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Comparative Study of Aqueous and Ethanolic Aromatic Malaysian Herbs Extracts Using Four Antioxidant Activity Assays

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ABSTRACT

Recently, much attention on medicinal herbs research has been focused on those herbs that are used for the prevention of various diseases. Herbs in addition to contributing taste and aroma to foods also contain a variety of bioactive substances which are responsible for their biological activities. In this study, Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and antioxidant activities of aromatic Malaysian herbs namely *Persicaria hydropiper* (L.) H. Gross, *Citrus hystrix* DC., *Murraya koenigii* Spreng., *Etlingera elatior* (Jack) R.M. Sm., *Cymbopogon citratus* Stapf. and *Kaempferia galanga* L., using water and ethanol as extraction solvents were determined. The antioxidant activities of these herbal extracts were determined by using four different assays, DPPH radical scavenging activity, Ferric Reducing Antioxidant Power (FRAP), β-carotene bleaching and Oxygen Radical Absorbance Capacity (ORAC) assays. Both extracts of *P. hydropiper* showed the highest TPC, TFC and reducing power in FRAP assay. In DPPH radical scavenging activity assay, the lowest EC_{50} values were shown by both extracts of *P. hydropiper* and *M. koenigii* and these two herbs showed no significant difference ($p>0.05$) with standards ascorbic acid and BHA/BHT combination. Among aqueous extracts, *P. hydropiper* and *M. koenigii* had the highest% inhibition in β-carotene bleaching assay and showed no significant difference ($p>0.05$) with BHA/BHT combination. However, among ethanolic extracts, *M. koenigii* had the highest% inhibition but all of the ethanolic extracts had lower percentage inhibition than BHA/BHT combination. In ORAC assay, aqueous *P. hydropiper, C. hystrix* and *M. koenigii* extracts showed significantly higher values ($p<0.05$) compared to other sample extracts, however, no significant difference ($p>0.05$) were observed among ethanolic extracts. For both aqueous and ethanolic extracts, there were strong correlation between TPC and TFC with DPPH radical scavenging activity, FRAP and β-carotene bleaching assays. The results provided evidence that the aromatic Malaysian herbs studied could be a potent source of natural antioxidants and can be used as functional food ingredients.

Key words: Aromatic Malaysian herbs, antioxidant activity, aqueous extract, ethanolic extract

INTRODUCTION

In recent years interest in human health is increasing. According to Hosein and Zinab (2007), the important physiological function of foods such as antioxidative action to protect living organisms from oxidative damages has received much attention and has been studied by many researchers. Oxidation is a chemical reaction where it transfer electron from a substance to an oxidizing agent.

The compound that can delay or inhibit the oxidation or other molecules is known as an antioxidant. Antioxidants terminate the chemical chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. Nowadays, synthetic antioxidants which are prepared from chemical process are added in foods to prevent or delay oxidation of food during their exposure to environmental factors such as air, light and temperature. However, they have several common biological effects to human body (Bulbul *et al.*, 2012). There has been considerable interest in finding for natural antioxidant from plant sources to replace the synthetic antioxidant. Wojdylo *et al.* (2007) have reported that the polyphenolic compounds which are commonly found in edible and inedible plants have multiple biological effects, including antioxidant activity.

Plants such as herbs have long been used in traditional medicine in various cultures throughout the world. Several aromatic Malaysian herbs such as *Persicaria hydropiper* (L.) H. Gross (daun kesum), *Citrus hystrix* DC. (daun limau purut), *Murraya koenigii* Spreng. (daun kari), *Etlingera elatior* (Jack) R.M. Sm. (bunga kantan), *Cymbopogon citratus* Stapf (serai makan) and *Kaempferia galanga* L. (daun cekur) were selected in this study. These aromatic herbs are well known among Malaysians and commonly used in Malaysian dishes to give unique flavour and aroma to food such as "asam pedas", curry and "laksa". Previously, Faujan *et al.* (2007) had investigated the antioxidant activities of aqueous extracts of *P. minus* (same family as *P. hydropiper*) and *M. koenigii* based on reducing power, Ferric Thiocyanate (FTC) and Thiobarbituric Acid (TBA) assays. They found that aqueous extracts of aromatic Malaysian herbs especially *P. minus* were potent source of natural antioxidants with similar characteristics to the synthetic antioxidant, BHT. Almey *et al.* (2010) investigated the Total Phenolic Content (TPC) and primary antioxidant activity in terms of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities of *C. hystrix* and *M. koenigii* extracts. They found that the extracting solvents significantly affected the total phenolic content and antioxidant activities of herbs.

