



Research Article

Antioxidant and anti-inflammatory activities of different parts of rhubarb (*Rheum rhabarbarum*) compared with *da huang* root (*R. officinale*)

Ho-Hyeon Lee^{1†}, Ju-Kyung Yu^{2†}, Yong-Sun Moon^{1*}

¹Department of Horticulture & Life Science, Yeungnam University, Gyeongsan 38541, Korea

²Syngenta Crop Protection LLC, Durham, NC 27709, USA

Abstract Rhubarb is known as “*da huang*” (*Rheum officinale*) in Asia. Its root is used in traditional remedies, particularly in Korea and China. In the West, rhubarb (*Rheum rhabarbarum*) stalk is used as a vegetable, but its beneficial properties are not known. In this study, we investigated the antioxidant and anti-inflammatory properties of *R. rhabarbarum* (“edible rhubarb”), while *R. officinale* (“*da huang*”) roots were used as control. For analysis, 200 mg of freeze-dried powder from different plant parts (leaf, stalk, and root of *R. rhabarbarum*, and root of *R. officinale*) were extracted with 50 mL of 70% EtOH for 24 h and condensed using vacuum evaporator. The antioxidant properties of the extracts were evaluated using ABTS assay. Edible rhubarb root extract showed strong ABTS⁺ free radical scavenging activity, which was equal to that of *da huang* root extract (20 mg/mL). However, the antioxidant activity of the leaf extract was higher than that of the stalk extract. The anti-inflammatory effect was determined using nitric oxide (NO) assay with LPS-induced RAW 264.7 macrophages. NO production by activated macrophages significantly decreased with an increasing concentration of extracts of the different edible rhubarb parts and *da huang* roots. Cell toxicity was determined using MTS assay. The extracts were not significantly toxic to RAW 264.7 cells. These findings confirm that edible rhubarb extracts have beneficial properties, but further research is required to assess its potential as a functional material, for example, a natural component of anti-inflammatory medicine.

Keywords rhubarb, *Rheum rhabarbarum*, antioxidant, anti-inflammatory, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS)



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[†]These authors contributed equally to this study.

***Corresponding author**
Yong-Sun Moon
Tel: +82-53-810-2946
E-mail: hangulmys@ynu.ac.kr

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1. Introduction

Rhubarb is a perennial plant from the genus *Rheum*. It has short and thick rhizome, and is characterized by a large inverted triangular leaf on a long petiole called the stalk (Clementi and Misiti, 2010). It is native to the mountainous and desert regions of the Tibetan Plateau in China, and also grows in Asia and North America, with approximately 60 species worldwide (Li et al., 2003; Wang et al., 2005). The root of *Rheum officinale* (called *da huang* in China) and *R. undulatum*

(called *jong da huang* in Korea) is commonly used in medicine (Khattak et al., 2020). Anthraquinone, rhein, emodin, aloe-emodin, chrysophanol, and physcion have been identified as the main medicinal components of rhubarb root (Aichner and Ganzera, 2015; Zheng et al., 2013). These compounds have strong anti-bacterial (Lu et al., 2011), anti-cancer (Huang et al., 2007), anti-inflammatory (Tang et al., 2007), and antioxidant (Cai et al., 2004) properties. Several studies have focused on the pharmacological functions of rhubarb root, such as its role in nerve cell protection in the brain (Li et al., 2019; Lu et al., 2015; Wang et al., 2016).

In the West, rhubarb (*R. rhabarbarum*) stalk, which is rich in dietary fiber, anthocyanins, and the flavonol quercetin and its derivatives, is mainly consumed raw, as a vegetable (Pussa et al., 2009; Takeoka et al., 2012), and in wine, jam, and juice. After adding sugar and heating, the sour stalk (pH 3.0-3.6) tastes sweet. Therefore, it is a popular ingredient of pies and tarts. Hence, rhubarb is commonly called a pie vegetable (Clementi and Misiti, 2010). While rhubarb leaf contains a large amount of oxalic acid and is not edible, it is commonly used as a natural pesticide (Pucher et al., 1938). Unlike medicinal rhubarb (*da huang*), which has been studied extensively, studies on the physiological and chemical activities of edible rhubarb are lacking.

Free radicals generated during normal metabolic processes in the body are highly reactive and easily oxidize the surrounding substances. Oxygen compounds derived from this process (for example, H_2O_2 , $OH\cdot$, OH^- , and O_2^-) are called reactive oxygen species (ROS) (Liou and Storz, 2010). A moderate amount of ROS prevents pathogen infection and facilitates intracellular signaling

(Valko et al., 2006). However, when ROS overwhelm the cellular antioxidant defenses, oxidative stress ensues (Charde et al., 2012), which results in oxidative and tissue damage, with cell destruction. This potentially harmful effect of ROS limits cellular antioxidant activity, enhancing cell aging and inflammation, and increasing the incidence of various cancers (Aruoma, 1998).

