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Antioxidant capacity and phenolic content of cocoa beans

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Abstract

This study investigated the antioxidant capacity and total phenolic content of cocoa beans from different countries, namely Malaysia, Ghana, Ivory Coast and Sulawesi. The antioxidant capacity of water and ethanolic extracts prepared from cocoa beans was measured by three different assays. To estimate the total phenolic content, the assay using Folin–Ciocalteu reagent was used. The water extract showed the higher value of antioxidant activity based on β-carotene bleaching assay, while the ethanolic extract showed the highest scavenging and ferric reducing activities. Ghanaian cocoa beans showed the highest antioxidant and scavenging activities, followed by Ivory Coast, Malaysian and Sulawesian. However, Malaysian and Sulawesian beans exhibited the highest ferric reducing activity, compared to the other beans. The highest phenolic content was found in Malaysian beans, followed by Sulawesian, Ghanaian and Ivory Coast. A positive correlation existed for both ethanolic ($r = 0.76$) and water extracts ($r = 0.78$) between phenolic content and ferric reducing activity. Our results showed that antioxidant capacity and phenolic content of Malaysian cocoa beans were comparable to Ghanaian, Ivory Coast, and Sulawesian beans.

Keywords: Cocoa beans; Antioxidant activity; Scavenging activity; Ferric reducing activity; Phenolic content

1. Introduction

Antioxidant components are microconstituents present in the diet that can delay or inhibit lipid oxidation, by inhibiting the intiation or propagation of oxidizing chain reactions, and are also involved in scavenging free radicals. Food such as fruits, vegetables and grains are reported to contain a wide variety of antioxidant components, including phenolic compounds. These compounds are found to be well correlated with antioxidant potential (Katalinic, Milos, Modun, Music, & Boban, 2004). Some epidemiological studies indicate a negative correlation between the intake of dietary flavonoids and coronary heart disease (Hertog, Fesken, Hollman, Katan, & Kromhout, 1993; Knekt, Jarvinen, Reunanen, & Maatela, 1996), cancer (Sun et al., 2002) and stroke (Keli, Hertog, Feskens, & Kromhout, 1996).

Phenolics or polyphenols have received considerable attention because of their physiological functions, including antioxidant, antimutagenic and antitumour activities (Kono, Shibata, Kodama, & Sawa, 1995; Saliva, Darmin, Fernandez, & Mitjavila, 1991). Cocoa bean and its products (cocoa liquor, cocoa powder, and dark chocolate) are food sources rich in phenolic compounds. Cocoa beans have a high phenolic content of about 12–18% (dry weight) in unfermented beans (Kim & Keeney, 1984). Dreosti (2000) reported that 60% of the total phenolics in raw cocoa beans are flavanol monomers (epicatechin and catechin) and procyanidin oligomers (dimer to decamer). These compounds were reported to be a potential candidate to combat free radicals, which are harmful to our body and food systems (Adamson et al., 1999; Sanbogi et al., 1998). In vitro studies demonstrated that these compounds have several biological activities, such as the ability to scav-

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enge superoxide radicals and hydroxyl radicals, reduce lipid peroxyl radicals and inhibit lipid peroxidation (Kanner, Frankel, & Granet, 1994; Salah et al., 1995; Vinson & Hontz, 1995).

Cocoa (*Theobroma cacao* L.) is an important crop in the economics of several countries such as Ghana, Ivory Coast, Nigeria, Indonesia and Malaysia. Malaysia is the fifth largest producer of cocoa beans in the world. It is one of the main producers of cocoa-based products in the world and the biggest in Asia. However, Malaysian beans are sold at a lower price compared to the West African beans, due to some weaknesses in its quality (low cocoa aroma, astringent and bitter taste). One of the factors which could cause this could be a high amount of phenolic substances. A study done by Natsume et al. (2000) reported that phenolic content in cocoa liquor varied with the country of origin. However, no report has been published on the antioxidant capacity of cocoa beans from different countries.

