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Effects of adding ethanol extracts from *Ulmus davidiana* to *Yackwa* base as an antioxidant during storage

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Abstract This study aimed to assess the antioxidative potential of adding ethanolic extracts from *Ulmus davidiana* to *Yackwa* (Korean fried cookie). The reducing power and antioxidant activity of *U. davidiana*-treated *Yackwa* were assessed against DPPH, nitric oxide, superoxide anions, superoxide dismutase, and ABTS radicals. Increasing the amount of *U. davidiana* extract in *Yackwa* dough resulted in improved antioxidant properties. *U. davidiana* extracts were effective antioxidants with a radical scavenging potency similar to the potency of butylated hydroxytoluene (BHT) and L-ascorbic acid. The DPPH and nitric oxide radical scavenging activity of *Yackwa* containing 0.2% *U. davidiana* resulted in increased oxidative stability relative to control dough. Although the antioxidant effects of *Yackwa* containing *U. davidiana* extract were lower than those of *Yackwa* containing BHT and L-ascorbic acid, these results suggest that *U. davidiana* extracts have potent antioxidant activity. Accordingly, *U. davidiana* extract is a potential additive for deep-fried foods, such as *Yackwa*, to improve their oxidative stability.

Keywords: *Ulmus davidiana, yackwa,* antioxidant activity, storage stability, functional food

Introduction

Yackwa is a Korean fried cookie that is a traditional holiday food. Various ingredients are used to make it, including wheat flour, water, honey, rice wine, and sesame oil. Renewed appreciation of traditional Korean foods has prompted a large increase in the production of *Yackwa*, and they have become a very popular food in the last 20 years. Although some stages of the *Yackwa* manufacturing process have been automated, many are still performed manually because of the delicate procedures required to produce high-quality *Yackwa*. Recently, Korean manufacturers have faced many problems distributing *Yackwa* because of their high fat content (12-29%) and porous structure, which exposes the fat to oxygen and leads to rapid oxidation. The shelf life of *Yackwa* depends on the rate at which lipid oxidation induces rancidity of the product (1). Deep fat frying causes hydrolysis, oxidation, and polymerization of the oil. Typical secondary products produced by these reactions include hydroperoxides, dimers, and polymers (2), which can cause the development of undesirable flavors and tastes. Oxidation produces hydroperoxides and, subsequently, low-molecular-weight volatile compounds such as aldehydes, ketones, carboxylic acids, and short-chain alkanes and alkenes. Dimers and polymers are also formed in oil via radical and Diels-Alder reactions during deep fat frying (3). In addition, lipid oxidation can lead to changes in the sensory and nutritional value, and even safety, of deep fried foods such as *Yackwa*. Generally, these negative changes reduce the consumer acceptance of oxidized foods (4). Lipid oxidation products such as free radicals, peroxides, aldehydes, and ketones are harmful to human health (5).

Antioxidants are food additives that can prevent the oxidation of lipids contained within food. They can minimize rancidity and delay the formation of toxic oxidation products to help maintain nutritional quality and increase shelf life. Therefore, various antioxidants are synthesized chemically and widely used to retard lipid oxidation during the storage of raw and precooked ground poultry, beef, pork products, and fried cookies (6). Examples include butylated hydroxyanisole (BHA), propylgallate, and butylated hydroxytoluene (BHT). However, synthetic antioxidants are associated with safety concerns, and their use as food additives is under government regulation. Therefore, natural antioxidants have become increasingly popular as safe alternatives (7). Many studies have assessed the antioxidant activity of various parts of plants (including fruits, spices, vegetables, and herbs), in addition to their potential applicability for food preservation (8,9). Extracts of such plants have been used traditionally to make food look and taste better, and also to prevent the deterioration of food quality during storage. Polyphenols (such as phenolic acids, lignins, and tannins), carotenoids, and vitamins are the major groups of biologically active compounds that are partially responsible for the total antioxidant capacity of plants. Total polyphenols are a good indicator of antioxidant capacity (9,10). Many herbs and spices (including basil, clover, fennel, ginger, garlic, nutmeg, oregano, rosemary, thyme, turmeric, and sage), in addition to other plant materials (such as apple, apricot, broccoli, cherry, peach, spinach, strawberry, and onion) that have high phenolic

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contents, are receiving increasing recognition from food manufacturers because they can preserve food quality and nutrients by helping to protect lipid contents from oxidative degradation (11-15).

Ulmus davidiana is a deciduous tree that is commonly found in Korea. Its root, stem, and bark have been utilized as traditional oriental medicines for the treatment of various diseases including inflammation, edema, gastric cancer, and mastitis. *U. davidiana* can also maintain and facilitate blood circulation (16). Some medicinal functions of *U. davidiana* have been determined and applied to the preparation of its extracts, as described in traditional Chinese and Korean medicine literature (17). Recently, *U. davidiana* was reported to potently inhibit lipid peroxidation and endogenous nitric oxide (NO)-induced apoptotic cell death (18). *U. davidiana* stems are used to treat inflammatory diseases, although the mechanism is poorly understood (19). Several reports have identified biologically active compounds, such as catechin glycoside uldavioside and catechin, in *U. davidiana* and also elucidated their biological actions (20). In addition, two kinds of neolignan glycosides and four kinds of lignan xylosides have been isolated from its roots, stem, and bark (21). Recently, Guo and Wang (22) reported that *U. davidiana* extracts exhibited strong antioxidant activities in assays assessing 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, hydroxyl radical scavenging, lipid peroxidation, and reducing power. Therefore, we hypothesized that *U. davidiana* would also have potent antioxidant activity, and that its constituents may exert synergistic antioxidant effects. To date, few attempts have been made to investigate the antioxidant properties of *U. davidiana*; therefore, limited data are available regarding the antioxidant content and activity of *U. davidiana* in food products.

