

Research Article

Lipid Peroxidation and Antioxidant Activities of the Aqueous Rhizome Extract of *Rheum officinale* Baillon

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Received 22 January 2018; Accepted 14 May 2018; Published 9 July 2018

Academic Editor: Jorge Barros-Velázquez

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Generation of reactive oxygen species (ROS) is associated with dysregulation of antioxidant defense mechanisms and incidence of human diseases. The specific aim of this study was to investigate the lipid oxidation and antioxidant activity of aqueous extract of *Rheum officinale* Baillon rhizome in order to evaluate its potential as a future novel natural antioxidant resource and a functional ingredient in food and pharmaceutical formations. Total phenolic and flavonoid contents of *Rheum* rhizome extract were dose dependently increased. Consistent with this, radical scavenging activities of *Rheum* rhizome extract as determined by 2,2-diphenyl-1-picrylhydrazyl assay and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity were significantly elevated as the concentration increased. In addition, the treatment of aqueous *Rheum* rhizome extract significantly increased ferric reducing and copper chelating activities. According to results of thiobarbituric acid reactive substance analysis, *Rheum* rhizome extract significantly delayed lipid oxidation. Preincubation with *Rheum* rhizome extract significantly inhibited *tert*-butyl hydroperoxide- (*t*-BHP-) induced ROS generation. Moreover, superoxide anion production was significantly lower in *Rheum* rhizome extract-treated RAW264.7 macrophage cells than *t*-BHP-incubated cells ($p < 0.05$). These findings suggest that *Rheum officinale* Baillon rhizome extract has a potential as an excellent natural antioxidant agent.

1. Introduction

Oxidative stress, which is an imbalance between the production of deleterious reactive oxygen species (ROS) and existing antioxidant defense system, plays a pivotal pathophysiological role in the development of liver disease, cancer, aging, autoimmune disorders, and cardiovascular and neurodegenerative diseases [1–5]. Overproduction of ROS such as hydroxyl radical (OH·), superoxide radical (O₂^{·-}), hydrogen peroxide (H₂O₂), and nitric monoxide (NO·) readily attacks the polyunsaturated fatty acids in the plasma membrane, resulting in the oxidative degradation of lipids [6, 7]. Consequently, lipid oxidation induces cellular and tissue damages through covalent binds, resulting in lipid peroxidation, DNA injury, inflammation, and subsequent cell death [8]. In addition, lipid oxidation in foods and food products lowers food quality, creating off-flavors and unhealthful compounds [9, 10]. Therefore, it is of great interest to prevent

ROS production and lipid oxidation for the improvement of quality and nutrition of foods in the agriculture and food industry.

Rhubarb (Dahuang) is an herbaceous perennial in the Polygonaceae family and its root, stems, and leaves have been used as Asian traditional herbal medicine for treatment of constipation, jaundice, gastrointestinal hemorrhages, and ulcers. Recent accumulating evidence shows that several species of the *Rheum* genus such as *Rheum emodi*, *Rheum undulatum* L., *Rhizoma Rhei*, *Rheum ribes*, *Rheum palmatum* L., and *Rheum rhaponticum* have antiallergic, antibacterial, antioxidant, anticancer, antiangiogenesis, and anti-inflammation properties [11–20]. In relation to antioxidant capacity, anthraquinone derivatives are considered to be one of the major biologically active constituents of aloe-emodin, rhein, emodin, chrysophanol, physcion, and danthron from the rhizomes of *Rheum undulatum* L. and *Rheum palmatum* L. [11, 12, 19, 21]. However, antioxidant

capacity of Korean rhubarb, *Rheum officinale* Baillon, has never been investigated in spite of the rapid growing interest in using natural antioxidants and functional ingredients in foods and dietary supplements. Given the close association between food quality, oxidative stress, and health outcomes, the demand for applying natural antioxidants either in the form of raw extracts or their chemical constituents in foods has been fueled by a growing consumer preference for healthy foods. Therefore, the specific purpose of the study was to investigate the inhibitory effect of aqueous extract from *Rheum officinale* Baillon on lipid oxidation and oxidative stress.

