

Article

New Triterpenoids from the Leaves of *Heritiera littoralis* and Their Anti-Inflammatory Activity

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Abstract: Six new triterpenoids, heritieras C–H (1–6), along with thirteen known triterpenoids (7–19), were isolated from the leaves of *Heritiera littoralis*. Their structures were identified by spectroscopic analysis, including 1D and 2D nuclear magnetic resonance (NMR), high-resolution electrospray ionization mass spectrometry (HRESIMS), and by comparison with the literature. Anti-inflammatory activity of the isolates was evaluated using the lipopolysaccharide (LPS) stimulated RAW 264.7 cell model. Among the isolated triterpenoid, compounds **1**, **12**, **16**, **17**, and **18** demonstrated inhibitory activity against nitric oxide (NO) release, in which compound **18** exhibited the best activity with an IC₅₀ value of 18.13 μM. The potential anti-inflammatory mechanism was investigated using molecular docking. The triterpenoids from *H. littoralis* could be served as potential candidates for the development of new anti-inflammatory agents.

Keywords: *Heritiera*; *Heritiera littoralis*; heritiera C; heritiera D; anti-inflammatory



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1. Introduction

Malvaceae is a family consisting of herbs, subshrubs, shrubs, or trees, with about 245 genera and 4300 species, of which about 59 genera and 264 species are found in China [1,2]. The genus *Heritiera* belongs to the family Malvaceae growing in tropical and subtropical regions of Asia. It consists of 35 species, of which 3 species can be found in provinces of Guangdong, Guangxi, Hainan, and Yunnan in China [3].

The genus *Heritiera* has a long history for medicinal use in China, especially for folk use [4,5]. *Heritiera littoralis* Dryand, a semi-mangrove plant, has been used as a traditional medicine by the Jing people [6]. This plant has been reported for its wide ranges of activities. The water extract of the bark from *H. littoralis* can be used to treat hematuria, diarrhea, and dysentery. The seeds are used to treat diarrhea. The twigs are served as toothbrushes [7,8]. Previous phytochemical investigations of *H. littoralis* revealed a few chemical compositions, such as flavonoids, terpenoids, phenylpropanoids, lignans, steroids, and triterpenoid saponins [9–14]. The constituents from *H. littoralis* have shown an impressive range of biological activities, including antibacterial, antioxidant [15], anti-inflammatory [16,17], and toxicant effects [11].

To further explore the anti-inflammatory ingredients from the leaves of *H. littoralis*, the chemical constitutions and pharmacological activities of *H. littoralis* were investigated. Nineteen compounds, including six new triterpenoids, heritieras C–H (1–6), and thirteen known triterpenoids (7–19) (Figure 1), were isolated from the extract of the leaves of *H. littoralis*. Furthermore, the isolates were evaluated for their anti-inflammatory activity against the production of the NO in LPS induced RAW 264.7 macrophage cells. The potential anti-inflammatory mechanism was further investigated using molecular docking. Herein, the isolation, purification, and determination of the isolates and their anti-inflammatory activity and molecular docking assay are described.

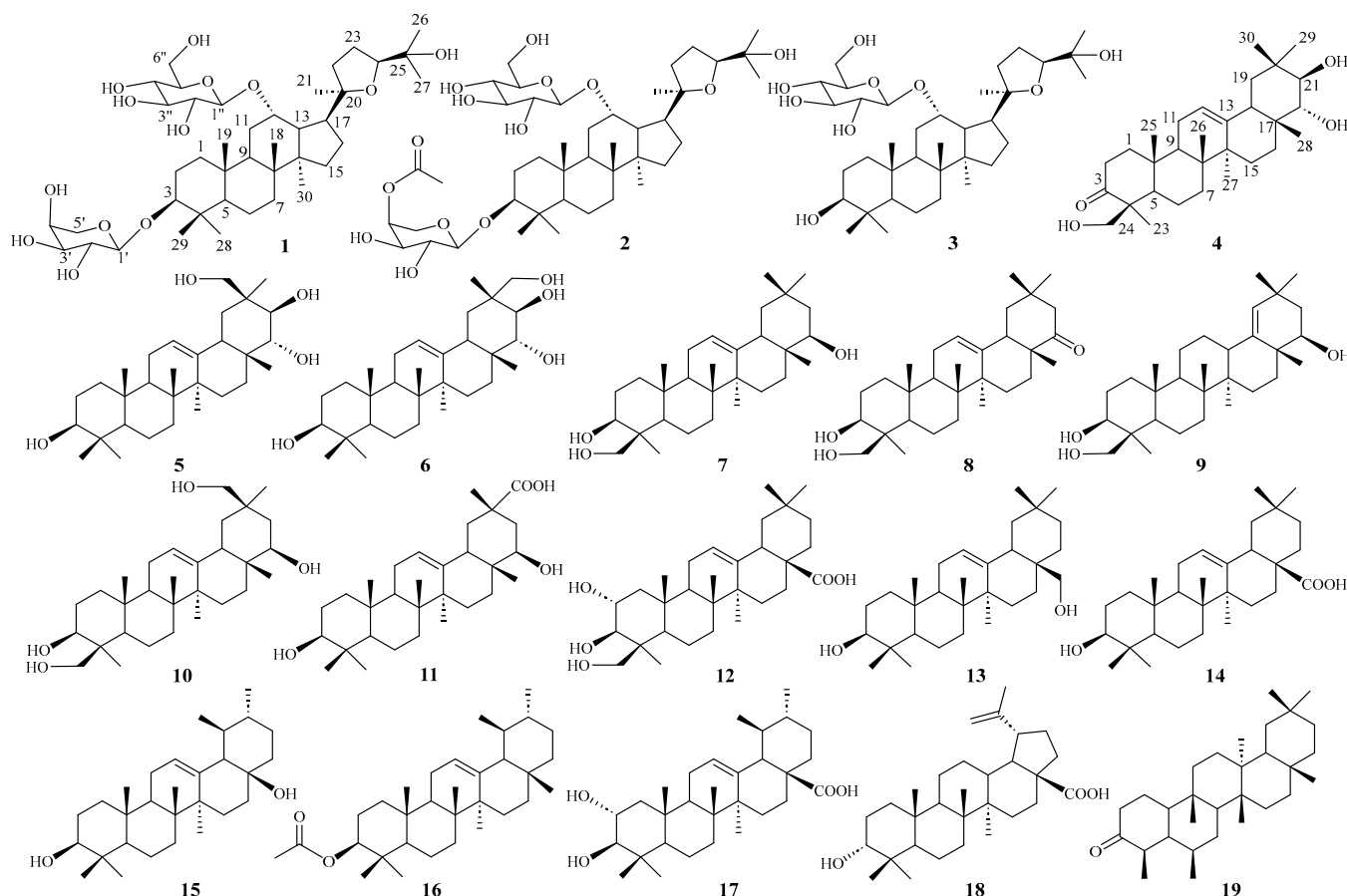


Figure 1. Structures of 1–19 from *H. littoralis*.

2. Results and Discussion

2.1. Elucidation of the Chemical Structures of Heritieras C–H (1–6)

Compound 1, a white amorphous powder, had the molecular formula of $C_{41}H_{70}O_{13}$ determined by HRESIMS m/z 793.4706 $[M + Na]^+$ (calcd. m/z 793.4714), indicating seven unsaturated degrees. The ^1H -NMR spectrum (Table 1) of 1 exhibited eight methyl singlets at δ_H 0.63 (3H, s, H-30), 0.95 (3H, s, H-28), 1.03 (3H, s, H-18), 1.16 (3H, s, H-21), 1.26 (3H, s, H-29), 1.41 (6H, s, H-19, 26), 1.48 (3H, s, H-27), and two anomeric protons, δ_H 4.72 (1H, d, $J = 6.4$ Hz) and δ_H 5.14 (1H, d, $J = 8.0$ Hz), as well as overlapping signals belonging to sugar moieties around δ_H 3.76–4.54. The ^{13}C -NMR, DEPT, and HSQC spectra of 1 displayed 41 carbon signals, including 30 carbons attributed to a triterpenoid skeleton [three oxygenated methines (δ_C 78.0, 81.8, 84.7), and two oxygenated quaternary carbon (δ_C 71.7, 86.9), four methines (δ_C 41.6, 50.0, 51.4, 54.4), four quaternary carbon (δ_C 38.5, 41.7, 41.9, 50.5), nine methylenes (δ_C 18.8, 22.3, 26.7, 27.1, 32.0, 34.6, 35.0, 36.1, 36.8), eight methyls (δ_C 17.2, 17.4 $\times$ 2, 23.6, 25.0, 26.7, 28.1, 30.5)]

