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STUDY ON THE CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF *Myrsine semiserrata* Wall. AND *Oligoceras eberhardtii* Gagnep.

SUMMARY OF CHEMISTRY DOCTORAL THESIS IN ORGANIC CHEMISTRY

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INTRODUCTION

According to the World Health Organization (WHO), about 7.6 million people die every year from cancer, typically lung cancer, liver cancer, colorectal cancer, breast cancer, Cervical cancer, stomach cancer, and prostate cancer account for more than 13% of deaths each year. Morbidity and mortality from cancer tend to increase. According to WHO estimates, the number of deaths from cancer worldwide will reach 11.8 million per year by 2030 [1]. In Vietnam, through statistics of cancer records in Hanoi, Ho Chi Minh City, and some provinces; It is estimated that each year in our country there are about 150 thousand new cancer patients and 75 thousand people die from cancer; this number tends to increase [2].

People have known about the use of chemical medicines since the 19th century to treat cancer, such as using potassium arsenite to treat myeloid leukemia until the 1930s [3]. To date, many natural compounds and products synthesized and semi-synthesized from natural compounds have been used effectively in the treatment and prevention of cancer and other diseases. people fight diseases and improve community health. A series of cancer treatment drugs use active ingredients isolated from nature, such as the group of compounds paclitaxel (Taxol®), a diterpenoid isolated from the red pine species Taxus brevifolia (Taxaceae), or some other compounds podophyllotoxin. , camptothecin, berbamine, beta-lapachone, betulinic acid, colchicine, curcumin, daphnoretin, ellipticine... and their semi-synthetic derivatives vinflunine, docetaxel (Taxotere®), ...[4,5].

Vietnam is a Southeast Asian country, belonging to the tropical monsoon climate region with two distinct seasons that change according to terrain, with lots of rain and high relative humidity, which are favorable conditions for plants to grow. Therefore, Vietnam has a rich and diverse flora with over 12,000 species, including over 3,200 plant species used as medicine in Folk Medicine; opens up great potential for research on natural compounds from Vietnamese plants [6].

Within the French-Vietnamese International Cooperation project "Phytochemistry Research of Vietnamese Vegetation"; some Vietnamese *Myrsine* and *Oligoceras* species have been collected and tested for preliminary activity. The results showed that the EtOAc extract of *Myrsine semiserrata* Wall. fruit has the ability to inhibit 57.19% of KB carcinoma cells at a concentration of 1 μg/mL. EtOAc extract of *Oligoceras eberhardtii Gagnep* fruit. capable of inhibiting 37.66% of KB cells at a concentration of 1.0 μg/mL. Until now, no domestic or international projects have been researching the chemical composition and biological activities of these two species, *Myrsine semiserrata* and *Oligoceras eberhardtii*.

Therefore, I chose the topic: **"Study on the chemical composition and biological activities of *Myrsine semiserrata* Wall. and *Oligoceras eberhardtii* Gagnep."**.

Objectives of the thesis:

- Determine the chemical constituents of two species *Myrsine semiserrata* and *Oligoceras eberhardtii* in Vietnam.
- Evaluate the cancer cytotoxicity and antibacterial activity of isolated compounds to search for biologically active compounds, as a scientific basis for applied research. Next, create health care products for the community.

The thesis includes:

1. Isolation and chemical structures determination of compounds from *Myrsine semiserrata* and *Oligoceras eberhardtii* species
2. Evaluate the cancer cytotoxicity and antibacterial activity of the isolated compounds.
3. Using atomistic simulation method to search for potential GSK-3β inhibitors from isolated compounds.

CHAPTER 1. OVERVIEW

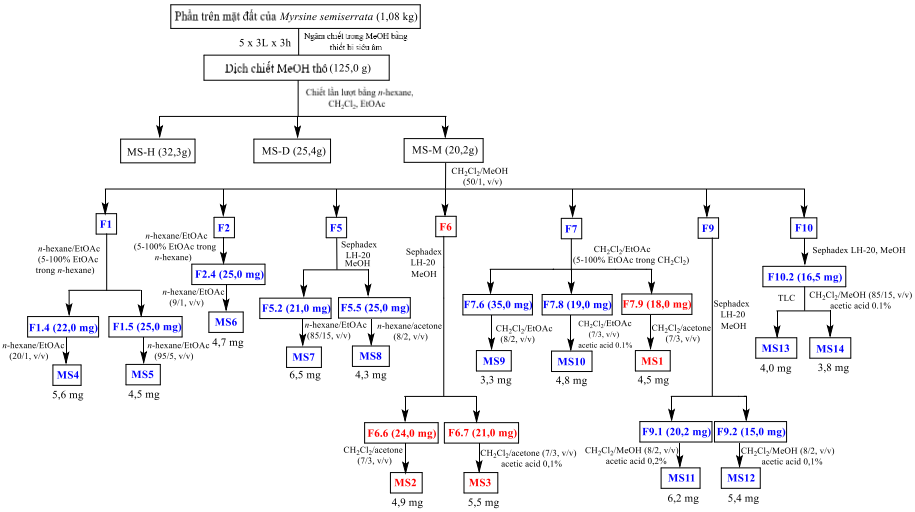


Figure 3.1. Isolation of compounds from *M. semiserrata*

3.1.2. Isolation of compounds from O. eberhardtii

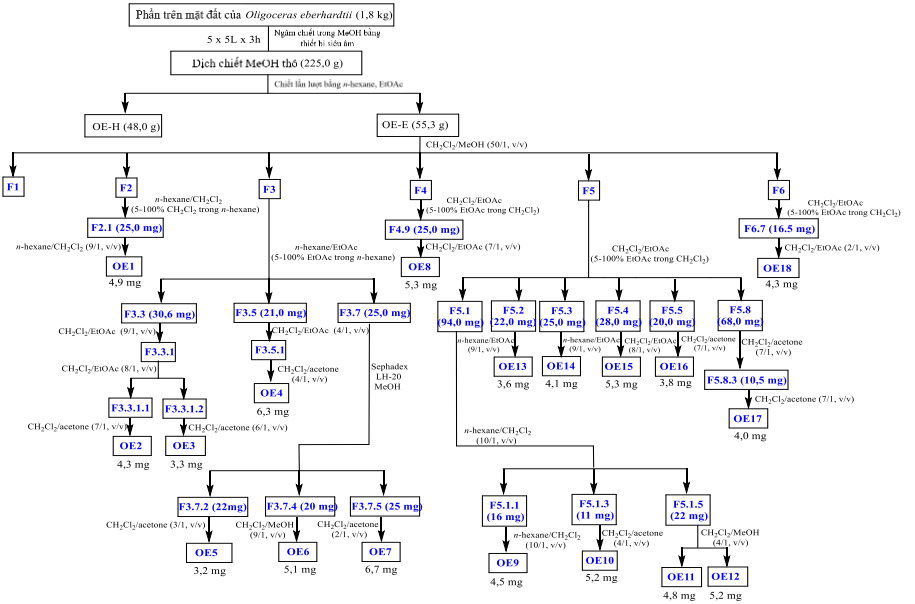


Figure 3.2. Isolation of compounds from *O. eberhardtii*

3.2. Physical properties and spectroscopy data of isolated compounds

3.2.1. Physical properties and spectroscopy data of isolated compounds from M. semiserrata

3.2.2. Physical properties and spectroscopy data of isolated compounds from O. eberhardtii

3.3. Activity of isolated compounds from *M. semiserrata* and *O. eberhardtii*

3.3.1. Cancer cell cytotoxic activity of isolated compounds from M. semiserrata and O. eberhardtii

Cytotoxicity tests were performed according to the method described in section 2.2.3.1. Compounds **MS1-MS14** isolated from *M. semiserrata* and **OE1-OE18** isolated from *O. eberhardtii* were tested for *in vitro* cytotoxic activity on 4 human cancer cell lines: lung cancer (A-549), liver cancer (HepG2), breast cancer (MCF-7), carcinoma (KB). The activity test results are shown in table 4.31.

