Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.06522

Assessment of antioxidant activities of HeukHarang a novel Korean *Lectuca sativa* L.

Seo Woo JANG[1#], Yong-Dae KIM[2#], Songmi LEE[3], Soon-Ho YIM[2*]

Abstract

In this study, antioxidant activities of HeukHarang a novel Korean *Lectuca sativa* L.extracts compair with Red skirt lettuce. The highest total polyphenol contents was 60% ethanol extracts of HeukHarang and total flavonoid contents was 40% ethanol extracts, 84.70 ± 0.11 mg TAE/10 g DW and 63.82 ± 0.09 mg QE/10 g DW, respectively. The highest FRAP activity and PMA were 60% and 80% ethanol extracts of heuharang, 1,876.3 ± 0.01 mM TEAC/10 g DW and 26.2 ± 0.0 mM AAEAC/10 g DW, respectively. Radical scavenging activity also HeukHarang ethanol extracts was higher than Red skirt lettuce. The highest ORAC value was 76.0 ± 0.43 mM TEAC/10 g DWthehd, 100% ethanol extracts of HeukHarang. As a result, the all antioxidant activities of Heuhalang ethanol extracts were higher than that of Red skirt lettuce as a control.

Keywords: *Lectuca sativa* L.; Korean lettuce; antioxidant; ORAC assay; FRAP; PMA.

Practical Application: Research about antioxidant activities and functional products of HeukHarang.

1 Introduction

A balanced diet is essential to ensure physical development and health. Numerous epidemiological studies have suggested high daily consumption of fruits and vegetables lowers the risk of several chronic diseases, such as cancer, cardiovascular disease and diabetes; the protective effects of fruit and vegetable consumption are mainly attributed to the presence of bioactive phytochemicals such as polyphenols (Arts & Hollman, 2005; Hooper & Cassidy, 2006; Russo et al., 2012; Chen & Chen, 2013).

Reactive Oxygen Species (ROS), hydroxyl radical, hydrogen peroxide and Superoxide anion etc., leads to DNA denaturation, lipid peroxidation, protein inactivation, and other cell dysfunction. These cause the cancers, diabetes, atherosclerosis and Parkinson's disease (Halliwell et al., 1992). ROS which are directly responsible for oxidative stress, are unstable and highly reactive. Furthermore, these are easily reacted with biomaterials and attacked biomolecules, caused damage to cells and tissues (Lee & Im, 2012). In particular, ROS, was produced by free Radicals (OH^{-}, O_2^{-}), causes aging, inflammation, carcinogenesis, and atherosclerosis (Cho et al., 2008). The human body has biological defense systems such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), glutathione (GSH) and glutathione S-transferase (GST) to remove these free radicals (Kil et al., 2015). Antioxidants can protect cells from oxidative damage by removing free radicals intermediates and prevent cell apoptosis (Lim, 2010). Synthetic antioxidants such as BHA and BHT have excellent antioxidant effects, however, showed various side effects caused by toxicity; thus, there is increasing interest in research on safe natural antioxidants (Park et al., 2012).

Lettuce (*Lactuca sativa* L.) is a leafy green vegetable that belongs to Asteraceae family and it is cultivated extensively for food consumption (Abdel Latef et al., 2020). The economically valuable vegetable crop lettuce is a minimally processed food product available throughout the entire year, and is a significant source of natural health promoting compounds (Yang et al., 2017).

Lettuce is of particular interest due to its high antioxidant and phytochemical content (Malejane et al., 2018). Most relevant phytochemicals found in lettuce include vitamin B, vitamin C, vitamin E, carotenoids, and polyphenols. The latter refers to a group of secondary metabolites responsible for the plants' defense system that has been described to have higher antioxidant capacity than, for example, vitamin C and E (Kim et al., 2016). Although their mechanisms of action are not yet fully understood (Fraga et al., 2019), a large number of epidemiological studies, and their associated meta-analyses, suggested that long-term consumption of diets rich in plant-derived polyphenols and antioxidants offer protection against development of diseases associated to metabolic syndrome, different types of cancer, osteoporosis, and neurodegenerative diseases (Pandey and Rizvi 2009). Chong et al. (2010) reviewed the evidence for the effects of fruit polyphenols on platelet function, blood pressure, vascular function, and blood lipids, all of them risk factors of cardiovascular diseases. The authors of that study concluded that despite the heterogeneity in the design of studies, the lack of controls, and the short intervention periods, there is evidence to suggest that flavonols, anthocyanins, and procyanidins are effective at reducing the above-mentioned risk factors.

