Study to find the best extraction solvent for use with guava leaves (*Psidium guajava* L.) for high antioxidant efficacy

Jongkwon Seo¹, Soojung Lee², Marcus L. Elam¹, Sarah A. Johnson¹, Jonghoon Kang³ & Bahram H. Arjmandi¹*

¹Department of Nutrition, Food and Exercise Sciences, College of Human Sciences, Florida State University, Tallahassee, Florida 32306
²Department of Food and Nutrition, Institute of Agriculture and Life Science, Gyeongsang National University, 501 Jinjudaero, Jinju 660-701, Korea
³Department of Biology, Valdosta State University, Valdosta, Georgia 31698

Keywords
Antioxidant, flavonoid, guava, hydroethanolic solvent, phenolic compound

Correspondence
Bahram H. Arjmandi, Department of Nutrition, Food and Exercise Sciences, College of Human Sciences, Florida State University, Tallahassee, Florida 32306. Tel: (850) 645 1517; Fax: (850) 645 5000; E-mail: barjmandi@fsu.edu

Funding Information
No funding information provided.

Received: 24 September 2013; Revised: 4 December 2013; Accepted: 12 December 2013

Abstract
The effects of guava leaves extracted using solvents of water, ethanol, methanol, and different concentrations of hydroethanolic solvents on phenolic compounds and flavonoids, and antioxidant properties have been investigated. The antioxidant capability was assessed based on 2,2-diphenyl-1-picrylhydrazyl radical and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical-scavenging abilities, reducing power, and nitric oxide- and nitrate-scavenging activities. The results demonstrated that the antioxidant ability of guava leaf extracts has a strong relationship with phenolic compound content rather than flavonoid content. Phenolic compound content of water extracted guava leaves was higher compared to pure ethanol and methanol extracts. However, phenolic compound content extracted using hydroethanolic solvent was higher than water, whereas 50% hydroethanolic was observed to be the most effective solvent showing high antioxidant ability.

Introduction
Medicinal plants have been used in the treatment and improvement of human diseases (Gutierrez et al. 2008; Nyirenda et al. 2012), and such plants with high antioxidant abilities can be used as natural medicines for preventing aging and chronic diseases (Kähkönen et al. 1999). In addition, these plants have various physiologically active substances with anticancer and antimicrobial abilities (Bhanot et al. 2011; Miyake and Hiramitsu 2011). The free radical-scavenging abilities of plants has been evaluated by in vitro models of scavenging activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), superoxide, hydroxyl radical, and nitric oxide radical, reducing power, lipid peroxidation levels, and antioxidant enzyme activities (Brand-Williams et al. 1995; Jayanthi and Lalitha 2011; Reddy et al. 2012). Reactive oxygen species (ROS), such as hydroxyl radical (·OH), superoxide anion (·O₂⁻), and hydrogen peroxide (H₂O₂), which are produced in the cell system, are known to cause oxidative damage. This damage may cause cellular injuries and
exacerbate several degenerative diseases associated with aging, cancer, and cardiovascular disease (Pham-Huy et al. 2008; Sharma and Singh 2012).

Guava (Psidium guajava L.), which is used as a traditional medicine, is found in countries with hot climates in areas such as South America, Europe, Africa, and Asia (Gutierrez et al. 2008). Its primary traditional uses include the alleviation of diarrhea and dehydration. Other reported uses include treatment of gastroenteritis, dysentery, stomach pain, diabetes mellitus, and wounds. In addition, it is known for its antioxidant, antibacterial, and anti-inflammatory properties (Qian and Nihorimbere 2004; Cheng et al. 2009; Han et al. 2011a). Guava leaves have phenolic compounds and flavonoids with high antioxidant activity. The main active substances in guava leaves are gallic acid, caffeic acid, guaijaverin (Gutierrez et al. 2008), tannins (Okuda et al. 1987), carotenoids (Mercadante et al. 1999), and triterpenoids (Shao et al. 2012). These substances have been extracted by using several solvents such as water (Moreno et al. 2000), ethanol, hydroethanol (Qian and Nihorimbere 2004), methanol (Chah et al. 2006), and hydromethanol (Bushra et al. 2012). However, there is a paucity of research investigating the most effective solvent for the antioxidant efficacy of guava leaves.

