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PHENOLIC CONSTITUENTS OF TWIGS AND LEAVES OF *WIKSTROEMIA INDICA* (L.) C.A. MEY. AND THEIR EFFECTS ON LPS-INDUCED IL-1β AND IL-10 CYTOKINE PRODUCTION IN RAW 264.7 CELL LINES

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Summary

Phenolic Constituents of Twigs and Leaves of *Wikstroemia indica* (L.) C.A. Mey. and their Effects on LPS-Induced IL-1β and IL-10 Cytokine Production in RAW 264.7 Cell Lines

Five phenolic compounds, including methyl 4-hydroxybenzoate (**1**), syringin (**2**), genkwanin (**3**), kaemferol-3-*O*-rhamnoside (**4**), and yuankanin (**5**), were isolated from the 70% EtOH extract of *W. indica* twigs and leaves. Their structures were identified using nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) datawith compound **2** being reported for the first time from this plant. The compounds were evaluated for their effects on IL-1β and IL-10 cytokine production in LPS-induced RAW 264.7 macrophages and **3 showed** considerably inhibitory activity against IL-1β with an IC_{50} value of 18.8 ± 2.46 μM.

Keywords: *W. indica*; *Phenolics; Flavonoids; IL-1β; IL-10.*

1. Introduction

The *Wikstroemia* Endl. genus comprises 94 reported species belonging to the Thymelaeaceae family; seven of them are found in Vietnam [1], [2]. *Wikstroemia indica* is a small shrub, found in tropical and subtropical regions of Asia, including China, India, and Southeast Asia, as well as Australia [3]. In Vietnam, this plant is commonly known as "Niet do" and grows wildly in all mountainous areas [4]. As a traditional Vietnamese herb, *W. indica* has a long history of use in folk medicine. It has been used to treat a variety of ailments, including asthma, parotitis, tonsillitis, and whooping cough [4]. The plant's medicinal properties are attributed to its diverse chemical composition, which includes flavonoids, coumarins, lignans, volatile oils, and polysaccharides [5]. In recent years, there has been growing interest in the scientific study of *W. indica*. It has been investigated as a potential source of natural products with various biological activities, including antibacterial, antiviral, anti-inflammatory, cytotoxic, and antifertility effects [5]. These studies have confirmed the traditional uses of the plant and have identified potential applications for its extracts and isolated compounds. Interleukin IL-1β is a key mediator in the inflammatory cascade, produced as a result of various inflammasome activation pathways, leading to the initiation and propagation of inflammatory responses [6]. Conversely, IL-10 serves as an anti-inflammatory cytokine that suppresses the production of various pro-inflammatory cytokines while promoting beneficial immune responses [7]. Enhancing IL-10 levels can mitigate excessive inflammatory responses and facilitate the healing process. Therefore, active compounds capable of inhibiting IL-1β production and promoting IL-10 synthesis hold significant promise for the treatment of inflammatory diseases.

In our ongoing investigations on natural products from Vietnamese medicinal plants, in the present study, we report the isolation of five phenolic compounds and their effects on IL-1β and IL-10 cytokine production in LPS-induced

RAW 264.7 macrophages of *W. indica* twigs and leaves.

2. Materials and methods

2.1. Plant material

W. indica twigs and leaves were collected in Viet Yen district, Bac Giang province, Vietnam, in September 2022 and identified by boganists Nguyen Quynh Nga and MSc.Phan Van Truong at the Medicinal Material Resources Center, NIMM, Vietnam. A voucher specimen (DV-080922) was deposited at the NIMM Herbarium.

2.2. General experimental procedures

Bruker 500 and 600 MHz FT-NMR spectrometers (Karlsruhe, Germany) with TMS as an internal standard were used for recording 1D and 2D NMR spectra. Open column chromatography (CC) was conducted using *silica gel* (Merck, 0.040–0.063 mm particle size), RP-C_{18} (Merck, 30–50 µm particle size), Diaion HP-20 (Mitsubishi Chemical Corp., Fuji, Tokyo, Japan), and MCI gel (Beijing Huideyi Technology Co., Ltd., CHP20P, 75–150 µm). Thin-layer chromatography (TLC) was carried out by using *silica gel* 60F_{254} (Merck, 0.25 mm) and RP-18F_{254} (Merck, 0.25 mm) plates. Spots were visualized under UV light (254 and 366 nm) or by spraying with 10% sulfuric acid in 96% ethanol (EtOH) followed by heating at 105°C. Semi-preparative HPLC was performed by HPLC 1525 Binary Pumps with a 2489 UV-Vis detector (Waters Corp, USA) and an ODS column (YMC-Pack-ODS-A, 250 × 10 mm i.d., 5 µM, Kyoto, Japan).

