

Full Length Research Paper

Antioxidant activities, flavonoids, ascorbic acid and phenolic contents of Malaysian vegetables

Yusuf Sumazian, Ahmad Syahida, Mansor Hakiman and Mahmood Maziah*

¹Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.

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Vegetable extracts of nine Malaysian vegetables; *Curcuma domestica*, *Kaempferia galanga*, *Piper betel*, *Piper sarmentosum*, *Polygonum minus*, *Cosmos caudatus*, *Centella asiatica*, *Hydrocotyle bonariensis* and *Barringtonia racemosa* were screened for their antioxidant properties. Total antioxidant activities of leaf or rhizome extract were assayed using different methods; ferric reducing antioxidant potential assay (FRAP), 1,1-diphenyl-2-picrylhydrazyl free radical scavenging assay (DPPH) and β -carotene bleaching assay. Another relevant antioxidant compounds studied were total ascorbic acid content, total flavonoid content and total phenolic content. All of assays studied extracted using aqueous and boiled aqueous except for β -carotene bleaching assay and total ascorbic acid content. DPPH free radical scavenging assay was found to be the best assay for total antioxidant while high activity of antioxidant also recorded in total phenolic content. FRAP assay showed that *C. domestica* contain the highest antioxidant activity in aqueous and boiled aqueous extract. Percent of inhibition using DPPH assay in aqueous extract showed that *K. galanga* was the highest while *P. betel* showed the highest in boiled aqueous extract. Total antioxidant activity using β -carotene bleaching assay was ranged from 8.05 - 19.04% while total ascorbic acid content ranged from 0.26 - 1.82 mg/g FW. For total flavonoid content, *C. domestica* and *P. minus* showed the highest for aqueous (4.05 mg/g FW) and boiled aqueous extract (6.28 mg/g FW), respectively. Total phenolic content in *C. caudatus* and *P. betel* showed the highest content with 6.43 and 12.35 mg/g FW in aqueous and boiled aqueous extract, respectively. Most of the assays exhibited higher antioxidant activities using boiled aqueous extraction compared to aqueous extract. Only FRAP assay showed high antioxidant using aqueous extraction but DPPH assay, total flavonoid content and total phenolic content respond better in boiled aqueous extraction. These findings showed that, most of Malaysian vegetables can exhibit high antioxidant activities using boiled aqueous.

Key words: Malaysian vegetables, antioxidant, ascorbic acid, flavonoid, phenolic.

INTRODUCTION

In recent decades, various species of plants have been used and consumed due to the presence of high antioxidant activities. The extracts of medicinal plants and natural products become a great source of antioxidant

and anti-ageing properties. Antioxidant is a protection agent which can terminate the initiation of oxidizing chain reactions and it can inhibit or delay oxidation by other molecules (Suchandra et al., 2007). Oxidation processes are important because it can control the production of free radicals and the unbalanced mechanism of antioxidant protection that can cause diseases and accelerated ageing (Dawidowicz et al., 2006). Free radicals can also initiate the oxidation of biomolecules

*Corresponding author. E-mail: maziahm@biotech.upm.edu.my.
Tel: +603 89466703. Fax: +603 89430913.

such as protein, amino acids, lipid and DNA which will lead into cell injury and death (Freidovich, 1999).

Several vegetables were selected in this study such as *Curcuma domestica*, *Kaempferia galanga*, *Piper betel*, *Piper sarmentosum*, *Polygonum minus*, *Cosmos caudatus*, *Centella asiatica*, *Hydrocotyle bonariensis* and *Barringtonia racemosa*. These vegetables were selected due to their medicinal value which have traditionally used as alternative medicine. *B. racemosa* can be used as treatment for itch, piles and typhoid fever (Deraniyagala et al., 2003). *K. galanga* rhizome is known to have carminative, diuretic, aromatic stomachic, insecticidal and incense which produced in the rhizome (Huang et al., 2008). *C. domestica* known for their yellow coloring agent and active compound called curcumin used for several ailments. *P. betel* is known for gastroprotective, anti-inflammatory and immunomodulatory properties (Mula et al., 2008). Chanwitheesuk et al. (2005) reported that *P. sarmentosum* has the potential to use as digestive tonic, carminative, expectorant, anti-hyperglycemia and anti-malaria while *C. asiatica* use as poultice for wound, scar and ulcer. It is likely that many more beneficial values of these vegetables but some not have been documented.

Synthetic antioxidants have been widely used in industries such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) although they promote negative health effects (Barlow, 1990). Therefore, medicinal plants are of great potential as a new source of pharmaceuticals, nutraceuticals, cosmeticeuticals and for alternative medicines. Validation must be provided to make sure the products are safe and effective. This study was undertaken to evaluate total antioxidant activities as well as compounds related to antioxidants such as ascorbic acid, flavonoid and phenolic. This study will result in new knowledge on antioxidant activities in selected Malaysian vegetables since there are only a few documented on this area of study.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrous chloride, ascorbic acid, 2,6-dichloroindophenol (DCPIP), β -carotene, Trolox and catechin were purchased from Sigma Co. St. Louis, Missouri, USA. Methanol, ethanol, linoleic acid, hydrochloric acid, sodium nitrite, sodium hydroxide, aluminium chloride, gallic acid, sodium carbonate, sodium hydroxide, Tween 20, chloroform and citric acid were purchased from Merck, Darmstadt, Germany. All the chemicals and reagents were of analytical grade.

Plant materials

All vegetables were bought from Department of Agriculture, Serdang, Selangor. The vegetables are *C. domestica* (CD), *K. galanga* (KG), *P. betel* (PB), *P. sarmentosum* (PS), *P. minus* (PM),

C. caudatus (CC), *C. asiatica* (CA), *H. bonariensis* (HB) and *B. racemosa* (BR). Rhizomes were used for *C. domestica* and *K. galanga* while the leaves were used for *P. betel*, *P. sarmentosum*, *P. minus*, *C. caudatus*, *C. asiatica*, *H. bonariensis* and *B. racemosa*.

