Research Journal of
Phytochemistry

ISSN 1819-3471

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Phytochemistry 6 (3): 61-74, 2012
ISSN 1819-3471 / DOI: 10.3923/rjphyto.2012.61.74

Influence of Different Extraction Parameters on Antioxidant Properties of *Carica papaya* Peel and Seed

[1]L.Y. Ng, [1]Y.K. Ang, [2]H.E. Khoo and [1]H.S. Yim

[1]Department of Food Science and Nutrition, Faculty of Applied Sciences, UCSI University, No. 1, Jalan Menara Gading, UCSI Heights, 56000 Kuala Lumpur, Malaysia

[2]Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Corresponding Author: H.S. Yim, Department of Food Science and Nutrition, Faculty of Applied Sciences, UCSI University, No. 1, Jalan Menara Gading, UCSI Heights, 56000 Kuala Lumpur, Malaysia Fax: +603-91023606

ABSTRACT

This study aimed to determine the effect of extraction media, solvent-to-water ratio and extraction time on Total Phenolics (TP) and antioxidant activities of the peel and seed of *Carica papaya* variety Sekaki. TP was determined by Folin-Ciocalteu's method, while antioxidant activities were evaluated using DPPH radical scavenging ability and Ferric Reducing/antioxidant Power (FRAP) assays. The ideal extraction conditions for papaya peel were 90% acetone (v/v) and 60 min extraction, which had the highest TP (15.18±0.07 μg mL^{-1}) expressed as gallic acid equivalents (GAE) in extract. The highest antioxidant activities measured using DPPH and FRAP assays were 37.3±1.2% and 19.70±0.47 μg mL^{-1} expressed as Trolox Equivalents (TE) in extract, respectively. Extraction with deionised water for 120 min was the best extraction condition for papaya seed, with the highest TP of 6.75±0.08 μg mL^{-1} and highest DPPH and FRAP of 57.3±0.4% and 16.05±0.14 μg mL^{-1}, respectively. Overall, based on the ideal extraction conditions chosen, optimum level of TP and antioxidant activities were obtained in papaya peel and seed extracts. The selected extraction conditions could be used for further studies and functional food product development.

Key words: Papaya, peel, seed, extraction time, antioxidant activity

INTRODUCTION

Plant extracts had been shown to possess various bioactivities, for example, *Luffa acutangula* Roxb. var. *amara* seed extract (Gill *et al*., 2011) and *Luffa cylindrical* seed oil (Yoganandam *et al*., 2010) showed potent antioxidative, anti-inflammatory and analgesic properties. Phenolic compounds are biologically active molecules that ubiquitously distributed as metabolites in plants (Luthria, 2008). Phenolic compounds are also well known for their antioxidant activities (Muchuweti *et al*., 2007; Gill *et al*., 2011). Since there is no single universal extraction method apphed for all food matrices, an optimized extraction procedure is very essential in order to increase the accuracy of the phenolic compounds assay from different food matrices. Several parameters such as extraction method, solvent type, solvent-to-water ratio, duration, pressure, temperature, solid-to-solvent ratio, or the particle size of sample matrix may significantly influence the extraction yield of phenolic compounds and their effects, either independent or interactive (Aghel *et al*., 2008; Chipurura and Muchuweti, 2010; Everette and Islam, 2012).

Selection of an extraction solvent is the preliminary parameter before an extraction is started. In selecting an extraction solvent, factors such as the purpose of extraction, polarity of the interested and undesirable components, cost, availability and safety need to be considered in order to maximize the extraction yield. Commonly used extraction solvents are ethanol, methanol, hexane and acetone whilst inorganic extraction solvents included water (Wang *et al*., 2008). The extraction efficiency can be improved by selecting the optimum solvent-to-water ratio, which therefore can increase the extraction yield. Extraction of phenolic compounds and antioxidants would be limited if some other components are being extracted due to certain solvent-to-water ratio. This might due to the specific solvent-to-water ratio that favors extraction of most analytes from the sample matrix other than the desired ones. Therefore, the solvent-to-water ratio is inconclusive and varies from one research to another (Wang *et al*., 2008).

Kermanshai *et al*. (2001) reported papaya seed extract to have an anthelmintic effect, which was able to reduce the activity of *Caenorhabditis elegans*. In folk medicine, papaya seed is involved in facilitating a good menstrual flow and is also used as emmenagogue, thirst quenchers, carminatives for bites and stings of poisonous insects (Adebiyi *et al*., 2003; Canini *et al*., 2007; Thomas *et al*., 2009). However, papaya seed possesses both positive and negative health effects depending on the way it is being utilized. As papaya seed has contributed to numerous positive health effects, papaya peel too possesses wound healing properties (Anuar *et al*., 2008). The extraction of papaya waste using different extraction media is crucial. Therefore, this study aims to determine the total phenolics and antioxidant activities of papaya seed and its peel. A best extraction method was determined based on five selected extraction media, different solvent-water ratios and a range of extraction times.

MATERIALS AND METHODS

Chemicals and reagents: Analytical grade methanol, acetone, glacial acetic acid, hydrochloric acid and Folin-Ciocalteu's reagent were purchased from Merck (Darmstadt, Hesse, Germany). Analytical grade ethanol and hexane and sodium carbonate anhydrous were obtained from Fisher Scientific (Loughborough, Leicestershire, UK). Sodium acetate anhydrous and TPTZ (2,4,6-tripyridyl-*s*-triazine) were procured from Ajax Finechem (Taren Point, New South Wales, Australia) and ACROS Organics (Geel, Antwerp, Belgium), respectively. Other chemicals were purchased from Sigma Chemicals (St. Louis, Missouri, USA).

Samples and sample preparation: Fully matured papaya (*Carica papaya* cv. Sekaki) (5 kg) was purchased from one of the selected night markets in Kuala Lumpur, Malaysia. The maturity of papaya was determined through the fully matured color of the peel. The papayas were washed under tap water and the seed and peel of papaya were separated from the pulp. Papaya peels were cut into small pieces of about 1 cm^2 before drying in a convection oven (Memmert, Schwabach, Germany) at 45°C. Papaya samples were milled using the miller (QUADRO® COMIL, Quadro Engineering, Waterloo, Canada) at 3873 rpm which yielded particle size of 813 micron. All samples were vacuum-packed using vacuum packager Model DZQ400/500 (Zhejian, China) into small packets of about 25 g in nylon-linear low-density polystyrene pouch (Flexoprint, Selangor, Malaysia).

