


COMPARATIVE ANALYSIS ON THE ANTIOXIDANT ACTIVITIES OF THREE *OPUNTIA*
SPP. FROM KOREA –IN HIGHLIGHT OF *O. HUMIFUSA* F. *JEOLLAENSIS*Keum Hee Hwang^{1*}, Hyo Jin Kim¹, Jeong Hyun Lim¹, Sung Soo Whang²¹Plant Resources Research Institute, Duksung Women's University, Seoul, 01369, Korea²Division of Science Education, Chonbuk National University, Jeonju 54896, Korea

ABSTRACT: To compare the potential antioxidant activities of three *Opuntia* spp., *O. humifusa* f. *jeollaensis* (Wang-gasi Chunnyuncho; OHJ), *O. humifusa* (Chunnyuncho; OH) and *O. ficus-indica* (Baiknyuncho; OFI), five antioxidant assays were performed using 70% methanol extraction on several plant parts. Significant differences were observed in five tested methods. Although there are some differences in the tested material volume among the plant parts, the total polyphenol contents of OHJ, OH, and OFI extracts were measured to be 38.87±3.01, 13.32±2.47, and 22.22±2.53 mg GAE/100g, respectively. Those of the total flavonoid contents were measured to be 98.11±0.58, 78.67±0.61, and 2.61±0.13 mg QE/100g, respectively. The average IC₅₀ values of OHJ, OH, and OFI alcoholic extracts against on DPPH radical scavenging activities, in testing 0.125, 0.25, 0.5, and 1 µg/ml concentrations in order, were 0.85, 0.90 and 1.18 µg/ml, respectively (ascorbic acid, 10.37 µg/ml). Those of the nitric oxide scavenging activity were 0.17, 0.12, and 0.10 µg/ml, respectively (ascorbic acid, 7.75 µg/ml). Finally, those of the reducing power were 0.49, 0.72 and 1.16 µg/ml, respectively (ascorbic acid, 4.62 µg/ml). We have found that a new form of *Opuntia* named *O. humifusa* f. *jeollaensis* contains the most amounts of polyphenols and flavonoids in comparing to OH and OFI well known as potential source of natural antioxidants so far. *Opuntia humifusa* f. *jeollaensis* also showed more potent antioxidant activities including on DPPH radical scavenging activities and the reducing power.

Key words - *Opuntia humifusa* f. *jeollaensis*, *Opuntia humifusa*, *Opuntia ficus-indica*, total polyphenols, total flavonoids, antioxidant activities

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INTRODUCTION

Free radicals play detrimental roles in peroxidation of lipid, denaturation of protein, tumor, transformation, mutation, aging, and cancer (Simic, 1988). To maintain a healthy life and to prevent deterioration in the quality of food by peroxidation of lipid, effective prevention of various diseases caused by free radical is necessary. Researchers are going on for the development of antioxidants that inhibits the generation and activity of free radicals (Choe, and Yang, 1982). Free radicals are produced in normal and/or pathological cell metabolisms. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis, and arteriosclerosis as well as in degenerative processes associated with ageing (Halliwell, and Gutteridge, 1985; Mahfuz, *et al.*, 2007). Antioxidant rich foods help in the prevention of cardiovascular diseases, cancer (Gerber, *et al.*, 2002; Serafini, *et al.*, 2002), and neurodegenerative diseases including Parkinson's and Alzheimer's disease (Di, and Esposito, 2003). Natural antioxidants like vitamin C, vitamin E, carotenes, phenolic acid, phytate and phytoestrogens are mostly derived from grains, fruits and vegetables, and have been identified to have the potential in reducing disease risk (Jacob, 1996; Knight, 1988).

