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Antioxidant properties of tropical juices and their effects on *in vitro* hemoglobin and low density lipoprotein (LDL) oxidations

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Abstract: Antioxidant properties of tropical juices, namely bambangan (*Mangifera pajang*), cocoa (*Theobroma cacao*) pulp and guava (*Psidium guajava*) juices and their effects on *in vitro* hemoglobin and low density lipoprotein (LDL) oxidations were determined. Total phenolics and its compounds in selected juices were determined using Folin-Ciocalteu assay and high performance liquid chromatography (HPLC), respectively. Evaluation of antioxidant properties was done using in vitro assays namely as diphenyl-1-picrylhydrazyl (DPPH) scavenging, β-carotene bleaching (BCB), hemoglobin and LDL oxidation assays. Guava and bambangan juices had the highest and lowest of total phenolic content (TPC), respectively. Nevertheless, bambangan juice showed the greatest scavenging activity on DPPH radical. Furthermore, antioxidant activity (AA) in BCB assay was in the order of cocoa pulp (79%) > bambangan (76%) > guava (47%) juices. Interestingly, the highest inhibition of malondialdehyde (MDA) formation was exhibited by bambangan juice (0.31 µM MDA) in hemoglobin oxidation, while guava juice (0.27 µM MDA) in LDL oxidation systems. The study indicated that bambangan juice may have a potential to be introduced as functional foods product because of its antioxidant properties.

Keywords: Antioxidant, polyphenols, oxidation, *Mangifera pajang*, *Theobroma cacao*, *Psidium guajava*

Introduction

Reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide and hydroxyl radical can cause damage to the nucleic acids, protein, lipids, and eventually can lead to generative diseases (Blomhoff, 2005). The imbalance of antioxidant systems can cause oxidative stress and seem to be associated with many diseases. In general, low density lipoprotein (LDL) and hemoglobin contain high amounts of polyunsaturated fatty acid (PUFA) content, which is important for biological human systems. Nevertheless, they are also susceptible to oxidative damage due to the presence of PUFA. Vendemiale *et al*. (1999) reported that the use of antioxidants, especially natural antioxidants are significant in preventing the lipid peroxidation.

It was suggested that regular consumption of fruit juices rich in polyphenols can enhance the protective effects against numerous degenerative diseases (Mullen *et al*., 2007). Moreover, drinking fruit juices was reported to reduce the risk of chronic diseases (Ruxton *et al*., 2006). Nowadays, there are many types of fruit juices available commercially in the market. The consumption of the fruit juices is increasing rapidly as they are convenient, nutritious and ready-to-drink.

Recently, the encouragement of research and development in biodiversity has resulted in the production of few new Malaysian local fruit juices. Bambangan juice is prepared from *Mangifera pajang*, one of the underutilized fruit that originate from the Borneo Island includes Sabah and Sarawak states of Malaysia. The fruit (Figure 1) has a yellow-orange pulp and its flesh that composed 60-65% of total fruit weight is used to produce juice. It has a delightful mango fragrance and very juicy. Bambangan tree can grow up to more than fifty feet high and the fruit is not only eaten ripe but also as young fruits by the local people. Previous studies reported that the fruit contained antioxidant capacity and considerable high phenolic and flavonoid contents (Khoo and Ismail, 2008; Abu Bakar *et al*., 2009; Ikram *et al*., 2009).