2.3. Extraction and isolation

The dried twigs and leaves of *W. indica* (5.0 kg) were refluxed with 70% ethanol (12 L × 3 h × 3 times) at 70°C. After filtering, extracts were combined and evaporated under reduced pressure to yield a residue (415.0 g, WI), which was further suspended in H_2O and successively extracted with *n*-hexane and ethyl acetate (EtOAc) to yield three extracts, including *n*-hexane extract (175.6 g, WIH), EtOAc extract (55.1 g, WIE), and water-soluble extract (150.5 g, WIW). WIE was separated on a *silica gel* CC using gradient elution with *n*-hexane–acetone (100%–10%, v/v) to obtain five fractions (E1 to E5). Fraction E1 (1.1 g) was chromatographed on an MCI CC eluted with a gradient of MeOH–H_2O (30–100%, v/v) to obtain **1** (11.6 mg). Fraction E2 (1.08 g) was chromatographed on an ODS CC using a solvent mixture of ACN–H_2O (50–100%, v/v) as an eluent to give **3** (16.8 mg). Fraction E3 (30 g) was separated by using an RP-C_{18} CC with a solvent mixture of MeCN–H_2O (30–100%, v/v) to obtain a subfraction E3.1 (30.8 mg). The subfraction E3.1 was purified by using an RP-C_{18} CC eluted with a solvent mixture of MeCN–H_2O (100%, v/v) to yield **4** (12 mg). WIW was separated by using a Diaion HP20 CC and a stepwise elution with 4.0 L each of 0%, 25%, 50%, and 75% MeOH in H_2O and 100% MeOH to yield five fractions (W0 to W4). Fraction W2 (39.1 g) was fractionated using *silica gel* CC and eluted with CH_2Cl_2–MeOH–H_2O (from 6 : 4 : 0.1 to 0 : 100: 0.1, v/v/v) to afford five subfractions (W2.1 to W2.5). Subfraction W2.5 (150.5 mg) was chromatographed on an RP-C_{18} CC with a solvent mixture of MeCN–H_2O (30–100%, v/v) to yield W2.5.1 (50 mg). The W2.5.1 was purified using semi-preparative HPLC (YMC-Pack ODS-A, 250 × 10 mm i.d., 5 µm, flow rate 2.0 mL/min) with an isocratic elution of 9.0% MeCN in H_2O to yield **2** (t_R = 28 min, 10.2 mg). Fraction W3 (32.8 g) was separated by using *silica gel* CC and eluted with EtOAc–MeOH–H_2O (from 85 : 15 : 5 to 0 : 100 : 5, v/v/v) to give six subfractions (W3.1 to W3.6). Subtraction W3.5 (0.95 g) was chromatographed on an MCI CC and eluted with a gradient of MeOH–H_2O (20–100%, v/v) to obtain four subfractions (W3.5.1 to W3.5.4). Subtraction W3.5.3 (450 mg) was chromatographed on an RP-C_{18} CC with a solvent mixture of MeCN–H_2O (20–100%, v/v) to yield a subfraction W3.5.3.1 (15 mg). Afterward, subfraction W3.5.3.1 was washed in MeOH several times to obtain **5** (6.2 mg).

Compound **1**: white powder; ^{1}H-NMR (600 MHz, CD_3OD): δ (ppm) 7.86 (2H, d, J = 8.4 Hz, H-2, H-6), 6.82 (2H, d, J = 8.4 Hz, H-3, H-5), 3.84 (3H, s, 7-OCH_3); ^{13}C-NMR (150 MHz, CD_3OD): δ (ppm) 122.3 (C-1), 132.7 (C-2, C-6), 115.2 (C-3, C-5), 163.5 (C-4), 168.7 (C-7), 52.2 (7-OCH_3).

