MINISTRY OF EDUCATION AND TRAINING
VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

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STUDY ON CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF *MAGNOLIA LAMDONGENSIS* AND *MAGNOLIA TIEPII* SPECIES

SUMMARY OF DISSERTATION ON SCIENCES OF MATTER

Major: Chemistry of Natural Compounds

Code: 9 44 01 17

Ha Noi - 2024

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

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The dissertation will be examined by Examination Board of Graduate University of Science and Technology, Vietnam Academy of Science and Technology at 14:00 p.m. December 10, 2024.

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INTRODUCTION

1. The urgency of the thesis

In the world, there have been many studies focusing on the chemical composition and biological activities of compounds isolated from the genus *Magnolia*. Given their great value in traditional health care systems, several species of this genus continue to be the subject of much pharmacological and phytochemical investigation over the past 20 years. However, there are not many studies on these directions for *Magnolia* species in Vietnam.

The process of investigating and screening medicinal resources in Lam Dong province in the direction of biological activity to develop high-value medicinal herbs has discovered and announced a number of species. *Magnolia lamdongensis* and *Magnolia tiepii* belonging to the genus *Magnolia* were announced in 2015, and there has been no publication on the chemical composition and biological activities of these two species. The results of research on plants and the biological activities of these two species will contribute to orienting the development of pharmaceutical raw materials, developing conservation and farming areas, and bringing about positive socio-economic effects. Extremely, providing pharmaceutical products to serve community health care. Therefore, I chose these two subjects to carry out the project "***Study on chemical composition and biological activities of Magnolia lamdongensis and Magnolia tiepii species***".

2. The objectives of the thesis

Research on the chemical composition and biological activities of *M. lamdongensis* species distributed in Lam Dong and *M. tiepii* species distributed in Khanh Hoa.

3. The main contents of the thesis

Isolation of compounds from the leaves of *M. lamdongensis* and *M. tiepii*.

- Determine the chemical structure of the isolated compounds.

- Survey the activities of some isolated compounds.

The layout of the thesis: The thesis consists of 146 pages with 36 tables, 107 pictures and 154 references. The thesis includes 4 chapters: Introduction (1 pages), Chapter 1: Overview (28 pages); Chapter 2: Materials and research methods (7 pages); Chapter 3: Experimental (16 pages); Chapter 4: Results and discussion (77 pages); Conclusion and recommendations (2 page); Articles related to the thesis (1 page); References (14 pages); Appendix (163 pages).

CHAPTER 1: OVERVIEW

Overview of plant characteristics, distribution, and domestic and international research on the chemical composition and biological activities of the *Magnolia* genus.

1.1. Introduction to *Magnolia*

1.1.1. Plant characteristics of Magnolia genus

1.1.2. The review of Magnolia genus in traditional medicine

1.1.3. The review of Magnolia chemical constituents

These three sections (1.1.1–1.1.3) introduce the plant characteristics, distribution, and uses of some *Magnolia* species according to traditional medicine and present research on chemical composition. Through the synthesis of documents, from the genus *Magnolia*, there are currently about 600 compounds isolated and presented in the following compound groups: alkaloid compounds (compounds **1**–**49**), lignans and neolignans (**50**–**318**), flavonoids (**319**–**344**), phenylethanoid glycosides (**345**–**377**), phenolic and phenolic glycosides (**378**–**437**), terpenoids (**438**–**574**), essential oil (**597-614**), and other compounds (**575**–**596**).

Among the compounds isolated from the genus *Magnolia*, lignan and neolignan compounds account for the majority.

1.1.4. The review of Magnolia biological activities

With the traditional medicinal effects of some *Magnolia* species, over the past two decades, many compounds extracted from this genus have been studied for their pharmacological effects. In addition to commonly tested bioactivities on natural compounds such as cytotoxic, anti-inflammatory, antibacterial, anti-oxidant, and anti-diabetic activities, scientists have also recorded neuroprotective effects, anti-allergic, anti-fungal, anti-malarial, etc., from isolated compounds.

1.2. Introduction about two plant were researched

Two species *Magnolia lamdongensis* and *Magnolia tiepii* were announced in 2015, of which *Magnolia lamdongensis* is endemic to Vietnam.

CHAPTER 2: PLANT MATERIALS AND METHODS

2.1. Plant materials

M. lamdongensis leaves were collected at Lam Ha district, Lam Dong province, in September 2020. *M. tiepii* leaves were collected at Khanh Vinh district, Khanh Hoa province, in May 2021. Scientific names were identified by Dr. Nong Van Duy at the Tay Nguyen Institute for Scientific Research.

2.2. Methods

2.2.1. Method of collecting research samples and identifying scientific names: Research samples are collected, pre-processed, photographed, made into specimens, scientific names determined, and information stored by botanical experts.

2.2.2. Sample processing methods and creating extracts for isolating compounds and testing biological activity: drying and weighing samples, total extraction, fractional extraction.

2.2.3. Isolation methods

This section presents methods for isolating pure compounds: thin-layer chromatography and column chromatography.

2.2.4. Methods for determination of chemical struture of compounds

This section showed the general methods to determine the chemical structure of the compounds are combination of physical parameters and modern spectroscopic methods including: Mass spectrometry (MS), magnetic resonance spectrum (1D, 2D-NMR), infrared (IR), Circular Dichroism (CD).

2.2.5. Methods for evaluation of biological activities

This section presents chemicals, equipment, and methods for testing antioxidant activity, NO production inhibitory activity, α-glucosidase enzyme inhibitory activity, and cytotoxic activity on some pure compounds.

CHAPTER 3: EXPERIMENTALS

3.1. Extraction of *M. lamdongensis*

This section presents the process of making methanol extracts and partitioned extract from *M. lamdongensis*.

3.2. Isolation compounds from *M. lamdongensis*

This section presents in detail the isolated procedure of 18 compounds from *M. lamdongensis*.

