**Pharmacogn.** Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

# Quantitative Analysis and *In vitro* Anti-inflammatory Effects of Gallic Acid, Ellagic Acid, and Quercetin from Radix Sanguisorbae

**Chang-Seob Seo, Soo-Jin Jeong[1], Sae-Rom Yoo, Na-Ri Lee, Hyeun-Kyoo Shin**

K-Herb Research Center, Korea Institute of Oriental Medicine, [1]KM Convergence Research Division, Korea Institute of Oriental Medicine, 1672 Yuseong-daero, Yuseong-gu, Daejeon 34054, Republic of Korea

Submitted: 13-08-2015 Revised: 29-09-2015 Published: 02-03-2016

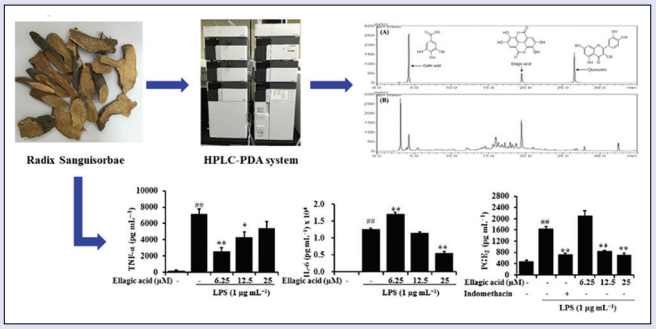
## ABSTRACT

**Background:** Radix Sanguisorbae has long been used to treat diarrhea, enteritis, duodenal ulcers, and internal hemorrhage. **Objective:** We investigated the *in vitro* anti-inflammatory effects of Radix Sanguisorbae and performed quantitative analyses of three marker components, namely gallic acid, ellagic acid, and quercetin, using high-performance liquid chromatography coupled with a photodiode array detector. **Materials and Methods:** The three marker components were separated using a reversed-phase Gemini $C_{18}$ analytical column maintained at 40°C by the gradient elution with two solvent systems. We examined the biological effects of the three marker compounds, gallic acid, ellagic acid, and quercetin, by determining their anti-inflammatory activities in the murine macrophage cell line RAW 264.7. **Results:** All of the marker compounds exhibited inhibitory effects on prostaglandin $E_2$ production in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages, with no cytotoxicity. Particularly, ellagic acid significantly inhibited production of the proinflammatory cytokines tumor necrosis factor alpha and interleukin-6 in LPS-treated RAW 264.7 cells. **Conclusion:** Our results suggest that ellagic acid is the most potent bioactive phytochemical component of radix Sanguisorbae in the treatment of inflammatory diseases.

**Key words:** Anti-inflammation, ellagic acid, quantitative analysis, radix Sanguisorbae

## SUMMARY

- Established high-performance liquid chromatography method was applied in the quantitative analysis of gallic acid, ellagic acid, and quercetin present in an extract from radix Sanguisorbae
- Among the three compounds, the ellagic acid (7.65 mg/g) is main component in radix Sanguisorbae
- Ellagic acid significantly inhibited production of the proinflammatory cytokines tumor necrosis factor alpha and interleukin-6 in lipopolysaccharide-treated RAW 264.7 cells.




**Abbreviations used:** HPLC: High-performance liquid chromatography, PDA: Photodiode array, TNF-α: Tumor necrosis factor alpha, IL: Interleukin, LPS: Lipopolysaccharide, $PGE_2$: Prostaglandin $E_2$, NSAIDs: Nonsteroidal anti-inflammatory drugs, COX: Cyclooxygenase.