Compound **2**: colorless amorphous powder; ^{1}H-NMR (600 MHz, CD_3OD): δ (ppm) 6.78 (2H, s, H-2, H-6), 6.57 (1H, d, J = 15.8 Hz, H-7), 6.35 (1H, dt, J = 15.8, 5.6 Hz, H-8), 4.25 (1H, dd, J = 5.6, 1.4 Hz, H-9), 4.89 (1H, overlapped, H-1′), 3.41–3.54 (3H, m, H-2′, H-3′, H-4′), 3.24 (1H, m, H-5′), 3.80 (1H, dd, J = 12.0, 2.4 Hz, H-6a), 3.69 (1H, dd, J = 12.0, 5.0 Hz, H-6b), 3.88 (6H, s, 3,5-OCH_3); ^{13}C-NMR (150 MHz, CD_3OD): δ (ppm) 135.3 (C-1), 105.5 (C-2, C-6), 154.4 (C-3, C-5), 135.9 (C-4), 131.3 (C-7), 130.1 (C-8), 63.6 (C-9), 57.1 (3,5-OCH_3), 105.4 (C-1′), 75.8 (C-2′), 78.4 (C-3′), 71.4 (C-4′), 77.9 (C-5′), 62.6 (C-6′).

Compound **3**: yellow amorphous powder; ^{1}H-NMR (600 MHz, acetone-d_6) and ^{13}C-NMR (150 MHz, acetone-d_6): δ (ppm) see Table 1.

Compound **4**: yellow amorphous powder; ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (150 MHz, CD_3OD): δ (ppm) see Table 1.

Compound **5**: pale-yellow powder; ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (150 MHz, CD_3OD): δ (ppm) see Table 1.

2.4. Cell culture

RAW 264.7 macrophages were cultured in DMEM with 10% fetal bovine serum, 100 units/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine. The culture conditions included a 5% CO_2 atmosphere and a temperature of 37°C as previously described [8]. The murine monocyte/macrophage RAW 264.7 (ATCC TIB-71) cell line was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and was maintained for no more than 25 passages.

2.5. Cell viability assay

Cells were plated in 96-well plates at 2×10^3 cells/well and cultured for 24 hours. They were then treated with compounds **1**–**5** (10 µM) for 48 hours. Cytotoxicity was assessed using the MTT assay as previously described [8]. Cell viability (%) was calculated by comparing the absorbance of treated cells to that of control cells exposed to the vehicle.

2.6. Assay for IL-1β and IL-10 cytokine-inducible activity in RAW 264.7 macrophages

RAW 264.7 macrophages (10^4 cells/well) were seeded in 96-well plates with 10% FBS and incubated at 37°C, 5% CO_2 for 12 hours. The cells were then stimulated with LPS (5 ng/mL) in the presence or absence of isolated compounds at different concentrations. After 24 hours, the supernatant was collected, and IL-1β and IL-10 levels were measured using an ELISA kit. Macrophages treated with 1% DMSO and LPS served as the control group. The experiment was performed in quintuplicate and analyzed independently [8]. ELISA kits for mouse IL-1β and IL-10 were purchased from Thermo Fisher.

2.7. Statistical analysis

Data are shown as mean ± SEM and analyzed using one-way ANOVA followed by the Tukey test in GraphPad Prism 9.5.0. A p-value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Structure identification

The ^{1}H-NMR spectrum of **1** showed characteristic signals corresponding to a 1,4-substituted benzene ring at δ_H 7.86 (2H, d, J = 8.4 Hz, H-2, H-6) and 6.82 (2H, d, J = 8.4 Hz, H-3, H-5), and a methoxy group at δ_H 3.84 (3H, s, 7-OMe). The ^{13}C-NMR spectrum demonstrated signals corresponding to eight carbons including a carbonyl ester (δ_C 168.7, C-7), a methoxy group (δ_C 52.2), and four signals of six carbons of a symmetrical benzene ring (δ_C 115.2, 122.3, 132.7, and 163.5). Analysis of the above-mentioned data and comparison of the spectral data with references [9] concluded that **1** was methyl 4-hydroxybenzoate (Fig. 1).

Compound **2** was obtained as a colorless amorphous powder. The ^{1}H-NMR spectrum analysis revealed the presence of a 1,2,4,6-substituted benzene ring at δ_H 6.78 (2H, s), a *trans* double bond at δ_H 6.57 (1H, d, J = 15.8 Hz, H-7) and 6.35 (1H, dt, J = 15.8, 5.6 Hz, H-8), an anomeric proton at δ_H 4.89, two methoxy groups at δ_H 3.88 (6H, s), an oxymethylene group at δ_H 4.25 (2H, dd, J = 5.6, 1.4 Hz). Further analysis of the ^{13}C-NMR and HSQC spectra of **2** revealed signals of 15 carbons including 6 carbons of a 1,2,4,6-substituted aromatic ring at δ_C 154.4 (x 2C), 135.9, 135.3, 105.4 (x 2C); two carbons of a *trans* double bond at δ_C 131.3 and 130.1; one oxymethylene at δ_C 63.6; two methoxy groups at δ_C 57.1 (x 2C); six carbons characteristic of a glucosyl moiety at δ_C 105.4, 78.4, 77.9, 75.8, 71.4, and 62.6. Based on the above analysis, the structure of **2** was elucidated as syringin [10] (Fig. 1).

