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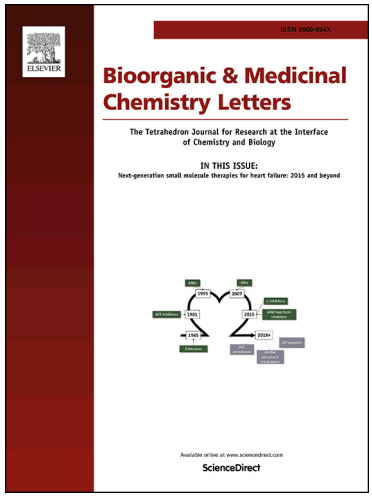

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Anti-allergic inflammatory components from *Sanguisorba officinalis* L.

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Abstract

Sanguisorba officinalis L. was well known as a traditional herbal medicine to treat inflammation and allergic skin diseases. The aim of this research was to indentify compounds with anti-allergic inflammatory property. Twenty-five compounds (**1**-**25**) were isolated from *S. officinalis* including two new compounds (**1** and **8**), and their chemical structures were identified by NMR and ESIMS analysis. Consequently, the anti-allergic inflammatory activities of these isolates were investigated by inhibiting β-hexosaminidase and IL-4 production in PMA/A23187-stimulated RBL-2H3 cells. Compounds **6**, **8**, **13**, **17**-**18** and **25** significantly inhibited β-hexosaminidase release and IL-4 production. Additionally, compounds **8**, **17** and **25** effectively suppressed the activation of NF-κB and NF-κB p65 translocation into the nucleus. Anti-inflammatory effects of isolated compounds were evaluated in LPS-stimulated RAW264.7 macrophages, and they showed dramatic inhibition on LPS-induced overproduction of nitric oxide (NO) and TNF-α. Consistently, the protein levels of iNOS and COX-2 were remarkably decreased by the single compounds **8**, **13** and **25**. These results showed that compounds **8**, **13** and **25** from *S. officinalis* may have a therapeutic potential for allergic inflammatory diseases.

Keywords: *Sanguisorba officinalis* L.; Rosaceae; anti-allergic inflammatory; NF-κB; iNOS and COX-2.

Sanguisorba officinalis L., a member of sub-family Rosoideae and family Rosaceae, is widely distributed in Asia, Western Europe and North America. Previous studies have reported that an ethanol extract of *S. officinalis* significantly inhibited LPS-stimulated nitric oxide (NO) and prostaglandin E_2 (PGE_2) production in RAW264.7 cells and played important roles in the inhibition of methicillin-resistant *Staphylococcus aureus*[1,2] Methanol extract of *S. officinalis* exhibited clear antiviral activities.[3] Aqueous extract of *S. officinalis* root on immediate-type allergic reactions was investigated through *in vivo* and *in vitro* tests.[4] However, the anti-allergic inflammatory effects of some single compounds isolated from *S. officinalis* have not been demonstrated.

An allergy occurs in individuals who have formed allergen-specific IgE antibodies following exposure and sensitization to specific allergens, such as pollen, dust, chemicals, variant proteins, or ultraviolet radiation.[5,6] With the allergy patients increasing annually, approximately 10 ~ 20 % of people in the world are affected by allergies.[7] Previous studies have reported that high serum IgE levels induce the activation of mast cells and cause an allergic reaction. Mast cells aggregate high-affinity IgE receptors (FcεRI) on their surfaces, which is important to the pro-inflammatory allergic response.[8-10] When an IgE-antigen binds with FcεRI, the receptor is activated, and a variety of biologically active mediators are released, causing allergic reactions, including the release of β-hexosaminidase, a common degranulation marker, histamine and inflammatory cytokines.[11] IL-4 is a critical cytokine for switching B cell antibody production to IgE and mast cell development.[12] RBL-2H3 cells originated from rat basophilic leukemia are a mast cell line and have been widely used to study IgE-FcεRI interactions and degranulation. Furthermore, RBL-2H3 cells are a useful model for *in vitro* screening of anti-allergy drug candidates.[7]

Inflammation is the first response of the immune system to infection or irritation. Lipopolysaccharide (LPS) is a principle component of the outer membrane of Gram-negative

bacteria, which can activate the inflammatory cells and stimulate the production of inflammatory mediators such as nitric oxide (NO), prostaglandin E_2 (PGE_2), tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6.[13] NF-κB is a ubiquitously expressed family of transcription factors controlling the expression of pro-inflammatory mediators involved in inflammation.[14]

In our efforts to search for natural components with anti-allergy property, two new compounds (**1** and **8**) with twenty-three known compounds (**2**-**7**, **9**-**25**) were isolated from *S. officinalis* (Fig. 1). Herein, we describe the isolation and structural determination of two new compounds, and twenty-three known compounds. Afterwards, we investigated the anti-allergic inflammatory effects of all isolated compounds.