**Correspondence:**

Dr. Hyeun-Kyoo Shin,
K-herb Research Center, Korea Institute of Oriental Medicine, 1672 Yuseong-daero, Yuseong-gu, Daejeon 34054, Republic of Korea.
E-mail: hkshin@kiom.re.kr

**DOI :** 10.4103/0973-1296.177908

**Access this article online**
**Website:** www.phcog.com
**Quick Response Code:**

## INTRODUCTION

Radix Sanguisorbae is derived from the root of *Sanguisorba officinalis* L. (*Rosaceae*) which is distributed widely in Korea, Japan, and China.[1] In these countries, it has long been used to treat diarrhea, enteritis, duodenal ulcers, and internal hemorrhage.[2-4] Phytochemical studies of *Sanguisorba* species have identified phenolic acids (e.g., gallic acid and ellagic acid), flavonoids (e.g., kaempferol and quercetin), triterpene glycosides (e.g., 3β, 19α-dihydroxyurs-12-en-28-oic acid), and dimeric triterpene glycosides (e.g., sanguidio sides A, B, C, and D).[4-8]

Inflammation is a self-protective response to various harmful stimuli such as damaged cells, pathogens, and irritants.[9] Radix Sanguisorbae is used to treat inflammatory diseases, and scientific evidence has been obtained to support its effects.[10] In addition, many studies have shown that gallic acid,[11,12] ellagic acid,[13,14] and quercetin[15,16] extracted from various herbal plants have anti-inflammatory effects. Based on previous research, we aimed to determine the bioactive chemical in radix Sanguisorbae that is responsible for its anti-inflammatory effect by testing the marker compounds gallic acid, ellagic acid, and quercetin in the murine macrophage cell line RAW 264.7. In addition, we performed quantitative analyses of two phenolic acids, gallic acid and ellagic acid, and one flavonoid, quercetin, to assess the quality of radix Sanguisorbae using high-performance liquid chromatography (HPLC) coupled with a photodiode array (PDA) detector.

## MATERIALS AND METHODS

### Plant material

Radix Sanguisorbae was purchased from HMAX (Jecheon, Korea) in October 2008. The botanical origin of this sample was taxonomically confirmed by Prof. Je-Hyun Lee, Dongguk University, Gyeongju, Republic of Korea. A voucher specimen (2008-ST-24) has been deposited

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

**For reprints contact:** reprints@medknow.com

**Cite this article as:** Seo CS, Jeong SJ, Yoo SR, Lee NR, Shin HK. Quantitative analysis and *In vitro* anti-inflammatory effects of gallic acid, ellagic acid, and quercetin from radix sanguisorbae. Phcog Mag 2016;12:104-8.


© 2016 Pharmacognosy Magazine | Published by Wolters Kluwer - Medknow

at the Herbal Medicine Formulation Research Group, Korea Institute of Oriental Medicine.

## Reagents and materials

Gallic acid (purity ≥97.5%), ellagic acid (purity ≥96.0%), and quercetin (purity ≥98.0%) were purchased from Sigma-Aldrich Co., (St Louis, MO, USA). HPLC-grade methanol, acetonitrile, and water were obtained from J.T. Baker (Phillipsburg, NJ, USA). Analytical reagent grade glacial acetic acid was procured from Merck (Darmstadt, Germany).

## Preparation of 70% ethanol extract

Dried radix Sanguisorbae (200 g) was extracted 3 times with 70% (v/v) ethanol (2.0 L) by sonication for 60 min. The extracted solution was filtered through filter paper, evaporated to dryness at 40°C using a Büchi R-210 rotary evaporator (Flawil, Switzerland) under vacuum, and then freeze-dried. The yield of the freeze-dried 70% ethanol extract was 12.15% (24.30 g).

## Quantitative analysis of the marker components in radix Sanguisorbae

The quantitative analysis was performed with a Prominence LC-20A series HPLC system (Shimadzu, Kyoto, Japan) which comprised a solvent delivery unit (LC-20 AT), online degasser (DGU-20$A_3$), column oven (CTO-20A), autosampler (SIL-20 AC), and PDA detector (SPD-M20A). Data processing was performed using LC Solution (version 1.24; Shimadzu, Kyoto, Japan). The analytical column used for separating the three marker compounds was a Gemini $C_{18}$ column (250 mm × 4.6 mm; particle size, 5 μm; Phenomenex, Torrance, CA, USA), which was maintained at 40°C. The mobile phases for chromatographic separation employed gradient elution with 1.0% v/v acetic acid in water (eluent A) and 1.0% v/v acetic acid in acetonitrile (solvent B). The gradient flow in the two-solvent system was as follows: 10–10% B (5 min), 10–50% B (30 min), 50% B (35 min), and 50–10% B (40 min). The analysis was performed at a flow rate of 1.0 mL/min using detection wavelengths of 254 nm for ellagic acid, 270 nm for gallic acid, and 370 nm for quercetin. The injection volume was 10 μL.