Extraction of antioxidants: Papaya sample (10.0 g) was weighed and added with 100 mL of extraction media (Table 1). Then, the mixture was placed onto the shaker (Green Seriker, Vision

Scientific, Bucheon, Korea) for 60-300 min. Sample was filtered through Whatman No.1 filter paper (GE Healthcare, Singapore) and collected into an amber reagent bottle, while the sample residues were re-extract using the respective extraction media. Pooled extracts were concentrated using rotary evaporator Rotavapor R-200 (BUCHI, Uster, Switzerland) at 45°C and oven dried at 45°C for overnight. The yield (%, w/w) of crude extract was determined by using the Eq. 1:

$$\text{Yield (\%)} = \frac{\text{Mass}_{\text{extract}}}{\text{Mass}_{\text{sample}}} \times 100 \quad (1)$$

Extraction parameters: Five different extraction media were selected for initial study. The extraction media were deionised water, methanol, ethanol, acetone and hexane. Extraction medium that yielded the highest total phenolics and antioxidant activities was further extracted based on five different percent of solvent used (10-90%), including deionised water and five different extraction times (60-300 min). All analyses were carried out at room temperature (25°C).

Folin-Ciocalteu's reagent assay: The Total Phenolics (TP) were assayed spectrophotometrically using the Folin-Ciocalteu's Reagent (FCR) method as described in Dubost *et al.* (2007) and Ferreira *et al.* (2007). Sample extracts (1 mL) with 5 different concentrations (50-500 μg mL^{-1}) were added with 4 mL of FCR reagent (previously prepared using 10-fold dilution), followed by the addition of 5 mL of 7.5% sodium carbonate solution in 100 mL of deionised water after 3 min.

The mixture was shaken vigorously using a vortex (Boeco, Hamburg, Germany) and incubated at room temperature in the dark for 30 min. Deionised water was used as blank. The absorbance was read at 765 nm using a visible spectrophotometer (PRIM, Secomam, Alès Gard, France). Total Phenolics (TP) were expressed as Gallic Acid Equivalents (GAE), in micrograms per milliliter of extract which was calculated based on the calibration curve of gallic acid (0.2-25 μg mL^{-1}). The calibration equation of gallic acid was:

$$y = 0.0165x\text{-}0.0003 \ (R^2 = 0.9972) \quad (2)$$

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay: DPPH radical-scavenging activity was done based on a method by Tsai *et al.* (2009). Briefly, DPPH reagent was prepared by dissolving 7.8 mg of DPPH powder in ethanol and top up to 100 mL. Sample extracts (2 mL) at various concentrations were placed in test tubes with 500 μL of DPPH reagent. The reaction mixture was shaken vigorously using vortex and incubated for 30 min at room temperature in the dark. The negative control was prepared without any addition of extract and ethanol was used as blank. The changes in the absorbance of samples were measured at 517 nm in a spectrophotometer. The DPPH radical scavenging activity of the sample was calculated based on the following Eq. 3:

$$\text{Scavenging activity (\%)} = [1 - \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbanc}_{\text{control}}}] \times 100 \quad (3)$$

Ferric reducing antioxidant power (FRAP) assay: Ferric Reducing Antioxidant Power (FRAP) was estimated based on a method described by Biglari *et al.* (2008) with slight modifications. Firstly, the working FRAP reagent was prepared by mixing 2.5 mL of 10 mmol L^{-1} TPTZ solution

in 40 mmol L^{-1} hydrochloric acid with 2.5 mL of 20 mmol L^{-1} $FeCl_3.6H_2O$ and 25 mL of 0.3 mol L^{-1} acetate buffer at pH 3.6. The freshly prepared FRAP reagent was incubated in a water bath (Model WB/OB 7-45, Memmert, Schwabach, Germany) at 37°C prior to use. The FRAP reagent (3 mL) was added into a test tube containing 100 μL of sample, followed by the addition of 300 μL of deionised water. The absorbance was read against reagent blank after 4 min. The FRAP value was expressed as Trolox Equivalent (TE) (in micrograms TE per milhliter of extract) based on the calibration curve of Trolox (5-400 μg mL^{-1}), where the calibration equation was:

$$y = 0.0057x-0.0214 \ (R^2 = 0.9984) \quad (4)$$

Statistical analysis: All experiments were carried out in triplicates and the data were analyzed using Statistical Package for Social Sciences (SPSS) for Windows version 16.0 (IBM, New York, USA). All the data were expressed as Mean±standard deviation. One-way Analysis of Variance (ANOVA) was used to analyze the mean value of data and Tukey's post-hoc multiple comparison tests were carried out to assess for any significant differences among the means. A statistical probability level of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Total phenolics and antioxidant activity on extraction parameters: The extraction yield was calculated, where papaya peel and seed comprised 13.5 and 17.8% of the fruit total weight, respectively (Table 1). This study investigated different parameters of extraction in order to obtain the best extraction method for papaya samples. The extraction yields (antioxidants and phenolics) from papaya samples may be influenced by many factors, such as the types of solvent, solvent-to-water ratio and extraction time (Cacace and Mazza, 2003; Dar and Sharma, 2011).

Solvent type

Papaya peel: In this study, the concentration of extraction solvents was fixed at 100% (v/v) while the extraction time was 2 h. The effect of each extraction parameter was studied using three different antioxidant assays namely, TP, DPPH and FRAP. The TP and antioxidant activities of papaya peel extracted using five different extraction media are shown in Table 2. The methanol extract of papaya peel had the highest TP as compared to other extraction media at all concentrations studied. Although, the acetone extract of papaya peel had the highest scavenging activity (DPPH assay), it was low in TP because most of the hydrophilic phenolic compounds are not solubilised in acetone, as acetone is water miscible (Matkovich and Christian, 1973). This observation is in agreement with Siddiq *et al.* (2005) that 100% acetone extract of *Moringa oleifera* leaves had the lowest antioxidant activity which is due to low polyphenolic content in the extract.

