Research Article

Extraction and Bioactivity Analysis of Major Flavones Compounds from *Scutellaria baicalensis* Using In Vitro Assay and Online Screening HPLC-ABTS System

Kwang Jin Lee, Pil Mun Jung, You-Chang Oh, Na-Young Song, Taesoo Kim, and Jin Yeul Ma

KM-Based Herbal Drug Development Group, Korean Institute of Oriental Medicine (KIOM), 1672 Yuseongdae-ro, Yuseong-gu, Daejeon 305-811, Republic of Korea

Correspondence should be addressed to Jin Yeul Ma; jyma@kiom.re.kr

Received 1 July 2014; Revised 18 August 2014; Accepted 18 August 2014; Published 1 September 2014

Academic Editor: Hassan Y. Aboul Enein

Copyright © 2014 Kwang Jin Lee et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The extraction efficiency of a number of solvent compositions for the improvement of bioactive compounds yield from *S. baicalensis* has been investigated. Also, free radical scavengers in the glycoside baicalin (BG), wogonoside (WG), aglycon baicalein (B), and wogonin (W) compounds of *S. baicalensis* were screened, identified, and quantified using coupled offline ABTS and online screening HPLC-ABTS assay. Increasing ethanol content fractions resulted in decreased extract yield of bioactive compounds. In this case, the best yield of 37.01 mg/g in BG, WG, B, and W compounds was obtained by a dipping method with an extraction time of 4 h. In addition, the yield (43.05%) and IC\(_{50}\) (34.04 μg/mL) determined through ABTS assay of the 60% aqueous ethanol extract were the most satisfactory of all solvent solutions tested. This result shows that an online screening HPLC-ABTS assay can be a powerful technique for the rapid characterization of bioactivity compounds in plant extracts. Moreover, their anti-inflammatory activities were evaluated via analyzed inhibitory effect on NO and inflammatory cytokine production. Furthermore, WG and W exhibited the strong inhibitory effects on inflammatory mediator production including NO, IL-6, and IL-1β in LPS-stimulated RAW 264.7 macrophages.

1. Introduction

*Scutellaria baicalensis* is one of the most widely used medicinal herbs for the treatment of various inflammatory diseases, such as hepatitis, tumors, and diarrhea in East Asian countries [1, 2]. *S. baicalensis* contains a variety of flavones, phenylethanoids, amino acids, sterols, and essential oils [3]. Its dried roots contain flavonoids such as baicalin, baicalein, wogonin, wogonin 7-O-glucuronide, oroxylin A, and oroxylin A 7-O-glucuronide [4]. These four major flavones: glycoside baicalin (BG, MW: 446.37, C\(_{21}\)H\(_{18}\)O\(_{11}\)), wogonoside (WG, MW: 460.39, C\(_{22}\)H\(_{20}\)O\(_{11}\)), aglycon baicalein (B, MW: 270.24, C\(_{15}\)H\(_{10}\)O\(_{5}\)), and wogonin (W, MW: 284.27, C\(_{16}\)H\(_{12}\)O\(_{5}\)) were reported to be the main bioactive components in *S. baicalensis* [5, 6]. Besides its anti-inflammation and anticancer properties, *S. baicalensis* is effective in treating bacterial and viral infections, reducing the total cholesterol level and decreasing blood pressure [7, 8]. The bioactivities of BG and B are much more than those of WG and W [6]. In previous studies of extraction methods, a variety of approaches have been developed for the extraction of useful components from *S. baicalensis*, for instance, soxhlet extraction (SE), heating reflux extraction (HRE), supercritical fluid extraction (SFE) [9], ultrasonic assisted extraction (UAE) [10], and microwave assisted extraction (MAE) [11]. Moreover, water, methanol, ethanol, and ethyl acetate are commonly used solvents for the extraction of bioactive compounds from plant materials and oriental medicine herbs (OMHs). Identification of the bioactivity compounds in *S. baicalensis* has been achieved through several methods, including thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), high-speed counter-current chromatography (HSCCC), capillary electrophoresis...
(CE), and micellar electrokinetic capillary chromatography (MEKC) [12]. In the past few years, online screening with a HPLC postcolumn assay involving the DPPH or ABTS radical technique has been developed, allowing bioactive compounds to be spectrophotometrically monitored. Also, this method was successfully applied for screening and identifying natural bioactive compounds from complex mixtures, especially for extracts of OMHs [13, 14].

