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베르노니아 시네레아 지상부의 화학 성분 및 항염증 활성

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Chemical Constituents from the Aerial Parts of *Vernonia cinerea* L. and Their Anti-Inflammatory Activity

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ABSTRACT

Background: Previous phytochemical studies of whole *Vernonia cinerea* L. plants have identified sesquiterpene lactones, sterols, and triterpenes, which possess anticancer, antifeedant, and antimalarial activities. However, there are no reports of other types of bioactive metabolites. Therefore, the present study aimed to identify phenolic compounds with anti-inflammatory activity in the aerial parts of the plant.
Methods and Results: Compounds were isolated from the aerial parts of *V. cinerea* using a silica and C-18 gel columns and semi-preparative HPLC instrument, and the structures of the compounds were determined using one- and two- dimension nuclear magnetic resonance spectroscopy and mass spectroscopy. The chloroform soluble extracts and isolated compounds were evaluated for their anti-inflammatory potential based on their ability to inhibit nitric oxide production and TNF-α induced NF-κB activity.
Conclusions: Phytochemical study of the aerial parts of *V. cinerea* led to the isolation of six phenolic compounds. Compound 1 was a major metabolite, and to the best of our knowledge, compounds 2 - 6 were isolated from *V.cinerea* for the first time. Among the isolates, compounds 1 and 3 exhibited TNF-α-induced NF-κB activity with IC_{50} values of 7.5 and 11.5 M, respectively, and the inhibitory activity of phenyl propanoid compound 3 on TNF-α-induced NF-κB was evaluated for the first time.

Key Words: *Vernonia cinerea* L., Anti-Inflammatory Activity, Asteraceae, Phenolic Compounds

INTRODUCTION

NF-κB is a transcription factor that is associated with cell apoptosis, differentiation, and migration. Upon activation, it may promote cell proliferation and prevent cell death through anti-apoptotic factors (Baldwin, 2001). Inhibition of NF-κB signalling has a potential application for either the treatment or prevention of cancer.

Nitric oxide (NO) is an inorganic gaseous molecule that is synthesized by the oxidation of l-arginine catalyzed by nitric oxide synthase (NOS) and is involved in a number of physiological and pathological processes in mammals (Moncada *et al*., 1991). In the NOS family, iNOS is expressed in a variety of cells including macrophages, endothelial cells, and smooth muscle cells in response to pro-inflammatory stimuli such as IL-1β, TNF-α, and LPS. NO plays an important role in the regulation of many physiological functions, such as host defence, neurotoxicity, and vasodilation (Anggård, 1994). However, excess production of NO has been implicated in immunological and

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inflammatory diseases including septicshock, rheumatoid arthritis, graft rejection, and diabetes (Anggård, 1994). Therefore, inhibition of NO increase is apparently an important therapeutic consideration in the development of anti-inflammatory and cancer agents.

The leaves, roots, and seeds of some species of *Vernonia* (Asteraceae) have been reported to have medicinal properties (Misra *et al*., 1984a). *Vernonia cinerea* L. is an annual herb that grows in south Asia. The plant is called 'Yaa Saam Wan' in Thailand. It is used as a tonic, stomachic, and astringent and also known to treat consumption, asthma, and bronchitis (Kone and Kande, 2012). Aqueous ethanolic extracts showed anti-ranikhet virus and anti-cancer activities (Beeran *et al*., 2014). The roots are used as an anthelmintic and diuretic (Dastur, 1977). The flowers are used to treat conjunctivitis, fever, and rheumatism (Kirtikar and Basu, 1975). There have been phytochemical reports on the diverse compounds from this species, including sesquiterpene lactones, sterols, and triterpenes (Kuo *et al*., 2003; Chea *et al*., 2006; Misra *et al*., 1984a, b). Some of these compounds have been shown to have anticancer (Kuo *et al*., 2003; Pratheeshkumar and Kuttan, 2011), antimalarial (Chea *et al*., 2006), and antifeedant activity (Tandon *et al*., 1998).

