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Anti-inflammatory activity and phytochemistry of the leaf extracts of *Baccaurea sylvestris* Lour

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Abstract. *Baccaurea* is a genus of plants in the family Phyllanthaceae. Many species of the *Baccaurea* had available bioactivities. They have been used in many countries from Indo-Malaysia to the Western Pacific. However, chemical components of this genus haven't been studied in detail. From the leaf extracts of *B. sylvestris* Lour collected Kbang district, Gia Lai province, Viet Nam, anti-inflammatory activity and phytochemistry of the leaf extracts of *Baccaurea sylvestris* Lour were reported. The *n*-hexane (BSH) and ethyl acetate (BSE) extracts inhibited NO production in lipopolysaccharide-induced RAW 264.7 cells with IC_{50} values of 14.90 ± 1.82 and 43.48 ± 4.92 µg/mL, respectively, without cell toxicity. From these extracts seven known compounds including friedelin (**1**), 3*β*-friedelanol (**2**), stigmast-4-en-3-one (**3**), (-)-epiafzelechin (**4**), 4-hydroxybenzaldehyde (**5**), 4-hydroxybenzoic acid (**6**) and 3,4,5-trimethoxyphenyl-*O*-*β*-D-glucopyranoside (**7**) were isolated. Their chemical structures were determined by ESI-MS and NMR spectral analyses and comparison with literature data. These compounds had available anti-inflammatory activity as previous reports.

Keywords: Baccaurea sylvestris, anti-inflammatory activity, friedelin, 3β-friedelanol, stigmast-4-en-3-one.

Classification numbers: 1.1.1, 1.1.6, 1.2.1.

1. INTRODUCTION

The genus *Baccaurea* (Phyllanthaceae) consists of flowering plants distributed from Indo-Malaysia to the Western Pacific. Phytochemical investigations from *Baccaurea* species have revealed a diversity of compounds [1 - 4]. *Baccaurea* plants have been reported to possess significant biological activities such as antioxidant [1], antifungal [2], antiinflammatory [3], and anticancer [4] activities. In Viet Nam, there are six *Baccaurea* species, namely *B. annamensis* Gagnep, *B. sylvestris* Lour, *B. oxycarpa* Gagnep, *B. ramiflora* Lour, *B. henii* Thin and *B. harmandiii* Gagn [5, 6]. *B. sylvestris* Lour grows mostly from Nghe An province to Binh Dinh

province [7]. So far, chemical and biological investigations of this plant have not been conducted. As part of our investigation on bioactive compounds from plants of Viet Nam, in the present study, we reported anti-inlammatory activity (Table 1) of *B. sylvestris* leaf extracts collected in Gia Lai province. Seven known compounds including friedelin (**1**), 3*β*-friedelanol (**2**), stigmast-4-en-3-one (**3**), (-)-epiafzelechin (**4**), 4-hydroxybenzaldehyde (**5**), 4-hydroxybenzoic acid (**6**) and 3,4,5-trimethoxyphenyl-*O*-*β*-D-glucopyranoside (**7**) (Fig. 1) were isolated and their structures determined.

2. MATERIALS AND METHODS

2.1. General Experimental Procedure

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer. ESI-MS spectra were measured on an Agilent 1100 Series LC/MSD Trap SL. Column chromatography (CC) was performed using silica gel 60 (230 - 400 mesh, Merck). Precoated silica gel 60 F254 (Merck) TLC was used for thin layer chromatography.

2.2. Plant material

The leaves of *B. sylvestris* Lour. (Phyllathaceae) were collected in March 2018 from Gia Lai province, and the scientific name was identified by Dr. Do Van Hai, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher specimen (PTH24032018) was deposited at the Institute of Ecology and Biological Resources.

2.3. Nitric oxide assay

In vitro anti-inflammatory activity of extracts on LPS-induced RAW 264.7 macrophages was investigated by measuring NO levels as previously described in the literature [8]. The RAW 264.7 cells were seeded to a 96-well plate at a concentration of 2×10^5 cells/well and grown in an incubator at 37 ^{o}C and 5 % CO_2 for 24 h. Next, the culture medium was replaced by DMEM without FBS for 3 h. Then, cells were treated with samples at different concentrations (100, 20, 4 and 0.8 µg/mL) for 2 h in the presence of LPS (10 µg/mL) for 24 h. N^G-Methyl-L-arginine acetate (L-NMMA) (Sigma) was used as the positive control and the cells treated with a diluted solution of DMSO (1.0 %) were used as the negative control. Nitrite (NO_2^-), an indicator of NO production, was determined using the Griess reagent system (Promega Cooperation, WI, USA). Briefly, 100 μL of medium was mixed with 50 μL of 1 % (w/v) sulfanilamide in 5 % (v/v) phosphoric acid and 50 μL of 0.1 % (w/v) *N*-1-naphthylethylenediamine dihydrochloride in a 96-well plate. The mixture in the plate was incubated at room temperature for 10 min. The optical density (OD) was measured at 540 nm using a Biotek microplate reader. The concentration of nitrite in each sample was calculated using the $NaNO_2$ standard curve. The ability of the sample to inhibit NO production was determined by the following formula:

% inhibition = 100 % - [concentration of NO_{sample}/concentration of NO_{LPS}] × 100.