Therefore, in this study, the phenolic compound and flavonoid content of water, ethanol, methanol, and hydroethanolic extracts of guava leaves were analyzed and evaluated with regard to antioxidant properties. The best extraction solvent for use with guava leaves for high antioxidant efficacy was selected.

Material and Methods

Chemicals and reagents

Ethanol and methanol were purchased from Duksan (Jinju, Korea). Folin–Ciocalteu reagent, caffeic acid, quercetin, DPPH, ABTS, potassium ferricyanide, trichloroacetic acid, ferrous chloride, sulfurilamide, phosphoric acid, and N-(1-naphthyl) ethylenediamine were purchased from Sigma (St. Louis, MO). Potassium acetate, sulfanilic acid, and naphthylamine were purchased from Yakuri (Osaka, Japan). All chemicals and reagents were of analytical grade. Guava leaves were obtained from Guava Korea Ltd. (Uiryeong-gun, Korea).

Preparation of water extracts

As described by Kandil et al. (1994), a sample of 100 g guava leaves in 1.5 L distilled water was boiled for 4 h. The sample was then filtered using Whatman filter paper No. 4. The filtrate was concentrated in a rotary evaporator at 60°C and dried using a freeze drier. The resulting extracts were stored at −18°C until the analysis.

Preparation of ethanol, methanol, and hydroethanolic extracts

The ethanol and methanol extracts were prepared by placing a sample of 100 g of guava leaves in 1.5 L pure ethanol (purity 94.0%) and 1.5 L pure methanol (purity 99.8%), respectively, for 4 days at room temperature. For the hydroethanolic extracts, hydroethanol solvents with water:ethanol in the ratios of 70:30, 50:50, 30:70, and 10:90 (v/v) were prepared for use in the extraction. After 4 days, the extracts were filtered using Whatman filter paper No. 4, and then the filtrates were concentrated using a rotary evaporator at 50°C. The resulting filtrates were dried using a freeze drier and stored at −18°C until further analysis.

Phenolic compound content assay

The Folin–Ciocalteu method (Ainsworth and Gillespie 2007) with a modification was used to determine the phenolic compound content of the samples. One milliliter of each extract was diluted with 2 mL distilled water and 0.5 mL of Folin–Ciocalteu reagent (Sigma Co.). After 3 min, 0.5 mL of 10% Na2CO3 solution was added to the mixture and the mixture was allowed to stand for 1 h at room temperature in a dark room. The absorbance was measured at 760 nm with a UV–visible spectrophotometer (Optizen 2120 UV; Mecasys Co., Ltd., Daejeon, Korea). A standard caffeic acid (Sigma Co.) solution (10–100 μg/mL) was used for the construction of a calibration curve. Results were expressed as mg caffeic acid/g extract. The tests were run in triplicate and averaged.

Flavonoid content assay

Flavonoid content was determined by Moreno’s method (Moreno et al. 2000). Each extract (1 mL) was added to a test tube containing 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 mol/L aqueous potassium acetate, and 4.3 mL of 80% ethanol. After 40 min at room temperature in a dark room, the absorbance was measured at 415 nm. Total flavonoid content was assessed using quercetin (Sigma Co.) as a standard (0–100 μg/mL).

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH)–scavenging assay

A series of water, ethanol, methanol, and hydroethanol guava leaf extracts (50, 100, 250, 500 and 1000 μg/mL) were prepared for an antioxidant assay. Scavenging activ-
ity of the extracts on DPPH was measured according to the method developed by Blois (1958). Varying concentrations of the guava leaf extract solutions (1 mL) were added to a DPPH methanol solution (5 mg/100 mL, 2 mL). The decrease in absorbance at 517 nm was measured with a UV-visible spectrophotometer. DPPH-scavenging activity (%) was calculated according to the following equation:

$$\text{DPPH}^+ \text{-scavenging activity} \% = \left[ 1 - \frac{A_{\text{sample}}}{A_0} \right] \times 100$$

where $A_{\text{sample}}$ is the absorbance of the sample solution in a steady state and $A_0$ is the absorbance of DPPH solution before adding the extract.