In a continuing research for anti-inflammatory agents, the chloroform ($CHCl_3$)-soluble extract of the aerial parts of *V. cinerea* exhibited inhibitory effects on the tumor necrosis factor alpha (TNF-α)-induced NF-κB activity and lipopolysaccharide (LPS)-induced nitric oxide (NO) production using murine macrophage RAW 264.7 cells. Although previous phytochemical studies on the whole plant of *V. cinerea* have reported anticancer, antimalarial, and antifeedant activity, there are no reports on other type of secondary metabolites with their anti-inflammatory activity. In particular, phenolic compounds, such as phenolic acids, flavonoids, and tannins are a large group of phytochemicals, existing ubiquitously in plants as secondary metabolites, which exhibit a wide range of biological and physiological functions, such as anti-allergenic, anti-inflammatory, anti-microbial and antioxidant activities (Balasundram *et al*., 2006; Manach *et al*., 2005; Middleton *et al*., 2000).

Accordingly, this research aims to find phenolic compounds with their anti-inflammatory activity, which have never been reported from the plant source. Here, we report the isolation and structure elucidation of secondary metabolites, as well as the inhibition on TNF-α-induced NF-κB activity and nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells.

MATERIALS AND METHODS

1. General experiments

UV spectra were recorded on a Shimadzu PharmaSpec-1700 UV-visible spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were measured on a Bruker Tensor-27 spectrophotometer (Bruker, Billerica, MA, USA). 1D and 2D NMR spectra were recorded on a Bruker AVANCE (400 MHz) spectrometer (Bruker, Billerica, MA, USA). Low- and high-resolution mass spectrometer analyses were performed with a BioTOF II ESI mass spectrometer (Bruker, Billerica, MA, USA). Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} (0.25 ㎜, Merck, Darmstadt, Germany). Silica gel (230 - 400 mesh, Merck, Darmstadt, Germany) and C-18 (YMCGEL ODS-A, 12 ㎚, S-150 ㎛, YMC Co., Ltd., Kyoto, Japan) were used for column chromatography. Semi-preparative HPLC (Beckman Coulter Inc., Brea, CA, USA) was conducted on a Beckman Coulter Gold-168 system equipped with a photodiode array detector using an Alltech reversed-phase Econosil C-18 column (10 ㎛, 10 × 250 ㎜, Alltech Inc., Nicholasville, KY, USA) with a flow rate of 2 ㎖/min.

2. Plant material

The aerial parts of *Vernonia cinerea* L. (Asteraceae) were collected from the Lampang Herb Conservation Club, Lampang Province, Thailand, in May 2011 and identified by comparison with the voucher specimen at the Forest Herbarium, Bangkok, Thailand. A voucher specimen (no. Vcw 002) was deposited at the Natural Product Chemistry Laboratory, College of Pharmacy, University of Hawaii at Hilo.

3. Extraction and isolation

The air dried aerial parts of *V. cinerea* (1 ㎏) were extracted with MeOH (2 times × 2 ℓ) at room temperature. The solvent was concentrated in vacuo to yield a MeOH extract (80 g), which was then suspended in distilled water (0.5 ℓ) and fractionated with chloroform ($CHCl_3$, 2 × 1 ℓ), ethyl acetate (EtOAc, 2 × 1 ℓ), and *n*-buthanol (BuOH, 2 × 1 ℓ), successively.

The $CHCl_3$ extracts (12 g) were subjected to silica gel column chromatography (CC; ∅ 10 ㎝; 230 - 400 mesh, 0.5 ㎏) using a gradient solvent system of hexane-ethyl acetate (100 : 0 to 30 : 70), to afford 57 fractions (C1-C57). The fractions (1 g) combined from C54 to C57 were subjected to silica gel CC (∅ 8 ㎝; 230 - 400 mesh, 80 g), with hexane-ethyl acetate (100 : 0 to 1 : 1) as the solvent system, yielding seven subfractions (C54S1 to C54S7). Subfraction C54S4 (0.5 g) was chromatographed on a sephadex LH-20 gel (50 g) column and eluted with H_2O-MeOH (100 : 0 to 50 : 50), to afford five subfractions (C54S4L1 to C54S4L5), and compound 3 (1.5 ㎎, 0.000019% recovery from the extract) was recrystallized from subfraction C54S4L2. Subfraction C54S4L3 (0.1 g) was subjected to preparative. HPLC (MeOH-H_2O = 40 : 60 to 100 : 0) to yield 6 (5 ㎎, t_R: 98 min, 0.00005%), 5 (2 ㎎, t_R: 105 min, 0.000025%), 4 (1 ㎎, t_R: 108 min, 0.000012%), and 2 (4 ㎎, t_R: 115 min, 0.00005%).