The value of IC_{50} (the half maximal inhibitory concentration) was calculated using Table Curve 2Dv4 computer software. All experiments were carried out in triplicate.

2.4. *In vitro* cytotoxic assay

The effects of extracts on the viability of cells were determined by sulforhodamine B (SRB) cytotoxic assay according to the Monks method [9]. Cells were grown in 96-well microliter pla-

tes containing 190 µL of medium (10 % DMSO) with 3.10^4 cells/well, then incubated at 37 °C with 5 % CO_2. After 24 h, the samples dissolved in DMSO (10 µL) were added to each well at concentrations of 100, 20, 4, and 0.8 µg/mL. The plate without samples served as a day 0 (time 0) control. The cells were continuously cultured for an additional 72 h. After incubating, cell monolayers were fixed with 20 % (wt/v) trichloroacetic acid, and stained for 30 min and washed with 5 % (v/v) acetic acid (three times) to remove excess SRB. The protein bound dye was dissolved in a 10 mM Tris base solution. Optical density (OD) was determined at 515 nm using an ELISA Plate Reader (Biotek).

$$(\%)\ \text{inhibition} = 100\ \% - (OD_{(sample)} - OD_{(0)})/(OD_{(c)} - OD_{(0)}) \times 100.$$

OD_{sample} is the average optical density value at 72 h; OD_0 is the average optical density value at time zero; and ODc is the average optical density value of the control sample which contains 10 % DMSO. IC_{50} values were calculated using Table Curve 2Dv4 software. All experiments were carried out in triplicate.

2.5. Extraction and isolation

The dried leaves of *B. sylvestris* (5 kg) were pulverized and extracted with 85 % aqueous MeOH (4 × 20 L) at room temperature (4 times × 24 h). The solvent was removed under reduced pressure to give a crude MeOH extract. The MeOH extract was suspended in H_2O (1 L), then successively partitioned with *n*-hexane and ethyl acetate (EtOAc). After removal of the solvent, *n*-hexane (BSH, 50 g), EtOAc (BSE, 15 g), and water (BSW, 45 g) extracts were obtained, respectively.

The BSH extract (50 g) was applied on a silica gel CC eluted with *n*-hexane/EtOAc (20/1; 15/1; 10/1; 5/1, v/v), to give four fractions (BH1–BH4). Fraction BH1 (4 g) was chromatographed on a silica gel CC eluted with *n*-hexane/CH_2Cl_2 (1.5/1, v/v) to give three subfractions (BH1.1-BH1.3). The subfraction BH1.2 (50 mg) was chromatographed on a silica gel CC eluted with *n*-hexane/CH_2Cl_2 (1.5/1, v/v) to give three subfractions (BH1.2.1-BH1.2.3). The subfraction BH1.2.3 (15 mg) was purified on a Sephadex LH-20 CC eluted with CH_2Cl_2/CH_3OH (2/8, v/v) to give compound **1** (7 mg). The fraction BH2 (8.5 g) was chromatographed on a silica gel column eluted with *n*-hexane/EtOAc (10/1, v/v) to give five subfractions (BH2.1-BH2.5). The subfraction BH2.2 (35 mg) was purified on a Sephadex LH-20 CC eluted with CH_2Cl_2/CH_3OH (1/9, v/v) to give compound **2** (17 mg) and the subfraction BH2.3 (27 mg) was purified on a Sephadex LH-20 CC eluted with CH_2Cl_2/CH_3OH (2/8, v/v) to give compound **3** (8 mg). The fraction BH3 (15.5 g) was chromatographed on a silica gel column eluted with CH_2Cl_2 to give four subfractions (BH3.1-BH3.4). The subfraction BH3.3 (250 mg) was subjected to a silica gel CC eluted with CH_2Cl_2/acetone (20/1) to afford four subfractions (BH3.3.1-BH3.3.4). The subfraction BH3.3.3 (57 mg) was purified on a Sephadex LH-20 CC eluted with MeOH to give compound **4** (16 mg).