Received 03 Jan., 2022
Accepted 18 Feb., 2022
[1] *Jeollanamdo Agricultural Research & Extension Services, Naju, Jeonnam, Korea*
[2] *Department of Pharmaceutical Engineering, Dongshin University, Naju, Jeonnam, Korea*
[3] *Department of Food and Nutrition, Dongshin University, Naju, Jeonnam, Korea*
*Corresponding author: virshyim@gmail.com
[#] *These authors contributed equally to this work.*

In this study, we investigated antioxidant effects of HeukHarang a novel Korean *Lectuca sativa* L. grown and harvested by the Jeollanamdo Agricultural Research & Extension Services. We extracted HeukHarang by various ethanol concentrations and investigated total polyphenol contents, total flavonoid contents and antioxidative activity evaluation. Furthermore, we intend to provide basic data to ascertain the availability of *L. sativa* L. as a natural antioxidant material.

2 Materials and methods

2.1 Experimental materials

HeukHarang, a noble lettuce *Lactuca sativa* L. sample was provided and used from Jeollanamdo Agricultural Research and Extension Services. Folin-Ciocalteu reagent, trolox, gallic acid, catechin, ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), DPPH (1,1-diphenyl-2-picrylhydrazyl), and L-ascorbic acid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The solvents and all reagents used special-grade reagents.

2.2 Sample extraction

Samples, HeukHarang and red lettuce as a control, pulverized with a grinder (NFM-3561SN, NUC Co., Daegu, Korea) were reflux extracted with distilled water, 20% ethanol, 40% ethanol, 60% ethanol, 80% ethanol, and 100% ethanol for 60 min at 100 °C and filtered. SpeedVac (COSMOS660-50L, Kyungseo Machines Co., Incheon, Korea) was used to concentrate and obtain yields. The concentrated sample was stored at 4 °C before use.

2.3 Total phenolic contents (TPC)

Total polyphenol content (TPC) was measured using the Folin-Ciocalteu colorimetric method (Yun et al., 2018), Hardinasinta et al. (2021) and gallic acid was used as a standard solution. Each extracts (500 μL) was reacted to Folin-Coicalteu reagent (500 μL) for 3 min at room temperature, added to 500 μ of 10% Na_2CO_3 and react for 1 hour, block the light, and then measured at 760 nm with an UV/VIS spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea). Total polyphenol contents (mg GAE/g) standard curve was obtained gallic acid ($y = 0.0378x + 0.3719$, $R^2 = 0.997$).

2.4 Total flavonoid contents (TFC)

Total flavonoid contents (TFC) were determined using the Moreno method (Moreno et al., 2000), and catechin was used as a standard solution. Each sample (200 μL) was diluted with 80% ethanol (800 μL). 100 μL of this sample solution was mixed with 5% $NaNO_2$ (60 μL) for 5 min at room temperature, then added 10% $AlCl_3$ (60 μL) 5 min at room temperature. After adding 1N NaOH (400 μL), absorbance was measured at 415 nm using a spectrophotometer (Mecasys Co., Daejeon, Korea). Total flavonoid content (mg CE/g) standard curve was obtained catechin ($y = 0.0025x + 0.0142$, $R^2 = 0.999$).

2.5 Ferric-reducing antioxidant power (FRAP)

The ferric-reducing antioxidant power (FRAP) activity was measured by modifying method of Benzie and Strain (1996). The FRAP solution was a mixture of 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), and 20 mM $FeCl_3 \cdot 6H_2O$. These samples were measured an UV/VIS spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea) at 595 nm. The FRAP (mg TE/g) standard curve was obtained Trolox ($y=0.0021x-0.0165$, $R^2=0.991$).

2.6 Phosphomolybdenum complex assay (PMA)

Spectrophotometric evaluation of antioxidant activity through the formation of a green phosphomolybdenum complex was carried out according to Prieto et al. (1999), El-Sheekh et al. (2021). Sample solutions (100 μL) were combined in an Eppendorf tube with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of aqueous solutions of each was measured at 695 nm against a blank. The antioxidant activity (mg AAE/g) standard curve was obtained L-ascorbic acid ($y = 0.0021x - 0.0165$, $R2 = 0.9910$) .

2.7 DPPH radical scavenging activity

DPPH radical scavenging activity is one of the methods for measuring the antioxidant activity. It was measured by applying the experimental methods of Yamaguchi et al. (1998). The sample solution (10 μL) and 0.2 mM DPPH (190 μL) were added into a 96-well plate at room temperature for 30 min, and the absorbance was measured at 515 nm using an ELISA reader (Thermo Fisher SCIENTIFIC, Multiskan Sky, Korea). The DPPH radical scavenging activity (mg TE/g) standard curve was obtained Trolox ($y = 0.0015x + 2.3996$, $R^2 = 0.997$).