Total antioxidant activity

Extraction for total antioxidant activities

Vegetables aqueous extract were obtained by employing the method of Wong et al. (2006) with minor modification. The vegetable parts were air dried separately for one week or until the weight is constant. A total of 0.1 g of dried rhizome or leaf of each vegetable was cut into small pieces and extracted with 25 ml distilled water. The homogenates were transferred into 100 ml conical flasks and allowed to stand at room temperature for one h in the dark with occasional agitation. Aqueous extract was obtained by filtering the mixture through Whatman No. 1 filter paper and the supernatant was used in the experiment. Boiled aqueous extract was prepared using the same procedure except that the homogenates was incubated in boiling water bath for one hour before the mixture was filtered through Whatman No. 1 filter paper and supernatant was used for subsequent studies.

Ferric reducing antioxidant potential (FRAP) assay

This assay was carried out as described by Kikuzaki and Nakatani (1993) with some modifications. A mixture of 4 mg sample in 4 ml of 99.5% ethanol, 4.1 ml of 2.5% linoleic acid in 99.5% ethanol, 8 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water was prepared in a screw-cap vial. This mixture was placed in an oven at 40°C in the dark. To 0.1 ml of this mixture, 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% ammonium thiocyanate were added. After 3 min, 0.1 ml of 2 M ferrous chloride in 3.5% hydrochloric acid was added to the reaction mixture. The absorbance was measured at 500 nm every 24 h until the reading of absorbance value of control reached its maximum value. The experiment was replicated three independent assays.

Free radical scavenging activity (DPPH) assay

The DPPH assay measures hydrogen atom (or one electron) donating activity and hence provides a measure of free-radical scavenging antioxidant activity. DPPH is a purple-coloured stable free radical and will form yellow color when it was reduced as diphenylpicrylhydrazine complex. The initial absorbance of methalonic DPPH was measured at 517 nm without any sample. An aliquot (0.2 ml) of extracts was mixed with 3 ml of methalonic DPPH solution. The change in absorbance at 517 nm was measured after 30 min of incubation in room temperature (Amin et al., 2006). The experiment was replicated three independent assays.

β -Carotene bleaching assay

Total antioxidant activity using β -carotene bleaching assay was assayed according to method described by Velioglu et al. (1998) using Trolox as standard. A mixture of β -carotene (0.2 mg in 1 ml chloroform), linoleic acid (0.02 ml) and Tween 20 (0.2 ml) were prepared and transferred into a round bottomed flask. The mixture was then added to 0.2 ml of aqueous extract or standard (as control). Chloroform was removed at room temperature under

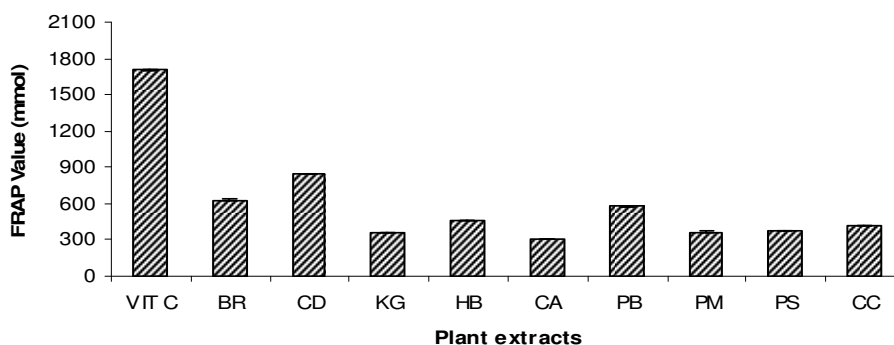


Figure 1. Total antioxidant content of aqueous extract of Malaysian plants using FRAP assay expressed as FRAP value. BR = *B. racemosa*, CD = *C. domestica*, KG = *K. galanga*, HB = *H. bonariensis*, CA = *C. asiatica*, PB = *P. betel*, PM = *P. minus*, PS = *P. sarmentosum* and CC = *C. caudatus*. Values are expressed as mean \pm SD (n = 3).

vacuum at reduced pressure using a rotary evaporator. Following evaporation, 50 ml of distilled water was added to the mixture, and then shaken vigorously to form an emulsion. Two ml aliquots of the emulsion were pipetted into test tubes and immediately subjected to thermal auto oxidation in a water bath at 50°C. The changes in absorbance were read at 20 min interval for 2 h at 470 nm using spectrophotometer. Antioxidant activity was expressed as percent of inhibition relative to the control. The experiment was replicated three independent assays.

Quantification of relevant antioxidant compounds

Total ascorbic acid (TAC)

Total ascorbic acid was measured according to Davies and Masten (1991). Each sample (leaf and rhizome) was extracted using 1% of phosphate citrate buffer pH 3.5 using chilled mortar and pestle. The homogenates was then centrifuged at 10000 rpm at 4°C for 10 min. The supernatant was collected and used for further analysis. The supernatant was added with 1 ml of 1.72 mM 2,6-dichloroindophenol (2,6-DCPIP) in 3 ml cuvette and the absorbance at 518 nm was measured immediately after mixing. The experiment was replicated three independent assays.

