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Antidiabetic and Antioxidant Capacities of Local Banana Peels Extract by Using Subcritical Water Extraction Technique

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Abstract

Banana peels has been shown to possess strong antioxidant which may exhibit protective responses against reactive oxygen species through free radicals scavenging and breaking the autoxidative chain reaction and restore the 'redox homeostasis' state. Subcritical water extraction method was used by using water as the solvent and increasing the temperature to between 100 and 374°C and keeping the pressure high enough to maintain the liquid state allowing the dielectric constant (ϵ) of water becomes like that of an organic solvent, like ethanol or methanol. The extract was collected by using temperatures of 100 °C, 150 °C, 180 °C and 200 °C with 30, 60, 90 and 120 minutes for the investigation of antioxidative compounds and antioxidant activity. The TPC ranged widely from 20.93 to 66.39 mg GAE/g for Pisang Tanduk and 43.64 to 151.40 mg GAE/g for Pisang Cavendish peel extract. While TFC of Pisang Tanduk ranged from 1.94 to 17.19 RE/g and for Pisang Cavendish it was as low from 3.80 to as high as 72.45 RE/g. Radical scavenging activities (inhibition of DPPH) of the extracts ranged from 36.96 to 85.60% for Pisang Tanduk and from 52.26 to 93.68%. Inhibition of ABST scavenging activity showed 97.14 to 99.03% inhibition for Pisang Tanduk. For Pisang Cavendish, it showed from 73.02 to 98.86% inhibition. Although both banana peel extracts appeared to have low TPC and TFC, its antioxidant activities were ranked moderate to high. This implies that antioxidative compounds other than phenolics and flavonoids were probably responsible for inhibition of DPPH.

Keywords: *Antioxidants, Banana peel, Phenolic compounds, Non-conventional extraction, Subcritical water extraction*

Introduction

Diabetes mellitus (DM) is a metabolic disorder that involves chronic hyperglycemia and alteration of carbohydrates, proteins and lipids

metabolism causing from defects in insulin secretion, insulin action, or both (Chowtivannakul et al., 2016; Obafemi et al.,

2017). There are two major type of diabetes which are Type 1 (insulin-dependent DM) and Type 2 (noninsulin-dependent DM) (Ahmed et al., 2018). Both of it affects more than 346 million people, of which Type 2 DM dominates 90% of these cases (Obafemi et al., 2017). Other than genetic susceptibility, environmental factors, viruses and infection, Type 1 DM is mainly caused by the lack of insulin due to the impaired functionality of the β -cells in the pancreas usually as a result of autoimmune destruction (Obafemi et al., 2017). Whereas Type 2 DM is caused by a combination of genetic factors which could be related to impaired insulin secretion and/or resistance, and also environmental factors such as obesity, overeating, lack of exercise, stress as well as aging, all of which can be noticeable. Occurrence of chronic diseases were recognized to be associated with the oxidative stress, where the reactive oxygen species (ROS) and reactive nitrogen species (RNS) including free radicals were continuously produced in human cells and led to oxidative damage to cell components (Lusia Barek, Hasmadi, Zaleha, & Mohd Fadzelly, 2015). Synthetic and naturally derived antioxidants exhibit tremendous protective responses against ROS through free radicals scavenging, metals chelating, quenching of single and breaking the autoxidative chain reaction and restore the 'redox homeostasis' state to its original level (Ismail et al., 2017). Moreover, evidences showed that natural antioxidants deliver better effectiveness as compared to synthetic antioxidants (Ismail et al., 2017). In recent years, several epidemiological studies are being carried out on bioactive compounds like phenolics and flavonoids due to their numerous health benefits regarding oxidative stress such as diabetes, cancer, inflammation and other related health problems (Alara, Abdurahman, & Olalere, 2017). Banana peel have been evaluated as inexpensive sources of antioxidants and recent studies demonstrated that banana peel generally includes higher phenolic compounds than that of banana pulps (Fatemeh, Saifullah, Abbas, & Azhar, 2012). The currently accepted scientific names for most groups of cultivated bananas are *Musa acuminata*, and although they are known as a weak primary antioxidant source, but a powerful secondary antioxidant source and it is one of the world's leading food crops with a

