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ABTS AND DPPH ANTIOXIDANT EFFECT OF EXTRACTS FROM GUAVA LEAVES AND FRUITS

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ABSTRACT

Guava (*Psidium guajava* L.) is an important fruit in tropical regions such as South Asia and South America. The aim of this study was to assess the antioxidant capacity of their extracts. Two antioxidant properties of guava leaves and fruits were analyzed using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activity. ABTS scavenging activity of fruits varied from 38.0% at 0.25 mg/ml for ethanol extract to 59.4% at 1.0 mg/ml. There was a significant difference between ethanol and water extracts (t = 5.842 for fruits and 9.571 for leaves, $p < 0.05$). DPPH scavenging activity of ethanol extract of leaves varied from 34.4% at 0.25 mg/ml for ethanol extract to 66.5% at 1.0 mg/ml. There was no significant difference in DPPH scavenging activity between leaves and fruits ($p > 0.05$). Although DPPH activity of ethanol extracts was slightly higher than that of water extracts, there was no significant difference ($p > 0.05$). Generally, the fruits had slightly higher antioxidant activity than leaves.

KEYWORDS: Antioxidant, ABTS, DPPH, guava (*Psidium guajava* L.).

INTRODUCTION

Psidium guajava L. belongs to family Myrtaceae which comprises approximately 150 species of shrubs and trees, many of which have edible fruits.[1] It is a popular tree fruit of the tropical and subtropical climates of the world and is native to tropical America stretching from Mexico to Peru. Guava is presently cultivated in most of the tropical and subtropical countries around the world.[2] Guava has been grown and utilized as an important fruit in tropical areas like India, Indonesia, Pakistan, Bangladesh, and South America.[3] The common guava has a fruit with a yellow skin and white, yellow, or pink flesh.[4] There are known for their sweet and tangy flavor and many uses. It is mostly eated raw (ripe or semi-ripe) or consumed in the form of juice, jams, and jellies.

Guava is an important food crop and medicinal plant in tropical and subtropical countries and is widely used as food and as folk medicine around the world. Guava leaves have exhibited beneficial effects as antioxidants,[5-6] antibacterial agents,[7-8] and as antidiabetic agents.[9]

There were many reports of the total antioxidant capacity of guava leaves and fruits.[5, 10]

Free radicals are produced by several endogenous and exogenous processes, and their negative effects are neutralized by antioxidant defenses.[11] Antioxidants are compounds that inhibit oxidation. Many antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase play an important role in scavenging free radicals and oxidants.

Among many antioxidants, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), and 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were commonly used to assess antioxidant activity in vitro. The DPPH assay method is based on the reduction of DPPH, a stable free radical. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry, so it can be useful to assess various products at a time.[12]

ABTS is a chemical compound used to observe the reaction kinetics of specific enzymes. A common use for it is in the enzyme-linked immunosorbent assay (ELISA) to detect for binding of molecules to each other. ABTS is also frequently used by the food industry and agricultural researchers to measure the antioxidant capacities of foods.[13] For example, polyphenol compounds, which widely exist in fruit, can quench free radicals inside human body, thus prevent oxidative damage by free radicals.

Antioxidants are found in certain foods and may prevent

some of the damage caused by free radicals by neutralising them.

The purpose of the present study is to evaluate guava leaves and fruits as sources of antioxidants for DPPH and ABTS radical to examine whether they are losing significant antioxidant activity or not for leaves or fruit.

MATERIALS AND METHODS

Sample extract

The plant materials were collected between February and April 2020 from agricultural fields in Korea. Leaves and fruit of guava was prepared from. The raw materials were ground using a Retsch GM 200 Mill (Fisher Bioblock, France). Ground plant material (100 g) with distilled water at 50°C under agitation. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA). The mixture was shaken vigorously for one hour at room temperature and left in the dark at room temperature for 20 min. The extracts were filtered with a 0.45 µm syringe filter. After filtration, the water was removed in a rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan) at 70°C. The extracts were centrifuged 5 minutes at 7,500 x g. To get dry powder, samples placed in a low temperature vacuum chamber. These powders were then used to determine antioxidant activities. All analyses were realized as much as possible in an area protected against light.

