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Phytochemical Constituents and Bioactivities of Aqueous Extract of Aromatic Herbs

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Abstract: Herbs have been used as a source of medicines since time immemorial. The purposes of this study were to determine total phenolic content, total flavonoid content and bioactivities including antioxidant and antibacterial activities of three types of aqueous aromatic herbal extracts: *Polygonum minus, Kaempferia galanga* L. and *Phaeomaria speciosa*. The antioxidant activities of herbs were analysed using two different mechanism methods namely DPPH radical scavenging and β-carotene bleaching assays. Disc diffusion assay was used to determine the antibacterial activities of herbs against eight pathogenic bacteria. Minimum inhibitory concentration (MIC) of herb extracts was determined. Aqueous *P.minus* extracts had higher total phenolic content, total flavonoid content and antioxidant activities in both assays. However, aqueous *P.speciosa* extracts showed most effective activity against Gram-positive and Gram-negative bacteria. The MIC was in range of 18.75-100 mg/mL. Aromatic herbs such as *P.minus* and *P.speciosa* were found to exhibit potential antioxidant and antibacterial agents.
Keywords: phytochemical, antioxidant, antibacterial, herbs, aqueous extracts.

Introduction

Medicinal plants are distributed throughout the world and widely used in everyday life as part of folk medicinal remedies. Bioactive compounds commonly found in fruits, vegetables, herbs and other plants have been shown to have possible health benefits with antioxidative, antimutagenic and anticarcinogenic inhibitory activities (1-3). The majority of the active compound in plants is phenolic compounds such as flavonoids which possessed antioxidant properties due to their high redox potential and wide range of biological activities. Therefore, the present study is intended to determine total phenolic content, total flavonoid content, antioxidant and antimicrobial activites of aqueous extracts of *Polygonum minus, Kaempferia galanga* L. and *Phaeomeria speciosa.*

Materials and Methods

Chemicals

Butylated hydroxyltoluene (BHT), butylated hydroxylanisole (BHA), 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-2-picrylhydrazyl (DPPH), β-carotene, linoleic acid, Tween 20, chloroform, nutrient agar and nutrient broth.

Test microorganisms

The bacterial cultures were grown on nutrient agar (Oxoid, UK) and stored at 4 ^{o}C. For the antibacterial evaluation, four Gram-positive bacteria, *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 43300), *Staphylococcus xylosus* (ATCC 29971), and *Micrococcus species* (ATCC 700405) and four Gram-negative bacteria: *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 6380) and *Cronobacter muytjensii* (ATCC 51329) were subcultured in the appropriate broths at 37 ^{o}C for 18-24 h.

Preparation of extract

Three aromatic herbs were collected from Kuala Selangor, Selangor, Malaysia. All herb samples were extracted using water. The portions of the fresh sample were cleaned using running tap water and dried using cabinet dryer (Vission Scientific) until constant weight. Then, the samples were crushed into fine particle 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). Then, the samples were filtered using Whatman No. 41 paper for both extractions. The samples were evaporated using a rotary evaporator (BUCHI) at 60 ^{o}C. The viscous samples were dried using freeze drier (Christ Martin, alpha 1-4 LD plus). The crude extracts were stored at -20 ^{o}C for further analysis.

Total phenolic content (TPC)

Total phenolic content of aqueous herb extracts were determined with slight modification according to Folin-Ciocalteu procedure (4). Total phenolic content of samples was determined as mg of gallic acid equivalent (GAE) per 1 g of extract weight using an equation obtained from the standard gallic acid calibration curve.

Total flavonoid content (TFC)

Total amount of flavonoid of aqueous herb extracts were determined using a method as described by (5). Total flavonoid content of samples was expressed in mg of quercetin equivalent (QE) per 1 g of extract weight.

DPPH free radical scavenging activity assay

The ability of antioxidant of aqueous herb extracts to scavenge DPPH radical by hydrogen donor were measured according to (6). EC_{50} is defined as the amount of extract necessary to decrease the initial DPPH radical concentration by 50% (7). The combination of BHA/BHT was used as standard.

β-carotene bleaching assay

The antioxidant activity of aqueous herb extracts was assayed based on the β-carotene bleaching method developed by (8) with some modifications. The combination of BHA/BHT was used as a standard. Antioxidant activity (AA) was expressed as percent of inhibition relative to the control.

Disc diffusion assay

The disc diffusion assay was conducted according to (9). The zones of inhibition was measured and expressed in milimeter. The inhibition zones were compared with the control disc containing standard antibiotic streptomycin (10µg) and chloramphenicol (10µg).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) determination of the extracts was performed by a serial broth dilution technique (3). The lowest concentration of herb extracts that will inhibit the visible growth of microorganisms after incubation is known as MIC.

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 ± SE using ANOVA, if justified by the statistical probably ($P<0.05$), by Duncan's new multiple range test. Differences were considered statistically significant if $P<0.05$.