This work investigates applications of offline ABTS IC\textsubscript{50} assays and online screening HPLC-ABTS assays for bioactivity screening, so that a more practical approach may be taken towards the use of online screening HPLC-ABTS assays for the rapid pinpointing of bioactivity peaks in chromatograms finds expression in experimental. Additionally, various solvent extraction techniques are compared in terms of their yields of the four major compounds: glycoside baicalin (BG), wogonoside (WG), aglycon baicalein (B), and wogonin (W) compounds in \textit{S. baicalensis}. And, their anti-inflammatory activities were evaluated via analyzed inhibitory effect on NO and inflammatory cytokine production.

2. Experimental

2.1. Reagents and Materials. The following reagents were used for radical-scavenging assays: ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulmonic acid), potassium persulfate, and trifluoroacetic acid (TFA) were purchased from Sigma Co. (USA). The standard chemicals of baicalin (BG), wogonoside (WG), baicalein (B), and wogonin (W) were obtained from Sigma Co. (USA). The dried root of \textit{S. baicalensis} was purchased from the Yeongcheon market (Gyeongsangbuk-do, South Korea) in March 2012. HPLC-grade ethanol, methanol, and acetonitrile were purchased from J. T. Baker (USA). The triple distilled water was filtered through a 0.2μm membrane filter prior to analysis.

2.2. Standard Sample Preparation. The high purity standard sample (higher than >95%) was prepared by dissolving 2 mg of the standard chemicals baicalin (BG), wogonoside (WG), baicalein (B), and wogonin (W) in 10 mL of methanol and adjusting the concentration to 200 ppm.

2.3. ABTS Sample Preparation. A 2 mM ABTS stock solution containing 3.5 mM potassium persulfate was prepared and was kept in the dark at room temperature for 16 h to allow the completion of radical generation and was then diluted with water (1:29, v/v).

2.4. Solvent Extraction. 5 g samples of the dry powder from the \textit{S. baicalensis} containing the four major flavonoids, BG, WG, B, and W, were loaded in 100 mL of pure 100% water, 100% ethanol, and 80%, 70%, and 60% aqueous ethanol solution 100 mL by dipping method for 4 h at 25°C. Each extract was filtered, concentrated under vacuum using rotavap evaporator, and refrigerated for 48 h. Then the samples were frozen dry and the total extraction yield was calculated. Each sample was filtered through a 0.2μm membrane filter prior to offline ABTS assay and online screening HPLC-ABTS analysis.

2.5. Extraction Yield. Each experiment was performed in two replicates and the data were subjected to calculations of means ± SD. The extract sample was expressed as a percentage of the weight. The extraction yield was measured using (1).

$$\text{Extraction yield (%)} = \frac{\text{Extracts dry weight}}{\text{Sample dry weight}} \times 100. \quad (1)$$

2.6. Offline ABTS Assay for Bioactivity Evaluation. The ABTS radical cation method [15] was modified to evaluate the free radical-scavenging effect of \textit{S. baicalensis} extracts. The ABTS reagent was prepared by mixing 5 mL of 7 mM ABTS with 88 μL of 140 mM potassium persulfate. The mixture was then kept in the dark at room temperature for 16 h to allow free radical generation and was then diluted with water (1:44, v/v). To determine the scavenging activity, 100μL ABTS reagent was mixed with 100 μL of sample in a 96-well microplate and was incubated room temperature for 6 min. After incubation, the absorbance was measured 734 nm using an ELISA reader (TECAN, Gröding, Austria), and 100% methanol was used as a control. The ABTS scavenging effect was measured using the following formula:

$$\text{Radical scavenging (%)} = \frac{(A) \text{ control} - (A) \text{ sample}}{(A) \text{ control}} \times 100. \quad (2)$$

The IC\textsubscript{50} ABTS values (the concentration of sample required to inhibition 50% of ABTS radicals) were obtained through extrapolation from regression analysis. The bioactivity was evaluated based on this IC\textsubscript{50} value.