A previous study from our laboratory screened for natural antioxidants in *U. davidiana* by isolating and identifying components with antioxidant activities. When *U. davidiana* was extracted using 70% ethanol and the extract was fractionated using ethyl acetate, the ethyl acetate fraction exhibited potent antioxidant activity; the major active components were (−)-catechin and its glycoside (23). Therefore, in the present study, we obtained extracts from *U. davidiana* using 70% ethanol. These extracts were then applied to *Yackwa* before the frying process to assess the antioxidant effects over different storage periods.

Materials and Methods

Chemicals and reagents

Potassium hexacyanoferrate (III), DPPH, nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide (NADH), trichloroacetic acid (TCA), ABTS [2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)], and trizma base were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethylenediamine tetracetic acid (EDTA) was purchased from Junsei Chemical (Tokyo, Japan). Pyrogallo was purchased from Yakuri Pure Chemical (Tokyo, Japan). All the other reagents used in this research, of analytical grade, were from Duksan Pure Chemicals (Seoul, Korea).

Plant materials and extraction procedures

U. davidiana roots (Jungsun, Gangwon, Korea) were purchased from a local herbal drugstore in Seoul, Korea. Air-dried *U. davidiana* root was crushed in a grinder (FM-681C, Hanil, Seoul, Korea) for 2 min; at every 15 s, the process was stopped for 15 s to keep the temperature of the samples from rising. For extraction, each sample (100 g) of *U. davidiana* was mixed with 500 mL of 70% ethanol. The mixture was stirred for 3 h at 80°C, and the stirring was repeated once again under the same condition. Then the stirred mixture was filtered through Whatman No. 2 filter paper (Whatman plc., Kent, UK). The filtrates were combined, and a rotary vacuum evaporator (N-1000, EYELA, Tokyo, Japan) was used to remove the solvent by evaporating at 50°C under low pressure. Any remaining water was removed by freeze-drying (Bondiro MCFD 8508 Freeze Dryer, Ilshin Co., Seoul, Korea).

Preparation of *Yackwa* and extraction

To prepare the *Yackwa*, 120 g of wheat flour, 15 g of sesame oil, 54 g of honey, 5 mL of rice wine, 5 mL of water, and 2 g of salt were mixed together and kneaded. BHT and L-ascorbic acid were added to the final concentrations allowed for deep fried food by the Korea Food Additives Code (24): 0.02% (w/v). The *Yackwa* containing *U. davidiana* extract were prepared by adding the extract at final concentrations of 0.05%, 0.1%, and 0.2%. These concentrations were based on a preliminary experiment performed previously (25), which demonstrated that the addition of the extract to *Yackwa* at these concentrations had similar effects to addition of BHT or L-ascorbic acid at the final concentrations allowed by the Korea Food Additives Code (24). The control *Yackwa* samples were prepared as described above, but without additives. After kneading, pieces of the dough, ~14 g each, were shaped using a *Yackwa* mold with a thickness of 0.7 cm and a diameter of 3.5 cm. The shaped pieces were fried for 15 min at 120±5°C using soybean oil (CJ, Seoul, Korea). After frying, the prepared *Yackwas* were placed in a basket made of bamboo for 30 min to remove any excessive oil from the surface. The *Yackwa* was then kept in an incubator for 5 days at 60°C without being wrapped. Three batches were made of each sample. At 1 day intervals, the *Yackwas* were removed from the incubator for extraction. A 5 g *Yackwa* was ground into a cream in a mortar and suspended in 10 mL of 95% ethanol. The suspension was incubated for 20 min in a shaking incubator (shaking incubator SI-900R, JEIO Tech., Suwon, Korea) at 2,500 rpm. Then, each suspension was filtered using Whatman No. 2 filter paper. *Yackwa* extract was obtained from the supernatant, and was diluted to the final concentrations of 0.05 and 0.5 kg/L, respectively, using 75% ethanol. Their antioxidant properties were then assessed.

Scavenging activity on DPPH radicals

The each extracts (0.05 kg/L) in ethanol (4 mL) were mixed with 1 mL of ethanolic solution containing the DPPH radicals, resulting in a final concentration of 0.15 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in

the dark. The absorbance was then measured at 517 nm against a blank (26). DPPH radical scavenging activity (%) was then calculated using the following equation:

DPPH radicals scavenging activity (%)
=[1−(absorbance of sample/absorbance of control)]×100