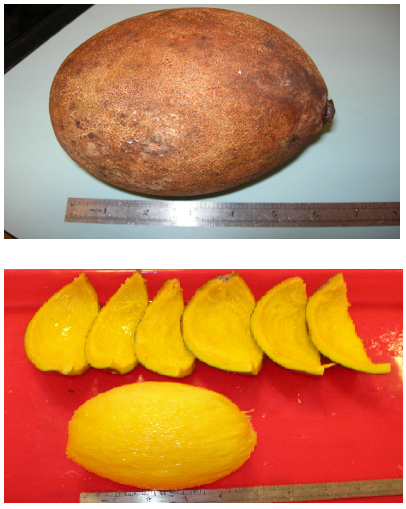

Figure 1. Bambangan fruit

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Cocoa (*Theobroma cacao*) pulp juice is a novel juice that recently developed by Malaysian Cocoa Board (MCB) in order to reduce the thickness of bean pulp before cocoa fermentation. The removal of some of the pulp prior to fermentation can produce cocoa with less acidity. Furthermore, the initiative to produce this juice was made since a large portion (90%) of the total pod weight was discarded as cocoa industrial waste (MCB, 2007). Previously, numerous studies on cocoa beans and its products concerning their polyphenol compounds and antioxidant capacity have been reported (Jonfia-Essien *et al*., 2008; Belščak *et al*., 2009).

However, studies examining the antioxidant potentials and polyphenolic compounds of tropical juices are relatively scarce. Moreover, there are limited published data on the bambangan and cocoa pulp juices. Research on these juices is important to understand the antioxidant values of these juices and their health-promoting properties. Therefore, this study was aimed to determine the antioxidant properties of three selected tropical fruit juices. In addition, their inhibition effects on human hemoglobin and LDL oxidations were also evaluated using *in vitro* biological assays. As for comparison, the commercial available white guava (*Psidium guajava*) juice product was selected since guava was well known to have high level in polyphenols and antioxidant activity (Patthamakanokporn *et al*., 2008).

Materials and Methods

Samples collection

Bambangan juice was obtained from Federal Agricultural Marketing Authority (FAMA), cocoa pulp juice was obtained from Malaysian Cocoa Board (MCB), while two local brands of guava juices namely as Fresh and Fresh Guava Juice Drink (Fresh and Fresh Foodtech Sdn. Bhd.) and Buonofresh′ Green Guava Juice (GD Marketing Sdn. Bhd.) were purchased from the hypermarket in Serdang, Selangor, Malaysia. Both guava juice products were homogenised (1:1, v/v) for few minutes. All juices were kept at 4ºC prior to extraction.

Chemicals and reagents

Phosphate buffered saline (PBS) tablets (Bio Basic Inc., Ontario, Canada), linoleic acid (Fluka, Castle Hill, Australia), chloroform (HmbG Chemicals, Hamburg, Germany), analytical grade methanol (System, Arkansas, USA), thiobarbituric acid (TBA) (Applichem GmbH, Darmstadt, Germany), trichloroacetic acid (TCA) (BDH Analar, Poole, England), copper (II) chloride ($CuCl_2$) (Hamburg Chemicals, Heilbronn, Germany) and potassium sodium tartrate (May and Baker, Dagenham , England) were purchased. Analytical grade gallic acid, sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), butylated hydroxytoluene (BHT), sodium azide (NaN3), OptiPrep (60% iodixanol), Hepes (N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]), bovine serum albumin (BSA), Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Tween 20 and high performance liquid chromatography (HPLC) grade polyphenol standards (gallic acid, ferulic acid, catechin, vanillic acid, protocatechuic acid, syringic acid and trans-cinnamic acid) were purchased from Sigma Chemicals (St. Louis, USA). Hydrochloric acid (HCl) fuming 37%, hydrogen peroxide (H_2O_2) solution 31%, sodium chloride (NaCl) and ethylenediaminetetraacetate acid (EDTA) were from Merck (Darmstadt, Germany). Copper (II) sulfate and HPLC grade solvents (methanol, acetic acid and water) were purchased from Fisher Scientific (New Hampshire, USA).

Sample extraction

The extraction method was according to Xu *et al*. (2008) with some modifications. Five millilitres of juice was extracted with 5 ml of 80% methanol for 30 min at room temperature using an incubator shaker (Heidolph Instruments Incubator1000, Schwabach, Germany). The mixture was centrifuged at 3000 rpm for 10 min using a Universal 32® centrifuge (Hettich, Zentrifugen, Germany). Then, the supernatant was stored at -80°C until further analysis. This extract was used for measuring the total phenolic content and antioxidant capacities.

