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PHYTOCHEMICAL INVESTIGATION AND THE INHIBITORY EFFECTS ON α-GLUCOSIDASE, XANTHINE OXIDASE ENZYMES OF *VERNONIA AMYGDALINA* AND *VERNONIA GRATIOSA*

SUMMARY DOCTORAL DISSERTATION

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Reviewer 1:
Reviewer 2:
Reviewer 3:

The thesis will be defended at the academy-level doctoral dissertation committee at the Vietnam Academy of Science and Technology.
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I. INTRODUCTION

Nowadays, the incidence of metabolic diseases tends to increase rapidly, typically diabetes and gout. The prevalence and severity of these diseases have increased and caused the burden of health-care costs. Their treatment is just symptomatic treatment by drugs whose prolonged use causes a variety of side effects. Medicinal plants come into the spotlight as alternative therapeutics in immune disorders because of their proven safety with potent immunomodulatory effects.

Meanwhile, Vietnam is a tropical country and rich in natural resources with more than 12000 plant species, and more than 4000 of them are used as folk medicine, but there are still many species that have not been studied both biological effects and chemical composition. Therefore, continuing to search for effective, safe, and low-cost herbal medicines is still necessary. *Vernonia* is a large genus of the Asteraceae family with about 1000 species, distributed mainly in countries in South America, North America, Africa, and Southeast Asia. According to the Vietnamese Dictionary of Medicinal Plants (Vo Van Chi, 2012), in Vietnam, 16 species of the genus *Vernonia* are used as medicine to treat diseases such as dysentery, fever, malaria, hepatitis, stomach pain, eczema, and snakebites. bites, burns,...Almost all studies on the genus *Vernonia* mainly focus on botany, chemical composition, and biological activity. The results show that they contain many classes of substances with various biological activities such as steroids, flavonoids, terpenoids, and polyphenols, ... However, research on the genus *Vernonia* in Vietnam is still quite limited. Therefore, I have conducted research: **"Phytochemical investigation and the inhibitory effects on α-glucosidase, xanthine oxidase enzymes of *Vernonia amygdalina* and *Vernonia gratiosa*''.**

1. Subjects and contents of thesis

Subjects of dissertation study: 02 species of *Vernonia* genus: *Vernonia amygdalina* and *Vernonia gratiosa* collected in Vietnam.

The main contents of the thesis:

1.1. Isolate compounds from two species *V. amygdalina* and *V. gratiosa*

1.2. Elucidate the chemical structure of isolated compounds from *V. amygdalina* and *V. gratiosa*

1.3. Evaluate the inhibitory effects of isolated compounds from *V. amygdalina* and *V. gratiosa* on α-glucosidase

1.4. Evaluate the inhibitory effects of isolated compounds from *V. amygdalina* and *V. gratiosa* on xanthine oxidase (XO).

2. New contribution of the dissertation

2.1. This is the first time to isolate and identify 07 new compounds from *V. amygdalina* (**LD1-LD7**) named as vernonioside K (**LD1**), vernonioside N (**LD2**)**,** vernonioside M

(LD3), vernonioside O **(LD4**), vernonioside L **(LD5**), vernonioside P (**LD6**), vernonioside Q (**LD7**) and 07 new compounds from *V. gratiosa* (**VG1 – VG7**) as: vernogratioside C (**VG1**), vernogratioside D (**VG2**), vernogratioside E (**VG3**), vernogratioside R (**VG4**), vernogratioside S **(VG5),** vernoratioside A (**VG6**), vernoratioside B (**VG7**).

2.2. The inhibition of the α-glucosidase enzyme activity by isolated compounds from *V. amygdalina* và *V. gratiosa* was evaluated for the first time. The results indicated that **LD1**, **LD5**, **LD14**, **LD12**, and **LD15** from *V. amygdalina* showed strong inhibitory effects with the IC_{50} values from **7.42 ± 0.95** µM to **78.56 ± 7.28** µM (compared with acarbose **127.53 ± 1.73** µM). In addition, **VG5** from *V. gratiosa* also exhibited significant inhibitory activity with an IC_{50} value of **47.08 ± 3.98** µM whereas compounds **VG-13** and **VG-15** weakly inhibited α-glucosidase enzyme (acarbose 146.64 ± 8.85 µM).

2.3. This is the first time to examine the inhibitory effects on XO of isolated compounds from *V. amygdalina* và *V. gratiosa*. As a result, **VG5, VG13** và **VG15** exhibited potential inhibition of XO enzyme with IC_{50} values from (**6.26 ± 0.60 to 47.65 ± 3.44** µM) (Positive control, allopurinol: (**1.12 ± 0.15** µM).

3. The layout of the thesis

The dissertation includes 148 pages with 39 tables and 94 figures. The layout of the thesis: Introduction (2 pages), Chapter 1: Overview (35 pages), Chapter 2: Objects and Methods (16 pages), Chapter 3: Results and discussion (56 pages), Conclusion (1 page), Recommendation (1 page), Publications (1 page), and references (6 pages), and Supporting Information.

II. CONTENTS OF THESIS

PREAMBLE: Indicate scientific sense, practicality, object, objectives and tasks of the dissertation research.

CHAPTER 1: OVERVIEW

1.1. General introduction about the Asteraceae family

1.2. Introduction of *Vernonia* genus

1.3. Overview of phytochemical studies of the *Vernonia* genus

1.4. Overview of biological studies of the *Vernonia* genus

CHAPTER 2: OBJECTS AND METHODS

2.1. Plant materials

Figure 2.1. *V. amygdalina* (Hanoi)

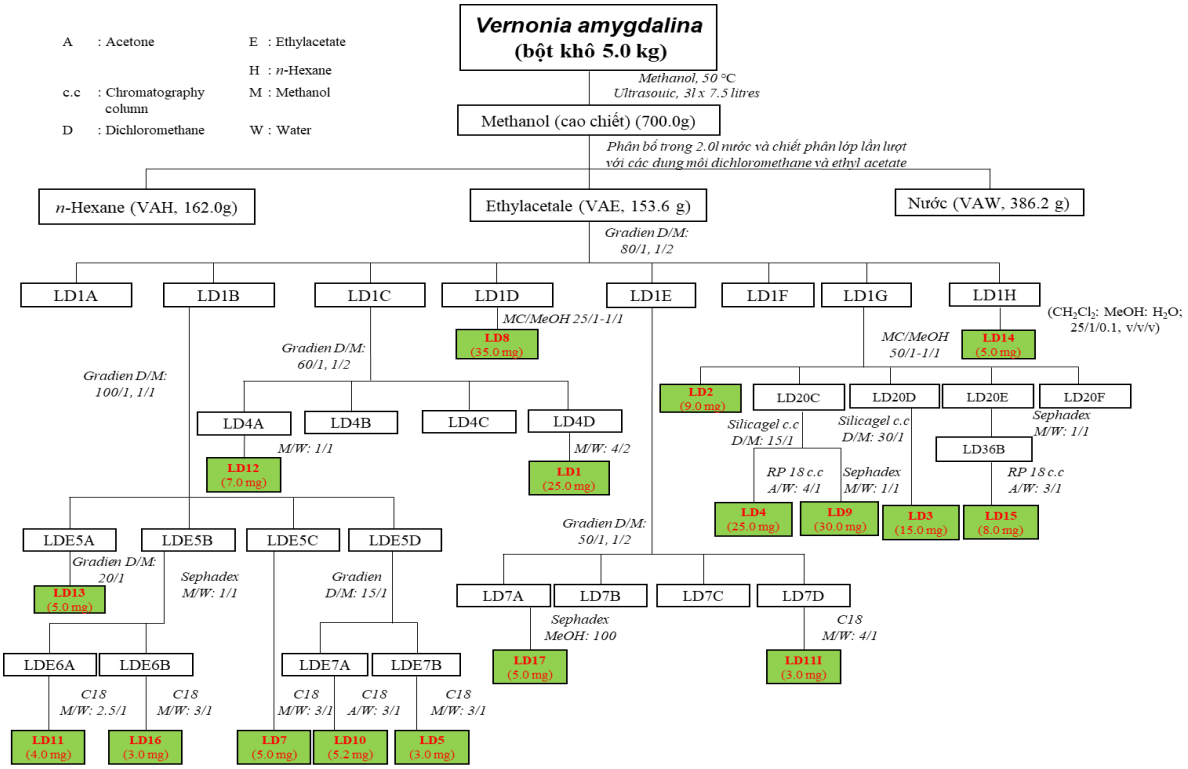
Figure 2.2. *V. gratiosa* (Quang Tri)

2.2. Methods

2.2.1. Extraction method

2.2.2. Isolation method

Combine chromatographic methods include thin-layer chromatography (TLC), and column chromatography (CC: silica gel, RP18 gel, LH-20 gel). Isolation schemes of



compounds from *V. amygdalina* and *V. gratiosa* were presented in Figures 2.3 and 2.4.

Figure 2.3. Isolation scheme of compounds from *V. amygdalina* in Vietnam

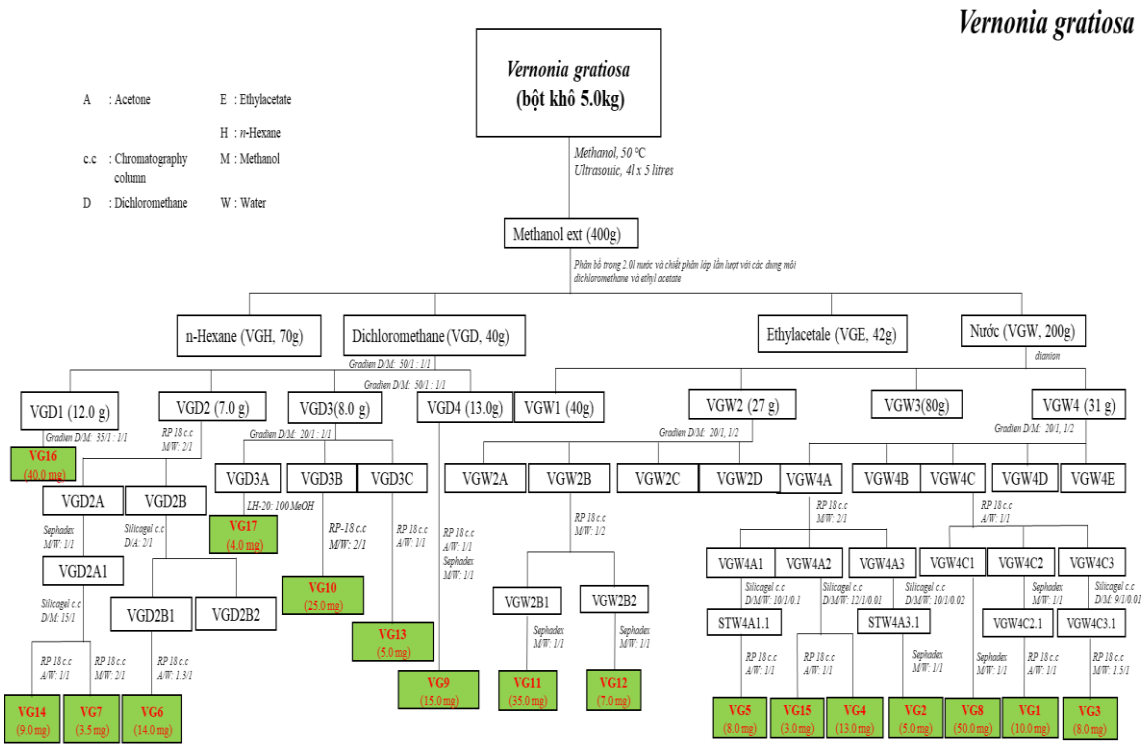


Figure 2.4. Isolation scheme of compounds from *V. gratiosa* in Vietnam

2.2.3. Structural elucidation of isolated compounds method

The general method to determine the chemical structure of the compound is a combination of physical parameters and modern spectroscopic methods including optical rotation ($[\alpha]_D$), mass spectrometry and high-resolution mass spectrometry, magnetic resonance spectrum (1D, 2D-NMR), CD spectrum.