NO scavenging assay

NO generated from sodium nitroprusside was used for NO scavenging assay based on the Griess reaction of Kato *et al*. (27). NO was generated spontaneously in aqueous solution of sodium nitroprusside at physiological pH (28). Then nitrite ions produced by interaction between oxygen and the generated NO were quantified using Griess reagent. NO scavengers compete with oxygen reducing the production of NO. The nitrite scavenging activity of 0.5 kg/L extracts at pH 1.2 was determined using Griess reagent and the absorbance at 520 nm. Sodium nitrite (1 mM) was mixed with extracts dissolved in suitable solvent systems. After incubating the mixture for 1 h at 37ºC, Griess reagent was added to the mixture for reaction. Nitrite diazotizes with sulphanilamide, and then is coupled with naphthylethylene-diamine to yield a chromophore. The absorbance due to the generated chromophore was measured at 520 nm and compared with that of the control prepared by mixing standard solutions of potassium nitrite with Griess reagent. The equation used to calculate nitrite scavenging activity (%) is as follows:

NO scavenging activity (%)=[1−(absorbance of 1 mM $NaNO_2$ added sample after standing for 1 h−absorbance of control)/(absorbance of 1 mM $NaNO_2$)]×100

Superoxide anion radical scavenging activity

The superoxide anion scavenging activity of the samples was measured by the method of Robak and Gryglewski (29). The following solutions were prepared in 0.1 M phosphate buffer (pH 7.4): 150 μM nitroblue tetrazolium (NBT), 60 μM phenazine methosulphate (PMS), and 468 μM NADH. Then these solutions, respectively, were mixed with 1 mL of each sample. Then the absorbance was determined at 560 nm. Superoxide anion radical scavenging can be expressed as follows:

Superoxide anion (O_2) radical scavenging activity (%)
=[1−(absorbance of sample/absorbance of control)]×100

Reducing power assay

The assay of reducing power was carried out using the procedure of Oyaizu (30). To 2.5 mL of the aqueous solution of each sample extract (0.5 kg/L), the same volume of 200 mM sodium phosphate buffer (pH 6.6) as well as the same volume of potassium hexacyanoferrate (III) solution (10 mg/mL in water) was added. After incubating the mixture for 20 min at 50ºC, it was mixed with 2.5 mL of TCA solution (100 mg/mL in water). Then the resulting mixture was centrifuged for 10 min at 1017×g. To 5 mL of the supernatant, the same volume of deionized water was added together with 1 mL of iron (III) chloride solution (1 mg/mL in water). The mixture, along with a blank, was subjected to the measurement of absorbance at 700 nm. Higher absorbance indicated higher reducing power (31).

ABTS radical scavenging assay

The total antioxidant activity of the fractions was measured based on the decolorization of ABTS radical cation (32). ABTS radicals were generated by chemical reaction with potassium persulfate. A 7 mM ABTS stock solution was prepared by dissolving ABTS in water. To produce ABTS radical cations ($ABTS^{+\cdot}$), the ABTS stock solution was mixed with 2.45 mM aqueous potassium persulfate solution. Then the mixture was placed in the dark for 12-16 h at room temperature prior to being used for the measurement of absorbance. Although ABTS began to be oxidized immediately, absorbance began to increase only after 6 h. When kept in a dark place, the ABTS radical cations produced in this way were stable at room temperature for at least 2 days. Radical generation was completed after 16 h of incubation at room temperature in a dark place. Then the mixture was diluted in ethanol (99.5%) until its absorbance at 734 nm reached 0.70±0.02. Assessment of scavenging activity was carried out by adding 0.9 mL of ABTS reagent to 0.1 mL of extract, followed by allowing 6 min of reaction time at room temperature, and then by measuring the absorbance of the mixture at 734 nm. Ethanol was used as a control. ABTS radical scavenging activity (%) was then calculated using the following equation:

ABTS radicals scavenging activity (%)
=[1−(absorbance of sample/absorbance of control)]×100

Superoxide dismutase (SOD) activity

SOD activity was determined according to the method of Kim *et al.* (33). Each extract (0.5 kg/L) in water (0.2 mL) was mixed with 3 mL of 50 mM tris-HCl buffer (50 mM tris[hydroxymethyl] amino-methane+10 mM EDTA, pH 8.5) and 0.2 mL of 7.2 mM pyrogallo, and the mixture was incubated 25ºC for 10 min. The reaction was stopped by the addition of 1 mL of 1 N HCl. The absorbance of the mixture was determined at 420 nm against a blank. The SOD activities can be expressed as follows:

SOD activity (%)
=[1−(absorbance of sample/absorbance of control)]×100

Statistical analysis

All results were expressed as means±standard deviations (SD) of three parallel measurements. The data were analyzed by two-way analyses of variance (ANOVA) using SPSS (Statistical Analysis Program, version 12.0, SPSS Inc., Chicago, IL, USA) to determine the effects of the each treatments. Significant differences between each treatment were determined by Duncan's multiple range tests ($p<0.05$). Correlations were obtained by Pearson correlation coefficient in bivariate correlations.

Results and Discussion

DPPH radical scavenging activity

The DPPH radical scavenging activities of *Yackwa* that contained *U. davidiana* were evaluated by DPPH assay. The scavenging effect of the *Yackwa* that contained of *U. davidiana* decreased as the storage time increased (Table 1). In the early stage, the DPPH radical scavenging activities of *Yackwa* that contained 0.05, 0.1, and 0.2% *U. davidiana*, BHT, and L-ascorbic acid treatment were 82.72, 83.47, 89.40, 84.77, and 88.94%, respectively, which was significantly higher than that of the control (77.12%, $p<0.001$). These activities decreased as the storage period increased; however, the DPPH radical scavenging activity of the control was less stable than that of the other samples for the entire study period ($p<0.001$). The *Yackwa* treated with 0.2% *U. davidiana* showed the highest DPPH radical scavenging activity at 5 days. Taken together, these results indicate that DPPH radical scavenging measurement can be used to determine the stability of *Yackwa* that contained *U. davidiana*.

