PAPER

THE EFFECT OF PEELING ON ANTIOXIDANT CAPACITY OF BLACK RADISH ROOT

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ABSTRACT

In this study, freeze-dried peeled and unpeeled root, as well as the juice from peeled and unpeeled root of black radish (*Raphanus sativus* L. var *niger*) cultivated in Mongolia were characterized for their DPPH· and $ABTS^{\cdot+}$ scavenging activity, reducing power, total phenolics, and flavonoids in order to evaluate the effect of the peel. The juice from the peeled root showed strong antioxidant potential may due to its high phenolic content. However, the ability of the dried unpeeled root extract to quench free radicals and reduce Fe^{3+} was higher than that of the dried peeled root extract.

Keywords: antioxidant capacity, black radish, peel, phenolic compounds, root

1. INTRODUCTION

Fruits and vegetables play a vital role in the prevention of degenerative diseases caused by oxidative stress and the improvement of general health as these contain vitamins, minerals, amino acids, dietary fibers, and phenolic compounds. For instance, the prevention of cancer and cardiovascular diseases has been strongly related to the intake of fresh fruits and vegetables rich in natural antioxidants. This suggests that a higher intake of such compounds will lower the risk of mortality from these diseases (WILLCOX *et al*., 2004).

Radish (*Raphanus sativus* Linn.) is an edible root vegetable of the *Brassicaceae* (*Cruciferae*) family with some other popular vegetables including white and red cabbage, broccoli, brussel sprouts, cauliflower, kohlrabi, rape, and mustard. Radish is originally from Europe and Asia. It grows in temperate climates at altitudes between 190 and 1240 m. It is 30-90 cm high and its roots are thick and of various sizes, forms, and colors (PEREZ GUTIERREZ and PEREZ, 2004). Radishes have different skin colors such as red, purple, through pink, black, yellow, and white, while its flesh is typically white (BANIHANI, 2017). The most popular varieties of the radish are red (*Raphanus sativus* L.), white (*Raphanus sativus* L. var *white*), and black (*Raphanus sativus* L. var *niger*). Among the people of Mongolia, black radish is less familiar than red and white radish. A few years ago, some Mongolians with diabetes firstly obtained the black radish root from Russia due to its positive effect on diabetic conditions. Furthermore, local farmers have been cultivating the black radish. It has a rough, black skin with hot-flavored. There are round and elongated varieties. Radishes are grown all over the world and mostly eaten raw as a crunchy vegetable, mainly in salads. These can be also brined, fermented (pickled), dried, and cooked in soups or stews. Recently, some people prefer to drink its juice due to its certain health benefits such as antioxidant, anti-microbial, anti-viral, anti-inflammatory, anti-tumorigenic, anti-mutagenic, anti-diabetic, anti-proliferative, hypocholesterolemic, antilithiasic, diuretic, nephroprotective, gastroprotective, and hepatoprotective. It is also very well known for its use in the treatment of bronchitis, diarrhea, gynecological disorders, and jaundice (JANJUA *et al*., 2013, AGARWAL and VARMA, 2014, BANIHANI, 2017, KUMAR and PATWA, 2018). In general, radish contains carbohydrates, sugars, dietary fibers, protein, various water-soluble vitamins (B_1, B_2, B_3, B_5, B_6, B_9, and C), and minerals (calcium, iron, magnesium, manganese, zinc, potassium, phosphorus, and fluoride). In addition to flavonoids, alkaloids, tannins, and phenolic compounds, the radish was found to have unique bioactive phytochemicals including glucosinolates and isothiocyanates (WANG *et al*., 2010, LUGASI and HOVARI, 2000, BANIHANI, 2017). Isothiocyanates are breakdown products resulting from the enzymatic hydrolysis of glucosinolates by the myrosinase. For instance, the most abundant glucosinolates in black radish and its juices are glucoraphasatin and glucoraphanin. These two glucosinolates and their degradation products (raphasatin and sulforaphane) are known for their antioxidant properties, which have been related to cancer and cholesterol gallstones prevention (CASTRO-TORRES *et al*., 2014, SARIKAMIS *et al*., 2015). In addition, radishes have a specific flavor and strong taste due to glucosinolates and their breakdown products.

