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Research Article

Screening for antioxidant activity in selected Thai wild plants

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Abstract

In recent years, intensive research on natural antioxidants derived from plants has grown due to their potential health-benefits and alternative antioxidants to replace synthetic antioxidants. The antioxidant properties of the twenty selected Thai wild plants were examined using three different assays; 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, Trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) assays. Their total phenolic contents (TPC) were also measured by Folin-Ciocalteu method. The TPC of ethanolic extracts ranged from 5.06 – 720.2 mg gallic acid equivalence (GAE)/g dry basis. The amount of plant material (dry basis) to decrease 50% of initial DPPH concentration (EC_{50}) ranged from 0.08 – 16.50 mg dry basis while the TEAC and FRAP ranged from 0.65 – 69.03 mM Trolox equivalence (TE)/g dry basis and 2.11 – 213.4 mg ascorbic acid equivalence (AscAE)/g dry basis, respectively. There was a strong correlation coefficient between FRAP and $1/EC_{50}$ (r = 0.9071) and FRAP and TEAC (r = 0.8783). The correlations coefficient between TPC and all three antioxidant assays ranged from 0.2973 – 0.7672, indicating that polyphenols in the ethanolic extracts were partly responsible for the antioxidant activities. Based on these results, the flower of *Cratoxylum cochinchinense* (Lour.) Blume and the leaves of *Schima wallichii* (DC.) Korth, possesed the highest antioxidant activities and thus could be potential rich sources of natural antioixdants and total phenolic content.

Keywords: *Cratoxylum cochinchinense, Schima wallichii*, DPPH, TEAC, FRAP, total phenolic content, Thailand.

Introduction

Phytochemicals exerting antioxidant actions are largely being recognized as of benefiting human health and disease prevention. These benefits may be a result of concerted actions of well-known

antioxidants such as vitamin C, vitamin E and β -carotene. Indeed, phenolic compounds are ubiquitously distributed in the plant kingdom and exhibit a wide range of medicinal properties, including anti-inflammatory, anti-carcinogenic viral, anti-allergic and immune-stimulating agents [1]. These protective effects have been mostly ascribed to their free radical scavenging, metal chelating and chain breaking effects. Many literature reports show a relatively strong correlation between the total phenolic content and the antioxidant capacity of plant extracts [2, 3, 4].

In recent years, intensive research on natural antioxidants derived from plants has grown due to their potential health-benefits in the search for replacements of synthetic antioxidants. The phenolic compounds and their antioxidant properties present in no fewer than 3,000 plant species, including some Thai plants, have been studied [5]. Various parts of many Thai wild plants are traditionally used as food or medicine and may contribute as potential sources for new natural antioxidants. Therefore, the aim of this study was designed for the evaluation of antioxidant activity of twenty selected Thai wild plants in order to identify new sources of natural antioxidants and to investigate the relationship between antioxidant properties and total phenolic content. These data will provide some useful information for healthier living, as well as the further screening of plants as potential sources of natural antioxidants.

Materials and Methods

Plant material

The twenty selected Thai wild plants were collected from the Royal Angkhang Agricultural Station Project of the Royal Project Foundation, Chiang Mai (Table 1).

Chemicals

All chemicals used were of at least analytical grade. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picryhydrazyl radical (DPPH), 2,4,6-tripyridyl-*s*-triazine (TPTZ), ascorbic acid and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). Folin – Ciocalteu reagent and sodium carbonate were purchased from Carlo Erba Reagenti SpA (Rodano, Italy). Iron (III) chloride hydrate was obtained from Fisher Scientific (Leicestershire, UK) and gallic acid was purchased from Fluka Chemical (Buchs, Switzerland).

Extraction

The method of Adebolo and Oladimeji [6], was adopted for extraction with little modification. Briefly, each sample was blended with 80% ethanol at a ratio of 1:10 (w/v) and incubated at 70°C for 15 min. The extract was centrifuged and then filtered. The alcoholic extract was kept in the freezer at –20°C.

DPPH free radical-scavenging assay

The method of Brand-Williams *et al*. [7], was adopted for evaluating the free radical scavenging with little modification. Briefly, 1 ml of a series of extract concentrations was mixed with 3 ml of 0.2 mM DPPH and then placed in the dark for 30 min. The 517 nm absorbance was measured and % inhibition and EC_{50} calculated (the concentration of the test compounds demonstrating 50% radical scavenging activity).

Table 1. List of selected Thai plants.

