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Full Length Research Paper

Determination of antioxidant property from some medicinal plant extracts from Thailand

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The evaluation of antioxidant property, total phenolic compounds and pigment content of 8 medicinal plants including: *Gynostemma pentaphyllum* Thunb., *Camellia sinensis* Ktze., *Cymbopogon citratus* Stapf., *Centella asiatica* (L.) Urban., *Andrographis paniculata* (Burm.) Wall. ex Nees, *Thunbergia laurifolia* Lindl., *Murdania loriformis* (Hassk.) Rolla Rao et Kammathy and *Acanthus ebracteatus* Vahl., was performed using 95% methanol as a solvent. *C. sinensis* methanolic extract showed the highest antioxidant activity with 0.34 µg/ml IC_{50} followed by the extract of *C. asiatica* and *G. pentaphyllum,* respectively. The extract of *A. paniculata* had the highest chlorophyll *a* yield. *C. sinensis* extract had the highest chlorophyll *b* content. While, the carotenoid constituent can be determined only in *G. pentaphyllum* methanolic extract. However, the highest level of total pigment was in *C. sinensis* extract. Correlation analysis found a statistically significant relationship with an exponential pattern between the amount of total phenolic compounds and the antioxidant property at correlation level was 0.9687. On the other hand, the total pigment yield did not have a correlation with antioxidant activity.

Key words: Pigment, phenolic compound, antioxidant, medicinal plant.

INTRODUCTION

Free radicals are normally created during energy production of cells. Among all of them are reactive oxygen species that can cause cell or tissue attack by biological molecules, such as lipids, proteins, enzymes, DNA and RNA (Hajar et al., 2010). The effect of free radicals has lead to the development of disorders including: cancer, autoimmune disorder, aging, rheumatoid arthritis, cataract, cardiovascular and Alzheimer's disease (Mohammad et al., 2010; Willcox et al., 2004; Anchana et al., 2005). Antioxidants play an important role in free radical scavenging by preventing and repairing cell damage from oxidative reaction, converting free radicals into less harmful molecules (Fernandez-Orozco et al., 2001).

There is an increasing interest in natural antioxidants because of the question about safety and toxicity of synthetic antioxidants (Amarowicz et al., 2000). In last decade, there were many studies dealing with natural antioxidant from plants and their application in health benefits (Huda-Faujan et al., 2009). Many natural antioxidants have already been isolated from different kinds of plant, such as oilseed, cereal crop, vegetables and herbs (Wettasinghe and Shahidi, 1999; Shon et al., 2003).

Among natural substances, phenolic compounds and pigments, such as chlorophylls and carotenoids are the major candidate for antioxidant property. All the phenolic classes have the structural characteristics of free radical scavengers and have potential as food antioxidants (Bandoniene and Murkovic, 2002). While, chlorophylls and carotenoids are the pigments that show the antioxidant properties with different levels (Ursula et al., 2005; Chen and Chan, 1996). Furthermore, antimutation and anticancer effects are the impressive properties of these compounds (Negishi et al., 1997; Dashwood et al., 1998).

The antioxidant effects of several plant materials have

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been reported (Al-Saikhan et al., 1995; Yen and Duh, 1995; Oomah and Mazza, 1996; Wang et al., 1996; Cao et al., 1996; Amarowicz et al., 1996). However, the relationship between pigment, phenolic contents and antioxidant activity of many medicinal plants is not available. Thus, the objective of this study was to determine the pigment and phenolic content and also assessed the antioxidant property of the methanolic extracts from some medicinal plants. Moreover, correlative analysis of these factors was evaluated.

MATERIALS AND METHODS

Chemicals

The chemicals used for the study were of analytical grade. Buthylated hydroxytoluene (BHT), 1,1-Diphenyl-2-picryl hydrozyl (DPPH) and gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ascorbic acid and Folin-Ciocaltue reagent were purchased from Merck Co. (Germany).

Plant material preparation and extraction

The plant parts of *Gynostemma pentaphyllum*, *Camellia sinensis*, *Cymbopogon citratus*, *Centella asiatica*, *Andrographis paniculata*, *Thunbergia laurifolia*, *Murdania loriformis* and *Acanthus ebracteatus* were collected from Thailand. Plant materials were cleaned and dried at room temperature followed by 60°C in hot air oven for 2 days. Dried materials were ground into powder. Five grams of each plant powder were extracted in 200 ml of 95% methanol by maceration with ultrasonicator for 2 h. The extraction was repeated twice. The combined organic solution was evaporated under vacuum to dryness, yielding the crude extract and then the extract was dissolved in 95% aqueous methanol for desirable concentration.