by the elevated postprandial blood glucose levels that typically begins as insulin resistance until the pancreas slowly loses its ability to produce insulin (Ahmed et al., 2018; Obafemi et al., 2017). Meanwhile, a major obstacle in the use of insulin are either its use by injecting, its price is relatively expensive or have undesirable side effects including haematological, coma and disturbance of liver and kidney (Khalil, 2004; PB PAPDI, 2013). Limiting of diabetes without any side effects is still a challenge to the medical system (Khalil, 2004). Therefore, search for new alternatives for antidiabetic drugs derived from natural plants are becoming more attractive because they contain substances that are safer in treating DM (Obafemi et al., 2017).

great source of minerals, vitamins, carbohydrates, flavonoids, phenolic compounds etc (Schmidt, Prestes, Kubota, Scapin, & Mazutti, 2015). Other research on banana peels extract such as from *Musa acuminata* which are common in Southeast Asia, indicated that it has wide range of medicinal properties in particular the high free radical scavenging activity (Schmidt et al., 2015). Banana peels are also commonly known to be rich in potassium (K), calcium (Ca), sodium (Na), iron (Fe), manganese (Mn), Copper (Cu), bromine, rubidium, strontium, zirconium and niobium (Schmidt et al., 2015). Banana peel also represents about 40% of total weight of the fresh fruit (Anhwange et al., 2008). These large amount of plant wastes which are highly perishable are being generated by food processing industries and are estimated to be around 700 million kilograms per year and could pose serious environmental threats to society (Selvamuthukumar & Shi, 2017). The total amount of phenolic compounds in banana peel has been reported from 0.90 to 3.0 g/100g dry weight (Fatemeh et al., 2012).

In extraction of bioactive compounds from plant sources, there are numerous methods to be used, one of them being through subcritical water extraction (SWE), that provides a vast benefit compare to the conventional method (Baharuddin, Nordin, Morad, & Rasidek, 2017). In the recovery of phytochemical from plant matrix, the method of extraction is very important, for example microwave-assisted

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extraction is more effective in the recovery of higher quality phenolics and flavonoids from plant matrix in a shorter time as compared to a conventional method like soxhlet, maceration and others (Alara et al., 2017). SWE is a 'green' extraction method using water as the solvent by increasing the temperature to between 100 and 374°C and keeping the pressure high enough to maintain the liquid state (below the critical pressure of 22 MPa) and allowing the physical properties of water to be change and specifically, the dielectric constant (ϵ) of water becomes similar to that of an organic solvent, like ethanol or methanol (Yan, Cao, & Zheng, 2017). Thus, this method avoids the use of organic solvent, giving high quality extracts, high extraction yield and faster extraction procedures which has been shown to successfully employed to extract antioxidant,

phenolic compounds, anthocyanins and other functional compounds from various foods, vegetables and natural matrices such as oregano, rosemary, grape pomace, grape skin and canola meat (Amir Hamzah, Morad, Nordin, Iliya Anisa, & Yusof, 2017; Herrero et al., 2010; Hiba et al., 2014; Rodríguez et al., 2006; Yan et al., 2017). One of the most important parameters affecting SWE efficiencies is the extraction temperature for when the temperature rises, there is a marked and systematic decrease in permittivity, an increase in the diffusion rate and a decrease in the viscosity and surface tension (Asl & Khajenoori, 2013). The main apparatus of a SWE machine consists of a high-pressure pump, heater, reactor and back-pressure regulators (Machmudah, 2015).