At all extract concentrations studied, papaya peels extracted using acetone showed significantly highest scavenging activity (DPPH assay), while deionised water showed significantly lowest. In

Table 1: Extraction yields of papaya peel and seed before and after drying

	Weight (g)		
Extract	Before drying	After drying	Yield (%, w/w)
Papaya peel	5.98	1.35	13.5
Papaya seed	6.76	1.78	17.8

Extraction yields are expressed as gram (g) and percentage (%) with respect to dry weight of papaya peel and seed

Table 2: Effect of extraction media on total phenolics and antioxidant activities of papaya peel

Extract concentration (μg mL^{-1})	Extraction medium	Total phenolics (μg mL^{-1})	DPPH scavenging activity (%)	FRAP value (μg mL^{-1})
50	dH_2O	0.03±0.03^{e}	5.4±0.3^{d}	4.18±0.23^{e}
	Hexane	0.40±0.22^{b}	22.2±1.1^{b}	7.56±0.37^{a}
	Acetone	0.07±0.06^{e}	49.9±0.5^{a}	4.81±0.48^{d}
	Methanol	1.49±0.13^{a}	14.9±0.5^{c}	5.33±0.12^{c}
	Ethanol	0.54±0.04^{b}	14.0±1.1^{c}	6.87±0.24^{b}
100	dH_2O	0.99±0.07^{c}	11.8±0.5^{e}	4.94±0.12^{e}
	Hexane	0.99±0.07^{c}	43.9±1.1^{b}	8.92±0.13^{b}
	Acetone	0.46±0.03^{d}	56.6±0.5^{a}	6.04±0.21^{d}
	Methanol	2.95±0.05^{a}	29.7±0.8^{c}	7.28±0.24^{c}
	Ethanol	1.25±1.12^{b}	28.1±0.9^{d}	9.78±0.59^{a}
200	dH_2O	2.75±0.06^{e}	14.9±0.8^{e}	6.74±0.28^{d}
	Hexane	1.55±0.13^{d}	65.8±0.7^{b}	12.55±0.28^{a}
	Acetone	1.12±0.11^{e}	75.1±0.5^{a}	10.17±0.58^{c}
	Methanol	5.16±0.17^{a}	48.5±0.6^{d}	9.89±0.45^{c}
	Ethanol	2.99±0.08^{b}	51.1±0.7^{c}	11.73±0.51^{b}
300	dH_2O	4.73±0.10^{b}	21.7±0.8^{e}	7.93±0.36^{e}
	Hexane	2.62±0.23^{d}	79.9±0.4^{b}	13.44±0.84^{a}
	Acetone	2.11±0.19^{e}	81.9±0.3^{a}	11.77±0.55^{b}
	Methanol	5.82±0.25^{a}	64.8±0.6^{d}	12.41±0.30^{b}
	Ethanol	4.11±0.35^{c}	68.6±0.7^{c}	13.44±0.15^{a}
500	dH_2O	8.06±0.10^{b}	-	-
	Hexane	3.32±0.29^{e}	82.5±0.4^{a}	-
	Acetone	4.15±0.16^{d}	-	-
	Methanol	9.39±0.09^{a}	-	-
	Ethanol	5.99±0.18^{c}	71.5±1.9^{b}	-

Values are presented as Mean±SD (n = 3), Values within a column with different superscript lower case letters are significantly different ($p<0.05$), Total phenolics are expressed as gallic acid equivalents in extract, FRAP values are expressed as Trolox eqnivalents in extract

comparison to other extraction media, the hexane extract had the average FRAP values; while at all concentrations, deionised water extracts had significantly lowest FRAP values. At the concentration of 100 μg mL^{-1}, ethanol was the solvent which showed the highest FRAP value, followed by hexane. However, scavenging activity (DPPH assay) of ethanol extracts was significantly higher than that of methanol extracts at both concentrations of 200 and 300 μg mL^{-1}. At an extract concentration of 500 μg mL^{-1}, scavenging activity (DPPH assay) and FRAP values were not able to be calculated for some of the extracts due to the excessively high extract concentrations. Therefore, acetone was selected as the best solvent for extraction of papaya peel.

DPPH assay was commonly used in assessing antioxidant activity of fruits and vegetables due to the high accuracy and simplicity (Tabart *et al*., 2009; Moon and Shibamoto, 2009). The acetone extract, which showed the highest scavenging activity measured by DPPH assay, has the polar index of 5.1, which is similar to methanol that showed the highest TP measured using Folin-Ciocalteu's reagent assay. In other words, this indicated that the type of phenolics extracted using acetone were potentially in hydrophilic and lipophilic forms. As revealed by Roudsari (2007), solvents with intermediate polarity are preferred to be used in extraction of phenolics and antioxidants as compared to those highly polar (deionised water) or non-polar (hexane) solvents.

Table 3: Effect of extraction media on total phenolics and antioxidant activities of papaya seed

