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Antioxidant activities of aqueous extracts of selected plants

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Abstract

The antioxidant properties of 25 edible tropical plants, expressed as Trolox equivalent antioxidant capacity (TEAC), were studied using DPPH (1,1-diphenyl-2-picrylhydrazyl free radical) scavenging and reducing ferric ion antioxidant potential (FRAP) assays. Their cupric ion chelating activities (CCA) and total polyphenol contents (TPC) were also determined. A strong correlation between TEAC values obtained for the DPPH assay ($TEAC_{DPPH}$) and those for the FRAP assay ($TEAC_{FRAP}$) implied that compounds in the extracts were capable of scavenging the DPPH free radical and reducing ferric ions. A satisfactory correlation of TPC with $TEAC_{DPPH}$ and $TEAC_{FRAP}$ suggested that polyphenols in the extracts were partly responsible for the antioxidant activities while its correlation with CCA was poor, indicating that polyphenols might not be the main cupric ion chelators. Principal component analysis (PCA) indicated that $TEAC_{DPPH}$, $TEAC_{FRAP}$ and TPC contributed to the total variation in the antioxidant activities of the plants.

Keywords: Antioxidant capacity; Tropical plants; DPPH free radical scavenging activity; Ferric reducing antioxidant power assay; Total polyphenol content; Cupric ions chelating activity; Principal component analysis

1. Introduction

Free radical reactions occur in the human body and food systems. Free radicals, in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology. An over-production of these reactive species can occur, due to oxidative stress brought about by the imbalance of the bodily antioxidant defense system and free radical formation. These reactive species can react with biomolecules, causing cellular injury and death. This may lead to the development of chronic diseases such as cancers and those that involve the cardio- and cerebrovascular systems. The consumption of fruits and vegetables containing antioxidants has been found to offer protection against these diseases. Dietary antioxidants can augment cellular defences and help to prevent oxidative damage to cellular components (Halliwell, 1989).

Besides playing an important role in physiological systems, antioxidants have been used in the food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats. These components in foods are readily oxidised by molecular oxygen and is a major cause of quality deterioration, nutritional losses, off-flavour development and discolouration. The addition of synthetic antioxidants, such as propyl gallate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone has been widely used industrially to control lipid oxidation in foods. However, the use of these synthetic antioxidants has been questioned due to their potential health risks and toxicity (Kalt & Kappus, 1993). The search for antioxidants from natural sources has received much attention and efforts have been put into identify compounds that can act as suitable antioxidants to replace synthetic ones. In addition, these naturally-occurring antioxidants can be formulated to give neutraceuticals that can help to prevent oxidative damage from occurring in the body. In this investigation, water was used as the extraction solvent to extract the hydrophilic antioxidants

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present in the plants. For use in foods, plant extracts made with water are nutritionally more relevant and would have obvious advantages in relation to certification and safety (Møller, Madsen, Aaltonen, & Skibsted, 1999). The use of a crude extract as an additive needs to be considered so that the sensory properties of the food product are not adversely affected.

Vitamins A, C and E and carotenoids are antioxidants derived from the diet. Another group of compounds, flavonoids, also possess antioxidant properties and may account for part of the benefits associated with the consumption of fruits and vegetables. Flavonoids belong to a large family of compounds with a common diphenylpropane structure ($C_6C_3C_6$) with different degrees of hydroxylation, oxidation and substitution. These compounds, also called polyphenols, commonly occur as glycosides in plants (Pietta, 2000). As antioxidants, 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, Tagliaferro, & Bobilya, 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 had 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). Currently, the study and availability of information on the antioxidant properties of many tropical plants are sporadic and lacking. The multitude and vastly differing methods used by various workers do not facilitate comparisons between various plants. In this study, the free radical scavenging activities of the plant extracts were followed via their reaction with the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical and their ferric ions reducing abilities were determined using the ferric ion reducing antioxidant potential (FRAP) assay. In this study, antioxidant activity is given as a quantity relative to that of Trolox. The usage of the Trolox equivalent antioxidant capacity (TEAC) parameter as well as the two popular assaying methods used in this investigation will offer the benefit of allowing comparisons to be made with other food and plant materials examined by other authors.

