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Phenolics, flavonoids content and antioxidant activities of 4 Malaysian herbal plants

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Abstract

Water and ethanolic extracts of four Malaysian local herbs, *Tenggek burung* (*Melicope Iunu-ankenda*), *Kesum* (*Polygonum minus*), Curry leave (*Murraya Koenigii*) and *Salam* (*Eugenia polyantha*) were investigated for their total phenolic content (TPC), total flavonoids content (TFC) and antioxidant activities (AA). Total phenolic content (TPC) of the herbs was determined using Folin-Ciocalteu reagent assay while the total flavonoid content (TFC) was determined based on aluminium chloride-flavonoid assay. The determination of AA was done using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activitiy and β-carotene bleaching assays (BCB). Different extraction solvents significantly affected the TPC, TFC and AA of all herbs studied ($p < 0.05$). Both Tenggek burung and Kesum showed highest TPC, TFC and AA regardless of extraction solvents compared to Curry leave and *Salam*. All herbs showed strong positive correlation between TPC and DPPH assay. However, negative and low correlation between TFC and AA were obtained for all herbs studied. This showed that phenolic compounds of certain structures were responsible for the AA of all the herbs in this study. In conclusion, all herbs in this study except curry leave could be inexpensive sources of good natural antioxidants with nutraceutical potential in food industry.

Introduction

World Health Statistics reported that from the estimated 57 million global deaths in 2008, 36 million (63%) were due to non-communicable diseases (NCDs) (WHO, 2011). Diet is a lifestyle factor that plays a major role in the primary and secondary prevention of chronic diseases. Consumption of fruits and vegetables is one of the nutritional recommendations that help to maintain human health (Lichtenstein *et al*., 2006; Halliwell, 2012) because our diet provides a huge amount of plant-derived phenolic compounds with antioxidant activity that helps to counter oxidative stress in our body (Hertog *et al*., 1993; Halliwell, 2012).

Malaysia is well known to have a rich biodiversity of many indigenous fruits and vegetables grown wildly in the region of Peninsular Malaysia, Sabah and Sarawak. There are more than 120 species representing various families of traditional vegetables of the Malays in Malaysia, locally called as '*ulam*' (Mansor, 1988). Many local herbs are cooked or eaten raw as salad while some are being boiled and the extracts are used for consumption among Malaysians.

To date, many studies has been done on many local Malaysian herbs such as turmeric (*Curcuma domestica*), betel leaf (*Piper betel*), pandan leaf (*Pandanus odorus*), '*asam gelugor*' (*Garnicia atroviridis*), '*mengkudu*' (*Morinda citrifolia*), '*pegaga*' (*Centella asiatica*), ginger (*Zingiber officinale*), cassava shoot (*Manihot asculenta*), *Cassia surattensis* and *Caesalpinia pulcherrima*. Herbs such as '*mengkudu*', ginger, pandan leaf, '*pegaga*', betel leaf, etc., showed high antioxidant activity (Jayamalar and Suhaila, 1998; Mohd. Zin *et al*., 2002; Zainol *et al*., 2003; Chew *et al*., 2009; Thoo *et al*., 2010). However, there are still some other herbs remain unexplored in-depth and hence, the knowledge gap is still exist.

Tenggek burung (*Melicopelunu ankeda*) which is belongs to *Rutaceae* family and genus of *Melicope*, where the leaves has been widely used by local people for the treatment of high blood pressure and giving freshness. It can be found in the temperate and tropical regions of East Asia (Rasadah and Zakaria, 1988). *Kesum* (*Polygonum minus*) is one of the herbs in *Polygonaceae* family, grows well in wet marshy places beside lakes and ponds. The genus *Polygonum* (*Polygonaceae*), comprising of about 300 species, is distributed worldwide, mostly in the northern temperate regions. Its leaves are being used to treat

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dandruff and constipation among the locals in India (Seidemann, 2005). As the leave possesses a strong peppery taste, hence it is being used as a kind of spice by the locals in Malay-style food Laksa and some other traditional dishes (Mansor, 1988).