Extract concentration ($\mu g\ mL^{-1}$)	Extraction medium	Total phenolics ($\mu g\ mL^{-1}$)	DPPH· scavenging activity (%)	FRAP value ($\mu g\ mL^{-1}$)
50	dH_2O	0.78±0.06^a	2.2±0.6^a	7.69±0.32^a
	Hexane	0.15±0.03^e	2.1±1.1^a	3.97±0.08^e
	Acetone	0.74±0.07^a	1.7±0.4^b	6.39±0.20^b
	Methanol	0.44±0.11^b	2.1±0.7^a	6.60±0.23^b
	Ethanol	0.14±0.00^e	2.8±1.1^a	4.11±0.18^e
100	dH_2O	1.61±0.09^a	13.8±0.9^a	9.17±0.51^a
	Hexane	0.29±0.03^e	1.6±0.4^d	4.08±0.07^e
	Acetone	1.01±0.09^b	3.8±0.5^c	6.91±0.21^c
	Methanol	0.79±0.08^c	3.9±0.2^c	7.63±0.33^b
	Ethanol	0.53±0.03^d	6.1±1.1^b	4.65±0.20^d
200	dH_2O	2.83±0.11^a	28.2±0.9^a	10.95±0.39^a
	Hexane	0.48±0.08^d	1.9±0.8^e	4.11±0.12^e
	Acetone	1.30±0.06^b	5.8±0.8^d	7.89±0.25^c
	Methanol	1.39±0.17^b	7.1±0.4^c	8.45±0.17^b
	Ethanol	0.92±0.08^c	11.1±0.5^b	5.53±0.28^d
300	dH_2O	4.40±0.09^a	32.4±0.7^a	12.47±0.49^a
	Hexane	0.87±0.12^d	1.4±0.4^d	4.14±0.08^e
	Acetone	1.50±0.09^c	8.9±1.4^c	8.78±0.09^c
	Methanol	2.91±0.14^b	8.9±0.9^c	9.95±0.42^b
	Ethanol	1.60±0.14^c	16.7±0.7^b	6.87±0.45^d
500	dH_2O	6.68±0.05^a	35.9±0.2^a	-
	Hexane	1.36±0.07^e	2.6±1.1^e	-
	Acetone	1.88±0.12^d	16.1±0.6^c	-
	Methanol	5.28±0.19^b	12.6±0.4^d	-
	Ethanol	2.31±0.17^c	28.7±0.9^b	-

Values are presented as Mean±SD (n = 3), Values within a column with different superscript lower case letters are significantly different ($p<0.05$), Total phenolics are expressed as gallic acid equivalents in extract, FRAP values are expressed as Trolox eqnivalents in extract

Furthermore, acetone is placed in the class 3 solvents, which has the lowest level of toxicity compared to many other toxic organic solvents (Al-Farsi and Lee, 2008). Hexane as a non-polar solvent showed the highest reducing power measured with FRAP assay. In addition, the solubility of hexane in water is very low and this potentially increases the complexity for the application involving water in the future.

Papaya seed: The results of TP and antioxidant activities of papaya seed are presented in Table 3. For papaya seed, the deionised water extract had the highest TP and antioxidant activity at all extract concentrations, except for scavenging activity at extract concentration of 50 $\mu g\ mL^{-1}$, where no significant difference was found between deionised water and hexane. At a concentration of 500 $\mu g\ mL^{-1}$, the deionised water extract from papaya seed was the most effective DPPH radical scavenger with inhibition of 35.9% as compared to other extracts. Generally, hexane extracts had the lowest TP and antioxidant activities. However, at concentration of 50 $\mu g\ mL^{-1}$, papaya seed extracted using acetone had significantly lowest scavenging activity (DPPH assay) compared to other extraction media.

The hexane extract of papaya peel was the least effective DPPH radical scavenger with inhibition of 2.6% with a significant difference at the sample concentration of 500 μg mL^{-1}, which was almost 14 times lower than the inhibition determined for the deionised water extract. Ethanol extracts were the second most effective DPPH radical scavenger after deionised water extracts at all concentrations except for 50 μg mL^{-1}. Acetone extracts showed lower scavenging activity than methanol extracts at low concentration but increased with increased sample concentration. However, ethanol was a weaker reducing agent (evaluated by Fe^{3+} reduction) as compared to methanol and acetone extracts. With comparison to methanol and acetone extracts, the ethanol extract had significantly lower TP at extract concentrations less than 300 μg mL^{-1}.

Accordingly, deionised water extracts of papaya seed showed the highest scavenging activity and reducing power, followed by methanol extracts. The result obtained has shown that the deionised water extract had the highest TP and antioxidant activities. Thus, deionised water was selected as the best extraction medium. Deionised water which is often used as a universal solvent has the highest polarity index as compared to other solvent used. This indicated that phenolics and other antioxidants extracted from papaya seed are hydrophilic rather than lipophilic. The fact is supported by the result that the hexane extract had the lowest TP and antioxidant activities as compared to other extraction media studied. Besides, deionised water is also cheaper in price and abundantly available.

The finding of this present study complied with the research study reported by Mariod *et al.* (2009) that water appeared to be the most effective extraction medium and hexane as the least effective solvent for the extraction of antioxidant compounds from black cumin seedcake. In the same manner, water was the better extraction medium in extracting flavonoids from tea compared to methanol and acetone (Hayouni *et al.*, 2007). Moreover, water is a good extraction medium in extracting the corn polyphenols instead of using 80% methanol or 70% ethanol (Tabart *et al.*, 2009). In another perspective, Yadav *et al.* (2008) reported that water extract of fenugreek seed had the highest hypoglycemic and anti-hyperglycemic activity in rats among all the extracts studied.

An extraction solvent system is generally selected based on the purpose of extraction, the polarity of interested and undesirable components, the overall cost, safety and availability (Yu *et al.*, 2002). It is reasonable to expect that different solvents would selectively extract different compounds based on their chemical structures, polarities and solubilities (Soon and Chiang, 2012). In addition, variation in antioxidant concentration and scavenging activities may be found in the extract from the same plant materials due to various factors, such as different degree of sun exposure to the plant, environmental factors and others (Dumas *et al.*, 2003). Indeed, the yields of hydrophilic and lipophilic antioxidants are dependable on the polarity of the solvent used (Durling *et al.*, 2007).

