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Investigation of The Antioxidant Activity of Cinnamon Bark Extracted with Different Solvents

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Abstract

The objective of this study was to evaluate the effect of methanol, ethyl acetate, and hexane for the extraction of cinnamon bark (Cinnamomum burmanni) (CB) on the contents of the total phenolic, total flavonoid and its 1,1,-diphonyl-1-picryl-hydrazyl (DPPH) radical scavenging activity. CB samples were ground with grinder machine in one mm sieve and extracted with treatments to determine parameter measured. All CB samples were extracted using different treatments. Parameter measured were total phenolic, total flavonoid and DPPH. CB was ground and extracted by using three treatments, data collected was statistical analysis and followed with Duncant Test, The highest total phenolic content found when CB extracted with methanol with the value of 111.43 mg gallic acids equivalent (GAE)/g DW while the lowest amount detected in hexane with the amount of 57.23 mg GAE/g dry weight (DW). It was significantly different ($P<0.05$) from those extracted with ethyl acetate and hexane. There was significant ($P<0.05$) different for the content of flavonoid among all treatment groups. Methanol was the highest solvent and hexane was the lowest with the amount of 286.27 and 64.73 mg rutin equivalent (RE)/g DW respectively. There was significantly different ($P<0.05$) for scavenging activity among all treatment. Using methanol in extracting CB showed the highest scavenging activity of 69.59 % among all other treatment groups. It could be concluded that Methanol was the best solution in extracting CB.

Keywords: antioxidant; cinnamon stick; DPPH; flavonoid; phenolic

INTRODUCTION

Preservation of palm oil decanter meal (PODM) as an alternative source of feed is very crucial. This is due to PODM is easily rancid when left on open air as the oxidation causes (Afdal et al., 2012). However PODM is a good potential source of feed for animal especially for ruminant. Therefore it is needed to reduce rancid using CB, an antioxidant agent for preserving of PODM.

Antioxidant is an inhibitor compound which is used to prevent

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or inhibit the oxidation process in food. It reacts with a free active radical compound so that this compound becomes a non active radical which is relatively stable. Antioxidant donates a hydrogen ion into a free radical which has been previously formed in lipid oxidation and reconverts it to the stable fatty acids (FA) (Hamilton & Allen, 1994). In this matter, antioxidant stabilizes free radical compound by completing an electron to the free radical. Antioxidant has been used in many fields such as in the food industry, animal feed industry, medicine etc. Antioxidant can be generated in the body and it can also be found in plants or as synthetic antioxidant.

CB contains active compounds, the antioxidants, which are useful for providing protection against an oxidative damage. Hence, they are able to prevent or to reduce the rancidity. This plant mainly contains cinnamaldehyde, the major component in bark and leaf (Hasanah et al., 2003). The composition of cinnamaldehyde in the cinnamon oil is around 80 - 95 %. Cinnamon Bark extract, considered safe and having no side effects, is being used as an antioxidant in food. Most research in CB has concentrated on the bark of this plant dealing with it's chemical composition (Azima et al., 2004; Hariana, 2008; Senanayake & Wijesekera, 2003; Shan et al., 2007; R. Wang et al., 2009). However, not much information is available on the antioxidant potential of CB or effects of various types of solvent for extraction of antioxidant from this plant.

Hence, the objective of this study was to evaluate the effectiveness of methanol, ethyl acetate, and hexane for the extraction of CB bark on the properties of antioxidant activity, including the contents of the total phenolic, total flavonoid and 1,1,-diphonyl-1-picryl-hydrazyl (DPPH) radical scavenging activity.

MATERIALS AND METHODS

Cinnamon Bark was originally found at a traditional market in West Sumatra, Indonesia. It was coarsely ground in a grinder machine (Retsch SM 100, Retech GmbH & Co KG, Germany) in 1 mm sieve at Laboratory of Animal Nutrition, Faculty of Agriculture, Universiti Putra Malaysia and kept it within a plastic container pending to be further used.