3.3. Physical properties and spectroscopic data of the isolated compounds from *M. lamdongensis*

*3.3.1. Compound **ML1**: rhamnetin 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-galacto-pyranoside*

*3.3.2. Compound **ML2**: oxytroflavoside F*

*3.3.3. Compound **ML3**: rhamnocitrin 3-O-β-neohesperidoside*

*3.3.4. Compound **ML4**: curcucomoside D*

*3.3.5. Compound **ML5**: astragalin*

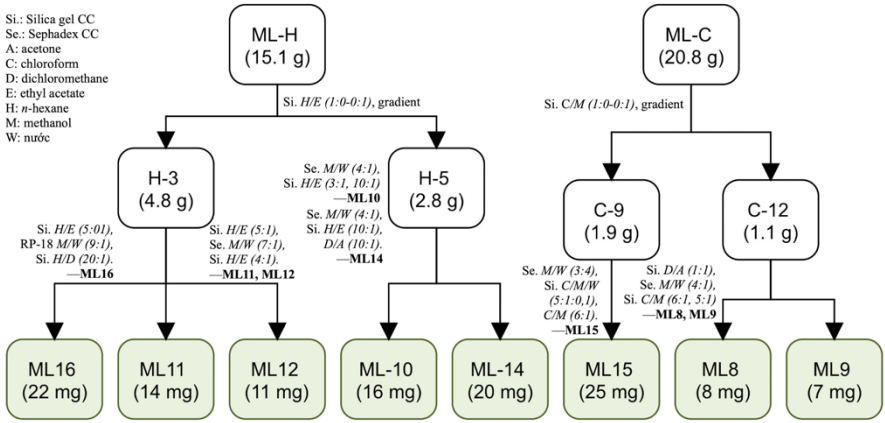


Figure 3.2-3.3. Schematic diagram of compounds isolated from ML-H and ML-C

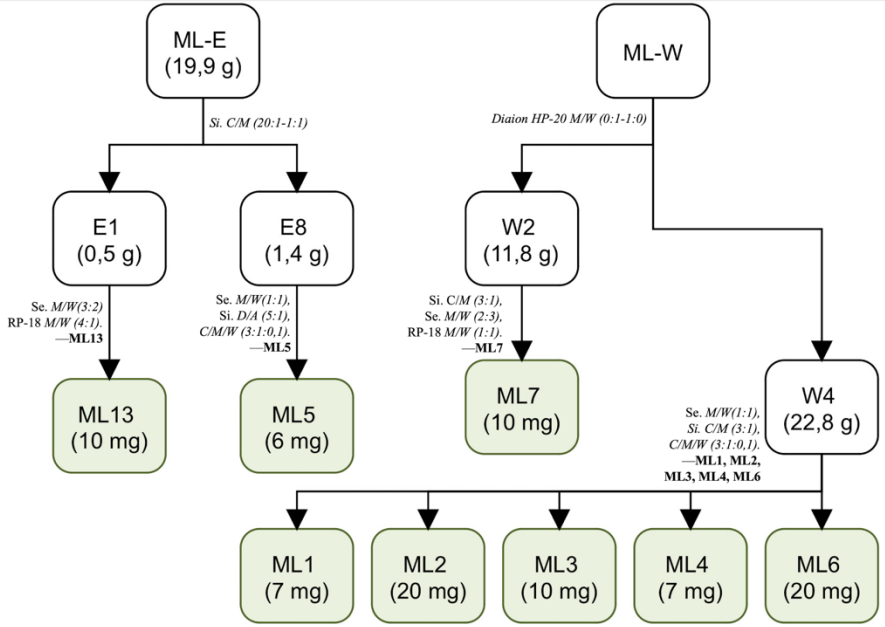


Figure 3.4-3.5. Schematic diagram of compounds isolated from ML-E and ML-W

*3.3.6. Compounds **ML6**: kaempferol 3-neohesperidoside and kaempferol 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside*

*3.3.7. Compounds **ML7**: quercetin 3-neohesperidoside and quercetin 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside*

*3.3.8. Compound **ML8**: 1-O-β-D-glucopyranosyl-(2S,3R,4E,8Z)-2-[(2-hydroxyocta-decanoyl)amido]-4,8-octadecadiene-1,3-diol*

*3.3.9. Compound **ML9**: 1-O-β-D-glucopyranosyl-(2S,3R,4E,8Z)-2-[(2-hydroxyoctadecanoyl)amido]-4,8-hexadecadiene-1,3-diol*

*3.3.10. Compound **ML10**: (-)-sesamin*

*3.3.11. Compound **ML11**: hinokinin*

*3.3.12. Compound **ML12**: dihydrosesamin*

*3.3.13. Compound **ML13**: (S)-eriodictyol*

*3.3.14. Compound **ML14**: stigmasterol*

*3.3.15. Compound **ML15**: daucosterol*

*3.3.16. Compound **ML16**: palmitic acid*

3.4. Extraction of *M. tiepii*

This section presents the process of making methanol extracts and partitioned extract from *M. tiepii*.

3.5. Isolation compounds from *M. tiepii*

This section presents in detail the isolated procedure of 20 compounds from *M. tiepii*.

3.6. Physical properties and spectroscopic data of the isolated compounds from *M. tiepii*

*3.6.1. Compound **MT1**: kaempferol 3-neohesperidoside*

*3.6.2. Compound **MT2**: nicotiflorin*

*3.6.3. Compound **MT3**: isoquercitrin*

*3.6.4. Compound **MT4**: magnoloside A*

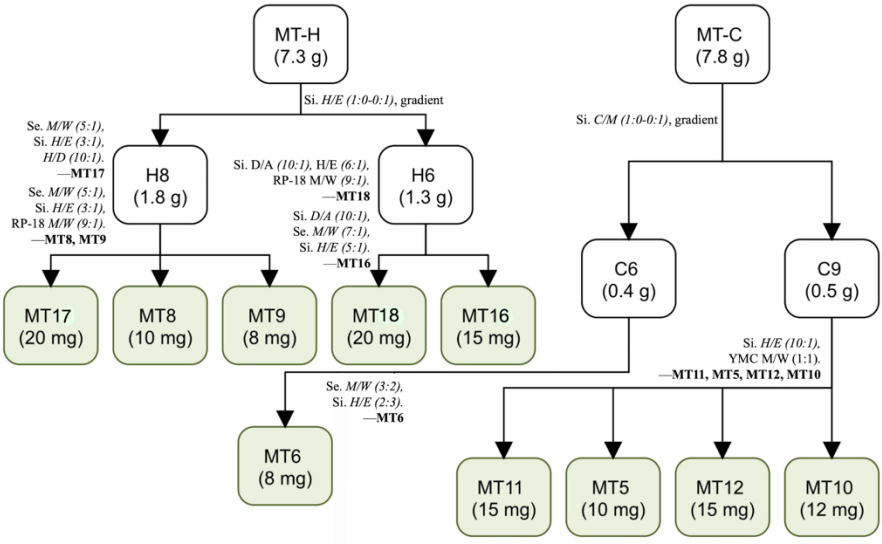
*3.6.5. Compound **MT5**: (+)-syringaresinol*