3.3.2. Using molecular docking simulations to predict the GSK-3β inhibition mechanism of potential active ingredients isolated from O. eberhardtii

Use molecular docking method and molecular dynamics calculations as described in 2.2.4 to determine the binding position and binding affinity of the isolated compounds **OE1 - OE18** with GSK-3*β*, while simultaneously simulating the activity of the GSK-3*β*+ inhibitor complex in solution. The results are shown in table 4.32-4.39.

3.3.3. Antimicrobial activity results of compounds isolated from M. semiserrata and O. eberhardtii

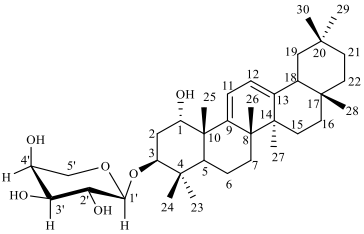
The isolated compounds **MS1-MS14** and **OE1-OE18** were evaluated for their ability to resist microbial strains tested under the international standard ATCC based on the multi-concentration dilution method as described in section 2.2.3.2. Test results are shown in table 4.40.

CHAPTER 4. DISCUSSION OF RESULTS

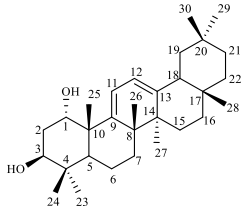
4.1. Structures elucidation of the isolated compounds

4.1.1. Structures elucidation of isolated compounds from the M. semiserrata

*4.1.1.1. Compound **MS1**: Myrsineoside A (3-O-α-L-arabinopyranosyl juglangenin A) (new compound)*



MS1



Juglangenin A

Figure 4.1. Chemical structure of compound **MS1** and reference compounds

Compound **MS1** was obtained as a white powder, and specific optical rotation was +17,9 (c 0.24, MeOH). On HR-ESI-MS of **MS1**, a pseudomolecular ion peak appears at m/z 611.3718 [M + K]+ (theoretical calculation for the molecular formula is $C_{35}H_{56}O_6K^+$ *m/z* 611.3708) allows determining the molecular formula of **MS1** as $C_{35}H_{56}O_6$ (with unsaturation △=8). The infrared spectrum of MS1 shows the absorption peak of the C=C double bond at 1638 cm^{-1}, 1559 cm^{-1}, and the hydroxyl group at 3441 cm^{-1}.

On the ^{1}H NMR spectrum of **MS1**, singlet signals of eight methyl groups appear at δ_H 0.90 (3H, s, H-30), 0.92 (3H, s, H-24), 0.93 (3H, s, H-28), 0.94 (3H, s, H-29), 1.09 (3H, s, H-27), 1.14 (3H, s, H-23), 1.19 (3H, s, H-26) and 1.26 (3H, s, H-25), along with the signal of two olefin protons at δ_H 5.73 (1H, d, *J* = 6.0 Hz, H-11) and 5.60 (1H, d, *J* = 6.0 Hz, H-12). Besides, the signal of two oxymethine protons was determined at δ_H 4.17 (1H, t, *J* = 3.0 Hz, H-1) and 3.69 (1H, dd, *J* = 4.8, 12, 0 Hz, H-3) and there is an overlapping signal in the aliphatic region (δ_H 3.52 – 3.86 ppm). Signal of two olefin protons at δ_H 5.73 (1H, d, *J* = 6.0 Hz, H-11) and 5.60 (1H, d, *J* = 6.0 Hz, H-12) with constant interaction *J* = 6.0 Hz along with the signal of 4 carbon olefins at δ_C 118.3 (C-11), 121.7 (C-12), 149.1 (C-13), 151.5 (C-9) on the ^{13}C NMR spectrum allows determining the presence of a conjugated diene system. In addition, the ^{1}H NMR spectrum of **MS1** also shows the appearance of a signal of an anomer proton at δ_H 4.31 (1H, d, *J* = 6.6 Hz, H-1') along with other signals of 3 groups oxymethine at δ_H 3.52 (1H, m, H-3'), δ_H 3.54 (1H, dd, *J* = 3.0; 12.2 Hz, Ha -5'), δ_H 3.58 (1H, dd, *J* = 6.6; 8.4 Hz, H-2'), δ_H 3.82

(1H, m, H-4') and 1 oxymethylene group at δ_H 3.86 (1H, dd, J = 2.5; 12.2 Hz, H_b-5'). Analysis of ^{13}C NMR and DEPT spectra with the help of HSQC interaction of MS1 shows the appearance of signals of 35 carbon atoms in which 30 carbon atoms are assigned to the aglycone part and signals for the sugar component are generated made from a 5-carbon sugar unit. Specifically, there are signals of 8 methyl groups at δ_C 16.9 (C-24), 20.7 (C-27), 21.9 (C-26), 24.1 (C-29), 26, 4 (C-25), 28.7 (C-23), 29.3 (C-28), 33.7 (C-30), 8 methylene groups at δ_C 18.5 (C-6), 26, 8 (C-15), 28.1 (C-16), 32.9 (C-7), 33.2 (C-2), 35.6 (C-21), 38.1 (C-22), 48.1 (C-19), 1 oxymethylene group at δ_C 66.5 (C-5'), 6 oxymethine groups at δ_C 74.2 (C-1), 85.3 (C-3), 107.4 (C-1'), 72.9 (C-2'), 74.4 (C-3'), 69.6 (C-4'), 2 sp^2 methine groups at δ_C 118.3 (C -11), 121.7 (C-12), 2 sp^3 methine groups at δ_C 45.9 (C-5), 47.1 (C-18) and 8 carbons not directly bonded to hydrogen. The above data along with the value of the interaction constants J ($J_{1',2'}$ = 6.6 Hz, $J_{2',3'}$ = 8.4 Hz) allows predicting the presence of a line origin α-arabinopyranose [123, 124].

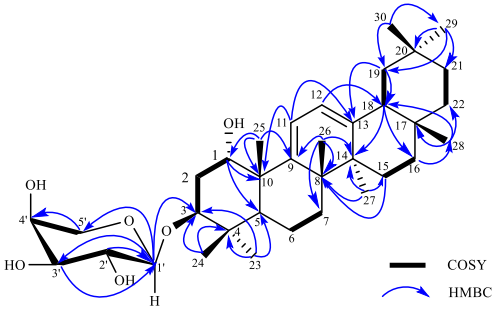


Figure 4.10. Key HMBC, COSY correlations of **MS1**

The direct bonds between protons and carbon of **MS1** were determined based on HSQC spectrum analysis. On the COSY spectrum of **MS1**, the presence of seven spin-spin interaction systems of adjacent protons is observed with bold lines in Figure 4.10, including H-1'/H-2'/H-3' /H-4'/CH_2-5', H-1/CH_2-2/H-3, H-5/CH_2-6/CH_2-7, H-11/H-12, CH_2-15/CH_2-16, H-18/CH_2-19 and CH_2-21/CH_2-22. The NMR spectrum data obtained from **MS1** and the above analyses suggest that compound **MS1** is a triterpenoid with an oleanane skeleton similar to the known compound juglangenin A [125]. The position of the sugar unit is determined based on the HMBC spectrum between the H-1' interaction of the sugar moiety (δ_H 4.31)

with the C-3 (δ_C 85.3) of the oleanane aglycon part, which allows us to determine Determine the location of the line base attached at C-3 (Figure 4.10).