Quercetin 3-*O*-*β*-D-glucopyranoside, 1 (30 ㎎, 0.00038%) was recrystallized in a solvent mixture, ($CHCl_3$: MeOH, 1 : 1) from the ethyl acetate portion.

1) Quercetin 3-*O*-*β*-D-glucopyranoside - Yellow amorphous powder, UV (MeOH) λ_{max} (log ε) 279 (4.0), 330 (3.7) ㎚; IR ν_{max} (KBr) 3325, 1655 cm^{-1}; ESI-MS *m/z* 465 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δH 7.48 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 7.46 (1H, d, *J* = 8.0 Hz, H-5'), 6.94 (1H, d, *J* = 2.0 Hz, H-2'), 6.82 (1H, d, *J* = 2.0 Hz, H-8), 6.48 (1H, d, *J* = 2.0 Hz, H-6), 5.12 (1H, d, *J* = 7.2 Hz, H-1"), 3.75 (1H, d, *J* = 10.4 Hz, H-6a"), 3.53 (1H, d, *J* = 10.4 Hz, H-6b"), 3.20-3.53 (4H, m, H-2" to H-5"), 12.96 (1H, s, OH-5); ^{13}C NMR (100 MHz, DMSO-d_6) δc 182.3 (C-4), 164.9 (C-7), 163.4 (C-5), 161.6 (C-9), 157.4 (C-2), 150.4 (C-4'), 146.2 (C-3'), 121.8 (C-1'), 119.6 (C-6'), 116.6 (C-5'), 114.0 (C-2'), 105.8 (C-10), 103.6 (C-1"), 100.3 (C-6), 95.1 (C-8), 77.6 (C-3"), 76.8 (C-5"), 73.5 (C-2"), 70.0 (C-4"), 61.0 (C-6").

2) (*E*)-4-(3,4-Dimethoxyphenyl)but-3-en-1-ol - Yellow amorphous powder, UV (MeOH) λ_{max} (log) 280 (4.1) ㎚; ESI-MS *m/z* 209 $[M+H]^+$; ^1H NMR (400 MHz, CD_3OD) δH 6.94 (1H, d, *J* = 2.0 Hz, H-2'), 6.91 (1H, dd, *J* = 7.4, 2.0 Hz, H-6'), 6.82 (1H, d, *J* = 7.4 Hz, H-5'), 6.44 (1H, d, *J* = 16.4 Hz, H-4), 6.09 (1H, dt, *J* = 16.4, 6.8 Hz, H-3), 3.77 (1H, t, *J* = 6.6 Hz, H-1), 2.50 (1H, q, *J* = 6.6 Hz, H-2), 3.92 (3H, s, 3'-OCH_3), 3.89 (3H, s, 4'-OCH_3); ^{13}C NMR (100 MHz, CD_3OD) δc 149.1 (C-4'), 148.8 (C-3'), 132.1 (C-4), 130.5 (C-1'), 124.2 (C-6'), 124.2 (C-3), 111.3 (C-5'), 108.8 (C-2'), 62.0 (C-1), 36.1 (C-2), 55.7 (4'-OCH_3), 55.6 (3'-OCH_3).

3) 3-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-propan-1-one - White amorphous powder, UV (MeOH) λ_{max} (log ε) 254 (3.6) ㎚; ESI-MS *m/z* 197 $[M+H]^+$; ^1H NMR (400 MHz, CD_3OD) δH 7.57 (1H, dd, *J* = 8.4, 2.0 Hz, H-6'), 6.98 (1H, d, *J* = 8.4 Hz, H-5'), 6.93 (1H, d, *J* = 2.0 Hz, H-2'), 4.04 (2H, t, *J* = 5.2 Hz, H-3), 3.98 (3H, s, 3'-OMe), 3.20 (2H, t, *J* = 5.2 Hz, H-2); ^{13}C NMR (100 MHz, CD_3OD) δc 199.0 (C-1), 151.0 (C-4'), 147.4 (C-3'), 131.2 (C-1'), 124.4 (C-6'), 115.8 (C-2'), 112.1 (C-5'), 59.0 (C-9'), 42.0 (C-8'), 56.7 (3'-OMe).

