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Antioxidant Properties of Three Banana Cultivars (*Musa acuminata* '*Berangan*', '*Mas*' and '*Raja*') Extracts

(Ciri-ciri Ekstrak Antioksidan Tiga Kultivar Pisang [*Musa acuminata* '*Berangan*', '*Mas*' dan '*Raja*'])

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ABSTRACT

The effect of solvent type in antioxidant compounds extraction from banana tissues was studied. The solvent system used was pure methanol, ethanol, acetone and their aqueous solution at 50% and 70% concentrations. Comparison among three common cultivars of banana in Malaysia (Berangan, Mas and Raja) had been done and their antioxidant activities were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging system, ferric reducing ability in plasma (FRAP) assays and total phenolic content (TPC) assays. Acetone 70% had the strongest antioxidant compounds extraction power as compared to other solvent. All banana samples were found to be low in primary antioxidant but powerful secondary antioxidant source of fruit. The ascending order of banana cultivars in term of their antioxidant activities in all antioxidant assays carried out were Berangan < Mas < Raja. FRAP-TPC assays were highly correlated (R^2>0.70) than FRAP-DPPH and TPC-DPPH assays due to the same mechanism that occurred in the reaction of FRAP and TPC assays.

Keywords: Antioxidant; banana; extraction solvent

ABSTRAK

Kesan jenis pelarut dalam pengekstrakan unsur antioksidan daripada tisu buah pisang telah dikaji. Sistem pelarut yang digunakan termasuklah metanol, etanol dan aseton tulen dan larutan akueus masing-masing pada kepekatam 50% dan 70%. Perbandingan antara tiga kultivar pisang yang lazim terdapat di Malaysia (Berangan, Mas dan Raja) telah dijalankan dan aktiviti antioksidan ditentukan melalui ujian sistem penindasan 2,2-diphenil-1-pikrilhidrazil (DPPH), sistem keupayaan penurunan ion ferik dalam plasma (FRAP) dan jumlah kandungan fenolik (TPC). Pelarut 70% aseton didapati mempunyai kuasa pengekstrakan unsur antioksidan yang paling kuat berbanding dengan pelarut lain. Semua sampel pisang dikenal pasti sebagai sumber buah yang rendah kandungan antioksidan primer tetapi kaya dengan antioksidan sekunder. Penyusunan secara tertib menaik daripada segi aktiviti antioksidan bagi kultivar pisang yang dikaji dalam semua ujian yang dijalankan adalah Berangan < Mas daripada Raja. Ujian FRAP-TPC dilaporkan mempunyai nilai kolerasi yang tinggi (R^2>0.70) berbanding dengan ujian FRAP-DPPH dan TPC-DPPH disebabkan oleh kewujudan mekanisme yang serupa dalam tindak balas ujian FRAP dan TPC.

Kata kunci: Antioksidan; pelarut pengekstrakan; pisang

INTRODUCTION

Much attention has been focused on the activity of natural antioxidants present in fruits because potentially these components may reduce the level of oxidative stress (Hassimotto et al. 2005), i.e. preventing free radicals from damaging proteins, DNA and lipids (Isabelle et al. 2010). Besides, they are also scientifically proven for their synergistic effects and protective properties against various degenerative disorders including cancer, stroke, cardiovascular, Alzhemier's disease and Parkinson's disease (Abdel Hameed 2009; Giasson et al. 2002; Kawasaki et al. 2008; Ndhlala et al. 2006).

Bananas are one of the most consumed fruits in tropical and subtropical regions (Alkarkhi et al. 2010). These edible fruit cultivars are a man-made complex based on two wild diplod species originating from South-East Asia: *Musa acuminata* Colla (AA), which is a highly polymorphous, with spindly plants that grow in clumps, and *Musa balbisiana* Colla (BB), a homogenous hardly plant with a massive psedo-trunk (Aurore et al. 2009). Generally they are broadly grouped into two categories, namely dessert banana and plantain. In Malaysia, the most important species of dessert bananas are *Mas* (AA) and *Berangan* (AA) while for plantain, there are *Awak* (AAB), *Nangka* (ABB) as well as *Raja* (ABB) (Hassan 2004).

Bananas have been classified as one of the antioxidative foods by Kanazawa and Sakakibara (2000). These tropical fruits have strong ability to protect themselves from the oxidative stress caused by the intense sunshine and high temperature by increasing their antioxidant levels. They are known as a weak primary antioxidant source but a powerful secondary antioxidant source (Haripyaree et al.

