

# Ecdysterols and triterpene glycoside from *Achyranthes aspera* L. and their NO production inhibitory activity

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**Abstract.** Phytochemical study on the methanolic extract of the aerial parts of *Achyranthes aspera* led to the isolation of six compounds including five ecdysterols, makisterone A (**1**), achyranthesterone A (**2**), 24(28)-dehydromakisterone A (**3**), podecdysone C (**4**), 20-hydroxyecdysone (**5**), and 3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-glucuronopyranosyl-oleanolic acid 28-*O*- $\beta$ -D-glucopyranosyl ester (**6**). Compounds **1-5** were first isolated from *A. aspera*. Their chemical structures were identified by 1D and 2D NMR spectra in comparison with the published data in literature. Compounds **2-5** significantly showed NO production inhibition effects in LPS-activated RAW264.7 cells with the IC<sub>50</sub> values ranging from 27.21 to 40.47  $\pm$  4.90  $\mu$ M compared to that of positive control compound, L-NMMA, 32.24  $\mu$ M.

**Keywords:** *Achyranthes aspera*, ecdysterol, amaranthaceae, nitric oxide inhibition.

**Classification numbers:** 1.1.1, 1.1.6

## 1. INTRODUCTION

*Achyranthes aspera* L., belongs to the genus *Achyranthes* (*Amaranthaceae*). It is a well-known medicine in traditional remedies in Viet Nam, China and India. *A. aspera* leaves has a bitter, sour taste, clearing heat, decongesting, low leprosy, strong blood circulation [1]. The root is used to treat colds, fever, malaria, dysentery, rheumatism, perforated nephritis, urinary incontinence, urinary incontinence, painful urination, dysmenorrhea, amenorrhea, menstrual irregularities, and fall injuries [1]. *A. aspera* is an intertropical species, distributed in India, China, Japan, Thailand, Laos, Cambodia and Viet Nam. It's also quite common in Viet Nam [1]. In our ongoing search for NO inhibition compounds from plants, the methanolic extract of *A. aspera* showed considerable effect with IC<sub>50</sub> value of 38.2  $\mu$ g/mL. Therefore, it was selected for further study. The phytochemical investigation on *A. aspera* reported to contain alkaloids, terpenoids, sterols and flavonoids [2]. Pharmacological studies on this species show that they exhibit a variety of effects such as: anti-inflammatory, anti-microbial, anti fertility, immunomodulatory, anti-hyperlipidemic, anti-diabetic, cardiovascular [2]. This paper reported the isolation of five ecdysterols (**1-5**) and a saponin (**6**) from *Achyranthes aspera*.

## 2. MATERIALS AND METHODS

### 2.1. Materials

The aerial parts of *Achyranthes aspera* L. were harvested in Me Linh Biodiversity Station, Vinh Phuc Province, Viet Nam (in February 2021), and taxonomically identified by Dr Nguyen The Cuong, at IEBER, Vietnam Academy of Science and Technology (VAST). A voucher specimen (code NCCT-P119) is kept at the Institute of Marine Biochemistry, VAST, Viet Nam.

### 2.2. Methods

The used equipment is provided in supporting information

(<https://vjs.ac.vn/index.php/jst/workflow/index/18462/4#workflow>).

### 2.3. Extraction and isolation

The sample powder (10.0 kg) and sonicated with MeOH (three times, each 20 L, 2h) to get extract (AC, 500 g). This was separated by dichloromethane, and ethyl acetate (in water) to give dichloromethane (AC.D, 324 g), ethyl acetate (AC.E, 87 g) extracts, and H<sub>2</sub>O layer (AC.W). AC.W was subjected to a Diaion HP-20 column, eluting with methanol/water (1/3, 1/1, 3/1, and 1/0) to give four fractions, AC.W1-AC.W4. AC.W4 (34 g) was isolated on a silica gel column eluting with CH<sub>2</sub>Cl<sub>2</sub>/MeOH/water (5/1/0.01, v/v) to yield five fractions AC1A-AC1E. AC1B was purified on an HPLC column eluting with 23% ACN to yield **3** (5.3 mg, t<sub>R</sub> 41.3 min). **2** (4.0 mg, t<sub>R</sub> 32.5 min) was yielded from AC1C by an HPLC using 18% ACN. AC1D was purified by pre-HPLC eluting with 20 % ACN to get **5** (13.9 mg, t<sub>R</sub> 37.8 min) and **1** (13.5 mg, t<sub>R</sub> 58.1 min). AC2E was purified by pre-HPLC eluting with 16 % ACN to obtain **4** (5.5 mg, t<sub>R</sub> 38.2 min). AC.D was subjected on a silica gel column (CC), eluted with dichloromethane/methanol (50/1, 25/1, 15/1, 5/1, 1/100, v/v) to give five fractions, AC2A- AC2E. AC2C was divided by a silica gel RP-18 CC, eluting with acetone/H<sub>2</sub>O (2/1, v/v) to get five fractions, AC2C1-AC2C5. AC2C5 was chromatographed on sephadex LH-20 eluting with methanol/water (1/1, v/v) to yield **6** (15.6 mg).