BORS *et al*. (2015) investigated the influence of the variety and vegetative stage on the total phenolic content and antioxidant capacity of radish. Significant differences were found in total phenolic content between radish varieties; the highest level was noticed in black radish, followed by red and white radish. Black and red radish had similar and significantly higher antioxidant capacity than white radish. Regarding the vegetative stage, the highest total phenolic content and antioxidant capacity were found in sprouts,

followed by seeds and roots. A positive and highly significant correlation (r^2=0.939) was determined between total phenolic content and antioxidant capacity of the radish varieties. LUGASI *et al*. (1998) reported that squeezed juice from black radish root exhibited strong hydrogen-donating ability, reducing power, copper (II)-chelating property and showed marked free radical (H_2O_2/·OH) scavenging activity. In their study, only one glucosinolate, namely glucotropaeolin, was detected in the juice by the HPLC analysis. In addition, a significant amount of polyphenols was detected in the juice. Therefore, the authors supposed that the degradation products of glucosinolates and the polyphenolic compounds are the main biologically active components of the sample. Several physiologically active compounds such as isothiocyanates, thiocyanates, indoles, nitriles, epithionitriles, cyano-epithioalkanes, oxazolidine–2–thiones, are released from hydrolysis of glucosinolates by the enzyme myrosinase (LUGASI *et al*., 1998, CASTRO-TORRES *et al*., 2014, BANIHANI, 2017).

In a study by NIKOLIC *et al*. (2012), the phenolic compounds content and DPPH radical scavenging capacity of black radish depended on root size in such a way that bigger root had a higher content of phenolic compounds and higher scavenging capacity. They also found a positive correlation between the phenolic compound content and radical scavenging capacity. Therefore, black radish roots with a weight higher than 100 g are recommended for human nutrition and health benefits. JANJUA *et al*. (2013) analyzed five different extracts of black radish root peel for important 14 phytochemicals, namely tannins, saponins, flavonoids, phlobatannins, anthraquinones, carbohydrates, reducing sugars, steroids, phytosterol, alkaloids, amino acids, terpenoids, cardiac glycosides, and chalcones. According to the proximate analysis, the black radish root peel contained 7% moisture and 93% dry matter which was composed of crude protein (28.57%), fats (27.76%) and carbohydrates (39.82%), while fibers were only 1.4% and ash content was 2.43%.

The objective of this study was to evaluate antioxidant activity and content of polyphenolic compounds in the black radish root cultivated in Mongolia. The antioxidant activities of the black radish root samples were investigated by using three different assays, namely DPPH, ABTS, and FRAP. Total phenolic content and total flavonoid content were also determined by spectrophotometrically.

2. MATERIALS AND METHODS

2.1. Chemicals and apparatus

Folin-Ciocalteu reagent, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,4,6-tripyridyl-*S*-triazine (TPTZ), gallic acid, quercetin, l-ascorbic acid, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (MO USA). All other chemicals and reagents were of analytical grade from local suppliers in Mongolia. The water used was purified in a Milli-Q system (Millipore, Bedford, MA, USA). Spectrophotometric determinations were carried out using a Shimadzu UV mini 1240 spectrophotometer.

2.2. Sample collection

Fresh black radish roots were purchased from a local produce market in Ulaanbaatar, Mongolia during the period September-October 2018. The radish roots were washed thoroughly under cold running tap water to remove surface impurities as well as to lower microbial load, spread on filter paper, cut-off their crown and tail. Afterward, the roots were halved with a stainless steel knife and separated into two parts. One part was peeled off with the knife. The other was processed with peel.

2.3. Sample preparation

Black radish root juice was obtained with the help of a laboratory-scale juice processor and then filtered using a sterilized muslin cloth. The obtained juice was stored at refrigeration temperature (4°C) and analyzed within 2 days. To obtain dried samples, the peeled and unpeeled black radish roots were sliced and freeze-dried for avoiding the loss of their juice rich in biologically active ingredients. The thoroughly dried samples were ground separately to a fine powder in a laboratory mill and then sifted through a mesh 0.5 mm in size. The powdered samples were stored in air-tight containers at 4°C until further use. To assay for antioxidant activity the powdered samples (1 g) were extracted with 50% (v/v) aqueous ethanol (50 mL) on a magnetic stirrer for 120 min at room temperature and centrifuged at 5000 rpm for 10 min at 4°C. The extracts were filtered with a Whatman No.5 filter paper and kept in dark at 4°C for further analysis.

