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ORIGINAL ARTICLE

Antioxidant, α-glucosidase inhibitory and anti-inflammatory effects of aerial parts extract from Korean crowberry (*Empetrum nigrum* var. *japonicum*)

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Abstract Crowberry (*Empetrum nigrum* L.) is a wild berry commonly found in the northern hemisphere. Crowberry fruits have been suggested as good resources for functional applications in the cosmetic and pharmaceutical industries, but the high polyphenolic content in crowberry leaves also indicates crowberry aerial parts as potential dietary health supplements. In this study, therefore, the biological activities of the aerial parts of Korean crowberry (*E. nigrum* var. *japonicum*) were investigated. Antioxidant activity was measured by three different assays on DPPH free radical scavenging, reducing power, and total antioxidant capacities. Dose-dependent antioxidant activities were exhibited by crude methanol extract and its fractions, suggesting that the crude methanol extract and EtOAc fraction possessed strong antioxidant activities and capacities. In addition, the crude methanol extract and EtOAc strongly inhibited α-glucosidase activity and suppressed the secretion of pro-inflammatory mediator and nitrite oxide from LPS-stimulated RAW 264.7 cells. These findings provide valuable evidence for the potential of such parts as good dietary

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sources of natural antioxidant, α-glucosidase inhibitory, and anti-inflammatory components, suggesting that using the non-edible parts (e.g., leaves and stems) of crowberry can be a potential natural avenue for improving human health.

1. Introduction

From ancient times to the present, plants have been used not only as food, but also as materials for alternative medical therapy because of their nutritional properties (Kim and Kwon, 2011). Experimental and epidemiological evidences ascribe a wide array of positive health effects to the phytochemicals present in plants (Tapsell et al., 2006; Zhang et al., 2013). These effects have prompted worldwide interest in investigations into the pharmaceutical properties of plants and in the analyses of their phytochemicals; such studies are intended to promote healthcare, and the studied plants have been used as conventional or complementary medicines because they present lower toxicity and side effects than do synthetic drugs (Hyun et al., 2014).

Crowberry (*Empetrum nigrum* L.) is a small genus of dwarf evergreen shrubs and a wild berry with considerable potential as herbal medicine given its high and diverse phenolic content (Ogawa et al., 2008; Koskela et al., 2010). In Korea, crowberry has been used to promote eternal youth and control inflammatory diseases, including cystitis, nephritis, and urethritis (Park et al., 2012). The total antioxidant content in crowberry fruits is greater than that in blueberry and raspberry fruits (Halvorsen et al., 2002). In addition, the extract of crowberry fruits restores the activity of cellular antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, and heme oxygenase-1 (Kim et al., 2009); these results suggest that crowberry extract can suppress hydrogen peroxide- and ultraviolet B-induced cell damage (Kim et al., 2009, 2013). Furthermore, crowberry fruits contain higher levels of flavonols, which is the sum of quercetin, myricetin, and kaempferol, than do onion, kale, and broccoli (Häkkinen et al., 1999). These properties indicate that crowberry fruits are good candidates as daily foodstuff that protects the body from oxidative stress. Flavonols and benzoic acid derivatives are the most abundant soluble phenolic compounds in crowberry leaf (Väisänen et al., 2013). Polyphenolic compounds have been shown to have an array of antioxidant effects *in vitro* and *in vivo* (Alinezhad et al., 2013; Williams et al., 2004; Wootton-Beard et al., 2011). Such antioxidant activity of polyphenolic compounds plays an important role in preventing many health problems, such as cardiovascular diseases, diabetes, cancer, and obesity (Kumar and Pandey, 2013; Vuong et al., 2014; Yang et al., 2014). Therefore, the level and composition of polyphenolic compounds in vegetable are closely correlated with their pharmaceutical properties (Wootton-Beard et al., 2011). A detailed analysis of leaf extract has suggested that crowberry leaf contains kaempferol, quercetin, stilbenes, chlorogenic acid, protocatechuic acid, and epicatechin, which have various biological activities (Väisänen et al., 2013). This finding indicates the potential of crowberry aerial parts as crude drug and dietary health supplements, yet a significant attention has been focused on the pharmacological properties of crowberry fruits (Halvorsen et al., 2002; Ogawa et al., 2008; Kim et al., 2009, 2013).

