C_{34}H_{44}O_{12}$ M 644.2833		**XM06: 1*H*-Dibenzo[b,e][1,4]dioxepin-11-one,3,8-dihydroxy-4-(methoxymethyl)-1,6-dimethyl** Formula: $C_{17}H_{16}O_6$ M 316.0947	
XM07: Moluccensin Y Formula $C_{33}H_{38}O_{13}$ M 642,2312		**XM08: Swietmanin H** Formula $C_{29}H_{34}O_9$ M 526,2203	
XM09: 3,30-diacetylphragmalin Formula $C_{33}H_{40}O_{13}$ M 644.2469		**XM10: Xyloccensin E** Formula $C_{35}H_{42}O_{14}$ M 686.2312	

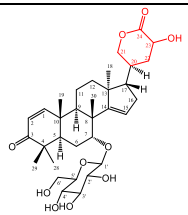
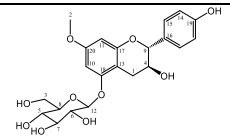
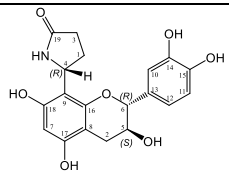
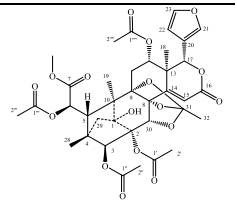
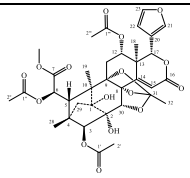
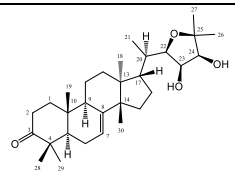
3.2. Structural Elucidation of Compounds Isolated from *Xylocarpus granatum*

Previous studies have indicated that *XG* is a widely studied species, with experimental evidence confirming that the genus *Xylocarpus* is characterized primarily by **triterpenoids**. The compounds isolated from *XG* collected in the mangroves of Quang Ninh, Vietnam, are in agreement with existing literature, belonging to the triterpenoid group—the taxonomic signature of the *Xylocarpus* genus.

Among the **11 isolated compounds**:

❖ **03 are novel compounds** that have not been reported in any other genus besides *Xylocarpus*, nor in any other family besides Meliaceae.

❖ **08 are known compounds**, all featuring **triterpenoid skeletons** consistent with those previously documented in scientific publications.

Code	Chemical structure	Code	Chemical structure
XG01: New Xylocagranoside A Formula $C_{33}H_{48}O_{10}$, M 604.3247		**XG02: New Xylocagranoside B** Formula $C_{22}H_{26}O_{10}$ M 450.1526	
XG03: Chất mới Xylocargranin A Formula $C_{19}H_{19}NO_7$, M 373.1162		**XG04: Xyloccensin P** Formula $C_{37}H_{42}O_{17}$ M 758.2422	
XG05: Xyloccensin Q Formula $C_{35}H_{40}O_{16}$, M 716.2316		**XG06: Odoratone** Formula $C_{30}H_{48}O_4$ M 472.3553	

XG07: Chisocheton F Formula $C_{28}H_{38}O_5$ M 454.2719	**XG08: Xylogranatin S** Formula $C_{34}H_{44}O_{11}$ M 628.2884
XG09: Xylogranatin C Formula $C_{29}H_{34}O_{10}$ M 542.2152	**XG10: Xylogranatin D** Formula $C_{29}H_{34}O_{10}$ M 542.2152
XG11: 9-O-methyl Xylogranatin R Formula $C_{28}H_{34}O_9$ M 514.2203	

3.3. Biological Activity Assays of Isolated Compounds

3.3.1. Antimicrobial Activity of Compounds Isolated from XM

The results indicated that 10 compounds exhibited inhibitory activity against 1 to 5 microbial strains. Notably, compound **XM06** displayed significant activity with **MIC values of 32–64 µg/mL** against all five tested strains.

