Food Anal. Methods (2011) 4:100–107
DOI 10.1007/s12161-010-9139-3

Antioxidant Activity of Pink-Flesh Guava (*Psidium guajava* L.): Effect of Extraction Techniques and Solvents

Khalid Hamid Musa · Aminah Abdullah · Khairiah Jusoh · Vimala Subramaniam

Received: 24 November 2009 / Accepted: 23 March 2010 / Published online: 20 April 2010

Abstract The effect of commonly used techniques and solvents in the antioxidant activities of pink-flesh guava fruit were studied. The extraction techniques compared were homogenization, shaking, sonication, magnetic stirring, and maceration for 1, 2, and 3 days. The solvent systems used were methanol, ethanol, and acetone at three different concentrations (50%, 70%, and 100%) and with 100% distilled water. The antioxidant activity of the fruit was evaluated using Folin–Ciocalteu index, ferric-reducing antioxidant power assay, and 1,1-diphenyl-2-picrylhydrazyl free radical-scavenging capacity. Ultrasonic and homogenization were the best techniques to extract the antioxidant from guava fruit. Homogenization technique was found to be the most convenient exhaustive and time-saving extraction technique. Results showed that the extracting solvent significantly ($P<0.05$) altered the antioxidant property estimations of pink-flesh guava fruit. Pure solvents were inefficient extraction media for antioxidant. Enhanced extraction yields were obtained from solvent containing higher water concentrations and 50% acetone is a recommended solvent for extracting antioxidants compounds from pink-flesh guava fruit. High correlations between phenolic compositions and antioxidant activities of pink-flesh guava extracts were observed. High levels of antioxidant activities were detected in pink-flesh guava, indicating that the fruit may serve as an excellent dietary source of natural antioxidants.

Keywords Pink-Flesh Guava · Antioxidant · Extraction Technique · Extraction Solvent

K. H. Musa (✉) · A. Abdullah
Food Science Program, School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia,
43600 Bangi, Selangor, Malaysia
e-mail: khalidsilik@yahoo.com

K. Jusoh
School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia,
43600 Bangi, Selangor, Malaysia

V. Subramaniam
Medicinal Plant Program, Biotechnology Division,
Forest Research Institute Malaysia,
52109 Kepong, Selangor, Malaysia

Introduction

Consumption of foods containing phytochemicals with potential antioxidant properties can reduce the risk of human diseases such as cancer, atherosclerosis, arthritis, diabetes, and other aging diseases (Temple 2000). Efforts have been undertaken to isolate, characterize, and extract antioxidant from natural plant sources. Extraction is the initial step in the isolation of bioactive components from plant materials. The aim of an extraction process is to obtain the maximum concentration of target compounds and of the highest antioxidant activity of the extracts (Spigno et al. 2007). Extraction is influenced by the chemical nature of the compounds, the extraction technique employed, and the presence of interfering substances (Chirinos et al. 2007).

The solvent extraction has been widely used to extract bioactive components from plants. Solvent extraction is a process designed to separate soluble antioxidant compounds by diffusion from a solid matrix (plant tissue) using a liquid matrix (solvent). It is noted that a solvent system for extraction is selected according to the purpose of extraction such as preparation or analysis, the nature of interested components, the physicochemical properties

of the matrix, the availability of reagents and equipments, cost, and safety concerns (Yu et al. 2002). The commonly used solvents for extracting antioxidant were methanol, ethanol, and acetone either singly or in combination with aqueous (Lim et al. 2007; Thaipong et al. 2006; Tachakittirungrod et al. 2007; Kahkonen et al. 1999; Velioglu et al. 1998; Zielinski and Kozlowska 2000). The polarities of the different organic solvent greatly influence the selection of a specific solvent for the extraction of a specific group of bioactive compounds.

Extraction of antioxidant is influence by the extraction technique employed (Chirinos et al. 2007). Various techniques have been applied to extract antioxidants from plant materials and other foodstuffs. The techniques commonly used were shaking (Jimenez-Escrig et al. 2001; Lapornik et al. 2005; Xu and Chang 2007), homogenization at high speed (Arnao et al. 2001; Naczk et al. 1992), ultrasonic (Palma and Taylor 1999), maceration (Ahn et al. 2002; Contini et al. 2008), stirring (Alothman et al. 2009), and microwave-assisted extraction (Hemwimon et al. 2007). The selections of particular extraction technique depend on the simplicity of the extraction technique and its convenience.

