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Full Length Research Paper

Antioxidant potential of ethanol extract of *Brassica rapa* L. root

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***Brassica rapa* L. (turnip) roots were extracted with 70% ethanol, and then sequentially fractionated into n-hexane, chloroform, and ethyl acetate fractions. The ethanol extract possessed antioxidant potentials such as free radical scavenging, nitrite scavenging, and lipid peroxidation inhibitory activities as well as reducing power. Among solvent fractions of turnip roots extract, ethyl acetate fraction exhibited significantly high activities in free radical scavenging ($p < 0.05$), reducing power ($p < 0.001$), and lipid peroxidation inhibition ($p < 0.05$) due to the highest level of total phenolic content. The antioxidant potential showed a positive correlation with total phenolic content.**

Key words: *Brassica rapa* L., ethanol extract, antioxidant potential, total phenolic content.

INTRODUCTION

Antioxidants can scavenge free radicals and nitrites, inhibit lipid peroxidation, and possess reducing power, thus protecting human body from oxidative stress (Arora and Chandra, 2011). Therefore, they have been considered to be effective in the prevention of aging, atherosclerosis, liver dysfunction, neurodegenerative disorder, and cancer (Senevirathne et al., 2006; Patil et al., 2009). Natural antioxidants, which are widely distributed in edible plants, have generated great attention due to their non-toxic side effects (Velioglu et al., 1998; Arora and Chandra, 2011).

The Brassicaceae family has been considered as economically important horticultural crops. *B. rapa* L. (syn. *Brassica campestris* L.), commonly known as turnip, has been one of the most popularly cultivated vegetables since prehistoric times (Fernandes et al., 2007; Sousa et al., 2008). Turnip edible parts are consumed as a raw, boiled and/or fermented vegetable all over the world.

They contain a variety of organic compounds with biological activity such as glucosinolates, phenylpropanoids, flavonoids, phenolics and organic acids (Fernandes et al., 2007). Turnip extract has been reported to possess hepatoprotective, nephroprotective, and anticancer effects (Rafatullah et al., 2006; Kim et al., 2006; Hong and Kim, 2008). The phenolic compounds such as kaempferol 3-*O*-soporoside-7-*O*-glucoside and isorhamnetin 3, 7-*O*-diglucoside were identified from leaves, stems and flower buds of turnip (Fernandes et al., 2007). In addition, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was screened in edible parts of a turnip (Fernandes et al., 2007). However, although turnip roots have been mainly consumed as a fermented vegetable food in Asia, their antioxidant potential has not yet been thoroughly studied.

In this communication, we investigated the antioxidant potential of ethanol extract of turnip roots by evaluating free radical scavenging, nitrite scavenging, and lipid peroxidation inhibitory activities, as well as reducing power.

MATERIALS AND METHODS

Extraction and solvent fractionation of *Brassica rapa* L. roots

B. rapa L. roots were harvested from the farms of Ganghwa island, South Korea in October 2009. The roots were cut into small pieces

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Table 1. Total phenolic content and antioxidant potential of *Brassica rapa* L. root.

Fraction	Yield (mg/100 g)	Color intensity[a]	Total phenolics (% as garlic acid)[b]	IC_{50} (mg/ml)	
				Free radical scavenging	Nitrite scavenging
70% Ethanol	2750	0.074	2.59 ± 0.160	0.23	0.124
n-Hexane	489	0.024	0.21 ± 0.008	1.65	0.252
Chloroform	356	0.034	0.56 ± 0.016	1.75	0.010
Ethyl acetate	153	0.076	1.08 ± 0.031	0.46	0.037
Residual	1540	0.168	0.63 ± 0.010	> 2.0	0.461

[a]Color intensity was expressed as an absorbance at 420 nm of 0.1%(w/w) solution in ethanol. [b]Data were expressed as mean ± SD.

and dried in the shade. The dried roots (100 g) were milled to a particle size of less than 400 μm and then extracted with 500 ml of 70% ethanol at room temperature for 24 h. After centrifugation (10000 × g, 20 min), the pellet was discarded and the supernatant was freeze-dried. The dried extract was dissolved in 200 ml of distilled water and then fractionated successively with n-hexane, chloroform and ethyl acetate of 600 ml, respectively. Three fractions and the final aqueous layer (residual water fraction) were evaporated and freeze-dried to eliminate residual solvents, respectively.

