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Antioxidative Activities of Water Extracts of Some Malaysian Herbs

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Abstract: This study was conducted on selected local herbs such as ulam raja (*Cosmos caudatus*), kesum *(Polygonum minus)*, selom *(Oenanthe javanica)*, pegaga *(Centella asiatica)* and curry leaves (*Murraya koenigii*) to investigate their antioxidative activities. The water extracts of the herbs were analysed for total phenolic content, reducing antioxidant power, ferric thiocyanate (FTC) and the thiobarbituric acid (TBA) test was also accried out. *Polygonum minus* showed the highest total phenolic content and reducing power among the herbs. Increasing the concentration of the extracts resulted in increased Fe^{3+} reducing antioxidant power for all the herbs. FTC and TBA tests on the extracts during seven days of storage showed that all the herbs extracts had the ability to reduce oxidation compared to the control ($P < 0.05$). From the FTC analysis, *Murraya koenigii* leaves was best in reducing the oxidation rate (67.67%) compared to the other herbs studied. Analysis of TBA showed that *Centella asiatica* extract had the highest antioxidant effect. However, both TBA and FTC analysis for these two herbs showed no significant difference ($P > 0.05$) from *Polygonum minus* and butylated hydroxyanisole (BHT) a synthetic antioxidant. Correlation analysis showed positive correlations between amount of total phenolic content and reducing power (r = 0.75) and antioxidative activities (r = 0.58) in linoleic acid emulsion system. This shows that antioxidative activities of these Malaysian herbal plants especially *Polygonum minus* may be a potential source of natural antioxidants with similar characteristics to the synthetic antioxidant, BHT.

Keywords: Antioxidative activities, Malaysian herbs, total phenolic content, reducing power, Thiobarbituric Acid (TBA)

INTRODUCTION

Plants are potential sources of natural antioxidants. They produce various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive (Lu and Foo, 1995). ROS which include free radicals such as superoxide anion radicals ($O^{\cdot}_2$), hydroxyl radicals ($OH^{\cdot}$) and non free-radical spesies such as H_2O_2 and single oxygen ($^1O_2$) are various forms of activated oxygen. These molecules are exacerbating factors in cellular injury and aging process (Gulçin *et al.*, 2003). In addition, ROS induce some oxidative damage to bio-molecules like lipids, nucleic acids, proteins and carbohydrates. Their damage causes aging, cancer and many other diseases (Aruoma, 1994). In foods, ROS can cause lipid peroxidation, which leads to the deterioration of the food (Miller and Rice-Evans, 1997). The oxidative deterioration of the lipid-containing

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food is responsible for the rancid odours and flavours during processing and storage, consequently decreasing the nutritional quality and safety of foods, due to the formation of secondary and potentially toxic compounds. The addition of antioxidants is one way of increasing the shelf life of foods (Cook and Samman, 1996).

Plant phenolics are probably had multifunctional antioxidants. These compounds were reported to quench oxygen-derived free radicals by donating a hydrogen atom or an electron to the free radical (Wanasundara and Shahidi, 1998). The antioxidant effect of phenolic compounds has been demonstrated in many systems through *in vitro* studies such as in human low density lipoprotein and liposomes (Leanderson *et al*., 1997).

Several studies had been conducted to evaluate the correlation between phenolic compounds and antioxidant activity. The antioxidant activity of Du-Zhong (*Eucomnia ulmoides*) (Yen and Hsieh, 1998), Mung Bean Hulls (Duh *et al*., 1997), ear mushrooms (Chao, 2001) and anise seed (*Pimpenella anisum* L.) (Gulçin *et al*., 2003) were found to correlate with the phenolic compounds. Studies on local Malaysian plants such as turmeric (*Curcuma domestica*), betel leaf (*Piper betel*), pandan leaf (*Panadanus odorus*), asam gelugur (*Garnicia atroviridis*), mengkudu (*Morinda citrifolia*), pegaga (*Centella asiatica*), ginger (*Zingiber officinale*), cassava shoot (*Manihot asculenta*) (Jayamalar and Suhaila, 1998; Mohd. Zin *et al*., 2002, Zainol *et al*. 2003; Noriham *et al*., 2004) have shown that they also exhibit good antioxidant activity. Wang *et al*., (1999) reported that the antioxidative properties of some vegetables and fruits are partly due to the low molecular weight phenolic compounds, which are known to be potent as an antioxidant.