## 3. RESULTS AND DISCUSSION

The <sup>1</sup>H NMR spectrum of **1** revealed one olefinic proton signal at δ<sub>H</sub> 5.83 (d, 2.4 Hz), five quaternary methyl groups at δ<sub>H</sub> 0.92, 0.99, 1.21, 1.16, and 1.19 (s, each), and one tertiary methyl group at δ<sub>H</sub> 0.96 (d, 6.6 Hz). The <sup>13</sup>C NMR and HSQC spectra of **1** indicated the presence of 28 carbons including one carbonyl (δ<sub>C</sub> 206.5), two olefin (δ<sub>C</sub> 122.1, CH and 168.0, C), three oxygenated quaternary (δ<sub>C</sub> 85.3, 78.0, and 73.8), three oxygenated methine (δ<sub>C</sub> 68.7, 68.5, and 75.4), two sp<sup>3</sup> quaternary (δ<sub>C</sub> 39.3, and 48.6), four sp<sup>3</sup> methine (δ<sub>C</sub> 51.8, 50.4, 41.7, and 35.1), seven methylene (δ<sub>C</sub> 37.4, 32.5, 21.5, 32.8, 31.8, 21.4, and 34.5), and six methyl groups (δ<sub>C</sub> 18.1, 24.4, 21.0, 26.1, 27.5, and 14.9) (Table 1, Figure 1). The above data were closely resembling those of ecdysone [3]. In addition, the HMBC interactions from methyl proton H<sub>3</sub>-19 (δ<sub>H</sub> 0.99) to C-1 (δ<sub>C</sub> 37.4), C-5 (δ<sub>C</sub> 51.8), C-9 (δ<sub>C</sub> 35.1), C-10 (δ<sub>C</sub> 39.3); from H-1 (δ<sub>H</sub> 1.45/1.81) to C-2 (δ<sub>C</sub> 68.7) and from H-5 (δ<sub>H</sub> 2.41) to C-3 (δ<sub>C</sub> 68.7), C-6 (δ<sub>C</sub> 206.5), C-7 (δ<sub>C</sub> 122.1 ) determined the carbon chemical shifts of two rings A and B, and two hydroxy groups at C-2 and C-3. The nonprotonated carbon bearing oxygen atom at δ<sub>C</sub> 85.3 matched perfectly for C-14 of ecdysone. This was further indicated by HMBC spectra. The HMBC interactions from H-18 (δ<sub>H</sub> 0.92) to C-

12 ( $\delta_C$  32.8)/C-13 ( $\delta_C$  48.6)/C-14 ( $\delta_C$  85.3)/C-17 ( $\delta_C$  50.4), from H-7 ( $\delta_H$  5.83) to C-5/C-9/C-14 not only indicated the hydroxy group at C-14 but also the NMR assignment of rings C and D (Figure 2).

