

Phytochemical Constituents and *In Vitro* Bioactivity of Ethanolic Aromatic Herb Extracts

(Kandungan Fitokimia dan Bioaktiviti *In Vitro* Etanol Ekstrak Aromatik Herba)

NURAIN AZIMAN, NORIHAM ABDULLAH*, ZAINON MOHD NOOR, KHAIRUSY SYAKIRAH ZULKIFLI
& WAN SAIDATUL SYIDA WAN KAMARUDIN

ABSTRACT

Phytochemical compounds, antioxidant and antibacterial activities of selected ethanolic aromatic Malaysian herbal extracts namely Persicaria hydropiper, Citrus hystrix, Murraya koenigii, Etlingera elatior, Cymbopogon citratus and Kaempferia galanga were screened and determined. Antioxidant activities were analysed using Ferric Reducing Antioxidant Power (FRAP), β -carotene bleaching and Oxygen Radical Absorbance Capacity (ORAC) assays. Disc diffusion assay was used to determine antibacterial activity against six bacteria strains. Alkaloids, flavonoids, saponins, tannins, terpenoids and steroids were detected in the herb extracts. P. hydropiper extract had the highest antioxidant activities in FRAP and ORAC assays in which 1676.67 mM TE/g EW and 11.20 mmol TE/g EW were obtained, respectively. However, M. koenigii extract showed 61.8% inhibition in β -carotene bleaching assay among samples but lower than BHA/BHT standard. M. koenigii extract showed the most effective antibacterial activity against three Gram-positive bacteria. Aromatic Malaysian herbs such as P. hydropiper and M. koenigii were found to exhibit high antioxidant and antibacterial activities.

Keywords: Antibacterial; antioxidant; ethanolic extracts; herbs; phytochemical

ABSTRAK

Penyaringan sebatian fitokimia, aktiviti anti-oksida dan anti-bakteria bagi ekstrak etanol herba aromatik terpilih di Malaysia iaitu Persicaria hydropiper, Citrus hystrix, Murraya koenigii, Etlingera elatior, Cymbopogon citratus dan Kaempferia galanga telah ditentukan. Aktiviti anti-oksida telah dianalisis menggunakan kaedah pengurangan kuasa anti-oksida Ferik (FRAP), pelunturan β -karotena dan kapasiti penyerapan radikal oksigen (ORAC). Kaedah cakera serapan telah digunakan untuk menentukan aktiviti anti-bakteria terhadap enam jenis bakteria. Alkaloid, flavonoid, saponin, tannin, terpenoid dan steroid telah dikesan di dalam ekstrak herba. Ekstrak P. hydropiper mempunyai aktiviti anti-oksida tertinggi iaitu 1676.67 mM TE/g berat ekstrak dan 11.20 mmol TE/g berat ekstrak, masing-masing dalam ujian FRAP dan ujian ORAC. Walau bagaimanapun, ekstrak M. koenigii menunjukkan perencatan tertinggi iaitu 61.8% dalam kaedah pelunturan β -karotena antara sampel, tetapi lebih rendah daripada standard BHA/BHT. Ekstrak M. koenigii juga menunjukkan aktiviti yang paling berkesan terhadap tiga bakteria Gram-positif. Aromatik herba di Malaysia seperti P. hydropiper dan M. koenigii telah didapati mempamerkan aktiviti-aktiviti pengantioksidan dan pengantibakteriaan yang tinggi.

Kata kunci: Anti-bakteria; anti-oksida; ekstrak etanol; fitokimia; herba

INTRODUCTION

Phytochemicals are natural bioactive compounds found in plants. Phytochemicals are divided into two groups; primary and secondary compounds. These classes are according to their functions in plant metabolism. Amino acids, sugars, proteins and chlorophyll are known as primary compounds while secondary compounds consists of alkaloids, terpenoids, phenolic compounds and many more (Krishnaiah et al. 2009).