Studies had demonstrated that the consumption of cocoa or chocolate reduced the risk of cardiovascular disease (Keen, 2001; Osakabe et al., 1998). Moreover, extracts prepared from cocoa powder and cocoa beans were shown to exhibit antihyperglycaemic effects on streptozotocin-induced diabetic rats (Amin, Faizul, & Azli, 2004b; Ruzaidi, Amin, Nawalyah, Hamid, & Faizul, 2005). Amin, Koh, and Asmah (2004a) demonstrated that an ethanolic extract prepared from Malaysian cocoa liquor exhibited a potential in decreasing the severity of hepatocarcinogenesis in rats. A review by Duke (2000) assumed that two spoons of cocoa in a cup of water or milk could be used as a palliative treatment of Parkinson's disease, mastitis, liver diseases, sexual dysfunction, fever, cystitis, cold, burns, asthma and bronchitis, diabetes and obesity.

Thus, this study was carried out to evaluate the antioxidant capacity of cocoa beans from different countries of origin, namely Malaysia, Ghana, Ivory Coast and Sulawesi. In addition, the correlation between antioxidant capacity and phenolic content was also determined. Besides flavour quality, data on antioxidant capacity of cocoa beans would be additional information to be considered when promoting the consumption of cocoa beans.

2. Materials and methods

2.1. Cocoa beans

2.1.1. General

Raw cocoa beans (dried and fermented) from Malaysia, Ghana, Ivory Coast and Sulawesi were purchased from KL Kepong (Cocoa Products) Sdn. Bhd, Selangor, Malaysia.

2.1.2. Chemicals

β-Carotene, linoleic acid, Tween 20, butylated hydroxytoluene (BHT), 2,2-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, Tris–HCl, 2,4,6-tripyridyl-s-triazine (TPTZ) and ferulic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA); Folin–Ciocalteu reagent, sodium bicarbonate, sodium acetate, glacial acetic acid, ferric chloride ($FeCl_3 \cdot 6H_2O$), ferrous sulphate ($FeSO_4 \cdot 7H_2O$) and ethanol were from Merck (Darmstadt, Germany); chloroform was from Fisher Scientific (Loughborough, UK).

2.1.3. Preparation of extracts

Raw cocoa beans were manually deshelled before grinding. The extract was prepared according to the method of Velioglu, Mazza, Gao, and Oomah (1998). Ground cocoa cotyledons were extracted with distilled water or 70% aqueous ethanol for 2 h at 50 °C using an orbital shaker (Unimax 1010, Heidolph, Germany). The ratio between sample to extraction medium was 1:25. The mixture was filtered through a filter paper (Whatman No. 1) using a Buchner funnel. The filtrate was considered as cocoa extract and used for the antioxidant assays.

2.2. β-Carotene–linoleate bleaching assay

The antioxidant activity of cocoa extract was assayed based on the β-carotene bleaching method developed by Velioglu et al. (1998). BHT was used as the standard. β-Carotene (0.2 mg in 1 ml chloroform), linoleic acid (0.02 ml) and Tween 20 (0.2 ml) were transferred into a round bottomed flask. The mixture was then added to 0.2 ml of cocoa extract or standard or ethanol (as control). Chloroform was removed at room temperature under vacuum at reduced pressure using a rotary evaporator (Unimax 1010, Heidolph, Germany). Following evaporation, 50 ml of distilled water was added to the mixture, then shaken vigorously to form an emulsion. Two millilitre aliquots of the emulsion were pipetted into test tubes and immediately placed in a water bath (Techne, Duxford Cambridge, UK) at 50 °C. The absorbance was read at 20 min intervals for 2 h at 470 nm, using a SECOMAM Anthelie Advanced 5 spectrophotometer. Degradation rate (DR) was calculated according to first order kinetics, using the following equation based on Al-Saikhan, Howard, and Miller (1995):

$$\ln(a/b) \times 1/t = \mathrm{DR}_{\mathrm{sample}} \text{ or } \mathrm{DR}_{\mathrm{standard}}$$

where ln is natural log, a is the initial absorbance (470 nm) at time 0, b is the absorbance (470 nm) at 20, 40, 60, 80, 100 or 120 min and t is the initial absorbance (470 nm) at time 0.