Compound **3** was isolated as a yellow amorphous powder. The ^{1}H-NMR spectrum of **3** showed signals of protons belonging to an AA′BB′ system at δ_H 7.96 (2H, d, J = 9.0 Hz, H-2′, H-6′) and 7.30 (2H, d, J = 9.0 Hz, H-3′, H-5′); two *meta*-coupled protons at δ_H 6.33 (1H, d, J = 2.4 Hz, H-6) and 6.69 (1H, d, J = 2.4 Hz, H-8), a singlet proton at δ_H 6.67 (1H, s, H-3), and a methoxy group at δ_H 3.93, suggesting that **3** has the structure of an apigenin framework. The ^{13}C-NMR spectrum of **3** showed signals of 16 carbons, including a ketone at δ_C 183.2. In addition, the ^{13}C-NMR spectrum also showed strong signals of the benzene ring with a substituent at the *para* position (δ_C 123.1 and 116.9), and a methoxy group at δ_C 56.4. The position of the methoxy group was determined through the HMBC interactions from the methoxy proton (δ_H 3.93), H-6, and H-8 to C-7 (δ_C 166.6) (Fig. 2). The NMR data of **3**, combined with comparison with reference [11], identified **3** as genkwanin (Fig. 1).

Table 1. NMR data of compounds **3**–**5**

No.	**3**		**4**		**5**	
	δ_H[a,b] (mult., J = in Hz)	δ_C[a,c]	δ_H[d,b] (mult., J = in Hz)	δ_C[d,c]	δ_H[d,b] (mult., J = in Hz)	δ_C[d,c]
2	-	163.1	-	159.3	-	162.6
3	6.67 (1H, s)	104.2	-	136.2	6.60 (1H, s)	106.8
4	-	183.2	-	179.7	-	180.3
5	-	162.1	-	163.2	-	159.6
6	6.33 (1H, d, 2.4)	98.7	6.23 (1H, brs)	99.9	6.92 (1H, d, 2.4)	104.4
7	-	166.6	-	166.0	-	164.5
8	6.69 (1H, d, 2.4)	93.2	6.41 (1H, brs)	94.8	6.95 (1H, d, 2.4)	97.2
9	-	158.7	-	158.6	-	160.7
10	-	106.0	-	105.9	-	110.3
1′	-	129.3	-	122.7	-	123.0
2′, 6′	7.96 (2H, d, 9.0)	123.1	7.79 (2H, d, 8.4)	131.9	7.86 (2H, d, 8.4)	129.4
3′, 5′	7.30 (2H, d, 9.0)	116.9	6.96 (2H, d, 9.0)	116.6	6.94 (2H, d, 8.4)	117.1
4′	-	165.4		161.6	-	166.1
7-OCH_3	3.93 (3H, s)	56.4	-	-	3.97 (3H, s)	56.8
			Rham		*Glc*	
1″			5.40 (1H, d, 1.2)	103.5	4.87 (1H, d, 7.8)	104.7
2″			4.24 (1H, brs)	71.9	3.63 (1H, t, 8.4)	74.7
3″			3.73 (1H, m)	72.2	3.35 (1H, t, 9.0)	77.7
4″			3.33 – 3.36 (2H, ovl)	73.2	3.52 (1H, t, 9.0)	71.1
5″				72.0	3.53 (1H, m)	77.2
6″			0.94 (3H, d, 5.4)	17.6	4.15 (1H, dd, 12.0, 1.8) 3.72 (1H, m)	70.5
					Xyl	
1‴					4.35 (1H, d, 7.2)	105.7
2‴					3.25 (1H, t, 8.4)	75.0
3‴					3.53 (1H, m)	77.5
4‴					3.44 (1H, t, 9.0)	71.5
5‴					3.86 (1H, dd, 12.0, 6.6) 3.22 (1H, t, 10.8)	66.9