2.8 ABTS radical scavenging activity

ABTS radical scavenging activity is one of the methods for measuring the antioxidant activity and was measured by applying the experimental method of Thaipong et al. (2006), Liu & Li (2021). A solution of 7.4 mM ABTS and 2.45 mM potassium persulfate was mixed at 37 °C for 24 h. Each sample (150 μL) and ABTS solution (1,350 μL) was reacted in the dark room for 5 min, and measured the UV/VIS spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea) at 734 nm. The ABTS radical scavenging activity (mg TE/g) standard curve was obtained Trolox ($y = 0.0001x + 0.9179$, $R^2 = 0.996$).

2.9 ORAC (oxygen radical absorbance capacity) assay

Oxygen radical absorbance capacity value was measured by modifying the experimental method of Talcott & Lee (2002). A sample solution with 75 mM phosphate buffer (pH 7.4) and 100 nM flurescein were treated at 37 °C for 30 min. The reaction was started to add the 34 mM 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH), and fluorescence was measured at 2-minutes intervals for 1 hour under the conditions of excitation 485 nm and emission 535 nm. At this time, the value of the

decreasing fluorescence was calculated by the area under the curve (AUC), and the ORAC value was calculated using the decreasing value of the trolox-specific fluorescence.

2.10 Statistical analysis

All experimental results are shown as the mean ± standard deviation (mean ± SD) measured three times. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), and one-way analysis of variance (ANOVA) was performed to test the statistical significance between each experimental group. In cases where differences were observed, Tukey's test was conducted post-test, with $p < 0.05$ considered statistically significant.

3 Result and discussion

3.1 Total polyphenol and flavonoid contents

Many hydroxyl groups (-OH) in polyphenols are easily bonded to various compounds, so they have excellent antioxidant and anti-cancer effects (Kim et al., 2012). Phenolic compounds contained in fruits provide general quality (such as color and taste) and health functions (such as antioxidant) of plants (Yildiz et al., 2021). They are important ingredients that can protect plants from infections by bacteria and viruses. In this study, total polyphenol contents present in 'HeukHarang' a noble *L. sativa* ethanol extracts compair with red lettuce as a control were measured using tannic acid as a reference substance (Table 1). Total polyphenol contents ranged from 23.20 to 84.70 mg TAE/10 g DW compair with red lettuce ranged from 23.80 to 75.80 mg TAE/10 g DW. The highest total polyphenol contents among 'HeukHarang' ethanol extracts was 60% ethanol extracts and water, 40%, 60%, 80% ethan0ol extracts were higher than red lettuce as significant (p<0.05) differences.

In the present study, total flavonoid contents of HeukHarang ranged from 31.70 to 63.82 mg QE/10 g DW compair with red lettuce ranged from 10.25 to 56.69 mg TAE/10 g DW. The highest total polyphenol contents among 'HeukHarang' ethanol extracts was 40% ethanol extracts and all extracts were higher than red lettuce as significant (p<0.05) differences.

A noble lettuce, HeukHarang shows the high contents of total polyphenol and flavonoid contents.

3.2 Ferric-reducing antioxidant power (FRAP) and phosphomolybdenum complex assay (PMA)

The FRAP method is a principle in which Fe^{3+} is reduced to Fe^{2+} by directly supplying electrons, and uses a principle in which the ferric tripyridyltriazine [Fe(III)-TPTZ] composite agent is reduced to ferrous tripyridyltriazine [Fe(II)-TPTZ] by an antioxidant (Choi et al., 2005). In this study, the ferric-reducing antioxidant power was measured and converted into TE/10 g DW using Trolox as a standard material and shown in Table 2. Ferric-reducing antioxidant power ranged from 131.1 to 1,876.3 mM TEAC/10 g DW compair with red lettuce ranged from 206.9 to 1,296.2 mM TEAC/10 g DW. The highest ferric-reducing antioxidant power among 'HeukHarang' ethanol extracts was 60% ethanol extracts and all extracts except water extracts were higher than red lettuce as significant (p<0.05) differences.

The PMA method is the principle that Mo(VI) is reduced to Mo(V) by extracts to react with phosphate to form a green phosphate/Mo(V) complex (Prieto et al., 1999). In this study, antioxidant activity was measured using L-ascorbic acid as a standard substance, and it was converted into AAEAC/10 g DW and shown in Table 2. Phosphomolybdenum reduced activity ranged from 11.6 to 26.2 mg AAEAC/10 g DW compair with red lettuce ranged from 1.1 to 4.7 mg AAEAC/10 g DW. The highest phosphomolybdenum reduced activity among 'HeukHarang' ethanol extracts was 80% ethanol extracts and all extracts were higher than red lettuce as significant (p<0.05) differences.