The objective of the present study was to determine and compare the total phenolic content and total flavonoid content of *P. hydropiper*, *C. hystrix*, *M. koenigii*, *E. elatior*, *C. citratus* and *K. galanga* which contribute to the antioxidant activities using two types of solvents; water and ethanol. Their abilities to scavenge free radicals by donating hydrogen, inhibitions of β-carotene peroxidation, reducing power and scavenge reactive oxygen radical were analysed.

MATERIALS AND METHODS

Chemicals: Butylated Hydroxyltoluene (BHT), Butylated Hydroxylanisole (BHA), ascorbic acid, ethanol, Folin-ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid, sodium nitrate ($NaNO_2$), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric chloride ($FeCl_3.6H_2O$), 2,4,6-tripyridyl-s-triazine (TPTZ), HCl, sodium acetate, glacial acetic acid, trolox, β-carotene, linoleic acid, Tween 20, chloroform, 2,2'-Azobis(2-methylpropionamidine) dihydrochloride (AAPH), AOX assay buffer and fluorescein solution.

Plant materials and extraction method: Six aromatic Malaysian herbs namely *Persicaria hydropiper* (L.) H. Gross, *Citrus hystrix* DC., *Murraya koenigii* Spreng., *Etlingera elatior* (Jack) R.M. Sm., *Cymbopogon citratus* Stapf and *Kaempferia galanga* L. were collected from Kuala Selangor, Selangor, Malaysia. The herb was authenticated by a botanist from Biodiversity Unit,

Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The voucher specimens for each herb were preserved under the reference number SK 2030/12 to SK 2035/12 at the herbarium of Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

All herb samples were extracted using water and ethanol as solvents. The herb samples were cleaned using running tap water and dried using a cabinet dryer (Vission Scientific) until constant weight. The samples were then crushed into fine particles using ultra centrifugal mill (Restch, zm 200) to uniform size of 0.5 mm. The samples were weighed and boiled using distilled water in a ratio of 1:30 (herb:water) for aqueous extraction. However, for ethanolic extraction, the samples were soaked and stirred in 95% ethanol in a ratio of 1:20 (herb:ethanol). The samples were then filtered using Whatman No. 41 filter paper for both extractions process. The samples were evaporated using a rotary evaporator (BUCHI) at 60°C for aqueous extraction and 50°C for ethanolic extraction. The viscous samples were lyophilised using a freeze drier (Christ Martin, alpha 1-4 LD plus). Both aqueous and ethanolic crude extracts were stored at -20°C for further analysis and the extraction yields of both extracts were determined.

Determination of total phenolic content (TPC): Total phenolic content of aqueous and ethanolic herbal extracts were determined according to method of Singleton and Rossi (1965) with slight modification. All samples and readings were prepared in triplicates and gallic acid was used as standard. Total phenolic content of herbal extracts was determined as mg of Gallic Acid Equivalent (GAE) per g of extract weight.

Determination of total flavonoid content (TFC): Total amount of flavonoid in aqueous and ethanolic herbal extracts were determined using method of Zhishen *et al.* (1999). Quercetin was used as a standard. Total flavonoid content of samples was expressed in mg of Quercetin Equivalent (QE) per g of extract weight.

DPPH radical scavenging activity assay: The ability of antioxidant in aqueous and ethanolic herbal extracts to scavenge DPPH radical by hydrogen donor were measured according to Braca *et al.* (2001). The percentage of scavenging effect of samples was calculated in following way:

$$\text{Scavenging effect (\%)} = 1 - \frac{\text{Abs. sample}}{\text{Abs. control}} \times 100$$

The graph of scavenging effect (%) was plotted against the corresponding concentration of the extract to obtain EC_{50} values. EC_{50} is defined as the amount of extract necessary to decrease the initial DPPH radical concentration by 50% (Othman *et al.*, 2007). Ascorbic acid and BHA/BHT combination were used as standards.