Materials and method

Chemicals and reagents

Deionised water (dH₂O), methanol (MeOH), formic acid (CH₂O₂), aluminium chloride (AlCl₃), sodium carbonate (Na₂CO₃), Folin–Ciocalteu reagent, Gallic acid, rutin, trolox, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS).

Plant materials

Two cultivars of banana, Pisang Tanduk and Pisang Cavendish were obtained from Selangor, Malaysia. The identities of each banana were checked by morphological examination and comparison with authentic herbarium specimens and information from Musa Germplasm Information System (MGIS). The voucher numbers of specimens were deposited at the Biodiversity Unit of Institute of Bioscience, Universiti Putra Malaysia (UPM).

Sample preparation

All the cultivar was chosen with degree of skin colour, C3 (more green than yellow), according to the Von Loesecke scale (Von Loesecke, 1950). Figure 1 shows the Von Loesecke colour scale. Each cultivar of banana was washed with distilled water and peeled. Banana pulp was removed, and the remaining peel was rewashed again with distilled water and then dried at 60°C for 24h. After drying, the peels were grounded using a heavy industrial grinder that the Molecular Medicine Laboratory in the Institute of Bioscience of UPM provided. The banana peel powder was stored at -20°C in polyethylene bags prior further study. All the extraction processes were carried out in triplicate, using fruit from different hands of bananas each time.

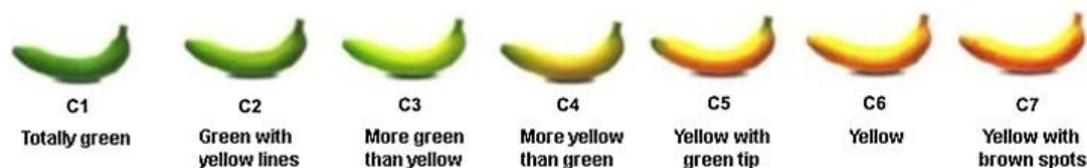


Figure 1: Banana skin colour degree using the Von Loesecke scale.

Subcritical water extraction (SWE)

By using Hawthorne, Grabanski, Martin, & Miller, 2000 with modification 50 g of banana peel powder were loaded into the vessel of the SWE machine. With a distilled water filled container being connected to the machine, the distilled water was slowly flowed through the preheating coil into the vessel through the machine at a flow rate of 20 g/min to fill the cell from bottom to top. The system outlet valve is then closed until the pressure builds to 100 bar (10 MPa) then the system was heated until 100, 150, 180 and 200 °C, each needed to be held for 30, 60, 90 and 120 minutes. The outlet valve is then used to maintain the pressure at 100 bar (still at 20 g/min) until the oven reaches the set point temperature. Once the extract was completed, the vessel was cooled with water to stop the extraction process. The extract was collected from the vessel and was filtered. The supernatant was collected and lyophilized using freeze-dryer that was provided by the Marine Laboratory in IBS. The lyophilized extract was stored at -80°C for further test.

Determination of total phenolic content (TPC)

The TPC were determined by using Folin-Ciocalteu method and Gallic acid was used as a standard equivalent by weighing 1 mg and dissolve in 1 ml of MeOH (Jaiswal & Abu-Ghannam, 2013). Sodium carbonate (Na₂ CO₃) solution was prepared by weighing 7.5 g of Na₂ CO₃ and dissolving it 100 mL of deionized water. Approximately, 60 ml of Folin–Ciocalteu reagent (Merck, Germany) were dissolved in 540 ml of deionized water (10% w/v). 500 µL of the 10% Folin–Ciocalteu reagent were added with 100 µL of banana peel extract solution/Gallic acid into a 1.5 mL centrifuge tube. Then 400 µL of 7.5% Na₂ CO₃ were added and the mixture was vortexed. The mixture was incubated at 40 °C for 1 hour under darkness. 200 µL of the mixture was pipetted into a 96 well plate from the 1.5 mL

centrifuge tube. The absorbance was measured at 765 nm using an ELISA reader. The TPC in banana peel extracts were expressed in terms of equivalent (mg of GAE/g of dried samples) using the formula;

$$C \text{ (GAE)} = QE \times \frac{V}{m}$$

Where;