3.3 DPPH and ABTS radical scavenging activity

Antioxidant activity was measured based on DPPH and ABTS free radical scavenging activities widely used to find antioxidants from various natural materials (Lee et al., 2014).

Table 1. Contents of total polyphenols and flavonoids in water and ethanol extracts of HeukHarang and red lettuce.

Sample		Total polyphenol contents (mg TAE[1)]/10 g DW[2)])	Total flavonoid contents (mg QE[3)]/10 g DW)
HeukHarang[4)]	**water**	**53.60 ± 0.03^{b}[5)6)]**	**50.80 ± 0.09^{d}**
	20% EtOH	**70.30 ± 0.20^{c}**	**43.72 ± 0.00^{c}**
	40% EtOH	**52.00 ± 0.05^{cd}**	**63.82 ± 0.09^{f}**
	60% EtOH	**84.70 ± 0.11^{d}**	**60.30 ± 0.00^{e}**
	80% EtOH	**73.80 ± 0.17^{c}**	**61.80 ± 0.00^{e}**
	100% EtOH	**23.20 ± 0.03^{a}**	**31.70 ± 0.00^{b}**
Red lettuce[7)]	**water**	**23.80 ± 0.04^{b}**	**10.25 ± 0.00^{a}**
	20% EtOH	**75.80 ± 0.06^{h}**	**34.37 ± 0.00^{h}**
	40% EtOH	**38.97 ± 0.06^{e}**	**41.61 ± 0.15^{i}**
	60% EtOH	**65.58 ± 1.41^{g}**	**56.69 ± 0.15^{j}**
	80% EtOH	**48.03 ± 0.06^{f}**	**33.37 ± 0.09^{g}**
	100% EtOH	**45.64 ± 0.08^{f}**	**10.86 ± 0.00^{a}**

[1)]TAE: tannic acid equivalent; [2)]DW: dry weight; [3)]QE: quercetin equivalent; [4)]Newly cultivated lettuce; [5)]Values are mean ± standard deviation (n=3); [6)]Values with different letters within the same row are significantly different by Duncan's multiple range test at p<0.05; [7)]Wild type lettuce.

DPPH free radical scavenging activity consists in the reduction of DPPH radical by substances present in the extraxts, leading to the formation of reduced DPPH-H, which changes in color from deep purple to loss of color (Chaves et al., 2017). DPPH free radial scavenging activities were measured using DPPH and expressed as trolox equivalent (Guldas et al., 2021). DPPH free radial scavenging activities of HeukHarang extracts used in this experiment were in the range of 727.4 to 804.9 mM TEAC/10 g DW and that of red lettuce were in the range of 299.1 to 341.9 mM TEAC/10 g DW. The highest DPPH free radical scavenging activity among 'HeukHarang' ethanol extracts was 20% ethanol extracts and all extracts were higher than red lettuce as significant (p<0.05) differences.

Another effective method to measure radical scavenging activity is the ABTS radical cation decolorization assay (Dulger Altiner, 2021). This method can be used to obtain results in a short time. It can be applied to both hydrophobic and hydrophilic sample (Kim et al., 2019). ABTS free radial scavenging activities of HeukHarang and red lettuce extracts were compared. Results are shown in Table 3. ABTS free radical scavenging activities of HeukHarang extracts were in the range of 2,305 to 4,405 mM AAEAC/10 g DW, with 60% ethanol extracts showing the highest activity, water, 80%, 100% ethaol extracts were higher than red lettuce. There were significant ($p < 0.05$) differences in ABTS free radical scavenging activity. These results suggest that antioxidants present in these extracts could eliminate highly reactive free radicals to prevent oxidation of important biomolecules such as unsaturated fatty acids, genes, and proteins (Fan et al., 2011).

3.4 ORAC assay

The ORAC method, adapted to use fluorescein as fluorescent probe, was used for determine antioxidant activity in vitro in biological samples and foods(Lucas-Abellán et al., 2008). The ORAC assay is the method to determine the radical chain breaking antioxidant capacity by generated AAPH value, thereby showing the advantage of a wide range of applications by reacting both the hydrophobic and hydrophilic components present in samples(Huang et al., 2005; Prior et al., 2003). Relative fluorescence intensity was more active at all extraction concentrations compared to Red skirt lettuce (Figure 1A). ORAC

Table 2. Ferric-reducing antioxidant power and Phosphomolybdenum in water and ethanol extracts of HeukHarang and red lettuce.