Table 1. NMR data for compounds **1-3** in CD<sub>3</sub>OD

	<b>1</b>		<b>2</b>		<b>3</b>	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
1	37.4	1.45 (dd, 6.6, 7.2) 1.81 (m)	37.4	1.44 (m)/ 1.81 (m)	37.4	1.45 (m)/ 1.82 (m)
2	68.5	3.97 (br d 2.4)	68.5	3.96 (br s)	68.5	3.97 (br s)
3	68.7	3.86 (ddd, 12.0, 4.2, 2.4)	68.7	3.85 (ddd, 12.0, 3.6, 3.6)	68.7	3.85 (ddd, 12.0, 3.6, 3.6)
4	32.5	1.89 (dd, 13.2, 4.2) 2.14 (ddd, 13.2, 13.2, 12.0)	31.8	1.89 (dd, 13.2, 4.2) 2.14 (ddd, 13.2, 13.2, 12.0)	32.5	1.62 (dd, 13.2, 4.2) 2.00 (ddd, 13.2, 13.2, 12.0)
5	51.8	2.41 (dd, 13.2, 4.8)	51.8	2.40 (m)	51.8	2.40 (m)
6	206.5	-	206.5	-	206.5	-
7	122.1	5.83 (d, 2.4)	122.2	5.82 (br s)	122.2	5.83 (br s)
8	168.0	-	167.9	-	167.9	-
9	35.1	3.17 (m)	35.0	3.15 (m)	35.1	3.18 (m)
10	39.3	-	39.3	-	39.3	-
11	21.5	1.72 (m)/ 1.82 (m)	21.6	1.79 (m)/ 2.08 (m)	21.0	1.83 (m)/ 2.04 (m)
12	32.8	1.73 (m)/ 1.78 (m)	32.8	1.72 (m)/ 1.75 (m)	32.9	1.72 (m)/ 1.78 (m)
13	48.6	-	48.4	-	48.5	-
14	85.3	-	85.3	-	85.3	-
15	31.8	1.63 (m)/ 2.00 (m)	31.6	1.62 (m)/ 1.98 (m)	31.8	1.62 (m)/ 1.98 (m)
16	21.4	1.77 (m)/ 2.01 (m)	21.4	1.70 (m)/ 2.07 (m)	21.5	1.72 (m)/ 1.83 (m)
17	50.4	2.37 (dd, 8.4, 8.4)	48.3	-	50.5	2.43 (m)
18	18.1	0.92 (s)	18.0	0.91 (s)	18.0	0.92 (s)
19	24.4	0.99 (s)	24.4	0.98 (s)	24.4	0.99 (s)
20	78.0	-	78.6	-	77.8	-
21	21.0	1.21 (s)	67.1	3.79 (d, 10.5) 3.83 (d, 10.5)	20.7	1.25 (s)
22	75.4	3.48 (d, 10.2)	79.3	3.45 (d, 10.2)	78.0	3.61 (dd, 10.2, 1.8)
23	34.5	1.20*/ 1.60 (m)	27.8	1.54 (m)/ 1.82 (m)	34.6	2.15 (m)/ 2.40 (m)
24	41.7	1.77 (m)	42.6	1.45 (m)/ 1.88 (m)	155.3	-
25	73.8	-	71.3	-	73.6	-
26	26.1	1.16 (s)	29.0	1.22 (s)	29.8	1.39 (s)
27	27.5	1.19 (s)	29.6	1.24 (s)	30.2	1.34 (s)
28	14.9	0.96 (d, 6.6)	-	-	110.4	4.98 (s)/ 5.15 (s)

The location of hydroxy groups at C-20 and C-22 was determined by the HMBC correlation between H-21 ( $\delta_H$  1.21) and C-17, C-20 ( $\delta_C$  78.0), C-22 ( $\delta_C$  75.4) and the values at C-20 and C-22. The HMBC correlations between H-28 ( $\delta_H$  0.96) and C-23 ( $\delta_C$  34.5), C-24 ( $\delta_C$  41.7) and C-25 ( $\delta_C$  73.8), between H-26 ( $\delta_H$  1.16)/H-27 ( $\delta_H$  1.19) and C-24/C-25 indicated one methyl group at C-24 and two methyl groups at C-25, and one hydroxy group at C-25. Proton H-

2 appeared as a doublet with small coupling constant ( $J = 2.4$  Hz) indicating  $\alpha$ /equatorial orientation of this proton. In addition, H-4<sub>ax</sub> appeared as a doublet of double of doublet (ddd) with all large  $J$  value (13.2, 13.2, 12.0 Hz) suggested a large value of  $J_{\text{H-3/H-4}}$  (12.0 Hz). This confirmed  $\alpha$ /axial orientation of H-3. The NMR data of **1** were compared with those of makisterone A (a compound previously isolated from *Leuzea carthamoides*) and found to match [3].