Antioxidant activity (AA) was expressed as percent of inhibition relative to the control, using the following formula:

$$\mathrm{AA} = \left(\frac{\mathrm{DR}_{\mathrm{control}} - \mathrm{DR}_{\mathrm{sample\ or\ standard}}}{\mathrm{DR}_{\mathrm{control}}}\right) \times 100$$

2.3. 2,2-diphenyl-2-picrylhydrazyl radical scavenging assay

The scavenging activity was estimated according to the method of Lai, Chous, and Chao (2001). An aliquot of cocoa extract (200 μl, 0.62–496 mg/ml in ethanol) or ascorbic acid (standard) (0.16–1.28 mg/ml) was mixed with

100 mM Tris–HCl buffer (800 μl, pH 7.4). Then 1 ml of 500 μM DPPH previously prepared in ethanol was added. The mixture was shaken vigorously and left to stand for 20 min at room temperature in a dark room. Absorbance was read using a spectrophotometer at 517 nm. The scavenging effect on of the DPPH radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \left(1 - \frac{\text{Absorbance of sample at 517 nm}}{\text{Absorbance of control at 517 nm}}\right) \times 100$$

EC_{50} value was determined from the plotted graph of scavenging activity against the concentration of cocoa extracts, which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Triplicate measurements were carried out, and their scavenging effect was calculated based on the percentage of DPPH scavenged.

2.4. Ferric reducing/antioxidant power (FRAP) assay

FRAP assay was determined based on the reduction of Fe^{3+}-TPTZ to a blue coloured Fe^{2+} TPTZ (Benzie & Strain, 1996). The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1, at to 37 °C. FRAP reagent (3 ml) was pipetted into test tubes. A total of 100 μl of sample and 300 μl of distilled water was then added to the same test tubes, and incubated at 37 °C for 4 min. Each sample was run in triplicate. Absorbance was measured at 593 nm. The FRAP value was calculated using the equation described by Benzie and Strain (1996). In the FRAP assay, the antioxidant potential of sample was determined from a standard curve plotted using $FeSO_4 \cdot 7H_2O$ at a concentration range between 200 and 1000 μM.

2.5. Total phenolic content

Total phenolic content was determined according to the method of Singleton and Rossi (1965). A sample (200 mg) was extracted with 2 ml of distilled water or 70% aqueous ethanol at room temperature for 2 h using an orbital shaker at 200 rpm. The mixture was centrifuged (Protech, Malaysia) at 1000*g* for 15 min. The supernatant (200 μl) was mixed with 1.5 ml of Folin–Ciocalteu reagent, and allowed to stand at room temperature for 5 min; then 1.5 ml of sodium bicarbonate solution (0.566 M) was added to the mixture. After 90 min, absorbance was read at 725 nm. Results were expressed as ferulic acid equivalents. The concentration used was in a range between 0.02 and 0.1 mg/ml.

2.6. Statistical analysis

All data were expressed as mean ± standard deviation. Data were analysed using one-way ANOVA using SPSS 11.5. Duncan's new multiple-range test was used to assess differences between means. Pearson's correlation test was used to assess correlations between means. A significant difference was considered at the level of $p < 0.05$.

3. Results and discussion

3.1. β-Carotene–linoleate bleaching

In the β-carotene bleaching assay, linoleic acid produces hydroperoxides as free radicals during incubation at 50 °C. The presence of antioxidants in the extract will minimise the oxidation of β-carotene by hydroperoxides. Hydroperoxides formed in this system will be neutralized by the antioxidants from the extracts. Thus, the degradation rate of β-carotene depends on the antioxidant activity of the extracts. There was a correlation between degradation rate and the bleaching of β-carotene; where the extract with the lowest β-carotene degradation rate exhibited the highest antioxidant activity. All extracts had a lower antioxidant activity than BHT (Figs. 1 and 2).

