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Comparison of Antioxidant Capacity and Nutritional Composition of three Cultivars of *Actinidia arguta*

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Abstract : *Actinidia arguta* extracts obtained from three cultivars ('Sae-Han', 'Dae-Sung', and 'Chil-Bo') were assayed for their antioxidant properties and nutritional composition. Antioxidant activity of the extracts was evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. Total phenolic contents of the extracts were determined by the Folin-Ciocalteu method. Vitamin C (L-ascorbic acid) content measured by a colorimetric method and reducing sugar content estimated by dinitrosalicylic acid (DNSA) method. Crude extracts from *A. arguta* 'Sea-Han' showed the most potent radical-scavenging activity showing 86.55% at 10 mg/ml. The DPPH radical scavenging activity of extracts and solvent fractions from Sea-Han cultivar was in decreasing order of EtOAc fraction>BuOH fraction>CH_2Cl_2 fraction>hexane fraction, among which EtOAc fraction showed the highest antioxidant activity (87.51% at 5 mg/ml). Total phenolic contents in *A. arguta* 'Sae-Han', 'Dae-Sung' and 'Chil-Bo', were 32.93, 28.23, and 25.60 mg/g, respectively. Vitamin C contents of them were 840.57, 578.81 and 730.10 ug/g, respectively.

Key words **:** Actinidia arguta, *antioxidant properties, total phenolics, vitamin C content, reducing sugar*

Introduction

Actinidia arguta, called hardy kiwifruit, has an edible smooth skin and contains high amounts of sugar and vitamin C (ascorbic acid) (Kim *et al.*, 2006). It is native to north China, Korea, and Japan. From the flower of *A. arguta*, liac alcohol epoxide was isolated and identified (Matich *et al.*, 2003). The Bower Actinidia (*A. arguta* (Sieb. & Zucc.) Planch. ex Miq.) is one of the valuable species due to its edible fruit, high content of nutritious substances, especially abundance vitamin C, distinctive flavor, and medicinal usage.

To make new cultivar with larger fruit and high yielding, we selected new *A. arguta* cultivars, 'Sea-Han', 'Dae-Sung', and 'Chil-Bo' and registered as a new variety denomination and certificated variety production and merchandising in 2006. However, we did not evaluated biological activities such as antioxidant activity, and nutritional composition of these cultivars. In this study, we evaluated antioxidant activity of extracts from *A. arguta.*

Oxidation can be defined as the transfer of electrons from one atom to another and its occurrence in living organisms is known to cause damage to DNA, protein and lipids (Maxwell, 1995; Afonso *et al.*, 2007). This damage appears to be one of the major ageing factors of living organisms such as cancer, cardiovascular disease, immune system decline and brain dysfunction etc. Antioxidants help to maintain the quality of many food products by preventing oxidation and can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction (Javanmardi *et al.*, 2003).

Although many antioxidants are used, most of them are synthetic antioxidants including, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Sherwin, 1990). However, using these synthetic antioxidants have been restricted due to their toxicity and are suspected to be carcinogenic. Therefore, the importance of searching for natural antioxidants, especially of plant origin, has greatly increased in recent years. Natural antioxidant can protect the human body from free radicals and delay the progress of many chronic diseases (Lai *et al.*, 2001). Hence, the studies on natural antioxidant have gained greater importance. Some researches have been suggested that antioxidant compounds can be found in wood, bark, stem, leaf, fruit, root, flower, and seed of many plants.

To our knowledge, however, antioxidant effects and total phenolic contents of *A. arguta* extracts have not previously been published. Because of the lack of such information, we report the antioxidant activities of extracts from three cultivars of *A. arguta*. Moreover, total phenolic

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contents, vitamin C contents, and reducing sugar contents from three cultivars of *A. arguta* were also investigated.

Materials and Methods

1. Materials

A. arguta, grown in the Korea Forest Institute (Suwon) was used. The weight and diameter of three cultivars (*A. arguta* 'Sae-Han', 'Dae-Sung', and 'Chil-Bo') are listed on Table 1.

2. Extraction and Fractionation

The dry fruit flesh was ground into powder and extracted with ethanol (EtOH) at 60°C for 30 min, and then the ethanol phase was dried under pressure to give the crude extract. The crude extract of Sea-Han fruit, which showed high antioxidant activity, was successively partitioned with organic solvents, *n*-hexane, dichloromethane, ethyl acetate, and butanol (Fig. 1).