Murraya koenigii (Curry leave) is a tropical to sub-tropical tree in the family of *Rutaceae* commonly known as curry patta in India. It is now widely found in all parts of India and Sri Lanka, China, Australia, the Pacific Islands and East Asia. It is one of the major spices that have been consumed in India for its characteristic flavor and aroma. It has been used as anagelsics, astringents, anti-dysentric or febrifuges in folk medicine in China and other Asian countries (Ito *et al*., 2006). Besides that, the root, leaves and bark of curry tree are used for the treatment of various ailments like snakebite, itchiness, general body aches, dropsy, morning sickness and vomiting (Kesari *et al*., 2007).

Eugenia polyantha (*Salam*) leave grows wildly in the western part of Peninsular Malaysia and in western Indonesia. It is known as serai kayu among the locals and comes from the family of *Mythaceae. Salam* is being used to treat diseases like diabetes, diarrhea and high blood pressure by utilizing its leaves, root, bark, stem and fruits (Seidemann, 2005). The leaves are slightly astringent or sour and the flavor develops more after frying. Usually it is eaten after boiling it (Seidemann, 2005).

Hence, the objectives of this investigation is to determine the total phenolics content (TPC) and total flavonoids content (TFC) and to characterize the antioxidant activity (AA) of four tropical plants, namely *Melicopelunu ankeda* (*Tenggek Burung*), *Polygonum minus* (*Kesum*), *Murraya koenigii* (curry leaves) and *Eugenia polyantha* (*Salam* leave). In addition, these herbs are commonly consumed as part of the Malaysian diet. Besides, the correlation between AA and TPC and TFC were also determined. Results from this study would also provide a better understanding on the AA of these plants so that it would be identified for further investigations and hence, developed into value-added foods and nutraceuticals for the benefit of mankind.

Materials and Methods

Materials

Fresh *Melicopelunu ankeda* (*Tenggek Burung*), *Polygonum minus* (*Kesum*), *Murraya koenigii* (curry) leaves and *Eugenia polyantha* (*Salam*) leaves were purchased from the wet market of Section 6, Shah Alam, Selangor, Malaysia. Upon arrival at the laboratory, samples were washed with water to remove debris and damaged portions. The leaves were stripped from the plants and freeze-dried. The dried leaves were stored in sealed polyethylene bags at 4°C until ready for extraction.

Chemical and reagents

β-carotene, 2,2-diphenyl-1-picrylhydrazyl (DPPH), aluminium chloride-6-hydrate, trichloroacetic acid (TCA), Tween 20, butylated hydroxytoluene (BHT), ascorbic acid, and rutin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Potassium ferricyanide, ferric chloride, phosphate buffer, ethanol, gallic acid, linoleic acid, Folin-Ciocalteau's reagent, sodium carbonate were purchased from Merck (Darmstadt, Germany). Chloroform was from Fischer Scientific (Loughborough, UK). All other chemicals were of analytical grade.

Preparation of extracts

The extract was prepared according to the method of Velioglu *et al.* (1998) with some modifications. One gram of the plant material was weighed, macerated, homogenized and extracted with distilled water or 80% ethanol for 2 h at 50°C using an orbital shaker at 200 rpm. The ratio of samples to extraction medium was 1:25. The mixture was filtered through a filter paper using a filter funnel. The filtrate was diluted 20 times to obtain the concentration of 2000 ppm and used for further analyses. The extraction solvent selected was 80% ethanol based on the results showed by Yoo *et al*. (2008) using some common Malaysian herbs while water is the usual solvent used by the general public when consuming these herbs (Wong *et al*., 2006).

Determination of total phenolic content

The total phenolic content (TPC) of the plant extracts was determined spectrophotometrically using Folin-Ciocalteau's reagent according to the method described by Kahkonen *et al.* (1999) with slight modifications. Three hundred microliter of samples (2000 ppm) was added into test tubes. Then, 1.5 ml of Folin Ciocalteau reagent (10 times dilution) and 1.2 ml of sodium carbonate (7.5 g/100 ml) were added. The contents of the tubes were mixed well and kept in the dark for 30 min. The absorbance was measured using spectrophotometer (PRIM, Secomam, Ales Gard, France) at 765 nm. The calibration equation of gallic acid standard curve was $y = 0.0106x + 0.0066$ ($R^2 = 0.9993$). TPC was expressed as g gallic acid equivalents (GAE) per 100 g of fresh material.