## Cell culture

The murine macrophage cell line RAW 264.7 was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were cultured in Dulbecco's modified Eagle's medium (Gibco Inc., Grand Island, NY, USA) which was supplemented with 5.5% heat-inactivated fetal bovine serum (Gibco Inc.,), penicillin (100 U/mL), and streptomycin (100 μg/mL) in a 5% $CO_2$ incubator at 37°C.

## Cell viability

Cell viability was assessed with the Cell Counting Kit-8 (CCK-8) assay (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. RAW 264.7 cells were incubated in 96-well plates with various concentrations of the test materials for 24 h. CCK-8 reagent was added to each well, before incubating for 4 h. The absorbance was measured at 450 nm with a Benchmark plus microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). The percentage of cell viability was calculated by the following formula: Cell viability (%) = (mean absorbance in test wells/mean absorbance in control wells) × 100.

## Measurement of tumor necrosis factor alpha, interleukin-6, and prostaglandin $E_2$ production

RAW 264.7 cells were treated with various concentrations of marker compounds from radix Sanguisorbae for 4 h before lipopolysaccharide (LPS) (1 μg/mL) stimulation. After incubation for 20 h, the supernatants were analyzed to determine the levels of tumor necrosis factor alpha (TNF-α) (BD Biosciences, Mountain View, CA, USA), interleukin-6 (IL-6) (BD Biosciences, Mountain View, CA, USA), and prostaglandin $E_2$ ($PGE_2$) (Cayman Chemical Co., Ann Arbor, MI, USA), according to the manufacturers' instructions.

## Statistical analysis

All of the values were expressed as the mean ± standard error mean of three independent samples of each compound from radix Sanguisorbae. One-way analysis of variance was used to identify significant differences between the treatment groups. Dunnett's test was used for multiple group comparisons. Differences were considered significant at $P < 0.05$.

# RESULTS AND DISCUSSION

We obtained good separation chromatograms using two solvent systems: 1.0% (v/v) acetic acid in distilled water (A) and 1.0% (v/v) acetic acid in acetonitrile (B), where we employed gradient elution. Figure 1a and b show typical HPLC chromatograms of the standards and the extract. Each compound in the HPLC chromatogram was identified by comparing the retention times and UV spectra with those of reference standards [Figure 1c]. The retention times of gallic acid, ellagic acid, and quercetin under the optimized conditions were 4.26, 19.42, and 26.52 min, respectively. The calibration curves of the three marker components exhibited good linearity with coefficients of determination ≥0.9997 in the seven different concentration ranges that we tested. Thus, the established HPLC analysis method was applied in the quantitative analysis of three compounds present in an extract from radix Sanguisorbae. The concentrations of gallic acid, ellagic acid, and quercetin were 2.37, 7.65, and 0.07 mg/g, respectively.

Before determining anti-inflammatory effects of the marker compounds, we examined the cytotoxic effects of radix Sanguisorbae in RAW 264.7 macrophages. Cells were treated with various concentrations of the marker compounds for 24 h and subjected to an assay using CCK-8. Gallic acid produced no cytotoxic effects at up to 10 μM in RAW 264.7 cells [Figure 2a]. Ellagic acid had no cytotoxic effects up to 5 μM, but it decreased the cell viability by 85.44 ± 0.60% and 40.48 ± 2.48% at 10 and 20 μM, respectively [Figure 2b]. Quercetin increased the proliferation rate at up to 10 μM but reduced the cell viability at 20 μM. No cytotoxicity was observed in RAW 264.7 cells treated with quercetin at up to 20 μM [Figure 2c]. Thus, nontoxic concentrations of the three marker compounds were used in the subsequent experiments.