2010; Lim et al. 2007; Yan et al. 2006). The antioxidant compounds identified in bananas include ascorbic acid, tocopherol, beta carotene, phenolic groups, dopamine and gallocatechin (Qusti et al. 2010; Someya et al. 2002).

The aim of an extraction process should be to provide for the maximum yield of substances and of the highest quality (concentration of target compounds and antioxidant power of the extracts) (Spigno et al. 2007). The solvent extraction has been widely used to extract bioactive components from plants (Musa et al. 2010). Among all the variables investigated (pre-treatment of the sample, solvent/sample ration, type of solvent, time and temperature of extraction) to ensure the efficiency of extraction, type of solvent has been the most studied factor (Spigno et al. 2007). The commonly used solvents for extracting antioxidant were methanol, ethanol and acetone either singly or in combination with aqueous solution (Alothman et al. 2009; Lim et al. 2007; Saravanan & Aradhya 2011; Wang et al. 1996 & Yan et al. 2006).

To date, no comparative study has been done on type of solvent extraction in the investigation of antioxidant activity for selected species of bananas. Exploring bananas as sources of bioactive phytochemicals offer enormous opportunities for the functional food industry. Therefore, this study aimed to determine the effect of solvent for extracting antioxidant compounds from banana tissues and to investigate the antioxidant properties of three bananas cultivars.

Materials and Methods

Chemicals

Folin-Ciocalteu phenol reagent, ferric chloride ($FeCl_3 \bullet 6H_2O$), and HCL were obtained from Merck (Darmstadt, Germany) and 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris (2-pyridyl)-*s*-triazine (TPTZ), gallic acid, Trolox and sodium acetate trihydrate were purchased from Sigma (USA). Sodium carbonate was purchased from RDH (Germany) while glacial acetic acid was from Mallinckrodt Baker (USA). All chemicals and reagents used in the study were of analytical grade.

Samples Collection and Preparation

All species of bananas with maturity index V (FAMA 2009) were collected fresh from Kajang Market in the state of Selangor, Malaysia. The maturity stage of bananas was standardized by visual (eyes) and mechanical color measurement (colorimeter, CR400, Minolta, Japan) of banana peels.

Extraction of Antioxidants

Bananas were peeled, cut into 1 cm slices and crushed in a food processor to produce uniform slurries. The slurry was prepared fresh to preserve the extracted antioxidant compounds. In the extraction process, about 1 g of banana slurries were weighed in universal bottles and 10 mL solvent was added. Solvents used were pure methanol, ethanol, acetone and their respective aqueous solution at 50% and 70% concentrations. Samples (banana slurries with solvents) were then homogenized using homogenizer (T 250, IKA, Germany) at 24,000 rpm for 1 min. All extracted samples were centrifuged by using tabletop centrifuge (MLX 210, Thermo-line, China) at 4750 *g* for 10 min. The supernatants were collected for further analysis.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Scavenging System

The determination of antioxidant activity through DPPH scavenging system was carried out according to the method of Musa et al. (2010). Stock solution was prepared by dissolving 40 mg DPPH in 100 mL methanol and kept at -20°C until used. About 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 spectrophotometer (UV 2450, Shimadzu, Japan). About 200 µL banana extracts with 3 mL methanolic DPPH solution prepared were kept overnight for scavenging reaction in the dark. An aliquot (250 µL) of samples (banana extracts with methanolic DPPH solution) and blank were then monitored at 516 nm wavelength on the next day with spectrophotometer (Epoch, Biotek, USA). Percentage of DPPH scavenging activity was determined as follow:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{A blank-A sample}}{\text{A blank}} \times 100\%,$$

where A is the absorbance

Ferric Reducing Ability in Plasma (FRAP)

The determination of antioxidant activity through FRAP was carried out according to the method of Musa et al. (2010). FRAP reagent was prepared fresh as using 300 mM acetate buffer, pH3.6 (3.1 g sodium acetate trihydrate, plus 16 mL glacial acid made up to 1:1 with distilled water); 10 mM TPTZ (2,4,6-tris (2-pyridyl)-*s*-triazine), in 40 mM HCLl; and 20 mM $FeCl_3 \bullet 6H_2O$ in the ratio of 10:1:1 to give the working reagent. About 1 mL FRAP reagent was added to 200 µL banana extracts and the absorbances were taken at 595 nm wavelength with spectrophotometer after 30 minutes. Calibration curve of Trolox was set up to estimate the activity capacity of samples. The result was expressed as mg of Trolox equivalents per 100 g of fresh sample (mg TE/100 g of FW).