To address this gap, we analyzed the biological activities of the aerial parts of Korean crowberry (*E. nigrum* var. *japonicum*).To better understand such biological activities, we determined the relationship between the amounts of phenols and flavonoids and the biological activities (including antioxidant and α-glucosidase inhibitory activities) of crude methanol (MeOH) extract and its fractions. We also determined the potential of Korean crowberry aerial parts as *in vitro* anti-inflammatory agents.

2. Materials and methods

2.1. Reagents

4-Nitrophenyl-α-D-glucopyranoside (*p*NPG), α-glucosidase (E.C. 3.2.1.20), acarbose, butylated hydroxytoluene (BHT), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade or better.

2.2. Plant materials and extraction

The aerial parts (leaves and stems) of crowberry were collected from Mt. Halla (in Jeju) at an altitude of about 3600 ft (N33.21.34.6/E126.27.47.5) in August 2012. The voucher specimen (JEJUA-5) was identified by Dr. Hyoun-Chol Kim and deposited in the Majors in Plant Resource and Environment at Jeju National University. The crowberry leaves and stems were air dried in darkness for 1 week at room temperature and ground into powder (approximately 0.5–1.0 mm), and then the ground material was exhaustively extracted with MeOH at room temperature for 24 h. After filtration, MeOH extract was evaporated using a rotary vacuum evaporator. The MeOH extract was suspended in water and sequentially partitioned with hexane, ethyl acetate (EtOAc), and n-butanol (BuOH). The remaining aqueous extract was used as aqueous fraction. Each fraction was evaporated using a vacuum evaporator, after which the dried MeOH extract and its fractions were re-dissolved in MeOH or water (for aqueous fraction) to a concentration of 1 mg/ml and stored at −20 °C until analysis.

2.3. Determination of total phenolic content

Total phenolic content was determined by Folin–Ciocalteu assay (Hyun et al., 2014). Briefly, the MeOH extract and its fractions were mixed with 50 μl of 2 N Folin–Ciocalteu reagent and incubated at room temperature for 5 min. After incubation, 0.3 ml of 20% sodium carbonate was added. The absorbance of the reaction mixtures was measured at 725 nm

by a spectrophotometer. Gallic acid was used as a standard, and the total polyphenol content of each sample was expressed in milligram gallic acid equivalent (mg GAE/g extract).

2.4. Determination of total flavonoid content

Total flavonoid content was determined by the colorimetric method (Hyun et al., 2014) using quercetin as a standard. Briefly, the test samples (0.5 ml) were mixed with 0.1 ml of 10% aluminum nitrate (w/v), 0.1 ml of 1 M potassium acetate, and 4.3 ml of 80% ethanol. After 40 min of incubation at ambient temperature, the absorbance of the supernatant was measured at 415 nm by using a spectrophotometer. The total flavonoid content was expressed as quercetin equivalent in milligram per gram extract (mg QE/g extract).

2.5. Determination of antioxidant activity

To determine the free radical scavenging activity of each sample, 0.15 M 1,1-diphenyl-2-picryl-hydrazyl (DPPH) in 4 ml of MeOH was mixed with different concentrations of each test sample. After incubation at 25 °C for 30 min, absorbance was measured at 517 nm using a spectrophotometer. The ability to scavenge the DPPH radical (three replicates per treatment) was calculated as described by Hyun et al. (2014).

The reducing power of each sample was determined according to the method of Hyun et al. (2014). Different concentrations of extracts (100, 200, and 300 μg/ml) were mixed with 0.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 0.5 ml of 1% potassium ferricyanide. The resultant mixture was incubated in a water bath at 50 °C. After 20 min, 2.5 ml of 10% trichloroacetic acid was added to the reaction mixture and centrifuged at 6500 rpm for 10 min. The supernatant (0.5 ml) was mixed with equal volumes of distilled water and 0.1 ml ferric chloride (0.1%, w/v). The intensity of the blue-green color was measured at 700 nm using a spectrophotometer.