In Korea, *O. ficus-indica* (Baiknyuncho) and *O. humifusa* (Chunnyuncho) are cultivated in large quantities. They are perennial plant and are members of *Opuntioideae* family. Though the origin of *O. ficus-indica* and *O. humifusa* in Korea can be traced back to South and Central America, when they were introduced into Korea is still unknown. *Opuntia ficus-indica* is grown only in Jeju Island where climate is sub-tropical, whereas *O. humifusa* is grown in mainland of Korea and is found to withstand severe cold temperature. A new forma of *Opuntia* named *O. humifusa* f. *jeolaensis* has phenotypic similarity to *O. humifusa* but differed from it by having flowers that has yellow petal with red color at the base and the presence of hard spine (Kim, et al., 2014). This new forma of *Opuntia* is widely cultivated in the Jeollabukdo province of Korea.

Not only being horticultural important, but also having medicinal properties, *Opuntia* spp. are increasingly becoming important in Korea (Go, et al., 2003; Stintzing, et al., 2005), so it is necessary to clarify their activity relationship. The extracts of *O. humifusa* has been shown to have many medicinal properties including protection of brain from glucose and oxygen deprivation (Huang, et al., 2008), hepatoprotective property (Park, et al., 2005; Ncibi et al., 2008), hypoglycemic property (Trejo-González, et al., 1996; Laurenz, et al., 2003; Kang, et al., 2013; Hahm, et al., 2011), antimicrobial activity (Jung, et al., 2012;), antioxidative property (Stintzing, et al., 2005; Lee, et al., 2005; Jung, et al., 2012; Yoon, et al., 2012; Kim, et al., 2011; Lee, and Lee, 2010), anti-inflammatory activity (Cho, et al., 2006), neuroprotective property (Go, et al., 2003), anticancer properties (Jung, et al., 2012; Lee, et al., 2013), anti-obesity activity (Kim, et al., 2011), chemopreventive effects (Kim, et al., 2013; Lee, et al., 2012; Hahm, et al., 2010), bone density increase effect (Kang, et al., 2012), clinical efficacy of facial masks (Yeom, et al., 2011) and wound healing property (Park, and Chun, 2001). They are also used in traditional oriental folk medicines to treat diabetes, indigestion, edema, burns, wounds, ect. (Ahn, 1998).

The aim of this study was to compare of the chemical and biological characteristics of the Wang-gasi Chunnyuncho, recently recorded as a new forma, with two kinds of *Opuntia* spp., Chunnyuncho and Baiknyuncho, widely cultivated in the south province of Korea. Total polyphenols, total flavonoids contents, and various antioxidative properties including DPPH radical scavenging activity, nitric oxide scavenging activity and reducing power were measured and compared.

EXPERIMENTAL

Plant materials - *Opuntia humifusa* f. *jeolaensis* (Wang-gasi-Chunnyuncho) buds (fresh weight, 12.3 g), flowers (42.61 g), fruits (131.44 g), stems (277.12 g) and *O. humifusa* (Chunnyuncho) flowers (38.58 g), fruits (106.49 g), and stems (302.56 g) were harvested from Jeollabuk-do, Korea. *Opuntia ficus-indica* (Baiknyuncho) stems (14.3 g) were harvested from Jeju-do. After collecting, all the plant samples were freeze dried immediately. A voucher specimen have been deposited in Duksung Womln's University, Seoul, South Korea. The freeze-dried plant samples were powdered and extracted with 70% MeOH 500-1500 ml at 95°C hot bath for 6 hours and filtered. The residues were re-percolated in three times. The evaporated extracts were freeze dried and preserved in minus 70°C deep freezer, until tested.

Chemicals - 2,2-diphenyl-1-picrylhydrazyl (DPPH), naphthylethylenediamine dichloride, phosphoric acid, gallic acid, quercetin, ascorbic acid were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Mlthanol was purchased from Duksan Chemicals Co. (Seoul, Korea). Sodium carbonate was purchased from Kanto Chemicals Co. (Tokyo, Japan). Potassium hexacyanoferrate (III), sodium nitroferricyanide, trichloroacetic acid were purchased from Samchun Chemicals Co. (Seoul, Korea). Aluminium chloride was purchased from Junsei Chemicals Co. (Tokyo, Japan). Other chemicals including phosphate buffer were purchased from Biosesang Co. (Seoul, Korea). All the chemicals used in this study were of analytical grade.