QE = concentration of Gallic acid solution established from the calibration curve

V = volume of extract (ml)

m = weight (g) of the dry extract

Determination of total flavonoid content (TFC)

By using Fatemeh et al., 2012 with modification the total flavonoid content of the plant extract was determined using aluminum chloride colorimetric method and using rutin as a standard solutions. Stock solution of rutin was prepared by weighing 1 mg of rutin with 1 mL of MeOH. Aluminium chloride (AlCl₃) solution was prepared by weighing 200 mg of AlCl₃ and was dissolved in 10 mL MeOH. For the analysis, 100 µl extract solution or standard solution was mixed with 100 µl AlCl₃ solution in a 96 well plate. The mixture was incubated at room temperature for 10 min under darkness. The absorbance of the mixture was measured at 435 nm using an ELISA plate reader. Rutin was used as the standard for the quantification of total flavonoid. Results were expressed as milligrams of rutin equivalent per gram extract (mg QE/g). Total content of flavonoid was calculated as follows:

$$\text{Total flavonoid content} = QE \times \frac{V}{m}$$

Where;

QE = concentration of rutin solution established from the calibration curve

V = volume of extract (ml)

m = weight (g) of the dry extract

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DPPH (2,2 diphenyl-1-picrylhydrazyl) radical scavenging activity

Free radical scavenging activity of the plant extract was determined by using DPPH assay according to the procedure described by Basma, Zakaria, Latha, & Sasidharan, 2011, with some modifications. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) 0.002% methanolic solution was prepared in a volumetric flask covered with aluminum foil. This assay was started by mixing 195 μ l of DPPH methanolic solution (final conc. DPPH is 0.2 M) with 50 μ l of extract in a 96 well plate. The plate was swirled slowly and left standing at room temperature for 60 minutes in darkness and the absorbance was measured using ELISA plate reader at 540 nm (Clarke, Ting, Wiart, & Fry, 2013). Trolox was used for the standard control. The antioxidant activity (AOA) was presented as the percentage inhibition against DPPH and was calculated using the following equation (Okoh, Asekun, Familoni, & Afolayan, 2014):

$$\% \text{AOA} = \left[\frac{(\text{Abscontrol} - \text{Abssample})}{\text{Abscontrol}} \right] \times 100$$

Where;

$Abs_{control}$ = the absorbance of control

Abs_{sample} = the absorbance of sample

ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) scavenging activity.

Scavenging activity of ABTS assay was determined by following the procedure of Lee, Yeom, Ha, & Bae, 2010, with some

Results and discussion

Total phenolic content of extract from banana peels

Phenolic compounds are important fruit constituents because they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Maisuthisakul et al., 2007). The total phenolic was determined and

modifications. ABTS solution was prepared by dissolving 6.62 mg of potassium persulphate in 10 mL of deionized water and mixing it with ABTS solution that was prepared by dissolving 38.4 mg of ABTS in 10 mL deionized water. The mixture was prepared 16 hours before-hand and the mixture was diluted with deionized water until the absorbance reading reached 0.7 ± 0.005 using a spectrophotometer. The sample was reacted with ABTS solution by adding 100 μ L sample and 100 μ L ABTS solution in a 96 well plate. The mixture was incubated for 1 hour in the room temperature and under darkness. The absorbance was measured at 735 nm using the ELISA plate reader. Results were expressed as in comparison with standard Trolox (Almeida et al., 2011). Percent activity was calculated using the following equation (Okoh et al., 2014):

$$\% \text{AOA} = \left[\frac{(\text{Abscontrol} - \text{Abssample})}{\text{Abscontrol}} \right] \times 100$$

Where;