Guava (*Psidium guajava* L.) belongs to the Myrtaceae family and is widespread throughout the tropical and subtropical areas. Guava is rich in antioxidants compounds and contains a high level of ascorbic acid ranging from 174.2 to 396.7 mg/100 g fresh fruit (Thaipong et al. 2006). Myricetin and apigenin were reported to be 549.5 and 579.0 mg/kg dry weight, respectively (Koo and Mohamed 2001). The objectives of this research were to investigate the effect of different extraction solvents and techniques on the antioxidant activity of pink-flesh guava fruit using the following assays Folin–Ciocalteu index (FCI), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay, and ferric-reducing antioxidant power (FRAP). Each of the above assays measures different aspects of the antioxidant activity of the fruit extracts.

Materials and Methods

Chemicals

Folin–Ciocalteu phenol reagent, ferric chloride ($FeCl_3 \cdot 6H_2O$), and HCl were obtained from Merck (Darmstadt, Germany) and 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), gallic acid and Trolox, and sodium acetate trihydrate were purchased from Sigma (USA). Sodium carbonate was purchased from RDH (Germany) while glacial acetic acid was from Mallinckrodt Baker (USA). All chemicals and reagents used in the study were of analytical grade.

Samples Collection and Preparation

Pink-fleshed guava fruits were collected from Setiawan in the state of Perak, North Malaysia. Samples were transfer in ice on the same date to the Food Analysis Laboratory, University Kebangsaan Malaysia. The estimated time of transportation was about 4 h. For the purposes of this study approximately 20 fruits pooled sample portion (taken from a 60 sample lot) were stored in freezer below −25°C until analysis.

Extraction of Antioxidants

Guava fruits were crushed (while still frozen) in a food processor to produce uniform slurry. The extraction procedure was conducted with (0.5 g) samples and 10 mL extracting solvent using one of the following techniques:

1. Homogenization for 1 min under high speed (24,000 rpm) using high-performance disperser (T 25 digital ULTRA-TURRAX®, IKA, Germany)
2. Shaking for 1 h (300 rpm) using shaker (Intertech, Taiwan)
3. Ultrasonic extraction for 1 h at ultrasonic bath (Soniclean, Thebarton, Australia)
4. Mixing with magnetic stirrer for 1 h (1,000 rpm) using magnetic stirrer (Heidolph, MR3001, K, Germany)
5. Maceration of the sample in the extraction solvent for 1, 2, or 3 days

All extracted samples were centrifuged using tabletop centrifuge (Kubota, Japan) for 10 min at 2,580×*g*. The supernatants were collected for further analysis.

In the second part of this work, the effect of different types of solvent was studied using the best technique selected in the first part. Solvents systems used were absolute methanol, ethanol, acetone, and their aqueous solutions at 50%, 70%, and 100% concentrations. All tests were performed at room temperature.

Determination of Folin–Ciocalteu Index

The FCI used were based on Slinkard and Singleton (1977). Pink-flesh guava fruit extract (0.1 mL), gallic acid (standard calibration), or extracting solvent was placed in a separate 10 mL vials, followed by the addition of 0.4 mL water and 0.5 mL diluted Folin–Ciocalteu reagent. The mixture were swirled and allowed to stand for 5 min followed by the addition of 1 mL of 7.5% (*w*/*v*) of sodium carbonate and samples were mixed. Solutions were allowed to stand for 2 h at room temperature and the absorbance was read at 765 nm wavelength using spectrophotometer (UNIKAM, UK). Results were expressed as milligrams of

gallic acid equivalents per 100 g of fresh sample (mg GAE/100 g of FW).