Determination of total polyphenol contents - Total polyphenol contents were estimated using the Folin-Ciocalteu colorimetric mlthod (Cai, et al., 2004) with a slight modification. Briefly, the appropriate dilutions of the filtered extracts were oxidized with Folin-Ciocalteu reagent and the reaction solution was neutralized with saturated sodium carbonate (20 g/ml). The absorbance of the resulting blue color was measured at 760 nm with a UV-VIS spectrophotometer after incubation for 1hr at room temperature. Quantification was conducted on the basis of the standard curve of gallic acid (400~10 µg/ml). Total polyphenol contents in three kinds of *Opuntia* spp. were expressed as µg gallic acid equivalents (GAE) per one g dry weight.

Determination of total flavonoid contents - Total flavonoids were determined using the method of Taga, et al. (1984) on the formation of complex flavonoid aluminum. A volume of 0.1 mL of 2% AlCl₃-mlthanol solution was mixed with 0.1 ml of the extract (1 µg/ml). The resultant mixture was incubated for 15 min for yellow color development which indicated the presence of flavonoid. The absorbance was measured at 420 nm using UV-VIS spectrophotometer. Total flavonoid contents in three kinds of *Opuntia* spp. were expressed as µg quercetin equivalent (QE) per one g dry weight.

DPPH free radical scavenging activity

The method of Shen *et al.* (2010) was used for the determination of scavenging activity of DPPH radical in the extract solution. A portion of 0.2 mM DPPH prepared in methanol was added to 0.5 and one mg of the plant extracts, and ascorbic acid was used as standard. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 min. The absorbance was measured by UV-VIS spectrophotometer at 520 nm. The scavenging ability of the plant on DPPH was calculated using the equation: DPPH scavenging activity (%) = $[(\text{Abs control} - \text{Abs sample}) / (\text{Abs control})] \times 100$, where Abs control is the absorbance of DPPH+ methanol; Abs sample is the absorbance of DPPH radical sample extract or standard.

Nitric oxide scavenging activity

The method of Marcocci *et al.* (1994) used for the determination of scavenging of nitric oxide in the extract solution. Scavenging of NO was determined using sodium nitroprusside (SNP) as NO donor. SNP (10 mM) in phosphate buffered saline was mixed with different concentrations of methanolic extract (125 to 500 µg/ml), ascorbic acid was used as standard (1.25 to 5 µg/ml) and incubated at 25°C for 150 min, then equal volume of Griess reagent (2% sulfanilamide in 4% phosphoric acid and 0.2% naphylethylenediamine dihydrochloride in 4% phosphoric acid) was add. The absorbance was immediately measured at 542 nm. The NO scavenging activity was calculated using the formula, percentage NO scavenging activity = $[(\text{Abs of Control} - \text{Abs of Sample}) / \text{Abs of Control}] \times 100$. Each experiment was carried out in triplicate and results were expressed as ml in % NO scavenging activity ± SD.

Reducing power assay

The reducing power was determined according to the method of Oyaizu (1986). 0.1 ml of the extract at eight kinds of concentration was mixed with 0.1 ml of 200 mM sodium phosphate buffer (pH 6.6) and 0.1 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20min. After adding 0.1 ml of 10% ferric chloride and the absorbance was measured reducing power. The increased absorbance of the reaction mixture indicated the increased reducing power. The assays were carried out in triplicate and the results were expressed as ml ± standard deviation (SD). Ascorbic acid was used as a standard.

Statistical analysis

All experiments were conducted in independent quadruplicate (n=4) and data were expressed as mean ± SD. Statistical significance was evaluated by one-way analysis of variance using SPSS Win program (Version 19.0, Cary, NC), and individual comparisons were determined using Duncan's multiple range tests at the p<0.05 level.