Determination of total phenolic content

Total phenolic compounds were determined according to Folin-Ciocaltue method (Velioglu et al., 1998). A 1.0 ml aliquot of sample was added to 1.5 ml of deionized water and 0.5 ml of 0.1 M Folin-Ciocaltue reagent, and the contents were mixed thoroughly. After 1 min, 1.0 ml of 20% sodium carbonate solution was added, and the mixture was again mixed thoroughly. The control contained all reaction reagents except the sample. After 30 min of incubation at 37°C, the absorbance was measured at 750 nm, and compared to gallic acid calibration curve. Total phenolics were estimated as gallic acid equivalent (GAE).

Determination of pigment content

The pigment of all samples was determined as described by Lichtenthaler and Wellburn (1985) with some modifications. Generally, 1000 µg/ml of methanolic extract was used for spectrophotometer measurement. The three wave lengths of absorbance at 470, 653 and 666 nm were recorded. Pigment content was calculated using the following equation:

$C_a = 15.65A_{666} - 7.340A_{653}$
$C_b = 27.05A_{653} - 11.21\ A_{666}$
$C_{x+c} = (1000\ A_{470} - 2.860C_a - 129.2\ C_b)/245$

Where, C_a is the chlorophyll *a* content; C_b is the chlorophyll *b* content and C_{x+c} is the carotenoid content. A_{470} is the absorbance at 470 nm, A_{653} is the absorbance at 653 nm and A_{666} is the absorbance at 666 nm.

Antioxidant assay

The scavenging effects of samples for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical were monitored according to the method of the previous report by Yen and Chen (1995). Briefly, 2.0 ml aliquot of test sample (in methanol) was added to 2.0 ml of 0.16 mM DPPH methanolic solution. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark, and its absorbance was read at 517 nm. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [1 - (A_{sample} - A_{sample\ blank})/ A_{control}] \times 100$$

Where, $A_{control}$ is the absorbance of the control (DPPH solution without sample); A_{sample} is the absorbance of the test sample (DPPH solution plus test sample) and $A_{sample\ blank}$ is the absorbance of the sample only (sample without DPPH solution). Synthetic antioxidants: BHT, gallic acid and ascorbic acid were used as positive controls.

Statistical analysis

Values expressed are means of three replicate determination ± standard deviation. All statistical analyses were carried out using SPSS 11 for Windows. To determine whether there were any differences between activities of samples, variance analysis was applied to the result. Values of $p \leq 0.05$ was considered as significant different ($\alpha = 0.05$)

RESULTS AND DISCUSSION

The methanolic extract and pigment content of 8 plants

From the same starting weight of dried plant material by using 95% methanol as a solvent, *C. sinensis* showed the highest extract yield (318 mg/g dw). The other extract yield can be sort descending: *C. citratus*, *C. asiatica*, *A. ebracteatus*, *G. pentaphyllum* and *A. paniculata*. All these plant extracts had higher content than *T. laurifolia* and *M. loriformis* with statistical significance (Table 1).

The data of Table 2 show that *A. paniculata* extract had the highest chlorophyll *a* yield. *C. sinensis* extract had the highest chlorophyll *b* content while, the carotenoid constituent can be determined only in *G. pentaphyllum* methanolic extract. However, the highest level of total pigment content is *C. sinensis*.

Plant species with different internal and/or external factors such as humidity, temperature, pH and nutrient condition affect the extract yield of samples (Bryant et al., 1983), especially, the essential element from growing area is the vital factor for growth and development of plant **(**Andrew et al., 1999; Arvidsson, 1999; Gremigni et al., 2001).

Table 1. Yield of methanolic extract from 8 medicinal plants.

Plant species	Methanolic extract (mg/g dw)
Gynostemma pentaphyllum	124.50 ± 4.68[d]
Camellia sinensis	318.86 ± 22.92[a]
Cymbopogon citratus	181.98 ± 4.30[b]
Centella asiatica	164.42 ± 2.46[c]
Andrographis paniculata	91.56 ± 3.00[e]
Thunbergia laurifolia	38.76 ± 2.34[f]
Murdania loriformis	22.55 ± 2.44[g]
Acanthus ebracteatus	126.05 ± 2.00[d]

Each value is presented as mean ± SD (n = 3). Means with different letters (a-g) differ statistically significance ($p \leq 0.05$).