*3.6.6. Compound **MT6**: (+)-pinoresinol*

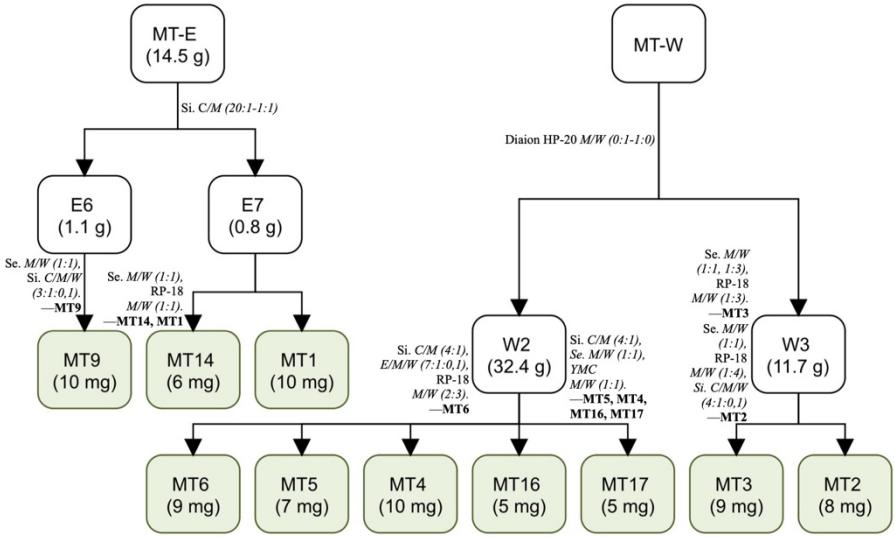
*3.6.7. Compound **MT7**: (-)-acanthoside B*

*3.6.8. Compound **MT8**: (9S)-9-O-methylcubebin*

*3.6.9. Compound **MT9**: (9R)-9-O-methylcubebin*



Fugure 3.7-3.8. Schematic diagram of compounds isolated from MT-H and MT-C



Fugure 3.9-3.10. Schematic diagram of compounds isolated from MT-E and MT-W

CHAPTER 4. RESULTS AND DISCUSSIONS

4.1. The result of isolation from *M. lamdongensis*

4.1-4.2. The result of isolation from *M. lamdongensis and M. tiepii*

◊ From the MeOH extract of *M. lamdongensis* has led to isolated 18 compounds:

- 10 flavonoids: **ML1**, **ML2**, **ML3**, **ML4**, **ML5**, **ML6a**, **ML6b**, **ML7a**, **ML7b**, **ML13**;
- 02 cerebrosides: **ML8**, **ML9**;
- 03 lignans: **ML10**, **ML14**, **ML15**;
- 02 sterols: **ML14**, **ML15**;
- 01 fatty acid compound: **ML16**.

Among them, compounds **ML1**, **ML2**, **ML6b**, **ML7a**, **ML7b**, **ML8**, **ML9**, **ML11**, and **ML13** were isolated for the first time from the *Magnolia* genus.

◊ From the MeOH extract of *M. tiepii* has led to isolated 18 compounds:

- 04 flavonoids: **MT1**, **MT2, MT3, MT15**;
- 01 phenylethanoid glycoside: **MT4**;
- 08 lignans: **MT5**, **MT6**, **MT7**, **MT8**, **MT9**, **MT10**, **MT16**, **MT17**;

- 03 megastigmanes: **MT11**, **MT12**, **MT13**;
- 01 phenolic glycoside: **MT14**;
- 01 sterol: **MT18**.

Among them, compounds **MT7**, **MT8**, **MT9**, **MT11**, **MT16**, and **MT17** were isolated for the first time from the *Magnolia* genus.

This section focuses on elucidating the structures of compounds isolated from two species, *M. lamdongensis* and *M. tiepii*, including groups of flavonoids, lignans, megastigmane…

◊ ***Flavonoids***

4.1.1. Compound ML1: rhamnetin 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside

Compound **ML1** was obtained as a yellow powder. The molecular formula $C_{28}H_{32}O_{16}$ was deduced from ESI-MS *m/z* 623.05 $[M+H]^+$.

The ^{1}H NMR spectra revealed the signals of three ABX-type protons [δ_H 7,77 (1H, d, J = 2,4 Hz, H-2′), δ_H 6,90 (1H, d, J = 8,4 Hz, H-5′), 7,63 (1H, dd, J = 8,4, 2,4 Hz, H-6′)] of B ring and meta-coupled protons at δ_H 6.27 (*d*, J = 1.8 Hz, H-6) and 6.55 (*d*, J = 1.8 Hz, H-8) of the A ring. Furthermore, the signals of two sugar anomeric protons could be discerned at δ_H 5.79 (*d*, J = 7.8 Hz, H-1″) and 5.24 (*d*, J = 1.6 Hz, H-1‴).

The ^{13}C NMR and DEPT spectra showed the presence of 28 carbon signals, in which, a methyl group at δ_C 17.46 and a methylene group at δ_C 62.57 suggested that two of the sugars were rhamnose and glucose, orderly.

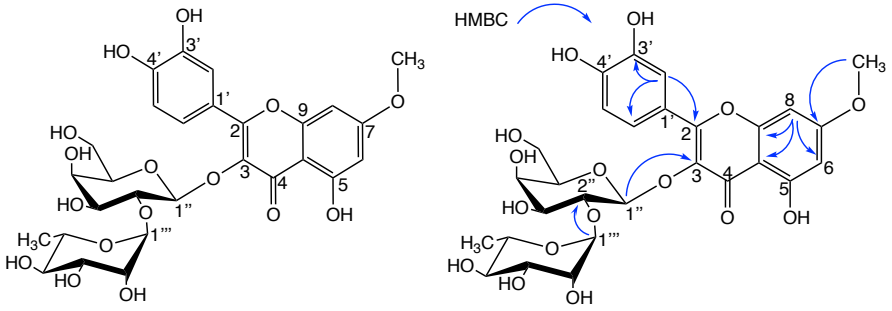


Figure 4.3. Structures and the key HMBC correlations of compound **ML1**

In the HMBC spectrum, the anomeric proton H-1‴ correlated with carbon C-2″ (δ_C 80.13), indicating that the Rha was located at the C-2″ position of the Glc moiety. The HMBC data also confirmed the correlation between H-1″ (Glc) proton with carbon C-3 (δ_C 134.56). Detailed analysis of the NMR spectra, compound **ML1** was identified as quercetin 3-neohesperidoside when compared to the published data.