Total flavonoid content

Total flavonoid content was assayed using aluminium chloride colorimetric assay employed the method of Zhishen et al. (1999). The plant extracts were added in 2 ml HCl in round bottom flask and were refluxed at 100°C for 30 min. The hydrophilic extract will be made up to 5 ml by using distilled water. To the mixture, 0.3 ml of 5% (w/v) NaNO₂ was added and 3 ml of 10% AlCl₃. At sixth min, 2 ml of 1 M NaOH was added to the mixture. The mixture was mixed well and the absorbance at 510 nm was read using spectrophotometer. Total flavonoid content was expressed as mg catechin (CE)/g dry weight (DW) of sample. The experiment was replicated three independent assays.

Total phenolic content

Total phenolic content of selected Malaysian vegetables was

determined using Folin-Ciocalteu assay as described by Singleton and Rossi, (1965). The vegetable extracts were mixed with 80% methanol containing 1% of HCl at room temperature. The mixture was vigorously shaken by replacing the mixture on the orbital shaker at 200 rpm. After that, the homogenate was centrifuged at 1000 x g for 15 min. The vegetable extracts was added with 0.2 ml of Folin-Ciocalteu reagent and at fourth min, 15% of Na₂CO₃ was added. The absorbance was read at 760 nm using a spectrophotometer. Total phenolic content was expressed as mg gallic acid equivalent (GAE)/g DW. The experiment was replicated three independent assays.

Statistical analysis

The data obtained were based on mean \pm SD of three independent experiments or three replicates.

RESULTS

Total antioxidant activity

Figure 1 showed the total antioxidant activity in aqueous extraction using FRAP assay. Vitamin C was used as positive control. The highest total antioxidant content among all vegetables studied is *C. domestica* (853.01 mmol) while second highest is *B. racemosa* (631.88 mmol) followed by *P. betel* (582.03 mmol). *H. bonariensis* (457.18 mmol), *C. caudatus* (419.07 mmol) and *P. sarmentosum* (377.41 mmol) showed moderate content of antioxidant, while the lowest total antioxidant content was found in *K. galanga* (362.23 mmol), *P. minus* (367.06 mmol) and *C. asiatica* (303.45 mmol). Figure 2 showed the results of FRAP assay using boiled aqueous extraction from the same vegetables as above. The antioxidative activity in decreasing order is as follows; *C. domestica* (623.87 mmol) > *B. racemosa* (432.65 mmol) > *K. galanga* (412.67 mmol) > *P. betel* (287.65 mmol) > *H. bonariensis* (229.78 mmol) > *C. caudatus* (200.65

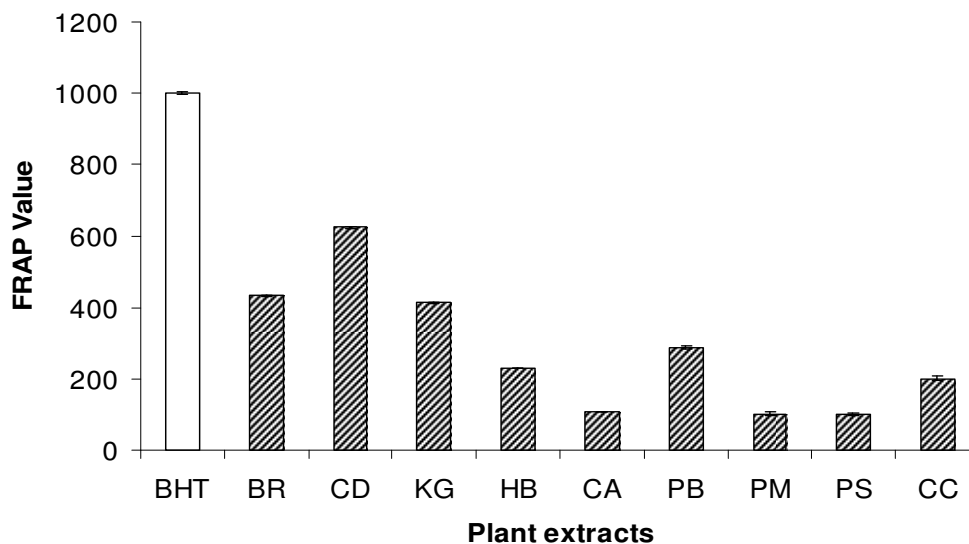


Figure 2. Total antioxidant content of boiled aqueous extract of Malaysian plants using FRAP assay expressed as FRAP value. BR= *B. racemosa*, CD= *C. domestica*, KG= *K. galanga*, HB= *H. bonariensis*, CA= *C. asiatica*, PB= *P. betel*, PM= *P. minus*, PS= *P. sarmentosum* and CC= *C. caudatus*. Values are expressed as mean \pm SD (n = 3).

mmol) > *C. asiatica* (106.54 mmol) > *P. minus* (100.65 mmol) > *P. sarmentosum* (98.76 mmol).

Figures 3 and 4 showed total antioxidant activity in Malaysian vegetables using DPPH free radical scavenging assay in aqueous and boiled aqueous extraction, respectively and the result expressed as percent of inhibition. Based on Figure 3, *K. galanga* scavenged the highest percentage of free radicals (83.27% of inhibition) compared to all Malaysian vegetables. *P. minus*, *C. asiatica*, *C. domestica*, *H. bonariensis* and *B. racemosa* showed high percent on inhibition with 81.49, 80.66, 79.12, 78.92 and 78.42% of inhibition, respectively. *C. caudatus* and *P. sarmentosum* showed low activity of antioxidant using DPPH assay with 24.81 and 15.44% of inhibition, respectively. The order of total antioxidant content using DPPH free radical scavenging assay expressed in percent of inhibition in Malaysian vegetables studied were as followed: *K. galanga* (83.27%) > *P. minus* (81.49%) > *C. asiatica* (80.66%) > *C. domestica* (79.12%) > *H. bonariensis* (78.92%) > *B. racemosa* (78.42%) > *P. betel* (61.99%) > *C. caudatus* (24.81%) > *P. sarmentosum* (15.44%). Figure 4 showed the same DPPH assay but using boiled aqueous to obtain the vegetable extract. The result showed that *P. betel* had the highest total antioxidant activity expressed as percentage of inhibition with 96.76%. This result followed by *P. minus* and *H. bonariensis* which also had high level of antioxidant with 81.77 and 77.57% of inhibition, respectively. Others vegetable extracts showed low antioxidant activity with

below than 40% (ranged from 16.81 - 38.22% of inhibition).