Herbs and spices are known to produce certain bioactive compounds which react with other organisms in the environment to exhibit antioxidant activity and inhibit bacterial and fungal growth. The majority of the active compounds are phenolics, vitamin C, vitamin E, tannins and carotenes (Aqil et al. 2006; Thitilertdecha et

al. 2008). Sources of natural antioxidants are primarily plant phenolics such as flavonoids that exhibit antioxidant, antimicrobial, anticarcinogenicity and other biological activities (Demiray et al. 2009; Mohan et al. 2008; Sengul et al. 2009). Antioxidant properties may also result from the chelation of transition metal ions by flavonoid compounds. The substances that inhibit the growth of pathogens and are least toxic to host cells are considered good candidates for development of new antimicrobial drugs.

Aromatic herbs, spices and roots have been used for thousands of century by many people in the world including Malaysia. These herbs have been improved not only as a way to rescue ancient traditions but also as an alternative solution to health problems. *Persicaria hydropiper* is a plant having a kind of nice, strong aroma

and locally known as 'daun kesum' in Malaysia. The boiled water of 'daun kesum' can be consumed to heal stomach ache and reduce dandruff problem, besides being used in cooking. 'Daun limau purut', the Malay name for kaffir lime or its scientific name *Citrus hystrix* is commonly used as a condiment in various Malay dishes especially 'Tom Yam'. It is well known for its medicinal properties such as to treat normal skin disorders. *Murraya koenigii* or curry leave belong to family Rutaceae, locally named as 'daun kari' is commonly used as a natural flavoring agent for various food preparations especially among the Malays and Indians. The leaves are used in indigenous medicine as a tonic for stimulant and stomach ache. *Etlintera elatior* is normally used in preparation of 'laksa' and 'nasi kerabu' because they impart exotic aroma into the dishes. This plant known as 'bunga kantan' is pinkish in colour and looks very pretty. *Cymbopogon citratus* which called as 'serai' or known as lemongrass had antioxidative properties as documented by Tachakittirungrod et al. (2007). *Kaempferia galanga* from the Zingiberaceae family also known as 'daun cekur' by the Malays are normally used as spice ingredient and food flavouring agent in cooking dishes. Traditionally, the fresh leaves are chewed for relieving sore throats and coughs. All of these herbs are easily available and are commonly used herbs among Malaysians.

To date there is a lack of data published on these plants especially on the antibacterial part and strong smell. This study aimed to screen the phytochemical compounds and to determine the antioxidant and antimicrobial activities of ethanolic extracts of *P. hydropiper*, *C. hystrix*, *M. koenigii*, *E. elatior*, *C. citratus* and *K. galanga*, moreover exploring Malaysian herbs as sources of bioactive compounds for the functional food industry.

MATERIALS AND METHODS

CHEMICALS

Ammonia, Mayer's reagent, ferric chloride, concentrated sulfuric acid (H_2SO_4), acetic anhydride, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), ascorbic acid, 95% ethanol, ferric chloride ($FeCl_3 \cdot 6H_2O$), 2,4,6-tripyridyl-s-triazine (TPTZ), sodium acetate, methanol, trolox, β -carotene, linoleic acid, Tween 20, 2,2-azobis-2-methyl-propanimidamide, dihydrochloride (AAPH), AOX assay buffer and fluorescein solution were purchased from Sigma-Aldrich (USA) and hydrochloric acid (HCl) was obtained from Merck (Darmstadt, Germany). Chloroform and glacial acetic acid were purchased from Friendemann Schmidt (Germany). Nutrient agar was obtained from Difco (Becton, Dickinson and company, France). Nutrient broth and standard antibiotic were obtained from Oxoid (UK).

PLANT MATERIALS AND EXTRACTION

Six aromatic Malaysian herbs namely *Persicaria hydropiper* (daun kesum), *Citrus hystrix* (daun limau purut), *Murraya koenigii* (daun kari), *Etlintera elatior*

(bunga kantan), *Cymbopogon citratus* (serai) and *Kaempferia galanga* (daun cekur) were collected from Kuala Selangor, Selangor, Malaysia. Samples were extracted using 95% ethanol as a solvent. We mainly used ethanol as an extraction solvent because this solvent is relatively safe and cheap for herbal medicine preparation compared with the other toxic organic solvent (Azizah et al. 2007; Phrompittayarat et al. 2007). The edible portions of the fresh sample were cleaned using running tap water and dried using cabinet dryer (Vission Scientific, Korea) overnight until constant weight. Then, the samples were crushed into fine particles using ultra centrifugal mill (Restch, Zm 200) to obtain uniform size which is 0.5 mm. The samples were soaked and stirred in 95% ethanol in a ratio of 1:20 (w/v). Then, the samples were filtered using Whatman No. 41 paper. The samples were evaporated using a rotary evaporator (BUCHI, Switzerland) at 50°C. The crude extracts were stored at -20°C for further analysis.