2. Materials and Methods

2.1. Chemicals. Potassium persulfate was purchased from Junsei Chemical (Tokyo, Japan). Pyrocatechol violet (PV) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from TCI (Tokyo, Japan). Dimethyl sulfoxide (DMSO) was purchased from Amresco Inc. (Solon, OH, USA). Fetal bovine serum (FBS) was obtained from Gibco Invitrogen (Grand Island, NY, USA). Dulbecco's modified Eagle's medium (DMEM) and penicillin-streptomycin (PS) were obtained from Welgene (Gyeongsan, Gyeongsangbuk-do, Republic of Korea). Gum Arabic, 1,1,3,3-tetramethoxypropane (TEP), sodium dodecyl sulfate (SDS), phosphotungstic acid, and 2-thiobarbituric acid (TBA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Perilla oil was food grade and procured from a local supplier. All other chemicals, unless otherwise noted, were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemical reagents used in this study were of the highest purity commercially available.

2.2. Sample Preparation. Dried *Rheum officinale* Baillon rhizome was obtained from a local market (Gyeongsan, Gyeongsangbuk-do, Republic of Korea). Rhizome was pulverized using a grinder. Hot water extraction was carried out in a 5 L round-bottomed flask fitted with a cooling condenser at $121 \pm 1^\circ\text{C}$ for 20 min. The cooled extract was filtered through double layers of filter paper (No. 1, Whatman International Ltd., UK). The filtered extract was centrifuged at $11,000 \times g$ for 20 min, and this process was repeated three times. Supernatant was collected and stored at 4°C for further analysis.

2.3. Measurement of Total Phenolic Contents. Total phenol concentration of *Rheum* rhizome extract was determined using the Folin-Ciocalteu method [22]. In brief, $10 \mu\text{L}$ of different concentrations of *Rheum* extract sample (0.25, 0.5, 1.0, 2.5, and 5.0 mg/mL) was added to a 96-well plate containing 10% Folin-Ciocalteu reagent ($20 \mu\text{L}$). After 5 min, $80 \mu\text{L}$ of 700 mM sodium carbonate was added to the mixture and incubated for 1 h at room temperature. The absorbance was measured by a microplate spectrophotometer (Epoch, BioTek, Winooski, VT, USA) at a wavelength of 765 nm. The concentration of phenolic compounds was calculated according to the following equation obtained from a standard curve of gallic acid. Total phenolic content

was expressed as mg of gallic acid equivalent (GAE) per mL of aqueous *Rheum* rhizome extract.

2.4. Measurement of Total Flavonoid Contents. Total flavonoid content was measured by the aluminum chloride colorimetric method. $25 \mu\text{L}$ of *Rheum* rhizome extract in the final concentration range of 0.25–5.0 mg/mL was added to a 96-well plate containing $125 \mu\text{L}$ of distilled water followed by the addition of $10 \mu\text{L}$ of 5% sodium nitrite solution. After 6 min, the reaction was carried out by addition of $15 \mu\text{L}$ of 10% (w/v) aluminum chloride. After 5 min, $50 \mu\text{L}$ of 1 mM sodium hydroxide and $275 \mu\text{L}$ of distilled water were added and mixed thoroughly. The absorbance was measured at a wavelength of 510 nm. The concentration of total flavonoid compounds was determined as compared to a standard curve of catechin as earlier described and expressed as mg of catechin per mL of aqueous *Rheum* rhizome extract.

2.5. DPPH Radical Scavenging Activity Assay. Free radical scavenging activity of *Rheum* extract was measured by DPPH assay. DPPH stock solution was freshly prepared in methanol at a concentration of 0.02%. An aliquot of $50 \mu\text{L}$ sample solution at a wide dose range from 0.25 to 5.0 mg/mL was mixed with $100 \mu\text{L}$ of DPPH reagent solution. After 30 min of incubation at room temperature in the dark, the absorbance of the mixture was measured at 510 nm against a blank. Scavenging activity was expressed as mg of ascorbic acid (AA) per mL of aqueous *Rheum* rhizome extract.