[a] acetone-d_6, [b] *600 MHz*, [c] *150 MHz*, [d] CD_3OD, *ovl: overlapped.*

Compound **4** was obtained as a yellow amorphous powder. The ^{1}H-NMR spectrum of **4** showed singlet signals at δ_H 6.23 (1H, brs, H-6) and 6.41 (1H, brs, H-8), typical for two protons at C-6 and C-8 positions of the ring A of a flavonol, and an anomeric proton at δ_H 5.40 (1H, d, J = 1.2 Hz). Two other doublet signals at δ_H 6.96 (2H, d, J = 9.0 Hz, H-3′, H-5′) and 7.79 (2H, d, J = 9.0 Hz, H-2′, H-6′) were characteristic of a *para*-substituted aromatic ring. The ^{13}C-NMR spectrum of **4** showed signals of 21 carbon atoms, of which 6 signals at δ_C 103.5 (C-1″), 71.9 (C-2″), 72.2 (C-3″), 73.2 (C-4″), 72.0 (C-5″), and 17.6 (C-6″) indicated the presence of an α-rhamnosyl moiety and 15 carbon signals belonging to an aglycone (kaempferol). The HMBC interaction between H-1″ (δ_H 5.40) and C-3 (δ_C 136.2) suggested the location of the α-rhamnopyranosyl moiety at C-3 of kaempferol (Fig. 2). Thus, it could be confirmed that **4** is kaempferol 3-*O*-α-L-rhamnopyranoside [12] (Fig. 1).

Compound **5** was isolated as a pale yellow powder. The ^{1}H- and ^{13}C-NMR data were similar to those of **3**, the only difference was the presence of two additional sugar moieties. The ^{1}H- and ^{13}C-NMR data were similar to those of **3**, except for the presence of two additional sugar moieties. The anomeric protons appeared at δ_H 4.87 (1H, d, J = 7.8 Hz, H-1″) and 4.35 (1H, d, J = 7.2 Hz, H-1‴), along with the protons of hydromethine and oxymethylene groups in the range of 3.22–4.15 ppm. The ^{13}C-NMR spectrum also showed signals from 11 carbons corresponding to the sugar moieties of hexose and pentose units. Comparison with the reference confirmed that these sugar moieties were β-glucopyranosyl and β-xylopyranosyl [13]. The aglycone was identified to be the same as that of **3** (genkwanin). The connection of the

sugar moieties to the aglycone was confirmed by the HMBC correlations from H-1″$_{Glc}$ (δ_H 4.87) to C-5 (δ_C 159.6), and H-1‴$_{Xyl}$ (δ_H 4.35) to C-6″$_{Glc}$ (δ_C 70.5) (Fig. 2). By comparison with literature values [13] and analysis of HSQC and HMBC data, the structure of **5** was determined as yuankanin (Fig. 1).

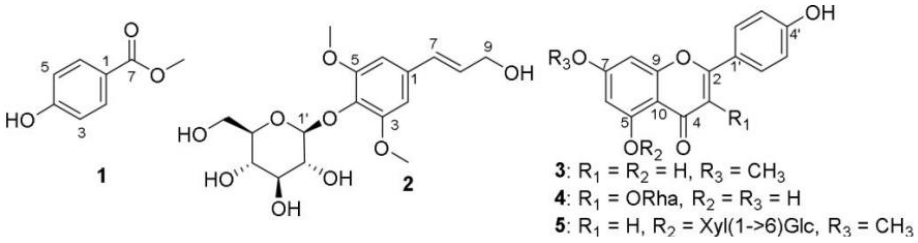

Fig. 1. Chemical structures of **1**–**5** isolated the aerial parts of *W. indica*

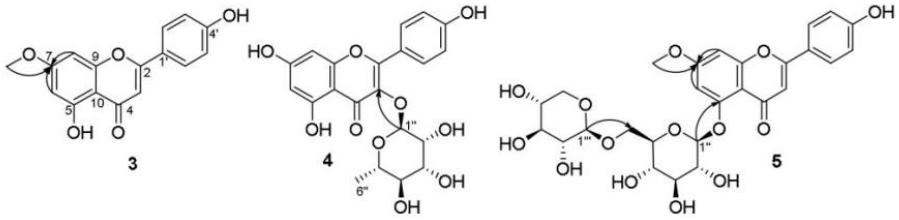

Fig. 2. Key HMBC correlations of compounds **3**–**5**

3.2. Bioactivity

To determine the effects on RAW 264.7 macrophage survival, five compounds **1**–**5** from *W. indica* were evaluated using MTT assays. The results are shown in Fig. 3. After 24 h incubation with 10 μM purified compounds, the cell survival rate was above 80%.