Total phenolic content (TPC)

The TPC was determined according to the method of Velioglu *et al*. (1998). The extract (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 Na_2CO_3 solution (0.566 M) was added to the mixture. After 90 min, absorbance was read at 725 nm using UV-visible spectrophotometer (Shimadzu Co., Kyoto, Japan). Gallic acid with different concentrations (20-100 µg/ml) was used as standard. Results were calculated through a standard calibration curve and expressed as gallic acid equivalents (GAE)/100 ml juice.

HPLC analysis of phenolic compounds

The samples extraction was according to the method of Belajová and Suhaj (2004) with slight modifications. Five millilitres of fruit juice was

extracted with 20 ml of 80% methanol. The mixture was homogenised for 10 min and then filtered through a 0.45 µm nylon membrane filter before injected into the HPLC. The analysis was performed using a HPLC system (Series 1100, Agilent Technologies, Santa Clara, USA) equipped with diode array detector. The analysis conditions were followed the method of He and Xia (2007). Sample (20 µl) was injected into the HPLC system. An eclipse XDB-C18 column (250 mm x 4.6 mm I.D) (Hewlett Packard, Palo Alto, USA) were used. Column temperature was set at 20 °C. Gradient elution was performed with 0.5% (v/v) acetic acid (solvent A) and methanol (solvent B) at flow rate of 0.6 ml/min. The linear gradient mode was as follows: 100% A and 0% B at the start, then to 10% A and 90% B at 20 min, remaining at 10% A and 90% B from 20-25 min, and falling back to 100% A and 0% B at 30 min. Polyphenols were detected at 280 nm. Identification of the compounds was based on the retention time and the UV–Vis spectra of standards. Seven phenolic compounds were tested namely gallic acid, catechin, ferulic acid, vanillic acid, trans-cinnamic acid, syringic acid and protocatechuic acid. Spiking test was used to confirm the individual peak. The standard curve was created by plotting the peak area of each standard versus concentrations (0.4-200 µg/ml). The results were expressed as mg/100 ml.

DPPH free radical scavenging assay

Scavenging activity of the juice extracts and gallic acid (standard) were determined according to the method described by Tang *et al*. (2002) with slight modifications. Two hundred microlitres of juice extract or standard was added to 1 ml DPPH (0.1 mM) in methanol. The mixture was then shaken vigorously and kept to stand in the dark room for 30 min at room temperature. The absorbance was read at 517 nm with solvent as blank. The reading was compared with the control that contained 200 µl of 80% methanol and 1 ml DPPH. The scavenging activity (%) was calculated according to the equation as follows:

$$\text{Scavenging activity (\%)} = [1 - A/B)] \times 100$$

Where, A = absorbance of sample at 517 nm, B = absorbance of control at 517 nm. The efficient concentration to reduce DPPH by 50% (EC_{50} value) was calculated from the plotted graph of scavenging activity against concentrations (10-80 µg/ml).

β-Carotene bleaching assay

Antioxidant activity (AA) of each sample was estimated by a β-carotene bleaching method (Velioglu *et al*., 1998; Lu & Foo, 2000). One millilitre of β-carotene solution (0.2 mg/ml chloroform) was pipetted into a round-bottom flask containing 0.02 ml of linoleic acid and 0.2 ml of Tween 20. The mixture was then evaporated at 40°C for 10 min using a rotary evaporator (Buchi Rotavapor R-200, Essen, Germany) to remove the chloroform. Then, the mixture was immediately diluted with 100 ml of distilled water and agitated vigorously to form an emulsion. Five millilitres aliquot of the emulsion was subsequently transferred into test tube containing 0.2 ml of juice extract. The tube was then gently mixed and placed in a water bath (Memmert GmbH, Schwabach, Germany) for 2 h at 50°C. The absorbance was measured at 470 nm at initial time (t = 0) against a blank, consisting of an emulsion without β-carotene. Solvent in the 5 ml of emulsion was used as control. The measurement was carried out at every 20 min intervals. Concentration of samples used was EC_{50} value calculated from DPPH assay, while BHT (standard) was at 200 ppm. AA was calculated based on a formula from Jayaprakasha *et al*.[18]

$$AA = [1- (A0 - At)/ (A^{o}0 - A^{o}t)] \times 100$$

Where, A0 and Aº0 are the absorbance values measured at initial time of the incubation for samples or standard and control respectively, while At and Aºt are the absorbance values measured in the samples or standard and control at t = 120 min.

