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Assessment of Antioxidant Capacity and Phenolic Content of Selected Commercial Beverages

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ABSTRACT

This study was aimed at assessing the antioxidant capacity and phenolic (free, bound, and total) contents in selected commercial beverages. Three different types of beverages commonly available in Malaysian supermarkets namely, cocoa, coffee and tea were selected. Phenolic contents were determined using a Folin-Ciocalteu assay. Antioxidant capacity (ferric reducing power and scavenging activity) was determined using FRAP and TEAC assays. Based on analysis of variance, coffee showed the highest amount of free phenolic compounds and antioxidant capacity compared to cocoa and tea ($p < 0.05$). The major phenolic compound detected in coffee was chlorogenic acid. Cocoa showed higher phenolic content than tea. However, cocoa and tea have similar catechin content and possessed comparable antioxidant capacity. The free phenolic content in the three beverages was found to be highly correlated with antioxidant capacity. In addition, moderate correlation was observed between total phenolic content and antioxidant capacity. On the other hand, there was no significant contribution of bound phenolic compounds towards antioxidant capacity. The contribution of antioxidant capacity in these beverages could be due to phenolic compounds in the free form. The study indicated that the beverages studied possessed varying degrees of antioxidant capacity and phenolic contents.

INTRODUCTION

Phytochemicals in foods, such as phenolic compounds, are a group of important components which have been reported to have the ability to reduce the risk of chronic diseases (Steinmetz & Potter, 1996). Fruits, vegetables, beverages, legumes and grains are rich in polyphenols (Pellegrini *et al*., 2003; Pulido, Hernandez-Gracia & Saura-Calixto, 2003; Adom, Liu & Sorrells, 2003). Epidemiological studies showed a negative correlation between the increased intake of fruits and vegetables and cardiovascular diseases and certain cancers (Sujatha, 2003; Knekt *et al*., 2002). Flavonoids belong to a polyphenol class commonly and widely distributed in our diet. They are reported to decrease certain risk factors for cardiovascular disease through different mechanisms such as by reducing LDL oxidation,

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free radical-quenching or metal chelating abilities (Wiseman, Balentine & Frei 1997). Geleijnse *et al.* (2002) and Arts *et al.* (2001) reported an inverse relationship between flavonoids (i.e., catechin) and ischemic heart disease.

Recently, Saura-Calixto & Goni (2006) reported that 68% of total dietary antioxidant capacity in the Spanish diet is derived from beverages. Beverages are considered to be important sources of phenolic compounds including phenolic acids. Several studies have found tea to be rich in catechin (Richelle, Tavazzi & Offord 2001); catechins, procyanidins, and anthocyanidins in cocoa and chocolate (Hammerstone, Lazarus & Schmitz, 2000) and chlorogenic acid, caffeic acid, ferrulic acid, and *p*-coumaric acid in coffee (Nardini *et al*., 2002). It has been reported that the health benefits of polyphenol compounds towards certain diseases could be due to its antioxidant capacity (Mamadou *et al*., 2006).

There are various types of beverages available in the Malaysian market including fruit and vegetable juices, cocoa, coffee, and tea. However, there is a lack of studies on the antioxidant capacity and phenolic contents of these beverages sold in Malaysia. Furthermore, little data have been reported on the contributions of free, bound, and total phenolic contents present in beverages towards antioxidant capacity. Information on the antioxidant capacity and phenolic contents of beverages may be useful to consumers and the food industry. Therefore, this study aimed to assess the antioxidant capacity and phenolic contents (free, bound, and total) of selected commercially available beverages as per serving size. The correlation between antioxidant capacity and phenolic contents was also carried out.

MATERIALS AND METHODS

Beverages

Three different types of beverages, namely cocoa coffee and tea bearing international brands were purposively selected for the study. The samples were purchased from different shopping malls at Kuala Lumpur, Malaysia. These beverages were chosen as they are commonly consumed by Malaysians. All the beverages were in ready-to-mix sachets, 3-in-1 mixture except for coffee (5 in 1 mixture).

Chemicals

2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride ($FeCl_3$), ferrous sulfate heptahydrate ($FeSO_4.7H_2O$), catechin, chlorogenic acid, 2,2'-azinobis (3-ethylbenzothiazoline 6-sulfonate; ABTS), Trolox, potassium peroxodisulfide, Folin-Ciocalteu reagent, and sodium carbonate (Na_2CO_3) were purchased from Sigma Co. (St. Louis, MO, USA).

