academicJournals

Vol. 7(47), pp. 3354-3359, 10 December, 2013
DOI: 10.5897/JMPR2013.5116
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

*Full Length Research Paper*

# Effect of extraction solvents on the phenolic content and antioxidant properties of two papaya cultivars

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Accepted 25 November, 2013

**In this study, three types of solvent extracts from two cultivars of papaya fruit (Hongkong and Eksotika) were used to examine the effects of extraction solvent on total phenolics content (TPC), total flavonoids content (TFC) and antioxidant activity by ferric reducing antioxidant power (FRAP), 1,1-diphenyl-2-picryl hydrazyl radical scavenging (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were determined spectrophotometrically. Results showed that extraction solvent had significant effects on TPC, TFC, and antioxidant activity of methanol and acetone extract. The highest content of TPC, TFC and antioxidant activity (FRAP, DPPH and ABTS) were found in 50% methanol and 50% acetone extracts. The TPC varied for both cultivars (Hongkong and Eksotika) from 16.35 to 46.65, 67.50 to 23.38 mg gallic acid/100 g fresh weight, and TFC were between 19.40 and 36.17, 39.81 and 21.04 mg quercetin/100 g fresh weight and antioxidant activity (FRAP from 124.84 to 90.23, 190.59 to 159.98 mg Trolox equivalents/100 g fresh weight), DPPH were between 47.82 and 28.72%, 74.56 and 38.57%) and (ABTS from 57.34 to 31.49% and 69.06 to 34.84%), respectively. The largest amount of TPC and TFC which leads to more effective radical scavenging effect was shown by 50% methanol extract. Moreover, amount of phenolic compounds and antioxidant activities increased in methanol and acetone extract. Therefore, a positive correlation occurred between antioxidant activity and phenolics compound. Methanol 50% and acetone 50% solvent showed the greatest capability in extracting antioxidants and inhibiting the free radicals produced. It was concluded that extraction solvent play important roles on the phenolics compounds and their antioxidant activity of papaya fruit extract.**

**Key words:** Papaya, extraction solvent, antioxidant activity, phenolic compound.

## INTRODUCTION

Papaya fruit (*Carica papaya* L.) belongs to the family of Caricaceae, is widespread throughout the tropical and subtropical areas. Papaya as in many fruits and vegetables is rich in antioxidant compounds. The fruit contains a high level of vitamin C, carotenoids such as *B*-carotene and lycopene. Peterson et al. (1982) reported that papaya fruit is a good natural source of ascorbic acid (vitamin C) and B-carotene (provitamin A). A study by Ralf et al. (2011) found that the most plentiful antioxidant of papaya are carotenoids of B-carotene, *β*-cryptoxanthin, lutein and lycopene and vitamin C was also reported at high level in papaya fruits. Antioxidant compounds play an important role in our body due to the positive effect on human health. Consumption of foods containing bioactive compound with potential antioxidant properties can decrease the risk of human disease such as cancer and heart diseases (Temple, 2000). Many studies have been made to isolate, characterize and extract antioxidant from natural plant sources. Plant phenolic compounds, and their secondary metabolites flavonoids and proanthocyanidins, have been frequently reported as the active bioactive components associated with antioxidant

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properties and health benefits (Pierson et al., 2012). Several studies revealed that phenolic compounds content differed with solvents polarities. For example, pure methanol was used for the extraction of tea polyphenols (Yao et al., 2006) and 50% acetone for extraction of wheat total phenolics (Zhou and Yu, 2004) which were found to be more effective than water. Extraction is the first stage to separate antioxidant compounds from plant materials. For the preparation of plant extracts, like those of papaya, water is certainly the safest and the most environmentally friendly and accessible solvent (Vuong et al., 2011). It is also significantly less expensive than organic solvents, which have been traditionally employed for plant bioactive extractions. Currently, no published information exists relating to the optimized use of water for isolating and analyzing the potential bioactive constituents of papaya fruit. The aim of this study were to examine the effect of different extracting solvents with different polarity on phenolics compound and antioxidant activity (Ferric reducing antioxidant power [FRAP], 2,2-diphenyl-1-picrylhydrazyl [DPPH] and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid [ABTS]) of two varieties of papaya (Hongkong and Eksotika).