and the other 11 carbons assigned to two sugar moieties [one L-arabinopyranose (δ_C 66.8, 69.6, 72.9, 75.2, 102.3) and one D-glucopyranose (δ_C 63.9, 72.9, 75.8, 78.3, 79.1, 102.7)]. The ^1H – ^1H COSY data revealed the partial structures $-\text{CH}_2(1)-\text{CH}_2(2)-\text{CH}(3)-$, $-\text{CH}_2(6)-\text{CH}_2(7)-$, $-\text{CH}(9)-\text{CH}_2(11)-\text{CH}(12)-\text{CH}(13)-\text{CH}(17)-\text{CH}_2(16)-\text{CH}_2(15)-$ and $-\text{CH}_2(22)-\text{CH}_2(23)-\text{CH}_3(24)-$ (Figure 2A). Detailed analysis of the abovementioned NMR data showed that the data of **1** was highly similar to those of ocotillol-II [18], indicating that **1** has the same basic dammarane triterpenoid skeleton as ocotillol-II. The differences from ocotillol-II are that **1** has an L-arabinopyranose connected at position 3 and a D-glucopyranose connected at position 12. Furthermore, the HMBC correlations from the anomeric protons δ_H 4.72 (H-1') to δ_C 81.8 (C-3) and δ_H 5.14 (H-1'') to δ_C 78.0 (C-12) suggested that the two sugars are situated at C-3 and C-12, respectively (Figure 2A). After acid hydrolysis of **1** using 1 M HCl, one set of carbon signals for an α -L-arabinopyranose and another set of carbon signals for a β -D-glucopyranose were identified by comparison on HPLC with authentic samples [19] (Figure S51). In addition, the relative stereochemistry was deduced from ROESY spectrum (Figure 2B). In the ROESY spectrum, the correlations between H-3/H-5/H₃-30/H-17 and H-12/H₃-18/H₃-19 revealed that H-3, 5, 30, and 17 were located on one side, whereas H-12, 18, and 19 were on the other side. According to biosynthetic characteristics of dammarane triterpenoid, H-18 and 19 are β -orientation, whereas H-30 is α -orientation [20]. Consequently, it can be deduced that H-3 is α -orientation and H-12 is β -orientation. In addition, the absolute configurations at C-20 and C-24 were determined to be *R* and *S*, respectively, by comparing with the ^{13}C NMR chemical shift in analogous epoxydammaranes [21,22]. Thus, the structure of **1** was deduced as (20*R*,24*S*)-20,24-epoxy-3 β ,12 α ,25-trihydroxy-12-*O*- β -D-glucopyranosyldammarane-3-*O*- α -L-arabinopyranoside (**1**), named heritiera C (Figure 1).

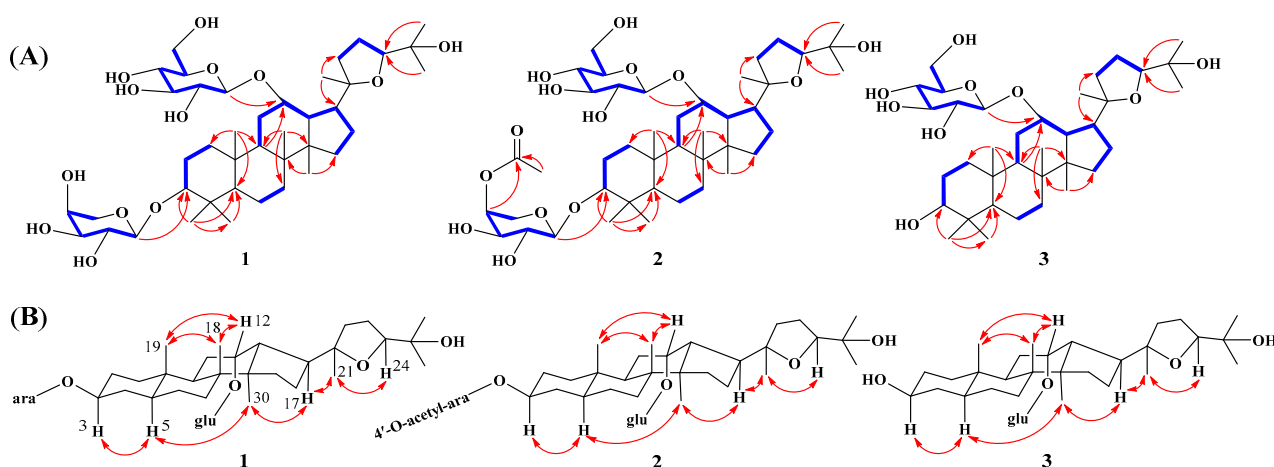


Figure 2. (A) ^1H – ^1H COSY (blue bold bonds) and key HMBC (red arrows) correlations of **1**–**3**. (B) ROESY (double dashed arrows) correlations of **1**–**3**.

Compound **2** was isolated as white amorphous powder. Its molecular formula was established as $\text{C}_{43}\text{H}_{72}\text{O}_{14}$ by HRESIMS m/z 835.4820 [$\text{M} + \text{Na}$] $^+$ (calcd. m/z 835.4820), which is 42 Da more than that of **1**. Extensive analysis of the NMR data (Table 1) revealed that **2** shares almost the same structure as **1**, the difference is one more acetyl group (δ_C 170.7, 20.9) at position 4' in **2**. The location of the acetyl group was confirmed by the HMBC correlations from δ_H 5.41 (H-4') to δ_C 170.7 ($-\text{OOCCH}_3$) (Figure 2A). The sugar units were determined to be α -L-arabinopyranose and β -D-glucopyranose based on the hydrolysis of **2** with 1 M HCl and the assays of HPLC (Figure S51). Moreover, the diagnostic ROESY correlations of H-3/H-5/H₃-30/H-17 suggested that H-3 is α -oriented, while the ROESY correlations of H-12/H-18/H-19 indicated that H-12 is β -oriented (Figure 2B). In addition,

to determine the absolute configuration of the chiral centers C-20 and C-24 in compound **2**, the carbon signals at δ_C 86.9 (C-20) and 84.6 (C-24) were compared with those of analogous dammaranes [21,22], suggesting that the chiral centers are 20*R* and 24*S*, respectively. Therefore, the structure of **2** was determined as (20*R*,24*S*)-20,24-epoxy-3 β ,12 α ,trihydroxy-12-*O*- β -D-glucopyranosyldammarane-3-*O*-[4'-*O*-acetyl]- α -L-arabinopyran-oside (**2**), named heritiera D (Figure 1).

Table 1. ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectral data of compounds **1–3** (pyridine- d_5 , δ in ppm, J in Hz).

	1		2		3	
NO.	δ_H (J in Hz)	δ_C (DEPT)	δ_H (J in Hz)	δ_C (DEPT)	δ_H (J in Hz)	δ_C (DEPT)
1	3.16, dt (10.3, 3.1); 2.08, m	36.1, CH ₂	3.20, dt (10.3, 3.1); 2.13, m	35.6, CH ₂	3.12, dt (10.3, 3.1); 2.16, m	36.0, CH ₂
2	2.07, m; 1.87, m	22.3, CH ₂	2.13, m; 1.86, m	22.0, CH ₂	2.08, m; 1.75, m	27.0, CH ₂
3	3.61, brs	81.8, CH	3.58, brs	81.5, CH	3.59, brs	75.7, CH
4		38.5, C		38.0, C		38.9, C
5	1.60, m	51.4, CH	1.64, m	50.9, CH	1.79, m	50.3, CH
6	1.57, m; 1.45, m	18.8, CH ₂	1.57, m; 1.45, m	18.3, CH ₂	1.54, m; 1.46, m	18.8, CH ₂
7	1.50, m; 1.16, m	36.8, CH ₂	1.52, m; 1.16, m	36.4, CH ₂	1.63, m; 1.21, m	36.8, CH ₂
8		41.7, C		41.5, C		41.8, C
9	1.91, m	54.4, CH	1.92, m	53.9, CH	2.05, m	54.7, CH
10		41.9, C		39.9, C		40.5, C
11	2.93, dt (8.5, 3.9); 1.53, m	35.0, CH ₂	2.93, dt (8.5, 3.9); 1.52, m	34.5, CH ₂	2.96, dt (12.2, 4.1); 1.54, m	34.8, CH ₂
12	4.47, m	78.0, CH	4.47, m	77.8, CH	4.54, m	77.2, CH
13	1.79, m	41.6, CH	1.79, m	41.2, CH	1.84, m	41.5, CH
14		50.5, C		50.0, C		50.5, C
15	1.37, m; 0.99, m	32.0, CH ₂	1.37, m; 0.99, m	31.5, CH ₂	1.44, m; 1.03, m	31.9, CH ₂
16	1.84, m; 1.76, m	26.7, CH ₂	1.86, m; 1.75, m	26.4, CH ₂	1.87, m; 1.64, m	26.8, CH ₂
17	1.88, m	50.0, CH	1.88, m	49.2, CH	1.90, m	49.7, CH
18	1.03, s	17.4, CH ₃	1.04, s	17.0, CH ₃	1.07, s	17.5, CH ₃
19	1.41, s	17.4, CH ₃	1.42, s	16.8, CH ₃	1.43, s	17.3, CH ₃
20		86.9, C		86.4, C		86.9, C
21	1.18, s	25.0, CH ₃	1.18, s	24.5, CH ₃	1.20, s	24.8, CH ₃
22	1.77, m; 1.57, m	34.6, CH ₂	1.75, m; 1.57, m	34.2, CH ₂	1.77, m; 1.60, m	34.7, CH ₂
23	2.05, m; 1.95, m	27.1, CH ₂	2.06, m; 1.95, m	26.7, CH ₂	2.05, m; 1.97, m	26.7, CH ₂
24	3.95, t (7.2)	84.7, CH	3.96, t (7.2)	84.2, CH	3.98, t (7.2)	84.6, CH
25		71.7, C		71.2, C		71.7, C
26	1.41, s	26.7, CH ₃	1.42, s	26.2, CH ₃	1.44, s	26.5, CH ₃
27	1.48, s	28.1, CH ₃	1.48, s	27.7, CH ₃	1.49, s	28.0, CH ₃
28	0.95, s	23.6, CH ₃	0.95, s	23.1, CH ₃	0.95, s	23.4, CH ₃
29	1.26, s	30.5, CH ₃	1.27, s	29.8, CH ₃	1.26, s	30.4, CH ₃
30	0.63, s	17.2, CH ₃	0.61, s	16.7, CH ₃	0.81, s	17.1, CH ₃
1'	4.72, d (6.4)	102.3, CH	4.73, d (6.5)	102.2, CH		
2'	4.43, dd (8.2, 6.4)	72.9, CH	4.31, m	75.1, CH		
3'	4.23, dd (8.2, 3.4)	75.2, CH	4.23, m	75.1, CH		
4'	4.37, m	69.6, CH	5.41, m	73.3, CH		
5'	4.33, m; 3.76, m	66.8, CH ₂	4.34, m; 3.53, m	63.5, CH ₂		
1''	5.14, d (8.0)	102.7, CH	5.14, d (8.0)	102.3, CH	5.17, d (7.8)	102.0, CH
2''	4.01, m	75.8, CH	4.02, m	75.3, CH	4.04, m	75.5, CH
3''	4.30, m	79.1, CH	4.29, m	78.7, CH	4.30, m	78.9, CH
4''	4.16, m	72.9, CH	4.15, m	72.3, CH	4.14, m	72.7, CH
5''	4.03, m	78.3, CH	4.04, m	77.8, CH	4.04, m	78.2, CH
6''	4.54, dd (11.3, 3.0); 4.37, dd (11.3, 5.5)	63.9, CH ₂	4.54, dd (11.3, 3.0); 4.37, dd (11.3, 5.5)	63.3, CH ₂	4.54, dd (11.3, 3.0); 4.37, dd (11.3, 5.5)	63.9, CH ₂
-OOCCH ₃				170.7, C		
-OOCCH ₃			1.98, s	20.9, CH ₃		