The total antioxidant capacity assay of the samples was evaluated by the phosphomolybdenum method according to the procedure described by Prieto et al. (1999). Different concentrations of each sample (100, 200, and 300 μg/ml) were mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The reaction mixture was incubated at 95 °C for 90 min. Subsequently, absorbance was measured at 695 nm using a spectrophotometer. Ascorbic acid (AsA) and butylated hydroxytoluene (BHT) were used for comparison.

2.6. α-Glucosidase inhibitory effect

A 150 μl reaction mixture containing 50 μl α-glucosidase (0.5 U/ml), 50 μl 0.2 M potassium phosphate buffer (pH 6.8), and 50 μl of the investigated samples was thoroughly mixed. After incubation at 37 °C for 15 min, 3 mM p-nitrophenyl glucopyranoside (pNPG) as a substrate was added to the mixture and incubated at 37 °C for 10 min. The reaction was terminated by the addition of 750 μl of 0.1 M sodium carbonate solution, and then absorbance at 405 nm was recorded using a spectrophotometer. The control sample used was the solvent mixture instead of the test sample. In the sample blank (without substrate) and control blank (without substrate), α-glucosidase was used with buffer in the mixtures. The inhibition of α-glucosidase activity in the sample was calculated as follows:

$$\text{Inhibition rate } (\%) = \left[1 - \left(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}\right)/\text{Abs}_{\text{control}}\right] \times 100,$$

where $\text{Abs}_{\text{sample}}$ represents the absorbance of the experimental sample, $\text{Abs}_{\text{blank}}$ denotes the absorbance of the blank, and $\text{Abs}_{\text{control}}$ represents the absorbance of the control. Different concentrations of extracts (0.5–300 μg/ml) were tested for analyzing IC_{50} value. The IC_{50} value was defined as the extract concentration required to inhibit 50% α-glucosidase activity under the assay conditions.

2.7. Cell culture

The murine macrophage cell line RAW 264.7 was purchased from the Korean Cell Line Bank (KCLB; Seoul, Korea). The RAW 264.7 cells were cultured in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY, USA) supplemented with 100 U/ml of penicillin–streptomycin and 10% fetal bovine serum (GIBCO, Grand Island, NY, USA) at 37 °C in a humidified chamber containing 5% CO_2.

2.8. Determination of nitrite oxide levels

The RAW 264.7 cells were plated at 1.5×10^5 cells/ml in 24-well plates, and then incubated for 24 h with or without lipopolysaccharide (LPS, 1 μg/ml) in the absence or presence of various concentrations (50, 100, and 200 μg/ml) of the test samples. The nitric oxide (NO) accumulated in the culture medium was determined by the Griess reaction method (Granger et al., 1996). The cell culture medium (100 μl) was mixed with 100 μl of Griess reagent [1% sulfanilamide (w/v) and 0.1% (w/v) naphthylethylenediamine in 2.5% phosphoric acid (v/v)] and incubated at room temperature for 10 min. Optical density was measured at 540 nm, and the NO levels were calculated from a standard curve generated from sodium nitrite.

2.9. Lactate dehydrogenase cytotoxicity assay

To detect cytotoxicity, lactate dehydrogenase (LDH) activity was determined using a non-radioactive cytotoxicity assay kit (Promega, Madison, WI, USA). The RAW 264.7 cells (1.5×10^5 cells/ml) were treated for 24 h at 37 °C with LPS (1 μg/ml) mixed with various concentrations of the test samples. After centrifugation at 3000 rpm for 10 min, the cell-free culture medium (50 μl) was incubated with 50 μl of reconstituted substrate mix from the non-radioactive cytotoxicity assay kit for 30 min at room temperature in the dark. Then, the reaction was terminated by the addition of 50 μl stop solution from the kit, and absorbance was measured at 490 nm using a microplate reader (Bio-TEK Instruments Inc., Vermont, WI, USA). Percentage cytotoxicity was determined as relative to the control group. All the experiments were performed in triplicate.