3.3.2. Antimicrobial Activity of Selected Compounds Isolated from XG

The experimental results showed that the tested compounds did not exhibit inhibitory activity against the microbial strains at the investigated concentrations.

3.3.3. Cytotoxic Activity of Compounds Isolated from XM

Compounds isolated from *XM* were evaluated against three cancer cell lines: breast cancer (**MCF-7**), hepatocellular carcinoma (**HepG2**), and

human cervical cancer (**HeLa**). The cell density was seeded at **10,000 cells/well/200 µL**, with results summarized in **Table 3.24**.

The results revealed that three compounds (**XM03, XM04, and XM05**) exhibited inhibitory effects on the growth of the studied cancer cell lines, with **IC_{50} values ranging from 26.77 µM to 54.19 µM**. The remaining samples did not show significant cytotoxicity in this assay, with IC_{50} values > 100 µM.

3.3.4. Cytotoxic Activity of Selected Compounds Isolated from XG

The cytotoxic activity of selected compounds from *XG* was evaluated against lung cancer (**A549**), breast cancer (**MCF-7**), and hepatocellular carcinoma (**HepG2**). The cell density was **10,000 cells/well/200 µL**, and the data are presented in **Table 3.25**.

Screening results showed that compounds **XG06** and **XG09** exhibited cytotoxic activity at the investigated concentrations. Other samples showed weak activity across the tested range.

3.4. Determination of purity of isolated compounds using the new method of qNMR

To use three isolated compounds from *XM* leaves as the standards for the LC-MS/MS analysis, these compounds have to be assessed for their purity. Therefore, this study applied the qNMR method to determine the purity of obtained compounds. The experiments were performed on a Bruker 400 MHz Advance III spectrometer with ethylene carbonate as the internal standard. The purity results are presented in **Table 3.26**. The data in Table 5 showed that three isolated compounds from the sample had high purity (more than 98%). It allowed us to conclude that the three isolated compounds in this study can be used as the standards for LC-MS/MS analysis.

Table 3.26. Purity results of XM07, XM09, and XM10 determined by qNMR.

Code	Name	Purity (%) (qNMR)
XM07	Moluccensin Y	98.07
XM09	3,30-Diacetylphragmalin	98.43
XM10	Xyloccensin E	98.36

The purity of the isolated compounds was determined by comparison with an internal standard, whose content had been pre-verified using the quantitative Nuclear Magnetic Resonance **(qNMR)** method. From the ^{1}H-NMR spectra of the isolates, characteristic hydrogen signals for the target analytes were identified, and an internal standard with signals specific to and non-overlapping with the analytes was selected.

3.5. Development of Qualitative and Quantitative Analytical Methods for the three compounds in *Xylocarpus moluccensis* Leaves

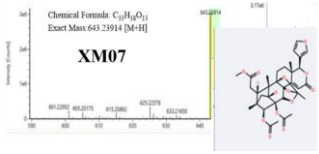
The low concentration of analytes in the herbal material, combined with the complex sample matrix and the limited detection capability of **LC-PDA**, poses significant challenges for quantifying trace constituents. Consequently, HPLC with a UV detector lacks the necessary sensitivity and specificity for trace analysis in complex matrices and must be replaced by a more sensitive and specialized method.

Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) has become a state-of-the-art analytical tool, offering high sensitivity and accuracy for analyzing natural constituents in plants. MS/MS data provides valuable information for structural identification and confirmation, as well as for proposing fragmentation pathways of precursor ions. Furthermore, MS/MS spectra facilitate both qualitative and quantitative analysis of

individual compounds in botanical materials. Thus, this study employed LC-MS/MS to qualitatively and quantitatively analyze three compounds with characteristic structures of the *Xylocarpus* genus: **XM07**, **XM09**, and **XM10**.

