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Antioxidant Activity and Total Phenolic Content of Some Medicinal Plants in Urticaceae Family

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ABSTRACT

Fifteen different extracts from the leaves of *Urtica d ioica*, *Pilea microphylla* and *Elatostema umbellatum* were examined for phenolics content and free radical scavenging capacity, to determine their potential as a source of natural antioxidants. The highest DPPH radical scavenging percentages were showed by the butanol and ethyl acetate extracts *Urtica dioica* that are 62.537 ± 5.069% and 62.177 ± 2.987% at concentration 1000 µg/ml, respectively. Total phenolic content ranged from 0.24 ± 0.15 to 100.30 ± 0.01 mg gallic acid/g dry weight. The ethyl acetate extract of *Urtica dioica* exhibited the lowest EC_{50} value (100.10 µg/ml). The result indicated that the antioxidant activity was not correlated with the phenolics content suggested that non-phenolic compounds might play major free radicals scavenging activity in studied plant materials.

Key Words: *Elatostema umbellatum*; *Pilea microphylla*; *Urtica dioica*; DPPH radical scavenging; Total phenolic content.

INTRODUCTION

There is no doubt that plants are a good source of biologically active natural products. In the investigation of bioactive natural compounds, it is essential to have access to simple biological tests to locate required activities [1]. The preservative effect of many plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues [2].

Phenolic compounds are naturally occurring substances in fruit, vegetables, nuts, seeds, flowers, and some herb beverages and are an integral part of the human diet. Several studies have indicated that the antioxidant activities of some fruits and vegetables were highly correlated with their total phenolic contents Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions [3,4]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [5].

Flavonoids, phenolic acids, and phenolic diterpenes [6,7], lignans are the examples of phenolic components with antioxidant properties.

Urticaceae is one of the plants family known in Asia as herbal medicine. Urticaceace includes a number of well-known, interesting and useful plants, including *Urtica dioica* (stinging nettles), *Pilea microphylla* (gunpowder plant) and *Elatostema umbellatum* [8].

There are some publications about the antioxidant activity of *Urtica dioica* in antioxidant activity but not much as these data. For *Pilea microphylla* and *Elatostema umbellatum* there are no report about their antioxidant activities and total phenolic contents. The purpose of this study was to evaluate three plants in Urticaceae family as new potential sources of natural antioxidants and phenolic compounds. Our study also demonstrates no possible relationship between phenolic content and antioxidant activity.

MATERIAL AND METHODS

Plant material

Leaves of *Urtica dioica* were collected from Asbechin village near Salmanshahr city in province of Mazandaran and also in Alborz mountain in north of Tehran province in Iran in August 2007. *Pilea microphylla* were collected in Penang island from USM main campus (Universiti Sains Malaysia) in March 2008. Leaves of *Elatostema umbellatum* were collected from Maxwell Hill in Taiping, Perak state in Peninsula of Malaysia. Voucher specimens have been deposited at the Herbarium of the School of Biological Sciences, Universiti Sains Malaysia in April 2008.

Plant extraction

The plant materials were washed, dried and blended to small pieces. The dried materials were then transferred into the soxhlet extractor with methanol as a solvent for 72 hours. The methanolic extracts were further partitioned in separating funnel using chloroform, diethyl ether, ethyl acetate and butanol as described by Mellidis and Papageorgiou [9] with a slight modification. The dried extracts were then weighed using microbalance and then were kept in 4°C.

Chemical reagents

The chemical reagents such as DPPH [2,2-diphenyl-1-picrylhydrazyl], Quercetin [3,3',4',5,7-Pentahydroxyflavone], gallic acid [3,4,5-Trihydroxybenzoic acid] and Folin-Ciocalteu`s phenol reagent, 2N were purchased from Sigma-Aldrich Co. (USA).

Determination of antioxidant activity: DPPH radical scavenging assay

Free radical scavenging activity of fifteen crude extracts of leaves of *Urtica dioica*, *Pilea microphylla* and *Elatostema umbellatum* (MeOH, Chlorofom, Diethyl ether, Ethyl acetate and Butanol extracts) were tested using DPPH method with final concentration 1000 µg/ml (Etanolic DPPH) (Sigma Chemical Co., USA) (300 µM) was used in the reaction mixture. Freshly prepared test samples (50 µl) were combined with DPPH solution (150 µl) in a 96 well micro-titer plate. DMSO was used as a negative control. The reaction mixture were incubated for 30 minutes at 37°C and the change in absorbance at 515 nm was measured using micro plate reader (Thermo Electron Corporation, Finland). All determinations were performed in triplicate. The obtained absorbance values were converted into the percentage of radical scavenging activity using the following equation:

Radical scavenging activity (%) = 100-[(AS/AC)× 100]

Where AS: absorbance of the sample
AC: absorbance of the negative control

Free radical scavenging potency as determined from EC_{50} value obtained. Lower EC_{50} value indicated strong free radical scavenging activity.