Compound **3** was isolated as white amorphous powder. Its molecular formula was established as C₃₆H₆₂O₉ by HRESIMS m/z 661.4291 [$M + \text{Na}$]⁺ (calcd. m/z 661.4292), which is 132 Da less than that of **1**. Extensive analysis of the NMR data (Table 1) revealed that **3** shares almost the same structure as **1**, the only difference is that compound **3** is one less arabinopyranose group at position 3 than **1**. Moreover, the HMBC correlations from δ_H 5.17 (H-1'') to δ_C 77.2 (C-12) suggested that the D-glucopyranose are situated

at C-12 (Figure 2A). The sugar unit was determined to be β -D-glucopyranose based on the hydrolysis of **3** with 1 M HCl and the assays of HPLC (Figure S49). Furthermore, the β -orientations of H-12 were then verified by the ROESY peaks of H-12/H-18/H-19 (Figure 2B). In addition, to determine the absolute configuration for chiral centers C-20/24 of **3**, the comparisons of the carbon signals of compound **3** at δ_C 86.9 (C-20) and 84.6 (C-24) with those of analogous dammaranes suggested its chiral centers to be 20*R* and 24*S* [21,22]. Consequently, the structure of **3** was identified as (20*R*,24*S*)-20,24-epoxy-3 β ,12 α ,25-trihydroxy-12-*O*- β -D-glucopyranosyldammarane (**3**), named heritiera E (Figure 1).

Compound **4** was isolated as white amorphous powder. Its molecular formula was established as $C_{30}H_{48}O_4$ by HRESIMS m/z 473.3633 $[M + H]^+$ (calcd. m/z 473.3631), suggesting seven unsaturated degrees. The ^1H -NMR spectrum (Table 2) of **4** exhibited seven methyl singlets at δ_H 1.06 (3H, s, H-26), 1.17 (3H, s, H-25), 1.22 (3H, s, H-27), 1.25 (3H, s, H-30), 1.29 (3H, s, H-28), 1.45 (3H, s, H-29), and 1.50 (3H, s, H-23), and one olefinic proton δ_H 5.35 (1H, t, $J = 3.6$ Hz, H-12). Its ^{13}C NMR spectrum (Table 2) revealed the presence of 30 carbon resonances, which were classified by DEPT and HSQC spectra, including one carbonyl carbon (δ_C 214.9), one double bond (δ_C 122.6, 145.0), three oxygenated carbons (δ_C 65.6, 75.0, 80.0), and seven methyl carbons (δ_C 16.1, 17.4, 21.2, 21.7, 22.7, 27.0, 31.9). Extensive analysis of the NMR data (Table 2) revealed that **4** shares almost the same structure as soyasapogenol A [23]. The difference between these two compounds is that soyasapogenol A has four oxygenated carbon signals, while **4** has three oxygenated carbon signals and a carbonyl carbon signal (δ_C 214.9), suggesting that one oxygenated carbon in soyasapogenol A has been oxidated to a carbonyl carbon in **4**. In the ^1H - ^1H COSY spectrum of **4**, the following partial structures were verified as $-CH_2(1)-CH_2(2)-$, $-CH(5)-CH_2(6)-CH_2(7)-$, $-CH(9)-CH_2(11)-CH(12)-$, $-CH_2(15)-CH_2(16)-$, $-CH(18)-CH_2(19)-$ and $-CH(21)-CH(22)-$ (Figure 3A). Furthermore, the key HMBC correlations from δ_H 2.37 and 2.83 (H-2), 1.50 (H₃-23), as well as 3.89 and 4.37 (H-24) to δ_C 214.9 (C-3) confirmed that the carbonyl carbon was situated at C-3 (Figure 3A). In addition, the relative configurations of **4** were determined by NOESY spectrum (Figure 3B). The key correlations between H-5/H-9/H-21/H₃-23/H₃-27/H₃-29 indicate that H-5, H-9, H-21, H₃-23, H₃-27, and H₃-29 are on the same side, suggesting that H-5, H-9, H-21, H₃-23, H₃-27, and H₃-29 are α -orientation. The correlations between H-18/H-22/H₂-24/H₃-25/H₃-26/H₃-28/H₃-30 suggest that H-18, H-22, H₂-24, H₃-25, H₃-26, H₃-28, and H₃-30 are on the same side, indicating that H-18, H-22, H₂-24, H₃-25, H₃-26, H₃-28, and H₃-30 are β -orientation. Consequently, according to the above analysis and the biosynthetic characteristics of oleanane triterpenoid [20], the absolute configuration of **4** can be determined as 21 β ,22 α ,24-trihydroxy-3-oxo-olean-12-ene (**4**), named heritiera F (Figure 1).

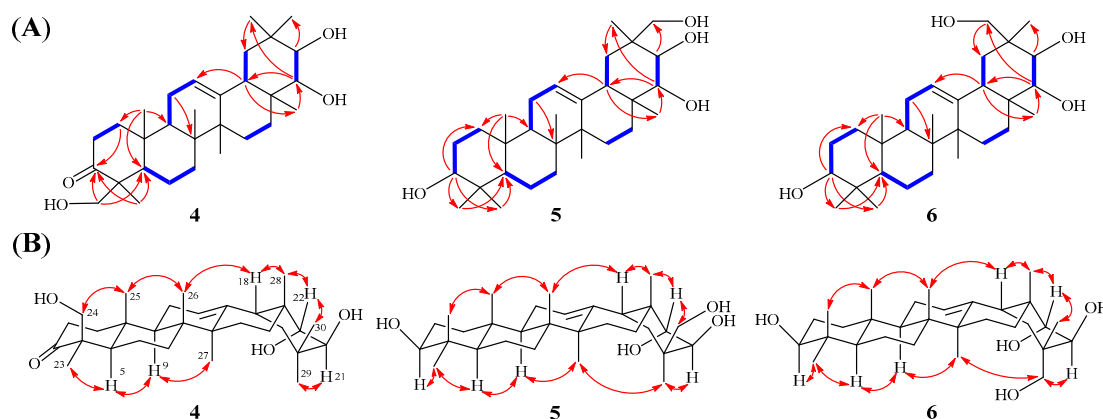


Figure 3. (A) ^1H - ^1H COSY (blue bold bonds) and key HMBC (red arrows) correlations of **4**–**6**. (B) NOESY (double dashed arrows) correlations of **4**–**6**.

Table 2. ^1H NMR and ^{13}C NMR spectral data of compounds **4–6** (pyridine- d_5 , δ in ppm, J in Hz).