Determination of Ferric Reducing/Antioxidant Power

The antioxidant capacity of each sample was estimated according to adapted procedure of Benzie and Strain (1996) with some modifications. FRAP reagent was prepared as using 300 mM acetate buffer, pH 3.6 [3.1 g sodium acetate trihydrate, plus 16-mL glacial acetic acid made up to 1 l with distilled water]; 10 mM TPTZ (2,4,6-tri(2-pyridyl)-*s*-triazine), in 40 mM HCl; and 20 m*M* $FeCl_3$ $6H_2O$ in the ratio of 10:1:1 to give the working reagent. FRAP reagent, 3,900 μL, prepared freshly and warmed at 37°C, was mixed with 100 μL test sample, standards, or extraction solvent as reagent blank. After 30 min the absorbance was measured at 595 nm wavelength. The result was expressed as milligrams of Trolox equivalents per 100 g of fresh sample (mg TE/g of FW).

Determination of Radical-Scavenging Activity

The decrease of the absorption at 516 nm wavelength of the DPPH solution after addition of the blank or sample extract was measured in a cuvette. An aliquot (3,900 μL) of methanolic DPPH solution (24 mg/L) was mixed with 100 μL of a sample solution (50 mg/mL). The absorption was monitored at the start and at 30 min. The percentage of DPPH scavenging activity was calculated using the following equation: Radical−scavenging activity = [Abs 516 nm (t = 0) − Abs516 nm $(t = t') \times 100/$Abs 516 nm $(t = 0)$].

Statistical Analysis

Data collected were analyzed statistically using MINITAB® (14.20) software. Correlation analyses was performed using Pearson's correlation coefficient (*r*).

Results and Discussion

Effect of Extraction Techniques on the Antioxidant Activity of Pink-Flesh Guava

Results of antioxidant activity using different extraction techniques are shown in Table 1. The results showed that FCI, DPPH, and FRAP varies with the extraction techniques. The average efficiency of the extraction techniques for FCI and DPPH values in pink-flesh guava fruit showed that both ultrasonic and homogenization were significantly higher ($P<0.05$) than other techniques. For FRAP values, ultrasonic was significantly higher ($P<0.05$) than other techniques including homogenization. Maceration for 24 h showed significantly ($P<0.05$) lower values for FCI, DPPH, and FRAP assay.

When comparing the time needed to achieved the extraction, ultrasonic takes longer time (1 h) while homogenization needs about 1 min to get the same activity for the extract. Extraction with magnetic stirrer technique (1,000 rpm for 1 h), in which extraction was enhanced by stirring effect, or shaking (300 rpm for 1 h) resulted in significantly ($P<0.05$) high antioxidant activity compared to maceration for 24 or 48 h but lower than maceration for 72 h. Even though, stirrer and shaker were

Table 1 Effect of extraction methods on the antioxidants activities from pink-flesh guava fruits determined by Folin–Ciocalteu index (FCI)[a], DPPH radical-scavenging activity[b], and ferric-reducing antioxidant power (FRAP)[c]

	Maceration			Homogenizer	Shaking	Magnetic Stirrer	Ultrasonic
	24h	48h	72h	1min	1h	1h	1h
FCI	320.80±4.19d	334.30±12.63cd	358.39±9.74b	383.13±10.88a	336.55±12.97cd	349.24±19.67bc	386.51±18.29a
RSD%[d]	1.31	3.78	2.72	2.84	3.85	5.63	4.73
DPPH	78.62±1.65c	80.05±2.34bc	81.19±0.18b	83.48±1.01a	82.95±2.40ab	83.05±2.26ab	84.86±0.65a
RSD%	2.10	2.92	0.22	1.20	2.89	2.72	0.77
FRAP	38.45±0.66c	36.13±0.32bc	38.67±1.17bc	41.61±1.56ab	40.34±0.73b	38.07±3.00bc	42.90±1.69a
RSD%	1.72	0.89	3.03	3.74	1.82	7.89	4.11

Results showed mean ± SD. Values in each row marked by the same letter are not significantly different at $P<0.05$

[a] Milligrams of gallic acid equivalent (GAE) per 100 g of fresh weight (FW)

[b] % compare to DPPH without added samples

[c] Milligrams of Trolox equivalent (TE) per gram fresh weight (FW)