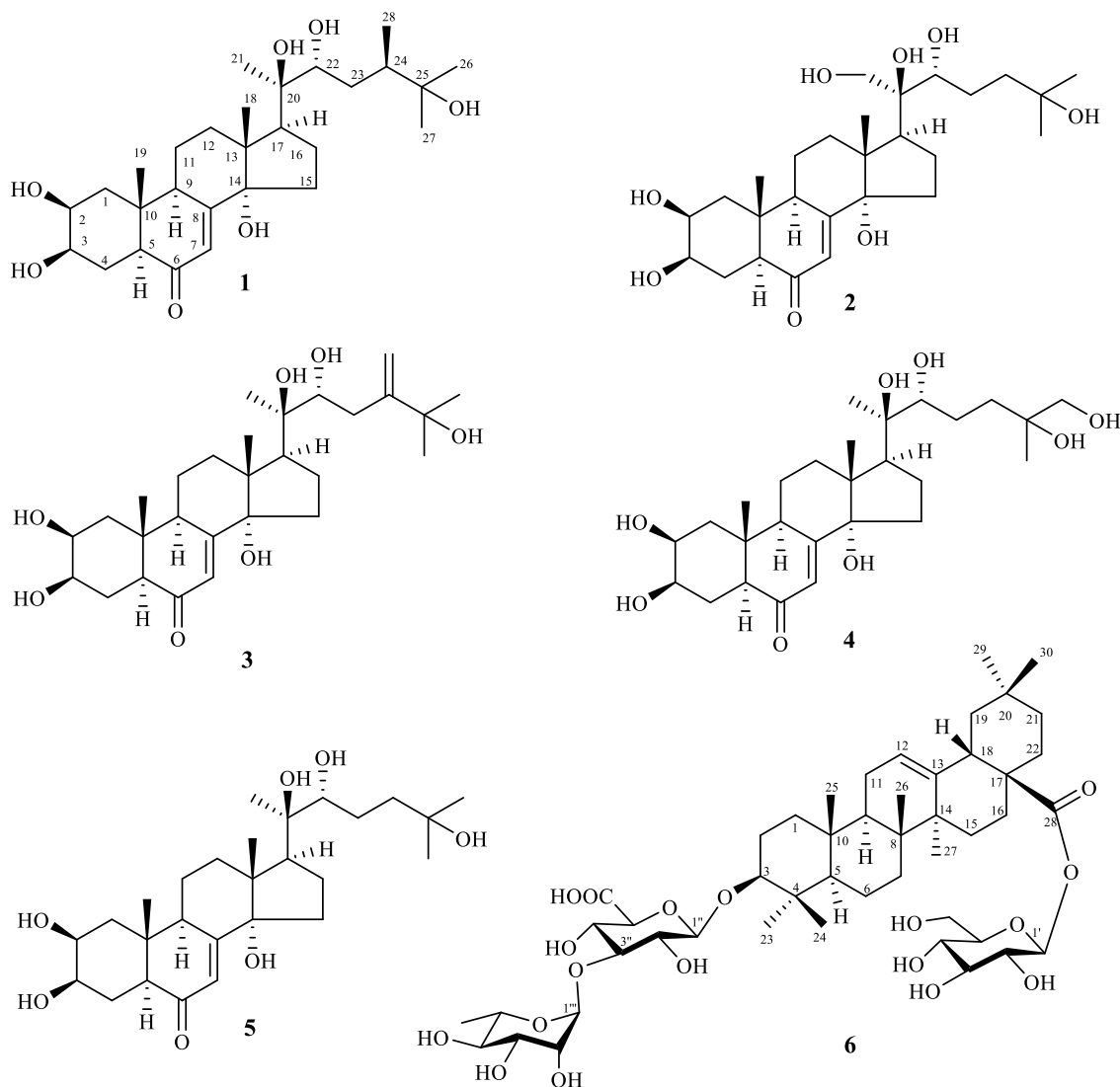


Figure 1. Chemical structures of **1-6**

The NMR spectra of **2** were remarkably similar to those of **1** except the C-21 methyl group signals in **1** ( $\delta_{\text{C}} 21.0/\delta_{\text{H}} 1.21$ ) were replaced by the oxygenated methylene signals ( $\delta_{\text{C}} 67.1/\delta_{\text{H}} 3.79$  (d, 10.5 Hz) and 3.83 (d, 10.5 Hz) as well as the lack of methyl group at C-24 in **2**. This was further confirmed by the analyzing  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, HSQC, and HMBC spectra of **2** in comparison with the corresponding data of **1** (Table 1). The HMBC correlations from H-21 ( $\delta_{\text{H}} 3.79/3.83$ ) to C-17 ( $\delta 48.3$ )/C-20 ( $\delta_{\text{C}} 78.6$ )/C-22 ( $\delta_{\text{C}} 79.3$ ) determined the hydroxylation of the