Hemoglobin oxidation assay

Human blood was applied for obtaining the hemoglobin and LDL as substrates for *in vitro* biological assays. Three healthy nonsmoking volunteers, ages 20–30 years were selected. Fasting peripheral venous blood samples (10 ml) were collected and transferred into EDTA tubes. The tube was immediately centrifuged at 3000 rpm and 4°C for 10 min. The plasma was taken out and applied for LDL oxidation assay. The study protocol was approved by the Medical Research Ethics Committee, Faculty Medicine and Health Sciences, Universiti Putra Malaysia (UPM), Malaysia [Approval No.: UPM/FPSK/PADS/T7-MJKEtikaPer/F01(JPD_JUN)(08)(05)]. A written informed consent was obtained from each volunteer.

Hemoglobin oxidation assay was followed a method described by Rodriguez *et al*. (2006) with modifications. The measurement was based on the malondialdehyde (MDA) formation from hemoglobin oxidation. The erythrocytes obtained were washed three times with 0.9% NaCl (2 ml), this step needs to be done within 15 min of blood withdrawal. After the third wash, packed erythrocytes were gently resuspended in PBS (pH 7.4) to obtain a 5%

hematocrit and preincubated at 37°C for 10 min in the presence of 1 mM NaN_3 to inhibit microbial growth. Subsequently, the mixture was divided into various aliquots of 1.6 ml for each experimental treatment. All treatment groups (0.2 ml of juice extract) and standards (BHT and gallic acid) were challenged with 0.2 ml of 0.01 M H_2O_2. The extracts used were standardised at concentration of EC_{50} value calculated from DPPH assay, while standards were at 200 ppm. However, negative and positive controls were done with solvent and H_2O_2 alone, respectively. After 60 min incubation at 37°C, cells were kept for 60 s in an ice bath.

MDA produced from hemoglobin oxidation was measured using TBA assay described by Buege and Aust (1978) with some modifications. One millilitre erythrocytes solution was vortexed with 1 ml of TCA: TBA: HCl reagent (15% TCA: 0.375% TBA: 0.25 mol HCl) (1:1:1, v/v/v) for 10 s. Then, the mixture was heated in boiling water for 15 min and cooled at room temperature for 10 min. Then, the mixture was centrifuged at 3000 rpm for 10 min. The absorbance of supernatant was read at 535 nm against blank. Lastly, the MDA level was calculated based on the following equation:

$$C = E/ kL$$

Where, C is concentration (M), E is the absorbance, k is molar extinction (1.56×10^5 $M^{-1}cm^{-1}$) and L is length of cuvette used. The results were expressed as µM MDA.

LDL oxidation assay

In this assay, isolation of human LDL was carried out prior to LDL oxidation measurement. The method was slightly modified from Graham *et al*. (1996). Four millilitres of plasma obtained from previous assay was mixed with 60% iodixanol (1 ml). After that, the mixture (4 ml) was transferred into Optiseal tubes using a syringe and overlaid with 0.5 ml of 20% iodixanol. Lastly, the tubes were overlaid with Hepes-buffer saline. LDL was isolated and concentrated from prepared plasma using an ultracentrifuge Type 100i Rotor (Beckman Coulter Optima L-100XP, Brea, USA). The ultracentrifugation was set at 16°C with 65 000 rpm for 3 h and slow acceleration at 305 g. The LDL–containing fraction can be seen as darker yellow-orange colour between various layer of other lipoprotein and triglycerides. This LDL-containing fraction was removed using a syringe through the side of the Optiseal tubes. The protein content of LDL fraction was measured by Lowry *et al*. (1951) method, using BSA as standard.