Jung *et al.* (34) previously assessed the antioxidant activity of *U. davidiana* extracts and demonstrated that the DPPH radical scavenging activity of *U. davidiana* methanol extracts was higher than that of their tested model systems for deep fried food. In addition, Lee and Han(35) demonstrated that the DPPH scavenging activity of methanol and ethanol extracts of the roots of *U. davidiana* was higher than that of other products. Therefore, these previous studies revealed that *U. davidiana* extracts have excellent free radical scavenging activity. Additional studies demonstrated that many flavonoids and related polyphenols contribute significantly to the antioxidant activity of medicinal plants (7). According to a previous study performed in our laboratory, *U. davidiana* ethanol root extracts had a total phenolic content of 168.32 mg gallic acid equivalents (GAE)/g and a total flavonoid content of 86.85 mg rutin equivalents (RE)/g (23). It is advantageous for extracts to have such high total flavonoid and total polyphenol contents because plants containing phenolic compounds, particularly flavonoids, have strong antioxidant properties (36). In addition, Balasundram *et al.* (37) reported that radical scavenging activity is an indicator of functionality and antioxidant activity, and this activity reflects the total polyphenol and flavonoid content in plant food.

The biological and chemical activities of polyphenol and flavonoid compounds are diverse, and include radical scavenging and potent antioxidant effects. Taken together, the findings of previous studies reporting that *U. davidiana* extracts have potent DPPH radical scavenging effects and the current observations that *U. davidiana* extracts with a high polyphenol and flavonoid content had higher DPPH radical scavenging ability than BHT and L-ascorbic acid when added to *Yackwas* suggest that the addition of *U. davidiana* extracts to deep fried foods, including *Yackwas*, could effectively suppress lipid oxidation by removing free radicals such as DPPH. These data suggest that *U. davidiana* extracts have hydrogen-donating ability, and therefore could potentially be used as antioxidants for scavenging or inhibiting free radicals.

NO scavenging activity

As shown in Table 2, the NO scavenging activities gradually decreased for all treatments during the storage time. In addition, all treatments had higher NO scavenging activities than the control over the 5 day study period, which indicates that each of the treatments was an effective antioxidant. In the early stage of the experiment, the NO scavenging activity ranged from 65-75% in the *U. davidiana* treatments, and from 74-78% in the BHT and L-ascorbic acid treatments. In addition, the nitric oxide scavenging activities of the control and the 0.05, 0.1, and 0.2% *U. davidiana* treatments were 25.53, 38.65, 47.75%, and 54.68%, respectively, after 5 days of storage. However, the scavenging activities of the BHT (60.11%) and L-ascorbic acid treatments (64.83%) were higher than those of the *U. davidiana* treatments. In addition, the highest scavenging activity was observed in the *Yackwa* samples at day 0. Furthermore, the nitric oxide scavenging activity increased with increasing concentrations of *U. davidiana*. Based on these results, treatment with 0.2% *U. davidiana* had almost the same scavenging effect as treatment with BHT and L-ascorbic acid.

The results suggest a high association between the phenolic contents, including flavonoids, of *U. davidiana* extract and its nitric oxide activity. These results are consistent with those reported by Lee and Han (35), in which the total phenolic content of *U. davidiana* extracts was positively correlated with DPPH radical and NO radical scavenging activity.

Table 1. Change in DPPH radical scavenging activity of *Yackwa* with different concentrations of *U. davidiana* extract during storage

Treatments	Storage days at 60°C					
	0	1	2	3	4	5
Control	77.12±1.73^{aF}	73.84±2.17^{aE}	67.94±2.69^{aD}	62.72±4.26^{aC}	57.58±0.68^{aB}	54.00±3.18^{aA}
0.05% extract	82.72±0.30^{bF}	81.35±0.94^{bE}	75.13±1.64^{bD}	71.62±1.51^{bC}	66.42±4.38^{bB}	59.87±1.11^{bA}
0.1% extract	83.47±0.45^{bF}	82.48±0.99^{bE}	74.05±1.08^{bD}	70.45±2.10^{bC}	67.46±3.83^{bB}	65.94±2.28^{bA}
0.2% extract	89.40±0.74^{eF}	88.72±0.36^{eE}	81.79±0.50^{eD}	79.80±1.94^{eC}	75.28±1.40^{eB}	70.56±2.53^{eA}
L-ascorbic acid	88.94±0.81^{dF}	85.96±0.84^{dE}	84.24±0.66^{dD}	74.84±2.25^{dC}	69.81±1.13^{dB}	66.30±0.57^{dA}
BHT	84.77±1.70^{cF}	83.17±0.09^{cE}	77.40±0.57^{cD}	75.40±0.64^{cC}	70.50±2.32^{cB}	67.48±0.62^{cA}

$^{a-e}$Means in the same column with different superscripts are significantly different ($p<0.001$).
$^{A-F}$Means in the same row with different superscripts are significantly different ($p<0.001$).