DPPH radical scavenging activity (%) =

$$\left[1 - \left\{ \frac{\Delta\text{ABS}(\text{sample} - \text{blank})}{\text{ABS}(\text{control})} \right\} \times 100 \right]. \quad (1)$$

2.6. ABTS Radical Scavenging Activity Assay. As described previously [23], ABTS stock solution (7.4 mM) was added to 2.6 mM potassium persulfate solution in equal quantities and kept for 16 h at room temperature in the dark to yield a dark colored solution containing ABTS radical cation. Before use, ABTS radical cation was diluted to an initial absorbance of 0.7 ± 0.02 at 734 nm. Free radical scavenging activity was determined by the addition of $10 \mu\text{L}$ of *Rheum* rhizome extract from 5 different final concentrations (0.25, 0.5, 1.0, 2.5, and 5.0 mg/mL) to $290 \mu\text{L}$ of ABTS working solution. After 6 min, the absorbance was determined at 734 nm. ABTS radical scavenging activity was expressed as mg of trolox equivalent (TE) per mL of aqueous *Rheum* rhizome extract.

ABTS radical scavenging activity (%) =

$$\left[1 - \left\{ \frac{\Delta\text{ABS}(\text{sample} - \text{blank})}{\text{ABS}(\text{control})} \right\} \times 100 \right]. \quad (2)$$

2.7. Ferric Reducing Antioxidant Power (FRAP) Assay. Reducing power was measured based on conversion of Fe(III) to Fe(II) using the method described by Benzie and Strain [24]. Briefly, FRAP working reagent was prepared by

addition of 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM ferric chloride ($\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$) solution at a 10:1:1 ratio. A wide dose range of 10 μL *Rheum* rhizome aqueous extract (0.25, 0.5, 1.0, 2.5, and 5.0 mg/mL) was mixed with 10 μL of distilled water, followed by the addition of prewarmed FRAP working reagent (100 μL). Optical density of the mixture was measured at a wavelength of 593 nm. Reducing power was described as the use of ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$).

2.8. Determination of Copper Chelating Activity. Cupric reducing antioxidant capacity of *Rheum* extract was determined according to the method of Megias [25]. In each well of a 96-well plate, 30 μL hot water extract sample which final concentration was different from 0.25 to 5.0 mg/mL was mixed with 6 μL of 4 mM PV, 290 μL of 50 mM sodium acetate buffer (pH 6.0), and 10 μL of 2 mM copper sulfate. In order to measure copper chelating activity, absorbance was determined at 632 nm according to the following equation:

$$\text{Copper chelating activity (\%)} = \left[1 - \left\{ \frac{\Delta\text{ABS}(\text{sample} - \text{blank})}{\text{ABS}(\text{control})} \right\} \times 100 \right]. \quad (3)$$

2.9. Measurement of Lipid Peroxidation. Oil emulsion was prepared by mixing raw perilla oil and rhubarb aqueous extract at the ratio of 3 to 1. Then, raw perilla oil emulsions were mixed with 10% gum arabic as an emulsifier and homogenized for 16 min by homogenizer (AM-8, Nissei, Japan). Lipid oxidation of the oil emulsion was induced at 30°C or 70°C using incubator (JISICO, Korea) and water bath (Shaking Water Bath, JEIO Tech, Korea), respectively. Heated raw perilla oil emulsions were collected at 0, 7, and 10 days of incubation. The lipid oxidation of raw perilla oil was analyzed by thiobarbituric acid reactive substance (TBARS) assay [26]. In brief, 10 μL of samples (rhubarb) at three different concentrations of 0, 0.25, and 5 mg/mL or standard solution (1,1,3,3-tetramethoxypropane, TEP) and 40 μL of 20 mM phosphate buffer (pH 7.0) were added to an Eppendorf tube on ice. In each tube, 50 μL of 3% sodium dodecyl sulfate (SDS), 200 μL of 0.1 N HCl, 30 μL of 10% phosphotungstic acid, and 100 μL of 0.7% of 2-thiobarbituric acid (TBA) were added. The tubes were firmly closed and boiled at 100°C for 30 min in water bath. The reaction mixture was mixed with 400 μL of *n*-butanol and then centrifuged at 3000 rpm for 10 min. Supernatants were collected and loaded in a 96-well plate. Fluorescence intensity was read at the excitation/emission wavelengths of 515 nm/555 nm using microplate reader (VICTOR Multi-label Plate Reader, PerkinElmer, Korea).

