Sains Malaysiana 42(9)(2013): 1253–1259

Antioxidant Activity in Crude Petroleum Benzene, Chloroform, Methanol and Water Extracts of Six Selected Vegetables

(Aktiviti Antioksida dalam Ekstrak Mentah Petroleum Benzena, Kloroform, Metanol dan Air daripada Enam Sayuran Terpilih)

SUMATHI MURTI*, NURHAYATI ZAINAL ABIDIN & ASHRIL YUSOF

ABSTRACT

*In this study, crude petroleum benzene, chloroform, methanol and water extracts of six selected vegetables namely, garlic chives (*Allium tuberosum*), celery (*Apium graveolens *(L.)), sweet potato leaves (*Ipomoea batatas *(L.)), curry leaves (*Murraya koenigii *(L.)), winged beans (*Psophocarpus tetragonolobus*) and sweet leaves (*Sauropus androgynus*) were tested for antioxidant activities using three bioassays namely 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, reducing power assay and metal chelating assay. Overall, among the 24 crude extracts tested, petroleum benzene and chloroform extract of* Murraya koenigii *(L.) showed higher free radical scavenging activities (IC_{50} = 0.02 mg/mL and 0.0225 mg/mL, respectively) when compared to ascorbic acid (IC_{50} = 0.00375 mg/mL) and strong reducing powers with absorbance value of 1.208 ± 0.006 and 1.833 ± 0.003 when compared to butylated hydroxyanisole with absorbance value of 2.625 ± 0.004 at the highest concentration tested, 1 mg/mL in both DPPH and reducing power assay, respectively. In metal chelating assay, methanol, petroleum benzene and chloroform extracts of* Murraya koenigii *(L.) showed moderate metal chelating activities of 88.60 ± 0.02%, 78.30 ± 0.23% and 73.61 ± 0.22%, respectively, at 1 mg/mL when compared to ethylenediaminetetraacetic acid which is 98.63 ± 0.13%. These findings suggested the important significance of* Murraya koenigii *(L.) consumption in prevention of diseases.*

Keywords: Curry leaves; DPPH radical scavenging assay; metal chelating assay; reducing power assay

ABSTRAK

*Dalam kajian ini, ekstrak mentah petroleum benzena, kloroform, metanol dan air daripada enam sayur-sayuran terpilih iaitu, daun kucai bawang putih (*Allium tuberosum*), saderi (*Apium graveolens *(L.)), daun ubi keledek (*Ipomoea batatas *(L.)), daun kari (*Murraya koenigii *(L.)), kacang botol (*Psophocarpus tetragonolobus*) dan daun manis (*Sauropus androgynus*) telah diuji untuk aktiviti antioksidan menggunakan tiga bioasai iaitu asai penyahbebas radikal 2, 2-difenil-1-picrylhydrazyl (DPPH), asai kuasa penurun dan asai pengkelat logam. Secara keseluruhannya, antara 24 ekstrak mentah yang diuji, ekstrak petroleum benzena dan kloroform* Murraya koenigii *(L.) menunjukkan aktiviti penyahbebas radikal yang tinggi (IC_{50} = 0.02 mg/mL dan 0.0225 mg/mL masing-masing) berbanding dengan asid askorbik (IC_{50} = 0.00375 mg/mL) dan kuasa penurun yang kuat dengan nilai keserapan 1.208 ± 0.006 dan 1.833 ± 0.003 berbanding dengan hydroxyanisole butylated dengan nilai keserapan 2.625 ± 0.004 pada kepekatan tertinggi yang diuji, 1 mg/mL pada kedua-dua asai DPPH dan kuasa penurun, masing-masing. Dalam asai pengkelat logam, ekstrak metanol, petroleum benzena dan kloroform* Murraya koenigii *(L.) menunjukkan aktiviti pengkelat logam yang sederhana 88.60 ± 0.02%, 78.30 ± 0.23% dan 73.61 ± 0.22% masing-masing pada 1 mg/mL berbanding dengan asid atelindiamintetrasetik (98.63 ± 0.13%). Penemuan ini menunjukkan kepentingan penggunaan* Murraya koenigii *(L.) dalam pencegahan penyakit.*