The BSE extract (15 g) was chromatographed on a silica gel CC eluted with *n*-hexane/EtOAc (2/1, v/v) to give two fractions, BE1 (11.2 g) and BE2 (1.5 g). The fraction BE1 was chromatographed on a silica gel CC eluted with *n*-hexane/EtOAc (4/1) to give three ~~smaller~~subfractions BE1.1-BE1.3. Subfraction BE1.1 was chromatographed on a silica gel CC eluted with *n*-hexane/EtOAc (4/1) to give five subfractions (BE1.1.1-BE1.1.5). The subfraction BE1.1.1 (35 mg) was purified on a silica gel CC eluted with *n*-hexane/EtOAc (4/1) to give compound **5** (7 mg). The subfraction BE1.1.4 (115 mg) was purified on a Sephadex LH-20 CC eluted with MeOH to give compound **6** (50 mg). Fraction BE2 was chromatographed on a silica gel CC eluted with CH_2Cl_2/EtOAc (1/1, v/v) to give four subfractions (BE2.1-BE2.4). The subfraction BE2.3 (27 mg) was purified on a Sephadex LH-20 CC eluted with MeOH to give compound

7 (9 mg).

Friedelin (1): white solid. ESI-MS: *m/z* 427 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 2.

3*β*-Friedelanol (2): white solid. ESI-MS: *m/z* 429 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 2.

Stigmast-4-en-3-one (**3**): white solid. ESI-MS: *m/z* 413 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 2.

(-)-Epiafzelechin (4): pale yellow solid. ESI-MS: *m/z* 297.0 [M+Na]+; $[\alpha]_D^{25}$ = -41(MeOH, *c* 0.1). ^{1}H-NMR (CD_3OD, 500 MHz): δ_H 4.89 (1H, s, H-2), 4.20 (1H, m, H-3), 2.76 (1H, dd, *J* = 2.5 Hz, 16.5 Hz, H-4a), 2.89 (1H, dd, *J* = 4.5 Hz, 16.5 Hz, H-4b), 5.95 (1H, d, *J* = 2.5 Hz, H-6), 5.97 (1H, d, *J* = 2.5 Hz, H-8), 6.80 (2H, d, *J* = 8.5 Hz, H-3′, H-5′), 7.34 (2H, d, *J* = 8.5 Hz, H-2′, H-6′). ^{13}C-NMR (CD_3OD, 125 MHz): δ_C 79.9 (C-2), 67.4 (C-3), 29.3 (C-4), 157.7 (C-5), 96.5 (C-6), 158.0 (C-7), 95.9 (C-8), 157.4 (C-9), 100.1 (C-10), 131.6 (C-1′), 129.1 (C-2′, 6′), 115.8 (C-3′, 5′), 157.9 (C-4′).

4-Hydroxybenzaldehyde (5): yellow solid. ESI-MS: *m/z* 123 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ_H 9.73 (1H, s, -CHO), 7.76 (2H, d, *J* = 8.5 Hz, H-2, H-6), 6.88 (2H, d, *J* = 8.5 Hz, H-3, H-5). ^{13}C-NMR (CD_3OD, 125 MHz): δ_C 192.7 (-CHO), 167.4 (C-4), 133.6 (C-2, C-6), 129.3 (C-1), 117.5 (C-3, C-5).

4-Hydroxybenzoic acid (6): pale yellow solid. ESI-MS: *m/z* 139 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ_H 7.89 (2H, d, *J* = 8.5 Hz, H-2, H-6), 6.84 (2H, d, *J* = 8.5 Hz, H-3, H-5). ^{13}C-NMR (CD_3OD, 500 MHz): δ_C 170.1 (-COOH), 163.3 (C-4), 133.0 (C-2, C-6), 116.0 (C-3, C-5).

3,4,5-Trimethoxyphenyl-*O*-*β*-D-glucopyranoside (7): white solid. ESI-MS: *m/z* 345 $[M-H]^-$; $[\alpha]_D^{25}$ = -17(MeOH, c 0.1). ^{1}H-NMR (CD_3OD, 500 MHz): δ_H 6.51 (2H, s, H-2, H-6), 4.83 (1H, overlapped, H-1′), 3.36-3.50 (4H, m, H-2′, H-3′, H-4′, H-5′), 3.68 (1H, dd, *J* = 6.5 Hz, 12.0 Hz, H-6′a), 3.94 (1H, dd, *J* = 2.5 Hz, 12.0 Hz, H-6′b), 3.83 (3H, s, 3-OMe, 5-OMe), 3.72 (3H, s, 4-OMe). ^{13}C-NMR (CD_3OD, 125 MHz): δ_C 156.0 (C-1), 96.2 (C-2, C-6), 154.8 (C-3, C-5), 134.5 (C-4), 103.2 (C-1′), 75.0 (C-2′), 78.1 (C-3′), 71.7 (C-4′), 78.4 (C-5′), 62.8 (C-6′), 56.6 (3,5-OMe), 61.2 (4-OMe).