In Malaysia, herbs are commonly eaten fresh as a vegetable (salad and *ulam*), especially among the Malay communities. Most of these herbs are believed to be associated with antioxidant activities and have many beneficial effects. Nowadays, there are numerous techniques that are available to evaluate antioxidant activities of compounds and of complex mixtures such as plant extracts (Anatolovich *et al*., 2002). Despite the existence of these various methods and techniques, one procedure alone cannot identify all possible mechanisms characterising an antioxidant (Frankel and Meyer, 2000). Therefore this study was conducted to evaluate the antioxidative activity of several Malaysian herbs using three different methods, and to evaluate the relationship between the antioxidative activity and total phenolic content of the herbs.

MATERIALS AND METHODS

Chemicals

Ammonium thiocyanate and ferrous chloride were purchased from Merck (Merck KGaA, Darmstadt, Germany). Ferric chloride, linoleic acid (99.5%), potassium ferricyanide, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Commercial rosemary (Rosemary Herbalox ®) was obtained from Kalsec UK Ltd. (Suffolk, UK).

Materials and Extraction Method

All herbs were obtained from a local wet market in Bandar Baru Bangi, Selangor, Malaysia. The herbs were ulam raja (*Cosmos caudatus)*, kesum *(Polygonum minus)*, selom *(Oenanthe javanica)*, pegaga *(Centella asiatica)* and curry leaves *(Murraya koenigii)*. The edible portions of the fresh samples were cleaned and washed using running tap water. Then excessive water was drained out and the samples were air-dried using a fan. Samples were blended with distilled water in a ratio of 3:1 (water: herb) and filtered using Whatman No. 1 paper. The filtrates were then evaporated using a vacuum evaporator at 50°C to give viscous mass. The crude extracts were weighed

and stored at 0-4°C for further experiments and analysis.

Determination of Total Phenolic Content

Total phenolic content was determined using Folin–ciocalteu reagent following the method of Singleton and Rossi (1965) with slight modification using tannic acid as a standard. Briefly, 1 ml of extract solution (5 mg ml^{-1}) was added in a 100 ml volumetric flask that contained about 60 ml distilled water. Then, 5 ml of Folin–ciocalteu reagent was added and the content of the flask thoroughly mixed. After 1-8 minutes, 15 ml Na_2CO_3 (20%) was added and the volume was made up to 100 ml using distilled water. The mixture was allowed to stand for 2 hours with intermittent shaking. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). Total phenolic content was determined as mg of tannic acid equivalent (TAE) using an equation obtained from the standard tannic acid calibration graph.

Reducing Antioxidant Power

The reducing antioxidant power of water extracts of the herbs was determined using the method of Oyaizu (1986). Different concentrations of herb water extracts (200 – 1200 ppm) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The supernatant (2.5 ml) was then mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance measured at 700 nm against a blank using UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). Increased absorbance of the reaction mixture indicates increase in reducing power.

Ferric Thiocyanate (FTC) Method

The antioxidant activities of the herbs water extracts were determined using the FTC method (Osawa and Namiki, 1981) with slight modification. Four milligrammes of each herb extract samples was dissolved in 4.0 ml ethanol (99.5%) and kept in a dark bottle (d = 40.0 mm, t = 75.0 mm). Each sample was then mixed with 4.1 ml linoleic acid (2.5% in ethanol 99.5%), 8 ml phosphate buffer (0.02 M, pH 7.0) and 3.9 ml distilled water to make up to 20 ml. BHT was used as a positive control and an empty bottle (no sample) was used as a negative control. The mixture / solution was incubated at 40 – 45°C. After incubation, 9.7 ml ethanol (75%) and 0.1 NH_4SCN (30%, as a colour reagent) was added to 0.1 ml of the solution. Precisely 3 min after the addition of 0.1 ml of $FeCl_2$ (0.002 M) in HCl 3.5% to the reaction mixture, the absorbance of the resulting red colour was measured at 500 nm using spectrophotometer (Jenway 6100, Dunmow, Essex, U.K) every 24 h until a day after the absorbance of the control reached maximum value (day seven). The inhibition of lipid peroxidation was calculated as follows:

$$\% \text{ Inhibition} = 100 - [(A_1 / A_o) \text{ X } 100]$$

Where A_o is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample extracts (Duh *et al*., 1999).

Thiobarbituric Acid (TBA) Test

The TBA test was conducted according to the combined method of Kikuzaki and Nakatani (1993) and Ottolenghi (1959). To 1 ml of the sample from the previous FTC method was added 2 ml of trichloroacetic acid and 2 ml of thiobarbituric acid solution. This mixture was then placed in a boiling water bath at 100°C for 10 min. After cooling, it was centrifuged at 3000 rpm for 20 min and absorbance of the supernatant was then measured at 532 nm using UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K).

Statistical Analysis

All analyses were run in triplicates. Data were analysed using analysis of variance (ANOVA)

($P < 0.05$) and the means separated by Duncan's multiple range tests using the Statistical Analysis System 6.21 software package (SAS, 1995).

RESULTS AND DISCUSSION

The Extraction Yield and Phenolic Content of the Extracts

The yield of herb water extracts and the concentration of total phenolic content (mg/ 100 g fresh weight) are shown in Table 1.

Table 1: Extraction yields and total phenolic content of herb water

Plants	Yield of extracts (%)	Total Phenolic (mg TAE/100 g fresh weight)
Cosmos caudatus	1.93^{c}	21.41^{b}
Centella asiatica	2.22^{bc}	3.72^{c}
Murraya koenigii	2.95^{b}	24.62^{b}
Polygonum minus	4.01^{a}	44.35^{a}
Oenanthe javanica	5.19^{a}	19.96^{b}

Values with the same lowercase within each column are not significantly different (P > 0.05)

From Table 1, the extraction yield shows that *Oenanthe javanica* extract was the highest (5.19%), while *Cosmos caudatus* was the lowest (1.93%) among the samples. Total phenolic content shows that *Polygonum minus* had the highest total phenolic content (44.35 mg TAE) and followed by *Murraya koeniigii*, *Cosmos caudatus* and *Oenanthe javanica* with mean values of 24.62, 21.41, 19.96 mg TAE respectively. *Centella asiatica* had the lowest total phenolic content.

Velioglu *et al.* (1998) reported that the total phenolic content in fruits, cereals and vegetables ranged from 213 to 10600 mg GAE/ dry weight, while Du-Zong (*Eucomnia ulmoides*) was between 8700 and 21000 mg GAE/ dry weight (Yen and Hsieh, 1998). Total phenolic content of *Moringa oleifera* in three different climates (India, Nicaragua and Niger) ranged from 2940 to 4250 mg GAE/ dry weight (Siddhuraju and Becker, 2003). Different levels reported in these studies may be attributed to the different plants, procedures and standards used to express the total phenolic contents used by the different investigators. The usage of Folin-Ciocalteu reagent was also measured based on the colour measurement which was non-specific on phenol. Perhaps there were other components that can react with the reagent such as ascorbic acid (Shahidi and Naczk, 1995). Besides, various phenolic compounds have different response to this assay (Singleton & Rossi, 1965). However, the measurement of colour changes after two hours storage could be used to determine the existence of phenol in samples.

Reducing Antioxidant Power

Reducing antioxidant power is a measure of the reductive ability, and it is evaluated by the transformation of Fe (III) to Fe (II) in the presence of the sample extracts (G¸lçin *et al.*, 2003). The reducing power of Malaysian herbs extracts, are summarised in Figure 1. From the figure, reducing power increased with an increase in extracts concentration.

