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STUDY ON CHEMICAL CONSTITUENTS AND SOME BIOLOGICAL ACTIVITIES OF TWO SPECIES *WEDELIA CHINENSIS* AND *WEDELIA TRILOBATA* IN THE ASTERACEAE FAMILY

Major: Organic Chemistry

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SUMMARY OF PHD THESIS IN CHEMISTRY

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Reviewer 1:
Reviewer 2:
Reviewer 3:

This thesis will be defended at the Graduate University of Science and Technology - Vietnam Academy of Science and Technology at hour date month 2023.

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INTRODUCTION

The use of plants as medicine has always been closely linked to the history and development of human society, with many advantages such as diverse chemical structures and biological activities, easy absorption and transformation in the body, as well as low toxicity. This is an advantage for us to exploit medicinal resources to serve life. Chemical studies oriented towards biological activity are considered the shortest and most effective way to selectively search for active compounds from natural resources. Vietnam is a country that is favored by nature and possesses an extremely rich plant system with over 12.000 higher plant species, of which an estimated 5.000 species are used in traditional medicine. Natural-originated compounds have received a lot of attention from scientists both inside and outside the country in researching and developing drugs for human treatment. The genus *Wedelia* has been and is being studied by scientists around the world. Some plants in this genus are used as traditional herbs worldwide, exhibiting many valuable activities such as cell toxicity, liver protection, fever reduction, pain relief, antibacterial, antibiotic, antioxidant, blood sugar lowering, and asthma treatment. The expanded chemical studies of the *Wedelia* genus have identified sesquiterpenes, diterpenes, triterpenes, triterpene saponins, flavonoids, etc. Our screening study found that the methanol extract of *W. chinensis* had anti-cancer activity (including against two lung cancer cell lines A549, H1975) and inhibited NO production related to inflammation. These preliminary results suggest that the *W. chinensis* plant, specifically, and other species in the *Wedelia* genus, in general, may contain important bioactive ingredients with potential for drug development research. This is a foundation for discovering

bioactive compounds with good biological activities such as anti-cancer and anti-inflammatory effects and studying their mechanisms of action. Chemical component research combined with biological activity testing will allow the acquisition of compounds with high selectivity and good biological activity. This is a successful method applied by advanced research groups worldwide.

Based on the above arguments, the NCS has chosen the topic "**Study on chemical constituents and some biological activities of two species *Wedelia chinensis* and *Wedelia trilobata* in the Asteraceae family"**

The aim of the thesis:

- Determine the chemical constituents of two species *W. chinensis* and *W. trilobata*.

- Evaluate anti-inflammatory activity, α-amylase and α-glucosidase inhibitory effects, and cytotoxic of isolated compounds as a basis for further application-oriented research.

The thesis content includes:

- Isolation of compounds from two species *W. chinensis* and *W. trilobata* by chromatographic methods.

- Determination of chemical structures of isolated compounds from two species *W. chinensis* and *W. trilobata*

- Evaluate anti-inflammatory activity, α-amylase and α-glucosidase inhibitory effects, and cytotoxic of the isolated compounds.

CHAPTER 1. OVERVIEW

1.1. General introduction to the genus *Wedelia*

1.1.1. Overview of the species Wedelia trilobata (L.) Hitchc.,

The plant commonly known as "Sai đat ba thuy" or "Sai đat kieng" in

Vietnamese is scientifically called *Wedelia trilobata* (L.) Hitchc. It is also known by the synonym *Sphagneticola trilobata* (L.) Pruski.

Previous studies have shown that the main components of *W. trilobata* include *ent*-kaurane diterpenes, eudesmane sesquiterpene lactones, and triterpenes, which exhibit various activities such as antibacterial, anti-tumor, hepatoprotective, and central nervous system inhibitory effects [7, 8].

1.1.2. Overview of the species *Wedelia chinensis* (Osbeck) Merr.

Wedelia chinensis (Osbeck) Merr, also synonymous with *Shagmeticola calendulacea* (L.) Pruski, belongs to the Asteraceae family, commonly known as the daisy family.

W. chinensis contains a variety of beneficial chemical components such as tannins, saponins, flavonoids, terpenoids, triterpenoid compounds, and phenolics. Previous studies in India on the *W. chinensis* herb did not find the presence of alkaloids, although investigations on the chemical composition of this species in China revealed the presence of alkaloids in the stems, leaves, and flowers [13]. Further studies on the biological activities of *W. chinensis* suggest that it has antioxidant, anti-inflammatory, analgesic, antibacterial, hepatoprotective, antidepressant, anticonvulsant, wound healing, sedative, and anticancer effects [10].

1.2. Research on the genus *Wedelia*

1.2.1. Studies on the chemical composition

1.2.1.1. Sesquiterpene composition

1.2.1.2. Diterpene composition

1.2.1.3. Triterpene and triterpene saponin composition

1.2.1.4. Flavonoid composition

1.2.1.5. Other components

1.2.2. Studies on the biological activities of species belonging to the genus Wedelia

1.2.2.1. Anti-cancer activity

1.2.2.2. Diabetes prevention and support activity

1.2.2.3. Antioxidant activity

1.2.2.4. Anti-inflammatory, antibacterial, and antifungal activity

1.2.2.5. Central nervous system inhibitory effects

1.2.2.6. Other biological activities

CHAPTER 2. METHODOLOGY AND EXPERIMENT

2.1. **Subjects**

2.1.1. Wedelia trilobata (L.) Hitch).

Wedelia trilobata (*W. trilobata*) was collected in Thai Binh province in September 2017. The scientific identification of the sample was performed by Dr Do Van Hai from the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. The voucher specimen 2016.55-W2 is deposited at the Department of Agro-Pharmaceutical Research, Center for Research and Technology Transfer.

2.1.2. Wedelia chinensis (Osbeck) Merr.

Wedelia chinensis (*W. chinensis*) was collected in Thai Binh province in September 2017. The scientific identification of the sample was performed by Dr Do Van Hai from the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. The voucher specimen 2016.55-W1 is deposited at the Department of Agro-Pharmaceutical Research, Center for Research and Technology Transfer.

2.2. **Methods**

2.2.1. Methods for compounds isolation

The combination of chromatographic methods used in this study includes thin-layer chromatography (TLC), preparative thin-layer chromatography (PTLC), and column chromatography (CC).