Ferric reducing antioxidant power (FRAP) assay: The ferric reducing antioxidant power of aqueous and ethanolic herbal extracts were determined according to method of Benzie and Strain (1996) with slight modifications. Trolox was used as a standard. The results were expressed in mM of Trolox Equivalent (TE) per g of extract weight.

β-carotene bleaching assay: The antioxidant activity of aqueous and ethanolic herbal extracts was determined based on the β-carotene bleaching method developed by Velioglu *et al.* (1998) with some modifications. Combination of BHA/BHT was used as a standard. Degradation Rate (DR) was calculated using the following equation:

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

where, in is natural log, a is the initial absorbance (470 nm) at time 0, b is the absorbance at time 20, 40, 60, 80, 100 and 120 min and t is the time intervals. Antioxidant Activity (AA) was expressed as percent of lipid peroxidation inhibition relative to the control, using the following formula:

$$AA\ (\%) = \frac{DR_{control} - DR_{sample/standard}}{DR_{control}} \times 100$$

Oxygen radical absorbance capacity (ORAC) assay: The ability of both herbal extracts to scavenge the reactive oxygen radical were determined using ORAC assay as described by Ou *et al.* (2001). The result was expressed in mmol Trolox Equivalents (TE) per g of extract weight.

Statistical analysis: All analyses were run in triplicates. Data were analysed by the Windows SAS program (Version 9.0, 2009). Data were expressed as Mean±SD using ANOVA. Differences were considered statistically significant if $p<0.05$.

RESULTS AND DISCUSSION

Extraction yield: The yield of both aromatic Malaysian herbal extracts is shown in Table 1 where *C. citratus* had the highest (19.22±1.58%) extraction yield among the aqueous extracts while *M. koenigii* possessed highest yield (17.55±1.73%) among the ethanolic extracts. Among the aqueous extracts, *P. hydropiper* and *K. galanga* had the lowest yield (5.09±0.04% and 5.52±0.68%, respectively). *P. hydropiper* also exhibited the lowest yield (6.62±0.23%) for ethanolic extracts, however significantly higher than the aqueous extracts. In comparison between the extracts, aqueous extracts of *C. hystrix* and *C. citratus* showed higher yield than ethanolic extracts. However, for other sample, ethanolic extracts had higher yield than the aqueous extracts.

Different amount in the yield among samples could be due to the factors such as cultivar origin, geographical and stage of harvest. Extractive value was highest in water and alcohol indicating

Table 1: Extraction yield of aqueous and ethanolic herbal extracts

	Yield of extracts (%)	
Samples	Aqueous extracts	Ethanolic extracts
Persicaria hydropiper	5.09±0.04^{Db}	6.62±0.23^{Ea}
Citrus hystrix	11.43±0.80^{Ba}	9.30±0.43^{Db}
Murraya koenigii	11.88±1.19^{Bb}	17.55±1.73^{Aa}
Etlingera elatior	7.92±0.21^{Cb}	10.82±0.42^{Ca}
Cymbopogon citratus	19.22±1.58^{Aa}	13.78±1.10^{Bb}
Kaempferia galanga	5.52±0.68^{Db}	8.46±0.57^{Da}

Values are expressed as Mean±Standard deviation (n = 3). Means with same capital letters within each column are not significantly different ($p>0.05$). Means with different small letters within each row are significantly different ($p<0.05$)

Table 2: Total phenolic content and total flavonoid content of aqueous and ethanolic herbal extracts