PHYTOCHEMICAL SCREENING

The herb extracts were subjected to phytochemical screening for secondary plant metabolites according to the methods described by Anyasor et al. (2010), Ayoola et al. (2008), Bansa and Adeyemo (2006) and Edeoga et al. (2005). The presence of alkaloids, flavonoids, saponins, tannins, terpenoids and steroids in the herb extracts were revealed.

SCREENING FOR ALKALOIDS

Crude extract (0.5 g) was diluted with 10 mL of 10% acetic acid in ethanol, boiled and filtered while hot. 2 mL of 10% dilute ammonia and 5 mL of chloroform were added to 5 mL of filtrate. The filtrate was shaken gently to extract the alkaloid base. The chloroform layer was extracted with 5% of HCl. The filtrate was treated with a few drops of Mayer's reagent and a white precipitate indicates the presence of alkaloids.

SCREENING FOR FLAVONOIDS

Crude extract (1 g) was added with 5 mL ethanol, boiled and filtered. A few drops of concentrated HCl and magnesium tape ribbon (1–2 cm) were added. Colours ranging from orange to red indicated flavones, red to crimson indicated flavonols and crimson to magenta indicated flavonones (Odebiyi & Sofowora 1978).

SCREENING FOR SAPONINS

Crude extract (2 g) was boiled in 20 mL of distilled water in a water bath and filtered. The filtrate was shaken vigorously for a stable persistent froth regarded as positive for the presence of saponins.

SCREENING FOR TANNINS

Crude extract (0.5 g) was boiled in 20 mL of water and then filtered. A few drops of 0.1% ferric chloride was added and

observed for brownish green or a blue-black colouration for the presence of tannins.

SCREENING FOR TERPENOIDS (SALKOWSKI TEST)

An extract (5 mL) was mixed in 2 mL of chloroform and concentrated H₂SO₄ (3 mL) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

SCREENING FOR STEROIDS

Acetic anhydride (2 mL) was added to 0.5 g crude extract with 2 mL H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

Ferric reducing antioxidant power of ethanolic herb extracts was determined according to Benzie and Strain (1996). Trolox was used as a standard. Exactly 8.7 mL of FRAP reagent (mixture of acetate buffer, TPTZ and Fe₂Cl₃·6H₂O) was added into 0.3 mL of the sample. The mixture was then incubated at 50°C for 1 h. The absorbance of samples was measured at 593 nm using UV-Vis Spectrophotometer (Perkin Elmer, Lambda 35, USA). The results were expressed in mM of Trolox equivalent (TE) per g of extract weight.

β-CAROTENE BLEACHING ASSAY

The antioxidant activity of ethanolic herb extracts was assayed based on β-carotene bleaching method developed by Velioglu et al. (1998) with some modifications. The combination of BHA/BHT was used as standard. About 6 mL of β-carotene (2 mg in 10 mL chloroform), 60 μL of linoleic acid and 600 μL of Tween 20 were transferred into a round bottomed flask. The mixture was then evaporated to remove chloroform at 40°C for 10 min using a rotary evaporator (BUCHI, Switzerland). About 300 mL of distilled water was added to the mixture then shaken vigorously to form an emulsion later. Then 5 mL aliquots of the emulsion were pipetted into test tubes containing 200 μL of standards or samples or control (ethanol) (0.2 mg/mL) and immediately placed in a water bath at 50°C. The absorbance was read at 20 min intervals for 2 h at 470 nm using UV-Vis Spectrophotometer (Perkin Elmer, Lambda 35). Degradation Rate (DR) was calculated using the following equation:

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

where \ln 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 inhibition relative to control, using the following formula:

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

OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC) ASSAY

The ability of ethanolic herb extracts to scavenge the reactive oxygen radical was determined using ORAC method (Ou et al. 2001). About 175 mg of AAPH (2,2'-azobis-2-methyl-propanimidamide, dihydrochloride) was completely dissolved in 2.7 mL of AOX Assay buffer to prepare AAPH working solution. Fluorescein working solution was prepared by dissolving 1.2 mL of fluorescein in 16.8 mL of AOX Assay buffer and was kept in dark conditions. Trolox standard was prepared as follows: 20 μL of 1.5 mM trolox was dissolved in 500 μL of AOX Assay buffer. The stock solution was diluted with the same AOX Assay buffer to 50, 25, 12.5, 6.25, 3.125, 1.563 and 0.781 μM working solutions. About 150 μL of fluorescein working solution was added to the blank assay plate. Then 25 μL of samples and trolox standards was added to each well followed by 25 μL of AAPH working solution. The kinetic fluorescence was determined using Gen5 micro plate reader (Biotek, Synergy HT). The result was expressed in mmol of Trolox equivalent (TE) per 1 g of extract weight.

TEST MICROORGANISMS

All of the test microorganisms were obtained from the Department of Microbiology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia. The bacterial cultures were grown on nutrient agar and stored at 4°C. For the antibacterial evaluation, three Gram-positive bacteria, *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 43300), *Staphylococcus xylosus* (ATCC 29971) and three Gram-negative bacteria: *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 14028) were subcultured in the appropriate broths at 37°C for 18-24 h.

DISC DIFFUSION ASSAY

Disc diffusion assay was conducted according to Mackeen et al. (1997). Nutrient agar plates were inoculated with bacterial organisms (optical density: 0.11, 625nm), approximately corresponding to 1-2 × 10⁸ CFU/mL with sterile swabs. Sterilized paper disc (Whatman AA discs, 6 mm) with 25 μL of 12.5, 25, 50 and 100 mg/mL of ethanolic herb extracts were aseptically placed apart from each other on each agar plate. The plates were incubated at 37°C for 18-24 h. The strength of activity was classified as strong for inhibition zone diameters ≥ 20 mm, moderate for diameters ranging from 10 to 19 mm and weak for diameters ranging from 1 to 9 mm (Shahidi Bonjar 2004). The inhibition zones were compared with the control disc containing standard antibiotic gentamicin (30 μg), streptomycin (10 μg) and chloramphenicol (10 μg).

RESULTS AND DISCUSSION

PHYTOCHEMICAL SCREENING

The phytochemical screening of the ethanolic herb extracts studied showed the presence of some bioactive compounds such as alkaloids, flavonoids, saponins, tannins, terpenoids and steroids (Table 1). Alkaloids were present in extracts of *P. hydropiper*, *C. hystrix*, *M. koenigii* and *E. elatior*. Flavonoids were found to be present in all samples except *E. elatior* extracts. Saponins were detected in all samples except *C. hystrix* extracts. *P. hydropiper*, *M. koenigii* and *E. elatior* were tested positive for tannins, while *P. hydropiper* and *C. citratus* extracts contained terpenoids. Steroids were found to be present in *P. hydropiper* and *M. koenigii* extracts only. Phytochemicals are known to be biologically active. These secondary metabolites exert antioxidant and antimicrobial properties through different mechanism. Most of the secondary metabolites were identified in the polar extracts (Gonzalez-Guevara et al. 2004).

Alkaloids which are one of the largest groups of phytochemicals in plant have amazing effects on humans based on their toxicity against cells of foreign organisms. Therefore, the compounds detected may be responsible for the antibacterial activity of the herb extracts. Flavonoids exhibited a wide range of biological activities such as antioxidant, anti-inflammatory, antimicrobial, anti-angionic, anticancer and anti-allergic (Anyasor et al. 2010; Chao et al. 2002; Igbinosa et al. 2009; Thitilertdecha et al. 2008). Saponins which are one of the active constituents involved in plant disease resistance because of their antimicrobial activity (Barile et al. 2007). Traditionally, saponins are subdivided into triterpenoid and steroid glycoside. Tannins are phenolic compound which act as primary antioxidants or free radical scavengers (Ayoola et al. 2008). According to Mohanta et al. (2007), several phenolic compounds like tannins present in cells of plant are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens. In addition, herbs that have tannins as their main components are astringent in nature. According to Sulaiman et al. (2008), the phytochemical screening of *K. galanga* leaves demonstrated the presence of flavonoids and saponins which supported these findings. The presence of these metabolites probably explains the various uses of this plant in traditional medicine.