Hydrochloric acids 6 M and three reagents including methanol, hexane and ethyl acetate were used to extract CB. Folin-Ciocalteu reagent, sodium carbonate, gallic acids were used to analyze the total phenolic content (Slinkard & Singleton, 1977). Rutin, $NaNO_2$, Aluminum chloride and NaOH were used to analyze total flavonoid (Zhishen et al., 1999). All chemical and reagent were bought from Sigma Aldrich, St. Louis, Missouri, USA

The solvents used to extract CB were methanol, ethyl acetate, hexane and hot water. Around 0.5 g of air dried powder of CB was placed into a 100 mL conical flask. 40 mL of three

different polarities of each solvent (hexane, ethyl acetate and methanol as different treatment) was poured, followed by 10 ml of 6 M HCl solution and stirred by using a magnetic stirrer for 30 minutes. The mixture was kept standing overnight. The next day the mixture were filtered using filter paper (Whatman No.1, England) and taken to dryness using vacuum Rotary Evaporator (Buchii, Switzerland) at 40 ^{0}C set up with the vacuum pump.

The extraction using hot water was a conventional method with slightly modification (Oliveira et al., 2009). Around five g of airdried powder of CB was put into a conical flask and added with 100 ml boiling water by using a magnetic stirrer for 15 minutes. Then, the extract was filtered over Whatman No. 1 paper and evaporated by using a vacuum rotary evaporator (Buchii, Switzerland). The crude extracts were then re-dissolved in 5ml of respective solvent to be used in test analysis.

The total amount of phenolic content of CB was determined following the Folin-Ciocalteu method (Folin & Ciocalteu, 1927). A series of concentration of gallic acid in µg/ml was prepared in distilled water. A standard curve was plotted as the absorbance at 765 nm versus the amount of standard phenolic (µg) used.

Five hundred microliter of crude extracts of CB was added to 2.5 mL of Folin-Ciocalteu reagent (diluted 1: 10) and 2.0 ml of 7.5 % of sodium carbonate (w/v). The mixture was vortex and incubated at 30 ^{o}C for 90 minutes. Then, the mixture was diluted to five times due to its concentrated mixture. The mixture was read at the absorbance 765 nm using a visible spectrophotometer (Spectronic 20 Genesys, USA). All samples were prepared in triplicate and in dark condition. The amount of total phenolic compound was calculated as mg of GAE from the calibration curve of gallic acid standard solution and expressed as mg gallic acid equivalent (GAE) /g dried weight (DW) of the sample material.

The amount of total flavonoid in the extracts was determined according to aluminum chloride colorimetric assay (Verzelloni et al., 2007; Zhishen et al., 1999). A series of concentration of rutin in µg/ml was prepared in distilled water. A standard curve was plotted as the absorbance at 510 nm versus the amount of standard flavonoid (µg) used. An aliquot (0.1 ml) of extracts was added to 0.3 ml 5% $NaNO_2$. After 5 min, 0.3 mL of 10% $AlCl_3$ was added. At 6 min, 2 ml 1 M NaOH was added and the total volume was made up to 5 ml with distilled water. The solution was mixed well and the absorbance was measured at 510 nm.

The determination of free radical scavenging activity of CB extracts was followed a method of Mishra et al., (2012). In brief, 0.1 mM solution of 1,1-diphenyl-2-picryl-hydrazil (DPPH) in methanol was prepared and 3 mL of this solution was added to 1 mL of each extracts with different concentrations (100,150, 200,250 and 300 µg/mL). The mixture was shaken

vigorously for a few second and incubated at room temperature for 30 min. Then the absorbance was measured at 517 nm using a visible spectrophotometer (Spectronic 20 Genesys, USA). The lower absorbance of the reaction mixture indicates higher free radical scavenging activity which calculated by the DPPH radical concentration using the following equation:

$$\text{Free radical scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100\%$$

Where A_0 was the absorbance of the control (methanol+DPPH) and A_1 was the absorbance of the sample. The respective extracts were analyzed for DPPH radical scavenging activity. The experimental design was a Completely Randomized Design with 5 treatments and three replications. Treatments included extraction of CB using methanol, hexane and ethyl acetate and two antioxidant controls butylated hydroxytoluen (BHT) and vitamin E for each treatment of A, B, C, D and E.