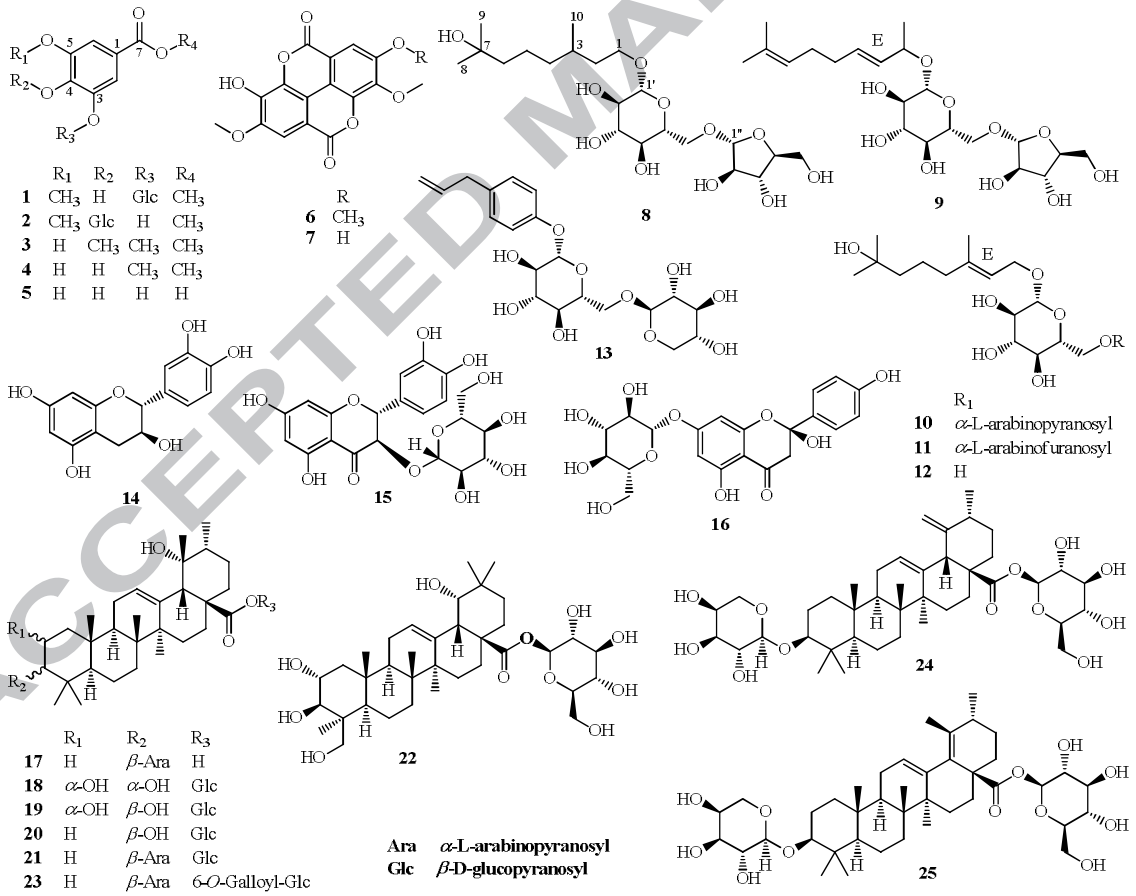


Figure 1. Structures of compounds **1**–**25** from *S. officinalis*.

Compound **1**[15] was isolated as white needles. The molecular formula was established as $C_{15}H_{20}O_{10}$ by a quasi-molecular ion peak $[M+Na]^+$ at m/z 383.0962 (calcd. 383.0949) in the HR-ESI-MS spectrum. The IR spectrum showed characteristic absorption bands of hydroxyl groups (3318 cm^{-1}), a carbonyl group (1696 cm^{-1}), an aromatic moiety (1603 cm^{-1}, 1514 cm^{-1}, 1408 cm^{-1}) and an ether group (1230 cm^{-1}). The ^{1}H-NMR spectrum of compound **1** (Table 1) revealed signals of two aromatic protons at δ_H 7.45 (d, J = 1.86 Hz, H-2) and 7.24 (d, J = 1.86 Hz, H-6), two methyl groups at δ_H 3.81 (s, 5-OMe) and 3.78 (s, 7-OMe), and one β-glucopyranosyl moiety from an anomeric proton at δ_H 4.73 (d, J = 7.26 Hz, H-1'). The ^{13}C-NMR spectrum of compound **1** (Table 1) showed fifteen carbon signals, which indicated the presence of one sugar unit along with nine carbon signals for an aglycone moiety. By further interpretation of HSQC spectra, nine signals were assigned to two methoxy signals at δ_C 56.0 (5-OMe) and 52.0 (7-OMe), two methine signals at δ_C 111.6 (C-2) and 107.8 (C-6), and five quaternary carbons at δ_C 166.0 (C-7), 147.9 (C-5), 145.3 (C-3), 141.5 (C-4) and 119.5 (C-1), while signals of the sugar unit were assigned at δ_C 102.7 (C-1'), 73.4 (C-2'), 75.9 (C-3'), 69.7 (C-4'), 77.3 (C-5'), 60.6 (C-6'). Additionally, HMBC correlations (Fig. 2) of δ_H 7.45 (H-2) and 7.24 (H-6) to δ_C 166.0 (C-7) indicated a carbonyl group attached to C-1. The correlations of δ_H 7.24 (H-6) and 3.81 (5-OMe) to δ_C 147.9 (C-5) confirmed the linkage position of a methoxy group to C-5. The key correlation from δ_H 4.73 (H-1') to δ_C 145.3 (C-3) indicated that glucose unit was linked at C-3 position. After acid hydrolysis of compound **1**, the sugar unit was determined to be D-glucose by GC analysis. The larger coupling constant (7.26 Hz) of the anomeric proton indicated the β-configuration of the glucosyl moiety. Thus, the structure of compound **1** was determined to be methyl 3-(β-D-glucopyranosyloxy)-4-hydroxy-5-methoxybenzoate.