2.7. Online Screening HPLC-ABTS Assay Analysis. The online radical-scavenging activity of \textit{S. baicalensis} was determined using the ABTS assay modified the methods used by Stewart et al. [16]. A 2 mM ABTS stock solution containing 3.5 mM potassium persulphate was prepared and was kept in the dark at room temperature for 16 h to allow the completion of radical generation and was then diluted with water (1:29, v/v). \textit{S. baicalensis} extract was injected into a Dionex Ultimate 3000 HPLC system (Thermo scientific). The chromatographic columns used in this experiment are commercially available; this is obtained from RS-tech (0.46 × 25 cm, 5μm,
Regents Regents Pump Pump Sample injection DAD detector Column VWD detector Passive split Reaction loop

Figure 2: Schematic of online screening HPLC-ABTS system.

C18, Daejeon, Korea). The injection volume was 10 μL, and the flow rate of the mobile phase was 1.0 mL/min. The wavelength of the UV detector was fixed at 275 nm. The run time was 80 min and the solvent program was the linear gradient method (90:10-60:40, A: B vol%). Figure 2 is a schematic showing the online coupling of HPLC to a DAD (Diode Array Detector) and the continuous flow ABTS assay. Online HPLC then arrived at a “T” piece, where ABTS was added. The ABTS flow rate was 0.5 mL/min, delivered by a Dionex Ultimate 3000 Pump. After mixing through a 1 mL loop which was maintained at 40°C, the absorbance was measured by a VIS detector at 734 nm. Data were analyzed using Chromeleon 7 software.

2.8. Cell Culture and Drug Treatment. RAW 264.7 cells were obtained from Korea Cell Line Bank (Seoul, Korea) and grown in RPMI 1640 medium containing 10% FBS and 100 U/mL of antibiotics sulfate. The cells were incubated in humidified 5% CO2 atmosphere at 37°C. To stimulate the cells, the medium was changed with fresh RPMI 1640 medium and LPS (200 ng/mL) [17, 18] was added in the presence or absence of four compounds (1, 3, 5, and 10 μM) for 24 h.

2.9. Cell Viability Assay. Cytotoxicity was analyzed using a cell counting kit (CCK, Dojindo, Japan). Four compounds were added to the cells and incubated for 24 h at 37°C with 5% CO2. CCK solutions were added to each well and the cells were incubated for another 1 h. Then the optical density was read at 450 nm using an ELISA reader (Infinity M200, Tecan, Männedorf, Switzerland).

2.10. Measurement of NO Production. NO production was analyzed by measuring the nitrite in the supernatants of cultured macrophage cells. The cells were pretreated with five compounds and stimulated with LPS for 24 h. The supernatant was mixed with a same volume of Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride, and 2.5% phosphoric acid) and incubated at room temperature (RT) for 5 min [17]. The absorbance at 570 nm was read.

2.11. Determination of TNF-α, IL-6, and IL-1β Cytokine Production. Cells were seeded at a density of 5 × 104 cells/mL in 24-well culture plates and pretreated with various concentrations of four compounds for 30 min before LPS stimulation. ELISA plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with capture antibody diluted in coating buffer (0.1 M carbonate, pH 9.5) and then washed five times with phosphate-buffered saline (PBS) containing 0.05% Tween 20. The nonspecific protein-binding sites were blocked with assay diluent buffer (PBS containing 10% FBS, pH 7.0) for more than 1 h. Promptly, samples and standards were added to the wells. After 2 h of incubation at RT or overnight at 4°C, the working detector solution (biotinylated detection antibody and streptavidin-HRP reagent) was added and incubated for 1 hour. Subsequently, substrate solution (tetramethylbenzidine) was added to the wells and incubated for 30 min in darkness before the reaction was stopped with stop solution (NH4PO4). The optical density was read at 450 nm [17].