ABTS radical scavenging activity

This assay involve the use of a pre-formed ABTS radical cation (2,2-azinobis 3-ethybenzothiozoline-6-sulphnolic acid diammonium salt, $ABTS^{+}$).[14] The stock solution of ABTS cation radical was dissolved in water to a 7 mM concentration, and 2.45 mM potassium persulfate was prepared. Two stock solutions were mixed and kept in the dark at room temperature for 16 h before use. Then, the solution yield a dark color. Working solution was prepared freshly before each assay by diluting the stock solution withphosphate buffered saline solution (PBS) (pH 7.4) for an absorbance of 0.7 ± 0.02 at 734 nm and equilibrated at 23℃. Free radical scavenging activity was assessed by mixing 300 µL of different fractions (25 -250 µg/mL, in respective solvents) with 3 mL of ABTS working solution. The decrease in absorbance was measured exactly one min after mixing the solution, and the final absorbance was noted up to 6 min at 734 nm using the Microplate Reader (VersaMax, California, USA). Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble vitamin E analog, serves as a positive control inhibiting the formation of the radical cation in a dose dependent manner.

DPPH free radical

The antioxidant activity of the seaweed extracts was measured based on the scavenging activity of DPPH free radical according to the method described by Brand-Williams et al.[15] with slight modifications. Each sample stock solution (1.0 mg/ml) was diluted to final concentrations of 0.1, 0.5, and 1.0 mg/ml, in water or ethanol. A solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of 80% ethanol. A stock solution of the compounds was prepared at 1 mg/ml in DMSO. The stock solution was diluted to varying concentrations in 96-well microplates. DPPH (final concentration 300 µM) was added to the solutions prepared with sample extracts and standard antioxidant substances. The plate was shaken to ensure thorough mixing before being wrapped with aluminum foil and placed into the dark. The radical scavenging reaction was carried out at 37 ºC in dark for 30 min. The optical density (OD) of the solution was read using the Microplate Reader (VersaMax, Califonia, USA) at the wavelength 515 nm. Corresponding blank sample was prepared and L-Ascorbic acid (0.1, 0.5, and 1.0 mg/ml) was used as reference standard (positive control). Inhibition of free radical scavenging activity was calculated using the following equation.

Statistical analysis

Data was conducted using Microsoft Excel and SPSS 21.0 for Windows (Chicago, IL, USA). A one-way and a two-way analysis of variance (ANOVA) followed by the Tukey post hoc test were used to analyze statistical significance ($p < 0.05$). The analysis was carried out at least in triplicate. The results were expressed as the mean±SD. Significance and confidence level were estimated at $p < 0.05$.

The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula.

Inhibition (%) = (IA-As)/IA×100

Where IA is the absorbance of the 100% initial and As is the absorbance of the sample. IA and As were the values which were subtracted the average absorbance of the blank wells.

RESULTS AND DISCUSSION

Table 1 was the optical density (OD) of ABTS by ethanol and water extracts of guava at different concentrations. The ethanol extract of guava fruit at quantities (0.25-1.0 mg/ml) scavenged the ABTS radicals on concentrations (Table 2). Various concentrations of fruit were higher than those of the leaves. ABTS scavenging activity of fruits varied from 38.0% at 0.25 mg/ml for ethanol extract to 59.4% at 1.0 mg/ml. The all groups did not show a statistically significant difference ($p > 0.05$). However, there was a significant difference between ethanol and water extracts ($t = 5.842$ for fruits and 9.571 for leaves, $p < 0.05$). The water extract values for leaves and fruits at 1.0 mg/ml were 10.8% and 19.6%, respectively. However, there was a significant difference between leaves and fruits ($p < 0.05$). Figure 2 was shown the rate of ABTS inhibitory of Trolox (positive control) and relative inhibitory rate for leaves and fruits on 1.0 mg/ml. The ethanol extract values for leaves and fruits were 71.5% and 91.9%, respectively. The water extract values for leaves and fruits were 13.0% and 23.6%, respectively.

Table 3 was the OD reesulst of DPPH by ethanol and water extracts of guava at different concentrations. The results of the DPPH radical assay for antioxidant activities expressed as ascorbic acid equivalent/mg dry extract for the guava extract were shown in Table 4. It was observed that inhibition percentage values go on increasing with enhancements in concentration of research guava extracts in the assay mixture. The DPPH activity of the fruit extracts was higher than that of leaf extracts. DPPH scavenging activity of ethanol extract of leaves varied from 34.4% at 0.25 mg/ml for ethanol extract to 66.5% at 1.0 mg/ml. DPPH scavenging activity of etanol extract of fruits varied from 18.4% at 0.25 mg/ml for ethanol extract to 59.8% at 1.0 mg/ml. There was no significant difference between leaves and fruits ($p > 0.05$). Although ethanol extracts were slightly higher in DPPH activity than water extracts, there was no significant difference ($p > 0.05$). Figure 2 was shown the rate of DPPH inhibitory of L-ascorbic acid (positive control) and relative inhibitory rate for potatoes on 1.0 mg/ml. The ethanol extract values for leaves and fruits were 79.7% and 71.8%, respectively. The water extract values for leaves and fruits were 70.7% and 61.6%, respectively.