3. Antioxidant activity

The antioxidant activity was evaluated by the DPPH (1, 1-diphenyl-2-picrylhydrazyl) method according to the procedure of Park *et al.* (2006). Ethyl alcohol soluble fraction (0.5 ml) of the samples at various concentrations (50, 100 and 125 ppm) was added to a solution of DPPH in EtOH (100 μM, 3 ml) and the reaction mixture were shaken vigorously. After incubating the mixtures for 10 min at room temperature, the remaining amounts of DPPH were determined by colorimetry (852A Diode Array Spectrophotometer, Hewlett Packard Co.) at 517 nm. The mixture of 0.5 ml EtOH and 3 ml DPPH solution was used as control. The mean values were obtained from triplicate experiments.

Table 1. The weight and diameter of *Actinidia arguta* fruit of three new cultivars.

A. arguta cultivar	Fruit Length (mm)	Fruit Width (mm)	Weight of Fruit (g)
'Sae-Han'	43.6	36.1	29.4
'Dae-Sung'	41.6	29.9	18.9
'Chil-Bo'	28.4	36.9	18.2

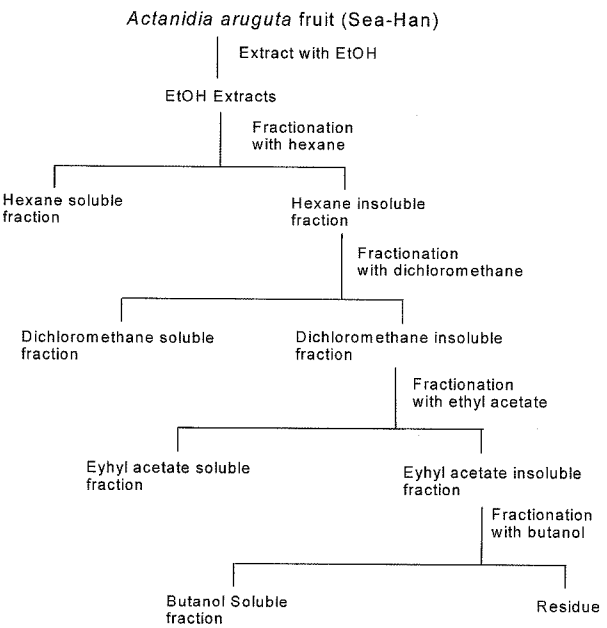

Figure 1. Flow diagram of sample preparation from *Actanidia arguta*.

4. Total phenolic contents

Total phenolic contents were measured according to the method of Cheung *et al.* (2003). Each sample (1 ml) was mixed with Folin and Ciocalteu's phenol reagent (1 ml, Sigma). After 3 min, 1 ml of saturated Na_2CO_3 was added to the mixture and it was made up to 10 ßȼ by adding distilled water. After the reaction solution was kept in the dark for 90 min, its absorbance was taken at 725 nm. A calibration curve was constructed with different concentrations of gallic acid (Wako pure chemical Industries) (0.01-0.1 mM) as a standard.

5. Vitamin C content

Vitamin C (L-ascorbic acid) was determined by a colorimetric method defined by Jagot and Dani (1982). A 0.5 g sample of dried fruits was weight and extracted with distilled water then filtered. 0.2 ml extracts was mixed with 0.8 ml 10% (w/v) trichloroacetic acid (TCA) at 4°C. After centrifugation at 3000 rpm for 5 min, 0.5 ml of supernatant was made up to 2 ml volume with distilled water. 0.2 ml 10% (v/v) Folin phenol reagent was then added to the mixture, and vigorously shaken. After 10 min of the reaction time, maximum absorbance was measured at 760 nm. The absorption maximum of the color developed by the interaction of ascorbic acid with Folin reagent is 760 mn.

6. Total soluble solids

The juice obtained from three cultivars of *A. arguta* fruit was used to measure the soluble solids content with a digital refractometer (Atago Co., Tokyo, Japan).

7. Reducing sugar content

Content of reducing sugars was estimated by dinitrosalicylic acid (DNSA) method of Miller (1959). Add 3 ml of DNSA reagent to 3 ml of sample and the mixture was heat at 90°C for 15 min to develop the red-brown colour. Subsequently, 1 ml of 40% potesium sodium tartrate solution was added to it and the mixture was cooled down. The absorbance of the solution was taken at 575 nm. A calibration curve was constructed with glucose at

different concentrations as a standard.

Results and Discussion

1. Antioxidant activity

Because the antioxidant activity of fruits is important for assessing their nutritional value (Rice-Evans et al., 1996), the free radical scavenging activity of three cultivars of *A. arguta* was measured using DPPH radical-scavenging assay. DPPH is a stable free radical. Antioxidants, on interaction with DPPH, transfer either electrons or hydrogen atoms to DPPH, thus neutralising the free radicals (Naik *et al.*, 2003). The colour of reaction mixture changes from purple to yellow when DPPH reacts with antioxidant and its absorbance at wavelength of 517 nm decreases.