2.2.2. Methods for structure elucidation of the isolated compounds

The general method for determining the chemical structure of compounds involves a combination of physical parameter determination and modern spectroscopic methods, including:

2.2.2.1. Mass Spectrometry (MS)

2.2.2.2. High-Resolution Electrospray Ionization Mass Spectrometry (HR-ESI-MS)

2.2.2.3. Nuclear Magnetic Resonance Spectroscopy (NMR)

2.2.2.4. Specific Rotation $[\alpha]_D$

2.2.2.5. Sugar determination method

2.2.2.6. Mosher's method for absolute configuration elucidation

2.2.3. Bioactivity assays

2.2.3.1. Anti-inflammation assay

2.2.3.2. α-Glucosidase inhibition assay

2.2.3.3. α-Amylase inhibition assay

2.2.3.4. Assay for cytotoxic evaluation

2.3. Thực nghiệm.

2.3.1. Isolation of compounds from *W. trilobata*

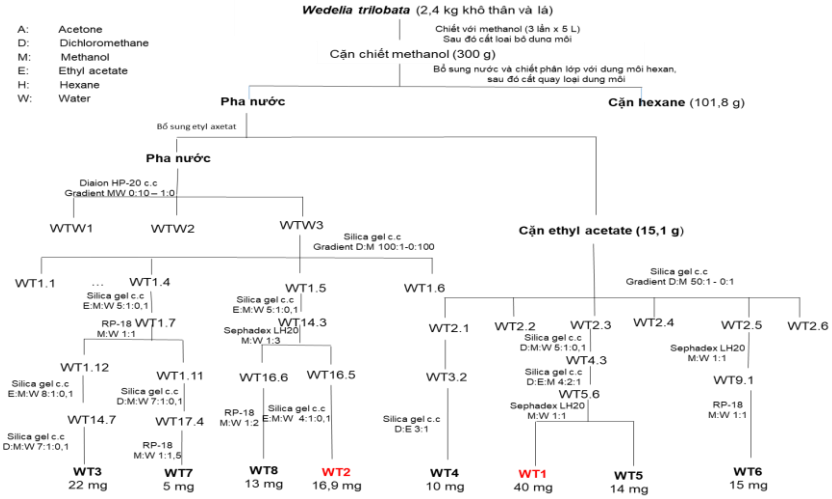


Figure 2.3. Isolation of compounds from *W. trilobata*

2.3.2. Isolation of compounds from W. chinensis

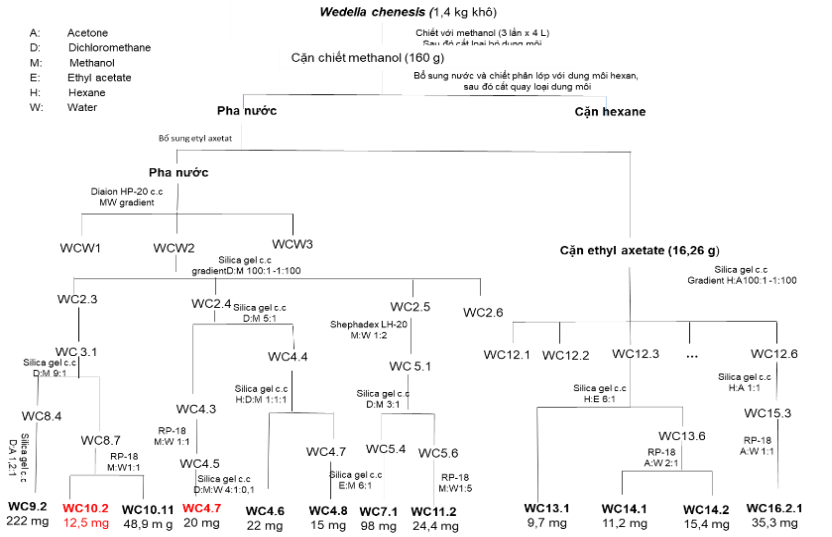


Figure 2.4. Isolation of compounds from *W. chinensis*

2.4. Physical properties and spectroscopy data of isolated compounds

2.4.1. Physical properties and spectroscopy data of isolated compounds from *Wedelia trilobata* (*W. trilobata*)

2.4.2. Physical properties and spectroscopy data of isolated compounds from *Wedelia chinensis* (*W. chinensis*)

2.5. Activity of isolated compounds from *W. chinensis* and *W. trilobata*

2.5.1. Anti-inflammatory activities of isolated compounds from *W. chinensis* and *W. trilobata*

The isolated compounds **WC1-WC12** and **WT1-WT8** were tested for their ability to inhibit nitric oxide (NO) production, following the method described in section 2.2.3.1. The results of the NO inhibition testing are presented in Table 3.21.

2.5.2. α-amylase and α-glucosidase inhibition of isolated compounds from *W. trilobata* and *W. chinensis*

The compounds **WT1-WT8** and **WC1-WC12** were evaluated for their α-amylase and α-glucosidase inhibition activity using the methods described in sections 2.2.3.3 and 2.2.3.2. The results are presented in Table 3.22.

2.5.3. Cytotoxic of isolated compounds from *W. chinensis* and *W. trilobata*

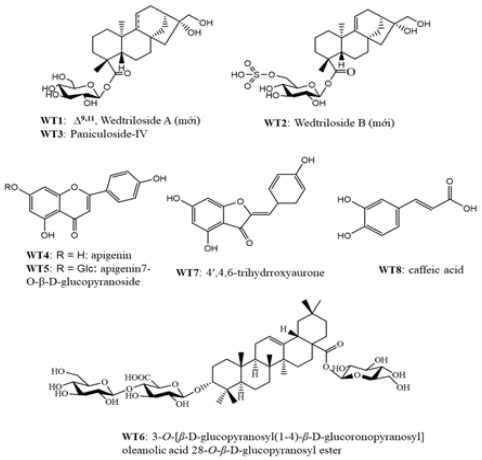
Cell cytotoxicity assays were performed using the method described in section 2.2.3.4. The results of the cytotoxicity activity testing are shown in Table 3.23.