Inflammation is a local defense mechanism that protects the body from physicochemical assault, such as bacteria and foreign substances, and involves various biochemical phenomena (Lordan et al., 2019). Macrophages are important immune control cells that are involved in the detection, phagocytosis, and destruction of bacteria and other harmful organisms, and also in repair processes, such as muscle regeneration, wound healing, and others (Caputa et al., 2019; St Pierre and Tidball, 1994). Inflammation is initiated by the release of ROS such as NO , NO_2 , and NO_3^- (Brune et al., 2003; Qian and Pollard, 2010). NO , an indicator of macrophage activation, is a free radical formed from l-arginine by NO synthase (NOS) (Rosenfeld et al., 2012). Upon excessive accumulation, reactive forms of nitrogen, such as NO , act as an oxidative trigger in the body, causing inflammatory reactions and cell damage (Sharma et al., 2007). Inflammatory cytokines and lipopolysaccharides promote the activation of nuclear factor- κB , which enhances the expression of inflammatory reaction-induced genes, such as inducible NOS (iNOS), and cyclooxygenase 2 (COX2) (Posadas et al., 2000). When NO reacts with ROS, it becomes a powerful oxidizer, causing various diseases, such as diabetes (Assmann et al., 2016), atherosclerosis (Forstermann, 2010), stomach cancer (Cheng and Fan, 2013), and uterine cancer (Elsehemy et al., 2016). There is substantial interest in the different effects of ROS

in the human body, and physiologically active substances that affect ROS generation and detoxification.

In the current study, we evaluated the antioxidant properties of the leaf, stalk, and root extracts of edible rhubarb (*R. rhabarbarum*). To investigate its potential as a medicinal material, we compared the antioxidant and anti-inflammatory properties of these extracts with those of *da huang* root extracts as a control. We show that edible rhubarb root has strong free-radical scavenging activity, equivalent to that of *da huang* root extract. It also reduces NO production by activated RAW 264.7 macrophages, indicating a promising anti-inflammatory activity. Hence, the potential use of edible rhubarb as a functional material should be investigated further.

2. Materials and methods

2.1. Plant material and sample preparation

Edible rhubarb (*R. rhabarbarum*) was grown in 30-cm pots in commercial soil with perlite in a greenhouse at the College of Applied Biotechnology Science, Yeungnam University (Gyeongsan, Korea). Three-year-old rhubarb was harvested, cleaned, and separated into roots, stalks, and leaves. Each part was individually squeezed to produce juice using a juice extractor (ZN35, Tefal, Rumilly, France) at room temperature (RT). The juice was purified using filter paper (No. 2, Advantec, Tokyo, Japan) and frozen at -20°C . Frozen juice was then individually freeze-dried to obtain a powder, for 48 h, using a freeze-dryer (FDS8508, Ilshinbiobase, Yangju, Korea). Dried Chinese *da huang* root was imported from China by Herb pharm (www.herb-pharm.co.kr) and dried Korean *da huang* (Dong Il, Yeong Cheon, Korea) was obtained from Hwa sang

dang store. Then, 3–5 g of dried roots were ground using a grinder (HR3752, Philips, Amsterdam, Netherlands) and then made into an even finer powder using liquid nitrogen. Next, 200 mg of individual samples were extracted with 70% EtOH in a shaking incubator (VS-203D, Vison Scientific Co., Daejeon, Korea) for 24 h at RT. The extracted samples were filtered with Whatman[®] filter papers (No. 2) and condensed using a rotary evaporator (EYEL4, Rikokai, Tokyo, Japan). The concentration of extract stocks was 50 mg/mL in 70% EtOH.

2.2. Analysis of ABTS⁺ free radical scavenging activity

Free-radical scavenging activity assay was performed using the method developed by Lee and Moon (2019). 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was from Wako Pure Chemical Ind. Ltd. (Osaka, Japan); potassium persulfate and dimethyl sulfoxide (DMSO) were from Sigma-Aldrich (St. Louis, MO, USA); and EtOH was from Merck (Darmstadt, Germany). For the assay, 7 mM of ABTS was dissolved in sterilized H₂O and potassium persulfate was added for a 2.45 mM solution. The solution was stored at RT for 16 h in the dark to generate ABTS free radicals. The ABTS⁺ stock was wrapped with aluminum foil and stored at -20°C . Immediately prior to the actual experiment, the ABTS⁺ stock was diluted using sterilized H₂O for an optical density (OD) value of 0.6 at 734 nm. When ABTS reacts with potassium persulfate, it is oxidized to turquoise-colored free radicals (ABTS⁺), which can be converted to ABTS by antioxidants. The prepared rhubarb extracts amples (50 mL) were placed in wells of a 96-well plate (3680, Corning Inc., Corning, NY, USA). Then, 50 mL of ABTS⁺ stock was added and allowed to react for 5 min at RT. Next, sample OD was measured at 734 nm using a microplatereader (UVM340, Biochrom,