**Scavenging activity on ABTS**

ABTS$^+$-scavenging activity was assessed according to the method described by Re et al. (1999). A mixture of ABTS (7.0 mmol/L) and potassium persulfate (2.45 mmol/L) in water was prepared and stored at room temperature for 12 h in a dark room to produce ABTS$^+$. The ABTS$^+$ solution in water was diluted to the level of absorbance of 1.50 at 414 nm for the analysis. Different concentrations of the extract solution (1 mL) were added to the diluted ABTS$^+$ solution (2 mL). The absorbance was recorded at 414 nm. The radical-scavenging activity was measured according to equation (1).

**Reducing power assay**

The reducing power was measured by the browning reaction method (Oyaizu 1986). Varying concentrations of the extract solutions (1.0 mL) were mixed with phosphate buffer (pH 6.6, 1.0 mL, 0.2 mol/L) and 1% aqueous potassium ferricyanide (1.0 mL). The mixture was incubated for 20 min at 50°C. An aliquot (1.0 mL) of 10% aqueous trichloroacetic acid was added to the mixture, which was subsequently centrifuged for 10 min at 5000 rpm. The upper layer of the solution (1.0 mL) was mixed with pure water (1.0 mL) and 0.1% aqueous FeCl$_3$ (1.0 mL), and the absorbance was measured at 700 nm.

**Nitric oxide radical-scavenging activity**

Nitric oxide radical (NO$^+$)-scavenging activity was measured by the Greiss reagent as described in a previous study (Sumanont et al. 2004). Sodium nitroprusside (5 mmol/L) was dissolved in phosphate buffer (pH 7.4, 2 mL), mixed with flavonoid solution (1 mL), and incubated at 25°C for 150 min. The Greiss reagent (0.5 mL) consisted of 2% sulfanilamide in 4% aqueous H$_3$PO$_4$, and 0.1% aqueous N-(1-naphthyl) ethylenediamide (1:1, v/v) was added to the sample solutions. The absorbance was measured at 542 nm. The percentage of scavenging activity was calculated according to equation (1).

**Nitrite-scavenging activity**

Nitrite-scavenging activity was evaluated based on the absorbance at 520 nm using a UV-spectrophotometer according to the method reported by Kato et al. (1987). One milliliter of 1 mmol/L NaNO$_2$ (Sigma Co.) solution was added to 1 mL of each sample, and the resulting mixtures were adjusted to pH 2.5 using 0.1 N HCl and 0.2 N citric acid solutions. Each sample was allowed to react at 37°C for 1 h, after which 1 mL of each sample was taken from the solution and mixed thoroughly with 3 mL of 2% acetic acid and 0.4 mL of the Griess reagent. The solutions were stored at room temperature for 15 min. The Griess reagent was prepared by mixing an equal amount of 1% sulfanilic acid (Sigma Co.) and 1% naphthylamine (Sigma Co.), which were made with 3% acetic acid. Nitrite-scavenging activity was calculated according to equation (1).

**Statistical analysis**

All experiments were carried out in triplicate. Values are presented as mean ± SD ($n = 3$). Statistical differences among the groups were determined by analysis of variance followed by Duncan’s multiple range test using the SPSS program (version 12.0; SPSS Inc., Chicago, IL) package. $P < 0.05$ was considered statistically significant.

**Results and Discussion**

**Phenolic compound and flavonoid content**

Total phenolic compound and flavonoid content of guava leaf extracts are listed in Figures 1, 2. The phenolic compound content of water extract was higher than that of the pure ethanol and pure methanol extracts (Fig. 1A). Furthermore, the phenolic compound content of the hydrophenolic extracts was higher than that of the water extract, and the highest content of phenolic compounds was in the 50% hydroethanolic extract (Fig. 2A). Among the three solvent extracts, the flavonoid content of the water and ethanol extracts was higher than that of the methanol extract (Fig. 1B). Among the four concentrations of the hydroethanolic extracts, the flavonoid content of the 70% hydroethanolic extract was the highest (Fig. 2B). This result is consistent with previous reports showing that the phenolic compound content of water extract was higher than in pure ethanol and pure methanol extracts.
Nyirenda et al. (2012) reported that polar compounds, such as phenolic compounds and flavonoids, were more soluble in aqueous solvents than in organic solvents. It was reported that the phenolic compound content of 50% hydroethanolic extract was higher than in the water extract of guava leaves (Qian and Nihorimbere 2004). Another study found that the order of increasing phenolic compound content of *Hieracium pilosella* was 50% hydroethanolic extract > 80% hydromethanolic extract > water extract (Stanojević et al. 2009). According to another research study, the phenolic compound content was highest in 40% hydroethanolic extract (Ito et al. 2012). Our results agree with several studies that examined the relationship between phenolic compounds and antioxidant capacity. A previous study found that antioxidant capacity varied according to the phenolic compound profile (Kosińska et al. 2012). Another study reported that there are positive correlations between the phenolic compound concentration and antioxidant ability (Kim et al., 2008).