[d] Relative standard deviation

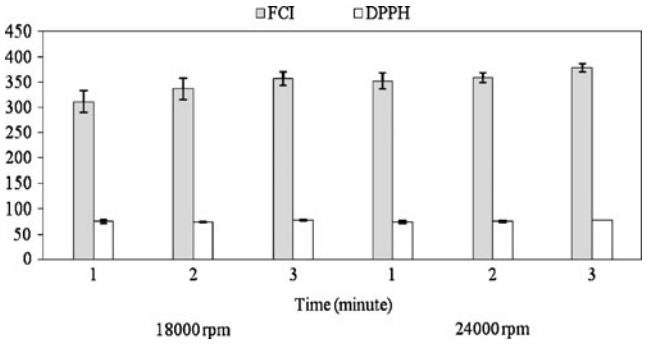

Fig. 1 Effect of homogenization speed (rounds per minute, *rpm*) and time (*minute*) on the antioxidants activities from pink-flesh guava fruits determined by Folin–Ciocalteu index (*FCI*) reported as milligrams of gallic acid equivalent per 100 g fresh weight and DPPH radical-scavenging activity reported as % compare to DPPH without added samples

based on mixing samples and solvents under effect of speed, yet the results are lower than homogenization. Probably this is due the very high speed of homogenization (24,000 rpm) compared with the speed for shaking (300 rpm) and stirring (1,000 rpm). Thaipong et al. (2006) extracted antioxidant from guava fruits using homogenization without reporting the speed. Alothman et al (2009) used the magnetic stirrer at a speed of 1,100 rpm for 3 h for the extraction of antioxidant from different tropical fruits including guava.

The antioxidant assays using maceration (although these values increased significantly with extraction time) were significantly ($P<0.05$) lower than those obtained for homogenization extraction, although the same solvent concentrations and the same temperature (room temperature) were used in both cases. Contini et al. (2008) used long maceration at room temperature for hazelnut by-products. Indicated that the use of long maceration at room temperature appeared to be as efficient as hot-extraction with regard to phenolic concentration of the extract. Tachakittirungrod et al. (2007) used maceration for 48 h (×3) for the extraction of antioxidant from different plants including guava. However, the results of this study showed that maceration technique resulted in the lowest value ($P<0.05$) of antioxidant activities compared to other techniques studied.

Extraction solvents behave differently with different extraction techniques. The mechanism of ultrasonic extraction involves two types of physical phenomena namely diffusion through the cell walls and washing out (rinsing) of the cell contents once the walls are broken (Vinatoru 2001). Ultrasonic enhanced extraction by intensification of mass transfer and easier access of the solvent to the cell (Jacques et al. 2007). Under the influence of homogenization technique, high speed (the key advantages of homogenization) plays an important role in extraction efficiency. High-speed mixing is known to affect the morphological changes in the plant sample matrix that caused the product to release more readily and thus enhances the extraction process. In maceration, the efficiency of extraction depends mainly on the solubility of the compound in the solvent, the mass transfer kinetics of the product, and the strength of the solute/matrix interactions. In maceration one would expect to obtain the same recovery (as homogenization) at much longer time. However, maceration after 3 days resulted in significantly ($P<0.05$) lower value of antioxidant activities compared to homogenization. Extracts that macerated for a longer period lost their antioxidant activities, possibly due to exposure to unfavorable conditions such as light and oxygen (Hemwimon et al. 2007) that lead to chemical degradation or to long-term oxidation.

Extraction time under different homogenization speed was reported (Fig. 1). Extraction at 24,000 rpm for 3 min showed the highest FCI value followed with 18,000 rpm for 3 min. However, when 18,000 rpm was used for 3 min the DPPH radical-scavenging value was higher ($P<0.05$) compared to 24,000 rpm for 3 min. However, homogenization at 24,000 rpm showed better repeatability in both

Table 2 Pearson's correlation coefficients of antioxidant activities of various extraction techniques

Correlation coefficient (*r*)	FRAP[b]	DPPH[c]
Maceration 24 h		
FCI[a]	0.864	0.961
FRAP		0.692
Maceration 48 h		
FCI	1.000	1.000
FRAP		0.999
Maceration 72 h		
FCI	0.943	0.961
FRAP		0.998
Homogenization		
FCI	0.962	0.967
FRAP		1.000
Shaking		
FCI	0.937	0.931
FRAP		0.744
Magnetic stirring		
FCI	0.864	0.998
FRAP		0.891
Ultrasonic		
FCI	0.998	0.785
FRAP		0.825