Besides, the thesis has also elucidated the structures of other flavonoid glycosides with an aglycone framework, such as kaempferol (**ML12**, **ML3**, **MT2**, **MT4**), rhamnocitrin (**ML4**, **ML5, ML7**), and quercetin (**ML13**, **MT14**). The characteristic feature of these flavonoid glycosides is the glycosylation structure at the C-3 position.

◊ ***Tetrahydrofurofuran lignans***

4.1.10. Compound ML10: (-)-sesamin

Compound **ML10** was isolated as a colorless needles.

The ^{1}H NMR spectra revealed the signals of three ABX-type protons [δ_H 6.84 (*dd*, *J* = 1.5, 0.5 Hz, H-2), 6.80 (*ddd*, *J* = 8.0, 1.5, 0.5 Hz, H-6), and 6.77 (*dd*, *J* = 8.0, 0.5 Hz, H-5)], protons of two dioxymethylene groups at δ_H 5,95 (2H, *s*, -O-CH_2-O-). The ^{1}H NMR also confirmed one oxymethine proton at δ_H 4.71 (1H, *d*, *J* = 4.5 Hz, H-7), one methine proton at δ_H 3.05 (1H, *ddd*, *J* = 4.5, 3.8, 2.1 Hz, H-8), and two oxymethylene protons at δ_H 4.23 (1H, *dd*, *J* = 9.2, 6.9 Hz, H-9_a), and 3.87 (1H, *dd*, *J* = 9.2, 3.8 Hz, H-9_b).

The ^{13}C NMR and DEPT spectra of **ML10** showed the presence of 10 carbon signals, including six aromatic carbons [δ_C 135.11 (C-1), 106.51 (C-2), 148.00 (C-3), 147.14 (C-4), 108.20 (C-5), and 119.36 (C-6)], one oxymethine carbon (δ_C 85.82, C-7), one methine carbon (δ_C 54.36, C-8), one oxymethylene carbon (δ_C 71.74, C-9), and one dioxymethylene carbon (δ_C 101.08). In the COSY spectra of **ML10** showed the correlation between H-7 and two protons H-8 and H-9. Besides, in the HMBC spectrum, proton H-7 (δ_H 4.71) correlated with carbon C-1 (δ_C 135.15), confirmed the C6–C3 unit (propylbenzene).

The ESI-MS gave a molecular ion peak at *m/z* 355.08 $[M+H]^+$, which was suggested the molecular formula $C_{20}H_{18}O_6$. This proved that the structure of **ML10** was duplicated with the remainder completely symmetrical. Detailed analysis of the NMR spectra as well as the CD spectrum of **ML10** showed the negative Cotton effects at 212 nm (Δε-4.19), 233 nm (Δε-4.05) and 290 nm (Δε-1.37), compound **ML10** was identified as (–)-sesamin when compared to the published data.

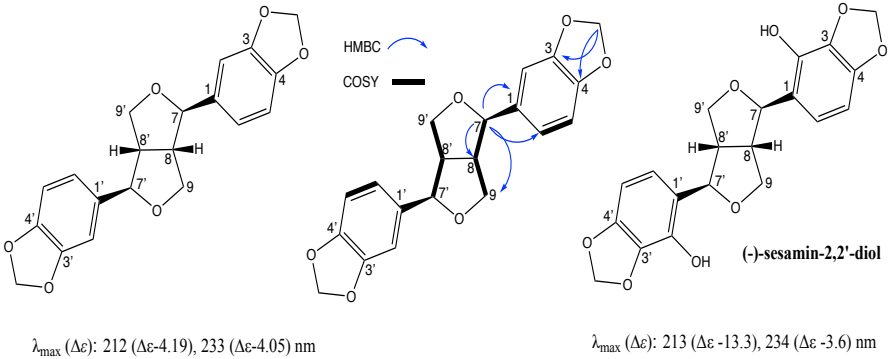


Figure 4.30. The structure and important HMBC, COSY corelations of compound **ML10**

In addition to compound **ML10** isolated from *M. lamdongensis*, there are also other tetrahydrofuran-type lignan compounds isolated from *M. tiepii*, such as **MT5**, **MT6**, and **MT7**. The common feature of these compounds is that the two propylbenzene units (C6-C3) is equivalent.

◊ ***Tetrahydrofuran lignans***

4.1.11. Compound ML11: hinokinin

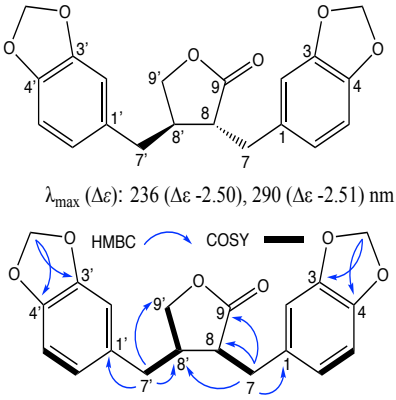
Compound **ML11** was obtained as a colorless oil.

The ESI-MS gave a molecular ion peak at *m/z* 355.08 $[M+H]^+$ and ^{13}C NMR spectrum of **ML11** indicated a molecular formula of $C_{20}H_{18}O_6$. The ^{13}C NMR spectrum displayed signals of 20 carbons, including 12 aromatic carbons [δ_C 108.31, 108.38, 108.84, 109.47, 121.57, 122.25, 131.37, 131.63, 146.40, 146.51, 147.91, 147.93]; 4 carbons of the tetrahydrofuran rings [δ_C 46.53 (C-8), 178.41 (C-9), 41.32 (C-8′), and 71.15 (C-9′)], two methylene

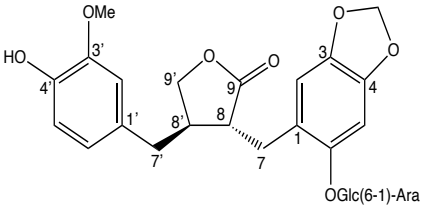
carbons [δ_C 34.88 (C-7) and 38.41 (C-7′)], and two dioxymethylene carbons [δ_C 101.02 and 101.03].