	Total phenolic content (mg GAE/g EW)		Total flavonoid content (mg QE/g EW)	
Samples	Aqueous extracts	Ethanolic extracts	Aqueous extracts	Ethanolic extracts
Persicaria hydropiper	148.83±13.77^{Ab}	172.67±2.93^{Aa}	424.17±34.67^{Ab}	598.33±12.83^{Aa}
Citrus hystrix	53.00±4.36^{Cb}	66.17±0.76^{Ca}	96.67±8.04^{Db}	241.32±4.14^{Ca}
Murraya koenigii	76.42±2.98^{Bb}	92.33±2.57^{Ba}	142.50±2.50^{Cb}	264.83±12.25^{BCa}
Etlingera elatior	64.00±3.46^{Ca}	32.17±6.43^{Ca}	184.17±1.44^{Bb}	277.50±8.66^{Ba}
Cymbopogon citratus	21.33±2.75^{Ea}	24.33±1.53^{Ea}	49.17±0.23^{Fb}	58.33±3.82^{Ea}
Kaempferia galanga	38.00±3.91^{Da}	38.33±3.21^{Da}	60.00±0.00^{Eb}	153.50±29.81^{Da}

Values are expressed as Mean±Standard deviation (n = 3). Means with different capital letters within each column are significantly different ($p<0.05$). Means with same small letters within each row are not significantly different ($p>0.05$)

the possibility of considerable amount of polar compound present in seeds (Shamsundar and Paramjyothi, 2010). According to Kumoro *et al.* (2009), methanol, ethanol and water have similar solubility properties because they contain hydroxyl group which is hydrophilic, however high percentage yield extract were obtained from extraction employing polar organic solvent.

Total phenolic content (TPC) in herb extracts: Total phenolic content was measured by reduction of Folin-ciocalteu during phenol oxidation. Folin-ciocalteu reagent, a mixture of phosphotungstic and phosphomolybdic acids is reduced to tungsten and molybdene (blue oxides) by electron transfer in alkaline condition which is obtained by adding sodium carbonate (Almey *et al.*, 2010). The intensity of blue colour reflects the quantity of phenol in the samples. A gallic acid was selected as standard due to its presence in almost all plants. Total phenolic content of aqueous and ethanolic aromatic Malaysian herbal extracts is shown in Table 2.

This study showed that TPC of both aqueous and ethanolic extracts of *P. hydropiper* were the highest among samples (148.83±13.77 and 172.67±2.93 mg GAE/g EW, respectively) followed by both aqueous and ethanolic *M. koenigii* extracts (76.42±2.98 and 92.33±2.57 mg GAE/g EW, respectively). However, *C. citratus* showed the lowest TPC values in both aqueous and ethanolic extracts (21.33±2.75 and 24.33±1.53 mg GAE/g EW, respectively). Results showed that TPC varied significantly from one plant to another. This study was supported by Zulfiker *et al.* (2011) who reported that methanolic *P. hydropiper* extract had the highest TPC (964.23 mg GAE/g EW). However, Almey *et al.* (2010) reported that ethanolic *M. koenigii* and *C. hystrix* extracts had TPC values of 12.31 and 6.65 mg GAE/100 g EW, respectively. Different plants, procedures and standards used to express the TPC and extrinsic factors like agronomic, environmental, handling and storage will give different level of TPCs (Faujan *et al.*, 2007; Balasundram *et al.*, 2006).

In comparison between the extracts, there was no significant difference ($p>0.05$) between the aqueous and ethanolic extracts of *E. elatior*, *C. citratus* and *K. galanga*. However, the ethanolic extracts of *P. hydropiper*, *C. hystrix* and *M. koenigii* showed higher TPC compared to the aqueous extracts. Albayrak *et al.* (2008) reported that Folin-ciocalteu method gives different responses to different phenolic compound depending on the chemical structures. The TPC obtained could be influenced by interference of other compounds as well as non-phenolic organic compound found in the plants (Maria and Daniela, 2009). According to Balasundram *et al.* (2005), precipitation of non-phenolic compound contributes to the highest TPC in ethanolic extracts.

Phenolic compounds undergo a complex redox reaction with Folin-ciocalteu reagent, however, some chemical group of amino acid, proteins and sugars could react with the reagent. In this study,

the herbs were dried before extraction and according to Prasad *et al.* (2009), ascorbic acid was lost during drying process and amino acids, sugars and proteins can be removed from the extraction solvents. Thus, interference from ascorbic acid or other compounds should be very little.