2.2.4. Evaluate inhibitory effects of compounds on α-glucosidase and xanthine oxidase

2.4. Physical and spectroscopic data

2.4.1. Physical and spectroscopic data from compounds of V. amygdalina

*2.4.1.1. Compound **LD1:** vernonioside K (new compound)*

White powder; $[\alpha]_D^{25}$: + 75° (c 0.2, MeOH); HR-ESI-MS *m/z*: 583.3248 $[M+Na]^+$. ^{1}H and ^{13}C NMR data: see Table 3.1

*2.4.1.2. Compound **LD2:** Vernonioside N (new compound)*

White powder; $[\alpha]_D^{25}$: +55° (c 0.2, MeOH); HR-ESI-MS *m/z*: 697.3385 $[M+Na]^+$. ^{1}H NMR (600 MHz, pyridine-d_5): δ_H 1.16 (1H, m, H-1), 1.78 (1H, m, H-1), 2.10 (1H, m, H-2), 1.67 (1H, m, H-2), 3.89 (1H, m, H-3), 1.48 (1H, q, J = 10.0, 20.0 Hz, H-4), 1.98 (1H, m, H-4), 1.25 (1H, m, H-5), 1.76 (1H, m, H-6), 1.80 (1H, m, H-6), 5.35 (1H, brs, H-7), 5.39 (1H, d, J = 5.0 Hz, H-11), 2.18 (1H, m, H-12), 3.00 (1H, dd, J = 5.5, 15.0 Hz, H-12), 2.19 (1H, m, H-14), 1.49 (1H, m, H-15), 1.76 (1H, m, H-15), 1.70 (1H, m, H-16), 2.14 (1H, m, H-16), 2.26 (1H, m, H-17), 0.75 (3H, s, H-18), 0.80 (3H, s, H-19), 2.92 (1H, m, H-20), 4.56 (1H, t, J = 5.5 Hz, H-22), 4.81 (1H, d, J = 4.5 Hz, H-23), 2.24 (1H, m, H-25), 1.12 (3H, d, J = 6.0 Hz, H-26), 1.11 (3H, d, J = 5.5 Hz, H-27), 1.33 (3H,

s, H-29), 3.23 (3H, s, OCH_3), 4.94 (1H, d, *J* = 7.8 Hz, H-1'), 3.99 (1H, t, *J* = 7.5 Hz, H-2'), 4.21 (1H, t, *J* = 7.5 Hz, H-3'), 4.16 (1H, t, *J* = 7.5 Hz, H-4'), 3.92 (1H, m, H-5'), 4.30 (1H, dd, *J* = 5.0, 10.0 Hz, H-6'), 4.49 (1H, dd, *J* = 2.0, 10.0 Hz, H-6'); ^{13}C NMR (150 MHz, pyridine-d_5): δ_C 34.8 (C-1), 29.9 (C-2), 77.1 (C-3), 34.3 (C-4), 39.0 (C-5), 30.1 (C-6), 120.6 (C-7), 136.3 (C-8), 143.7 (C-9), 35.9 (C-10), 119.3 (C-11), 41.2 (C-12), 42.5 (C-13), 51.6 (C-14), 23.4 (C-15), 28.7 (C-16), 46.5 (C-17), 12.2 (C-18), 19.3 (C-19), 45.6 (C-20), 176.7 (C-21), 76.3 (C-22), 86.1 (C-23), 84.3 (C-24), 32.3 (C-25), 17.0 (C-26), 18.1 (C-27), 111.2 (C-28), 16.7 (C-29), 48.0 (OCH_3), 102.0 (C-1'), 74.9 (C-2'), 78.2 (C-3'), 71.4 (C-4'), 77.5 (C-5'), 62.5 (C-6').

*2.4.1.3. Compound **LD3:** Vernonioside M (new compound)*

White powder; $[\alpha]_D^{25}$: +45° (c 0.2, MeOH); HR-ESI-MS *m/z*: 697.3385 $[M+Na]^+$. ^{1}H NMR (600 MHz, pyridine-d_5): $\boldsymbol{\delta_H}$ 1.23 (1H, m, H-1), 1.85 (1H, m, H-1), 1.80 (1H, m, H-2), 3.95 (1H, m, H-3), 1.40 (1H, q, *J* = 10.0, 20.0 Hz, H-4), 2.00 (1H, m, H-4), 1.25 (1H, m, H-5), 1.14 (1H, m, H-6), 1.80 (1H, m, H-6), 5.37 (1H, brs, H-7), 5.48 (1H, d, *J* = 5.0 Hz, H-11), 2.18 (1H, m, H-12), 3.22 (1H, dd, *J* = 5.5, 15.0 Hz, H-12), 2.28 (1H, m, H-14), 1.47 (1H, dd, J = 5.0, 10.0, H-15), 1.81 (1H, m, H-15), 1.70 (1H, m, H-16), 2.14 (1H, m, H-16), 2.16 (1H, m, H-17), 0.71 (3H, s, H-18), 0.82 (3H, s, H-19), 2.85 (1H, m, H-20), 4.73 (1H, t, *J* = 5.5 Hz, H-22), 4.80 (1H, d, *J* = 4.5 Hz, H-23), 2.02 (1H, m, H-25), 0.92 (3H, d, *J* = 6.0 Hz, H-26), 1.17 (3H, d, *J* = 5.5 Hz, H-27), 1.33 (3H, s, H-29), 3.23 (3H, s, OCH_3), 4.94 (1H, d, *J* = 7.8 Hz, H-1'), 3.99 (1H, t, *J* = 7.5 Hz, H-2'), 4.21 (1H, t, *J* = 7.5 Hz, H-3'), 4.16 (1H, t, *J* = 7.5 Hz, H-4'), 3.95 (1H, m, H-5'), 4.30 (1H, dd, *J* = 5.0, 10.0 Hz, H-6'), 4.49 (1H, dd, *J* = 2.0, 10.0 Hz, H-6'); ^{13}C NMR (150 MHz, pyridine-d_5): δ_C 34.9 (C-1), 29.9 (C-2), 77.1 (C-3), 34.3 (C-4), 39.4 (C-5), 30.1 (C-6), 120.6 (C-7), 136.3 (C-8), 143.8 (C-9), 35.9 (C-10), 119.4 (C-11), 41.7 (C-12), 42.5 (C-13), 51.6 (C-14), 23.3 (C-15), 28.5 (C-16), 45.9 (C-17), 12.1 (C-18), 19.3 (C-19), 48.4 (C-20), 176.0 (C-21), 79.7 (C-22), 79.5 (C-23), 83.5 (C-24), 31.3 (C-25), 16.7 (C-26), 17.7 (C-27), 107.6 (C-28), 15.1 (C-29), 48.0 (OCH_3), 102.1 (C-1'), 74.9 (C-2'), 78.1 (C-3'), 71.4 (C-4'), 78.1 (C-5'), 62.5 (C-6').

*2.4.1.4. Compound **LD4:** Vernonioside O (new compound)*

White powder; $[\alpha]_D^{25}$: +48.0° (c 0.2, MeOH); Phổ HR-ESI-MS *m/z*: 667.3308 $[M+Cl]^-$. ^{1}H NMR (600 MHz, pyridine-d_5): $\boldsymbol{\delta_H}$ 1.23 (1H, m, H-1), 1.80 (1H, m, H-1), 1.67 (1H, m, H-2), 1.22 (1H, m, H-2), 3.93 (1H, m, H-3), 1.99 (1H, q, *J* = 10.0, 20.0 Hz, H-4α), 2.00 (1H, m, H-4β), 1.22 (1H, m, H-5), 1.23 (1H, m, H-6), 1.78 (1H, m, H-6), 5.43 (1H, brs, H-7), 5.48 (1H, d, *J* = 5.0 Hz, H-11), 2.13 (1H, d, *J* = 14.5 Hz, H-12), 3.09 (1H, dd, *J* = 5.5, 14.5 Hz, H-12), 2.22 (1H, m, H-14), 1.44 (1H, m, H-15), 1.74 (1H, m, H-15), 1.57 (1H, dd, *J* = 7.5, 20.0 Hz, H-16), 2.37 (1H, m, H-16), 2.23 (1H, m, H-17), 0.76 (3H, s, H-18), 0.80 (3H, s, H-19), 2.87 (1H, m, H-20), 4.80 (1H, t, *J* = 4.0 Hz, H-22), 4.93 (1H, d, *J* = 4.0 Hz, H-23), 2.05 (1H, m, H-25), 1.07 (3H, d, *J* = 6.0 Hz, H-26), 1.18 (3H, d, *J* = 5.5 Hz, H-27), 4.27 (1H, m, H-28), 1.28 (3H, d, *J* = 5.5 Hz H-29),

4.96 (1H, d, *J* = 6.5 Hz, H-1'), 3.99 (1H, t, *J* = 7.5 Hz, H-2'), 4.21 (1H, t, *J* = 7.5 Hz, H-3'), 4.16 (1H, t, *J* = 7.5 Hz, H-4'), 3.11 (1H, m, H-5'), 4.50 (1H, dd, *J* = 12.5 Hz, H-6'), 4.49 (1H, dd, *J* = 5.0, 12.5 Hz, H-6'); ^{13}C NMR (150 MHz, pyridine-d_5): δ_C 34.8 (C-1), 29.9 (C-2), 78.1 (C-3), 35.3 (C-4), 39.0 (C-5), 30.1 (C-6), 120.6 (C-7), 136.8 (C-8), 143.8 (C-9), 35.9 (C-10), 119.3 (C-11), 41.4 (C-12), 42.5 (C-13), 51.6 (C-14), 23.3 (C-15), 28.7 (C-16), 46.8 (C-17), 14.4 (C-18), 19.9 (C-19), 46.6 (C-20), 177.1 (C-21), 79.1 (C-22), 8.29 (C-23), 82.0 (C-24), 30.7 (C-25), 17.1 (C-26), 17.8 (C-27), 81.0 (C-28), 14.0 (C-29), 102.0 (C-1'), 74.9 (C-2'), 78.1 (C-3'), 71.4 (C-4'), 77.1 (C-5'), 62.5 (C-6').

*2.4.1.5. Compound **LD5:** vernonioside L (new compound)*

White powder; $[\alpha]_D^{25}$: +45.7° (c 0.2, MeOH); HR-ESI-MS *m/z*: 669.3402 $[M+Cl]^-$. ^{1}H NMR (600 MHz, pyridine-d_5): δ_H 1.15 (1H, m, H-1), 1.77 (1H, m, H-1), 1.84 (1H, m, H-2), 2.15 (1H, m, H-2), 3.90 (1H, m, H-3), 1.17 (1H, q, *J* = 10.0, 20.0 Hz, H-4), 1.77 (1H, m, H-4), 1.24 (1H, m, H-5), 1.65 (1H, m, H-6), 2.08 (1H, m, H-6), 5.33 (1H, s, H-7), 5.54 (1H, s, H-11), 2.47 (1H, m, H-12), 2.20 (1H, dd, *J* = 6.6, 17.4 Hz, H-12), 2.23 (1H, m, H-14), 1.52 (1H, m, H-15), 1.82 (1H, m, H-15), 1.47 (1H, m, H-16), 2.33 (1H, m, H-16), 1.99 (1H, m, H-17), 0.75 (3H, s, H-18), 0.80 (3H, s, H-19), 1.97 (1H, m, H-20), 5.80 (1H, s, H-21), 4.55 (1H, m, H-22), 4.59 (1H, m, H-23), 4.53 (1H, m, H-24), 1.79 (1H, m, H-25), 1.05 (3H, d, *J* = 5.0 Hz, H-26), 1.15 (3H, d, *J* = 5.0 Hz, H-27), 4.53 (1H, m, H-28), 1.17 (3H, d, *J* = 6.5 Hz, H-29), 4.94 (1H, d, *J* = 6.5 Hz, H-1'), 3.96 (1H, m, H-2'), 4.22 (1H, m, H-3'), 4.18 (1H, m, H-4'), 4.22 (1H, m, H-5'), 4.31 (1H, m, H-6'), 4.57 (1H, m, H-6'); ^{13}C NMR (150 MHz, pyridine-d_5): δ_C 34.9 (C-1), 29.9 (C-2), 77.1 (C-3), 34.9 (C-4), 39.0 (C-5), 30.0 (C-6), 120.7 (C-7), 136.4 (C-8), 143.9 (C-9), 35.9 (C-10), 118.9 (C-11), 41.5 (C-12), 42.4 (C-13), 51.3 (C-14), 23.6 (C-15), 27.8 (C-16), 44.9 (C-17), 12.7 (C-18), 19.3 (C-19), 50.7 (C-20), 98.9 (C-21), 87.4 (C-22), 82.5 (C-23), 81.9 (C-24), 31.5 (C-25), 17.4 (C-26), 17.6 (C-27), 84.5 (C-28), 14.5 (C-29), 48.0 (OCH_3), 102.0 (C-1'), 74.9 (C-2'), 78.1 (C-3'), 71.4 (C-4'), 78.1 (C-5'), 62.5 (C-6').

*2.4.1.6. Compound **LD6**: vernonioside P (new compound)*

Yellow oil; $[\alpha]_D^{25}$: –31.6 (c 0.2, $CHCl_3$); HR-ESI-MS *m/z*: 551.3118 $[M + Cl]^-$. ^{1}H NMR (600 MHz, pyridine-d_5): $\boldsymbol{\delta_H}$ 1.33 (1H, m, H-1), 2.00 (1H, m, H-1), 1.28 (1H, m, H-2), 1.71 (1H, m, H-2), 3.35 (1H, m, H-3), 1.44 (1H, m, H-4), 1.86 (1H, brd, *J* = 1.5, 10.5 Hz, H-4), 1.41 (1H, m, H-5), 1.30 (1H, m, H-6), 1.92 (1H, m, H-6), 5.40 (1H, brs, H-7), 5.53 (1H, d, *J* = 5.0 Hz, H-11), 2.24 (1H, m, H-12), 2.34 (1H, dd, *J* = 5.5, 16.8 Hz, H-12), 2.42 (1H, m, H-14), 1.67 (1H, d, *J* = 5.5 Hz, H-15), 1.95 (1H, m, H-15), 5.18 (1H, t, *J* = 5.5 Hz, H-16), 2.26 (1H, m, H-17), 0.59 (3H, s, H-18), 0.93 (3H, s, H-19), 1.72 (1H, m, H-20), 1.05 (1H, d, *J* = 5.5 Hz, H-21), 0.94 (1H, m, H-22), 4.09 (1H, dd, *J* = 4.0, 7.0 Hz, H-23), 4.06 (1H, dd, *J* = 4.0, 6.5 Hz, H-24), 1.35 (3H, s, H-26), 1.37

(3H, s, H-27), 3.02 (1H, q, *J* = 5.0, 9.5 Hz, H-28), 1.56 (3H, d, *J* = 5.0 Hz, , H-29), 2.05 (3H, s, $OCOCH_3$), ^{13}C NMR (150 MHz, pyridine-d_5): δ_C 35.9 (C-1), 30.9 (C-2), 71.4 (C-3), 34.7 (C-4), 40.6 (C-5), 31.0 (C-6), 122.1 (C-7), 136.3 (C-8), 145.3 (C-9), 37.0 (C-10), 119.2 (C-11), 43.7 (C-12), 43.8 (C-13), 50.1 (C-14), 38.4 (C-15), 80.9 (C-16), 64.0 (C-17), 13.0 (C-18), 19.9 (C-19), 33.0 (C-20), 20.2 (C-21), 39.9 (C-22), 71.4 (C-23), 70.4 (C-24), 72.0 (C-25), 28.4 (C-26), 26.9 (C-27), 57.0 (C-28), 14.3 (C-29), 21.5 ($OCOCH_3$), 172.8 ($OCOCH_3$).