The free radical scavenging activities of three cultivars of *A. arguta* are shown in Fig. 2. The free radical scavenging activities of three cultivars, *A. arguta* 'Sea-Han', 'Dae-Sung', and 'Chil-Bo' were 86.54, 74.64 and 59.12% at 10 mg/ml, respectively. The antioxidant activity of the fruits of *A. arguta* cultivars appeared to be concentration dependent.

The crude extract of *A. arguta* 'Sea-Han' fruit, which showed high antioxidant activity, was successively fractionated with organic solvents, *n*-hexane, dichloromethane, ethyl acetate, and butanol (Fig. 1). Fig. 3 shows free radical scavenging activities of the solvent fractions of *A. arguta* 'Sea-Han', which has highest activity. The DPPH radical scavenging activity was in decreasing order of EtOAc fraction> BuOH fraction> CH_2Cl_2 fraction> hexane fraction, among which EtOAc fraction showed the highest antioxidant activity of 87.51% at 5 mg/ml. *n*-Hexane fraction shows poor antioxidant activity. These results suggest that the antioxidant activity of *A. arguta* 'Sea-Han' is partially attributable to the EtOAc fraction and also agree with the results of Park *et al.* (2005) with *Callistemon citrinus* extracts.

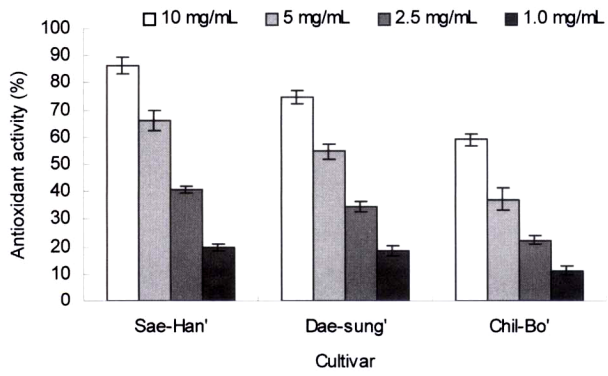

Figure 2. Free radical scavenging activity of three cultivars of *A. arguta*.

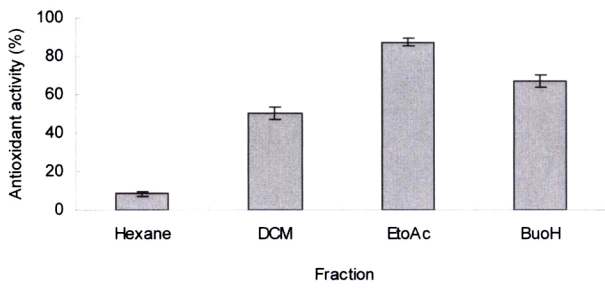

Figure 3. Free radical scavenging activities of solvent fractions of the Sea-Han cultivar of *A. arguta*. The values are mean ± SD (n=3).

2. Total phenolics content

Generally, the antioxidant activity increases with the increase in the total phenolic contents. The total phenolic contents of three cultivars of *A. arguta* are presented in Fig. 4. Total phenolic content in fruit of three cultivars of *A. arguta* 'Sea-Han', 'Dea-Sung', and 'Chil-Bo' were 32.93, 28.23, and 25.60 mg/g, respectively.

Total phenolic contents of the 'Sea-Han' cultivar were in the decreasing order of EtOAc fraction (49.10 mg/g) > CH_2Cl_2 fraction (41.10 mg/g) > BuOH fraction (30.12 mg/g) > *n*-hexane fraction (9.11 mg/g) (Fig. 5). From the results shown in Fig. 5, we can suggest that higher yields of phenolic compounds were obtained with increasing polarity of the solvent (Lee *et al.*, 2004).

3. Vitamin C content

Vitamin C (ascorbic acid) is usually selected as an index of the nutrient quality because of its liable nature as compared to the other nutrients in food (Lee and

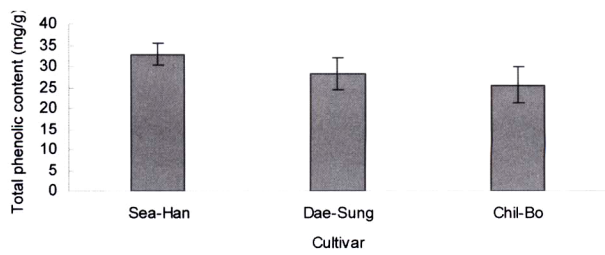

Figure 4. The total phenolic contents of three cultivars of *A. arguta*. The values are mean ± SD (n=3).