3.5.1. predicted fragmentation

While there are no prior reports on the fragmentation of **XM07**, **XM09**, and **XM10**, the research group proposed fragmentation mechanisms based on data obtained from the **Xevo G2-XS QTOF (Waters, USA)** system. The proposed mechanisms for **XM07**, **XM09**, and **XM10** are illustrated in Figures 3.53, 3.54, and 3.55, respectively; quantitative and qualitative fragmentation patterns are provided in Appendix 4. These mechanisms were validated by experimental spectral scanning, which confirmed the high reliability of the proposed pathways. Precursor ion qualitative mass spectra were acquired on the **UPLC-MS/MS Xevo G2-XS QTof** system.



Ký hiệu chất phân tích	Mảnh ion	m/z	
XM07	Định lượng	643,3270 →	583,2251
	Định tính	643,3270 →	357,2057
	Định tính	643,3270 →	95,0036

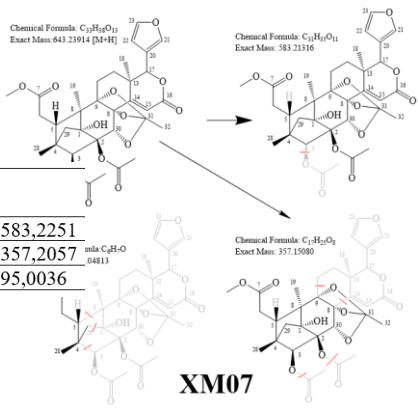
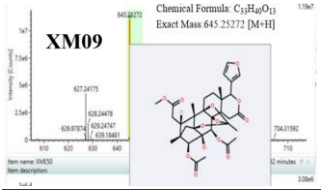
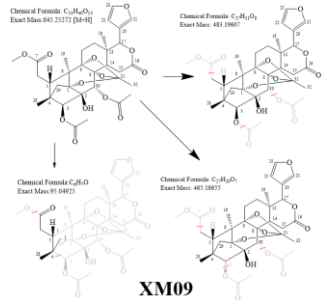


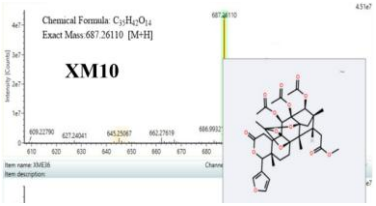
Figure 3.53. MS spectrum of parent ion and predicted fragmentation of **XM07**

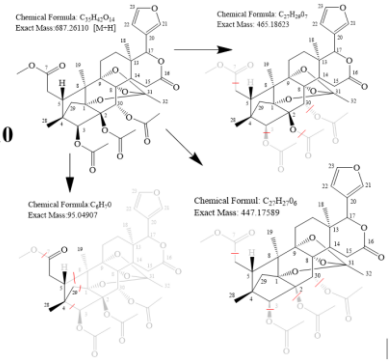




Ký hiệu chất phân tích	Mảnh ion	m/z
XM09	Định lượng	645,4065 → 483,2574
	Định tính	645,4065 → 465,2359
	Định tính	645,4065 → 95,0053

Figure 3.54. MS spectrum of parent ion and predicted fragmentation of **XM09**





Ký hiệu chất phân tích	Mảnh ion	m/z
XM10	Định lượng	687,4809 → 465,2508
	Định tính	687,4809 → 95,0046
	Định tính	687,4809 → 447,1207

Figure 3.55. MS spectrum of parent ion and predicted fragmentation of **XM10**

The mass spectra of all analytes exhibited precursor ions consistent with their molecular formulas. The product ions (daughter ions) obtained from the daughter scan were also in agreement with fragmentation data from previous studies. Through the optimization process, the product ion with the highest signal intensity for each compound was selected as the quantifier ion to ensure methodological sensitivity, while other product ions at various collision energies were selected as qualifier ions.