One ANOVA followed by Duncan test were done for statistical analysis. All statistical tests were conducted at the 95% confidence level using a SAS program version 9.2 (SAS Institute Inc., Cary, NC). Proximate analysis including dry matter, ash, organic matter, crude protein and ether extract was done according to the procedure of AOAC (A.O.A.C., 1990). Analysis of fiber content including neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin followed procedure Soest, (1990). Mineral analysis together with Cr, Fe, Mn, Ni and Cu was analyzed using atomic absorption spectrometry (AAS).

RESULTS AND DISCUSSIONS

Phenolic compounds are usually distributed throughout the plant kingdom. It is a group of aromatic compound having one or more hydroxyl group. It is also known as carboxylic acids. This compound is an important ingredient of food products of plant origin and it is directly related to the sensory CBteristic of foods such as flavor, astringency, and color (Ribéreau-Gayon et al., 2006). Phenolic is also categorized as primary antioxidant. Pokorný, (2001) explained that phenolic, an antioxidant, deactivates the lipid free radical and stabilizes hydroperoxide. It prevents the decomposition of hydroperoxides into free radicals. The phenolic content of a recovery extract depends on the solvent used in extracting of the plant samples.

The phenolic content of CB in this experiment was shown as mg equivalent gallic acid (GEA)/g dry weight (DW) of sample by referring to a standard curve of gallic acid $y = 0.0055x + 0.0835$, $R^2 = 0.9926$ (Figure 1).

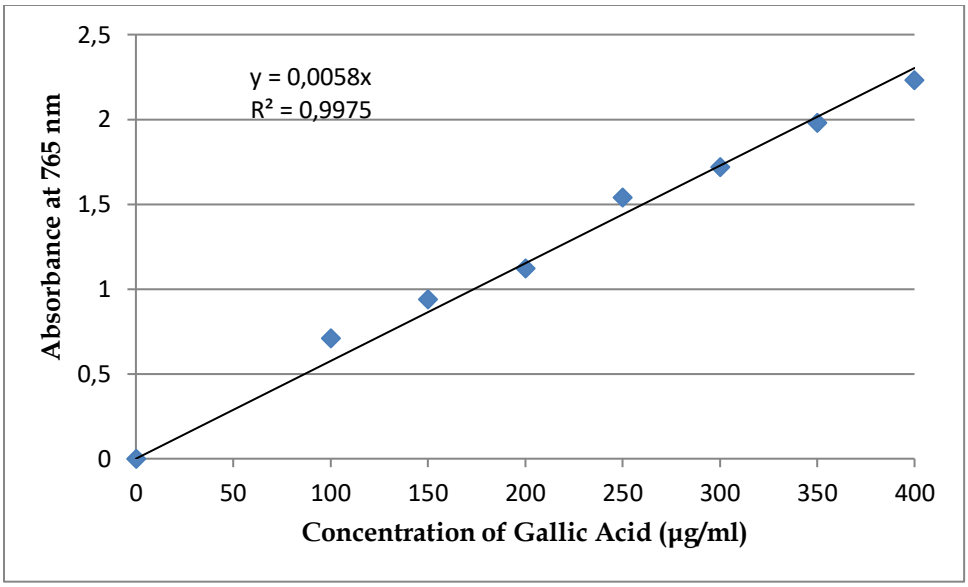

Figure 1. Standard curve of total phenolic for *Cinnamomum burmannii* extracts

The total phenolic content of CB extracted by different solvents presented in Table 1. The highest total phenolic content found when CB extracted with methanol with the value of 111.43 mg GAE/g DW while the lowest amount detected in hexane with the amount of 57.23 mg GAE/g DW. The methanolic extract showed significantly different ($P<0.05$) from those extracted with ethyl acetate and hexane. This can be explained by high polarity of methanol compared to other solvents. Phenolic contents and antioxidant activities varied considerably as a function of solvent polarity (Trabelsi et al., 2010). Trabelsi et al., (2010) also found that the pure methanol provided the highest content of phenolic in extracting *Limoniastrum monopetalum* leaves. This finding is in agreement with Hall, (2001) who stated that methanol was the best solvent for extracting phenolic materials. The content of phenolic of CB followed the order of methanol > ethyl acetate > water > hexane.