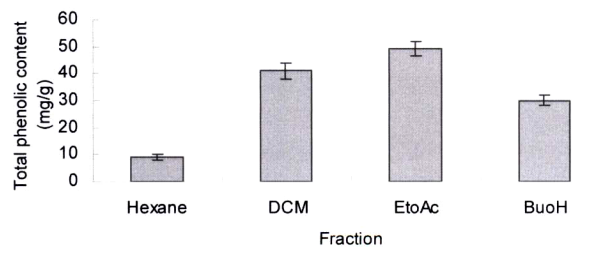

Figure 5. The total phenolic contents of solvent fractions of the 'Sea-Han' cultivar of *A. arguta*. The values are mean ± SD (n=3).

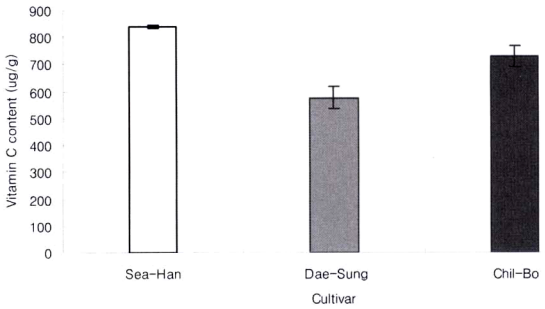

Figure 6. Vitamin C content of three cultivars of *A. arguta*. The values are mean ± SD (n=3).

Kader, 2000). It is also reported that chemical contributors to antioxidant activity in fruit are numerous including vitamin C (Navarro *et al.*, 2006).

Therefore, vitamin C contents of three cultivars of *A. arguta* are presented in Fig. 6. Vitamin C content in the fruit of three cultivars, 'Sea-Han', 'Dea-Sung', and 'Chil-Bo' were 840.57, 578.81 and 730.10 μg/g, respectively. Therefore, it appears that *A. arguta* studied in this work is a good source of vitamin C in the diet.

Vitamin C is considered as one of the most important water-soluble antioxidant and can directly scavenge superoxide radical, singlet oxygen, hydrogen peroxide, and hydroxyl radical (Klimczak *et al.*, 2007).

According to clinical and epidemiological studies, the recommended daily acceptance for vitamin C is suggested to be 100-120 mg/day to achieve cellular saturation and optimum risk reduction of heart disease, stroke, and cancer in healthy individuals (Naidu, 2003). The vitamin C content in *A. arguta* 'Sea-Han' is 84.05 mg/100g: it can deliver about 70.04-80.05% of recommended daily intake of vitamin C.

4. Soluble solid content and reducing sugar content

The results of total soluble solids and reducing sugar content of the three cultivars of *A. arguta* are shown in Fig. 7. The level of total soluble solids was higher in *A. arguta* 'Dae-Sung' as compared to 'Sea-Han' and 'Chil-Bo'. Reducing sugar contents of three cultivars, *A. arguta* 'Sea-Han', 'Dea-Sung', and 'Chil-Bo' were 225.64, 251.79, and 175 mg/g, respectively. Because the contents of reducing sugar and the levels of total soluble solids contribute to fruit sweetness, the studies on total soluble solids and reducing sugar content are important to assess their tastes and quality of *A. arguta* (Dhumal *et al.*, 2007).

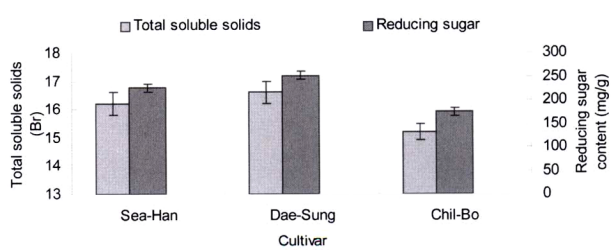

Figure 7. Total soluble solids and reducing sugar contents of three cultivars of *A. arguta*. The values are mean ± SD (n=3).

Conclusion

The results of present work indicated that the extracts from three cultivars of *A. arguta* had different levels of antioxidant activity. The antioxidant activity of three cultivars of *A. arguta* studied decreased in the order 'Sea-Han', 'Dea-Sung', 'Chil-Bo'. The present study also reveals the difference in nutritional composition that may lead to their different applications. Vitamin C content in *A. arguta* 'Sea-Han' is higher than that of others. However, the components responsible for the antioxidant activity are unclear. Therefore, it is suggested that further study could be performed on the isolation and identification of the antioxidant components from *A. arguta*.

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