Determination of total phenolic compounds

The amounts of phenolics in the selected medicinal plant extracts were determined with Folin-Ciocalteu reagent using the method of Salvi [10] with a little bit modified. 0.5 ml of each sample was dissolved in 1.0 ml DMSO (3 replicates), 1.0 ml of 10% dilution of Folin-Ciocalteu reagent and after 3 minutes 3 ml of Na_2CO_3 (1%, w/v) were added and the resulting mixture was incubated at room temperature for 2 hours. The absorbance of all samples was measured at 760 nm using a spectrophotometer (Model U-1900 Spectrophotometer Hitachi High Technology Corporation 2006). The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg L^{-1}. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dry weight), which is a common reference compound.

Statistical analysis

Results were tested for statistical significance by GraphPad Prism. Differences were considered statistically significant at the $p < 0.05$ level and One Way ANOVA.

RESULT AND DISCUSSION

Antioxidant activity

The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity. DPPH is one of the compounds that possess a proton free radical and shows a maximum absorption at 517 nm. When DPPH encounter proton radical scavengers, its purple colour fades rapidly. This assay determines the scavenging of stable radical species of DPPH by antioxidants. According to Table 1, the antioxidant activity of *Urtica dioica* extracts increases as the solvent polarity increases. The weakest antioxidant activities were found within chloroform extracts except for *Pilea microphylla.*

Table 1. Percentage of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity values for each plant extract evaluated at concentration 1000 µg/ml.

	DPPH scavenging activity (%) Plant extract				
	Methanol	**Chloroform**	**Diethyl ether**	**Ethyl acetate**	**Butanol**
Urtica dioica	27.103 ± 3.787^{b}	5.800 ± 0.191^{a}	25.783 ± 3.218^{b}	62.177 ± 2.987^{d}	62.537 ± 5.069^{d}
Pilea microphylla	46.990 ± 4.692^{c}	47.163 ± 4.983^{c}	60.687 ± 2.465^{d}	8.620 ± 0.955^{d}	18.340 ± 2.340^{b}
Elatostema umbellatum	17.513 ± 4.491^{c}	0	0	54.390 ± 2.960^{cd}	23.610 ± 4.926^{b}

Each value represented the mean ± SD of three replicates (n = 3). Values with different letters are significantly different (P <0.05) based on One Way and Tukey HSD test.

Thus the extracts were screening of the radical scavenging activity at different concentrations. The ethyl acetate extract of *Urtica dioica* showed the lowest EC_{50} value (100.10 µg/ml). This was followed by methanol extract of *Pilea microphylla* with EC_{50} at 121.90 µg/ml (Table 2).

Table 2. EC_{50} values of each plant extracts.

Samples	***Urtica dioica***	***Pilea microphylla***	***Elatostema umbellatum***
Methanol extract	-	121.90	-
Chloroform extract	-	215.30	-
Diethyl ether extract	-	373.50	-
Ethyl acetate extract	100.10	-	501.20
Butanol extract	399.20	-	-
Quercetin *	4.95	4.95	4.95

*The final concentration for quercetin was 125 µg/ml

Total phenolic content

The concentration of total phenolic compounds in all plant extracts was expressed as milligrams of gallic acid equivalents (GAE) per mg dry weight of plant (concentration of gallic acid standard prepared similarly). Total phenolic contents of *Urtica dioica* , *Pilea microphylla* and *Elatostema u mbellatum* were studied using Folin-Ciocalteu method. Absorbance of samples were measured at 760 nm and the amount of total phenolics in mg GAE/g extract were then analyzed and interpreted.

By manipulating the regression equation of gallic acid calibration curve (y = 9.936x + 0.015, R^2 = 0.9822), the total phenolic content of each extract was calculated and expressed as gallic acid equivalent (GAE) to facilitate the comparison [11]. Therefore, in this work, we calculated the total phenolic contents in units of mg gallic acid equivalent of phenolic compound as shown in Figure 1 (y = -0.172x + 36.70, r^2 = 0.033) . A wide range of total phenolics content was found in studied. Their content ranged from 0.24 ± 0.15 to 100.30 ± 0.01 mg/g gallic acid equivalent (GAE) of extracts, with an average of 50.27 mg/g. As shown in Table 3 the chloroform extract of *Elatostema umbellatum* had the highest contents of phenolic compounds (100.3 ± 0.01 mg/g GAE) followed by ethyl acetate extract of *E. umbellatum* (60.6 ± 0.1 mg/g GAE), diethyl ether extract of *Pilea microphylla* (55.7 ± 0.2 mg/g GAE) and methanol extract of *E. umbellatum* (52.0 ± 0.07 mg/g GAE). Result showed significant differences ($p < 0.05$) in total phenolics content among the fifteen samples (Table 3).