Compound **8**[15] was isolated as a yellowish viscous liquid with its molecular formula to be determined as $C_{21}H_{40}O_{11}$ based on an HR-ESI-MS peak at *m/z* 491.2459 $[M + Na]^+$ (calcd

for 491.2463). The IR spectrum showed typical absorption bands for hydroxyl groups (3368 cm^{-1}), and a distinctive absorption band for an ether group (1039 cm^{-1}). Acid hydrolysis of compound **8** gave D-glucose and L-arabinose. The ^{1}H and ^{13}C NMR spectrum of compound **8** (Table 1) showed signals of a monoterpene moiety, which contained three methyl groups at δ_H 1.17 (s, H-8 and H-9) and 0.92 (d, J = 6.60 Hz, H-10); δ_C 29.4 (C-8), 29.3 (C-9) and 20.2 (C-10), four methylene groups at δ_H 1.68 (m, H-2a) and 1.43 (m, H-2b), 1.33 (m, H-4a) and 1.13 (m, H-4b), 1.42 (m, H-5a) and 1.31 (m, H-5b), 1.44 (m, H-6a) and 1.41 (m, H-6b); δ_C 38.0 (C-2), 38.9 (C-4), 22.8 (C-5), and 45.1 (C-6); a methine group at δ_H 1.62 (m, H-3); δ_C 30.9 (C-3), an oxygenated methylene at δ_H 3.55 (dt, J = 9.42, 7.14 Hz, H-1a) and 3.94 (m, H-1b); δ_C 69.5 (C-1) and an oxygenated quatenary carbon at δ_C 71.6 (C-7). The ^{1}H-^{1}H COSY spectrum (Fig. 2), correlations between the oxygenated methylene group (H-1) and methylene group (H-2), together with the spin system from H-2 through a methine group H-3 to H-6 and continuing to H-10, established the segment of $–OCH_2$(1)-CH_2-CH(3)-[CH_3(10)]-CH_2(4)-CH_2(5)-CH_2(6). Furthermore, the HMBC correlations (Fig. 2) of δ_H 0.92 (H-10) to δ_C 38.0 (C-2) and 38.9 (C-4) indicated a methyl group was located at C-3, while the correlations between δ_H 1.17 (H-8/H-9) to δ_C 71.6 (C-7) and 45.1 (C-6) implied that two methyl groups were attached to C-7. Based on both 1D-NMR and 2D-NMR data, the monoterpene moiety was identified as hydroxycitronellol.[31] The coupling constant of the anomeric proton at δ_H 4.25 (d, J = 7.86 Hz, H-1') indicated the β-configuration of glucosyl moiety. The HMBC correlations of δ_H 4.25 (H-1') to δ_C 69.5 (C-1) and δ_H 3.55 (H-1a) and 3.94 (H-1b) to δ_C 104.6 (C-1') confirmed the glucose was connected to C-1 of the aglycone. Moreover, the HMBC correlations of δ_H 4.02 (H-6'a) and 3.61 (H-6'b) to δ_C 110.0 (C-1''), and δ_H 4.96 (H-1'') to δ_C 68.2 (C-6') implied the arabinose was linked to C-6' of the glucose, which was further confirmed by the downfield shift by about 5 ppm of the signal of δ_C 68.2 (C-6'). The coupling constant of the anomeric proton at δ_H 4.96 (d, J = 1.26 Hz, H-1''), as well as signal at δ_C 86.0

(C-4'') and 83.2 (C-2'') indicated that arabinose moiety was an α-arabinofuranose structure.[31] Therefore, the structure of compound **8** was elucidated as 7-hydroxy-3,7-dimethyloctyl-6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside.

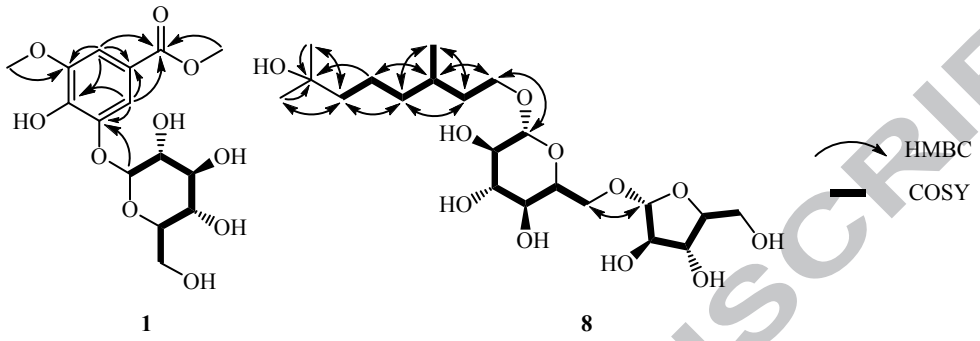
Table 1. The ^{1}H and ^{13}C NMR spectroscopic data of compounds **1** and **8**