4) 1*H*-Indole-3-carbaldehyde - White amorphous powder, ESI-MS *m/z* 146 $[M+H]^+$; ^1H NMR (400 MHz, CD_3OD) δH 9.90 (1H, s, CHO), 8.85 (1H, dd, *J* = 6.8, 1.2 Hz, H-4), 8.17 (1H, s, H-2), 7.48 (1H, dd, *J* = 6.8, 1.2 Hz, H-7), 7.28 (2H, m, H-5/H-6); ^{13}C NMR (100 MHz, CD_3OD) δc 185.5 (CHO), 138.0 (C-2), 137.0 (C-1a), 124.3 (C-6), 124.0 (C-3a), 122.5 (C-5), 121.5 (C-4), 117.0 (C-3), 112.2 (C-7).

5) *trans*-Cinnamic acid - Yellow amorphous powder, UV (MeOH) λ_{max} (log) 280 (4.0) ㎚; ESI-MS *m/z* 149 $[M+H]^+$; ^1H NMR (400 MHz, CD_3OD) δH 7.72 (1H, d, *J* = 16.0 Hz, H-3), 7.60 (2H, m, H-2'/H-6'), 7.42 (3H, m, H-3'/H-4'/H-5'), 6.50 (1H, d, *J* = 16.0 Hz, H-2); ^{13}C NMR (100 MHz, CD_3OD) δc 172.0 (C-1), 147.9 (C-3), 115.3 (C-3), 134.2 (C-1'), 129.3 (C-3'/C-5'), 125.5 (C-2'/C-6'), 127.4 (C-4').

6) Uracil - White amorphous powder, ESI-MS *m/z* 113 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δH 5.45 (1H, d, *J* = 7.6 Hz, H-5), 7.39 (1H, d, *J* = 7.6 Hz, H-6); ^{13}C NMR (100 MHz, DMSO-d_6) δc 164.7 (C-4), 151.7 (C-2), 142.5 (C-6), 100.4 (C-5).

4. Tumor necrosis factor-α (TNF-α) activated nuclear factor-kappa B (NF-κB) assay

Human embryonic kidney cells 293 Panomics (Fremont, CA, USA) were employed for monitoring changes occurring along the NF-κB pathway (Kondratyuk *et al*., 2012). Stable constructed cells were seeded into 96-well plates at 20×10^3 cells per well. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen Co., Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (FBS), 100 units/㎖ penicillin, 100 ㎍/㎖

streptomycin, and 2 mM l-glutamine. After 48 h incubation, the medium was replaced and the cells were treated with various concentrations of test substances. TNF-α (Human, Recombinant, *E. coli*, Calbiochem, San Diego, CA, USA) was used as an activator at a concentration of 2 ng/㎖ (0.14 nM). The plate was incubated for 6 h. Spent medium was discarded and the cells were washed once with PBS. Cells were lysed using 50 ㎕ (for 96-well plate) reporter lysis buffer from Promega, by incubating for 5 min on a shaker, and stored at −80℃. The luciferase assay was performed using the Luc assay system from Promega (Promega Co., Madison, WI, USA). The gene product, luciferase enzyme, reacts with luciferase substrate, emitting light which was detected using a luminometer (LUMIstar Galaxy BMG, Ortenberg, Germany). Data for NF-κB constructs are expressed as IC_{50} values (i.e. concentration required to inhibit TNF-activated NF-κB activity by 50%). As a positive control, two known NF-κB inhibitors were used: TPCK, IC_{50} = 3.8 μM and BAY-11, IC_{50} = 2.0 μM.

5. Inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells (iNOp) assay

The level of nitrite, the stable end product of NO, was estimated as described previously (Park *et al*., 2011). Briefly, RAW 264.7 cells were seeded and incubated in 96-well culture plates at 37℃, 5% CO_2 in a humidified air for 24 h. The cultured medium was replaced with phenol red-free medium containing various concentrations of compounds for 15 min prior to 1 ㎎/㎖ of LPS exposure for 20 h. The amount of nitrite in the culture media was measured by using Griess reagent. Under the same experimental conditions, SRB assays were performed to evaluate the cytotoxic effect of compounds toward RAW 264.7 cells. L-N^G-monomethyl arginine citrate (l-NMMA), as a positive control of this assay showed an IC_{50} value of 25.1 μM.