2.12. Statistical Analysis. The results are expressed as mean ± SD values for the number of experiments. Statistical significance was compared each treated group with the control
Table 1: Yield of extraction solvent composition and bioactivity of the *S. baicalensis* extracts by free radical-scavenging activity ABTS IC$_{50}$ assay.

| Extraction solvent | Extraction yield (%) | IC$_{50}$ (µg/mL) |
|--------------------|----------------------|-------------------|
| Water 100%         | 18.36 ± 0.25a        | 302.17 ± 37.40b   |
| EtOH 100%          | 1.97 ± 0.01          | 80.69 ± 2.10      |
| EtOH 80%           | 23.00 ± 0.90         | 44.87 ± 4.76      |
| EtOH 70%           | 33.10 ± 0.10         | 39.22 ± 2.74      |
| EtOH 60%           | 43.05 ± 0.47         | 34.04 ± 3.22      |

*a*Each value in mean ± SD (*n* = 2); *b*each value in mean ± SD (*n* = 3).

and determined by Student’s *t*-tests. Each experiment was repeated at least three times to yield comparable results. Values with *P* < 0.01 and *P* < 0.001 were considered significant.

3. Result and Discussion

3.1. Extraction from *S. baicalensis*. Components from OMHs can be extracted using various extraction methods, and the extraction efficiency and component contents vary according to the extraction method. This study investigated the extraction efficiency, composition, and bioactivity of components from *S. baicalensis* using various solvent extractions. Different solvents used for the optimization of the extraction of major flavone compounds from *S. baicalensis* were water, ethanol, and 80%, 70%, and 60% aqueous ethanol. Increasing ethanol percentage decreased the yield of extracted bioactive compounds. The extraction efficiencies obtained using 60%, 70%, and 80% aqueous ethanol are 43.05%, 33.10, and 23%, respectively. Moreover, the yield and bioactivity (radical scavenging) obtained using pure water and ethanol extractions were lower than those obtained using mixed aqueous ethanol solvents (Table 1).

3.1.1. Offline ABTS Assay. ABTS is one of the compounds that have a proton free radical, with a characteristic absorption, which decreases significantly upon exposure to proton radical scavengers. It is well accepted that the ABTS radical scavenging by bioactivity is attributable to their hydrogen-donating ability. According to Figure 3, the ABTS radical-scavenging activity of 100% water, 100% ethanol, and 80%, 70%, and 60% aqueous ethanol. Increasing ethanol percentage decreased the yield of extracted bioactive compounds. The extraction efficiencies obtained using 60%, 70%, and 80% aqueous ethanol are 43.05%, 33.10, and 23%, respectively. Moreover, the yield and bioactivity (radical scavenging) obtained using pure water and ethanol extractions were lower than those obtained using mixed aqueous ethanol solvents (Table 1).

![Figure 3: Free radical-scavenging activity of *S. baicalensis* extracts by ABTS assay.](image1)

*Figure 3: Free radical-scavenging activity of *S. baicalensis* extracts by ABTS assay. (●) Water, (○) 100% EtOH, (●) 80% EtOH, (△) 70% EtOH, and (▲) 60% EtOH, results are mean ± S.D (*n* = 3).*

3.1.2. Online HPLC-ABTS Assay Analysis. The HPLC separated analyses react after column with the ABTS and the reduction is detected as a negative peak by a VIS absorbance detector at 734 nm. As the ABTS radical is much more water soluble than DPPH, the ABTS assay is more widely used for a significant difference between two experimental methods. Consequently, this study shows that 60% aqueous ethanol has the highest bioactivity, while the 100% water extract has the lowest value. Thus, it is considered that using a water-ethanol mixture extract is suitable for water-ethanol mixture extract is higher than that of water or ethanol extract.