Determination of total flavonoid content

The total flavonoid content was determined based on the formation of flavonoid-aluminium as described by Djeridane *et al.* (2006). One mililiter of sample extract (2000 ppm) was mixed with 1 ml of 2% aluminium chloride-6-hydrate solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured using spectrophotometer (PRIM, Secomam, Ales Gard, France) at 430 nm. Rutin was used as a standard to plot the calibration curve. The amount of flavonoids was expressed as rutin equivalents (RE).

DPPH radical scavenging activity assay

Free radical scavenging activity against 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical was measured using the method described by Oboh (2005). Then 1 ml of sample extract (2000 ppm) was added into 2 ml of 0.15 mM of DPPH and mixed thoroughly with vortex mixer (VORTEX V-1, BPECO, Germany). The mixture was allowed to stand in dark for 30 min before measuring the absorbance at 517 nm using spectrophotometer (PRIM, Secomam, Ales Gard, France) against ethanol blank and distilled water as negative control. Ascorbic acid was used as a comparative standard. Antioxidant activity (AA) was expressed as the percentage of DPPH decrease.

$$AA\ (\%) = \frac{(A_{control} - A_{sample}) \times 100}{A_{control}}$$

β-carotene bleaching assay

β-carotene bleaching (BCB) assay was conducted using a method by Velioglu *et al.* (1998) with slight modifications. The working reagent was prepared as followed: 0.2 mg of β-carotene was dissolved in 1 ml of chloroform and then mixed with 0.02 ml of linoleic acid and 0.2 ml of Tween 20 in a round bottom flask. Subsequently, the mixture was evaporated using a rotary evaporator at 50°C. After evaporation, 50 ml of distilled water was added to the mixture and then shaken vigorously to form an emulsion. The absorbance of the mixture taken at 0 min was measured at 470 nm. Next, 2 ml of the emulsion were pipette into test tubes containing 0.2 ml of sample extract or standard (BHT) or control (ethanol 80%) and immediately placed in a water bath (WB/OB 7-45, Germany) at 50°C. The absorbance was read at 20 min interval until 120 min of incubation using UV-VIS spectrophotometer (PRIM, Secomam, Ales Gard, France) at 470 nm. The β-carotene bleaching rate of the sample was calculated based on the formula as below:

$$\ln (a/b) \times 1/t = DR_{sample/standard} \text{ or } DR_{control}$$

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 time at 20, 40, 60, 80, 100 or 120 min.

AA was calculated as percentage of inhibition relative to control using equation below:

$$\%\ AA = 100 \times (R_{control} - R_{sample}) / R_{control}$$

Where, $R_{control}$ and R_{sample} are the bleaching rates of β-carotene in the emulsion without antioxidant and with sample extract, respectively.

Statistical analysis

All data were expressed as mean ± standard deviation and were done in triplicate independent analyses. Data were analyzed using one-way ANOVA using SPSS version 20 (SPSS Inc., Chicago, Illinois, USA). One-way analysis of variance (ANOVA) followed by Bonferroni's test for comparison, as a post hoc test to analyze the difference between the four herbs while independent samples t-test was used to determine the difference between ethanolic and water extracts of a particular sample. Pearson correlation was used to assess the relationships between TPC and TFC and AA (DPPH and BCB). The significance level was set at $p < 0.05$.

Results and Discussion

Total polyphenol contents

TPC of ethanolic extracts were in the order of *Tenggek Burung* > *Kesum* > *Salam* > Curry leaves while TPC of the water extracts were in the order of *Salam* > *Tenggek Burung* > *Kesum* > Curry leaves (Figure 1). For water extracts, *Salam* demonstrated the highest TPC and was significantly different ($p < 0.05$) with all the other herbs. TPC of *Tenggek Burung* was significantly higher ($p < 0.05$) than *Kesum*. However, there was no significant difference ($p > 0.05$) observed between *Tenggek Burung* and *Kesum* in ethanolic extracts. Curry leaves showed the lowest TPC for both water and ethanolic extracts.