Position	**1**[a]		Position	**8**[b]	
	δ_C[d]	δ_H[c] (*J* in Hz)		δ_C[d]	δ_H[c] (*J* in Hz)
1	119.5		1	69.5	3.55 dt (9.42, 7.14)
					3.94 m[e]
2	111.6	7.45 d (1.86)	2	38.0	1.68 m[e], 1.43 m[e]
3	145.3		3	30.9	1.62 m[e]
4	141.5		4	38.9	1.33 m[e], 1.13 m[e]
5	147.9		5	22.8	1.42 m[e], 1.31 m[e]
6	107.8	7.24 d (1.86)	6	45.1	1.44 m[e], 1.41 m[e]
7	166.0		7	71.6	
5-OMe	56.0	3.81 s	8	29.4	1.17 s
7-OMe	52.0	3.78 s	9	29.3	1.17 s
1'	102.7	4.73 d (7.26)	10	20.2	0.92 d (6.60)
2'	73.4	3.31 m[e]	1'	104.6	4.25 d (7.86)
3'	75.9	3.30 m[e]	2'	75.2	3.16 dd (9.12, 7.86)
4'	69.7	3.20 m[e]	3'	78.1	3.35 t (9.12)
5'	77.3	3.29 m[e]	4'	72.0	3.27 t (9.12)
6'	60.6	3.69 d (10.32), 3.51 m[e]	5'	76.7	3.44 m[e]
			6'	68.2	4.02 dd (11.22, 2.40)
					3.61 dd (11.22, 6.00)
			1''	110.0	4.96 d (1.26)
			2''	83.2	4.00 dd (3.24, 1.26)
			3''	79.0	3.82 dd (5.88, 3.24)
			4''	86.0	3.97 dt (5.88, 3.48)
			5''	63.2	3.73 dd (11.88, 3.48)

	3.63 dd (11.88, 3.48)

Assignments were done by HMQC and HMBC experiments; *J* values (Hz) are in parentheses.

[a]Measured in DMSO-d_6, [b]Measured in methanol-d_4, [c]600 MHz, [d]150 MHz, [e]overlapped



1 **8**

Figure 2. The Key ^{1}H-^{1}H COSY and HMBC correlations of compounds **1** and **8**.

Together with the two new compounds (**1** and **8**), twenty-three other compounds were identified as methyl 4-(β-D-glucopyranosyloxy)-3-hydroxy-5-methoxybenzoate (**2**)[16], methyl 3,4-*O*-dimethylgallate (**3**)[17], methyl 3-methoxy-4,5-dihydroxybenzoate (**4**)[17], gallic acid (**5**)[18], 3,3',4-tri-*O*-methylellagic acid (**6**)[19], 3,4'-di-*O*-methylellagic acid (**7**) [19], geranyl 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside (**9**)[20], rhodioloside E (**10**)[21], (2*E*)-7-hydroxy-3,7-dimethyl-2-octenyl 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside (**11**)[22], (2*E*)-7-hydroxy-3,7-dimethyl-2-octenyl-β-D-glucopyranoside (**12**)[23], miyaginin (**13**)[24], (+)-catechin (**14**)[18], taxifolin 3-*O*-β-D-glucopyranoside (**15**)[25], (2*S*)-hydroxynaringenin-7-*O*-β-D-glucopyranoside (**16**)[26], ziyuglycoside II (**17**)[19], kaji-ichigoside F_1 (**18**)[27], rosamultin (**19**)[27], 28-*O*-β-D-glucopyranosyl pomolic acid (**20**)[19], ziyuglycoside I (**21**)[19], sericoside (**22**)[28], 3β-[(α-L-arabinopyranosyl)oxy]-19α-hydroxyurs-12-en-28-oic acid 28-(6-*O*-galloyl-β-D-glucopyranosyl) ester (**23**)[29], 3β-[(α-L-arabinopyranosyl)oxy]urs-12,19(29)-dien-28-oic acid β-D-glucopyranosyl ester (**24**)[29], 3β-[(α-L-arabinopyranosyl)oxy]urs-12,18-dien-28-oic acid

β-D-glucopyranosyl ester (**25**)[30]. Their structures were elucidated by spectroscopic data, chemical evidence, and comparisons with previous reports (Fig. 1).

To study the anti-allergic inflammatory activity, the effects of isolated compounds on cell viability were evaluated by MTS to ensure that the decreased level of mast cell granules and IL-4 production in RBL-2H3 cells was not due to the cell death in each condition. Treatment with test samples for 24 h at the concentrations of 20 μM and 5 μM produced no significant cytotoxic effects except compound **6**, which showed the significant cytotoxic to RBL-2H3 cells as compared with the control (Fig. 3A). Test samples also showed no cellular toxicity against RAW264.7 cells except compound **6** which showed little toxicity to cells (Fig. 3B).