The ^{1}H NMR spectrum of **ML11** showed the signals of two ABX aromatic proton systems [δ_H 6.63 (1H, *d*, *J* = 1.8 Hz, H-2), 6.73 (1H, *d*, *J* = 7.9 Hz, H-5), and 6.60 (1H, *dd*, *J* = 7.9, 1.8 Hz, H-6)] and [6.45 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.70 (1H, *d*, *J* = 8.2 Hz, H-5′), and 6.46 (1H, *dd*, *J* = 8.2, 1.8 Hz, H-6′)], four protons of two dioxymethylene groups at δ_H 5.93 and 5.94 (each 2H, *m*), a tetrahydrofuran moiety with four protons including: two methine protons at δ_H 2.53 (1H, *ddd*, *J* = 9.5, 7.3, 5.1 Hz, H-8) and 2.45 (1H, ddd, *J* = 9.5, 7.3, 4.6 Hz, H-8′), two oxymethylene protons at δ_H 4.13 (1H, *dd*, *J* = 9.2, 6.9 Hz, H-9$_a$′) and 3.86 (1H, *dd*, *J* = 9.2, 7.1 Hz, H-9$_b$′).

According to the HMBC experiment, protons of methylenedioxy groups correlated with aromatic carbons at δ_C 147.93 (C-3), 146.51 (C-4), 147.91 (C-3′), 146.40 (C-4′). The HMBC data also confirmed the correlation between protons H-6 and H-6′ with carbons C-7 (δ_C 34.88) and C-7′ (δ_C 38.41), respectively, which indicated the presence of a benzyl fragment that is attached to a tetrahydrofuran ring, resulting in a lignan-type skeleton. By comparison of the NMR data of **ML11** with those of the published data [22], **ML11** was assigned as hinokinin.



λ_{max} ($\Delta\varepsilon$): 236 ($\Delta\varepsilon$ -2.50), 290 ($\Delta\varepsilon$ -2.51) nm



Rubrifloside B

λ_{max} ($\Delta\varepsilon$): 237 ($\Delta\varepsilon$ –4.53), 288 ($\Delta\varepsilon$ –11.94) nm

Figure 4.33. The structure and important HMBC, COSY corelations of compound **ML11**

The tetrahydrofuran structure is also recorded in compounds **ML12** (from *M. lamdongensis*), **MT8**, **MT9**, and **MT10** (from *M. tiepii*).

◊ ***Megastigmanes***

4.2.11. Compound MT11: dehydrovomifoliol

Compound **MT11** was obtained as an amorphous powder.

The ^{1}H NMR, ^{13}C NMR, and DEPT spectra indicated that **MT11** was a megastigmane. The remaining 13 signals, including two carbonyl carbons (δ_C 197,42 and 197,01), four olefinic carbons (δ_C 127.81, 160.40, 145.04, 130.41), one sp3 carbon (δ_C 79.31), one methylene carbon (δ_C 49.73), one quaternary carbon (δ_C 41.46), and four methyl carbon (δ_C 18.68, 22.95, 24.36, 28.37).

The ^{1}H NMR spectrum displayed signals for four methyl groups [δ_H 1.89 (3H, d, J = 1.2 Hz), 2.31 (3H, *s*), 1.11 (3H, *s*), 1.03 (3H, *s*)], a pair of isolated methylene protons centered [δ_H 2.50 (1H, *d*, J = 17.1 Hz), 2.34 (1H, *d*, J = 17.1 Hz)], and three olefinic protons [δ_H 6.83 (1H, *d*, J = 15.6 Hz), 6.47 (1H, *d*, J = 15.6 Hz), 5.96 (1H, brs)].

In the HMBC spectrum, the correlations were observed between the protons and carbons: H-2 and C-1, C-3, C-4, C-6, C-11; H-4 and C-2, C-6, C-13; H-7 and C-5, C-6, C-8, C- 9; H-8 and C-6, C-7, C-9; H-10 and C-7, C-8, C-9; H-11, H-12 and C-1, C-2, C-6; H-13 and C-4, C-5, C-6, indicated that the structure of **MT11** is 6,9-dihydroxy-4,7-megastigmadien-3-one. Thus, compound **MT11** was identified as dehydrovomifoliol by comparison with the reported data.

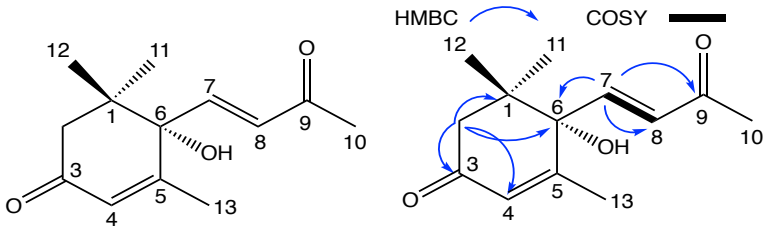


Figure 4.72. The structure and important HMBC, COSY corelations of compound **MT11**

Besides compound **MT11**, from the leaves of M. *tiepii*, two other megastigmane compounds were isolated: **MT12** and **MT13**.

4.3. Results of biological activity testing

4.3.1. Results for the antioxidant activity test

MeOH extracts from two species, *M. lamdongensis* (ML-M) and *M. tiepii* (MT-M), along with some isolated compounds, including **ML1**, **ML2**, **MT1**, **MT2**, and **MT4**, were tested. Antioxidant activity is based on the ability to scavenge free radicals generated by DPPH.

Antioxidant activity test results showed that the two extracts ML-M and MT-M have antioxidant ability with SC_{50} values of 120.62 and 396.30 μg/mL, respectively.

Two compounds, **ML1** (rhamnetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside) and **MT4** (magnoloside A), showed antioxidant activity with SC_{50} values of 46.73 and 295.28 μg/mL, respectively (table 4.29). The remaining compounds all gave negative results at the tested concentration (400 μg/mL).

Table 4.29. Results for the antioxidant activity test

Sample	Concentration (μg/mL)	Scavenging capacity (SC, %)	SC_{50} (μg/mL)
Positive (+)	50	81.26±0.53	13.42
Negative (-)	-	0	-
ML-M	200	64,25±0,54	120,62
MT-M	400	51,85±1,12	396,30
ML1	400	66,41±0,96	295,28
MT4	400	76,07±0,50	46,73

Negative (-): DPPH/EtOH + DMSO,

positive (+): DPPH/EtOH + ascorbic acid

4.3.2. Results of testing NO production inhibitory activity on RAW264.7 cells

The thesis has investigated the inhibitory activity of LPS-stimulated NO production on RAW264.7 macrophages of several compounds, including **ML1**, **ML2**, **MT2**, **MT7**, and **MT11**.

Test results showed that compounds **MT2** and **MT11** exhibited anti-inflammatory activity by evaluating their ability to inhibit NO production on RAW264.7 cells with IC_{50} values of 236.18 and 202.74 µg/mL, respectively, compared to the cardamonin standard (IC_{50} 167.4 µg/mL), these two samples were not cytotoxic to RAW264.7 cells at a concentration of 256 µg/mL. The remaining samples did not show NO production inhibitory activity.