Wong *et al.* (2006) reported that 11 mg gallic acid equivalent (GAE) per g dry basis (db) in the water extract of *Salam* leave from Singapore while our results demonstrated higher TPC in the water extract of *Salam* leave (14.6 mg GAE/g db). Cheung *et al.* (2003) demonstrated that the TPC in organic extracts was higher in water extracts for mushroom extracts. Our results demonstrated that the TPC of *Tenggek Burung* and *Kesum* was higher than their water

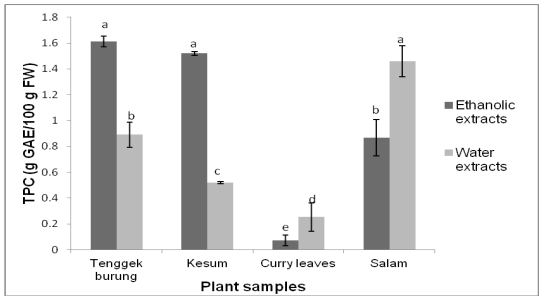

Figure 1. Total Phenolic Content (TPC) of *Tenggek Burung*, *Kesum*, curry leaves and *Salam* leave extracts. Concentration of sample was 2000 ppm (0.02 g/ml). Values were presented as mean ± standard deviation of triplicate independent analyses. Different lower case letters (a-d) on the bars in each sample denote significantly different ($p < 0.05$).

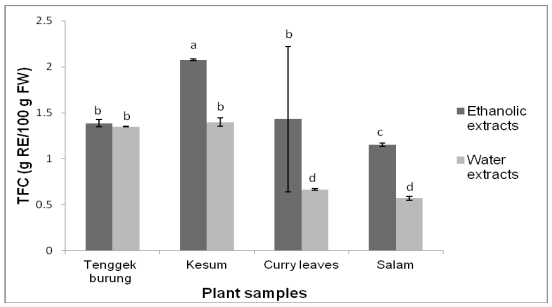

Figure 2. Total Flavonoid Content (TFC) of *Tenggek Burung*, *Kesum*, curry leaves and *Salam* leave extracts. Concentration of sample was 2000 ppm (0.02 g/ml). Values were presented as mean ± standard deviation of triplicate independent analyses. Different lower case letters (a-d) on the bars in each sample denote significantly different ($p < 0.05$).

extracts whereas the water extract of curry leave and *Salam* demonstrated higher TPC than their ethanolic extracts.

Besides that, phenolic compounds, depending on the number of phenolic groups, react differently to the Folin-Ciocalteu reagent (Singleton *et al*., 1999). Zhang *et al.* (2007) proposed that the extraction of phenolic compounds from a sample is directly related to the compatibility of the compounds with the solvent system according to the 'like-dissolves-like' principle. The present results could be due to the fact that plant phenolics demonstrate an wide range of solubility in solvents with different polarity (Rice-Evans *et al*., 1997). Hence, Thoo *et al.* (2010) concluded that no single ethanol concentration was able to recover all the phenolic compounds from a particular sample.

Total flavonoid contents

From Figure 2, total flavonoid content (TFC) of the ethanolic extracts were in the order of *Kesum* > Curry leave > *Tenggek Burung* > *Salam* while TFC of the water extracts were in the order of *Kesum* > *Tenggek Burung* > Curry leave > *Salam*. *Kesum* showed the highest TFC while *Salam* leaves showed the lowest TFC for both water and ethanolic extracts. Figure 2 also showed that the TFC of ethanolic extracts always demonstrated value higher than water extracts for all samples. This finding was not consistent with the findings by Wang *et al.* (2009) showing that higher TFC was found in water extracts of glossy privet fruit compared to ethanolic extracts.

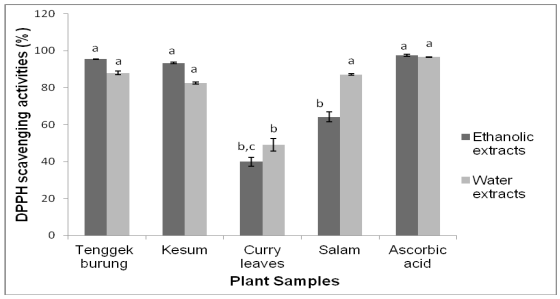

Figure 3. DPPH scavenging activities of *Tenggek Burung*, *Kesum*, curry leaves and *Salam* leave extracts. Concentration of sample was 2000 ppm (0.02 g/ml). BHT (200 ppm) was used as a comparative standard. Values were presented as mean ± standard deviation of triplicate independent analyses. Different lower case letters (a-b) on the bars in each sample denote significantly different ($p < 0.05$). Coefficients of variance was less than 2%.