Various cytokines play key roles in mediating inflammatory responses.[17] Thus, we measured the production of the cytokines, and TNF-α and IL-6, which are related to humoral and cellular inflammation, respectively. An inflammatory reaction was induced by treating RAW 264.7 cells with LPS, which significantly increased TNF-α production. Gallic acid and quercetin had no significant effects on TNF-α production in LPS-stimulated RAW 264.7 cells [Figure 3a and c, respectively]. By contrast, ellagic acid significantly inhibited LPS-stimulated TNF-α production at 6.25 and 12.5 μM, but not at 25 μM [Figure 3b]. LPS also significantly enhanced IL-6 production in RAW 264.7 cells [Figure 4]. Ellagic acid had a significant inhibitory effect on LPS-induced IL-6 production at 25 μM, but not at lower concentrations of 6.25 and 12.5 μM [Figure 4b]. Quercetin significantly suppressed LPS-stimulated IL-6 production at concentrations of 6.25–25 μM [Figure 4c]. However, gallic acid did not affect IL-6 production in LPS-treated RAW 264.7 cells [Figure 4a].

We also examined whether gallic acid, ellagic acid, and quercetin from radix Sanguisorbae can regulate the proinflammatory mediator $PGE_2$, which is known to control multiple aspects of inflammation.[18] In this experiment, indomethacin was used as a positive control.[19] As expected, LPS markedly increased the level of $PGE_2$ in RAW 264.7 cells. By contrast, indomethacin significantly reduced LPS-induced $PGE_2$ production.

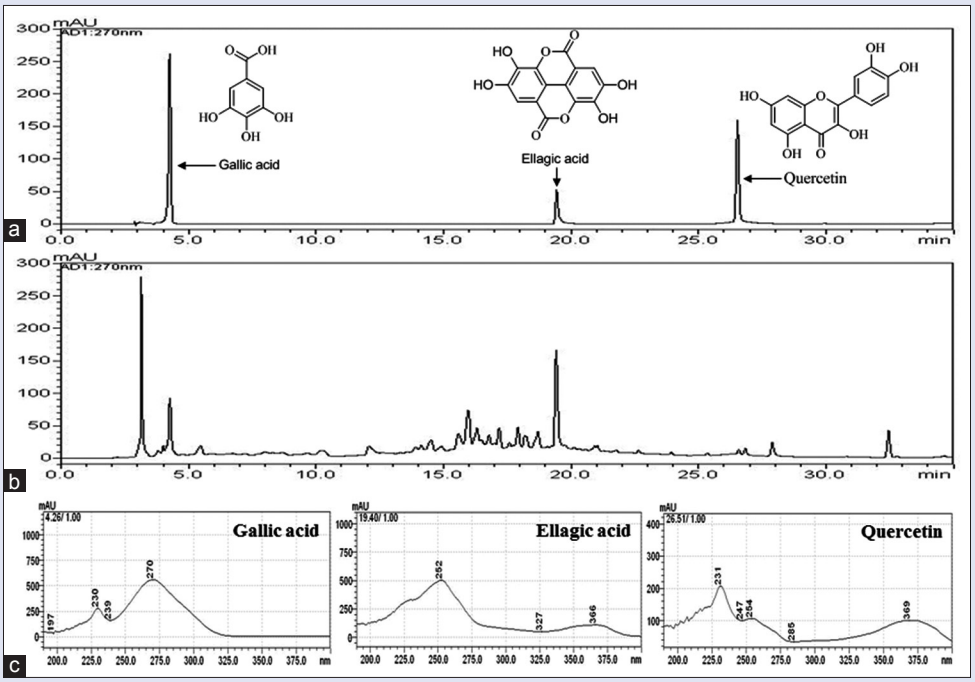



**Figure 1:** (a) Typical high-performance liquid chromatography chromatogram of the standard mixture, (b) 70% ethanol extract of radix Sanguisorbae, and (c) ultraviolet spectra of marker components