Guava leaves rich source of various health-promoting micro- and macronu- trients as well as bioactive compounds. Guava extracts at lower concentations exhibited high antioxidant capacity with high total phenol and flavonoid content.[8] Guava extracts at higher concentrations showed prooxidant action with high total phenol and flavonoid content, and strong antiviral and antibacterial activity.[8] Water and ethanol of extracts Guava leaves showed effects on scavenging hydroxyl radicals and inhibiting lipid peroxidation.[16]

These polysaccharides demonstrate various physicochemical, biological, and pharmacological properties, such as antioxidant, anti-inflammatory, antidiabetic, immunomodulatory, and antitumor activities.[17] In an advanced study, silver nanoparticles were synthesized by utilizing crude polysac- charides of GLs, and showed showed high DPPH radical- and ABTS radical cation-scavenging activity.[16]

GL extracts have been extensively studied for their high levels of antioxidant. However, the degree of antioxidant varied depending on the guava leaf, stem, and extraction methods.

Table 1: The optical density (OD) of ABTS by ethanol extracts of guava at different concentrations.

Tissues	No.	Concentration (mg/ml)								
		0.25	**0.50**	**1.00**	**0.25**	**0.50**	**1.00**	**0.25**	**0.50**	**1.00**
Leaves	1st	0.094	0.113	0.158	0.096	0.105	0.162	0.099	0.114	0.165
	2nd	0.106	0.122	0.158	0.107	0.119	0.170	0.110	0.122	0.169
	3rd	0.117	0.128	0.165	0.115	0.125	0.177	0.120	0.126	0.175
	Mean	0.106	0.121	0.160	0.106	0.116	0.170	0.110	0.121	0.170
Fruits	1st	0.147	0.198	0.333	0.184	0.275	0.346	0.203	0.252	0.489
	2nd	0.178	0.185	0.228	0.180	0.221	0.253	0.171	0.203	0.343
	3rd	0.162	0.178	0.187	0.173	0.195	0.207	0.153	0.179	0.259
	Mean	0.162	0.187	0.249	0.179	0.230	0.269	0.176	0.211	0.364

Table 2: The degree of inhibition (%) of ABTS by guava at different concentrations.

Tissues	Concentration (mg/ml)	Solvent		*t*-test
		Ethanol	**Distilled water**	
Leaves	0.25	37.95±0.98	7.32±2.69	5.842*
	0.50	43.27±2.05	8.04±1.30	
	1.00	59.39±0.63	10.78±2.27	
Fruits	0.25	60.69±1.38	8.11±3.58	9.571*
	0.50	67.48±2.87	14.68±3.84	
	1.00	76.42±4.05	19.64±3.36	
F-test		2.702	1.551	

Data represented the mean ± SD from three replicates.

Table 3: The optical density (OD) of DPPH ethanol extracts of guava at different concentrations.

Tissues	No.	Concentration (mg/ml)								
		0.25	0.50	1.00	0.25	0.50	1.00	0.25	0.50	1.00
Leaves	1st	0.111	0.123	0.159	0.108	0.15	0.184	0.106	0.16	0.221
	2nd	0.093	0.173	0.204	0.103	0.152	0.184	0.100	0.158	0.237
	3rd	0.089	0.098	0.191	0.103	0.151	0.185	0.099	0.157	0.256
	Mean	0.098	0.131	0.185	0.105	0.151	0.184	0.102	0.158	0.238
Fruits	1st	0.067	0.079	0.108	0.089	0.104	0.201	0.079	0.086	0.119
	2nd	0.101	0.129	0.173	0.078	0.092	0.187	0.076	0.076	0.155
	3rd	0.076	0.162	0.196	0.088	0.081	0.171	0.081	0.098	0.191
	Mean	0.081	0.123	0.159	0.085	0.092	0.186	0.079	0.087	0.155

Table 4. The degree of inhibition (%) of DPPH by guava at different concentrations

Tissues	Concentration (mg/ml)	Solvent		*t*-test
		Ethanol	Distilled water	
Leaves	0.25	34.37±3.41	20.63±2.75	1.802
	0.50	57.41±2.28	31.25±6.32	
	1.00	66.47±5.75	43.52±7.15	
Fruits	0.25	18.35±2.05	16.99±2.70	0.743
	0.50	33.54±12.6	26.99±0.62	
	1.00	59.79±3.24	37.61±1.95	
F-test		1.007	0.517	

Data represented the mean ± SD from three replicates.