Total flavonoid content (TFC) in herb extracts: Flavonoids are naturally occurring substances in plants. Mechanisms of action of flavonoid are through scavenging and chelating process (Ebrahimzadeh *et al.*, 2008). Total flavonoid content was measured using $AlCl_3$ calorimetric method and according to Chang *et al.* (2002) this method determines flavones and flavonols. The $AlCl_3$ forms acid stable complexes with the C-4 keto group or either C-3 or C-5 hydroxyl group of flavones and flavonols (Konyahoglu *et al.*, 2005). In addition, $AlCl_3$ forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids (Chang *et al.*, 2002).

In this study, total flavonoid content of each herbal extract was expressed as mg Quercetin Equivalent (QE) as shown in Table 2. The results showed that the flavonoid content differs among samples. TFC of *P. hydropiper* was highest for both aqueous and ethanolic extracts (424.17±34.67 and 598.33±12.83 mg QE/g EW, respectively) followed by *E. elatior*, *M. koenigii*, *C. hystrix*, *K. galanga* and *C. citratus*.

In comparison between extracts, ethanolic extract of all herbs were significantly higher ($p<0.05$) in TFC than aqueous extracts. The results are comparable to those obtained by Luis *et al.* (2009), who reported that the ethanolic extracts of three species of Portuguese shrubs were higher than the aqueous extracts. Flavonoid is the largest group of plant phenolics. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups. Flavonoid distribution in plants depends on several factors that include variation in plant family/order and within species (Balasundram *et al.*, 2006).

DPPH radical scavenging activity assay: In DPPH radical scavenging activity assay, the odd electron of DPPH free radical (purple colour) is stabilized (turn yellow colour) by hydrogen donor from the herbal extracts. Table 3 shows the EC_{50} value of aqueous and ethanolic herbal extracts. Ascorbic acid and BHA/BHT combination were used as standards in this assay. The EC_{50} values are inversely proportional to the ability of the extracts to act as DPPH scavengers (antioxidant activity).

From the results it may be postulated that herbal extracts reduce the radicals to the corresponding hydrazine when it reacts with the hydrogen donor. The DPPH radicals react with suitable reducing agent, the electrons become paired off and the solution loss colour

Table 3: EC_{50} of aqueous and ethanolic herbal extracts

	EC_{50} (mg mL^{-1})	
Samples/Standards	Aqueous extracts	Ethanolic extracts
Persicaria hydropiper	0.30 ± 0.00^{Aa}	0.31 ± 0.01^{Aa}
Citrus hystrix	0.59 ± 0.04^{Bb}	3.49 ± 0.45^{Da}
Murraya koenigii	0.33 ± 0.01^{Ab}	0.42 ± 0.01^{Aa}
Etlingera elatior	0.56 ± 0.02^{Bb}	3.47 ± 0.42^{Da}
Cymbopogon citratus	2.53 ± 0.11^{Db}	3.10 ± 0.20^{Ca}
Kaempferia galanga	1.89 ± 0.17^{Ca}	2.06 ± 0.11^{Ba}
Ascorbic acid	0.28 ± 0.00^{A}	
BHA/BHT		0.30 ± 0.00^{A}

Values are expressed as Mean±Standard deviation (n = 5). Means with different capital letters within each column are significantly different ($p<0.05$). Means with same small letters within each row are not significantly different ($p>0.05$)

stoichiometrically depending on the number of electrons taken up (Sawant *et al.*, 2009). The extracts that required the lowest concentration to promote 50% of inhibition in this sudy were both aqueous and ethanolic *P. hydropiper* (0.30±0.00 and 0.31±0.01 mg mL^{-1}, respectively) and *M. koenigii* extracts (0.33±0.01 and 0.42±0.01 mg mL^{-1}, respectively) with no significant difference ($p>0.05$). Thus, the antioxidant activities of these extracts were postulated to be greater than other samples. In comparison with standards, activity of these two herbs exhibited no significant difference ($p>0.05$) with the standards ascorbic acid and BHA/BHT combination. For aqueous extracts, there was no significant difference ($p>0.05$) in DPPH radical scavenging activity between *C. hystrix* and *E. elatior*. However, these herbs had a significantly higher ($p<0.05$) activity compared to *K. galanga* and *C. citratus*. Among ethanolic extracts, *K. galanga* had a significantly higher ($p<0.05$) activity than *C. citratus*, whereas *C. hystrix* and *E. elatior* exhibited the lowest activity but no significant difference existed ($p>0.05$) between them.