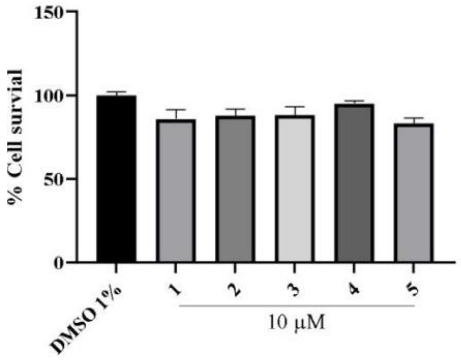

Fig. 3. Effect of compounds (**1**–**5**; 10 μM) from *W. indica* on RAW 264.7 macrophage survival. Samples were incubated with RAW 264.7 macrophages in 96 well plates (30000 cells/well) for 24 h. The blank was incubated with dimethyl sulfoxide. MTT (10 mg/mL) in phosphate-buffered saline (100 μL) was incubated with the cells for 45 min until the reaction was completed. The absorbance was measured at 570 nm.

Compounds **1**–**5** were screened for their effect on IL-1β and IL-10 cytokine production in LPS-induced RAW 264.7 macrophages. As shown in Fig. 4A, the tested compounds at the concentration of 10 μM displayed an inhibitory effect on IL-1β production in RAW 264.7 macrophages with different potency, and IL-1β concentrations ranged from 88.82 to 322.92 pg/mL compared to the control group treated with LPS 5 ng/mL (IL-1β concentration of 405.4 pg/mL). Among these compounds, compounds **1** and **3** showed stronger inhibition of IL-1β secretion than others, with IL-1β concentrations of 88.83 pg/mL and 94.35 pg/mL, respectively. Furthermore, the effect of these compounds on the stimulation of IL-10 production in LPS-induced RAW 264.7 macrophages was evaluated and shown in Fig. 4B. Again, all compounds at the concentration of 10 μM stimulated IL-10 secretion in LPS-induced RAW 264.7 macrophages, with IL-10 levels ranging from 131.07 to 233.98 pg/mL, compared to the control group treated with LPS alone (IL-10 concentration of 109.43 pg/mL). Of the compounds, **3** showed better effects than the others on both IL-1β inhibition and IL-10 stimulation. So, further evaluation of this compound was conducted on IL-10 and IL-1β

cytokines at different concentrations (1–30 μM). The results (Fig. 5) showed that the stimulatory effect on IL-10 production and inhibitory effect on IL-1β production of **3** gradually increased with the test concentrations in both RAW 264.7 macrophages and LPS-induced RAW 264.7 macrophages. Compound **3** inhibited IL-1β production in LPS-induced RAW 264.7 macrophages with an IC_{50} value of 18.8 ± 2.46 μM.

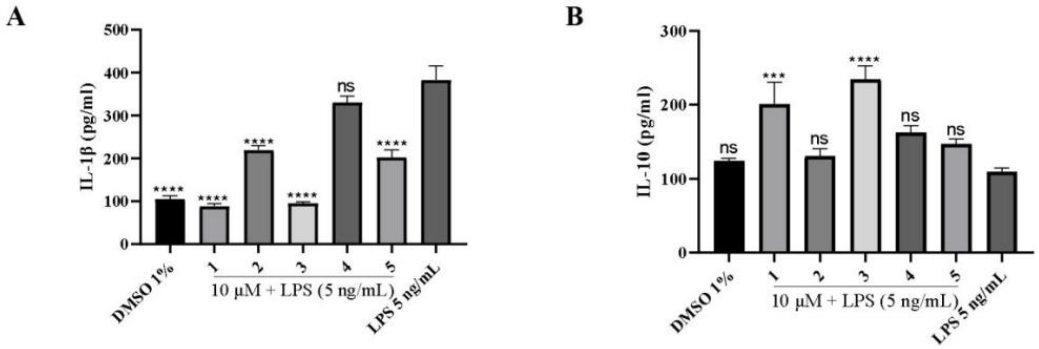

Fig. 4. Modulatory effects of compounds **1–5** on IL-1β (A) and IL-10 (B) cytokine production in LPS-induced RAW 264.7 macrophages. Values represent the mean ± SEM (n = 5). Statistical significance was analyzed by one-way ANOVA followed by the Tukey test. *ns*: not statistically significant, ***$p < 0.001$, and ****$p < 0.0001$ compared to the cells treated with LPS only.