Table 2. Change in nitric oxide scavenging activity of *Yackwa* with different concentrations of *U. davidiana* extract during storage

Treatments	Storage days at 60°C					
	0	1	2	3	4	5
Control	55.61±2.51^{aF}	47.12±2.47^{aE}	37.41±3.52^{aD}	34.03±2.56^{aC}	27.94±0.35^{aB}	25.53±2.51^{aA}
0.05% extract	65.26±4.10^{bF}	62.11±1.94^{bE}	55.15±2.61^{bD}	48.03±1.80^{bC}	42.68±0.78^{bB}	38.65±2.61^{bA}
0.1% extract	72.68±0.35^{cF}	67.31±1.59^{cE}	61.22±0.57^{cD}	59.10±2.51^{cC}	50.76±1.84^{cB}	47.75±2.28^{cA}
0.2% extract	74.55±0.73^{dF}	72.09±1.52^{dE}	65.04±0.85^{dD}	63.28±2.50^{dC}	57.54±1.14^{dB}	54.68±1.71^{dA}
L-ascorbic acid	78.19±1.66^{fF}	76.70±0.23^{fE}	74.43±0.58^{fD}	70.03±1.52^{fC}	67.05±1.64^{fB}	64.83±1.82^{fA}
BHT	73.92±0.17^{eF}	69.43±0.95^{eE}	67.18±3.61^{eD}	69.56±0.35^{eC}	65.32±1.05^{eB}	60.11±2.15^{eA}

$^{a-f}$Means in the same column with different superscripts are significantly different (p<0.001).
$^{A-F}$Means in the same row with different superscripts are significantly different (p<0.001).

Table 3. Change in superoxide anion radical scavenging activity of *Yackwa* with different concentrations of *U. davidiana* extract during storage by using PMS-NADH method

Treatments	Storage days at 60°C					
	0	1	2	3	4	5
Control	44.74±1.43^{aF}	35.64±0.76^{aE}	25.94±0.64^{aD}	20.09±0.40^{aC}	11.57±0.50^{aB}	7.56±1.50^{aA}
0.05% extract	50.39±1.97^{bF}	42.48±0.84^{bE}	34.83±1.01^{bD}	25.60±1.84^{bC}	20.26±0.86^{bB}	13.57±0.15^{bA}
0.1% extract	61.46±1.53^{cF}	65.36±0.51^{cE}	44.79±0.64^{cD}	33.41±1.67^{cC}	25.18±0.70^{cB}	17.33±0.99^{cA}
0.2% extract	72.63±1.53^{eF}	65.36±0.51^{eE}	62.29±0.50^{eD}	53.88±2.46^{eC}	37.52±2.59^{eB}	28.16±2.21^{eA}
L-ascorbic acid	72.63±5.24^{dF}	61.73±1.42^{dE}	54.93±1.17^{dD}	42.20±0.85^{dC}	32.49±3.22^{dB}	32.53±3.20^{dA}
BHT	92.53±1.20^{fF}	89.01±2.95^{fE}	80.28±5.67^{fD}	71.19±1.83^{fC}	61.58±14.79^{fB}	36.47±2.82^{fA}

$^{a-f}$Means in the same column with different superscripts are significantly different (p<0.001).
$^{A-F}$Means in the same row with different superscripts are significantly different (p<0.001).

Superoxide anion radical scavenging activity

The superoxide anion scavenging activities of the *Yackwas* that contained *U. davidiana* are shown in Table 3. Each of the treatments exerted scavenging activities on the superoxide anion radical. The superoxide anion scavenging activities of the *Yackwas* that contained *U. davidiana* increased markedly as the concentration increased and decreased markedly with increased storage time. Furthermore, the superoxide anion radicals present in each of the treatments at each treatment time differed significantly (p<0.001). On day 0, the superoxide anion radical scavenging activities of the control, 0.05, 0.1, and 0.2% *U. davidiana*, BHT, and L-ascorbic acid treatments were 44.74, 50.39, 61.46, 72.63, 92.53, and 72.63%, respectively. In addition, superoxide anion scavenging activity, which was reported for the 0.2% *U. davidiana* treated *Yackwas* (28.16%) on day 5, was significantly slightly lower (p<0.001) than that of the BHT and L-ascorbic acid treatment (36.47 and 32.53%, respectively). The next highest values (17.33, 13.57, and 7.56%) were observed in the 0.1 and 0.05% *U. davidiana* treated *Yackwas* and the control, respectively. Although the activities of the *Yackwas* containing 0.2, 0.1 and 0.05% *U. davidiana* were lower than those that were treated with BHT and L-ascorbic acid, they were significantly higher (p<0.001) than that of the control. These results indicate that the *U. davidiana* treatments showed generally strong antioxidant properties. In addition, it should be noted that slight lower scavenging activities were observed in *U. davidiana* ethanol extract than in that of synthetic antioxidants such as BHT and natural antioxidants such as L-ascorbic acid.

Significantly higher activities were observed in the extract of *U. davidiana* when compared with the activities of *Hemlpteleae davidii* extract in studies conducted by Lee *et al.* (38) and Kim *et al.* (39). Although those studies reported good superoxide anion radical activities for the ethanol extract of *U. davidiana* and water and ethanol extracts of *Hemipteleae davidiana* cortex, the activities were lower than those observed in other studies conducted to evaluate *U. davidiana* extract. In the current study, *Yackwas* containing *U. davidiana* extract exhibited good superoxide anion scavenging activity at all tested concentrations. This result is consistent with previous studies, suggesting that this extract is a natural antioxidant.