Table 4.30. Results of testing NO production inhibitory activity on RAW264.7 cells

Sample	Concentration	Inhibition NO (%)	Cell survival (%)	IC_{50} (µg/mL)
Negative (-)	1%	-	104,76±0,15	
Positive (+)	81 µg/mL	45.85±2.12	86.47±0.21	167.4
	810 µg/mL	86.93±0.96	71.8±0.51	
LPS	1 µg/mL	0.0±0.9	100.0±0.13	
MT2	256 µg/mL	53.06 ± 0.37	100.07 ± 0.93	**236,18**
	128 µg/mL	32.65 ± 0.12	102.04 ± 0.83	
	64 µg/mL	20.41 ± 0.09	103.58 ± 0.44	
MT11	256 µg/mL	59.59 ± 0.18	99.10 ± 0.11	**202,74**
	128 µg/mL	36.73 ± 0.37	99.18 ± 0.51	
	64 µg/mL	24.49 ± 0.07	100.45 ± 0.13	

Negative (-): DMSO, positive (+): Cardamonin

4.3.3. Results of α-glucosidase enzyme inhibitory activity tests

Table 4.11. Results of *α*-glucosidase enzyme inhibitory activity tests

Sample	Concentration (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)
Positive (+)	100	63.05±1.28	93.34
ML1	400	79.49±0.92	179.86
ML2	400	67.65±0.74	316.88
MT13	400	80.32±1.26	117.58

Positive *(+): Voglibose*

Some isolated compounds, including two flavonoids (**ML1**, **ML2**) and megastigmane glycoside (**MT13**), were evaluated for their *α*-glucosidase enzyme inhibitory activity (table 4.31). The results of the *α*-glucosidase

enzyme inhibitory activity test showed that samples **ML1**, **ML2**, and **MT13** all showed α-glucosidase enzyme inhibitory activity at the tested concentrations with IC_{50} values of 179.86, 316.88 và 117.58 µg/mL, respectively.

4.3.4. Results of the cytotoxic activity test

Cytotoxic activity test results show that MeOH extracts of *M. lamdongensis* (ML-M) and *M. tiepii* (MT-M) leaves have no cytotoxic activity against liver cancer cell lines (Hep-G2) at tested concentrations.

For purified compounds including **ML10**, **ML11**, **MT1**, **MT2**, and **MT11**, only compound **ML11** gave weak positive results on all three cell lines, Hep-G2, RD, and HeLa, with IC_{50} values ranging from 45.89 to 75.97 µM (table 4.33). The remaining compounds all showed negative results for toxicity tests on these strains at the tested concentrations.

Table 4.33. Results of the cytotoxic activity test

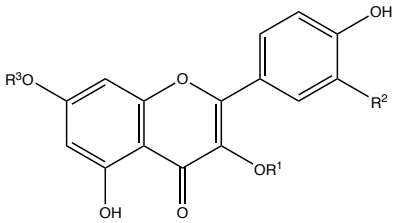
Sample	**Cell lines IC_{50} (µM)**		
	Hep-G2	RD	HeLa
ML11	75.97±3.19	60.44±3.39	45.89±3.37

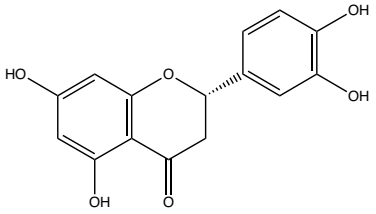
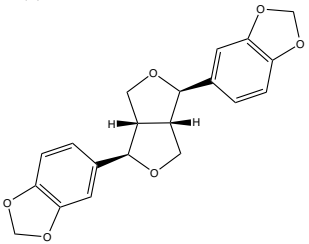
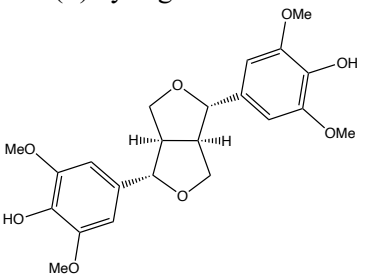
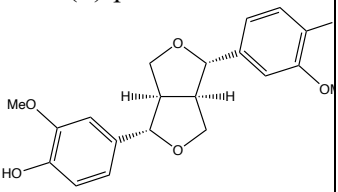
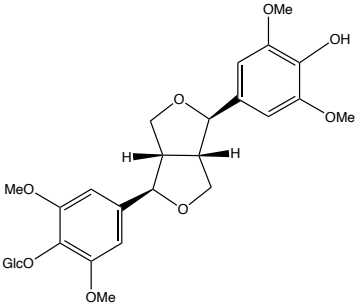
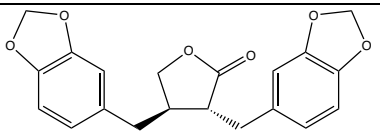
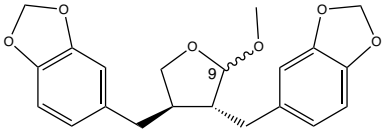
Negative (-):DMSO, positive (+): Ellipticine

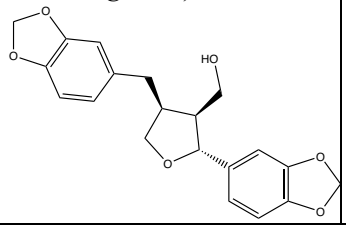
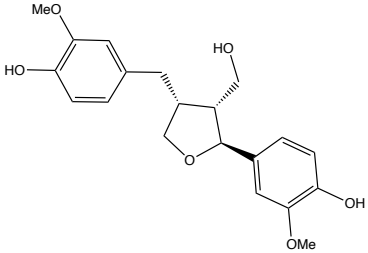
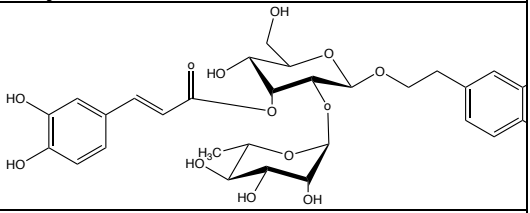
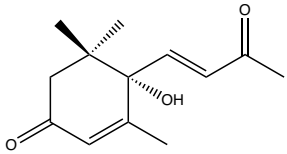
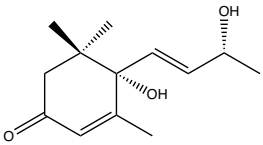
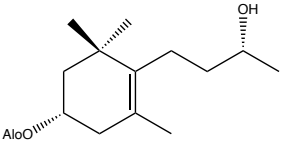
4.4. Summary of research results

From the leaves of two species *M. lamdongensis* and *M. tiepii*, **32** compounds have been isolated, mainly belonging to the classes of flavonoids, lignans, megastigmanes, phenols, and sterols.

