

Cardiovascular Protective Properties of Kiwifruit Extracts *in Vitro*

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It is currently accepted that the consumption of fruit-derived antioxidants such as vitamin C, carotenoids, and flavonoids provides a preventive effect against cardiovascular disease. The purpose of the present study was to investigate potential cardiovascular protective properties of aqueous and 70% ethanol extracts from kiwifruit by analyzing the antioxidative, antihypertensive, hypocholesterolemic, and fibrinolytic activities *in vitro*. Aqueous and 70% ethanol extracts at 50 mg/ml showed DPPH-radical scavenging activities of 72.31% and 70.75%, respectively. Total antioxidant activity in linoleic acid emulsion was 85–88% at 10 mg/ml and 96–98% at 50 mg/ml of kiwifruit extract. Inhibitory activities against angiotensin I-converting enzyme of kiwifruit extracts were 21–26% at 10 mg/ml and 46–49% at 50 mg/ml, and inhibitory activities on HMG-CoA reductase were 13–14% at 10 mg/ml and 19–30% at 50 mg/ml. Fibrinolytic activity of kiwifruit was also observed at a high concentration of 100 mg/ml in both aqueous and 70% EtOH extracts. Based on our results, kiwifruit have potential cardiovascular protective properties *in vitro*.

Key words kiwifruit; antioxidant; angiotensin I-converting enzyme (ACE) inhibition; HMG-CoA; fibrinolytic activity

Actinidia deliciosa (Actinidiaceae), the well-known as just “kiwi” is a plant native to the eastern Asia. Commercial planting began in New Zealand in the late 1930s, and for the past three decades kiwifruit has been increasingly available worldwide.¹⁾ In Korea, seedlings of kiwifruit were introduced in 1977 for the first time, and Korean kiwifruit have found their way onto the market since 1981. Kiwifruit contain many medicinally useful compounds such as vitamin C, carotenoids, flavonoids, minerals, and others.²⁾ It is currently accepted that the consumption of fruit-derived antioxidants such as vitamin C, carotenoids, and flavonoids has a preventive effect against certain cancers and cardiovascular disease.^{3–5)} Kiwifruit have also been used for the treatment of cancers in Chinese folk medicine.⁶⁾ For example, many different cancers, especially cancers of the digestive system (mainly stomach cancer), lung, and liver, have been treated with kiwifruit prescriptions due to its cytotoxic and antioxidant activities.^{6,7,10–12)}

Atherosclerosis is a complex multifactorial process involving oxidation of cholesterol and the intracellular accumulation of oxidized cholesterol, high blood pressure, and platelet aggregation. Many natural products with bioactive constituents such as antioxidants can ameliorate this process and prevent cardiovascular disease at many steps in the process.¹³⁾ There are very few studies on the cardiovascular protective effects such as inhibition of angiotensin I-converting enzyme (ACE) and HMG-CoA reductase, or fibrinolytic activity of fruit, even though they have been considered to prevent cardiovascular disease. Kiwifruit is likely to be able to affect the process of atherosclerosis by virtue of diverse constituents such as phenolic antioxidants, dietary fibers, minerals, etc. However, to the best of our knowledge, studies of the cardiovascular protective properties of kiwifruit have not been reported. The purpose of the present study was to investigate the potential cardiovascular protective properties of crude kiwifruit extract by analyzing the antioxidative, antihypertensive, hypocholesterolemic, and fibrinolytic activities *in vitro*.

MATERIALS AND METHODS

Preparation of Kiwifruit Extracts Kiwifruit was purchased from a local supermarket. It was ground without the peel and extracted with distilled water or 70% ethanol (kiwi : solvent = 1 : 10, w/w) at 100 °C for 3 h. The aqueous or 70% ethanol extracts were concentrated under reduced pressure, lyophilized to obtain powder, and then used as tests samples. The powder of kiwifruit extracts was stored at 4 °C until assay.

Determination of Antioxidant Activity with the DPPH Radical-Scavenging Method The hydrogen-donating or radical-scavenging ability of kiwifruit extracts was measured using the stable radical α, α -diphenyl- β -picrylhydrazyl (DPPH).¹⁴⁾ A methanolic solution (50 μ l) of the extracts was placed in a cuvette, and 2 ml of a 6×10^{-5} M methanolic solution of DPPH was added. Absorbance measurements commenced immediately at 515 nm using a spectrophotometer (Jasco V-530, Japan). The decrease in absorbance was determined after 70 min when the absorbance stabilized. The absorbance of the DPPH radical without kiwifruit extracts, *i.e.*, the control, was measured daily. The percent inhibition of the DPPH radical in the samples was calculated according to the formula of Yen and Duh.¹⁵⁾

$$\% \text{ inhibition} = [(A_{C(0)} - A_{A(t)}) / A_{C(0)}] \times 100$$

where $A_{C(0)}$ is the absorbance of the control at $t=0$ min and $A_{A(t)}$ is the absorbance of the antioxidant at $t=70$ min. Vitamins C and E were used as positive controls.