Kesum and Curry leaves showed higher TFC value compared to their TPC value irrespective of the solvents used in the extraction. This might be due to different compounds extracted using different solvents having different solubilities. This result was in line with the study by Wang *et al.* (2009) showing that ethanolic extracts of glossy privet fruit contained more myricetin, quercetin, oleanolic acid and ursolic acid while its water extracts contained more gallic acid and caffeic acid. From the results of TPC and TFC, it was clear that ethanolic extracts exhibited a significantly higher TPC and TFC content compared with water extracts for most samples. This was because ethanol as an organic solvent was able to denature polyphenol oxidases and was more efficient in degrading cell wall, thus able to extract more endocellular materials compared to water (Prior *et al.*, 2005).

Antioxidant activity

DPPH Free Radical Scavenging Activity

This assay was based on the reduction of DPPH radicals which causes an absorbance drop at 517 nm where the purple colour changed into yellow colour. Antioxidants, on interactions with DPPH, transferring an electron from a hydrogen atom to DPPH free radical, hence neutralizing its free radical activity (Prior *et al*., 2005; Huang *et al.*, 2005). *Tenggek*

Burung had the highest DPPH radical scavenging activity while curry leave showed the lowest activity regardless of the solvents used. For ethanolic extracts, DPPH free radical scavenging activities were in the order of Ascorbic acid > *Tenggek Burung* > *Kesum* > *Salam* > curry leaves while for water extracts, it was in the order of Ascorbic acid > *Tenggek Burung* > *Salam* > *Kesum* > curry leaves (Figure 3).

In both ethanolic and water extracts, curry leave demonstrated significantly lower ($p < 0.05$) DPPH free radical scavenging activities than all other herbs. Both water and ethanolic extracts of *Tenggek Burung* and *Kesum* demonstrated no significant difference ($p > 0.05$) in DPPH radical scavenging activities. Water extracts of *Salam* leaves and curry leaves yielded significantly higher ($p < 0.05$) scavenging activities than their ethanolic extracts while ethanolic extracts of *Tenggek Burung* and *Kesum* showed significantly higher ($p < 0.05$) scavenging activities than their water extracts. Water extracts of *Salam* leaves and *Tenggek Burung* showed comparable AA with Ascorbic acid standard (Figure 3).

Our results was not consistent with a study conducted by Ningappa *et al.* (2007) who showed that ethanolic extracts of curry leaves (*Murayya koenigii*) yielded higher AA than its water, hexane and chloroform extracts. This could be because of the nature of the sample being analyzed. Our study used fresh sample while they were using processed curry powder. On the other hand, DPPH had a better solubility in organic solvent especially in ethanol compared to aqueous solvents, being an important limitation when interpreting the role of hydrophilic antioxidants (Prior *et al*., 2005). Our result was consistent with most of the reports suggesting that binary solvent system was more effective than mono solvent system in extracting antioxidant compounds (Azizah *et al.*, 2007; Yoo *et al*., 2008; Wang *et al.*, 2009; Thoo *et al*., 2010; Sah *et al*., 2012; Essaidi *et al*., 2013). Higher AA should be found in alcoholic extracts compared to water extracts because alcoholic solvent maximizes the interaction of DPPH radicals with antioxidants present in the sample (Spigno *et al*., 2007).