4 ^a			5 ^a		6 ^b	
NO.	δ_{H} (J in Hz)	δ_{C} (DEPT)	δ_{H} (J in Hz)	δ_{C} (DEPT)	δ_{H} (J in Hz)	δ_{C} (DEPT)
1	1.33, m; 1.78, m	40.8, CH ₂	1.00, m; 1.58, m	39.5, CH ₂	1.01, m; 1.60, m	39.5, CH ₂
2	2.37, m; 2.83, m	36.0, CH ₂	1.86, m; 1.86, m	28.5, CH ₂	1.86, m; 1.86, m	28.5, CH ₂
3		214.9, C	3.46, dd (11.2, 5.0)	78.4, CH	3.46, dd (11.2, 5.0)	78.4, CH
4		55.6, C		42.5, C		39.5, C
5	1.39, m	58.2, CH	0.88, m	56.1, CH	0.86, m	56.1, CH
6	1.18, m; 1.64, m	20.4, CH ₂	1.44, m; 1.63, m	19.2, CH ₂	1.45, m; 1.61, m	19.2, CH ₂
7	1.30, m; 1.49, m	33.3, CH ₂	1.35, m; 1.56, m	33.2, CH ₂	1.33, m; 1.55, m	33.2, CH ₂
8		40.6, C		39.8, C		39.8, C
9	1.68, m	47.9, CH	1.68, m	48.4, CH	1.69, m	48.4, CH
10		37.4, C		37.6, C		37.6, C
11	1.68, m; 1.91, m	24.6, CH ₂	1.90, m; 1.90, m	24.3, CH ₂	1.92, m; 1.94, m	24.3, CH ₂
12	5.35, t (3.6)	122.6, CH	5.36, t (3.4)	123.4, CH	5.41, t (3.6)	123.0, CH
13		145.0, C		144.8, C		145.1, C
14		42.4, C		41.1, C		42.5, C
15	0.97, m; 1.84, m	26.9, CH ₂	1.00, m; 1.90, m	26.9, CH ₂	1.01, m; 1.90, m	27.0, CH ₂
16	1.01, m; 1.96, m	27.8, CH ₂	1.10, m; 2.06, m	28.0, CH ₂	1.05, m; 2.14, m	27.8, CH ₂
17		39.6, C		39.8, C		41.5, C
18	2.61, dd (13.8, 4.3)	44.3, CH	2.89, dd (13.8, 4.3)	44.6, CH	2.75, dd (13.8, 4.3)	43.7, CH
19	1.34, m; 2.09, m	47.6, CH ₂	1.81, m; 2.06, m	43.9, CH ₂	1.40, m; 2.58, m	41.4, CH ₂
20		37.0, C		40.6, C		40.7, C
21	3.87, s	75.0, CH	4.15, d (3.4)	76.6, CH	4.51, d (3.5)	70.8, CH
22	3.78, t (3.0)	80.0, CH	3.84, s	79.8, CH	3.87, s	80.2, CH
23	1.52, s	21.2, CH ₃	1.26, s	29.1, CH ₃	1.25, s	29.1, CH ₃
24	3.89, d (11.1) 4.37, d (11.1)	65.6, CH ₂	1.08, s	16.9, CH ₃	1.08, s	17.0, CH ₃
25	1.17, s	16.1, CH ₃	0.96, s	16.1, CH ₃	0.98, s	16.1, CH ₃
26	1.06, s	17.4, CH ₃	1.31, s	17.4, CH ₃	1.08, s	17.5, CH ₃
27	1.22, s	27.0, CH ₃	1.06, s	27.1, CH ₃	1.33, s	27.1, CH ₃
28	1.29, s	22.7, CH ₃	1.34, s	22.8, CH ₃	1.35, s	22.7, CH ₃
29	1.46, s	21.7, CH ₃	1.44, s	27.4, CH ₃	3.66, d (10.1) 4.07, d (10.1)	72.0, CH ₃
30	1.25, s	31.9, CH ₃	4.33, d (10.3) 4.42, d (10.3)	67.4, CH ₂	1.54, s	17.9, CH ₂

^a **4** and **5** were recorded in 600 MHz; ^b **6** was recorded in 400 MHz.

Compound **5** was isolated as white amorphous powder. Its molecular formula was established as C₃₀H₅₀O₄ by HRESIMS m/z 497.3606 [M + Na]⁺ (calcd. m/z 497.3607), suggesting six unsaturated degrees. The ^1H -NMR spectrum (Table 2) of **5** exhibited seven methyl singlets at δ_{H} 0.96 (3H, s, H-25), 1.06 (3H, s, H-27), 1.08 (3H, s, H-23), 1.26 (3H, s, H-24), 1.31 (3H, s, H-26), 1.34 (3H, s, H-28) and 1.44 (3H, s, H-29), and one olefinic proton δ_{H} 5.36 (1H, t, J = 3.4 Hz, H-12). Its ^{13}C NMR spectrum (Table 2) revealed the presence of 30 carbon resonances, which were classified by DEPT and HSQC spectra, including one double bond (δ_{C} 123.4, 144.8), four oxygenated carbons (δ_{C} 67.4, 76.6, 78.4, 79.8), and seven methyl carbons (δ_{C} 16.1, 16.9, 17.4, 22.8, 27.1, 27.4, 29.1). Extensive analysis of the NMR data (Table 2) revealed that **5** shares almost the same structure as abrisapogenol L [24], the difference being in that **5** possesses a methyl group at C-24 instead of a CH₂OH at C-24 in abrisapogenol L. In the ^1H – ^1H COSY spectrum of **5**, the partial structures revealed are as follows: –CH₂(1)–CH₂(2)–CH(3)–, –CH(5)–CH₂(6)–CH₂(7)–, –CH(9)–CH₂(11)–CH(12)–, –CH₂(15)–CH₂(16)–, –CH(18)–CH₂(19)– and –CH(21)–CH(22)– (Figure 3A). In addition, the key HMBC correlations from δ_{H} 3.46 (H-3) and 0.88 (H-5) to δ_{C} 16.9 (C-23) and 29.1 (C-24) confirmed that two methyl groups of C-23 and C-24 were linked to C-4 in **5**. (Figure 3A). Furthermore, the relative configurations of **5** were determined by NOESY spectrum (Figure 3B). In the NOESY spectrum of **5**, the correlations of H-3/H-5/H-9/H-

21/H₃-27/H₃-29 suggest that H-3, H-5, H-9, H-21, H₃-27 and H₃-29 are on the same side, indicating H-3, H-5, H-9, H-21, H₃-27 and H₃-29 are α -orientation. The NOESY correlations of H-18/H-22/H₃-28/H₂-30 suggest that H-18, H-22, H₃-28 and H₂-30 are on the same side, indicating H-18, H-22, H₃-28 and H₂-30 are β -orientation. According to the above analysis, the structure of **5** was deduced as 3 β ,21 β ,22 α ,30-tetrahydroxy-olean-12-ene (**5**), named heritiera G (Figure 1).

Compound **6** was isolated as white amorphous powder. Its molecular formula was established as C₃₀H₅₀O₄ by HRESIMS m/z 497.3596 [M + Na]⁺ (calcd. m/z 497.3607), which is the same as that of **5**. The 1D NMR data (Table 1) for **5** and **6** are highly similar, the difference being in C-21 (δ_H 4.51, δ_C 70.8), C-29 (δ_H 3.66 and 4.07, δ_C 72.0) and C-30 (δ_H 1.54, δ_C 17.9) in **6** instead of C-21 (δ_H 4.15, δ_C 76.6), C-29 (δ_H 1.44, δ_C 27.4), and C-30 (δ_H 4.33 and 4.42, δ_C 67.4) in **5**. From the above analysis, it can be inferred that **6** possesses a methyl group at C-29 and a CH₂OH at C-30 instead of a CH₂OH at C-29 and a methyl group at C-30. In the NOESY spectrum of **6**, the correlations of H-3/H-5/H-9/H α -16/H-21/H₃-27/H₂-29 suggest that H-3, H-5, H-9, H α -16, H-21, H₃-27, and H₂-29 are on the same side, indicating H-3, H-5, H-9, H α -16, H-21, H₃-27, and H₂-29 are α -orientation. The NOESY correlations of H-18/H-22/H₃-28/H₃-30 suggest that H-18, H-22, H₃-28, and H₃-30 are on the same side, indicating H-18, H-22, H₃-28, and H₃-30 are β -orientation (Figure 3B). Thus, the structure of **6** was deduced as 3 β ,21 β ,22 α ,29-tetrahydroxyl-olean-12-ene (**6**), named heritiera H (Figure 1).

By comparing the measured NMR (¹H and ¹³C) and MS data to those reported in the literature, the known triterpenoids were identified as soyasapogenol B (**7**) [25], soyasapogenol E (**8**) [26], soyasapogenol H (**9**) [27], wistariasapogenol B (**10**) [28], triptotriterpenic acid B (**11**) [29], 2 α ,3 β ,24-trihydroxy olean-12-en-28-oic acid (**12**) [30], erythrodiol (**13**) [31], oleanolic acid (**14**) [32], uvaol (**15**) [33], β -amyrin acetate (**16**) [34], 2 α -hydroxy ursolic acid (**17**) [35], betulinic acid (**18**) [13], and friedelin (**19**) [36] (Figure 1).