Besides, the HMBC spectrum shows the interaction between Me-23 (δ_H 1.14) and Me-24 (δ_H 0.92) with C-3 (δ_C 85.3), C-4 (δ_C 40.4), C-5 (δ_C 45.9) indicates that these two methyl groups are attached to C-4. HMBC interaction between Me-25 (δ_H 1.28) with C-1 (δ_C 74.2), C-5 (δ_C 45.9), C-10 (δ_C 45.7), and C -9 (δ_C 151.5); between Me-26 (δ_H 1.19) with C-8 (δ_C 43.7), C-9 (δ_C 151.5) and C-14 (δ_C 42.1); between Me-27 (δ_H 1.09) with C-8 (δ_C 43.7), C-13 (δ_C 149.1), C-14 (δ_C 42.1) and C-15 (δ_C 26.8); between Me-28 (δ_H 0.93) with C-16 (δ_C 28.1), C-17 (δ_C 33.2), C-18 (δ_C 47.1) and C-22 (δ_C 38.1) allows determining the positions of four methyl groups attached directly at C-10, C-8, C-14 and C-17 of the oleanane aglycon part, respectively (Figure 4.10). Interaction between protons of groups Me-29 (δ_H 0.94) and Me-30 (δ_H 0.90) with C-19 (δ_C 48.1), C-20 (δ_C 31.9), and C-21 (δ_C 35.6) and the mutual interaction of these two methyl groups allows determining the position of the two methyl groups attached at C-20. In addition, on the HMBC spectrum, the long-distance interactions between H-11 (δ_H 5.73), H-12 (δ_H 5.60), Me-25 (δ_H 1.26), Me- 26 (δ_H 1.19) to C-9 (δ_C 151.5), and from H-11 (δ_H 5.73) and Me – 27 (δ_H 1.09) to C-11 (δ_C 24.1) allows identification of the olean-9(11),12-diene skeleton of **MS1**.

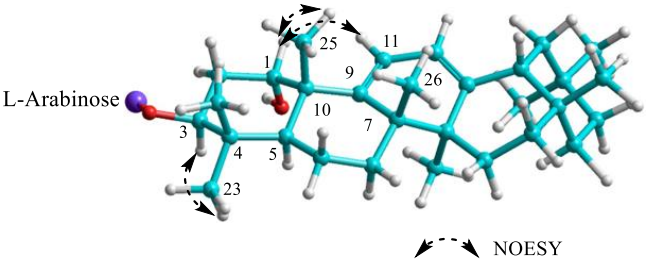


Figure 4.12. Key NOESY correlations of **MS1**

The relative configuration of compound **MS1** was determined based on the interaction constant values and the NOESY spectrum. On the NOESY spectrum, interaction signals appear between H-1 (δ_H 4.17) and H-11 (δ_H 5.73)/Me-25 (δ_H 1.26), between Me-24 (δ_H 0.92) and Me-25 (δ_H 1.26), from which we conclude that H-1 is β-oriented. The α-oriented C-3 position proton was identified by

interactions on the NOESY spectra of H-3 (δ_H 3.69) and Me-23 (δ_H 1.14). Furthermore, the separation of H-1 (t, J = 3.0 Hz) and H-3 (dd, J = 4.8; 12.0 Hz) further confirms the hydroxy group at C-1 and the O-glycosides at C-3 are oriented α and β, respectively. Analysis of interactions on the NOESY spectrum of MS1 shows interactions between Hα-3/H-3' and H-1' suggesting protonation at the α-oriented C-1' site. Besides, when comparing the chemical shift at C-3 (δ_C 85.3) of **MS1** (which has a low field shift) with the ^{13}C NMR spectrum value at 73.0 (C-3) of juglangenin A [125] shows the conformation of the *O*-glycoside group at position C-3 in **MS1**. The above information shows that the structure of compound **MS1** is similar to juglangenin A, except for the appearance of a sugar unit in MS1 instead of the OH group at C-3 in juglangenin A.

Table 4.1. NMR spectral data of **MS1** and reference compounds

C	$^{\#}\delta_C$[d]	δ_C[a,c]	δ_H[b,c] (J in Hz)	C	$^{\#}\delta_C$[d]	δ_C[a,c]	δ_H[b,c] (J in Hz)
1	72,8	74,2	4,17 (t, 3,0)	19	46,7	48,1	1,09 (m) 1,72 (m)
2	31,7	33,2	2,04 (m) 2,22 (m)	20	31,1	31,9	-
3	73,0	85,3	3,69 (dd, 4,8; 12,0)	21	34,6	35,6	1,15 (m) 1,40 m)
4	39,0	40,4	-	22	37,0	38,1	1,31 (m) 1,52 (m)
5	44,3	45,9	1,49 (m)	23	28,7	28,7	1,14 (s)
6	17,4	18,5	1,59 (m) 1,70 (m)	24	15,6	16,9	0,92 (s)
7	32,4	32,9	1,40 (br ddd, 2,4; 2,4; 15,0) 1,76 (m)	25	25,7	26,4	1,26 (s)
8	42,5	43,7	-	26	21,0	21,9	1,19 (s)
9	150,3	151,5	-	27	20,3	20,7	1,09 (s)
10	45,0	45,7	-	28	28,2	29,3	0,93 (s)
11	117,0	118,3	5,73 (d, 6,0)	29	23,7	24,1	0,94 (s)
12	119,9	121,7	5,60 (d, 6,0)	30	33,2	33,7	0,90 (s)
13	149,0	149,1	-	Ara			
14	40,7	42,1	-	1'		107,4	4,31 (d, 6,6)
15	25,6	26,8	1,09 (m) 1,92 (m)	2'		72,9	3,58 (dd, 6,6; 8,4)
16	27,0	28,1	0,91 (m)	3'		74,4	3,52 (m)

			2,06 (m)				
17	32,2	33,3	-	4'		69,6	3,82 (m)
18	45,8	47,1	2,19 (m)	5'		66,5	3,54 (dd, 3,0; 12,2) 3,86 (dd, 2,5; 12,2)

[a]150MHz, [b]600 MHz, [c]CD_3OD, [d]100 MHz, [#]δ_C: NMR spectral data of juglangenin A measured in $CDCl_3$ [125].

Hydrolyze compound **MS1** in HCl environment to obtain simple sugars. The sugar group in **MS1** is determined to be L-arabinose with polar rotation angle [α]24D = +102 (c 0.1, H_2O) and R_f = 0.53 consistent with the value of the specific polar rotation angle of sugar L-arabinose on previously published documents [123,124] and compared on TLC thin plates of standard L-arabinose sugar. From all the above spectral data analysis, it is possible to determine the structure of compound **MS1** as 3-*O*-*α*-L- arabinopyranosyl juglangenin A, this is a new compound and is specifically named myrsineoside A.