2.10. Statistical analysis

All the experiments were conducted for three independent replicates, and data were expressed as mean ± standard

deviation. Statistical analyses were performed by ANOVA. Duncan's test was used to determine the significance of differences between the groups. Differences at $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Total phenolic and flavonoid contents in crowberry aerial part extract

In plants, phenolic and flavonoid compounds have acted as plant defense mechanisms against pathogens, parasites, and predators (Baidez et al., 2007); these compounds have exhibited antioxidant, anticancer, antimicrobial, antiviral, and anti-inflammatory activities (Kumar and Pandey, 2013; Vuong et al., 2014; Yang et al., 2014). In this study, the total phenolic content (TPC) and total flavonoid content (TFC) in the crude MeOH extract from crowberry aerial parts and its fractions were determined from the calibration curves of gallic acid ($y = 0.0079x + 0.0093$, $R^2 = 0.9998$) and quercetin ($y = 0.0026x - 0.0024$, $R^2 = 0.9996$), respectively. The highest TPC was exhibited by the crude MeOH extract (1170 ± 4 mg GAE/g), followed by the BuOH (950 ± 76 mg GAE/g) and EtOAc (817 ± 25 mg GAE/g) fractions, whereas the lowest TPC was exhibited by the hexane fraction (Table 1). The highest TFC was exhibited by the EtOAc fraction (346 ± 5.3 mg QE/g extract), followed by MeOH and BuOH. These results indicate that extraction using polar solvents more highly enriched polyphenolic content than did extraction using solvents with low polarity; thus, the selection of solvents for fraction extraction is an important factor in obtaining desirable phenolic compositions (Phang et al., 2013).

3.2. Antioxidant properties of extract prepared from crowberry aerial parts

Oxidative stress caused by reactive oxygen species plays a major role in many disease processes, including those of cardiovascular diseases, coronary heart disease, atherosclerosis, and insulin resistance (Alfadda and Sallam, 2012). Protecting the body against the consequences of oxidative stress may be achieved by improving the manner by which the available antioxidant nutrition in food is maximized. In this regard, scientific studies have suggested that antioxidant compounds from natural sources, such as fruits and vegetables, have higher bioavailability and lower side effects than do synthetic antioxidant agents (Benedetti et al., 2004; Atawodi, 2005). To investigate the antioxidant activity of crowberry extract, we determined the antioxidant activities of the crude MeOH extract and its fractions on the basis of DPPH free radical scavenging activity, reducing power, and total antioxidant capacity. As shown in Fig. 1, the antioxidant activities of the crude MeOH extract and its fractions increased in a dose-dependent manner. The highest free radical scavenging activity was observed in the EtOAc fraction (RC_{50} = 5.08 ± 0.15 μg/ml), followed by the crude MeOH extract (RC_{50} = 9.60 ± 0.69 μg/ml) and the BuOH fraction (RC_{50} = 10.72 ± 0.66 μg/ml extract) (Table 2). Using a reducing power assay, we then determined Fe^{3+}–Fe^{2+} transformation in the presence of the crude MeOH extract and its fractions. This assay is a robust and useful method for measuring antioxidant capacity

Table 1 Total phenolic content and total flavonoid content in (crowberry aerial parts) methanol crude extract and its fractions.

Extract and fractions	Total phenol (mg GAE/g)[a]	Total flavonoid (mg QE/g)[b]
MeOH extract	1170 ± 4[c]	117.1 ± 2.6
Hexane fraction	111 ± 10	8.9 ± 0.5
EtOAc fraction	817 ± 25	346.6 ± 5.3
BuOH fraction	950 ± 76	68.4 ± 2.6
Aqueous fraction	160 ± 8	10.8 ± 0

[a] Total phenolic content was analyzed as the gallic acid equivalent (GAE) mg/g of the extract; values are the average of triplicates.
[b] Total flavonoid content was analyzed as the quercetin equivalent (QE) mg/g of the extract; values are the average of triplicates.
[c] Each value represents the mean ± SD, and the means were significantly different as calculated from a paired Duncan's test, with $p < 0.05$.