LDL oxidation assay was carried out based on a method described by Tsoukatos *et al*. (1997) with some modifications. Firstly, pooled LDL-containing fractions were suspended in PBS (1.7 ml, pH 7.4) in a final volume of 2 ml containing 80 µg/ml LDL protein. A 4 µM $CuCl_2$ (0.1 ml) was then added to induce oxidation. Meanwhile, 0.2 ml of juice extract or standards (BHT and gallic acid) were also added and the mixture were incubated at 37°C for 3 h. Negative and positive controls were done using solvent and $CuCl_2$ alone, respectively. The oxidation was terminated by adding 1 ml of EDTA (0.01% final concentration) and left until cool. The MDA levels produced from copper-induced LDL oxidation was measured using TBA method as mentioned above.

Statistical analysis

All the values were expressed as means ± standard deviation (SD) of triplicate measurements. Data were statistically analysed using statistical software, SPSS version 15.0 for windows (SPSS Inc, Chicago, Illinois, USA). One-way analysis of variance (ANOVA) was used to determine differences in means among groups and Pearson correlation test was applied to find correlations between phenolics content and antioxidant capacities. The level of significance was set at $p < 0.05$.

Results and Discussions

Total phenolic content (TPC)

Guava juice contained the significant highest TPC (24.64 ± 0.51 mg GAE/100 ml) compared to other juices studied. Meanwhile, TPC values in cocoa pulp and bambangan juices were 16.33 ± 0.25 and 10.01 ± 0.14 mg GAE/100 ml, respectively. Previously, guava fruit was reported as a good source of phenolic compounds (Thaipong *et al*., 2006). However, lower phenolic content of bambangan juice can be attributed to the fact that 78% of its total phenolic content occurs in the kernel and 17% occurs in the peel (Abu Bakar *et al*., 2009). Both of these fruit parts were excluded during juice preparation. A similar result was reported by Loots *et al*. (2006) who showed that low phenolics in grape juice was due to the elimination of grape's seeds and peel during juice making. A low TPC value was also related to the phenolic contents that retain in the discarded fibrous tissue of the fruit during juice pressing (Brasil *et al*., 1995). Accordingly, all the fruit juices studied were made from the pulps that naturally contained structural fibrous tissues. Slighty lower phenolics in cocoa pulp juice could be affected by the selection of appropriate cocoa varieties or clones. The juice

quality was depends on the selection of cocoa types where three-quarter ripe and yellowish-green pods was the most suitable period to yield cocoa pulp juice (MCB, 2007).

Contents of individual phenolic compounds

Even as total phenolics may be a useful marker of nutritional advantage, the actual profile of phenolics within the juices seems to be more significant. Thus, the individual phenolic compounds of the juices were studied using HPLC. Figure 2 shows the chromatographic separation of phenolic compounds in the juices. In bambangan juice, the prominent compound was unambiguously identified as gallic acid. Besides, vanillic acid and trans-cinnamic acid were detected in this juice. The phenolic compounds found in guava juice were gallic acid, catechin, vanillic acid, trans-cinnamic acid and ferulic acid. Moreover, various compounds were identified in cocoa pulp juice and two main phenolics were protocatechuic acid and syringic acid. However, there are few unknown compounds found in guava and cocoa pulp juices which needed more commercial available polyphenol standards to identify them.