*4.1.1.2. Compound **MS2**: Myrsineoside B (3-O-α-L-arabinopyranosyl castanopsol) (new compound)*

*4.1.1.3. Compound **MS3**: Myrsineoside C (3-O-β-D-xylopyranosyl castanopsol) (new compound)*

*4.1.1.4. Compound **MS4**: Lupeol acetate*

*4.1.1.5. Compound **MS5**: Taraxerone*

*4.1.1.6. Compound **MS6**:* Kazinol B

*4.1.1.7. Compound **MS7**: Kazinol A*

*4.1.1.8. Compound **MS8**: 4'-O-methyl-8-prenylnaringenin*

*4.1.1.9. Compound **MS9**: Cucurbitacin D*

*4.1.1.10. Compound **MS10**: Cucurbitacin H*

*4.1.1.11. Compound **MS11**: Eclalbasaponin II*

*4.1.1.12. Compound **MS12**: Spergulacin*

*4.1.1.13. Compound **MS13**: Kaempferol 3-O-α-L-rhamnopyranoside*

*4.1.1.14. Compound **MS14**: Quercetin-3-O-α-L-rhamnopyranoside*

4.1.2. Structures elucidation of isolated compounds from the O. eberhardtii

*4.1.2.1. Compound **OE1**: Lupeol acetate*

*4.1.2.2. Compound **OE2**: 5,7,3'-Trihydroxy-6,4',5'-trimethoxyflavone*

Compound **OE2** was isolated as a pale-yellow powder. On the 1H NMR spectrum of **OE2**, signals of three methoxy groups appear at δ_H 3.89

(3H, s, 6-OCH_3), 3.91 (3H, s, 4'-OCH_3), 3.96 (3H, s, 5'-OCH_3) with four sp^2 methine groups at δ_H 6.59 (1H, s, H-8), 6.65 (1H, s, H-3), 7.09 (1H, d, J = 2 ,4 Hz, H-2'), 7.13 (1H, d, J = 2.4 Hz, H-6').

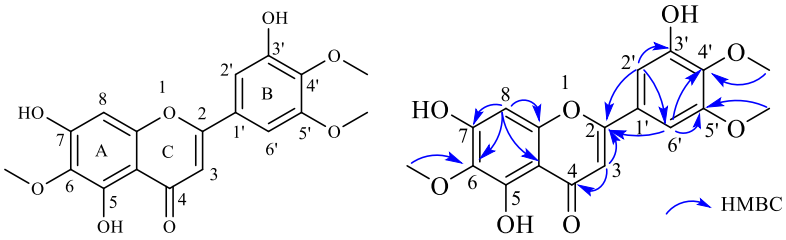


Figure 4.50. Chemical structure and key HMBC, COSY correlations of **OE2**

Table 4.15. NMR spectral data of **OE2** and reference compounds

C	$^{*}\delta_C$ [d, c]	δ_C [a,c]	δ_H [b,c] (*J* in Hz)
2	165,7	165,7	-
3	105,1	105,1	6,65 (s)
4	184,2	184,3	-
4a	105,9	105,9	-
5	153,9	154,0	
6	132,9	132,9	-
7	154,7	154,7	-
8	95,4	95,4	6,59 (s)
8a	158,9	158,9	-
1'	127,8	127,8	-
2'	103,1	103,2	7,09 (d, 2,4)
3'	155,0	155,1	-
4'	141,2	141,3	-
5'	152,3	152,3	-
6'	108,8	108,8	7,13 (d, 2,4)
4'-OCH_3	61,1	61,1	3,91 (s)
5'-OCH_3	56,7	56,7	3,96 (s)
6-OCH_3	60,9	60,9	3,89 (s)

[a]150 MHz, [b]600 MHz, [c]CD_3OD, [d]125 MHz, [*]δ_C of 5,7,3'-trihydroxy-6,4',5'-trimethoxyflavone measured in CD_3OD [139]

The ^{13}C NMR spectrum of **OE2** shows the presence of signals of 15 carbon atoms including ten non-hydrogen bonded aromatic carbon atoms at δ_C 105.1 – 165.7, one carbonyl group at δ_C 184.3 (C-4), two sp2 methine carbons at δ_C 95.4 (C-8) of ring A, δ_C 105.1 (C-3) of ring C and two methine carbons of ring B at δ_C 103.2

(C-2') and 108.8 (C-6'). From the above spectral data, it is suggested that **OE2** is a flavone. In addition, on the COSY spectrum, no spin-spin interaction systems of neighboring protons were observed. At the same time, on the HMBC spectrum, interactions of H-3 (δ_H 6.65) with C-2 (δ_H 6.65) appeared (δ_C 165.7), C-4 (δ_C 184.3), C-4a (δ_C 105.9); H-8 (δ_H 6.59) with C-6 (δ_C 132.9), C-7 (δ_C 154.7), C-8a (δ_C 158.9), C-4a (δ_C 105.9); H-2' (δ_H 7.09) with C-2 (δ_C 165.7), C-3' (δ_C 155.1), C-4' (δ_C 141.3) and C-6' (δ_C 108.8); H-6' (δ_H 7.13) with C-2 (δ_C 165.7), C-4' (δ_C 141.3), C-5' (δ_C 152.3) confirms the position of C-3, C-8, C-2′, C-6′ respectively. In addition, the positions of methoxy groups 4'-OCH_3, 5'-OCH_3, and 6-OCH_3 at C-4′, C-5′, and C-6 were assigned based on the observed HMBC interactions from the protonation of the methoxy groups 4'-OCH_3 with C-4', 5'-OCH_3 with C-5' and 6-OCH_3 with C-6. Comparing the spectral data of **OE2** with previously published documents on the compound 5,7,3'-trihydroxy-6,4',5'-trimethoxyflavone [139] shows consistency in all positions. Thus, it can be confirmed that **OE2** is 5,7,3'-trihydroxy-6,4',5'-trimethoxyflavone.

*4.1.2.3. Compound **OE3**: 5,7,2',5'-tetrahydroxy-6,3',4'- trimethoxyflavone*

*4.1.2.4. Compound **OE4**: Dehydrovomifoliol*

*4.1.2.5. Compound **OE5**: Protocatechuic acid*

*4.1.2.6. Compound **OE6**: Chrysocriol-7-O-β-D-glucopyranoside*

*4.1.2.7. Compound **OE7**: 7Z-roseoside*

*4.1.2.8. Compound **OE8**: 5,7,3'-trihydroxy-6,4',5'- trimethoxyflavanone*

*4.1.2.9. Compound **OE9**: Lup-20(29)-ene*

*4.1.2.10. Compound **OE10**: 23-deoxojessic acid*

*4.1.2.11. Compound **OE11**: Cucurbitacin F*

*4.1.2.12. Compound **OE12**: 3β-(β-D-glucosyloxy)-16α,23α-epoxycucurbita-5,24-diene-11-one*