ANTIOXIDANT ACTIVITIES OF HERBS

The antioxidant activity can be measured using several methods but three methods were chosen in this study. Ferric reducing antioxidant power is to measure the ability of antioxidant presence in the herb extracts to reduce the ferric ion Fe^{3+} -TPTZ complex to blue coloured ferrous ion Fe^{2+} -TPTZ by electron donor in acidic medium. The reducing power (FRAP value) of herb extracts are summarized in Figure 1. The figure shows ethanolic extracts of *P. hydropiper* exhibited the significantly highest (1676.67 ± 124.23 mM TE/g EW) antioxidant activity, followed by *C. hystrix*, *E. elatior*, *M. koenigii*, *C. citratus* and *K. galanga*. However, there were no significant differences among *C. hystrix*, *E. elatior* and *M. koenigii*. Sumazian et al. (2010) found that the FRAP value of *P. hydropiper* extract was 367.06 mmol by using water as solvent. The difference in results obtained might possibly be due to the different extraction solvents and methods. The presence of phytochemical compound such as alkaloid, flavonoid, saponin, tannin and terpenoid compounds might contribute to antioxidants and hence exhibits higher FRAP values. Besides, several studies reported the phenolic compound in spices and herbs significantly contributed to their antioxidant properties. The highest reducing power of *P. hydropiper* extracts may be mainly due to the flavonoid compound according to Miean and Mohamed (2001) which reported that flavonoid compounds, such as myricetin and quercetin were existed in *P. hydropiper* extracts. In other studies, Tachakittirungrod et al. (2007) reported the comparison between *C. hystrix* and *C. citratus* extracts. The studies reported that the ethanolic *C. hystrix* extracts exhibited higher FRAP value (781 mM TE/g EW) compared to the ethanolic *C. citratus* extracts (631 mM TE/g EW).

β -carotene bleaching assay is to measure the ability of a compound to inhibit the oxidation of β -carotene. The procedure depends on the hydroperoxides that were produced by linoleic acid during incubation at 50°C. These hydroperoxides have the ability to oxidize β -carotene. The presence of antioxidants in the herb extracts will hinder the extent of β -carotene bleaching by neutralizing the linoleate free radical and other free radical formed in the system. The percentage inhibition of ethanolic herb extracts is presented in Figure 2. From this study, oxidation of β -carotene was effectively inhibited by *M. koenigii* which showed the

TABLE 1. Phytochemical screening of ethanolic aromatic Malaysian herb extracts

Samples	Alkaloids	Flavonoids	Saponins	Tannins	Terpenoids	Steroids
<i>Persicaria hydropiper</i>	+	+	+	+	+	+
<i>Citrus hystrix</i>	+	+	-	-	-	-
<i>Murraya koenigii</i>	+	+	+	+	-	+
<i>Etligeria elatior</i>	+	-	+	+	-	-
<i>Cymbopogon citratus</i>	-	+	+	-	+	-
<i>Kaempferia galanga</i>	-	+	+	-	-	-