Total phenolic content and antioxidant potential

The total phenolic content of each fraction was determined at a wavelength of 725 nm using the Folin-Ciocalteu spectrophotometric method as described by Ismail et al. (2004). The contents were expressed as gallic acid equivalents (GAE).

Free radical scavenging activity was measured at a wavelength of 517 nm using a stable DPPH free radical according to the method of Chan et al. (2007). DPPH free radical scavenging activity was expressed as IC_{50} value which is the effective concentration of a sample required to scavenge DPPH free radicals. Nitrite scavenging activity was measured at a wavelength of 520 nm using $NaNO_2$ and Griess reagent according to the method of Choi et al. (2008). Then, pH value of reaction mixtures was adjusted to 1.2. L-ascorbic acid was used as a positive control. Nitrite scavenging activity was expressed as IC_{50} value which is the effective concentration of a sample required to scavenge 50% nitrites.

Lipid peroxidation inhibitory activity was determined by measuring the peroxidation of linoleic acid, similar to the method of Shyamala gowri and Vasantha (2010). Briefly, 400 μg of each fraction in 10 ml ethanol were added with 2.5% linoleic acid in 10 ml ethanol and then lipid peroxide was measured at a wavelength of 500 nm every 12 h for 3 days at 40°C. A lower absorbance of the reaction mixture indicated stronger inhibitory effect of lipid peroxidation. Reducing power was determined according to the method of Senevirathne et al. (2006). A higher absorbance of the reaction mixture at a wavelength of 700 nm indicated stronger reducing power.

All the aforementioned experiments were conducted in triplicate. Data were expressed as mean ± standard deviation (SD). Data were analyzed by using one-way ANOVA followed by Duncan's multiple range tests using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Ethanol extract of *B. rapa* L. roots was divided into n-hexane, chloroform, and ethyl acetate fractions to determine the total phenolic content and antioxidant potential. The phenolic content and color intensity were increased in the order of ethyl acetate > chloroform > n-hexane fractions (Table 1). The total phenolic content of each fraction showed a positive correlation with relative polarity and dielectric constant of solvents. However, 25% of total phenolics in ethanol extract of *Brassica rapa* still remained in the residual water fraction. The color intensity at 420 nm indicated the quantity of phenolic compounds (Benerjee and Bonde, 2011). Phenolic compounds of plant origin are known to possess antioxidant activity due to their ability to chelate metals and inhibit lipid peroxidation (Choi et al., 2008). The highest content of total phenolics was 10.8 ± 0.31 mg GAE/g of ethyl acetate fraction, approximately 2.0-fold than that of chloroform fraction. The results were consistent with the report that ethyl acetate fraction of *Mangifera indica* leaves has the highest total phenolic content (Badmus et al., 2011). Also it was reported that extremely non-polar solvents as well as water were less efficient to extract the phenolic compounds from plant sources (Pérez et al., 2007; Azlim Almey et al., 2010; Benerjee and Bonde, 2011).

DPPH free radical scavenging activity of extracts represents their hydrogen-donating ability to serve as free radical inhibitors or scavengers (Patil et al., 2009). The DPPH radical scavenging activity of all fractions gradually increased in a concentration-dependent manner. The radical scavenging activities were high in the order of ethyl acetate > n-hexane > chloroform fractions (Table 1). The radical scavenging activity of ethyl acetate fraction was significantly higher than that of the other two fractions ($p<0.05$). IC_{50} value of ethyl acetate fraction for DPPH radical scavenging was 0.46 mg/ml, which corresponded to its highest total phenolic content. The phenolics and their derivatives are considered to be responsible for free radical scavenging (Wang et al., 2008).

Nitrite causes the oxidization of hemoglobin which can lead to methemoglobinemia and the formation of nitrosoamine which can increase the risk for cancer (Choi et al., 2008). Certain antioxidants could scavenge the

