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Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents

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ABSTRACT

The antioxidant capacity and phenol content of three tropical fruits pulps, namely, honey pineapple, banana and Thai seedless guava, were studied. Three solvent systems were used (methanol, ethanol and acetone) at three different concentrations (50%, 70% and 90%) and with 100% distilled water. The antioxidant capacity of the fruit extracts was evaluated using a ferric reducing/antioxidant power assay and the free radical-scavenging capacity was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical-scavenging assays. The efficiency of the solvents used to extract phenols from the three fruits varied considerably. The polyphenol content of Thai seedless guava was 123 to 191 gallic acid equivalents/100 g (GAE/100 g), that of *pisang mas* was 24.4 to 72.2 GAE/100 g, and that of honey pineapple was 34.7 to 54.7 GAE/100 g. High phenol content was significantly correlated with high antioxidant capacity.

1. Introduction

There is strong evidence that free radicals are responsible for the damage of lipids, proteins and nucleic acids in cells (Leong & Shui, 2002), leading to several physiological and pathological abnormalities, such as inflammation, cardiovascular diseases and ageing. Recent studies indicate that frequent consumption of fruits is associated with lower risk of stroke and cancer (Bae, Lee, & Guyatt, 2008; Beecher, 1999; Kawasaki, Hurt, Mistree, & Farrar, 2008; Wright et al., 2008). This protective effect is related to the plant antioxidant microconstituents contained in the plant parts. Different fruits exhibit different antioxidant capacities according to their polyphenol content, vitamin C, E, carotenoids and flavonoids (Saura-Calixto & Goni, 2006).

Of late, considerable attention has been focussed on the polyphenols and flavonoids from different plant sources. Flavonoids are polyphenols with diphenylpropane ($C_6C_3C_6$) skeletons. They are considered to be the largest group of naturally occurring phenols and it is estimated that 2% of all the carbon photosynthesized by plants is converted into flavonoids (Vijayakumar, Presannakumar, & Vijayalakshmi, 2008). Flavonoids are widely distributed in the plant kingdom with a huge diversity of structures (Iwashina, 2000). They exist as four major classes: 4-oxoflavonoids (flavones, flavonols), anthocyanins, isoflavones, and flavan-3-ol derivatives (catechin and tannins) (Rhodes & Price, 1996). Several studies have emphasized that flavonoids from different botanical sources can act as powerful antioxidants, even more so than can the traditional vitamins (Lewis, Fields, & Shaw, 1999; Vijayakumar et al., 2008; Vinson, Dabbagh, Serry, & Jang, 1995).

There are several methods established for the extraction of polyphenols from plant materials. Those methods vary in solvents and conditions used. The extraction method is essential for the accurate quantification of antioxidant content and capacity. This fact makes it hard to compare data from literature reports, due to the reason mentioned earlier (Santas, Carbó, Gordon, & Almajano, 2008). Naczk and Shahidi (2006) identified a group of factors that influence the quantification of phenolics in plant materials. The chemical nature of the phenolic compounds, the extraction method employed and the assay method were some of those factors.

Tropical fruits are well known to be associated with many medicinal properties. In this study we chose three different types of tropical fruits commonly grown in Malaysia, namely, honey pineapple, a local cultivar of banana (known as *pisang mas*) and guava – the Thai seedless cultivar. To our knowledge, no reports are available on the antioxidant capacity of these 3 indigenous Malaysian fruits.

The main objectives of this study were (i) to determine the polyphenolic content and antioxidant capacity of these three tropical fruits and (ii) to examine the efficiency of different solvent systems for the extraction of polyphenols. The phenolic compounds were extracted from the fruits by using three conventional solvents, namely, methanol, ethanol and acetone with different proportions of water.

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2. Materials and methods

2.1. Plant materials

Fresh fruit samples with no apparent physical or microbial damage were collected separately, at different times, from different local markets in Penang, Malaysia. Samples included honey pineapple (*Ananas comosus* Merr.), a local type of banana (*Musa paradasiaca*) and guava (*Psidium guajava* L.), the Thai seedless cultivar. All the fruits were of eating quality, and were identically selected in terms of shape, size, colour, and ripening stage.