The objectives of this investigation are to determine the total polyphenol contents and characterise the free radical scavenging, ferric ion reducing and cupric ion chelating capabilities of some tropical plants consumed in the Southeast Asian region. Principal component analysis (PCA) is used to identify the total variation in the antioxidant activities of the plants by the methods used. Results from this preliminary study will provide a better understanding of the antioxidant properties of these plants and allow the identification of plants with high antioxidant activity for further investigation and development into value-added foods and neutraceuticals.

2. Materials and methods

2.1. Plant material

Twenty-five plants were obtained fresh from Singapore wet-markets on six separate occasions between June 2002 and May 2003 ($n = 6$). The plants that were investigated include Asian pennywort (*Centella asiatica*, Umbelliferae), betel (*Piper betel*, Piperaceae), cekur manis (*Sauropus androgynus*, Euphorbiaceae), Chinese boxthorn (*Lycium chinense*, Solanaceae), curry tree (*Murraya koenigii*, Rutaceae), coriander (*Coriandrum sativum* L., Umbelliferae), daun salam (*Eugenia polyantha*, Myrtaceae), horseradish tree (*Moringa pteriosperma*, Moringaceae), ketumbar jawa (*Eryngium foetidum* L., Umbelliferae), laksa (*Polygonium hydropiper*, Polygonaceae), local celery (*Apium graveolens* L., Umbelliferae), melinjau (*Gnetum genom*, Gnetaceae), mint (*Mentha arvensis* L., Labiatae), petai (*Parkia speciosa*, Leguminosae), pucuk paku (*Diplazium esculentum*, Polypodiaceae), red amaranthus (*Amaranthus gangeticus*, Amaranthaceae), roselle (*Hibiscus sabdariffa*, Malvaceae), rue (*Ruta graveolens* L., Rutaceae), spring onion (*Allium fistulosum*, Liliaceae), sweet potato (*Ipomea babatas*, Convolvulaceae), tapioca (*Manihot esculenta* L., Euphorbiaceae), Thai basil (*Ocimum basilicum* L., Labiatae), ulam raja (*Cosmos caudatus*, Compositae), West Indian pea tree (*Sesbania grandiflora*, Leguminosae) and wild lime (*Citrus hystrix*, Rutaceae).

Upon arrival at the laboratory, samples were washed with water to remove debris and damaged portions were removed. The leaves of the samples were stripped from the plants and dried according to the method described by Mohd Zin and co-workers (2002) in a convection oven at 45 °C for 48 h until there was no change in weight. The dried leaves were stored in sealed polyethylene bags with silica gel included as a dessicant. The samples were kept in a refrigerator at 4 °C until ready for extraction.

2.2. Chemicals

All chemicals used were of at least analytical grade. 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH), tetramethylmurexide ammonium salt (TMM) and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were obtained from Sigma Chemicals Co. (St. Louis, USA). Gallic acid and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Acros Organics (New Jersey, USA). Anhydrous sodium carbonate (Na_2CO_3), cupric sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), Folin–Ciocalteu phenol reagent, hydrochloric acid (HCl), glacial acetic acid, methanol, potassium chloride (KCl) and sodium acetate trihydrate were obtained from Merck (Darmstadt, Germany) and hexamine was bought from Aldrich Co. (Milwaukee, USA).

2.3. Extraction

Dried leaves were ground using a domestic blender and 0.5 g of this material was extracted using 25 ml of deionised water. The mixture was allowed to stand at room temperature for 1 h in the dark, with occasional agitation. The aqueous extract was obtained by filtering the mixture through Whatman No. 1 filter paper and used for analysis without further treatment.

2.4. Assaying methods

2.4.1. Total polyphenols contents determination

An aliquot of 100 μl of an extract was mixed with 2.5 ml of Folin–Ciocalteu phenol reagent (10x dilution) and allowed to react for 5 min. Then 2.5 ml of saturated Na_2CO_3 solution was added and allowed to stand for 1 h before the absorbance of the reaction mixture was read at 725 nm. The total polyphenol contents (TPC) of the extract was expressed as mg gallic acid equivalents per gram of plant material on dry basis (db).

