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Quantification of total phenolic compound and *in vitro* antioxidant potential of fruit peel extracts

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Abstract

This study was undertaken to evaluate the potential of fruit waste materials as source of natural antioxidant. The fruit peels including mango, guava and papaya peel were used in this study. The total phenolic content (TPC) was determined by Folin-Ciocalteu assay while antioxidant activities were determined by using ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, ferric thiocynate (FTC) and thiobarbituric acid (TBA) assays. These antioxidant activities were compared to synthetic antioxidants, BHA/BHT combination and ascorbic acid. The results demonstrated that TPC ranged from 3.23 to 15.84 g GAE/100 g extract. Mango peels extract exhibited highest TPC compared to guava peel and papaya peel extract. In the FRAP assay, mango peel extract at 200 ppm, guava peel extract at 400 ppm and papaya peel extract at 1200 ppm, exhibited reducing power comparable to the permissible amount of BHA/BHT at 200 ppm. At concentration of 250 µg/ml, the DPPH radical scavenging activity of extracts and standards decreased significantly in the order of mango peel extract > guava peel extract > BHA/BHT > ascorbic acid > papaya peel extract. For the FTC assay, the antioxidant activity of mango peel extract was significantly higher than ascorbic acid, guava peel and papaya peel extract but lower than BHA/BHT while in the TBA assay, percentage inhibition of BHA/BHT and ascorbic acid were found to be higher than fruit peel extracts. The quantitative analysis for flavonoids showed the presence of catechin, epicatechin and kaempferol in the peel extracts.

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Introduction

Malaysia has a rich array of luscious local fruits. Edible fruits in this country are over hundred known species. Generally, there are two types of fruits, the seasonal fruits and non-seasonal fruits. The famous seasonal fruits includes mango (*Mangifera indica*), durian (*Durio zibethinus* murray), rambutan (*Nephelium lappaceum*), mangosteen (*Garcinia mangostana*), langsat (*Lansium domesticum*) and others. The seasonal fruits are generally available in the market normally at the beginning, middle and towards the end of the year. There are also many non-seasonal fruits such as guava (*Psidium guajava*), papaya (*Carica papaya*), watermelon (*Citrullus lanatus*), pineapple (*Ananas comosus*) and others.

In the recent years, more attention has been paid to the antioxidants contained in fruits. Guo *et al*. (2003) claimed that high fruit intakes were associated with reduced mortality and morbidity of cardiovascular disease and some types of cancer. One of possible mechanism was attributed to the antioxidant activity presented by the fruits. Besides classical antioxidants including vitamin C, vitamin E and β-carotene, phenolic compounds had been identified as important antioxidants contained in fruits. Some phenolic compounds are more powerful as antioxidants than vitamin C and vitamin E *in vitro* (Guo *et al*., 2003). However, fruits are diverse in antioxidant composition and antioxidant activity and those with high antioxidant activity generally contain more antioxidants. Interestingly, the peel fractions of some fruits possess higher antioxidant activity than the pulp fractions. Study by Li *et al.* (2006) reported that pomegranate peel exhibited higher antioxidant activity compared to its pulp. The peel fractions of fruits may potentially contain more antioxidants quantitatively or qualitatively than the pulp fractions. Therefore this study was carried out with the aim to evaluate the total phenolic content and antioxidant activities of selected local fruit peels as a source of natural antioxidant.

Materials and Method

Mango (Chokanan), guava (Kampuchea) and papaya (Eksotika) of commercial maturity were obtained from wet market in Shah Alam, Selangor. The TPC were determined according to the Folin-Ciocalteu method (Singleton and Lamuela-Raventos, 1999) with some modifications by using gallic acid as a standard for phenolic compound. Each prepared

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standard solution was poured into 10 ml volumetric flask. Folin-Ciocalteu reagent (0.5 ml) was added and the contents of the flask mixed thoroughly. After 3 min, Na_2CO_3 (1.5 ml, 20%, w/v) was added and shaken. Finally, the mixture was brought up to 10 ml by adding distilled water and shaken once again. The mixture was allowed to stand at room temperature (20ºC) for 2 hours. The blue colour produced was measured spectrophotometrically at 760 nm after the reading being auto-zero with blank solution. Ten mg of each extract was dissolved in 2 ml distilled water. Then, 0.1 ml of the mixture was transferred into 10 ml volumetric flask and 0.5 ml of Folin-Ciocalteu reagent was added and mixed gently. The following step was similar to the procedures for the preparation of standard gallic acid.