*4.1.2.13. Compound **OE13**: Lupeol*

*4.1.2.14. Compound **OE14**: 3-(E)-Coumaroyltaraxerol*

*4.1.2.15. Compound **OE15**: 4'-O-methyl-8-prenylnaringenin*

*4.1.2.16. Compound **OE16**: 6,7,8-trimethoxycoumarin*

*4.1.2.17. Compound **OE17**: 3-hydroxy-4-methoxybenzoic acid*

*4.1.2.18. Compound **OE18**: Vomifoliol*

4.1.3. Results of isolated compounds (MS1-MS14, OE1-OE18) from M. semiserrata and O. eberhardtii

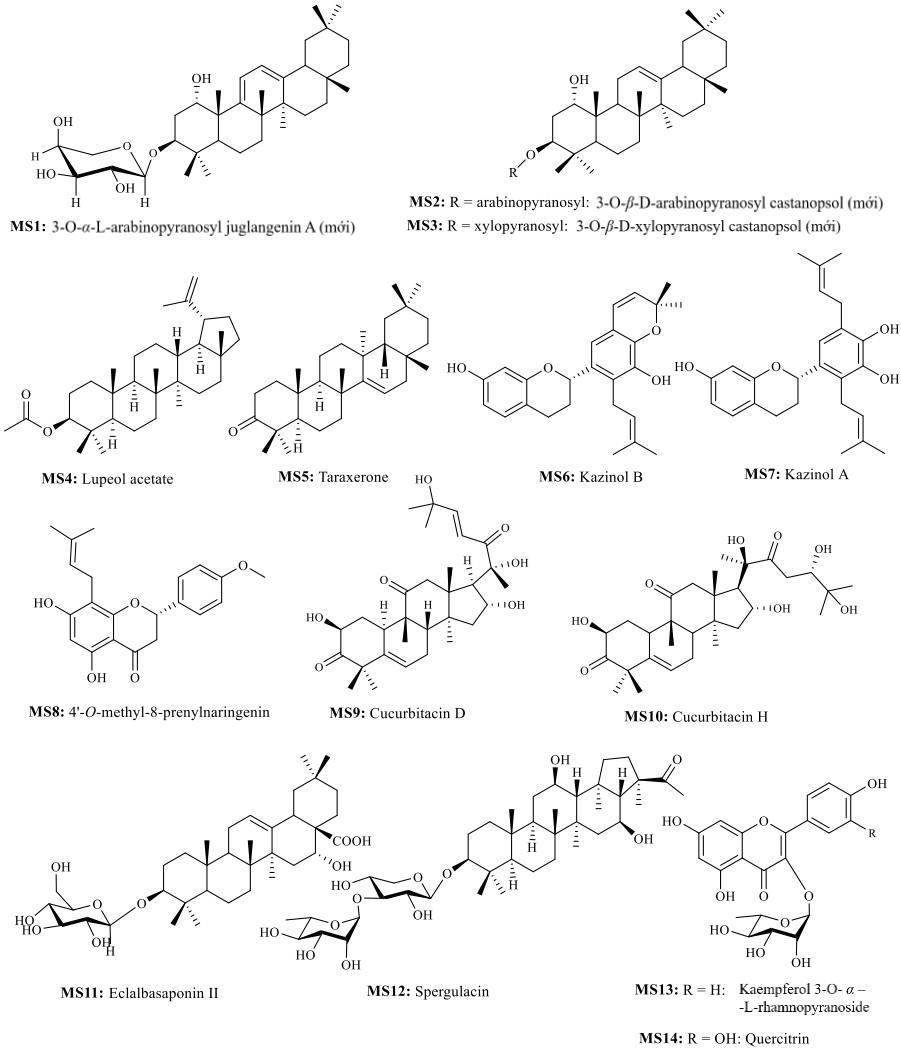


Figure 4.70. Isolated compounds **MS1-MS14** from *M. semiserrata*

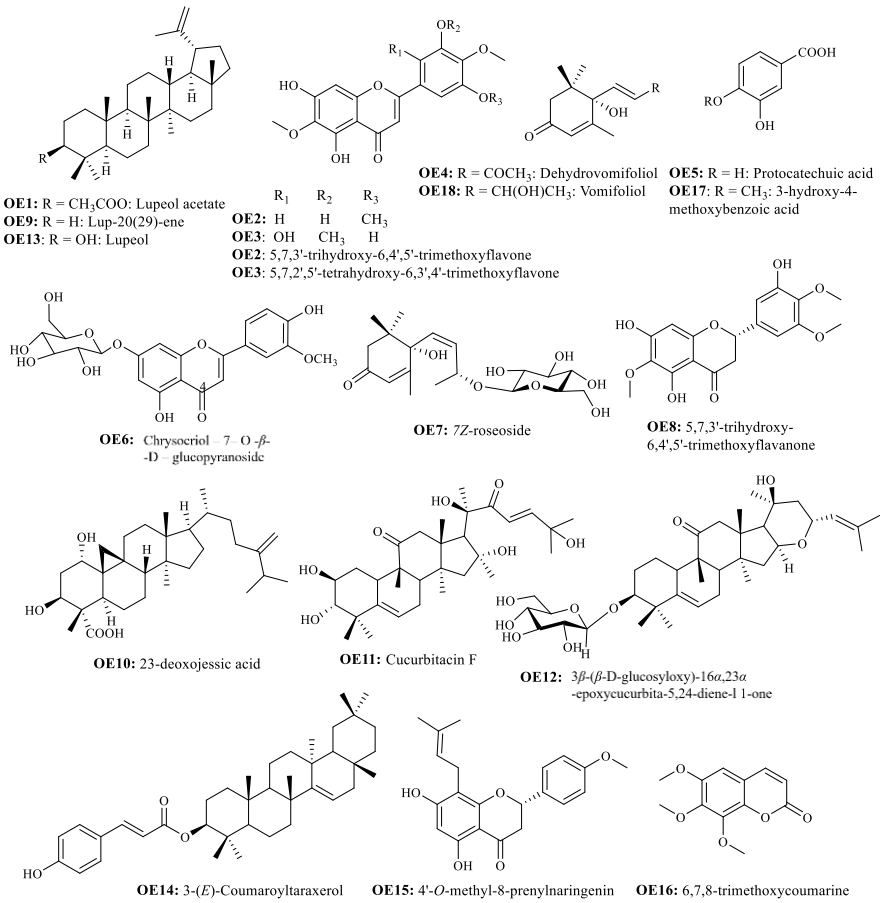


Figure 4.71. Isolated compounds **OE1-OE18** from *O. eberhardtii*

4.2. Evaluation of biological activity of compounds isolated from *M. semiserrata* and *O. eberhardtii*

4.2.1. Cytotoxic activity of compounds isolated from M. semiserrata and O. eberhardtii

Results of evaluating the in vitro cytotoxic activity of compounds isolated from M. semiserrata and *O. eberhardtii* on 4 human cancer cell lines including lung cancer (A549), liver cancer (HepG2), breast cancer (MCF-7), carcinoma (KB) (Table 4.31) shows that: Compound cucurbitacin D (**MS9**) isolated from M. semiserrata exhibits strong cytotoxicity against cancer lines. KB, HepG2, MCF-7

letters with corresponding IC_{50} values of 2.05 ± 0.05; 2.32 ± 0.07; 5.45 ± 0.15 µM while cucurbitacin H (**MS10**) only showed strong cytotoxicity against carcinoma lines (IC_{50} = 6.76 ± 0.15).

Table 4.31. Results of testing the cytotoxicity of compounds isolated from *M. semiserrata* and *O. eberhardtii*

No.	IC_{50} (µM)			
	KB	HepG2	A549	MCF7
MS1	74,87 ± 2,27	129,82 ± 0,84	71,06 ± 3,74	123,81 ± 1,92
MS2	32,80 ± 0,77	79,18 ± 1,09	23,39 ± 2,02	73,58 ± 1,18
MS3	104,95 ± 2,66	177,21 ± 0,39	43,57 ± 1,41	192,01 ± 4,37
MS4	-	-	-	-
MS5	-	-	-	-
MS6	49,11 ± 2,70	61,98 ± 3,72	72,22 ± 0,15	196,53 ± 5,07
MS7	-	-	-	-
MS8	137,65 ± 2,90	-	-	-
MS9	2,05 ± 0,05	2,32 ± 0,07	72,38 ± 3,44	5,45 ± 0,15
MS10	6,76 ± 0,15	11,21 ± 0,34	185,12 ± 1,04	37,56 ± 1,22
MS13	-	-	-	-
MS14	-	-	-	-
OE1	-	-	-	-
OE2	-	-	-	-
OE3	142,84 ± 8,08	196,03 ± 3,48	157,66 ± 2,15	197,58 ± 3,96
OE6	-	-	-	-
OE8	-	-	-	-
OE9	-	-	-	-
OE12	41,32 ± 2,13	36,42 ± 1,84	38,34 ± 1,28	102,32 ± 1,60
OE13	122,18 ± 3,46	160,51 ± 1,74	101,54 ± 2,35	140,79 ± 2,55
OE14	-	-	-	-
OE15	-	-	-	-
Ellipticine*	1,78 ± 0,02	1,75 ± 0,02	1,75 ± 0,02	1,78 ± 0,02