Antioxidant Activity in Linoleic Acid Emulsion The total antioxidant activity was determined according to the method of Yen and Hsieh.¹⁶⁾ Each extract in 0.5 ml of distilled water was mixed with linoleic acid emulsion (2.5 ml, 0.02 M, pH 7.0) and phosphate buffer (2 ml, 0.2 M, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier, and 50 ml of phosphate buffer, and then the mixture was homogenized. The reaction mixture was incubated at 37 °C. Aliquots of 0.1 ml were taken at 0 and 24 h during incubation. The degree of oxidation was measured according to the thiocyanate

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method¹⁷⁾ by sequentially adding ethanol (4.7 ml, 75%), ammonium thiocyanate (0.1 ml, 30%), sample solution (0.1 ml), and ferrous chloride (0.1 ml, 0.02 M in 3.5% HCl). The mixture was allowed to stand for 3 min, and then the peroxide value was determined by reading the absorbance at 500 nm using a spectrophotometer (Jasco V-530, Japan). A control was performed with linoleic acid but without the extracts. Vitamin E was used as a positive control.

ACE Inhibition Activity The inhibitory activity against ACE was assayed using the method of Cushman and Cheung.¹⁸⁾ The assay mixture contained potassium phosphate buffer 100 mM (pH 8.3), sodium chloride 300 mM, hippuryl-L-histidyl-L-leucine (HHL) 5 mM, and enzyme prepared from rabbit lung acetone powder (Sigma), 0–10 mU per 0.25 ml of assay volume. An appropriate amount of the extracts was added to the assay mixture, incubated for 30 min at 37 °C, and then terminated by adding 1 N HCl 0.25 ml. The hippuric acid liberated was extracted with 1.5 ml of ethyl acetate, and 1.0 ml of the extract was evaporated by heating at 120 °C for 30 min in an oil bath. The residue was then dissolved in 1.0 ml of distilled water. The absorbance was measured at 228 nm using a spectrophotometer (Jasco V-530, Japan). Captopril (Sigma) was used as a positive control.

HMG CoA Reductase Inhibition Activity HMG-CoA reductase was partially purified according to the method of Kleinsek *et al.*¹⁹⁾ Briefly, male Sprague-Dawley rats (180–200 g) were fed an AIN-96 diet containing 2% cholestyramine for 5 d prior to killing at the mid-dark period, which is the diurnal high point of enzyme activity. After the animals were decapitated, the livers were excised and immediately homogenized in 2 ml/g liver of ice-cold buffer A (potassium phosphate 50 mM, sucrose 0.2 M, and dithiothreitol 2 mM, pH 7.0) with a glass-Teflon Potter-Elvehjem homogenizer. The homogenate was centrifuged at 15000 *g* for 10 min, and the postmitochondrial supernatant solution was centrifuged at 100000 *g* for 75 min. The supernatant solution was decanted, and the microsomes were washed by resuspension of the pellet in buffer A containing EDTA 50 mM (1 ml per gram of liver) and homogenized as before. The homogenate was centrifuged at 100000 *g* for 60 min, and the washed microsomal pellets were slow-frozen in a –20 °C freezer for a minimum of 2 h. The microsomal pellets were thawed at room temperature. Three milliliters of buffer B (potassium phosphate 50 mM, 0.1 M sucrose, dithiothreitol 2 mM, KCl 50 mM, and EDTA 30 mM, pH 7.0) was added and homogenized. After standing for 15–30 min at room temperature, the suspension was centrifuged at 100000 *g* for 60 min at 20 °C. The supernatant containing soluble HMG-CoA reductase was used for the enzyme inhibitory activity assay as the crude enzyme. The reaction mixture for activity assay contained potassium phosphate 50 μ M, dithiothreitol 2 μ M, NADPH 0.3 μ M, HMG-CoA 0.15 μ M, and enzyme (pH 7.0). The reaction mixture was preincubated in a cuvette without HMG-CoA for 5 min at 37 °C. The assay for enzyme activity was then carried out with the addition of HMG-CoA to the reaction mixture in a recording spectrophotometer. The initial velocity of the reaction was measured, and the net rate of NADPH oxidation was determined by subtracting the rate of its oxidation in the absence of HMG-CoA from the rate observed with both substrates present.