β-carotene bleaching assay

β-carotene bleaching (BCB) inhibition assay is a good model for membrane based lipid peroxidation. Linoleic acid produces radicals during incubation at 50°C. The presence of different antioxidants in the extracts can hinder the extent of β-carotene bleaching by neutralizing the linoleate radicals formed in the system (Shon *et al*., 2003; Miraliakbari and Shahidi, 2008). Thus, the extracts with the lowest β-carotene degradation rate had the highest AA. For ethanolic extracts, the AA was in the order of BHT > *Tenggek Burung* > *Kesum* > *Salam* > Curry leave while the AA of water extracts was in the order of *Salam* > BHT > Curry leave > *Kesum* > *Tenggek Burung* (Figure 4). The AA of water extracts of curry leave, *Salam* and *Kesum* were significantly higher ($p < 0.05$) than their ethanolic extracts respectively while the ethanolic extracts of *Tenggek Burung* demonstrated significantly higher AA ($p < 0.05$) than its water extract.

Table 1. Pearson correlation coefficients (r) between antioxidant activities (obtained from DPPH and BCB assays) with total phenolic content (TPC) and total flavonoids content (TFC) of four herbal plants

Samples	(a)DPPH		(b)BCB	
	TPC	TFC	TPC	TFC
Tenggek burung (*Melicopelunu ankenda*)	0.976*	0.389	-0.033	0.102
Kesum (*Polygonum minus*)	0.9674*	0.5814	-0.0596	0.2107
Salam (*Eugenia polyantha*)	0.975*	-0.626*	0.475	-0.986**
Curry leave (*Murraya koenigii*)	0.998**	0.4660	0.5002	-0.5227

*Significant at $p < 0.05$
**Significant at $p < 0.01$

Both curry leaves and *Salam* leaves have significantly lower ($p < 0.05$) BCB activities than both *Tenggek Burung* (84.7%) and *Kesum* (83.4%). No significant difference ($p > 0.05$) observed between water extracts of *Salam* compared to BHT (Figure 4), this imply that *Salam* has comparable AA with BHT. From the results, it was clear that water extracts of all samples studied showed higher AA than their ethanolic extracts except *Tenggek Burung*. The high AA of *Salam* and *Tenggek Burung* extracts measured by both DPPH free radical scavenging and BCB assay may be attributed to the high TPC and cooperative effect of phenolics and/or synergistic effect of phenolic acids present (Bilia *et al*., 2008; Thoo *et al*., 2010).

Water-soluble antioxidant compounds other than flavonoids and phenolics from the plants seemed to inhibit the oxidation of β-carotene in the β-carotene-linoleate system better than compounds soluble in ethanol. Hassimotto *et al*. (2005) classified antioxidant capacity as high (>70%), intermediate (40-70%) or low (<40%) levels of oxidation inhibition. Hence, all water extracts were considered as having high inhibition levels. Azizah *et al.* (2007) reported that the AA of water extracts of cocoa beans was higher compared with its ethanolic extracts. This happened because of the different solubilities and polarities of different antioxidant compounds in the samples.

Correlation between AA with TPC and TFC

Polyphenols have been reported to be responsible for the AA of plant extracts. Usually, extracts that contain a high amount of polyphenols also show high AA (Wong *et al*., 2006). With reference to Table 1, all samples showed a strong positive correlation between

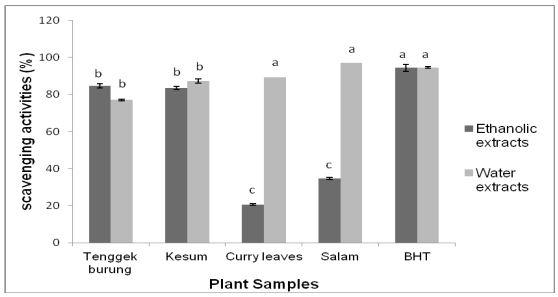

Figure 4. Antioxidant activity (as measured by BCB inhibition rate) of *Tenggek Burung*, *Kesum*, curry leaves and *Salam* leave extracts. Concentration of sample was 2000 ppm (0.02 g/ml). BHT (200 ppm) was used as a comparative standard. Values were presented as mean ± standard deviation of triplicate independent analyses. Different lower case letters on the bars in each sample denote significantly different ($p < 0.05$). Coefficients of variance were less than 2%.