Preparation of sample

All the beverages (cocoa, coffee, and tea were prepared in serving sizes according to the manufacturer's instruction. Beverages were prepared using hot water (68°C). One sachet of beverage (20 g, except for cocoa beverages, 30 g) was torn off and infused into 200 ml of hot water. The beverage was then stirred with a glass rod for about 2 min. All the beverages were freshly prepared before analysis.

Determination of antioxidant capacity

Ferric reducing/antioxidant power (FRAP) assay

This assay followed the method described by Benzie & Strain (1996) and Katalinic *et al*. (2005). In principle, FRAP assay measures the change in absorbance

at 593 due to the formation of a blue-coloured complex that formed between ferrous ion (Fe^{2+}) and 2,4,6-tripyridiyl-s-triazine (TPTZ). Prior to this, colourless ferric ion (Fe^{3+}) was oxidized to ferrous ion (Fe^{2+}) by the action of electron donating antioxidants. Freshly prepared FRAP reagent was warmed at 37°C in a water bath which gives the initial reading ($A_{initial}$; t = 0 min). This reagent was prepared by mixing 10 mmole of 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, 20 mM $FeCl_3$ and 0.3 M acetate buffer (pH 3.6) in the ratio of 1:1:10. For sample, 100 µl of beverages (prepared as per serving size) were added to 100 µl of deionized water and 1.8 ml of FRAP reagent. The mixture was incubated at 37°C for 4 min. Absorbance was read at 593 nm using a UV-Vis Spectrophotometer (Prim, Secomam, France). FRAP value was calculated based on the following equation. A reducing ability in FRAP assay was calculated with reference to the reaction given by a $FeSO_4.7H_2O$ at concentrations ranging from 100 – 1000 µM. The values were expressed as µmol of Fe^{2+} equivalents per 200 ml beverages.

$$\text{FRAP value} = A_{final} - A_{initial}$$

A_{final} - Final absorbance at 593 nm (4 min)

$A_{initial}$ - Initial absorbance at 532 nm (0 min)

Scavenging activity assay

Scavenging activity of the beverages was determined based on the inhibition of the radical cation of 2,2'-azinobis (3-ethylbenzothiazoline 6-sulfonate; ABTS) as described by Katalinic *et al*. (2005) with slight modifications on the sample volume, increasing it from 20 µl to 100 µl. The assay was carried out at room temperature.

ABTS solution was prepared by adding 0.04 g of ABTS in 10 ml of distilled water. The ABTS radical cation ($ABTS^{\cdot+}$) was produced by dissolving initially prepared ABTS solution in 2.45 mM potassium peroxodisulfate, and the mixture was allowed to stand at room temperature for 12 h. The resulting ABTS solution was further diluted with distilled water to an absorbance of 0.70 ± 0.02 at 734 nm before use.

Firstly, 20 ml of diluted ABTS solution was added to 100 µl of beverages or Trolox as the standard, and the reaction mixture was incubated at room temperature for 6 min. Subsequently, the decrease in absorbance at 734 nm was determined using UV-Vis spectrophotometer (Prim, Secomam, France). Percentage inhibition of the radical cation $ABTS^{\cdot+}$ was calculated based on the following equation.

$$\text{Inhibition (\%)} = [(A - B) / A] \times 100$$

A - Absorbance of control (t = 0 min)

B - Absorbance of beverages/Trolox (t = 6 min)

The decrease in absorbance due to the inhibition of radical cation was determined using the standard curved plotted using a Trolox at concentrations ranging from 20 – 100 µM. The values were expressed as µmol of Trolox equivalents per 200 ml beverages.

Determination of phenolic (free, bound and total) contents

Extraction of free and bound phenolic contents

Total phenolics were determined directly from appropriately diluted beverages with distilled water. Extraction of free and bound phenolics followed the method of Adom, Liu & Sorrells (2003). Each beverage (50 ml) was centrifuged at 2,500 g for 10 min (Rotofix 32, Hettich Zentrifugen,

Germany). An aliquot (1 ml) of supernatant was transferred into 100 ml volumetric flask and then made up to 100 ml with distilled water. The mixture was used to determine free phenolic contents.