Total antioxidant activity using β -carotene bleaching assay results expressed as percent of total antioxidant activity was showed in Figure 5. According to Figure 5, the percent of total antioxidant activity was ranged from 8.05 - 19.04% of antioxidant. *H. bonariensis*, *C. asiatica* and *P. sarmentosum* showed the highest percent of total antioxidant activity among the vegetables studied with 19.04, 18.35 and 17.6% of antioxidant, respectively. This result followed with *K. galanga*, *P. betel* and *C. caudatus* which showed moderate amount of total antioxidant activity compared to *H. bonariensis*, *C. asiatica* and *P. sarmentosum* with 15.90, 15.45 and 13.15% of antioxidant, respectively. Low antioxidant activity was found in *P. minus*, *C. domestica* and *B. racemosa* with 12.55, 11.15 and 8.05% of antioxidant, respectively.

Figure 6 showed that total ascorbic acid content in selected Malaysian vegetables. According to Figure 6, the results indicates that total ascorbic acid content was found the highest in *P. sarmentosum* with 1.82 mg/g FW, compared to the control (0.90 mg/g) while the lowest total ascorbic acid content can be detected in *B. racemosa* with 0.26 mg/g FW. That makes *P. sarmentosum* contains 7 folds of total ascorbic acid content compared to that of *B. racemosa*. *P. betel* and *H. bonariensis* contain 1.01 mg/g FW and 0.70 mg/g FW, respectively of total ascorbic acid content while *B. racemosa*, *K. galanga*, and *C. domestica* which ranged from 0.26 - 0.41 mg/g FW resulted in the lowest total ascorbic acid

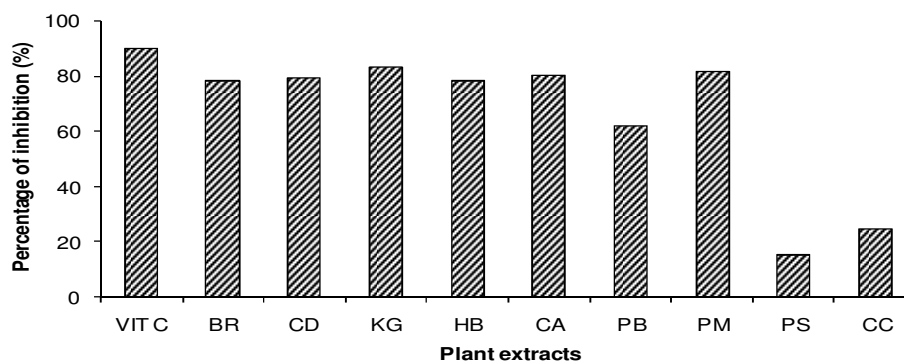


Figure 3. Total antioxidant content of aqueous extract of Malaysian plants using DPPH free radical scavenging assay expressed as percent of inhibition. BR = *B. racemosa*, CD = *C. domestica*, KG = *K. galanga*, HB = *H. bonariensis*, CA = *C. asiatica*, PB = *P. betel*, PM = *P. minus*, PS = *P. sarmentosum* and CC = *C. caudatus*. Values are expressed as mean \pm SD (n = 3).

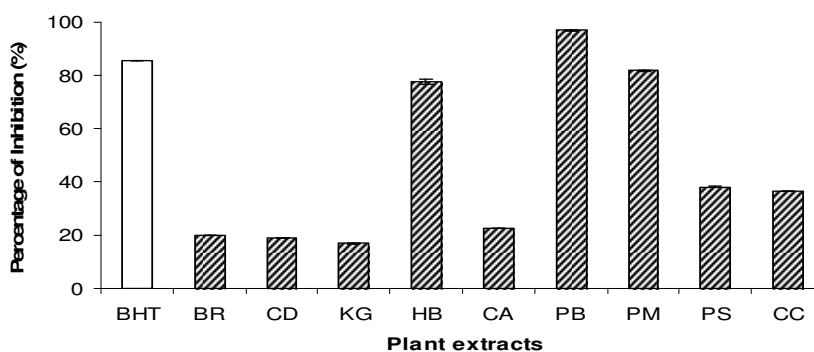


Figure 4. Total antioxidant content of boiled aqueous extract of Malaysian plants using DPPH free radical scavenging assay expressed as percent of inhibition. BR = *B. racemosa*, CD = *C. domestica*, KG = *K. galanga*, HB = *H. bonariensis*, CA = *C. asiatica*, PB = *P. betel*, PM = *P. minus*, PS = *P. sarmentosum* and CC = *C. caudatus*. Values are expressed as mean \pm SD (n = 3).