## MATERIALS AND METHODS

Papaya (*C. papaya* L. cv. Hongkong and Eksotika) fruits at the mature-green stage of ripening (green with 75% yellow) were collected from Pusat Flora Cheras, Jabatan Pertanian, and Hulu Langat Semenyih in Selangor, Malaysia. The fruits were selected to ensure uniformity in size (800 to 1000 g) and color as well as to ensure freedom from diseases and infection. The selected fruits were transferred on the same day to the University Kebangsaan Malaysia Food Laboratory, Bangi.

### Extraction process

The papaya samples were peeled, cut into 1 cm slices, and placed in a food processor to form uniform slurries using a Waring blender model HGBZWTS3. The fruit samples were prepared fresh for the preservation of the extracted antioxidant compounds. For this process, approximately 1 g of papaya slurries was weighed in universal bottles, into which 10 ml solvent was added. The solvents include pure acetone, ethanol, and methanol, as well as their respective aqueous solutions at 50 and 70% concentrations. The samples (papaya slurries with solvents) were then homogenized (T 250, IKA, Germany) at 24,000 rpm for 1 min. All extracted samples were centrifuged using a tabletop centrifuge (MLX 210, Thermo-line, China) at 4750 g for 10 min. The supernatants were collected for further analysis.

### Content of antioxidant compounds

#### *Total phenol content (TPC)*

Antioxidant activity was determined using TPC based on the method of Musa et al. (2011). Approximately, 0.4 ml distilled water and 0.5 ml diluted Folin–Ciocalteu reagent were added to 100 µl papaya extracts. The samples (papaya extracts with Folin–Ciocalteu reagent) were set aside for 5 min before 1 ml 7.5% sodium carbonate (w/v) was added. The absorbances were taken at 765 nm wavelength using a spectrophotometer after 2 h. The calibration curve of gallic acid (GA) was used for the estimation of sample activity capacity. The result was recorded in terms of mg of GA equivalents per 100 g of fresh sample (mg GA/100 g of FW).

#### *Total flavonoid content (TFC)*

The TFC was determined by the colorimetric method as described by Abu Bakar et al. (2009). A total of 0.5 ml of the extract was mixed with 2.25 ml of distilled water in a test tube, followed by the addition of 0.15 ml of 5% (w/v) $NaNO_2$ solution. After 6 min, 0.3 ml of a 10% $AlCl_3 \cdot 6H_2O$ solution was added, and the reaction was allowed to stand for another 5 min before 1.0 ml of 1 M NaOH was added. The mixture was mixed well by vortexing, and the absorbance was measured immediately at 510 nm using a spectrophotometer (Epoch, Biotek, USA). The results were expressed as milligrams of quercetin equivalents (QE) per 100 g of fresh sample (mg QE/100 g of FW).

### Determination of antioxidant activity

#### *Ferric reducing antioxidant power (FRAP)*

Musa et al. (2011) proposed the idea of determining antioxidant activity through FRAP. First, 300 mM acetate buffer FRAP reagent was prepared fresh as follows: pH 3.6 (3.1 g sodium acetate trihydrate plus 16 ml glacial acid made up to 1:1 with distilled water); 10 mM 2,4,6-tris (2-pyridyl)-*s*-triazine (TPTZ) in 40 mM HCl; and 20 mM FeCl3·6H2O in the ratio of 10:1:1 to provide the working reagent. In addition, approximately 1 ml FRAP reagent was added to 100 µl papaya extracts, and the absorbances were taken at 595 nm wavelength using a spectrophotometer after 30 min. The calibration curve of Trolox was established to approximate sample activity capacity. The result was recorded as mg of Trolox equivalents (TEs) per 100 g of fresh sample (mg TE/100 g of FW).