Total phenolic content of the extracts

It has been recognized that the phenolic compounds are class of antioxidant agents which act as free radical terminators (Shahidi and Wanasundara, 1992). The Folin-Ciocaltue reagent method is actually not an antioxidant test but instead an assay for the quantity of oxidizable substance, that is, phenolic compounds (Wangensteen et al., 2004). Figure 1 shows the content of total phenolic compounds ranged from 4.05 to 48.81 mg GAE/g extract. *C. sinensis* with 48.81 mg GAE/g extract of total phenolic content had the highest amount of this substance among the plants in this research. The compounds such as phenolic substances, which contain hydroxyls, are responsible for the radical scavenging effect in the plants (Das and Pereira, 1990; Young, 1981). According to our study, the high contents of these phytochemicals in *C. sinensis* can explain its high radical scavenging activity.

Antioxidant assay

DPPH radical-scavenging activity

DPPH is a useful reagent for investigating the free radical-scavenging activities of compounds. In the DPPH test, the extracts were able to reduce the stable radical $DPPH^{\bullet}$ to the yellow-coloured diphenylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction (Shon et al., 2003). As seen in Table 3, the methanolic extract of all plants exhibited a concentration-dependent DPPH radical scavenging activity. At 500 µg/ml, *G. pentaphyllum*, *C. sinensis* and *C. asiatica* showed the same level of scavenging potential with positive control: ascorbic acid and gallic acid but better than BHT significantly. At concentrations lower than 500 µg/ml, almost all plant extracts had lower scavenging effect than control except *C. sinensis* and *C. asiatica* at 50 µg/ml and *C. sinensis* at 5 µg/ml as shown in Table 3.

IC_{50}

The efficiency of antioxidant activity can be assessed by IC_{50} of the extract. The lower the IC_{50} extract have, the better the antioxidant property the extract contain. *C. sinensis* had the lowest IC_{50} but not statistically significant with *C. asiatica* (Figure 2). Both of them are the same significant level as positive controls. While, *A. ebracteatus* and *M. loriform* showed the highest IC_{50} value.

Correlation between the content of phenolic compounds and antioxidant activity has been described (Wangensteen et al., 2004). A high correlation between total phenolic compound content and antioxidant activity, in term of IC_{50}, of all plant extracts was found with $r^2 = 0.9687$, $p \leq 0.001$ (Figure 3). On the other hand, the total pigment yield does not have a correlation with antioxidant activity (data not shown).

Conclusion

The highest total phenolic compound level and also total pigment content of *C. sinensis* extract compared with other plants in this research corresponded with the highest DPPH radical scavenging activity of this extract. *C. asiatica* had the second order of total phenolic compound content in comparison with all extract but its pigment content was quite low. However, the DPPH radical scavenging activity of *C. asiatica* showed the second order among all plant extracts. Analysis of correlation found a statistically significant relationship with an exponential pattern between the amount of total phenolic compounds and DPPH radical scavenging at highly correlation level. In contrast, the total pigment yield did not have a correlation with DPPH radical scavenging activity.

Table 2. Pigment content of 8 medicinal plant extracts.

Plant species	Pigment content (µg/g dw)		
	Chlorophyll *a*	Chlorophyll *b*	Carotenoid
Gynostemma pentaphyllum	134.27 ± 15.98^{bc}	122.04 ± 12.77^{e}	34.59 ± 1.26
Camellia sinensis	146.91 ± 11.57^{b}	1942.03 ± 123.91^{a}	-
Cymbopogon citratus	82.81 ± 6.63^{d}	170.49 ± 36.84^{cd}	-
Centella asiatica	93.24 ± 4.21^{d}	137.89 ± 4.01^{de}	-
Andrographis paniculata	225.09 ± 27.52^{a}	241.11 ± 29.99^{b}	-
Thunbergia laurifolia	32.64 ± 3.96^{e}	58.88 ± 9.46^{f}	-
Murdania loriformis	47.88 ± 7.01^{e}	50.78 ± 3.89^{f}	-
Acanthus ebracteatus	116.58 ± 3.21^{c}	132.39 ± 1.96^{de}	-

Each value is presented as mean ± SD (n = 3). Means with different letters (a to g) differ statistically significance ($p \leq 0.05$).