RESULTS AND DISCUSSION**Total polyphenol and flavonoid contents**

The yields of 70% methanolic extracts from the three *Opuntia* spp., Wang-gasi Chunyuncho (OHJ), Chunyuncho (OH) and Baiknyuncho (OFI), were presented in Table 1. The total phenolic contents (TPC) of OHJ, OH, and OFI extracts were determined through a linear gallic acid standard curve ($y=0.0059x+0.099$, $R^2=0.9964$) and expressed as microgram of gallic acid equivalents (GAE) per 1 gram of dry plants extracts (µg GAE/1g extract). TPC of buds, flowers, fruits and stems of OHJ extracts, the flowers, fruits and stems of OH and the stems of OFI were observed (p<0.05) to be 49.89±6.96, 71.71±2.39, 11.53±3.53, 22.34±2.59, 12.49±3.09, 15.65±1.69, 11.83±2.63, 22.22±2.53 mg GAE/100 g of extract, respectively (Table 1). In this study, the total flavonoids contents (TFC) of OHJ, OH, and OFI extracts were evaluated by aluminium colourimetric assay, using quercetin as a standard compound ($y=0.0074x+0.0601$, $R^2=0.9856$) and then expressed as microgram of quercetin equivalents (QE) per 1 gram of dry plants extracts (µg QE/1g extract). TFC of buds, flowers, fruits and stems of OHJ extracts, the flowers, fruits and stems of OH and the stems of OFI extracts were observed (p<0.05) to be 211.53±1.02, 149.51±1.13, 15.85±0.08, 15.51±0.08, 200.17±1.41, 17.44±0.28, 18.41±0.15, 2.61±0.13 mg QE/100 g extract, respectively (Table 1). Polyphenol and flavonoid compounds constitute the primary class of natural antioxidants present in the plant kingdom, and they are endowed with free radical scavenging and antioxidant activities (Amin, and Yazdanparst, 2007). Diverse biological activities (e.g. anti-inflammatory, anti-carcinogenic, and anti-atherosclerotic activities) were exhibited (Shetty, *et al.*, 1995).

DPPH free radical scavenging activity

As a kind of stable free radical, DPPH can accept an electron of hydrogen radical to become a stable diamagnetic molecule, which is widely used to investigate radical scavenging activity. The antioxidants can react with DPPH, a deep-violet colored stable free radical, converting it into a yellow colored α, α -diphenyl- β -picrylhydrazine. The discoloration of the reaction mixture can be quantified by measuring the absorbance at 520 nm, which indicates the radical scavenging ability of the antioxidant (Braca, *et al.*, 2001). OHJ extracts evenly showed the most antioxidant potential to scavenge DPPH radicals in every part of plant (Fig. 1). Methanol extracts of OHJ, OH, and OFI were measured at concentrations of 0.125, 0.25, 0.5, 1 µg/ml. DPPH radical scavenging activity of the methanol extract of OHJ, OH, and OFI increased depending on the sample concentration.

In comparison with ascorbic acid (IC_{50} , $10.19 \pm 1.05 \mu\text{g/ml}$) of OHJ ($0.57 \pm 0.08 \sim 1.13 \pm 0.39 \mu\text{g/ml}$), OH ($0.55 \pm 0.06 \sim 1.19 \pm 0.24 \text{ mg/ml}$) and OFI ($1.18 \pm 0.27 \mu\text{g/ml}$) were shown to have reliable IC_{50} values in Table 2. According to recent reports, glasswort seed extract (Kang, *et al.*, 2011), dried jujube (Kim, *et al.*, 2011) and fruits extracts of *Cratagus pinnatifida* Major (Choi, and Hwang 2013) showed higher antioxidant effect than those of Vitamin C and Vitamin E, even activity their IC_{50} values of DPPH radical scavenging activity were showed to be around 800, 500 and $48.5 \mu\text{g/ml}$, respectively. OHJ, OH, and OFI also showed potential DPPH free radical scavenging activity. As shown in Table 2, the IC_{50} values of buds of OHJ and fruits of OH against on the DPPH radical scavenging activities were showed to be 0.57, and $0.55 \mu\text{g/ml}$, respectively.