(Moein et al., 2008). The assay results show that the reducing power of the EtOAc and MeOH extracts was higher than that of the other fractions (Fig. 1A). The 100-μg/ml EtOAc fraction exhibited an OD_{700} value of 0.53 ± 0.02, and the 300-μg/ml hexane fraction displayed an OD_{700} value of 0.18 ± 0.01. The hexane fraction revealed the lowest total antioxidant capacity, whereas the EtOAc fraction showed a relatively higher total antioxidant capacity than did the other samples (Fig. 1B). These results indicate that the EtOAc fraction exerted a stronger radical scavenging effect than did the other fractions. In fact, the EtOAc fraction of crowberry has been demonstrated to suppress hydrogen peroxide- and ultraviolet B-induced cell damage and γ-ray-induced apoptosis via the scavenging of reactive oxygen species and the enhancement or restoration of endogenous antioxidant enzyme (Kim et al., 2009, 2011, 2013). The EtOAc fraction and crude MeOH extract used in the current work showed better free radical scavenging activities than did BHT (RC_{50} = 43.74 ± 1.78 -μg/ml BHT), but exhibited lower reducing power than did BHT (Table 2 and Fig. 1A). These findings indicate that the antioxidant activity of crowberry extract may be due to its proton-donating abilities. It is widely assumed that the antioxidant property of plant extracts is related with the levels of total phenolic and flavonoid contents (Zou et al., 2012). In fact, polyphenolic compounds including kaempferol, quercetin, quercitrin, rutin, catechin, epicatechin, and others have been showing antioxidant effects *in vitro* and *in vivo* (Nabavi et al., 2012; Yu et al., 2012). For example, in the liver of rats, quercetin specifically elevates the activities of antioxidant enzymes including catalase, cupper/zinc superoxide dismutase, glutathione peroxidase, glutathione reductase, and Glutathione S transferase (Yu et al., 2012). These indicate that polyphenolic compounds are beneficial for the development of antioxidant agents. Therefore, to determine antioxidant agents in the aerial parts of Korean crowberry, it will be interesting to analyze the composition of phenolic compounds.

3.3. α-Glucosidase inhibitory activity of crowberry aerial part extract

Diabetes mellitus (DM) is a complex disease characterized by a metabolic disorder due to absolute or relative insulin deficiency, which leads to imbalanced carbohydrate, lipid, and protein