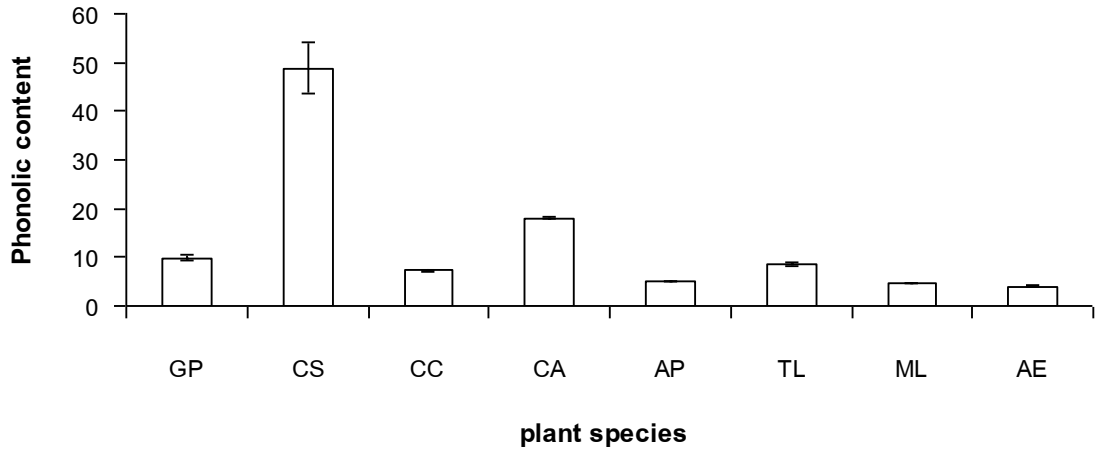

Figure 1. Total phenolic content (mg GAE/g extract) of 8 plant methanolic extracts are expressed as gallic acid equivalence (GAE). Each bar is presented as mean ± SD (n = 3). Means with different letters (a to f) differ statistically significance ($p \leq 0.05$). Plant extracts: GP, *Gynostemma pentaphyllum*; CS, *Camellia sinensis*; CC, *Cymbopogon citratus*; CA, *Centella asiatica*; AP, *Andrographis paniculata*; TL, *Thunbergia laurifolia*; ML, *Murdania loriformis*; AE, *Acanthus ebracteatus*.

Table 3. Antioxidant activity of plant methanolic extracts at different concentration.

Plant species	Scavenging effect (%)				
	0.05 (µg/ml)	0.5 (µg/ml)	5 (µg/ml)	50 (µg/ml)	500 (µg/ml)
Ascorbic acid	1.43 ± 1.25^{cdefE}	8.05 ± 1.07^{cdefD}	57.13 ± 0.99^{cC}	89.98 ± 0.72^{bB}	97.02 ± 0.45^{abA}
BHT	0.95 ± 1.05^{cdefD}	2.56 ± 1.35^{hijD}	47.88 ± 1.22^{dC}	71.97 ± 1.09^{cB}	93.56 ± 0.54^{defA}
Gallic acid	7.93 ± 0.72^{bE}	30.11 ± 0.99^{bD}	82.05 ± 0.99^{bC}	93.98 ± 0.55^{abB}	98.03 ± 0.47^{abA}
G. pentaphyllum	1.27 ± 0.48^{cdefD}	5.40 ± 1.48^{efghD}	18.16 ± 4.07^{gC}	67.87 ± 3.83^{cB}	98.79 ± 0.79^{abA}
C. sinensis	22.59 ± 2.55^{aC}	65.29 ± 2.04^{aB}	98.62 ± 0.52^{aA}	99.15 ± 0.30^{aA}	99.35 ± 0.63^{aA}
C. citratus	0.61 ± 0.25^{deD}	2.05 ± 0.35^{hijD}	25.03 ± 4.15^{efC}	34.55 ± 4.49^{eB}	95.85 ± 1.16^{bcdeA}
C. asiatica	2.95 ± 0.52^{cdeE}	6.68 ± 0.86^{defgD}	21.74 ± 1.38^{fgC}	94.24 ± 1.94^{abB}	98.04 ± 0.98^{abA}
A. paniculata	0.36 ± 0.18^{efD}	1.25 ± 0.47^{jD}	3.34 ± 0.27^{ijC}	32.92 ± 1.53^{eB}	93.08 ± 1.37^{efA}
T. laurifolia	0.36 ± 0.12^{efC}	0.43 ± 0.21^{jC}	1.35 ± 0.12^{jC}	44.71 ± 5.96^{dB}	94.46 ± 0.56^{cdefA}
M. loriformis	1.41 ± 0.38^{cdefD}	3.87 ± 0.49^{ghijD}	6.57 ± 1.11^{hiC}	18.97 ± 1.95^{fB}	91.50 ± 2.69^{fA}
A. ebracteatus	0.20 ± 0.07^{fD}	1.56 ± 0.44^{ijCD}	2.30 ± 0.38^{ijC}	20.22 ± 2.23^{fB}	93.79 ± 0.91^{defA}

Each value is presented as mean ± SD (n = 3). Means within column with different letters (a to g) differs statistically ($p \leq 0.05$). Means within column with different letters (A to G) differs statistically ($p \leq 0.05$).