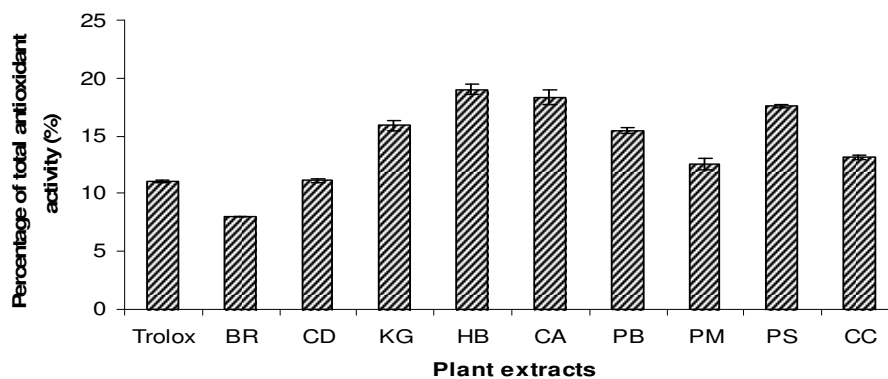


Figure 5. Total antioxidant content of Malaysian plants using β -carotene bleaching assay expressed as percent of total antioxidant activity. Results are expressed as trolox equivalents. BR = *B. racemosa*, CD = *C. domestica*, KG = *K. galanga*, HB = *H. bonariensis*, CA = *C. asiatica*, PB = *P. betel*, PM = *P. minus*, PS = *P. sarmentosum* and CC = *C. caudatus*. Values are expressed as mean \pm SD (n = 3).

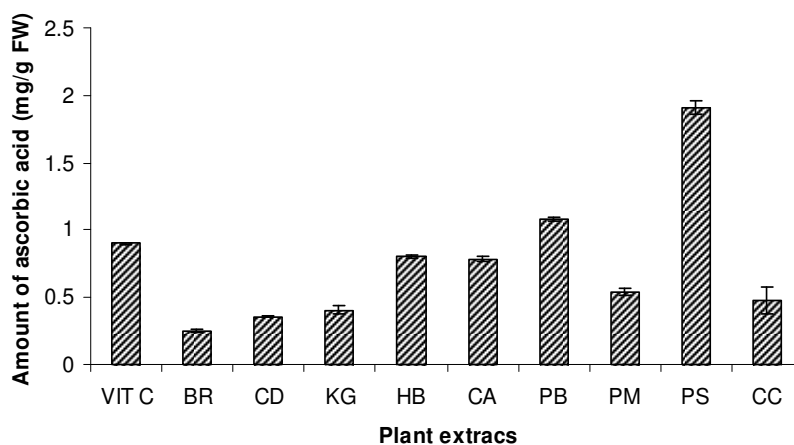


Figure 6. Total ascorbic acid content of Malaysian plants. Results are expressed as vitamin C equivalents. BR = *B. racemosa*, CD = *C. domestica*, KG = *K. galanga*, HB = *H. bonariensis*, CA = *C. asiatica*, PB = *P. betel*, PM = *P. minus*, PS = *P. sarmentosum* and CC = *C. caudatus*. Values are expressed as mean \pm SD (n = 3).

content among the vegetables studied. On the other hand, vegetables such as *C. asiatica*, *P. minus* and *C. caudatus* showed moderate activity of total ascorbic acid content with 0.79, 0.54 and 0.48 mg/g FW, respectively.

Figures 7 and 8 showed the total flavonoid content for aqueous and boiled aqueous extraction, respectively. From the results for aqueous extraction (Figure 7), the ranged for total flavonoid content was from 0.42 - 4.05 mg/g DW. *C. domestica* showed the highest while *B. racemosa* showed the lowest flavonoid content with 4.05 and 0.42 mg/g DW, respectively. *H. bonariensis*, *C. caudatus* and *P. sarmentosum* showed high flavonoid content with 3.32, 3.13 and 3.05 mg/g DW, respectively. For other vegetables, flavonoid content was less than 3 mg/g DW (ranged from 0.42 - 2.86 mg/g DW). The decreasing order of total flavonoid content in aqueous extract is as followed; *C. domestica* (4.05 mg/g DW) > *H. bonariensis* (3.32 mg/g DW) > *C. caudatus* (3.13 mg/g DW) > *P. sarmentosum* (3.05 mg/g DW) > *K. galanga* (2.86 mg/g DW) > *P. minus* (2.46 mg/g DW) > *C. asiatica* (2.09 mg/g DW) > *P. betel* (0.75 mg/g DW) > *B. racemosa* (0.42 mg/g DW). Figure 8 on the other hand showed total flavonoid content in boiled aqueous extract. *P. minus* was the highest among other vegetables studied with 6.28 mg/g DW. The second highest was found in *P. sarmentosum* with 2.03 mg/g DW. The other vegetables showed low total flavonoid content in boiled aqueous extract which ranged from 0.29 - 0.78 mg/g DW.

Figures 9 and 10 showed total phenolic content in aqueous and boiled aqueous extraction, respectively. For aqueous extraction (Figure 9), total phenolic content was ranged from 1.89 - 6.43 mg/g DW. The highest total phenolic content was detected in *C. caudatus* while the

lowest in *K. galanga*. *P. sarmentosum* and *P. betel* also showed high phenolic content with 6.35 and 4.01 mg/g DW, respectively. *C. asiatica*, *H. bonariensis*, *P. minus* and *C. domestica* was in the ranged of 3.00 - 4.00 mg/g DW of total phenolic content while *B. racemosa* and *K. galanga* showed phenolic activity lower than 3.00 mg/g DW with 2.93 and 1.89 mg/g DW, respectively. For boiled aqueous extract (Figure 10), the highest phenolic content was found in *P. betel* (12.35 mg/g DW) while *K. galanga* (2.78 mg/g DW) was found the lowest phenolic content in both extracts. *P. minus*, *C. asiatica* and *H. bonariensis* showed high phenolic content with 10.92, 9.34 and 9.25 mg/g DW, respectively. For the other vegetables, the activity was lower than 9.00 mg/g DW but still high compared to that of aqueous extract. Total phenolic content can be ordered in decreasing of value as followed; *P. betel* (12.35 mg/g DW) > *P. minus* (10.92 mg/g DW) > *C. asiatica* (9.34 mg/g DW) > *H. bonariensis* (9.25 mg/g DW) > *C. caudatus* (8.76 mg/g DW) > *P. sarmentosum* (7.66 mg/g DW) > *C. domestica* (4.51 mg/g DW) > *B. racemosa* (3.32 mg/g DW) > *K. galanga* (2.78 mg/g DW).