The reductive potential of extracts was determined according to the method of Oyaizu (1986). The different concentrations of extracts (200 ppm-1200 ppm) in 1 ml of distilled water were mixed with 5.0 ml phosphate buffer (0.2 M, pH 6.6, mixture of K_2HPO_4 and KH_2PO_4) and 5 ml of 1% potassium ferricyanide. The mixtures were incubated at 50°C for 20 min. After incubation, 5.0 ml of 10% trichloroacetic acid was added to the mixture, followed by centrifuging at 3000 rpm for 10 min. The upper layer (5 ml) was mixed with 5.0 ml distilled water and 1.0 ml of 0.1% ferric chloride and the absorbance was measured at 725 nm by using spectrophotometer (PerkinElmer Lambda 36, USA). Distilled water was used as a blank. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid (Vitamin C) and combination of BHA/BHT were used as standards.

The scavenging activity on DPPH radical of extracts was determined by modifying the methods of Tagashiri and Ohtake (1998). Stock solutions of extracts were prepared as 100 mg/ml in dimethyl sulfoxide. The solutions were diluted to different concentrations (3.9-250 µg/ml in methanol) in a 96-well microtitre plate. Then 5 µl of DPPH solution (prepared as 2.5 mg/ml in methanol) was added to each well. The plate was shaken gently and placed in the dark for 30 min at room temperature (37ºC). The absorbance was then measured at 517 nm using microtitre plate reader (BioTek, USA). Ascorbic acid and combination of BHA/BHT were used as standards. The radical scavenging activity in percent was calculated using the following formula:

$$\text{Scavenging effect (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Another assay (FTC) was conducted by using the linoleic acid system (Kikuzaki and Nakatani, 1993). The extracts (4 mg) were mixed with 99.5% ethanol (4 ml), 2.5% linoleic acid in 99.5% ethanol (4.1 ml), 0.02M phosphate buffer (pH 7.0, 8 ml) and distilled water (3.9 ml) and kept in screw cap vial under dark condition at 40°C. The final concentration of sample was 0.02% w/v. Aliquots (0.1 ml) were drawn from the incubation solution and mixed with 75% ethanol (9.7 ml) and 30% ammonium thiocyanate (0.1 ml). Precisely, 3 min after addition of 0.1 ml of 0.02M ferrous choride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the red colour was measured at 500 nm each 24 hour interval until 1 day after absorbance of the control reached maximum value. The control and standard were subjected to the same procedures as the sample, except that for the control, only the solvent was added, and for the standard, 4 mg sample was replaced with 4 mg of BHA/BHT and ascorbic acid.

For TBA Assay, the same samples prepared for FTC assay were used. Two ml of sample solution was added with 1 ml of 20% of trichloroacetic acid and 2 ml of thiobarbituric acid solution. The final sample concentration was 0.02% w/v. Then, the mixture was placed in water bath (Memmert, Germany) for 10 minutes. After cooling, it was centrifuged at 3000 rpm for 20 minutes. Absorbance of the supernatant was measured at 532 nm by using UV-vis spectrophotometer (PerkinElmer Lambda 36, USA). Antioxidant activity was recorded based on absorbance on the final day. In FTC and TBA method, antioxidant activity was described by percent inhibition:

$$\text{Percent inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The hydrolysis of extracts was carried out according to the method of Hertog *et al.* (1993) with modification. Eighty mg of each extract was dissolved in 24 ml methanol and homogenised until all extract dissolved. Then, 16 ml of distilled water and 10 ml of 6 M HCL were added into the solution. The mixture was thermostated for 2 hours at 95ºC. The final solution was filtered using a 0.45 µm nylon membrane filter prior to HPLC analysis. Single standards were prepared by accurately weighing out the commercial standard and followed by dissolution in methanol. Each solution was sonicated for 5 minutes. A standard mixture was prepared by serial dilution of single standard with concentration of 800, 600, 400, 200, 100, 50 and 20 ppm.

Results and Discussion

Table 1 shows the TPC in the fruit peel extracts expressed as gram of gallic acid equivalent/100 g crude extract. Among the extracts studied, mango peel extract (15.84 ± 1.66 g GAE/100 g crude extract) possessed the highest phenolic compound ($p < 0.05$), followed by guava peel extract (7.21 ± 0.61 g GAE/100 g CE) and papaya peel extract (3.23 ± 0.05 g GAE/100 g CE). A higher phenolic compound of mango peel and guava peel was in agreement with the findings by Ajila *et al.* (2007a) and Hassimoto *et al*. (2005) and are related to the protection of the plant against UV light. Other study has indicated that pomegranate peel and apple peel also possessed high phenolic compounds which were 24.94 g GAE/100 g CE and 309 mg GAE/100 g FW, respectively (Li *et al.*, 2006; Drogoudi *et al.*, 2008).