*2.4.1.7. Compound **LD7**: Vernonioside Q (new compound)*

Amorphous white powder; $[\alpha]_D^{25}$: –37.1 (c 0.2, $CHCl_3$); HR-ESI-MS: *m/z* 713.3690 $[M+Cl]^-$. ^1H NMR (600 MHz, pyridine-d_5): $\boldsymbol{\delta_H}$ 1.32 (1H, m, H-1), 1.98 (1H, m, H-1), 1.30 (1H, m, H-2), 1.96 (1H, m, H-2), 3.71 (1H, m, H-3), 1.30 (1H, m, H-4), 1.96 (1H, m, H-4), 1.40 (1H, m, H-5), 1.30 (1H, m, H-6), 1.96(1H, m, H-6), 5.38 (1H, brs, H-7), 5.50 (1H, d, *J* = 5.5 Hz, H-11), 2.24 (1H, d, *J* = 16.8 Hz, H-12), 2.34 (1H, dd, *J* = 5.5, 16.8 Hz, H-12), 2.39 (1H, m, H-14), 0.89 (1H, m, H-15), 1.51 (1H, m, H-15), 4.06 (1H, dd, *J* = 4.0, 7.0 Hz, H-16), 1.47 (1H, dd, *J* = 5.0, 9.0 Hz, H-17), 0.50 (3H, s, H-18), 0.90 (3H, s, H-19), 1.67 (1H, d, *J* = 6.0 Hz, H-20), 1.53 (1H, d, *J* = 5.0 Hz, H-21), 0.91 (1H, m, H-22), 5.16 (1H, d, *J* = 6.0 Hz, H-23), 1.34 (3H, s, H-26), 1.36 (3H, s, H-27), 3.00 (1H, q, *J* = 5.0, 9.5 Hz, H-28), 1.03 (3H, d, *J* = 5.5 Hz, H-29), 2.03 (3H, s, $OCOCH_3$), 4.39 (1H, d, *J* = 6.5 Hz, H-1'), 3.13 (1H, d, *J* = 1.5, 8.0 Hz, H-2'), 3.26 (1H, m, H-3'), 3.27 (1H, m, H-4'), 3.34 (1H, m, H-5'), 3.64 (1H, d, *J* = 4.5, 10.0 Hz, H-6'), 3.85 (1H, d, *J* = 1.5, 10.0 Hz, H-6'); ^{13}C NMR (150 MHz, pyridine-d_5): δ_C 35.9 (C-1), 30.6 (C-2), 78.9 (C-3), 34.7 (C-4), 40.5 (C-5), 31.0 (C-6), 122.1 (C-7), 136.3 (C-8), 145.3 (C-9), 37.1 (C-10), 119.2 (C-11), 43.7 (C-12), 43.8 (C-13), 50.1 (C-14), 39.9 (C-15), 80.9 (C-16), 64.0 (C-17), 13.0 (C-18), 19.9 (C-19), 33.0 (C-20), 20.2 (C-21), 39.9 (C-22), 71.4 (C-23), 70.4 (C-24), 72.0 (C-25), 28.4 (C-26), 26.9 (C-27), 57.0 (C-28), 14.3 (C-29), 21.5 ($OCOCH_3$), 172.9 ($OCOCH_3$), 102.4 (C-1"), 75.2 (C-2"), 77.9 (C-3"), 71.7 (C-4"), 78.1 (C-5"), 62.8 (C-6").

*2.4.1.8. Compound **LD8**: (22R,23S,24R,28S)-28-methoxy-7,8,9,11-tetradehydro-3β-16α,21,24 tetrahydroxy-21,23:22,28-diepoxy-5α- stigmastane).*

*2.4.1.9. Compound **LD9:** Vernoamyoside E*

*2.4.1.10. Compound **LD10:** Vernonioside B_2*

*2.4.1.11. Compound **LD11:** Vernoniacum B*

*2.4.1.12. Compound **LD12:*** (23*S*,24*R*,28*S*)-3*β*,22*α*-dihydroxy-7,8,9,11-tetradehydro-24,28-epoxy-5*α*-stigmastane-21,23-carbolactone

*2.4.1.13. Compound **LD13:** Vemonioside B_1*

*2.4.1.14. Compound **LD14**: Veramyoside H*

*2.4.1.15. Compound **LD15**: Veramyoside J*

*2.4.1.16. Compound **LD16**: Vernoamyoside A*

*2.4.1.17. Compound **LD17:** α-spinasterol*

2.4.2. Physical and spectroscopic data from compounds of V. gratiosa.

*2.4.2.1. Compound **VG1:** Vernogratiosides A (new compound)*

White powder; $[\alpha]_D^{25}$: –28 (c 0.02, MeOH); HR-ESI-MS *m/z*: 831.3892 $[M+Cl]^-$. ^1H and ^{13}C NMR data: see Table 3.4

*2.4.2.2. Compound **VG2 :** Vernogratioside B (new compound)*

White powder; $[\alpha]_D^{25}$: –32 (c 0.2, MeOH); CD (c 5×10^{-4}, MeOH); HR-ESI-MS *m/z*: 779.4212 $[M-H]^-$ và *m/z* $[M+Cl]^-$ 815.3984. ^1H NMR (600 MHz, pyridine-d_5): δ_H 1.36 (1H, m, H-1), 2.00 (1H, m, H-1), 1.60 (1H, m, H-2), 2.02 (1H, m, H-2), 3.72 (1H, m, H-3), 1.39 (1H, m, H-4), 1.90 (1H, m, H-4), 1.40 (1H, m, H-5), 1.95 (1H, m, H-6), 5.43 (1H, brs, H-7), 5.52 (1H, d, *J* = 6.5 Hz, H-11), 2.02 (1H, m, H-12), 2.24 (1H, m, H-12), 2.23 (1H, m, H-14), 1.14 (1H, m, H-15), 1.64 (1H, m, H-15), 1.52 (1H, m, H-16), 1.92 (1H, m, H-16), 2.19 (1H, m, H-17), 0.63 (3H, s, H-18), 0.94 (3H, s, H-19), 2.57 (1H, m, H-20), 1.84 (1H, m, H-22), 2.10 (1H, m, H-22), 1.85 (1H, m, H-23), 2.05 (1H, m, H-23), 1.85 (1H, m, H-25), 1.00 (3H, d, *J* = 7.0 Hz, H-26), 1.03 (3H, d, *J* = 7.0 Hz, H-27), 3.95 (1H, q, *J* = 6.5 Hz, H-28), 1.19 (3H, d, *J* = 6.5 Hz, H-29), 4.56 (1H, d, *J* = 7.5 Hz, H-1'), 3.42 (1H, dd, *J* = 8.0, 9.0 Hz, H-2'), 3.29 (1H, m, H-3'), 3.34 (1H, m, H-4'), 3.58 (1H, m, H-5'), 3.68 (1H, d, *J* = 5.5, 12.0 Hz, H-6'), 3.87 (1H, d, *J* = 2.0, 12.0 Hz, H-6'), 4.51 (1H, d, *J* = 8.0 Hz, H-1''), 3.64 (1H, m, H-2''), 3.53 (1H, m, H-3''), 3.88 (1H, m, H-4''), 3.56 (1H, m, H-5''), 3.77 (2H, m, H-6''); ^{13}C NMR (150 MHz, pyridine-d_5): δ_C 36.0 (C-1), 30.6 (C-2), 78.9 (C-3), 35.1 (C-4), 40.6 (C-5), 31.0 (C-6), 121.6 (C-7), 137.3 (C-8), 145.4 (C-9), 37.1 (C-10), 119.5 (C-11), 41.2 (C-12), 43.6 (C-13), 52.6 (C-14), 23.7 (C-15), 26.6 (C-16), 50.5 (C-17), 12.3 (C-18), 20.0 (C-19), 41.8 (C-20), 178.4 (C-21), 23.7 (C-22), 23.0 (C-23), 91.14 (C-24), 36.17 (C-25), 17.2 (C-26), 17.6 (C-27), 78.8 (C-28), 17.9 (C-29), 101.4 (C-1'), 83.6 (C-2'), 77.7 (C-3'), 71.5 (C-4'), 77.7 (C-5'), 62.7 (C-6'), 106.2 (C-1''), 73.5 (C-2''), 74.7 (C-3''), 70.0 (C-4''), 77.1 (C-5''), 62.1 (C-6'').

*2.4.2.3. Compound **VG3:** Vernogratioside C (new compound)*

White powder; $[\alpha]_D^{25}$: –33° (c 0.2, MeOH); HR-ESI-MS: *m/z* $[M+H]^+$ 797.4316 và $[M+Na]^+$ 819.4140. ^1H NMR (600 MHz, pyridine-d_5): δ_H 1.48 (1H, m, H-1), 1.85 (1H, m, H-1), 1.70 (1H, m, H-2), 2.09 (1H, m, H-2), 3.73 (1H, m, H-3), 1.43 (1H, m, H-4), 1.82 (1H, m, H-4), 1.48 (1H, m, H-5), 1.21 (1H, m, H-6), 2.02 (1H, m, H-6), 1.49 (1H, m, H-7), 1.84 (1H, m, H-7), 3.06 (1H, m, H-8), 5.46 (1H, m, H-11), 1.84 (1H, m, H-12), 2.17 (1H, m, H-12), 2.12 (1H, m, H-14), 2.11 (1H, m, H-15), 2.60 (1H, m, H-15), 1.82

(1H, m, H-17), 0.75 (3H, s, H-18), 1.26 (3H, s, H-19), 2.57 (1H, m, H-20), 1.65 (1H, m, H-22), 2.10 (1H, m, H-22), 1.85 (1H, m, H-23), 2.04 (1H, m, H-23), 1.94 (1H, m, H-25), 1.00 (3H, d, *J* = 7.0 Hz, H-26), 1.03 (3H, d, *J* = 7.0 Hz, H-27), 3.95 (1H, q, *J* = 6.5 Hz, H-28), 1.19 (3H, d, *J* = 6.5 Hz, H-29), 4.55 (1H, d, *J* = 7.5 Hz, H-1'), 3.41 (1H, dd, *J* = 7.5, 9.0 Hz, H-2'), 3.29 (1H, m, H-3'), 3.32 (1H, m, H-4'), 3.58 (1H, m, H-5'), 3.67 (2H, d, *J* = 5.5, 12.0 Hz, H-6'), 4.51 (1H, d, *J* = 8.0 Hz, H-1''), 3.62 (1H, dd, *J* = 8.0, 9.5 Hz, H-2''), 3.52 (1H, dd, *J* = 8.0, 9.5 Hz, H-3''), 3.87 (1H, m, H-4''), 3.55 (1H, m, H-5''), 3.77 (2H, d, *J* = 6.5 Hz, H-6''); ^{13}C NMR (150 MHz, pyridine-d_5): δ_C 35.4 (C-1), 30.2 (C-2), 79.3 (C-3), 36.1 (C-4), 44.0 (C-5), 27.0 (C-6), 26.6 (C-7), 54.2 (C-8), 145.3 (C-9), 39.1 (C-10), 119.8 (C-11), 39.6 (C-12), 42.5 (C-13), 49.7 (C-14), 46.1 (C-15), 212.2 (C-16), 47.1 (C-17), 12.5 (C-18), 18.0 (C-19), 41.1 (C-20), 178.4 (C-21), 23.9 (C-22), 23.0 (C-23), 91.2 (C-24), 36.2 (C-25), 17.2 (C-26), 17.6 (C-27), 71.9 (C-28), 17.9 (C-29), 101.4 (C-1'), 83.7 (C-2'), 77.8 (C-3'), 71.5 (C-4'), 77.9 (C-5'), 62.7 (C-6'), 106.2 (C-1''), 73.7 (C-2''), 74.7 (C-3''), 70.1 (C-4''), 77.1 (C-5''), 62.3 (C-6'').