methyl group at C-21. The carbon chemical shifts of C-17/C-20/C-21/C-22 were very similar to those of achyranthesterone A suggested 20*R*,22*α*-OH-configuration [4]. Both two hydroxyl groups at C-2 and C-3 were suggested to be  $\beta$ -orientation, similar to **1** from the small *J* value of H-2 (broad singlet) and the large  $J_{\text{H-3/H-4}}$  value (12.0 Hz). Thus, **2** was identified as achyranthesterone A, which was previously isolated from *Achyranthes bidentata* [4].

Table 2. NMR data for compounds **4** and **5** in CD<sub>3</sub>OD.

C	<b>4</b>		<b>5</b>	
	$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$
1	37.4	1.45 (dd, 6.6, 7.2) 1.81 (m)	37.4	1.45 (dd, 6.6, 7.2) 1.81 (m)
2	68.5	3.97 (br d 2.4)	68.5	3.97 (brs)
3	68.7	3.86 (ddd, 12.0, 4.2, 2.4)	68.7	3.86 (brd, 12.0)
4	32.5	1.89 (dd, 13.2, 4.2) 2.14 (td, 13.2, 12.0)	32.5	1.89 (dd, 13.2, 4.2) 2.14 (td, 13.2, 12.0)
5	51.8	2.40 (m)	51.8	2.41 (dd, 13.2, 4.8)
6	206.5	-	206.5	-
7	122.2	5.83 (d, 2.4)	122.1	5.83 (d, 2.4)
8	168.0	-	168.0	-
9	35.1	3.19 (m)	35.1	3.17 (m)
10	39.3	-	39.3	-
11	21.5	1.77 (m)/ 2.00 (m)	21.5	1.72 (m)/1.82 (m)
12	32.8	1.72 (m)/ 1.78 (m)	32.8	1.73 (m)/1.78 (m)
13	48.7	-	48.6	-
14	85.3	-	85.3	-
15	31.8	1.61 (t, 10.8)/ 2.00 (m)	31.8	1.63 (m)/2.00 (m)
16	21.5	1.70 (m)/1.82 (m)	21.5	1.77 (m)/2.01 (m)
17	50.5	2.41 (m)	50.5	2.37 (dd, 8.4, 8.4)
18	18.0	0.91 (s)	18.0	0.91 (s)
19	24.4	0.99 (s)	24.4	0.99 (s)
20	77.9	-	77.9	-
21	21.1	1.20 (s)	21.1	1.21 (s)
22	78.5	3.35 (m)	78.4	3.48 (d, 10.2)
23	26.5	1.30 (m)/ 1.70 (m)	27.4	1.54 (m)/1.82 (m)
24	37.1	1.48 (m)/1.81 (m)	42.4	1.45 (m)/1.88 (m)
25	73.6	-	71.3	-
26	70.7	3.39 (s)	29.7	1.22 (s)
27	23.6	1.16 (s)	29.0	1.22 (s)

The NMR spectra of **3** were remarkably similar to those of **1** except that the methyl doublet signals in **1** were disappeared and the additional signals due to one vinyl group [ $\delta_{\text{C}}$  155.3 and  $\delta_{\text{C}}$  110.4/ $\delta_{\text{H}}$  5.15 (s) and 4.98 (s)] in **3**. These changes were further confirmed by HSQC and HMBC spectra. In the HMBC spectra of **3**, H<sub>3</sub>-26/H<sub>3</sub>-27 correlated with C-25 ( $\delta$  73.6) and C-24 ( $\delta$  155.3) and H<sub>2</sub>-28 correlated with C-23 ( $\delta$  34.6)/C-24 ( $\delta$  155.3)/C-25 ( $\delta$  73.6), and H<sub>3</sub>-21 ( $\delta$  1.25) correlated with C-17 ( $\delta$  50.5)/C-20 ( $\delta$  77.8)/C-22 ( $\delta$  78.0) (Figure 2) indicating two hydroxyl

groups at C-20 and C-22, and the double bond at C-24/C-28. All the NMR data of **3** were compared to those of 24(28)-dehydromakisterone A (a compound previously isolated from *Leuzea carthamoides*) and found to match [5].