Results and Discussion

Table 1 showed the total phenolic content, total flavonoid content and antioxidant activities of aqueous aromatic herb extracts. From this study, the TPC of aqueous *P.minus* extracts were the highest among samples (148.83 ± 13.77 mg GAE/ g EW) followed by *P.speciosa* and *K.galanga* L. extracts (64.00 ± 3.46 and 38.00 ± 3.91 mg GAE/g EW, respectively). Aqueous *P.minus* extracts also showed the highest of TFC among samples (424.17 ± 34.67 mg QE/g EW). The results showed that the TPC and TFC varied significantly from one plant to another. Different plants, procedures, standards used to express the TPC and extrinsic factor (agronomic, environmental, handling and storage) will be the reason of the variation results obtained (10-11).

There were many assays used to determine the antioxidant activities with different mechanisms such as radical scavenging,

decompositions of peroxides, prevention of chain initiation and binding of transition metal ion catalyst. Antioxidants were able to reduce free radical by donating an electron and hydrogen atom. 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 herb extracts. The free radical scavenging activity of extracts was presented in EC_{50} values. BHA/BHT was used as standard in this assay. The EC_{50} values are inversely proportional to the ability of the extracts to act as DPPH scavengers (antioxidant activity). The herb extracts that required the lowest concentration to promote 50% of inhibition in this sudy were aqueous *P.minus* (304.32 ± 1.85 µg/mL) and no significant difference (P>0.05) with BHA/BHT standard (296.84 ± 4.26 µg/mL). Thus, the antioxidant activities of these extracts are postulated to be greater than other samples. The β-carotene bleaching assay is to measure the ability of a compound to inhibit the oxidation of β-carotene. The presence of antioxidants in the herb extracts will hinder the extent of β-carotene bleaching by electron donor to neutralize the linoleate free radical and other free radical formed in the system. From this study, oxidation of β-carotene was effectively inhibited by *P.minus* extracts which showed the highest percentage (67.22 ± 7.62%) among sample extracts, however this herb was no significant difference (P>0.05) compared BHA/BHT standard (70.1 ± 3.33%). *K.galanga L* and *P.speciosa* extracts were showed no significant difference (P>0.05). The values of antioxidant activity are classified as high (> 70% inhibition), moderate (40-70% inhibition) and low (< 40% inhibition) (12). Thus, *P.minus* extracts possess moderate antioxidant activities. Based on both assays, herb extracts showed positive correlation between antioxidant activity and total phenolic content and total flavonoid content. The high content of phenolic and flavonoid compounds in *P.minus* extracts might contribute to the highest termination of chain radical reactions by donating electron and hydrogen atoms to the peroxy radical. Phenolics are the largest group of phytochemicals and have been said to account for most of the antioxidant activity of plant extracts (13) and flavonoid is the largest group of plant phenolics. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups. Our result is in agreement with finding by (14) who reported positive linear correlation between total phenolic content and antioxidant activities of a number of medicinal plant extracts. 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) (15). From this study, indicated that *P.minus* extracts exhibited high activity for both assays, thus, this herb able to reduce free radical through electron and hydrogen atom transfer mechanisms.

The antibacterial activity of four different concentrations of aqueous herb extracts was assayed against eight strains of pathogenic bacteria as shown in Table 2 and Table 3 using disc diffusion assay. 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 (16). As shown in Table 2, *P.minus* extracts at 0.625 to 2.5 mg/disc concentration showed moderate inhibition against *S.aureus* and the highest concentration of *P.minus* extracts showed weak inhibition against *S.xylosus*.

Table 1: Total phenolic content, total flavonoid content and antioxidant activities of aqueous aromatic herb extracts.

Sample	Total phenolic content (mgGAE/gEW)	Total flavonoid content (mgQE/gEW)	Antioxidant activities	
			EC_{50} (µg/mL)	β-carotene bleaching (Inhibition (%))
BHA/BHT	-	-	296.84 ± 4.26^{g}	70.10 ± 3.33^{j}
P.minus	148.83 ± 13.77^{a}	424.17 ± 34.67^{d}	304.32 ± 1.85^{g}	67.22 ± 7.62^{j}
K.galanga L.	38.00 ± 3.91^{c}	60.00 ± 0.00^{f}	1891.21 ± 172.38^{i}	38.24 ± 5.33^{k}
P.speciosa	64.00 ± 3.46^{b}	184.17 ± 1.44^{e}	562.38 ± 16.50^{h}	45.35 ± 4.54^{k}

Values are expressed as mean ± standard deviation (n=3). Means with a-c small letters are significantly different (p<0.05) for total phenolic content. Means with d-f small letters are significantly different (p<0.05) for total flavonoid content. Means with g-i same small letters are not significantly different (P>0.05) for EC_{50}. Means with j-k same small letters are not significantly different (P>0.05) for inhibition in β-carotene bleaching assay.