Fibrinolytic Activity Assay Fibrinolytic activity was

estimated using the modified fibrin plate assay of Astrup and Mullertz,²⁰⁾ using plasmin as a standard (Fig. 1). The fibrinogen solution (10 ml of 0.6% human fibrinogen in potassium phosphate buffer 20 mM, pH 7.0) was pipetted into Petri dishes (100×15 mm). After mixing with 0.1 ml of thrombin solution (100 NIH U/ml in potassium phosphate buffer 20 mM, pH 7.0, containing NaCl 0.1 M), the dishes were left for 30 min at room temperature to form the fibrin layer. Samples (50 μ l) were dropped onto the fibrin layer and incubated at 37 °C for 8 h. Fibrin plate clearance was monitored by measuring two perpendicular diameters of the cleared zone. Fibrinolytic activities were expressed as the clear zone area.

RESULTS AND DISCUSSION

Aqueous or 70% ethanol extracts of kiwifruit showed antioxidant activities at all concentrations investigated, as shown in Table 1. At the concentration of 50 mg/ml, aqueous and 70% EtOH extracts showed DPPH radical-scavenging activities of 72.31% and 70.75%, respectively, when those of vitamin C and E were 95.71% and 94.78%, respectively. Total antioxidant activity in linoleic acid emulsion was 87.55% and 85.68% at 10 mg/ml of aqueous and 70% ethanol extracts. At 50 mg/ml, kiwifruit extracts showed antioxidant activity of 96–98%, which was similar to that of vitamin E (96.29% at 0.1 mg/ml). There are a few previous studies on the antioxidant activity of kiwifruit. According to Wang *et al.*,²¹⁾ the antioxidant activity of commonly consumed fruit has been rated in the order of plum > kiwi > apple > pear. Collins *et al.*¹²⁾ demonstrated significant antioxidant activity of kiwifruit *in vitro*. The oxidation of low-density lipoprotein (LDL) cholesterol has been proposed as an important step in the formation of atherosclerotic lesions. The role of antioxidants as potential antiatherogenic compounds has been also recognized. Many studies have demonstrated that polyphenolic flavonoids derived from plants used in the human diet medicinally have antioxidant properties.²²⁾

The inhibitory activities against ACE and HMG-CoA reductase of kiwifruit are shown in Table 2. Aqueous and 70% EtOH extracts showed ACE inhibition activities of 21–26% at 10 mg/ml and 46–49% at 50 mg/ml, when that of captopril, as a positive control, was 99%. ACE is of great importance for controlling blood pressure through the renin-angiotensin system. Hypertension is a known risk factor for the development of atherosclerosis. For that reason, interest in

Table 1. Antioxidant Activity of Kiwifruit Extracts

Extract	Concentration (mg/ml)	DPPH radical-scavenging activity (%) ^{a)}	Total antioxidant activity (%) ^{a)}
Aqueous	1	4.70±2.16	16.53±2.66
	10	21.55±3.02	87.55±2.37
	50	72.31±2.79	96.55±3.26
70% EtOH	1	5.78±1.12	11.14±1.44
	10	21.80±2.23	85.68±3.07
	50	70.75±2.03	97.72±2.11
Vitamin C	0.1	95.71±3.11	—
Vitamin E	0.1	94.78±3.09	96.29±2.13

Values are means of triplicate determinations±S.D. ^{a)} Antioxidant activity is expressed as relative activity compared with negative control.

Table 2. Inhibitory Activity of Kiwifruit Extracts on ACE and HMG-CoA Reductase

Extract	Concentration (mg/ml)	ACE inhibitory activity (%) ^{a)}	HMG-CoA reductase inhibitory activity (%) ^{a)}
Aqueous	1	2.48±1.46	≤0
	10	25.60±2.66	14.36±1.83
	50	46.85±3.78	29.80±8.83
70% EtOH	1	1.66±2.12	≤0
	10	21.49±1.58	13.20±2.83
	50	49.18±2.24	19.59±8.31
Captopril	0.01	99.00±2.11	—
Lovastatin	0.01	—	≥100

Values are means of triplicate determinations±S.D. a) Inhibitory activity is expressed as relative activity compared with negative control.