Table 4.34. The results of isolating compounds

Flavonoids	
ML1 rhamnetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside (Isolated for the first time from *Magnolia*)	 **ML1:** R^1=Gal-(2-1)-Rha, R^2=OH, R^3=CH_3 **ML2**: R^1=Gal-(2-1)-Rha, R^2=H, R^3=CH_3 **ML3:** R^1=Glc-(2-1)-Rha, R^2=H, R^3=CH_3 **ML4:** R^1=Ara-(2-1)-Rha, R^2=H, R^3=CH_3 **ML5=MT15:** R^1=Glc, R^2=H, R^3=H **MT1=ML6a:** R^1=Glc-(2-1)-Rha, R^2=H, R^3=H **ML6b:** R^1=Gal-(2-1)-Rha, R^2=H, R^3=H **ML7a:** R^1=Glc-(2-1)-Rha, R^2=OH, R^3=H **ML7b:** R^1=Gal-(2-1)-Rha, R^2=OH, R^3=H **MT2:** R^1=Glc-(6-1)-Rha, R^2=H, R^3=H **MT3:** R^1=Glc, R^2=OH, R^3=H
ML2 oxytroflavoside F (Isolated for the first time from *Magnolia*)	
ML3 rhamnocitrin 3-*O*-*β*-neohesperidoside (Isolated for the first time from *Magnolia*)	
ML4 curcucomoside D	
ML5=MT15 astragalin	
MT1=ML6a kaempferol 3-neohesperidoside	
ML6b kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside (Isolated for the first time from *Magnolia*)	
ML7a quercetin 3-neohesperidoside (Isolated for the first time from *Magnolia*)	
ML7b quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside (Isolated for the first time from *Magnolia*)	
MT2 nicotiflorin	
MT3 isoquercitrin	

<table>
<tr><td>ML13 (S)-eriodictyol
(Isolated for the first time from Magnolia)</td><td>  </td></tr>
<tr><td colspan="2">Lignans</td></tr>
<tr><td>ML10 (-)-sesamin
  </td><td>MT5 (+)-syringaresinol
  </td></tr>
<tr><td>MT6 (+)-pinoresinol
  </td><td>MT7 (-)-acanthoside B
(Isolated for the first time from Magnolia)
  </td></tr>
<tr><td>ML11=MT16 hinokinin
(Isolated for the first time from Magnolia)</td><td>  </td></tr>
<tr><td>MT8 (9S)-9-O-methylcubebin
(Isolated for the first time from Magnolia)</td><td rowspan="2"> 
MT8: 9S
MT9: 9R</td></tr>
<tr><td>MT9 (9R)-9-O-methylcubebin</td></tr>
</table>

(Isolated for the first time from *Magnolia*)	
ML12=MT17 dihydrosesamin (Isolated for the first time from *Magnolia*) 	**MT10** lariciresinol 
Phenylethanoid	
MT4 magnoloside A	
Megastigmanes	
MT11 dehydrovomifoliol (Isolated for the first time from *Magnolia*)	
MT12 blumenol A	
MT13 manglieside C	
Cerebrosides	
ML8 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-[(2-hydroxyoctadecanoyl)amid	**ML9** 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-[(2-hydroxyhexadecanoyl)amido]-4,8-octadecadiene-1,3-diol

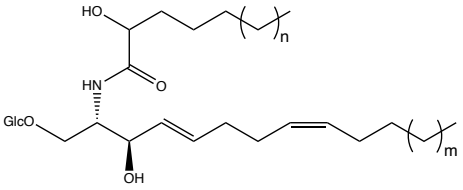
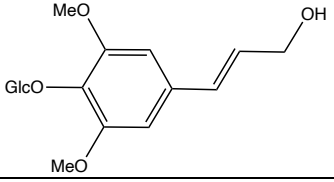
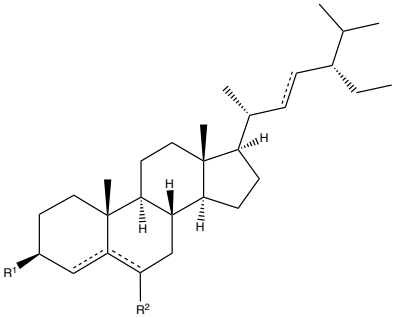
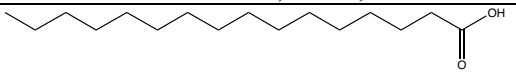
o]-4,8-octadecadiene-1,3-diol (Isolated for the first time from *Magnolia*)	(Isolated for the first time from *Magnolia*)
	**ML8:** m=7, n=12 **ML9:** m=7, n=10
Phenolic, sterol, and fatty acid	
MT14 syringin	
ML14 stigmasterol **ML15** daucosterol **MT18**β-sitosterol	 **ML14:** R^1=OH, R^2=H, $\Delta^{5,6}$, $\Delta^{22,23}$ **ML15:** R^1=OGlc, R^2=H, $\Delta^{5,6}$ **MT18:** R^1=OH, R^2=H, $\Delta^{5,6}$
ML16 palmitic acid	

Table 4.2. Summary of biological activity results

No.	Compounds	Bioactivitives
1	**ML1**	Inhibits α-glucosidase enzyme, IC_{50} = 179.86 µg/mL.
2	**ML2**	Inhibits α-glucosidase enzyme, IC_{50} = 316.88 µg/mL.
3	**MT13**	Inhibits α-glucosidase enzyme, IC_{50} = 117.58 µg/mL.
4	**MT2**	Inhibition of NO production in RAW264.7 cells, IC_{50} = 236.18 µg/mL.
5	**MT11**	Inhibition of NO production in RAW264.7 cells, IC_{50} = 202.74 µg/mL.
6	**ML1**	Antioxidant on DPPH system, SC_{50} = 295,28 µg/mL.
7	**MT4**	Antioxidant on DPPH system, SC_{50} = 46.73 µg/mL.
8	**ML11**	Cytotoxic, Hep-G2 (IC_{50} = 75.97±3.19 µM), RD (IC_{50} = 60.44±3.39 µM), and HeLa (IC_{50} = 45.89±3.37 µM).

