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Total Phenolics and Antioxidant Activities of *Pouteria campechiana* Fruit Parts

(Fenolik Jumlah dan Aktiviti Antioksida Sebatian Bahagian Buah *Pouteria campechiana*)

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ABSTRACT

This study aimed to evaluate the total phenolics and antioxidant capacities of the seeds, pulp and peel of Pouteria campechiana *fruit using three extraction solvents (water, 70% methanol and 70% ethanol). Among them, 70% ethanol exhibited the best solvent for yielding highest total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities. The results showed that 70% ethanol extract from the peel contained the highest TPC (2304.7 mg gallic acid equivalent/100 g dw) while the pulp has the highest TFC (6414.03 mg rutin equivalent/100 g dw). The antioxidant activities of the pulp and peel ethanolic extracts were high as determined using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation assay (49.60 and 49.56 mmoL TE/100 g dw) and ferric reducing antioxidant power assay (43.88 and 42.94 Fe^{2+}/100 g dw) but not for seeds. However, their diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities were ~88%. Thus, the pulp and peel of* P. campechiana *fruit can be utilized as natural source for antioxidant components.*

Keywords: Antioxidant activity; Pouteria campechiana*; solvent extraction; total phenolics*

ABSTRAK

Kajian ini bertujuan untuk menentukan fenolik jumlah dan keupayaan antioksidan daripada biji, isi dan kulit buah Pouteria campechiana *dengan menggunakan tiga pelarut pengekstrakan (air, 70% metanol dan 70% etanol). Antara pelarut tersebut, 70% etanol adalah yang terbaik untuk mendapatkan kandungan fenolik jumlah (TPC), kandungan flavonoid jumlah (TFC) dan mempunyai aktiviti antioksidan yang tertinggi. Keputusan kajian menunjukkan bahawa ekstrak 70% etanol daripada kulit buah mengandungi TPC tertinggi (2304.7 mg persamaan asid galik 100 g bk), manakala isinya mengandungi TFC yang tertinggi (6414.03 mg persamaan rutin/100 g bk). Aktiviti antioksidan ekstrak etanol isi dan kulit yang ditentukan oleh asai radikal kation ABTS (49.60 and 49.56 mmoL TE/100 g bk) dan asai kuasa antioksidan penurunan ferik (43.88 and 42.94 Fe^{2+}/100 g bk) adalah tinggi tetapi tidak untuk bijinya. Aktiviti merantas radikal DPPH adalah ~88%. Maka, isi dan kulit buah* P. campechiana *boleh digunakan sebagai sumber semula jadi untuk mendapatkan sebatian antioksidan.*

Kata kunci: Aktiviti antioksidan; jumlah fenolik; pelarut pengestrakan; Pouteria campechiana

Introduction

Pouteria campechiana (PC) is one of the tropical fruits belonging to *Sapotaceae* family. It is commonly called 'canistel' in English, while in Malay it is named as 'Buah kuning telur'. PC is a native to Central America and it is widely distributed around the tropical and subtropical regions in South America and Asia (Silva et al. 2009). The fruit is ovoid in shape measuring between 7.5 and 12.5 cm in length and 5 and 7.5 cm in breadth (Ma et al. 2004). The skin of the ripe fruit is yellow-orange in color, whereas the unripe fruit is green. Although the yellow pulp is edible either eaten fresh or after process, it is not commonly consumed among Malaysians and remains underutilized.

Malaysia is one of the countries rich in diversity of underutilized fruits where these fruits are rarely eaten, unknown and unfamiliar (Ikram et al. 2009). Many unexplored indigenous species have not received much attention by people but they may potentially be the superior food owing to their excellent nutritional or phytochemical components (Kong et al. 2010). This study could also provide information for a better perceptive in nutraceutical and other functional properties of the PC fruit. All data established should help in enhancing the commercial value of the species and further contribute to its conservation.