2.2. Chemicals and reagents

Folin–Ciocalteu's (FC) phenol reagent, sodium nitrite, sodium acetate, aluminium chloride, ferrous sulphate, ferric chloride, sodium carbonate, methanol, ethanol, and acetone were purchased from R & M Chemicals (Essex, UK). 2,4,6-Tris (1-pyridyl)-5-triazine (TPTZ), and 1,1-diphenyl-2-picrylhydrazyl (DPPH·) were purchased from the Fluka company (Switzerland). Gallic acid and catechin were supplied by Sigma–Aldrich (St. Louis, MO, USA). All chemicals and reagents used in the study were of analytical grade.

2.3. Extraction of polyphenols

First, fruits were washed with clean sterile water and peeled. After that, 100 g of fruit pulps were diced into small cubes and blended for 3 min and then extracted with 300 ml of organic solvent with magnetic stirring on a hot plate (magnet 4.5 × 0.5 cm; hotplate Stable Temperature, Cole Parmer Instrumental Company, Bunker Court, USA) at 1100 rpm for 3 h at room temperature (25 ± 1 °C). The fruit extracts were then filtered using a clean muslin cloth and centrifuged (KUBOTA 5100 Centrifuge, Japan) at 4750 g for 15 min. After that, the supernatant was concentrated at 50 °C using a rotary evaporator (IKA- WERKE- RV06ML, Stanfer, Germany). The crude extracts were collected after 3 h and stored at 4 °C in the dark. Light exposure was avoided throughout the extraction process. The extraction process was carried out in duplicate, using two different fruit samples each time.

Three different solvent-water extraction systems were used (methanol, ethanol and acetone) at three different concentrations in distilled water (50, 70, and 90%) and 100% distilled water (H_2O).

2.4. Determination of total phenolic content

Total phenolic contents (TP) of the fruit extracts were determined using FC assay which was described by Singleton and Rossi (1965). 40 μl of properly diluted fruit extract solution were mixed with 1.8 ml of FC reagent. The reagent was pre-diluted, 10 times, with distilled water. After standing for 5 min at room temperature, 1.2 ml of (7.5% w/v) sodium carbonate solution were added. The solutions were mixed and allowed to stand for 1 h at room temperature. Then, the absorbance was measured at 765 nm, using a UV–visible spectrophotometer (Shimadzu UV-1601PC, Japan). A calibration curve was prepared, using a standard solution of gallic acid (20, 40, 60, 80 and 100 mg/l, $r^2 = 0.997$). Results were expressed on fresh weight basis (fw) as mg gallic acid equivalents/100 g of sample.

2.5. Determination of total flavonoids

Total flavonoid contents (TF) of the fruit extracts were determined according to the colorimetric assay developed by Zhishen, Mengcheng, and Jianming (1999). One ml of properly diluted fruit extract was mixed with 4 ml of distilled water. At zero time, 0.3 ml of (5% w/v) $NaNO_2$ was added. After 5 min, 0.3 ml of (10% w/v) $AlCl_3$ was added. At 6 min, 2 ml of 1 M solution of NaOH were added. After that, the volume was made up to 10 ml, immediately by the addition of 2.4 ml of distilled water. The mixture was shaken vigorously and the absorbance of the mixture was read at 510 nm. A calibration curve was prepared using a standard solution of catechin (20, 40, 60, 80 and 100 mg/l, $r^2 = 0.996$). The results were also expressed on a fresh weight basis as mg catechin equivalents (CEQ) / 100 g of sample.

2.6. Ferric reducing/antioxidant power assay (FRAP assay)

FRAP assay was performed according to a modified method described by Benzie and Strain (1999). Briefly, a 40 μl aliquot of properly diluted fruit extract was mixed with 3 ml of FRAP reagent. Then, the reaction mixture was incubated at 37 °C for 4 min. After that, the absorbance was determined at 593 nm against a blank that was prepared using distilled water and incubated for 1 h instead of 4 min. FRAP reagent should be pre-warmed at 37 °C and should always be freshly prepared by mixing 2.5 ml of a 10 mM 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ) solution in 40 mM HCl with 2.5 ml of 20 mM $FeCl_3.\ 6H_2O$ and 25 ml of 0.3 M acetate buffer, pH 3.6. A calibration curve was prepared, using an aqueous solution of ferrous sulphate $FeSO_4.\ 7H_2O$ (200, 400, 600, 800 and 1000 μM, $r^2 = 0.997$). FRAP values were expressed on a fresh weight basis as micromoles of ferrous equivalent Fe (II) per gramme of sample.