2.4. DPPH free radical scavenging assay

$DPPH^{\cdot}$ scavenging ability of the samples was determined as described by ADEDAPO *et al*. (2009) with a minor modification. Briefly, 2 mL of 0.135 mM DPPH in ethanol (99.7%) were mixed with 100 μL of the sample solution. After 30 min in the dark, absorbance was measured at 517 nm against a blank (ethanol was used instead of the sample solution). Lower absorbance indicates higher scavenging activity. Percentage inhibition was calculated by comparing the absorbance of the test sample and the blank. Results were also expressed as Trolox equivalents (TE) by using a calibration curve (r^2=0.9973) of Trolox (0-300 μM).

2.5. ABTS radical cation ($ABTS^{\cdot+}$) scavenging assay

To evaluate $ABTS^{\cdot+}$ scavenging ability of the samples, the method of RE *et al*. (1999) was adopted. Firstly, to produce a stable $ABTS^{\cdot+}$ an aqueous solution of 7 mM ABTS was oxidized with 2.45 mM potassium persulfate for 12-16 h in the dark at room temperature. Before analysis, the $ABTS^{\cdot+}$ solution was diluted with distilled water to an absorbance of 0.75±0.05 at 734 nm. A 2 mL of $ABTS^{\cdot+}$ solution was mixed with 20 μL of the sample solution and absorbance was read at 734 nm exactly after 7 min. The percentage of $ABTS^{\cdot+}$ decolorization was calculated by comparing the absorbance of the test sample and the blank. The blank was prepared by replacing the sample solution with distilled water. The TE against $ABTS^{\cdot+}$ was also calculated using the calibration curve (r^2=0.9993) constructed with the standard Trolox (0.1-1.5 mM) under the same experimental conditions.

2.6. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay, a method for measuring total reducing power of electron-donating substances, was performed as previously described (BENZEI and STRAIN, 1996) with a slight modification. FRAP reagent containing 5 mL of 2,4,6-tripyridyl-*S*-triazine (TPTZ, 10 mM) in 40 mM HCl, 5 mL of ferric chloride hexahydrate (20 mM), and 50 mL of acetate buffer (300 mM, pH 3.6) was freshly prepared and warmed at 37°C before use. Sample solutions (100 μL) were allowed to react with 3 mL of the FRAP reagent for 30 min in the dark. Absorbance of the colored product was measured at 593 nm. A higher absorbance of the reaction mixture indicates greater reducing power. Aqueous solutions of ascorbic acid (0-0.1 mg/mL) were used for calibration (r^2=0.9998). The values were expressed as the concentration of ascorbic acid (vitamin C) that is most effective natural antioxidant having the ferric reducing ability.

2.7. Determination of total phenolic content

The total phenolic content of the black radish root samples was evaluated using the Folin-Ciocalteu colorimetric method (SINGLETON *et al*., 1999). In a test tube, each 20 μL of sample solution was mixed with 1.58 mL of distilled water and 100 μL of 1.8 N Folin-Ciocalteu phenol reagent and allowed to stand for 5 min. Then 300 μL of 20% aqueous sodium carbonate solution was added and shaken vigorously by a vortex. After incubation at room temperature for 2 h in the dark, the absorbance of the green-blue complex was measured at 765 nm. The results of total phenolic compounds were expressed as gallic acid equivalents (GAE), based on a calibration curve (r^2 = 0.9996) of gallic acid in the concentration range of 0.1 to 1.0 mg/mL.

2.8. Determination of total flavonoid content

Estimation of the total flavonoids in the black radish root samples was carried out using the procedure reported by ADEDAPO *et al*. (2009). Equal volume of sample solution and 2% anhydrous aluminium chloride in 50% (v/v) ethanol were mixed well and after 1 h at room temperature absorbance was measured at 420 nm. A yellow color indicates the presence of flavonoids. The interference background of the sample solution was corrected by preparing the test without aluminium chloride. The total flavonoid content was estimated from a calibration curve (r^2=0.9986) plotted at 420 nm with quercetin (10-50 μg/mL) and expressed as quercetin equivalents (QE).

2.9. Statistical analysis

All the measurements were taken five times and expressed as mean value ± standard deviation. The data were analyzed using one-way ANOVA for mean differences. Statistical significance was declared at $p < 0.05$.