Figure 1. Phenolic compound and flavonoid content of guava leaf extracts for each extract solvent. Phenolic compound content of guava leaf extract (A), Flavonoid content of guava leaf extract (B). The results are expressed as mean ± SD. The significance of differences was determined by one-way analysis of variance using SPSS version 12.0. A $P < 0.05$ indicates that the difference is significant.

Figure 2. Phenolic compound and flavonoid content of guava leaf extracts for each concentration of hydroethanolic solvent. Phenolic compound content of guava leaf hydroethanolic extracts (A), flavonoid content of guava leaf hydroethanolic extracts (B). H.E., hydroethanolic extract. The results are expressed as mean ± SD. The significance of differences was determined by one-way analysis of variance using SPSS version 12.0. A $P < 0.05$ indicates that the difference is significant.

**DPPH- and ABTS•*-scavenging activity and reducing power**

The antioxidant properties of three solvent extracts were evaluated by in vitro tests including DPPH- and ABTS•*-scavenging activity and reducing power (Table 1). In all measurements, the antioxidant capacity was observed to be significantly higher in the water extract that had the highest content of phenolic compounds, which suggests a positive correlation between the antioxidant capacity and the phenolic compound content. The antioxidant activities increased depending on the concentration of the extracts. Furthermore, the antioxidant activity of the hydroethanolic extracts was higher than that of the water extracts and was highest for 50% hydroethanolic extract (Table 2).

Our results are consistent with previous reports. It was shown that DPPH- and ABTS•*-scavenging activity and reducing power of guava leaves in the water extract were higher than in purely ethanol, methanol, hexane, and ethyl acetate extracts (Aktumsek et al. 2013). Furthermore, the activity of 50% hydroethanolic extract was
observed to be even higher than that of the water extract (Qian and Nihorimbere 2004). It was reported that DPPH- and ABTS⁺-scavenging activities were significantly correlated with the total abundance of phenolic compounds (Tayade et al. 2013). Antioxidant activity is strongly correlated with reducing power, which increased depending on the concentration and reaction time of the extracts (Kwon et al. 2013). Our results clearly suggest that the antioxidant abilities of guava leaves, such as DPPH- and ABTS⁺-scavenging activity and reducing