Common name	Botanical name	Part used
Phak Hia	*Artemisia dubia* Wall. ex DC. (Syn. *A. vulgaris* L. var. *indica* Maxim.)	Stem and leaves
NangLaeo	*Aspidistra sutepensis* K. Larsen	Flower
Ya Puen Laem Nok Sai	*Bidens bipinnata* , L.	Stem and leaves
Peen nok sai	*Bidens pilosa* Linn.	Stem and leaves
Rachawadi pa	*Buddieia asiatica* Lour	Stem and leaves
Khi lek	*Cassia siamea* Britt.	Flower
Phak plap	*Commelina diffusa* Burm.f.	Stem and leaves
Ya Khamai	*Conyza sumatrensis* (Retz.) Walker	Stem and leaves
Tio kliang	*Cratoxylum cochinchinense* (Lour.) Blume	Flower
Khruea Kham	*Cuscuta australis* R. Br.	Stem
Phak Kut Khao	*Diplazium esculentum* (Retz.) Sw.	Stem and leaves
Khae Pa	*Dolichandrone serrulata* (DC.) Seem.	Flower
Som Jee	*Embelia ribes* Burm.f.	Leaves
Som Kui	*Embelia sessiliflora* Kurtz	Leaves
Sa Riam Dong	*Melicope pteleifolia* (Champ.ex Benth.) Hartley	Leaves
Phak Samui	*Micromelum minutum Wight & Arn*	Stem and leaves
Mara Khinok	*Momordica charantia* L.	Leaves
Talo	*Schima wallichii* (DC.) Korth.	Leaves
Yanang	*Tiliacora triandra* (Colebr.) Diels	Leaves
Som Pi	*Vaccinium sprengelii* (G.Don) Sleum.	Leaves

Trolox equivalent antioxidant capacity (TEAC) assay

TEAC was evaluated based on the method described by Zhou and Yu [8]. The TEAC values were descried as mM Trolox equivalence (TE)/g dry basis.

Ferric-reducing antioxidant power assay (FRAP)

FRAP was done according to the method of Benzie and Strain [9]. The results were expressed as milligrams ascorbic acid equivalence (AscAE)/g dry basis.

***Determination of total phenolic content** (TPC)*

TPC was determined using Folin-Ciocalteu method [10]. The results are shown as milligrams of gallic acid equivalence (GAE)/g dry basis.

Statistical analysis

All results were obtained in triplicate and data were expressed as mean±SD. Analysis of variance was performed using ANOVA procedures. Significant differences between means were determined by Duncan multiple range test (DMRT) comparison test at a level of $P < 0.05$. Correlations among the methods were established by regression analysis.

Results and Discussion

Total phenolic content and antioxidant activities

The antioxidant activities and TPC of the twenty ethanolic Thai wild plant extracts are shown in Table 2. From these results it can be seen that the TPC of ethanolic extracts ranged from 5.06 – 720.2 mg GAE/g dry basis. The flower of *Cratoxylum cochinchinense* was found to have the highest TPC (720.2 mg GAE/g dry basis), followed by *Schima wallichii* and *Vaccinium sprengelii*.

Table 2. Antioxidant activities and total phenolic content of twenty selected Thai wild plant extracts.

Botanical name	TPC (mg GAE/g dry basis)	EC_{50} (mg dry basis)	TEAC (mM TE/g dry basis)	FRAP (mg AscAE/g dry basis)
Artemisia dubia Wall. ex DC. (Syn. *A. vulgaris* L. var. *indica* Maxim.)	14.24±1.11 $^{a-c}$	5.31±0.40 ^{g}	1.88±0.05 $^{a-c}$	11.74±0.05 $^{a-d}$
Aspidistra sutepensis K. Larsen	5.06±0.00 ^{a}	16.50±1.23 ^{j}	1.34±0.01 $^{a-b}$	2.11±0.04 ^{a}
Bidens bipinnata , L.	34.18±2.82 $^{d-e}$	0.96±0.03 $^{a-d}$	5.79±0.29 $^{e-f}$	24.22±3.37 $^{e-f}$
Bidens pilosa Linn.	24.62±1.87 $^{b-e}$	0.82±0.00 $^{a-d}$	11.22±0.15 ^{h}	26.91±1.70 ^{f}
Buddieia asiatica Lour	19.17±1.56 $^{a-d}$	3.87±0.09 ^{f}	3.40±0.46 $^{c-d}$	16.18±0.77 $^{c-d}$
Cassia siamea Britt.	28.90±1.95 $^{c-e}$	1.49±0.04 $^{c-e}$	4.55±0.06 $^{d-e}$	21.16±0.28 $^{d-f}$
Commelina diffusa Burm.f.	19.73±0.12 $^{a-d}$	1.56±0.13 $^{d-e}$	5.93±0.03 $^{e-f}$	11.06±0.29 $^{a-c}$
Conyza sumatrensis (Retz.) Walker	15.66±1.14 $^{a-c}$	2.17±0.18 ^{e}	7.32±0.06 $^{f-g}$	11.92±0.26 $^{a-d}$
Cratoxylum cochinchinense (Lour.) Blume	720.2±23.5 ^{i}	0.09±0.00 ^{a}	16.91±0.54 ^{j}	84.07±1.63 ^{i}
Cuscuta australis R. Br.	33.21±1.45 $^{d-e}$	0.93±0.02 $^{a-d}$	6.36±0.02 ^{f}	28.15±3.33 ^{f}
Diplazium esculentum (Retz.) Sw.	19.48±0.66 $^{a-d}$	1.58±0.07 $^{d-e}$	8.33±0.39 ^{g}	8.18±0.01 $^{a-c}$
Dolichandrone serrulata (DC.) Seem.	13.25±0.28 $^{a-c}$	15.46±1.32 ^{i}	0.65±0.01 ^{a}	12.51±0.66 $^{b-d}$
Embelia ribes Burm.f.	57.89±2.94 ^{f}	0.35±0.01 $^{a-b}$	27.77±1.13 ^{k}	60.75±0.16 ^{g}
Embelia sessiliflora Kurtz	65.08±3.11 ^{f}	0.20±0.00 $^{a-b}$	34.40±0.19 ^{l}	74.49±0.66 ^{h}
Melicope pteleifolia (Champ.ex Benth.) Hartley	40.31±0.55 ^{e}	5.67±0.07 ^{g}	0.69±0.01 ^{a}	6.38±0.14 $^{a-c}$
Micromelum minutum Wight & Arn	61.15±1.40 ^{f}	1.20±0.02 $^{b-d}$	13.40±0.08 ^{i}	24.07±0.25 $^{e-f}$
Momordica charantia L.	6.94±0.25 ^{a}	0.58±0.02 $^{a-d}$	2.42±0.03 $^{b-c}$	3.38±0.08 $^{a-b}$
Schima wallichii (DC.) Korth.	206.1±20.3 ^{h}	0.08±0.00 ^{a}	69.03±2.93 ^{n}	213.4±17.9 ^{j}
Tiliacora triandra (Colebr.) Diels	8.60±0.04 $^{a-b}$	6.86±0.13 ^{h}	0.67±0.01 ^{a}	5.15±0.09 $^{a-b}$
Vaccinium sprengelii (G.Don) Sleum.	95.42±6.26 ^{g}	0.53±0.01 $^{a-c}$	56.06±0.13 ^{m}	63.67±0.27 ^{g}