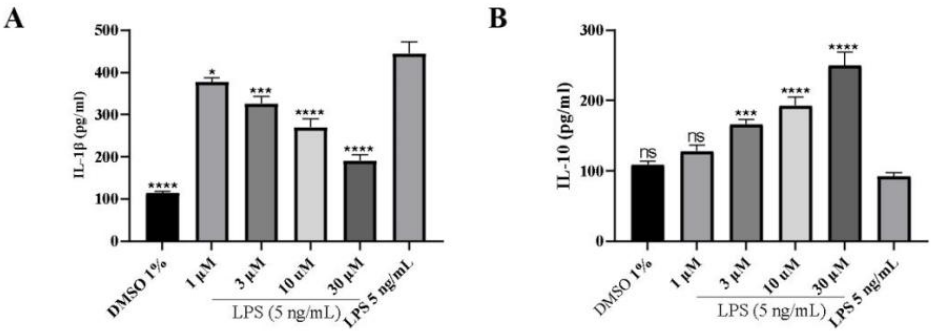

Fig. 5. Modulation effects of **3** at 1–30 μM for 12 h on LPS-induced IL-1β (A) and IL-10 (B) cytokine production in RAW 264.7 macrophages. Values represent the mean ± SEM (n = 5). Statistical significance was analyzed by one-way ANOVA followed by the Tukey test. *$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$ compared to the cells treated with LPS only.

The isolated compounds include a simple phenolic compound (**1**), a phenylpropanoid (**2**), and three flavonoids (**3**–**5**). All flavonoids demonstrated effects on the production of cytokines IL-1β and IL-10 in LPS-induced RAW 264.7 macrophages. This finding aligns with the study conducted by Lee et al. (2020), which examined the immunomodulatory and anti-inflammatory properties of *W. indica*. Their research indicated that the ethanolic extract of *W. indica* effectively reduced inflammatory symptoms in a mouse model of atopic dermatitis induced by 2,4-dinitrochlorobenzene (DNCB) while decreasing serum levels of IL-4 and IgE [14]. In subsequent studies carried out by the same researchers, quercitrin, a main constituent isolated from *W. indica*, had the strongest antiallergic action against antigen-induced β-hexosaminidase release and IL-4 mRNA expression, which are indicators of degranulation in RBL-2H3 cells [15]. Among the isolated flavonoids, compounds **4** and **5** are glycosylated flavones that exhibited lower activity compared to compound **3**. Notably, compound **5** demonstrated a stronger effect than compound **4**, possibly due to differences in the positioning of the sugar moieties. Genkwanin (**3**), a non-glycosylated flavone, showed significant inhibition of the production of IL-1β while markedly enhancing IL-10 levels compared to the other compounds. Previous studies have indicated

that genkwanin exerts its anti-inflammatory effects through various mechanisms. Importantly, a recent study has shown that genkwanin exhibits substantial anti-inflammatory activity *in vitro* by robustly downregulating pro-inflammatory mediators such as *i*NOS, TNF-α, IL-1β, and IL-6 [16]. In another research, El Menyiy et al. (2023) have demonstrated that this compound reduces serum levels of TNF-α, IL-6, and nitric oxide (NO), while significantly increasing IL-10 levels [17]. Collectively, these findings indicate that *W. indica* not only reduces the production of pro-inflammatory cytokines but also enhances the levels of anti-inflammatory cytokines, demonstrating a dual action in regulating inflammation and supporting immune function.

4. Conclusion

Chemical investigation of the 70% EtOH extract of *W. indica* twigs and leaves led to the isolation of five phenolic compounds, namely methyl 4-hydroxybenzoate (**1**), syringin (**2**), genkwanin (**3**), kaempferol-3-*O*-rhamnoside (**4**), and yuankanin (**5**). The structures of compounds were elucidated by using NMR and MS data, and comparison with data in references. The isolated compounds were screened for their effects on the production of the IL-1β and IL-10 cytokines in LPS-induced RAW 264.7 macrophages. The results showed that **3** could be a potential modulator for both cytokines. Further testing at different concentrations revealed that **3** considerably inhibited the production of IL-1β with an IC_{50} value of 18.8 ± 2.46 μM.