Cambridge, UK). ABTS⁺ scavenging ability was determined using the following equation:

$$\text{ABTS}^+ \text{ free radical scavenging activity (\%)} \\ = \{1 - (S - SC) / (BS - BC)\} \times 100$$

S: sample (sample / ABTS⁺)

SC: control (H₂O / ABTS⁺)

BS: blank of sample (sample / H₂O)

BC: blank of control (H₂O / H₂O)

2.3. RAW 264.7 macrophage preparation

RAW 264.7 macrophages were obtained from the National Institute for Korean Medicine Development (NIKOM; Gyeongsan, Korea). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM; HyClone, Cytiva, Marlborough, MA, USA) containing 10% fetal bovine serum (FBS; HyClone, Cytiva) and 1% penicillin-streptomycin (Welgene, Gyeongsan, Korea), in a T25 flask (CytoOne T25 TC flask, USA Scientific, Ocala, FL, USA). The cells were incubated at 37°C in a 5% CO₂ incubator (MCO-17AIC, Sanyo, Osaka, Japan). When they reached 70-80% confluency, they were collected using a cell scraper (Celltreat, Pepperell, MA, USA). The collected cells were mixed with 10 mL of trypan blue (Sigma-Aldrich) at a 1:1 ratio and stained for 5 min. The cells were counted using a hemocytometer. Different concentrations of macrophages were prepared in DMEM for further experiments, as described.

2.4. NO detection using Griess assay

NO production by RAW 264.7 macrophages was measured using a modified Griess assay (Kim et al., 2013). The cells were placed in a 96-well cell plate (cell culture plate, Thermo Fisher Scientific, Waltham, MA, USA) and diluted in DMEM to 5 × 10⁴ cells well. Each well containing 160 μL of cells was

incubated in a 5% CO₂ incubator at 37°C for 24 h, and then subcultured in fresh DMEM. The cells were treated with 20 μL of 1 mg/mL LPS (Sigma-Aldrich) for 6 h to activate them. Induced cells were treated with 20 μL of each rhubarb extract and incubated in a 5% CO₂ incubator at 37°C for 12 h. Next, 100 μL of the cell solution were transferred to a new 96-well plate for the Griess assay (Promega, Madison, WI, USA). Cells were treated with 50 μL of 1% sulfanilamide solution (in 5% phosphoric acid) for 5 min in the dark, followed by the addition of 50 μL of 0.1% *N*-1-naphthyl-ethylene-diamine dihydrochloride (NED) solution for an additional 15 min. Sample absorbance was read at 540 nm using a microplate reader (Epoch, BioTek, Winooski, VT, USA). The NO production in each sample was normalized by the absorbance value of LPS-treated cells as a control. The readings with the Korean rhubarb root sample were used as a reference.

2.5. Evaluation of macrophage viability using the MTS assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay is routinely used to test cell viability, cell proliferation, or cytotoxicity. However, it has several shortcomings, such as cell loss during washing and harvesting, and the utilization of DMSO, which is toxic to eukaryotic cells. 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay compensates for these shortcomings and can be used to measure cell viability more accurately than MTT assay, using a colored formazan dye that is soluble in cell culture media. Accordingly, MTS assay was conducted using the CellTiter 96[®] AQueous One kit (G3580, Promega). RAW 264.7 macrophages (180 μL) were dispensed in a 96-well

microplate (1×10^4 cells well). The macrophages were stabilized at 37°C for 24 h in a 5% CO_2 incubator. Then, 20 mL of rhubarb extract were added per well, and the cells incubated for additional 16 h. Next, 10 mL of MTS were added per well and the incubation continued for 3 h to allow red formazan formation. Sample absorbance was measured using a microplate reader (Epoch, BioTek) at 490 nm. To investigate cell toxicity of the rhubarb extracts, cell viability was also determined without extract addition. The readings with the Korean rhubarb root sample were used as a reference.