Sample		FRAP (mM TEAC[1)]/10 g DW[2)])	PMA (mg AAEAC[3)]/10 g DW)
HeukHarang[4)]	**water**	**131.1 ± 0.01^{d}**[5)6)]	**12.6 ± 0.30^{e}**
	20% EtOH	**1,337.2 ± 0.01^{e}**	**11.6 ± 0.30^{d}**
	40% EtOH	**1,752.2 ± 0.03^{f}**	**12.9 ± 0.00^{f}**
	60% EtOH	**1,876.3 ± 0.01^{g}**	**21.7 ± 0.30^{g}**
	80% EtOH	**1,340.5 ± 0.02^{e}**	**26.2 ± 0.00^{j}**
	100% EtOH	**556.3 ± 0.01^{b}**	**22.1 ± 0.30^{h}**
Red lettuce[7)]	**water**	**297.5 ± 0.01^{c}**	**1.1 ± 0.30^{a}**
	20% EtOH	**1,065.5 ± 0.02^{i}**	**2.1 ± 0.30^{f}**
	40% EtOH	**1,121.9 ± 0.02^{j}**	**1.6 ± 0.00^{c}**
	60% EtOH	**1,296.2 ± 0.02^{k}**	**1.3 ± 0.00^{b}**
	80% EtOH	**934.9 ± 0.01^{h}**	**3.9 ± 0.79^{i}**
	100% EtOH	**206.9 ± 0.01^{a}**	**4.7 ± 1.07^{k}**

[1)]TEAC: Trolox equivalent antioxidant capacity; [2)]DW: dry weight; [3)]AAEAC: Ascorbic Acid equivalent antioxidant capacity; [4)]Newly cultivated lettuce; [5)]Values are mean ± standard deviation (n=3); [6)]Values with different letters within the same row are significantly different by Duncan's multiple range test at p<0.05; [7)]Wild type lettuce.

Table 3. Antioxidant activities of noble Korean lettuce.

Sample		DPPH (mM TEAC[1)]/10 g DW[2)])	ABTS (mM AAEAC[3)]/10 g DW)
HeukHarang[4)]	**water**	**781.6 ± 0.05^{e}**[5)6)]	**3,305.0 ± 0.00^{h}**
	20% EtOH	**804.9 ± 0.00^{b}**	**2,815.0 ± 0.06^{i}**
	40% EtOH	**792.4 ± 0.00^{d}**	**3,755.0 ± 0.06^{g}**
	60% EtOH	**754.1 ± 0.00^{g}**	**4,405.0 ± 0.06^{e}**
	80% EtOH	**742.7 ± 0.00^{i}**	**4,305.0 ± 0.00^{f}**
	100% EtOH	**727.4 ± 0.00^{j}**	**2,305.0 ± 0.06^{j}**
Red lettuce[7)]	**water**	**303.0 ± 0.00^{f}**	**502.0 ± 0.00^{k}**
	20% EtOH	**318.1 ± 0.00^{c}**	**6,142.0 ± 0.06^{c}**
	40% EtOH	**322.3 ± 0.00^{b}**	**6,482.0 ± 0.00^{b}**
	60% EtOH	**341.9 ± 0.00^{a}**	**7,822.0 ± 0.06^{a}**
	80% EtOH	**322.1 ± 0.00^{b}**	**3,542.0 ± 0.06^{d}**
	100% EtOH	**299.1 ± 0.00^{h}**	**262.0 ± 0.06^{l}**

[1)]TEAC: Trolox equivalent antioxidant capacity; [2)]DW: dry weight; [3)]AAEAC: Ascorbic Acid equivalent antioxidant capacity; [4)]Newly cultivated lettuce; [5)]Values are mean ± standard deviation (n=3); [6)]Values with different letters within the same row are significantly different by Duncan's multiple range test at p<0.05; [7)]Wild type lettuce.