Kata kunci: Asai kuasa penurun; asai pengkelat logam; asai penyahbebas radikal DPPH; daun kari

INTRODUCTION

Epidemiological studies have shown that there is a positive association between intake of vegetables and the reduction of cardiovascular diseases (Hu 2003) and certain cancers (Riboli & Norat 2003). It is generally assumed that the main dietary constituents contributing to these protective effects are the antioxidant components (Agudo et al. 2007; Kong et al. 2010; Venkat Ratnam et al. 2006). It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defences or by supplementing with proven dietary antioxidants such as Vitamins C and E (Jenner 2003; Laggner et al. 2005; Lee & Jeong 2007; Stanner et al. 2004). Researchers have reviewed extensively on antioxidant activities of aqueous extracts of sweet leaves, curry leaves and their local celery using 2, 2-diphenyl-1-picrylhydrazyl DPPH assay and ferric reducing antioxidant power (FRAP) assay (Arulselvan & Subramaniam 2007; Wong et al. 2006). The present study deals with six exclusive lists of such plants based on information collected from various literatures dealing with plants found in Malaysia.

Allium tuberosum or garlic chives is a relatively new vegetable in the English-speaking world but well-known in Asian cuisines. Garlic chives are often used in Chinese herbal medicines to treat fatigue, control excessive bleeding and as an antidote for ingested poisons (Chevallier 1996). The leaves and bulbs are applied to insect bites, cuts and wounds, while the seeds are used to treat kidney, liver and digestive system problems.

Apium graveolens (L.) is a plant species in the family Apiaceae. *Apium graveolens* which is used worldwide as a vegetable, either for the crisp petiole (leaf stalk) or the fleshy taproot. In temperate countries, celery is also grown for its seeds. The use of celery seed in pills for relieving pain was described by Aulus Cornelius Celsus ca. 30 AD. The seeds are also used to treat arthritis and urinary tract infections. The essential oils from celery have a sedative and anticonvulsant effect and are used in the treatment of hypertension (Chevallier 1996).

Ipomoea batatas (L.) or the sweet potato leaves is from a dicotyledonous plant which belongs to the Convolvulaceae family. Amongst the approximately 50 genera and more than 1000 species of this family, only I. batatas is a crop plant whose large, starchy, sweet tasting tuberous roots are an important root vegetable (Purseglove 1991; Woolfe 1992). In total, it contains 15 different compounds that could help prevent heart disease, diabetes, infection and some types of cancer (Edmond & Ammerman 1971).

Murraya koenigii (L.) or the curry leaves is from a tropical to sub-tropical tree in the Rutaceae family, which is native to India. It produces the leaves known as curry leaves or sweet neem leaves. The leaves are highly valued as seasoning in Asian cooking. In India, the curry leaf is used to prevent conditions such as nausea and stomach upsets. It is also used in treating skin irritations and poisonous bites. Its oil is invaluable as repellents and to cure skin disorders common to the tropics. The leaves of *Murraya koenigii* are also used as an herb in Ayurvedic medicine (Arulselvan & Subramanian 2007).

Psophocarpus tetragonolobus or the winged bean is a tropical legume plant native to Papua New Guinea. The plant is one of the best nitrogen fixers with nodulation accomplished by the soil bacterium Rhizobium. Each of these parts of the winged bean provides a source of Vitamin A, Vitamin C, calcium, iron and other vitamins (Venketeswaran et al. 1990).

Sauropus androgynus, also known as *katuk*, star gooseberry, or sweet leaf, is a shrub grown in some tropical regions as a leaf vegetable. It is one of the most popular leaf vegetables in South Asia and Southeast Asia and is notable for high yields and palatability. The shoot tips have been sold as tropical asparagus. It is among only a few floras containing Vitamin K (Kao et al. 1999).

Many studies have reported the phytochemical constituents of these plants, however, till date, no research has been conducted on their anti-oxidant properties. Since the plants are widely consumed in Asia, the findings of this study would be health beneficial.