Total Phenolic Content (TPC)

The determination of antioxidant activity through TPC was carried out according to the method of Musa et al. (2010). About 200 µL banana extracts was added with 0.4 mL distilled water and 0.5 mL diluted Folin-Ciocalteu reagent. The samples (banana extracts with Folin-Ciocalteu reagent) were left for 5 min before 1 mL 7.5% sodium

carbonate (w/v) was added. The absorbances were taken at 765 nm wavelength with spectrophotometer after 2 hours. Calibration curve of gallic acid was set up to estimate the activity capacity of samples. The result was expressed as mg of gallic acid equivalents per 100 g of fresh sample (mg GA/100 g of FW).

STATISTICAL ANALYSIS

Data collected were analyzed statistically using Statistical Packages for the Social Sciences (SPSS) software. Correlation analyses ware performed using Pearson's correlation coefficient (R^2). Significant level used was $p<0.05$ for all data analyzed.

RESULTS AND DISCUSSION

EFFECT OF SOLVENT TYPE ON EXTRACTION OF ANTIOXIDANTS

Solvents such as methanol, ethanol, acetone, propanol, ethyl acetate and dimethylformamide often used in the extraction of antioxidant compounds from fresh fruits/ vegetables at different concentration (Alothman et al. 2009). Solubility of antioxidant compounds in solvent was proven to have strong influence on the recovery of those compounds during the extraction processes. In other words, polarity of solvents indirectly played a vital role in extraction process since it would increase 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, screening process was important to justify the best solvent in antioxidant compounds extraction so that the maximum antioxidant activity for a certain sample could be identified.

Results indicated that the recovery of antioxidant compounds depends very much on the type and polarity of solvent used. The best solvent for DPPH assays was 70% ethanol for all banana cultivars studied (Table 1). However, the 70% acetone solvent seemed to have the best extraction power among all the solvent systems used for FRAP and TPC assays (Table 2 & 3). This observation was similar to the result reported by Alothman et al. (2009) where these authors found out that acetone 70% was the best solvent in antioxidant compounds extraction for TPC assays but second effective solvent for FRAP and DPPH assays. Saravanan and Aradhya (2011) concluded that acetone is the most powerful solvent in extracting the antioxidant compounds from banana tissues while Wang et al. (1996) used pure acetone (100% concentration) in extracting antioxidant compounds from several kinds of fruit tissues including banana for their studies. In contrast, Yan et al. (2006) used ethanol 50% in their extraction of antioxidant compounds from banana tissues. However, no screening of solvents had been carried out by two latter groups of authors thus no comparison of the efficiency of acetone and ethanol as extraction solvents could be done.

The difference in the extraction ability of solvents with several types and concentrations might be possibly due to the change in relative polarity in those solvents (Musa et al. 2010). Most of the pure solvents (acetone, ethanol and water) had weak extraction powers in this study. The better extraction power of aqueous solvent suggests that the mixing of non polar solvent and water may increase the polarity index of solvents and further enhance the extraction power of the particular solvent. This finding was consistent with the study of Musa et al. (2010) where they concluded that the increase in polarity for a solvent to a certain level (up to 50% water) will contribute to the solubility of the antioxidant compounds in solvent. This explained why the extraction power of distilled is the weakest solvent for these three types of bananas as compared to other solvents.

ANTIOXIDANT PROPERTIES OF BANANAS

Antioxidant can be generally categorized into two main groups, namely primary antioxidants and secondary antioxidants. DPPH assays often used to measure the ability of primary antioxidants in plants where these primary antioxidants react to scavenge the free radical from DPPH solution hence suppress the formation of initiation chain of free radical and destroy the propagation chain by donating hydrogen atom or electron so that the free radical can be changed to a more stable form of products (Nurliyana et al. 2010 & Yan et al. 2006). This indirectly contributes to the discoloration of DPPH solution from purple to yellow.