*2.4.2.4. Compound **VG4:** Vernogratioside R (new compound)*

Amorphous white powder; $[\alpha]_D^{25}$: –35° (c 0.2, MeOH); HR-ESI-MS: *m/z* $[M+Cl]^-$ 873.4033. ^{1}H NMR (600 MHz, CD_3OD): δ_H 1.36 (1H, m, H-1), 2.00 (1H, m, H-1), 1.60 (1H, m, H-2), 2.02 (1H, m, H-2), 3.73 (1H, m, H-3), 1.40 (1H, m, H-4), 1.90 (1H, m, H-4), 1.42 (1H, m, H-5), 1.21 (1H, m, H-6), 1.95 (1H, m, H-6), 5.43 (1H, brs, H-7), 5.56 (1H, brs, H-11), 2.17 (1H, m, H-12), 2.52 (1H, m, H-14), 1.81 (1H, m, H-15), 2.04 (1H, m, H-15), 5.28 (1H, t, *J* = 7.5 Hz, H-16), 2.62 (1H, m, H-17), 0.60 (3H, s, H-18), 0.94 (3H, s, H-19), 2.65 (1H, m, H-20), 1.85 (1H, m, H-22), 1.96 (1H, m, H-22), 1.84 (1H, m, H-23), 2.13 (1H, m, H-23), 1.87 (1H, m, H-25), 1.01 (3H, d, *J* = 7.0 Hz, H-26), 1.04 (3H, d, *J* = 6.5 Hz, H-27), 3.95 (1H, q, *J* = 6.5 Hz, H-28), 1.15 (3H, d, *J* = 6.5 Hz, H-29), 4.56 (1H, d, *J* = 7.5 Hz, H-1'), 3.42 (1H, d, *J* = 8.5 Hz, H-2'), 3.30 (1H, m, H-3'), 3.34 (1H, m, H-4'), 3.57 (1H, m, H-5'), 3.67 (1H, m, H-6'), 3.87 (1H, m, H-6'), 4.52 (1H, d, *J* = 8.0 Hz, H-1''), 3.63 (1H, m, H-2''), 3.52 (1H, m, H-3''), 3.86 (1H, m, H-4''), 3.55 (1H, m, H-5''), 3.77 (2H, m, H-6''); ^{13}C NMR (150 MHz, CD_3OD): δ_C 35.9 (C-1), 30.5 (C-2), 79.8 (C-3), 35.1 (C-4), 40.5 (C-5), 30.9 (C-6), 122.2 (C-7), 136.1 (C-8), 145.4 (C-9), 37.1 (C-10), 119.0 (C-11), 41.2 (C-12), 44.3 (C-13), 49.9 (C-14), 33.4 (C-15), 77.4 (C-16), 56.7 (C-17), 13.9 (C-18), 20.0 (C-19), 10.2 (C-20), 178.3 (C-21), 23.9 (C-22), 25.2 (C-23), 90.7 (C-24), 37.2 (C-25), 16.8 (C-26), 17.5 (C-27), 71.8 (C-28), 17.8 (C-29), 101.3 (C-1'), 73.5 (C-2'), 74.7 (C-3'), 70.0 (C-4'), 77.0 (C-5'), 62.7 (C-6'), 106.2 (C-1''), 73.5 (C-2''), 74.7 (C-3''), 70.0 (C-4''), 77.0 (C-5''), 62.1 (C-6'').

*2.4.2.5. Compound **VG5:** Vernogratioside S (new compound)*

Amorphous yellow powder.; $[\alpha]_D^{25}$: +45.7° (c 0.2, MeOH); HR-ESI-MS: *m/z* 817.4067 $[M+Cl]^-$. ^{1}H NMR (600 MHz, CD_3OD): δ_H 1.34 (1H, m, H-1), 2.00 (1H, m, H-1), 1.61 (1H, m, H-2), 2.01 (1H, m, H-2), 3.73 (1H, m, H-3), 1.39 (1H, m, H-4), 1.92 (1H, m, H-4), 1.40 (1H, m, H-5), 1.34 (1H, m, H-6), 1.98 (1H, m, H-6), 5.42 (1H, brs, H-7), 5.49 (1H, brs, H-11), 2.16 (1H, m, H-12), 2.12 (1H, m, H-14), 1.63 (1H, m, H-15), 2.07 (1H, m, H-15), 2.07 (1H, m, H-16), 1.40 (1H, m, H-16), 1.82 (1H, m, H-17), 0.60 (3H, s, H-18), 0.94 (3H, s, H-19), 2.24 (1H, m, H-20), 1.82 (1H, d, *J* = 9.0 Hz, H-22), 1.16 (1H, m, H-23, H-24), 1.16 (3H, s, H-26), 1.17 (3H, s, H-27), 1.63 (1H, m, H-28), 0.99 (3H, t, *J* = 7.2 Hz, H-29), 4.55 (1H, d, *J* = 7.5 Hz, H-1'), 3.41 (1H, dd, *J* = 7.5, 8.5 Hz, H-2'), 3.30 (1H, m, H-3'), 3.36 (1H, m, H-4'), 3.57 (1H, t, *J* = 8.5 Hz, H-5'), 3.67 (1H, dd, *J* = 5.5, 12.0 Hz, H-6'), 3.87 (1H, dd, *J* = 2.5, 12.0 Hz, H-6'), 4.51 (1H, d, *J* = 7.5 Hz, H-1''), 3.63 (1H, dd, *J* = 8.0, 10.0 Hz, H-2''), 3.52 (1H, dd, *J* = 3.0, 10.0 Hz, H-3''), 3.88 (1H, m, H-4''), 3.53 (1H, m, H-5''), 3.76 (2H, d, *J* = 6.0 Hz, H-6''); ^{13}C NMR (150 MHz, CD_3OD): δ_C 36.0 (C-1), 30.6 (C-2), 79.9 (C-3), 35.1 (C-4), 40.5 (C-5), 31.0 (C-6), 121.7 (C-7), 137.3 (C-8), 145.3 (C-9), 37.1 (C-10), 119.4 (C-11), 41.1 (C-12), 43.2 (C-13), 52.5 (C-14), 33.3 (C-15), 28.6 (C-16), 53.9 (C-17), 11.6 (C-18), 19.9 (C-19), 50.1 (C-20), 178.5 (C-21), 23.8 (C-22), 29.6 (C-23), 52.8 (C-24), 74.7 (C-25), 27.3 (C-26), 27.1 (C-27), 24.7 (C-28), 14.3 (C-29), 101.4 (C-1'), 83.7 (C-2'), 77.8 (C-3'), 71.5 (C-4'), 77.7 (C-5'), 62.7 (C-6'), 106.2 (C-1''), 73.5 (C-2''), 74.5 (C-3''), 70.0 (C-4''), 77.1 (C-5''), 62.1 (C-6'').

*2.4.2.6. Compound **VG6:** Vernoratioside A (new compound)*

Amorphous white powder; HR-ESI-MS: *m/z*: 741.3583 [M + Cl]; $[\alpha]_D^{25}$: +44.5 (c 0.1, MeOH); ^{1}H NMR (600 MHz, pyridine-d_5): δ_H 1.38 (1H, m, H-1), 2.02 (1H, m, H-1), 1.73 (1H, m, H-2), 2.00 (1H, m, H-2), 3.73 (1H, m, H-3), 1.35 (1H, m, H-4), 1.90 (1H, m, H-4), 1.43 (1H, m, H-5), 1.94 (2H, m, H-6), 5.43 (1H, m, H-7), 3.06 (1H, m, H-8), 5.55 (1H, m, H-11), 2.18 (1H, m, H-12), 2.25 (1H, m, H-12), 2.52 (1H, m, H-14), 1.76 (1H, m, H-15), 5.35 (1H, t, *J* = 7.0 Hz, H-16), 2.13 (1H, m, H-17), 0.65 (3H, s, H-18), 0.94 (3H, s, H-19), 2.25 (1H, m, H-20), 4.93 (1H, d, *J* = 6.5 Hz, H-21), 4.47 (1H, t, J = 4.5 Hz, H-22), 4.39 (1H, brd, *J* = 4.0 Hz, H-23), 1.88 (1H, m, H-25), 0.97 (3H, d, *J* = 6.0 Hz, H-26), 1.03 (3H, d, *J* = 6.5 Hz, H-27), 3.81 (1H, q, *J* = 6.5 Hz, H-28), 1.18 (3H, d, *J* = 6.5 Hz, H-29), 2.00 (3H, s, 16-CH_3COO), 3.48 (3H, s, 21-OCH_3); ^{13}C NMR (150 MHz, pyridine-d_5): δ_C 36.0 (C-1), 30.6 (C-2), 78.9 (C-3), 35.0 (C-4), 40.5 (C-5), 31.0 (C-6), 122.3 (C-7), 136.3 (C-8), 145.3 (C-9), 37.1 (C-10), 119.0 (C-11), 41.1 (C-12), 44.1 (C-13), 50.1 (C-14), 34.2 (C-15), 79.1 (C-16), 55.0 (C-17), 13.5 (C-18), 19.9 (C-19), 52.7 (C-20), 110.6 (C-21), 83.8 (C-22), 83.7 (C-23), 83.6 (C-24), 31.1 (C-25),

17.5 (C-26), 18.1 (C-27), 82.7 (C-28), 14.1 (C-29), 102.4 (C-1'), 75.1 (C-2'), 78.1 (C-3'), 71.7 (C-4'), 77.9 (C-5'), 62.8 (C-6'), 172.3 (16-CH_3COO), 21.6 (16-CH_3COO), 56.7 (21-OCH_3).

2.4.2.7. Compound **VG7**: Vernoratioside B (new compound)

Amorphous white powder; $[\alpha]_D^{25}$:+ 30.6 c 0.2, MeOH); HR-ESI-MS: *m/z* 727.3466 $[M+Cl]^-$. ^1H NMR (600 MHz, pyridine-d_5): δ_H 1.39 (1H, m, H-1), 2.04 (1H, m, H-1), 1.63 (1H, m, H-2), 2.00 (1H, m, H-2), 3.72 (1H, m, H-3), 1.36 (1H, m, H-4), 1.90 (1H, m, H-4), 1.43 (1H, m, H-5), 1.95 (2H, m, H-6), 5.44 (1H, m, H-7), 5.59 (1H, brd, *J* = 5.5 Hz, H-11), 2.24 (2H, m, H-12), 2.53 (1H, m, H-14), 1.80 (1H, m, H-15), 5.30 (1H, t, *J* = 7.0 Hz, H-16), 1.92 (1H, m, H-17), 0.64 (3H, s, H-18), 0.94 (3H, s, H-19), 1.78 (1H, m, H-20), 5.54 (1H, s, H-21), 4.31 (1H, m, H-22), 4.90 (1H, m, H-23), 1.90 (1H, m, H-25), 0.92 (3H, d, *J* = 7.0 Hz, H-26), 0.93 (3H, d, *J* = 7.5 Hz, H-27), 4.10 (1H, q, *J* = 6.5 Hz, H-28), 1.12 (3H, d, *J* = 8.0 Hz, H-29), 2.00 (3H, s, 16-CH_3COO); ^{13}C NMR (150 MHz, pyridine-d_5): δ_C 35.9 (C-1), 30.6 (C-2), 78.9 (C-3), 35.0 (C-4), 40.5 (C-5), 31.0 (C-6), 122.3 (C-7), 136.3 (C-8), 145.2 (C-9), 37.2 (C-10), 119.2 (C-11), 41.1 (C-12), 44.1 (C-13), 50.1 (C-14), 34.3 (C-15), 79.2 (C-16), 55.1 (C-17), 14.0 (C-18), 19.9 (C-19), 53.0 (C-20), 101.7 (C-21), 77.3 (C-22), 83.6 (C-23), 91.9 (C-24), 30.6 (C-25), 17.4 (C-26), 18.0 (C-27), 81.2 (C-28), 20.1 (C-29), 102.4 (C-1'), 75.2 (C-2'), 78.1 (C-3'), 71.7 (C-4'), 77.9 (C-5'), 62.8 (C-6'), 172.3 (16-$CH_3\underline{C}OO$), 21.7 (16-$\underline{C}H_3COO$).

*2.4.2.8. Compound **VG8: VE1***

*2.4.2.9. Compound **VG9**: Vernoniacum B*

*2.4.2.10. Compound **VG10:** Kaempferol*

*2.4.2.11. Compound **VG11**: Quercetin 3-0-methyl ether*

*2.4.2.12. Compound **VG12:** Quercetin*

*2.4.2.13. Compound **VG13:** Apigenin*

*2.4.2.15. Compound **VG14**: Syringaresinol-β-D-glucoside*

*2.4.2.16.Compound **VG15**: 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3 hydroxy-1-(E)-propenyl)-2,6-dimethoxy phenoxy]propyl-β-D-glucopyranoside*

*2.4.2.17. Compound **VG16**: 11β,13-dihydrovernolide*

*2.4.2.18. Compound **VG17**: 5-(methoxymethyl)-1H-pyrrole-2-carbaldehyde*

CHAPTER 3: RESULTS AND DISCUSSION

3.1. Determination of chemical structures of isolated compounds from *V. amygdalina*

17 compounds (**LD1**-**LD17**) were isolated from the leaves of *V. amygdalina*, including 07 new stigmastane steroids (**LD1- LD7)** and 10 known ones (**LD8 – LD17)**. Their structures were elucidated by the NMR, HR-ESI-MS, and previous published data.