| Solvents | 50 | 100 | 250 | 500 | 1000 |
|----------|----|-----|-----|-----|------|
| DPPH     |    |     |     |     |      |
| Water    | 28.12 ± 0.21ab | 51.51 ± 1.09bc | 89.00 ± 0.52bc | 92.79 ± 0.15bd | 93.86 ± 0.06bd |
| Ethanol  | 18.97 ± 1.66ac | 35.57 ± 2.87bc | 71.80 ± 0.53bd | 92.78 ± 0.35bd | 92.95 ± 0.08bd |
| Methanol | 18.76 ± 3.58ac | 24.33 ± 1.20ab | 49.88 ± 1.63aA | 88.07 ± 2.22aA | 90.29 ± 2.05aA |
| ABTS     |    |     |     |     |      |
| Water    | 37.17 ± 0.37bc | 64.27 ± 0.23bc | 97.18 ± 0.00cC | 98.29 ± 0.13bc | 98.74 ± 0.07bc |
| Ethanol  | 21.12 ± 0.38bc | 41.05 ± 3.77bb | 81.01 ± 1.12cC | 91.27 ± 0.26cC | 94.26 ± 0.19bb |
| Methanol | 16.25 ± 2.87bc | 25.89 ± 3.73bc | 50.17 ± 3.48bc | 82.22 ± 1.89bc | 85.09 ± 2.72bc |
| Reducing power | 0.19 ± 0.00cC | 0.28 ± 0.00cC | 0.51 ± 0.01cC | 0.83 ± 0.01cC | 1.35 ± 0.00cC |
| Ethanol  | 0.12 ± 0.01ab  | 0.16 ± 0.01ab  | 0.24 ± 0.00ab  | 0.40 ± 0.00aA  | 0.69 ± 0.02aA  |
| Methanol | 0.16 ± 0.00ab  | 0.21 ± 0.01ab  | 0.40 ± 0.01aA  | 0.67 ± 0.03aA  | 1.15 ± 0.02aA  |
| NO       |    |     |     |     |      |
| Water    | 12.54 ± 1.42ab | 14.45 ± 2.30abA| 18.05 ± 2.52aA | 27.32 ± 2.76aA | 35.20 ± 2.13aA |
| Ethanol  | 27.29 ± 0.71ab | 34.62 ± 0.37bc | 36.22 ± 2.32bc | 39.76 ± 0.09bc | 41.67 ± 0.65bc |
| Methanol | 25.33 ± 1.89ab | 28.63 ± 1.42bb | 29.38 ± 1.62bb | 29.56 ± 1.59bb | 35.44 ± 2.63bc |
| NO₂      |    |     |     |     |      |
| Water    | 15.52 ± 2.03ab | 19.26 ± 1.96bc | 33.45 ± 0.54ab | 56.61 ± 1.28ab | 82.99 ± 0.64ab |
| Ethanol  | 3.23 ± 0.17ab  | 13.91 ± 1.34bc | 34.18 ± 0.70ab | 61.87 ± 1.23ab | 80.50 ± 1.17ab |
| Methanol | 14.64 ± 1.83ab | 17.36 ± 0.92bc | 43.45 ± 1.78bc | 53.57 ± 1.09ab | 68.96 ± 1.66ab |

The results are expressed as mean ± SD. The significance of differences was determined by one-way analysis of variance using SPSS version 12.0. A *P < 0.05 indicates that the difference is significant. *Means with different superscripts in the same row show significant difference. A-DMeans with different superscripts in the same column show significant difference. NS, not significant.
power, are closely dependent on the contents of the phenolic compounds.

**Nitric oxide (NO) radical- and nitrite (NO$_2$)-scavenging activity**

Nitric oxide-scavenging activity of the ethanol extract with a high content of flavonoids was significantly higher than that of the water or methanol extract, while nitrite scavenging abilities of the three solvent extracts did not differ significantly (Table 1). In the test using the mixed solvents, both nitric oxide- and nitrite-scavenging abilities were significantly higher in the 50% hydroethanolic extract that had the highest content of phenolic compounds (Table 2).

In previous studies for the flavonoid content of *Impatiens balsamina*, potato peel, sugar beet pulp, and sesame cake, the flavonoid content of purely methanol extract was higher than that of the content of water or purely ethanol extracts (Su et al. 2012). However, the flavonoid content of 50% hydroethanolic extract was higher than that of other extracts, such as water and 80% methanol extracts (Stanojević et al. 2009). Furthermore, the nitrite-scavenging activity of plum with high flavonoid content in 80% ethanol extracts was higher than that of two other kinds of plum that had low levels of flavonoid content (Kim et al. 2012).

According to this study, the water extract with a high content of phenolic compounds showed high antioxidant abilities in the DPPH$^-$ and ABTS$^{++}$-scavenging activity and in the reducing power assay. Ethanol extract with a high flavonoid content showed high antioxidant activities in the nitric oxide radical- and nitrite-scavenging ability assay. In the antioxidant ability tests of hydroethanolic extracts, as measured by DPPH$^-$ and ABTS$^{++}$-scavenging activity, reducing power, and nitric oxide and nitrite-scavenging activity, the activity of 50% hydroethanolic extract was the highest among the three different solvents and the other hydroethanolic extracts. This comparison strongly suggests that the best extraction solvent for high antioxidant efficacy of guava leaves is the 50% hydroethanolic solvent.