CHAPTER 3: RESULTS AND DISCUSSION

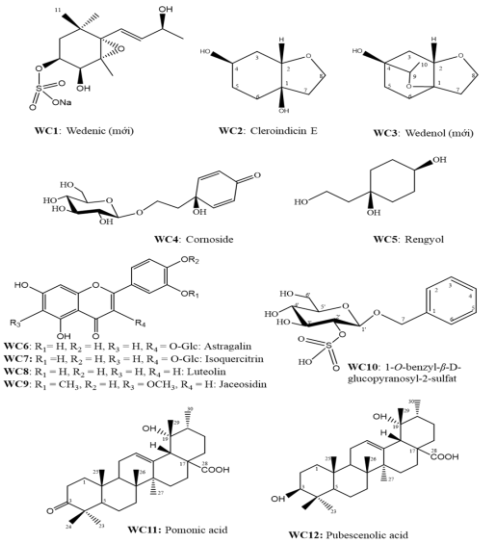
❖ From the two species, *W. trilobata* and *W. chinensis*, a total of 20 compounds have been isolated and elucidated the structures, which included 4 new compounds. Specifically:

❖ 8 compounds were isolated from *W. trilobata* (Figure 3.47), including 2 new compounds namely wedtriloside A (**WT1**) and wedtriloside B (**WT2**), together with 6 known compounds: paniculoside-IV (**WT3**), apigenin (**WT4**), apigenin7-*O*-*β*-D-glucopyranoside (**WT5**), 3-*O*-[*β*-D-glucopyranosyl(1-4)-*β*-D-glucoronopyranosyl] oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester (**WT6**), 4′,4,6-trihydrroxyaurone (**WT7**), and caffeic acid (**WT8**)

❖ 12 compounds were isolated from *W. chinensis* (Figure 3.48) including 2 new compounds namely wednenic (**WC1**) and wednenol (**WC3**) together with 10 known compounds: cleroindicin E (**WC2**), cornoside (**WC4**), rengyol (**WC5**), kaempferol-3-*O*-D-glucoside (**WC6**), quercetin-3-*O*-*β*-D-glucoside (**WC7**), luteolin (**WC8**), jaceosidin (**WC9**), 1-*O*-benzyl-*β*-D-glucopyranosyl-2-sulfat (**WC10**), pomonic acid (**WC11**), ilexgenin B (**WC12**).



Hình 3.47. Chemical structures of isolated compounds **WT1-WT8** from *W. Trilobata*



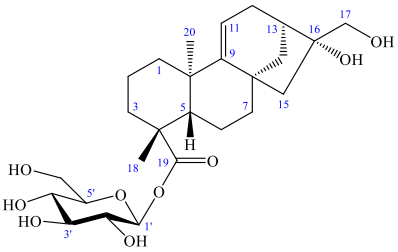
Hình 3.48. Chemical structures of isolated compounds **WC1-WC9** from *W. chinensis*

3.1. Structures elucidation of the isolated compounds

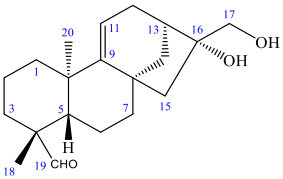
3.1.1. Structures elucidation of isolated compounds from the W. trilobata

*3.1.1.1. Compound **WT1**: Wedtriloside A (new compound)*

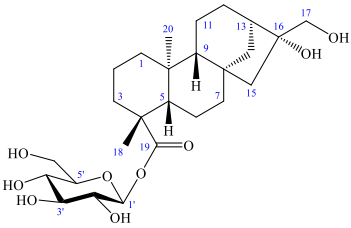
Compound **WT1** was obtained as a white, amorphous powder, with a negative optical rotation $[\alpha]_D^{24}$ -61.6 (c 0.15, MeOH). Its molecular formula was found to be $C_{26}H_{40}O_9$ via the HR-QTOF-MS ion at *m/z* 541.2659 $[M+HCOO]^-$ (theoretical calculation for the molecular formula $C_{27}H_{41}O_{11}$, M = 541.2649).



WT1



16*α*,17-dihydroxy-*ent*-9(11)-kaurene-19-al



Paniculoside-IV

Figure 3.1. Chemical structure of compound **WT1** and reference compounds.

The major IR absorption bands indicated a double bond (1630 cm^{-1}), a carboxyl group (1727 cm^{-1}), and hydroxyl groups (3423 cm^{-1}). Analysis of the ^1H NMR, signals of two methyl groups appear at δ_H 1.24 (3H, s, H-18) and 1.02 (3H, s, H-20), along with a signal of an olefinic proton at δ_H 5.20 (1H, t, J = 3.0 Hz, H-11). Additionally, signals of an oxymethylene proton are identified at δ_H 3.51 (1H, d, J = 11.5 Hz, H_a-17) and 3.55 (1H, d, J = 11.0 Hz, H_b-17). Furthermore, the proton spectrum of **WT1** shows the presence of an anomeric proton signal at δ_H 5.47 (1H, d, J = 8.0 Hz, H-1') along with six other signals of a sugar moiety at δ_H 3.36 (1H, dd, J = 8.0, 9.0 Hz, H-2'), δ_H 3.43 (1H, dd, J = 9.0, 9.0 Hz, H-3'), δ_H 3.38 (1H, t, J = 9.0 Hz, H-4'),

δ_H 3.40 (1H, m, H-5'), 3.83 (1H, dd, J = 2.0, 11.5 Hz, H_a-6'), and 3.71 (1H, dd, J = 4.5, 11.5 Hz, H_b-6'). Based on this data along with the values of the coupling constants J ($J_{1',2'}$ = 8,0 Hz, $J_{2',3'}$ = 9.0 Hz, and $J_{3',4'}$ = 9.0 Hz), the presence of a β-glucopyranose sugar moiety can be predicted..

Analysis of the ^{13}C NMR spectrum of **WT1** reveals the presence of signals from 26 carbon atoms, including 2 signals from methyl groups, 9 signals from methylene groups with 2 methylene groups directly attached to oxygen at δ_C 68.9 (C-17) and 62.4 (C-6'), 9 signals from methine groups resonating in the range of 45.0 to 114.9 ppm, and 6 signals from non-hydrogenated carbon atoms. The ^{13}C NMR spectrum also shows the presence of a resonating methine group at δ_C 114.9 and an unhydrogenated carbon at δ_C 159.0 ppm, suggesting the presence of a C=C double bond. Based on the obtained spectral data and the aforementioned analysis, **WT1** is suggested to be an *ent*-kaurane diterpenoid compound. This aligns perfectly with the synthetic characteristics of diterpenoid compounds previously discovered from the *Wedelia* genus [99].