The NMR spectra of **4** closely resembling those of **2** except for some slight changes of chemical shifts at the side chain. The downfield shift of the oxygenated methylene carbon at C-21 (from  $\delta$  67.1 in **2** to 70.7 in **4**) and the upfield shift of C-27 (from 29.0 in **2** to 23.6 in **4**) suggested that the hydroxy group was moved from C-21 to C-26 in **4**. Therefore, the NMR assignments of **4** were compared directly to those of podecdysone C (20*R*,26-dihydroxyecdysone) and found to match [6].

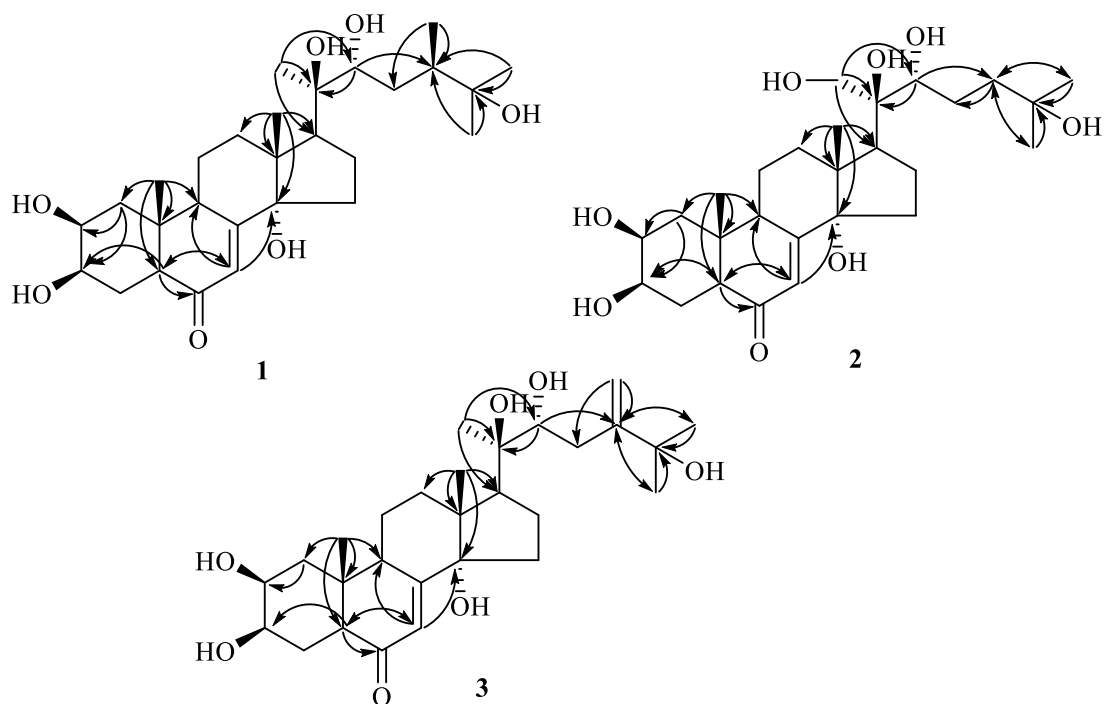


Figure 2. Important HMBC interactions of **1** and **2**.

The NMR spectra of **5** were very similar to those of **2** except that the quaternary oxygenated methylene carbon at C-21 in **2** ( $\delta_C$  67.1/ $\delta_H$  3.79 and 3.83) was replaced by a methyl group in **5** ( $\delta_C$  21.1/ $\delta_H$  1.21). Comparing the NMR data of the side chain between **4** and **5** showed that these compounds had the same data at C-17 and from C-20 to C-23, but the data of C-24, C-25, C-26, and C-27 were different (Table 2) [3]. The above evidence suggested that compound **5** was 20-hydroxy-ecdysone. Therefore, the NMR data of **5** were compared to those of 20-hydroxyecdysone (a compound previously isolated from *Leuzea carthamoides*) and found to match [3].