(*): Control substance, (-): Compounds have IC_{50} > 200 µM

MS10 showed moderate cytotoxic activity against two lines HepG2 (IC_{50} = 11.21 ± 0.34) and MCF7 (IC_{50} = 37.56 ± 1.22 µM). Both compounds showed weak toxicity to the A549 cell line. Two new compounds myrsineoside B (**MS2**) and myrsineoside C (**MS3**) also showed moderate cytotoxicity against the A549 cell line at IC_{50} values of 23.39 ± 2. 02 and 43.57 ± 1.41 µM, respectively showed weaker toxicity against HepG2 and MCF7 cell lines. Besides, the new compound MS2 also shows moderate cytotoxicity to carcinoma lines with an IC_{50} value of 32.80 ± 0.77 µM while **MS3** shows weak cytotoxicity to cell lines this cancer. In addition, the results in Table 4.31 also show that the new compound myrsineoside A (**MS1**) and two known compounds Kazinol B (**MS6**), 4′-*O*-methyl-8-prenylnaringenin (**MS8**) also have toxic effects. The cells were weak against the four cancer cell lines tested above while the remaining substances (**MS4**, **MS5**, **MS7**, **MS13**, **MS14**) isolated from *M. semiserrata* were not toxic to the cancer cell lines tested.

Compound 3*β*-(*β*-D-glucosyloxy)-16*α*,23*α*-epoxycucurbita-5,24-diene-11-one (**OE12**) isolated from *O.eberhardtii* exhibits moderate cytotoxic activity against three lines of carcinoma, liver cancer and lung cancer with corresponding IC_{50} values of 41.32 ± 2.13; 36.42 ± 1.84 and 38.34 ± 1.28 µM compared to the positive control ellipticine (IC_{50} = 1.78 µM), in addition to two compounds lupeol (**OE13**) and 5,7,2', 5'-tetrahydroxy-6,3',4'-trimethoxyflavone (**OE3**) showed weak toxic activity against all four cancer cell lines tested with IC_{50} values in the range of 101.54-197.58 µM. The remaining compounds did not show cytotoxic activity at the studied concentrations on all four cancer cell lines tested.

The compounds isolated from *M. semiserrata* with good activity are all triterpenoids. Two compounds **MS9** and **MS10** with cucurbitacin skeletons show better activity than the new saponins with oleanane skeletons **MS1**, **MS2**, and **MS3**. The cytotoxic activity of the compound cucurbitacin D has been studied a lot since the early twentieth century, mainly isolated from the Cucurbitaceae family. The anti-cancer activity is strongly expressed in different cell lines such as breast, lung, liver, uterine, pancreatic cancer, ...[157]. Interestingly, the compound cucurbitacin D (MS9) first isolated from M. semiserrata showed much stronger inhibition of breast and liver cancer cells than cucurbitacin D isolated from Cucumis prophetarum (IC_{50}: 26.7 µM (MCF-7), 5.0 µM (HepG2)) [158]. For cucurbitacin H, there are few studies on cytotoxic activity, only a recent study in 2020 by the authors Neha Kapoor

and colleagues showed that cucurbitacin H is one of the ingredients of the drug that can against SARS-CoV-2 [159]. This shows that in the current research of the researcher, cucurbitacin H (**MS10**) isolated from *M. semiserrata* has strong anti-cancer activity with very valuable carcinoma cell lines, promising further research in the future. treatment of this type of cancer.

4.2.2. Using molecular docking simulations to predict the GSK - 3β inhibition mechanism of potential active ingredients isolated from O. eberhardtii

4.2.2.1. Test the computational suitability of the mVina version with 11 known GSK-3β inhibitors

4.2.2.2. Using molecular docking models and computational molecular dynamics simulations for substances isolated from O. eberhardtii

a) Using molecular docking in calculations for substances isolated from O. eberhardtii

Table 4.36. Binding affinity of 18 compounds isolated from *Oligoceras eberhardtii* to GSK-3β (ΔG_{Dock} kcal. mol^{-1})

No.	Compound	ΔG_{Dock}	No.	Compound	ΔG_{Dock}
1	**OE1**	-8,2	11	**OE11**	-7,9
2	**OE2**	-8,6	12	**OE12**	-9,4
3	**OE3**	-8,4	13	**OE13**	-9,1
4	**OE4**	-6,5	14	**OE14**	-8,5
5	**OE5**	-6,8	15	**OE15**	-7,7
6	**OE6**	-8,7	16	**OE16**	-7,5
7	**OE7**	-6,3	17	**OE17**	-7,0
8	**OE8**	-5,7	18	**OE18**	-6,2
9	**OE9**	-6,4	19	**indirubin-3'-monoxime**	-10,5
10	**OE10**	-8,6			

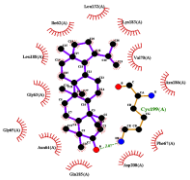
The binding affinity of 18 compounds isolated from *Oligoceras eberhardtii* to GSK-3β was predicted using mVina (table 4.36). In particular, the binding free energy ranges from -9.4 to -5.7 kcal. mol^{-1}, average value is -7.6 ± 0.2 kcal.mol^{-1}. Among these 18 compounds, two compounds including lupeol (**OE13**) and 3β-(β-D-glucosyloxy)-16α,23α-epoxycucurbita-5,24-diene-11-one (**OE12**) are thought to be able to inhibit GSK-3β has the closest binding affinity to CHEMBL365229, which is the experimental substance with the greatest binding affinity. Specifically, the results are presented in table 4.37. Based on previously

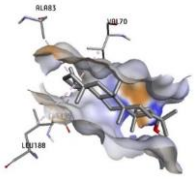
published documents, it can be seen that the active region of GSK-3β protein is composed of several important amino acids including Ile62, Asp133, Val135, Gln185, Asn186, Cys199, Asp200. In drug development studies, inhibitors are often aimed at forming bonds with these amino acids to effectively block enzyme activity by interfering with substrate recognition and catalytic mechanisms, ultimately affecting cellular function and downstream signaling. In this study, the binding profile between the two ligands and GSK-3β was analyzed using PyMOL software [48] and is shown in figure 4.74. Among these, compound OE12 forms a hydrogen bond with amino acid Cys199 of GSK-3β (Table 4.37). Additional hydrophobic interactions were observed due to Val70, Ala83, and Leu188 forming with OE12. Meanwhile, Asn64, Phe67, Arg141, Lys183, and Asp200 are amino acids that create hydrophobic interactions with OE13, the bond between the ligand and protein is further reinforced through 4 hydrogen bonds with amino acids Lys183, Gln185, Asn186, Asp 200.

