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Tricin derivatives as anti-inflammatory and anti-allergic constituents from the aerial part of *Zizania latifolia*

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Methanol extract of *Zizania latifolia* was partitioned with EtOAc, *n*-BuOH, and H₂O. From the EtOAc layers, a new flavonolignan along with a known flavone and three known flavonolignans, triclin (1), salcolin A (2), salcolin B (3), and salcolin C (4), were isolated through repeated silica gel and ODS column chromatography. The chemical structure of the new flavonolignan was determined to be triclin-4'-*O*-[erythro- β -guaiacyl-(7''-*O*-methyl)-glyceryl] ether and was named salcolin D (5) based on physicochemical and spectroscopic data, including FT-NMR and ESI-MS. All compounds were isolated for the first time from this plant. Compounds 2–5, triclin derivatives, all exhibited higher anti-inflammatory and anti-allergy activities than triclin. In particular, salcolin D (5) was shown to have the strongest inhibitory activity against LPS-induced NO production in RAW 264.7 cells as well as β -hexosaminidase release in IgE-sensitized RBL-2H3 cells. These results suggest that the presence of triclin derivatives conveys allergy and inflammation treatment ability to *Z. latifolia*.

Key words: *Zizania latifolia*; new flavonolignan; triclin; salcolin; anti-allergy; anti-inflammation

Zizania latifolia Turcz, known as wild rice, is a fascicular and perennial aquatic plant that grows in warm water. It is distributed in Japan, Vietnam, Korea, and southern China, particularly south of the Yellow River. *Z. latifolia* Turcz differs botanically from the three wild rice species grown in North America, namely *Z. aquatica*, *Z. palustris*, and *Z. texana*. Moreover, grains of wild rice have been used to treat a variety of ailments in Chinese medicinal practice. Reports have elucidated the rich nutrient content, particularly quality protein,

dietary fiber, vitamin B1, B2, and E contents, of wild rice species.¹⁾

Recently, it has been demonstrated that cultivated grains of wild rice possess a variety of biological activities, such as anti-obesity activity,²⁾ suppressive effects against hyperlipidemia and oxidative stress,³⁾ and improvement of high-fat/cholesterol diet-induced insulin resistance in rats.⁴⁾ On the other hand, studies on the aerial part of *Z. latifolia* are scarce, except for the inhibitory effect of *Z. latifolia* rhizoma on apoptosis induced by H₂O₂ in Neuro2A cells⁵⁾ as well as the anti-fatigue effect of *Z. caudiflora* (Turczaninow) Nakai through a forced swimming test.⁶⁾

Inflammation is a complex process regulated by a cascade of nitric oxide (NO), cytokines, prostaglandins, and growth factors produced by activated macrophages. Macrophages play a major role in innate immunity, initiating and propagating defensive reactions against pathogens. Excess NO production by activated macrophages and other cells exposed to endotoxins contributes to rheumatoid arthritis, atherosclerosis, asthma, and pulmonary fibrosis. Thus, agents that down-regulate NO production would be beneficial in the treatment of inflammation.^{7–13)} In addition, mast cells play an important role in acute inflammation and allergic responses.^{14,15)} The rat basophilic leukemia cell line RBL-2H3 is a mucosal mast cell line as well as the major model system for antigen-induced degranulation.¹⁶⁾ Several studies have shown that allergic inflammation mediated by mast cells is suppressed by plant phenolics or flavonoids, including kaempferol, quercetin, myricetin, rutin,¹⁷⁾ chlorogenic acid,¹⁸⁾ and EGCG.¹⁹⁾

Previously, we have demonstrated that methanol extract of the aerial part of *Z. latifolia* Turcz exhibits anti-allergic effects through inhibition of compound 48/80-induced degranulation, antigen-induced β -hexosaminidase release, as well as PMA plus A23187-induced

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Abbreviations: c.c, column chromatography; LPS, lipopolysaccharide; NO, nitric oxide.

TNF- α production in RBL-2H3 mast cells.²⁰) However, *Z. latifolia* Turcz has not yet been completely examined in terms of its functional properties and phytochemical components. Thus, this study was initiated to investigate the bioactive compounds in *Z. latifolia* Turcz. In this paper, we describe the procedure for the isolation and structure determination of a flavone, triclin (1), three flavonolignans, salcolin A (2), salcolin B (3), and salcolin C (4), and a new flavonolignan, salcolin D (5) (Fig. 1). These compounds were isolated for the first time from aerial parts of *Z. latifolia* and were identified as active components with anti-inflammatory and anti-allergic effects.

Materials and methods

Plant sample. Dried aerial parts of *Z. latifolia* were purchased from store located at Yeongcheon City, Gyeongbuk, Korea. A voucher specimen (KHU-0130301) was deposited at the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea.