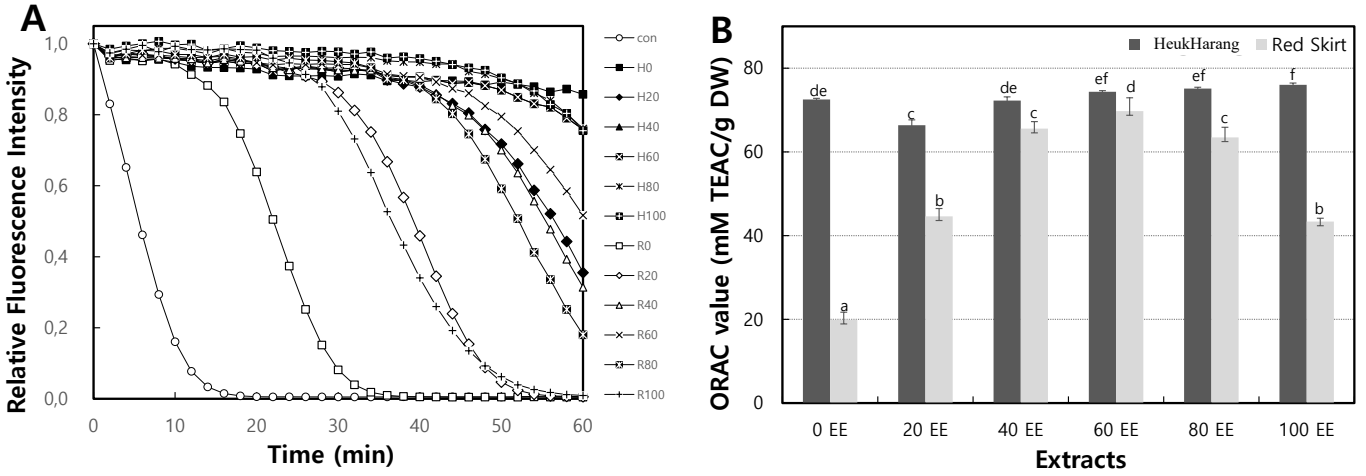

Figure 1. Relative fluorescence intensity (A) and ORAC value (B) of various ethanol extracts from HeukHarang and Red skirt. (A) Each marks are indicated at various ethanol extract; (B) The values are indicated as means ± standard deviation of three independent experiments. Means with different letters (a-f) are significantly different (p<0.05). 0EE; distilled water extracts, 20EE; 20% ethanol extracts, 40EE; 40% ethanol extracts, 60EE; 60% ethanol extracts, 80EE; 80% ethanol extracts, 100EE; 100% ethanol extracts.

value of HeukHarang extracts used in this experiment were in the range of 66.4 ± 1.3 to 76.0 ± 0.mM TEAC/g DW and that of red skirt lettuce as a control were in the range of 19.9 ± 1.8 to 69.7 ± 3.2 mM TEAC/g DW. The highest ORAC value among 'HeukHarang' ethanol extracts was 100% ethanol extracts and all extracts were higher than red skirt lettuce as significant (p<0.05) differences (Figure 1B).

4 Conclusion

To examine the antioxidant characteristics of newly developed Korean lettus, HeukHarang, samples were extracted with different ethanol extraction concentrations. Total antioxidant activity, as Total phenolic contents, total flavonoid contents, Ferric-reducing antioxide power, phosphomolybdenum complex assay, DPPH radical scavenging activity, ABTS radical scavenging activity and ORAC value, of HeukHarang, showed higher than that of Red skirt lettus. The 60% ethanol extract was the most active.

References

Abdel Latef, A. A. H., Dawood, M. F. A., Hassanpour, H., Rezayian, M., & Younes, N. A. (2020). Impact of the static magnetic field on growth, pigments, osmolytes, nitric oxide, hydrogen sulfide, phenylalanine ammonia-lyase activity, antioxidant defense system, and yield in lettuce. *Biology*, 9(7), 172-189. http://dx.doi.org/10.3390/biology9070172. PMid:32709036.

Arts, I. C., & Hollman, P. C. (2005). Polyphenols and disease risk in epidemiologic studies. *The American Journal of Clinical Nutrition*, 81(1, Suppl.), 317S-325S. http://dx.doi.org/10.1093/ajcn/81.1.317S. PMid:15640497.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma(FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1), 70-76. http://dx.doi.org/10.1006/abio.1996.0292. PMid:8660627.

Chaves, V. C., Calvete, E., & Reginatto, F. H. (2017). Quality properties and antioxidant activity of seven strawberry (*Fragaria* x *ananassa* duch) cultivars. *Scientia Horticulturae*, 225, 293-298. http://dx.doi.org/10.1016/j.scienta.2017.07.013.

Chen, A. Y., & Chen, Y. C. (2013). A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention. *Food Chemistry*, 138(4), 2099-2107. http://dx.doi.org/10.1016/j.foodchem.2012.11.139. PMid:23497863.

Cho, S. H., Choi, Y. J., Rho, C. W., Choi, C. Y., Kim, D. S., & Cho, S. H. (2008). Reactive oxygen species and cytotoxicity of Bamboo (*Phyllostachys pubescens*) sap. *Korean Journal of Food Preservation*, 15, 105-110.