+ = presence, - = absence

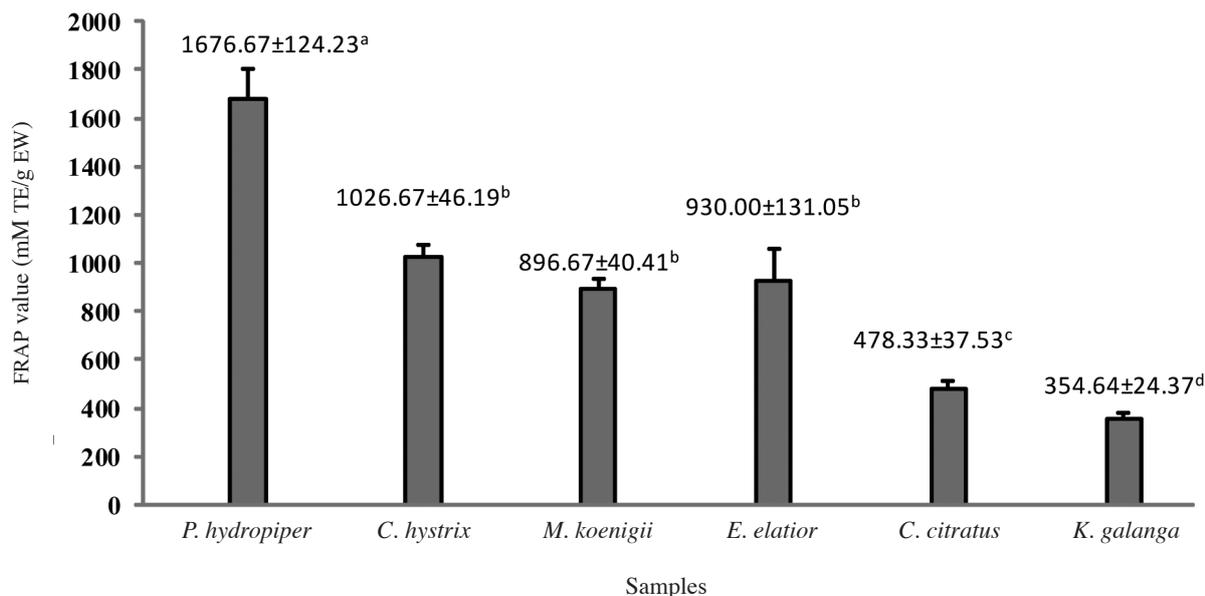


FIGURE 1. Antioxidant activity of ethanolic aromatic Malaysian herb extracts determined by FRAP assay. Values are expressed as mean \pm standard deviation ($n=3$). Means with same small letters are not significantly different ($p>0.05$)

significantly highest percentage ($61.80 \pm 3.14\%$) 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 $29.68 \pm 4.71\%$ inhibition, respectively. However, all of ethanolic herb extracts, showed lower antioxidant activity compared with BHA/BHT standard ($70.1 \pm 3.33\%$). Our results were different to the finding of Mishra et al. (2009) where the maximum inhibition of methanol extract of *M. koenigii* was 77.8% and close with the BHT positive control (74.1%). According to Hassimotto et al. (2005), the values of antioxidant activity are classified as high ($> 70\%$ inhibition), moderate (40-70% inhibition) and low ($< 40\%$

inhibition). Thus, *M. koenigii* and *P. hydropiper* extracts showed moderate antioxidant activities. However, the *M. koenigii* extract was significantly higher than *P. hydropiper* extract.

ORAC assay is used to measure the relative antioxidant activity of sample using fluorescence-based technology of detection. A peroxy radical (ROO^\cdot) is formed from the breakdown of AAPH (2,2'-azobis-2-methylpropanimidamide, dihydrochloride) at 37°C . The peroxy 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