Instruments and reagents. Preparative-liquid chromatography (prep-LC) (LC-Forte/R, YMC, Japan) was carried out using cartridge YMC-DispoPack (ODS-25,

80 g, and 120 g, YMC, Japan). Silica gel (230-400 mesh, Merck, Germany) and Sephadex LH-20 (Merck, Germany) were used for column chromatography. Thin-layer chromatography (TLC) analysis was performed using on a Kiesel gel 60F₂₅₄ plate (Merck Co., Darmstadt, Germany) and a RP-18 F_{254s} plates (Merck Co., Darmstadt, Germany), and detection was performed by a UV lamp at 254 and 365 nm and 10% H₂SO₄ solution by spraying and heating. ¹H and ¹³C NMR along with 2D NMR data were obtained on a Bruker AVANCE II 400 (¹H NMR at 400 MHz, ¹³C NMR at 100 MHz) spectrometer (Bruker, Germany) in pyridine-*d*₅ with TMS as internal standard. ESI/MS was obtained on a 6530 Accurate-Mass Q-TOF LC/MS (Agilent Technologies, Germany). All the reagent grade chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Isolation of the bioactive compounds from *Z. latifolia*.

The dried powder (5 kg) was extracted at room temperature with 80% MeOH (45 L). The concentrated extract was partitioned with water (3 L), EtOAc (3 L $\times$ 3) and *n*-BuOH (3 L $\times$ 3), successively, to give the EtOAc extract (97 g, ZLE), *n*-BuOH extract (240 g, ZLB), and H₂O extract (57 g, ZLW). The EtOAc extract was applied to a silica gel c.c. (12 $\times$ 14 cm) and eluted with CHCl₃ : MeOH (50:1 $\rightarrow$ 30:1 $\rightarrow$ 20:1 $\rightarrow$ 12:1 $\rightarrow$ 10:1 $\rightarrow$ 7:1 $\rightarrow$ 5:1 $\rightarrow$ 3:1 $\rightarrow$ 1:1, each 3 L) monitoring by TLC to produce 13 fractions (ZLE-1 to ZLE-13). Fraction ZLE-8 (4.7 g) was proceeded by prep-LC cartridge: YMC-DispoPack AT (ODS-25: 120 g, particle size: 25 μ m). The fractions were analyzed by UV detector at 254 and 356 nm, the mobile phase consisted of A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile with a flow rate of 10 mL/min. The gradient consisted of 10% B held for 20 min to 30% B at a 0.20%/min rate, from 30% B to 50% B at a 0.20%/min rate, from 50% B to 70% B at a 0.2%/min rate, and from 70% B to 90% B at a 0.30%/min rate. Total 14 fractions (ZLE-8-1 to ZLE-8-14) were yielded. Fraction ZLE-8-4 (327 mg) was proceeded by prep-LC cartridge: YMC-DispoPack AT (ODS-25 : 80 g, particle size : 25 μ m), using A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile as a mobile phase at a flow rate of 10 mL/min, and the detector was maintained at 254 and 356 nm. The gradient consisted of 10% B to 35% B at a 0.5%/min rate, from 35% B to 60% B at a 0.6%/min rate. Compound 3 (27 mg, RT = 14.2 min), compound 1 (21 mg, RT = 15.7 min), and compound 2 (32 mg, RT = 17.8 min) were obtained. ZLE-8-6 (231 mg) was proceeded by prep-LC cartridge: YMC-DispoPack AT (ODS-25: 80 g, particle size: 25 μ m), using A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile as a mobile phase at a flow rate of 10 mL/min, and the detector was maintained at 254 and 356 nm. The gradient consisted of 15% B to 40% B at a 0.5%/min rate, from 40% B to 7% B at a 0.6%/min rate. Compound 4 (52 mg, RT = 21.1 min) and compound 5 (21 mg, RT = 23.4 min) were obtained.

Tricin (1) pale yellow powder (CH₃OH); negative ESI/MS *m/z* 329 [M-H]⁻; IR (CaF₂ plate) 3413, 2941, 2842, 1664, 1611 cm⁻¹; ¹H NMR (400 MHz, pyridine-*d*₅) δ 7.42 (2H, s, H-2', 6'), 7.01 (1H, s, H-3), 6.87

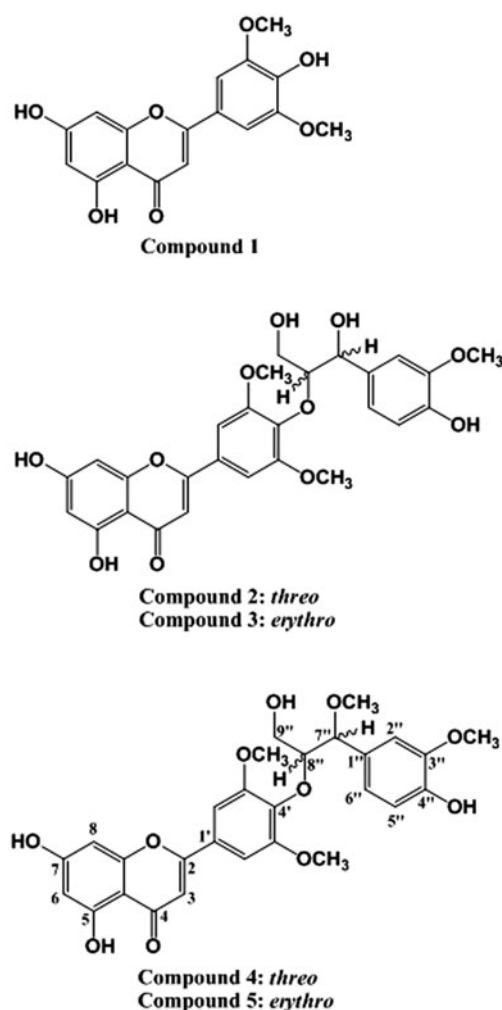


Fig. 1. Chemical structures of the isolated compounds 1–5 from the *Z. latifolia*.