According to Silva et al. (2009), PC fruit possesses good antioxidant potential attributed to the presence of phenolic compounds such as gallic acid and catechin in the acetone extract of the fruit (Ma et al. 2004). Different type of solvents can affect the efficiency of phenolic compounds extraction (Prasad et al. 2011). To the best of our knowledge, very limited studies were found on the influence of different solvents on the antioxidant performances of seeds, pulp and peel of PC fruit. Hence, this study was aimed to seek for the most suitable solvent for extraction of phenolic compounds from seeds, pulp and peel of PC fruit and also to demonstrate good antioxidant capacity of the extracts.

MATERIALS AND METHODS

SAMPLING AND SAMPLE PREPARATION

Fruits of PC were sampled (~500 g) from University Agriculture Park, Universiti Putra Malaysia, Selangor, Malaysia. The fruits were washed under tap water and separated into different parts; seeds, pulp and peel for lyophilization. The lyophilized sample was ground into powder and sieved through a 0.25 mm mesh siever.

The moisture contents of seed, pulp and peel of PC were determined using a direct drying method described by Tee et al. (1996) using an air oven (Memmert Universal, Schwabach, Germany) at 105°C. The weights of PC samples were taken using a digital balance with an accuracy of 10^{-4} g. The length, width and pulp thickness of PC fruit were measured using a vernier caliper, while the circumference of the fruit was measured using a household measurement tape.

EXTRACTION OF ANTIOXIDANT

The extraction method was modified from Liu et al. (2008a). Lyophilized sample was extracted with different solvents namely, distilled water, 70% methanol and 70% ethanol at the ratio of 1:20 (w/v). One gram of lyophilised sample was added with 20 mL of solvent containing 1.2 M HCl. The extraction was conducted using an orbital shaker (Heidolph Unimax 1010, Schwabach, Germany) at 200 rpm, 60ºC for 2 h. After extraction, the extract was filtered through Whatman paper No. 4. All chemicals used were analytical reagent grade.

DETERMINATION OF TOTAL PHENOLIC CONTENT

The method for determination of total phenolic content (TPC) was slightly modified from Singleton and Rossi (1965). A properly diluted sample (2 mL) was added with 1 mL of Folin-Ciocalteau reagent and incubated for 5 min. After that, 4 mL saturated Na_2CO_3 solution (60 g/L) was added to the mixture and final volume of the mixture was made up to 10 mL using distilled water. The reaction mixture was allowed to stand for 2 h in the dark and the absorbance was read at 760 nm using UV-1601 spectrophotometer (Shimadzu Corporation, Victoria, Australia) against distilled water as blank. TPC of the samples was expressed as gallic acid equivalent (GAE) per 100 g sample in dry weight (dw).

DETERMINATION OF TOTAL FLAVONOID CONTENT

The total flavonoid content (TFC) was determined following the method previously described by Liu et al. (2008a). An appropriately diluted sample (2 mL) was mixed with 0.2 mL of 5% $NaNO_2$. After 5 min of incubation, 0.2 mL of 10% $AlCl_3$ was added to the mixture and allowed to stand for 6 min. Then, 2 mL of 1 M NaOH was added and the reaction mixture was made up to a total volume of 5 mL using distilled water. Absorbance was measured at 510 nm against distilled water as blank. TFC of the samples was expressed as rutin equivalent (RE) per 100 g sample in dry weight (dw).

DETERMINATION OF ABTS RADICAL CATION ASSAY

The method of Re et al. (1999) was adopted with some modifications. Briefly, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) salt was dissolved in water to a concentration of 7 mM. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS solution with 2.45 mM potassium persulfate (final concentration). The reaction mixture was incubated at room temperature in the dark for 12 to 16 h to generate free radicals. Absorbance was measured at 734 nm after 6 min against distilled water as blank. Percentage of antioxidant capacity was calculated following (1).

$$\text{Antioxidant activity}\,(\%) = \frac{\left(A_{ABTS} - A_{\text{sample or standard}}\right)}{A_{ABTS}} \times 100, \quad (1)$$

where, A_{ABTS} is the absorbance of ABTS radical cation without sample or standard; $A_{\text{sample or standard}}$ is the absorbance of ABTS radical cation with sample or standard.