Nitric oxide scavenging activity

Despite the possible beneficial effects of NO, its contribution to oxidative damage is increasingly becoming evident. This is due to the fact that NO can react with superoxide to form the peroxynitrite anion, which is a potentially strong oxidant that can decompose to produce $\cdot\text{OH}$ and NO_2 (Beckman and Koppenol, 1996, Pacher, *et al.*, 1996). NO released from SNP has a strong NO^+ character which can alter the structure and function of many cellular components. OHJ, OH and OFI extract showed antioxidant potential to scavenge Nitric oxide radical (Fig. 2). Methanol extracts of OHJ, OH, and OFI were measured at concentrations of 0.125, 0.25, 0.5, 1 $\mu\text{g/ml}$. The extracts of various parts of OHJ, OH, and OFI showed nitric oxide radical scavenging activity depend on the concentration, their IC_{50} values were presented in Table 2. In comparison with ascorbic acid (IC_{50} , $6.88 \pm 0.71 \mu\text{g/ml}$), the extracts of the stems of OFI and OH, were showed strongest antioxidant activity than the fruits of OHJ and OH. Their IC_{50} values against on the nitric oxide scavenging activities were showed in the order of their size, 0.11 ± 0.01 , 0.11 ± 0.00 , 0.12 ± 0.01 , and $0.13 \pm 0.02 \text{ mg/ml}$, respectively.

Table 1. Total polyphenol and flavonoid contents of three *Opuntia* spp. cultivated in Korea

| Contents | <i>Opuntia humifusa</i> f. <i>jeolaensis</i> ⁶⁾ | | | | <i>Opuntia humifusa</i> ⁶⁾ | | | <i>Opuntia ficus-indica</i> ⁶⁾ |
|--|--|-------------------|------------------|------------------|---------------------------------------|------------------|------------------|---|
| | BUDS | FLOWERS | FRUITS | STEMS | FLOWERS | FRUITS | STEMS | STEMS |
| Total plant materials(g) ¹⁾ | 12.30 | 42.61 | 131.44 | 277.12 | 38.58 | 106.49 | 302.56 | 14.30 |
| 70% methanolic extracts(g) ²⁾ | 4.74 | 25.17 | 15.11 | 69.58 | 4.51 | 14.76 | 41.93 | 6.90 |
| Yields(% W/W) ³⁾ | 38.54 | 59.07 | 11.51 | 25.11 | 11.69 | 13.86 | 13.86 | 48.25 |
| Total polyphenols (mg GAE/100g extracts) ⁴⁾ | 49.89 ± 6.96 | 71.71 ± 2.39 | 11.53 ± 3.53 | 22.34 ± 2.59 | 12.49 ± 3.09 | 15.65 ± 1.69 | 11.83 ± 2.63 | 22.22 ± 2.53 |
| Total flavonoids (mg QE/100g extracts) ⁵⁾ | 211.53 ± 1.02 | 149.51 ± 1.13 | 15.85 ± 0.08 | 15.51 ± 0.08 | 200.17 ± 1.41 | 17.44 ± 0.28 | 18.41 ± 0.15 | 2.61 ± 0.13 |

1) Freeze dried plants weights

2) Freeze dried powder weights

3) Based on Freeze dried powder weight

4) Based on gallic acid as a standard

5) Based on quercetin as a standard

6) *Opuntia humifusa* f. *jeolaensis* (Wang-gasi Chunmyuncho); *Opuntia humifusa* (Chunmyuncho); *Opuntia ficus-indica* (Bailayuncho)

Reducing power assay

Antioxidant activity is reported to be concomitant with the reducing power, or the capability of reducing oxidized intermediates of the lipid peroxidation processes (Ordonez, *et al.*, 2006), and the reducing activity is generally associated with the presence of reductions (Duh, 1998) which have been shown to exert an antioxidant effect by donating a hydrogen atom and thereby breaking the free radical chain. The reducing power of OHJ, OH, and OFI extracts showed a dose-dependent response. Compared to the positive control (ascorbic acid: reducing power, 1.88 ± 0.14 at 10 $\mu\text{g/ml}$), OHJ, OH, and OFI extracts (reducing power, $1.77 \pm 0.09 \sim 3.43 \pm 0.28$, $1.57 \pm 0.76 \sim 2.79 \pm 0.17$, and 1.47 ± 0.12 at one $\mu\text{g/ml}$) showed high reducing power (Table 2).