2.4.2. DPPH free radical scavenging assay

The DPPH free radical scavenging activity of each sample was determined using the Ultraspec 3000 UV/vis Spectrophotometer (Pharmacia Biotech Ltd., Cambridge CB4 4FJ, UK) according to the method described by Leong and Shui (2002). Briefly, a 0.1 mM solution of DPPH in methanol was prepared. The initial absorbance of the DPPH in methanol was measured at 515 nm and did not change throughout the period of assay. An aliquot (40 μl) of an extract (with appropriate dilution, if necessary) was added to 3 ml of methanolic DPPH solution. The change in absorbance at 515 nm was measured at 30 min. The antioxidant capacity based on the DPPH free radical scavenging ability of the extract was expressed as μmol Trolox equivalents per gram of plant material on dry basis.

2.4.3. Ferric reducing antioxidant potential assay

The ability to reduce ferric ions was measured using a modified version of the method described by Benzie and Strain (1996). An aliquot (200 μl) of an extract (with appropriate dilution, if necessary) was added to 3 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10 mM TPTZ solution and 1 part of 20 mM $FeCl_3 \cdot 6H_2O$ solution) and the reaction mixture was incubated in a water bath at 37 °C. The increase in absorbance at 593 nm was measured at 30 min. The antioxidant capacity based on the ability to reduce ferric ions of the extract was expressed as μmol Trolox equivalents per gram of plant material on dry basis.

2.4.4. Cupric ions chelation assay

Plant extracts were first diluted 10 times with hexamine–HCl buffer containing 10 mM KCl at pH 5.0. One millilitre of this sample was mixed with 1 ml of 400 μM of $CuSO_4$ (made using the same buffer). Subsequently, 100 μl of 2 mM TMM solution was added to this mixture. Absorbance of the final reaction mixture was recorded at 460 and 530 nm and the ratio of the absorbance at 460 and 530 nm was computed. The absorbance ratio was then converted to corresponding free cupric ion concentration using a standard curve of absorbance ratio against concentration of free cupric ion. The difference between the total amount of cupric ions present and the free cupric ions would give the concentration of chelated cupric ions. The amount of cupric ions chelated was calculated as a percentage of the total amount of cupric ions chelated (Wettasinghe & Shahidi, 2002).

2.5. Statistical analysis

SAS systems (Windows Release Version 8.02) was used for statistical analysis of the experimental data. Principal component analysis (PCA) was conducted on all 150 data points to understand the covariance structure and identify relationships between the variables. Factor analysis is performed to reduce and explain the variability of the data. The correlation matrix is used to standardise the variables which are not measured on the same scale. Factor analysis is used to understand the correlation between the variables instead as the dimension of the variables are small. The Varimax method is used to produce orthogonal transformations to the reduced factors so as to better identify the high and low correlations.

3. Results and discussion

3.1. Total polyphenol contents

The total polyphenol content (TPC) of the plant extracts were determined using the Folin–Ciocalteu phenol reagent. From Fig. 1, local celery had the lowest amount of polyphenols while the highest was observed in petai. Generally, extracts that contain a high amount of polyphenols also exhibit high antioxidant activity. Aqueous extracts of mint, laksa, tapioca, daun salam, sweet potato, cekur manis and betel leaves which showed high antioxidant activities also had high TPC. Curry tree and petai were exceptions as they had high TPC but did not exhibit high antioxidant activities as observed in other plants.

The Folin–Ciocalteu phenol reagent is used to obtain a crude estimate of the amount of phenolic compounds present in an extract. Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the reagent. However, the assay has been shown not specific to just polyphenols but to any other substance that could be oxidised by the Folin reagent and various researchers have reported the poor specificity of the assay (Escarpa & González, 2001; Singleton, Orthofer, & Lamuela-Raventos, 1999). In addition, phenolic compounds, depending on the number of phenolic groups they have, respond differently to the Folin–Ciocalteu rea-gent (Singleton et al., 1999).