2.6. Statistical analyses

All experiments were conducted in triplicate. Statistical analysis was performed using IBM SPSS Statistics version 21.0 (IBM, New York, NY, USA). One-way analysis of variance (ANOVA) was used, followed by Duncan's multiple range test for multiple comparisons. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Rhubarb extracts in 70% EtOH show high free-radical scavenging ability

The ABTS^+ -scavenging ability of different concentrations of medicinal Chinese *da huang* root (MCR), medicinal Korean *da huang* root (MKR), and edible rhubarb root (ERR) in 70% ethanol extract is shown in Fig. 1. At relatively low concentrations (0.5–5 mg/mL), the free radical reduction rate of MCR was significantly higher than that of MKR and ERR ($p < 0.05$). However, no statistical difference in the free-radical scavenging capacities of MCR and MKR was noted at a concentration of 10 mg/mL. We were unable to unambiguously conclude that

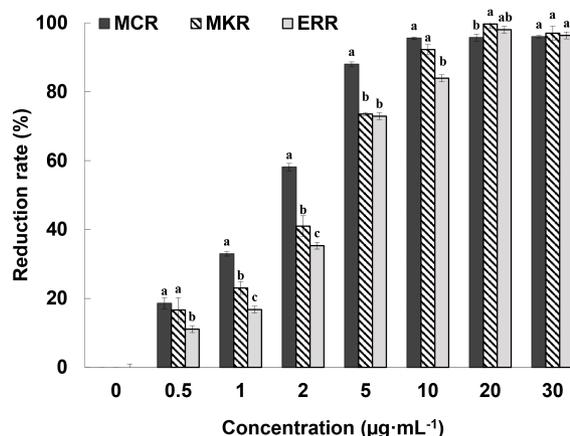


Fig. 1. ABTS free radical scavenging ability of 70% ethanol extracts of rhubarb roots. The different letters indicate statistical differences at $p < 0.05$ using Duncan's multiple range test. Each value represents the mean \pm SD of triplicates. MCR, medicinal Chinese *da huang* root; MKR, medicinal Korean *da huang* root; ERR, edible rhubarb root.

the free-radical scavenging ability of MCR is higher than that of MKR as the age of plants used for the preparation of MCR and MKR was unspecified by the manufacturer. At concentrations of 20 mg/mL and above, MCR and MKR both demonstrated 100% reduction of free radicals, thus confirming the lack of statistically significant difference between the samples. In addition, the free-radical reduction rate of ERR was the same as that of MCR and MKR, indicating high antioxidant capacity of ERR.

The free-radical scavenging ability of 70% EtOH extracts of ERR, edible rhubarb leaf (ERL), and edible rhubarb stalk (ERS) is shown in Fig. 2. ERR showed 100% ABTS^+ reduction ability at concentrations of 25 mg/mL and above, whereas ERL and ERS showed 100% ABTS^+ reduction rate at concentrations above 200 mg/mL and 300 mg/mL, respectively. Using DPPH assay, Raudsepp et al. (2013) reported that the antioxidant activity depends on the extraction solvent used, with the highest activity for blue honeysuckle berries extracted with buffered water and Siberian rhubarb

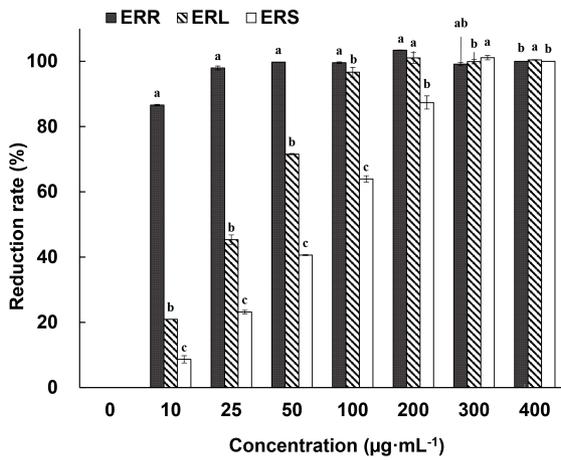


Fig. 2. ABTS free radical scavenging ability of different parts of edible rhubarb. The different letters indicate statistical differences at the $p < 0.05$ using Duncan's multiple range test. Each value represents the mean \pm SD of triplicates. MCR, medicinal Chinese *da huang* root; MKR, medicinal Korean *da huang* root; ERR, edible rhubarb root.