6. Statistical analysis

All data were expressed as means ± SD. Statistical analysis was performed using SPSS software (version 18.0, SPSS Inc., Chicago, IL, USA). Significant differences between the groups were determined using the analysis of variance (ANOVA) followed by Duncan Post Hoc Test (DPHT). $p < 0.05$ were considered as statistical significance.

RESULTS AND DISCUSSION

1. Structure elucidation of compounds 1 - 6

Repeated chromatography of the aerial parts of *V. cinerea* on silica gel and YMC-pack RP-C_{18} columns led to the isolation of six known compounds (1 - 6) (Fig. 1).

Compound 1 was obtained as a light yellow amorphous powder and its molecular weight was evaluated as *m/z* 465 $[M + H]^+$ by the positive ESI-MS spectrometry. The UV spectrum showed absorption maxima at 330 and 279 ㎚ indicating a conjugated double bond and a carbonyl group, respectively. The IR spectrum displayed characteristic absorption bands at 3,325 and 1,655 ㎝$^{-1}$, corresponding to the hydroxy group (s) and conjugated C = O group (s), respectively. The ^{13}C NMR spectrum revealed 15 carbon signals, along with six glucose unit carbons at [δc 103.6 (C-1"), 77.6 (C-3"), 76.8 (C-5"), 73.5 (C-2"), 70.0 (C-4"), 61.0 (C-6")], suggested that 1 is a glycosylated flavone skeleton (Han *et al*., 2004). The ^1H NMR spectrum showed a downfield shifted OH proton at δ_H 12.96 (1H, s, OH-5) due to the hydrogen bond with a carbonyl group (C-4), and two aromatic doublet protons at δ_H 6.82 (1H, d, J = 2.0 Hz, H-8) and 6.48 (1H, d, J = 2.0 Hz, H-6), suggesting a di-substituted A-ring. In addition, the ^1H NMR spectrum displayed ABX type aromatic system protons at [δ_H 7.48 (1H, dd, J = 8.0, 2.0 Hz, H-6'), 7.46 (1H, d, J = 8.0 Hz, H-5'), and 6.94 (1H, d, J = 2.0 Hz, H-2')] in B-ring and glycose unit protons at [δ_H 5.12 (1H,

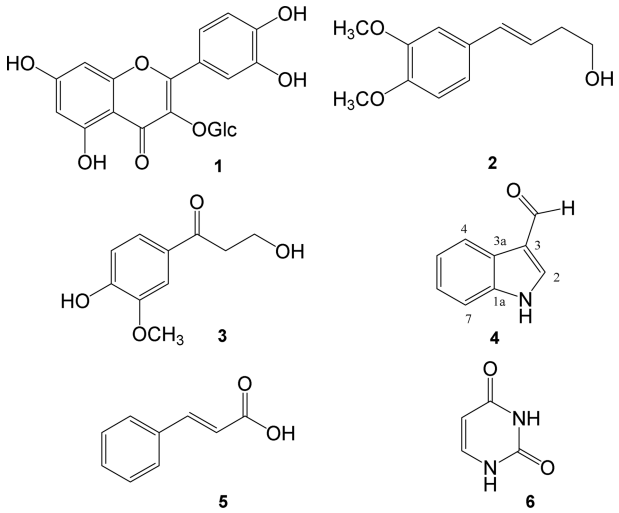
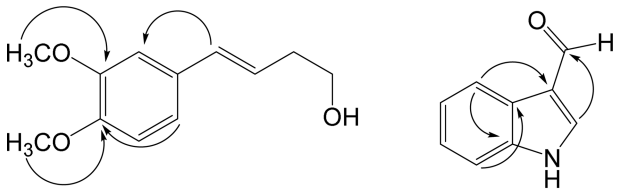


Fig. 1. Structures of compounds 1 - 6 isolated from *V. cinerea*.

d, J = 7.2 Hz, H-1"), 3.75 (1H, d, J = 10.4 Hz, H-6"a), 3.53 (1H, d, J = 10.4 Hz, H-6"b), 3.20 - 3.53 (4H, m, H-2" to H-5")]. The heteronuclear multiple bond correlation (HMBC) correlation of the anomeric proton (H-1") with C-3 indicated the position of glycose unit at C-3. Accordingly, compound 1 was assigned as quercetin 3-*O*-*β*-D-glucopyranoside (1), by comparison of its physicochemical data with literature values (Choi *et al*., 2000).