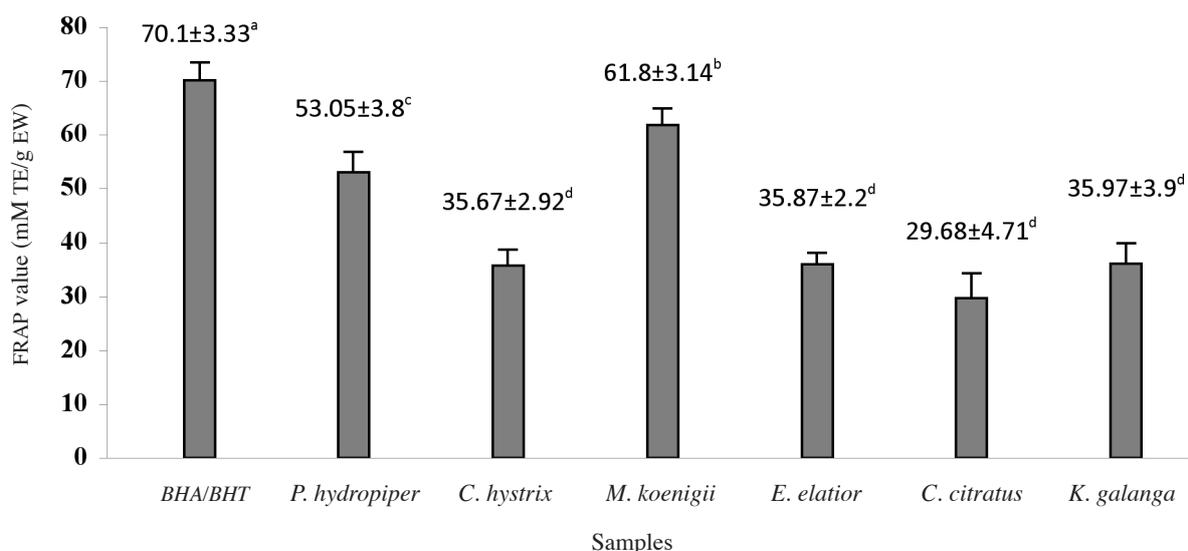


FIGURE 2. Percentage inhibition of BHA/BHT standard and ethanolic aromatic Malaysian herb extracts as determined by β -carotene bleaching assay. Values are expressed as mean \pm standard deviation ($n=3$). Means with same small letters are not significantly different ($p>0.05$)

antioxidant in sample will inhibit the fluorescence decay by a hydrogen atom transfer mechanism. Trolox was used as a positive control. Table 2 shows the ORAC value of ethanolic herb extracts which was expressed as mmol TE/g. The ORAC values for the ethanolic herbs ranged from 8.88 mmol of TE/g to 11.20 mmol of TE/g of extract weight. The ethanolic herbs extract with the highest ORAC values were *P. hydropiper* (11.20 ± 2.0 mmol TE/g EW), followed by *C. hystrix* (10.51 ± 1.94 mmol TE/g EW), *M. koenigii* (9.93 ± 1.89 mmol TE/g EW), *C. citratus* (9.78 ± 1.78 mmol TE/g EW), *K. galanga* (9.59 ± 0.4 mmol TE/g EW) and *E. elatior* (8.88 ± 1.12 mmol TE/g EW). However, there were no significant difference ($p>0.05$) among all ethanolic herb extracts studied. The flavonoids, saponins and tannins compounds which were detected in almost all samples after phytochemical screening might also contribute to the termination of chain radical reactions by donating hydrogen atoms to the peroxy radical.

From this study, the results for each assay differ from one to another. From the observation, the factor might be

TABLE 2. ORAC value of ethanolic aromatic Malaysian herb extracts

Samples	ORAC value (mmol TE/g extract weight)
<i>Persicaria hydropiper</i>	11.20 ± 2.00 ^a
<i>Citrus hystrix</i>	10.51 ± 1.94 ^a
<i>Murraya koenigii</i>	9.93 ± 1.89 ^a
<i>Etligeria elatior</i>	8.88 ± 1.12 ^a
<i>Cymbopogon citratus</i>	9.78 ± 1.78 ^a
<i>Kaempferia galanga</i>	9.59 ± 0.4 ^a

Values are expressed as mean ± standard deviation ($n = 3$). Means with same small letters within each row are not significantly different ($p>0.05$)

due to the different assays have different principles and different compounds detected for each assay. According to Gursoy et al. (2009), two factors that affect the results obtained by different antioxidant assays are some phytochemicals available in the extracts that may contain high molecular weight antioxidants or antioxidants bound

TABLE 3. Antibacterial activity of ethanolic aromatic Malaysian herb extracts against the Gram-positive and Gram-negative bacteria based on disc diffusion assay