CONCLUSIONS AND RECOMMENDATIONS

1. CONCLUSIONS

This is the first publication in Vietnam as well as in the world on the chemical composition and bioactivities of the leaves of *Magnolia lamdongensis* distributed in Lam Ha district, Lam Dong province and *Magnolia tiepii* distributed in Khanh Vinh district, Khanh Hoa province.

From the leaves of two studied species, **32** compounds were isolated and identified and the cytotoxic activity of the extracts and some selected compounds were evaluated, namely:

1.1. Chemical constituent

From leaves plant *Magnolia lamdongensis* 18 compounds were isolated including: rhamnetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside (**ML1**), oxytroflavoside F (**ML2**), rhamnocitrin 3-*O*-*β*-neohesperidoside (**ML3**), curcucomoside D (**ML4**), astragalin (**ML5**), kaempferol 3-neohesperidoside (**ML6a**), kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside (**ML6b**), quercetin 3-neohesperidoside (**ML7a**), quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)- *β*-D-galactopyranoside (**ML7b**), 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-[(2- hydroxyoctadecanoyl)amido]-4,8-octadecadiene-1,3-diol (**ML8**), 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-[(2-hydroxyoctadecanoyl)amido]-4,8-hexadecadiene-1,3-diol (**ML9**), (-)-sesamin (**ML10**), hinokinin (**ML11**), dihydrosesamin (**ML12**), (*S*)-eriodictyol (**ML13**), stigmasterol (**ML14**), daucosterol (**ML15**), and palmitic acid (**ML16**). Among them, compounds **ML1**, **ML2**, **ML6b**, **ML7a**, **ML7b**, **ML8**, **ML9**, **ML11**, and **ML13** were isolated for the first time from the *Magnolia* genus.

From leaves plant *Magnolia tiepii* 18 compounds were isolated including: kaempferol 3-neohesperidoside (**MT1**), nicotiflorin (**MT2**), isoquercitrin (**MT3**), magnoloside A (**MT4**), (+)-syringaresinol (**MT5**), (+)-pinoresinol (**MT6**), (-)-acanthoside B (**MT7**), (9*S*)-9-*O*-methylcubebin (**MT8**), (9*R*)-9-*O*-methylcubebin (**MT9**), lariciresinol (**MT10**),

dehydrovomifoliol (**MT11**), blumenol A (**MT12**), manglieside C (**MT13**), syringin (**MT14**), astragalin (**MT15**), hinokinin (**MT16**), dihydrosesamin (**MT17**), *β*-sitosterol (**MT18**). Among them, compounds **MT7**, **MT8**, **MT9**, **MT11**, **MT16**, and **MT17** were isolated for the first time from the *Magnolia* genus.

1.2. Biological activity

Test samples ML-M, MT-M, **ML1**, and **MT4** exhibited antioxidant activity on the DPPH system at the tested concentrations, with SC_{50} values of 120.62, 396.30, 295.28, 46.73 μg/mL, respectively.

Two compounds, **MT2** and **MT11**, showed anti-inflammatory activity by evaluating their ability to inhibit NO production on RAW264. 7 cells with IC_{50} values of 236.18 and 202.74 μg/mL, respectively. These two samples were not cytotoxic to RAW264.7 cells at a concentration of 256 μg/mL.

Compounds **ML1**, **ML2**, and **MT13** exhibited *α*-glucosidase enzyme inhibitory activity at tested concentrations with IC_{50} values of 179.86, 316.88, and 117.58 μg/mL, respectively.

Compound **ML11** showed weak cytotoxic activity on three cell lines of Hep-G2, RD, and HeLa with IC_{50} values of 75.97±3.19, 60.44±3,39, 45.89±3.37 μM, respectively.

2. RECOMMENDATIONS

- Research on chemical composition and biological activities of other species of the genus *Magnolia* in Vietnam.

- Continue to further research the biological activity of the isolated compounds in other activity tests.

NEW FINDINGS OF THE THESIS

1. The thesis provides the first results on the chemical composition of the leaves of *M. lamdongensis*. From the leaves of *M. lamdongensis* collected in Lam Ha district, Lam Dong, 18 compounds were isolated and identified, including 9 compounds isolated for the first time from the genus *Magnolia*.

2. The thesis also provided the first results on the chemical composition of the leaves of *M. tiepii*. From the leaves of *M. tiepii* collected in Khanh Vinh district, Khanh Hoa province, 18 compounds were isolated, including 6 compounds isolated for the first time from the genus *Magnolia*.

3. The thesis provides the first results on the antioxidant activity, inhibition of NO production, inhibition of the α-glucosidase enzyme, and cytotoxicity of some compounds isolated from the leaves of two species, *M. lamdongensis* and *M. tiepii*.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **Pham Van Huyen**, Nguyen Huu Huong Duyen, Nguyen Thi Thu Hien, Tran Thi Ngoc Hanh, Nguyen Thi Dieu Thuan, Nguyen Huu Toan Phan (2024), *Chemical constituents of Magnolia tiepii*, Chemistry of Natural Compounds, 60(3), 520-522. DOI: 10.1007/s10600-024-04368-6.
2. **Pham Van Huyen**, Nguyen Huu Huong Duyen, Nguyen Thi Thu Hien, Tran Thi Ngoc Hanh, Nguyen Thi Dieu Thuan, Nguyen Huu Toan Phan (2023), *Flavonoid glycosides from the leaves of Magnolia lamdongensis*, Chemistry of Natural Compounds, 59(4), 773-775. DOI: 10.1007/s10600-023-04108-2.
3. **Pham Van Huyen**, Le Thi Tuong An, Trinh Thi Luong, Nguyen Huu Huong Duyen, Tran Thi Ngoc Hanh, Nguyen Thi Thu Hien, Nguyen Thi Dieu Thuan, Nguyen Huu Toan Phan (2021), *Quercetin derivatives of the leaves of Magnolia lamdongensis*, 11(10), 1-4. DOI: 10.9790/9622-1110040104.
4. **Pham Van Huyen**, Tran Thi Ngoc Hanh, Tran Ngoc Huyen Vi, Nguyen Huu Huong Duyen, Nguyen Thi Thu Hien, Nguyen Thi Dieu Thuan, Nguyen Huu Toan Phan (2022), *Flavonoid glycosides from the leaves of Magnolia tiepii (Magnoliaceae)*, 9(11), 13-16.
5. **Pham Van Huyen**, Nguyen Thi Thu Hien, Tran Thi Ngoc Hanh, Nguyen Thi Dieu Thuan, Nguyen Huu Huong Duyen, Nguyen Huu Toan Phan (2023), *Chemical constituents of Magnolia tiepii leaves*, Journal of Analytical Sciences, 29(3), 142-147.