Solvent-to-water ratio

Papaya peel and seed: Antioxidant and phenolic compounds of papaya peel were extracted using different percentages of acetone (solvent-to-water ratio), ranging from 10% (v/v) to 90% (v/v). Different percentages of acetone were obtained by addition of different amounts of water, which was able to further improve the extracting efficiency (Yu *et al.*, 2002). A suitable solvent-to-water ratio is able to extract polar to non-polar antioxidant compounds, depending on the amount of polar and non-polar antioxidants from the selected sample. In this study, five different solvent-to-water ratios were used to extract phenolics and other antioxidant compounds from the papaya peel. For

Table 4: Effect of percentage of acetone on total phenolics and antioxidant activities of papaya peel

Extract concentration (μg mL^{-1})	Percentage of acetone	Total phenolics (μg mL^{-1})	DPPH scavenging activity (%)	FRAP value (μg mL^{-1})
50	10%	0.74±0.06^{d}	4.9±0.6^{e}	4.21±0.22^{cd}
	30%	0.77±0.12^{e}	4.5±1.1^{e}	3.93±0.00^{d}
	50%	0.55±0.04^{e}	6.7±0.6^{b}	4.30±0.16^{c}
	70%	1.26±0.03^{b}	6.4±0.8^{b}	4.81±0.26^{b}
	90%	1.77±0.05^{a}	14.9±0.8^{a}	5.47±0.17^{a}
100	10%	1.58±0.04^{c}	13.4±0.6^{b}	5.12±0.24^{c}
	30%	1.40±0.15^{d}	12.5±0.8^{c}	4.71±0.28^{d}
	50%	1.45±0.03^{d}	12.9±0.6^{c}	5.84±0.18^{b}
	70%	2.35±0.04^{b}	14.0±0.6^{b}	6.13±0.36^{b}
	90%	3.59±0.04^{a}	26.0±0.7^{a}	7.38±0.09^{a}
200	10%	2.97±0.04^{c}	22.3±1.2^{c}	6.76±0.51^{d}
	30%	3.04±0.17^{c}	21.0±0.9^{d}	6.37±0.24^{d}
	50%	2.76±0.03^{d}	24.5±0.4^{b}	7.34±0.25^{c}
	70%	4.50±0.06^{b}	22.4±0.4^{c}	8.65±0.37^{b}
	90%	6.79±0.14^{a}	33.4±0.4^{a}	9.66±0.41^{a}
300	10%	4.35±0.07^{d}	27.5±1.0^{e}	8.41±0.13^{d}
	30%	4.48±0.15^{cd}	24.5±1.1^{d}	8.18±0.30^{d}
	50%	4.50±0.05^{c}	29.4±0.6^{b}	9.33±0.17^{c}
	70%	6.81±0.11^{b}	27.4±0.5^{c}	11.08±0.42^{b}
	90%	10.68±0.09^{a}	35.8±0.5^{a}	13.38±0.54^{a}
500	10%	7.37±0.12^{e}		-
	30%	7.43±0.07^{e}	-	-
	50%	7.45±0.03^{e}	-	-
	70%	10.62±0.26^{b}	-	-
	90%	15.62±0.24^{a}	-	-

Values are presented as Mean±SD (n = 3), Values within a column with different superscript lower case letters are significantly different ($p<0.05$), Total phenolics are expressed as gallic acid equivalents in extract, FRAP values are expressed as Trolox eqnivalents in extract

papaya seed, water was chosen as the best extraction medium. Therefore, the determinations of TP and antioxidant activities using different solvent-to-water ratios were not performed.

TP and antioxidant activities of papaya peel extracted by different percentages of acetone are shown in Table 4. The results showed that for DPPH assay, highest extraction capability was recorded at 90% acetone followed by 70% acetone at all extract concentrations. At 50 μg mL^{-1} extract concentrations, both 50 and 70% of acetone used had a scavenging activity (DPPH assay) higher than 10 and 30% acetone used. At the sample concentration of 100 μg mL^{-1}, the scavenging activity of the 50% acetone extract was lower than with 10% acetone.

The highest value of TP was recorded for 90% acetone, followed by 70% acetone and both were significantly different from all other percentages studied. However, 50% acetone used showed the significantly lowest TP at extract concentrations of less than 300 μg mL^{-1} compared to other ratio of acetone used. On the other hand, 30% acetone used had the significantly lowest TP and antioxidant activity, except for the FRAP value at extract concentrations of 200 and 300 μg mL^{-1}. For FRAP assay, the highest value was recorded for 90% acetone, followed by 70% acetone at all extracts concentrations. The 50% acetone used showed moderate FRAP values as compared with 90 and 70% acetone used in all extract concentrations. Significantly lower FRAP values were

obtained for both 10 and 30% acetone used compared to higher percentages of acetone used. Overall, the results showed that 90% acetone used had the significantly highest effect on TP and antioxidant activities. Thus, 90% acetone (v/v) was considered the best solvent-to-water ratio used.

As reported by Hayouni *et al.* (2007), they found that aqueous acetone gave the highest levels of polyphenol in the fruits of *Quercus coccifera* L. (Fagaceae) and *Juniperus phoenicea* L. (Cupressaceae). They also revealed that the variations in the yield of various extracts were highly correlated with the different polarities of various compounds exist in a sample extract. Aqueous acetone was found to be more effective than methanol and water for extraction of total phenolics from black currant leaves (Tabart *et al.*, 2009). Al-Farsi and Lee (2008) also reported that polar antioxidants were well extracted with aqueous acetone. In addition, acetone is useful in dissolving the protein-bound phenolic complexes and therefore, considered as a good solvent to extract phenolics from protein-rich sample. Therefore, an acetone-water mixture is a good solvent in the extraction of less polar phenolics and highly methoxylated aglycone forms of polyphenols (Gonzalex-Montelongo *et al.*, 2010).

Extraction time: Extraction time is one of the crucial factors for extraction of phenolics and antioxidant compounds since these compounds are potentially prone to degradation if exposed to ambient conditions for long duration. Thoo *et al.* (2010) revealed that excess extraction time lead to reduction of phenolic and antioxidant yields. The selection of the best extraction time was based on the highest radical scavenging activities, which depicted by DPPH and supported by TP as well as FRAP assays.

Papaya peel: The TP and antioxidant activities of papaya seed extracted using 90% acetone at five different extraction times (60-300 min) are shown in Table 5. The result showed that 60 min of extraction yielded the highest scavenging activity (DPPH assay) at all extract concentrations (50-300 μg mL^{-1}) except 100 μg mL^{-1}. An extraction time of 120 min had shown the significantly lowest scavenging activity at all sample concentrations, while the highest scavenging activity was found at 240 min extraction at the sample concentration of 100 μg mL^{-1}. As observed, 180-300 min extraction showed moderately high scavenging activity.