2.2. Anti-Inflammatory Assay of the Isolates

Cell viability was measured by an MTT assay. The RAW 264.7 cell viability assays showed that the survival rate remained greater than 90% after treatment with all isolates at different concentrations from 0 to 50 μ M. All isolates were evaluated for their anti-inflammatory effects on the production of NO in the RAW 264.7 macrophage cell line exposed to the inflammatory stimulus by LPS. Dexamethasone was used as a positive control. The effects of active compounds (IC₅₀ < 50 μ M) on the production of NO by LPS-induced RAW 264.7 cells are shown in Table 3.

Table 3. Anti-inflammatory effects of active compounds (IC₅₀ < 50 μ M) on the production of the NO in LPS-stimulated RAW 264.7 Cells ^a.

Compounds	IC ₅₀ (μ M)
1	47.12 $\pm$ 0.61
12	25.23 $\pm$ 0.76
16	45.31 $\pm$ 0.45
17	39.98 $\pm$ 0.42
18	18.13 $\pm$ 0.32
Dexamethasone ^b	16.37 $\pm$ 0.82

^a All values are means of three independent experiments. Values present mean $\pm$ SD of triplicate experiments.

^b Dexamethasone as positive control.

The results showed that compound **18** substantially inhibited the release of NO, with IC₅₀ value of 18.13 μ M. The value is slightly lower than that of positive control, dexamethasone, with IC₅₀ value of 16.37 μ M. Compounds **1**, **12**, **16**, and **17** showed moderate effects with IC₅₀ values of 47.12, 45.31, 39.98, and 25.23 μ M, respectively. Compounds **2–11**, **15**,

and **19** showed no significant effects against LPS-induced nitric oxide production in RAW 264.7 macrophages.

2.3. Predicted Binding Modes of Compounds and iNOS, COX-2 Using Molecular Docking Analysis

NO serves as an indicator of inflammatory response, and elevated levels of NO within tissues signify the overexpression of critical proteins, iNOS and COX-2 [37,38]. Specifically, iNOS plays a role in catalyzing the production of NO within the inflammatory signaling pathway [39]. Studies have shown that the interaction between iNOS/COX-2 and certain small molecules can halt the inflammatory process and prevent the overproduction of downstream inflammatory mediators [40]. In an effort to elucidate the potential mechanisms behind NO inhibition, the binding interactions between bioactive compounds and iNOS/COX-2 were examined.

The compounds (**1**, **12**, **16**, **17**, **18**, and dexamethasone) were subjected to molecular docking. Results from molecular docking study revealed that compounds **1**, **12**, **16**, **17**, **18**, and dexamethasone had strong interactions with the iNOS/COX-2 protein (Figures 4, 5 and S53–S62) and the binding residues and the logarithms of free binding energies were collated in Tables S1 and S2. The results show that **18** docks effectively into the active sites of iNOS and COX-2, with binding energies of -9.0 kcal/mol, which is comparable to those of dexamethasone (positive control, with binding energies of -9.5 and -9.6 kcal/mol, respectively). Further simulations were conducted to model the docking sites of compound **18** with iNOS and COX-2. The results show that compound **18** binds to the amino acid residues TRP-457 and GLY-365 of the iNOS protein through hydrogen bonds, and to the amino acid residues ARG-1044 and GLN-1370 of the COX-2 protein through hydrogen bonds as well.

Comparison their IC_{50} values with the results of molecular docking, it was discovered that the IC_{50} values of the active compounds correlate with the trends observed in molecular docking study. Compounds with higher activity have lower Logarithms of free binding energies (FBE). For example, compound **18** and dexamethasone form hydrogen bonds with the amino acid residue GLY-365 in the iNOS protein to show lower IC_{50} values and free binding energies. Meanwhile, compounds **12**, **17**, and **18** all feature an α -oriented hydroxyl group (OH) on ring A and demonstrate excellent activities. They are likely the critical factor contributing to their activities.

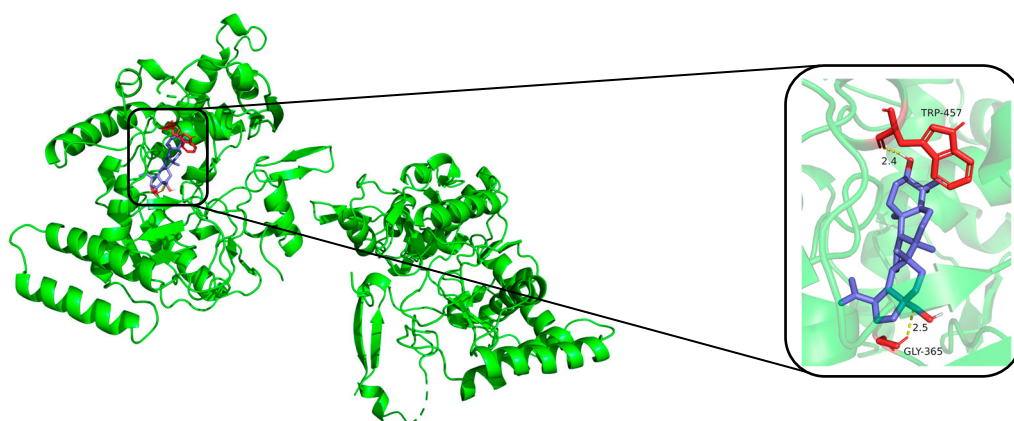


Figure 4. Molecular docking result of compound **18** with iNOS protein. Molecular docking simulation obtained at lowest energy conformation, highlighting potential hydrogen contacts of compound **18** (iNOS protein is shown in green, compound **18** are in purple). For clarity, only interacting residues are labeled. Hydrogen bonds between amino acid residues and compounds are represented by yellow dashed lines. These figures were created by PyMOL (3.0.0).

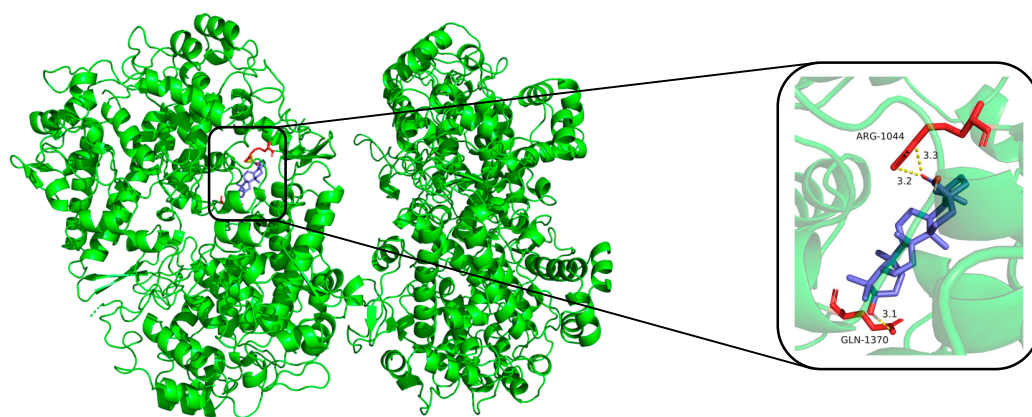


Figure 5. Molecular docking result of compound **18** with COX-2 protein. Molecular docking simulation obtained at lowest energy conformation, highlighting potential hydrogen contacts of compound **18** (COX-2 protein is shown in green, compound **18** are in purple). For clarity, only interacting residues are labeled. Hydrogen bonds between amino acid residues and compounds are represented by yellow dashed lines. These figures were created by PyMOL.

3. Experimental

3.1. General Experimental Procedures

UV spectra were acquired with a TU-1901 spectrophotometer. NMR experiments were conducted on Bruker Advance 600 or 500 MHz spectrometers. Optical rotations were acquired based on a JASCO P-2000 polarimeter. HRESIMS were recorded on Agilent 6545 Q-TOF LC-MS mass spectrometer. Semi-preparative HPLC separations were conducted on an Agilent 1260 instrument equipped with a DAD detector and a C₁₈ column; analytical HPLC was conducted on an Agilent 1260 instrument equipped with a DAD detector and an Agilent C₁₈ column. OD values of 96-well were measured with an imark Bio-Rad plate microplate reader.

3.2. Plant Material

The dried leave (5.0 kg) of *H. littoralis* was obtained from Beilun Estuary in Guangxi Province, China, in September 2018 and identified by professor Qiuping Zhong (Guangxi Institute of Botany). A voucher specimen (No. ID-20180910) is deposited at the Guangxi key laboratory of green chemical materials and safety technology, Beibu Gulf University, China.