The NMR data of **6** showed seven methyl singlets ( $\delta_C/\delta_H$ : 28.6/1.07, 17.8/0.86, 16.1/0.97, 17.1/0.82, 26.3/1.17, 33.5/0.93, 24.0/0.95), one methyl doublet of the rhamnose molecule ( $\delta_C/\delta_H$ : 18.0/1.26 (d,  $J = 6.0$  Hz)), one  $>C=CH$  double bond ( $\delta_C/\delta_H$ : 144.9 and 123.8/5.27), two carboxyl carbons ( $\delta_C$  178.1 and 177.8), and signals of three sugar moieties (Table 3). The above evidence suggested the aglycone of **6** was oleanolic acid and the sugar moieties were a rhamnose, a glucose, and a glucuronopyranose [7]. The upfield shift of the anomeric carbon at  $\delta_C$  95.7

together with the downfield shift of anomeric proton at  $\delta_{\text{H}}$  5.40 (which was indicated by HSQC spectrum) suggested one glucose sugar linked to C-28 by an ester linkage. The terminal rhamnose was identified by the typical signals at  $\delta_{\text{C}}$  102.6, 74.1, 72.3, 72.3, 69.9 (5CH) and 18.0 (CH<sub>3</sub>), and the remaining sugar signals at  $\delta_{\text{C}}$  178.1 (C=O), 106.6 (CH), 83.9 (CH), 77.3 (CH), 76.0 (CH), 72.5 (CH) were similar to those of a glucuronopyranose which had a glycosyl linkage at C-3' [7]. The strong downfield shift of C-3 carbon ( $\delta_{\text{C}}$  91.1) indicated one sugar moiety linked to C-3 by an ether linkage, as similar to most of natural saponins. The large coupling constant values of two anomeric protons at  $\delta$  4.38 and 5.40 suggested two  $\beta$ -form of the glycosyl linkages, whereas the very small J value of H-1'' and H-2'' (rhamnose) suggested for  $\alpha$ -form of the glycosyl linkage. All the NMR data of **6** were compared to those of 3-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-glucuronopyranosyl-oleanolic acid 28-O- $\beta$ -D-glucopyranosyl ester and found to match. This compound was previously isolated from *Achyranthes aspera* [7].

Table 3. NMR data for compound **6** in CD<sub>3</sub>OD

C	$\delta_{\text{C}}$	$\delta_{\text{H}}$	C	$\delta_{\text{C}}$	$\delta_{\text{H}}$
1	39.9	1.00 (m)/1.62 (m)	28	177.8	-
2	28.9	1.70 (m)/1.98 (m)	29	33.5	0.93 (s)
3	91.1	3.20 (dd, 12.0, 4.2)	30	24.0	0.95 (s)
4	40.2	-	3-O-GluA		
5	57.1	0.80 (d, 12.0)	1'	106.6	4.38 (d, 7.8)
6	19.3	1.41 (m)/1.56 (m)	2'	76.0	3.50 (t, 9.0)
7	34.0	1.30 (m)/1.50 (m)	3'	83.9	3.60 (d, 9.0)
8	40.7	-	4'	72.5	3.53 (t, 9.0)
9	48.0	1.60 (m)	5'	77.3	3.38 (dd, 9.0, 7.5)
10	37.9	-	6'	178.1	-
11	25.0	1.90 (m)	3'-O-Rha		
12	123.8	5.27 (br s)	1''	102.6	5.20 (br s)
13	144.9	-	2''	72.3	3.97 (br s)
14	42.9	-	3''	72.3	3.74 (dd, 9.0, 3.0)
15	26.9	1.10 (m)/1.82 (m)	4''	74.1	3.40 (t, 9.0)
16	24.6	1.63 (m)/2.03 (m)	5''	69.9	4.12 (m)
17	47.2	-	6''	18.0	1.26 (d, 6.0)
18	42.6	2.87 (dd, 13.5, 4.5)	28-O-Glc		
19	48.8	1.18 (m)/1.73 (m)	1'''	95.7	5.40 (d, 8.4)
20	31.5	-	2'''	73.9	3.23 (dd, 9.0, 8.4)
21	34.9	1.23 (m)/1.42 (m)	3'''	78.3	3.37 (t, 9.0)
22	33.1	1.53 (m)/1.76 (m)	4'''	71.1	3.52 (t, 9.0)
23	28.6	1.07 (s)	5'''	78.7	3.42 (m)
24	17.8	0.86 (s)	6'''	62.5	3.70 (dd, 11.5, 5.0) 3.83 (dd, 11.5, 2.0)
25	16.1	0.97 (s)	28	178.1	-
26	17.1	0.82 (s)	29	33.5	0.93 (s)
27	26.3	1.17 (s)	30	24.0	0.95 (s)