(*R. rhaponticum*) stalk in 30% EtOH and rhubarb roots showed the highest antibacterial effect. Ozturk et al. (2007) used the DPPH assay to test edible rhubarb (*R. ribes*) consumed in Turkey. In that study, methanol extracts of the root and stalk were tested, at a concentration of 100 mg/mL. In the cited study, the free-radical scavenging activity

of the stalk extract (87.1%) was higher than that of the root extract (60.1%) ($p < 0.05$), which differs from the observations described in the current study. This difference can be explained by the findings of Komatsu et al. (2006), who noted several differences in the active ingredients of rhubarb associated with the differences in cultivation area and genetic differences. We propose that the differences noted herein could be explained by the different subspecies tested (*R. rhabarbarum* vs. *R. ribes*), or extraction solvents used (ethanol vs. methanol).

3.2. Edible rhubarb extracts in 70% EtOH suppress NO production by LPS-induced RAW 264.7 macrophages

As shown in Fig. 3(A), MKR suppressed NO production by activated macrophages to 35% that of the positive control at a concentration of 30 mg/mL, and ERR suppressed NO production to 38% at a concentration of 40 mg/mL. These results are encouraging, as both MKR and ERR suppressed NO production to the negative control level. When activated RAW 264.7 macrophages were exposed to 70% EtOH extracts of ERL and ERS, we observed

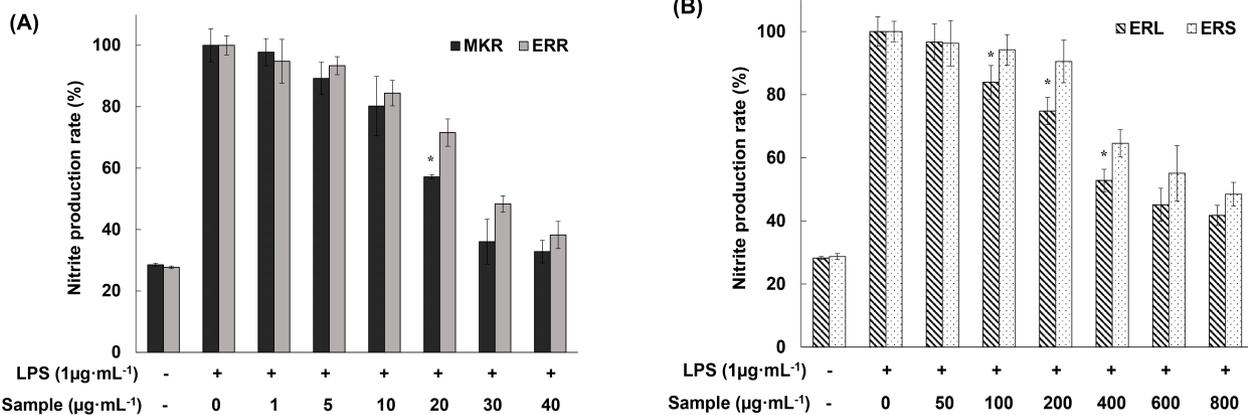


Fig. 3. Anti-inflammatory properties of rhubarb using NO assay. (A) Inflammation was induced by LPS in RAW 264.7 cells (5×10^4 cells well). After a 12 h treatment, NO production rate was measured. Anti-inflammatory effect of rhubarb root were compared with Korean *da huang* root as a control. (B) Rhubarb leaf (ERL) and stalk (ERS) were analyzed. The asterisk (*) indicates statistical differences at $p < 0.05$ by t-test. Each value represents the mean \pm SD of triplicates. MKR, medicinal Korean *da huang* root; ERR, edible rhubarb root; ERL, edible rhubarb leaf; ERS, edible rhubarb stalk.

suppression of NO production at a concentration of 100 mg/mL and above, with the suppression efficiency depending on the extract concentration (Fig. 3(B)). Collectively, these experiments revealed similar suppression level of NO production in activated RAW 264.7 macrophages by extracts of all edible rhubarb parts, and also demonstrated high free-radical scavenging ability of these extracts. Choi et al. (2014) reported that stilbene derivatives in 70% EtOH extract of MKR (*R. undulatum*) down regulate the expression of iNOS in RAW 264.7 cells with an enhanced anti-inflammatory effect. Accordingly, we propose that the ERR extract contains phenolic compounds or derivatives, and that these compounds inhibit NO production and, consequently, support the anti-inflammatory effect of the extract.

As described above, at the phytochemical level, 70% EtOH extracts of ERR, ERL, and ERS showed high antioxidant activity. Next, to confirm their anti-inflammatory effect at the cellular level, we investigated their ability to inhibit NO production by LPS-activated RAW 264.7 macrophages. LPS is a known toxin produced by gram-negative bacteria that induces inflammatory responses in cells. In the current study, 100% NO production in positive control (LPS added) was detected indicating an inflammatory induction, while 30% NO production was detected in negative control (no LPS added).