At all extract concentrations, TP was found to be the highest at 240 min extraction, while 120 min extraction resulted in significantly lowest TP. The TP was found to have a similar trend for all extract concentrations (50-500 μg mL^{-1}), where an increasing trend was found for TP in the following manner: 120<60<180<300<240 min. For the FRAP assay, 240 min extraction showed the highest FRAP values at extract concentrations of 100 and 300 μg mL^{-1}. Highest FRAP values were also obtained from 300 min extraction at extract concentration of 200 μg mL^{-1} and 180 min extraction at extract concentration of 50 μg mL^{-1}. An extraction time of 120 min had the significantly lowest FRAP values for all extract concentrations (50-300 μg mL^{-1}) except at extract concentration of 100 μg mL^{-1}, no significant difference was found for the FRAP values between 60 and 120 min extraction.

Prolongation of extraction duration potentially increases the loss of phenolics and antioxidants by exposure to light and oxygen. This was in agreement with Durling *et al.* (2007) that phenolic compounds in dried sage (*Salvia officinalis*) become unstable and degraded at long extraction time. As shown in the Table 5, 60 min extraction for Folin-Ciocalteu's reagent and FRAP assays did not appear to be the optimum extraction time. The differences are potentially due to the variation of

Table 5: Effect of extraction time on total phenolics and antioxidant activities of papaya peel

Extract concentration (μg mL^{-1})	Extraction time (min)	Total phenolics (μg mL^{-1})	DPPH scavenging activity (%)	FRAP value (μg mL^{-1})
50	60	1.39±0.03^{b}	17.9±0.7^{a}	5.47±0.17^{a}
	120	1.32±0.03^{c}	12.8±0.6^{b}	4.48±0.34^{b}
	180	1.70±0.03^{a}	17.2±0.4^{a}	5.61±0.13^{a}
	240	1.73±0.03^{a}	17.6±0.9^{a}	5.55±0.53^{a}
	300	1.72±0.00^{a}	17.8±1.2^{a}	5.39±0.43^{a}
100	60	3.04±0.05^{c}	26.8±0.4^{b}	6.41±0.22^{cd}
	120	2.76±0.03^{d}	22.7±0.4^{d}	5.84±0.32^{d}
	180	3.45±0.03^{b}	26.7±0.4^{b}	7.17±0.18^{b}
	240	3.63±0.05^{a}	29.3±0.6^{a}	7.96±0.61^{a}
	300	3.49±0.03^{b}	25.5±0.7^{c}	6.83±0.73^{bc}
200	60	5.86±0.03^{c}	38.5±0.5^{a}	9.62±0.43^{bc}
	120	5.41±0.08^{d}	30.2±0.5^{d}	7.95±0.33^{d}
	180	6.46±0.10^{b}	33.7±0.5^{c}	9.39±0.14^{c}
	240	6.87±0.09^{a}	35.5±0.7^{b}	10.05±0.48^{b}
	300	6.56±0.06^{b}	33.2±0.6^{c}	11.81±0.65^{a}
300	60	8.80±0.13^{d}	41.9±0.5^{a}	12.84±0.39^{ab}
	120	8.22±0.09^{e}	34.9±0.5^{d}	11.45±0.60^{c}
	180	9.83±0.05^{c}	36.9±0.6^{b}	12.58±0.23^{b}
	240	10.44±0.10^{a}	37.6±0.7^{b}	13.44±0.42^{a}
	300	10.00±0.09^{b}	35.9±1.0^{c}	12.86±0.61^{ab}
500	60	14.65±0.05^{d}	-	-
	120	14.04±0.14^{e}	-	-
	180	16.27±0.11^{c}	-	-
	240	16.97±0.10^{a}	-	-
	300	16.54±0.09^{b}	-	-

Values are presented as Mean±SD (n = 3), Values within a column with different superscript lower case letters are significantly different ($p<0.05$), Total phenolics are expressed as gallic acid equivalents in extract, FRAP values are expressed as Trolox eqnivalents in extract

solubility of phenolics and other antioxidants in organic solvent and the interaction of phenolics with other constituents in the sample (Thoo *et al*., 2010). Consequently, the final equilibrium to be achieved between the solute concentration in the papaya peel solid matrix and in the 90% acetone (v/v) solution was differed.

Papaya seed: The results for TP and antioxidant activities of papaya seed extracted using 60-300 min at 5 different extracts concentrations are shown in Table 6. Results from DPPH assay showed that papaya seed extract had the highest scavenging activity for 120 min extraction at all extract concentrations except for 50 μg mL^{-1}. On the other hand, the significantly lowest scavenging activity was recorded for 180 min extraction at extract concentrations more than 50 μg mL^{-1} as compared to other extraction times. Besides, 300 min extraction showed the highest scavenging activity at the sample concentration of 50 μg mL^{-1} but the scavenging activity was reduced when the papaya seed extract concentrations increase. Conversely, 240 min extraction showed higher scavenging activity at extract concentrations more than 50 μg mL^{-1} compared to other extraction time. Moreover, 60 min extract showed moderate scavenging activity at all papaya seed extract concentrations.

Table 6: Effect of extraction time on total phenolics and antioxidant activities of papaya seed