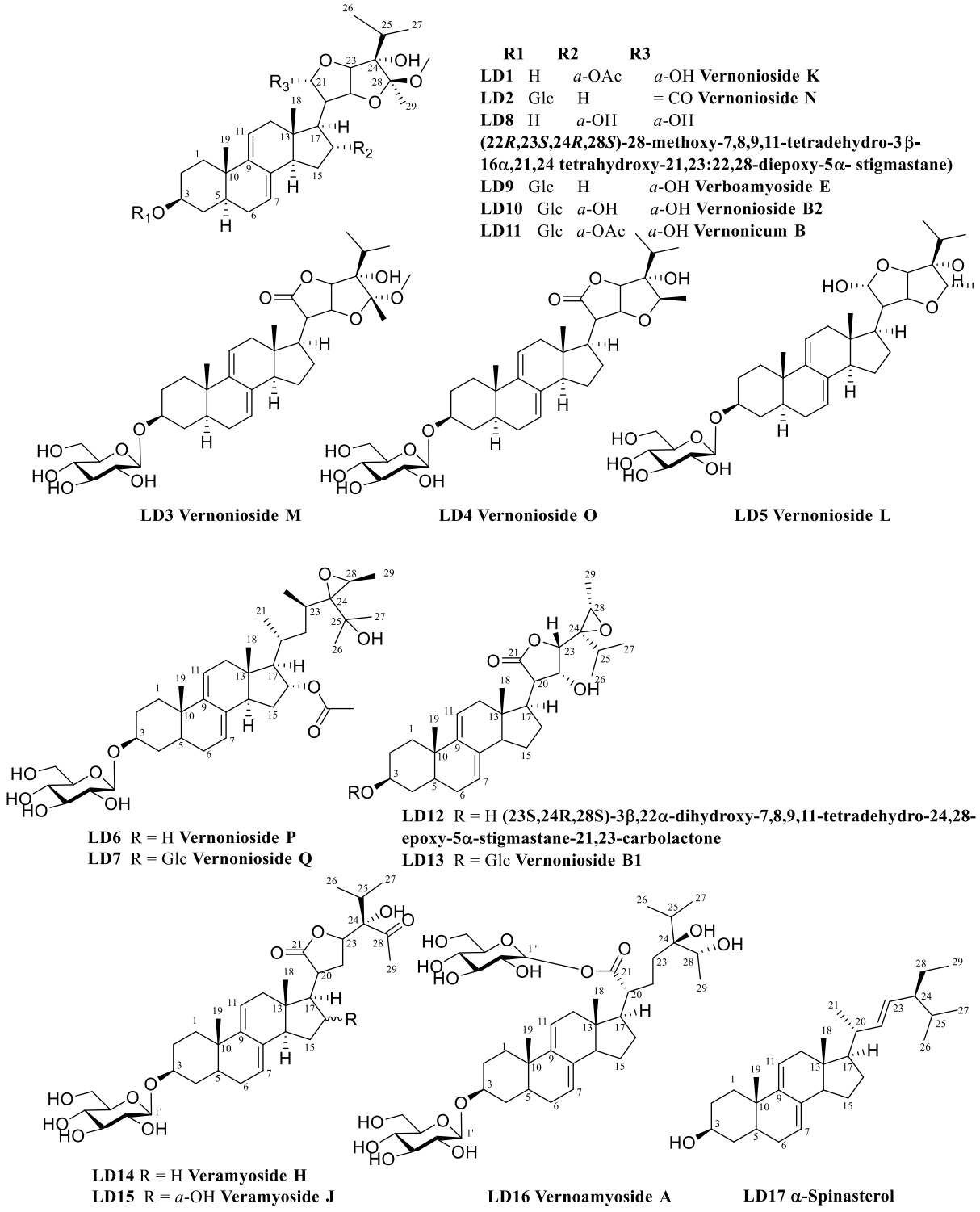
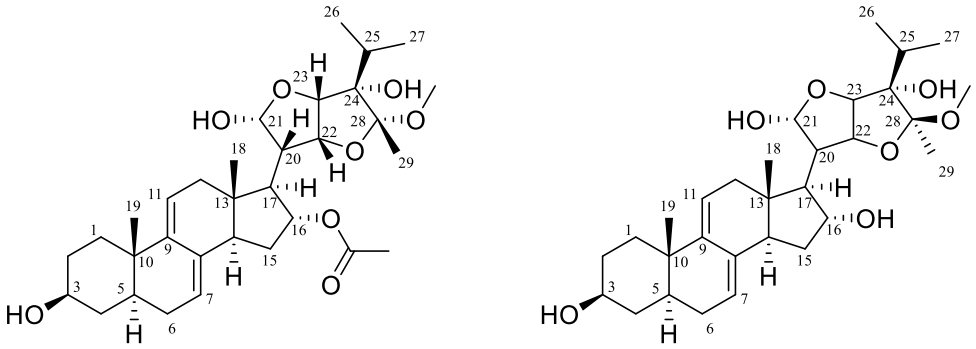


Figure 3.1. Chemical structures of isolated compounds from *V. amygdalina*

3.1.1. Compound LD1: Vernonioside K (new compound)

Compound **LD1** was obtained as a white amorphous solid. Electrospray ionization high-resolution time-of-flight mass spectrometry (ESI-HR-TOF-MS) revealed a quasi-molecular ion peak at *m/z* 583.3248 [M + Na]+ (calcd. for $C_{32}H_{48}NaO_8^+$, 583.3241), suggesting a molecular formula of $C_{32}H_{48}O_8$. The ^1H and ^{13}C nuclear magnetic resonance (NMR) data of **LD1** showed the characteristic signals of $\Delta^{7,9(11)}$ stigmastane-type steroidal skeleton. The ^1H NMR spectrum of **LD1** revealed the presence of two olefinic protons [δ_H 5.33 (1H, brs, H-7), 5.44 (1H, d, J = 6.0 Hz, H-11)], a distinctive H-

3 multiplet [δ_H 3.78 (1H, m, H-2)], an isopropyl group [δ_H 1.11 (3H, d, J = 6.6 Hz, H-26), 1.23 (3H, d, J = 6.6 Hz, H-27)], two angular methyl protons [δ_H 0.61 (3H, s, H-18), 0.86 (3H, s, H-19)], another methyl proton [δ_H 1.59 (3H, s, H-29)], an acetylate methyl group [δ_H 2.21 (3H, s, 16-OAc)], and a methoxyl group [δ_H 3.29 (3H, s, 28-OCH_3)]. ^{13}C NMR showed 29 carbon signals, including six quaternary carbons, six methylene groups, four olefinic carbons, an acetyl group, five methyl groups, and a methoxy group. The planar structure of **LD1** was further supported by the heteronuclear multiple bond correlation (HMBC) spectrum. The HMBC correlations between H-11 and C-8/C-9/C-10/C-13 and between H-7 and C-5/C-8/C-9/C-15 indicated that the two double bonds were at 7(8) and 9(11) positions. The HMBC cross-peaks from H-16 to δ_C 170.8 (CH_3COO) indicated that the acetyl group was located at C-16. For the side chain, the HMBC spectrum showed the connection of H-26/H-27 and C-24, suggesting that the isopropyl moiety was attached to C-24. Additionally, the positions of methoxy and methyl groups at C-28 were deduced by the HMBC correlation of 28-OCH_3/H-29 with C-28. The HMBC cross-peaks from H-20 to C-21/C-22, from H-21 to C-22/C-23, from H-22 to C-20/C-23, and from H-23 to C-21/C-24 confirmed the presence of two furan rings, which were connected *via* C-22 and C-23. Finally, the side chain was attached to C-17 by the HMBC correlation of H-17 and C-20. The nuclear Overhauser effect spectroscopy (NOESY) correlations between H-3 and H-5, H-14 and H-17, and H-18 and H-16 and H-19, indicated that rings A/B and C/D fused in trans; H-16, H-18, and H-19 were in the β configuration; and H-3, H-5, and H-17 were in the α configuration. Furthermore, the NOESY correlation between H-17 and 28-OCH3 indicated that these protons adopted the α configuration, whereas correlations between H-20 and H-18/H-21/H-27, between H-23 and H-22/H-27/H-29 indicated that these protons were in the β configuration. Therefore, the stereochemistry of the side chain was determined as shown in Figure 3.2. Finally, the structure of compound **LD1** was elucidated and named vernonioside K.



LD1 **LD1a**

Figure 3.2. Chemical structures of compounds **LD1** and reference compound **LD1a**

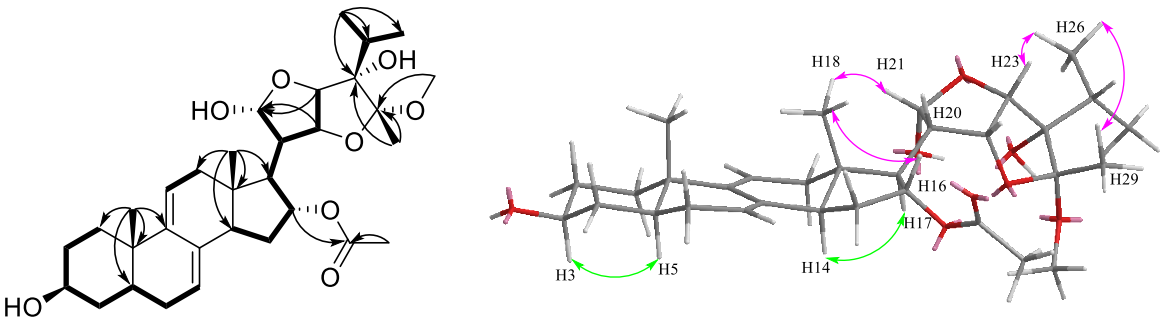
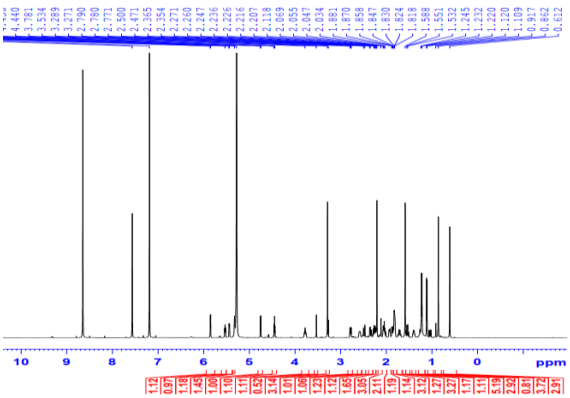


Figure 3.3. Key COSY, HMBC, and NOESY correlations of compound **LD1**

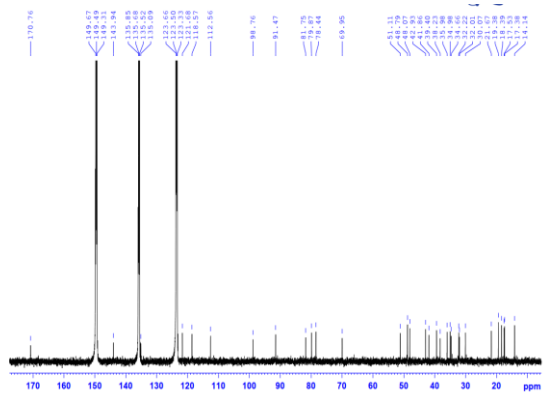
Table 3.1. ^{1}H (500 MHz), ^{13}C NMR (125 MHz) spectroscopic data of **LD1** and reference compound

C	$^{\#}\delta_C$ [a]	δ_C [a]	δ_H [a] (mult., J = Hz)
1	35.2	35.0	1.23, m *
2	32.5	32.0	1.71, m *
3	70.2	70.0	3.78, m
4	38.8	38.2	1.54, q (12.0)
			1.49, d, (12.0)
5	39.6	39.4	1.40, m
6	30.4	30.1	1.82, m *
7	121.5	121.7	5.33, brs
8	135	135.1	
9	144.2	143.9	
10	36.2	36.0	
11	118.6	118.6	5.44, d (6.0)
12	41.8	41.9	2.35, dd (16.8, 6.6)
			2.49, d (17.4)
13	43.7	42.9	
14	49.2	48.8	2.58, m
15	35.3	34.7	1.92, m *
16	76.3	78.4	5.53, t (6.0)
17	56.1	51.1	2.79, dd (11.4, 5.4)
18	14.6	14.1	0.61, s
19	19.7	19.4	0.86, s
20	48.6	48.8	2.58, m
21	99.2	98.8	2.22, m *
22	81.0	79.9	4.45, t, (6.0)
23	91.2	91.5	4.75, d (6.0)
24	82.0	81.8	
25	32.4	32.2	2.27, m *
26	17.5	17.4	1.11, d (6.6)
27	18.5	18.4	1.23, d (6.6)
28	113.4	112.7	
29	17.5	17.5	1.59, s
28-OCH_3	48.5	48.1	3.29, s
CH_3COO		170.8	
$\underline{C}H_3OO$		21.7	2.21, s

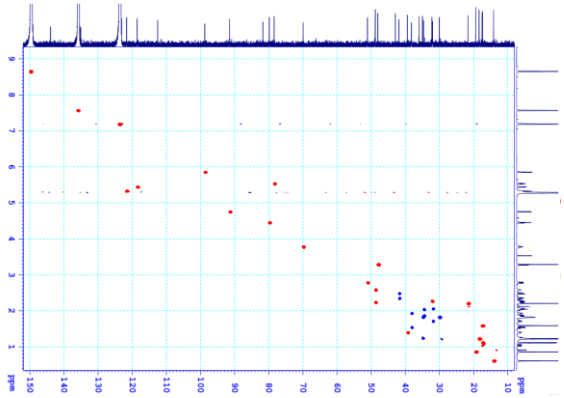
[a]pyridine-d_5, $^{\#}\delta_C$ LD1a. (*) overlapped