In this study, two extraction mediums were used for preparing the cocoa extracts. A previous study had reported that antioxidant activity and the yield of phenolic content was influenced by different extracting solvents (Sun & Ho, 2005). For example, a water extract of *Terminalia chebuta* showed good antioxidant activity, compared to methanolic extracts of *Lycopersicon esculentum* (Cai, Qiong, Mei, & Harold, 2004). Moreover, from a toxicological point of view, ethanol and water are safer than acetone, methanol and other organic solvents (Oktay, Gulcin, & Kufrevioglu, 2003). The antioxidant activity of cocoa ethanolic extracts followed the order of Ghana ≈ Ivory Coast ≈ Malaysia > Sulawesi (Table 1). There was a significant difference ($p < 0.05$) between the antioxidant activity of Sulawesian beans and other beans. No significant difference was found between Ivory Coast and Ghanaian beans. Malaysian cocoa extract showed a significant difference ($p < 0.05$) in antioxidant activity compared to the all other extracts except for Ivory Coast.

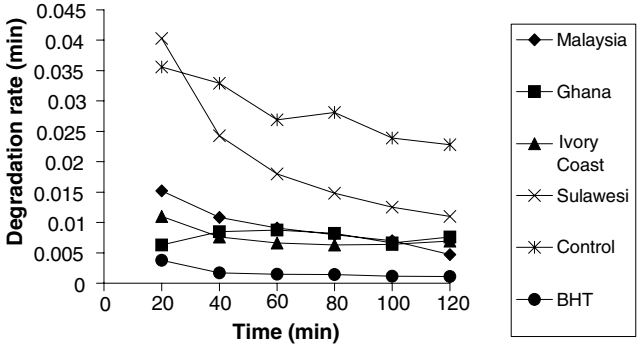

Fig. 1. Degradation rate of raw cocoa bean (ethanolic extract) assayed by β-carotene bleaching method ($n = 3$). Concentration sample was 0.04 g/ml (40 000 ppm). BHT at 200 ppm was used as the standard. Coefficients of variance (CV) are less than 11%.

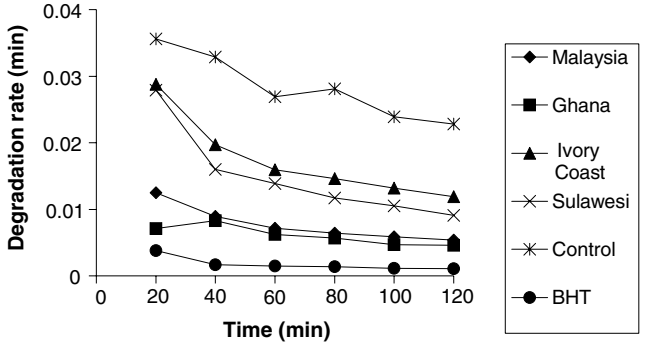

Fig. 2. Degradation rate of raw cocoa bean (water extract) assayed by β-carotene bleaching method ($n = 3$). Concentration of sample was 0.04 g/ml (40 000 ppm). BHT at 200 ppm was used as the standard. Coefficients of variance (CV) are less than 18%.