The reducing power the reducing capability of a compound may serve as a significant indicator of its potential antioxidant activity (Miri, *et al.*, 1995), thus the significant antioxidant activity of OHJ, OH, and OFI appears to be at least partially related to its reducing power activity. Prasad *et al.* (2009) reported that reducing power depends on the presence of hydroxyl groups in the phenolic compounds, which act as electron donors. According to Lee and Goh (2001), the reducing power of red wine containing 1,667~2,537 $\mu\text{g/ml}$ of total polyphenolic compounds and that of white wine containing 247-339 mg/L of total polyphenolic compounds are in the range from 3.1~3.4 and 1.5~1.7 $\mu\text{g/ml}$, respectively. Overall reducing power trend was similar to those of DPPH radical scavenging activities. The abundance of TPC might play an important role in the high reducing power OHJ, OH, and OFI extracts.

Table 2. Antioxidant activities of three *Opuntia* spp. cultivated in Korea expressed by IC₅₀ values

| Species and Part of <i>Opuntia</i> | | DPPH free radical scavenging activity ⁵⁾ | Nitric oxide scavenging activity ⁵⁾ | Reducing power ^{3,5)} |
|---|---------|---|--|--------------------------------|
| <i>Opuntia humifusa</i> f. <i>jolaensis</i> ¹⁾ (Wang-gasi Chunyuncho) | BUDS | 0.57±0.08 | 0.17±0.03 | 3.43±0.28 |
| | FLOWERS | 0.89±0.30 | 0.17±0.06 | 2.18±0.23 |
| | FRUITS | 0.79±0.28 | 0.13±0.02 | 3.34±0.47 |
| | STEMS | 1.13±0.39 | 0.13±0.02 | 1.77±0.09 |
| <i>Opuntia humifusa</i> ¹⁾ (Chunyuncho) | FLOWERS | 0.95±0.12 | 0.12±0.02 | 1.65±0.19 |
| | FRUITS | 0.55±0.06 | 0.12±0.01 | 2.79±0.17 |
| | STEMS | 1.19±0.24 | 0.11±0.00 | 1.57±0.76 |
| <i>Opuntia ficus-indica</i> ¹⁾ (Baiknyuncho) | STEMS | 1.18±0.27 | 0.11±0.01 | 1.47±0.12 |
| Ascorbic acid ²⁾ | | 10.19±1.05 | 6.88±0.71 | 1.88±0.14 ⁴⁾ |

1) units : mg/ml

2) units : $\mu\text{g/ml}$

3) reducing power at 1 mg/ml of extracts

4) reducing power at 10 $\mu\text{g/ml}$ of ascorbic acid

5) means of 4 or 5 replicated measurements \pm SD, $p < 0.05$.