$Abs_{control}$ = the absorbance of control

Abs_{sample} = the absorbance of sample

Statistical analysis

All the experiments were performed in triplicate, and the results were expressed as mean \pm SD (standard deviation) and analyzed by Analysis of variance (ANOVA). Statistical analysis was performed using SPSS 17.0 and Excel 2016.

carried out using Folin-Ciocalteu method and was expressed as gallic acid equivalent. The amounts of total phenolic content of each extract from the banana peels was expressed as means \pm standard deviation of triplicate analyses and are shown in Table 1 and 2. There is variation in total phenol content from different parameters of Pisang Tanduk

cultivar ranging from 31.6 mg GAE/g dry extract weight to 69.5 mg GAE/g dry extract weight. The results showed the parameter of

200°C and 90 minutes' extract contained the highest phenolic contents in comparison to other parameters.

Table 1
Total phenolic content* of pisang tanduk.

Time	TPC			
	100 °C	150 °C	180 °C	200 °C
	mg GAE/g	mg GAE/g	mg GAE/g	mg GAE/g
30 minutes	31.64 ± 0.96	39.81 ± 1.76	35.49 ± 1.35	20.93 ± 1.01
60 minutes	31.82 ± 0.96	27.82 ± 0.27	44.37 ± 1.32	56.94 ± 2.37
90 minutes	51.96 ± 1.01	36.91 ± 1.03	44.75 ± 2.15	69.51 ± 1.60
120 minutes	40.74 ± 0.89	46.59 ± 1.64	45.68 ± 1.65	66.39 ± 1.02

*Values are means ± SD of three measurements.

Table 2
Total phenolic content* of pisang cavendish.

Time	TPC			
	100 °C	150 °C	180 °C	200 °C
	mg GAE/g	mg GAE/g	mg GAE/g	mg GAE/g
30 minutes	49.45 ± 3.84	72.93 ± 2.16	89.21 ± 3.21	57.65 ± 3.30
60 minutes	67.36 ± 2.90	70.72 ± 1.31	62.11 ± 4.49	74.60 ± 2.59
90 minutes	55.93 ± 8.48	55.03 ± 2.49	67.17 ± 8.86	113.03 ± 2.23
120 minutes	43.64 ± 5.78	73.06 ± 1.51	113.44 ± 9.00	151.40 ± 1.62

*Values are means ± SD of three measurements.

In general, this ranking shows that by extracting at a higher temperature through subcritical method, Pisang Cavendish and Tanduk peel had higher TPC than of lower temperatures. The TPC of banana peel have been reported in the literatures as high as 1.1 g GAE/100g of dry matter (Sultana et al., 2008) when extracted with ethanol, and 907 mg CEQ/100 g when extracted with water-chloroform (Someya et al., 2002) and 1.4 g GAE/100 g when extracted with methanol (Gonzalez-Montelongo et al., 2010). Through the present data, this show that phenolic content may be extracted by using the subcritical water extraction technique without using unnecessary hazardous compound such as methanol, ethanol and chloroform.

Total flavonoid content of extract from banana peels

From Tables 3 and 4, total flavonoid content (TFC) of both banana peels sample from Pisang Tanduk and Pisang Cavendish showed to increase following the increase in temperature and time. For Pisang Tanduk, in parameter of 120 minute at 200 ° C showed the highest TFC like that of Pisang Cavendish. By comparing Table 3 and 4, Pisang Cavendish peels also showed that the TFC of the banana peels extracts always demonstrated value higher than Pisang Tanduk peel extract. This finding was not consistent with the findings by Wang et al. (2009) showing that higher TFC was found in Pisang Tanduk peel extracts compared to Pisang Canvendish extract.

The difference in TFC might be due to different compounds extracted using different temperatures resulting in changing the dielectric constant of the water, thus having different solubilities. From the results of TPC

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and TFC, it was clear that Pisang Cavendish peel extracts exhibited a significantly higher

TPC and TFC content compared with Pisang Tanduk peel extracts for most parameter.