2.10. In Vitro RAW264.7 Macrophage Study

2.10.1. Measurement of Cell Viability. RAW264.7 macrophage cells were cultured at 37°C and 5% CO_2 in DMEM with 10% FBS and 1% PS. Cell viability was determined by a modified

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [27]. RAW264.7 cells were seeded on a 24-well plate at a density of 1×10^5 cells/well and cultured overnight. After 24 h treatment of *Rheum* extract with different concentrations (0, 0.1, 0.5, and 1 mg/mL), RAW264.7 cells were incubated 0.5 mM *tert*-butyl hydroperoxide (*t*-BHP) for 1 h. Further incubation with MTT dye solution (0.5 mg/mL MTT solution and medium at a ratio of 1:5) was executed for 1 h. After dissolving purple formazan into DMSO, the absorbance of the product was measured at 540 nm. The percentage of cell viability was calculated by the following equation:

$$\text{Cell viability (\% of control)} = \frac{\text{OD}_{\text{sample-blank}}}{\text{OD}_{\text{Control-blank}}} \times 100. \quad (4)$$

2.10.2. Determination of Cell Toxicity. Effect of aqueous *Rheum* rhizome extract on cell toxicity was measured by neutral red assay [28]. RAW264.7 cells (1×10^5 cells/well in a 24-well plate) were treated with different concentrations of aqueous *Rheum* extract (0, 0.1, 0.5, and 1 mg/mL) for 24 h, followed by treatment of 0.5 mM *t*-BHP for an additional 1 h. Culture medium was changed to 0.004% neutral red solution dissolved in medium and incubated for 3 h. Lysis buffer containing distilled water, ethanol, and acetic acid at a ratio of 50:49:1 was added to each well and the plate was rocking for 15 min. The optical density at 540 nm was measured.

2.10.3. Measurement of Intracellular Reactive Oxygen Species (ROS) Levels. The level of intracellular ROS was determined using 2',7'-dichlorofluorescein diacetate (DCFH-DA) [29]. RAW264.7 cells were planted on a 96-well plate at a density of 5×10^4 cells/well and incubated at 37°C and 5% CO_2 for 24 h. After 24 h of treatment of *Rheum* extract (0, 0.1, 0.5, and 1 mg/mL), the medium was changed to PBS containing 120 μM DCFH-DA with or without 0.5 mM *t*-BHP and incubated for 60 min at 37°C. The absorbance was determined at 488 nm excitation and 525 nm emission by using a fluorescence plate reader (VICTOR X3, PerkinElmer, Turku, Singapore).

2.10.4. Measurements of Superoxide Anion Production. Preincubated RAW264.7 cells with *Rheum* extract (0, 0.1, 0.5, and 1 mg/mL) for 24 h were incubated with or without 0.5 mM *t*-BHP for 1 h. NBT solution (0.5 mg/mL NBT solution and medium at a ratio of 1:5) was added and further incubated at 37°C and 5% CO_2 for 7 h. Medium was removed, and lysis buffer including DMSO and 2 M potassium hydroxide at a ratio of 1:1 was added. The absorbance was measured at 570 nm.

2.11. Statistical Analysis. The data are presented as the mean \pm standard deviation (SD) of at least three independent triplicate experiments. Data were analyzed by the ANOVA procedure using the Statistical Analysis System (SAS 9.4) software. Differences among groups were determined using Bonferroni procedure at the 5% level.