Compounds **1-6** were tested for their inhibitory effect on NO production in LPS-activated RAW264.7 cells as described in the previous papers [8]. These isolates did not show cytotoxic activity according to the MTT test, and were further evaluated for their NO production inhibitory activity. Interestingly, four ecdysterols (**2-5**) showed significant inhibition with IC<sub>50</sub> values of 27.21 ~40.47  $\mu$ M, compared to positive control compound, L-NMMA, which showed an IC<sub>50</sub> value of 32.24  $\mu$ M. Whereas, compounds **1** and **6** have no activity with IC<sub>50</sub> values over 100  $\mu$ M. These results suggested that ecdysterol components in *A. asperea* may play important role in NO inhibition activity of this plant and further studies on the anti-inflammatory activity of these ecdysterols should be conducted.

Table 4. NO inhibition effects in LPS-activated RAW264.7 cells of compounds **1-6**.

Compounds	NO inhibition (IC <sub>50</sub> , $\mu$ M)
<b>1</b>	> 100
<b>2</b>	40.47 $\pm$ 4.90
<b>3</b>	27.21 $\pm$ 1.23
<b>4</b>	33.44 $\pm$ 1.53
<b>5</b>	27.89 $\pm$ 2.11
<b>6</b>	> 100
<b>L-NMMA *</b>	32.24 $\pm$ 2.30

\*positive control compound

#### 4. CONCLUSIONS

From the results of NO production inhibitory activity screening, the methanol extract of *A. asperea* was phytochemical studied and led to the isolation of five ecdysterols (**1-5**) and one saponin. Their chemical structures were evident by 1D and 2D NMR spectra in comparison with the previous reported data. The isolates were further evaluated their NO inhibition effects in LPS-activated RAW264.7 cells. As the results, the ecdysterols **2-5** were significant inhibitors with IC<sub>50</sub> values of 27.21 ~ 40.47  $\mu$ M, compared to positive control compound, L-NMMA, which showed an IC<sub>50</sub> value of 32.24  $\mu$ M.

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**Declaration of competing interest.** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### REFERENCES

1. Chi V. V. - Dictionary of Vietnamese medicinal plants, Medical Publishing House, Ha Noi, 2018.



2. Goyal B., Goyal R., Mehta A. - Phyto-pharmacology of *Achyranthes aspera*: A Review, *Pharmacognosy Rev.* **1** (2007) 143-150.
3. Jaroslav P., Milos B., Karel V., Vera L., Juraj H. - Ecdysterols from the roots of *Leuzea carthamoides*, *Phytochemistry* **37** (1994) 707-711. 10.1016/S0031-9422(00)90343-1
4. Meng D. L., Li X., Wang J. H., Li W. - A new phytosterone from *Achyranthes bidentata* Bl, *J. Asian Nat. Prod. Res.* **7** (2005) 181-184. doi.org/10.1080/102860203100001625094
5. Nanqun Z., Hiroe K., Bret C. V., Nobuji N., Mukund V. K., Robert T. R., Chi-Tang H. - Ecdysteroids of quinoa seeds (*Chenopodium quinoa* Willd.), *J. Agric. Food Chem.* **49** (2001) 2576-2578. doi.org/10.1021/jf0014462
6. Apichart S., Boon-ek Y., Nart P. - Naturally occurring 20,26-dihydroxyecdysone exists as two C-25 epimers which exhibit different degrees of moulting hormone activity, *Tetrahedron* **54** (1998) 14565-14572. doi. 10.1002/chin.199909222
7. Olaf K., Ernst H., Martin G. S., Josef R., Franz B., Efre M., Dawit A. Asfaw D. -Three saponins, a steroid, and a flavanol glycoside from *Achyranthes aspera*, *Monatshefte fur Chemie* **131** (2000) 195-204. doi.org/10.1007/PL00010306
8. Ha T. T. T., Dung N. T., Tai B. H., Kiem P. V. - Polypunctosides E-K: seven new steroidal saponins from *Polygonatum punctatum* Royle ex Kunth and their nitric oxide production inhibitory activities, *J. Nat. Med.* **77** (2023) 238-249. doi.org/10.1007/s11418-022-01663-w