Current study revealed that all phenolic compounds identified can be divided into three groups: hydroxybenzoic acids (gallic acid, vanillic acid, syringic acid and protocatechuic acid), hydroxycinnamic acids (ferulic acid and trans-cinnamic acid) and flavan-3-ols (catechin). Table 1 shows the contents of individual phenolic compounds in the fruit juices tested. Bambangan juice exhibited the highest content of gallic acid (1.69 mg/100 ml) and it was comparable to bayberry juices (0.6-2.4 mg/100 ml) (Fang *et al*., 2009). Moreover, the protocatechuic acid content in cocoa pulp juice (0.69 mg/100 ml) was higher than bayberry juices (0.40 mg/100 ml) (Fang *et al*., 2009). Higher catechin, vanillic acid and ferulic acid contents were found in guava juice. These compounds may provide the antioxidant activity of guava juice. Recently, ferulic acid and its derivatives which present in various plant species has been focused deeply on their antioxidant contributions (Anselmi *et al*., 2004).

Table 1. Phenolic compounds contents of fruit juices

Phenolic compounds (mg/100 ml)	Bambangan	Cocoa pulp	Guava
Gallic acid	1.69 ± 0.07	0.22 ± 0.03	0.51 ± 0.09
Catechin	nd	1.36 ± 0.42	4.49 ± 1.13
Vanillic acid	0.49 ± 0.04	nd	0.71 ± 0.20
Ferulic acid	nd	0.95 ± 0.15	1.10 ± 0.27
Trans-cinnamic acid	0.20 ± 0.08	0.23 ± 0.08	0.23 ± 0.08
Protocatechuic acid	nd	0.69 ± 0.14	nd
Syringic acid	nd	0.62 ± 0.10	nd

Values were expressed as mean ± SD (n = 3). nd: Not detected.

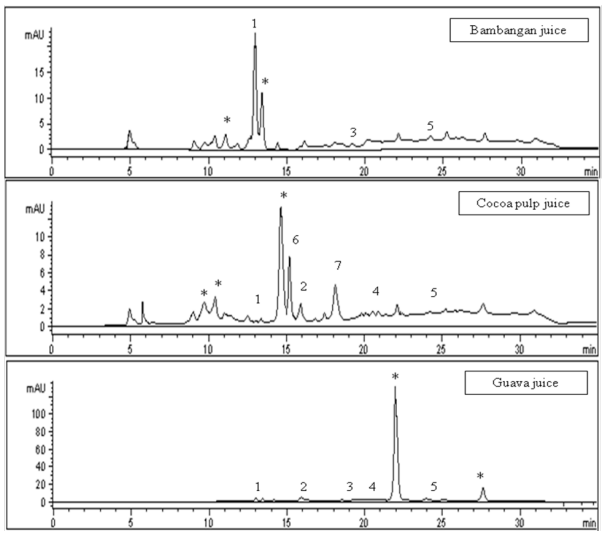

Figure 2. HPLC chromatograms of phenolic compounds in fruit juices. Detection was done at 280 nm. Peak: (1) gallic acid, (2) catechin, (3) vanillic acid, (4) ferulic acid, (5) trans-cinnamic acid, (6) protocatechuic acid, (7) syringic acid; (*) unidentified

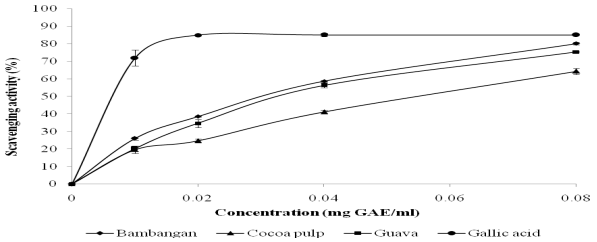

Figure 3. Scavenging activity of fruit juices on DPPH free radicals. Results were expressed as mean ± SD (n = 3). Gallic acid was used as the standard

DPPH free radical scavenging activity

The comparison of DPPH free radical scavenging activity of the studied fruit juices and gallic acid are shown in Figure 3. The highest scavenging activity was showed by bambangan juice, subsequently to guava and cocoa pulp juices. The scavenging ability against DPPH radicals was high even at lower juice concentrations, especially for bambangan and guava juices. At highest concentration of juices (0.08 mg GAE/ml), the scavenging activity almost reached a plateau when comparing with the standard (gallic acid). The ascending order of EC_{50} value was bambangan juice (0.030 mg GAE/ml) < guava juice (0.033 mg GAE/ml) < cocoa pulp juice (0.055 mg GAE/ml). The lowest EC_{50} value was acquired by bambangan juice, indicating it has the highest antioxidant capacity to scavenge DPPH radicals. A comparable result was obtained in orange juice (0.31 mg/ml) and wine (0.46 mg/ml) as reported by Kelebek *et al*. (2009).