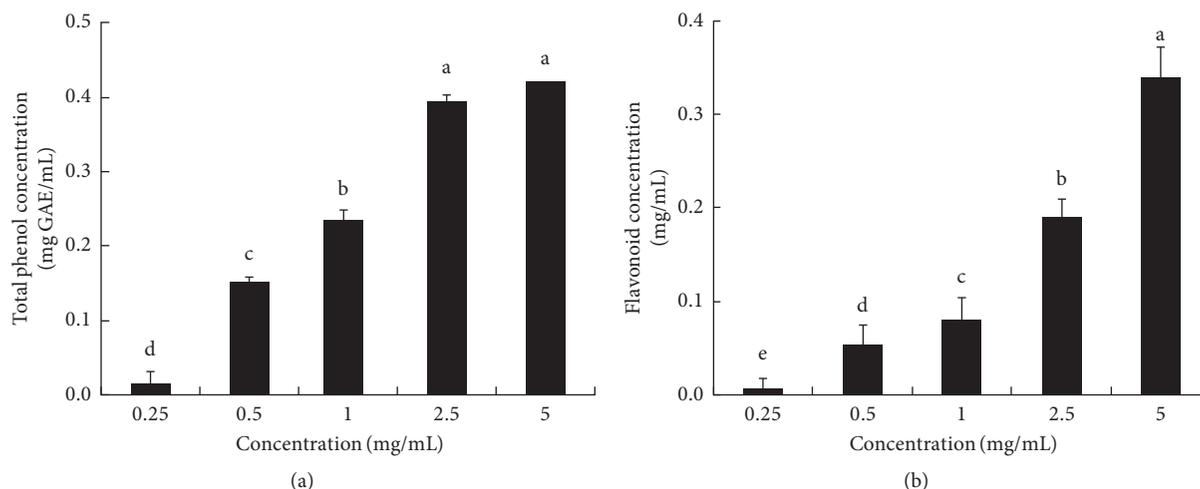


FIGURE 1: Effect of aqueous *Rheum* rhizome extracts on total phenol (a) and flavonoid (b) contents. The concentrations of total phenolic compounds and flavonoids are calculated according to standard curves of gallic acid and catechin and expressed as milligrams of gallic acid equivalent (GAE) and catechin per milliliter of aqueous *Rheum* rhizome extract. Values are expressed as the mean \pm standard deviation (SD) ($n = 9$). Bars with different letters indicate significant differences ($p < 0.05$).

TABLE 1: Radical scavenging and metal chelating activities of aqueous *Rheum* extracts.

| Concentration of <i>Rheum</i> extract (mg/mL) | DPPH (mg AA/mL) | ABTS (mg TE/mL) | FRAP (mM Fe ²⁺ /mL) | Copper chelating (% of control) |
|---|------------------------------|------------------------------|--------------------------------|---------------------------------|
| 0.25 | 0.02 \pm 0.01 ^a | 0.38 \pm 0.01 ^d | 0.02 \pm 0.02 ^c | 2.68 \pm 4.86 ^a |
| 0.50 | 0.05 \pm 0.01 ^b | 0.76 \pm 0.02 ^c | 0.08 \pm 0.03 ^{bc} | 8.80 \pm 4.93 ^{ab} |
| 1.00 | 0.07 \pm 0.01 ^c | 0.88 \pm 0.01 ^b | 0.13 \pm 0.04 ^b | 13.98 \pm 3.85 ^b |
| 2.50 | 0.11 \pm 0.02 ^d | 0.94 \pm 0.00 ^a | 0.21 \pm 0.01 ^a | 23.61 \pm 5.72 ^c |
| 5.00 | 0.13 \pm 0.02 ^e | 0.94 \pm 0.00 ^a | 0.33 \pm 0.09 ^a | 45.01 \pm 2.88 ^d |

Values are expressed as the mean \pm standard deviation (SD) from at least three experiments ($n = 9$). Different letters indicate a significant difference among groups according to Bonferroni procedure ($p < 0.05$). AA, ascorbic acid; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; TE, Trolox equivalent.