![Figure 4: Chromatogram of online screening HPLC-ABTS radical scavenging of standard chemical compounds.](image2)

*Figure 4: Chromatogram of online screening HPLC-ABTS radical scavenging of standard chemical compounds.*
evaluation of water bioactivities. Combined UV (positive signals) and ABTS quenching (negative signals) chromatograms of the different *S. baicalensis* extracts and standard chemical (200 ppm) are presented in Figures 4 and 5. Several eluted flavonoids in the extract were detected, including BG, WG, B, and W giving a positive signal on the UV detector (275 nm). Among them, the others showed hydrogen-donating capacity (negative peak) towards the ABTS radical at the applied concentration. These results revealed that the method can be applied for a quick screening of bioactivity, or more precisely, of radical-scavenging activity of compounds. In this case, BG and B were determined chromatographically, confirming their bioactivity though WG or W was not detected. This means that BG and B have the high bioactivity, whereas WG and W have the low bioactivity. In addition, Gao et al. [6] elicited similar results using a DPPH assay method. It shows
Table 2: Comparison of extract efficiency by online screening HPLC-ABTS in positive and negative peak area.

| Extraction solvent (%) | Compounds | $R_t$ (min) | Positive peak | Negative peak |
|------------------------|-----------|-------------|---------------|---------------|
|                        |           | Average peak area (mAU) | Standard deviation (SD±) | RSD (%) | Yield (%) | Total extraction amount (mg) | Average peak area (mAU) | Standard deviation (SD±) | RSD (%) |
| 100% Water             | BG        | 54.76       | 12.80          | 2.54          | 19.86       | 1.07          | 0.21                      | 6.01                      | 0.01                      | 0.08       |
|                        | WG        | 45.53       | 0.20           | 0.22          | 114.26      | 0.01          | 0.01                      | ND                       | ——                       |
|                        | B         | 36.42       | 8.71           | 10.32         | 118.52      | 0.96          | 0.13                      | 2.76                      | 3.91                      | 141.42     |
|                        | W         | 70.11       | 2.93           | 1.32          | 45.18       | 0.23          | 0.04                      | ND                       | ——                       |
| 100% Ethanol           | BG        | 54.77       | 505.72         | 26.58         | 5.26        | 12.84         | 0.90                      | 166.45                    | 1.79                      | 1.08        |
|                        | WG        | 45.48       | 468.99         | 24.32         | 5.19        | 11.91         | 1.21                      | ND                       | ——                       |
|                        | B         | 36.18       | 803.00         | 126.99        | 15.81       | 20.15         | 1.32                      | 161.15                    | 15.61                     | 9.69        |
|                        | W         | 70.18       | 164.19         | 13.56         | 8.26        | 4.16          | 0.24                      | ND                       | ——                       |
| 80% Aqueous ethanol    | BG        | 54.74       | 545.35         | 31.60         | 5.80        | 8.41          | 11.42                     | 179.00                    | 13.59                     | 7.59        |
|                        | WG        | 45.47       | 1258.84        | 232.65        | 18.48       | 19.10         | 38.08                     | ND                       | ——                       |
|                        | B         | 36.60       | 1492.57        | 372.90        | 24.98       | 22.46         | 28.79                     | 244.52                    | 49.46                     | 20.23       |
|                        | W         | 70.03       | 292.71         | 16.91         | 5.78        | 4.51          | 4.99                      | ND                       | ——                       |
| 70% Aqueous ethanol    | BG        | 54.76       | 753.16         | 26.72         | 3.55        | 11.34         | 22.70                     | 211.85                    | 21.91                     | 10.34       |
|                        | WG        | 45.50       | 1249.30        | 208.61        | 16.70       | 18.53         | 54.40                     | ND                       | ——                       |
|                        | B         | 36.68       | 1634.98        | 384.09        | 23.49       | 24.07         | 45.40                     | 267.07                    | 53.40                     | 20.00       |
|                        | W         | 70.06       | 300.91         | 16.19         | 5.38        | 4.52          | 7.39                      | ND                       | ——                       |
| 60% Aqueous ethanol    | BG        | 54.78       | 1049.88        | 34.23         | 3.26        | 15.12         | 41.46                     | 246.00                    | 29.69                     | 12.07       |
|                        | WG        | 45.51       | 1246.60        | 104.08        | 8.35        | 17.78         | 71.14                     | ND                       | ——                       |
|                        | B         | 36.59       | 1710.77        | 194.39        | 11.35       | 24.26         | 62.26                     | 272.92                    | 56.03                     | 20.53       |
|                        | W         | 70.05       | 314.99         | 6.94          | 2.20        | 4.55          | 10.13                     | ND                       | ——                       |

aRSD: relative standard deviation; bND: not detected.