DISCUSSION

Total antioxidant activity

The antioxidant studies of different vegetables in Malaysia have been done but there is lack of published data on this particular field. This study focused on total antioxidant activity in Malaysian local vegetables which can be easily found and edible. Total antioxidant activity

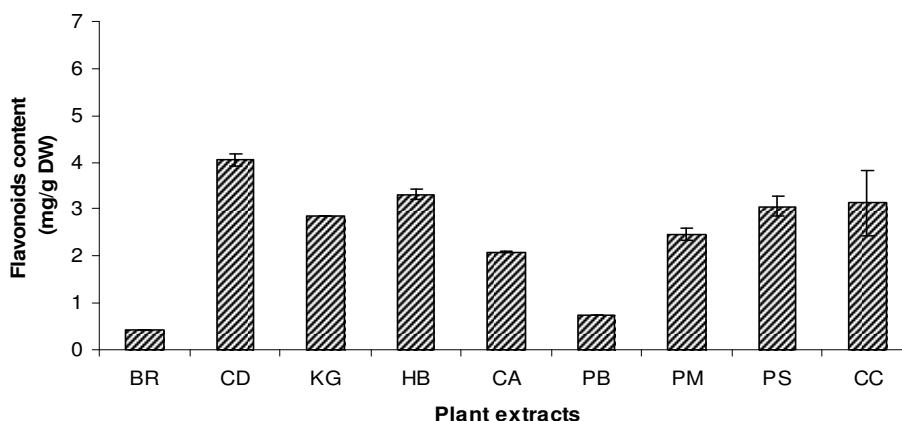


Figure 7. Total flavonoid content of aqueous extract of Malaysian plants using aluminium chloride colometric assay. BR = *B. racemosa*, CD = *C. domestica*, KG = *K. galanga*, HB = *H. bonariensis*, CA = *C. asiatica*, PB = *P. betel*, PM = *P. minus*, PS = *P. sarmentosum* and CC = *C. caudatus*. Values are expressed as mean \pm SD (n = 3).

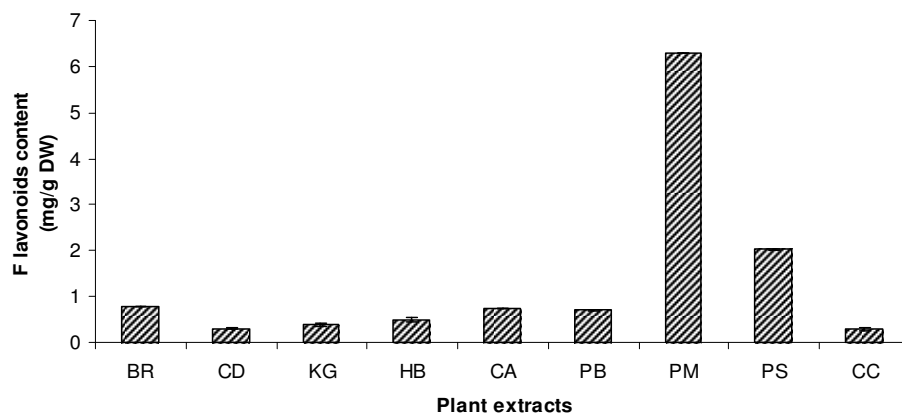


Figure 8. Total flavonoid content of boiled aqueous extract of Malaysian plants using aluminium chloride colometric assay. BR = *B. racemosa*, CD = *C. domestica*, KG = *K. galanga*, HB = *H. bonariensis*, CA = *C. asiatica*, PB = *P. betel*, PM = *P. minus*, PS = *P. sarmentosum* and CC = *C. caudatus*. Values are expressed as mean \pm SD.

can be measured using several methods but three methods chosen to measure total antioxidant which are ferric reducing antioxidant potential assay (FRAP), DPPH free radical scavenging assay (DPPH) and β -carotene bleaching assay. Based on the results, all methods that were used successfully exhibited high antioxidative activities. According to Chan et al. (2009), the study using DPPH method on *K. galanga* showed that fresh weight of the plant exhibited total antioxidant activities better than freeze dried and air dried plant extract (fresh weight > freeze dried > air dried). In this study, vegetable samples were air dried before extracted and analyzed. The results showed that total antioxidant activities were successfully exhibited in air-dried samples. According to Dasgupta

and De (2007) *C. asiatica* was found to have low activity of total antioxidant activities among the vegetable extracts studied. It was in contrary with this study since *C. asiatica* exhibit consistently high total antioxidant activities. From this study the results for each assay differ from one to another. From the observation the factor maybe due to the different assays have different principles and different antioxidant compounds detected for each assay. Of all the assays used, DPPH free radical scavenging assay was considered the best method to study total antioxidant since all of the data were close to that of trolox (standard). This is in agreement with Norhaiza et al. (2009) which found that DPPH free radical scavenging assay was better than FRAP assay and