The resulting residue was used to determine bound phenolic content. The residue was digested with 5 ml of 2 M NaOH in the presence of 10 mmoles of EDTA and 1% (w/v) ascorbic acid at room temperature under nitrogen for 1 hr. EDTA and ascorbic acid were used as metal chelator to reduce degradation of bound phenolic compound. Then, the mixture was neutralized with appropriate volume of 2 M HCl. Subsequently, 5 ml of n-hexane was added to remove lipid fraction. The mixture was then transferred into a separating funnel and shaken vigorously for 3 min. The lower layer of the mixture was transferred into a beaker. The defatting process was repeated twice. The lipid-free solution was extracted 5 times with ethyl acetate (100%). Then, ethyl acetate was evaporated to dryness using a rotary evaporator (Rotavor R-200, Buchi, Switzerland). Lipid-free solution was appropriately diluted before determination of bound phenolic contents.

Folin –Ciocalteu assay

Free, bound, and total phenolic contents were determined using a Folin-Ciocalteu assay based on the formation of blue-green complexes between phenolic compounds and Folin-Ciocalteu reagent as described by Velioglu *et al*. (1998) with slight modifications by increasing the sample volume from 100 µl to 550 µl.

Firstly, 550 µl of beverage was added to 0.75 ml of diluted Folin-Ciocalteu reagent. Then, the mixture was kept at room temperature for 5 min. Subsequently, 750 µl of 6% (w/v) sodium carbonate (Na_2CO_3) was added to it. The solution was cooled at room temperature for 90 min. Absorbance was read at 725 nm using a UV-Vis spectrophotometer (Prim, Secomam, France). Catechin was used as the standard. The concentrations used in the range of 10-60 µM. Free, bound, and total phenolics were expressed as mg of catechin equivalent (CE) per 200 ml of beverages.

Determination of major phenolic compounds

Extraction of phenolic compounds

The content of major phenolic compounds was quantified in each beverage following the method described by Goto *et al*. (1996) and Nishitani & Sagesaka (2004). All beverages were extracted using water-acetonitrile (1:1, v/v) for 1 hr at room temperature. The resulting extracts were then filtered through a 0.45 µm nylon membrane filter before HPLC analysis.

Estimation of phenolic compounds

Quantification was done using a Hewlett Packard HPLC Series 1100, Agilent Technologies, USA equipped with degasser, quaternary pump, autosampler and diode array detector. A stationary reversed phase C_{18}, 250 mm x 4.6 mm I.D stainless steel column was used (Hewlett Packard, USA). Water/methanol/phosphoric acid (85:15:0.1) was used as isocratic mobile phase, and the flow rate was set at 1.5 ml/min. Beverages and standards (50 µl) were injected onto the HPLC system. The wavelength used was 280 nm and were operated at 30ºC. Chlorogenic acid and catechin with concentrations ranging from 100-200 µg/ml were used as external standards. Chlorogenic acid and catechin were calculated from the curve generated by plotting the peak area of each authentic standard versus concentration. The results were expressed as gram chlorogenic acid or catechin in each serving of the beverage.

Statistical analysis

Data were expressed as mean ± standard deviation of two replications. One-way ANOVA (SPSS version 12.0) and Tukey's post hoc test were used to determine the mean differences for antioxidant capacity, phenolic (free, bound, and total) contents, and individual phenolic contents of beverages at a significance level of $p < 0.05$. Pearson's correlation (r-value) was used to determine correlation between antioxidant capacity and phenolic contents.

RESULTS

Results in Table 1 shows that bound, free, and total phenolic contents were in the range of 0.1–2, 4-6, 10–24, and 4-7 mg CE equivalent per serving size respectively. Cocoa beverage had significantly ($p < 0.05$) highest amount of bound phenolic compounds followed by tea and coffee. Coffee had significantly higher ($p < 0.05$) free phenolic content, followed by tea and cocoa. On the other hand, cocoa had significantly higher total phenolic content compared to coffee and tea. The present study showed a significant and positive correlation ($r = 0.79$; $p < 0.01$) between free and total phenolic contents. However, no significant correlation ($r = 0.31$; $p > 0.05$) was found between bound and total phenolic contents. The major phenolic compound obtained in coffee beverage was chlorogenic acid with the range 1.01 to 1.05 mg per serving. Catechin was found as the major phenolic compound in both cocoa and tea with the range 0.4 to 0.41 mg per serving.