DETERMINATION OF FERRIC REDUCING ANTIOXIDANT POWER

This assay was carried out according to Benzie and Strain (1996) with slight modifications. Three reagents were prepared as ferric reducing antioxidant power (FRAP) reagent: 300 mM acetate buffer at pH3.6, 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3$. Then, FRAP reagent was prepared by mixing acetate buffer, TPTZ solution and $FeCl_3$ solution at a ratio of 10:1:1 (v/v/v). An appropriately diluted sample extract (50 µL) was added with 3 mL of the FRAP reagent and incubated at 37ºC for 30 min. Absorbance was read at 593 nm against distilled water as blank.

DETERMINATION OF DPPH RADICAL SCAVENGING ACTIVITY

The method used was slightly modified from the method described by Brand-Williams et al. (1995). Sample extract (0.1 mL) was added with 3.9 mL of 100 µM 2,2-diphynyl-1-picrylhydrazyl (DPPH) solution in ethanol. After 30 min of incubation, absorbance of the reaction mixture was read at 515 nm against ethanol as blank. The results were expressed as percentage (%) of DPPH radical scavenging activity calculated using (2):

$$\text{Scavenging activity}\,(\%) = \frac{\left(A_{\text{control}} - A_{\text{sample}}\right)}{A_{\text{control}}} \times 100, \quad (2)$$

where, A_{control} is the absorbance of DPPH radicals without sample and A_{sample} is the absorbance of DPPH radicals with sample.

STATISTICAL ANALYSIS

All analyses were done in triplicates. The results were expressed as means ± standard deviations. The data were analyzed using SPSS statistical software version 15 (SPSS Inc, Illinois, USA). The one-way analysis of variance (ANOVA) and Tukey's honestly significant difference test were used to compare the means among groups. The relationship between antioxidant components and antioxidant capacities were investigated via Pearson correlation test. The level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

PHYSICAL PROPERTIES

The description about the physical properties of PC fruit may be useful for future application especially in industrial processing. The physical parameters measured from the fruits are shown in Table 1. PC fruits pulp thickness was approximately 1.1 cm and constituted about 77.11% of total fruit weight. Meanwhile, seeds and peel accounted about 16.5% and 6.39% of total fruit weight, respectively. Seeds composed of 50.17% of moisture while pulp and peel contained 46.41% and 48.28% of moisture, respectively.

TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENTS

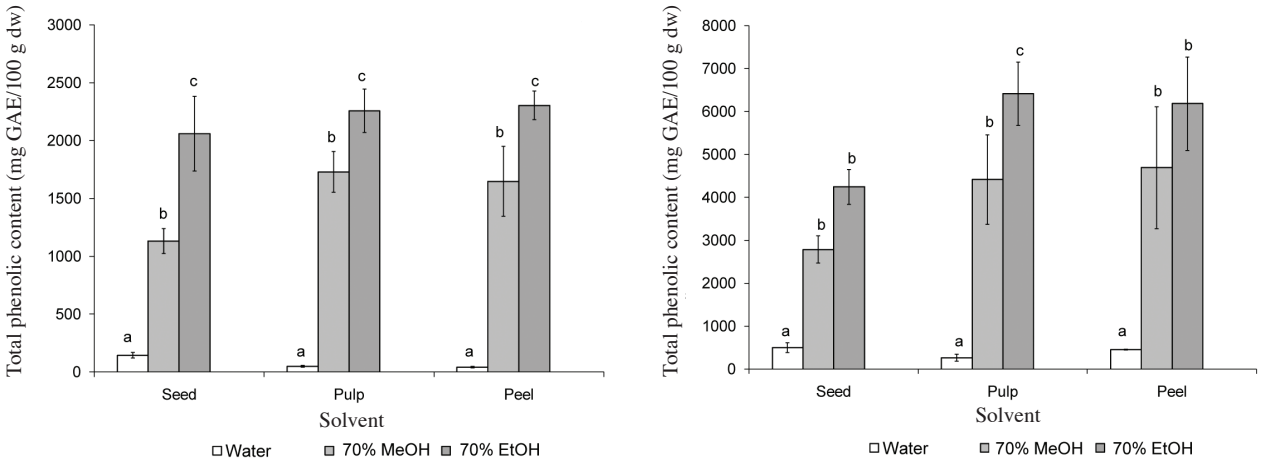
In this study, 70% ethanol demonstrated as the best extraction solvent with the highest TPC and TFC for all the fruit parts (Figure 1). TPC values of the ethanolic extracts in an ascending order was 2060, 2257.8 and 2304.7 mg GAE/100 g dry weight (dw) for peel, pulp and seeds, respectively. On the other hand, TFC values were 6414, 6180.6 and 4242.3 mg RE/100 g dw for pulp, peel and seeds, respectively.