The objectives of the study were to evaluate the crude petroleum benzene, chloroform, methanol and water extracts of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus* and *Sauropus androgynus* for:

radical scavenging activities using DPPH radical scavenging assay; reducing ability using the reducing power assay and metal chelating ability using the metal chelating assay.

Materials and Methods

Material

The leaves of *Allium tuberosum* (garlic chives), *Apium graveolens* (L.) (celery), *Ipomoea batatas* (L.) (sweet potato leaves), *Murraya koenigii* (L.) (curry leaves), *Psophocarpus tetragonolobus* (winged bean) and *Sauropus androgynus* (sweet leaves) were analysed for antioxidant potentials.

Plant preparation

The selected vegetables leaves were washed and dried in a hot oven at 60°C for three days. The dried samples were weighed using (Mettler AJ100) before it was ground into fine powder.

Plant extraction

In a separate conical flask, 200 mL of petroleum benzene, chloroform, methanol and water, respectively, was added into each 20 g of the sample's powder. The flasks were then placed in an incubated shaker at 200 rpm for three days at room temperature 34 ± 2ºC.

The mixture was then filtered using the 24 cm Whatman filter paper into a round bottom flask and concentrated using a rotary evaporator at 40-50°C. The crude extracts were weighed and kept in vials wrapped with aluminium foil to reduce the risk of oxidation. The residues were weighed to calculate the percentage of solubility (Laetitia et al. 2008).

Determination of 2, 2'–diphenyl–1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH free radical scavenging activity of the selected vegetable extracts was appraised using previously described method (Iqbal et al. 2005). Briefly, 5.0 mL of freshly prepared solution of DPPH (0.025 g/L) were added to 1.0 mL of extract containing 25 µg/mL of dry extract in methanol. The absorbance of the reaction mixture was recorded at 517 nm using a spectrophotometer (Hitachi) and DPPH radical scavenging activity was calculated. The ascorbic acid was used as the positive reference standard in the DPPH radical scavenging assay.

REDUCING POWER ASSAY

The reducing power of the prepared extracts was determined using the method of Oyaizu (1986) with minor modifications. Extracts of 1 mg, 0.5 mg, 0.25 mg, 0.125 mg and 0.0625 mg were dissolved in 1.0 mL of methanol to which was added 2.5 mL of 0.2 M phosphate buffer (pH6.6) and 2.5 mL of a 1% (W/V) potassium ferricyanide (Sigma). The mixtures were incubated in water bath at 50°C for 20 min. After that, 2.5 mL of a 10% (W/V) trichloroacetic acid solution (Sigma) was added and the mixture was then centrifuged at 1000 rpm for 10 min. A 2.5 mL aliquot of the upper layer was combined with 2.5 mL of distilled water and 0.5 mL of a 1% 9 W/V) solution of ferric chloride. The absorbance of reaction mixture was read spectrophotometrically at 700 nm. The increased absorbance of the reaction mixture indicates greater reducing power. The mean values from three independent test runs were calculated for each extract.

METAL CHELATING ASSAY

The chelation of ferrous ions by the extracts and standard was evaluated using the method of Dinis et al. (1994). $FeCl_2$ in methanol (2 mM) and Ferrozine in distilled water (1 mM) were prepared. EDTA was used as a positive reference standard for the metal chelating assay.

To determine the chelation activity, the reaction mixtures of crude extracts, $FeCl_2$ and Ferrozine was shaken vigorously and left standing at room temperature for 10 min. The absorbance was measured at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated.

RESULTS AND DISCUSSION

All four petroleum benzene, chloroform, methanol and water extracts at different concentrations of 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.0625 mg/mL and control (without crude extract) were tested for their ability as antioxidants using the following assays:

DPPH FREE RADICAL SCAVENGING ACTIVITY

DPPH assay has been widely used to evaluate the free radical-scavenging effectiveness of various antioxidant substances in food systems (Cotelle et al. 1996). Table 1 indicates the IC_{50} value of the positive crude extracts of the selected local vegetables. The values are extrapolated from dose response curve using mean of triplicates ± S.E. for each concentration. IC_{50} (the amount of antioxidant material required to scavenge 50% of free radical in the assay system) of standard was observed as 3.75 µg/mL. There was an inverse relationship between IC_{50} and antioxidant activity. Among the 24 crude extracts tested, curry leaves showed higher scavenging activities when compared with ascorbic acid. Thus, curry leaves showed free radical scavenging activity where its antioxidant activity may be attributed to proton donating ability and they are able to scavenge the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) to 1,1-diphenylhydrazine (stable DPPH). The presence of the antioxidants leads to the disappearance of the radical chromogens in DPPH (Mathew & Abraham 2006). The intensity of the yellow colour depends on the amount and nature of radical scavenger in the sample and standard compounds. The colour changes from purple to transparent, slight yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm (Huang et al. 2005). The ascorbic acid was selected as the standard reference in this DPPH assay because it is a potent antioxidant known to scavenge a wide variety of reactive oxygen species (ROS) (Lutsenko et al. 2002).

REDUCING POWER ASSAY

The reducing power analyses the ability to reduce Fe^{3+} to Fe^{2+}. The reducing potential of all 24 crude extracts at the highest concentration tested that is 1 mg/mL are shown in Table 2 in descending order. The higher the absorbance reading of the crude extracts, the stronger the ability of the extracts to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron. Overall, out of the 24 crude extracts tested, crude chloroform and petroleum benzene extracts of curry leaves

TABLE 1. The IC_{50} value of the positive crude extracts of selected local vegetables

Local Vegetables	IC_{50} Values (µg/mL)			
	Crude petroleum benzene extract	Crude chloroform extract	Crude methanol extract	Crude water extract
Allium tuberosum	-	467.5	-	192.5
Apium graveolens (L.)	-	-	-	25
Ipomoea batatas (L.)	-	500	292.5	75
Murraya koenegii (L.)	21.25	22.5	-	32.5
Psophocarpus tetragonolobus	400	-	117.5	27.5
Sauropus androgynous (L.)	36.25	125	145	307.5

TABLE 2. The reducing power of all 24 crude extracts tested at the highest concentration tested, 1 mg/mL in descending order

Reducing capacities/power	Absorbance reading at 1 mg/mL	Crude extract of selected vegetables
0.700-1.999	1.833 ± 0.003	Curry leaves + chloroform
	1.529 ± 0.004	Sweet leaves + water
	1.208 ± 0.006	Curry leaves + petroleum benzene
0.400-0.699	0.696 ± 0.001	Celeries + methanol
	0.686 ± 0.003	Sweet potato leaves + water
	0.656 ± 0.004	Sweet leaves + petroleum benzene
	0.493 ± 0.005	Sweet potato leaves + petroleum benzene
	0.482 ± 0.003	Winged beans + methanol
	0.481 ± 0.001	Sweet potato leaves + chloroform
	0.443 ± 0.002	Sweet leaves + methanol
	0.432 ± 0.004	Sweet potato leaves + methanol
0.100-0.399	0.322 ± 0.002	Celeries + water
	0.295 ± 0.003	Celeries +petroleum benzene
	0.288 ± 0.002	Celeries + chloroform
	0.285 ± 0.003	Garlic chives + methanol
	0.282 ± 0.002	Winged beans + water
	0.272 ± 0.002	Winged beans + petroleum benzene
	0.243 ± 0.002	Garlic chives + petroleum benzene
	0.235 ± 0.003	Curry leaves + water
	0.232 ± 0.002	Sweet leaves + chloroform
	0.230 ± 0.004	Garlic chives + water
	0.225 ± 0.002	Garlic chives +chloroform
	0.223 ± 0.002	Winged beans + chloroform
	0.192 ± 0.002	Curry leaves + methanol