3. RESULTS AND DISCUSSION

3.1. Anti-inflamatory activity of leaf extracts

Nitric oxide (NO) participates in various responses of host resistance to various pathogens, and the overproduction of NO causes pathological problems related to inflammation. RAW 264.7 cells activated by LPS produce large amounts of NO. Therefore, LPS-induced production of NO in murine macrophage RAW 264.7 cells was used for screening useful anti-inflammatory agents [8]. As shown in Table 1, the BSH extract possessed potent NO inhibitory activity against lipopolysaccharide (LPS)-induced nitric oxide release with an IC_{50} value of 14.90 ± 1.82 µg/mL, followed by the BSE extract (IC_{50} = 43.48 ± 4.92 µg/mL), whereas the BSW extract was inactive in the NO inhibitory assay (IC_{50} > 100 µg/mL) [8]. The cell survival rates of the extracts were observed at active concentrations, ranging from 81 to 96 % at 100 µg/mL, exhibiting the safety of the BSH and BSE extracts and effective suppression of inflammatory responses.

Table 1. NO-inhibitory activities of leaf extracts.

Concentration (µg/mL)	BSH		BSE		BSW		L-NMMA	
	% inhibition	% cell survival	% inhibition	% cell survival	% inhibition	% cell survival	% inhibition	% cell survival
100	72.79	81.13	55.35	101.17	36.90	96.14	103.28	89.94
20	52.54	94.30	46.12	102.56	29.68	97.71	72.62	98.86
4	37.50		31.08		18.45		25.27	
0.8	22.46		18.65		1.40		12.03	
IC_{50}	**14.90 ± 1.82**	-	**43.48 ± 4.92**	-	**>100**	-	**8.83 ± 0.75**	-

3.2. Structural elucidation of the isolated compounds

Compound **1** was isolated as a white solid. The ^{1}H-NMR spectrum showed characteristic signals of a friedelane skeleton with seven methyl singlets at δ_H 0.72, 0.87, 0.95, 1,00, 1.01, 1.05, and 1.18 (each peak, 3H, s) and a methyl doublet at δ_H 0.88 (d, J = 6.5 Hz, H_3-23). The ^{13}C-NMR and DEPT spectra showed signals of 30 carbons, including one ketone carbonyl at δ_C 213.1 (C-3); eight methyls at δ_C 6.8 (C-23), 14.7 (C-24), 18.0 (C-25), 20.3 (C-26), 18.7 (C-27), 32.1 (C-28), 35.0 (C-29), and 31.8 (C-30); eleven methylenes; four methines and six quaternary carbons at δ_C 28.2 (C-20), 30.0 (C-17), 37.5 (C-9), 38.3 (C-14), 39.7 (C-13), and 42.1 (C-5).

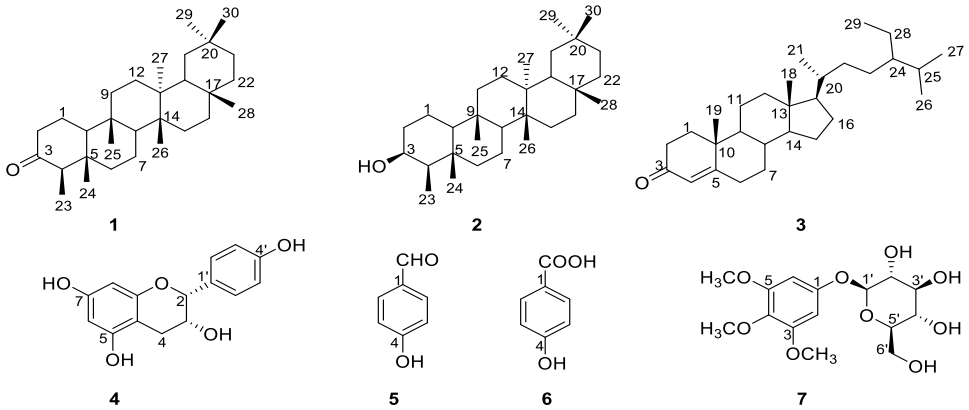

Figure 1. Chemical structures of compounds **1-7**.

The assignments of carbon atoms were determined by HMBC spectrum. The HMBC correlations of H_3-23 (δ_H 0.88) and C-4 (δ_C 58.2)/C-5 (δ_C 42.1), H_3-24 (δ_H 0.73) and C-4 (δ_C 58.2)/(C-5 (δ_C 42.1)/C-6 (δ_C 41.3)/C-10 (δ_C 59.5), H_3-25 (δ_H 0.87) and C-8 (δ_C 53.1)/C-9 (δ_C 37.5)/C-10 (δ_C 59.5)/C-11 (δ_C 35.6), H_3-26 (δ_H 1.01) and C-8 (δ_C 53.1)/C-13 (δ_C 39.7)/C-14 (δ_C 38.3)/ C-15 (δ_C 32.4), H_3-27 (δ_H 1.05) and C-12 (δ_C 30.5)/C-13 (δ_C 58.2)/C-18 (δ_C 40.8), H_3-28 (δ_H 1.18) and C-17 (δ_C 58.2)/C-18 (δ_C 40.8)/C-21 (δ_C 32.8), H_3-29 (δ_H 0.95) and C-19 (δ_C 35.4)/C-20 (δ_C 28.2)/C-30 (δ_C 31.8), H_3-30 (δ_H 1.00) and C-19 (35.4)/C-20 (δ_C 28.2)/C-29 (δ_C 35.0) were observed. In addition, the HMBC correlations between H-2a (δ_H 2.38) and C-3 (δ_C