2.7. DPPH free radical-scavenging assay

The antioxidant capacity of the fruit extracts was also studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the method proposed by De Ancos, Sgroppo, Plaza, and Cano (2002). An aliquot (10 μl) of fruit extract was mixed with 90 μl of distilled water and 3.9 ml of 25 mM DPPH· methanolic solution. The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. The absorbance was measured later, at 515 nm, against a blank of methanol without DPPH·. Results were expressed as percentage of inhibition of the DPPH radical. Percentage of inhibition of the DPPH radical was calculated according to the following equation:

$$\%\ \text{inhibition of DPPH}^{\cdot} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100$$

where Abs control is the absorbance of DPPH· solution without extracts.

2.8. Statistical analysis

Data were analysed using SPSS software. Analysis of variance (ANOVA) and Duncan's multiple range method were used to compare any significant differences between solvents and samples. Values were expressed as means ± standard deviations. Differences were considered significant at $P < 0.05$. All the analyses were carried out in triplicates.

3. Results and discussion

3.1. Polyphenol content

Table 1 shows the total phenolic content (TP) of the fruit extracts measured using Folin-Ciocalteu's colorimetric method. TP of the fruits ranged from 34.7 GAE/100 g (fw) to 54.7 GAE/100 g (fw) for honey pineapple, while it ranged from 24.4 GAE/100 g (fw) to 72.2 GAE/100 g (fw) for *pisang mas*, and from 123 GAE/

Table 1
Total phenolic[A] content and total flavonoid[A] content of fruits extracts obtained from different solvent extraction systems.

Solvent	Pineapple		Banana *pisang mas*		Guava	
	Total phenolics (mg GAE/100 g fresh weight)	Total flavonoids (mg CEQ/100 g fresh weight)	Total phenolics (mg GAE/100 g fresh weight)	Total flavonoids (mg CEQ/100 g fresh weight)	Total phenolics (mg GAE/100 g fresh weight)	Total flavonoids (mg CEQ/100 g fresh weight)
100% H_2O	34.7 ± 3.44^{a}	3.34 ± 0.54^{d}	27.0 ± 1.96^{ab}	13.7 ± 1.55^{e}	153 ± 2.73^{c}	20.5 ± 4.33^{c}
Methanol (%)						
90	35.6 ± 1.16^{a}	1.37±a 0.11^{a}	37.5 ± 0.58^{d}	4.70 ± 0.19^{a}	109 ± 1.00^{a}	13.9 ± 0.57^{a}
70	51.3 ± 1.92^{d}	1.24 ± 0.03^{a}	34.4 ± 0.34^{d}	5.24 ± 0.26^{a}	155 ± 2.12^{c}	18.2 ± 0.59^{bc}
50	39.0 ± 1.42^{b}	3.70 ± 0.07^{e}	29.3 ± 2.17^{c}	6.85 ± 0.10^{b}	119 ± 2.53^{b}	14.5 ± 0.50^{ab}
Ethanol (%)						
90	39.5 ± 1.08^{b}	2.24 ± 0.10^{b}	24.4 ± 2.45^{a}	7.03 ± 0.47^{b}	185 ± 3.02^{f}	39.5 ± 2.23^{d}
70	54.7 ± 1.79^{e}	4.14 ± 0.26^{f}	28.8 ± 4.10^{c}	9.35 ± 0.35^{c}	157 ± 6.18^{c}	37.1 ± 0.83^{d}
50	44.1 ± 0.83^{c}	2.44 ± 0.05^{bc}	35.3 ± 4.39^{d}	7.04 ± 1.67^{b}	123 ± 4.45^{b}	15.3 ± 1.65^{ab}
Acetone (%)						
90	50.7 ± 2.43^{d}	3.24 ± 0.08^{d}	72.2 ± 2.03^{f}	23.7 ± 0.75^{f}	191 ± 4.35^{f}	40.6 ± 2.23^{de}
70	41.6 ± 1.64^{c}	2.58 ± 0.05^{c}	45.8 ± 1.90^{e}	11.5 ± 0.10^{d}	166 ± 3.12^{d}	40.9 ± 5.16^{de}
50	54.1 ± 0.79^{de}	1.48 ± 0.06^{a}	35.9 ± 0.80^{d}	9.76 ± 0.26^{c}	180 ± 5.79^{e}	44.6 ± 3.43^{e}

[A] Values are means (n = 6) ± SD. Values with the same superscript letter are not statistically significant at the 5% level.

100 g (fw) to 191 GAE/100 g (fw) for the Thai seedless guava. Therefore, guava extracts had higher polyphenol contents when compared with the other two fruits. These results are on a par with some earlier observations on similar fruits obtained from different geographical origins (Jiménez-Escrig, Rincón, Pulido, & Saura-Calixto, 2001; Mahattanatawee et al., 2006; Sun, Chu, Wu, & Liu, 2002).