3. Results and Discussion

3.1. Total Phenolic and Flavonoid Contents. Polyphenolic compounds including flavonoids are known as powerful antioxidants due to their hydroxyl groups and radical scavenging activities. These compounds may contribute directly to antioxidant capacity, thus having protective functions against oxidative damage and health benefits [30–33]. In order to investigate antioxidant activity of *Rheum* aqueous extract, we first examined concentrations of total phenols and flavonoids. As shown in Figure 1(a), total phenolic contents of rhizome extract significantly increased in a dose-dependent manner with a significant increase at 0.5 mg/mL. Total flavonoid concentration reached a maximum at 5 mg/mL, about 30 folds higher than 0.25 mg/mL (Figure 1(b)). These findings suggest that flavonoids and phenols may be important components of *Rheum officinale* Baillon, and its radical scavenging activity could be attributed to the presence of these constituents.

3.2. Radical Scavenging and Metal Chelating Activities. Excess generation of free radicals or ROS causes oxidative stress and disease [1–5]. Increased use of naturally occurring antioxidants is considerably regarded as an effective and safe

way to prevent ROS-induced diseases. Therefore, we determined the antioxidant effect of *Rheum* extract based on radical scavenging. The electron donation ability of rhizome extract from *Rheum* was determined by DPPH purple-colored solution bleaching assay. Aqueous *Rheum* extract significantly increased the degree of color change in a dose-dependent manner, indicating significant free radical scavenging activity (Table 1). In addition, the extent of decolorization measured as the percentage inhibition of ABTS radical cation was assessed after addition of *Rheum* rhizome extract (Table 1). Aqueous extract of *Rheum* rhizome significantly increased both DPPH and ABTS radical scavenging activities in a dose-dependent manner from a concentration of 0.25 to 5 mg/mL, indicating strong free radical scavenging activity.

Copper(I) and iron(II) are regarded as catalysts for the generation of highly reactive hydroxyl radicals, which cause cell or tissue damages, and consequently diseases [6, 7]. Therefore, copper chelating capacity and ferric reducing activity are important markers for antioxidant activity of natural resources and functional ingredients [34–36]. As shown in Table 1, both ferric reducing capacity and copper chelating activity were significantly increased by *Rheum* extract in a dose-dependent manner, in ranges of 0.02–0.32 mM ferric levels and 3–35% copper chelating compared

TABLE 2: Inhibitory effect of rhubarb aqueous extract on lipid oxidation over storage time at 30°C and 70°C.

| Storage time (days) | Storage temperature (°C) | Concentration of rhubarb (mg/mL) | | |
|---------------------|--------------------------|----------------------------------|---------------------------|---------------------------|
| | | 0 | 2.5 | 5 |
| 0 | — | 50 ± 7.44 ^{Ad} | 47 ± 4.08 ^{Bc} | 45 ± 1.95 ^{Bd} |
| | 30 | 125 ± 3.32 ^{Ac} | 77 ± 4.49 ^{Bc} | 74 ± 3.17 ^{Bc} |
| 7 | 70 | 199 ± 21.92 ^{Ab} | 192 ± 11.04 ^{Aa} | 136 ± 7.81 ^{Bab} |
| | 30 | 237 ± 0.92 ^{Aab} | 130 ± 4.30 ^{Bb} | 119 ± 7.14 ^{Bb} |
| 10 | 70 | 277 ± 8.37 ^{Aa} | 232 ± 21.86 ^{Aa} | 161 ± 9.98 ^{Ba} |

Data are expressed as the mean ± standard deviation (SD) from at least three experiments ($n = 9$). Different letters (A, B, C) within same row indicate a significant difference groups with the same storage time and temperature ($p < 0.05$). Small letters (a–e) demonstrate significant difference at a given concentration of rhubarb aqueous extract ($p < 0.05$).