**Conclusion**

This study intended to find the best extraction solvent for high antioxidant efficacy of guava leaves using various solvents. The phenolic compound content of water extract was higher than pure ethanol and methanol extract. Furthermore, the phenolic compound content of hydroethanolic extracts was higher than water extracts. The antioxidant activity of hydroethanolic extracts was higher than that of the water extracts and was significantly high in the 50% hydroethanolic extract that had the highest content of phenolic compounds.

**Acknowledgment**

This research was supported by High Value-added Food Technology Development Program of Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (20090237). The authors wish to thank Guava Korea Ltd. (South Korea) and Uiryeong Guava Agricultural Union Corporation (South Korea) for their support.

**Conflict of Interest**

None declared.

**References**

Ainsworth, E. A., and K. M. Gillespie. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat. Protoc. 2:875–877.

Aktumsek, A., G. Zengin, G. O. Guler, Y. S. Cakmak, and A. Duran. 2013. Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic *Centaurea* L. species. Food Chem. Toxicol. 55:290–296.

Bhanot, A., S. Rohini, and M. N. Noolvi. 2011. Natural sources as potential anti-cancer agents: a review. Int. J. Phytomed. 3:09-26.

Blois, M. S. 1958. Antioxidant determinations by the use of a stable free radical. Nature 181:1199–1200.

Brand-Williams, W., M. E. Cuvelier, and C. L. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm.-Wiss. Technol. 28:25–30.

Bushra, S., H. Zaib, A. Muhammad, and M. Adil. 2012. Investigation on the antioxidant activity of leaves, peels, stem bark, and kernel of mango (*Mangifera indica* L.). J. Food Sci. 77:849–852.

Chah, K. F., C. A. Eze, C. E. Emuelosi, and C. O. Esimone. 2006. Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants. J. Ethnopharmacol. 104:164–167.

Cheng, F. C., S. C. Shen, and J. S. B. Wu. 2009. Effect of guava (*Psidium guajava* L.) leaf extract on glucose uptake in rat hepatocytes. J. Food Sci. 74:132–138.

Gutierrez, R. M., S. Mitchell, and R. V. Solis. 2008. *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology. J. Ethnopharmacol. 117:1–27.

Han, E. H., Y. P. Hwang, J. H. Choi, J. H. Yang, J. K. Seo, Y. C. Chung, et al. 2011a. *Psidium guajava* extract inhibits thymus and activation-regulated chemokine (TARC/CCL17) production in human keratinocytes by inducing heme oxygenase-1 and blocking NF-kB and STAT1 activation. Environ. Toxicol. Pharmacol. 32:136–145.

Ito, T., M. Kakino, S. Tazawa, T. Watarai, M. Oyama, H. Maruyama, et al. 2012. Quantification of polyphenols and...
pharmacological analysis of water and ethanol-based extracts of cultivated agarwood leaves. J. Nutr. Sci. Vitaminol. 58:136–142.

Jayanthi, P., and P. Lalitha. 2011. Reducing power of the solvent extracts of Eichhornia crassipes (Mart.) Solms. Int. J. Pharm. Pharm. Sci. 3:126–128.

Kähkönen, M. P., A. I. Hopia, H. J. Vuorela, J. P. Rauha, K. Pihlaja, T. S. Kujala, et al. 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47:3954–3962.

Kandil, O., N. M. Radwan, A. M. M. Amer, and H. A. El-Banna. 1994. Extracts and fractions of thymus capitatus exhibit antimicrobial activities. J. Ethnopharmacol. 44:19–24.

Kato, H., I. E. Lee, N. V. Chyuen, S. B. Kim, and F. Hayase. 1987. Inhibitory of nitrosamine formation by nondialyzable melanoids. Agric. Biol. Chem. 51:1333–1338.

Kim, K. M., P. Seguin, J. K. Ahn, J. J. Kim, S. C. Chun, E. H. Kim, et al. 2008. Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. J. Agric. Food Chem. 56:7265–7270.

Kim, S. N., M. R. Kim, S. M. Cho, S. Y. Kim, J. B. Kim, and Y. S. Cho. 2012. Antioxidant activities and determination of phenolic compounds isolated from oriental plums (Sodam, Oshiwase, and Formosa). Nutr. Res. Pract. 6:277–285.