3.5.2. Analytical conditions

The MS/MS analysis was operated under the following conditions: ionization interface (ESI) voltage, + 0.5 kV; nitrogen gas was used for the ESI source with a flow rate of 900 L/h at 350 °C. At the cone, the source temperature was set to 150 °C, and a nitrogen gas flow of 30 L/h was used to minimize solvent interference. Also, a multiple reaction monitoring (MRM) mode was carried out with transition parameters in Table 3.28, including the fragment ions, cone voltage, and fragmentation voltage.

Table 3.28. Optimized MS/MS parameters for the target compounds

Code	Mảnh ion	m/z		Cone (V)	CE (V)
XM07	Assay	643.3270	583.2251	20	8
	Identification	643.3270	357.2057	20	32
	Identification	643.3270	95.0036	20	46
XM09	Assay	645.4065	483.2574	6	14
	Identification	645.4065	465.2359	6	16
	Identification	645.4065	95.0053	6	48
XM10	Assay	687.4809	465.2508	40	16
	Identification	687.4809	95.0046	40	42
	Identification	687.4809	447.1207	40	20
IS		287.1539	242.1559	25	18

3.5.3. Selection of internal standard

Do fluctuations in ion current during mass spectrometry, internal standards—either isotopic (ideal but often unavailable) or structurally similar analogs—are essential to improve analytical precision and control. Based on the chemical formulas, logP (partition coefficient), and pKa values of the three candidates: Etodolac ($C_{17}H_{21}NO_3$; LogP = 2.5; pK_a = 4.65), Atorvastatin ($C_{33}H_{35}FN_2O_5$; LogP = 6.36; pK_a = 4.46), and Ezetimibe ($C_{24}H_{21}F_2NO_3$; LogP = 4.5; pK_a = 9.48), mass spectrometric scans were performed to evaluate their signal intensities and compatibility with the chromatographic and MS detection conditions. The spectral scans and signal intensity measurements for Atorvastatin, Etodolac, and Ezetimibe were conducted, with the results presented in Figure 3.56. The results indicated that the most intense signals for all tested internal standards corresponded to the protonated precursor ion $[M+H]^+$. However, the signal intensity for Etodolac (IS) was significantly higher than that of Ezetimibe. While Atorvastatin yielded a strong signal, its retention time was excessively long compared to Etodolac, despite being prepared at the same concentration of 5 µg/mL. Consequently, Etodolac was selected for further investigations.

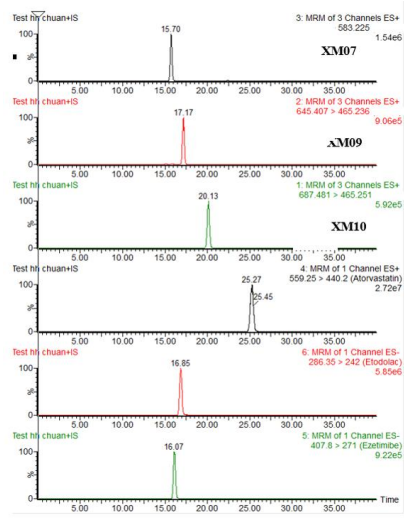


Figure 3.56. Chromatogram of the internal standards

3.5.4. Investigation of Chromatographic Conditions

Selection of stationary phase

Given the non-polar nature of the analytes, this study investigated several chromatographic columns featuring non-polar bonded phases commonly used in analysis, such as **C18**, **C8**, and **C6-phenyl**. The chromatographic results in Figure 3.57 indicate that the C6-phenyl column (Figure 3.57-4a) exhibited strong interactions with the analytes and for the C18 stationary phase (Figure 3.57-4b) resulting in asymmetrical peaks (tailing factor b/a > 2.0) peak splitting, the peaks were poorly resolved. The C8 column (150 mm length) (Figure 3.57-4d) provided complete resolution and symmetrical peaks for all analytes; however, the analysis time was excessively long. In contrast, the C8 column (50 mm length) (Figure 3.57-4c) achieved baseline separation with symmetrical peaks (b/a < 2.0) and a significantly shorter run time. Therefore, the C8 column was selected as the stationary phase for this method.