Extract concentration (μg mL^{-1})	Extraction time (min)	Total phenolics (μg mL^{-1})	DPPH scavenging activity (%)	FRAP value (μg mL^{-1})
50	60	0.76±0.03^{a}	4.2±1.1^{c}	5.47±0.17^{a}
	120	0.58±0.03^{b}	9.0±0.8^{b}	5.43±0.09^{a}
	180	0.31±0.03^{e}	2.4±0.7^{d}	4.40±0.09^{e}
	240	0.43±0.03^{d}	1.0±0.6^{e}	4.94±0.17^{b}
	300	0.47±0.03^{c}	10.4±0.8^{a}	4.38±0.13^{c}
100	60	1.51±0.03^{a}	11.3±0.8^{c}	6.44±0.21^{a}
	120	1.47±0.05^{a}	21.5±1.4^{a}	6.25±0.08^{b}
	180	0.82±0.03^{d}	5.7±0.5^{d}	5.53±0.06^{d}
	240	1.01±0.05^{c}	10.1±0.9^{c}	5.90±0.08^{c}
	300	1.25±0.03^{b}	14.8±1.6^{b}	4.83±0.06^{e}
200	60	2.75±0.09^{a}	21.5±0.6^{b}	8.96±0.58^{a}
	120	2.79±0.06^{a}	33.0±1.3^{a}	8.94±0.13^{a}
	180	1.87±0.07^{d}	15.4±0.4^{c}	7.50±0.09^{e}
	240	2.22±0.05^{c}	20.6±0.8^{b}	8.04±0.13^{b}
	300	2.54±0.03^{b}	32.6±1.1^{a}	7.07±0.31^{d}
300	60	4.02±0.11^{b}	31.9±0.9^{b}	10.32±0.45^{c}
	120	4.35±0.08^{a}	37.4±1.4^{a}	12.41±0.15^{a}
	180	2.68±0.08^{e}	21.8±0.4^{d}	8.86±0.06^{e}
	240	3.28±0.06^{d}	29.1±0.6^{c}	10.95±0.34^{b}
	300	3.71±0.07^{c}	36.3±0.9^{a}	9.41±0.36^{d}
500	60	6.39±0.08^{b}	37.2±1.4^{c}	-
	120	7.06±0.12^{a}	42.6±1.7^{a}	-
	180	4.60±0.04^{e}	28.8±0.7^{d}	-
	240	5.59±0.06^{d}	36.1±1.3^{c}	-
	300	6.07±0.04^{c}	40.5±0.7^{b}	-

Values are presented as Mean±SD (n = 3), Values within a column with different superscript lower case letters are significantly different ($p<0.05$), Total phenolics are expressed as gallic acid equivalents in extract, FRAP values are expressed as Trolox eqnivalents in extract

The papaya seed extracts had the highest TP was recorded for 120 min extraction at all sample concentrations except for 50 μg mL^{-1}. On the other hand, significantly lowest TP was found for 180 min extraction for all extract concentrations. Nonetheless, 60 min extraction showed the high TP at 50 μg mL^{-1}. Besides, moderate levels of TP were found for 240 and 300 min extraction at all concentrations of papaya extract. For the FRAP assay, the highest FRAP value was recorded for 120 min extraction at extract concentrations less than 300 μg mL^{-1}. Significantly lower FRAP values were found for 180 min extraction at 100-300 μg mL^{-1} extract concentration of papaya seed. Generally, the other extraction times had moderate FRAP values for papaya seed at extract concentrations of 50-300 μg mL^{-1}.

In this study, 120 min extraction generally showed the highest effect on TP and antioxidant activities. Therefore, 120 min extraction was considered as economic and practical, which was also chosen as the best extraction time for the sample of papaya seed. Conversely, 180 min appeared to be the least optimum extraction time for TP and antioxidant activities. An increase in extraction time might give rise to possible degradation (Garcia-Salas *et al*., 2010). However, a short extraction time might yield a small amount of phenolic compounds.

Each single phenolic compound has a different kinetic rate to dissolve in extraction solvent and hence caused the variation of extraction time (Vasco *et al*., 2008). Variation in the extraction time

for the papaya seed extract is highly due to the relation with variety degree of phenolic polymerization, solubility of phenolics, types of phenolic present and the interaction between phenolics and other constituents in a particular extract (Thoo *et al.*, 2010). These factors had lead to variation in extraction time in order to reach equilibrium between solid matrix (papaya seed) and deionised water used. A similar study has been reported by Arora *et al.* (2011) that the DPPH scavenging effect in methanolic extract of *Cucumis melo* seed (at extract concentrations of 50, 100, 200 and 300 μg mL^{-1}) were 10 times higher than the values found our *Carica papaya* seed methanol extract.

CONCLUSION

The best extraction conditions for *Carica papaya* peel were 90% acetone and 60 min extraction. These conditions have yielded TP (expressed as GAE) ranged between 1.39 and 14.65 μg mL^{-1}. The highest antioxidant capacities measured using DPPH assay were between 17.9 and 41.9%; and highest FRAP values (expressed as TE) were 5.47 and 12.84 μg mL^{-1}. While the best extraction conditions for *Carica papaya* seed were deionised water and 120 min extraction. Papaya seed extract had TP ranged between 0.58 and 7.06 μg mL^{-1}. The highest DPPH radical scavenging activity were between 9.0 and 42.6%, while highest FRAP value were between 5.43 and 12.41 μg mL^{-1}. Based on these extraction conditions, maximum extraction yields could be obtained for future studies of *Carica papaya* wastes (peel and seed). The best extraction parameters studied will be useful for nutraceutical development.

REFERENCES

Adebiyi, A., P.G. Adaikan and R.N.V. Prasad, 2003. Tocolytic and toxic activity of papaya seed extract on isolated rat uterus. Life Sci., 74: 581-592.

Aghel, N., Z. Ramezani and S. Beiranvand, 2008. Hesperidin from *Citrus sinensis* cultivated in Dezful, Iran. Pak. J. Biol. Sci., 11: 2451-2453.

Al-Farsi, A.M. and C.Y. Lee, 2008. Optimization of phenolics and dietary fiber extraction from date seeds. Food Chem., 108: 977-985.

Anuar, N.S., S.S. Zahari., I.A. Taib and M.T. Rahman, 2008. Effect of green and ripe *Carica* papaya epicarp extracts on wound healing and during pregnancy. Food Chem. Toxicol., 46: 2384-2389.

Arora R., M. Kaur and N.S. Gill, 2011. Antioxidant activity and pharmacological evaluation of *Cucumis melo* var. agrestis methanolic seed extract. Res. J. Phytochem., 5: 146-155.

Biglari, F., A.F.M. AlKarkhi and M.E. Azhar, 2008. Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. Food Chem., 107: 1636-1641.

Cacace, J.E. and G. Mazza, 2003. Optimization of extraction of anthocyanins from black currants with aqueous ethanol. J. Food Sci., 68: 240-248.

Canini, A., D. Alesiani, G.D. Arcangelo and P. Tagliatesta, 2007. Gas chromatography mass spectrometry analysis of phenolic compounds from *Carica papaya* L. leaf. J. Food Compos. Anal., 20: 584-590.