showed the highest reducing powers when compared with the standard reference; butylated hydroxyanisole (BHA). This finding suggest that these plants are electron donor and might contain high amounts of reductones, which could react with free radicals, convert them to more stable products and terminate radical chain reactions. Chan et al. (2008) reported that the high antioxidant activities of chloroform extracts of leafy materials were probably due to the extracted tannins and photosynthetic pigments. The chloroform crude extracts of curry leaves contained tannins may serve as a significant indicator of its potential antioxidant activity (Meir et al. 1995). Mishra et al. (2009) reported the presence of phenolics, flavonoids and condensed tannins in both chloroform and petroleum benzene extracts of *Murraya koenigii* (L.) leaves. Some low molecular weight phenolics are pro-oxidants in Fenton-driven systems, apparently because the phenolics are able to redox cycle the metal ion required for radical formation (Aruoma et al. 1993). A group of researchers tested tannins which do not act as pro-oxidants in the Fenton systems and in fact react very rapidly to quench the hydroxyl radical. They also examined the antioxidant capabilities of tannin-protein complexes (Riedl & Hagerman 2001). The presence of reductants such as antioxidant substances in the crude extracts caused the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form (Fe^{2+}). The formation of Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Chung et al. 2002). The colour change from yellow to various shades of green and blue depending on the reducing power of each antioxidant samples.

METAL CHELATING ASSAY

The metal chelating assay analyses the ability to chelate ferrous ion. Table 3 indicates chelating capacity on ferrous ion of all 24 crude extracts tested, at the highest concentration tested, 1 mg/mL from highest chelating ability to the lowest. The values represent the mean of triplicates ± S.E. for each concentration. Petroleum benzene extract of garlic chives and celery, water extract of celery and chloroform extracts of sweet potato leaves showed moderate metal chelating activity at 1 mg/mL when compared with EDTA which is 98.63 ± 0.13%. Metal chelating capacity is significant since antioxidants in the extract reduced the concentration of the catalyzing transition metal in lipid peroxidation (Duh et al. 1999). It was reported that chelating agents, which form alpha bonds with a metal and therefore are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gordon 1990). Methanol extracts of curry leaves were shown to be the strongest chelator where they interfered with the formation of ferrous and ferrozine complex suggesting that they had chelating activity and captured ferrous ion before ferrozine. This is supported by Ningappa et al. (2008) who reported that methanol extract of curry leaves chelated ferrous ions effectively as antioxidants,

TABLE 3. The chelating capacity on ferrous ion of all 24 crude extracts tested, at the highest concentration tested, 1 mg/mL in descending order

Chelating capacity on ferrous ion	Percentage of inhibition of ferrozine-Fe^{2+} complex at 1 mg/mL	Crude extract of selected vegetables
(≥ 80.00%)	88.60 ± 0.20	Curry leaves + methanol
	88.56 ± 0.19	Sweet potato leaves + chloroform
	87.46 ± 0.28	Celeries + water
	81.09 ± 0.27	Garlic chives + petroleum benzene
	81.02 ± 0.14	Celeries + petroleum benzene
(50.00 - 79.99%)	79.78 ± 0.13	Winged beans + chloroform
	79.40 ± 0.14	Sweet potato leaves + petroleum benzene
	78.30 ± 0.23	Curry leaves + chloroform
	78.05± 0.25	Garlic chives +chloroform
	77.02 ± 0.14	Winged beans + petroleum benzene
	73.61 ± 0.22	Curry leaves + petroleum benzene
	73.45 ± 0.17	Celeries + chloroform
	70.99 ± 0.14	Sweet leaves + chloroform
	70.68 ± 0.22	Garlic chives + methanol
	67.02 ± 0.33	Winged beans + water
	66.95 ± 0.14	Sweet leaves + petroleum benzene
	66.81 ± 0.26	Curry leaves + water
	59.52 ± 0.34	Celeries + methanol
	58.94 ± 0.26	Winged beans + methanol
	56.14 ± 0.34	Sweet potato leaves + water
	52.23 ± 0.03	Garlic chives + water
	50.02 ± 0.36	Sweet leaves + methanol
(< 50%)	48.14 ± 0.42	Sweet leaves + water
	46.03± 0.30	Sweet potato leaves + methanol