Figure 1 illustrates the reductive capabilities of different extracts compared with BHA/BHT and ascorbic acid. Increase in absorbance of the reaction mixture indicated the reducing power of the samples. Among the samples studied, BHA/BHT showed the highest antioxidant activity. At 200 ppm, reductive potential of the samples decreased in the order: ascorbic acid (4.9803) > mango peel (1.5487) > BHA/BHT (1.5050) > guava peel (1.0120) > papaya peel (0.4460). However, reducing power of the samples at 1200 ppm exhibited in the different order: BHA/BHT (5.487) > mango peel (5.237) > guava peel (4.5167) > ascorbic acid (4.3607) > papaya peel (1.5323). The mango peel extract showed higher antioxidant activity than guava peel and papaya peel extracts ($p < 0.05$). The water extracts of mango peel at 200 ppm (1.5487), as well as guava peel at 400 ppm (1.5807) and papaya peel at 1200 ppm (1.5323), exhibited greater reducing power, comparable to 200 ppm (1.5050) of BHA/BHT. Ajila *et al.* (2007b) repeated that the reducing power increased with the concentration of acetone extract of two types of Indian mango peel. A similar trend was also found by Duh and Yen (1997) showing the reductive potential of three herbal water extracts increased with increasing amount of samples. Vitamin C showed an increasing concentration up to a certain extent (400 ppm) and then leveled off with moderate decrease. This is probably because under certain curcimstances, vitamin C acts either as antioxidant or prooxidant depending on its concentration where at lower concentration, it works as antioxidant while at higher concentration, it behaves as prooxidant (Schaefer *et al.*, 1995).

Figure 2 depicts a free radical scavenging activity of water extracts of fruit peel and standards. Among the extracts studied, mango peel extract showed

Table 1. Flavonoid content in fruit peel extracts as determined by HPLC

Samples	Concentration of flavonoid (mg/g crude extract)							
	Catechin	Epicatechin	Kaempferol	Quercetin	Myricetin	Rutin Hydrate	Naringin	Apigenin
Mango peel	810.74	21.83	20.88	ND	ND	ND	ND	ND
Guava peel	1523.79	ND	ND	ND	ND	ND	ND	ND
Papaya peel	740.22	9.75	ND	ND	ND	ND	ND	ND

[a] ND; Not detected

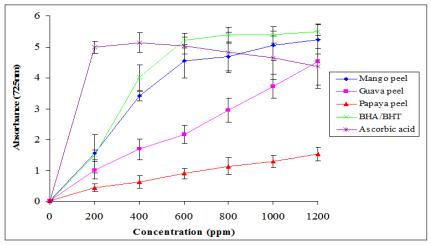

Figure 1. Ferric reducing antioxidant power of fruit peel extracts.

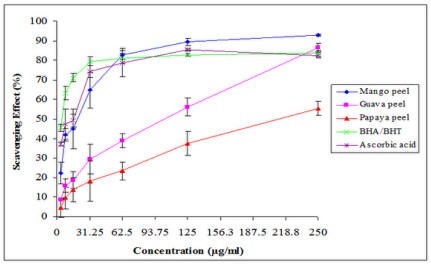

Figure 2. Free radical scavenging activity of fruit peel extracts.

the strongest DPPH scavenging activity followed by guava peel and papaya peel extract ($p < 0.05$). The DPPH scavenging effect of water extracts and standards on the DPPH radical decreased significantly in the order of mango peel > guava peel > BHA/BHT > ascorbic acid > papaya. These results demonstrated that at 250 ug/ml concentration, mango peel extract possessed a stronger scavenging activity compared to BHA/BHT and ascorbic acid ($p < 0.05$), indicating that the extract has effective activities as hydrogen donors and antioxidants by reacting with the lipid radical. The scavenging effect of the extract could be related to its phenolic compounds since gallic acid equivalent of total phenolics was estimated as 15.84 ± 1.66 g GAE/100 g CE. According to Ajila *et al.* (2007b) ripe mango peel contained higher amount of anthocyanins and carotenoids compared to unripe mango peel while raw mango peel had high amount of polyphenol content.