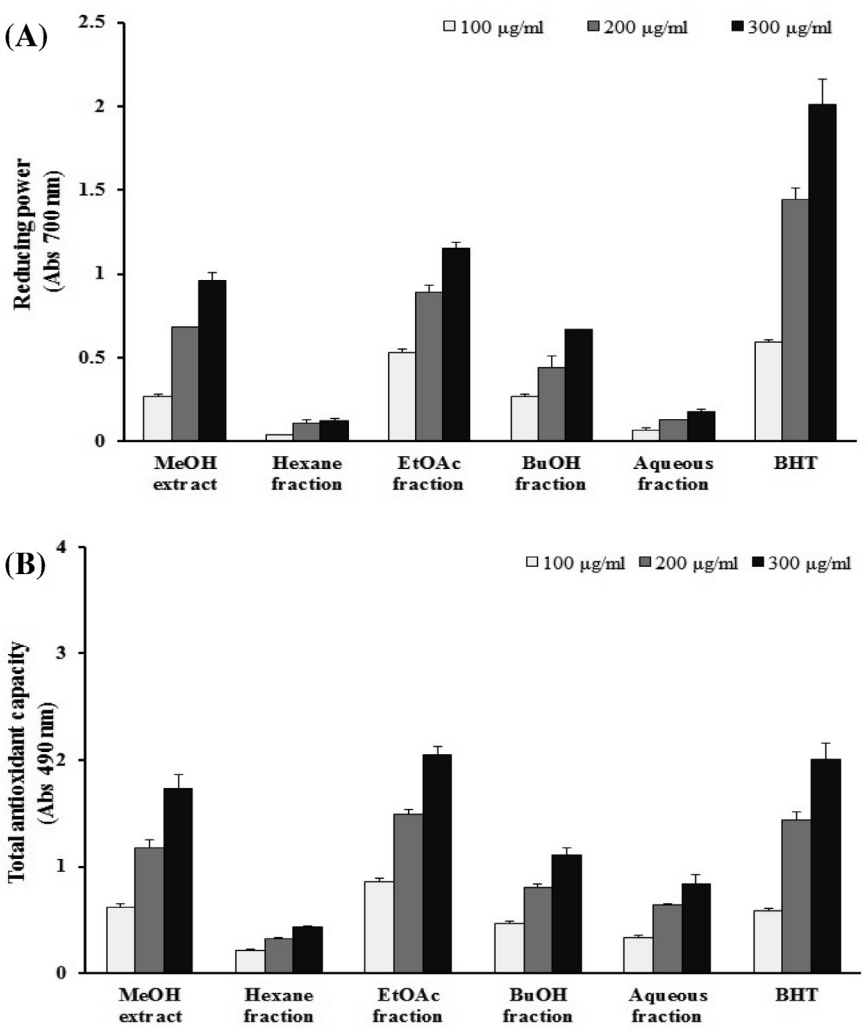

Figure 1 Antioxidant activity of crowberry (*E. nigrum* var. *japonicum*) aerial part extract. The antioxidant activity was measured on the basis of electron donation ability, as determined by reducing power (A), and total antioxidant capacity (B). Values are the average of triplicate experiments and represented as mean ± SD.

Table 2 DPPH free radical scavenging activity and α-glucosidase inhibition activity of crowberry aerial part extract.

Extract and fractions	IC_{50} (μg/ml)[a]	RC_{50} (μg/ml)[b]
MeOH extract	5.4 ± 0.3	9.60 ± 0.69
Hexane fraction	>100	52.32 ± 1.94
EtOAc fraction	0.6 ± 0.0	5.08 ± 0.15
BuOH fraction	4.3 ± 0.6	10.72 ± 0.66
Aqueous fraction	>100	77.99 ± 1.78
Acarbose	118.34 ± 6.09	
BHT		43.74 ± 1.78

[a] RC_{50}: Amount required for a 50% reduction of DPPH free radicals after 30 min.
[b] IC_{50}, 50% inhibition of α-glucosidase activity under assay conditions.

metabolism (Balaji et al., 2013; Kazeem et al., 2013); many DM patients suffer from serious complications, including nephropathy, retinopathy, neuropathy, and cardiovascular diseases (Jeong et al., 2013). The polyphenolic compounds in plants inhibit the activities of carbohydrate hydrolyzing enzymes, such as α-amylase and α-glucosidase, because of their ability to bind with proteins (Mai et al., 2007). As alternative antidiabetic drugs, several α-amylase and α-glucosidase inhibitors have recently been isolated from medicinal plants (Wang et al., 2013; Xiao et al., 2013). In the present study, the potential of crowberry as an antidiabetic supplement was investigated by using *p*NPG as a substrate in determining the α-glucosidase inhibitory activities of the crude MeOH extract and its fractions (Table 2). The EtOAc fraction with the highest inhibitory activity exhibited the lowest IC_{50} value at 0.6 ± 0.0 μg/ml. The second lowest values were produced by the BuOH fraction (IC_{50} = 4.3 ± 0.6 μg/ml) and the crude MeOH extract (IC_{50} = 5.4 ± 0.3 μg/ml). The hexane and aqueous fractions showed inhibitory effects that were lower than those displayed by the other samples. The high-level α-glucosidase inhibitory activity of the EtOAc fraction indicates that crowberry aerial parts extract can potentially reduce postprandial hyperglycemia by delaying carbohydrate digestion.