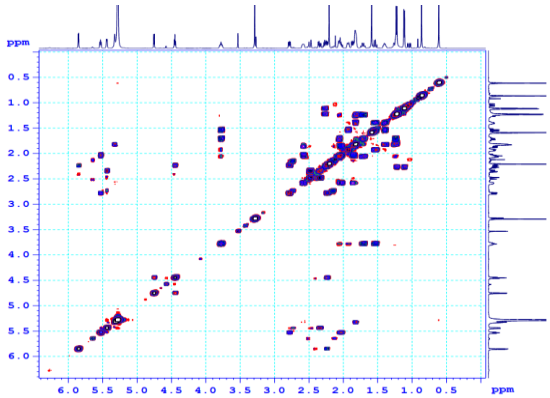
^{1}H-NMR spectrum of compound **LD1**



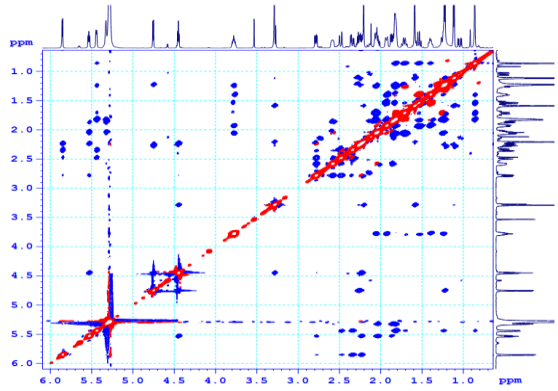
^{13}C-NMR spectrum of compound **LD1**



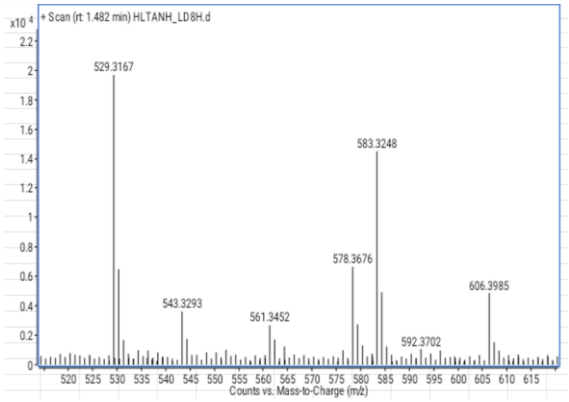
HSQC spectrum of compound **LD1**



COSY spectrum of compound **LD1**



ROESY spectrum of compound **LD1**



HR-ESI-MS spectrum of **LD1**

3.1.2. Compound LD6: Vernonioside P (new compound)

Compound **LD6** was isolated as a white crystalize. The molecular formula of **LD6** was determined as $C_{31}H_{48}O_6$ by the HR-ESI-MS spectrum at *m/z* 551.3118 $[M+Cl]^-$ (calcd. for $C_{29}H_{39}O_4Cl^-$, 551.3139). The ^{1}H NMR data of **LD6** showed typical signals of a stigmastane steroid skeleton and was similar to those of compounds **LD1**-**LD5**, except for the structure of the side chain part. The ^{1}H NMR spectrum of **LD6** displayed the presence of two doublet methyl groups [δ_H 1.05 (1H, d, J = 5.5 Hz, H-21), 1.56 (1H, d, J = 5.0 Hz, H-29)], two *tert*-methyl group [δ_H 1.35 (3H, s, H-26), 1.37 (3H, s, H-27)], two oxymethine groups [δ_H 4.93 (1H, dd, J = 4.0, 7.0 Hz, H-23), 3.02 (1H, q, J = 4.0, 9.5 Hz, H-28)], and a methine and a methylene group in the upfield region [1.72 (1H, m, H-20), 0.94 (2H, m, H-22)] for the side chain, which was confirmed by the COSY

correlations of H-20/ H-21/ H-22/ H-23 as well as HMBC correlation of H-21 and C-20/ C-22, H-23 với C-24/ C-28/ C-25, H-29 and C-24/ C-28, H-26/H-27 and C-24/ C-25. The relative configuration of **LD6** was identified by the NOESY spectrum. The ROESY spectrum revealed the cross-peaks from H-16/ H-18, H-18/ H-19, H-18/ H-20 suggesting the β configuration of these protons. Besides, the correlations of H-3 /H-5, H-14/ H-17, H-17/ H-21, H-21/ H-23, H-23/ H-28 were observed in the NOESY spectrum of **LD6**, which indicated that protons H-3, H-5, H-14, H-17, H-21, H-23, and H-28 had α-form. Therefore, the structure of **LD6** was established as shown in Figure 3.4 and named vernonioside P.

Table 3.2. ^{1}H (125 MHz) và ^{13}C NMR-(500 MHz) spectroscopic data of **LD6** and reference compound

C	#δ_C[a]	δ_C[a]	δ_H[a](mult., *J* in Hz)
1	35.9	35.9	1.33, m/ 2.00, m
2	30.6	30.9	1.28, m/ 1.71, m
3	78.9	71.4	3.35, m
4	34.7	34.7	1.44, m/ 1.86, br d (1.5, 10.5)
5	39.9	40.6	1.41, m
6	30.6	31.0	1.30, m
7	122.1	122.1	5.40, br s
8	136.9	136.3	-
9	145.2	145.3	-
10	37.1	37.0	-
11	119.2	119.2	5.53, d (5.5)
12	40.5	43.7	2.24, d (16.8)
13	43.6	43.8	-
14	50.1	50.1	2.42, m
15	34.7	38.4	1.67, d (5.5)/ 1.95, m
16	70.0	80.9	5.18, t (5.5)
17	57.0	64.0	1.49, dd (9.0, 5.0)
18	14.2	13.0	0.59, s
19	19.9	19.9	0.93, s
20	33.0	33.0	1.72, m
21	13.0	20.2	1.05, d (5.5)
22	47.8	39.9	0.94, m
23	80.9	71.4	4.09, dd (7.0, 4.0)
24	71.0	70.4	4.06, dd (6.5, 4.0)
25	70.0	72.0	-
26	28.4	28.4	1.35, s
27	26.9	26.9	1.37, s
28	63.9	57.0	3.02, q (9.5, 5.0)
29	14.3	14.3	1.56, d (5.0)
$OCO\underline{C}H_3$	21.5	21.5	2.05, s
$O\underline{C}OCH_3$	172.8	172.8	
1'	102.4		
2'	75.1		
3'	77.9		
4'	71.7		
5'	78.1		
6'	62.8		

[a] in pyridine, #δ_C reference compound

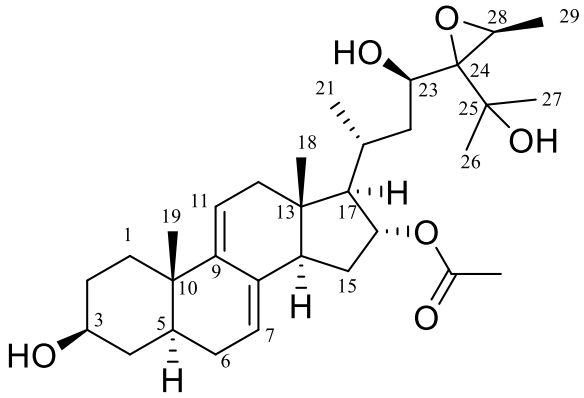
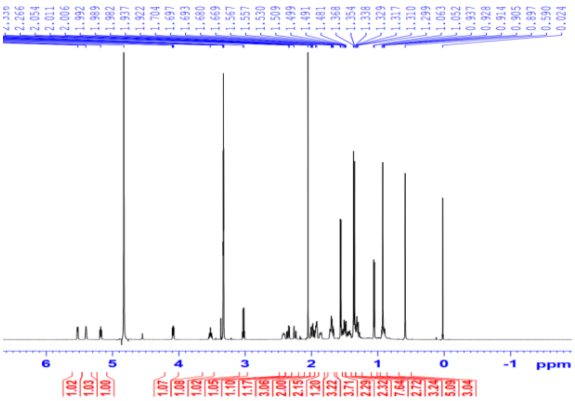
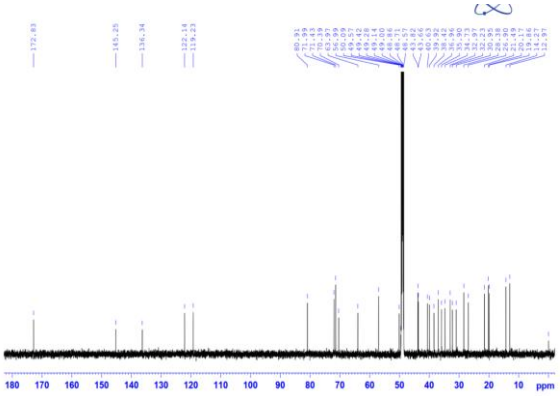


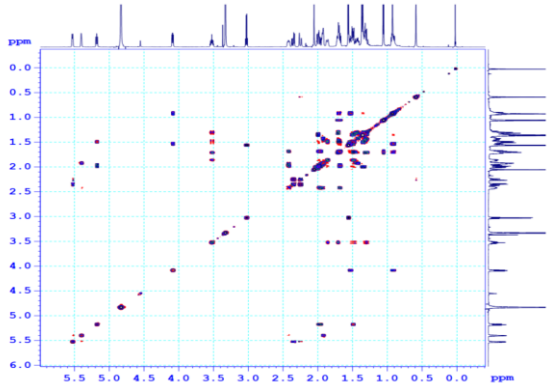
Figure 3.4. Chemical structure of compound **LD6**



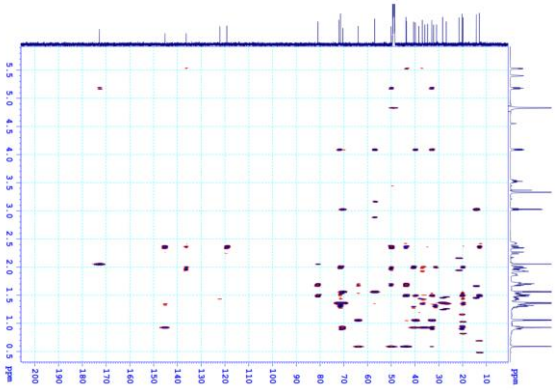
^{1}H NMR spectrum of **LD6**



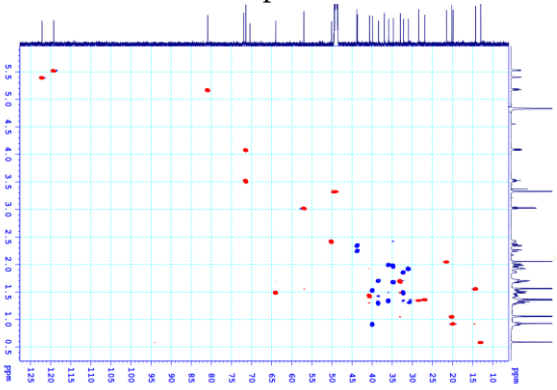
^{13}C NMR spectrum of **LD6**



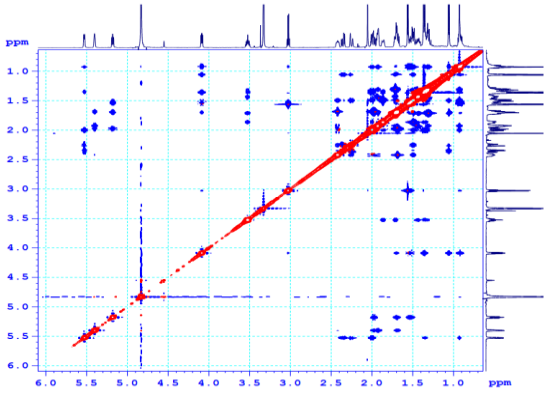
COSY NMR spectrum of **LD6**



HMBC spectrum of **LD6**

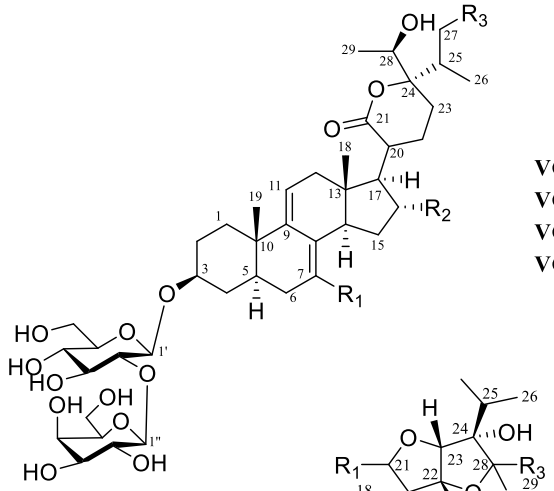


HSQC spectrum of **LD6**

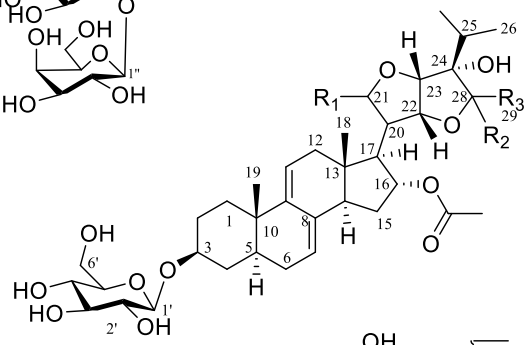


ROESY spectrum of **LD6**

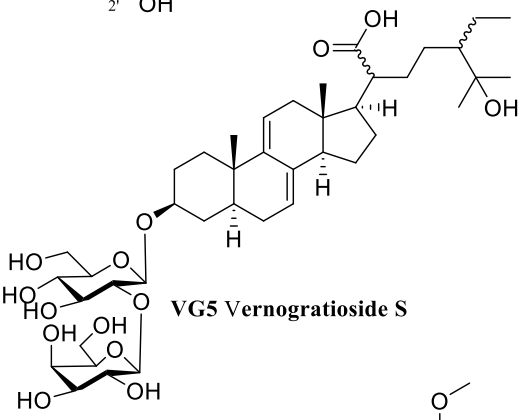
3.2. Identification of chemical structures of isolated compounds from *Vernonia gratiosa*



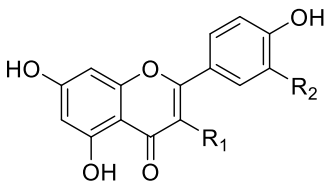
	R_1	R_2	R_3	
VG1	H	H	OH	**Vernogratioside A**
VG2	H	H	CH_3	**Vernogratioside B**
VG3	H_2	C=O	CH_3	**Vernogratioside C**
VG4	H	*a*-OAc	CH_3	**Vernogratioside R**



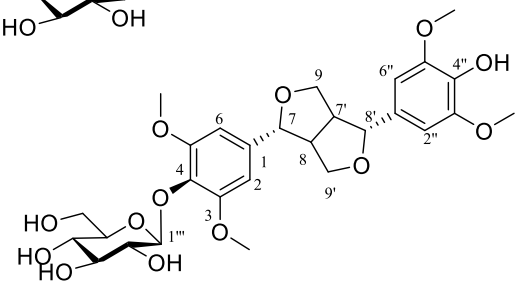
VG5 R_1 = β-OCH_3, R_2 = H, R_3 = β-CH_3 **Vernoratioside A**
VG6 R_1 = α-OH, R_2 = H, R_3 = α-CH_3 **Vernoratioside B**
VG8 R_1 = α-OH, R_2 = H, R_3 = β-CH_3 **VE_1**
VG7 R_1 = α-OH, R_2 = α-CH_3, R_3 = β-OCH_3 **Vernoniacum B**