213.1)/C-10 (δ_C 59.0); H-2b (δ_H 2.29) and C-1 (δ_C 22.3), H-4 (δ_H 2.27) and C-1 (δ_C 22.3)/C-3 (δ_C 213.1)/C-5 (δ_C 42.1)/C-10 (δ_C 59.0)/C-23 (δ_C 6.8)/C-24 (δ_C 14.7) confirmed the position of the ketone group. The molecular formula of **1** as $C_{30}H_{50}O$ was determined by NMR and ESI-MS spectra, that showed a *quasi*-molecular ion peak at *m/z* 427 $[M+H]^+$. Based on spectral analysis of **1** and comparison with data in the literature [10], compound **1** was identified as friedelin (friedelan-3-one). Friedelin was first isolated from the bark of cork trees [10]. This compound exhibited potent anti-inflammatory activity with a maximum inhibition of 52.5 % at a dose of 40 mg/kg in an assay using the carrageenan-induced rat paw oedema model [11].

Table 2. The NMR spectral data for compounds **1**–**3**[#].

Position	**1**		**2**		**3**	
	δ_C [a]	δ_H [b] (mult., *J* in Hz)	δ_C [a]	δ_H [b] (mult., *J in* Hz)	δ_C [a]	δ_H [b] (mult., *J in* Hz)
1	22.3	1.96 (m), 1.69 (m)	15.8	1.55[c], 1.43 (m)	35.7	2.01 (m), 1.32 (m)
2	41.5	2.38 (m), 2.29 (m)	36.1	1.55[c], 1.34[c]	33.9	2.42 (m), 1.68 (m)
3	213.1	-	72.8	3.73 (brd, 2.0)	199.6	-
4	58.2	2.27 (q, *J* = 7.0)	49.2	1.25[c]	123.7	5.72 (s)
5	42.1	-	37.1	-	171.7	-
6	41.3	1.75 (m), 1.27 (m)	41.8	1.74 (dt, 13.0, 3.0), 097[c]	32.9	2.39 (m), 2.26 (m)
7	18.2	1.48 (m), 1.40 (m)	17.6	1.39 (m)	32.1	1.83 (m), 1.01 (m)
8	53.1	1.40 (m)	53.2	1.27[c]	35.7	1.51 (m)
9	37.5	-	38.4	-	53.8	0.92 (- [c])
10	59.5	1.55 (m)	61.4	0.90 (brd, 12.5)	38.6	-
11	35.6	1.46 (m), 1.28 (m)	35.4	1.55[c], 1.20 (m)	21.1	1.50 (m), 1.42 (m)
12	30.5	1.35 (m)	30.7	1.34[c], 1.31[c]	39.7	2.02 (m), 1.15 (m)
13	39.7	-	37.9	-	42.4	-
14	38.3	-	39.7	-	55.9	1.0 (m)
15	32.4	1.51 (m), 1.29 (m)	32.4	1.47[c], 1.27[c]	24.2	1.60 (m), 1.30 (m)
16	36.0	1.57 (m), 1.38 (m)	36.1	1.43[c], 1.14[c]	28.2	1.86 (m), 1.28 (m)
17	30.0	-	30.0	-	56.0	1.11 (m)
18	42.8	1.56 (m)	42.9	1.54 (m)	11.9	0.71 (s)
19	35.4	1.37 (m), 1.20 (m)	35.2	1.90 (dt, 10.5, 2.5), 1.55[c]	17.4	1.18 (m)
20	28.2	-	28.2	-	36.1	1.36 (m)
21	32.8	1.48 (m), 1.28 (m)	32.9	1.47[c], 1.27[c]	18.7	0.92 (d, 6.5)
22	39.3	1.49 (m), 0.94 (m)	39.3	1.47[c], 0.91[c]	34.0	2.32 (m), 1.02 (m)
23	6.8	0.88 (d, 6.5)	11.6	0.94 (d, 7.5)	26.1	1.16 (m)
24	14.7	0.72 (s)	16.4	0.97 (s)	45.9	0.91 (m)
25	18.0	0.87 (s)	18.3	0.86 (s)	29.2	1.66 (m)
26	20.3	1.01 (s)	20.1	0.99 (s)	19.8	0.84 (d, 7.0)
27	18.7	1.05 (s)	18.7	1.01 (s)	19.0	0.82 (d, 6.5)
28	32.1	1.18 (s)	31.8	1.17 (s)	23.1	1.28 (m), 1.22 (m)
29	35.0	0.95 (s)	35.0	0.95 (s)	12.0	0.85 (t, 7.5)
30	31.8	1.00 (s)	32.1	1.00 (s)		-

[#] recorded in $CDCl_3$; [a]125 MHz; [b]500 MHz; [c]overlapped.