Table 3
Total flavonoid content* of pisang tanduk.

Time	TFC			
	100 °C	150 °C	180 °C	200 °C
30 minutes	3.26 ± 0.23	4.02 ± 0.43	4.85 ± 0.97	2.43 ± 0.08
60 minutes	1.94 ± 2.06	2.16 ± 0.08	5.90 ± 0.10	7.95 ± 0.16
90 minutes	2.99 ± 0.04	4.37 ± 0.19	5.86 ± 0.22	15.37 ± 0.62
120 minutes	2.06 ± 0.12	5.24 ± 0.27	5.84 ± 0.17	17.19 ± 0.68

*Values are means ± SD of three measurements.

Table 4
Total flavonoid content* of pisang cavendish.

Time	TFC			
	100 °C	150 °C	180 °C	200 °C
30 minutes	7.94 ± 1.36	8.17 ± 1.40	25.43 ± 1.96	45.13 ± 3.88
60 minutes	3.80 ± 3.80	22.43 ± 0.57	37.51 ± 2.55	56.26 ± 1.31
90 minutes	11.31 ± 1.41	19.64 ± 3.02	30.90 ± 0.88	46.25 ± 0.96
120 minutes	7.76 ± 0.57	43.58 ± 0.49	57.55 ± 1.31	72.45 ± 0.76

*Values are means ± SD of three measurements.

In general, the TFC was higher in Cavendish prepared from the green fruits had higher TFC than those obtained from the ripe fruits. In all types and stage of ripeness, it is evident that the peel always presented higher TFC than the pulp. The variation in TPC and TFC among different plant materials might be attributed to factors such as natural chemical composition, maturity at harvest, soil state and conditions of post-harvest storage (Huang et al., 2005).

DPPH radical scavenging activity

The antioxidant activity reflected by the DPPH radical scavenging assay increasing as the temperature and time increases, similar to the total phenolic content in both the extracts in Table 5 and 6. The percent inhibition of DPPH was from 36.96 to 85.60%. This suggests that the total phenolics content measured by the Folin–Ciocalteu assay may

be more important in contributing to the antioxidant activity than the physico-chemical nature of the individual phenolics in the extracts. Furthermore, it could also indicate that a critical concentration of phenolics is enough to obtain the desired antioxidant activity after which there is a saturation effect and the presence of additional phenolics does increase the antioxidant activity. The differences in antioxidant activity in a particular assay are largely a function of the ratio of hydrophilic and hydrophobic nature of phenolics. DPPH assay essentially measures the antioxidant activity of the water soluble phenolics and it is possible that the amount of hydrophilic phenolics having antioxidant activity in the DPPH assay using the two species of banana peels may be similar. In order to clarify these possibilities additional antioxidant assays were used to find the differences in phenolic profile-related antioxidant activity of these two banana peel samples.

Table 5
DPPH* of pisang tanduk.

Time	DPPH inhibition (%)			
	100 °C	150 °C	180 °C	200 °C
30 minutes	50.47 ± 1.60	50.43 ± 7.52	59.48 ± 6.23	36.96 ± 0.56
60 minutes	44.22 ± 0.84	51.72 ± 0.85	60.34 ± 0.77	70.62 ± 0.36
90 minutes	50.50 ± 2.61	53.56 ± 0.85	66.70 ± 0.09	70.62 ± 0.17
120 minutes	57.29 ± 2.04	59.02 ± 1.44	66.70 ± 2.04	85.60 ± 0.33

*Values are means ± SD of three measurements.

Table 6
DPPH* of pisang cavendish.