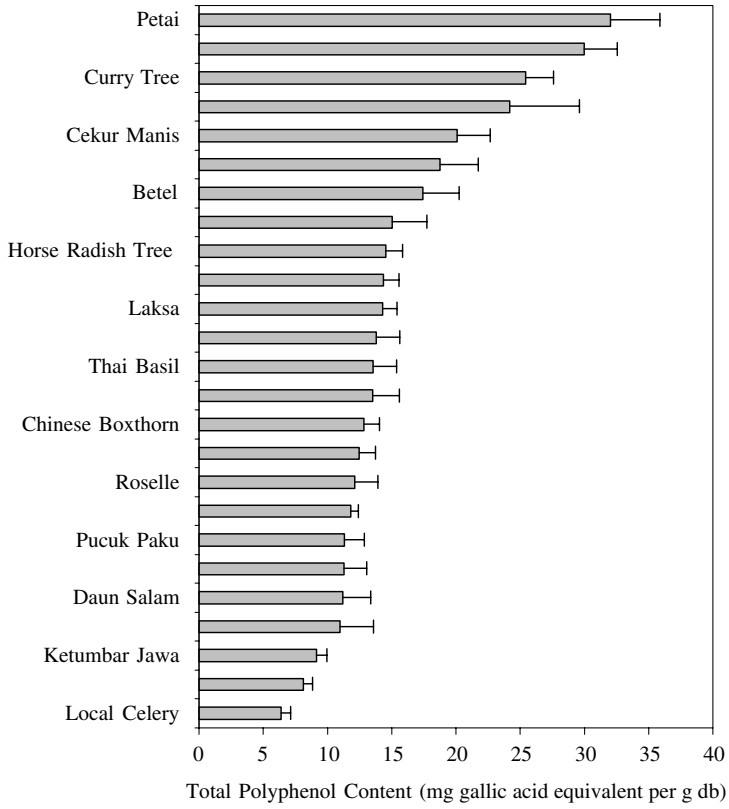

Fig. 1. Total polyphenol contents of 25 tropical plants based on the results obtained from the Folin–Ciocalteu phenol assay ($n = 6$, error bars represent standard deviation).

Hence, this may explain the observation in petai and curry tree, where they high TPC values did not correspond to a high antioxidant activity.

3.2. Antioxidant activity

3.2.1. DPPH free radical scavenging activity

The stable radical DPPH has been used widely for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, plant and fruit extracts and food materials. The assay is based on the reduction of DPPH radicals in methanol which causes an absorbance drop at 515 nm. In this study, the antioxidant activity was expressed as Trolox equivalents per gram of plant material on a dry basis as it is a more meaningful and descriptive expression than assays that express antioxidant activity as the percentage decrease in absorbance. As such the results provide a direct comparison of the antioxidant activity with Trolox.

The DPPH free radical scavenging activity of 25 plant extacts are shown in Fig. 2. Local celery showed the lowest DPPH free radical scavenging activity while ulam raja leaves had the highest activity. Other than ulam raja; red amaranthus, mint, laksa, tapioca, daun salam, sweet potato, cekur manis and betel leaves also showed relatively high DPPH free radical scavenging activities.

3.2.2. Ferric ion reducing activity

The ability of the plants extracts to reduce ferric ions was determined using the FRAP assay developed by Benzie and Strain (1996). An antioxidant capable of donating a single electron to the ferric-TPTZ (Fe(III)-TPTZ) complex would cause the reduction of this complex into the blue ferrous-TPTZ (Fe(II)-TPTZ) complex which absorbs strongly at 593 nm.

The trend for ferric ions reducing activities of the 25 plants did not vary markedly from their DPPH free radical scavenging activities, when a comparison betweeen Figs. 1 and 2 was made. Similar to the results obtained for the DPPH free radical scavenging assay; mint, laksa, tapioca, daun salam, sweet potato, cekur manis and betel leaves showed relatively strong ferric ion reducing activities. Interestingly, $TEAC_{FRAP}$ values were consistently higher than those obtained for $TEAC_{DPPH}$. Gil, Tomás-Barberán, Hess-Pierce, and Kader (2002) reported a similar trend where the DPPH free radical scavenging activities of stone fruits, expressed as ascorbic acid equivalents, were higher than their corresponding ferric ion reducing activities.