Compound **2** was obtained as a white solid. The ^{1}H-NMR spectrum of **2** also displayed signals of a friedelane structure with seven methyl singlets at δ_H 0.97 (s, H_3-24), 0.86 (s, H_3-25), 0.99 (s, H_3-26), 1.01 (s, H_3-27), 1.17 (s, H_3-28), 0.95 (s, H_3-29), 1.00 (s, H_3-30) and a methyl doublet at δ_H 0.94 (d, *J* = 7.5 Hz, H_3-23). In addition, an oxymethine proton was found at δ_H 3.73

(br d, J = 2.0 Hz, H-3). The small coupling constant of H-3 (J = 2.0 Hz) confirmed H-3 in the *equatorial* orientation (H-3*α*) and the hydroxyl group in the *axial* orientation (3*β*-OH) [12]. The ^{13}C-NMR and DEPT spectra of **2** showed signals of 30 carbons, including eight methyls, eleven methylenes, five methines, and six quaternary carbons. Different from compound **1**, the carbonyl carbon was replaced by an oxymethine carbon at δ_C 72.8 in the ^{13}C-NMR spectrum of compound **2**. Similar to compound **1**, the positions of the methyls were confirmed by the HMBC correlations between H_3-23 (δ_H 0.94) and C-3 (δ_C 72.8)/C-4 (δ_C 49.2)/C-5 (δ_C 37.1), H_3-24 (δ_H 0.97) and C-4 (δ_C 49.2)/C-5 (δ_C 37.1)/C-6 (δ_C 41.8)/C-10 (δ_C 61.4), H_3-25 (δ_H 0.86) and C-8 (δ_C 53.1)/C-9 (δ_C 38.4)/C-10 (δ_C 61.4)/C-11 (δ_C 35.4), H_3-26 (δ_H 0.99) and C-8 (δ_C 53.2)/C-14 (δ_C 39.7)/C-15 (δ_C 32.4), H_3-27 (δ_H 1.01) and C-12 (δ_C 30.7)/C-13 (δ_C 37.9)/C-14 (δ_C 39.7)/C-18 (δ_C 42.9), H_3-28 (δ_H 1.17) and C-16 (δ_C 36.1)/C-17 (δ_C 30.0)/C-18 (δ_C 42.9)/C-22 (δ_C 39.3), H_3-29 (δ_H 0.95) and C-19(δ_C 35.2)/C-20 (δ_C 28.2)/C-21 (δ_C 32.9)/C-30 (δ_C 32.1), H_3-30 (δ_H 1.00) and C-19 (δ_C 35.2)/C-20 (δ_C 28.2)/C-21 (δ_C 32.9)/C-29 (δ_C 35.0). The ESI-MS spectrum of **2** gave a *quasi*-molecular ion peak at *m/z* 429 $[M+H]^+$, combined with NMR data, that suggested the molecular formula of **2** as $C_{30}H_{52}O$. Compound **2** was determined as 3*β*-friedelanol based on NMR and MS spectral analysis and comparison with data published in the literature [13]. 3*β*-Friedelanol was reported to have anti-inflammatory activity by inhibiting 51 % of carrageenan-induced rat paw oedema at a dose of 30 mg/kg [14].