The TPC attained by different solvents from the same source of fruit part were significantly different to each other. The observed trends for both TPC and TFC were analogous for all fruit parts where 70% ethanol > 70% methanol > aqueous extracts. The current study showed that 70% ethanol extract gave high TPC and TFC contradictory to the previous report by Ma et al. (2004). These variations might be attributed to differences in growing environment, season, soil, rainfall and usage of fertilizers (Manach et al. 2004). Although the methanolic extracts possessed higher TPC and TFC than water extracts but they were about 23 to 45% lesser than the ethanolic extracts. Musa et al. (2011) reported that combination of aqueous and organic solvents such as methanol or ethanol has improved effects on TPC as compared with water or organic solvent alone.

TABLE 1. Physical properties of *Pouteria campechiana* fruit[1]

Sample	Weight (g)	Length (cm)	Width (cm)	Circumferences (cm)	Pulp thickness (cm)
Fruit	118.09 ± 35.48	7.43 ± 0.21	5.40 ± 0.62	17.90 ± 2.44	1.10 ± 0.10
Fruit part	Weight (g)	Portion (%)	Moisture (%)		
Seeds	19.77 ± 7.32	16.50 ± 1.50	50.17 ± 4.70		
Pulp	90.83 ± 27.47	77.11 ± 0.12	46.41 ± 1.64		
Peel	7.16 ± 0.83	6.39 ± 1.59	48.28 ± 3.04		

[1] Data are shown as mean ± standard (n=3)



(a) (b)

Note: [a-c] Different letters indicate significant difference among the solvents (p<0.05)

FIGURE 1. (a) Total phenolic content and (b) total flavonoid content of seeds, pulp and peel of *Pouteria campechiana* in different extraction media

Our findings revealed that PC fruit peel and pulp were potential sources of polyphenolic compounds, where both fruit parts gave remarkable TPC and TFC in the ethanolic as well as methanolic extracts. As observed, the ability of aqueous ethanol to solubilize phenolic or flavonoid compounds in PC fruit was stronger than aqueous methanol. Nonetheless, both efficiency and effectiveness of extraction solvents are depending on type of analytes exist and sample matrixes (Garcia-Salas et al. 2010). Antolovich et al. (2000) also reported that many flavonoids have low solubility in aqueous media. This fact can be the reason to explain for low TFC obtained in the water extract.

Interestingly, when TPC of ethanolic extract of seeds, pulp and peel (1026.52, 1209.96 and 1192.01 mg GAE/100 g fw, respectively) were expressed in fresh weight (fw), higher TPC values were noted than that of Brazillian *Pouteria caimito* fruit (616 mg GAE/100 g fw) and other Brazillian exotic fruits (de Assis et al. 2009). PC fruit showed higher TPC in comparison to 62 types of common fruits (11 to 585 mg GAE/100 g fw) including orange, guava, cherries, persimmon, pomegranate, cherry tomato, apple, banana, mango, pear, blueberry, grape, papaya and watermelon (Fu et al. 2011). The TPC of PC fruit parts are comparable to some of the Malaysian underutilized fruits (6 to 3185 mg GAE/100 g dw) (Ikram et al. 2009). Out of 52 Malaysian underutilized fruits studied (Ikram et al. 2009), TPC of 48 underutilized fruits that extracted by 80% methanol were lower than the TPC of PC pulp (1730 mg GAE/100 g dw) extracted by 70% methanol. Nevertheless, the lower moisture content found in the PC species that may contribute to higher calculated TPC in fresh weight should not be ignored. As Folin-Ciocauteu reagent method was employed for TPC measurement, other reducing agents such as ascorbic acid, sugars and aromatic amines may also affect the results (Magalhães et al. 2008).