Fig. 3A

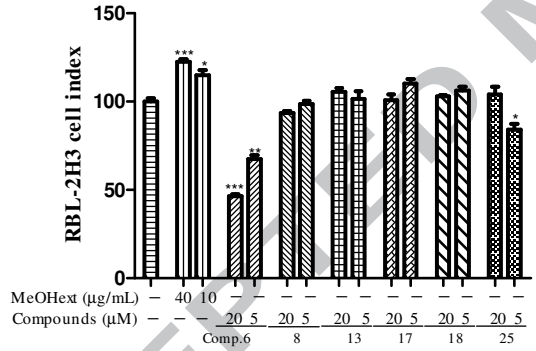


Fig. 3B

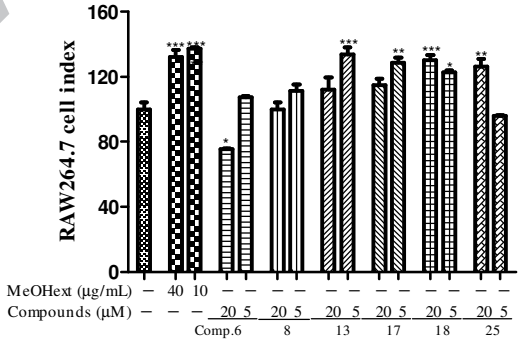


Figure 3. Effect on the viability of RBL-2H3 cells and RAW264.7 cells; (A) RBL-2H3 cells seeded into 96-well plate overnight were treated with samples for 24 h, and the cell viability was tested by MTS assays. (B) RAW264.7 cells were treated with samples for 24 h, and cell viability was assayed by MTS assays. The data are representative of three independent experiments and expressed as mean ± SEM (*P < 0.05; **P < 0.01; ***P < 0.001 versus with control group).

Mast cells contain a large amount of cytoplasmic granules, which activation caused the

process of degranulation.[32] The enzyme β-hexosaminidase is stored in the secretory granules of mast cells and is released along with chemical mediators when mast cells are stimulated. The release of β-hexosaminidase can be used to quantify the extent of degranulation.[33] Twenty-five isolated compounds were tested on the β-hexosaminidase assay to determine whether these compounds regulate the degranulation of mast cells. Results showed that methanol extract and five compounds (**8**, **13**, **17**-**18** and **25**) significantly reduced the β-hexosaminidase level released from the PMA/A23187-stimulated RBL-2H3 cells (Fig. 4).

Fig. 4

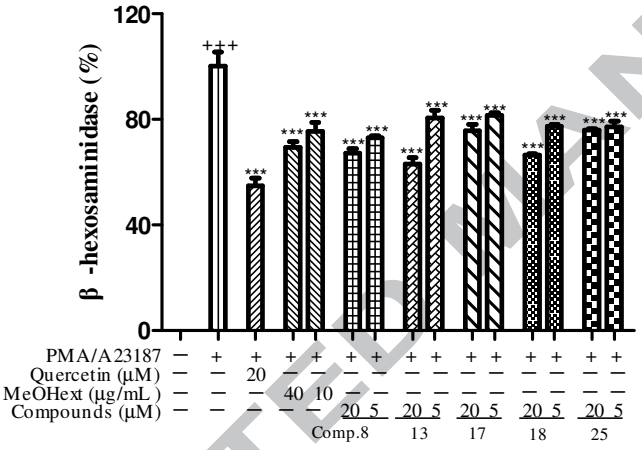


Figure 4. Effect on β-hexosaminidase secretion in RBL-2H3 cells; RBL-2H3 cells incubated into 48-well plate overnight were pretreated with samples for 1 h prior to stimulation with 30 ng/mL of PMA plus 350 ng/mL of A23187 for 1 h. The supernatants were collected for the detection of β-hexosaminidase. Experiments were conducted in triplicate and expressed as mean ± SEM (***$P < 0.001$ versus with PMA/A23187 group).

Modulation of inflammatory cytokines from mast cells is one of the key indicators of reduced allergic symptoms.[34] We examined whether five compounds (**8**, **13**, **17**-**18** and **25**) could regulate pro-inflammatory cytokines IL-4 in RBL-2H3 cells. Treatment with samples

dose-dependently blocked IL-4 secretion induced by DNP-BSA or PMA/A23187 in RBL-2H3 cells (Fig. 5A and 5B).