The ethanolic extracts of *C. hystrix, M. koenigii, E. elatior* and *C. citratus* exhibited higher activity than aqueous extracts. However, aqueous and ethanolic extracts of *P. hydropiper* and *K. galanga* were not significantly different ($p>0.05$) in DPPH radical scavenging activity. Different DPPH radical scavenging activity of each sample in each extract implies that the extracting solvent used would affect the radical scavenging potency which is due to the different polarities of each antioxidant compound present in the sample (Ismail and Tan, 2002). The possible reason for the higher EC_{50} values of the plants could be due to the presence of other reducing agents not reactive towards DPPH radical.

In addition, there was strong correlation between total phenolic content and scavenging ability for both aqueous ($R^2 = 0.883$) and ethanolic ($R^2 = 0.952$) extracts. However, the relationship between total flavonoid content and antioxidant ability showed moderate correlation for aqueous extracts ($R^2 = 0.786$) and strong correlation for ethanolic extracts ($R^2 = 0.846$). This fact is more obvious for ethanolic extracts which probably indicates that ethanol isolates phenolic compounds with more pronounced antioxidant properties. According to Rohman *et al.* (2010), antiradical scavenging activity revealed a moderate relationship with the total phenolic content ($R^2 = 0.645$) and with total flavonoid content ($R^2 = 0.709$). However, some coefficients were not high and this could be due to the fact that some natural antioxidants such as vitamin C and carotenoids are not phenolic compounds and that each antioxidant compound may show different activity with different assays.

Many researchers reported that other phytochemicals such as ascorbic acid, tocopherol and pigment also contribute to total antioxidant activity. However, content of bioactive compounds in vegetables greatly varies in the amount, depending on the species, variety, time of the year and degree of ripeness (Pengseng *et al.*, 2010).

Ferric reducing antioxidant power (FRAP) assay: Ferric reducing antioxidant power assay is used to measure the ability of antioxidant presence in herbal extracts to reduce the Fe^{3+}-TPTZ complex to blue coloured of Fe^{2+}-TPTZ by electron donor at acidic medium. The reducing power (FRAP value) of herbal extracts are summarized in Fig. 1.

The aqueous extract of *P. hydropiper* exhibited the highest antioxidant potential (3244.67±78.95 mM TE/g EW) followed by *M. koenigii*>*C. hystrix* = *E. elatior*>*K. galanga*> *C. citratus*. As for ethanolic extract, *P. hydropiper* also exhibited the highest antioxidant potential (1676.67±124.23 mM TE/g EW), followed by *C. hystrix, E. elatior, M. koenigii, C. citratus* and *K. galanga*. Generally, the presence of phenolic compounds contributes to the reducing properties which exert their action by breaking the free radical chain through donation of a hydrogen atom.

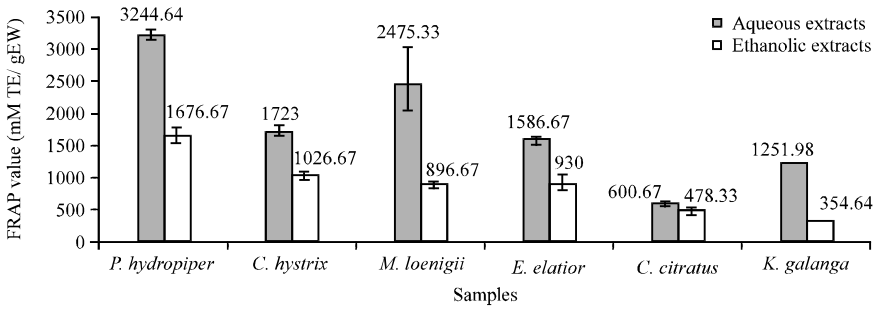

Fig. 1: Antioxidant potential of aqueous and ethanolic herbal extracts assayed by FRAP assay. Values are expressed as Mean±Standard deviation (n = 3)

In this study, the aqueous herbal extracts showed significantly higher antioxidant potential compared to ethanolic extracts. This finding is in agreement with the previous study reported by Ismail and Tan (2002), where aqueous extract of Nori and Kumbu exhibited higher antioxidant activity compared to their ethanolic extract. This study was also supported by Cheung *et al.* (2003) who reported that water extract had higher antioxidant activity than methanolic extract in mushroom (*L. edodes* and *V. volvacea*).