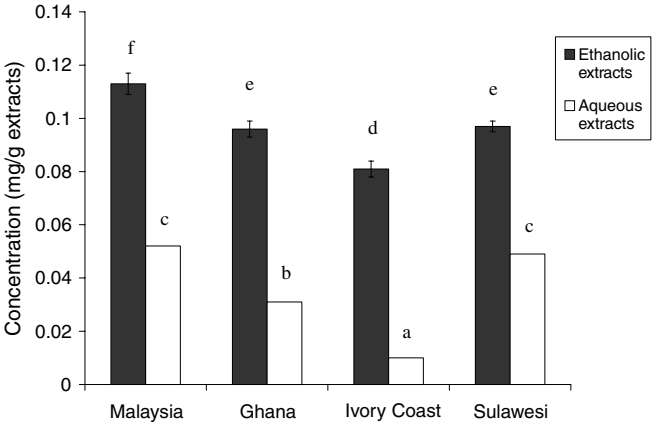

Fig. 3. Total phenolic content of cocoa beans extract. Concentration of sample was 0.01 g/ml. Results are expressed as ferulic acid equivalents. Values are expressed as mean ± standard deviation ($n = 3$). Means with different letters were significantly different at the level of $p < 0.05$.

Table 1
Antioxidant activity (%) of raw cocoa beans assayed by β-carotene–linoleate bleaching

Countries of origins	Antioxidant activity (%)	
	Ethanol	Water
Malaysia	67.6 ± 1.8d	72.7 ± 1.5bc
Ghana	74.1 ± 2.5bc	74.9 ± 2.4b
Ivory Coast	71.02 ± 1.53cd	83.69 ± 1.07a
Sulawesi	26.1 ± 1.8e	51.4 ± 3.0f

Concentration sample was 0.04 g/ml. Values are expressed as mean ± standard deviation ($n = 3$). Means with different letters were significantly different at the level $p < 0.05$.

Water extracts of Ivory Coast cocoa were significantly higher ($p < 0.05$) in antioxidant activity, followed by Ghana, Malaysia and Sulawesi. However, analysis of variance showed no significant difference between Ghanaian and Malaysian beans. All water extracts of cocoa beans showed higher antioxidant activity compared to ethanolic extracts. Water-soluble antioxidant compounds of cocoa beans seem to inhibit the oxidation of β-carotene in a β-carotene–linoleate system better than compounds soluble in ethanol. This finding was supported by a study on mushroom (*L. edodes* and *V. volvacea*), which showed water extracts having higher antioxidant activity than methanolic extracts (Cheung, Peter, & Vincent, 2003). Amin and Tan (2002) reported that water extracts of seaweeds (*Laminaria* sp.) had higher antioxidant activity, compared to ethanolic extracts. Antioxidant components present in water extracts such as amino acids, phenolic acids and flavanoids in propolis (Nagai, Reiji, Hachiro, & Nobutaka, 2003), pyrocatechol in nettle (Gulcin, Irfan, Oktay, & Mehmet, 2004), catechin in tea, wine, grape, apple, pear and peach (Dreosti, 2000; Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994; Spanos & Wrolstad, 1990; Cheng & Crisoto, 1995) have high antioxidant activity. In cocoa beans, antioxidant compounds soluble in water could be (−)-epicatechin, (+)-catechin, and quercetin (Sanbogi, Suzuki, & Sakane, 1997). Other factors that also influence the antioxidants activity are antioxidants concentration, extraction medium, temperature, pH of medium (Gazzani, Papetti, Massolini, & Daglia, 1998), chemical structures and position in the molecule (Prior, Wu, & Schaich, 2005).

The total phenolic content of the cocoa bean is shown in Fig. 3. Malaysian beans had the highest phenolic content, followed by Sulawesi, Ghana and Ivory Coast for both water and ethanol extracts. Several studies showed a correlation between antioxidant activity and phenolic content (Nagai et al., 2003; Velioglu et al., 1998; Yang, Lin, & Mau, 2002). However, based on β-carotene bleaching assay, the study showed no correlation between antioxidant activity and phenolic content for both extracts. Our finding is in agreement with Amarowicz, Wanasundara, Wanasundara, and Shahidi (1993), who reported that flaxseed with the lowest phenolic content, exhibited the highest antioxidant activity. In addition, the antioxidant activity of pecan, cashew nut (Tsuda, Makino, Kato, Osawa, & Kawakishi, 1993) and buckwheat (Sun & Ho, 2005) was inversely correlated with phenolic content. Based on the β-carotene bleaching assay, phenolic compounds of cocoa beans weakly inhibited the oxidation of β-carotene by hydroperoxides. A high antioxidant activity could also be due to other compounds besides phenolics which are soluble in water and ethanol.