Figure 6: Effect of four compounds on (a) cell viability and LPS-induced (b) NO production in RAW 264.7 cells. RAW 264.7 cells were pretreated with four compounds for 30 min before incubation with LPS for 24 h. (a) Cytotoxicity was evaluated by a CCK. (b) The culture supernatant was analyzed for nitrite production. As a control, the cells were incubated with vehicle alone. Data shows mean ± SE values of triplicate determination from independent experiments. * $P < 0.01$ and ** $P < 0.001$ were calculated from comparing with LPS-stimulation value.

the details of an online HPLC-ABTS assay system which analyzes the extracts from solvents (Figures 5(a)–5(d)). The retention time ($R_t$) of glycoside BG ($R_t$: 54.74–54.87 min) and WG ($R_t$: 45.47–45.53 min) and aglycone B ($R_t$: 36.18–36.68 min) and W ($R_t$: 70.03–70.18 min) was reported by this work. The 60% aqueous ethanol extract resulted in the greatest total extraction amount (BG: 41.46, WG: 71.14, B: 62.26, and W: 10.13 mg) were the total extraction amount highest them 70% aqueous ethanol extract (BG: 22.70, WG: 54.40, B: 45.40, and W: 7.39 mg), 80% aqueous ethanol extract (BG:
Figure 7: Effect of four compounds on the production of (a) TNF-α, (b) IL-6, and (c) IL-1β cytokine in macrophages. Cells were pretreated with four compounds for 30 min before being incubated with LPS for 24 h. Production of cytokines was measured by ELISA. Data shows mean ± SE values of duplicate determinations from three independent experiments. *P < 0.01 and **P < 0.001 were calculated from comparing with LPS-stimulation value.

3.2. Anti-Inflammatory Activities Screening

3.2.1. Effect of Four Compounds on RAW 264.7 Cell Viability. We evaluated the cytotoxicity of four compounds using a CCK to determine the optimal concentration that would be effective for anti-inflammation with minimum toxicity. As shown in Figure 6(a), baicalin shows little toxicity at concentrations of 10 μM. Also, baicalein contains strong toxicity on macrophage viability at 3 μM or more. Wogonoside and wogonin did not affect cell viability up to 10 μM, indicating two compounds are not toxic to cells.

3.2.2. Effect of Four Compounds on NO Production in LPS-Stimulated RAW 264.7 Macrophages. We evaluated the effects of four compounds on NO secretion in LPS-stimulated RAW 264.7 cells. The cells were pretreated with four compounds at various concentrations prior to LPS stimulation and NO production was measured. As a positive control, we employed 10 μM dexamethasone, which is widely employed as an anti-inflammatory agent. As shown in Figure 6(b), baicalin shows slightly inhibitory effect on NO secretion upon LPS stimulation at 5 μM. Baicalein repressed NO secretion at concentrations of 3 μM or more. However, this inhibitory effect was related with strong cytotoxicity of baicalein [18]. Wogonoside and wogonin were strongly inhibited NO production in a dose-dependent manner with statistical significance.

3.2.3. Effect of Four Compounds on LPS-Induced Inflammatory Cytokines Production. Next, we investigated the inhibitory effect of four compounds on the production of inflammatory cytokines, which is another parameter of inflammation. In
this study, we examined the effect of four compounds on TNF-α, IL-6, and IL-1β cytokine production. As shown in Figure 7(a), all compounds did not inhibit TNF-α production at all concentrations. As shown in Figure 7(b), wogonoside and wogonin were significantly inhibited IL-6 cytokine secretion at concentrations of 3 μM or more with statistical significance. Consistent with IL-6 results, wogonoside and wogonin showed inhibitory effect on IL-1β cytokine production in a dose-dependent fashion (Figure 7(c)).