(1H, d, $J = 2.0$ Hz, H-8), 6.74 (1H, d, $J = 2.0$ Hz, H-6), 3.84 (6H, s, OCH₃', 5'). ¹³C NMR (100 MHz, pyridine-*d*₅) δ 182.5 (C-4), 165.7 (C-7), 164.4 (C-2), 162.9 (C-5), 158.3 (C-9), 149.1 (C-3', 5'), 141.1 (C-4'), 121.1 (C-1'), 104.9 (C-2', 6'), 104.7 (C-10), 104.2 (C-3), 99.7 (C-6), 94.8 (C-8), 56.2 (C-3', 5'-OCH₃).

Salcolin A (**2**) yellow amorphous powder (CH₃OH); negative ESI/MS m/z 525 [M-H]⁻; IR (CaF₂ plate) 3369, 2938, 1652, 1611, 1588, 1512, 1495, 1356, 1263, 1159, 1124, 841 cm⁻¹; ¹H NMR (400 MHz, pyridine-*d*₅) δ 7.59 (1H, br. s, H-2''), 7.48 (1H, dd, $J = 8.0$, 1.2 Hz, H-6''), 7.31 (2H, s, H-2', 6'), 7.27 (1H, d, $J = 8.0$ Hz, H-5''), 7.00 (1H, s, H-3), 6.85 (1H, d, $J = 2.0$ Hz, H-8), 6.74 (1H, d, $J = 2.0$ Hz, H-6), 5.78 (1H, d, $J = 6.0$ Hz, H-7''), 4.93 (1H, q, $J = 6.0$, 4.0 Hz, H-8''), 4.44 (1H, dd, $J = 12.0$, 4.0 Hz, H-9''e), 4.06 (1H, dd, $J = 12.0$, 4.0 Hz, H-9''a), 3.81 (6H, s, OCH₃', 5'), 3.72 (3H, s, OCH₃''). ¹³C NMR (100 MHz, pyridine-*d*₅) δ 183.9 (C-4), 167.3 (C-7), 165.0 (C-2), 163.9 (C-5), 159.8 (C-9), 155.1 (C-3', 5'), 149.6 (C-3''), 148.7 (C-4''), 142.2 (C-4'), 135.1 (C-1''), 128.1 (C-1'), 121.9 (C-5''), 117.2 (C-6''), 113.0 (C-2''), 107.1 (C-3), 106.3 (C-10), 106.0 (C-2', 6'), 101.3 (C-6), 96.3 (C-8), 90.4 (C-8''), 74.9 (C-7''), 63.1 (C-9''), 57.7 (C-3', 5'-OCH₃), 57.0 (C-3''-OCH₃).

Salcolin B (**3**) yellow amorphous powder (CH₃OH); negative ESI/MS m/z 525 [M-H]⁻; IR (CaF₂ plate) 3364, 2933, 1649, 1607, 1590, 1495, 1457, 1358, 1121, 832 cm⁻¹; ¹H NMR (400 MHz, pyridine-*d*₅) δ 7.54 (1H, d, $J = 1.2$ Hz, H-2''), 7.38 (1H, dd, $J = 8.0$, 1.2 Hz, H-6''), 7.31 (2H, s, H-2', 6'), 7.27 (1H, d, $J = 8.0$ Hz, H-5''), 7.00 (1H, s, H-3), 6.84 (1H, d, $J = 2.0$ Hz, H-8), 6.74 (1H, d, $J = 2.0$ Hz, H-6), 5.71 (1H, d, $J = 4.8$ Hz, H-7''), 5.07 (1H, q, $J = 4.8$, 3.6 Hz, H-7''), 4.65 (1H, dd, $J = 12.0$, 5.2 Hz, H-9''e), 4.33 (1H, dd, $J = 12.0$, 3.2 Hz, H-9''a), 3.82 (6H, s, OCH₃', 5'), 3.73 (3H, s, OCH₃''). ¹³C NMR (100 MHz, pyridine-*d*₅) δ 183.9 (C-4), 167.4 (C-7), 165.1 (C-2), 164.4 (C-5), 159.8 (C-9), 155.3 (C-3', 5'), 149.6 (C-3''), 148.6 (C-4''), 141.7 (C-4'), 135.6 (C-1''), 128.0 (C-1'), 121.8 (C-6''), 117.3 (C-5''), 112.9 (C-2''), 107.1 (C-3), 106.3 (C-10), 106.1 (C-2', 6'), 101.3 (C-6), 96.3 (C-8), 89.4 (C-8''), 75.0 (C-7''), 62.9 (C-9''), 57.7 (C-3', 5'-OCH₃), 57.1 (C-3''-OCH₃).