3.2. Scavenging activity on 2,2-diphenyl-2-picrylhydrazyl radical

The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity. DPPH is one of the compounds that possess a proton free radical and shows a maximum absorption at 517 nm. When DPPH encounters proton radical scavengers, its purple colour fades rapidly. This assay determines the scavenging of stable radical species of DPPH by antioxidants.

For ethanolic extracts, Ghanaian beans showed the highest scavenging effect, whereas Sulawesian beans exhibited the lowest activity among the beans (Fig. 4). Ivory Coast beans exhibited a slightly higher scavenging effect than Malaysian beans. However there was no significant difference between these beans.

The scavenging activity of aqueous extracts was in the order of Ghana > Malaysia > Ivory Coast > Sulawesi. There were significant differences ($p < 0.05$) among the beans. Malaysian beans exhibited a significant higher ($p < 0.05$) scavenging effect compared to Ivory Coast beans (Fig. 5).

The scavenging activity of both cocoa extracts on DPPH radicals rapidly increased from 0.62 to 2.5 mg/ml. Results showed that scavenging activity was increased as the concentration of extract increased until a plateau was reached after 2.5 mg/ml. At a concentration of 4.96 mg/ml, ethanolic extracts showed higher scavenging activity than aqueous extracts (Figs. 4 and 5). A study by Cheung et al. (2003) on mushroom (*V. volvaco*) found the scavenging activity of methanolic extracts was significantly higher than aqueous extracts.

EC_{50} value was determined from the plotted graph of scavenging activity against the concentration of cocoa extracts, which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50% (Table 2). The lowest EC_{50} indicates the strongest ability of the extracts to act as DPPH scavengers. For ethanolic extracts, Malaysian and Ghanaian beans had the same EC_{50}, which was slightly lower than Ivory Coast beans. However, no significant difference existed between these beans. The EC_{50} value of Sulawesian beans for both ethanolic and water extracts was not obtained.

Of the water extracts, Ghanaian beans showed the lowest EC_{50}, compared with the other beans. Malaysian beans had a significantly higher ($p < 0.05$) EC_{50} compared to Ghanaian beans. Analysis of variance revealed a significant difference ($p < 0.05$) between EC_{50} values of ethanolic and water extracts. Sun and Ho (2005) reported a significant correlation between total phenolics and scavenging ability of buckwheat extracts on DPPH radicals. However, our study showed no correlation between scavenging ability and total phenolics. This finding was similar to the results obtained from the β-carotene bleaching assay. A study by Yu et al. (2002) found no correlation between scavenging activity and the total phenolic content. Our results indicated that high scavenging ability on DPPH radicals could not be due to phenolic compounds in the cocoa bean extracts.

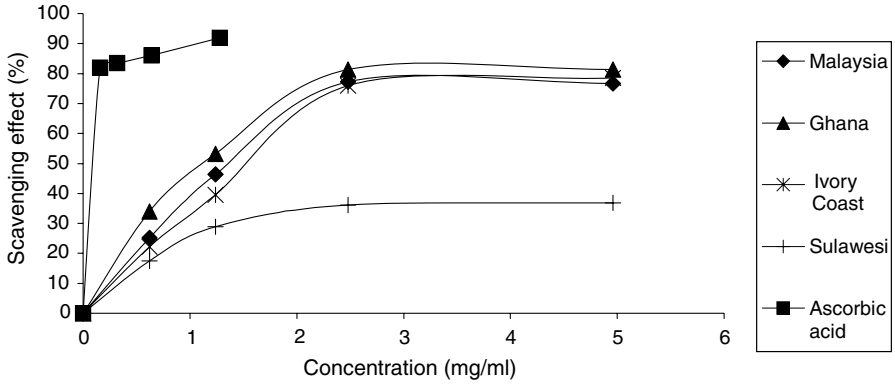

Fig. 4. Scavenging effect of ethanolic extract on DPPH radicals. Values are expressed as mean ± standard deviation ($n = 3$). Ascorbic acid was used as the standard. Coefficients of variance (CV) are less than 12%.