4. Conclusions

This study provides the comparison of free radical scavengers in the extracts of S. baicalensis by an offline ABTS assay and an online screening HPLC-ABTS assay. The results showed the effect of solvent composition on total extraction yield from S. baicalensis. Based on our investigations, total extract yield and bioactivity decreased with ethanol increasing in the solvent mixture. In addition, the bioactivities of BG and B were determined to be much greater than those of WG or W. The yield (43.05%) and IC_{50} (34.04 μg/mL) determined through ABTS assay of the 60% aqueous ethanol extract were the most satisfactory of all solvent solutions tested. And, this confirms the feasibility of assessing the bioactivity of specific phytochemicals using the online screening HPLC-ABTS assay. There was a very small margin of error between the results of the offline ABTS assay and those of the online screening HPLC-ABTS assay. Moreover, their anti-inflammatory activities were evaluated via analyzed inhibitory effect on NO and inflammatory cytokine production. Furthermore, WG and W exhibited the strong inhibitory effects on inflammatory mediator production including NO, IL-6, and IL-1β in LPS-stimulated RAW 264.7 macrophages. In conclusion, these compounds could be developed as a new anti-inflammatory therapeutic agent without cytotoxicity. These results will be compiled as a database, for use in investigating the constituents of natural products and the resources of pharmaceutical, nutrition, and cosmetic products.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This study was achieved at KM-Based Herbal Drug Development Group, KIOM. The authors also acknowledge the support from the “Study on drug efficacy enhancement using bioconversion for herbal medicines” (K14050) project.

References

[1] K. Ishimaru, K. Nishikawa, T. Omoto, I. Asai, K. Yoshihira, and K. Shimomura, “Two flavone 2′-glucosides from Scutellaria baicalensis,” Phytochemistry, vol. 40, no. 1, pp. 279–281, 1995.

[2] H. G. Park, S. Y. Yoon, J. Y. Choi et al., “Anticonvulsant effect of wogonin isolated from Scutellaria baicalensis,” European Journal of Pharmacology, vol. 574, no. 2-3, pp. 112–119, 2007.

[3] H. Cao, Y. Jiang, J. Chen et al., “Arsenic accumulation in Scutellaria baicalensis Georgi and its effects on plant growth and pharmaceutical components,” Journal of Hazardous Materials, vol. 171, no. 1–3, pp. 508–513, 2009.

[4] C. Zhang, Y. Zhang, J. Chen, and X. Liang, “Purification and characterization of baicalin-β-D-glucuronidase hydrolyzing baicalin to baicalein from fresh roots of Scutellaria viscidula Bge,” Process Biochemistry, vol. 40, no. 5, pp. 1911–1915, 2005.

[5] M. Himeji, T. Ohtsuki, H. Fukazawa et al., “Difference of growth-inhibitory effect of Scutellaria baicalensis-producing flavonoid wogonin among human cancer cells and normal diploid cell,” Cancer Letters, vol. 245, no. 1-2, pp. 269–274, 2007.

[6] Z. Gao, K. Huang, X. Yang, and H. Xu, “Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of Scutellaria baicalensis Georgi,” Biochimica et Biophysica Acta—General Subjects, vol. 1472, no. 3, pp. 643–650, 1999.

[7] C. Li, L. Zhang, G. Lin, and Z. Zuo, “Identification and quantification of baicalein, wogonin, oxorinyl A and their major glucuronide conjugated metabolites in rat plasma after oral administration of Radix scutellariae product,” Journal of Pharmaceutical and Biomedical Analysis, vol. 54, no. 4, pp. 750–758, 2011.

[8] H.-B. Li and F. Chen, “Isolation and purification of baicalein, wogonin and oxorinyl A from the medicinal plant Scutellaria baicalensis by high-speed counter-current chromatography,” Journal of Chromatography A, vol. 1074, no. 1-2, pp. 107–110, 2005.