flavonoids have been reported to be able to interfere with the activities of enzymes involved in reactive oxygen species generation, quenching free radicals, chelating transition metals and rendering them redox inactive in the Fenton reaction (Heim et al. 2002). Antioxidant compounds present in plant extracts are therefore multi-functional and their activity and mechanism would largely depend on the composition and conditions of the test system. Many authors have stressed the need to perform more than one type of antioxidant activity measurement to take into account the various mechanisms of antioxidant action (Frankel & Meyer 2000; Prior & Cao 1999). Transition metal ions such as those of copper and iron are important catalysts for the generation of highly reactive hydroxyl radicals via the Fenton reaction in both *in vivo* and *in vitro* systems. Ligands that bind to metal ions can alter the redox potentials of these ions, which would render the ions catalytically silent. As secondary antioxidants, compounds can act as effective ligands that sequester copper and ferrous ions by 'wrapping' themselves around these ions. These ligands could help intercept and suppress radicals formed via catalysis from fuelling a chain reaction (Aruoma et al. 1987). Free hydroxyl groups in the flavonoid ligands chelating the central metal ion may scavenge free radicals.

Vegetables commonly consumed by the population of Malaysia include cabbage, Chinese mustard, onions, long beans, swamp cabbage and spinach. Food balance sheet data for Malaysia showed that per capita of vegetables supply per year was 37.60 kg (FAO 2005). The actual vegetable consumption data are lacking. Mubarik (1996) reported that in 1991, Malaysians consumed 4.65 kg cabbage, 2.90 kg swamp cabbage, 2.45 kg spinach and 0.77 kg kale. The recent studies showed that these vegetables have high antioxidant capacity (Amin et al. 2004, 2006; Amin & Lee 2005).

Chevallier (1996) also reported curry leaf has medicinal value as traditionally used in eastern Asia. Interest in greater use of curry leaf has been stimulated since its high antioxidant potency was reported and this antioxidant activity is attributed due to mahanimbine, murrayanol and mahanine from *M. koenigii* (Ningappa et al. 2008; Tachibana et al. 2003). Chowdhury et al. (2001) reported that these alkaloids shown to have antimicrobial activity against gram positive and negative bacteria and fungi. The use of curry leaf powder as antioxidant in ground and pre-cooked muscle foods on oxidative stability has been poorly reported.

CONCLUSION

The results of the study implied the enormous nutritive value of the six plant species and their significance in the prevention of the free radical-induced diseases. This study suggested that *Murraya koenigii* (L.) has natural antioxidant potential which can be developed into a good

herb with medicinal properties other than its general use of being a spice. The observations may be used to substantiate the scientific reasoning that free radical-scavenging is indeed the factor of these plants in the treatment or prevention of the onset of deadly disorders like arthritis, breast cancer and atherosclerosis. The conclusions if established by *in vivo* studies on biological systems can open up new avenues in the search for natural antioxidants that can be employed successfully in further clinical trials.

ACKNOWLEDGEMENTS

The authors wish to acknowledge University of Malaya for the financial assistance received through the Postgraduate Research Fund Grant PS249/2009C.

REFERENCES

Agudo, A., Cabrera, L., Amiano, P., Ardanaz, E., Barricarte, A., Berenguer, T., Chirlaque, M.D., Dorronsoro, M., Jakszyn, P., Larranaga, N., Martinez, C., Navarro, C., Quiros, J.R., Sanchez, M.J., Tormo, M.J. & Gonzalez, C.A. 2007. Fruit and vegetable intakes, dietary antioxidant nutrients, and total mortality in Spanish adults: Findings from the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Spain). *American Journal of Clinical Nutrition* 85(6): 1634-1642.

Amin, I., Zamaliah, M.M. & Chin, W.F. 2004. Total antioxidant activity and phenolic content in selected vegetables. *Fd. Chem*. 87: 581-586.

Amin, I. & Lee, W.Y. 2005. Effect of different blanching times on antioxidant properties in selected cruciferous vegetables. *J. Sci. Fd. Agric*. 98: 2314-2320.

Amin, I., Norazaidah, Y. & Emmy Hainida, K.I. 2006. Antioxidant activity and phenolic content of spinach species (*Amaranthus* sp). *Fd. Chem*. 94: 47-52.

Arulselvan, P. & Subramanian, S.P. 2007. Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra structural changes of pancreatic beta-cells in experimental diabetes in rats. *Chem. BioL. Interact*. 165(2): 155-164.

