

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

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

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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 $88.60 \pm 0.02\%$, $78.30 \pm 0.23\%$ and $73.61 \pm 0.22\%$, respectively, at 1 mg/mL when compared to ethylenediaminetetraacetic acid which is $98.63 \pm 0.13\%$. 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 kacang 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-picrylhidrazyl (DPPH), asai kuasa penurunan 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 penurunan 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 penurunan, masing-masing. Dalam asai pengkelat logam, ekstrak metanol, petroleum benzena dan kloroform Murraya koenigii (L.) menunjukkan aktiviti pengkelat logam yang sederhana $88.60 \pm 0.02\%$, $78.30 \pm 0.23\%$ dan $73.61 \pm 0.22\%$ masing-masing pada 1 mg/mL berbanding dengan asid atelindiamintetrasetik ($98.63 \pm 0.13\%$). Penemuan ini menunjukkan kepentingan penggunaan Murraya koenigii (L.) dalam pencegahan penyakit.

Kata kunci: Asai kuasa penurunan; 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 $34 \pm 2^\circ\text{C}$.

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 \pm 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 $\mu\text{g/mL}$. 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 ($\mu\text{g/mL}$)			
	Crude petroleum benzene extract	Crude chloroform extract	Crude methanol extract	Crude water extract
<i>Allium tuberosum</i>	-	467.5	-	192.5
<i>Apium graveolens</i> (L.)	-	-	-	25
<i>Ipomoea batatas</i> (L.)	-	500	292.5	75
<i>Murraya koenigii</i> (L.)	21.25	22.5	-	32.5
<i>Psophocarpus tetragonolobus</i>	400	-	117.5	27.5
<i>Sauropus androgynous</i> (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 Fe³⁺/ ferricyanide complex to the ferrous form (Fe²⁺). The formation of Fe²⁺ 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,

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.

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