3.3. Rhubarb extracts in 70% EtOH do not impair viability of LPS-induced RAW 264.7 macrophages

In this study, we verified that edible rhubarb root (ERR) has the same, high, antioxidant properties and the ability to inhibit NO production by activated macrophages as the roots of medicinal rhubarb (*da huang*) used in Korea (MKR) and China

(MCR). We also confirmed that the extracts of each part of edible rhubarb exert anti-inflammatory effect on activated macrophages most likely by reducing the production of inflammatory cytokines and various inflammatory mediators that initiate the inflammatory response.

To check whether the decrease in NO production by activated macrophages observed upon exposure to rhubarb extracts was associated with cell death caused by toxins present in the extracts or the antioxidant activity of the extracts, we analyzed cell viability using the MTS assay.

We observed 85% cell viability upon exposure to 70% EtOH extracts of MKR and ERR at concentrations of 30 mg/mL and lower (Fig. 4(A)). This demonstrated that these extracts are not cytotoxic at certain concentrations, and that the inhibition of NO production by activated macrophages was associated with the anti-inflammatory effects of these extracts but not with cytotoxicity. In addition, we observed over 90% cell viability upon exposure to 70% EtOH extracts of ERL and ERS at concentrations of 100 μ g/mL and lower (Fig. 4(B)). Cell proliferation was assessed as the concentration of the extracts increased.

4. Conclusions

Even though many studies related to rhubarb have been published, most of them focus on *da huang* (*R. officinale*), used as an oriental medicinal herb, and not on *R. rhabarbarum*. Since edible parts of these plants are different, i.e., roots for *da huang* and stalks for edible rhubarb, we compared specific activities of the extracts of these different parts to examine the potential of their usage. We showed that the free-radical scavenging activity of the root of edible rhubarb is pronounced and as

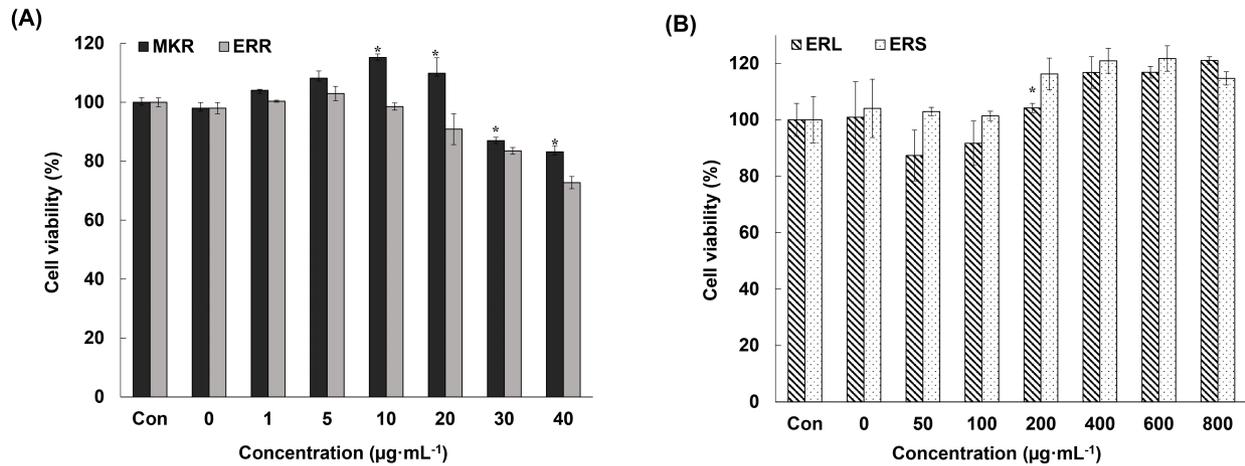


Fig. 4. Cell viability of rhubarb using MTS assay. (A) Inflammation was induced by LPS in RAW 264.7 cells (5×10^4 cells/well). After 3 h incubation, color change of MTS from clear to red due to live mitochondrial activity was measured. Cell viability of rhubarb root was compared with Korean *da huang* as a control. (B) Rhubarb leaf and stalk were analyzed. The asterisk (*) indicates statistical differences at $p < 0.05$ by t-test. Each value represents the mean \pm SD of triplicates. Con, untreated healthy cell as control; MKR, medicinal Korean *da huang* root; ERR, edible rhubarb root; ERL, edible rhubarb leaf; ERS, edible rhubarb stalk.

high as that of *da huang* root. In addition, edible rhubarb extracts dramatically reduced NO production by activated macrophages, indicating a good anti-inflammatory effect. Hence, the edible rhubarb could potentially become a new vegetable crop in Korea. In addition, leftover (leaves and stalks) from *da huang* root harvest could be used as potential functional materials.