Compound 2 was obtained as a yellow amorphous powder. The ESI-MS spectrum of 2 gave a molecular ion [M + H] at *m/z* 209. The NMR and heteronuclear single-quantum correlation (HSQC) spectra of 2 displayed ABX-type aromatic protons at [δH 6.94 (1H, d, J = 2.0 Hz, H-2'), 6.91 (1H, dd, J = 7.4, 2.0 Hz, H-6'), and 6.82 (1H, d, J = 7.4 Hz, H-5')] with six aromatic carbons at [δc 149.1 (C-4'), 148.8 (C-3'), 130.5 (C-1'), 124.2 (C-6'), 111.3 (C-5'), and 108.8 (C-2')], a *trans*-olefinic group at δ_H 6.44 (1H, d, J = 16.4 Hz)/δc 132.1 (C-4) and at 6.09 (1H, dt, J = 16.4, 6.8 Hz/δc 124.2 (C-3), and two methoxy groups at δ_H 3.92/δc 55.6 (3'-OCH_3) and at δ_H 3.89/δc 55.7 (4'-OCH_3). The position of two methoxy signals at (δ_H 3.92 and 3.89) was confirmed at C-3' and C-4', respectively, through the HMBC analysis (Fig. 2). The remaining methylene at δ_H 2.50 (1H, q, J = 6.6 Hz)/δc 36.1 (C-2) and an oxygen bearing methylene group at δ_H 3.77 (1H, t, J = 6.6 Hz)/δc 62.0 (C-1) were confirmed as a part of the *trans*-olefinic group, on the basis of the HMBC correlations from H-1 to C-3 and from H-2 to C-4. In addition, three-bond HMBC correlations of the olefinic proton (H-4) with C-2'/C-6' and of H-2'/H-6' with C-4 were shown as in Fig. 2, indicating a but-3-en-1-ol group was attached at C-1' of the phenyl group. Accordingly, the structure of 2 was identified as (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-1-ol (Masuda and Jitoe, 1995).

Compound 4 was obtained as a white amorphous powder. The MS spectrum showed a molecular ion peak at *m/z* 146 $[M + H]^+$. The NMR and HSQC spectra of 4 displayed signals for a tri-substituted olefinic group at δH 8.17 (1H, s)/δc (138.0, C-2) and δc (117.0, C-3), an aromatic quaternary carbon at δc 124.0 (C-3a), a downfield shifted aromatic quaternary at δc 137.0 (C-1a) due to a nitrogen atom attachment, and four protonated aromatic signals at [δH 8.85 (1H, dd, J = 6.8, 1.2 Hz)/δc 121.5 (C-4), 8.17 (1H, s)/δc 138.0 (C-2), 7.48 (1H, dd, J = 6.8, 1.2 Hz)/δc 112.2 (C-7), δh 7.28 (1H, m)/δc 122.5 (C-5), and δh 7.28 (1H, m)/δc 124.3 (C-6)], indicated the presence of an indole skeleton (Wang *et al*., 2013). In addition, the NMR spectra revealed an aldehyde group signal at δH 9.90 (1H, s)/δc 185.5 (CHO). The HMBC spectrum displayed two- to three-bonds correlations from H-2 to aldehyde carbon (CHO)/C-3a and from H-4 to C-3/C-1a/C-3a (Fig. 2), confirmed the identification of compound 4 as 1*H*-indole-3-carbaldehyde, previously reported from *Laminaria japonica* (Wang *et al*., 2013).



2 4

Fig. 2. Key ^{1}H-^{13}C HMBC correlations for 2 and 4.