Table 1 shows that the DPPH scavenging percentages for *Berangan*, *Mas* and *Raja* banana were 6.2-36.8%, 4.6-48.9% and 3.2-63.1%, respectively depending on the types of solvent used. In other words, *Raja* banana had the best ability in DPPH scavenging system, followed by *Mas* and *Berangan*. This result was lower than that of the finding of Alothman et al. (2009) although same types of solvents were used (water, acetone, ethanol and methanol) where they reported that the DPPH scavenging percentages for *Mas* bananas was 24.4-72.2%. Meanwhile, Sulaiman et al. (2011) found that the order of banana cultivars according to DPPH scavenging percentages were Raja (0.68-1.65 mg TE/g FW) < Mas (0.63-1.73 mg TE/g FW) < Berangan (0.53-2.15 mg TE/g FW) by using hexane, chloroform and methanol as solvents. The difference in results obtained might possibly due to the different extraction methods and solvents (Chirinos et al. 2007; Uma et al. 2010).

FRAP assays are widely used to determine the efficiency of antioxidant compounds in plants to compete with the FRAP reagent and reduce the ferric to ferrous. Antioxidant compounds that are able to function in this approach are categorized as secondary antioxidants where they suppress the radical formation and prevent oxidative damage. In addition, secondary antioxidants are also active in metal chelating and oxygen scavenging. Reduction of ferric in FRAP reagent will lead to the formation of blue-colored-product, ferrous-2,4,6-tris (2-pyridyl)-*s*-triazine (TPTZ) complex.

TABLE 1. Mean (n = 3) percentage of DPPH scavenging activity of three different banana cultivars

Solvent	*Berangan*	*Mas*	*Raja*
Water	6.9^{e}	4.6^{d}	24.3cd
Acetone			
50%	16.9^{cd}	41.7^{ab}	62.8a
70%	36.8^{a}	42.5^{ab}	54.6^{ab}
100%	7.1^{e}	15.1^{cd}	3.2^{d}
Ethanol			
50%	15.8^{d}	43.8^{a}	42.2^{abc}
70%	27.5^{b}	48.9^{a}	63.2^{a}
100%	20.6^{cd}	19.9^{bcd}	36.2^{bc}
Methanol			
50%	36.12^{a}	35.7^{abc}	55.6^{ab}
70%	22.0^{c}	57.4^{a}	12.1^{d}
100%	6.2^{e}	41.1^{ab}	56.8^{ab}

Different alphabet within the same column indicates significant different ($p<0.05$)

In FRAP assays, *Raja* banana had highest Trolox equivalent (TE) content, 140.8-1607.2 mg TE/100 g FW, followed by *Mas* (233.6-485.8 mg TE/100 g FW) and *Berangan* (39.4-403.7 mg TE/100 g FW), again, depending on the types of solvent used (Table 2). However, Sulaiman et al. (2011) reported the higher value of TE in same cultivar of bananas except *Raja* where the TE content for *Berangan*, *Mas* and *Raja* bananas were 26-1393 mg TE/100 g FW, 98-1030 mg TE/100 g FW and 14-860 mg TE/100 g FW respectively. On the other hand, Alothman et al. (2009) found that *Mas* banana contains 0.59-3.30 µmol ferum (II)/g FW in FRAP assays.

TPC assays depend on the mechanism that involved oxidation and reduction reaction as FRAP assays. This mechanism can be correlated with the redox properties of antioxidant compounds in plants. The antioxidant compounds will react with Folin-Ciocalteu reagent and thus measure the concentration of phenolic groups (Nurliyana et al. 2010). In this study, the formation of deep blue color of sample solution indicate that the sample contain high phenolic concentration while the formation of light blue color of sample solution indicate that the phenolic content in the sample is low.

In consistent with the result from DPPH and FRAP assays, Raja bananas was reported, again, to have the higest gallic acid (GA) content in TPC assays, 254.3-2178.6 mg GA/100 g FW), followed by Mas (154.3-726.4 mg GA/100 g FW) and Berangan (58.6-767.3 mg GA/ 100 g FW) (Table 3). In contrast with the findings of Alothman et al. (2009) and Sulaiman et al. (2011), the GA content is higher in these three banana cultivars. Alothman et al. (2009) reported that *Mas* banana has 27.0-72.7 mg GA in 100 g of bananas while according to Sulaiman et al. (2011), the GA contents in *Berangan, Mas* and *Raja* banana were 13-263 mg GA/100 g FW, 14-160 mg GA/100 g FW and 20-133 mg GA/100 g FW.