The probable reason for the lower $TEAC_{DPPH}$ values of the plants could be due to the presence of compounds not reactive towards DPPH. Antioxidant compounds such as polyphenols may be more efficient reducing agents for ferric iron but some may not scavenge DPPH free radicals as efficiently due to steric hindrance. In this study, local celery

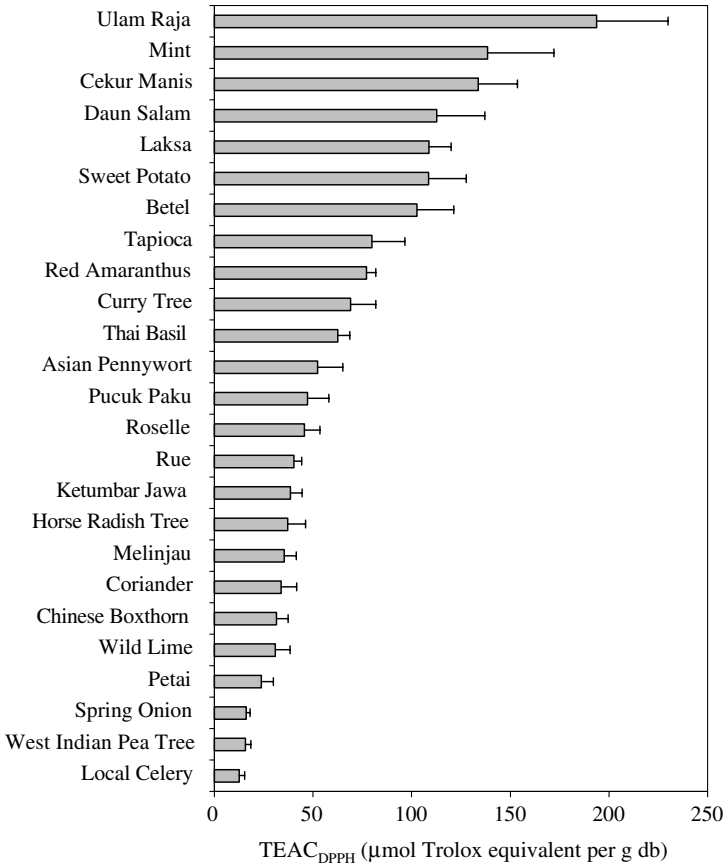

Fig. 2. Antioxidant activities of 25 tropical plants based on their abilities to scavenge DPPH free radicals ($n = 6$, error bars represent standard deviation).

had the lowest and ulam raja had the highest ferric ions reducing activity.

To date, the potent antioxidant properties of ulam raja has not been reported. Various workers also reported high antioxidant activities for some of the plants studied in this investigation using other assaying methods. Chu, Chang, and Hsu (2000) reported strong DPPH free radical scavenging activity and ferric ions reducing power in sweet potato leaves. Betel leaves had been reported to be an effective antioxidant where it was more potent than BHT and BHA as an extract of the leaves retarded rancidity of butter cakes and extended their shelf life (Lean & Mohamed, 1999). Peng et al. (2003) identified 10 flavonoid compounds and Yagi, Uemura, and Okamura (1994) found three hydrophilic sulfated flavonoids in laksa leaves which exhibited potent antioxidant properties. Betalains responsible for the intense red color of red amaranthus were also powerful scavengers of the DPPH free radical and some of them were found to be stronger than rutin, catechin and ascorbic acid (Cai, Sun, & Corke, 2003). Miean and Mohamed (2001) reported a high total flavonoid content in spring onion leaves. However, this amazingly high flavonoid content and an expected high antioxidant activity were not observed in spring onion leaves in this study.

3.3. Cupper ion chelation activity

The TMM indicator has been used to determine the ability of components in food samples and plant extracts to sequester the free metal ions. In this study, cupric ions were chosen over ferrous ions because the latter were less redox stable at ambient conditions. 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, Grootveld, & Halliwell, 1987). Free hydroxyl groups in the flavonoid ligands chelating the central metal ion may scavenge free radicals.