### DPPH radical scavenging activity

Based on the method of Musa et al. (2011), the antioxidant activity was assessed using a DPPH scavenging system. The stock solution was obtained by dissolving 40 mg DPPH in 100 ml methanol, which was stored at -20°C until further use. Approximately 350 ml stock solution was mixed with 350 ml methanol to obtain the absorbance of 0.70±0.01 unit at 516 nm wavelength by using a spectrophotometer (Epoch, Biotek, USA). In the dark, approximately 100 µl papaya extracts with 1 ml prepared methanolic DPPH solution was stored overnight for scavenging reaction. The percentage of DPPH scavenging activity was determined based on the following equation:

$$\text{DPPH scavenging activity (\%)} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

where A is the absorbance.

#### *ABTS assay*

The ABTS radical cation was generated by the interaction of ABTS (250 µM) and $K_2S_2O_8$ (40 µM). After the addition of 990 µl of ABTS solution to 10 ml of fruit extract, the absorbance at 734 nm was monitored. The percentage decrease of the absorbance was calculated and plotted as a function of the concentration of the extracts and Trolox for the standard reference data (Özgen et al., 2006). The following formula was used:

**Table 1.** Mean (n = 3) total phenol and total flavonoids content of two papaya cultivars (Hongkong and Eksotika).

| Solvent | TPC mg/100 g FW | | TFC mg/100 g FW | |
|---|---|---|---|---|
| | Hongkong | Eksotika | Hongkong | Eksotika |
| **Acetone** | | | | |
| 50% | 42.10 ± 1.60$^{Bb}$ | 56.08 ± 2.45$^{Ac}$ | 33.38 ± 0.16$^{Bb}$ | 35.33 ± 0.49$^{Ab}$ |
| 70% | 38.50 ± 2.56$^{Bc}$ | 44.81 ± 2.13$^{Ad}$ | 28.36 ± 0.54$^{Bc}$ | 30.24 ± 0.67$^{Ac}$ |
| 100% | 20.80 ± 1.17$^{Be}$ | 28.01 ± 1.81$^{Af}$ | 19.40 ± 0.72$^{Be}$ | 24.49 ± 0.47$^{Ad}$ |
| **Ethanol** | | | | |
| 50% | 41.18 ± 2.18$^{Bb}$ | 54.95 ± 1.63$^{Ac}$ | 32.46 ± 1.00$^{Bb}$ | 34.40 ± 0.96$^{Ab}$ |
| 70% | 35.09 ± 2.13$^{Bd}$ | 40.50 ± 2.18$^{Ae}$ | 29.60 ± 0.93$^{Ac}$ | 31.06 ± 0.54$^{Ac}$ |
| 100% | 19.07 ± 1.37$^{Be}$ | 27.48 ± 1.23$^{Af}$ | 23.42 ± 1.42$^{Ad}$ | 24.11 ± 0.71$^{Ad}$ |
| **Methanol** | | | | |
| 50% | 46.65 ± 1.45$^{Ba}$ | 67.50 ± 2.28$^{Aa}$ | 36.17 ± 0.62$^{Ba}$ | 39.81 ± 0.93$^{Aa}$ |
| 70% | 45.50 ± 1.72$^{Ba}$ | 65.62 ± 0.56$^{Ab}$ | 35.07 ± 0.71$^{Ba}$ | 38.48 ± 0.49$^{Aa}$ |
| 100% | 21.67 ± 2.68$^{Be}$ | 26.72 ± 1.92$^{Af}$ | 24.04 ± 0.67$^{Ad}$ | 25.58 ± 0.54$^{Ad}$ |
| **Water** | 16.35 ± 2.35$^{Bf}$ | 23.38 ± 2.93$^{Ag}$ | 20.32 ± 0.79$^{Ae}$ | 21.04 ± 0.63$^{Ae}$ |

$^{A-B}$Different letters within the same row indicate significant differences (P<0.05). $^{a-f}$Different letters within the same column indicate significant differences (P<0.05).

Percentage of reduction power = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$

where A is the absorbance.

#### Statistical analysis

Data were expressed as the means of three independent experiments. Statistical comparisons of the results were performed by one-way analysis of variance (ANOVA) using SPSS ver.19. Significant differences (P<0.05) among the solvent extraction were analyzed by Duncan 'triplicates range test (Bryman and Cramer, 2012).

