ANTIOXIDANT ACTIVITY OF KIWIFRUIT (*ACTINIDIA CHINENSIS*)

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Summary

Kiwifruit (Actinidia spp.) are a relatively new, but economically important crop grown in many different parts of the world. In present study antioxidant activity of Kiwifruits (*Actinidia chinensis* L., syn. *A. deliciosa*) were examined employing different in vitro assay systems. The extract showed moderate antioxidant activity in some models. In DPPH radical-scavenging model, extract show potent activity (IC_{50} was 204.0 ± 8.3 μg ml^{-1}). The extract showed good Fe^{2+} chelating activity. IC_{50} was 174.5 ± 11.2 μg ml^{-1}. EDTA showed very strong activity (IC_{50} = 4.2 ± 0.2 μg ml^{-1}). The extract showed weak reducing power between 16 and 256 μg ml^{-1} but its activity was dose dependent. Vitamin C showed very strong reducing power. Also it was capable of scavenging H_2O_2 in a concentration dependent manner. IC_{50} for scavenging of H_2O_2 was 432.3 ± 19.7 μg ml^{-1}. The IC_{50} values for vitamin C and quercetin were 16.7 ± 0.6 and 41.2 ± 1.7 μg ml^{-1}, respectively. The total amount of phenolic and flavonoid content of extract were determined by employing Folin-Ciocalteau and aluminum chloride colorimetric assays.

Key words: Antioxidant activity, Kiwi, DPPH, Iron chelating ability, *Actinidia chinensis*, *Actinidia deliciosa*.

Introduction

The genus *Actinidia* Lindl. is large, containing between 50 and 70 species of climbing plants originating mainly in southern China [1]. *Actinidia chinensis* L., (syn. *A. deliciosa*) is one of the commercially most important crops of New Zealand and other countries such as Chile, China and Italy [2,3]. Kiwi is also used extensively as a fruit in Iran [4].

Although kiwifruits are normally consumed as fresh fruit, studies have demonstrated that, for example, Californian grown kiwifruits can be successfully canned or frozen [5]. A wide range of compounds has been detected within the *Actinidia* genus leading to distinct and different flavors in the fruit [6]. These compounds include polyphenolics, acids, alcohols and volatile compounds such as terpenes and esters. Flavor is also influenced by the sugar to acid balance, with some growers currently receiving a premium for sweeter fruit. The extensive range of *Actinidia* fruit colors is caused by the presence or absence of chlorophyll, anthocyanins and carotenoids [6]. Healthful attributes of kiwifruit include its high ascorbic acid levels [2], quinic acid levels [7], and the presence of triterpenes and folic acid [6]. The fruits of the kiwi contain significant amounts of vitamin C. Ascorbic acid content exists within the fruits of this genus, ranging from 40 to over 1500 mg per 100 g fruit fresh weight [8]. Recently a new vitamin E has been isolated from kiwi fruit and its antioxidant capacity has been compared with that of tocopherols using five different methods [9]. Also high amounts of polysaccharides have been described in kiwi [10,11]. Kiwifruits are used for treatment of many different types of cancers, e.g., stomach, lung, and liver cancer [12] in eastern medicine. Recent studies showed that extracts of kiwi fruits inhibit cancer cell growth [13] and exhibit cell protection against oxidative DNA damage in vitro [14]. Simultaneously Phytochemical investigations focused upon isolated kiwi fruit ingredients. The composition of kiwi fruits in regard to vitamins, elementals, proteins, polyphenols, and lipophilic constituents has been well described. In spite of many papers published about biological activities of kiwi fruit, little is currently known about the antioxidant activity of this fruit. In the present study, the antioxidant effects of Kiwi fruits were investigated by different in vitro assay systems. According to previous studies free radicals have been shown to play an important role in the initiation and/or progression of various diseases such as cancer, and cardiovascular disease [15].

Materials and methods

Plant materials and preparation of extract: The fresh kiwi fruits were obtained from a commercial supplier during the summer. Fruits were sliced and dried in a ventilated incubator at 45°C for 5 days. The dried fruit was then powdered. One hundred grams of powder was extracted at room temperature by percolation method using methanol. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained, which was then freeze-dried for complete solvent removal.

Chemicals: Ferrozine, Trichloroacetic acid (TCA), 1,1-diphenyl-2-picryl hydrazyl and Hydrogen peroxide were purchased from Sigma Chemicals Co. (St Louis, MO, USA). Gallic acid, Quercetin, Butylated hydroxyanisole, Ascorbic acid, Potassium ferricyanide, EDTA and Ferric chloride were purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade or purer.

Determination of total phenolic and flavonoid contents: Total phenolic content was determined by the Folin-Ciocalteau method [16]. Briefly, the extract sample (0.5 ml) was mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent for 5 min and 2.0 ml of 75 g l^{-1} sodium carbonate were then added. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. Results were expressed as gallic acid equivalents/ml . Total flavonoids were estimated using the method of Ebrahimzadeh et al.

[17]. Briefly, 0.5 ml solution of plant extract was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (Perkin Elmer). Total flavonoid content was calculated as Quercetin/ ml from a calibration curve.

Antioxidant activity

DPPH radical-scavenging activity: The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the samples [18,19]. Different concentrations of sample were added, at an equal volume, to ethanolic solution of DPPH (100 μM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamin C, BHA and quercetin were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Reducing power determination: The reducing power of extract was determined according to recently published paper [20]. Briefly, 2.5 ml of sample (25-800 μg ml^{-1}) in water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as positive control.