3. RESULTS AND DISCUSSION

BORS *et al*. (2015) investigated the influence of the variety and vegetative stage on the total phenolic content and antioxidant capacity of radish. NIKOLIC *et al*. (2012) examined the

influence of black radish root size on the content and radical scavenging capacity of phenolic compounds. In this study, we evaluated the effect of the peel on the antioxidant activity of the juice and the ethanol extract prepared from black radish root cultivated in Mongolia. The juice obtained from black radish root and the ethanol extract of the freeze-dried root showed potent antioxidant activity in different test systems and contained a significant amount of polyphenols. Peeling increased the antioxidant capacity of black radish root juice significantly ($p<0.05$). On the other hand, the ethanol extract of the dried peeled and unpeeled root exerted statistically similar antioxidant potential.

3.1. DPPH· scavenging activity

It is well known that the DPPH· scavenging ability is attributed to the hydrogen donating ability of phytocompounds. A comparison of DPPH· scavenging abilities of the black radish root samples is shown in Fig. 1. Trolox, a synthetic water-soluble antioxidant analogue of vitamin E, was used as a reference compound, and DPPH· scavenging potentials expressed as TE of the studied samples were listed in Table 1. The black radish root juice were examined for their DPPH· scavenging ability after dilution by 10 times with distilled water. The strong DPPH· scavenging capacity of 60.9% was detected in the juice from peeled root, while the juice from unpeeled root exerted weak DPPH· scavenging capacity of 21.9% (Fig. 1), which reflects approximately 2.8-fold difference. However, DPPH· scavenging activities of the peeled and unpeeled root extracts were comparable to each other. At a concentration of 50 mg/mL, the percentage scavenging of the peeled root extract was 61.8% whereas that of the unpeeled root extract was 65.7%. The black radish root extracts quenched DPPH· with a mean of 414 μmol TE/100 g dry weight (Table 1).

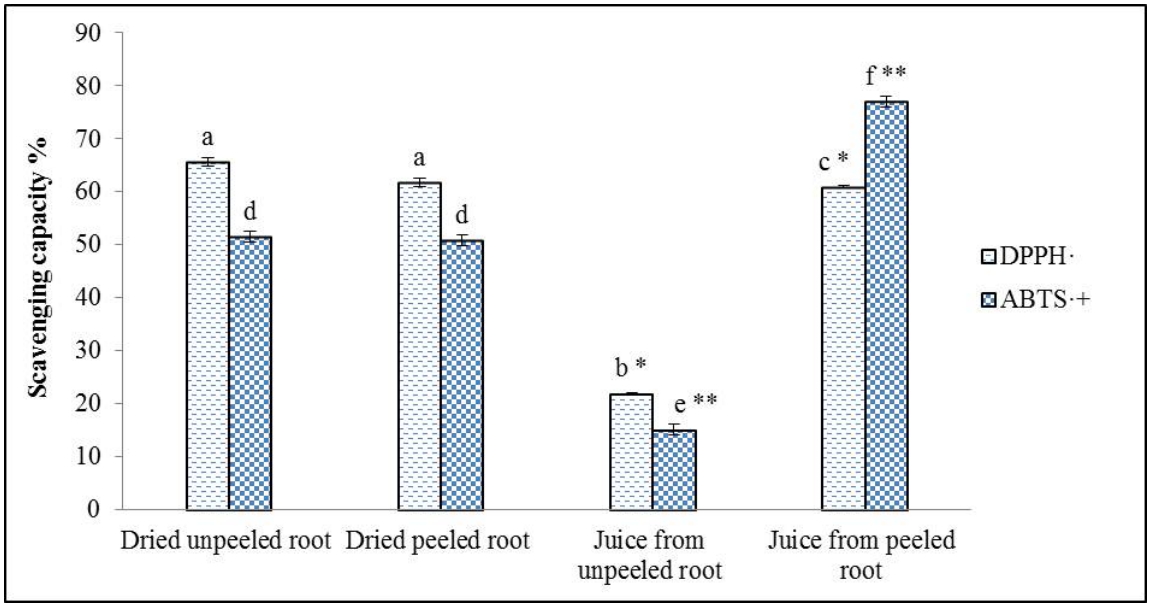

Figure 1. DPPH· and ABTS·· scavenging activity of the black radish root samples (%). Values are mean ± standard deviations of five parallel determinations. The vertical bars represent the standard deviations for each data point. Values with different superscript letters are significantly different ($p < 0.05$). *- diluted 10 times; **- diluted 5 times.