Table 4.37. Docking calculation results of two potential compounds

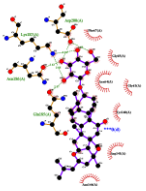
No.	Compound	ΔG_{Dock}	Amino acids participate in hydrogen bonding	Amino acids participate in Van der Waals bonds
1	**OE12**	-9,4	Lys183, Gln185, Asn186, Asp 200	Asn64, Phe67, Asp200
2	**OE13**	-9,1	Cys199	Val70, Ala83, Leu188

(A)





(B)



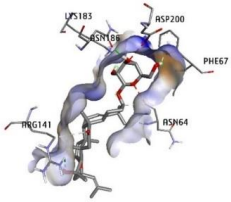


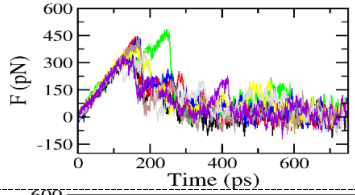
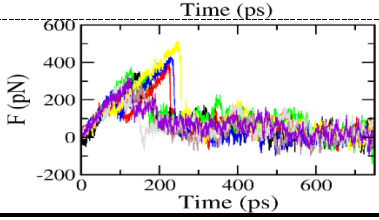
Figure 4.74. Binding profiles between **OE12** (A), **OE13** (B) and GSK-3β

b) Using computational molecular dynamics simulations for isolates from O. eberhardtii

Through linear regression, the expected binding free energy of the ligand to GSK-3β can be calculated as follows:

$$\Delta G_{FPL}^{Pre} = 0.035 \times W - 14.628$$

Table 4.38. Graph showing the pulling force causing bond breakage of two potential inhibitors during movement away from the GSK-3β binding site

TT	Hợp chất	Công kéo
1	**OE12**	
2	**OE13**	

Based on the evaluation step with experimental substances performed previously, it can be concluded that the FPL model is capable of calculating relatively accurately the binding affinity of the ligand to GSK-3β. Therefore, the free energy of ligand binding of **OE12** and **OE13** to GSK-3β was evaluated using the FPL method. The binding configuration of the two initial compounds was refined through MD simulation for 20ns. After 5ns of MD simulations, the complex has reached a steady state (table 4.38). Then, the final binding configuration of the complex was chosen as input data for the FPL simulation. The graph of force causing bond rupture was almost identical to previous experimental substances, showing the accuracy of the calculation model. It can be seen that the traction force quickly increases to a maximum value before suddenly decreasing to zero. The average value of the traction force causing a bond fracture and the pulling work are provided in Table 4.37. The predicted binding free energies are -9.87 and -10.46 kcal.mol^{-1}. The predicted k_i of potential compounds **OE13** and **OE12** are 64.43 and 23.95, respectively (table 4.39).

Table 4.39. FPL simulation results of two potential compounds

No.	Compound	$\Delta G_{\mathbf{Dock}}$	$\Delta F_{\mathbf{Max}}$	W	$\Delta G_{\mathbf{FPL}}^{\mathbf{Pre}}$	Prediction k_i (nM)
1	**OE12**	-9,4	653,1 ± 23,6	119,1 ± 3,6	-10,46	23,95
2	**OE13**	-9,1	792,5 ± 31,9	135,9 ± 2,7	-9,87	64,43

[a] *Unit of energy: kcal. mol*$^{-1}$

Therefore, it can be argued that these two compounds have the ability to block the function of GSK-3β. With the experimental values determined above with 4 cancer cell lines, it shows that both compounds **OE12** and **OE13** have cytotoxic activity, in which **OE12** has moderate cytotoxic activity. average but stronger than **OE13**. This shows that theoretical calculations are completely consistent with experimental values, and molecular docking methods and molecular dynamics simulations can be used to investigate the activity of substances isolated from other species.

4.2.3. Antimicrobial activity for compounds isolated from *M. semiserrata* and *O. eberhardtii*

Six compounds were isolated from *M. semiserrata* (**MS1-MS3**, **MS7**, **MS13**, **MS14**), and eleven compounds were isolated from *O. eberhardtii* (**OE1-OE3**, **OE6**, **OE8**, **OE9**, **OE11**, **OE12-OE15**) was diluted in DMSO at decreasing concentration ranges: 256µg/ml, 128µg/ml, 64µg/ml, 32µg/ml, 16µg/ml, 8µg/ml, 4µg/ml and 2µg/ml to test the activity respectively. with three Gram-negative bacterial strains (*Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* ATCC13076), three Gram-positive strains (*Enterococcus faecalis* ATCC299212, *Stapphylococus aureus* ATCC25923, *Bacillus cereus* ATCC 14579) and one yeast strain *Candida albicans* ATCC10231. The results obtained in table 4.39 show that most of the compounds isolated from *O. eberhardtii* species do not show antibacterial ability against the above bacterial and fungal strains, only **OE3** (5,7,2 ',5'-tetrahydroxy-6,3',4'-trimethoxyflavone) has quite strong resistance to *Enterococcus faecalis* strain at MIC = 32 µg/ml.

For the substances isolated from *M. Semiserrata*, mainly saponin and flavonoid compounds show tested anti-microbial ability, specifically three compounds myrsineoside B (**MS2**), myrsineoside C (**MS3**), and Kaempferol 3-O-α-L-rhamnopyranoside (**MS13**) shows resistance to gram-positive strains,

showing no resistance to gram-negative strains. Specifically, compound MS3 showed strong resistance to all 3 gram-positive bacteria compared to the antagonist at MIC = 32 µg/ml, and **MS13** showed strong resistance to 01 gram-positive strain, *Enterococcus faecalis*, equivalent to the antagonist *Streptomycin* at MIC=256 µg/ml; **MS2** showed quite good resistance to two gram-positive bacteria *Enterococcus faecalis* and *Bacillus cereus* compared to the antagonist at MICs of 128 and 64 µg/ml, respectively. Thus, it can be seen that two new compounds myrsineoside B (MS2) and myrsineoside C (**MS3**) have good potential for application in the treatment of some infections such as endocarditis, urinary tract infections, and prostatitis.

Table 4.39. Antimicrobial activity of compounds isolated from *M. semiserrata* and *O. eberhardtii* (MIC µg/ml)

No.	Compound	Gram-positive			Gram-negative			Yeast
		Enterococcus faecalis ATCC2 99212	*Staphylococus aureus* ATCC2 5923	*Bacillus cereus* ATCC 14579	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC27 853	*Salmonella enterica* ATCC 13076	*Candida albicans* ATCC 10231
1	**OE3**	32	-	-	-	-	-	-
2	**MS2**	128	-	64	-	-	-	-
3	**MS3**	32	32	32	-	-	-	-
4	**MS13**	256	-	256	-	-	-	256
5	**Streptomycin***	*256*	*128*	*128*	*32*	*256*	*128*	-
6	**Cyclohexamide***							*32*

(*): control substance, (-): MIC of compound > 256 µg/ml

*Compounds **OE1**, **OE2**, **OE6**, **OE8**, **OE9**, **OE12** – **OE15**, **MS1**, **MS7**, **MS14** all have MIC values greater than 256 µg/ml.*

CONCLUSIONS

Using combined chromatography and modern spectroscopic methods, there was a comparison with spectral data of similar compounds in reference documents, from two species *Myrsine semiserrata* and *Oligoceras eberhardtii* I

isolated and identified. Structures of 32 compounds and evaluation of some biological activities of these compounds. Specifically:

- ***Chemical constituents:***

1. From *M. semiserrata*, 14 compounds (**MS1-MS14**) were isolated and determined, including 3 new compounds and 11 known compounds. The three new compounds were named: 3-*O*-*α*-L- arabinopyranosyl juglangenin A (**MS1**), 3-*O*-*β*-D- xylopyranosyl castanopsol (**MS3**), 3-*α*-L-arabinopyranosyl castanopsol (**MS2**) and 11 known compounds include: Lupeol acetate (**MS4**), taraxerone (**MS5**), kazinol B (**MS6**), kazinol A (**MS7**), 4′-O-methyl-8-prenylnaringenin (**MS8**), cucurbitacin D (**MS9**), cucurbitacin H (**MS10**), eclalbasaponin II (**MS11**), spergulacin (**MS12**), kaempferol 3-*O*-*α*-L-rhamnopyranoside (**MS13**), quercitrin (**MS14**).