[a] Folin–Ciocalteu index

[b] DPPH radical-scavenging activity

[c] Ferric-reducing antioxidant power

Table 3 Effect of different extraction solvents on the antioxidants activities from pink-flesh guava fruits determined by Folin–Ciocalteu index (FCI)[a], DPPH radical-scavenging activity[b], and ferric-reducing antioxidant power (FRAP)[c]

		FCI	RSD%[d]	DPPH	RSD%	FRAP	RSD%
Acetone	50%	330.36±14.31a	4.33	85.10±0.59a	0.70	38.19±0.38a	1.01
	70%	329.04±13.62a	4.14	86.79±0.08a	0.10	37.61±0.89a	2.37
	100%	251.81±22.11c	8.78	73.14±2.78c	3.80	29.59±2.04d	6.89
Ethanol	50%	270.48±6.20b	2.29	83.29±1.00a	1.20	35.01±0.95b	2.71
	70%	237.11±9.07c	3.82	75.86±0.98bc	1.30	31.37±0.87cd	2.77
	100%	131.69±5.85e	4.45	43.89±1.52f	3.46	22.51±0.62e	2.77
Methanol	50%	253.01±13.65bc	5.39	70.86±1.36d	1.91	32.91±1.16bc	3.52
	70%	249.04±6.25c	2.51	79.61±2.64b	3.31	33.10±1.01bc	3.06
	100%	203.98±9.23d	4.53	67.79±2.93d	4.33	29.26±1.25cd	4.26
Water		210.36±12.99d	6.18	60.71±2.61e	4.30	27.24±0.84d	3.08

Values in each column marked by the same letter are not significantly different at $P<0.05$. Results showed mean ± SD

[a] Milligrams of gallic acid equivalent (GAE) per 100 g of fresh weight (FW)

[b] % compare to DPPH without added samples

[c] Milligrams of Trolox equivalent (TE) per gram of fresh weight (FW)

[d] Relative standards deviation

FCI (2.32%) and DPPH (0.07%) assays compared to homogenization at 18,000 rpm.

Correlations and Repeatability for Antioxidant Assays of Extraction Techniques

The repeatability of the each extraction techniques was performed by calculating the relative standards deviations (RSD %). All extraction techniques showed good repeatability since all RSD % were lower than 10% for all assays. The relative standard deviations for FCI were between 1.31% for maceration (24 h) and 5.65% for magnetic stirring. As for DPPH the relative standard deviations were between 0.22% for maceration (72 h) and 2.92% for maceration (48 h), while for FRAP, the relative standard deviations were between 0.89%for maceration (48 h) and 7.89% for magnetic stirring.

The correlations (Table 2) between polyphenols (FCI) and values for antioxidant activity (DPPH and FRAP) were high. Generally, the correlation between FCI and FRAP were higher when compared to the correlation between FRAP and DPPH or FCI and DPPH. In maceration (48 h), the correlation between FCI and FRAP or DPPH were the highest (1.000). Using magnetic stirrer or maceration (24 h) showed the lowest correlation (0.864) between FCI and FRAP while homogenization showed the lowest correlation (0.692) between FCI and DPPH. Homogenization also showed the highest correlation (1.000) between DPPH and FRAP. Shaking extract showed the lowest correlation (0.744) between FRAP and DPPH. Data in the literature about the relation between concentration of phenolic compounds and antioxidant activity are contradictory. Some authors observed high correlations (Kahkonen et al. 1999; Luximon-Ramma et al. 2003; Thaipong et al. 2006; Tachakittirungrod et al. 2007) while others showed no or weak direct correlation (Akowuah et al. 2005).