Aruoma, O.I., Grootveld, M. & Halliwell, B. 1987. The role of iron in ascorbate-dependent deoxyribose degradation. Evidence consistent with a site-specific hydroxyl radical generation caused by iron ions bound to the deoxyribose molecule. *Journal of Inorganic Biochemistry* 29: 289-299.

Aruoma, O.I., Murcia, A., Butler, J. & Halliwell, B. 1993. Evaluation of the antioxidant and prooxidant actions of gallic acid and its derivatives. *J. Agric. Food Chem*. 41: 1880-1885.

Chan, L.W., Cheah, E.L., Saw, C.L., Weng, W. & Heng, P.W. 2008. Antimicrobial and antioxidant activities of *Cortex Magnoliae Officinalis* and some other medicinal plants commonly used in South-East Asia. *Chinese Medicine* 3: 15.

Chevallier, A. 1996. *The Encyclopedia of Medicinal Plants*. London: Dorling Kindersley Publishers Ltd.

Chowdhury, B.K., Jha, S., Bhattacharyya, P. & Mukherjee, J. 2001. Two new carbazole alkaloids from *Murraya koenigii*. *Indian Journal of Chemistry* 40: 490-494.

Chung, Y.C., Chang, C.T., Chao, W.W., Lin, C.F. & Chou, S.T. 2002. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. *J. Agric. Food Chem*. 50: 2454-2458.

Cotelle, N., Bemier, J.L., Catteau, J.P., Pommery, J., Wallet, J.C. & Gaydou, E.M. 1996. Antioxidant properties of hydroxyl-flavones. *Free Radic. Biol. Med*. 20: 35-43.

Dinis, T.C.P., Madeira, V.M.C. & Almeida, L.M. 1994. Action of phenolic derivatives (acetoaaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxylradical scavengers. *Archieves of Biochemistry and Biophysics* 315: 161-169.

Duh, P.D., Tu, Y.Y. & Yen, G.C. 1999. Antioxidant activity of water extract of harng Jyur (*Chrysanthemum morifolium* Ramat). *Lebensm. Wiss TechnoL*. 32(5): 269-277.

Edmond, J.B. & Ammerman, G.R. 1971. *Sweetpotatoes: Production, Processing, and Marketing*. Westport, Connecticut: AVI Publishing Company.

Frankel, E.N. & Meyer, A.S. 2000. The problems of using one dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture* 80: 1925-1941.

Gordon, M.H. 1990. The mechanism of antioxidant action *in vitro*. In *Food Antioxidants*, edited by Hudson, B.J.F. London: Elsevier Applied Science.

Heim, K.E., Tagliaferro, A.R. & Bobilya, D.J. 2002. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *Journal of Nutritional Biochemistry* 13: 572-584.

Hu, F.B. 2003. Plant-based foods and prevention of cardiovascular disease: An overview. *American Journal of Clinical Nutrition* 78: 544S-551S.

Huang, D.J., Chen, H.J., Lin, C.D. & Lin, Y.H. 2005. Antioxidant and antiproliferative activities of water spinach (*Ipomea aquatica Forsk*) constituents. *Botanical Bulletin of Academia Sinica* 46: 99-106.

Iqbal, S., Bhanger, M.I. & Anwar, F. 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. *Food Chem*. 93: 265-272.

Jenner, P. 2003. Oxidative stress in Parkinson's Disease. *Ann. Neurol*. 53(3): 26-38.

Kao, C.H., Ho, Y.J., Wu, C.L. & Chang Lai, S.P. 1999. Using 99mTc-DTPA radioaerosol inhalation lung scintigraphies to detect the lung injury induced by consuming *Sauropus androgynus* vegetable and comparison with conventional pulmonary function tests. *Respiration* 66(1): 46-51.

Kong, K.W., Amin Ismail, Tan, C.P. & Nor Fadilah Rajab. 2010. Optimization of oven drying conditions for lycopene content and lipophilic antioxidant capacity in a by-product of the pink guava puree industry using response surface methodology. *LWT - Food Science and Technology* 43(5): 729-735.

Laetitia, M.D., Gaetan, L.F. & Christian, M. 2008. Radical scavenging, antioxidant and antimicrobial activities of halophytic species. *Journal of Ethnopharmacology* 116(2): 258-262.