3.3. Extraction and Isolation

Dried leaves (5.0 kg) of *H. littoralis* were extracted with H₂O-EtOH (20 L each, 25:75) under the extraction tank (4 h each), The filtrates were combined and dried under reduced pressure to yield a residue (0.52 kg). The concentrates were suspended in H₂O (1 L) and partitioned with EtOAc (5 × 1 L) and *n*-BuOH fraction (5 × 1 L). The EtOAc extract (233 g) was eluted by silica gel CC (200~300 mesh) using petroleum ether (PE) and ethyl acetate (95:5 to 50:50 *v/v*) as eluents and six fractions (Frs. 1–6) were obtained based on TLC assay. Fr. 2 (43.1 g) was subjected to silica gel CC (200~300 mesh) and eluted sequentially with PE-CH₂Cl₂ (100:0, 90:10, 80:20, 50:50, and 0:100, *v/v*) to yield five fractions (Fr. 2.1–Fr. 2.5). Fr. 2.3 was purified by recrystallization to yield **19** (12.6 mg). Fr. 2.4 was further separated using Sephadex LH-20 (PE:CH₂Cl₂:MeOH = 1:2:1, *v/v/v*) to obtain compound **16** (5.2 mg). Six fractions (Fr. 3.1–Fr. 3.6) were obtained by further separation of Fr. 3 (24.3 g) on an ODS column eluted with H₂O-MeOH (40:60 to 0:100, *v/v*). Fr. 3.3 was subjected to silica gel CC (200~300 mesh) and eluted sequentially with CH₂Cl₂-MeOH (100:0, 98:2, 96:4, 94:6, 90:10, and 1:1, *v/v*) to yield five fractions (Fr. 3.3.1–Fr. 3.3.5), according to TLC monitoring. Fr. 3.3.2 was purified by semipreparative HPLC (5 μm, 9.3 × 250 mm, 2.5 mL/min, 197 nm),

eluted with H₂O-MeCN (27:73, *v/v*) to yield **11** (*t_R* 14.2 min, 7.3 mg), **13** (*t_R* 23.8 min, 9.4 mg), and **14** (*t_R* 32.9 min, 12.3 mg). After purification over Sephadex LH-20 CC eluted with MeOH, Fr. 3.3.3 was purified by semipreparative HPLC (5 μ m, 9.3 $\times$ 250 mm, 2.5 mL/min, 197 nm), to obtain compounds **4** (*t_R* 16.7 min, 5.6 mg), **7** (*t_R* 20.1 min, 39.1 mg), and **9** (*t_R* 28.6 min, 4.6 mg). Fr. 3.4 was purified by semipreparative HPLC (5 μ m, 9.3 $\times$ 250 mm, 2.5 mL/min, 210 nm), eluted with H₂O-MeOH (15:85, *v/v*) to yield **18** (*t_R* 36.7 min, 8.6 mg). Fr. 4 (20.7 g) was further separated using Sephadex LH-20 (MeOH) to yield five fractions (Fr. 4.1–Fr. 4.4). Fr. 4.1 (6.1 g) was submitted to ODS column eluted with H₂O-MeOH (40:60 to 10:90, *v/v*) to give **12** (*t_R* 15.4 min, 7.2 mg), **15** (*t_R* 18.6 min, 6.5 mg), and **17** (*t_R* 26.1 min, 12.1 mg). Fr. 4.2 (3.5 g) was submitted to Sephadex LH-20 column eluted with MeOH to give **8** (20.1 mg). Compounds **5** (*t_R* 16.7 min, 4.8 mg), **6** (*t_R* 25.2 min, 13.7 mg), and **10** (*t_R* 15.4 min, 54.0 mg) were afforded by semipreparative HPLC purification of Fr. 4.3 (5 μ m, 9.3 $\times$ 250 mm, 2.5 mL/min, 196 nm), eluted with H₂O-MeCN (23:77, *v/v*). Fr. 4.4 purified by semipreparative HPLC (H₂O:MeOH = 40:60, *v/v*) separations to yield **3** (*t_R* 23.5 min, 5.2 mg). Fr. 5 (23.1 g) was purified successively by ODS column eluted with H₂O-MeOH (50:50 to 10:90, *v/v*) and a Sephadex LH-20 column (H₂O:MeOH = 80:20, *v/v*) to give **1** (8.2 g) and **2** (4.5 mg).

3.4. Characterization of the Isolates

Heritiera C (**1**): white amorphous powder; $[\alpha]_D^{25}$ – 10.7 (*c* 0.10, MeOH); HRESIMS *m/z* 793.4914 [*M* + Na]⁺, calcd for C₄₁H₇₀O₁₃Na). For ¹H (pyridine-*d*₅, 600 MHz) and ¹³C NMR (pyridine-*d*₅, 150 MHz) spectroscopic data, see Table 1; all significant data are given in the electronic Supplementary Materials (Figures S1–S8).

Heritiera D (**2**): white amorphous powder; $[\alpha]_D^{25}$ – 23.5 (*c* 0.10, MeOH); HRESIMS *m/z* 835.4820 [*M* + Na]⁺, calcd for C₄₃H₇₂O₁₄Na). For ¹H (pyridine-*d*₅, 600 MHz) and ¹³C NMR (pyridine-*d*₅, 150 MHz) spectroscopic data, see Table 1; all significant data are given in the Supplementary Materials (Figures S9–S16).

Heritiera E (**3**): white amorphous powder; $[\alpha]_D^{25}$ – 17.2 (*c* 0.10, MeOH); HRESIMS *m/z* 661.4291 [*M* + Na]⁺, calcd for C₃₆H₆₂O₉Na). For ¹H (pyridine-*d*₅, 600 MHz) and ¹³C NMR (pyridine-*d*₅, 150 MHz) spectroscopic data, see Table 1; all significant data are given in the Supplementary Materials (Figures S17–S24).

Heritiera F (**4**): white amorphous powder; $[\alpha]_D^{25}$ + 15.4 (*c* 0.10, MeOH); HRESIMS *m/z* 473.3633 [*M* + H]⁺, calcd for C₃₀H₄₈O₄). For ¹H (pyridine-*d*₅, 600 MHz) and ¹³C NMR (pyridine-*d*₅, 150 MHz) spectroscopic data, see Table 2; all significant data are given in the electronic Supplementary Materials (Figures S25–S32).

Heritiera G (**5**): white amorphous powder; $[\alpha]_D^{25}$ + 27.4 (*c* 0.10, MeOH); HRESIMS *m/z* 497.3607 [*M* + Na]⁺, calcd for C₃₀H₅₀O₄Na). For ¹H (pyridine-*d*₅, 600 MHz) and ¹³C NMR (pyridine-*d*₅, 150 MHz) spectroscopic data, see Table 2; all significant data are given in the Supplementary Materials (Figures S33–S40).

Heritiera H (**6**): white amorphous powder; $[\alpha]_D^{25}$ + 20.2 (*c* 0.10, MeOH); HRESIMS *m/z* 497.3596 [*M* + Na]⁺, calcd for C₃₀H₅₀O₄Na). For ¹H (pyridine-*d*₅, 600 MHz) and ¹³C NMR (pyridine-*d*₅, 150 MHz) spectroscopic data, see Table 2; all significant data are given in the Supplementary Materials (Figures S41–S48).

Soyasapogenol B (**7**): colorless crystals; ¹H NMR (400 MHz, CDCl₃) δ : 5.14 (1H, t, *J* = 3.7 Hz, H-12), 4.10 (1H, d, *J* = 11.0 Hz, H-3), 3.49 (1H, s, H-22), 1.13 (3H, s, H-23), 1.00 (3H, s, H-25), 0.92 (3H, s, H-26), 0.84 (3H, s, H-27), 0.82 (3H, s, H-28), 0.80 (3H, s, H-29), 0.75 (3H, s, H-30); ¹³C NMR (100 MHz, CDCl₃) δ : 38.3 (C-1), 25.7 (C-2), 80.4 (C-3), 42.0 (C-4), 55.8 (C-5), 18.4 (C-6), 33.1 (C-7), 39.5 (C-8), 47.7 (C-9), 37.3 (C-10), 23.6 (C-11), 122.3 (C-12), 143.7 (C-13), 42.3 (C-14), 28.3 (C-15), 27.2 (C-16), 36.6 (C-17), 45.0 (C-18), 46.0 (C-19), 30.4

(C-20), 41.1 (C-21), 76.3 (C-22), 22.4 (C-23), 64.5 (C-24), 16.1 (C-25), 16.8 (C-26), 25.0 (C-27), 28.4 (C-28), 32.2 (C-29), 19.6 (C-30).