The other isolates were identified as, 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-propan-1-one (3) (Jones *et al*., 2000), *trans*-cinnamic acid (5) (Davidse *et al*., 1990), and uracil (6) (Kitajima *et al*., 1999), by comparison of their physical and spectral data with published values. Among the isolates, compound 1 was obtained as a major metabolite. To the best of our knowledge, compounds 2 - 6 were isolated for the first time from this plant source.

2. Evaluation of anti-inflammatory activity

The $CHCl_3$-soluble portion from the aerial parts of *V. cinerea* exhibited potent inhibitory activity against TNF-α-induced NF-κB activity and NO production in LPS-stimulated RAW 264.7 cells with inhibition rates of 62.3 and 63.3%, respectively. Compounds 1 - 3 isolated from the $CHCl_3$ extract were also evaluated for their anti-inflammatory potential based on their ability to inhibit TNF-α-induced NF-κB activity and NO production, while compounds 4 - 6 could not be evaluated in this manner, due to their insufficient amounts available for testing. As shown in Table 1, when treated with a fixed concentration of 50 μM, quercetin glycoside, 1 and phenyl propanoid, 3 exhibited moderate TNF-α-induced NF-κB inhibitory activity with IC_{50} values of 7.5 and 11.5 μM, respectively, compare to that of the positive control, tosyl phenylalanyl chloromethyl ketone (TPCK, IC_{50} = 3.8 M), whereas compounds 1 - 3 showed weak inhibitory activity on NO production.

Table 1. Inhibition effect of compounds 1 - 3 on the TNF-α-induced NF-κB activity and NO production in LPS-stimulated RAW 264.7 cells.

Compounds	Nitrite assay (NO)			NF-κB		
	%Inhibitory[1)]	%Survive[2)]	IC_{50} (M)	%Inhibitory	%Survive	IC_{50} (M)
Aerial parts	56.2 ± 1.5*	> 100	17.6 ± 0.8	35.5 ± 0.6*	> 100	nd
Roots	44.8 ± 2.5*	> 100	nd[3)]	8.7 ± 3.2*	> 100	nd
CH_2Cl_2 partition	63.3 ± 3.7*	> 100	nd	62.3 ± 5.6*	> 100	nd
BuOH partition	55.4 ± 4.4*	> 100	nd	34.1 ± 1.5*	> 100	nd
1	23.8 ± 4.8	86.1 ± 5.7	nd	68.3 ± 8.8	80.0 ± 6.2	7.5 ± 2.5
2	15.1 ± 10.6	> 100	nd	61.0 ± 8.4**	94.6 ± 11.3	nd
3	26.7 ± 7.3**	> 100	nd	57.0 ± 6.6**	80.0 ± 8.6	11.5 ± 1.2
4	–[4)]	–	–	–	–	–
5	–	–	–	–	–	–
6	–	–	–	–	–	–
l-NMMA[5)]			25.1 ± 2.3			
TPCK[6)]						3.8 ± 0.6
BAY-11[6)]						2.0 ± 0.3

1) % inhibition at 50 μM, 2) % survival at concentration of 50 μM, 3) nd; Not determined, 4) –; not tested, 5) positive control for NO, 6) positive control for NF-κB. Data represent means ± SD of triplicate determinations from three separate experiments. Significantly different at $*p < 0.05$ and $**p < 0.01$ between groups.

Compound 3 has been reported to have weak activity on NO production (Min and Cuong, 2013), but inhibitory activity of 3 on TNF-α-induced NF-κB was evaluated first time in this study. In addition, quercetin and its derivatives have been found to possess NF-κB inhibitory and antioxidant effects (Kim *et al*., 2013; Lee *et al*., 2013).

In a previous research for cancer chemopreventive agents, major secondary metabolites, sesquiterpene lactones isolated from the flower of *V. cinerea* exhibited potent inhibitory effects on the tumor necrosis factor alpha (TNF-α)-induced NF-κB activity and lipopolysaccharide (LPS)-induced nitric oxide production using murine macrophage RAW 264.7 cells (Youn *et al*., 2012). Although the sesquiterpene lactones have been reported to have a potential on the cancer chemopreventive activity in the previous study, the anti-inflammatory activity of several phenolic compounds isolated from the aerial parts of *V. cinerea* can be attributed, at least in part, to inhibition of TNF-α-induced NF-κB activity.

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