Compound **3** was isolated as a white solid. The ^{1}H-NMR spectrum of compound **3** revealed typical signals of a sterol with two methyl singlets at δ_H 0.71 (s, H_3-18) and 1.81 (s, H_3-19); three methyl doublets at δ_H 0.92 (d, J = 6.5 Hz, H_3-21), 0.84 (d, J = 6.5 Hz, H_3-26) and 0.82 (d, J = 6.5 Hz, H_3-27) and a methyl triplet at δ_H 0.89 (t, J = 7.5 Hz, H_3-29). In addition, an olefinic proton at δ_H 5.72 (1H, s) was observed. The ^{13}C-NMR spectrum of **3** showed signals of 29 carbons, including a carbonyl carbon at δ_C 199.6 (C-3); two olefinic carbons at δ_C 123.7 (C-4) and 171.7 (C-5) and six methyls at δ_C 11.9 (C-18), 12.0 (C-29), 17.4 (C-19), 18.7 (C-21), 19.0 (C-27), 19.8 (C-26). The HMBC correlations between H_2-1 (δ_H 2.01 and 1.32) and C-3 (δ_C 199.6)/C-5 (δ_C 171.7), H-2 (δ_H 2.42 and 1.32) and C-3 (δ_C 199.6), H-4 (δ_H 5.72) and C-6 (δ_C 32.9)/C-10 (δ_C 38.6) revealed positions of the carbonyl and olefinic groups. The HMBC correlations between H_3-19 (δ_H 1.18) and C-1 (δ_C 31.7)/C-5 (δ_C 171.7)/C-9 (δ_C 53.8)/C-10 (δ_C 38.6), H_3-18 (δ_H 0.71) and C-12 (δ_C 39.7)/C-13 (δ_C 42.4)/C-17 (δ_C 56.0), H_3-21 (δ_H 0.92) and C-17 (δ_C 56.0)/C-20 (δ_C 36.1)/C-21 (δ_C 18.7)/C-22 (δ_C 34.0), H_3-26 (δ_H 0.84) and C-24 (δ_C 45.9)/C-25 (δ_C 29.5)/C-27 (δ_C 19.0), H_3-27 (δ_H 0.82) and C-24 (δ_C 45.9)/C-25 (δ_C 29.5)/C-26 (δ_C 19.8), H_3-29 (δ_H 0.89) and C-24 (δ_C 45.9)/C-28 (δ_C 23.1) proved positions of the methyls. The molecular formula of **3** as $C_{29}H_{48}O$ was determined by NMR spectroscopic data and the *quasi*-molecular ion peak at *m/z* 413 $[M+H]^+$ in the ESI-MS spectrum. Compound **3** was identified as stigmast-4-en-3-one by NMR spectral analysis and comparison with those in the previous paper [15]. This compound was reported to possess anti-inflammatory activity against NO release with an IC_{50} value of 15.9 µM [16].

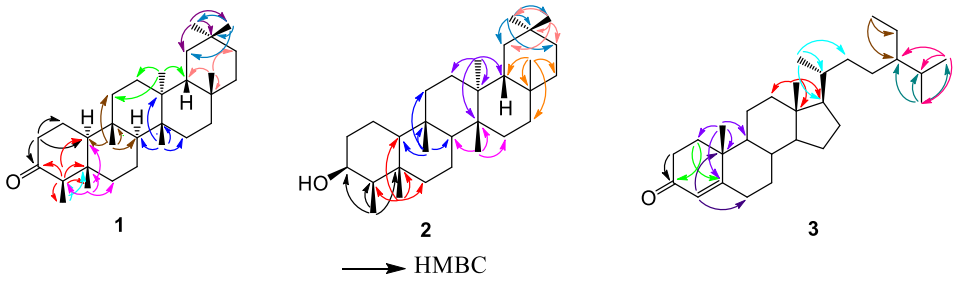

Figure 2. Key HMBC correlations of compounds **1**-**3**.

Compound **4** was isolated as a pale yellow solid. The ^{1}H-NMR spectrum revealed characteristic signals of a flavanol with 2 *meta*-aromatic protons at δ_H 5.97 (1H, d, J = 2.5 Hz, H-8) and 5.95 (1H, d, J = 2.5 Hz, H-6); four aromatic protons of an A_2B_2 system at δ_H 6.80 (2H, d, J = 8.5 Hz, H-3′, H-5′) and 7.34 (2H, d, J = 8.5 Hz, H-2′, H-6′); two oxymethine protons at δ_H 4.89 (1H, s, H-2) and 4.20 (1H, brs, H-3), and a methylene group at δ_H 2.76 (1H, dd, J = 2.5, 16.5 Hz, H-4a) and 2.90 (1H, dd, J = 4.5, 16.5 Hz, H-4b). The ^{13}C-NMR spectrum showed signals of 15 carbons, including 12 aromatic carbon signals, two oxymethine carbons at δ_C 79.9 (C-2) and 67.4 (C-3), and a methylene carbon at δ_C 29.3 (C-4). The small coupling constants of H-2 and H-3 confirmed the 2,3-*cis* configuration [17]. The molecular formula of **4** as $C_{15}H_{14}O_5$ was deduced from NMR spectral data combined with a *quasi*-molecular ion peak at ESI-MS 297 $[M+Na]^+$. Based on spectral analysis and the optical rotation ($[\alpha]_D^{25}$ = -41(MeOH, *c* 0.1), compound **4** was determined as **(-)**-epiafzelechin. The NMR and optical data were in agreement with those in the literature [18]. (-)-Epiafzelechin was reported to exhibit anti-inflammatory activity on carrageenin-induced mouse paw edema at a dose of 100 mg/kg [19].