Soyasapogenol E (**8**): white amorphous powder; ^1H NMR (600 MHz, CDCl_3) δ : 5.29 (1H, t, $J = 3.7$ Hz, H-12), 4.20 (1H, d, $J = 11.1$ Hz, H-24), 3.44 (1H, dd, $J = 11.8, 4.5$ Hz, H-24), 3.34 (1H, d, $J = 11.1$ Hz, H-3), 1.25 (3H, s, H-27), 1.22 (3H, s, H-23), 0.99 (6H, s, H-26, 29), 0.93 (3H, s, H-30), 0.89 (3H, s, H-25), 0.85 (3H, s, H-28); ^{13}C NMR (150 MHz, CDCl_3) δ : 38.5 (C-1), 27.7 (C-2), 80.9 (C-3), 42.9 (C-4), 55.9 (C-5), 18.5 (C-6), 33.0 (C-7), 39.8 (C-8), 47.7 (C-9), 36.8 (C-10), 23.9 (C-11), 123.7 (C-12), 141.7 (C-13), 41.9 (C-14), 25.4 (C-15), 27.3 (C-16), 47.7 (C-17), 47.7 (C-18), 46.8 (C-19), 34.3 (C-20), 51.0 (C-21), 217.3 (C-22), 25.5 (C-23), 22.5 (C-23), 64.6 (C-24), 16.2 (C-25), 16.8 (C-26), 25.2 (C-28), 32.1 (C-29), 20.7 (C-30).

Soyasapogenol H (**9**): colorless crystals; ^1H NMR (600 MHz, pyridine- d_5) δ : 4.98 (1H, s, H-19), 4.52 (1H, d, $J = 11.0$ Hz, H-24), 4.00 (1H, dd, $J = 12.3, 3.8$ Hz, H-22), 3.69 (1H, dd, $J = 11.0, 4.3$ Hz, H-3), 2.39 (1H, d, $J = 11.6$ Hz, H-24), 1.54 (3H, s, H-23), 1.34 (3H, s, H-30), 1.14 (3H, s, H-29), 1.08 (6H, s, 25, 26), 0.92 (3H, s, H-28), 0.87 (3H, s, 27); ^{13}C NMR (150 MHz, pyridine- d_5) δ : 39.6 (C-1), 28.1 (C-2), 80.5 (C-3), 43.7 (C-4), 56.9 (C-5), 19.5 (C-6), 35.5 (C-7), 40.7 (C-8), 52 (C-9), 37.7 (C-10), 22.0 (C-11), 27.3 (C-12), 39.1 (C-13), 43.3 (C-14), 29.0 (C-15), 35.7 (C-16), 41.3 (C-17), 143.3 (C-18), 129.7 (C-19), 34.1 (C-20), 42.7 (C-21), 75.8 (C-22), 23.9 (C-23), 64.9 (C-24), 18.9 (C-25), 17.8 (C-26), 15.4 (C-27), 16.6 (C-28), 32.5 (C-29), 30.5 (C-30).

Wistariasapogenol B (**10**): white amorphous powder; ^1H NMR (600 MHz, pyridine- d_5) δ : 3.95 (2H, m, H-24, 29), 3.81 (4H, m, H-24, 29), 3.73 (1H, m, H-22), 3.69 (1H, dd, 1.5, 5.9, H-3), 1.58 (3H, s, H-30), 1.29 (3H, s, H-28), 1.25 (3H, s, H-27), 1.09 (3H, s, H-23), 1.01 (3H, s, H-26), 0.94 (3H, s, H-25); ^{13}C NMR (150 MHz, pyridine- d_5) δ : 39.3 (C-1), 28.8 (C-2), 80.5 (C-3), 43.6 (C-4), 56.7 (C-5), 19.5 (C-6), 33.9 (C-7), 40.4 (C-8), 48.5 (C-9), 37.4 (C-10), 24.4 (C-11), 123.1 (C-12), 145.0 (C-13), 42.7 (C-14), 26.8 (C-15), 28.8 (C-16), 38.5 (C-17), 45.6 (C-18), 42.4 (C-19), 36.3 (C-20), 39.3 (C-21), 75.6 (C-22), 24.4 (C-23), 65.0 (C-24), 16.6 (C-25), 17.4 (C-26), 26.2 (C-27), 21.7 (C-28), 70.6 (C-29), 28.9 (C-30).

Triptotriterpenic acid B (**11**): white amorphous powder; ^1H NMR (600 MHz, pyridine- d_5) δ : 5.44 (1H, t, $J = 3.6$ Hz, H-12), 4.04 (1H, dd, $J = 7.4, 3.2$ Hz, H-22), 3.46 (1H, dd, $J = 11.2, 4.9$ Hz, H-3), 1.30 (3H, s, H-30), 1.27 (3H, s, H-28), 1.26 (3H, s, H-27), 1.08 (3H, s, H-23), 1.08 (3H, s, H-24), 1.00 (3H, s, H-26), 0.88 (1H, s, H-25); ^{13}C NMR (150 MHz, pyridine- d_5) δ : 38.4 (C-1), 29.1 (C-2), 78.4 (C-3), 40.0 (C-4), 56.1 (C-5), 19.2 (C-6), 33.6 (C-7), 40.4 (C-8), 48.4 (C-9), 37.7 (C-10), 25.4 (C-11), 123.6 (C-12), 144.7 (C-13), 42.8 (C-14), 26.7 (C-15), 21.4 (C-16), 39.5 (C-17), 45.1 (C-18), 41.9 (C-19), 43.0 (C-20), 38.2 (C-21), 75.7 (C-22), 29.3 (C-23), 17.0 (C-24), 16.2 (C-25), 17.6 (C-26), 28.5 (C-27), 25.9 (C-28), 181.92 (C-29), 24.3 (C-30).

$2\alpha,3\beta,24$ -trihydroxy olea-12-en-28-oic acid (**12**): white amorphous powder; ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 5.15 (1H, brs, H-12), 4.24 (1H, m, H-2), 3.90 (1H, brs, H-3), 3.71 (1H, d, $J = 10.7$ Hz, H-24), 3.20 (1H, d, $J = 10.7$ Hz, H-24), 1.09 (3H, s, H-27), 0.93 (3H, s, H-23), 0.87 (6H, s, H-25, 30), 0.85 (3H, s, H-29), 0.67 (3H, s, H-26); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ : 41.3 (C-1), 64.7 (C-2), 72.6 (C-3), 41.8 (C-4), 48.3 (C-5), 18.0 (C-6), 32.8 (C-7), 47.1 (C-9), 37.7 (C-10), 23.2 (C-11), 121.5 (C-12), 143.9 (C-13), 43.8 (C-14), 27.2 (C-15), 22.9 (C-16), 45.7 (C-17), 40.8 (C-18), 45.5 (C-19), 30.4 (C-20), 33.4 (C-21), 32.2 (C-22), 22.6 (C-23), 63.8 (C-24), 16.8 (C-25), 16.5 (C-26), 25.7 (C-27), 178.6 (C-28), 32.9 (C-29), 23.4 (C-30).

Erythrodiol (**13**): white amorphous powder; ^1H NMR (600 MHz, CDCl_3) δ : 5.19 (1H, d, $J = 3.3$ Hz, H-12), 3.56 (1H, d, $J = 11.0$ Hz, H-28), 3.22 (1H, d, $J = 11.0$ Hz, H-28), 3.12 (1H, m, H-3), 1.16 (3H, s, H-27), 0.99 (3H, s, H-23), 0.94 (3H, s, H-24), 0.93 (3H, s, H-30), 0.88 (3H, s, H-29), 0.86 (3H, s, H-26), 0.78 (3H, s, H-25); ^{13}C NMR (150 MHz, CDCl_3) δ : 38.7 (C-1), 26.1 (C-2), 79.1 (C-3), 38.9 (C-4), 55.3 (C-5), 18.5 (C-6), 32.7 (C-7), 39.9 (C-8), 47.7 (C-9), 37.0 (C-10), 22.1 (C-11), 122.5 (C-12), 144.3 (C-13), 41.8 (C-14), 28.2 (C-15), 23.7 (C-16), 37.0 (C-17), 42.4 (C-18), 46.6 (C-19), 31.1 (C-20), 34.2 (C-21), 31.2 (C-22), 27.3 (C-23), 15.7 (C-24), 15.6 (C-25), 16.8 (C-26), 25.7 (C-27), 69.8 (C-28), 33.3 (C-29), 23.6 (C-30).

Oleanolic acid (**14**): white amorphous powder; ^1H NMR (400 MHz, CDCl_3) δ : 5.27 (1H, d, $J = 3.5$ Hz, H-12), 3.12 (1H, m, H-3), 1.16 (3H, s, H-27), 0.99 (3H, s, H-23), 0.93 (3H, s, H-30), 0.90 (3H, s, H-29), 0.91 (3H, s, H-25), 0.77 (3H, s, H-24), 0.74 (3H, s, H-26); ^{13}C NMR (100 MHz, CDCl_3) δ : 38.5 (C-1), 27.3 (C-2), 79.1 (C-3), 38.9 (C-4), 55.3 (C-5), 18.5 (C-6), 32.7 (C-7), 39.4 (C-8), 47.7 (C-9), 37.0 (C-10), 23.1 (C-11), 122.8 (C-12), 143.7 (C-13), 41.8 (C-14), 27.8 (C-15), 23.7 (C-16), 46.7 (C-17), 41.1 (C-18), 46.0 (C-19), 30.8 (C-20), 33.9 (C-21), 32.6 (C-22), 28.2 (C-23), 15.7 (C-24), 15.6 (C-25), 17.3 (C-26), 26.1 (C-27), 183.6 (C-28), 33.3 (C-29), 23.6 (C-30).