Choi, S. Y., Lin, S. H., Ha, T. Y., Kim, S. R., Kang, K. S., & Hwang, I. K. (2005). Evaluation of the estrogenic and antioxidnat activity of some edible and medicianl plant. *Korean Journal of Food Science Technology*, 37(4), 549-556.

Chong, M. F. F., Macdonald, R., & Lovegrove, J. A. (2010). Fruit polyphenols and CVD risk: a review of human intervention studies. *British Journal of Nutrition*, 104(Suppl. 3), S28-S39. http://dx.doi.org/10.1017/S0007114510003922. PMid:20955648.

Dulger Altiner, D. (2021). Physicochemical, sensory properties and *in-vitro* bioaccessibility of phenolics and antioxidant capacity of traditional noodles enriched with carob (*Ceratonia siliqua* L.) flour. *Food Science and Technology*, 41(3), 587-595. http://dx.doi.org/10.1590/fst.21020.

El-Sheekh, M. M., El-Shenody, R. A. E. K., Bases, E. A., & El Shafay, S. M. (2021). Comparative assessment of antioxidant activity and biochemical composition of four seaweeds, Rocky Bay of Abu Qir in Alexandria, Egypt. *Food Science and Technology*, 41(Suppl. 1), 29-40. http://dx.doi.org/10.1590/fst.06120.

Fan, Z. L., Wang, Z. Y., & Liu, J. R. (2011). Cold-field fruit extracts exert different antioxidant and antiproliferative activities in vitro. *Food Chemistry*, 129(2), 402-407. http://dx.doi.org/10.1016/j.foodchem.2011.04.091. PMid:30634244.

Fraga, C. G., Croft, K. D., Kennedy, D. O., & Tomás-Barberán, F. A. (2019). The effects of polyphenols and other bioactive on human health. *Food & Function*, 10(2), 514-528. http://dx.doi.org/10.1039/C8FO01997E. PMid:30746536.

Guldas, M., Ziyanok-Demirtas, S., Sahan, Y., Yildiz, E., & Gurbuz, O. (2021). Antioxidant and anti-diabetic properties of *Spirulina*

platensis produced in Turkey. *Food Science and Technology*, 41(3), 615-625. http://dx.doi.org/10.1590/fst.23920.

Halliwell, B., Gutteridge, J. M. C., & Cross, C. E. (1992). Free radicals, antioxidants, and human disease: where are we now? *The Journal of Laboratory and Clinical Medicine*, 119(6), 598-620. PMid:1593209.

Hardinasinta, G., Mursalim, M., Muhidong, J., & Salengke, S. (2021). Determination of some chemical compounds of bignay (*Antidesma bunius*) fruit juice. *Food Science and Technology*, 41(4), 974-979. http://dx.doi.org/10.1590/fst.27720.

Hooper, L., & Cassidy, A. (2006). A review of the health care potential of bioactive compounds. *Journal of the Science of Food and Agriculture*, 86(12), 1805-1813. http://dx.doi.org/10.1002/jsfa.2599.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistrybehind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 53(6), 1841-1856. http://dx.doi.org/10.1021/jf030723c. PMid:15769103.

Kil, K. J., Doh, E. S., Chang, J. P., Lee, S. H., & Yoo, J. H. (2015). Antibacterial and antioxidant activities of bio-fermented *Sanguisorbae Radix* Extract. *Kor J Herbol*, 30(4), 29-35. http://dx.doi.org/10.6116/kjh.2015.30.4.29..

Kim, E. J., Choi, J. Y., Yu, M. R., Kim, M. Y., Lee, S. H., & Lee, B. H. (2012). Total polyphenols, total flavonoid contents, and antioxidant activity of Korean natural and medicinal plants. Kor. *Journal of Food Science and Technology*, 44, 337-342.

Kim, H. H., Heo, M. R., Lee, S., & Yim, S. H. (2019). Validation of analytical method and antioxidant properties of *Eriobotrya japonica* Lindl. leaf extract according to extraction solvent. *Korean Journal of Food Science Technology*, 51, 301-308.

Kim, M. J., Moon, Y., Tou, J. C., Mou, B., & Waterland, N. L. (2016). Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). *Journal of Food Composition and Analysis*, 49, 19-34. http://dx.doi.org/10.1016/j.jfca.2016.03.004.

Lee, G. Y., & Im, D. Y. (2012). Skin-related biological activities of the extract and its fractions from *Puerarariae Flos. Journal of Society of Cosmetic Scientists of Korea*, 18, 858-864.