The antioxidant capacity was determined by reducing power and scavenging activity methods. The antioxidant capacity in coffee from both methods was significantly higher ($p < 0.05$) than those in tea and cocoa beverages. Figures 1 and 2 showed positive and significant correlations between the free phenolic contents in the beverages and the reducing power ($r = 0.77$) and the scavenging activity ($r = 0.87$). However, the bound phenolic content was inversely related to reducing power and scavenging activity ($r = -0.56$ and $r = -0.43$, respectively), while the total phenolic content showed moderate correlation with scavenging activity ($r = 0.58$) and a low correlation ($r = 0.37$, $p < 0.05$) with the reducing power.

DISCUSSION

There is accumulating evidence indicating beverages as one of the major sources of phenolic compounds in the diet (Saura-

Table 1. Free and bound phenolic content of beverages expressed as mg catechin equivalent (CE)

Type of Beverage	*Free*	*Bound*	*Major phenolic compound**	*Total phenolic Content*
Tea	4.07 ± 1.21	0.11 ± 0.06	0.41 ± 0.01	4.18 ± 1.64
Coffee	6.32 ± 1.69	0.10 ± 0.01	1.03 ± 0.03	6.43 ± 2.1
Cocoa	5.25 ± 1.53	1.96 ± 0.70	0.41 ± 0.01	7.21 ± 0.28

Values are expressed as mean ± S.E.M. Bound phenolic of cocoa was significantly different ($p < 0.05$) compared to coffee and tea. One serving is equivalent to 200 ml. Asterisk (*) indicates that the results are expressed as mg catechin for tea and cocoa and as chlorogenic acid for coffee beverages

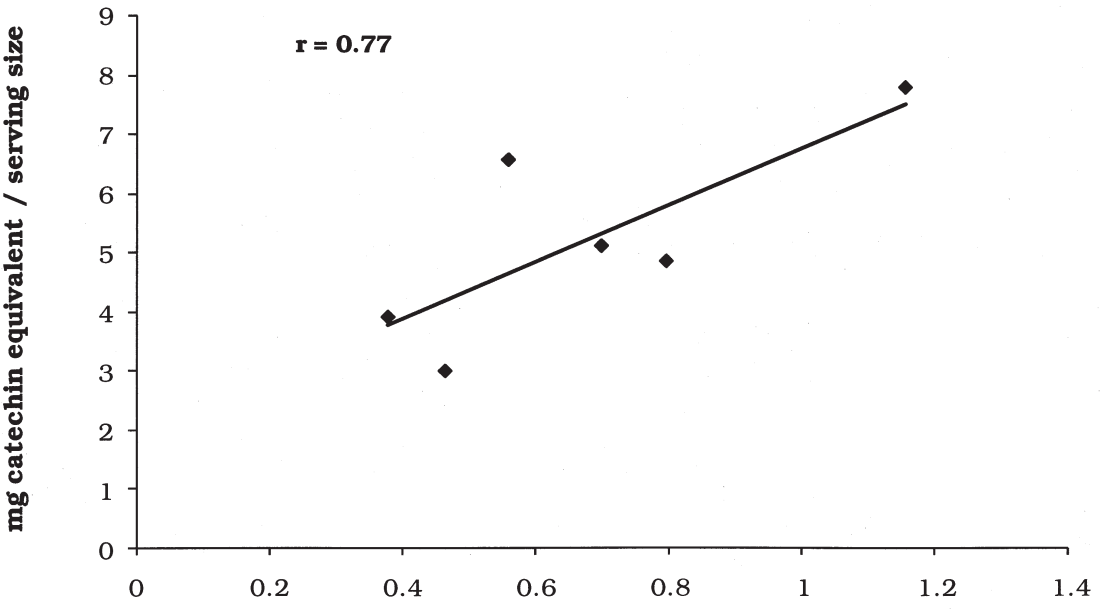

Figure 1. Relationship between free phenolic and reducing power of beverages. The correlation was significantly different at the level of $p < 0.01$