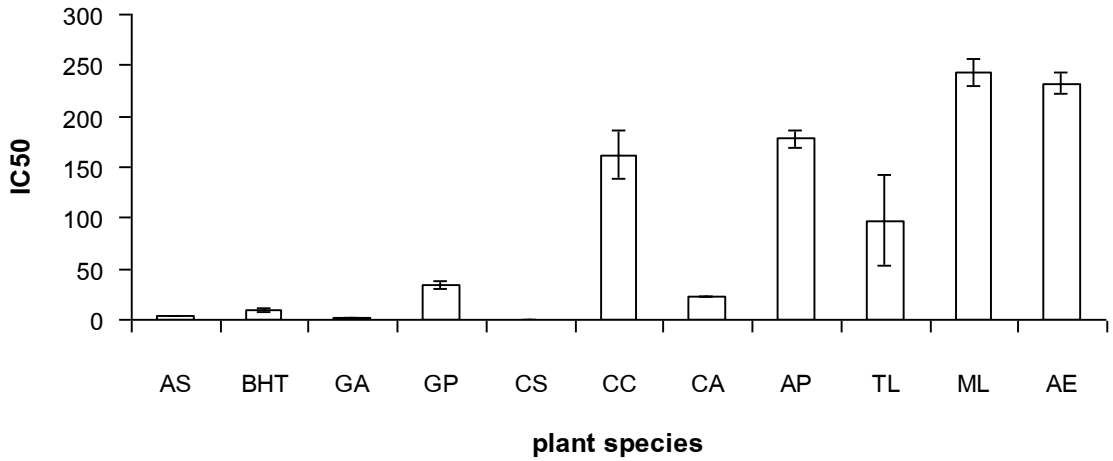

Figure 2. IC_{50} (µg/ml) values of plant extracts for free radical scavenging activity by DPPH radical Each bar is presented as mean ± SD (n = 3). Means with different letters (a to e) differ statistically significance ($p \leq 0.05$). Plant extracts: GP, *Gynostemma pentaphyllum*; CS, *Camellia sinensis*; CC, *Cymbopogon citratus*; CA, *Centella asiatica*; AP, *Andrographis paniculata*; TL, *Thunbergia laurifolia*; ML, *Murdania loriformis*; AE, *Acanthus ebracteatus* and standard substances: AS, accorbic acid, BHT, butylated hydroxy toluene; GA, gallic acid.

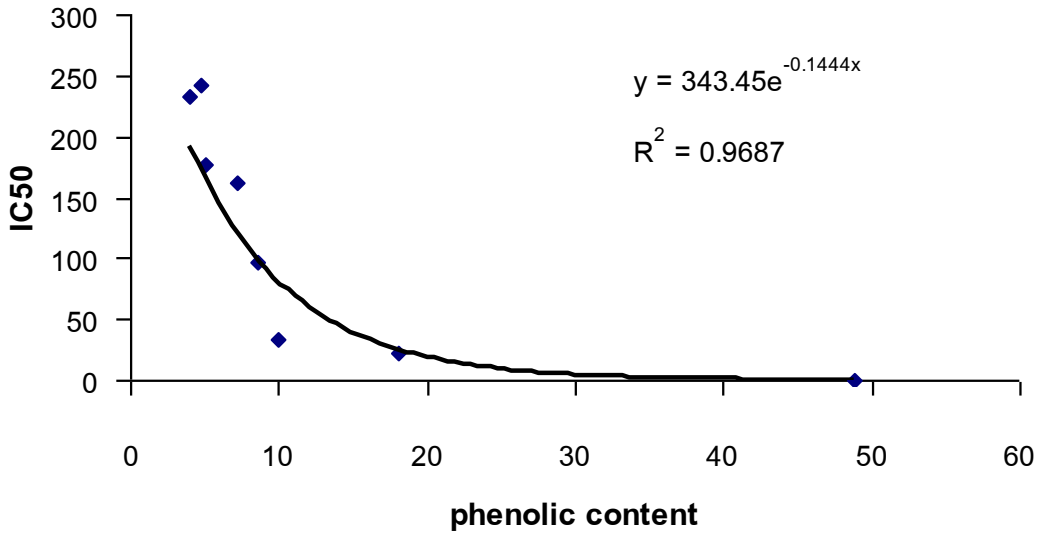

Figure 3. Correlation between phenolics compound content (mg GAE/g extract) and IC_{50} (µg/ml), indicator for antioxidant property, of all tested plant methanolic extracts.

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