The total flavonoid (TF) content of these fruits was determined. Guava fruits also had the highest TF content, followed by *pisang mas* and honey pineapple (Table 1). Correlation analysis was performed on the polyphenolic content analysis methods for the three fruits. The correlations between TP and TF assays were 0.853 and 0.763 for guava and *pisang mas*, respectively, which were highly significant at the 0.01 level. On the other hand, the correlation between TP and TF was insignificant, in the case of pineapple (r = 0.031), at the 0.05 level. These results indicate that the flavonoids are an important phenolic group in representing the antioxidant capacity of guava and *pisang mas* but not of pineapple, where it could be related to other antioxidant compounds contained in pineapple fruit pulp.

3.2. Effect of solvent system

Earlier, solvents, such as methanol, ethanol, acetone, propanol, ethyl acetate and dimethylformamide, have been commonly used for the extraction of phenolics from fresh produce at different concentrations in water (Antolovich, Prenzler, Robards, & Ryan, 2000; Luthria & Mukhopadhyay, 2006). The recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. Furthermore, solvent polarity will play a key role in increasing phenolic solubility (Naczk & Shahidi, 2006). Therefore, it is hard to develop a standard extraction procedure suitable for the extraction of all plant phenols. Usually, the least polar solvents are considered to be suitable for the extraction of lipophilic phenols unless very high pressure is used.

From the results shown in Table 1, it is evident that the recovery of phenolic compounds was dependent on the solvent used and its polarity (for all three fruits). For pineapple extracts, acetone (50%) and ethanol (70%) gave the highest yield of TP without significant differences between them. Acetone (70%) could recover the highest yield of TP (72.2 ± 2.03) in *pisang mas* extracts with significant difference when compared with all other solvent systems used. The highest yield of guava TP was obtained using acetone (90%) or ethanol (90%), with no significant differences between them (p < 0.05). Ethanol and water mixtures are commonly used for the extraction of phenols from plant materials (Bahorun, Luximon-Ramma, Crozier, & Aruoma, 2004; Durling et al., 2007). This is due to the wide range of phenols that the aqueous ethanol mixtures can dissolve. Furthermore, ethanolic mixtures have acceptability for human consumption models. On the other hand, acetone–water mixtures are good solvent systems for the extraction of polar antioxidants (Lu & Foo, 1999; Luximon-Ramma, Bahorun, & Crozier, 2003; Sun et al., 2002).

3.3. Antioxidant capacity

There are a huge varieties of antioxidants contained in fruits. Therefore, measuring the antioxidant capacity of each compound separately becomes very difficult. Several methods have been developed to estimate the antioxidant capacity of different plant materials (Guo et al., 2003). Usually, those methods measure the ability of antioxidants, in a particular plant material, to scavenge specific radicals, by inhibiting lipid peroxidation or chelating metal ions. In this study, two different methods have been used to evaluate the antioxidant capacity of the extracts of the three fruits extracts; they are ferric reducing/antioxidant power assay (FRAP assay) and DPPH free radical-scavenging assay.

FRAP assay is commonly used to study the antioxidant capacity of plant materials. The antioxidant capacity of fruits extracts is determined by the ability of the antioxidants in these extracts to reduce ferric iron to ferrous in FRAP reagent, which consists of 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ) prepared in sodium acetate buffer, pH 3.6. The reduction of ferric iron in FRAP reagent will result in the formation of a blue product (ferrous – TPTZ complex) whose absorbance can be read at 593 nm.

1,1-Diphenyl-2-picrylhydrazyl (DPPH$^{\cdot}$) is a stable organic nitrogen radical. DPPH radical is commercially available. The assay of the scavenging of DPPH radical is widely used to evaluate the antioxidant capacity of extracts from different plant materials. The percentage of inhibition of DPPH$^{\cdot}$ within the assay time will reflect the antioxidant capacity of the extract assessed. The assay time would vary from 10–20 min up to about 6 h (Gil, Tomas-Barberan, Hess-Pierce, Holeroft, & Kader, 2000).

The antioxidant capacities of the fruit extracts tested varied. Guava extracts exhibited high FRAP and DPPH$^{\cdot}$ values which can be interpreted as the highest antioxidant capacity among the three fruits studied (Table 2). Leong and Shui (2002) and Mahattanatawee et al. (2006) reported similar results, showing that guava had a high antioxidant capacity in comparison with the other types of fruits in both studies.