Salcolin C (**4**) yellow amorphous powder (CH₃OH); negative ESI/MS m/z 539 [M-H]⁻; IR (KBr) 3436, 2957, 2926, 2531, 1649, 1589, 1495, 1358 cm⁻¹; ¹H NMR (400 MHz, pyridine-*d*₅) δ 7.34 (2H, s, H-2', 6'), 7.33 (1H, d, $J = 1.2$ Hz, H-2''), 7.25 (1H, d, $J = 8.0$ Hz, H-5''), 7.23 (1H, dd, $J = 8.0$, 1.6 Hz, H-6''), 7.01 (1H, s, H-3), 6.86 (1H, d, $J = 2.0$ Hz, H-8), 6.75 (1H, d, $J = 2.0$ Hz, H-6), 5.08 (1H, d, $J = 6.8$ Hz, H-7''), 5.00 (1H, q, $J = 6.8$, 4.0 Hz, H-8''), 4.26 (1H, dd, $J = 12.0$, 3.6 Hz, H-9''e), 3.89 (1H, dd, $J = 12.0$, 3.6 Hz, H-9''a), 3.83 (6H, s, OCH₃', 5'), 3.59 (3H, s, OCH₃''), 3.30 (3H, s, OCH₃-7'').

¹³C NMR (100 MHz, pyridine-*d*₅) δ 183.6 (C-4), 167.1 (C-7), 165.0 (C-2), 164.0 (C-5), 159.5 (C-9), 154.8 (C-3', 5'), 149.5 (C-3''), 148.9 (C-4''), 142.7 (C-4'), 131.3 (C-1''), 127.1 (C-1'), 122.3 (C-6''), 117.1 (C-5''), 112.7 (C-2''), 106.6 (C-3), 105.9 (C-10), 105.6 (C-2', 6'), 101.0 (C-6), 96.0 (C-8), 87.9 (C-8''), 86.0 (C-7''), 63.1 (C-9''), 57.6 (C-3', 5'-OCH₃), 57.3 (C-3''-OCH₃), 56.6 (C-7''-OCH₃).

Salcolin D (**5**) yellow amorphous powder (CH₃OH); positive HRESI-MS m/z 541.1691[M+H]⁺, calcd for m/z C₂₈H₂₉O₁₁ 541.1704; IR (KBr) 3656, 2667, 2521, 2502, 1687, 1509, 1425, 1398, 1115 cm⁻¹; ¹H NMR (400 MHz, pyridine-*d*₅) δ 7.33 (1H, d, $J = 1.6$ Hz, H-2'), 7.29 (2H, s, H-2', 6'), 7.26 (1H, d, $J = 8.4$ Hz, H-5''), 7.19 (1H, dd, $J = 8.0$, 1.6 Hz, H-6''), 6.99 (1H, s, H-3), 6.85 (1H, d, $J = 2.0$ Hz, H-8), 6.73 (1H, d, $J = 2.0$ Hz, H-6), 4.99 (1H, d, $J = 4.0$ Hz, H-7''), 4.97 (1H, m, H-8'), 4.50 (1H, dd, $J = 12.8$, 2.8 Hz, H-9''e), 4.25 (1H, dd, $J = 12.8$, 2.8 Hz, H-9''a), 3.79 (6H, s, OCH₃', 5'), 3.76 (3H, s, OCH₃''), 3.39 (3H, s, OCH₃-7'').

¹³C NMR (100 MHz, pyridine-*d*₅) δ 183.9 (C-4), 167.4 (C-7), 165.1 (C-2), 164.3 (C-5), 159.8 (C-9), 155.2 (C-3', 5'), 149.8 (C-3''), 149.1 (C-4''), 141.5 (C-4'), 131.7 (C-1''), 127.9 (C-1'), 122.9 (C-6''), 117.3 (C-5''), 113.3 (C-2''), 107.0 (C-3), 106.3 (C-10), 106.0 (C-2', 6'), 101.3 (C-6), 96.3 (C-8), 88.1 (C-8''), 84.7 (C-7''), 62.5 (C-9''), 58.3 (C-3', 5'-OCH₃), 57.6 (C-3''-OCH₃), 57.0 (C-7''-OCH₃).

Cell culture. RAW 264.7 cells, a murine macrophage cell line, and RBL-2H3 cells, a rat basophilic leukemia cell line, were purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea). RAW 264.7 cells were cultured in DMEM (Gibco-BRL, Rockville, MD, USA) containing 1% antibiotics (penicillin/streptomycin) and 10% heat-inactivated fetal bovine serum (FBS, Gibco BRL, NY, USA). RBL-2H3 cells were grown in MEM (Gibco BRL, NY, USA) with 10% FBS and 1% penicillin/streptomycin. Both cell lines were grown in a 37 °C humidified incubator with 5% CO₂ in air.

Determination of cell viability. To determine the cytotoxicity of the components, cell viability was determined by the MTT assay,²³ in which active mitochondria reduce MTT into formazan dye. RBL-2H3 cells were seeded at a density of 3×10^4 cells/well in 96-well microplates. Next day, cells were treated with various concentrations (0.1–10 μ g/mL) of the components at 37 °C. After 48 h, 100 μ L of MTT (5 mg/mL) was added to each well, and plates were incubated at 37 °C for 4 h. One hundred microliters of DMSO was added to each well to dissolve the cells. The plates were kept at room temperature for 5 min, and the absorbance was measured at 550 nm using a multiwell spectrophotometer (Molecular Devices, Sunnyvale, CA). Cytotoxicity of the components in IgE-sensitized RBL-2H3 cells was also assessed by the MTT assay. The cells (3×10^4 cells/well) were seeded and incubated with 450 ng/mL DNP-specific IgE at 37 °C overnight before treatment with the components.