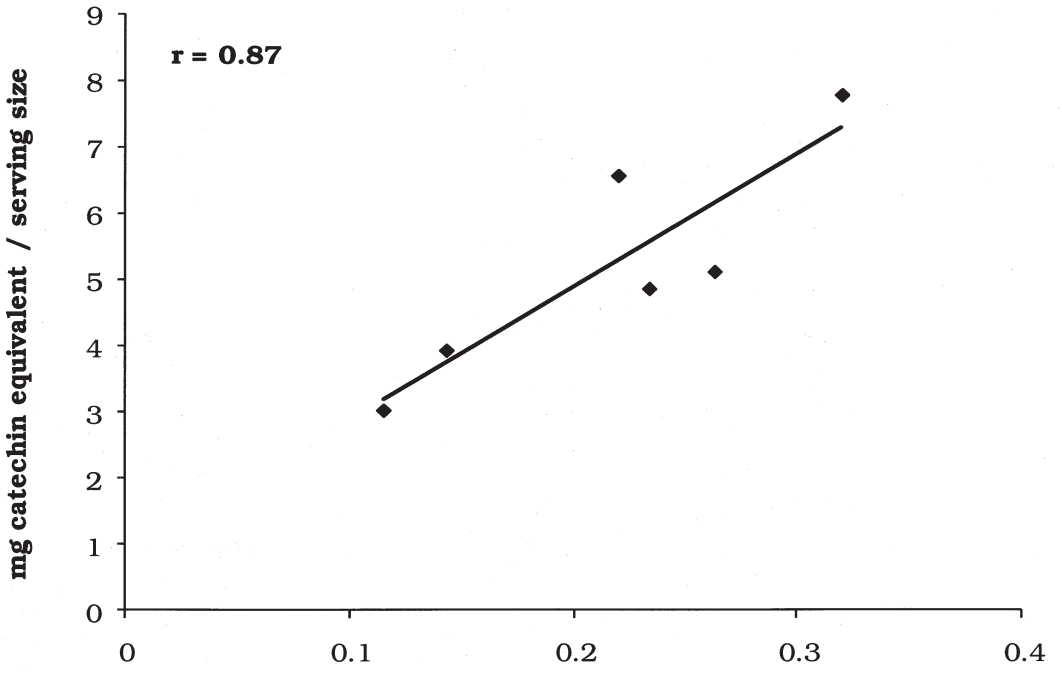

Figure 2. Relationship between free phenolic and scavenging activity. The correlation was significantly different at the level of $p < 0.01$

Calixto & Goñi, 2006; Pellegrini *et al*., 2003; Pulido, Hernandez-Gracia & Saura-Calixto, 2003). Among beverages, coffee was reported to have the highest antioxidant capacity compared to other beverages such as tea, beer, and red wine (Pulido, Hernandez-Gracia & Saura-Calixto, 2003). However, Auger *et al.* (2004) reported that tea has the highest antioxidant compound (catechin and procyanidins) compared to coffee and cocoa beverages. Adom, Liu & Sorrels (2003) reported that bound phenolic content significantly contributed towards total phenolic and flavonoids contents compared to free phenolic compounds. Furthermore, Nardini & Ghiselli (2004) showed that free forms of phenolic compounds are rarely present in plants. To date, most of the present methods do not consider the contribution of bound phenolic towards total phenolic content. Crozier *et al*. (2000) reported that most of the phenolic compounds are always found in glycosylated form, i.e., conjugated to a sugar. With that, most of phenolic compounds are present in bound form, link to cell-wall structural components, such as cellulose, lignin, and proteins through ester bonds. No study has been reported on the correlation between phenolic especially free and bound forms and antioxidant capacity.

The present study has shown that coffee exhibited the highest but insignificant amount of free phenolic content among the beverages. It is also rich in total phenolic, but relatively low in bound phenolic compounds. Phenolic content in the studied beverages could be influenced by several factors such as amount of coffee powder added and complex synergism between these compounds, as determined by Folin-Ciocalteu assay. George *et al*. (2005) reported that other reducing compounds such as vitamin C and sugar in beverages could significantly contribute to the estimation of total phenolic content.

Coffee showed the highest antioxidant capacity as measured by its reducing power (FRAP) and scavenging activity (TEAC) compared to cocoa and tea beverages (Table 2). There was a significant correlation ($p < 0.01$) between free phenolic and antioxidant capacity. However, there was no significant contribution of bound phenolic towards antioxidant capacity. The high correlation was likely due to the contribution of free form phenolics such as chlorogenic acids, which was in the range of 1.01 to 1.05 mg per serving size. However, other phenolics such as caffeic acid, *p*-coumaric acid, and ferrulic acid present in coffee could also contribute to the phenolic content (Nardini *et al*., 2002).