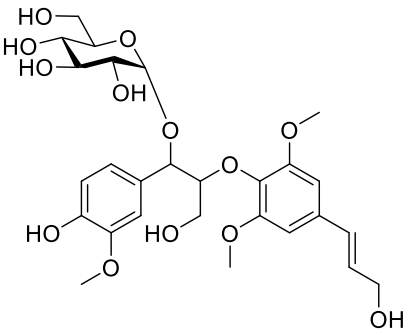
VG5 Vernogratioside S



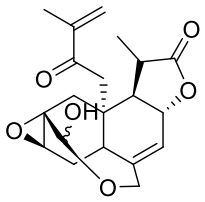
	R_1	R_2	
VG10	OH	H	**Kaempferol**
VG11	OCH_3	OH	**Quercetin 3-*O*-methyl ether**
VG12	OH	OH	**Quercetin**
VG13	H	H	**Apigenin**



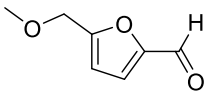
VG14 Syringaresinol-β-D-glucoside



VG15 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3 hydroxy-1-(E)-propenyl)-2,6-dimethoxy phenoxy]propyl-β-D-glucopyranoside



VG16 11β,13-dihydrovernolide



VG17 5-(methoxymethyl)-1H-pyrrole-2-carbaldehyde

Figure 3.5. Chemical structures of isolated compounds from *V. gratiosa*

3.3.1. Hợp chất VG1; Vernogratioside A (New compound)

Table 3.4. ^{1}H (500 MHz) và ^{13}C-NMR (125 MHz) spectroscopic data of **VG1**

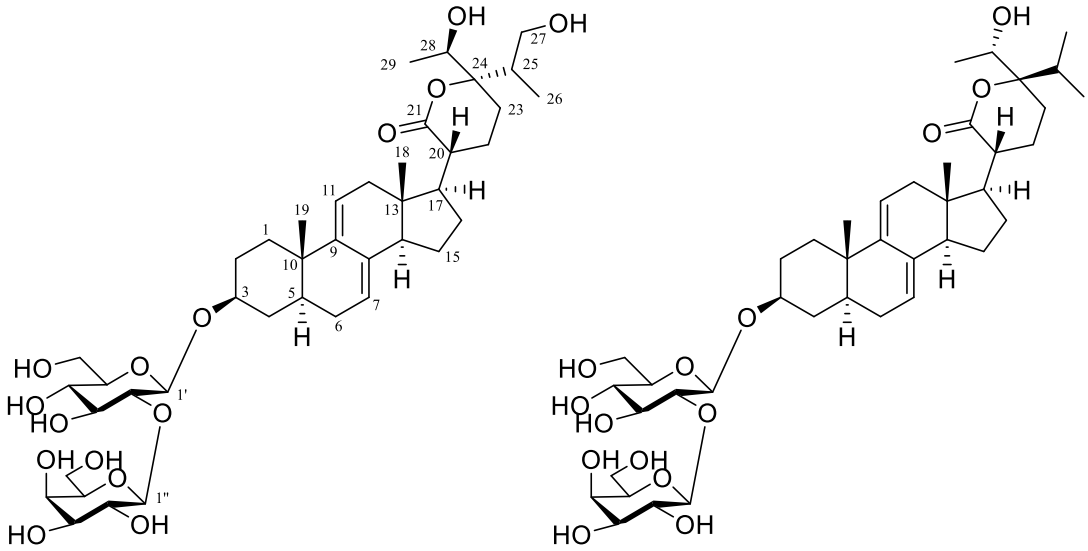
C	**VG1**			C			
	$^{\#}\delta_C$[a]	δ_C[a,b]	δ_H[a, c] (Độ bội *J* = Hz)		$^{\#}\delta_C$[a]	δ_C[a, b]	δ_H[a, c] (Độ bội *J* = Hz)
1	36,0	36.0	1.33, m, 2.00, m	21	178.	177.	
2	30,6	30.6	1.61, m, 2.02, m	22	23.7	27.4	1.14, m, 1.55, m
3	79,9	79.9	3.72, m	23	23.0	23.5	1.60, m
4	35,1	35.1	1.40, m, 1.90, m	24	91.1	91.1	
5	40,6	40.6	1.39, m	25	36.2	43.4	2.12, m
6	31,0	31.0	1.96, m	26	17.2	12.6	1.06, s
7	121,6	121.7	5.43, s	**27**	**17.6**	**63.9**	**3.45, m, 3.80, m**
8	137,3	137.2		28	71.8	71.1	3.91, m
9	145,4	145.4		29	17.9	17.6	1.26, d (6.5)
10	37.1	37.1		1′	101.	101.	4.55, d (7.5)
11	119.5	119.4	5.50, d (6.5)	2′	83.7	83.6	3.42, m
12	41.2	41.0	1.96, m, 2.23, m	3′	77.7	77.7	3.29, m
13	43.6	43.2		4′	71.5	71.5	3.34, m
14	52.6	52.8	1.15, m	5′	77.7	77.8	3.60, m
15	23.7	23.7	1.81, m, 1.48, m	6′	62.7	62.7	3.68, m, 3.89, m
16	26.6	27.4	1.98, m, 1.50, m	1″	106.	106.	4.51, d (8.0)
17	50.4	50.7	1.79, m	2″	73.5	73.5	3.64, m
18	12.3	11.9	0.61, s	3″	74.7	74.7	3.53, m
19	20.0	19.9	0.95, s	4″	70.0	70.0	3.89, m
20	41.7	41.9	2.55, m	5″	77.0	77.0	3.55, m
21	178.4	177.4		6″	62.1	62.1	3,77, m

[a] đo trong CD_3OD, $^{\#}\delta_C$ hợp chất tham khảo

Compound **VG1** was yielded as a white amorphous powder with the molecular formula $C_{41}H_{64}O_{15}$, which was identified from its HR-ESI-MS at *m/z* [M+Cl]- 831.3892; (calcd for $C_{41}H_{64}ClO_{15}^-$, 831.3939). The NMR data of **VG1** were determined based on 1D, 2D NMR, and ECD analyses, and by comparison with previously reported vernocuminosides. These data showed that **VG1** is a $\Delta^{7,9(11)}$ stigmastan type steroid saponin with a δ-lactone ring system. Indeed, the ^{1}H NMR representation of **VG1** displayed signals of two olefinic protons [δ_H 5.43 (1H, s, H-7) and 5.50 (1H, brd, *J* = 5.5 Hz, H-11)], a distinctive H-3 multiplet [δ_H 3.72 (1H, m, H-3), two angular methyls [δ_H 0.66 (3H, s, H-18) and 0.94 (3H, s, H-19)], a propanyl-1-ol unit [δ_H 2.12 (1H, m, H-25), 1.06 (3H, d, *J* = 7.0 Hz, H-26), and 3.45 (2H, m, H-27)], and another doublet methyl [δ_H 1.26 (3H, d, *J* = 6.5 Hz, H-29)]. In addition, two sets of proton signals

for glucopyranosyl and galactopyranosyl units, along with their anomeric protons [δ_H 4.55 (1H, d, J = 7.5 Hz, H-1′) and 4.51 (1H, d, J = 8.0 Hz, H-1″), were shown in the ^{1}H NMR spectrum. The large coupling constants (J = 7.5 Hz between H-1′ and H-2′, J = 8.0 Hz between H-1″ and H-2″) supported *β*-linkage of the sugar moieties. The ^{13}C NMR data of **VG1** revealed 42 carbon resonances, containing 29 for the aglycone moiety and 12 for the two sugar units. The ^{13}C NMR data showed the existence of a carbonyl [δ_C 177.4 (C-21), four olefinic carbons [δ_C 121.7 (C-7), 137.2 (C-8), 145.4 (C-9), 119.4 (C-11)], two oxygenated methine carbons [δ_C 79.9 (C-3), 71.1 (C-28), and four methyl carbons [δ_C 11.9 (C-18), 19.9 (C-19), 12.6 (C-26), 17.6 (C-29)] for the aglycone moiety. The existence of the *δ*-lactone unit in the side chain of **VG1** was deduced by the connectivities of H-20/H-22/ H-23, H-25/H-26/H-27, and H-28/H-29, together with the HMBC correlations between H-22 and C-21/C-24, H-26 and C-24/C-25/C-27, and H-29 and C-24/C-28. The HMBC correlations from H-17 to C-20/C-21 allowed us to determine the location of the *δ*-lactone unit at C-17 of the aglycone of **VG1**. The HMBC from H-1′ (*δ*H 4.55) to C-3 (δ_C 79.9) demonstrated that the *β*-D-glucosyl group was connected to C-3. The position of the galactopyranosyl moiety at C-2′ was estimated from a downfield shift of C-2′ (*δ*C 83.6) in **VG1** compared to C-2′ (*δ*C 75.1) of glucose in vernocuminoside H, as well as the long-range HMBC correlation of H-1″ (δ_H 4.51) with C-2′ (δ_C 83.7). The comparison between the NMR values of 1 and the reported NMR data showed that **VG1** has similar NMR values to vernocuminoside I (Ver I), which was recently purified from the stem bark *V. cumingiana* Benth [20]. The main difference is the replacement of an oxygenated methylene group in **VG1** by a methyl group in Ver I. This was also confirmed by the HMBC correlations of H-27 with C-24, C-25, and C-26. Thus, the planar structure of **VG1** was deduced. The stereochemistry of **VG1** was defined based on NOESY correlations. In particular, the NOESY cross-peaks H-3/H-5, H-14/H-17, and H-18/H-19 indicated that A/B and C/D fused in *trans*, H-18 and H-19 had *β*-configurations, and H-3, H-5, and H-17 had α-configurations. In addition, the NOESY correlation of H-18/H-20 showed the relationship between the lactone ring E and the β-orientation of H-20 showed the relationship between the lactone ring E and the positioning of H-20 in a β-structure. The stereochemistry of C-24 and C-28 (Fig. 2) was determined from the NOESY cross-peaks from H-26 to H-28. The stereochemistry of C-24 was deduced based on the ECD spectrum. The ECD spectrum of **VG1** showed the opposite signals to those of vernocuminoside H, a new saponin reported from the *Vernonia* genus. Indeed, the circular dichroism spectrum of **VG1** showed λmax (mdeg) 221 (+2.82), and 243 (+10.78) nm. Thus, the absolute configuration of C-24 was assigned as *S*-form based on the established correlation between the absolute configuration and the Cotton effect's sign. Moreover, the stereochemistry of C-28 in **VG1** has been remaining due to the small amount. Finally, the identification of the sugar residues as D-glucose and D-galactose was established through the absolute configurations obtained from the acid hydrolysis of **VG1**. This was further confirmed by comparing the results with authentic samples through TLC analysis.

As a result, compound **VG1** was found to be a new compound and was named vernogratioside A.



VG1 **VG1a**

Figure 3.6. Chemical structure of compound **VG1** and reference compound **VG1a**

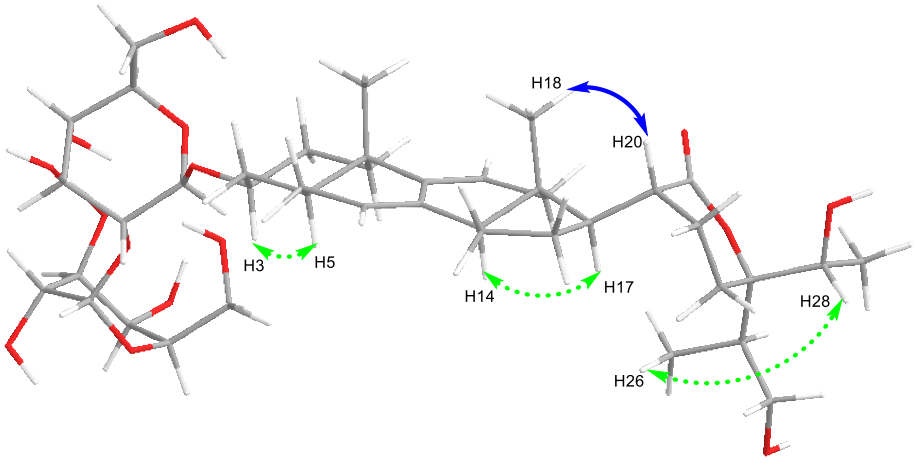
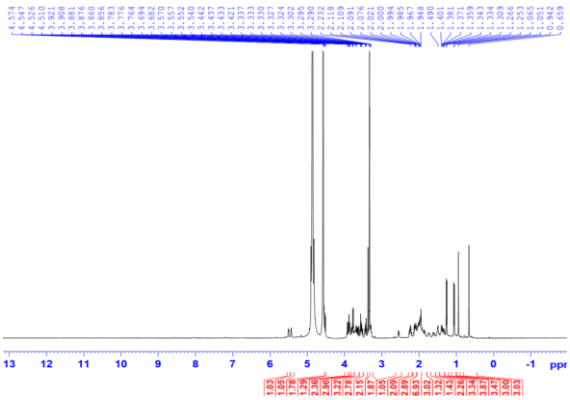
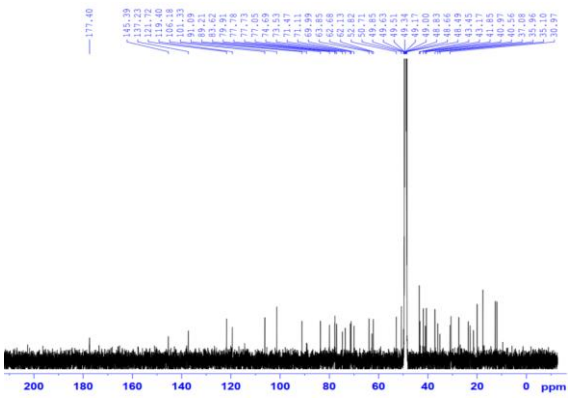