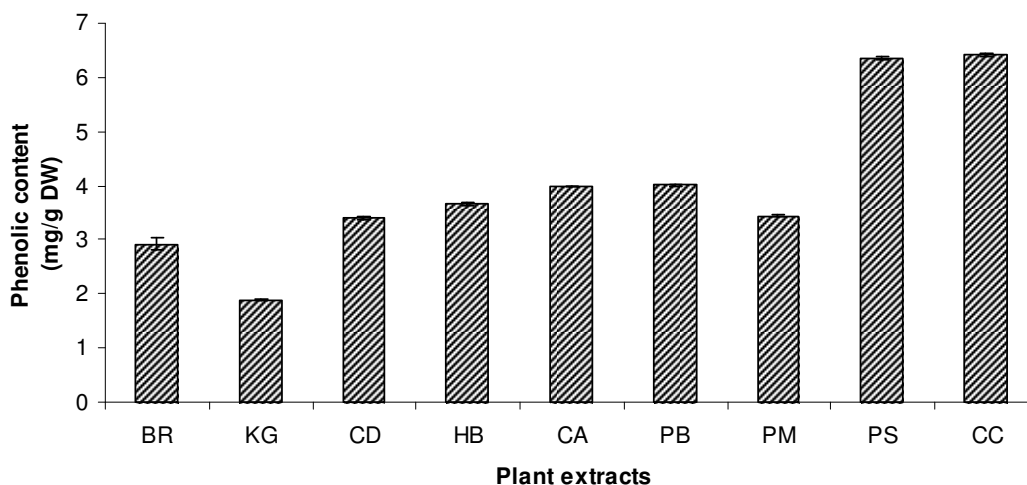


Figure 9. Total phenolic content of aqueous extract of Malaysian plants using Folin-Ciocalteu assay. BR = *B. racemosa*, CD = *C. domestica*, KG = *K. galanga*, HB = *H. bonariensis*, CA = *C. asiatica*, PB = *P. betel*, PM = *P. minus*, PS = *P. sarmentosum* and CC = *C. caudatus*. Values are expressed as mean \pm SD (n = 3).

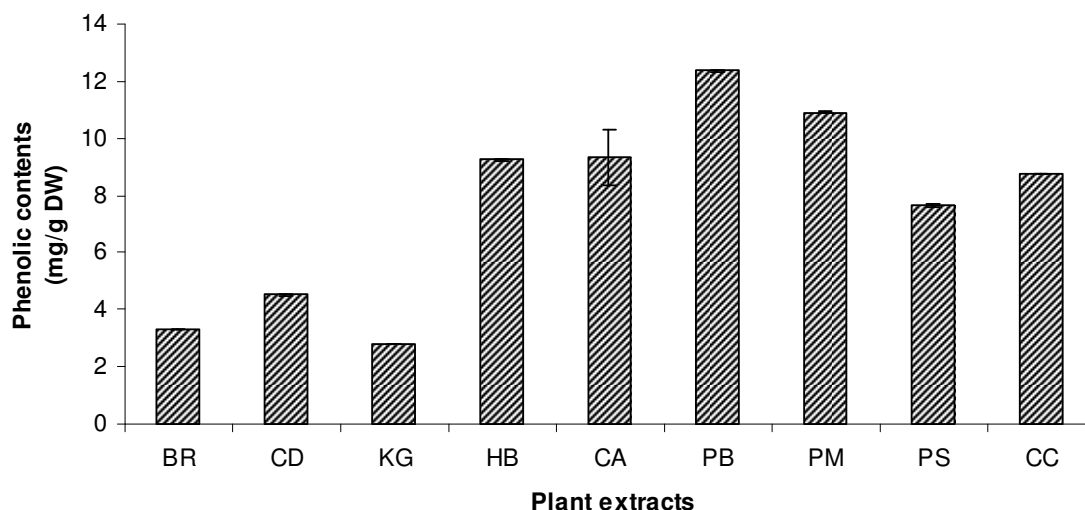


Figure 10. Total phenolic content of boiled aqueous extract of Malaysian plants using Folin-Ciocalteu assay. BR = *B. racemosa*, CD = *C. domestica*, KG = *K. galanga*, HB = *H. bonariensis*, CA = *C. asiatica*, PB = *P. betel*, PM = *P. minus*, PS = *P. sarmentosum* and CC = *C. caudatus*. Values are expressed as mean \pm SD (n = 3).

β -carotene bleaching assay. Norhaiza et al. (2009) made comparison studies of all of three methods mentioned to compare the antioxidant activities of two varieties of *Labisia pumila*; var. *alata* and var. *pumila* against the control (BHT) and found out that DPPH free radical scavenging assay gave the highest result for all samples tested. DPPH free radical scavenging assay is more indirect assay compare to FRAP assay because DPPH assay measured the inhibition of reactive species (free

radicals) generated in the reaction mixture and these results depend on the type of reactive species used (Cao et al., 1996). The observed antioxidant of extracts may be due to the neutralization of free radical (DPPH), either transfer of hydrogen atom or by transfer of an electron (Naik et al., 2003). β -Carotene bleaching assay showed the lowest percentage of activities in all assays for total antioxidant activities. Extraction using aqueous and boiled aqueous extract showed different pattern of

activities. For FRAP assay, average aqueous extract showed higher antioxidant activities compared to boiled aqueous extract. DPPH assay showed same pattern with FRAP assay but the highest antioxidant activities were found in boiled aqueous extract.