Table 3. Total phenolic content of each plant extract expressed gallic acid equivalent (GAE).

Plants	Type of extract	Total phenolic content (mg GAE/g dry extract)*
Urtica dioica	Methanol	21.80 ± 0.11^{cd}
	Chloroform	36.40 ± 0.00^{e}
	Diethyl ether	17.20 ± 0.13^{b}
	Ethyl acetate	18.70 ± 0.15^{bc}
	Butanol	25.00 ± 0.16^{d}
Pilea microphylla	Methanol	15.20 ± 0.15^{b}
	Chloroform	36.40 ± 0.01^{e}
	Diethyl ether	55.70 ± 0.16^{g}
	Ethyl acetate	42.10 ± 0.22^{f}
	Butanol	23.90 ± 0.20^{d}
Elatostema umbellatum	Methanol	52.00 ± 0.07^{g}
	Chloroform	100.30 ± 0.01^{i}
	Diethyl ether	21.50 ± 0.05^{cd}
	Ethyl acetate	60.60 ± 0.14^{h}
	Butanol	0.24 ± 0.15^{a}

Values are mean ± standard deviation of triplicate analyses. Values of the column, are statistically different ($p < 0.05$) as measured by Tukey`s HSD test.

* milligram gallic acid equivalent per gram dry extract

According to Pourmorad *et al.* (2006) [12], the amount of phenol content in methanol extract from *Urtica dioica* was 24.1 ± 1 mg/g. The result in Table 3 confirmed their result (21.8 ± 0.11 mg/g).

Aqueous nettle (*Urtica urens*) extract had a low ferric reducing/antioxidant power (3 vs > 20mM), but a moderate phenol antioxidant coefficient (2.2 vs > 3 mM/1) as compared with the aqueous leaves extract *Melissa officinalis* [13].

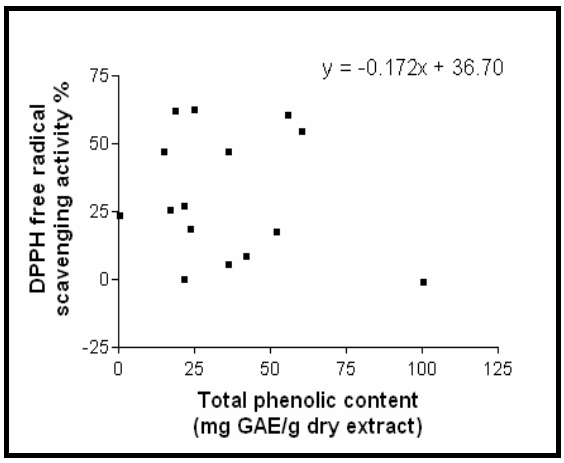

Figure 1. Correlation between the antioxidant activity and total phenolic contents.

When the antioxidant activities were compared in a linear correlation model (Pearson`s test), there were no significant correlation with total phenolic content, with an estimated coefficient of determination, (r^2) value of 0.0332, at $p < 0.05$.

The present study showed that Iranian *Urtica dioica*, which are often included in some Iranian dishes as a ingredient - especially in north of Iran as a traditional ingredient added to dishes - are strong radical scavengers among other Urticaceae family and can be considered as good sources of natural antioxidants for side dishes, commercial and medicinal uses. However, further study is needed.

CONCLUSION

According to the evaluation of percentage of free radical-scavenging activity of five different extracts from 3 plants in Urticaceae family, the butanol and ethyl acetate extracts of *Urtica dioica* and the diethyl ether extract of *Pilea microphylla* were found to have a higher antioxidant activity than other extracts. Based on the EC_{50} value, the ethyl acetate extract of *Urtica dioica* showed the best activity.

The present study suggests that *Urtica dioica* as a potential source of natural antioxidants. However, the toxicity of plant extracts with high antioxidant activity should be tested, to confirm their safety for use as food additives.

Also this result suggests that phenolic compounds do not make a major contribution to the antioxidant activity of the extracts. There were no correlation between the antioxidant activity and total phenolic contents.

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