Chipurura, B. and M. Muchuweti, 2010. Effect of irradiation and high pressure processing technologies on the bioactive compounds and antioxidant capacities of vegetables. Asian J. Clin. Nutr., 2: 190-199.

Dar, B.N. and S. Sharma, 2011. Total phenolic content of cereal brans using conventional and microwave assisted extraction. Am. J. Food Technol., 6: 1045-1053.

Dubost, N.J., B.X. Ou and R.B. Beelman, 2007. Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. Food Chem., 105: 727-735.

Dumas, Y., M. Dadomo, G. Di Lucca and P. Grolier, 2003. Effects of environmental factors and agricultural techniques on antioxidantcontent of tomatoes. J. Sci. Food Agric., 83: 369-382.

Durling, N.E., O.J. Catchpole, J.B. Grey, R.F. Webby, K.A. Mitchell, L.Y. Foo and N.B. Perry, 2007. Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol-water mixtures. Food Chem., 101: 1417-1424.

Everette, J.D. and S. Islam, 2012. Effect of extraction procedures, genotypes and screening methods to measure the antioxidant potential and phenolic content of orange-fleshed sweetpotatoes (*Ipomoea batatas* L.). Am. J. Food Technol., 7: 50-61.

Ferreira, I.C.F.R., P. Baptista, M. Vilas-Boas and L. Barros, 2007. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. Food Chem., 100: 1511-1516.

Garcia-Salas, P., A. Morales-Soto, A. Segura-Carretero and A. Fernandez-Gutierrez, 2010. Phenolic-compound-extraction systems for fruit and vegetable samples: Review. Molecules, 15: 8813-8826.

Gill, N.S., R. Arora and S.R. Kumar, 2011. Evaluation of antoxidant, anti-inflammatory and analgesic potential of the *Luffa acutangula* Roxb. var. amara. Res. J. Phytochem., 5: 201-208.

Gonzalex-Montelongo, R., M.G. Lobo and M. Gonzalez, 2010. The effect of extraction temperature, time and number of steps on the antioxidant capacity of methanolic banana peel extracts. Separ. Purif. Technol., 71: 347-355.

Hayouni, E.A., M. Abedrabba, M. Bouix and M. Hamdi, 2007. The effects of solvents and extraction method on the phenolic contents and biological activities *in vitro* of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. Food Chem., 105: 1126-1134.

Kermanshai, R., B.E. McCarry., J. Rosenfeld., P.S. Summers., E.A. Weretilnyk and G.J. Sorger, 2001. Benzyl isothiocyanate is the chief or sole anthelmintic in papaya seed extracts. Phytochem., 57: 427-435.

Luthria, D.L., 2008. Influence of experimental conditions on the extraction of phenolic compounds from parsley (*Petroselinium crispum*) flakes using a pressurized liquid extractor. Food Chem., 107: 745-752.

Mariod, A.A., R.M. Ibrahim., M. Ismail and N. Ismail, 2009. Antioxidant activity and phenolic content of phenolic rich fractions obtained from black cumin (*Nigella sativa*) seedcake. Food Chem., 116: 306-312.

Matkovich, C.E. and G.E. Christian, 1973. Salting-out of acetone from water-basis of a new solvent extraction system. Anal. Chem., 45: 1915-1921.

Moon, J.-K. and T. Shibamoto, 2009. Antioxidant assays for plant and food components. J. Agric. Food Chem., 57: 1655-1666.

Muchuweti, M., E. Kativu, C.H. Mupure, C. Chidewe, A.R. Ndhlala and M.A.N. Benhura, 2007. Phenolic composition and antioxidant properties of some spices. Am. J. Food Technol., 2: 414-420.

Roudsari, M.H., 2007. Subcritical water extraction of antioxidant compounds from canola meal. M.Sc. Thesis, University of Saskatchewan, Saskatoon.

Siddiq, A., F. Anwar, M. Manzoor and A. Fatima, 2005. Antioxidant activity of different solvent extracts of *Moringa oleifera* leaves under accelerated storage of sunflower oil. Asian J. Plant Sci., 4: 630-635.

Soon, L.K. and L.K. Chiang, 2012. Influence of different extraction solvents on lipophilic extractives of *Acacia* hybrid in different wood portions. Asian J. Applied Sci., 5: 107-116.

Tabart, J., C. Keversa, J. Pincemail, J.O. Defraigne and J. Dommesa, 2009. Comparative antioxidant capacities of phenolic compounds measured by various tests. Food Chem., 113: 1226-1233.

Thomas, G.E., H.G. Rodolfo, M.D. Juan, S.F. Georgina, C.G. Luis, R.B. Ingrid and G.T. Santiago, 2009. Proteolytic activity in enzymetic extracts from *Carica* papaya L. cv. Maradol harvest by-products. Process Biochem., 44: 77-82.

Thoo, Y.Y., S.K. Ho, J.Y. Liang, C.W. Ho and C.P. Tan, 2010. Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (*Morinda citrifolia*). Food Chem., 120: 290-295.

Tsai, S.Y., S.J. Huang, S.H. Lo, T.P. Wu, P.Y. Lian and J.L. Mau, 2009. Flavour components and antioxidant properties of several cultivated mushrooms. Food Chem., 113: 578-584.

Vasco, C., J. Ruales and A. Kamal-Eldin, 2008. Total phenolic compounds and antioxidant capacities of major fruits from ecuador. Food Chem., 111: 816-823.

Wang, J., B. Sun, Y. Cao, Y. Tian and X. Li, 2008. Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. Food Chem., 106: 804-810.

Yadav, M., R. Tomar, GBKS. Prasad, S. Jain and H. Yadav, 2008. Complementary hypoglycemic and anti-hyperglycemic activity of various extracts of fenugreek seeds in rats. Asian J. Biochem., 3: 182-187.

Yoganandam, G.P., K. Ilango, K. Sunil and A. Elumalai, 2010. *In vitro* antioxidant activity of *Luffa cylindrical* seed oil. J. Global Pharma. Technol., 2: 93-97.

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