Table 3. Fibrinolytic Activity of Kiwifruit Extracts

Extract (mg/ml)	1	10	50	100
Aqueous	—	—	0.41±0.00	3.32±1.00
70% EtOH	—	—	0.38±0.05	2.35±0.21

Values are means of triplicate determinations±S.D. Fibrinolytic activity is expressed as the clear zone area (cm²).

identifying food sources as ACE inhibitors has increased. There have been studies of the ACE-inhibitory activity of medicinal plants or herbs.^{23,24)} Recently, Aviram and Dornfeld²⁵⁾ have reported that pomegranate juice has dose-dependent inhibitory effects on serum ACE activity *in vivo* and *in vitro*, and suggested that pomegranate juice consumption offers protection against cardiovascular disease. It is also necessary to investigate the inhibitory effects of kiwifruit juice on ACE activity *in vivo*. The inhibitory activity of kiwifruit extracts on HMG-CoA reductase, a key enzyme in cholesterol biosynthesis was 13–14% at 10 mg/ml and 19–30% at 50 mg/ml, when lovastatin showed inhibitory activity of over 100% at 0.01 mg/ml. Recently, studies for the screening of natural products as HMG-CoA reductase inhibitors have been increasing. Liu *et al.*²⁶⁾ reported that some crude extracts from traditional Chinese herbs were effective HMG-CoA reductase inhibitors, and Ha *et al.*²⁷⁾ observed the inhibitory effects of some cereals on HMG-CoA reductase. However, no study has reported the inhibitory activity of commonly consumed fruit on HMG-CoA reductase. Thus the inhibitory activity of kiwifruit extracts on HMG-CoA reductase deserves attention, although the inhibitory activity was not high.

The fibrinolytic activity of kiwifruit is summarized in Table 3 and Fig. 2. We observed fibrinolytic activity at levels of 50 mg/ml and 100 mg/ml in both aqueous and 70% ethanol extracts. The fibrinolytic activity of kiwifruit water extracts at 100 mg/ml was equal to the activity of plasmin at 12.6 μ g (Fig. 1). An epidemiologic study showed that frequent intakes of fruit and vegetables was associated with increased activity of the fibrinolytic system and thus a reduced risk of thromboembolic and cardiovascular disease in individuals who exhibit this food intake pattern. These results urge us to investigate the fibrinolytic activity of other commonly consumed fruit.

Based on our results, kiwifruit has potential use as a cardiovascular protective agent. The cardiovascular protective properties of kiwifruit extracts observed in the present study

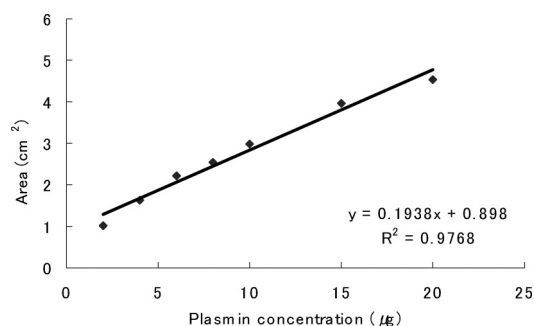


Fig. 1. Fibrinolytic Activity of Plasmin by Concentration

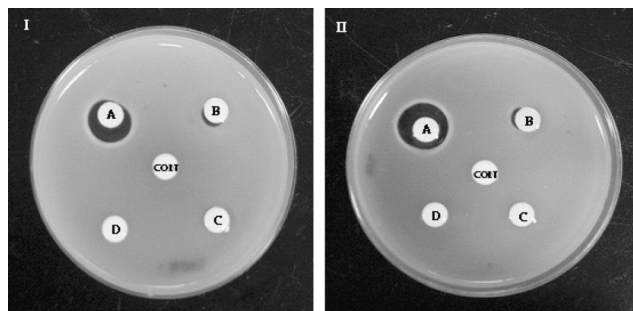


Fig. 2. Fibrinolytic Activity in 70% Ethanol or Water Extract of Kiwifruit by Concentration

I, 70% EtOH extract; II, water extract; A, 100 mg/ml; B, 50 mg/ml; C, 10 mg/ml; D, 1 mg/ml; CON, control.

are considered to be due to antioxidants such vitamin C, carotenoids, and polyphenols or flavonoids in which kiwifruit are rich, but further studies are needed for the identification of compounds with specific cardiovascular protective activity. In addition, in the present study, the activities of kiwifruit were observed *in vitro* but not *in vivo*, and therefore *in vivo* studies are needed for confirmative data.

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