TABLE 2. Mean (n = 3) ferric reducing ability in plasma (FRAP) (mg trolox equivalent/100 g of banana) of three different banana cultivars

Solvent	*Berangan*	*Mas*	*Raja*
Water	39.4^{a}	233.6^{f}	141.4^{d}
Acetone			
50%	384.9^{a}	447.3^{b}	1122.4^{b}
70%	403.7^{a}	471.1^{ab}	1607.2^{a}
100%	136.9^{bcd}	261.0^{f}	236.3^{d}
Ethanol			
50%	127.1^{cd}	360.2^{d}	165.5^{d}
70%	157.1^{bc}	403.2^{c}	441.8^{c}
100%	116.5^{d}	236.6^{f}	140.8^{d}
Methanol			
50%	165.5^{b}	305.3^{e}	275.5^{cd}
70%	160.1^{bc}	457.5^{ab}	212.3^{d}
100%	144.2^{bcd}	485.8^{a}	1002.7^{b}

Different alphabet within the same column indicates significant different ($p<0.05$)

In brief, all cultivars of bananas studied showed low DPPH scavenging percentage, but high TE and GA contents. This confirmed the antioxidant properties of bananas as a weak primary antioxidant but a powerful secondary antioxidant source of food (Haripyaree et al. 2010; Lim et al. 2007; Yan et al. 2006).

CORRELATION OF DPPH, FRAP AND TPC ASSAYS

FRAP and TPC assays showed the same trend as discussed earlier. This statement is proven by the high correlation between these two assays for three cultivars of banana studied (Table 4). In contrast, the correlations between DPPH-FRAP and DPPH-TPC assays were low. The high correlation between FRAP-TPC assays can be explained by the similar mechanism occurred in both assays (Alothman et al. 2009), i.e. oxidation and reduction (redox) reaction in the antioxidant compounds from bananas tissues. This finding was supported by Akowuah et al. (2005) and Sulaiman et al. (2011). Besides, other researchers also concluded that FRAP-TPC assays had the higher correlation as compared to other antioxidant assays, including DPPH assays (Maizura et al. 2011; Thaipong et al. 2006). Maizura et al. (2011) reported higher correlation between FRAP and TPC assays (R^2=0.91) compared to TPC and DPPH assays (R^2=0.86) when using herb and plant extract.

CONCLUSION

This result of this study showed that the type of solvent used had significant effect ($p<0.05$) on the antioxidant compounds for extraction of banana fruits. Acetone 70% was observed to exhibit the properties of good extraction ability as compared to other solvent in the antioxidant assays carried out. *Raja* banana was reported to have the highest antioxidant activity in all assays, followed by *Mas* and *Berangan*. All of the three bananas cultivars were found to be low in primary antioxidant content but high in secondary antioxidant content. The FRAP and TPC assays showed better correlation value due to the similar mechanism occurred in both assays.

TABLE 3. Mean (n = 3) total phenolic content (mg gallic acid/100 g of banana) of three different banana cultivars

Solvent	*Berangan*	*Mas*	*Raja*
Water	58.6^{h}	154.3^{f}	254.3^{d}
Acetone 50%	648.2^{b}	615.1^{b}	1436.9^{b}
Acetone 70%	767.3^{a}	726.4^{a}	2178.6^{a}
Acetone 100%	439.5^{c}	517.7^{c}	684.7^{cd}
Ethanol 50%	321.2^{fg}	519.5^{c}	376.0^{d}
Ethanol 70%	389.1^{d}	677.7^{a}	708.2^{cd}
Ethanol 100%	375.1^{de}	586.4^{b}	685.6^{cd}
Methanol 50%	304.7^{g}	447.3^{d}	478.6^{cd}
Methanol 70%	348.2^{ef}	525.8^{bc}	395.1^{d}
Methanol 100%	403.8^{d}	696.89^{a}	1139.5^{bc}

Different alphabet within the same column indicates significant different ($p<0.05$)

TABLE 4. Pearson's correlation coefficients of antioxidants activities of different banana cultivars

Correlation coefficient (R^2)	FRAP[b]	DPPH[c]
Berangan		
TPC[a]	0.89	0.34
FRAP		0.39
Mas		
TPC	0.72	0.25
FRAP		0.67
Raja		
TPC	0.94	0.51
FRAP		0.66

[a] Total Phenolic Content (TPC)
[b] Ferric Reducing Ability in Plasma (FRAP)
[c] 2,2-diphenyl-1-picrylhydrazyl (DPPH) Scavenging System

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