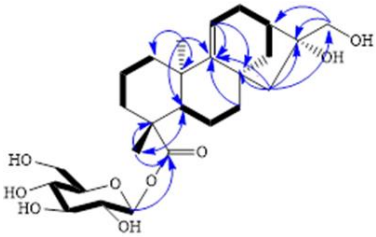


Figure 3.9. Key HMBC (H→C), COSY (H—H) correlations of **WT1**

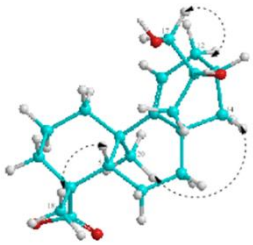


Figure 3.11. Key NOESY (H⇠⇢H) correlations of **WT1**

The direct bonds between protons and carbons in **WT1** were determined based on the analysis of the HSQC spectrum. The position

of the sugar moiety was determined through the HMBC spectrum, showing the interaction between the H-1' signal of the sugar moiety (δ_H 5.47, J = 8.0 Hz) and C-19 (δ_C 177.7) of the *ent*-kaurane aglycone, which allowed us to determine the attachment of the sugar moiety directly to the C-19 position (Figure 3.9)

Furthermore, the HMBC spectrum revealed interactions between H-20 (δ_H 1.02) and C-1 (δ_C 42.2), C-5 (δ_C 48.1), C-9 (δ_C 159.0), and C-10 (δ_C 39.9); and between H-18 (δ_H 1.28) and C-4, C-5, and C-19, allowing us to determine the positions of the two methyl groups directly attached to C-10 and C-4 of the *ent*-kaurane aglycone (Figure 3.9). The interaction between H-17 (δ_H 3.51) and C-16 (δ_C 85.6), C-15 (δ_C 55.8), and C-13 (δ_C 45.0) confirmed the attachment of the CH_2-17 group to C-16. Several proton-proton interactions were observed in the COSY spectrum, such as H-11 (δ_H 5.20) with H-12 (δ_H 2.22, δ_H 1.48) and H-13 (δ_H 2.16); H-5 (δ_H 1.66) with H-6 (δ_H 2.56, δ_H 1.98) and H-7 (δ_H 2.03, δ_H 1.51); and H-1 (δ_H 1.20, 1.95) with H-2 (δ_H 1.93, 1.50) and H-3 (δ_H 2.23, 1.07) (Figure 3.9).

The α configuration of the methylene group at position C-14 was determined by the interaction observed in the NOESY spectrum between H_a-14 (δ_H 1.45) and H_3-20 (δ_H 1.02). Additionally, the NOESY spectrum showed an interaction between H_3-18 (δ_H 1.28) and H-5 (δ_H 1.66). However, there was no interaction observed between δ_H 1.28 (H_3-18)/δ_H 1.66 (H-5) and H_3-20 (δ_H 1.02), indicating a β configuration for H_3-18 and H-5. Analyzing the NOESY interactions of **WT1**, the interaction between H_β-12 and H_a-17 suggests an α configuration for the hydroxyl group at position C-16. Furthermore, comparing the chemical shift values at C-16 (δ_C 85.6) and C-17 (δ_C 68.9) of **WT1** with the ^{13}C NMR spectrum values at δ_C 84.6 (C-16) and 68.4 (C-17) of 16α,17-dihydroxy-*ent*-9(11)-kaurene-19-al [100] indicates consistency at both positions. In contrast, the compound 16β,17-hydroxy-*ent*-kauran-19-oic acid-β-D-glucopyranosyl ester shows corresponding chemical shift values at the two positions of δ_C 79.8 (C-16) and δ_C 70.3 (C-17) [99, 100].

Based on the given information, the structure of compound **WT1** is similar to 16α,17-dihydroxy-*ent*-9(11)-kaurene-19-al, except for the presence of a carbonyl group in **WT1** instead of an aldehyde group in 16α,17-dihydroxy-*ent*-9(11)-kaurene-19-al, and the presence of a glucose moiety at position C-19. Hydrolysis of compound **WT1** in an alkaline environment yields a monosaccharide. The sugar moiety

in **WT1** is identified as D-glucose with a specific rotation $[\alpha]^{24}_{D}$ = +10.5 (c 0.15, H_2O), which is consistent with the specific rotation value of D-glucose reported previously [90, 99].

Table 3.1. NMR spectral data of **WT1** and reference compounds.

C	$^{\#}\boldsymbol{\delta}_C$[d]	$\boldsymbol{\delta}_C$[a,c]	$\boldsymbol{\delta}_H$[b,c] mult. (*J* in Hz)
1	39.9	42.2	1.20 m/1.95 m
2	19.2	21.2	1.93 m/1.50 m
3	35.1	39.4	2.23 m/1.07 m
4	48.3	46.2	-
5	45.9	48.1	1.66 dd (8.5. 11.0)
6	17.5	19.6	2.56 m/1.98 m
7	29.6	31.3	2.03 m/1.51 m
8	42.6	43.9	-
9	156.6	159.0	-
10	38.3	39.9	-
11	113.8	114.9	5.20 t (3.0)
12	30.1	31.2	2.22 m/1.48 m
13	44.2	45.0	2.16 m
14	42.9	44.2	2.06 m/1.45 m
15	55.0	55.8	1.96 m/1.54 m
16	84.6	85.6	-
17	68.4	68.9	3.51 d (11.0) 3.55 d (11.0)
18	24.2	28.4	1.24 s
19	206.6	177.7	-
20	23.7	24.5	1.02 s
1'		95.4	5.47 d (8.0)
2'		74.0	3.36 dd (8.0, 9.0)
3'		78.5	3.43 dd (9.0, 9.0)
4'		71.1	3.38 t (9.0)
5'		78.7	3.40 m
6'		62.4	3.83 dd (2.0, 11.5) 3.71 dd (4.5, 11.5)

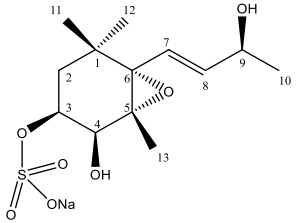
[a]125MHz, [b]500 MHz, [c]CD_3OD,[d]75 MHz $^{\#}\delta_C$: Data of 16*α*,17-dihydroxy-*ent*-9(11)-kaurene-19-al measured in CD_3Cl_3 [90].