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Conflict of interests

The authors declare no potential conflicts of interest.

Author contributions

Conceptualization: Moon YS. Data curation: Lee HH. Formal analysis: Lee HH, Moon YS. Methodology: Lee HH. Writing - original draft: Lee HH, Yu JK. Writing

- review & editing: Yu JK, Moon YS.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

ORCID

Ho-Hyeon Lee (First author)

<https://orcid.org/0000-0002-0893-3927>

Ju-Kyung Yu (First author)

<https://orcid.org/0000-0002-8304-9031>

Yong-Sun Moon (Corresponding author)

<https://orcid.org/0000-0002-1697-4518>

References

- Aichner D, Ganzera M. Analysis of anthraquinones in rhubarb (*Rheum palmatum* and *Rheum officinale*) by supercritical fluid chromatography. *Talanta*, 144, 1239-1244 (2015)
- Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. *J Am Oil Chem Soc*, 75, 199-212 (1998)

- Assmann TS, Brondani LA, Bouas AP, Rheinheimer J, de Souza BM, Canani LH, Bauer AC, Crispim D. Nitric oxide levels in patients with diabetes mellitus: A systematic review and meta-analysis. *Nitric Oxide*, 61, 1-9 (2016)
- Brne B, Zhou J, Knethen AV. Nitric oxide, oxidative stress, and apoptosis. *Kidney Int*, 63, S22-S24 (2003)
- Cai Y, Sun M, Xing J, Corke H. Antioxidant phenolic constituents in roots of *Rheum officinale* and *Rubia cordifolia*: Structure-radical scavenging activity relationships. *J Agric Food Chem*, 52, 7884-7890 (2004)
- Caputa G, Flachsmann LJ, Cameron AM. Macrophage metabolism: A wound-healing perspective. *Immunol Cell Biol*, 97, 268-278 (2019)
- Charde M, Shukla A, Bukhariya V, Mehta J, Chakole R. Herbal remedies as antioxidants: An overview. *Int J Pharmacol Res*, 1, 25-34 (2012)
- Cheng J, Fan XM. Role of cyclooxygenase-2 in gastric cancer development and progression. *World J Gastroenterol*, 19, 7361-7368 (2013)
- Choi RJ, Chun J, Khan S, Kim YS. Desoxyrhapontigenin, a potent anti-inflammatory phytochemical, inhibits LPS-induced inflammatory responses via suppressing NF- κ B and MAPK pathways in RAW 264.7 cells. *Int Immunopharmacol*, 18, 182-190 (2014)
- Clementi EM, Misiti F. Potential health benefits of rhubarb. In: *Bioactive Foods in Promoting Health: Fruits and Vegetables*, Watson R, Preedy V (Editors), Academic Press, Cambridge, MA, USA, p 407-423 (2010)
- Day BJ. Catalase and glutathione peroxidase mimics. *Biochem Pharmacol*, 77, 285-296 (2009)
- Elsehemy A, Postovit LM, Fu Y. Nitric oxide signaling in human ovarian cancer: A potential therapeutic target. *Nitric Oxide*, 54, 30-37 (2016)
- Forstermann U. Nitric oxide and oxidative stress in vascular disease. *Eur J Physiol*, 459, 923-939 (2010)
- Huang Q, Lu G, Shen HM, Chung MCM, Ong CN. Anti-cancer properties of anthraquinones from rhubarb. *Med Res Rev*, 27, 609-630 (2007)
- Khattak AK, Hassan SM, Mughal SS. General overview of phytochemistry and pharmacological potential of *Rheum palmatum* (Chinese rhubarb). *Innovare J Ayuvedic Sci*, 8, 1-5 (2020)
- Kim DH, Hwang EY, Son JH. Anti-inflammatory activity of *Carthamus tinctorious* seed extracts in RAW 264.7 cells. *J Life Sci*, 23, 55-62 (2013)
- Komatsu K, Nagayama Y, Tanaka K, Ling Y, Cai SQ, Omote T, Meselhy MR. Comparative study of chemical constituents of rhubarb from different origins. *Chem Pharm Bull*, 54, 1491-1499 (2006)
- Lee HH, Moon YS. Assessment of important factors influencing consistent and accurate ABTS assay. *J Korean Soc Food Sci Nutr*, 48, 390-393 (2019)
- Li A, Bao B, Grabovskaya-Borodina AE, Hong SP, McNeil J, Mosyakin SL, Ohba H, Park CW. Ulmaceae through Basellaceae. In: *Flora of China*, Wu ZY, Raven PH, Hong DY (Editors), Missouri Botanical Garden Press, St. Louis, MO, USA, p 277-350 (2003)
- Li X, Chu S, Liu Y, Chen N. Neuroprotective effects of anthraquinones from rhubarb in central nervous system disease. *Evid Based Complementary Altern Med*, 2019, 1-12 (2019)
- Liou GY, Storz P. Reactive oxygen species in cancer. *Free Radic Res*, 44, 479-496 (2010)
- Lordan R, Tsoupras A, Zabetakis I. Inflammation. In: *The Impact of Nutrition and Statins on Cardiovascular Diseases*, Zabetakis I, Lordan R, Tsoupras A (Editors), Academic Press, Cambridge, MA, USA, p 23-51 (2019)
- Lu C, Wang H, Lv W, Xu P, Zhu J, Xie J, Liu B, Lou Z. Antibacterial properties of anthraquinones extracted from rhubarb against *Aeromonas hydrophila*. *Fish Sci*, 77, 375-384 (2011)
- Lu K, Zhang C, Wu W, Zhou M, Tang Y, Peng Y. Rhubarb extract has a protective role against