Determination of LPS-induced NO production from RAW 264.7 cells. RAW 264.7 cells were seeded in 96-well plates (1×10^5 cells/well) overnight and treated with various concentrations of test components (in 0.1% DMSO) in the presence of LPS (final concentration, 100 ng/mL) at 37 °C for 24 h. After LPS stimulation for 24 h, NO production in the cell culture

medium was measured by the Griess Reagent System.²⁴⁾ Briefly, the culture supernatant (100 μ L) was mixed with same volume of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine in 2.5% phosphoric acid) for 10 min, and absorbance was measured at 540 nm. The NO concentration was calculated from a standard curve of NaNO₂.

Determination of antigen-induced β -hexosaminidase release from RBL-2H3 cells. The inhibitory effect of the extract on the release of β -hexosaminidase in RBL-2H3 cells was evaluated as reported by Matsuda et al.²⁵⁾ with modifications. In brief, RBL-2H3 cells seeded in 24-well plates (2×10^5 cells/well) were sensitized with anti-DNP IgE (450 ng/mL) at 37 °C overnight. The cells were washed with Siraganian buffer and then incubated in 160 μ L of the incubation buffer at 37 °C for 20 min. Then, the cells were treated with 20 μ L of extract for 10 min, followed by addition of 20 μ L of antigen (DNP-BSA, 10 μ g/mL) at 37 °C for 10 min to stimulate the cells to granulate. The reaction was stopped by cooling in an ice bath for 10 min. Twenty-five microliters of the supernatant was transferred into a 96-well plate and incubated with 25 μ L of substrate (1 mM *p*-nitrophenyl-*N*-acetyl- β -D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37 °C for 1 h. The reaction was stopped by adding 200 μ L of stop solution (0.1 M Na₂CO₃/NaHCO₃, pH 10.0), and the absorbance was measured at 405 nm using a microplate reader. The extract was dissolved in 0.1% DMSO and added to incubation buffer.

Statistical analysis. All experiments were performed three to five times. Data are expressed as the mean $\pm$ standard error of the mean (SEM) or standard deviation. A significant difference from the respective controls for each experimental test condition was assessed using one-way analysis of variance with application of Duncan's multiple range test using IBM SPSS version 20 (SPSS, Chicago, IL). A *p* value of <0.05 was considered significant.

Results

Isolation and identification of tricin derivatives

Aerial parts were extracted in 80% MeOH, and concentrated extracts were successively partitioned using EtOAc, *n*-BuOH, and H₂O. Successive repeated SiO₂ and ODS-A column chromatographies of the EtOAc fraction afforded purified a flavone and four flavonolignans, including a new flavonolignan. They were respectively identified as 4',5,7-trihydroxy-3',5'-dimethoxyflavone, tricin (1), tricin-4'-*O*-(*threo*- β -guaiacylglyceryl) ether, salcolin A (2), tricin-4'-*O*-(*erythro*- β -guaiacylglyceryl) ether, salcolin B (3), and tricin-4'-*O*-[*threo*- β -guaiacyl-(7''-*O*-methyl)-glyceryl] ether, salcolin C (4) based on interpretation of the spectroscopic data, including NMR, MS, IR, and were confirmed by comparison of the data with those reported in the literature.^{21–23)} All compounds were isolated for the first time from *Z. latifolia*.

Compound (5) (yellow amorphous powder) was developed by TLC, followed by spraying with 10% H₂SO₄ and heating to a dark yellow color. The molecu-