Chlorogenic acid is a combination of caffeic and quinic acid through an ester linkage. Although coffee is rich in chlorogenic acid, caffeic acid can also be esterified to sugar, organic acid, and lipids (Clifford, 1999). Thus, overestimation of free phenolic content in coffee could be

Table 2. Scavenging activity and reducing power in each serving of beverage

Type of Beverage	*TEAC (μmol Trolox equivalent)*	*FRAP (μmol Fe^{2+} equivalent)*
Cocoa	0.18 ± 0.02^{a}	0.47 ± 0.05^{a}
Tea	0.19 ± 0.04^{b}	0.58 ± 0.07^{b}
Coffee	0.28 ± 0.03^{c}	$0.98 \pm 0.10^{a,b}$

Values are expressed as mean ± S.E.M. Values sharing same superscripts are significantly different ($p < 0.05$) within the same column. One serving is equivalent to 200 ml.

due to other phenolic acids. Moreover, manufacturer practices such as roasting of coffee beans could lead to changes in chemical composition and biological activities of coffee bean, thus resulting in the formation of new compounds through Maillard reaction, carbohydrate caramelization, and pyrolysis of organic compounds (Daglia *et al.*, 2000). These conditions, at least in part, could influence phenolic content as well as antioxidant capacity in beverages.

The present work has shown that cocoa had a comparable amount of free phenolic compounds compared to coffee and tea. Furthermore, it exhibited significantly ($p < 0.05$) highest amount of bound and total phenolic compounds compared to the other two beverages (Table 1). It is important to note that antioxidant properties could be influenced by factors such as antioxidant concentration, extraction medium, temperature, and pH of medium (Gazzani *et al.*, 1998). Moreover, Amin, Zamaliah & Chin (2004) and Amin & Lee (2005) revealed that a thermal treatment could significantly decrease the antioxidant properties and phenolic content. These factors, at least in part, could affect the phenolic content and antioxidant capacity of beverages.

This study indicated that cocoa possessed the lowest antioxidant capacity among the studied beverages. This could be due to the types of phenolic compounds present in cocoa. Although cocoa is rich in bound and total phenolic contents, they were not found to have significant contribution towards antioxidant capacity. Furthermore, no correlation was observed between bound and total phenolic contents. Sanbogi, Suzuki & Sakane (1997) reported that free phenolic compounds present in cocoa could be (–)-epicatechin, (+)-catechin, and quercetin. Therefore, catechin in the free form could strongly contribute towards antioxidant capacity as reported by Azizah *et al.* (2007). This postulation was supported with a high correlation ($p < 0.05$) between free phenolic content and antioxidant capacity.

Numerous studies have reported that tea has significant amounts of phenolic compounds namely catechins (epicatechin, epigallocatechin, epicatechin gallate, and epigallo catechin gallate), flavonols (myricetin, kaempferol, and quercetin) and proanthocyanidins (Amra *et al.*, 2006; Ali *et al.*, 2005). In this study, tea exhibited the lowest free and total phenolic content compared to the other 2 beverages studied (Table 1). In addition, tea had bound phenolic content similar to cocoa beverage. This finding was similar to that obtained by Saura-Calixto and Goni (2006), who showed that total phenolic content of tea to be lower compared to other beverages (coffee, red wine, beer, and orange juice). This could be due to the differences in extraction temperatures and times. Recently, Amra *et al.* (2006) reported that longer extraction at high temperature could reduce the catechin content. Moreover, Jose, Joaquim & Rita (1999) reported the highest amount of catechins extracted at 70°C, and declined above this temperature in various types of tea.

Antioxidant capacity of tea was comparable to that of cocoa, and this could be related to the properties of phenolic compounds. Richelle, Tavazzi & Offord (2001) showed that antioxidant activity of beverages was related to the concentrations of antioxidant compounds present.

CONCLUSIONS

The study indicated that coffee exhibited the highest antioxidant capacity and free phenolic content compared to tea and cocoa. Cocoa had high content of bound and total phenolic content among these beverages, while tea had lower phenolic content but a similar antioxidant capacity compared to that of cocoa, probably due to their catechin content. The results also indicated that free phenolic content strongly contributed to total phenolic

content and antioxidant capacity in the beverages. No significant contribution was found between bound phenolic towards antioxidant capacity and total phenolic content. Thus, the contribution of phenolic contents to antioxidant capacity per serving size was in the order of free > total > bound phenolic compunds.

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