## RESULTS AND DISCUSSION

### Content of antioxidant compounds

#### *TPC and TFC*

Table 1 showed significant difference (p<0.05) in the total phenolic and total flavonoids of the two papaya fruit varieties. Eksotika variety gave the highest phenolic content (67.50 mg/GAE/100 g DW) when compared with Hongkong variety. The content of phenolic compounds in different solvent extracts (acetone, ethanol and methanol) of the fruits in the two varieties of *C. papaya* is shown in Table 1. With increase in solvent polarity, TP and TF content increased in extract. High content of TP (7.05 mg/g DW) and TF (39.06 mg/g DW) were obtained from methanol extract in Eksotika. After methanol 50%, acetone 50% had high content of phenolic compounds in extract. In both varieties, the phenolic contents and total flavonoid content in the Eksotika were more than the Hongkong. As found in this study, in a mixture with no aqueous content, the extraction efficiency was low and negative. It is clear that the addition of some amount of water enhance the extraction efficiency. One possible reason for the increased efficiency with the presence of some water might be due to the increase in bulge of plant material by water, which increased the contact surface area between the plant matrix and the solvent (Rostagno, 2003). Our results is similar to that reported by Ali et al. (2011), where methanol solvent was most effective in extracting phenolic components from ginger fruit. Turkmen et al. (2006) reported that solvent with different polarity had significant effect on phenolics compound and antioxidant activity in higher content in more polar solvents (Siddhuraju et al., 2003; Sultana et al., 2007). The phenolics compounds often associated with other biomolecules (polysaccharides, proteins, terpenes, chlorophyll, inorganic compounds, etc) and solvent must be found suitable for the extraction. Research conducted by Musa et al. (2011) confirmed the ineffectiveness of acetone, methanol and water for the extraction of total phenols of grapes seeds (*Vitis vinifera*).

### Antioxidant activity

Frequently used solvents for antioxidant compound extraction (from fresh fruits/vegetables at different concentrations) include acetone, ethanol, methanol, propanol, and ethyl acetate (Mahattanatawee et al., 2006; Alothman et al., 2009). The solubility of antioxidant

**Table 2.** Mean (n = 3) Antioxidant activity (FRAP, DPPH and ABTS) of two papaya cultivars (Hongkong and Eksotika.

| Solvent | FRAP mg/100 g FW | | DPPH % | | ABTS% | |
|---|---|---|---|---|---|---|
| | Hongkong | Eksotika | Hongkong | Eksotika | Hongkong | Eksotika |
| **Acetone** | | | | | | |
| 50% | $124.84 \pm 1.82^{Ba}$ | $190.59 \pm 1.97^{Aa}$ | $44.75 \pm 1.49^{Bb}$ | $72.37 \pm 1.30^{Ab}$ | $57.34 \pm 1.14^{Ba}$ | $69.06 \pm 1.14^{Aa}$ |
| 70% | $115.90 \pm 1.97^{Bc}$ | $181.84 \pm 1.30^{Ac}$ | $41.13 \pm 1.59^{Bc}$ | $69.78 \pm 1.05^{Ac}$ | $52.65 \pm 1.41^{Bc}$ | $60.62 \pm 1.41^{Ac}$ |
| 100% | $97.05 \pm 3.15^{Be}$ | $171.14 \pm 1.52^{Ae}$ | $32.93 \pm 2.10^{Be}$ | $53.34 \pm 3.07^{Ae}$ | $35.77 \pm 0.81^{Be}$ | $41.40 \pm 0.81^{Ae}$ |
| **Ethanol** | | | | | | |
| 50% | $114.83 \pm 1.56^{Bc}$ | $174.56 \pm 1.67^{Ad}$ | $38.69 \pm 1.61^{Bd}$ | $70.54 \pm 0.79^{Abc}$ | $48.43 \pm 1.41^{Bd}$ | $56.40 \pm 1.41^{Ad}$ |
| 70% | $125.96 \pm 1.24^{Ba}$ | $191.33 \pm 2.26^{Aa}$ | $37.38 \pm 1.56^{Bd}$ | $64.55 \pm 1.22^{Ad}$ | $47.96 \pm 2.15^{Bd}$ | $55.12 \pm 2.15^{Ad}$ |
| 100% | $98.01 \pm 0.78^{Be}$ | $161.35 \pm 1.35^{Af}$ | $29.73 \pm 0.56^{Bf}$ | $54.81 \pm 1.44^{Ae}$ | $35.30 \pm 0.81^{Be}$ | $42.80 \pm 0.81^{Ae}$ |
| **Methanol** | | | | | | |
| 50% | $120.95 \pm 2.09^{Bb}$ | $187.69 \pm 1.87^{Ab}$ | $47.82 \pm 0.90^{Ba}$ | $74.56 \pm 0.67^{Aa}$ | $54.06 \pm 0.96^{Bb}$ | $66.71 \pm 0.96^{Ab}$ |
| 70% | $116.33 \pm 2.21^{Bc}$ | $182.51 \pm 1.22^{Ac}$ | $42.90 \pm 1.71^{Bc}$ | $71.74 \pm 1.29^{Ab}$ | $51.24 \pm 1.38^{Bc}$ | $65.78 \pm 1.38^{Ab}$ |
| 100% | $102.53 \pm 0.91^{Bd}$ | $173.49 \pm 1.88^{Ad}$ | $33.31 \pm 1.49^{Be}$ | $54.64 \pm 1.42^{Ae}$ | $36.71 \pm 0.81^{Be}$ | $41.87 \pm 0.81^{Ae}$ |
| **Water** | $90.23 \pm 2.83^{Bf}$ | $159.98 \pm 2.32^{Af}$ | $28.72 \pm 0.97^{Bf}$ | $38.57 \pm 1.90^{Af}$ | $31.49 \pm 0.54^{Bf}$ | $34.84 \pm 0.54^{Af}$ |