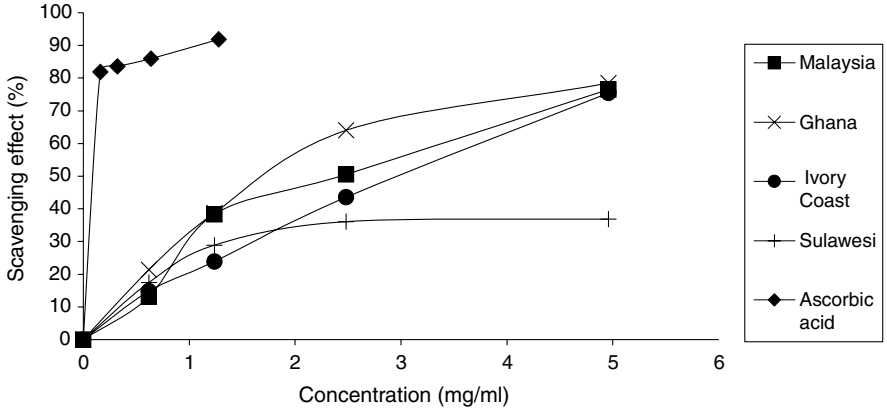

Fig. 5. Scavenging effect of water extract on DPPH radicals. Values are expressed as mean ± standard deviation ($n = 3$). Ascorbic acid was used as the standard. Coefficients of variance (CV) are less than 20%.

Table 2
Scavenging activity (EC_{50}) of cocoa beans on DPPH radicals

Countries of origins	EC_{50}(DPPH) mg/ml	
	Ethanolic extracts	Water extracts
Malaysia	1.3 ± 0.01b	2.4 ± 0.1d
Ghana	1.3 ± 0.01b	1.7 ± 0.01c
Ivory Coast	1.5 ± 0.1bc	2.9 ± 0.0e
Sulawesi	ND	ND

Values are expressed as mean ± standard deviation ($n = 3$). Ascorbic acid was used as a standard. EC_{50} value is defined as the amount antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Means with different letters were significantly different ($p < 0.05$, ANOVA). ND = not detected.

3.3. Ferric reducing activity based on FRAP assay

The ethanolic extract of Sulawesian beans exhibited the highest antioxidant potential among the extracts (Fig. 6), based on the FRAP assay. Malaysian and Sulawesian beans had higher antioxidant potential compared to Ghanaian and Ivory Coast beans. For water extracts, Sulawesian beans also exhibited the highest antioxidant potential, followed by Ghanaian, Malaysian and Ivory Coast. All water extracts exhibited significant difference ($p < 0.05$) in antioxidant potential, except for Ghanaian and Malaysian beans.

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex and producing a coloured ferrous tripyridyltriazine (Fe^{2+}–TPTZ) (Benzie & Strain, 1996, 1999). Generally, the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain through donating a hydrogen atom (Gordon, 1990; Duh, Du, & Yen, 1999). According to Benzie and Strain (1996), the reduction of Fe^{3+}–TPTZ complex to blue coloured of Fe^{2+}–TPTZ occurs at low pH.