Fig. 5A

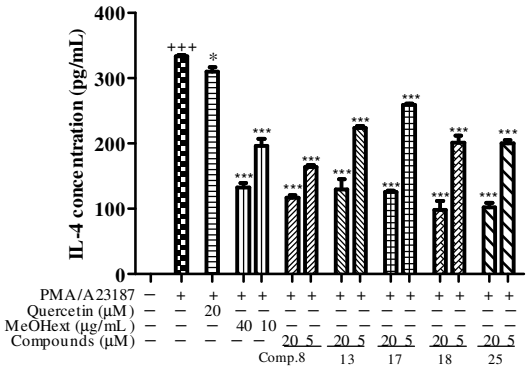


Fig. 5B

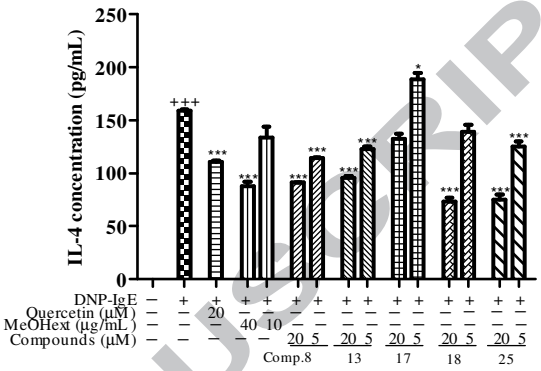


Figure 5. Effect on IL-4 production in RBL-2H3 cells; (A) RBL-2H3 cells incubated into 48-well plate overnight were pretreated with samples for 2 h prior to stimulation with 30 ng/mL of PMA plus 350 ng/mL of A23187 for 18 h. The supernatants were collected and IL-4 cytokine was tested by using ELISA kit. Experiments were conducted in triplicate and expressed as mean ± SEM (***$P < 0.001$ versus with PMA/A23187 group). (B) RBL-2H3 cells were sensitized with IgE overnight at 37 °C, followed by pretreatment with drug for 2 h before stimulation with 1 μg/mL DNP-BSA for 18 h. The supernatants were analyzed for IL-4 production (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ versus with DNP-BSA group).

NF-κB is a transcription factor related to inflammation. The effects on NF-κB activation was tested in 293T cells. PMA increased NF-κB transcription, which was decreased by methanol extract, and compounds (**8**, **17** and **25**) pretreatment in a dose-dependent manner. Methanol extract (40 μg/mL) and compounds **8**, **17** and **25** (20 μM) showed the significant reduction on the activation of NF-κB transcription (Fig. 6A). Additionally, RAW264.7 cells were pretreated with samples or Bay11-7082 (20 μM) for 2 h prior to addition of LPS (1 μg/mL). The effect of methanol extract, and compounds (**8**, **13**, **17**-**18** and **25**) on LPS-

induced NF-κB pathway was tested. LPS caused the translocation of NF-κB from the cytosol to the nucleus, which was inhibited by the methanol extract, and compounds (**8**, **17** and **25**) (Fig. 6B).

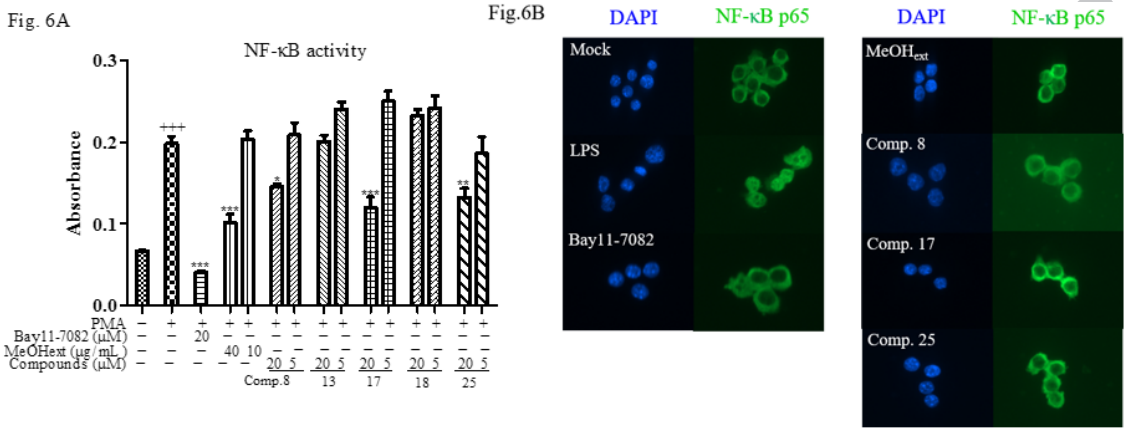


Figure 6. Effect on NF-κB transcription and translocation; (A) Effect on PMA-induced NF-κB activation in 293T cells. The 293T cells were transfected with a reporter plasmid, pNF-κB-SEAP. Experiments were conducted in triplicate and expressed as mean ± SEM (***P* < 0.01; ****P* < 0.001 versus with PMA group). (B) Effect on NF-κB translocation into nucleus. RAW264.7 cells in eight-well glass chamber plate were pre-incubated with samples or Bay 11-7082 for 2 h prior to addition of LPS (1 μg/mL). The cells were immunostained with a polyclonal anti- NF-κB p65 antibody and Alexa Fluor 488-conjugated 2nd antibody. Bay 11-7082 was used as a control for the NF-κB inhibitor.

RAW264.7 cells were pretreated with samples for 2 h prior to addition of LPS (500 ng/mL). TNF-α cytokine was assayed using ELISA kit. Methanol extract (40 μg/mL) and compounds **8**, **13**, **17**-**18** and **25** (20 μM) decreased the production of TNF-α in LPS-activated RAW264.7 cells, especially compounds **8** and **17** significantly decreased the production of TNF-α (Fig. 7A). NO production in LPS-activated RAW264.7 cells was also decreased by the treatment of samples, especially the methanol extract (40 μg/mL) and compounds **8**, **13**, **17**

and **25** (20 μM) showed the significant decrease on NO production (Fig. 7B).