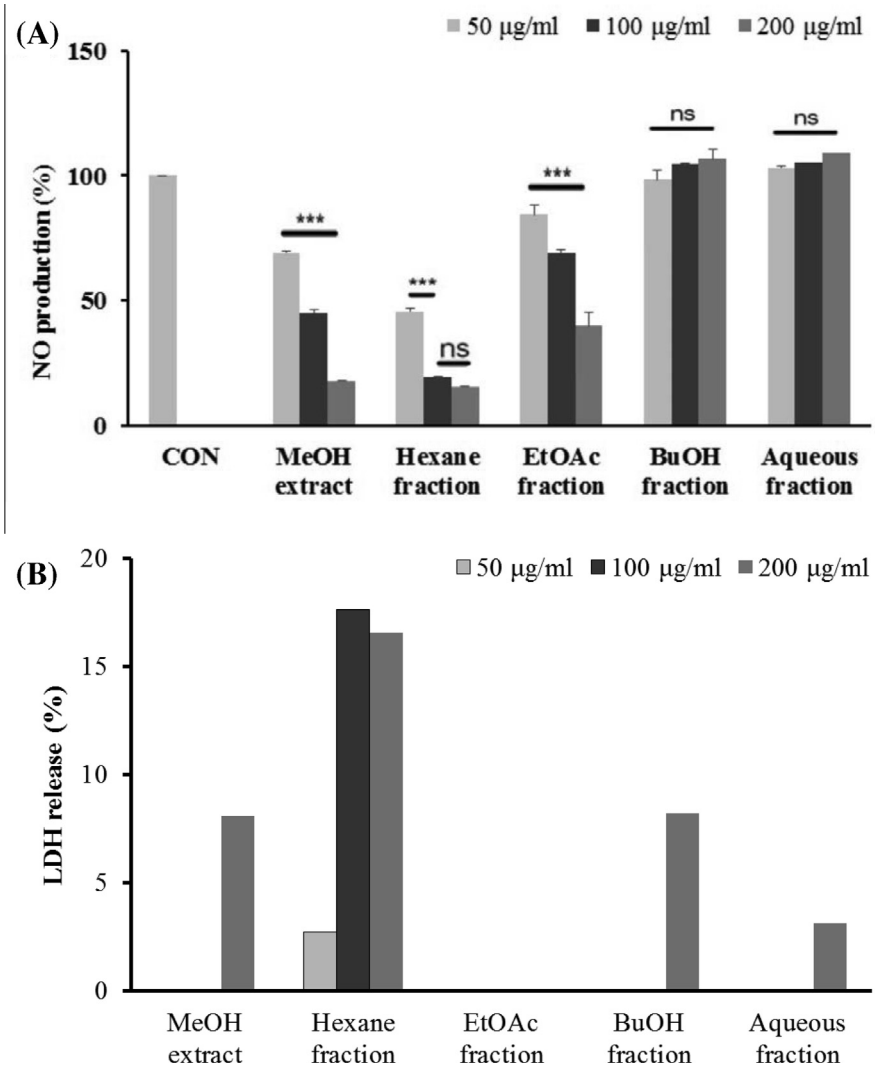

Figure 2 Effects of the crude extract and its fractions on NO production in LPS-stimulated RAW 264.7 cells. (A) NO production of RAW 264.7 cells stimulated by LPS. RAW 264.7 cells were incubated with LPS in the presence of the indicated concentration of the crude extract and its fractions for 24 h. The amounts of NO were determined using the Griess reagent in the culture medium. (B) Influence of the crude extract and its fraction on the viability of LPS-stimulated RAW 264.7 cells. Cytotoxicity was determined by the LDH method. Values are the mean ± SD of triplicate experiments; $^{***}p < 0.001$.