Kosińska, A., K. Magdalena, I. Estrella, T. Hernandez, B. Bartolome, and G. A. Dykes. 2012. Phenolic compound profiles and antioxidant capacity of Persea americana Mill. Peels and seeds of two varieties. J. Agric. Food Chem. 60:4613–4619.

Kwon, T. H., T. W. Kim, C. G. Kim, and N. H. Park. 2013. Antioxidant activity of various solvent fractions from edible brown alga, Eisenia bicyclis and its active compounds. J. Food Sci. 78:679–684.

Mercadante, A. Z., A. Steck, and H. Pfander. 1999. Carotenoids from Guava (Psidium guajava L.): isolation and structure elucidation. J. Agric. Food Chem. 47:145–151.

Miyake, Y., and M. Hiramin. 2011. Isolation and extraction of antimicrobial substances against oral bacteria from lemon peel. J. Food Sci. Technol. 48:635–639.

Moreno, M. I. N., M. I. Isla, A. R. Sampietro, and M. A. Vattuone. 2000. Comparison of the free radical scavenging activity of propolis from several regions of Argentina. J. Ethnopharmacol. 71:109–114.

Nyirenda, K. K., J. D. K. Saka, D. Naidoo, V. J. Maharaj, and C. J. F. Muller. 2012. Antidiabetic, anti-oxidant and antimicrobial activities of Fadoga angulanta extracts from Malawi. J. Ethnopharmacol. 143:372–376.

Okuda, T., T. Yoshida, T. Hatano, K. Yazaki, Y. Ikegami, and T. Shingu. 1987. Guavins A, C and D, complex tannin from Psidium guajava. Chem. Pharm. Bull. 35:443–446.

Oyaizu, M. 1986. Studies on products of the browning reaction. Antioxidative activities of browning reaction products prepared from glucosamine. Jpn. J. Nutr. 44:307–315.

Pham-Huy, L. A., H. He, and C. Pham-Huy. 2008. Free radicals, antioxidants in disease and health. Int. J. Biomed. Sci. 4:89–96.

Qian, H., and V. Nihorimbere. 2004. Antioxidant power of phytochemicals from Psidium guajava. J. Zhejiang Univ. Sci. 5:676–683.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol. Med. 26:1231–1237.

Reddy, N. S., S. Navanesan, S. K. Sinniah, N. A. Wahab, and K. S. Sim. 2012. Phenolic content, antioxidant effect and cytotoxic activity of Lea indica leaves. BMC Complement Altern. Med. 12:128–134.

Shao, M., Y. Wang, X. J. Huang, C. L. Fan, Q. W. Zhang, X. Q. Zhang, et al. 2012. Four new triterpenoids from the leaves of Psidium guajava. J. Asian Nat. Prod. Res. 14:348–354.

Sharma, S. K., and A. P. Singh. 2012. In vitro antioxidant and free radical scavenging activity of Nardostachys jatamansi DC. J. Acupunct. Meridian Stud. 5:112–118.

Stanojević, L., M. Stanković, V. Nikolić, L. Nikolić, D. Ristić, J. Canadanovic-Brunet, et al. 2009. Antioxidant activity and total phenolic and flavonoid contents of Hieracium pilosella L. extracts. Sensors 9:5702–5714.

Su, B. L., R. Zeng, J. Y. Chen, C. Y. Chen, J. H. Guo, and C. G. Huang. 2012. Antioxidant and antimicrobial properties of various solvent extracts from Impatients balsamina L. stems. J. Food Sci. 77:614–619.

Sumanont, Y., Y. Murakami, M. Tohda, O. Vajragupta, K. Matsumoto, and H. Watanabe. 2004. Evaluation of the nitric oxide radical scavenging activity of manganese complexes of curcumin and its derivative. Biol. Pharm. Bull. 27:170–173.

Tayade, A. B., P. Dhar, M. Sharma, R. S. Chauhan, O. P. Chaurasia, and R. B. Srivastava. 2013. Antioxidant capacities, phenolic contents, and GC/MS analysis of Rhodiola imbricate edgew. root extracts from trans-himalaya. J. Food Sci. 78:402–410.