Comparing the spectral data of **WT1** with the compound 16*α*,17-dihydroxy-*ent*-kauran-19-oic acid-*β*-D-glucopyranosyl ester (Paniculoside-IV) [99] shows similarity at most positions, except for the presence of an additional double bond at positions C-9/C-11 in **WT1**. Based on all the spectral analysis, it can be concluded that compound **WT1** is 16*α*,17-dihydroxy-*ent*-9(11)-kaurene-19-oic acid-*β*-D-glucopyranosyl ester. This is a novel compound and is named wedtriloside A.

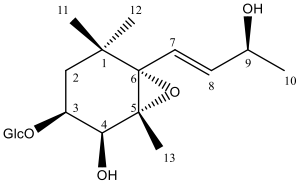
3.1.2. Structure elucidation of compounds from the W. chinensis

*3.1.2.1. Compound **WC1**: Wednenic (new compound)*

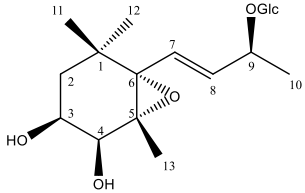
Compound **WC1** was obtained as a white powder with a negative optical rotation $[\alpha]_D^{24}$: -26.5 (c 0.25, MeOH). The molecular formula of **WC1** is $C_{13}H_{22}O_7SNa$, was determined by HRESIMS, with a protonated molecular ion peak at *m/z* 345.0987 $[M + H]^+$ and a sodium adduct molecular ion peak at *m/z* 367.0801 $[M + Na]^+$. The fragment ion peak at *m/z* 225.1482 $[M - SO_4Na]^-$ in the (-)HRESIMS spectrum showed the presence of a sulfate group in **WC1**.



Chemical structure of compound **WC1**



1: (3*S*,5*R*,6*S*,9*S*,7*E*)-megastigman-7-ene-5.6-epoxy-3,9-diol 3-*O*-*β*-D-glucopyranoside



2: (3*S*,4*S*,5*R*,6*S*,9*S*,7*E*)-megastigman-7-ene-5,6-epoxy-3,4,9-triol 9-*O*-*β*-D-glucopyranoside

Figure 3.2.2. Chemical structure of compound **WC1** and reference compounds.

The ^{1}H NMR spectrum of **WC1** shows signals corresponding to four methyl groups at δ_H 1.24 (3H, d, J = 6.0 Hz, H-10), δ_H 1.03 (3H, s, H-12), δ_H 1.13 (3H, s, H-11), and δ_H 1.28 (3H, s, H-13). Additionally, a signal of a methylene proton pair is identified at δ_H 1.47 (1H, dd, J = 3.5, 12.5 Hz, H_a-2) and δ_H 1.84 (1H, t, J = 12.5 Hz, H_b-2).

The ^{1}H NMR of **WC1** also reveals the presence of three oxygenated methine groups at δ_H 4.40 (1H, ddd, J = 3.0, 3.5, 12.5 Hz, H-3), δ_H 4.27 (1H, dd, J = 1.0, 3.0 Hz, H-4), and δ_H 4.31 (1H, dd, J = 6.0, 12.5 Hz, H-9). Signals of two olefinic protons are identified at δ_H 5.92 (1H, dd, J = 1.0, 16.5 Hz, H-7) and δ_H 5.69 (1H, dd, J = 6.0, 16.5 Hz, H-8). The large coupling constant (J = 16.5 Hz) between H-7 and H-8 confirms the E configuration of the double bond between C-7 and C-8. In the ^{13}C NMR and DEPT of **WC1**, signals for 13 carbon atoms are observed, including signals for four methyl groups identified at δ_C 23.7 (C-10), 24.8 (C-11), 29.5 (C-12), and 17.1 (C-13). A methylene group is present at δ_C 37.9 (C-2), two oxygenated methine groups at δ_C 71.6 (C-4) and 68.5 (C-9), and one oxygenated methine group linked to a sulfate moiety at δ_C 75.5 (C-3). Signals for three hydroxyl-

free carbon atoms are observed at δ_C 35.5 (C-1), 69.4 (C-5), and 71.3 (C-6), along with a pair of trans-olefinic carbon atoms at δ_C 125.6 (C-7) and 139.3 (C-8). Based on the spectral data, **WC1** is suggested to be a megastigmane compound. The connectivity between C-2/C-3/C-4 and C-7/C-8/C-9 is determined based on the HSQC and COSY spectra, which show interactions between adjacent protons such as H-2 (δ_H 1.47, 1.84)/H-3 (δ_H 4.64)/H-4 (δ_H 4.27) and H-7 (δ_H 5.92)/H-8 (δ_H 5.69)/H-9 (δ_H 4.31)/H-10 (δ_H 1.24) (Figure 3.21). The interactions between H-4, H-7, H-8, H-12, and H-13 with C-6 allow for the determination of the bond positions between C-7 and the methyl groups. The spectral signals of compound **WC1** are similar to the data of (3*S*,4*S*,5*R*,6*S*,9*S*,7*E*)-megastigman-7-ene-5,6-epoxy-3,4,9-triol 9-*O*-*β*-D-glucopyranoside, with slight differences at positions C-3 and C-4 due to the additional bonds with the natrisulfonate group and hydroxyl, which are determined through interactions observed in the HMBC spectrum.