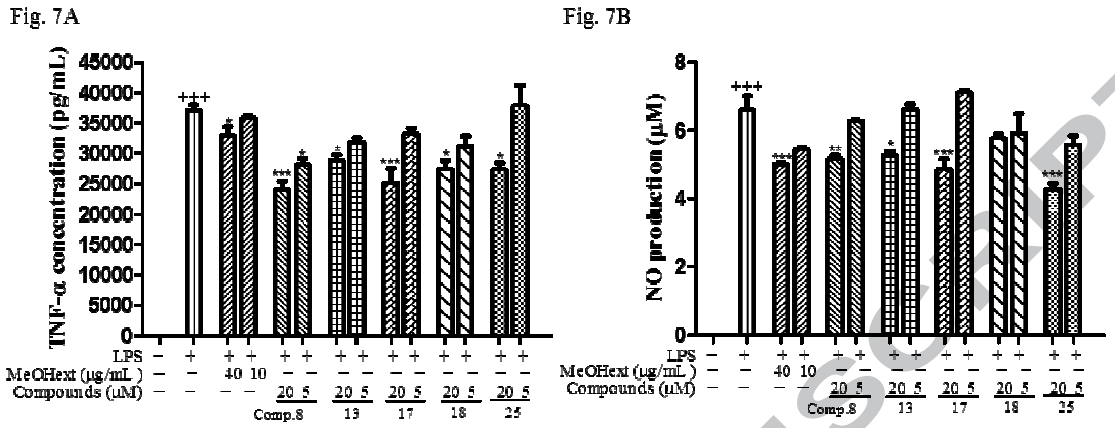


Figure 7. Effect on the production of inflammatory mediators in LPS-activated RAW264.7 cells; RAW264.7 cells were pretreated with samples for 2 h prior to addition of LPS for 24 h. TNF-α in the supernatants was measured by ELISA (A) and NO in the supernatants was detected by Griess reagent (B). Experiments were conducted in triplicate and expressed as mean ± SEM (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ versus with LPS group).

Pro-inflammatory enzymes, such as iNOS and COX-2, cause inflammation. Thus, we also examined whether isolated compounds could affect iNOS and COX-2 protein expressions by Western blot analysis. RAW264.7 cells were pretreated with test samples or celecoxib (20 μM) for 2 h prior to addition of LPS (200 ng/mL). Stimulation of the RAW264.7 cells with LPS resulted in accumulation of iNOS and COX-2 proteins, as determined by Western blot analysis. Compounds **8**, **13** and **25** significantly reduced the levels of iNOS protein at the concentration of 20 μM. Celecoxib (20 μM), methanol extract (40 μg/mL), compounds **8**, **13** and **25** (20 μM) reduced the levels of COX-2 protein expression in LPS-activated RAW264.7 cells (Fig. 8).

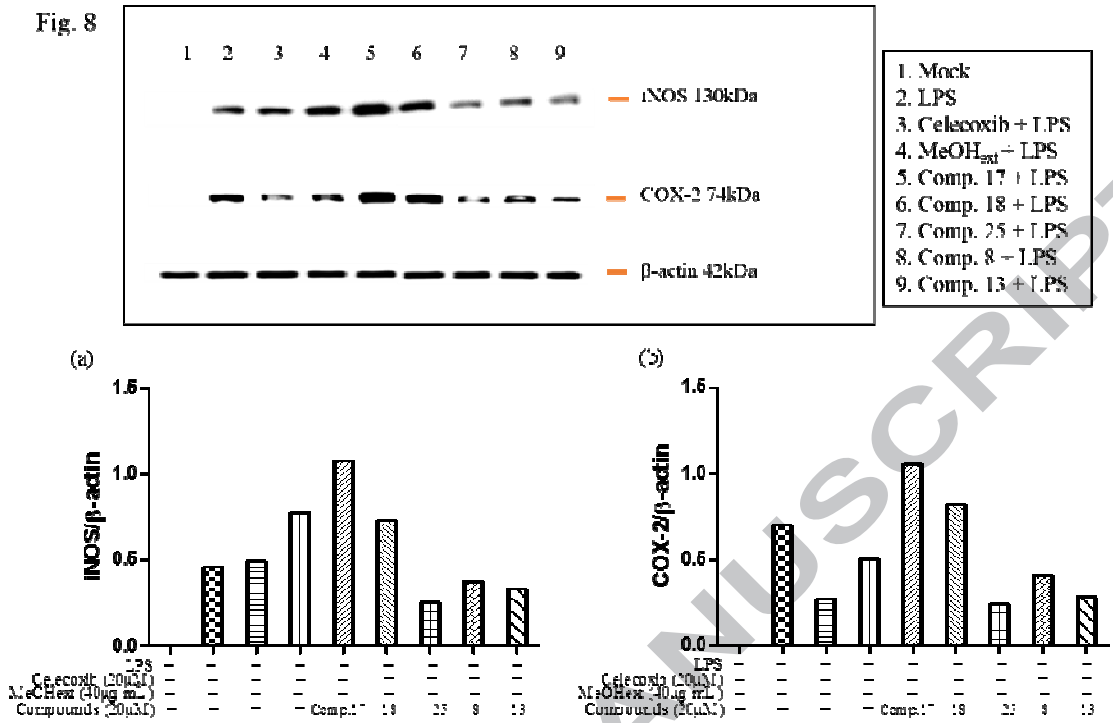


Figure 8. Effect on the expression of COX-2 and iNOS in LPS-activated RAW264.7 cells; RAW264.7 cells were pretreated with samples for 2 h, and then LPS was added to the cells for 18 h. The protein levels of iNOS and COX-2 were detected by Western blot analysis. Celecoxib was used as a control for the COX-2 inhibitor, β-actin was used as the control protein.