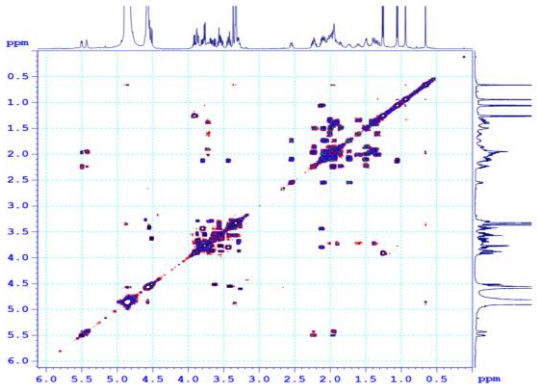
Figure 3.7. Key NOESY correlations of **VG1**



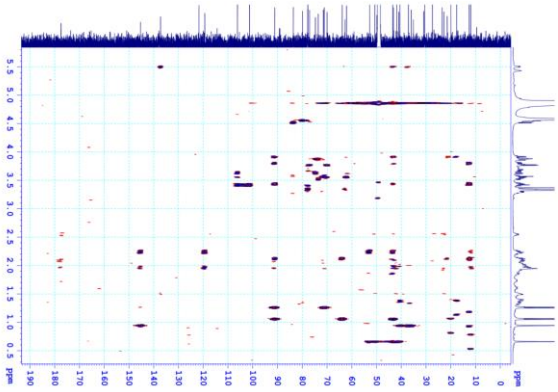
^{1}H-NMR spectrum of **VG1**



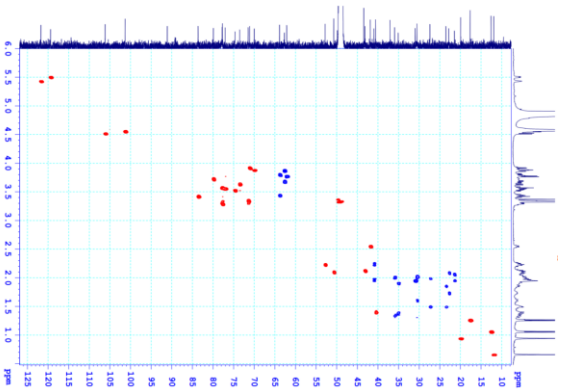
^{13}C-NMR spectrum of **VG1**



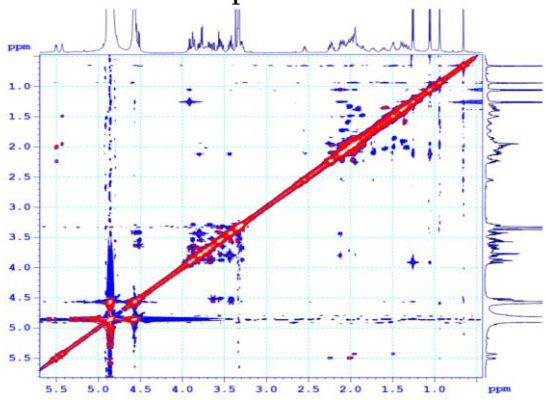
COSY spectrum of **VG1**



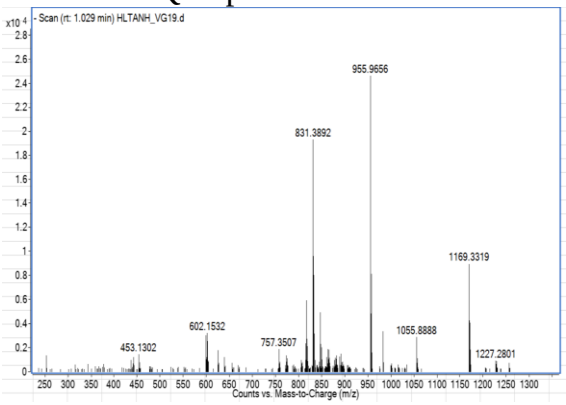
HMBC spectrum of **VG1**



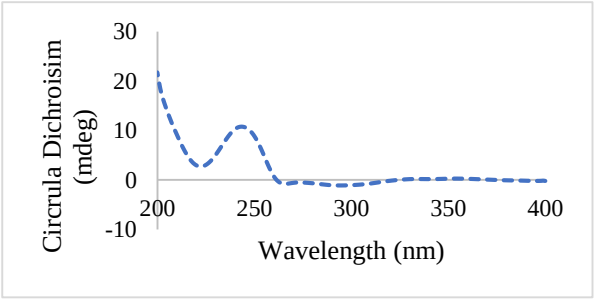
HSQC spectrum of **VG1**



NOESY spectrum of **VG1**



HR-ESI-MS spectrum of **VG1**



Experimental CD spectrum of **VG1**

3.4. BIOLOGICAL ACTIVITY OF ISOLATED COMPOUNDS FROM *V. AMYGDALINA* VÀ *V. GRATIOSA*

3.5.1. Anti-α-glucosidase activity of isolated compounds from V. amygdalina

Table 3.6. Inhibitory effects of isolated compounds from *V. amygdalina* on α-glucosidase

	LD1	**LD2**	**LD3**	**LD5**	**LD4**
IC_{50}	78.56 ± 7.28	>500	>500	**14.74 ± 1.57**	>500
	LD6	**LD7**	**LD8**	**LD14**	**LD16**
IC_{50}	>500	>500	>500	**48.55 ± 4.31**	>500
	LD12	**LD15**	**LD17**	**Acarbose**	

IC_{50}	**72.41 ± 7.56**	**7.42 ± 0.95**	>500	**127.53 ± 1.73**	

3.5.2. Anti-α-glucosidase activity of isolated compounds from V. gratiosa

Table 3.7. Inhibitory effects of isolated compounds from *V. gratiosa* on α-glucosidase

	VG1	**VG2**	**VG3**	**VG15**	**VG5**
IC_{50}	*>500*	*>500*	*>500*	**47.08 ± 3.98**	**424.79 ± 37.83**
IC_{50}	**VG6**	**VG7**	**VG8**	**VG13**	**VG14**
IC_{50}	*>500*	*>500*	*>500*	**477.52 ± 20.84**	*>500*
IC_{50}	**VG4**	**VG17**	**Acarbose**		
IC_{50}	*>500*	**>500**	**146.64 ± 8.85**		

3.5.3. Anti-xanthine oxidase activity of isolated compounds from V. amygdalina

Table 3.8. Inhibitory effects of isolated compounds from *V. amygdalina* on xanthine oxidase

	LD1-LD17
IC_{50}	***>500***

3.5.4. Anti-xanthine oxidase activity of isolated compounds from V. gratiosa

Table 3.9. Inhibitory effects of isolated compounds from *V. gratiosa* on xanthine oxidase

	VG1	**VG2**	**VG3**	**VG4**
IC_{50}	*>500*	*>500*	*>500*	*>500*
	VG5	**VG6**	**VG7**	**VG8**
IC_{50}	**47.65 ± 3.44**	*>500*	*>500*	*>500*
	VG15	**VG14**	**VG13**	**Allopurinol**
IC_{50}	**26.92 ± 1.04**	*>500*	**6.26 ± 0.60**	**1.12 ± 0.15**

CONCLUSION

1. Phytochemical study

Seventeen compounds (**LD1**-**LD7**) were isolated from the leaves of *V. amygdalina*, including seven new stigmastane steroids (**LD1-LD7**), named as (**LD1-**vernonioside K); (**LD2-**Vernonioside N)**,** (**LD3-**Vernonioside M)**;** (**LD4-**Vernonioside O)**;** (**LD5-**Vernonioside L); (**LD6**- vernonioside P); (**LD7**-Vernonioside Q) and ten known ones: (**LD8**-(22*R*,23*S*,24*R*,28*S*)-28-methoxy-7,8,9,11-tetradehydro-3*β*-16*α*,21,24 tetrahydroxy-21,23:22,28-diepoxy-5*α*- stigmastane); (**LD9-**Vernoamyoside E); (**LD10-vernonioside B2)**; (**LD11-**Vernoniacum B); (**LD12-**(23*S*,24*R*,28*S*)-3*β*,22*α*-dihydroxy-7,8,9,11-tetradehydro-24,28-epoxy-5*α*-stigmastane-21,23-carbolactone); (**LD13-**vemonioside B_1); (**LD14**: Veramyoside H); (**LD15**- Veramyoside J); (**LD16**-Vernoamyoside A); (**LD17- *α***-spinasterol).

Seventeen compounds were isolated from the aerial parts of *V. gratiosa*, including seven new compounds (**VG1 – VG7**) as Vernogratiosides A (**VG1**); Vernogratioside B (**VG2**); Vernogratioside C (**VG3**); Vernogratioside R (**VG4**); Vernogratioside S **(VG5);** Vernoratioside A (**VG6**); Vernoratioside B (**VG7**); and ten known compounds: VE1 (**VG8**); Vernoniacum B (**VG9**); Kaempferol (**VG10**); Quercetin 3-o-methyl ether (**VG11**); Quercetin (**VG12**); Apigenin (**VG13**); Syringaresinol-*β*-D-glucoside (**VG14**); 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3 hydroxy-1-(*E*)-propenyl)-2,6-dimethoxy phenoxy]propyl-*β*-D-glucopyranoside (**VG15**); 11*β*,13-dihydrovernolide (**VG16**); 5-(methoxymethyl)-1*H*-pyrrole-2-carbaldehyde (**VG17**).

2. Biological activities

From *V. amygdalina*, compounds **LD1**, **LD5**, **LD14**, **LD12**, and **LD15** showed strong inhibitory effects on α-glucosidase with IC_{50} values from **7.42 ± 0.95** μM to **78.56 ± 7.28** μM (compared to positive control, acarbose **127.53 ± 1.73** μM). Besides, compound **VG5** from *V. gratiosa* also significantly inhibited α-glucosidase with IC_{50} values of **47.08 ± 3.98** μM (acarbose **146.64 ± 8.85** μM).

Compounds **VG5, VG13**, and **VG15** also exhibited the potential inhibitory effects on xanthine oxidase activity with IC_{50} values of (**6.26 ± 0.60 - 47.65 ± 3.44** μM) (allopurinol là: **1.12 ± 0.15** (μM). The others did not show any activities.

RECOMMENDATION

Our results suggest that **LD5, LD14, LD12, LD1** from *V. amygdalina* **,** and **VG15** from *V. gratiosa* may potentially have a use as a therapeutic compound for the treatment or prevention of diabetes disease. In addition, compounds **VG15** và **VG3** may be a promising candidate for the treatment of gout disease. However, comprehensive and in-depth research about the molecular mechanism of α-glucosidase enzymes of compounds **LD5**, **LD14**, **LD12**, **LD1**, and **VG15**, as well as tests on *in vivo* models and clinical trials are required. At the same time, more research is needed to evaluate the toxicity of these compounds in both *in vitro* and *in vivo* models. Furthermore, repeated studies need to be conducted to confirm the xanthine oxidase inhibitory effect of compounds **VG15** and **VG3**, as well as to find out their target mechanism of action.

LIST OF PUBLICATIONS

1. Cong Pham Van, Hieu Ngo Van, Minh Bui Quang, Nam Duong Thanh, Dan Nguyen Van, Tuan Do Thanh, Ngoc Tran Minh, Hien Nguyen Thi Thu, Trung Nguyen Quang, Thao Do Thi, Loan Pham Thanh, Hien Do Thi Thu, and Anh Hoang Le Tuan; "Stigmastane-type steroid saponins from the leaves of *Vernonia amygdalina* and their α-glucosidase and xanthine oxidase inhibitory activities"; 2023; *Natural Product research*; SCIE; IF-2.488; https://doi.org/10.1080/14786419.2023.2188589

2. Pham Van Cong, Hoang Le Tuan Anh, Nguyen Quang Trung, Bui Quang Minh, Ngo Viet Duc, Nguyen Van Dan, Nguyen Minh Trang, Nguyen Viet Phong, Le Ba Vinh, Le Tuan Anh & Ki Yong Lee; "Isolation, structural elucidation and molecular docking studies against SARS-CoV-2 main protease of new stigmastane-type steroidal glucosides isolated from the whole plants of *Vernonia gratiosa"*; *Natural Product research;* SCIE; IF-2.488; https://doi.org/10.1080/14786419.2022.2042534

3. Pham Van Cong, Hoang Le Tuan Anh, Le Ba Vinh, Yoo Kyong Han, Nguyen Quang Trung, Bui Quang Minh, Ngo Viet Duc, Tran Minh Ngoc, Nguyen Thi Thu Hien, Hoang Duc Manh, Le Thi Lien8, Ki Yong Lee; "Alpha-Glucosidase Inhibitory Activity of Saponins Isolated from *Vernonia gratiosa* Hance"; *Journal of Microbiology Biotechnology*. 2023; 33(6): 797-805; SCIE; IF-3.27, https://doi.org/10.4014/jmb.2212.12040

4. Pham Van Cong, Ngo Van Hieu, Bui Quang Minh, Ngo Viet Duc, Vu Thi Trang, Nguyen Thi Thu Hien, Nguyen Viet Khanh, Tran Thi Phuong Anh, Ton That Huu Dat, Le Tuan Anh, Hoang Le Tuan Anh; "Constituents of *Vernonia gratiosa* Hance and their *α*-glucosidase and xanthine oxidase inhibitory activities"; *Vietnam Journal of Chemistry*, 2022, 60(5)**,** 653-659; ESCI, IF-0.74; doi: 10.1002/vjch.202200019