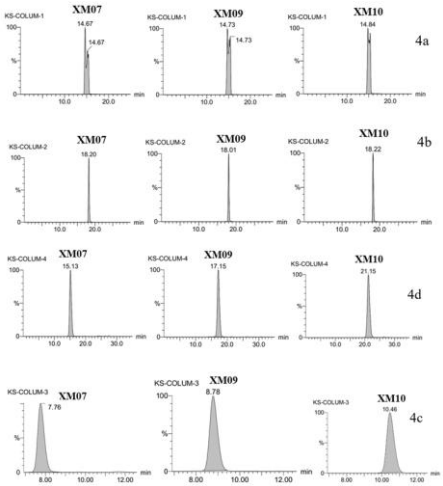


Figure 3.57. Chromatograms for stationary phase investigation

Selection of mobile phase

The investigation was conducted using a standard mixture containing analytes **XM37**, **XM50**, and **XM36** in methanol at a concentration of 5 μL. The mass spectrometry parameters were maintained as previously optimized, with the initial chromatographic conditions as follows:

Mobile phases with 40% Methanol caused high backpressure, while Acetonitrile with salt buffers led to low signal and poor elution. A mobile phase of 0.1% Formic acid with Acetonitrile (60:40) was selected due to its successful detection of analytes at approximately 11 minutes and higher signal intensity compared to the acetic acid system.

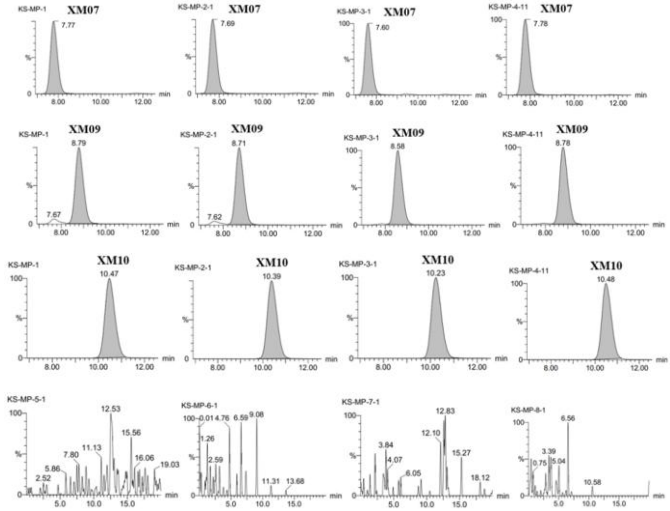


Figure 3.58. Chromatogram of the mobile phase

Proposed chromatographic conditions are as follows:

- ✓ Column: Zorbax XDB-C8, 80Å, 4.6 x 50 mm, 3.5 µm
- ✓ Column temperature: 35 °C
- ✓ Mobile phase: 0.1% Formic acid (A) – Acetonitrile (B) (60:40, v/v)
- ✓ Gradient program:

Time (min)	Flow rate (ml)	A (%)	B (%)
0	0,6	60	40
12.40	0,6	60	40
12.50	0,6	20	80
17.00	0,6	20	80
17.01	0,6	60	40
20.00	0,6	60	40