Table 4 The polarities indices of organic solvent–water mixtures

Solvent	Acetone			Ethanol			Methanol			Water
%	100	70	50	100	70	50	100	70	50	100
Polarity index	5.1	6.27	7.05	5.2	6.34	7.1	5.1	6.27	7.05	10

Calculated from Eq. 1

Table 5 Pearson's correlation coefficients of antioxidant activities of various extraction solvents

Correlation coefficient (r)	FRAP[b]	DPPH[c]
Acetone 50%		
FCI[a]	0.976	0.959
FRAP		0.888
Acetone 70%		
FCI	0.895	0.725
FRAP		0.942
Acetone 100%		
FCI	0.995	0.898
FRAP		0.905
Ethanol 50%		
FCI	0.959	0.976
FRAP		0.876
Ethanol 70%		
FCI	0.856	0.906
FRAP		0.589
Ethanol 100%		
FCI	0.828	0.961
FRAP		0.856
Methanol 50%		
FCI	0.929	0.981
FRAP		0.950
Methanol 70%		
FCI	0.977	0.946
FRAP		0.878
Methanol 100%		
FCI	0.844	0.939
FRAP		0.760
WATER		
FCI	0.995	0.787
FRAP		0.729

[a] Folin–Ciocalteu index

[b] DPPH radical-scavenging activity

[c] Ferric-reducing antioxidant power

Effect of Extraction Solvent on the Antioxidant Activity of Pink-Flesh Guava

Comparative study was carried out to establish extractive efficiency of various solvents on the antioxidant activity of pink-flesh guava fruit (Table 3). The results showed that FCI, DPPH, and FRAP values were sensitive to extraction solvents whereby in pure solvents, acetone gave the highest extraction efficiency followed by methanol, water, and ethanol, respectively. Aqueous organic solvents were found to give the highest values. Both, 50% acetone and 70% acetone were the best solvents for obtaining extracts with higher antioxidant activities in pink-flesh guava. However, with 50% ethanol the FCI and FRAP values were significantly ($P<0.05$) lower than both 50% and 70% acetone, unlike DPPH value where the three solvents showed no significant differences ($P<0.05$).

Comparing antioxidant activities from this study and other published data is difficult due to the fact that the content of antioxidant compounds can be influenced by extracting solvent, variety, source of the materials (geographical location). Lim et al. (2007) used 50% ethanol for the extraction of Malaysian seeded and seedless white guava fruits. Thaipong et al (2006) extracted antioxidant from Thai pink guava fruits with methanol. Luximon-Ramma et al (2003), Jimenez-Escrig et al. (2001), and Vasco et al. (2008) extracted antioxidant from Spanish and Mauritius guava fruits using two solvents system consist of acetone and methanol. Tachakittirungrod et al. (2007) extracted antioxidants with 95% ethanol from Thai guava fruit, stem, and leaf. Alothman et al. (2009) compared different solvents for the extraction of antioxidant from Thai seedless guava fruit. They reported that 90% acetone extracts showed higher antioxidant activity than other solvents and water showed the lowest activity. As can be seen from these results, the efficiency of solvents to extract the antioxidant compounds differ among different fruits and among different varieties of the same fruit. It is very hard to develop a standards extraction solvent suitable for the extraction of all plant antioxidant compounds.

Effect of Solvent Polarity on the Antioxidant Activity of Pink-Flesh Guava

Variations in the value of antioxidant activities of different extracts might attribute to the change in relative

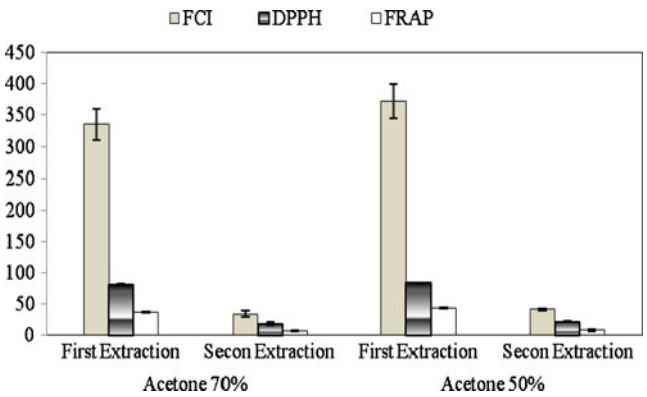

Fig. 2 Effect of repeated extraction on the antioxidants activities from pink-flesh guava fruits determined by Folin–Ciocalteu index (*FCI*) reported as milligrams of gallic acid equivalent per 100 g fresh weight, DPPH radical-scavenging activity reported as % compare to DPPH without added samples, and ferric-reducing antioxidant power (FRAP) reported as milligrams of Trolox equivalent (TE) per gram fresh weight

polarity of different solvents used. As found in this investigation, in a mixture with no aqueous content, the extraction efficiency was low and unfavorable. It is clear that the addition of some amount of water enhances the extraction efficiency. The values for polarity index of a mixture of two solvents, P_m, were calculated from Eq. 1 (Hemwimon et al. 2007).

$$P_m = \emptyset_1 P_1 + \emptyset_2 P_2 \quad (1)$$

where $\emptyset_1$ and $\emptyset_2$ are the volume fractions of solvents 1, and solvent 2, respectively, and P_1 and P_2 are polarity indices of solvent 1 and solvent 2, respectively.