Reducing power

The reducing abilities of the *U. davidiana* treatments were compared with those of the BHT, L-ascorbic acid and control treatments. As shown in Table 4, the reducing ability of each of the treatments was decreased at 60°C. After 5 days, all treatments resulted in antioxidant effects of varying degrees when compared with the control. The treatments showed comparable reducing powers in the following order: control<0.05% *U. davidiana* extract<0.1% *U. davidiana* extract<0.2% *U. davidiana* extract<L-ascorbic acid<BHT, with the values of 1.23, 1.56, 1.78, 1.83, 2.15, and 2.40%, respectively. Furthermore, the antioxidant activities of L-ascorbic acid and BHT were considerably higher than those of the other treatments, with the activity of L-ascorbic acid being more effective than *U. davidiana* extract but less effective than BHT. Similar to the antioxidant activity, the reducing power of the *U. davidiana* treatments increased with increasing amounts of *U. davidiana* and decreased with

Table 4. Change in reducing power of *Yackwa* with different concentrations of *U. davidiana* extract during storage

Treatments	Storage days at 60°C					
	0	1	2	3	4	5
Control	3.03±0.08^{aE}	2.46±0.10^{aD}	2.09±0.16^{aD}	1.85±0.02^{aC}	1.69±0.02^{aB}	1.23±0.00^{aA}
0.05% extract	3.16±0.04^{bE}	2.77±0.09^{bD}	2.76±0.09^{bD}	1.96±0.02^{bC}	1.85±0.04^{bB}	1.56±0.06^{bA}
0.1% extract	3.40±0.07^{cE}	2.95±0.03^{cD}	2.95±0.03^{cD}	2.15±0.04^{cD}	1.94±0.03^{cC}	1.78±0.02^{cB}
0.2% extract	3.50±0.07^{dE}	3.03±0.04^{dD}	3.15±0.03^{dD}	2.17±0.01^{dC}	2.09±0.03^{dB}	1.83±0.05^{dA}
L-ascorbic acid	3.75±0.29^{eE}	3.12±0.07^{eD}	3.36±0.14^{eD}	2.53±0.33^{eC}	2.28±0.22^{eB}	2.15±0.24^{eA}
BHT	4.13±0.13^{fE}	3.26±0.07^{fD}	3.50±0.03^{fD}	2.92±0.06^{fC}	2.47±0.05^{fB}	2.40±0.03^{fA}

$^{a-f}$Means in the same column with different superscripts are significantly different (p<0.001).
$^{A-E}$Means in the same row with different superscripts are significantly different (p<0.001).

Table 5. Change in ABTS radical scavenging activity of *Yackwa* with different concentrations of *U. davidiana* extract during storage

Treatments	Storage days at 60°C					
	0	1	2	3	4	5
Control	17.76±4.39^{a}	17.37±4.03^{a}	16.57±4.43^{a}	14.94±4.10^{a}	15.50±4.27^{a}	15.41±3.64^{a}
0.05% extract	24.12±3.96^{b}	24.31±3.77^{b}	23.94±3.53^{b}	22.00±4.48^{b}	22.19±4.51^{b}	22.23±3.39^{b}
0.1% extract	27.78±4.85^{bc}	28.19±4.52^{bc}	27.50±4.56^{bc}	25.29±5.26^{bc}	24.80±4.14^{bc}	24.83±5.42^{bc}
0.2% extract	30.17±6.38^{c}	30.68±6.76^{c}	31.14±7.20^{c}	26.49±6.12^{c}	26.13±4.43^{c}	26.32±5.79^{c}
L-ascorbic acid	35.75±7.50^{d}	35.93±7.21^{d}	36.33±7.80^{d}	32.41±6.39^{d}	33.50±5.48^{d}	31.68±5.90^{d}
BHT	47.77±6.15^{e}	48.27±8.09^{e}	47.74±8.01^{e}	42.00±10.05^{e}	43.29±5.28^{e}	43.20±8.11^{e}

$^{a-e}$Means in the same column with different superscripts are significantly different (p<0.001).

increasing storage time. In this study, the *U. davidiana* treatments generally had higher reducing powers, which suggests that the reducing activity of the tested treatments contributed significantly to their antioxidant effects. This indicates that it is likely that there are reductones in *U. davidiana*.

Guo and Wang (16) reported that each of the *U. davidiana* extracts evaluated, with the exception of the chroloform extract, had greater reducing power than that of α-tocopherol at the same concentration. *U. davidiana* was previously shown to be a source of phenolic compounds (16), and similar differences in the total reducing phenolic contents of different crop have also been reported (40). The reducing properties of triterpenes and phytosterols are generally derived from the reductones they contain (41). Reductones donate a hydrogen atom, which breaks the free radical chain to exert antioxidant effects. Reductones also react with certain peroxide precursors, thereby preventing peroxide formation (41). The current data describing the reducing capacity of *U. davidiana* extracts suggest that the reductone-associated and hydroxide groups of compounds could act as electron donors to terminate radical chain reactions by reacting with free radicals to produce stable molecules. This effect may be explained by the study of Lee and Kim (42), which revealed that *U. davidiana* extracts contain triterpene esters, together with the current observations regarding the strong reducing power of *Yackwas* containing *U. davidiana*. Therefore, it is likely that the *U. davidiana* extracts contributed significantly to the antioxidant effects observed in the current study.