Compound **5** was obtained as a yellow solid. The ^{1}H-NMR spectrum showed a signal of an aldehyde group at δ_H 9.73 (1H, s) and four A_2B_2 system aromatic protons at δ_H 7.76 (2H, d, J = 8.5 Hz, H-2; H-6) and 6.88 (2H, d, J = 8.5 Hz, H-3; H-5). The ^{13}C-NMR spectrum showed signals of seven carbons, including one carbonyl carbon at δ_C 192.7, and six aromatic carbons at δ_C 167.4 (C-4), 133.6 (C-2, C-6), 129.3 (C-1), and 117.5 (C-3, C-5). The ESI-MS spectrum of **5** gave a *quasi*-molecular ion peak at *m/z* 123 $[M+H]^+$, that indicated molecular formula of **5** as $C_7H_6O_2$. Compound **5** was identified as 4-hydroxybenzaldehyde by comparing NMR data with those in the literature [20]. 4-Hydroxybenzaldehyde was tested for anti-inflammatory activity against NO production with significant inhibition (39 %) at 0.5 mM concentration [21].

Compound **6** was isolated as a pale yellow solid. Similar to **5**, the ^{1}H-NMR spectrum of **6** displayed signals of A_2B_2 system aromatic protons at δ_H 7.89 (2H, d, J = 8.5 Hz, H-2, H-6), 6.84 (2H, d, J = 8.5 Hz, H-3, H-5). The ^{13}C-NMR spectrum showed signals of seven carbons including one carboxyl group at δ_C 170.1 and six aromatic carbons at δ_C 163.3 (C-4), 144.7 (C-1), 133.0 (C-2, C-6), and 116.0 (C-3, C-5). The molecular formula of **6** as $C_7H_6O_3$ was determined by NMR and a *quasi*-molecular ion peak at ESI-MS 139 $[M+H]^+$. Compound **6** was identified as 4-hydroxybenzoic acid [20]. 4-Hydroxybenzoic acid was investigated for anti-inflammatory effect due to its ability to reduce TNF-α and IL-6 levels in RAW 264.7 cells at 1 μM concentration [22].

Compound **7** was obtained as a white solid. The ^{1}H-NMR spectrum showed 2 aromatic proton signals at δ_H 6.51 (2H, s, H-2, H-6); three methoxy groups at δ_H 3.83 (6H, s, 3,5-OMe), 3.72 (3H, s, 4-OMe) and signals of a glucose moiety at δ_H 4.83 (1H, overlapped, H-1′), 3.36-3.50 (4H, m, H-2′, H-3′, H-4′, H-5′), 3.68 (1H, dd, J = 6.5 Hz, 12.0 Hz; H-6′a), 3.94 (1H, dd, J = 2.5 Hz, 12.0 Hz; H-6′b). The ^{13}C-NMR spectrum displayed signals of 15 carbons including six aromatic carbons at δ_C 156.0 (C-1), 154.8 (C-3, C-5), 134.5 (C-4), 96.2 (C-2, C-6), six carbons of glucopyranosyl ring at δ_C 103.2 (C-1′), 78.1 (C-3′), 78.4 (C-5′), 74.9 (C-2′), 71.7 (C-4′), 62.8 (C-6′) and three methoxy groups at δ_C 61.2 (4-OMe) and 56.6 (3-OMe,5-OMe). The molecular formula of **7** as $C_{15}H_{22}O_9$ was assigned by NMR combined with a *quasi*-molecular ion peak at *m/z* 345 $[M-H]^-$. Compound **7** was found to match 3,4,5-trimethoxyphenol-1-*O*-*β*-D-glucopyranoside by comparing NMR data of **7** with those in the literature [23, 24]. In addition, the optical rotation of compound **7** ($[\alpha]_D^{25}$ = -17(MeOH, c 0.1)) was similar to previous publications ($[\alpha]_D^{25}$ = -22,3(MeOH, c 0.38)) [23, 25]. Thus, the structure of **7** was determined as 3,4,5-trimethoxyphenol-1-*O*-*β*-D-glucopyranoside. This compound was reported to have a week anti-inflammatory effect on NO release with an IC_{50} of 90.72 ± 1.93 μM when it was tested on RAW264.7 cells [26].

4. CONCLUSIONS

This is the first study on the biological activity and phytochemistry of *B. sylvestris*. The *n*-hexane (BSH**)** and ethyl acetate (BSE) leaf extracts exhibited anti-inflammatory activity with IC_{50} values of 14.90 ± 1.82 and 43.48 ± 4.92 µg/mL, respectively. From these extracts, seven known compounds were isolated and their structures were determined as friedelin (**1**), 3*β*-friedelanol (**2**), stigmast-4-en-3-one (**3**), (-)-epiafzelechin (**4**), 4-hydroxybenzaldehyde (**5**), 4-hydroxybenzoic acid (**6**), and 3,4,5-trimethoxyphenyl-*O*-*β*-D-glucopyranoside (**7**). Previously, compounds **1-7** were reported as effective anti-inflammatory agents. So, the results of the study are the basis for developing an anti-inflammatory herbal functional food from the leaves of *B. sylvestris*.

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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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