The ability to chelate cupric ions varied widely for the plants tested – daun salam chelated the least amount of cupric ions while petai chelated the most. The cupric ions chelating abilities of the plants used in this study, given in Fig. 4, had not been reported before. The ability to

chelate cupric ions gives an indication whether compounds found in a particular extract contain potential secondary antioxidants.

There is significant overlap of the values for each variable, as observed in Figs. 1–4, although the mean values showed a trend. A large variation with significant overlap in similar type of parameters was also encountered in a study of common vegetables conducted by Ou, Huang, Hampsch-Woodill, Flanagan, and Deemer (2002). This type of result appears to be quite common due to natural variation when samples were obtained at random at different occasions. For market surveys, samples were usually obtained without knowing exactly their specific cultivation information and postharvest storage conditions. Antioxidant activity had been reported to be highly dependent on cultivation conditions as these influence the biosynthesis of antioxidant phytochemicals. Differences in cultivar (Wang & Stretch, 2002), storage conditions (Kalt, Forney, Martin, & Prior, 1999), soil and weather conditions (Howard, Pandjaitan, Morelock, & Gil, 2002), cultivation methods (Asami, Hong, Barrett, & Mitchell, 2003) and stage of growth at harvest are several such factors. Zheng and Wang (2001) attempted to minimise natural variation by obtaining their herb samples in a single month from a single location.

4. Statistical analysis

4.1. Correlations

4.1.1. Correlations of $TEAC_{DPPH}$ and $TEAC_{FRAP}$ with TPC

Polyphenols have been reported to be responsible for the antioxidant activities of botanical extracts. Both DPPH free radical scavenging and FRAP assays have been used to measure antioxidant activity and the results of DPPH and FRAP assays should correlate with those of TPC.

With reference to Table 1, the correlations of TPC against the antioxidant activity based on the DPPH and FRAP assays involving all 25 plants were only satisfactory. Zheng and Wang (2001) reported excellent correlationships for medicinal plants and culinary herbs when antioxidant activity (determined using the oxygen radical absorbance assay, ORAC assay) was compared with TPC. A satisfactory correlation between antioxidant activities with that of TPC could be attributed to an error introduced in the assays used to determine the extracts' ability to scavenge

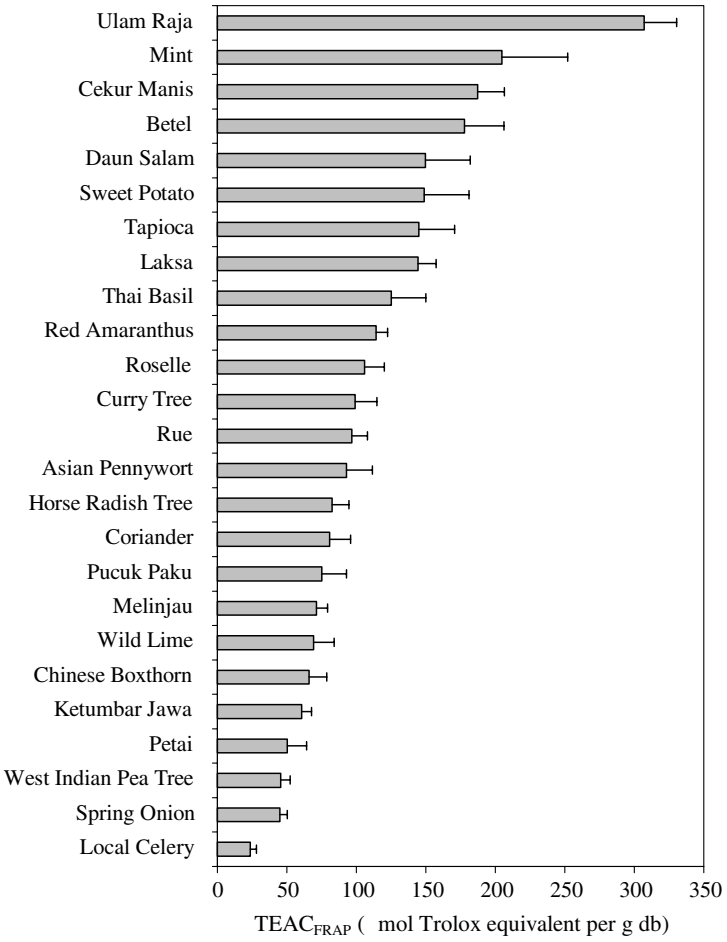

Fig. 3. Antioxidant activities of 25 tropical plants based on their abilities to reduce the ferric ion-TPTZ complex ($n = 6$, error bars represent standard deviation).