ANTIOXIDANT ACTIVITIES

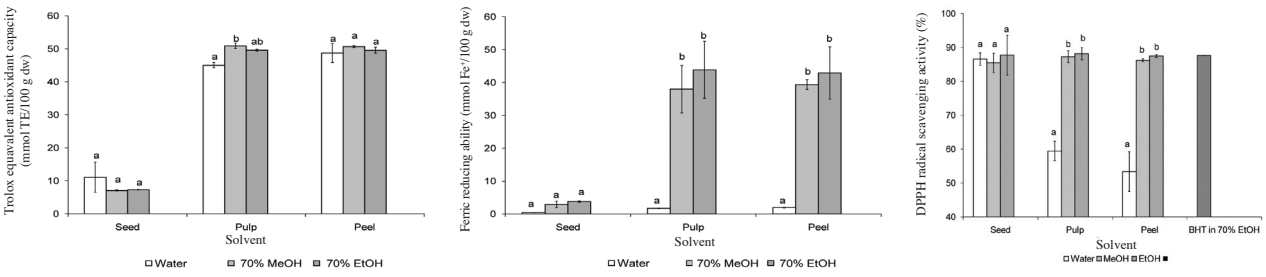
In order to have a better understanding on the antioxidative characteristic of PC extracts, different antioxidant assays were selected. High reactivity of PC extracts towards DPPH and ABTS radicals indicated their capabilities to function as hydrogen or electron donors that are significant in preventing lipid peroxidation. On the other hand, FRAP assay exhibited the abilities of extracts to reduce ferrous (Fe^{3+}) to ferric (Fe^{2+}) ions (Visavadiya et al. 2009).

The high antioxidant activities observed in the extracts may be attributed to the TPC and TFC. Overall, antioxidant activities noted do not follow the order of TPC and TFC. The antioxidant activities of both ethanolic and methanolic extracts did not differ significantly with each other in the seeds, pulp and peel of PC as determined using ABTS, FRAP and DPPH assays (Figure 2). As shown in Figure 2, BHT with concentration of 30 µg/mL had DPPH radical scavenging activity (%) comparable with the ethanolic extract of samples.

The present study revealed that 70% methanol and 70% ethanol are the best two extraction solvents used for obtaining the highest antioxidant activities. Musa et al. (2011) reported that antioxidant activities of pink guava ethanolic and methanolic extracts based on FRAP and DPPH assays were higher than their water counterpart. Besides, methanolic extracts of *Canarium odontophyllum* fruit parts assessed by Trolox equivalent antioxidant capacity (TEAC) assay exhibited the greatest antioxidant capacity as compared with water and ethanolic extracts (Khoo et al. 2012).

CONCLUSION

Pulp and peel were the main sources of antioxidant constituents of PC fruits. It appears that 70% ethanol was the more effective extraction solvent than 70% methanol, since it provided excellent antioxidant profiles and is a green solvent that is less hazardous and environmental friendly at the same time. These findings are important for the utilization of PC fruits as a natural source for nutraceutical and functional properties. Further studies are warranted for extra evidences on the beneficial aspects of PC ethanolic pulp and peel extracts.



(a) (b) (c)

Note: [a–b] Different letters indicate significant difference among the solvents ($p<0.05$)

FIGURE 2. (a) Trolox equivalent antioxidant capacity, (b) ferric reducing antioxidant power and (c) DPPH radical scavenging activity of seeds, pulp and peel of *Pouteria campechiana* in different extraction media

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