- radiation-induced brain injury and neuronal cell apoptosis. *Mol Med Rep*, 12, 2689-2694 (2015)
- Ozturk M, Ozturk FA, Duru ME, Topu G. Antioxidant activity of stem and root extracts of Rhubarb (*Rheum ribes*): An edible medicinal plant. *Food Chem*, 103, 623-630 (2007)
- Posadas I, Terencio MC, Guillen I, Ferrndiz ML, Coloma J, Paya M, Alcaraz MJ. Co-regulation between cyclo-oxygenase-2 and inducible nitric oxide synthase expression in the time-course of murine inflammation. *Naunyn-Schmiedeberg's Arch Pharmacol Pharmacol*, 361, 98-106 (2000)
- Pucher GW, Wakeman AJ, Vickery HB. The organic acids of rhubarb (*Rheum hybridum*): III. The behavior of the organic acids during culture of excised leaves. *J Biol Chem*, 126, 43-54 (1938)
- Pussa T, Raudsepp P, Kuzina K, Raal A. Polyphenolic composition of roots and petioles of *Rheum rhaponticum* L. *Phytochem Anal*, 20, 98-103 (2009)
- Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell*, 141, 39-51 (2010)
- Raudsepp P, Anton D, Roasto M, Meremae K, Pedastsaar P, Maesaar M, Raal A, Laikoja K, Pussa T. The antioxidative and antimicrobial properties of the blue honeysuckle (*Lonicera caerulea* L.), Siberian rhubarb (*Rheum rhaponticum* L.) and some other plants, compared to ascorbic acid and sodium nitrite. *Food Control*, 31, 129-135 (2013)
- Rosenfeld R, Garcin E, Arvai A, Tainer J, Getzoff E. Nitric oxide synthases in motion. *Nitric Oxide*, 27, S25 (2012)
- Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology*, 15, 252-529 (2007)
- St Pierre BA, Tidball JG. Differential response of macrophage subpopulations to soleus muscle reloading after hindlimb suppression. *J Appl Physiol*, 77, 290-297 (1994)
- Takeoka GR, Dao L, Harden L, Pantoja A, Kuhl JC. Antioxidant activity, phenolic and anthocyanin contents of various rhubarb (*Rheum* spp.) varieties. *Int J Food Sci Technol*, 48, 172-178 (2012)
- Tang T, Yin L, Yang J, Shan G. Emodin, an anthraquinone derivative from *Rheum officinale* Baill, enhances cutaneous wound healing in rats. *Eur J Pharmacol*, 567, 177-185 (2007)
- Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact*, 160, 1-40 (2006)
- Wang A, Yang M, Liu J. Molecular phylogeny, recent radiation and evolution of gross morphology of the rhubarb genus *Rheum* (Polygonaceae) inferred from chloroplast DNA trnL-F sequences. *Ann Bot*, 96, 489-498 (2005)
- Wang Y, Fan X, Tang T, Fan R, Zhang C, Huang Z, Peng W, Gan P, Xiong X, Huang W, Huang X. Rhein and rhubarb similarly protect the blood-brain barrier after experimental traumatic brain injury via gp91phox subunit of NADPH oxidase/ROS/ERK/MMP-9 signaling pathway. *Sci Rep*, 6, 1-13 (2016)
- Zheng QX, Wu HF, Guo J, Nan HJ, Chen SL, Yang JS, Xu XD. Review of rhubarbs: Chemistry and pharmacology. *Chin Herb Med*, 5, 9-32 (2013)