Table 4 showed the polarities of the solvent used in this study. As can be seen from the table, the increased in the ratio of water increases the polarity index of the mixture. Each solvent seemed to have distinct specificities in the extraction of antioxidants. This fact is in accordance with polarity of the solvent used for the extraction and its solubility and content of antioxidants in the fruit (Turkmen et al. 2006). Thus, there appears to be an optimal solvent composition for extraction using homogenization. From the results showed in Table 3 along with Table 4, it may be suggested that a certain degree of increase in the solvent polarity (up to 50% water) could enhance the solubility of antioxidant compounds in the mixture.

Correlations and Repeatability for Antioxidant Assays of Extraction Solvents

All solvents for all antioxidant assays used in this study showed good repeatability as relative standards deviation (RSD %) whereby the lower RSD% the better the repeatability. However, the organic aqueous solvents mixture showed the better repeatability compared to pure organic solvents or water. The relative standard deviations for FCI were between 2.29% for 100% ethanol and 8.78% for 100% acetone. As for DPPH the relative standard deviations were between 0.22% for 70% acetone and 2.92% for water. The relative standards deviation for FRAP were between 1.01% for 50% acetone and 6.89% for 100% acetone.

The results of FCI, DPPH, and FRAP assays used in the present investigation were compared and correlated with each other (Table 5). The higher the DPPH and FRAP values the higher the FCI. It is logical that antioxidant activities were related to the active component in the extract. For FCI and FRAP the highest correlation (0.995) was observed in 100% acetone and water, while 100% methanol showed the lowest value. FCI and DPPH in 50% methanol showed the highest correlation (0.981) while 70% acetone showed the lowest value (0.725). As for FRAP and DPPH the correlation in 50% methanol showed the highest correlation (0.950) while 70% ethanol showed the lowest value (0.589). However, the chosen solvent for antioxidant extraction (50% acetone) showed high correlation between the antioxidant assays compared to the second choice (70% acetone).

Effect of Repeated Extraction on the Antioxidant Activity of Pink

Fresh pink guava samples were extracted two times to determine the effect of repeated extraction of 50% and 70% acetone on the antioxidant activity. The results showed that first extraction results in significantly higher ($P<0.05$) recovery than the second extraction (Fig. 2). Repeated extraction allowed for additional recovery but the time, cost, and potential error introduced while performing a second extraction would not be justified. Second extraction required more chemicals that may contribute negatively towards the environment. Furthermore, the potential error introduced while performing a second extraction would not be justified. Therefore, it was unnecessary to use a second extraction to improve recovery.

Conclusion

This study indicated that the extracts obtained from pink-flesh guava fruits have remarkable antioxidant activities, the extent of which depends on the extraction technique and solvent. Homogenization technique is a simple procedure (one-step extraction) which gives high antioxidant activities while requiring the shortest extraction time (less than 3 min) when compared with the other extraction techniques. Antioxidant extraction depends on the solubility of antioxidant compounds of plant material in the extraction solvent. Acetone was the best solvent compared to methanol, ethanol, or water. The addition of water to organic solvent increased the effectiveness of the extraction. The 50% aqueous acetone was used in this study for best recovery of antioxidant compounds. This aqueous organic mixing allows more scope in the choice of solvent to be used in an extraction process possibly leading to an economic process and improved environmental, health, and safety considerations.

Acknowledgment This research was supported by National University of Malaysia under project nos. STGL 004-2006 and FRGS-ST-01-2006. The authors thank Ms. Zaidah Abu Bakar and Mr. Johar Puteh for technical advice during sample collection.