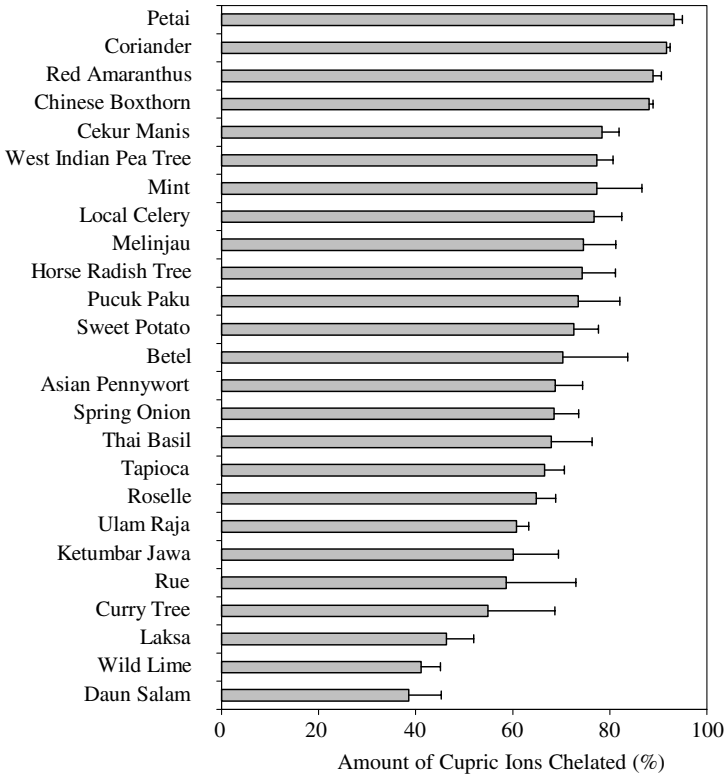

Fig. 4. The cupric ion chelating activities of 25 tropical plants. ($n = 6$, error bars represent standard deviation).

Table 1
Correlation coefficients, R, for relationships between assays

	$TEAC_{DPPH}$	$TEAC_{FRAP}$	TPC
$TEAC_{FRAP}$	0.94953		
TPC	0.56562	0.54803	
Cupric ion chelation activity	−0.19634	−0.22648	0.15887

DPPH free radicals or reduce ferric ions. In these assays, which are based on the measurement an endpoint, one could actually be measuring the antioxidant activity of the reaction by-products, rather than the compounds present in the original mixture. This has been reported by Arts, Dallinga, Voss, Haenen, and Bast (2003) using another commonly used assay involving the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical cation ($ABTS^{\cdot+}$). Another source of error could arise from the lack of specificity of the Folin–Ciocalteau phenol reagent that was used in the determination of total phenolic compounds, as described earlier.

4.1.2. Correlations of cupric ions chelation activities with other assays

Cupric ions chelation activities of the plants did not show any significant correlation with any of the three assays (Table 1). Phenolic compounds have been reported to be chelators of free metal ions. However, a poor correlation of cupric ion chelating activity with TPC of all plants was observed and this might indicate that phenolic compounds might not be the main chelators of copper ions. In a complex mixture, organic acids, amino acids and sugars can be sequesters of transition metal ions. In addition, the ability for phenolic compounds to chelate metal ions depend on the availability of properly oriented functional groups (van Acker et al., 1996). A sample high in polyphenols might not chelate metal if the polyphenols present did not have suitable groups that could chelate the cations. Bidentate ligands are more powerful scavengers of metal cations than monondentate ligands, for example, catechol binds ferric ions tightly whereas phenol does not (Hider, Liu, & Khodr, 2001). When a phenolic group is conjugated with a carbohydrate group, as in naturally-occurring phenolic glycosides, it can no longer bind metals (Hider et al., 2001). So far, no clear relationship had been reported between free radical scavenging activity, ferric ions reducing abilities with metal ion chelation activities.