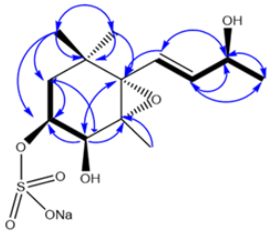


Figure 3.26. Key HMBC (H→C), COSY (H—H) correlations of **WC1**

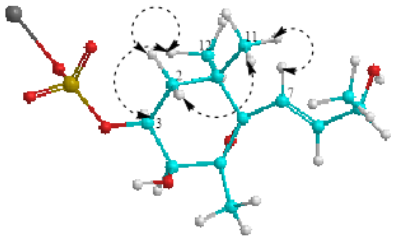


Figure 3.31. Key NOESY (◄- - -►) correlations of **WC1**

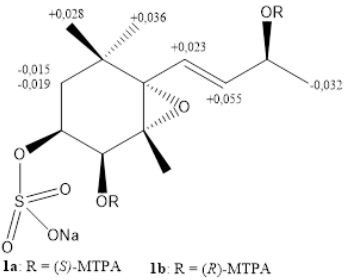


Figure 3.32. $\Delta\delta_{H\ (S-R)}$ values for MTPA esters of **WC1**

Table 3.9. NMR spectral data of **WC1** and reference compounds

C	$^{1}\boldsymbol{\delta}_{C}$[d.c]	$^{2}\boldsymbol{\delta}_{C}$[d.c]	$\boldsymbol{\delta}_{C}$[a.c]	$\boldsymbol{\delta}_{H}$[b.c] mult. (*J* = Hz)
1	35.9	35.5	35.5	-
2	45.5	40.5	37.9	1.47 dd (3.5, 12.5) 1.84 t (12.5)
3	73.0	66.7	75.5	4.60 ddd (3.0, 3.5, 12.5)
4	38.5	73.3	71.6	4.27 d (3.0)
5	67.8	69.7	69.4	-
6	71.3	71.6	71.3	-
7	125.7	129.6	125.6	5.92 dd (1.0, 16.5)
8	139.0	136.2	139.3	5.69 dd (6.0, 12.5)
9	68.7	74.4	68.5	4.31 dd (6.0, 12.5)
10	23.9	22.4	23.7	1.24 d (6.0)
11	29.8	29.8	29.5	1.13 s
12	25.2	24.9	24.8	1.03 s
13	20.3	17.8	17.1	1.28 s

[a]125MHz, [b]500 MHz, [d]100 MHz, [c]CD_3OD. $^{1}\boldsymbol{\delta}_{C}$ data of (3*S*,5*R*,6*S*,7*E*,9*S*) megastigman-7-ene-5,6-epoxy-3,9-diol 3-*O*-*β*-D-glucopyranoside measured in CD_3OD [101]. $^{2}\boldsymbol{\delta}_{C}$ data of (3*S*,4*S*,5*R*,6*S*,9*S*,7*E*)-megastigman-7-ene-5,6-epoxy-3,4,9-triol9-*O*-*β*-D-glucopyranoside measured in CD_3OD [96].

The α-configuration of H-3 is determined through the interaction between H-3 and H_a-2, H-12, as well as the interaction of H-7 and H_b-2 with H-11 and H-8 on the NOESY spectrum. Furthermore, the coupling constant J = 12.5 Hz of H-3 with H_a-2 also allows for the determination of the axial position of H-3. Additionally, the NOESY interaction between H-4 and H-3 confirms the equatorial bond of H-4 as well as the α-configuration at these positions. The coupling constants of H-3 [δ_H 4.60 (1H, ddd, J = 3.0, 3.5, 12.5 Hz)] and H-4 [δ_H 4.27 (1H, dd, J = 1.0, 3.0 Hz)] match the corresponding parameters of the compound (3*S*,4*S*,5*R*,6*S*,9*S*,7*E*)-megastigman-7-ene-5,6-epoxy-3,4,9-triol 9-*O*-*β*-D-glucopyranoside measured in the same solvent [δ_H 3.79 (1H, ddd, J = 3.0, 3.0, 12.0 Hz, H-3) and 3.88 (1H, dd, J = 1.0, 3.0 Hz, H-4)]. These findings lead to the conclusion that the two compounds have similar configurations at C-3 and C-4 [111].

The chemical shifts of C-5 (δ_C 69.4) and C-6 (δ_C 71.3) exhibit similar values to the ^{13}C NMR spectrum data (measured in CD_3OD) of (3*S*,4*S*,5*R*,6*S*,9*S*,7*E*)-megastigman-7-ene-5,6-epoxy-3,4,9-triol 9-*O*-*β*-D-glucopyranoside [δ_C 69.7 (C-5) and 71.6 (C-6)] [111] and differ from the spectral data of a compound with a 5*S*,6*R* configuration, namely (3*S*,4*S*,5*S*,6*R*,7*E*,9*S*)-5,6-epoxy-3,4,9-trihydroxy-7-megastigmen-3-*O*-*β*-D-glucopyranoside [$_C$ 68.2 (C-5) and 70.4 (C-6)] [112]. Therefore, the configuration of **WC1** can be determined as 5*R*, 6*S*. To determine the absolute configuration at C-9, we synthesized the (*S*)- and (*R*)-MTPA esters of **WC1** (see section 2.2.2.6). By comparing the Δδ values of the (*S*)- and (*R*)-MTPA esters (Figure 3.32), the configuration at C-9 of **WC1** is determined to be the *S* configuration according to the Mosher's rule [92, 110, 113, 114].

Based on the spectral analysis data mentioned above, the structure of compound **WC1** is determined to be (3*S*,4*S*,5*R*,6*S*,9*S*,7*E*)-megastigman-5,6-epoxy-7-ene-4,9-diol-3-natri sulfonate. It is a new compound named wedenic.

3.2. Biological activity evaluation of compounds isolated from *W. trilobata* and *W. chinensis*

3.2.1. Inhibition activity of the compounds on NO production

The results of evaluating the inhibitory activity of compounds isolated from *W. trilobata* and *W. chinensis* on NO production show that compounds **WC9** and **WC11** exhibited significant inhibition of NO production in RAW264.7 cells with corresponding IC_{50} values of 10.72 ± 1.06 and 10.91 ± 0.67 µM respectively (Table 3.21). Additionally, compounds **WC12** and **WT4** showed noteworthy inhibition of NO production in RAW264.7 macrophages with corresponding IC_{50} values of 26.92 ± 1.12 and 21.9 ± 0.90 µM. respectively. Compound **WT6** displayed weak inhibitory activity against NO production in RAW264.7 cells with an IC_{50} value of 78.5 ± 0.97 µM, while the remaining compounds did not exhibit any inhibitory activity on NO production in RAW264.7 cells.