[9] M.-C. Lin, M.-J. Tsai, and K.-C. Wen, “Supercritical fluid extraction of flavonoids from Scutellaria Radix,” Journal of Chromatography A, vol. 830, no. 2, pp. 387–395, 1999.

[10] Y. Jin, Y. S. Kim, Y. D. Cheng, and K. J. Lee, “Extraction of kurarinone and leachianone A from Sophora flavescens ait using ultrasonic wave,” Asian Journal of Chemistry, vol. 24, no. 7, pp. 2917–2920, 2012.

[11] H. Wang, L. Chen, Y. Xu et al., “Dynamic microwave-assisted extraction coupled on-line with clean-up for determination of caffeine in tea,” LWT—Food Science and Technology, vol. 44, no. 6, pp. 1490–1495, 2011.

[12] K. J. Lee, S. D. Choi, and J. Y. Ma, “Phytochemical analysis of curcumin from turmeric by RP-HPLC,” Asian Journal of Chemistry, vol. 25, no. 2, pp. 995–998, 2013.

[13] Y.-J. Li, J. Chen, and P. Li, “Identification and quantification of free radical scavengers in the flower buds of Lonicera species by online HPLC-DPPH assay coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry,” Biomedical Chromatography, vol. 26, no. 4, pp. 449–457, 2012.

[14] H. A. G. Niederländer, T. A. van Beek, A. Bartasiute, and I. I. Koleva, “Antioxidant activity assays on-line with liquid chromatography,” Journal of Chromatography A, vol. 920, no. 2, pp. 121–134, 2008.

[15] N. Pellegrini, M. Ying, and C. Rice-Evans, “Screening of dietary carotenoids and carotenoid-rich fruits extract for antioxidant activities applying 2, 2′-azobis (3-ethylbenzothione-6-sulfonic acid) radical cation decolorization assay,” Methods in Enzymology, vol. 299, pp. 384–389, 1999.

[16] A. J. Stewart, W. Mullen, and A. Crozier, “On-line high-performance liquid chromatography analysis of the antioxidant activity of phenolic compounds in green and black tea,” Molecular Nutrition and Food Research, vol. 49, no. 1, pp. 52–60, 2005.

[17] H.-J. Choi, O.-H. Kang, P.-S. Park et al., “Mume Fructus water extract inhibits pro-inflammatory mediators in...
lipopolysaccharide-stimulated macrophages,” *Journal of Medicinal Food*, vol. 10, no. 3, pp. 460–466, 2007.

[18] Y.-C. Oh, W.-K. Cho, Y. H. Jeong et al., "Anti-inflammatory effect of Sosihotang via inhibition of nuclear factor-κB and mitogen-activated protein kinases signaling pathways in lipopolysaccharide-stimulated RAW 264.7 macrophage cells,” *Food and Chemical Toxicology*, vol. 53, pp. 343–351, 2013.

[19] J.-H. Park, R.-Y. Kim, and E. Park, “Antioxidant and α-glucosidase inhibitory activities of different solvent extracts of skullcap (*Scutellaria baicalensis*),” *Food Science and Biotechnology*, vol. 20, no. 4, pp. 1107–1112, 2011.

[20] L.-L. Dong, Y.-J. Fu, Y.-G. Zu et al., “An enhanced preparation and purification of the major antioxidants baicalein and wogonin from *Scutellariae* radix,” *Food Chemistry*, vol. 133, no. 2, pp. 430–436, 2012.

[21] C. Li, L. Zhou, G. Lin, and Z. Zuo, "Contents of major bioactive flavones in proprietary traditional Chinese medicine products and reference herb of *Radix Scutellariae*,” *Journal of Pharmaceutical and Biomedical Analysis*, vol. 50, no. 3, pp. 298–306, 2009.

[22] H.-B. Li, Y. Jiang, and F. Chen, “Separation methods used for *Scutellaria baicalensis* active components,” *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, vol. 812, no. 1-2, pp. 277–290, 2004.