3.4. Anti-inflammatory activity of crowberry aerial part extract

Inflammation is the normal immune response to infection or tissue injury, and it plays a causative role in the development of various diseases, including rheumatoid arthritis, asthma, and atherosclerosis (Mueller et al., 2010). Although a number of steroidal or non-steroidal anti-inflammatory drugs have been developed, plant-derived drugs have been key medicines for the management of inflammatory diseases (Fürst and Zündorf, 2014). Pharmacological statistics show that 12 out of the 40 anti-inflammatory drugs approved between 1983 and 1994 worldwide are based on the phytochemicals present in plants (Daniela et al., 2012). Crowberry has long been used in folk medicine to treat inflammatory diseases (Park et al., 2012). Thus far, however, no scientific evidence supports the anti-inflammatory activity of the plant. Macrophages are a major source of various pro-inflammatory mediators, such as NO and prostaglandin E2; mediation occurs typically under pathological conditions and in response to LPS (Lopes et al., 2012). Therefore, the inhibition of NO production mediated by inflammatory stimuli is relevant to the prevention of inflammatory diseases (Biesalski, 2007). To evaluate the anti-inflammatory activity of crowberry aerial part extract, the RAW 264.7 cells were stimulated with LPS in the presence of the crude MeOH extract and its fractions at various concentrations. After this, the NO production in the LPS-stimulated cells was determined. As shown in Fig. 2A, the hexane fraction significantly inhibited excessive NO production in the LPS-stimulated RAW 264.7 cells. The hexane fraction at 100 and 200 μg/ml inhibited NO production by greater than 81% and 84%, respectively, over the level generated by the mock control. The crude MeOH extract and EtOAc fraction at 200 μg/ml inhibited LPS-induced NO production by 82% and 60%, respectively. As determined by LDH assay, which

Table 3 Correlations between the biological activities and total phenolic and flavonoid contents of crowberry aerial parts.

	Correlation R^2	
	TPC	TFC
Free radical scavenging activity	0.921***	0.890***
Reducing power	0.868***	0.807***
Total antioxidant capacity	0.786***	0.734***
α-glucosidase inhibitory activity	−0.635**	−0.404
NO production	−0.268	−0.433

The methanol crude extract (from the crowberry aerial parts) and its fractions were used in the correlation.
** Significance at $p < 0.01$.
*** Significance at $p < 0.001$.

measures the activity of LDH released from dead cells into the culture medium, the number of viable activated macrophages remained unaltered by the crude MeOH extract and its fractions (Fig. 2B). This result indicates that the inhibitory effect of EtOAc fraction on NO production is not attributed to cytotoxic effects, but that the inhibition of NO production by a high concentration of the hexane fraction may be due to cytotoxicity. Taken together, these findings suggest that crowberry aerial part extract suppresses the secretion of pro-inflammatory mediator from LPS-stimulated cells.

3.5. Correlation between phytochemical content and biological activity

As previously stated, plant-derived polyphenolic compounds are currently of particular interest to medical and food nutrition research because of their pharmaceutical properties (Es-Safi et al., 2007; Wahle et al., 2010; Gülçin, 2012; Chua et al., 2013). Aside from being radical scavengers, polyphenolic compounds can function as effective enzyme action inhibitors and immune modulators (Havsteen, 2002; Cimino et al., 2012; Weng and Yen, 2012). To analyze the correlation between the phytochemical content and biological activity of crowberry extract, the Pearson correlation coefficient for TPC and TFC was employed. As shown in Table 3, antioxidant activity showed a good correlation with TPC and TFC, whereas the coefficient of correlation between TFC and antidiabetic activity and NO production was nonsignificant. This finding indicates that polyphenolic compounds are major contributors to the antioxidant activity of crowberry aerial part extract. In addition, the absence of correlation between phytochemical content and NO production suggests that non-polyphenolic compounds that possess strong anti-inflammatory activities (e.g., polysaccharides) (Joseph et al., 2011; Rodrigues et al., 2012) are the active compounds in crowberry aerial part extract. Furthermore, some of the polyphenols found in the extract may be extremely active even in small number quantities because of their structural characteristics. This feature causes a nonlinear relationship between polyphenolic content and NO production (Table 3).

4. Conclusion

We analyzed the antioxidant, α-glucosidase inhibitory, and anti-inflammatory activities of crowberry aerial part extract. The results serve as initial insight into the pharmacological properties of crowberry aerial parts and provide valuable evidence for the potential of such parts as good dietary sources of natural antioxidant, α-glucosidase inhibitory, and anti-inflammatory components that improve human health. Further works are needed to better understand the mechanism of its preventive and/or protective effects. In addition, it will be required to investigate the isolation of the bioactive compounds, mechanisms of action, *in vivo* test, and safety and long-term side effects of bioactive compounds. Results may be useful for further studies on Korean crowberry for its applications in pharmaceutical industries.

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