Samples	Conc. (mg/disc)	Microorganisms (Inhibition zone diameter, mm)					
		Gram-positive bacteria			Gram-negative bacteria		
		BS	SA	SX	EC	PA	ST
<i>P. hydropiper</i>	2.5	11.0±0.8	-	-	-	-	-
	1.25	11.0±1.0	-	-	-	-	-
	0.625	11.0±2.0	-	-	-	-	-
	0.3125	9.0±1.0	-	-	-	-	-
<i>C. hystrix</i>	2.5	11.0±0.8	-	-	-	-	-
	1.25	11.0±1.0	-	-	-	-	-
	0.625	11.0±2.0	-	-	-	-	-
	0.3125	9.0±1.0	-	-	-	-	-
<i>M. koenigii</i>	2.5	16±3.0	14.5±2.1	8.5±0.4	-	-	-
	1.25	13.9±2.1	14.3±2.5	8.5±0.3	-	-	-
	0.625	12.6±2.1	12.5±0.7	8.5±0.5	-	-	-
	0.3125	11.4±1.7	9.5±0.7	8.0±1.0	-	-	-
<i>E. elatior</i>	2.5	11.7±2.1	-	-	-	-	-
	1.25	-	-	-	-	-	-
	0.625	-	-	-	-	-	-
	0.3125	-	-	-	-	-	-
<i>C. citratus</i>	2.5	-	-	-	-	-	-
	1.25	-	-	-	-	-	-
	0.625	-	-	-	-	-	-
	0.3125	-	-	-	-	-	-
<i>K. galanga</i>	2.5	-	-	-	-	-	-
	1.25	-	-	-	-	-	-
	0.625	-	-	-	-	-	-
	0.3125	-	-	-	-	-	-
Gentamicin	0.03	25.7±0.6	19.0±3.1	31.0±2.1	20.0±2.0	18.3±5.5	21.3±2.8
Streptomycin	0.01	23.0±2.0	13.0±0.0	20.0±1.2	14.0±2	12.0±1.4	14.5±0.7
Chloram-phenicol	0.01	2.0±1.0	10.7±1.6	15.0±1.0	17.0±2.8	-	18.0±0.4
Ethanol	-	-	-	-	-	-	-

Values are expressed as mean ± standard deviation ($n = 3$). “-”, no inhibition zone. BS=*Bacillus subtilis* (ATCC 6633), SA=*Staphylococcus aureus* (ATCC 43300), SX=*Staphylococcus xylosus* (ATCC 29971), EC=*Escherichia coli* (ATCC 11229), PA=*Pseudomonas aeruginosa* (ATCC 27853), and ST=*Salmonella typhimurium* (ATCC 14028).

to complex molecules and some of phenolic compounds might not have antioxidant properties.

ANTIMICROBIAL ACTIVITIES OF HERB

The antibacterial activity of four different concentrations of ethanolic herb extracts was assayed against six strains of pathogenic bacteria is shown in Table 3. As shown in the table, all concentration of herb extracts of *M. koenigii* showed the most effective inhibition against all tested Gram-positive bacteria. However, all concentration of *P. hydropiper* and *C. hystrix* extracts and also the highest concentration of *E. elatior* extracts showed antibacterial activity against *B. subtilis*. Extracts from many species of *Persicaria* has been found to possess antimicrobial activity (Borchardt et al. 2008). Among the Gram-positive and Gram-negative bacteria tested, the Gram-positive bacteria were more susceptible to the extracts. This can be explained by the fact that the outer membrane of Gram-negative bacteria is known to present a barrier to the penetration of numerous antibiotic molecules and the periplasmic space contains enzymes which are able to degrade exogenous molecules (Tanaka et al. 2006). These antibacterial activities are likely due to the presence of the secondary metabolites in the extracts. The flavonoids, saponins and tannins compounds which were present in the *M. koenigii* are well known as general antimicrobial properties. Tannins have been found to inhibit synthesis of protein by form irreversible complexes with proline rich protein (Igbinsosa et al. 2009). These factors might be the reasons for the variation in the results obtained.

CONCLUSION

Screening for phytochemicals of six aromatic plants studied showed the presence of bioactive compounds but of different kinds from one to another. *Persicaria hydropiper* and *Murraya koenigii* exhibited good antioxidant and antibacterial activities indicating the potential of these plants as a source of functional ingredients that can be used in food and pharmaceutical industries.

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Food Technology Programme
Faculty of Applied Sciences
Universiti Teknologi MARA
40450, Shah Alam, Selangor
Malaysia

*Corresponding author; email: noriham985@salam.uitm.edu.my

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