At 200 ppm, *Polygonum minus* and *Cosmos caudatus* had the highest ability to reduce Fe (III) and was not significantly different from BHA ($P > 0.05$). The absorbance of *Polygonum minus*, *Cosmos caudatus* and BHA were 0.809, 0.773 and 0.966 respectively at 200 ppm. *Oenanthe javanica* extract had the lowest ability to reduce Fe (III) (0.174) and was not significantly different ($P > 0.05$) to *Centella asiatica*, rosemary and BHA. At 1200 ppm, *Polygonum minus* showed the highest ability to reduce Fe (III) and not significantly different to BHA ($P > 0.05$).

From Figure 1, all the herbs extracts showed increasing reducing ability as the concentration of extracts increased. This result is similar to that reported by G¸lçin *et al.* (2003) and Noriham *et al.*, (2004), who demonstrated antioxidative activity on four types of Malaysian plants and *Pimpinella anisum* seeds extracts.

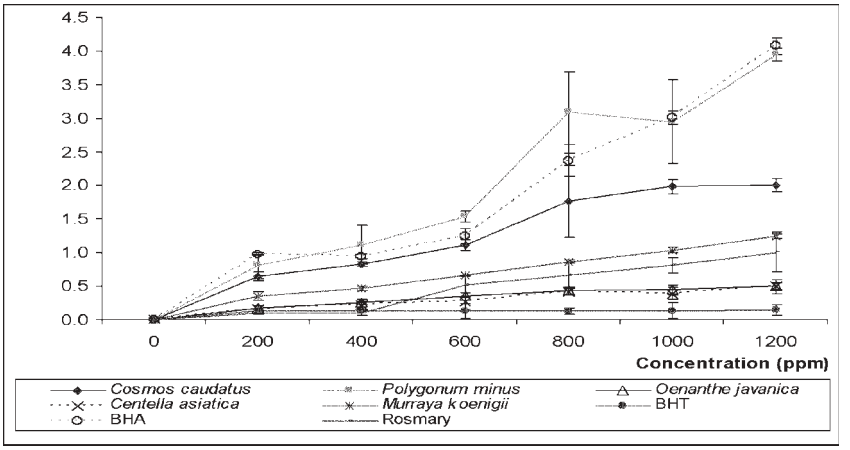

Figure 1: Reducing power of water extracts of selected Malaysian herbs

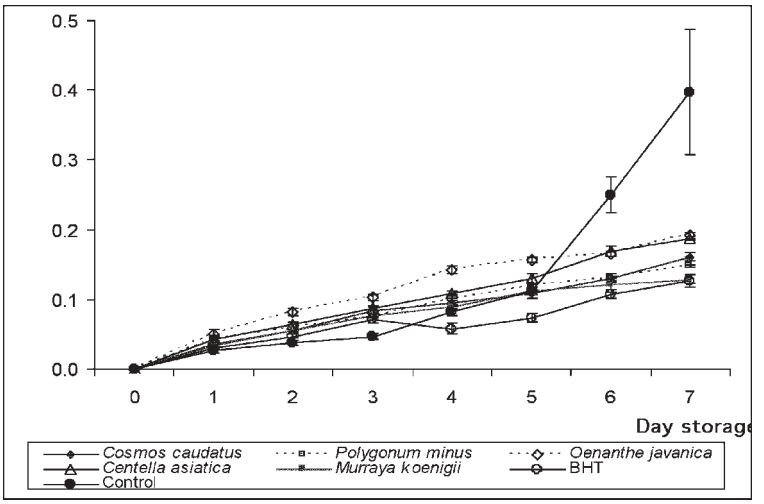

Figure 2: Analysis FTC of water extracts of selected Malaysian herbs

The ability to reduce Fe (III) may be attributed to hydrogen donation from phenolic compounds (Shimada *et al.*, 1992) which is also related to the presence of reductant agents (Duh, 1998). Correlation analysis carried out showed a positive correlation (r = 0.75) between phenolic compounds and reducing power.