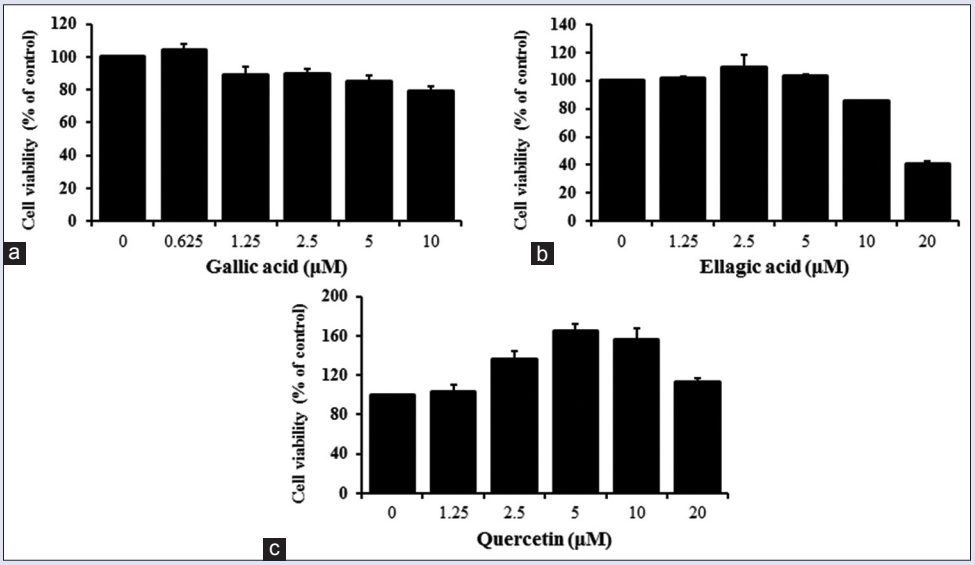



**Figure 2:** (a-c) Cytotoxicity of gallic acid, ellagic acid, and quercetin from radix Sanguisorbae in RAW 264.7 cells. Cells were seeded into 96-well plates and treated with various concentrations of gallic acid, ellagic acid, or quercetin for 24 h. Cell viability was assessed using a Cell Counting Kit-8 assay

Gallic acid, ellagic acid, and quercetin also significantly inhibited $PGE_2$ production in LPS-treated RAW 264.7 cells [Figure 5]. Overall, these results suggest that ellagic acid may be a major bioactive compound that is responsible for the anti-inflammatory effects of radix Sanguisorbae.

Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen, and naproxen, are generally used to treat inflammation. NSAIDs target cyclooxygenase (COX) which synthesizes prostaglandin. However, recent studies suggest that a multitarget approach should be considered in the development of anti-inflammatory drugs because inflammation is a complex disease.[20] Natural products that contain multiple components are attractive candidates as multitarget drugs. Indeed, many herbal plants and their bioactive compounds have been shown to produce anti-inflammatory effects by targeting various inflammatory molecules, including COX, nuclear factor kappa B, mitogen-activated protein kinases, proinflammatory cytokines, and mediators.[21-23] In addition, the low frequency of side effects and low toxicity of natural products would be helpful in addressing the safety problem associated with NSAIDs. Thus, further studies should also