Falvonoid is also categorized as a natural compound. It consists of many kinds of chemically phenolic structures. It is usually found in fruits, vegetables, cereals, barks, roots, stems and flower. Flavonoid is a part of phenolic compound having the antioxidant properties like the vitamin A, E and C (Boots et al., 2008; Valko et al., 2006). Flavonoids are one of the main phenoliCB studied, due to their documented potent antioxidant activities (Rice-Evans et al., 1996), some are more potent than the well known antioxidant vitamins (Razali et al., 2012).

The flavonoid content of this experiment was evaluated by using aluminum chloride colorimetric method, and the results were expressed as mg rutin equivalents / g DW of sample by referring to a standard curve of rutin $y = 0.0022x + 0.0213$, $R^2 = 0.9966$ (Figure 2).

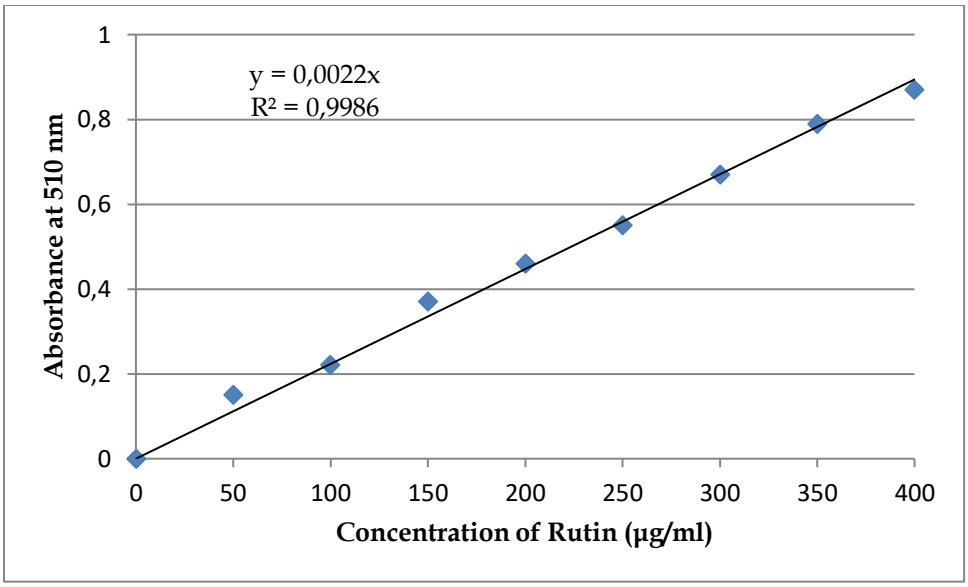

Figure 2. Standard curve of total flavonoid for *Cinnamomum burmannii* extracts

Table 1. Total phenolic, flavonoic and DPPH of CB extracted by different solvents

Solvent	Phenolic (mg GAE/g DW)	Flavonoid (mgRE/gDW)	DPPH (%)
Ethyl acetate	86.20^{b}±0.29	87.53^{b}±0.60	8.76^{f}±0.02
Hexane	57.23^{d}±0.54	64.73^{d}±0.15	13.04^{e}±0.06
Methanol	111.43^{a}±0.58	286.27^{a}±0.72	69.59^{b}±0.24
Hot water	75.07^{c}±0.39	80.90^{c}±0.21	34.98^{c}±1.09
Vitamin E	-	-	25.43^{d}±0.25
BHT	-	-	91.85^{a}±0.02
P	<0.0001	<0.0001	<0.0001

Different superscript within the same coulomb is significantly different (P<0.05)