There are some reports describing DPPH· scavenging activity of black radish root and its juice, but the results varies depending on the experimental condition. For instance, DPPH· scavenging capacity of 80% (v/v) ethanol extract from black radish root ranged from 88.3% to 55.6% at a concentration of 5.5 mg/ml depending on the root size and the appropriate IC_{50} values were 1.59 and 3.54 mg/mL, respectively (NIKOLIC *et al*., 2012). According to the BORS *et al*. (2015), black radish root showed weak activity (12.23%) to scavenge DPPH· at the relatively high concentration (the dry extract prepared from 500 mg freeze-dried powder was recovered in 3 mL methanol). The squeezed juice from black radish has a significant scavenging activity to quench DPPH· with an IC_{50} value of 0.54 mL (LUGASI *et al*., 1998).

3.2. $ABTS^{\cdot+}$ scavenging activity

The antioxidant capacity of the samples was evaluated by $ABTS^{\cdot+}$ assay because proton radical scavenging is an important attribute of antioxidants. $ABTS^{\cdot+}$, a protonated radical, is reduced in the presence of hydrogen-donating antioxidants. Besides being one of the fastest, $ABTS^{\cdot+}$ method also provides good solubility, which allows the analyses of both lipophilic and hydrophilic compounds (RE *et al*., 1999). Fig. 1 showed the percentage inhibition of $ABTS^{\cdot+}$ by the studied samples, whereas Table 1 presented the scavenging abilities expressed as TE. The black radish root juice diluted previously 5 times with distilled water was analyzed by $ABTS^{\cdot+}$ assay. The juice from the peeled black radish root quenched 76.9% of $ABTS^{\cdot+}$ in the reaction mixture (Fig. 1), which is about 5.2-fold greater than that found in the juice obtained from the unpeeled root (14.9%). The same as the DPPH· scavenging activity, peeled and unpeeled root extracts showed similar ability to scavenge $ABTS^{\cdot+}$ with a mean of 1510.7 μmol TE per 100g dry weight (Table 1). At 50 mg/mL, the scavenging percentages were 50.7% and 51.4% for the peeled and unpeeled black radish root extracts, respectively (Fig. 1). To our best knowledge, there is almost no data on $ABTS^{\cdot+}$ scavenging potential of black radish root samples.

3.3. Ferric reducing antioxidant power

The reducing power of the black radish root samples was analyzed using the FRAP assay by comparing with reducing power of ascorbic acid (Table 1). The formation of blue-colored TPTZ–Fe^{2+} complex from colorless oxidized TPTZ–Fe^{3+} by the action of the electron-donating antioxidants under acidic condition (pH 3.6) was recorded at 593 nm. The reducing ability of the plant samples could be attributed to the number of hydroxyl groups in the phenolic and flavonoid compounds. The studied samples from black radish root intensively reduced Fe^{3+} to Fe^{2+}. It was found that 1 g (dry weight) of black radish root and 1 mL of its juice showed reducing power equal to that estimated in 3.5-3.7 and 0.8-1.0 mg of pure ascorbic acid, respectively. In a study by LUGASI *et al*. (1998), the reducing effect of 1 mL of black radish root juice was the same as that of 0.73 μmol ascorbic acid. It is equal to 0.13 mg ascorbic acid per 1 mL of the juice. According to HANLON and BARNES, the reducing power of the freeze-dried unpeeled root of black radish (Nero Tondo variety) was 23.7 μmol ascorbic acid/g which is equal to 4.17 mg ascorbic acid/g. It was comparable to our result.

Similarly to DPPH· and ABTS·+ scavenging ability, the dried unpeeled root extract and the juice obtained from peeled root showed higher reducing power. However, the margin of difference between reducing power of the juice obtained from the peeled and unpeeled root was narrow. The results indicated that phytoconstituents in black radish are capable of donating electrons that can react with free radicals to convert them into more stable compounds and reduce the oxidized intermediates of lipid peroxidation process.

Table 1. Antioxidant potential of the black radish root samples.

Sample	DPPH· scavenging activity	ABTS·+ scavenging activity	Fe^{3+} reducing power
Dried peeled root	401.47±5.54[1]	1500.45±21.24[1]	349.59±4.24[3]
Dried unpeeled root	426.52±4.91[1]	1520.95±109.58[1]	372.23±6.53[3]
Juice from peeled root	198.05±0.83[2]	569.66±4.41[2]	100.49±4.95[4]
Juice from unpeeled root	71.98±0.43[2]	109.36±1.16[2]	83.90±1.46[4]

[1]Expressed as μmol Trolox equivalents/100g.
[2]Expressed as μmol Trolox equivalents/100mL.
[3]Expressed as mg ascorbic acid/100g.
[4]Expressed as mg ascorbic acid/100mL.
Values are mean ± standard deviations of five parallel determinations.