ABTS radical scavenging activity

The method used to assess ABTS radical scavenging depends on the absorbance of the ABTS radical cation, which can be measured at 734 nm. Decolorization of ABTS reflects the ability of antioxidant compounds to inhibit radical activity by providing hydrogen atoms or electrons to the radicals. In the presence of an antioxidant reductant, the color of the radical disappears as the radical is reduced to ABTS (32). The ABTS radical scavenging of each of the treatments is presented in Table 5. In the early stage of the storage period, the ABTS radical scavenging activities of *Yackwas* treated with 0.05, 0.1, and 0.2% *U. davidiana*, BHT, and L-ascorbic acid were 24.12, 27.78, 30.17, 47.77, and 35.75%, respectively, which were significantly higher than that of the control (p<0.001). In addition, the ABTS radical scavenging activities of the control, as well as *Yackwas* treated with 0.05, 0.1, and 0.2% *U. davidiana*, BHT and L-ascorbic acid were 15.41, 22.23, 24.83, 26.32, 43.20, and 31.68%, respectively, on day 5. Taken together, these results showed that there was increased ABTS scavenging activity with increased *U. davidiana* concentration for all treatments.

The total phenolic content of *U. davidiana* was relatively high, and the ABTS radical scavenging activities of the samples were high enough to suggest that it, together with other nutrients, could be used as a nutraceutical. These results are important because the antioxidant scavenging effects of *U. davidiana* extract are mostly associated with their component antioxidants, which largely consist catechins and their glycosides (23,43). The strong antioxidant activity of catechins is partly attributed to their phenolic structures, which have the ability to form complexes with metal ions to prevent their participation in peroxidase reactions (44,45). Pietta *et al*. (9) reported that green tea ethanol extracts containing 70% catechins had the highest ability to scavenge $ABTS^{+\cdot}$

Table 6. Change in superoxide dismutase (SOD) activity of *Yackwa* with different concentrations of *U. davidiana* extract during storage

Treatments	Storage days at 60°C					
	0	1	2	3	4	5
Control	30.17±1.45^{aF}	26.20±2.41^{aE}	22.23±2.38^{aD}	15.18±0.56^{aC}	10.67±1.20^{aB}	3.89±0.66^{aA}
0.05% extract	36.86±1.14^{bF}	33.55±0.80^{bE}	28.56±1.03^{bD}	19.21±0.58^{bC}	15.48±1.48^{bB}	10.00±1.99^{bA}
0.1% extract	42.66±1.54^{cF}	35.55±1.75^{cE}	31.49±0.96^{cD}	25.98±1.00^{cC}	18.61±2.21^{cB}	11.51±1.44^{cA}
0.2% extract	53.88±0.76^{dF}	51.08±0.46^{dE}	45.54±0.26^{dD}	41.54±1.44^{dC}	29.87±0.78^{dB}	24.33±0.95^{dA}
L-ascorbic acid	60.33±0.41^{eF}	59.17±0.06^{fE}	47.00±0.75^{eD}	44.70±0.41^{fC}	36.29±0.64^{eB}	30.09±0.62^{eA}
BHT	66.21±2.25^{fF}	53.63±4.53^{eE}	51.88±1.94^{fD}	43.93±1.13^{eC}	39.40±1.71^{fB}	35.69±0.93^{fA}

$^{a-f}$Means in the same column with different superscripts are significantly different (p<0.001).
$^{A-F}$Means in the same row with different superscripts are significantly different (p<0.001).

Table 7. Correlation between preparation treatments and antioxidant activities of *Yackwa*[1)]

		Preparation treatments		Antioxidant activities					
		U. davidiana extract ratio	Storage time	DPPH	NO·	O_2^-	SOD	RP	ABTS
Preparation treatments	*U. davidiana* extract	1.000							
	Storage time	0.260	1.000						
Antioxidant activities	DPPH	0.534***	-0.779***	1.000					
	NO·	0.714***	-0.635***	0.905***	1.000				
	O_2^-	0.545***	-0.789***	0.930***	0.903***	1.000			
	SOD	0.615***	-0.740***	0.935***	0.908***	0.954***	1.000		
	RP	0.313**	-0.904***	0.880***	0.831***	0.908***	0.872***	1.000	
	ABTS	0.732***	-0.159	0.522***	0.668***	0.528***	0.564***	0.424***	1.000

[1)]The values represent the correlation coefficient (r) between preparation treatments, oxidation stabilities, and antioxidant activities (DPPH, NO, O_2^-, SOD, RP, and ABTS). RP, reducing power. **p<0.01; ***p<0.001.

radicals; their activity was considerably higher than that of ginseng, *Gingko biloba*, or grape skin and seed extracts. In addition, the high $ABTS^{+\cdot}$ radical scavenging activity of the plant extracts was attributable to not only catechins but also other components such as phenolic compounds. Therefore, the present study provided new information regarding the antioxidant activity of *U. davidiana* extracts and revealed that they have potential as a natural antioxidant-rich food additive.