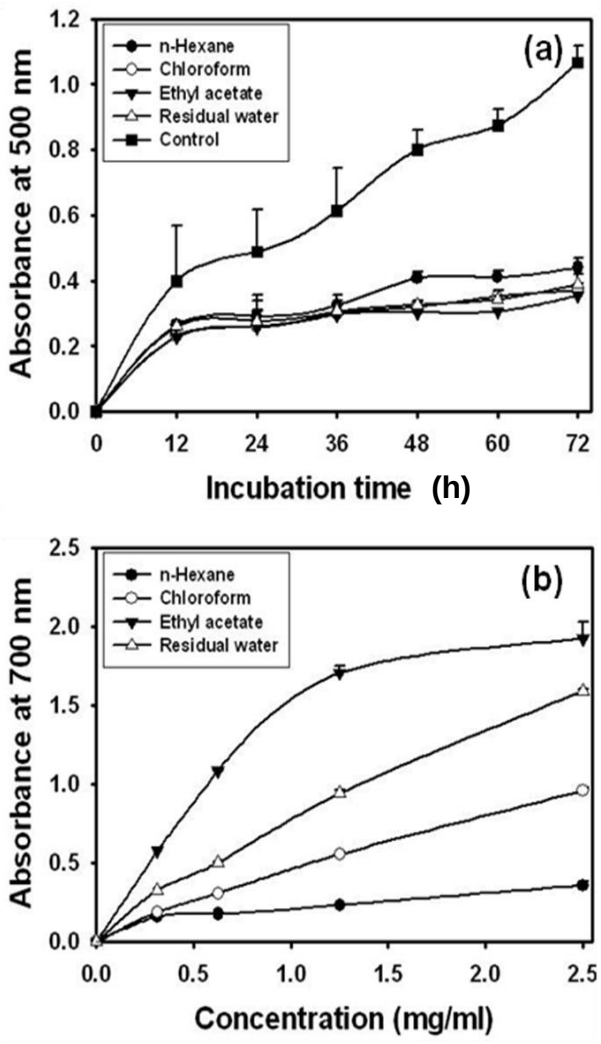

Figure 1. (a) Lipid peroxidation inhibitory activity; (b) reducing power of ethanol extract of *Brassica rapa* L. root. The ethanol extract was sequentially divided into n-hexane, chloroform, ethyl acetate, and residual water fractions. Data were expressed as mean ± SD.

toxic nitrite because of their reducing power (Wang et al., 2008). Each fraction of ethanol extract of *B. rapa* exhibited a concentration-dependent nitrite scavenging activity. The nitrite scavenging rates were high in the order of chloroform > ethyl acetate > n-hexane fractions (Table 1). IC_{50} values for nitrite scavenging were 0.010 mg/ml in chloroform fraction and 0.037 mg/ml in ethyl acetate fraction, respectively. The nitrite scavenging activity of chloroform and ethyl acetate fractions was higher than that of positive control compound L-ascorbic acid with an IC_{50} value of 0.054 mg/ml. In addition, the nitrite scavenging activities were measured under acidic condition (pH 1.2) which is similar to pH of stomach. It suggests that the ethanol extract of *Brassica rapa* L. roots might inhibit the production of carcinogenic nitrosamine *in vivo* (Choi et al., 2008).

Lipid peroxidation inhibitory activities were high in the order of ethyl acetate ≥ chloroform > n-hexane fractions (Figure 1a). The lipid peroxidation in the control group greatly increased as a function of the incubation time. However, it was significantly restrained by the addition of each fraction ($p < 0.05$) as compared to that of control. The inhibitory activity showed a positive correlation with total phenolic content. The lipid peroxidation is initiated by the reaction between unsaturated lipids and reactive oxygen species, leading to the formation of lipid radical and lipid peroxide which cause damage to the cell membrane (Badmus et al., 2011). Antioxidants can inhibit the lipid peroxidation by scavenging free radicals and chelating catalytic metals (Shyamala gowri and Vasantha, 2010). The ethanol extract of *B. rapa* L. roots had the inhibitory activity against lipid peroxidation.

Reducing powers were high in the order of ethyl acetate > chloroform > n-hexane fractions (Figure 1b). It showed a positive correlation with relative polarity and dielectric constant of solvents. Each fraction of ethanol extract of *B. rapa* exhibited a concentration-dependent reducing power. The reducing power of ethyl acetate fraction was significantly higher ($p < 0.001$) than that of other fractions. The reducing power of natural sources, which is considered as their electron transfer ability, is associated with their phenolic content and antioxidant activity (Shyamala gowri and Vasantha, 2010; Badmus et al., 2011). The ethanol extract of *B. rapa* L. roots showed the reducing ability of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions.

It is concluded that the ethanol extract of *B. rapa* L. roots possessed the antioxidant activity such as free radical scavenging, nitrite scavenging, and lipid peroxidation inhibitory activities as well as reducing power. Furthermore, the ethyl acetate fraction of ethanol extract of *B. rapa* exhibited the highest antioxidant potential. Total phenolic content of *B. rapa* L. roots showed a positive correlation with their antioxidant activity. Further studies are needed to isolate and identify the individual compounds responsible for the antioxidant activity.

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