lar weight was determined to be 540 based on the pseudomolecular ion peak at *m/z* 539 [M-H][−] in the negative ESI/MS. In the ¹H NMR spectrum, three olefine methine proton signals at δ 7.33 (1H, d, *J* = 1.6 Hz, H-2''), 7.26 (1H, d, *J* = 8.4 Hz, H-5''), and 7.19 (1H, dd, *J* = 8.0, 1.6 Hz, H-6'') due to a 1,2,4-trisubstituted benzene ring and five olefine methine proton signals at δ 7.29 (2H, s, H-2', 6'), 6.99 (1H, s, H-3), 6.85 (1H, d, *J* = 2.0 Hz, H-8), and 6.73 (1H, d, *J* = 2.0 Hz, H-6) owing to a flavonoid moiety were observed. In the oxygenated region, two oxygenated methylene proton signals at δ 4.99 (1H, d, *J* = 4.0 Hz, H-7'') and 4.97 (1H, m, H-8''), an oxygenated methylene proton signal at δ 4.50 (1H, dd, *J* = 12.8, 2.8 Hz, H-9''e) and 4.25 (1H, dd, *J* = 12.8, 2.8 Hz, H-9''a), and four methoxy proton signals at δ 3.79 (6H, s, OCH₃', 5'), 3.76 (3H, s, OCH₃''), 3.39 (3H, s, OCH₃-7'') were observed. The relative configuration of chiral carbons, C-7'' and C-8'', in compound (5) was deduced to be *erythro* type from the coupling constant (*J* = 4.0 Hz) between the oxygenated methine signals of H-7'' (δ _H 4.99) and H-8'' (δ _H 4.97). It was reported that the *J*_{H-7'',8''} coupling constant of the proton resonance in guaiacylglyceryl moiety can be used to distinguish the *erythro* and *threo* forms, *J* value to be 4.0–5.0 and 6.0–7.0 Hz, respectively.^{24,26,27)} The ¹³C NMR spectrum showed 28 carbon signals including four methoxy carbons [δ 58.3 (C-3', 5'-OCH₃), 57.6 (C-3''-OCH₃), 57.0 (C-7'-OCH₃)], confirming that compound (5) was flavonoid with a phenylpropanoid moiety. In the low-magnetic-field region, a conjugated ketone carbon δ 183.9 (C-4) and seven oxygenated olefine quaternary carbons [δ 167.4 (C-7), 165.1 (C-2), 164.3 (C-5), 159.8 (C-9), 155.2 (C-3', 5'), 141.5 (C-4')], two olefine quaternary carbons [δ 127.9 (C-1'), 106.3 (C-10)], and five olefine methine carbon [δ 107.0 (C-3), 106.0 (C-2', 6'), 101.3 (C-6), 96.3 (C-8)] signals were observed, which indicated the presence of a flavones moiety. In addition, two oxygenated olefine quaternary carbon [δ 149.8 (C-3''), 149.1 (C-4'')], one olefine quaternary carbon δ 131.7 (C-1''), and three olefine methine carbon [δ 122.9 (C-6''), 117.3 (C-5''), 113.3 (C-2'')], two oxygenated methine [δ 88.1 (C-8''), 84.7 (C-7'')] and one oxygenated methylene carbon δ 62.5 (C-9'') signals were observed, which indicated the presence of a phenylpropanoid moiety. In the HMBC spectrum the oxygenated methine proton δ _H 4.97 (1H, m, H-8'') showed a correlated cross peak with an oxygenated olefine quaternary carbon δ _C 141.5 (C-4'), indicating the flavone and phenylpropanoid moieties were linked between δ _C C-8'' and δ _C C-4' via an ether linkage, and a cross-peak between an oxygenated methine proton signal at δ _H 4.99 (1H, d, *J* = 4.0 Hz, H-7'') and a methoxy carbon signal at δ _C 57.0, indicating the methoxy group is linked to the C-7'' hydroxy of the phenylpropanoid moiety (Fig. 2). Finally, the structure of compound (5) was determined to be tricin-4'-*O*-[*erythro*- β -guaiacyl-(7''-*O*-methyl)-glyceryl] ether, that is 7''-methoxy salcolin B. The high resolution positive ESI-MS showed the pseudomolecular ion peak [M+H]⁺ at *m/z* 541.1691, which was calculated as 541.1704 for C₂₈H₂₉O₁₁. As the structure of compound (5) has not been reported until now, it was named salcolin D.

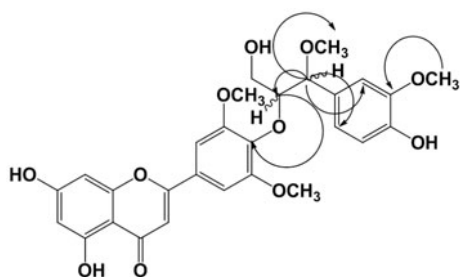


Fig. 2. Key gHMBC correlations for compound 5.

Inhibition of LPS-induced NO production in RAW 264.7 cells by components isolated from Z. latifolia

To test the anti-inflammatory effects of the components from *Z. latifolia*, NO production and cytotoxicity were measured in LPS-stimulated RAW 264.7 cells and the values of LPS alone were used as controls. RAW 264.7 cells were incubated with LPS (100 ng/mL) in the presence of various compounds for 24 h. Fig. 3(b) shows that the inhibition rates of NO production for all components in the culture media significantly increased in a dose-dependent manner. Salcolin D (5) showed the strongest inhibition rates of 0.0 ± 3.18 , 13.86 ± 2.51 , 21.47 ± 2.62 , and $59.14 \pm 0.78\%$ at 0.1, 1, 5, and 10 $\mu\text{g/mL}$, respectively. In other words, at a concentration of 10 $\mu\text{g/mL}$, inhibition rates of triclin (1), salcolin

A (2), salcolin B (3), salcolin C (4), and salcolin D (5) were 23.29 ± 3.21 , 32.23 ± 1.93 , 34.25 ± 2.76 , 43.76 ± 2.82 , and $59.14 \pm 0.78\%$, respectively (Fig. 3(b)). We also tested cell viability by MTT assay to ensure that reduction of NO production was not due to cell death. Neither the tested compounds nor 0.1% DMSO significantly affected cell viability under the conditions used here (Fig. 3(a)).