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References

1. https://www.worldfloraonline.org/taxon/wfo-4000040707.
2. Do H. B., Dang Q. C., Bui X. C., Nguyen T. D., Do T. D., Pham V. H. (2006), *Medicinal plants and animals in Vietnam*, Science and Technics Publishing House, Hanoi, 477-479.
3. Suroowan S., Mahomoodally F. (2020), *Chapter 45 - Wikstroemia indica*, in *Underexplored Medicinal Plants from Sub-Saharan Africa*, Namrita Lall, Editor Academic Press. 295-301.
4. Vo V. C. (1997), *Vietnamese medicinal plant dictionary*, Medical Publishing House, Hanoi, 875.
5. Li Y. M., Zhu L., Jiang J. G., Yang L., Wang D. Y. (2009), Bioactive components and pharmacological action of *Wikstroemia indica* (L.) CA. Mey. and its clinical application, *Current Pharmaceutical Biotechnology*, 10(8), 743-752.
6. Dinarello C. A. (1996), Biologic basis for interleukin-1 in disease, *Blood*, 87(6), 2095-147.
7. Moore K. W., de Waal Malefyt R., Coffman R. L., O'Garra A. (2001), Interleukin-10 and the interleukin-10 receptor, *Annual Review of Immunology*, 19, 683-765.
8. Nguyen T. M., Do T. H., Nguyen T. T., Vu T. D., Tran T. H., Nguyen H. T., Doan T. P., Oh W. K., Park J. H. (2024), Effects of coumarins from roots of *Paramignya scandens* (Griff.) Craib on LPS-induced IL-1β and IL-10 cytokine production in RAW 264.7 macrophages, *Natural Product Sciences*, 30(1), 30-57.
9. Bui T. T. L., Dang H. P., Nguyen T. N. (2015), Investigation of chemical constituents of the chloroform extract of the stem of *Paramignya trimera* (Oliver) Burkill (Rutaceae), *Journal of Analytical Sciences*, 20, 297-302.
10. Dong H., Wu M., Wang Y., Du W., He Y., Shi Z. (2021), Total syntheses and anti-inflammatory activities of syringin and its natural analogues, *Journal of Natural Products*, 84(11), 2866-2874.
11. Gomes R. A., Ramirez R. R., Maciel J. K. D. S., Agra M. D. F., Souza M. D. F. V. D., Falcão-Silva V. S., Siqueira-Junior J. P. (2011), Phenolic compounds from *Sidastrum micranthum* (A. St.-Hil.) fryxell and evaluation of acacetin and 7,4'-di-*O*-methylisoscutellarein as motulator of bacterial drug resistence, *Química Nova*, 34, 1385-1388.
12. Fukunaga T., Nishiya K., Kajikawa I., Watanabe Y., Suzuki N., Takeya K., Itokawa H. (1988), Chemical studies on the constituents of *Hyphear tanakae* Hosokawa from different host trees, *Chemical and Pharmaceutical Bulletin*, 36(3), 1180-1184.
13. Soltani S., Koubaa I., Dhouib I., Khemakhem B., Marchand P., Allouche N. (2023), New specific α-glucosidase inhibitor flavonoid from *Thymelaea tartonraira* leaves: Structure elucidation, biological and molecular docking studies, *Chemistry & Biodiversity*, 20(3), e202200944.
14. Lee S. Y., Park N. J., Jegal J., Jo B. G., Choi S., Lee S. W., Uddin M. S., Kim S. N., Yang M. H. (2020), Suppression of DNCB-induced atopic skin lesions in mice by *Wikstroemia indica* extract, *Nutrients*, 12(1), 173. doi: 10.3390/nu12010173.
15. Jegal J., Park N. J., Lee S. Y., Jo B. G., Bong S. K., Kim S. N., Yang M. H. (2020), Quercitrin, the main compound in *Wikstroemia indica*, mitigates skin lesions in a mouse model of 2,4-dinitrochlorobenzene-induced contact hypersensitivity, *Evidence-Based Complementary and Alternative Medicine*, 2020(1), 4307161.
16. Gao Y., Liu F., Fang L., Cai R., Zong C., Qi Y. (2014), Genkwanin inhibits proinflammatory mediators mainly through the regulation of miR-101/MKP-1/MAPK pathway in LPS-activated macrophages, *PLoS One*, 9(5), e96741.
17. El Menyiy N., Aboulaghras S., Bakrim S., Moubachir R., Taha D., Khalid A., Abdalla A. N., Algarni A. S., Hermansyah A., Ming L. C. (2023), Genkwanin: An emerging natural compound with multifaceted pharmacological effects, *Biomedicine & Pharmacotherapy*, 165, 115159.