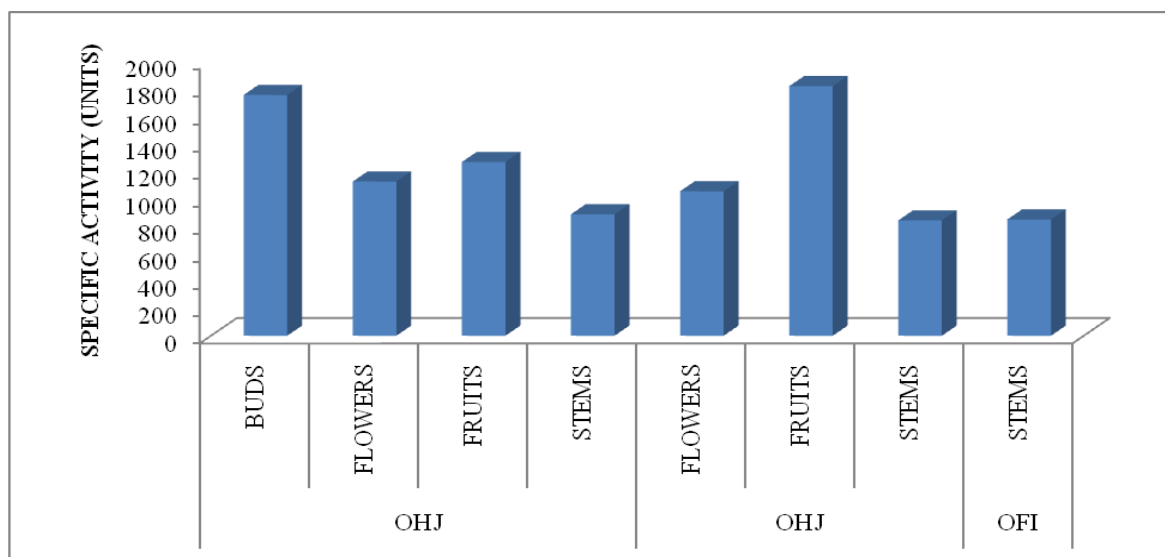


Figure 1. Comparison of antioxidant activity tested by DPPH radical scavenging activity of the plant parts of three *Opuntia* spp., cultivated in south province of Korea, expressed by specific activity. Specific activity was expressed as an unit which means the amounts of IC₅₀ values in $\frac{1}{2}$ one g of a dried test sample. All experiments were conducted in independent quadruplicate (n=4) and data were expressed as mean \pm SD. Statistical significance was evaluated at the $p < 0.05$ level.