Suppression of β -hexosaminidase release in IgE-stimulated RBL-2H3 cells by components isolated from Z. latifolia

The ability of each extract to inhibit β -hexosaminidase release from IgE-sensitized RBL-2H3 cells induced by DNP-BSA is shown in Fig. 4(b). The values of DNP-BSA alone were used as controls. Release of β -hexosaminidase was inhibited most effectively by salcolin D (5) (inhibition rates of 42.83 ± 3.15 , 89.43 ± 2.36 , and $82.97 \pm 6.96\%$ at 0.1, 1, and 10 $\mu\text{g/mL}$, respectively). In addition, inhibition rates of triclin (1), salcolin A (2), salcolin B (3), salcolin C (4), and salcolin D (5) at 1 $\mu\text{g/mL}$ were 24.34 ± 2.89 , 25.06 ± 1.36 , 27.69 ± 5.61 , 70.55 ± 0.60 , and $89.43 \pm 2.36\%$, respectively (Fig. 4(a)). The compounds were not cytotoxic to IgE-sensitized RBL-2H3 cells under these conditions (Fig. 4(a)). The extracts were also not toxic to cells not treated with IgE (data not shown).

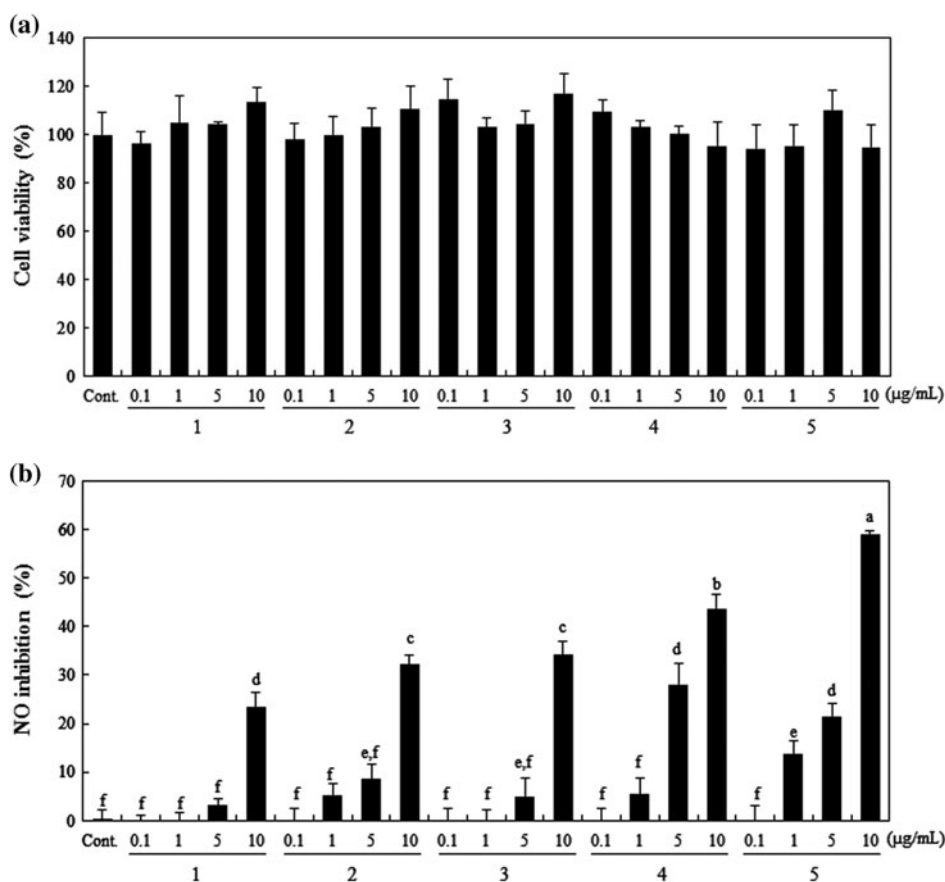


Fig. 3. Cytotoxicity and inhibitory effects of compounds from *Z. latifolia* on NO (production from RAW 264.7 cells stimulated by LPS).

Notes: NO production was assayed in the culture media of cells stimulated with LPS (100 ng/mL) for 24 h. Values are mean $\pm$ SEM of three independent experiments. Values with different superscript letters are significantly different ($p < 0.05$), as determined by analysis of variance by Duncan's multiple range test.