3.6. Method Validation

Table 3.36. Summary results of method validation

Parameter	result		
System suitability	RSD ≤ 5.3%.		
Specificity	The validation results confirmed that the LOQ sample had clear, identifiable peaks at the target analytes' retention times while the blank sample showed no corresponding peaks, with its maximum response signal at those retention points not exceeding 20% of the active compounds' signal at their working concentrations. **XM07** (7.86 min), **XM09** (8.90 min), **XM10** (10.64 min), **IS** (8.99 min)		
Limit of Detection (LOD) and Limit of Quantitation (LOQ)		**LOD** and **LOQ**	
	XM07	0.009 ppm – 0.03 ppm	
	XM09	0.015 ppm – 0.05 ppm	
	XM10	0.036 ppm – 0.12 ppm.	
Linear Range	$R^2 \geq 0.995$;		
	XM07	: 0.03 – 7.50 ppm	$R^2 = 0.9978$
	XM09	: 0.05 – 12.50 ppm	$R^2 = 0.9961$
	XM10	: 0.12 – 30.00 ppm	$R^2 = 0.9954$
Accuracy	**XM07**	: 97.75 – 100.59 %	RSD : 1.21 %
	XM09	: 97.11 – 101.27 %	RSD : 1.61 %
20%	**XM10**	: 96.32 – 99.46 %	RSD : 1.20 %
	XM07	: 101.45 – 103.32 %	RSD : 0.63 %
	XM09	: 100.58 – 101.59 %	RSD : 0.37 %
100%	**XM10**	: 102.57 – 104.13 %	RSD : 0.51 %
	XM07	: 101.99 – 104.13 %	RSD : 0.82 %
	XM09	: 100.63 – 102.89 %	RSD : 0.88 %
150%	**XM10**	: 101.26 – 102.73 %	RSD : 0.53 %
Intra-day and Inter-day Precision	**XM07** (%)	: 0.0134 ± 0.00034	RSD : 2.35 %
		: 0.0128 ± 0.00025	RSD : 1.76 %
	XM09 (%)	: 0.0225 ± 0.0011	RSD : 4.24 %
		: 0.0216 ± 0.00083	RSD : 3.49 %
	XM10 (%)	: 0.0598 ± 0.0023	RSD : 3.47 %
		: 0.0571 ± 0.0016	RSD : 2.56 %
Stability of Active Compounds (24 hours/25 °C)	**XM07** (%)	: 0.0129 ± 0.00061	RSD : 4.32 %
	XM09 (%)	: 0.0219 ± 0.0011	RSD : 4.62 %
	XM10 (%)	: 0.0569 ± 0.0030	RSD : 4.81 %

3.7. Application of the Analytical Procedure for Qualitative and Quantitative Determination of XM07, XM09, and XM10

The qualitative and quantitative analysis of **XM07**, **XM09**, and **XM10** in herbal samples revealed that the content of **XM07** ranged from 0.012% to 0.015%, **XM09** from 0.019% to 0.025%, and **XM10** from 0.059% to 0.074% (Table 3.37). Regarding the samples of *Xylocarpus granatum*, as well as the EXG-methanol extract (MeOH) and EXG-ethyl acetate extract, none of these three target compounds were detected.

Table 3.37. Analytical results of selected samples

Test \ **Sample code**	**EXM1**	**EXM2**	**EXM3**	**EXM4**	**EXM5**
1. Identification XM07	Positive	Positive	Positive	Positive	Positive
2. Identification XM09	Positive	Positive	Positive	Positive	Positive
3. Identification XM10	Positive	Positive	Positive	Positive	Positive
4. Assay XM07 (%)	**0.0135** ± 0.00036 **RSD = 1.66**	**0.0159** ± 0.00065 **RSD = 1.82**	**0.0123** ± 0.00024 **RSD = 0.86**	**0.0143** ± 0.0010 **RSD = 3.17**	**0.0144** ± 0.00057 **RSD = 1.78**
5. Assay XM09 (%)	**0.0232** ± 0.0015 **RSD = 4.00**	**0.0250** ± 0.0010 **RSD = 2.60**	**0.0194** ± 0.00061 **RSD = 1.99**	**0.0195** ± 0.0020 **RSD = 4.55**	**0.0183** ± 0.00087 **RSD = 2.11**
6. Assay XM10 (%)	**0.0601** ± 0.0024 **RSD = 1.78**	**0.0741** ± 0.0040 **RSD = 2.40**	**0.0589** ± 0.0016 **RSD = 1.22**	**0.0646** ± 0.0037 **RSD = 2.57**	**0.0643** ± 0.00074 **RSD = 0.51**
7. Identification					
EXG- MeOH extract		Moluccensin Y :		**Not detected**	
		3,30-Diacetylphragmalin:		**Not detected**	
		Xyloccensin E:		**Not detected**	
EXG- Etylacetate extract		Moluccensin Y:		**Not detected**	
		3,30-Diacetylphragmalin:		**Not detected**	
		Xyloccensin E:		**Not detected**	