The effects of water extracts of fruit peel on peroxidation of linoleic acid as compared to synthetic antioxidants are illustrated in Figure 3. In the early stages, the autoxidation of linoleic acid without added extracts was accompanied by a rapid increase of peroxide value at day 1. Significant differences ($p < 0.05$) were found between the control and the linoleic acid containing extracts, which slowed the rate of peroxide formation. The water extract of mango peel exhibited the highest activity than guava and papaya peel extract. There was a significant difference ($p <$

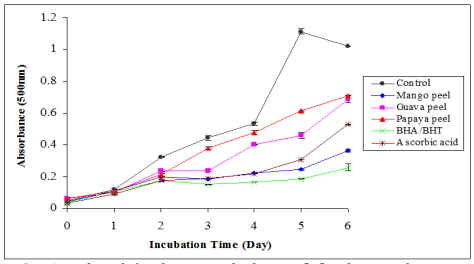

Figure 3. Antioxidative activity of fruit peel extracts as measured by F_{TC} method.

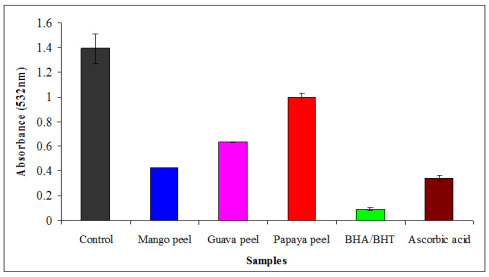

Figure 4. Antioxidative activity of fruit peel extracts as measured by TBA method.

0.05) between the antioxidative activities of mango peel extract and BHA/BHT. Lower absorbance value in the FTC method indicated a higher level of antioxidant activity. The absorbance value of the control increased gradually and reached maximum levels on day 5 and finally dropped on day 6. The control had the highest ($p < 0.05$) absorbance value (1.112) followed by papaya peel extract (0.6149), guava peel extract (0.4569), ascorbic acid (0.3063), mango peel extract (0.2447) and BHA/BHT (0.1864). Based on the results, mango peel extract exhibited 77.99% inhibition in the linoleic acid peroxidation system, which was significantly ($p < 0.05$) higher than ascorbic acid (72.46%) but lower than BHA/BHT (83.24%). Meanwhile, the antioxidant activities of guava peel and papaya peel extract were 58.91% and 44.7%, respectively ($p < 0.05$).

In TBA assay, formation of secondary product is the basis for evaluating the extent of lipid peroxidation. Figure 4 shows that the control sample had the highest ($p < 0.05$) absorbance value (1.3903), followed by papaya peel extract (0.9991), guava peel extract (0.632), mango peel extract (0.4273), ascorbic acid (0.3401) and BHA/BHT (0.0893). Based on the results, BHA/BHT had the highest percentage inhibition of 93.58% followed by ascorbic acid (75.54%), mango peel extract (69.27%), guava peel extract (54.54%) and papaya peel extract (29.45%). A significant difference was found between the total antioxidant activity of water extracts of fruit peel compared with BHA/BHT and ascorbic acid. In this assay, guava and papaya peel extracts showed lower antioxidant activity compared to mango peel extract. The differences in antioxidative activities could be due to several factors, such as different mechanisms involved in the two determination methods, structures of the different phenolic compounds, the antioxidative mechanisms exhibited by the compounds and possibly due to the synergistic effects of different compounds (Abdul Hamid *et al.*, 2003).

The results for quantitative analysis of flavonoids using HPLC of all samples are shown in Table 2. Catechin, epicatechin and kaempferol were identified in the samples with catechin being the most abundant flavonoid detected in guava peel extract (1523 mg/g crude extract) followed by mango peel and papaya peel extracts (810.74 and 740.22 mg/g CE, respectively). Epicatechin was present in mango peel extract (21.83 mg/g CE) and papaya peel extract (9.75 mg/g CE) but none was detected in guava peel extract. Kaempferol was detected only in mango peel extract with the concentration of 20.88 mg/g CE. In contrast, Berardini *et al.* (2005) reported that quercetin was detected in the mango peel extract. This could be probably due to the different extraction method employed and species of mango used in the study. In addition to flavonoid, other complex phenolic compounds in fruit peel extracts may also contribute to the antioxidant activity.

Conclusion

In conclusion TPC of mango peel extract showed highest value followed by guava and papaya peel extract. Antioxidant activities from the four assays indicated that mango peel extract possessed potent antioxidant properties. Major phenolic compounds as detected by HPLC which contribute to the antioxidant activities were catechin, epicatechin and kaempferol.

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