Modern research has shown that *Sanguisorba* plants exhibit a wide range of pharmacological activities, such as hemostatic, anti-inflammatory, anti-allergy, antibacterial, anti-oxidant, hypoglycemic, neuroprotective and anticancer effects.[35] The present study showed that methanol extract and single compounds **6**, **8**, **13**, **17**-**18** and **25** isolated from *S. officinalis* profoundly affected PMA plus A23187-induced systemic allergic reaction and anti-DNP IgE-induced local allergic reaction. Methanol extract and single compounds **6**, **8**, **13**, **17**-**18** and **25** inhibited PMA plus A23187-mediated β-hexosaminidase release and PMA plus A23187 or anti-DNP IgE-mediated cytokine IL-4 production. But Compound **6** showed the

cytotoxicity to RBL-2H3 cells after 24 h treatment, that's why compound **6** looked like having the effects on the reduction of β-hexosaminidase release and cytokine IL-4 production. During the pathogenesis of allergic disease, IL-4 is crucial for the induction of IgE synthesis and mast cell development.[12] Therefore, we simply speculate that anaphylactic degranulation of mast cells is suppressed by methanol extract and single compounds **8**, **13**, **17**-**18** and **25**.

NF-κB participates in regulating the expression of cytokines that are involved in the inflammatory response.[36] Inhibition of this signaling pathway may explain the potent activity of isolated compounds as suppressors of inflammatory cytokines. In this study, methanol extract and single compounds **8**, **17** and **25** significantly suppressed the activation of NF-κB P65 and NF-κB translocation into the nucleus (Fig. 6), indicating that NF-κB pathway pla yed a significant role in the anti-inflammatory effects of isolated compounds.

Macrophages play an important role in the specific and non-specific immune responses during the inflammation process. After macrophages are activated by LPS, large amounts of the inflammatory mediators will be released.[37] Therefore, LPS induced macrophages have usually been used for assessing the anti-inflammatory effects of various agents. We investigated the effects of isolated compounds on the inflammatory mediators of LPS-stimulated RAW264.7 macrophage cells. We found that pretreatment with tested compounds significantly inhibited the production of NO and TNF-α induced by LPS (Fig. 7). NO is a free radical produced from L-arginine by nitric oxide synthases (NOS), the high level of NO might cause inflammatory damage to a target tissue during an infection.[38-40] Therefore, the regulation of NO release via inhibiting iNOS is helpful to alleviate the inflammatory destruction. COX-2 is an inducible isoform of cyclooxygenase, and mainly exerts its important role in the inflammation.[41, 42] We also found that compounds **8**, **13** and **25** significantly decreased iNOS and COX-2 expression levels in RAW264.7 cells induced by

LPS (Fig. 8).

In summary, our findings demonstrated that *S. officinalis* especially compounds **8**, **13** and **25** are potential therapeutic candidates for anti-allergic inflammatory drugs.

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Supplementary material

^{1}H, ^{13}C NMR, HMQC, HMBC, COSY, and HR-ESI-MS spectra of compounds **1** and **8**; General experimental procedures, Bio-assay are available as Supplementary material.

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15. Physical and spectroscopic data of new compounds: Methyl 3-(β-D-glucopyranosyloxy)-4-hydroxy-5-methoxybenzoate (**1**): White needles; $[\alpha]_D^{25}$ –40.6° (c = 0.1, MeOH); IR (KBr): νmax 3318, 1696, 1603, 1514, 1408, 1230 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR data (DMSO-d_6, 150 MHz), see Table 1; HR-ESI-MS: m/z 383.0962 $[M+Na]^+$ (calcd. for 383.0949, $C_{15}H_{20}O_{10}Na$). 7-Hydroxy-3,7-dimethyloctyl-6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside (**8**): Yellowish viscous liquid; $[\alpha]_D^{25}$ –79.0° (c = 0.1, MeOH); IR (KBr): νmax 3368, 1039 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR data (CD_3OD, 150 MHz), see Table 1; HR-ESI-MS: m/z 491.2459 $[M+Na]^+$ (calcd. for 491.2463, $C_{21}H_{40}O_{11}Na$).

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Highlights:

- Two new compounds and twenty-three known compounds were isolated and identified from *Sanguisorba officinalis* L.
- Anti-allergic inflammatory activities of these 25 isolates were evaluated, and potent activities were found.
    - These results showed that *S. officinalis* may have a therapeutic potential for allergic inflammatory diseases.