* **EXM1 – EXM5** : Sample code

This study introduces an advanced LC-MS/MS method for simultaneously determining Moluccensin Y, 3,30-Diacetylphragmalin, and Xyloccensin E. The method employs a C8 column and MRM technique with a simplified mobile phase, significantly reducing analysis time and matrix effects. Comprehensive validation according to AOAC and ICH guidelines confirmed its reliability for both qualitative and quantitative analysis of Limonoids in medicinal plants. The procedure is suitable for quantifying these three compounds in *Xylocarpus moluccensis* leaves. Notably, these analytes were not detected in *Xylocarpus granatum* samples or other tested extracts.

CONCLUSIONS AND RECOMMENDATIOS

❖ CONCLUSIONS

The dissertation investigated the chemical constituents of *Xylocarpus granatum* and *Xylocarpus moluccensis* collected from mangrove forests in Vietnam—two species that have remained relatively understudied in the country. The findings are summarized as follows:

1. Chemical composition

✓ Using various chromatographic techniques, **21 compounds** were isolated from *Xylocarpus granatum* and *Xylocarpus moluccensis*, including **07 new compounds** published internationally:

➢ **04 new compounds from *X. moluccensis*: XM01** (Xylomoluccenoid D), **XM02** (Xylomoluccenoid C), **XM03** (Xylomoluccenoid B), and **XM04** (Xylomoluccenoid A).

➢ **03 new compounds from *XG*: XG01** (Xylocagranoside A), **XG02** (Xylocagranoside B), and **XG03** (Xylocargranin A).

✓ **06 known triterpenoids** were isolated from *XM*: **XM05** (Xylomolin S), **XM06** (1H-Dibenzo[b,e][1,4]dioxepin-11-one, 3,8-dihydroxy-4-(methoxymethyl)-1,6-dimethyl), **XM07** (Moluccensin Y), **XM08**

(Swietmanin H), **XM09** (3,30-Diacetylphragmalin), and **XM10** (Xyloccensin E).

✓ **08 known triterpenoids** were isolated from *XG*: **XG04** (Xyloccensin P), **XG05** (Xyloccensin Q), **XG06** (Odoratone), **XG07** (Chisocheton F), **XG08** (Xylogranatin S), **XG09** (Xylogranatin C), **XG10** (Xylogranatin D), and **XG11** (9-O-methyl Xylogranatin R).

✓ **XM10 (Xyloccensin E)** was isolated from *XM* for the first time.

✓ **XM06** and **XM08** were isolated from the genus *Xylocarpus* for the first time.

✓ Pure isolates from *XM* leaves, including **Moluccensin Y, 3,30-Diacetylphragmalin, and Xyloccensin E**, were utilized as reference standards to establish qualitative and quantitative analytical procedures.

✓ The purity of these three reference standards was determined via **qNMR** as follows: **Moluccensin Y (98.07%)**, **3,30-Diacetylphragmalin (98.36%)**, and **Xyloccensin E (98.43%)**.

✓ A state-of-the-art **LC-MS/MS** method was developed and validated for the simultaneous determination of these three characteristic compounds. The procedure complies with **AOAC** and **ICH** guidelines for analytical method validation and can be applied for the quantification of these constituents in *XM*.

2. Biological Activity

✓ **Antimicrobial Activity:** The 11 isolated compounds from *XM* exhibited inhibitory activities against 1 to 5 microbial strains. Notably, **XM06** showed significant activity with **MIC values of 32–64 µg/mL** against five strains: *E. faecalis* ATCC29212, *S. aureus* ATCC25923, *B. cereus* ATCC14579, *P. aeruginosa* ATCC27853, and *C. albicans* ATCC10231.