Table 3.21. Evaluation of the evaluation of inhibitory activity on NO production in RAW264.7 cells by isolated compounds from *W. chinensis* and *W. trilobata*

Compound	IC_{50} (µM)
WC9	10.72 ± 1.06
WC11	10.91 ± 0.67
WC12	26.92 ± 1.12
WT4	21.9 ± 0.90
WT6	78.5 ± 0.97
Cardamonin *	2.12 ± 0.05

* Cardamonin is used as a control compound

3.2.2. Inhibition activity on α-amylase and α-glucosidase.

The results of evaluating the inhibitory ability on *α*-amylase and *α*-glucosidase are shown in Table 3.22 indicating that most of the compounds have the ability to inhibit *α*-amylase and *α*-glucosidase. Among them, the most impressive inhibition activity on *α*-amylase and *α*-glucosidase is exhibited by compound **WT6** with corresponding IC_{50} values of 52.08 and 190.4 μM, respectively, which is stronger than the positive control acarbose (IC_{50} = 67.8 and 450.56 μM) (Table 3.22).

Table 3.22. Evaluation of the inhibitory activity on *α*-amylase and *α*-glucosidase enzymes by the compounds isolated from *W. trilobata* and *W. chinensis*

	IC_{50} (μM)	
Compounds	**α-amylase**	**α-glucosidase**
WT1	112.20 ± 2.87	-
WT2	87.10 ± 1.89	-
WT4	-	27.54 ± 1.12
WT6	52.08 ± 0.56	190.40 ± 2.01
WT8	181.97 ± 2.62	173.78 ± 2.37
WC1	436.8 ±28.6	915.6 ±36.5
WC9	112.8 ±15.1	785.9 ±12.7
WC11	420.7 ±25.2	-
WC12	395.6 ±18.3	821.4 ±55.2
Acabose*	67.80 ± 0.32	450.56 ± 2.31

(-): No inhibition. (*): Control compound.

New compounds (**WT1** and **WT2**) isolated from *W. trilobata*. showed significant inhibitory activity against *α*-amylase with IC_{50} values of 112.20 and 87.10 μM, respectively. Compound **WT8** exhibited moderate inhibitory activity against α-amylase with an IC_{50}

value of 181.97 µM but showed stronger inhibition against *α*-glucosidase with an IC_{50} value of 173.8 µM. surpassing the positive control acarbose (IC_{50} = 450.56 µM). The study also revealed that compound **WT4** demonstrated potent inhibition of *α*-glucosidase with an IC_{50} value of 27.54 µM, surpassing the control compound acarbose (IC_{50} = 450.56 µM). On the other hand, compounds isolated from *W. chinensis* showed mostly moderate and weak inhibition activity against *α*-glucosidase and *α*-amylase respectively.

3.2.3. Cytotoxic

The results of evaluating the cytotoxic of the isolated compounds showed that two compounds **WT3** and **WT4** isolated from *W. trilobata* exhibited moderate cytotoxicity against lung cancer and liver cancer cell lines with corresponding IC_{50} values of 36.31 ± 1.15; 49.3 ± 1.03 µM and 31.77 ± 1.34; 34.6 ± 0.74 µM respectively and weak cytotoxicity against the remaining cell lines. The compounds **WC11** and **WC9** isolated from *W. chinensis* demonstrated moderate cytotoxic activity against the prostate cancer cell line with IC_{50} values of 25.12 ± 1.07 µM and 30.20 ± 1.23 µM respectively compared to the positive control camptothecin (IC_{50} = 4.65 µM). Additionally, **WC9** showed weak cytotoxicity against the other tested cancer cell lines with IC_{50} values ranging from 45.71 to 78.89 µM. On the other hand, **WC11** exhibited moderate cytotoxicity against the A-549 cell line. with an IC_{50} value of 36.31 ± 1.15 µM. but showed weak to non-toxic effects against the Hep3B and MCF-7 cell lines. Compound **WC12** showed weak to moderate cytotoxicity against the tested cancer cell lines. with IC_{50} values ranging from 31.77 to 53.70 µM. The remaining

compounds did not show cytotoxic activity at the studied concentrations against all four tested cancer cell lines.

Table 3.23. Evaluation of the cytotoxic activity testing of compounds isolated from *W. chinensis* and *W. trilobata*.

Compounds	**IC_{50} (μM)**			
	A549	Hep3B	MCF-7	PC3
WC9	78.89 ± 2.11	79.43 ± 1.19	45.71 ± 1.32	30.20 ± 1.23
WC11	36.31 ± 1.15	69.18 ± 1.45	67.61 ± 2.08	25.12 ± 1.07
WC12	31.77 ± 1.34	53.70 ± 1.23	37.15 ± 1.18	53.70 ± 2.36
WT3	36.31 ± 1.15	49.3 ±1.03	87.61 ± 1.08	55.6 ± 1.17
WT4	31.77 ± 1.34	34.6 ± 0.74	79.8 ± 1.18	61.0 ± 1.94
Camptothecin *	4.65 ± 0.14	0.34 ± 0.014	0.80± 0.02	0.97 ± 0.008

(*): Positive control

CONCLUSIONS

By using a combination of chromatographic and modern spectroscopic methods a comparison was made with spectral data of similar compounds in the reference literature. Isolated and determined the structure of 20 compounds from two species *Wedelia chinensis* and *Wedelia trilobata* and some biological activities of these compounds were evaluated. Specifically:

1. From the species *W. trilobata*, 08 compounds (**WT1-WT8**) have been isolated and structurally elucidated, of which 2 compounds are new and named wedtriloside A (**WT1**) and wedtriloside B (**WT2**) and 6 known compounds were Paniculoside-IV (**WT3**), apigenin (**WT4**), apigenin7-O-β-D-glucopyranoside (**WT5**), 3-O-[β-D-glucopyranosyl(1-4)-β-D-glucoronopyranosyl] oleanolic acid 28-O-β-D-glucopyranosyl ester (**WT6**), 4′.4.6-trihydrroxyaurone (**WT7**) and caffeic acid (**WT8**).