The relationship between total phenolic content and FRAP assay showed strong correlation for both aqueous and ethanolic extracts (R^2 = 0.950 and R^2= 0.879, respectively). Total flavonoid content also showed strong correlation with FRAP assay for both aqueous and ethanolic extracts (R^2 = 0.860 and R^2 = 0.945, respectively). These correlations indicate that the phenolic compounds especially flavonoid could be the main contribution of antioxidant potential for all aromatic Malaysian herbs studied. This is in agreement with the previous findings where many phenolic compounds in plants are good sources of natural antioxidants. This result was in agreement with Othman *et al.* (2007) and Faujan *et al.* (2007), who found a strong correlation between total phenolic content and FRAP assays. According to Verzelloni *et al.* (2007), the total antioxidant activity of red wine is highly correlated with the flavonoid content (R^2 = 0.988).

β-carotene bleaching assay: Lipid peroxidation inhibition activity was determined using β-carotene bleaching assay. This procedure depends on the hydroperoxides that were produced by linoleic acid during incubation at 50°C. These hydroperoxides free radical have the ability to oxidize β-carotene. The presence of antioxidants in herbal extracts will minimize the oxidation of β-carotene molecules by hydroperoxides. Therefore, the antioxidant activity of the extracts could be estimated using this assay. The results of % lipid peroxidation inhibition activity of aqueous and ethanolic herbal extracts are shown in Fig. 2.

From this study, antioxidant activity (% inhibition) of *P. hydropiper* (67.22±7.62%) and *M. koenigii* (66.07±2.99%) were highest among aqueous extracts and there were no significant difference ($p>0.05$) with the positive control, BHA/BHT combination (70.10±3.33%). The % inhibition followed by *C. hystrix, E. elatior, K. galanga* and *C. citratus* with 45.57±3.98, 45.35±4.54, 38.24±5.33 and 28.21±2.06%, respectively. For ethanolic extract, *M. koenigii* showed the highest percentage (61.80±3.14%) of inhibition followed by *P. hydropiper, K. galanga, E. elatior, C. hystrix* and *C. citratus* with 53.05±3.80, 35.97±3.90, 35.87±2.20, 35.67±2.92 and 29.68±4.71% of inhibition, respectively. However, all of ethanolic herbal extracts, showed lower antioxidant activity compared to the standard BHA/BHT combination.

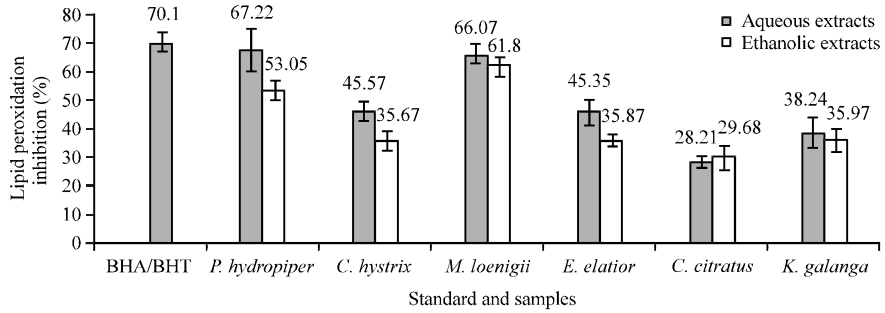

Fig. 2: % lipid peroxidation inhibiton of aqueous and ethanolic herb extracts assayed by β-carotene bleaching assay. Values are expressed as Mean±SD (n = 3)