Lee, H. H., Moon, Y. S., Yun, H. K., Park, P. J., & Kwak, E. J. (2014). Contents of bioactive constituents and antioxidant activities of cultivated and wild Raspberries. *Weonye Gwahag Gisulji*, 32(1), 115-122. http://dx.doi.org/10.7235/hort.2014.13114.

Lim, S. M. (2010). Resistance to reactive oxygen species and antioxidant activities of some strains of lactic scid bacteria from the mustard leaf kimchi. *Korean Journal of Microbiology*, 46, 375-382.

Liu, Y., & Li, S.-M. (2021). Extraction optimization and antioxidant activity of *Phyllanthus urinaria* polysaccharides. *Food Science and Technology*, 41(Suppl. 1), 91-97. http://dx.doi.org/10.1590/fst.11320.

Lucas-Abellán, C., Mercader-Ros, M. T., Zafrilla, M. P., Fortea, M. I., Gabaldón, J. A., & Núñez-Delicado, E. (2008). ORAC-fluorescein assay to determine the oxygen radical absorbance capacity of resveratrol complexed in cyclodextrins. *Journal of Agricultural and Food Chemistry*, 56(6), 2254-2259. http://dx.doi.org/10.1021/jf0731088. PMid:18303815.

Malejane, D. N., Tinyani, P., Soundy, P., Sultanbawa, Y., & Sivakumar, D. (2018). Deficit irrigation improves phenolic content and antioxidant activity in leafy lettuce varieties. *Food Science & Nutrition*, 6(2), 334-341. http://dx.doi.org/10.1002/fsn3.559. PMid:29564100.

Moreno, M. I. N., Isla, M. I., Sampietro, A. R., & Vattuone, M. A. (2000). Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. *Journal of Ethnopharmacology*, 71(1-2), 109-114. http://dx.doi.org/10.1016/S0378-8741(99)00189-0. PMid:10904153.

Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, 2(5), 270-278. http://dx.doi.org/10.4161/oxim.2.5.9498. PMid:20716914.

Park, H. J., Kang, S. A., Lee, J. Y., & Cho, Y. J. (2012). Antioxidant activities of extracts from medicinal plants. *Kor J Food Preserv*, 19(5), 744-750. http://dx.doi.org/10.11002/kjfp.2012.19.5.744.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, 269(2), 337-341. http://dx.doi.org/10.1006/abio.1999.4019. PMid:10222007.

Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B., & Jacob, R. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC) of plasmaand other biological and food samples. *Journal of Agricultural and Food Chemistry*, 51(11), 3273-3279. http://dx.doi.org/10.1021/jf0262256. PMid:12744654.

Russo, M., Spagnuolo, C., Tedesco, I., Bilotto, S., & Russo, G. L. (2012). The flavonoid quercetin in disease prevention and therapy: facts and fancies. *Biochemical Pharmacology*, 83(1), 6-15. http://dx.doi.org/10.1016/j.bcp.2011.08.010. PMid:21856292.

Talcott, S. T., & Lee, J. H. (2002). Ellagic aicd and flavonoid antioxidant content of muscadine wine and juice. *Journal of Agricultural and Food Chemistry*, 50(11), 3186-3192. http://dx.doi.org/10.1021/jf011500u. PMid:12009984.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins, B. D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, 19(6-7), 669-675. http://dx.doi.org/10.1016/j.jfca.2006.01.003.

Yamaguchi, T., Takamura, H., Matoba, T., & Terao, J. (1998). HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Bioscience, Biotechnology, and Biochemistry*, 62(6), 1201-1204. http://dx.doi.org/10.1271/bbb.62.1201. PMid:9692204.

Yang, X., Cui, X., Zhao, L., Guo, D., Feng, L., Wei, S., Zhao, C., & Huang, D. (2017). Exogenous glycine nitrogen eEnhances accumulation of glycosylated flavonoids and antioxidant activity in lettuce (*Lactuca sativa* L.). *Frontiers of Plant Science*, 8, 2098-2114. http://dx.doi.org/10.3389/fpls.2017.02098. PMid:29326732.

Yildiz, E., Guldas, M., & Gurbuz, O. (2021). Determination of in-vitro phenolics, antioxidant capacity and bio-accessibility of Kombucha tea produced from black carrot varieties grown in Turkey. *Food Science and Technology*, 41(1), 180-187. http://dx.doi.org/10.1590/fst.00320.

Yun, H. Y., Lim, S. J., Park, H. J., & Shin, Y. G. (2018). Correlation between antioxidant compounds and activities of 'Hibiscus sabdariffa' teas from different origins. *Journal of the East Asian Society of Dietary Life*, 28(1), 40-46. http://dx.doi.org/10.17495/easdl.2018.2.28.1.40.