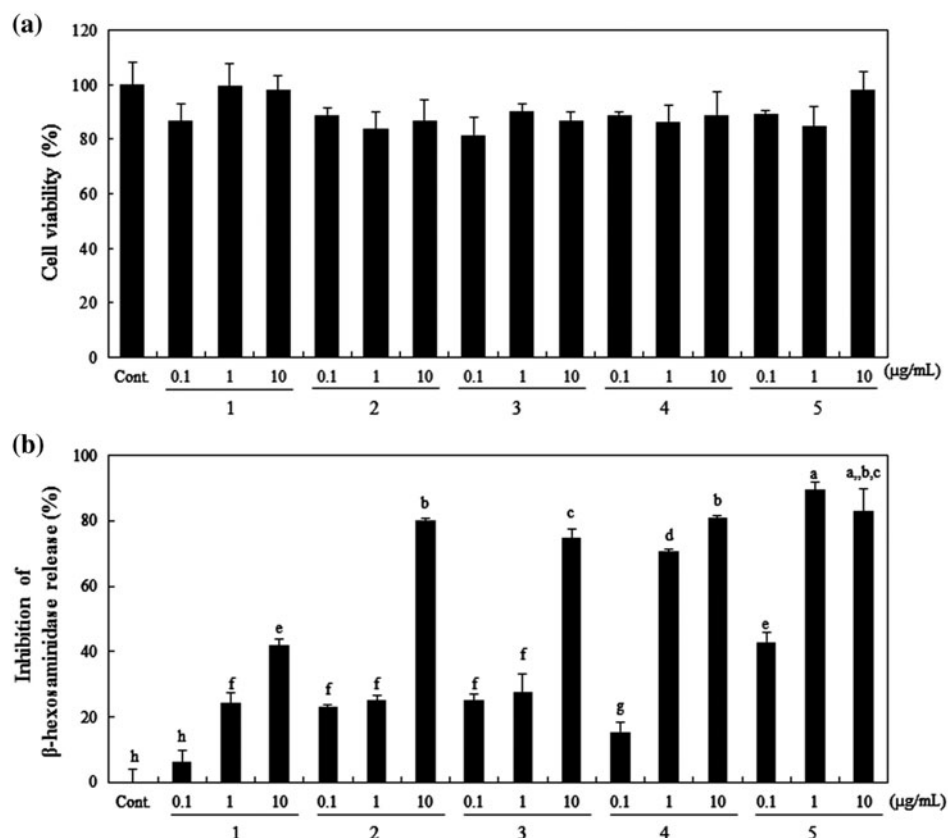


Fig. 4. Cytotoxicity and inhibitory effects of compounds from *Z. latifolia* on β -hexosaminidase release from RBL-2H3 cells induced by IgE with DNP-BSA.

Notes: RBL-2H3 cells (2×10^5 cells) were sensitized with 450 ng/mL of DNP-specific IgE overnight and pretreated with varying doses of compounds for 10 min. Cells were stimulated with 10 μ g/mL of DNP-BSA for 10 min. β -Hexosaminidase release was assayed in the culture media of cells. The value represents mean $\pm$ SEM of three independent experiments. Values with different superscript letters are significantly different ($p < 0.05$), as determined by analysis of variance by Duncan's multiple range test.

Discussion

Z. latifolia Turcz., which belongs to the tribe Oryzeae within Gramineae, is a perennial aquatic plant grown in lakes, pond, or wetlands, and it is cultivated mainly in regions of southeastern Asia, including Russia, Korea, Japan, and China.²⁸⁾ In China, culms and rhizomes are used as a tonic to treat anemia and fever in addition to heart, kidney, and liver disorders.²⁹⁾ Although various reports have been carried out on the nutritional aspects of the grain, little attention has been given to the biological efficacy of the aerial part of this plant.

Previously, we found that ethanol extract of *Z. latifolia* suppresses β -hexosaminidase release, a marker of mast cell degranulation, in RBL-2H3 cells. Thus, extract of the aerial parts was fractionated into ethylacetate, butanol, and water fractions in order to identify the active anti-inflammatory and anti-allergic constituents of this plant. Here, we present for the first time one new flavonolignan, one flavone, and three flavonolignans isolated from the ethylacetate fraction of the aerial parts of *Z. latifolia* as active constituents with inhibitory activities against NO production and histamine release *in vitro*. These five compounds were isolated using prep-LC equipped with cartridges of various size and were identified on the basis of spectroscopic data analysis, including NMR, UV, and ESI-MS. Four flavonolignans, including a new flavonolignan,

showed structural similarities in that they are derived from triclin and are diastereomers of each other. Salcolin A (2) and salcolin B (3) are diastereomers distinguished by *threo* and *erythro*. Salcolin C (4) and salcolin D (5) are also *threo* and *erythro*, respectively. Further studies are needed to determine the absolute chemistry of flavonolignans because spectroscopic data including NMR, MS and IR are not able to make a sharp distinction between RS or SR of erythro and SS or RR of threo. Thus, alpha and beta configuration of structures were omitted like several references.^{30,31)} Among the isolated compounds, the new flavonolignan salcolin D showed the strongest anti-inflammatory and anti-allergic activities. Therefore, we believe that salcolin D is the active constituent responsible for the efficacy of *Z. latifolia*.

Several previous studies have reported that compounds (1–4) isolated from *Oryzae sativa* L. and *Zea* may have anti-inflammatory effects.^{32,33)} Tricin is reported to inhibit COX activity in mice, PGE2 generation in colon cells and murine plasma, and adenoma formation.³⁴⁾ It has been suggested that triclin derivatives exert their anti-inflammatory potential mainly through COX enzyme activity with little effect on COX-2 protein expression.³⁵⁾ From the comparison of five triclin derivatives, salcolin D, a newly identified flavonolignan, was confirmed as an effective compound with better anti-inflammatory and anti-allergic activities than triclin, a potent COX inhibitor. The result

suggested that methoxyl group in guaiacylglycerol moiety was important for anti-inflammatory effect, but the relationship with stereochemistry was not clear. These results suggest that salcolin D can be useful as a potential anti-allergic inflammatory agent, and *Z. latifolia* was ascertained as a possible therapeutic source.

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