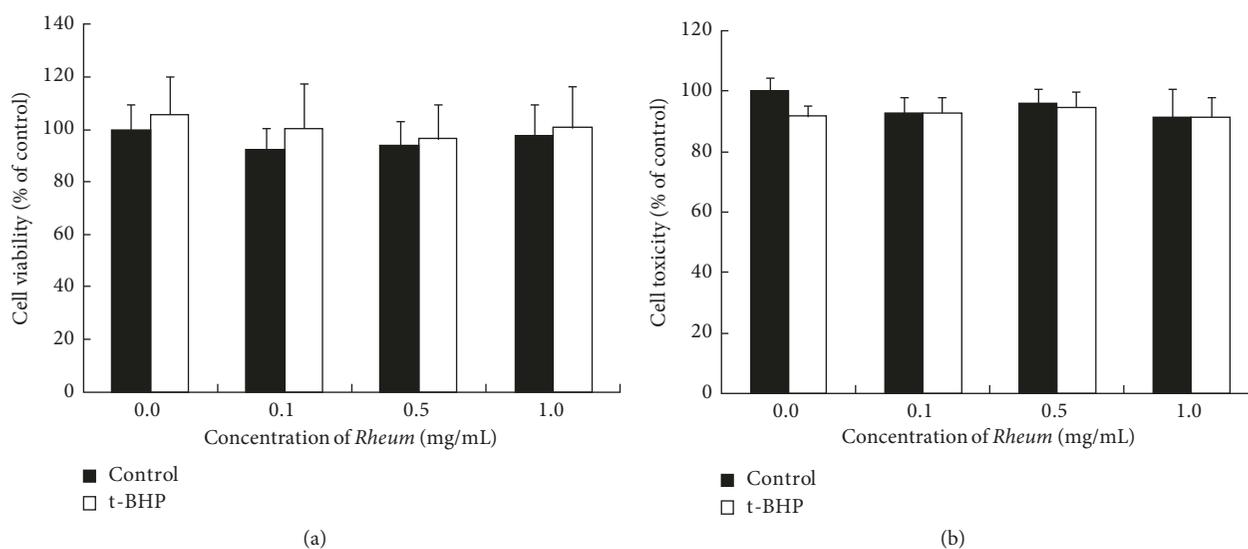


FIGURE 2: Effects of *Rheum* rhizome extract on cell viability (a) and cell toxicity (b) in 0.5 mM *tert*-butyl hydroperoxide- (*t*-BHP-) induced RAW264.7 macrophages. Each value represents the mean ± standard deviation (SD) from at least three experiments ($n = 9$). Bars with different letters differ among aqueous *Rheum* rhizome extract treatment groups ($p < 0.05$).