A higher antioxidant capacity in guava juice was expected because it contained the highest TPC. Higher phenolic contents generally indicated stronger antioxidant capacities (Rice-Evans & Miller, 1996). Surprisingly, bambangan juice acquired lowest TPC but exhibited the highest scavenging activity. This may contribute by other antioxidant constituents that

contain in this juice. In addition, other methanol-soluble compounds like methylxanthines and certain pigments in this juice may react with the DPPH radicals (Belščak *et al*., 2009).

β-Carotene bleaching activity

The β-carotene bleaching (BCB) rates of the studied fruit juices and standard are shown in Figure 4. The slowest decrement in BCB activity was observed in bambangan and cocoa pulp juices. These juices also gave a better inhibition of β-carotene bleaching compared to BHT. In addition, antioxidant activities of cocoa pulp, bambangan and guava juices were 78.90 ± 5.70%, 75.91 ± 3.44% and 46.74 ± 4.56%, respectively. BHT had slightly lower in antioxidant activity (AA) (77.02 ± 0.99%) compared to cocoa pulp juice. The AA of cocoa pulp and bambangan juices was good as other commercial fruit juices such as sour cherry (94.4%), red grape (87.0%), strawberry (85.9%) and cherry (71.0%) juices (Kulisic-Bilusic *et al*., 2009).

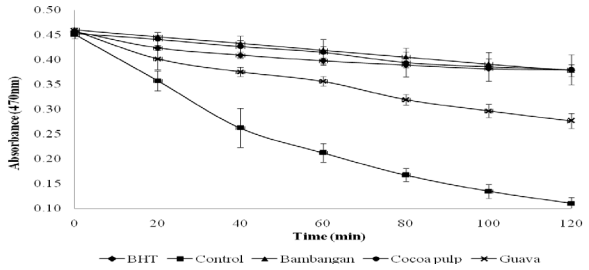

Figure 4. Absorbance of fruit juices and standard in β-carotene bleaching assay. Results were expressed as mean ± SD (n = 3). BHT (200 ppm) was used as the standard

Inhibition effects on hemoglobin oxidation

Inhibition effects of the studied fruit juices and standards on hemoglobin oxidation are presented in Figure 5. Although the juices were less effective in prevention of hemoglobin oxidation as the standards, but they gave a significant protective effect towards the free radicals compared to the control group. The most effective inhibition was found in bambangan juice (0.703 ± 0.015 μM MDA), while a similar result was found in cocoa pulp (0.736 ± 0.008 μM MDA) and guava (0.740 ± 0.010 μM MDA) juices.

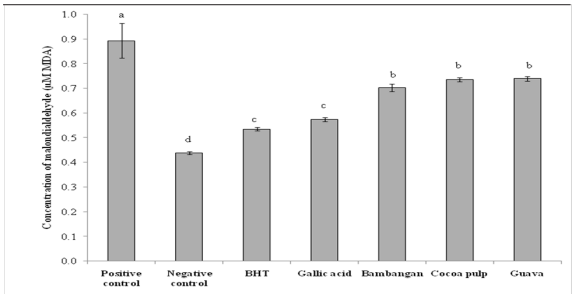

Figure 5. Inhibition effects of fruit juices and standards on hemoglobin oxidation. Results were expressed as mean ± SD (n = 3). Gallic acid and BHT were used as the standard. Means with different letters showed a significant different at the level of $p < 0.05$

The inhibition effects of the juices on hemoglobin oxidation may due to presence of certain bioactive phenolic compounds. For instance, polyphenol hydroxytyrosol (3,4-dihydroxyphenyl) ethanol in olive oil had been reported as a powerful inhibitor in peroxidation of red blood cells (RBCs) (Manna *et al*., 1999). In human lipoproteins *in vitro* assays, particular issues about the cell membrane structure-function relationship and the oxidative treatment should be considered (Manna *et al*., 1999). Furthermore, they suggested further determination regarding the effects of H_2O_2-induced oxidation on RBC membrane is important to elucidate the antioxidant properties of studied sample.