SOD activity

SOD activities of treatments were higher on day 0 and 5 days when compared to the control (Table 6). In the early stage, the SOD activities of the 0.05, 0.1, and 0.2% *U. davidiana* extracts, BHT, and L-ascorbic acid were 36.86, 42.66, 53.88, 66.21, and 60.33%, respectively, which were higher than that of the control (30.17%). At the end of the storage period, the SOD activities of the control as well as samples treated with 0.05, 0.1, and 0.2% *U. davidiana* extracts, L-ascorbic acid, and BHT treatments were 3.89, 10.00, 11.51, 24.33, 30.09, and 35.69%, respectively, which was similar to the antioxidant activity determined above. Therefore, higher SOD activity was observed in response to BHT, and its SOD activity was approximately 10-fold higher than that of the control. In addition, the SOD activity increased with increasing concentrations of *U. davidiana* extracts and decreased with increasing storage time. Lee and Han (35) have reported that solvent extracts of the root of *U. davidiana* showed a higher SOD activity at a concentration of 500 mg/mL than the SOD activity of solvent extracts of *U. davidiana* bark. Conversely, Kim (46) reported that compounds from *U. davidiana* extract showed SOD activities that were lower than that of the L-ascorbic acid at a concentration of 10 mM. However, in spite of these results, *U. davidiana* extract may contain SOD activity and could therefore reduce the damage of free radicals.

Correlation between preparation treatments and antioxidant activities of *Yackwa*

Calculated coefficients of correlations between preparation treatments, oxidation stabilities, and antioxidant activities of *Yackwa* are shown in Table 7. The additional rate of *Yackwa* was significantly positively correlated with DPPH radical scavenging (r=0.534, p<0.001), nitric oxide scavenging (r=0.714, p<0.001), superoxide anion radical scavenging (r=0.545, p<0.001), SOD activity (r=0.615, p<0.001), reducing power (r=0.313, p<0.01), and ABTS radical scavenging (r=0.732, p<0.001). The storage time of *Yackwa* was negatively correlated with DPPH radical scavenging (r= –0.779, p<0.001), nitric oxide scavenging (r= –0.635, p<0.001), superoxide anion radical scavenging (r= –0.789, p<0.001), SOD activity (r= –0.740, p<0.001), and reducing sugar (r= –0.904, p<0.001). The most positive influential variables for additional rate were ABTS radical scavenging and nitric oxide scavenging. These results suggest that long-term storage has the potential to alter the antioxidant activity of *Yackwas*. Significant

antioxidant effects are desirable for *Yackwas*. Previous studies analyzing *U. davidiana* revealed the following phytochemical components: catechin rhamnoside, (+)-catechin, catechin apiofuranoside (43), sesquiterpene *O*-naphthaquinones (18), triterpene esters (42), and lignan and neolignan glycosides (20). The bioactive ingredients in *U. davidiana* may have potential medicinal activity, such as antioxidant effects. For example, catechin, a well-known flavonoid, has been reported to possess reducing power and the ability to inhibit free radicals such as DPPH (46). In particular, (−)-catechin and its glycoside isolated from the ethyl acetate fraction of *U. davidiana* showed similar inhibitory effects on both free radicals and DPPH reducing power. In addition, Lee and Kim (42) isolated triterpene esters, which have reducing power, from *U. davidiana*. Because *U. davidiana* extract contains components with strong antioxidant effects, the addition of *U. davidiana* extracts to *Yackwa* may inhibit lipid oxidation. Therefore, adjusting antioxidant parameters, which are related to the ratio of the added components and *Yackwa* storage time, by increasing the amount of added *U. davidiana* could produce high-quality *Yackwa* that can be stored for prolonged periods. Taken together, the results of this study indicate that the degree of oxidative degradation of *Yackwa* stored for different times depends on the amount of *U. davidiana* added.

Conclusions

In conclusion, DPPH radical scavenging, superoxide anion radical scavenging, nitrite oxide scavenging, ABTS radical scavenging activities, reducing power, and SOD activities may account for antioxidant abilities of *U. davidiana Yackwa*. The assay results indicated that *U. davidiana* could be a good antioxidant source. Overall, the antioxidant activity increased with the increasing concentrations of *U. davidiana* and decreased with the increasing storage time. Generally, antioxidant activity of compounds according to various antioxidant assays decreased in the order as follows, control > 0.05% > 0.1% > *U. davidiana* 0.2% > L-ascorbic acid > BHT treatments. From the results of this study, the *U. davidiana Yackwa* were highly effective for the antioxidant assayed, with the exceptions of ABTS radical scavenging activity. *U. davidiana* contains several antioxidant substances that are formed during reactions between sugar and free amino groups of proteins through the Maillard reaction during food processing. Furthermore, the radical scavenging effect and reducing power of *U. davidiana* remained high even after a long storage period, which could delay the onset of lipid autoxidation and increase food storage time. Accordingly, *U. davidiana* could be utilized as an additive to foods that contain oil to improve their oxidative stability. However, additional studies are necessary to assess the antioxidant activity of foods treated with *U. davidiana* after consumption, in addition to potential beneficial effects on human health. These results suggest that *U. davidiana* is a healthy food product with radical scavenging and antioxidant activities.

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