Time	DPPH inhibition (%)			
	100 °C	150 °C	180 °C	200 °C
30 minutes	69.04 ± 0.69	72.92 ± 0.23	94.18 ± 0.87	78.23 ± 0.67
60 minutes	69.04 ± 0.45	79.96 ± 1.04	83.78 ± 1.53	76.26 ± 0.34
90 minutes	52.26 ± 0.65	64.98 ± 0.78	70.85 ± 0.67	86.96 ± 1.86
120 minutes	63.31 ± 0.57	70.33 ± 0.54	89.94 ± 1.98	93.68 ± 0.53

*Values are means ± SD of three measurements.

ABTS free radical scavenging activity

The ability of banana peel extracts to scavenge ABTS radicals has been demonstrated (Okonogi et al., 2007). Therefore, ABTS scavenging activity of each sample was reported as the percentage of ABTS inhibition, with a higher value is associated to a stronger antioxidant activity. All extracts showed free radical scavenging properties (Table 7 and 8). The inhibition of ABTS radical of the banana peel ranged from 93.26 to 98.69%. The ABTS scavenging activity was increases as temperature increases. The extracts prepared from Cavendish and Tanduk banana peel exhibited the high scavenging activity. It is evident that the extract obtained from the peel had high antioxidant. Typically for plant materials, ABTS inhibition would follow a similar

order of the TPC and TFC, i.e. as the concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds increases, the ABTS radical scavenging activity also increases (Alothman et al., 2009; Gonzalez-Montelongo et al., 2010). However, the TPC and TFC shows low values in this study. Even though values were quite low in terms of TPC and TFC, their antioxidant activities are very high. These results imply that antioxidative compounds other than phenolics and flavonoids were also involved in inhibiting the ABTS radicals. Compounds such as ascorbic acid, β-carotene, α-carotene and different xanthophylls (Subagio et al., 1996; Kondo et al., 2005) have been detected in banana and may have contributed to the antioxidant activity of the extracts.

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Table 7
ABTS* of pisang tanduk

Time	ABTS inhibition (%)			
	100 °C	150 °C	180 °C	200 °C
30 minutes	97.31 ± 0.23	98.00 ± 5.63	98.17 ± 0.56	98.09 ± 0.51
60 minutes	97.14 ± 0.85	98.60 ± 0.53	98.69 ± 0.75	99.03 ± 1.24
90 minutes	98.17 ± 0.64	98.26 ± 0.78	98.60 ± 0.33	98.09 ± 0.17
120 minutes	97.57 ± 0.19	98.09 ± 0.84	98.69 ± 1.98	98.00 ± 0.94

*Values are means ± SD of three measurements.

Table 8
ABTS* of pisang cavendish

Time	ABTS inhibition (%)			
	100 °C	150 °C	180 °C	200 °C
30 minutes	93.26 ± 0.72	96.71 ± 0.55	98.69 ± 0.85	98.09 ± 0.24
60 minutes	90.33 ± 0.73	98.60 ± 0.46	98.08 ± 0.87	97.39 ± 3.98
90 minutes	73.02 ± 0.32	90.24 ± 0.54	98.17 ± 7.32	98.43 ± 0.43
120 minutes	79.74 ± 0.27	94.47 ± 0.48	98.69 ± 1.52	98.86 ± 0.14

*Values are means ± SD of three measurements.

Conclusion

The present study indicated that Banana peel is the potent source of novel bioactive compounds like flavonoids and polyphenols with wide range of medicinal properties in particular the high free radical scavenging activity. Total amount of phenolic compound was at the highest in 200 °C for 90 minutes and flavonoid compounds was maximum in parameter at 200 °C for 120 minutes. All Pisang Tanduk and Pisang Cavendish peel extracts that were extracted through the subcritical water extraction technique were very strong antioxidant, using DPPH and

ABTS assays. Because of antioxidant activity, Banana peel may show good biological activities and may be effective in various diseases such as DM and may also offers innovative strategies to develop natural drug and supplements against the diseases. The free radical scavenging properties of the extracts were not directly related to the TPC and TFC, suggesting the presence of other antioxidative compounds that had also contributed to the inhibition of DPPH radicals.

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