$^{A-B}$ Different letters within the same row indicate significant differences ($P<0.05$). $^{a-f}$Different letters within the same column indicate significant differences ($P<0.05$).

compounds in solvent was found to have a significant effect on the recovery of compounds at the time of extraction. Thus, the polarity of solvents has an indirect function in the extraction process, because it can raise the solubility of antioxidant compounds (Alothman et al., 2009). It was impossible to develop a standard solvent that was suitable for the all kinds of antioxidant compounds extraction from plants. Thus, the screening process is important to identify the best solvent for a specific extraction procedure and thus complete the optimal antioxidant task for a certain sample.

## Ferric reducing antioxidant power (FRAP)

For measurement of the reductive ability, the $Fe^{3+}$- $Fe^{2+}$ transformations in the presence of papaya extracts sample was investigated. Table 2 shows FRAP values for two different cultivars (Hongkong and Eksotika). The result ranged from 90.23 to 124.84 mg /100 g FW in Hongkong, 190.59 to 159.98 mg/100 g FW. Significant differences ($P<0.05$) in FRAP values were found between the different fruit variety. Both ethanol 70% and acetone 50% were the best solvent for finding extracts with higher antioxidant activity. The FRAP value obtained by ethanol 70% was higher significantly ($P<0.05$) than the extract obtained by acetone and methanol 70%. However, for FRAP values sample extracted by ethanol 70% were not significantly ($P<0.05$) different from acetone 50%, while both were significantly ($P<0.05$) higher than methanol for both cultivars (Hongkong and Eksotika) of papaya fruit. When comparing the results from this study with other study, values from different sources seriously differ. The FRAP mean value in this study showed that both cultivars (Hongkong and Eksotika) fruits were higher than that of Lim et al. (2007) for fruits 106 ± 28 mg TE/100 g FW. The better extraction power of aqueous solvent indicates that the mixing of a non-polar solvent with water may increase the polarity index of solvents, thereby consequently enhancing the extraction power of a certain solvent. Our findings are consistent with those of Musa et al. (2011), who found that the increase in polarity of a solvent (up to 50% water) enhances the solubility of antioxidant compounds.