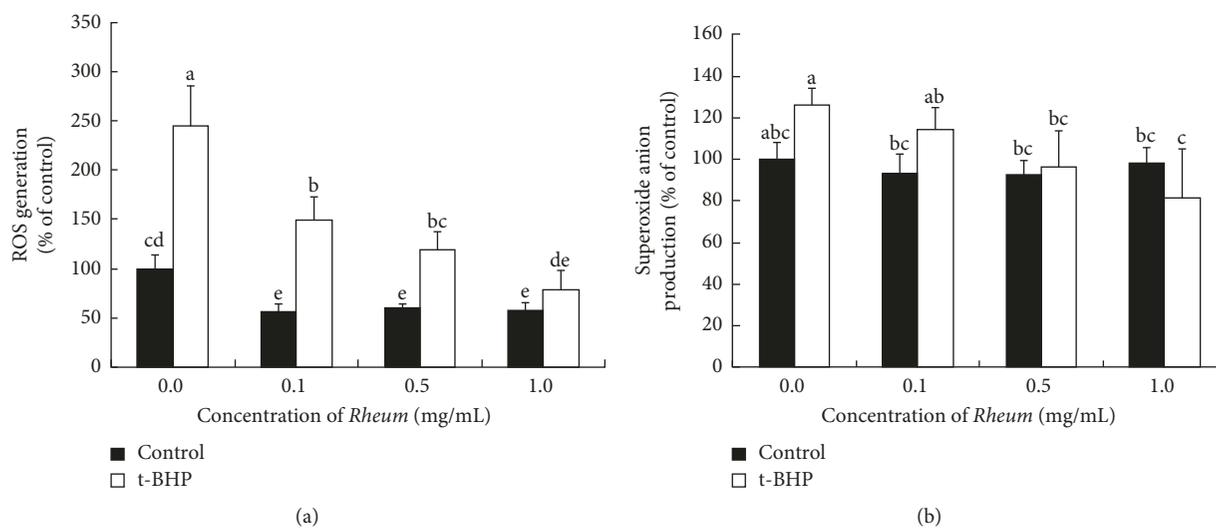


FIGURE 3: Effects of *Rheum* rhizome extract on production of intracellular reactive oxygen species (a) and superoxide anion (b) in 0.5 mM *tert*-butyl hydroperoxide- (*t*-BHP-) induced RAW264.7 macrophages. The value of each bar represents the mean ± standard deviation (SD) ($n = 9$). Means sharing the same letter are not significantly different at the 5% level.

to control, respectively. These results demonstrated that *Rheum* rhizome extract has antioxidant capacity mediated by ferric reducing and copper chelating activities.

3.3. Lipid Oxidation. Increased oxidation process in the foods contributes to food quality deterioration by increasing oxidative rancidity and deleterious food product as well as losing color and nutrient value [9, 10]. Thereby, the ways in which antioxidants inhibit oxidation of food and increase the antioxidant efficacy have attracted much attention. In the present study, inhibitory effect of aqueous *Rheum* extract on lipid oxidation was measured using thiobarbituric acid reactive substances assay. Malondialdehyde, the end product of lipid oxidation, was significantly generated as storage time and temperature were increased (Table 2). Even though lipid peroxidation in oil emulsion mixture was significantly increased according to storage temperature and times, treatment of aqueous extract of *Rheum* rhizome significantly suppressed malondialdehyde levels in the raw perilla oil emulsions in a dose-dependent manner. This result supported that aqueous extract of *Rheum officinale* Baillon inhibits lipid oxidation.

3.4. Antioxidant Properties of *Rheum* Aqueous Extract in *t*-BHP-Treated RAW264.7 Murine Macrophages. Antioxidant effect of *Rheum officinale* Baillon was determined in *in vitro* cell culture model, *t*-BHP treated RAW264.7 macrophage cells. First, we measured the cytotoxic effect of *Rheum officinale* Baillon rhizome extract. After 24 h exposure to 0.1, 0.5, and 1 mg/mL of *Rheum* extract, viabilities of RAW264.7 cells were not statistically different from control, regardless of *t*-BHP treatment (Figure 2(a)). In addition, cell toxicity was not altered by either *Rheum* extract or *t*-BHP treatment (Figure 2(b)). These results indicate that *Rheum* extract at any concentration ranging from 0.1 to 1 mg/mL had no significant cytotoxicity on RAW264.7 cell viability.

As an indicator of oxidative stress, increased production of ROS promotes the pathogenesis of multiple diseases [1–5]. In order to evaluate the effect of *Rheum* extract on oxidative process, measurement of ROS and superoxide anion production were carried out in *t*-BHP-treated RAW264.7 cells. In the presence of *Rheum* extract, *t*-BHP-induced ROS (Figure 3(a)) and superoxide anion production (Figure 3(b)) were significantly diminished in a dose-dependent manner, compared to *t*-BHP only-treated cells.

4. Conclusions

In the present study, analysis of radical scavenging abilities, metal chelating activities, and total phenolic and flavonoid contents showed that aqueous extract from *Rheum officinale* Baillon rhizome could be a potent source of natural antioxidants. In addition, *Rheum* aqueous extract significantly inhibited lipid oxidation in a dose-dependent manner. To the best of our knowledge, this is the first study to suggest that aqueous extract of Korean Rhubarb (*Rheum officinale* Baillon) rhizome may be useful as a natural antioxidant due to its antioxidant capacities to prevent ROS generation and delay oxidant degradation of lipids.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

The authors greatly appreciate the technical assistance from Darae Lee and Naeun Jeon, Yeungnam University. This work was supported by the 2014 Yeungnam University Research Grant.

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