Table 1 shows the flavonoid content of experimental CB. There was significant (P<0.05) different on the content of flavonoid among all treatments. Methanol showed the highest flavonoid and hexane was the lowest with the amount of 286.27 and 64.73 mg/g DW respectively. This result shows methanol was the best solvent in extracting flavonoid. This result is also in agreement with Havsteen, (2002) and Hendra, (2010) who successfully extracted flavonoid from different material. Some reports show the differences and inconsistent results. Khokhar & Magnusdottir, (2002) reported that aqueous ethanol was superior to methanol and acetone for extracting flavonoids from tea. Wang & Helliwell, (2001) reported that aqueous ethanol was superior to methanol and acetone for extracting flavonoids from tea. However, Hendra, (2010) stated that among polar solvents, methanol has been often a useful compromise that permits the extraction of the majority of flavonoids. It is fascinating to

highlight that methanol is a good solvent to extract flavonoids from CB since there is no other information concerning the solvent effect on the extraction of flavonoid from CB to compare with.

The results indicate that the total flavonoids content was the dominant content of phenolic. Yanishlieva-Maslarova, (2001) reported that flavonoids constitute a large group of naturally occurring plant phenols. It might be possible that flavonoids are the major compound group among the natural phenolic compounds which several structures are well-known (Lin & Tang, 2007). The high phenolic and flavonoid contents found in CB may contribute to their antioxidant activities. The majority of natural antioxidants are phenolic compounds and the most important groups of natural antioxidants are the tocopherols, flavonoids and phenolic acids (Yanishlieva-Maslarova, 2001). This is a common statement since flavonoids or other phenolic contents that have many important effects on plants, animals and human biochemistry and physiology as well, acting as enzyme inhibitors of toxic substances, and against infection (Middleton et al., 2000).

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity is one of the popular methods to detect the activity of the antioxidant in food (Yu, 2001). The antioxidant activity is assessed by the scavenging of synthetic radicals in polar organic solvents, e.g. methanol, at room temperature. The scavenging activity of DPPH radical is followed by monitoring the decrease in absorbance at 517 nm which occurs due to reduced by the antioxidant or react with a radical species.

Table 1 shows the DPPH radical scavenging activity of experimental CB with the references of vitamin E and BHT. There was significantly different ($P<0.05$) scavenging activity among all treatment and 2 references. Using methanol in extracting CB was the highest scavenging activity of 69.59 % among other treatments. However in comparison with references, the scavenging activity of methanol extracted CB was higher than that of vitamin E but lower than that of BHT.

This finding might be related to the high content of total phenolic and flavonoid when using methanol to extract CB. The phenolic compounds exhibit the antioxidant activity as a result of their capacity to scavenge free radicals (Seyoum et al., 2006). Naczk et al., (2005) reported that the significant correlations between the total phenol content and antioxidant activity determined by DPPH and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays. This might be that phenolic, as antioxidants, has the ability to donate hydrogen ions or electron which stabilizes the radicals (Ammar et al., 2009). Although (Brettonnet et al., 2010) stated that the relationship between antioxidant activity and the phenolic content in some food was still unclear. This might be due to the existing of many kinds of phenolic compound existing on the food.

Flavonoid might be played an important role in determining the antioxidant activity. This is in agreement with (Williams et al., 2004) who stated flavonoid was an antioxidant, scavengers, in a wide range of reactive oxygen species and inhibitors of lipid peroxidation. (Ammar et al., 2009) also added that flavonoids showed to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and other various free radicals that are probably involved in several diseases. There were many researches are concerning the relationship between flavonoid and the antioxidant activities (Bors et al., 2001; Cai et al., 2006; Cotelle, 1996) with the variable result shown.

CONCLUSION

It could be concluded that methanol is the best solvent for extracting phenolic and flavonoid compounds of CB. Using methanol for CB extraction provided the highest total content phenolic or flavonoid and this result also shows the highest activity of antioxidant measured with the DPPH radical scavenging activity. Therefore methanolic extract of CB was recommended to be used as antioxidant agents, especially for preserving of animal feed.

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