4.1.3. Correlations of $TEAC_{DPPH}$ with $TEAC_{FRAP}$

As shown in Table 1, the strong correlation between the mean values of $TEAC_{DPPH}$ and $TEAC_{FRAP}$ of all plant samples deserves detailed attention. This could be explained from the basic concept that antioxidants are reducing agents. Antioxidants are compounds capable of donating a single electron or hydrogen atom for reduction. However, not all reducing agents are antioxidants. In this study, the strong correlation between the mean values of $TEAC_{DPPH}$ and $TEAC_{FRAP}$ indicated that compounds present in the aqueous extracts capable of reducing DPPH radicals were also able to reduce ferric ions. Arnous, Makris, and Kefalas (2000) reported a strong correlation between DPPH free

radical scavenging ability and ferric ion reducing ability in wines. Pulido, Bravo, and Saura-Calixto (2000) reported that, in general, the ferric ion reducing ability of antioxidants correlates with the results from other methods used to estimate antioxidant capacity. The antioxidant activity of polyphenols determined using different free radical methods showed similar results to those obtained using the FRAP assay suggests that the reducing ability of polyphenols seemed to be an important factor dictating free radical scavenging capacity of these compounds.

4.2. Principal component analysis

Principal component analysis (PCA) was performed to understand how the four parameters, namely, DPPH free radical scavenging ability, ferric ion reducing power, TPC and CCA, contribute to antioxidant activity of the plant extracts. Factor analysis indicated that three parameters were the main contributors to the variation of antioxidant activity. The most significant component – DPPH free radical scavenging ability contributed the largest variation of approximately 60%, while ferric ion reducing ability and TPC accounted for approximately 28% and 10%, respectively, to the total variation. CCA did not contribute significantly to the total variation as it only indicated the ability of the extracts to chelate free copper ions which is indirectly responsible for antioxidant capacity.

A factor rotation using the Varimax method was performed and two factor loadings were obtained that accounted for the total covariance of the plant extracts. From Fig. 5, DPPH free radical scavenging ability, ferric ion reducing power and TPC were shown to be highly loaded on Factor 1 (PC1). DPPH free radical scavenging ability and ferric ion reducing power were found to be similarly loaded, which indicated the two properties are closely related to antioxidant activity. TPC was also highly loaded on PC1, which suggests phenolic compounds are good antioxidants. Factor 2 (PC2), on the other hand, loads highly on the plant extracts' ability to chelate cupric ions and moderately on TCP. PC2 clearly indicates a high amount of phenolic compounds does not necessary translates into a high cupric ion sequestering activity, that is, secondary antioxidant properties are not directly related to primary antioxidant property.

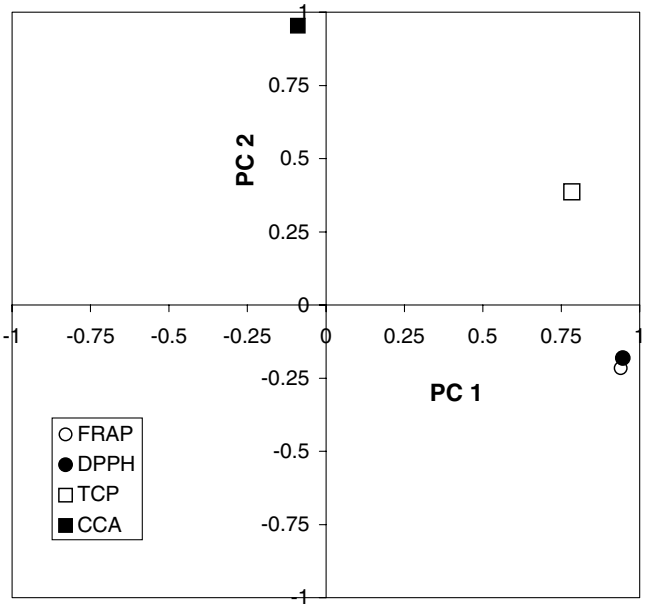

Fig. 5. Principal component analysis of FRAP, DPPH, TCP and CCA.

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