Table 2
Antioxidant capacity[A] of fruits extracts obtained from different solvent extraction systems.

Solvent	Pineapple		Banana *pisang mas*		Guava	
	FRAP (μmol Fe (II)/g fresh weight)	(%) DPPH· inhibition	FRAP (μmol Fe (II)/g fresh weight)	(%) DPPH· inhibition	FRAP (μmol Fe (II)/g fresh weight)	(%) DPPH· inhibition
100% H_2O	2.23 ± 0.53^{b}	12.7 ± 1.28^{a}	0.59 ± 0.15^{a}	36.8 ± 2.93^{a}	29.8 ± 3.79^{f}	82.3 ± 1.88^{b}
Methanol (%)						
90	4.92 ± 0.11^{e}	89.7 ± 0.32^{e}	3.23 ± 0.14^{ed}	65.6 ± 4.10^{c}	19.1 ± 0.90^{b}	68.6 ± 4.26^{a}
70	5.30 ± 0.08^{f}	89.9 ± 0.63^{e}	2.70 ± 0.24^{ed}	71.2 ± 1.71^{d}	22.6 ± 0.34^{c}	69.2 ± 4.29^{a}
50	4.13 ± 0.04^{d}	67.7 ± 1.17^{b}	2.57 ± 0.10^{d}	32.8 ± 1.20^{a}	15.9 ± 0.73^{a}	67.5 ± 3.05^{a}
Ethanol (%)						
90	1.72 ± 0.16^{a}	90.8 ± 0.56^{e}	3.10 ± 0.77^{ed}	35.8 ± 4.11^{a}	30.8 ± 1.37^{f}	86.9 ± 4.85^{b}
70	3.41 ± 0.18^{c}	87.5 ± 0.74^{d}	2.92 ± 0.59^{ed}	79.1 ± 2.50^{e}	31.9 ± 0.95^{f}	84.0 ± 3.04^{b}
50	2.17 ± 0.09^{b}	78.9 ± 1.01^{c}	3.30 ± 0.79^{e}	64.1 ± 3.14^{c}	$25.6 \pm 1.90d^{e}$	81.9 ± 1.65^{b}
Acetone (%)						
90	7.90 ± 0.05^{h}	93.7 ± 0.58^{f}	5.26 ± 0.35^{f}	68.0 ± 0.51^{cd}	30.1 ± 0.32^{f}	94.6 ± 3.13^{c}
70	7.17 ± 0.11^{g}	87.9 ± 1.01^{d}	1.93 ± 0.22^{c}	65.9 ± 2.12^{c}	26.9 ± 0.66^{e}	93.4 ± 1.97^{c}
50	8.38 ± 0.05^{i}	87.8 ± 1.47^{d}	1.22 ± 0.02^{b}	52.3 ± 1.48^{b}	24.2 ± 0.40^{cd}	93.7 ± 3.49^{c}

[A] Values are means ($n = 6$) ± SD. Values with the same superscript letter are not statistically significant at the 5% level.

FRAP and DPPH· assays showed the same trends. This is proved by the significant correlations between FRAP values and DPPH· values for all the fruits in the current study. Correlation values were 0.659 and 0.438 for guava and honey pineapple, respectively (significant at $P < 0.01$ level) and 0.398 for *pisang mas* (significant at $P < 0.05$). This correlation could be due to the same mechanism that FRAP and DPPH· methods rely on. This mechanism concerns the ability of the antioxidants to reduce certain radicals (ferric iron and DPPH radical). Another significant correlation between TP and antioxidant capacity of fruits extracts (FRAP and DPPH· values) was obtained. These correlations confirm that the phenolic compounds are the main microconstituents contributing to the antioxidant activities of these fruits.

4. Conclusion

The recovery of phenols was dependent on the fruit type and the solvent system used. Acetone (50%) and ethanol (70%) were the most efficient solvents for extracting phenols from honey pineapple, while acetone (70%) was the most efficient solvent system for the extraction of phenols from *pisang mas*; both acetone (90%) and ethanol (90%) efficiently extracted phenols from the Thai seedless guava. There was a good correlation between total phenol content and antioxidant capacity of the fruit extracts. The higher the total phenolic content of the fruits, the higher were the FRAP and DPPH· values. The phenol content, flavonoid content, and antioxidant capacity were highest in Thai seedless guava (among the three fruits).

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