Table 4: ORAC value of aqueous and ethanolic herbal extracts

	ORAC value (mmol TE/g EW)	
Samples	Aqueous extracts	Ethanolic extracts
Persicaria hydropiper	7.59±0.49^{Ab}	11.20±2.00^{Aa}
Citrus hystrix	7.96±0.20^{Ab}	10.51±1.94^{Aa}
Murraya koenigii	7.79±0.04^{Ab}	9.93±1.89^{Aa}
Etlingera elatior	6.37±0.53^{Bb}	9.78±1.78^{Aa}
Cymbopogon citratus	6.07±0.99^{Bb}	8.88±1.12^{Aa}
Kaempferia galanga	5.77±0.89^{Bb}	10.59±0.40^{Aa}

Values are expressed as Mean±Standard deviation (n = 3). Means with same capital letters within each column are not significantly different ($p>0.05$). Means with different small letters within each row are not significantly different ($p<0.05$)

In comparison between extracts, aqueous extract of *P. hydropiper*, *C. hystrix* and *E. elatior* seem to inhibit the oxidation of β-carotene better than compounds which are soluble in ethanol. This finding was supported by Othman *et al.* (2007) who showed aqueous extract of cocoa beans had higher antioxidant activity than ethanolic extract using the same assay.

Based on β-carotene bleaching assay, both aqueous and ethanolic extracts showed a positive moderate correlation between antioxidant activity and total phenolic content (R^2 = 0.863 and R^2 = 0.742, respectively) and total flavonoid content (R^2 = 0.745 and R^2 = 0.611, respectively).

Oxygen radical absorbance capacity (ORAC) assay: This method is used to measure the relative antioxidant activity of sample using fluorescence-based technology of detection. A peroxyl radical (ROO^-) is formed from the breakdown of AAPH (2,2'-Azobis(2-methylpropionamidine) dihydrochloride) at 37°C. The peroxyl radical will oxidize fluorescein (3',6'-dihydroxy-spiro[isobenzofuran-1[3H], 9'[9H]-xanthen]-3-one) to generate a product without fluorescence. The presence of antioxidant in sample will inhibit the fluorescence decay by a hydrogen atom transfer mechanism. Trolox was used as a positive control. Table 4 shows ORAC values of aqueous and ethanolic herbal extracts which were expressed as mmol TE/g EW.

The ORAC values for the aqueous and ethanolic herbal extracts ranged from 5.77-11.20 mmol of TE/g extract weight. The aqueous herbal extracts with the highest ORAC values were *C. hystrix* (7.96±0.20 mmol TE/g EW), followed by *M. koenigii* (7.79±0.04 mmol TE/g EW) and *P. hydropiper* (7.59±0.49 mmol TE/g EW). However, there were no significant difference ($p>0.05$) in ORAC values

among these herbs. As for ethanolic herbal extract, the highest ORAC value was *P. hydropiper* (11.20±2.00 mmol TE/g EW) however no significant difference ($p>0.05$) in ORAC values among samples were observed.

This study showed that the ethanolic herbal extracts were significantly higher in ORAC values than aqueous herbal extracts. The ORAC values and total phenolic content of both aqueous and ethanolic extracts had moderate correlation coefficient of 0.516 and 0.672, respectively. Moreover, there were weak relationship between the ORAC values and total flavonoid content of both aqueous and ethanolic extracts ($R^2 = 0.380$ and $R^2 = 0.473$, respectively). Present result is in agreement with findings by Zheng and Wang (2001) who reported positive linear correlation between total phenolic content and antioxidant activity of selected medicinal herb extracts such as *Aloe vera, Thymus vulgaris* and *Ginkgo biloba*.

CONCLUSION

The extracting solvents significantly affected the percentage yield, TPC, TFC and antioxidant activities of six aromatic Malaysian herbs studied namely *P. hydropiper, C. hystrix, M. koenigii, E. elatior, C. citratus* and *K. galanga*. The highest TPC, TFC and antioxidant activities were detected in both aqueous and ethanolic extracts of *P. hydropiper,* followed by *M. koenigii* and *E. elatior*. Further researches on the identification of phenolic compounds responsible for the antioxidative activities of the extracts are now in progress.

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