Uvaol (**15**): white amorphous powder; ^1H NMR (500 MHz, CDCl_3) δ : 5.14 (1H, t, $J = 3.6$ Hz, H-12), 3.52 (1H, t, $J = 8.2$ Hz, H-28a), 2.89 (d, $J = 15.5$ Hz, 1H), 2.78 (1H, d, $J = 15.5$ Hz, H-28b), 1.25 (3H, s, H-27), 1.10 (s, 3H, H-25), 1.00 (s, 3H, H-23), 0.99 (s, 3H, H-26), 0.95 (s, 3H, H-29), 0.93 (s, 3H, H-30), 0.79 (s, 3H, H-24); ^{13}C NMR (125 MHz, CDCl_3) δ : 38.9 (C-1), 27.4 (C-2), 79.2 (C-3), 38.2 (C-4), 55.3 (C-5), 18.5 (C-6), 32.9 (C-7), 39.5 (C-8), 47.8 (C-9), 43.5 (C-10), 23.4 (C-11), 125.1 (C-12), 138.7 (C-13), 42.2 (C-14), 26.1 (C-15), 23.5 (C-16), 37.0 (C-17), 54.2 (C-18), 40.2 (C-19), 39.6 (C-20), 35.3 (C-21), 30.8 (C-22), 28.3 (C-23), 15.8 (C-24), 15.8 (C-25), 16.9 (C-26), 23.5 (C-27), 70.2 (C-28), 17.5 (C-29), 21.5 (C-30).

β -amyrin acetate (**16**): white amorphous powder; ^1H NMR (500 MHz, CDCl_3) δ : 4.50 (1H, m, H-3), 5.25 (1H, t, $J = 3.6$ Hz, H-12), 1.10 (3H, s, H-24), 0.90 (3H, s, H-25), 0.85 (3H, s, H-26), 0.89 (3H, s, H-27), 0.99 (3H, s, H-28), 1.05 (3H, s, H-29), 0.91 (3H, s, H-30), 0.93 (3H, s, H-31), 2.03 (3H, s, H-32). ^{13}C NMR (125 MHz, CDCl_3) δ : 38.4 (C-1), 23.7 (C-2), 81.1 (C-3), 37.8 (C-4), 55.4 (C-5), 18.4 (C-6), 32.7 (C-7), 39.8 (C-8), 47.6 (C-9), 37.0 (C-10), 23.6 (C-11), 121.8 (C-12), 145.4 (C-13), 41.9 (C-14), 26.3 (C-15), 27.1 (C-16), 32.6 (C-17), 47.4 (C-18), 46.9 (C-19), 31.2 (C-20), 34.8 (C-21), 37.3 (C-22), 16.9 (C-23), 28.2 (C-24), 15.7 (C-25), 16.9 (C-26), 26.1 (C-27), 23.8 (C-28), 33.4 (C-29), 28.5 (C-30), 171.2 (C=O), 21.4 ($\text{CH}_3\text{-C=O}$).

2α -hydroxy ursolic acid (**17**): white amorphous powder; ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ : 5.16 (1H, dd, $J = 15.2, 5.6$ Hz, H-12), 1.08 (3H, s, H-27), 0.92 (9H, m, H-23, 24, 26), 0.88 (1H, s, H-25), 0.74 (3H, s, H-29), 0.70 (3H, s, H-30); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ : 47.1 (C-1), 67.5 (C-2), 82.3 (C-3), 39.5 (C-4), 54.8 (C-5), 18.2 (C-6), 32.6 (C-7), 38.9 (C-8), 46.8 (C-9), 36.3 (C-10), 23.0 (C-11), 124.5 (C-12), 138.2 (C-13), 41.7 (C-14), 27.5 (C-15), 23.8 (C-16), 47.0 (C-17), 52.4 (C-18), 38.4 (C-19), 37.6 (C-20), 30.2 (C-21), 36.3 (C-22), 28.8 (C-23), 17.1 (C-24), 17.0 (C-25), 17.2 (C-26), 23.3 (C-27), 178.3 (C-28), 16.4 (C-29), 21.5 (C-30).

Betulinic acid (**18**): white amorphous powder; ^1H NMR (500 MHz, CDCl_3) δ : 4.78 (1H, brs, H-29a), 4.61 (1H, brs, H-29b), 3.19 (1H, dd, $J = 11.2, 4.9$ Hz, H-3), 1.69 (3H, s, H-30), 0.97 (3H, s, H-23), 0.96 (3H, s, H-26), 0.93 (3H, s, H-25), 0.82 (3H, s, H-24), 0.75 (3H, s, H-27); ^{13}C NMR (125 MHz, CDCl_3) δ : 38.7 (C-1), 27.4 (C-2), 79.0 (C-3), 38.9 (C-4), 55.6 (C-5), 18.3 (C-6), 34.3 (C-7), 40.7 (C-8), 50.5 (C-9), 37.2 (C-10), 20.8 (C-11), 25.5 (C-12), 38.4 (C-13), 42.4 (C-14), 29.5 (C-15), 32.1 (C-16), 56.3 (C-17), 46.9 (C-18), 49.2 (C-19), 150.5 (C-20), 29.7 (C-21), 37.0 (C-22), 28.0 (C-23), 15.4 (C-24), 16.0 (C-25), 16.1 (C-26), 14.7 (C-27), 77.0 (C-28), 109.7 (C-29), 19.4 (C-30).

Friedelin (**19**): white amorphous powder; ^1H -NMR (500 MHz, CDCl_3) δ : 0.72 (3H, s, H-3), 0.87 (3H, s), 0.90 (3H, s), 0.95 (3H, s), 0.98 (3H, s), 1.01 (3H, s), 1.05 (3H, s), 1.18 (3H, s); ^{13}C NMR (125 MHz, CDCl_3) δ : 22.2 (C-1), 41.7 (C-2), 213.1 (C-3), 58.2 (C-4), 42.2 (C-5), 41.4 (C-6), 18.2 (C-7), 53.2 (C-8), 37.6 (C-9), 59.5 (C-10), 36.1 (C-11), 30.5 (C-12), 39.8 (C-13), 38.4 (C-14), 35.2 (C-15), 32.6 (C-16), 30.0 (C-17), 42.9 (C-18), 35.3 (C-19), 28.1 (C-20), 32.9 (C-21), 39.4 (C-22), 7.0 (C-23), 14.7 (C-24), 18.0 (C-25), 20.2 (C-26), 18.6 (C-27), 32.2 (C-28), 35.5 (C-29), 31.9 (C-30).

3.5. Enzymatic Hydrolysis of Compounds 1–3

Acidic hydrolysis of compounds **1–3** were carried out according to the method described previously [18]. The configuration of the sugar moiety was determined by com-

paring their retention times with the derivatives of authentic samples. D-glucose and L-arabinose were confirmed by comparison with those of authentic samples t_R : 6.5 min (D-glucose); 6.8 min (L-arabinose) (Figures S49–S51).

3.6. Anti-Inflammatory Assay

All compounds showed no cytotoxicity on RAW 264.7 cells at the concentrations from 0 to 50. All isolates from the leaves of *H. littoralis* were subjected to MTT assays to assess the cell viability of LPS stimulated RAW 264.7 cell model. Anti-inflammatory activity of terpenoids were evaluated using LPS stimulated RAW 264.7 cell model. The productions of NO were determined by Griess analysis [41,42].

3.7. Molecular Docking Studies

Molecular docking simulations were performed using the software AutoDock Vina along with AutoDock Tools (ADT 1.5.6) using the hybrid Lamarckian Genetic Algorithm (LGA) [43]. The three-dimensional (3D) crystal structures of iNOS (PDB code, 3E6T) and COX-2 (PDB code, 1PXX) were obtained from the RCSB Protein Data Bank, which resolution was 2.5 Å [44]. The standard 3D structures (PDB format) of selected compounds for molecular docking were constructed by chem3D Pro 22.0 software, whose configurations were determined by their NOESY spectra and Chem3D modeling. The cubic grid box of 20 Å size (x, y, z) with a spacing of 1.000 Å and grid maps were built. All of the other parameters were used according to default settings of AutoDock Vina. Results differing by less than 2.0 Å in positional root mean-square deviation (RMSD) were clustered together, and the results of the most favorable free energy of binding were chosen as the resultant complex structures.

4. Conclusions

Six new compounds, heritieras C–H (1–6), and thirteen known triterpenoids (7–19) were isolated from the leaves of *H. littoralis*. Five triterpenoids decreased secretions of NO on LPS stimulated RAW 264.7 cells. Among these compounds, compound 18 substantially inhibit the release of NO. The results from molecular docking revealed the preliminary anti-inflammatory mechanism due to the bindings of iNOS/COX-2 with bioactive triterpenoids. Our research disclosed that *H. littoralis* is potentially useful as a medicine for the treatment of inflammation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules30010131/s1>, Experimental details, including figures and tables of this manuscript, are provided as Supplementary Material.

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