2. From *O. eberhardtii*, 18 known compounds were isolated and determined, including: lupeol acetate (**OE1**), 5,7,3'-trihydroxy-6,4',5'-trimethoxyflavone (**OE2**), 5, 7,2',5'-tetrahydroxy-6,3',4'-trimethoxyflavone (**OE3**), dehydro vomifoliol (**OE4**), protocatechuic acid (**OE5**), chrysoeriol-7-*O*-*β*-D-glucopyranoside (**OE6**), 7Z-roseoside (**OE7**), 5,7,3'-trihydroxy-6,4',5'-trimethoxyflavanone (**OE8**), lup 20(29)-ene (**OE9**), 23-deoxojessic acid (**OE10**), cucurbitacin F (**OE11**), 3*β*-(*β*-D-glucosyloxy)-16*α*,23*α*-epoxycucurbita-5,24-diene-11-one (**OE12**), lupeol (**OE13**), 3-(E)-coumaroyltaraxerol (**OE14**), 4′-O-methyl-8-prenylnaringenin (**OE15**), 6,7,8-trimethoxycoumarine (**OE16**), 3-hydroxy-4-methoxybenzoic acid (**OE17**), vomifoliol (**OE18**).

- ***Biological activities:***

3. Conducted evaluation of the cytotoxic activity of compounds isolated from *M. semiserrata* (**MS1-MS14**) and *O. eberhardtii* (**OE1-OE18**) on 4 human cancer cell lines: lung cancer (A549), liver cancer (HepG2), breast cancer (MCF-7), carcinoma (KB). The results showed that compound MS9 isolated from M. semiserrata showed strong cytotoxicity against the KB carcinoma line, HepG2 liver cancer, and MCF7 breast cancer with IC_{50} values of 2. 05 ± 0.05; 2.32 ± 0.07; 5.45±0.15 µM, respectively. **MS10** exhibited strong cytotoxicity against KB (IC_{50} = 6.76 ± 0.15) and moderate cytotoxicity against HepG2 and MCF7 with IC_{50} values of 11.21 ± 0.34, 37.56 ± 1.22 µM, respectively. The two new compounds **MS2** and **MS3** also showed moderate cytotoxicity against the A549 lung cancer cell line at IC_{50} values of 43.57±1.41 and 23.39 ±2.02 µM, respectively.

Compound **OE12** isolated from *O.eberhardtii* showed moderate cytotoxic activity against three lines of KB carcinoma, HepG2 liver cancer, and A549 lung cancer with corresponding IC_{50} values of 41. 32 ± 2.13, 36.42 ± 1.84 and 38.34 ± 1.28μM.

4. The antibacterial activity of compounds isolated from two species *M. semiserrata* and *O. eberhardtii* has been studied. The results showed that the compounds isolated from *O. eberhardtii* did not show antibacterial ability against the tested bacterial and fungal strains. Compounds **OE3**, **MS2**, **MS3**, **MS13** showed relatively good antibacterial effects in testing, in which **MS3** showed strong resistance to 3 gram-positive bacteria *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* ATCC13076 with the following values: The corresponding MICs were all 32 μg/ml.

5. Using molecular docking simulation and molecular dynamics simulation for potential GSK-3*β* inhibitors isolated from *O. eberhardtii*, the results identified two compounds **OE12** and **OE13** that have the ability to inhibit the functional activity of GSK-3β are consistent with experimental values.

RECOMMENDATION

Compound **MS9** isolated from *M. semiserrata* shows strong cytotoxicity against KB carcinoma line, HepG2 liver cancer, and MCF7 breast cancer lines, and **MS10** shows strong cytotoxicity against KB lines. Carcinoma can continue to research the cytotoxic mechanism to guide the applicability of this compound.

Through the use of simulation calculations, it has been confirmed that 2 compounds **OE12** and **OE13** cability to inhibit GSK-3*β* in cancer treatment following experiments, so this method can be used to perform searches. compounds isolated from *M. semiserrata* have the ability to inhibit GSK-3*β*.

NEW CONTRIBUTIONS OF THE THESIS

For the first time, the chemical composition and biological activities of two species *Myrsine semiserrata* and *Oligoceras eberhardtii* were studied in Vietnam and around the world.

1. Chemical constituents

From *Myrsine semiserrata* and *Oligoceras eberhardtii* species, 32 compounds were isolated and determined, including 3 new compounds from *M. semiserrata* including myrsineoside A, myrsineoside B, myrsineoside C, and 18 compounds discovered for the first time isolated from the *Oligoceras* genus.

2. Biological activities

In vitro carcinogenic cytotoxic activity against 4 cancer cell lines KB, HepG2, A549, and MCF7 of 2 species *Myrsine semiserrata* and *Oligoceras eberhardtii* was studied. The results showed that the compound cucurbitacin D isolated from M. semiserrata showed the best cytotoxicity against the KB carcinoma line, HepG2 liver cancer, and MCF7 breast cancer with IC_{50} values of 2, respectively. 05 ± 0.05; 2.32 ± 0.07; 5.45 ± 0.15 µM. Two new compounds myrsineoside B, and myrsineoside C isolated from *M. semiserrata* and 3*β*-(*β*-D-glucosyloxy)-16*α*,23*α*-epoxycucurbita-5,24-diene-11-one isolated from *O. eberhardtii* can also be used. showed moderate cytotoxicity against lung cancer cell line A549 with IC_{50} values of 43.57 ± 1.41; 23.39 ± 2.02 and 38.34 ± 1.28 µM.

For the first time, using molecular docking and molecular dynamics simulations for potential GSK - 3*β* inhibitors isolated from *O. eberhardtii*, the results resulted in the identification of two compounds **OE12** (3*β*-(*β*-D-glucosyloxy)-16*α*,23*α*-epoxycucurbita-5,24-diene-11-one) and **OE13** (lupeol) have the ability to inhibit the function of GSK-3*β* in accordance with experimental values.

LIST OF PUBLISHED ARTICLES

1. **Nguyen Thi Binh Yen**, Nguyen Thuy Linh, Vu Van Nam, Trieu Quy Hung, Doan Thi Mai Huong, Pham Van Cuong, 2024, Searching for potential GSK-3β inhibitors from *Oligoceras eberhardtii* Gagnep. Using atomistic simulations, *Vietnam Journal of Science and Technology* (Accepted).
2. **Thi Binh Yen Nguyen,** Thuy Linh Nguyen, Van Nam Vu, Quy Hung Trieu, Thi Mai Huong Doan, Van Cuong Pham, 2024, A new cytotoxic saponin from the ethyl acetate extract of *Myrsine semiserrata* Wall., *Vietnam Journal of Chemistry*, 2024, 1 (DOI: 10.1002/vjch.202400143)
3. **Nguyen Thi Binh Yen**, Trieu Quy Hung, Pham Van Cuong, Doan Thi Mai Huong, Nguyen Thuy Linh, Tran Van Hieu, Nguyen Manh Hung, Flavonoids from the aerial parts of *Oligoceras eberhardtii* Gagnep. and their cytotoxic evaluation, *Vietnam Journal of Chemistry and Applications*, 3B(71)/9-2024, 72-78.