Antioxidant Capacity

Figure 2 shows the FTC of various types of water extracts of the Malaysian herbs studied. The FTC method measures the amount of peroxide value at the start of the lipid peroxidation, where ferric ion is formed upon reaction of peroxide with ferrous chloride. The ferric ion then unites with ammonium thiocyanate producing ferric thiocyanate, a red-coloured substance. The darker the colour, the higher is the absorbance.

The results in Figure 2 shows that all samples were oxidized when stored for seven days at 40-45°C. Initially, the absorbance of *Cosmos caudatus, Murraya koenigii* and BHT was

significantly different ($P < 0.05$) compared to the control. After seven days, it had been shown that all samples effectively inhibit linoleic acid oxidation. The percentage of inhibition of linoleic acid was in the order *Murraya koenigii* > *Polygonum minus* > *Cosmos caudatus* > *Centella asiatica* > *Oenanthe javanica* with percentage values of 67.7%, 63.2%, 59.7%, 53.1%, and 52.1% respectively. Even though the percentage inhibition for *Murraya koenigii* was the highest, the result was not statistically different ($P > 0.05$) from *Polygonum minus* and all the other herbs extracts except for the control.

Noriham *et al.*, (2004) reported that the percentage of inhibition of *Polygonum minus*, *Zingiber officinale*, *Melicope lunu-ankenda* and *Manihot esculenta* was 85.5%, 68.0%, 68.4% and 54.7% respectively. The antioxidant activities also increased, as concentration of the plant samples increased (Nagai *et al.*, 2003). The correlation analysis between total phenolic content and FTC analysis was positive (r = 0.58). These phenolic compounds may donate hydrogen and can terminate the free radical reaction chain by changing it to stable compounds (Amarowicz *et al.*, 2000).

Thiobarbituric Acid (TBA) Test

FTC is used to measure the production of peroxide compounds at the initial stage of oxidation while TBA test is used to measure the secondary product of oxidation such as aldehyde and ketone (Farag *et al.*, 1989).

Table 2 shows the absorbance of herb extracts using the TBA test at 7 days of storage. It shows that the absorbance of *Centella asiatica* was the lowest (0.037) and not statistically different from *Polygonum minus, Murraya koenigii* and *Cosmos caudatus*. The absorbance of the control sample obviously showed the highest reading ($P < 0.05$). This could indicate that the amount of peroxidation was greater than that at the secondary stage. Secondary products such as malonaldehyde was not stable for a long period of time. It is turned into alcohol and acid, which cannot be detected by a spectrophotometer (Asmah *et al.*, 2000).

Table 2: The TBA of the water extracts of selected Malaysian herbs

Samples	Absorbance reading (A=532 nm) at 7 days of storage
Cosmos caudatus	0.052^{bc}
Centella asiatica	0.037^{c}
Murraya koenigii	0.059^{bc}
Polygonum minus	0.060^{bc}
Oenanthe javanica	0.062^{b}
BHT	0.065^{b}
Control	0.172^{a}

Values with the same lowercase within each column are not significantly different (P > 0.05)

CONCLUSION

Among all the local herbs extract analysed, kesum (*Polygonum minus*) showed the highest total phenolic content (44.35 mg TAE / 100 g fresh weight) and the greatest ability in reducing Fe^{3+}. However, curry leaves (*Murraya koeniigi*) and pegaga (*Centella asiatica*) showed the best antioxidant activity for the FTC and TBA methods, respectively. However, the results for *Murraya koeniigi* and *Centella asiatica* for the FTC and TBA tests were not statistically different ($P > 0.05$) from that for *Polygonum minus*. Total phenolic content correlated positively with reducing power (r = 0.75) and antioxidative activities (r = 0.58) in linoleic acid emulsion system. This shows that antioxidative activities of these Malaysian herbs studied may be a potential source of natural antioxidants in foods.

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