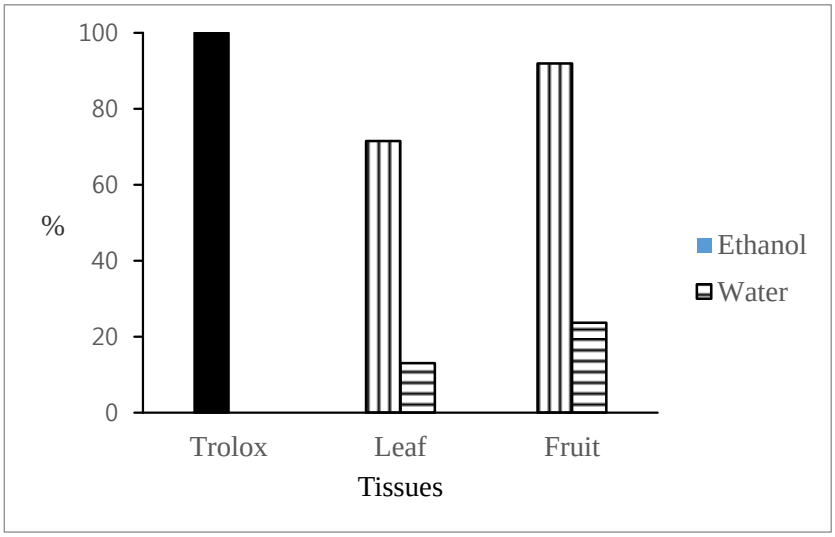

Figure 1: The rate of ABTS inhibitory of Trolox (positive control) and relative inhibitory rate for guava on 1.0 mg/ml.

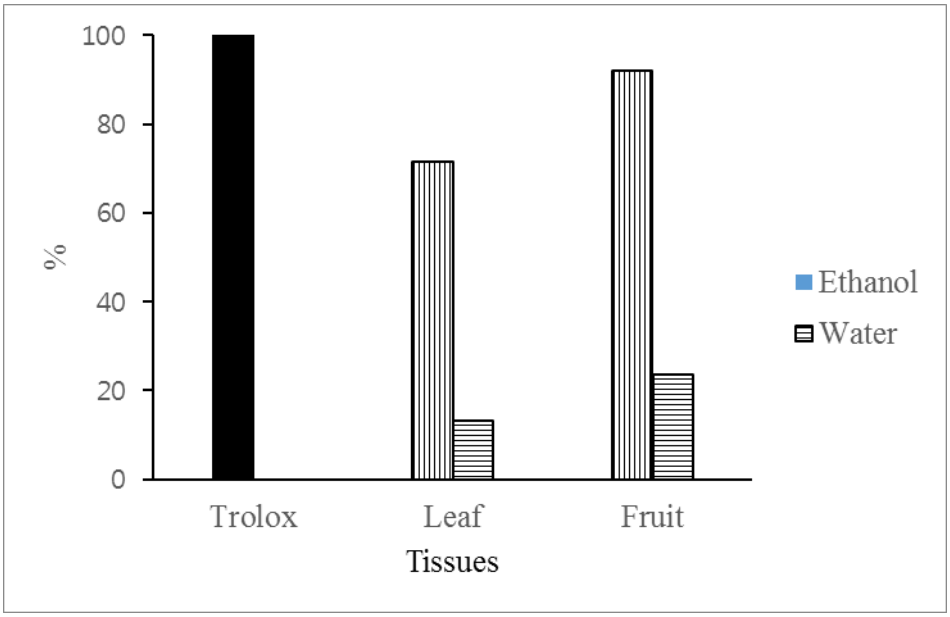

Figure 2: The rate of DPPH inhibitory of L-ascorbic acid (positive control) and relative inhibitory rate for guava on 1.0 mg/ml.

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