Inhibition effects on LDL oxidation

Figure 6 shows the inhibition effects of the studied juices and standards on LDL oxidation. Apparently, guava juice had the highest inhibition of MDA formation (0.27 ± 0.00 μM MDA) among the juices. For bambangan and cocoa pulp juices, inhibition effects were 0.30 ± 0.01 μM MDA and 0.33 ± 0.04 μM MDA, respectively. A good observation was achieved as the MDA level of guava juice was closer to the BHT. Guava juice was the most efficient inhibitor of LDL oxidation, it may due to the presence of flavonoids in the fruit (Miean & Mohamed, 2001). It was noted that flavonoid compounds exhibited a good protective effects against copper-induced LDL oxidation (Chirinos *et al*., 2008). The same finding was found in red wine, composed of flavan-3-ols compounds showed high inhibition against LDL oxidation (Kerry & Abbey, 1997). On the other hand, ferulic acid in guava juice (Figure 3) may not responsible for the inhibition effect against LDL oxidation. A study done by Cirico & Omaye (2006) found that ferulic acid in guava may not effective and even promote the copper-induced LDL oxidation.

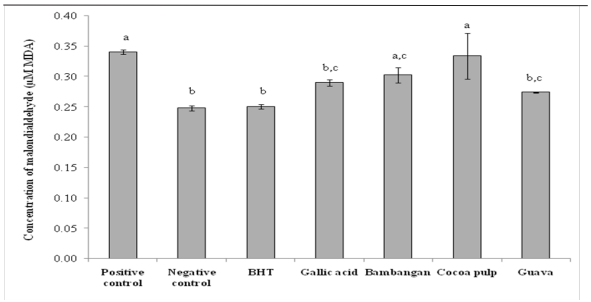

Figure 6. Inhibition effects of fruit juices and standards on LDL oxidation. Results were expressed as mean ± SD (n = 3). Gallic acid and BHT were used as the standard. Means with different letters showed a significant different at the level of $p < 0.05$

Correlations between total phenolics and antioxidant capacities

Correlations were done to link the total phenolics and antioxidant capacities of fruit juices measured

by different assays. TPC values were negatively correlated with antioxidant activity (AA) of fruit juices (r = -0.825). However, there might be other compounds that may contribute to their antioxidant activities. This was in agreement with Thaipong *et al*. (2006), who also found a negative correlation (r = -0.79) between TPC and AA value in guava. A significant correlation between TPC values and inhibition effects against hemoglobin oxidation (r = 0.757), may due to the contribution of polyphenols in studied juices as antioxidants. A strongly positive correlation was found between AA values and inhibition effects on LDL oxidation (r = 0.784). This may due to both assays have the same principle in protecting the lipid peroxidation. This was in agreement with Kulisic-Bilusic *et al*. (2009), showing that a strong correlation was found between AA value of fruit juices and their inhibition on copper-induced LDL oxidation.

Conclusions

The present study found that bambangan juice demonstrated the greatest scavenging activity on DPPH and inhibiting hemoglobin oxidation although it had the lowest TPC among the juices. Furthermore, antioxidant activity of this juice in retardation of β-carotene bleaching was more than 70%. Meanwhile, the highest TPC in guava juice might contribute to its protective effect against LDL oxidation. According to biological *in vitro* assays, certain unknown compounds in bambangan and guava juices may play significant roles as inhibitor of lipid peroxidation. Overall, bambangan juice could be regarded as a potential new functional food due to its health-promoting properties. However, further investigation of the health benefits using *in vivo* and human study is warranted.

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