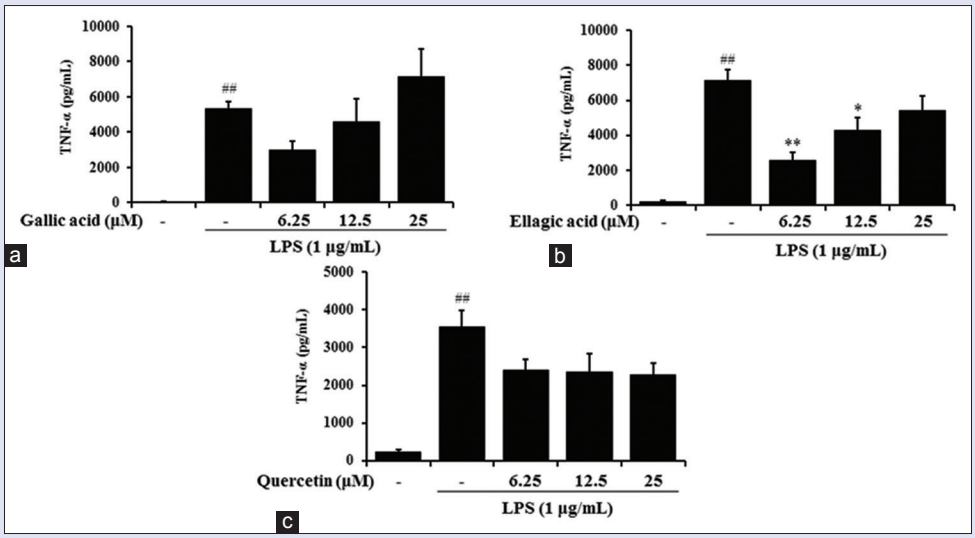



**Figure 3:** (a-c) Effect of gallic acid, ellagic acid, and quercetin from radix Sanguisorbae on lipopolysaccharide-stimulated TNF-α production in RAW 264.7 cells. TNF-α production was measured in the culture medium of cells that had been pretreated with various concentrations (6.25, 12.5, or 25 μM) of each compound for 4 h and then stimulated with lipopolysaccharide (1 μg/mL) for 20 h. Each bar represents the mean of three independent experiments. $^{\#\#}P < 0.01$ versus untreated control, and $^{*}P < 0.05$ and $^{**}P < 0.01$ versus lipopolysaccharide-treated cells

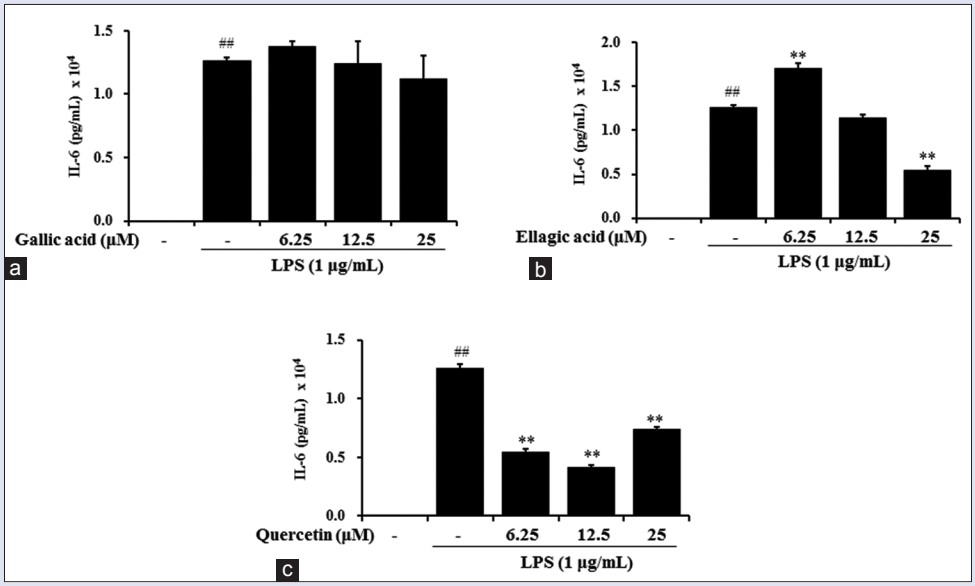



**Figure 4:** (a-c) Effects of gallic acid, ellagic acid, and quercetin from radix Sanguisorbae on lipopolysaccharide-stimulated IL-6 production in RAW 264.7 cells. IL-6 production was measured in the culture medium of cells that had been pretreated with various concentrations (6.25, 12.5, or 25 μM) of each compound for 4 h and then stimulated with lipopolysaccharide (1 μg/mL) for 20 h. Each bar represents the mean of three independent experiments. $^{\#\#}P < 0.01$ versus untreated control; and $^{**}P < 0.01$ versus lipopolysaccharide-treated cells

identify the multitargeting efficacy of the active anti-inflammatory compound ellagic acid from radix Sanguisorbae.

## CONCLUSION

We successfully established a rapid, accurate, and convenient HPLC–PDA method for the quantitative analysis of two phenols, gallic acid and ellagic acid, and one flavonoid, quercetin, in radix Sanguisorbae. Among these components, we found that ellagic acid was the most abundant, i.e., 7.65 mg/g. Our HPLC–PDA method may be helpful for the quantitative analysis of radix Sanguisorbae. Furthermore, our findings suggest the potential role of ellagic acid from radix Sanguisorbae as an anti-inflammatory drug.

### Financial support and sponsorship

This research was supported by a grant (No. K15251) from the Korea Institute of Oriental Medicine.