Laggner, H., Hermann, M., Sturm, B., Gmeiner, B.M. & Kapiotis, S. 2005. Sulfite facilitates LDL lipid oxidation by transition metal ions: A pro- oxidant in wine? *FEBS Letters* 579(28): 6486-6492.

Lee, K.J. & Jeong, H.G. 2007. Protective effects of kahweol and cafestol against hydrogen peroxide- induced oxidative stress and DNA damage. *Toxicology Letters* 173(2): 80-87.

Lutsenko, E.A., Carcamo, J.M. & Golde, D.W. 2002. Vitamin C prevents DNA mutation induced by oxidative stress. *J. Bio*L. *Chem*. 277: 16895-16899.

Mathew, S. & Abraham, T.E. 2006. Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various *in vitro* models. *Food Chem*. 94: 520-528.

Meir, S., Kanner, J., Akiri, B. & Hada, S.P. 1995. Determination and involvement of aqueous reducing compounds in oxidative defense system of various senescing leaves. *J. Agric. Food. Chem.* 43: 1813-1819.

Mishra, J., Yousuf, A., Singh, R. & Aradhana. 2009. Phytochemical investigation and *in-vitro* antioxidant potential of leaves of *Murraya koenigii*. *International Journal of Integration Biology* 7(3): 171-174.

Mubarik, A. 1996. *Dynamics of Vegetable Production, Distribution and Consumption in Asia*. Asian Research and Development Center.

Ningappa, M.B., Dinesha, R. & Srinivas, L. 2008. Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extracts. *Food Chemistry* 106: 720-728.

Oyaizu, M. 1986. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepapared from glucosamine. *Japanese Journal of Nutrition* 446: 307-315.

Prior, R.L. & Cao, G. 1999. *In vivo* total antioxidant capacity: Comparison of different analytical methods. *Free Radical Biology and Medicine* 27: 1173-1181.

Purseglove, J.W. 1991. *Tropical Crops: Dicotyledons*. (Longman Scientific and Technical). New York, USA: John Wiley and Sons, Inc. (Original work published 1968).

Riedl, K.M. & Hagerman, A.E. 2001. Tannin-protein complexes as radical scavengers and radical sinks. *Journal of Agricultural and Food Chemistry* 49: 4917-4923.

Riboli, E. & Norat, T. 2003. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *American Journal of Clinical Nutrition* 78(3): 559S-569S.

Stanner, S.A., Hughes, J., Kelly, C.N. & Buttriss, J.A. 2004. Review of the epidemiological evidence for the 'antioxidant hypothesis'. *Public Health Nutrition* 7: 407-422.

Tachibana, Y., Kikuzaki, H., Lajis, N.H. & Nakatani, N. 2003. Comparison of antioxidative properties of carbazole alkaloids from *Murraya koenigii* leaves. *Journal of Agriculture and Food Chemistry* 51: 6461- 6467.

Wong, S.P., Lai, P.L. & Jen, H.W.K. 2006. Antioxidant activities of aqueous extracts of selected plants. *Food Chem.* 99: 775-783.

Woolfe, J.A. 1992. *Sweet Potato: An Untapped Food Resource*. Cambridge, UK: Cambridge Univ. Press.

Venkat Ratnam, D., Ankola, D.D., Bhardwaj, V., Sahana, D.K. & Ravi Kumar, M.N.V. 2006. Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. *Journal of Controlled Release* 113(3): 189-207.

Venketeswaran, S., Dias, M.A.D.L. & Weyers, U.V. 1990. The winged bean: A potential protein crop. In *Advances in New Crops,* edited by Janick, J. & Simon, J.E. Portland: Timber Press.

Sumathi Murti* & Nurhayati Zainal Abidin
Institute of Biological Science
Faculty of Science
University of Malaya
50603 Kuala Lumpur
Malaysia

Ashril Yusof
Sport Centre
University of Malaya
50603 Kuala Lumpur
Malaysia

*Corresponding author; email: m_sumi2001@yahoo.com

Received: 2 August 2012
Accepted: 2 February 2013