TPC and DPPH free radical scavenging activity. High scavenging activity on DPPH radicals could be due to the low molecular weight phenolic compounds in the samples studied. Paixão *et al.* (2007) reported that DPPH is known to react specifically with low molecular weight phenolic compounds. However, the low positive correlation found from AA and TFC for *Tenggek burung* and *Kesum* could be due to only flavonoids of certain structures and particularly certain hydroxyl position in the molecule that will determine the antioxidant properties (Miliauskas *et al.*, 2004). Several studies found no correlation between scavenging activity and TPC (Othman *et al*., 2007; Yu *et al.*, 2002). However, several researchers found strong positive correlation between TPC and AA for different plants (Maksimović *et al*., 2005; Yu *et al*., 2005; Thoo *et al*., 2010).

A low correlation between AA with that of TPC could be due to an error introduced in the assays (Wong *et al*., 2006). These assays which were based on the measurement of an end product, one could actually be measuring the AA of the reaction by-products, rather than the compounds present in the original mixture (Halliwell, 2009). Another source of error could be due to the lack of specificity of the Folin-Ciocalteau phenol reagent. It was well known to be unable to differentiate between phenol type and non-phenolic substances, giving an inaccurate and overestimation of TPC (Ikawa *et al*., 2003; Prior *et al*., 2005).

The correlations of TPC and TFC against the AA based on β-carotene bleaching assay in this study were low or negative. This showed that the phenolic compounds or flavonoids from the plants in this study weakly inhibited the oxidation of β-carotene by hydroperoxides. Perhaps, other bioactive compounds and the reaction by-products were responsible for the AA determined. This remains to be determined in the future. Our findings were consistent with the study conducted by Othman *et al.* (2007) who showed that there was no correlation between AA and TPC for cocoa bean extracts. However, Thoo *et al.* (2010) reported strong correlation between TPC and TFC with DPPH for *mengkudu* while Sah *et al*. (2012) reported moderate positive correlation between TPC with DPPH in lemongrass leave.

Research suggested the need to perform more than one type of AA measurement to consider the various mechanisms of antioxidant action and limitation of each assay (Huang *et al.*, 2005). For a natural product to be use in foods, plant extracts made with water are nutritionally more relevant and have more advantages in relation to food safety (Wong *et al*., 2006).

Both *Tenggek burung* and *Kesum* showed high AA with both DPPH and BCB inhibitory activity (Figure 3 and 4). Hence, we could postulate that the phenolic compounds in *Tenggek burung* and *Kesum* were hydrophobic in nature. Interestingly, both ethanolic extracts of both *Salam* and curry leave showed low AA assayed by both DPPH and BCB. This could be due to some errors introduced in the assays to determine the extracts' ability to scavenge DPPH free radicals or linoleate radical (Wong *et al*., 2006). Curry leave seems to be not a good source of natural antioxidants compared to other samples in this study. Different solvents affected the TPC, TFC and AA of all four samples significantly. Factors influencing the recovery and AA of specific sample were antioxidants concentration, extraction medium (time and polarity), temperature, pH of medium, chemical structures and position in the molecule (Prior *et al.*, 2005; Zhang *et al.*, 2007; Thoo *et al*., 2010). Hence, a high yield of individual phenolic compounds will not exhibit a high AA as it is dependent on the synergistic effects of the extracted phenolic compounds (Thoo *et al.*, 2010). Extraction conditions can greatly influence AA in plants. All the extraction parameters should be optimized as they are important for optimization of sample extraction procedures (Michiels *et al*., 2012).

Moreover, *in vitro* antioxidant assays are based on chemical reactions *in vitro* where it is different to actual physiological systems. However, the results of *in vitro* AA assays should not compromise their value in guiding human research (Huang *et al*., 2005). Further work is also required for the isolation and identification of individual phenolic compounds present in all these herbal samples to determine the mechanisms involved in the AA of these herbal extracts.

Conclusions

This study demonstrated that all samples except Curry leave are good sources of natural antioxidants. All samples showed significant positive correlations with DPPH assay while low and negative correlations showed between BCB and TPC. All samples had significant negative and low correlations between TFC and both DPPH and BCB. Water extracts of all the leaves studied showed higher AA than 80% ethanol. Extraction solvent significantly affected the TPC, TFC and AA of all four samples. Future in-depth studies are needed to identify and verify the bioactive compounds contributing to AA in these plants.

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