### Conflicts of interest

There are no conflicts of interest.

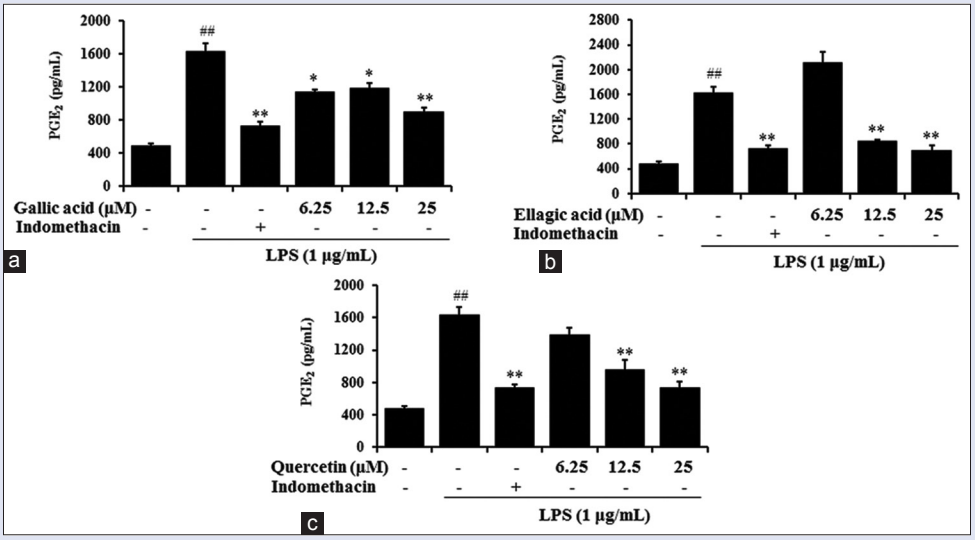



**Figure 5:** (a-c) Effects of gallic acid, ellagic acid, and quercetin from radix Sanguisorbae on lipopolysaccharide-stimulated prostaglandin $E_2$ production in RAW 264.7 cells. Prostaglandin $E_2$ production was measured in the culture medium of cells that had been pretreated with various concentrations (6.25, 12.5, or 25 μM) of each compound for 4 h and then stimulated with lipopolysaccharide (1 μg/mL) for 20 h. Indomethacin (1.25 ng/mL) was used as the positive control. Each bar represents the mean of three independent experiments. $^{\#\#}P < 0.01$ versus untreated control; and $^{*}P < 0.05$ and $^{**}P < 0.01$ versus lipopolysaccharide-treated cells