Sulawesian and Malaysian beans are well known to have a low cotyledon pH, while Ghanaian has medium pH, and

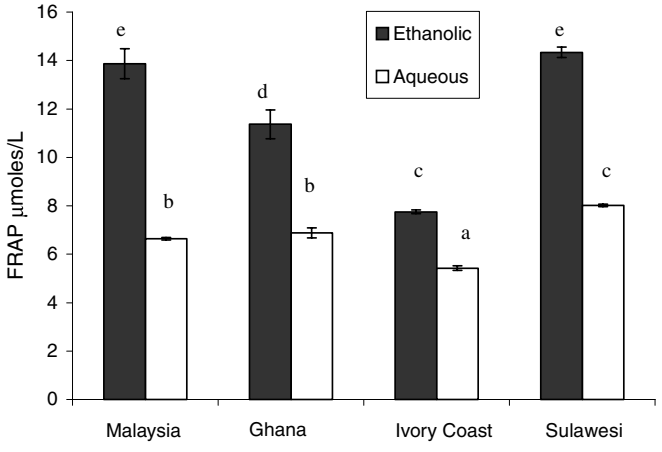

Fig. 6. Antioxidant potential of cocoa extracts assayed by FRAP assay. Concentration of sample was 0.04 g/ml. Values are expressed as mean ± standard deviation ($n = 3$). Means with different letters were significantly different at the level of $p < 0.05$.

Ivory Coast has high pH (Misnawi, Jinap, Nazamid, & Jamilah, 2002). The highest antioxidant potential of Malaysian beans could be due to the highly acidic (low pH) nature of the bean cotyledon, which many influence the pH of the assay medium.

In addition, there was positive correlation between FRAP assay and phenolic content for both the ethanolic ($r = 0.764$) and aqueous extracts ($r = 0.782$). This result was in agreement with Benzie and Stezo (1999), who found a strong correlation between total phenolic content and FRAP assay. Gardner, White, McPhail, and Duthie (2000) have assessed the synthetic free radical potassium nitrosodisulfonate (by using electron spin resonance) and Fe^{3+} (by using FRAP) and found a strong correlation with phenolic content. In this study, phenolic compounds of cocoa beans exhibited high reducing power on Fe^{3+}–TPTZ. Rice-Evans, Miller, and Paganga (1997) reported that phenolic compounds have redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. The redox potential of phenolic compounds plays an important role in determining the antioxidant capacity (Rice-Evans et al., 1997). Analysis of variance showed that ethanolic extracts of cocoa beans had significant stronger ($p < 0.05$) reducing power than water extracts. This could be due to the high amount of total phenolics present in ethanolic extracts, compared to aqueous extracts. Cheung et al. (2003) reported that the amount of phenolic compounds in organic extracts was higher than in water extracts.

Our results revealed that the antioxidant capacity and phenolic content of Malaysian cocoa beans was comparable to Ivory Coast, Ghanaian and Sulawesian beans, indicating that Malaysian beans have a similar antioxidant capacity to other beans. The extracting solvent significantly affected the total phenolic content and antioxidant capacity of cocoa beans. Ethanolic extracts showed the highest antioxidant capacity when determined by the DPPH and FRAP assays, while aqueous extracts showed the highest antioxidant activity when evaluated by the β-carotene bleaching assay. Moreover, the highest total phenolic amount was found in the ethanolic extracts. Based on the antioxidant assays, it is thus suggested that phenolic compounds present in cocoa extracts have strong scavenging ability and ferric reducing power rather than β-carotene-bleaching activity. This could be due to the antioxidant mechanisms of phenolic compounds towards free radicals. Beside phenolic compounds, the presence of methyl xanthine (theobromine and caffeine) and anthocyanins in cocoa beans might influence the antioxidant capacity. In addition, these compounds are miscible in water or water–ethanol.

However, there are several methodological limitations for antioxidant determinations (Kaur & Kapoor, 2001). The most widely used methods for measuring antioxidant activity are those that involve the generation of radical species, where the presence of antioxidants determines the disappearance of radicals (Cao, Alessio, & Cutler, 1993). It is pertinent to use different assays in an efficient extraction

medium, instead of relying on a single assay to assess and compare the antioxidant capacity.

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