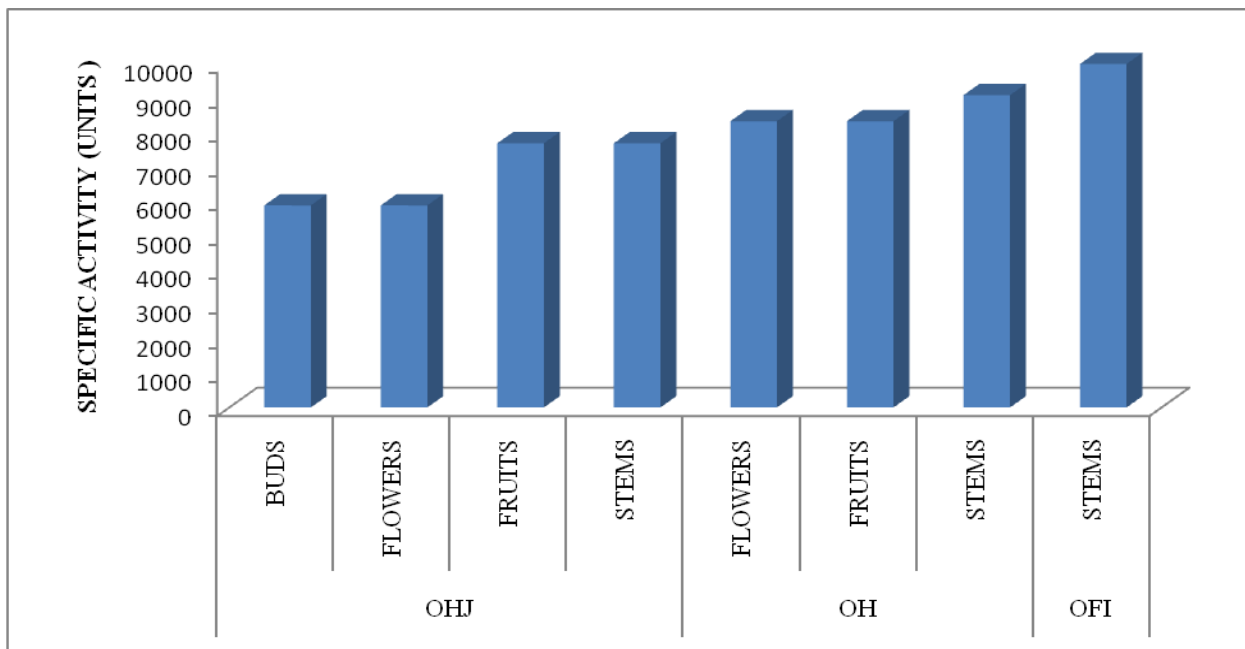


Figure 2. Comparison of antioxidant activity tested by NO scavenging activity of the plant parts of three *Opuntia* spp., cultivated in south province of Korea, expressed by specific activity. Specific activity was expressed as an unit which means the amounts of IC₅₀ values in 1 g of a dried test sample. All experiments were conducted in independent quadruplicate (n=4) and data were expressed as mean±SD. Statistical significance was evaluated at the p<0.05 level.

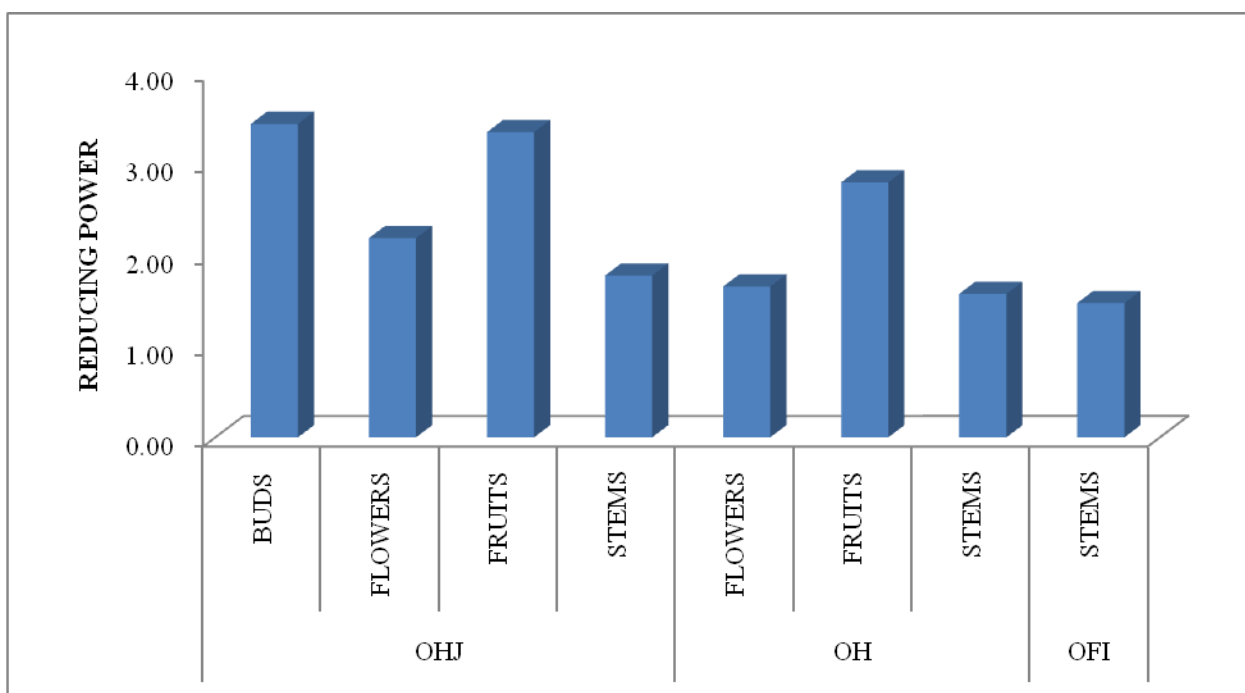


Figure 3. Comparison of antioxidant activity tested by reducing power of the plant parts of three *Opuntia* spp., cultivated in south province of Korea. All experiments were conducted in independent quadruplicate (n=4) and data were expressed as mean±SD. Statistical significance was evaluated at the p<0.05 level.

ACKNOWLEDGMENTS

This research was supported by the National Research Foundation of Korea(NRF) grant funded by the Ministry of Science, ICT & Future Planning(NRF-2010-0021753)

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ISSN : 0976-4550

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