✓ **Cytotoxicity:** Compounds from *XM* were tested against lung (SK-LU-1), breast (MCF-7), liver (HepG2), and cervical (HeLa) cancer cell lines. Results showed that three compounds—**XM01 (new), XM02 (new), and XM05**—exhibited growth inhibition across the tested lines (HeLa, MCF-7, HepG2) with **IC_{50} values ranging from 26.77 to 54.19 µM**.

✓ Cytotoxicity screening of compounds from *XG* revealed that **XG06 (Odoratone)** and **XG09 (Xylogranatin C)** displayed cytotoxic effects against A549, MCF-7, and HepG2 lines with IC_{50} values of 27.54–46.99 µM. Other compounds did not show significant activity in these assays.

❖ RECOMMENDATIONS

Further studies are warranted on the genus ***Xylocarpus***, specifically *XG* and *XM*, which are prominent mangrove species in Vietnam. In particular, more intensive research should focus on compounds exhibiting potent biological activities, such as the novel compounds **XM01 (Xylomoluccenoid A)**, **XM02 (Xylomoluccenoid B)**, and **XM05 (Xylomolin S)**. These compounds have shown promising growth inhibition against studied cancer cell lines (**HeLa**, **MCF-7**, and **HepG2**) with IC_{50} values ranging from 14.32 to 54.19 µM.

Future investigations should include neurotoxicity assays and sub-chronic toxicity studies for the novel compounds internationally published for the first time (**XM01, XM02, XM03, XM04, XG01, XG02, and XG03**). Such studies aim to further uncover the therapeutic potential and safety profiles of these two Vietnamese mangrove species. Furthermore, it is necessary to continue the phytochemical investigation of the remaining fractions from the crude extracts of both *XG* and *XM*.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **Thi Bich Ha Tran**, Van Thanh Nguyen, Phuong Thao Nguyen, Thanh Hoang Le, Nguyen Trung Nguyen Le, Thi Ngoc Hanh Le, Dai Lam Tran, Dang Quang Ta, Viet Hung Tran*, 2025, Simultaneous quantification of 3 typical limonoids in the Vietnam mangrove *Xylocarpus moluccensis* leaves by LC-MS/MS, Journal of Liquid Chromatography & Related Technologies, 48:11-15, 313-323.
https://doi.org/10.1080/10826076.2025.2478104.
(Journal of Liquid Chromatography & Related Technologies, ISSN 1082-6076, CHEMISTRY, ANALYTICAL – SCIE, (IF = 1.4 ; Q3)

2. Nguyen Phuong Thao, **Tran Thi Bich Ha***, Kieu Thi Phuong Linh, Pham Thi Mai Huong, Pham Thanh Binh, Vu Thanh Trung, Nguyen The Cuong, Tran Viet Hung, Nguyen Van Thanh, 2026, Xylocargranosides A–B and Xylocargranin A from the mangrove *Xylocarpus granatum*, Journal of Natural Medicines, 2026, 80 (2), 418-428.
https://doi.org/10.1007/s11418-025-01993-5.
(Journal of Natural Medicines, ISSN 13403443, SCIE, IF = 2.9; Q1)

3. **Tran Thi Bich Ha**, Tran Viet Hung, Nguyen Phuong Thao, Kieu Thi Phuong Linh, Pham Thi Mai Huong, Pham Thanh Binh, Vu Thanh Trung, Nguyen The Cuong, Nguyen Van Thanh*, Xylomoluccenoids A–D from the mangrove Xylocarpus moluccensis leaves, 2026, (Elsevier - Fitoterapia, SCIE, IF = 2.6; Q2) Manuscript Number: FITOTE-D-26-01361. Reviewed, awaiting acceptance.