References

Ahn HS, Jeon TI, Lee JY, Hwang SG, Lim Y, Park DK (2002) Antioxidative activity of persimmon and grape seed extract: in vitro and in vivo. Nutr Res 22:1265–1273

Akowuah GA, Ismail Z, Norhayati I, Sadikun A (2005) The effects of different extraction solvents of varying polarities on polyphenols

of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity. Food Chem 93:311–317

Alothman M, Baht R, Karim AA (2009) Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chem 115:785–788

Arnao MA, Cano A, Acosta M (2001) The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem 73:239–244

Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power the FRAP assay. Anal Biochem 239:70–76

Chirinos R, Rogez H, Campos D, Pedreschi R, Larondelle Y (2007) Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruız and Pavon) tubers. Sep Purification Technology 55:217–225

Contini M, Baccelloni S, Massantini R, Anelli G (2008) Extraction of natural antioxidants from hazelnut (*Corylus avellana* L.) shell and skin wastes by long maceration at room temperature. Food Chem 110:659–669

Hemwimon S, Pavasant P, Shotipruk A (2007) Microwave-assisted extraction of antioxidative anthraquinones from roots of *Morinda citrifolia*. Separ Purif Tech 54:44–50

Jacques RA, Freitas LS, Perez VF, Dariva C, Oliveira AP, Oliveira JVO, Caramao EB (2007) The use of ultrasound in the extraction of Ilex paraguariensis leaves: a comparison with maceration. Ultrason Sonochem 14:6–12

Jimenez-Escrig A, Rincon M, Pulido R, Saura-Calixto F (2001) Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. J Agr Food Chem 49:5489–5493

Kahkonen MP, Hopia AI, Heikki JV, Ranha JP, Pihleja K, Kujalal TS (1999) Antioxidant level of plant extracts containing phenolic compounds. J Agric Food Chem 47:3954–3962

Koo MH, Mohamed S (2001) Flavonoid (myricetin, quercetin, kaempferol, luteolin and apigenin) content of edible tropical plants. J Agric Food Chem 49:3106–3112

Lapornik B, Prosek M, Wondra AG (2005) Comparison of extracts prepared from plant by-products using different solvents and extraction time. J Food Eng 71:214–222

Lim YY, Lim TT, Tee JJ (2007) Antioxidant properties of several tropical fruits: a comparative study. Food Chem 103:1003–1008

Luximon-Ramma A, Bahorun T, Crozier A (2003) Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. J Sci Food Agric 83:496–502

Naczk M, Wanasundara PKJPD, Shahidi F (1992) Spectrophotometric quantification method of sinapic acid in hexane-extracted and methanol-ammonia-water-treated mustard and rapeseed meals. J Agr Food Chem 40:444–448

Palma M, Taylor LT (1999) Extraction of polyphenolic compounds from grape seeds with near critical carbon dioxide. J Chromatogr 849:117–124

Slinkard K, Singleton V (1977) Total phenol analysis; automation and comparison with manual methods. AJEV 28:49–55

Spigno G, Tramelli L, De Faveri DM (2007) Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. J Food Eng 81:200–208

Tachakittirungrod S, Okonogi S, Sombat Chowwanapoonpohn S (2007) Study on antioxidant activity of certain plants in Thailand: mechanism of antioxidant action of guava leaf extract. Food Chem 103:381–388

Temple NJ (2000) Antioxidants and disease: more questions than answers. Nutr Res 20:449–459

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH (2006) Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compos Anal 19:669–675

Turkmen N, Sari F, Velioglu YS (2006) Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. Food Chem 99:835–841

Vasco C, Ruales J, Kamal-Eldin A (2008) Total phenolic compounds and antioxidant capacities of major fruit from Ecuador. Food Chem 111:816–823

Velioglu YS, Mazza G, Gao L, Oomah BD (1998) Antioxidant level and total phenolics in selected fruits, vegetables and grain products. J Agric Food Chem 46:4113–4117

Vinatoru M (2001) An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrason Sonochem 8(2001):303–313

Xu SK, Chang C (2007) A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. J Food Sci 72:S159–S166

Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M (2002) Free radical scavenging properties of wheat extracts. J Agric Food Chem 50:1619–1624

Zielinski H, Kozlowska H (2000) Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. J Agric Food Chem 48:2008–2016