Metal chelating activity: The chelating of ferrous ions by the extract was determined by the method of Ebrahimzadeh et al. [21,22]. Briefly, the sample (25-400 μg ml^{-1}) was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated as $[(A_0 - A_s)/A_s] \times 100$, where A_0 was the absorbance of the control, and A_s was the absorbance of the extract/standard. Na_2EDTA was used as positive control.

Scavenging of hydrogen peroxide: Briefly, a solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. 1.4 ml of extracts (25-800 μg ml^{-1}) in distilled water was added to 0.6 ml of 40 mM hydrogen peroxide solution. The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide (UV- Visible EZ201, Perkin Elmer: USA). The percentage of hydrogen peroxide scavenging by the extracts and standard compounds was calculated as follows: % Scavenged (H_2O_2) = $[(A_o - A_1)/A_o] \times 100$ where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the sample of extract and standard [23].

Statistical analysis: Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($p < 0.05$) and the means separated by Duncan's multiple range tests. The IC_{50} values were calculated from linear regression analysis.

Results and discussion

Total phenol and flavonoid contents: The total concentration of phenolic compounds in the extracts was expressed as microgrammes of gallic acid equivalents (GAE) per milliliter by using an equation that was obtained from standard gallic acid graph as: Absorbance = 0.0054 × total phenols [μg gallic acid] + 0.0628 ($R^2 = 0.987$). The total phenolic content of extract was 9.3 ± 0.4 μg gallic acid equivalent/ ml. Total flavonoid contents in the extract was expressed as microgrammes of quercetin equivalents per milliliter by using an equation that was obtained from standard quercetin graph as: Absorbance = 0.0063 × total flavonoids [μg quercetin] ($R^2 = 0.999$). The total flavonoid content of extract was 7.9 ± 0.63 μg quercetin equivalent/ ml. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities [21]. Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases [21].

DPPH radical-scavenging activity: The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples [24]. DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [25]. IC_{50} for DPPH radical-scavenging activity was 204.0 ± 8.3 μg ml^{-1}. The IC_{50} values for ascorbic acid, quercetin and BHA were 3.7 ± 0.1, 3.9 ± 0.2 and 29.3 ± 5.9 μg ml^{-1}, respectively.

Reducing power: In the reducing power assay, the presence of reductants (antioxidants) in the sample would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Fig. 1 shows the dose– response curves for the reducing power of the extract. It was found that the reducing power of extract also increased with the increase of its concentration. Vitamin C used as a standard antioxidant. There was significant difference among the extract and vitamin C in reducing power ($p< 0.001$).

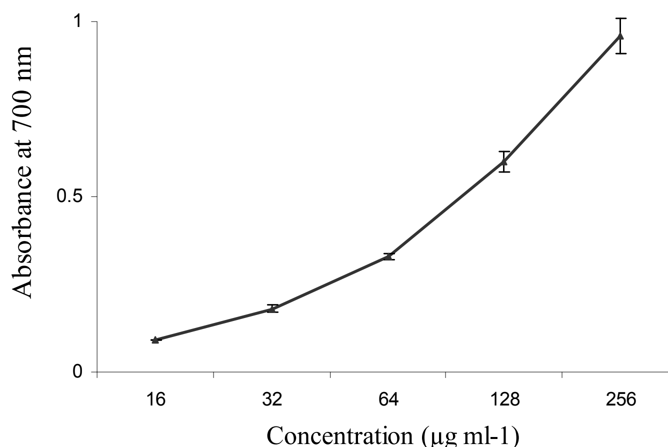

Fig 1. Reducing power of kiwi fruit extract. Vitamin C showed very potent activity (because of high differences, its data not shown).

Fe^{2+} chelating activity: The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular disease [26]. Because Fe^{2+} also has been shown to cause the production of oxy-radicals and lipid peroxidation, minimizing Fe^{2+} concentration in Fenton reactions affords protection against oxidative damage. The chelating of ferrous ions by the extract was estimated by our recently published paper [17]. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complexes decreases. In this assay, both extract and EDTA interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. The absorbance of Fe^{2+}– ferrozine complex was decreased dose-dependently, *i.e.* the activity was increased on increasing concentration from 25 to 400 µg ml^{-1}. Metal chelating capacity was significant since the extract reduced the concentration of the catalyzing transition metal in lipid peroxidation. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion [26]. The extract showed good Fe^{2+} chelating activity. IC_{50} was 174.5 ± 11.2 µg ml^{-1}. EDTA showed very strong activity (IC_{50} = 4.2 ± 0.2 µg ml^{-1}).

Hydrogen peroxide scavenging: Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water [17]. The ability of the extracts to effectively scavenge hydrogen peroxide, determined according to the method of Mahmoudi *et al.,* [23], where they are compared with that of quercetin as standard. The differences in H_2O_2 scavenging capacities between the extracts may be attributed to the structural features of their active components, which determine their electron donating abilities. Extract was capable of scavenging H_2O_2 in a concentration dependent manner. IC_{50} for scavenging of H_2O_2 was 432.3 ± 19.7 µg ml^{-1}. The IC_{50} values for vitamin C and quercetin were 16.7 ± 0.6 and 41.2 ± 1.7 µg ml^{-1}, respectively. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems [23].

Conclusions

The extract of Kiwifruits (*Actinidia chinensis* L., syn. *A. deliciosa*) exhibited different levels of antioxidant activity in all the studied models. Future investigations of in vivo models of antioxidant activity are needed.

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