2. From the species *W. chinensis*, 12 compounds have been isolated and structurally elucidated, of which 2 compounds are new and named named wednenic (**WC1**) and wednenol (**WC3**) and 10 known compounds were Cleroindicin E (**WC2**), cornoside (**WC4**).

rengyol (**WC5**), kaempferol-3-*O*-*β*-D-glucoside (**WC6**), quercetin-3-*O*-*β*-D-glucoside (**WC7**), luteolin (**WC8**), jaceosidin (**WC9**), 1-*O*-benzyl-*β*-D-glucopyranosyl-2-sulfate (**WC10**), pomonic acid (**WC11**), and ilexgenin B (**WC12**).

3. NO production inhibition in RAW264.7 macrophage cells of the isolated compounds from both species *W. trilobata* and *W. chinensis* was investigated. The results showed that **WC9** and **WC11** exhibited relatively good inhibition of NO production in RAW264.7 cells with corresponding IC_{50} values of 10.72 ± 1.06 and 10.91 ± 0.67 μM, while the remaining compounds only showed weak inhibitory activity.

4. *α*-amylase and *α*-glucosidase inhibitions of the compounds isolated from *W. chinensis* and *W. trilobata* on were investigated. The results showed that most of the compounds isolated from *W. trilobata* exhibited the ability to inhibit *α*-amylase and *α*-glucosidase enzymes. Specifically, compounds **WT4** and **WT6** showed significant inhibition of *α*-glucosidase with corresponding IC_{50} values of 27.54 ± 1.12 μM and 190.40 ± 2.01 μM respectively, which were stronger than the positive control acarbose (IC_{50} = 67.8 μM and 450.56 μM). Furthermore, compound **WT6** also exhibited strong inhibition of *α*-amylase with an IC_{50} value of 52.08 μM. However, the compounds isolated from *W. chinensis* only showed moderate to no inhibition of *α*-amylase and *α*-glucosidase enzymes.

5. The cytotoxic on cancer cell lines of compounds **WC1**-**WC12** and **WT1**-**WT8** was evaluated. The results showed that compounds **WC9**, **WC11** and **WC12** exhibited cytotoxic effects on all four tested cell lines at a moderate level. In contrast, compounds **WT3** and **WT4** isolated from *W. trilobata* only showed cytotoxic activity against the Hep3B and PC3 cell lines at a moderate level with corresponding IC_{50} values of 49.3, 55.6, 34.6 and 61.0 μM. The remaining compounds did not show any activity.

RECOMMENDATION

The results in this thesis indicate that the isolated compound **WT4** from *W. trilobata* has a potent inhibitory effect on *α*-glucosidase, indicating the potential for further evaluation of its *in vivo* enzyme inhibition activity to determine its applicability.

Some isolated compounds from *W. trilobata* exhibit good inhibitory activity against both *α*-amylase and *α*-glucosidase enzymes, suggesting further investigation into their blood sugar-lowering

effects.

The isolated compound **WC9** from *W. chinensis* shows relatively good inhibition activity against NO production, with an IC_{50} value of 10.72 ± 1.06 µM compared to the positive control cardamonin (IC50 = 2.12 ± 0.05 µM). Further research is needed to understand the mechanism of NO production inhibition by this compound.

NEW CONTRIBUTIONS OF THE THESIS

1. Research on chemical constituents

Two species of the genus *Wedelia, Wedelia trilobata* (L.) Hitch and *Wedelia chinensis* (Osbeck) Merr, were isolated and the structures of 20 compounds were determined, including 4 new compounds and 5 compounds isolated for the first time from the genus *Wedelia*:

- The four new compounds are wedtriloside A, wedtriloside B, wednenic and wednenol.
- The five compounds isolated for the first time from the genus *Wedelia* are 3-*O*-[*β*-D-glucopyranosyl(1-4)-*β*-D-glucoronopyranosyl] oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester, cornoside, rengyol, and 1-*O*-benzyl-*β*-D-glucopyranosyl-2-sulfate.

2. Research on biological activities

For the first time in Vietnam, *in vitro* experiments were conducted to test the inhibitory potential of compounds isolated from two species, *Wedelia trilobata* (L.) Hitch and *Wedelia chinensis* (Osbeck), on *α*-amylase and *α*-glucosidase enzymes. The results revealed that the compound apigenin 3-*O*-[*β*-D-glucopyranosyl(1-4)-*β*-D-glucuronopyranosyl] oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester exhibited stronger inhibition against *α*-glucosidase compared to the positive control acarbose. Additionally, the same compound showed stronger inhibition against *α*-amylase than acarbose as well.

LIST OF PUBLISHED ARTICLES

1. **Nguyen Thi Luyen**, Pham Thanh Binh, Pham Thi Tham, Ta Manh Hung, Nguyen Hai Dang, Nguyen Tien Dat, Nguyen Phuong Thao. Wedtrilosides A and B. two new diterpenoid glycosides from the leaves of *Wedelia trilobata* (L.) Hitchc. with α-amylase and α-glucosidase inhibitory activities. Bioorganic Chemistry. **2019** (85), 319 -324; IF: 5.3

2. Nguyen Phuong Thao, Pham Thanh Binh, **Nguyen Thi Luyen**, Ta Manh Hung, Nguyen Hai Dang, Nguyen Tien Dat. α-Amylase and α-Glucosidase Inhibitory Activities of Chemical Constituents from *Wedelia chinensis* (Osbeck.) Merr. Leaves. Journal of Analytical Methods in Chemistry. **2018**, ID: 2794904; IF: 2.594

3. Nguyen Phuong Thao, Pham Thanh Binh, **Nguyen Thi Luyen**, Nguyen Duy Cong, Nguyen Hai Dang, Nguyen Tien Dat. Anti-inflammatory and cytotoxic activities of constituents from *Wedelia trilobata* (L.) Hitchc. Vietnam J. Chem. **2019**, 57 (1), 121-127.

4. **Nguyễn Thị Luyến**, Phạm Thanh Bình, Nguyễn Duy Công, Bùi Thị Thúy Luyện, Nguyễn Hải Đăng, Nguyễn Tiến Đạt, Nguyễn Phương Thảo. Nghiên cứu thành phần hóa học cây Sài đất (*Wedelia chinensis* (Osbeck.) Merr.). Tạp chí dược học. **2018**, 510, 25-29.