3.4. Total phenolic content

Plant polyphenols are the significant group of compounds acting as free radical scavenging or primary antioxidants; therefore, it is justifiable to determine phenolic content in the plant extract. These can also chelate metal ions that could catalyze the formation of reactive oxygen species, which promotes lipid peroxidation (IQBAL *et al*., 2015). In many studies, phenolic compounds have demonstrated higher antioxidant activity than antioxidant vitamins and carotenoids (VELIOGLU *et al*., 1998). Total phenolic contents (expressed as GAE) in the black radish root samples are given in Table 2. The amount of total phenolics in the juice ranged from 103 to 146 mg/100mL. LUGASI *et al*. (1998) detected by the Folin-Denis method relatively low polyphenol content (25.5 mg/100 mL juice) in black radish root juice. In the case of the dried black radish root, total phenolic content was in the range of 750-791 mg/100 g (7.5-7.9 mg/g) dry weight. The lower level of total phenolic content was obtained by BORS *et al*. (2015) in black radish root (4.75 mg of GAE/g dry weight) and by HANLON and BARNES (2011) in the freeze-dried unpeeled root of the Nero Tondo black radish (2.4 μg of GAE/g). Total phenolic content in the unpeeled root was higher than those of the peeled root. These results revealed that black radish root skin contained a considerable amount of phenolic ingredients. However, the juice obtained from the unpeeled root contained lower amount of total phenolics compared to the peeled root juice. It indicated that the phenolic constituents present in the peel could not be extracted into the juice or these substances may be degraded during processing and storage by the action of any factors such as an enzyme. JANJUA *et al*. (2013) reported that phytochemicals of black radish root peel were not well dissolved in water as well as methanol. Moreover, the lowest yield was of water extract.

3.5. Total flavonoid content

Flavonoids exert potent antioxidant activity by several different mechanisms, such as scavenging of free radicals, chelation of metal ions, and inhibition of enzymes responsible for free radical generation. Depending on their structure, flavonoids are able to scavenge practically all known reactive oxygen species (PANDA, 2012). LUGASI and HOVARI (2000) demonstrated that radish contains a significant amount of flavonoids such as kaempferol, quercetin, myricetin, apigenin, and luteolin. Before that, some researchers observed 7 mg/kg (0.7 mg/100g) flavonoids including quercetin and myricetin in the radish root (LUGASI *et al*., 1998).
The amount of total flavonoid content in the black radish root samples was assessed by the aluminium chloride assay. Total flavonoid content in the juice prepared from the peeled root was 0.81 mg QE/100 mL, while total flavonoids were not detected in the juice from unpeeled root (Table 2). Dried peeled and unpeeled root of black radish contained comparable amounts of total flavonoids with a mean of 15.8 mg QE per 100 g of dry weight.

Table 2. Total phenolics and flavonoids of the black radish root samples.

Sample	Total phenolic content	Total flavonoid content
Dried peeled root	749.60±14.59[1]	16.00±0.04[3]
Dried unpeeled root	791.20±46.71[1]	15.69±0.09[3]
Juice from peeled root	146.00±3.42[2]	0.81±0.00[4]
Juice from unpeeled root	103.10±2.28[2]	–

[1]Expressed as mg gallic acid equivalents/100g.
[2]Expressed as mg gallic acid equivalents/100mL.
[3]Expressed as mg quercetin equivalents/100g.
[4]Expressed as mg quercetin equivalents/100mL.
Values are mean ± standard deviations of five parallel determinations.

4. CONCLUSIONS

The juice obtained from the peeled and unpeeled root showed considerable differences in antioxidant activity when it was calculated by the three different methods used in this study. The juice prepared from the peeled root showed strong antioxidant potential may due to its high phenolic content. However, the juice from the unpeeled root contained a relatively low amount of total phenolics and had no flavonoid content. It was supposed that the phenolic constituents including flavonoids present in the peel could not be extracted into the juice or these substances may be degraded. Although dried black radish root samples contained significantly different amounts of total phenolics, they showed statistically similar free radical scavenging activities and reducing power. Thus, the antioxidant activities of the dried root samples are probably from the combined action of phenolics and other constituents. The results of this study show that the root of black radish cultivated in Mongolia contained a significant amount of biologically active phenolics with antioxidant properties. Thus, the black radish root may serve as a potential source in functional food development.

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