## DPPH radical scavenging activity

Table 2 shows free radical scavenging activity values for two different varieties (Hongkong and Eksotika) of papaya fruits. The results showed that Eksotika variety is having significantly ($P<0.05$) higher scavenging activity compared to Hongkong variety. The results in Table 2 showed that antioxidant activity were sensitive to extraction solvents; generally acetone gave the highest extraction recovery. DPPH values of papaya fruit in both cultivar (Hongkong and Eksotika) extracted decrease with increase in the organic solvent concentration, until the concentration reached 100%. Aqueous organic solvent were found to give the highest values. Methanol 50% was the best solvent for obtaining extracts with high antioxidant activates in both cultivars of papaya fruit followed significantly ($p>0.05$) with acetone 50%. The results are higher than those of Grant et al. (2009), where the DPPH scavenging percentages for papaya ranged from 19.21 to 33.63%, despite the use of the same types of solvents (water and methanol). The difference in findings might possibly be attributed to the different extraction methods and solvents used (Uma et al., 2010). The different results obtained from the previous studies

**Table 3.** Correlation coefficients of antioxidants activities of different papaya cultivars.

| Correlation coefficient ($R^2$) | FRAP | DPPH | ABTS |
|---|---|---|---|
| **Hongkong** | | | |
| TPC | 0.89 | 0.92 | 0.96 |
| TFC | 0.87 | 0.85 | 0.91 |
| | | | |
| **Eksotika** | | | |
| TPC | 0.72 | 0.86 | 0.94 |
| TFC | 0.79 | 0.89 | 0.94 |

may be attributed to different cultivars, growing conditions, maturity stage at harvest, or the storage conditions and time elapsed before the fruits were analyzed. Sample preparation method may also influence the results.

### ABTS radical-scavenging activity assay

This method is widely used to evaluate antioxidant activities within a relatively short time compared with other methods. Also, the ABTS radical has been used to confirm results obtained with DPPH, because both possess similar antioxidant mechanisms. Significant differences ($P<0.05$) in ABTS values were found between the different fruit variety. As shown in Table 2, significant difference ($p < 0.05$) in ABTS was observed. Eksotika variety gave the highest ABTS (69.06) when compared with Hongkong (57.34). In this study, different solvents such as acetone, ethanol and methanol have been used for the extraction of antioxidant activity from different papaya varieties. The effects of these solvents in extracting antioxidant activity are shown in Table 2. This indicated the possible influence of extracting solvent on antioxidant activity. Among all the papaya fruits extracts, 50% acetone was found to be the most efficient solvent for extracting ABTS when compared with all other solvent systems used, the level of these ABTS ranged from Eksotika variety (69.06 to 34.84%) and Hongkong variety (57.34 to 31.49%). However, ethanol is the least effective solvent for extracting their antioxidant compounds from papaya fruit for both in both cultivar (Hongkong and Eksotika). Comparing antioxidant activity from this study and other published data is difficult due to the fact that content of antioxidant compounds can be influenced by extracting solvent, cultivar and location. Grant et al. (2009) reported that ABTS radical of papaya fruit was 25.6% unripe and 34.4% ripe.

### Correlation of TPC and TFC with FRAP, DPPH, and ABTS assays

A correlation analysis among phenolic compounds (TPC and TFC) assays, and antioxidant activity (FRAP, DPPH and ABTS) was performed regardless of the extraction solvent used. A high correlation (Table 3) was found between TPC, TFC and antioxidant activity (FPAP, DPPH and ABTS) for both cultivars (Hongkong and Eksotika). Thus, it can reasonably be concluded that in the extract, antioxidant activity is related to the active component. Findings of researches of correlation analyses among TPC, TFC, and antioxidant activities (FRAP, DPPH, and ABTS) are high (Mahattanatawee et al., 2006). There have been significant effects on the antioxidant activities of papaya fruit based on the solvent.

## Conclusion

The recovery of phenolic compounds and antioxidant activity was dependent on the extracting solvent used and the cultivars of papaya fruit. 50% methanol was the most efficient solvent for extracting phenolics compound and DPPH from the papaya fruit, while 50% acetone presented the highest antioxidant activity (FRAP and ABTS) when compared with all other solvents. The addition of 50% water to methanol, acetone or ethanol can enhance the extracting power and antioxidant activity estimation, especially methanol and acetone. This correlation showed that phenolic compounds are the main micro constituents contributing to the antioxidant activity of papaya.

## ACKNOWLEDGEMENT

This research was supported by STGL 004-2007 and BKBP-FST– K004092 and DPP-2013-037.

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