Relevant antioxidant activities

Relevant antioxidant compounds such as total ascorbic acid, total flavonoid and total phenolic content was successfully analyzed from local Malaysian vegetables. Studies by Chanwitheesuk et al. (2005) found that total ascorbic acid content in *C. asiatica*, *P. sarmentosum* and *K. galanga* showed higher activity than total ascorbic acid in Figure 6. Chanwitheesuk et al. (2005) also reported that vegetable derived from Piperaceae (*P. sarmentosum*) and Zingiberaceae (*K. galanga*) family showed high correlation of total ascorbic acid content with total antioxidant content with the correlation value, $r = 0.99$ and 0.63 , respectively. In this study, *P. sarmentosum* showed the highest total ascorbic acid content all of Malaysian vegetables studied but there is no correlation between total ascorbic acid and total antioxidant activities. According to Bahorun et al. (2004), it is normal when total ascorbic acid do not correlate with the total antioxidant activities since total ascorbic acid made little or no contribution to the total antioxidant activities of vegetables. Total flavonoid content in aqueous and boiled aqueous extraction showed different activities. The activities of total flavonoid content in boiled aqueous extraction showed that only *P. minus* and *P. sarmentosum* vegetable extracts can stand high temperature. For the rest of plant extracts very low activity of total flavonoid content was recorded which is below than 1.00 mg/g DW. For aqueous extraction for total flavonoid content most of the vegetables studied showed high activity compared to that of boiled aqueous extraction. Tilak et al. (2004) studied total flavonoid and total phenolic in *Curcuma longa* using different extraction such as boiled aqueous for 10 mins, 30 mins, boiled ethanol for 10 min, stirring aqueous for 1 h and stirring ethanol for 1 h. Tilak et al. (2004) found that boiled ethanol extract for 10 mins gave the highest activity (7.869 mg trolox eq/g FW) of total flavonoid and the second highest was found in 1 h stirred ethanol extraction with 7.835 mg trolox eq/g FW. For total phenolic activity the same pattern can be observed with boiled ethanol extract for 10 mins and 1 h stirred ethanol extraction was found to be the best extraction method with 1434.78 and 991.11 mg trolox equivalent/g FW, respectively. Chan et al. (2009) reported that freeze-dried extract of *K. galanga* exhibited higher total phenolic activity compared to air-dried extract with 112 and 39 mg GAE/g sample, respectively. Tachakittirungrod et al. (2007) reported total

phenolic content of guava leaf extracts from methanol, butanol, ethyl acetate and hexane and found out that extraction using methanol exhibited the highest activity of total phenolic followed by butanol, ethyl acetate and hexane with 5.271 , 1.943 , 1.810 and 1.619 mg GAE/ml, respectively. Most of the vegetables studied showed antioxidant activities but varies from one assay to the other.

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REFERENCES

- Amin I, Norazaidah Y, Emmy Hainida KI (2006). Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species. Food Chem. 94: 47-52.
- Bahorun T, Luximon-Ramma A, Crozier A, Aruoma OI (2004). Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. J. Sci. Food Agric. 84: 1553-1561.
- Barlow SM (1990). Toxicological aspects of antioxidants used as food additives. In: Hudson B, Editor, Food Antioxidants, Elsevier Applied Science, London pp. 253-308.
- Cao G, Sofic E, Prior RL (1996). Antioxidant capacity of tea and common vegetables. J. Agric. Food Chem. 4: 3426-3431.
- Chan EWC, Lim YY, Wong SK, Lim KK, Tan SP, Lianto FS, Yong MY (2009). Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. Food Chem. 113: 166-172.
- Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N (2005). Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. Food Chem. 92: 491-497.
- Dasgupta N, De B (2007). Antioxidant activity of some leafy vegetables of India: A comparative study. Food Chem. 101(2): 471-474.
- Davies SHR, Masten SJ (1991). Spectrophotometric method for ascorbic acid using dichlorophenolindophenol: elimination of the interference due to iron. Anal. Chim. Acta 248: 225-227.
- Dawidowicz AL, Wianowska D, Baraniak B (2006). The antioxidant properties of alcoholic extracts from *Sambucus nigra* L. (antioxidant properties of extracts). LWT 39: 308-315.
- Deraniyagala SA, Ratnasooriya WD, Goonasekara CL (2003). Antinociceptive effect and toxicological study of the aqueous bark extract of *Barringtonia racemosa* on rats. J. Ethnopharmacol. 86: 21-26.
- Freidovich, I (1999). Fundamental aspects of reactive oxygen species or what's the matter with oxygen?. Ann. New York Academy Sci. 893: 13.
- Huang L, Yagura T, Chen S (2008). Sedative activity of hexane extract of *Kaempferia galanga* L. and its active compounds. J. Ethnopharmacol. 120: 123-125.
- Kikuzaki H, Nakatani N (1993). Antioxidant effects of some ginger constituents. J. Food Sci. 58: 1407-1410.
- Mula S, Banerjee D, Patro BS, Bhattacharya S, Barik A, Bandyopadhyay SK, Chattopadhyay S (2008). Inhibitory property of the Piper betel phenolics against photosensitization-induced biological damages. Biorg. Med. Chem. 16: 2932-2938.
- Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohoni PP, Biyani MK (2003). Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochem. 63: 97-104.

- Norhaiza M, Maziah M, Hakiman M (2009). Antioxidative properties of leaf of a popular herb, *Labisia pumila*. J. Med. Plant Res. 3(4): 217-223.
- Singleton VL, Rossi JA (1965). Colorimetry of total phenolics with phoshomolybdic-phosphotungstic acid reagent. Am. J. Soc. Enol. Viticult. 16: 144-158.
- Suchandra C, Zareena N, Gautam S, Soumyakanti A, Prasad SV and Arun S (2007). Antioxidant activity of some phenolic constituents from green pepper (*Piper nigrum* L.) and fresh nutmeg mace (*Myristica fragrans*). Food Chem. 101: 515-523.
- Tachakittirungrod S, Okonogi S, Chowwanapoonpohn S (2007). Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. Food Chem. 103: 381-398.
- Tilak JC, Banerjee M, Mohan H, Devasagayam TPA (2004). Antioxidant availability of turmeric in relation to its medicinal and culinary uses. Phytother. Res. 18: 798-804.
- Velioglu YS, Mazza G, Gao L, Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food Chem. 46: 4113-4117.
- Wong SP, Lai PL, Jen HWK (2006). Antioxidant activities of aqueous extracts of selected plants. Food Chem. 99: 775-783.
- Zhishen J, Mengcheng T, Jianming W (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64: 555-559.