Table 2: Antibacterial activity of aqueous aromatic herb extracts against the Gram-positive bacteria based on disc diffusion assay

Samples	Conc. (mg/disc)	Diameter of Inhibition zone (mm)			
		Gram-positive bacteria			
		BS	***SA***	***SX***	***MS***
P.minus	2.5	-	16.0±2.8	7.0±0.2	-
	1.25	-	12.5±1.5	-	-
	0.625	-	10.0±1.4	-	-
	0.3125	-	-	-	-
K.galanga L.	2.5	-	-	-	-
	1.25	-	-	-	-
	0.625	-	-	-	-
	0.3125	-	-	-	-
P.speciosa	2.5	8.0±0.0	10.0±1.1	14.0±1.5	18.0±1.5
	1.25	-	8.0±0.9	11.0±1.4	9.0±0.8
	0.625	-	-	-	-
	0.3125	-	-	-	-
Streptomycin	0.01	23.0±2.0	13.0±0.0	20.0±1.2	13.0±0.3
Chloramphenicol	0.01	20.0±1.0	10.7±1.6	15.0±1.0	25.3±4.4
Water	-	-	-	-	-

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) and MS=*Micrococcus species* (ATCC 700405).

Table 3: Antibacterial activity of aqueous aromatic herb extracts against the Gram-negative bacteria based on disc diffusion assay

Samples	Conc. (mg/disc)	Diameter of Inhibition zone (mm)			
		Gram-negative bacteria			
		EC	***PA***	***PV***	***CM***
P.minus	2.5	-	8.0±0.2	-	9±0.8
	1.25	-	7.5±1.1	-	-
	0.625	-	7.5±0.1	-	-
	0.3125	-	-	-	-
K.galanga L.	2.5	-	-	-	-
	1.25	-	-	-	-
	0.625	-	-	-	-
	0.3125	-	-	-	-
P.speciosa	2.5	8.0±0.8	9.0±0.9	11.0±1.1	15.0±1.5
	1.25	6.5±0.0	8.0±0.9	10.0±0.8	10.0±0.7
	0.625	-	-	-	8.0±0.5
	0.3125	-	-	-	-
Streptomycin	0.01	23.0±2.0	13.0±0.0	19.0±2.1	13.0±0.3
Chloramphenicol	0.01	20.0±1.0	10.7±1.6	20.7±1.2	25.3±4.4
Water	-	-	-	-	-

Values are expressed as mean ± standard deviation (n=3). " – ", no inhibition zone. EC=*Escherichia coli* (ATCC 11229), PA=*Pseudomonas aeruginosa* (ATCC 27853), PV=*Proteus vulgaris* (ATCC 6380) and CM=*Cronobacter muytjensii* (ATCC 51329).

However, *P.speciosa* extracts exhibited most effective inhibition of all Gram-positive bacteria studied at a concentration of 1.25 and 2.5 mg/disc. As given in Table 3, among the four selected Gram-negative bacteria, maximum zone of inhibition was formed by *P.speciosa* extracts which showed inhibition against all Gram-negative bacteria followed by *P.minus* extracts which showed inhibition against *P.aeruginosa* and *C.muytjensii*. However, no inhibition was observed for *K.galanga* L. extracts. The antibacterial activity of *P.minus* and *P.speciosa* extracts may probably due to the high content of phenolic and flavonoid in these herbs. The presence of flavonoid, phenolic and tannin in *Orithazh thamarai chooranam* extracts could be responsible in their antimicrobial properties (17). Generally, Gram-negative bacteria are more resistance than Gram-positive bacteria (3). But in this study, *P.speciosa* extracts showed antibacterial activity against both Gram-positive and Gram-negative bacteria. This might be due to the fact that the microorganisms possess a mechanism for detoxifying the active principles in the extract (18). From this study, the antibacterial activities of these herbs were not as effective as the commercial antibiotics streptomycin and chloramphenicol. This might be because of mode of extraction. Previous study demonstrated the organic extracts were more effective than aqueous extracts. This may be due to the better solubility of the active components in organic solvents (19). As shown in Table 4, the MIC value of herb extracts was in range of 18.75-100 mg/mL.

Conclusions

The high antioxidant and antibacterial activities of herbs appeared to be attributed to its phenolic and flavonoid content. Aqueous extracts of *Polygonum minus* exhibited good antioxidant and *Phaeomeria speciosa* exhibited good antibacterial activities indicating the potential of these plants as a source of functional ingredients that can be used in food products. Further works on the identification of phenolic compounds responsible for the antioxidant and antibacterial activities of the extracts are now in progress.

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Table 4: Minimum Inhibitory Concentration (MIC) of aqueous aromatic herb extracts

Samples	Minimum Inhibitory Concentration (mg/mL)							
	Gram-positive bacteria				Gram-negative bacteria			
	BS	***SA***	***SX***	***MS***	***EC***	***PA***	***PV***	***CM***
P.minus	-	18.75	100	-	-	25	-	100
***K.galanga* L.**	-	-	-	-	-	-	-	-
P.speciosa	75	50	50	50	50	50	50	25

Values are expressed as mean (n=3). " – ", = not determine. BS=*Bacillus subtilis* (ATCC 6633), SA=*Staphylococcus aureus* (ATCC 43300), SX=*Staphylococcus xylosus* (ATCC 29971), MS=*Micrococcus species* (ATCC 700405), EC=*Escherichia coli* (ATCC 11229), PA=*Pseudomonas aeruginosa* (ATCC 27853), PV=*Proteus vulgaris* (ATCC 6380) and CM=*Cronobacter muytjensii* (ATCC 51329).

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