## REFERENCES


1. Bae KH. The Medicinal Plants of Korea. Seoul: Kyo-Hak Publishing Co. Ltd.; 2000. p. 233.
2. Cheng DL, Cao XP. Pomolic acid derivatives from the root of *Sanguisorba officinalis*. Phytochemistry1992;31:1317-20.
3. Zhang L, Koyyalamudi SR, Jeong SC, Reddy N, Smith PT, Ananthan R, *et al.* Antioxidant and immunomodulatory activities of polysaccharides from the roots of *Sanguisorba officinalis*. Int J Biol Macromol 2012;51:1057-62.
4. Nguyen TT, Cho SO, Ban JY, Kim JY, Ju HS, Koh SB, *et al.* Neuroprotective effect of Sanguisorbae radix against oxidative stress-induced brain damage: *In vitro* and *in vivo*. Biol Pharm Bull 2008;31:2028-35.
5. Ban JY, Nguyen HT, Lee HJ, Cho SO, Ju HS, Kim JY, *et al.* Neuroprotective properties of gallic acid from Sanguisorbae radix on amyloid beta protein (25-35)-induced toxicity in cultured rat cortical neurons. Biol Pharm Bull 2008;31:149-53.
6. Ayoub NA. Unique phenolic carboxylic acids from *Sanguisorba minor*. Phytochemistry 2003;63:433-6.
7. Mimaki Y, Fukushima M, Yokosuka A, Sashida Y, Furuya S, Sakagami H. Triterpene glycosides from the roots of *Sanguisorba officinalis*. Phytochemistry 2001;57:773-9.
8. Liu X, Shi B, Yu B. Four new dimeric triterpene glucosides form *Sanguisorba officinalis*. Tetrahedron 2004;60:11647-54.
9. Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. Chronic inflammation: Importance of NOD2 and NALP3 in interleukin-1 beta generation. Clin Exp Immunol 2007;147:227-35.
10. Lee NH, Lee MY, Lee JA, Jung DY, Seo CS, Kim JH, *et al.* Anti-asthmatic effect of *Sanguisorba officinalis* L. and potential role of heme oxygenase-1 in an ovalbumin-induced murine asthma model. Int J Mol Med 2010;26:201-8.
11. Liu KY, Hu S, Chan BC, Wat EC, Lau CB, Hon KL, *et al.* Anti-inflammatory and anti-allergic activities of Pentaherb formula, Moutan Cortex (Danpi) and gallic acid. Molecules 2013;18:2483-500.
12. Hsiang CY, Hseu YC, Chang YC, Kumar KJ, Ho TY, Yang HL. *Toona sinensis* and its major bioactive compound gallic acid inhibit LPS-induced inflammation in nuclear factor-κB transgenic mice as evaluated by *in vivo* bioluminescence imaging. Food Chem 2013;136:426-34.
13. Mo J, Panichayupakaranant P, Kaewnopparat N, Nitiruangjaras A, Reanmongkol W. Topical anti-inflammatory and analgesic activities of standardized pomegranate rind extract in comparison with its marker compound ellagic acid *in vivo*. J Ethnopharmacol 2013;148:901-8.
14. Rogerio AP, Fontanari C, Borducchi E, Keller AC, Russo M, Soares EG, *et al.* Anti-inflammatory effects of *Lafoensia pacari* and ellagic acid in a murine model of asthma. Eur J Pharmacol 2008;580:262-70.
15. Kim B, Choi YE, Kim HS. *Eruca sativa* and its flavonoid components, quercetin and isorhamnetin, improve skin barrier function by activation of peroxisome proliferator-activated receptor (PPAR)-α and suppression of inflammatory cytokines. Phytother Res 2014;28:1359-66.
16. Shaik YB, Castellani ML, Perrella A, Conti F, Salini V, Tete S, *et al.* Role of quercetin (a natural herbal compound) in allergy and inflammation. J Biol Regul Homeost Agents 2006;20:47-52.
17. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta 2011;1813:878-88.
18. Phipps RP, Stein SH, Roper RL. A new view of prostaglandin E regulation of the immune response. Immunol Today 1991;12:349-52.
19. Rhind SG, Gannon GA, Suzui M, Shephard RJ, Shek PN. Indomethacin inhibits circulating $PGE_2$ and reverses postexercise suppression of natural killer cell activity. Am J Physiol 1999;276(5 Pt 2):R1496-505.
20. Koeberle A, Werz O. Multi-target approach for natural products in inflammation. Drug Discov Today 2014;19:1871-82.
21. Seo HJ, Huh JE, Han JH, Jeong SJ, Jang J, Lee EO, *et al.* Polygoni rhizoma inhibits inflammatory response through inactivation of nuclear factor-kappa B and mitogen activated protein kinase signaling pathways in RAW 264.7 mouse macrophage cells. Phytother Res 2012;26:239-45.
22. Oh WJ, Jung U, Eom HS, Shin HJ, Park HR. Inhibition of lipopolysaccharide-induced proinflammatory responses by *Buddleja officinalis* extract in BV-2 microglial cells via negative regulation of NF-κB and ERK1/2 signaling. Molecules 2013;18:9195-206.
23. Choi RJ, Chun J, Khan S, Kim YS. Desoxyrhapontigenin, a potent anti-inflammatory phytochemical, inhibits LPS-induced inflammatory responses via suppressing NF-κB and MAPK pathways in RAW 264.7 cells. Int Immunopharmacol 2014;18:182-90.




Hyeun-Kyoo Shin

## ABOUT AUTHOR

**Hyeun-Kyoo Shin**, Korean medicine doctor, is a principal researcher at K-herb Research Center, Korea Institute of Oriental Medicine. Her major is Korean medicine. He is interested in the area of traditional herbal medicines.