The difference in letter on column represents a different statistic at $P < 0.05$.

The number of plants (dry basis) to decrease 50% of initial DPPH concentration (EC_{50}) indicated that the lower amount the plant exhibits, the higher the antioxidant activity. The other assays, the TEAC and FRAP, were calculated from their standard curve. The higher amount of their values means higher antioxidant activity. Therefore, EC_{50} value should invert with TEAC and FRAP values. From the results, EC_{50} ranged from 0.08 – 16.50 mg dry basis while 0.65 – 69.03 mM TE/g dry basis and 2.11 – 213.4 mg AscAE/g dry basis in TEAC and FRAP, respectively.

The leaves of *S. wallichii* appeared to have the highest antioxidant activities with the highest TEAC and FRAP values and the lowest EC_{50} value, followed by *C. cochinchinense* and *V. sprengelii*. The profound antioxidant activity of *S. wallichii* could be attributed to known saponins and tannins. Yoshida *et al*. [11] reported that ten polyphenol compounds including camelliin B and two new hydrolyzable tannins, named schimawalin A and B, were isolated from the dried flowers. For *C. cochinchinense*, the results of this research were similar to those reported by Tang *et al*. [12], where high antioxidant activity was found in the roots of *C. cochinchinense* using the ABTS assay. In more up-to-date findings, new xanthones and other known compounds exhibiting effective antioxidant properties, were isolated from the roots [13]. In contrast was the flower of *Aspidistra sutepensis*, showing the lowest antioxidant activities among the three different assays.

Generally, extracts with a high amount of phenolic compounds also exhibit high antioxidant activity [14]. Ethanolic extracts of *C. cochinchinense*, *S. wallichii*, *V. sprengelii*, *Embelia sessiliflora*, *Micromelum minutum* and *Embelia ribes* which showed high antioxidant activities also had high TPC. Comparing between the antioxidant assay and TPC, *S. wallichii* had the highest antioxidant activities, while the highest TPC was obtained in *C. cochinchinense*.

In contrast to these results was *Melicope pteleifolia*, which exhibited high TPC but did not show high antioxidant activity as observed in other plants. It could be explained by the different response of various phenolic compounds in the antioxidant assays. According to Frankel *et al*. [15], the molar responses of each assay is roughly proportional to the number of the phenolic hydroxyl groups in a given substrate, but the reducing capacity is enhanced when two phenolic hydroxyl groups are oriented ortho or para.

Correlations of TPC with antioxidant activities

In this study, there were distinct correlation coefficients (r) between studied parameters (TPC, $1/EC_{50}$, TEAC and FRAP) in the twenty selected Thai wild plant extracts (Table 3). Significant linear correlations were obtained between FRAP and $1/EC_{50}$ (r = 0.9071), and FRAP and TEAC (r = 0.8783) which indicated that both methods showed the same trend. A strong correlation between FRAP and $1/EC_{50}$, and FRAP and TEAC implied that antioxidants in these plants were capable of scavenging free radicals ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$) and reducing oxidants (ferric ions). Many studies have demonstrated the ferric ion reducing ability of antioxidants and correlates with the results from other methods used to estimate antioxidant capacity [14, 16, 17]. This could be explained by the basic concept that antioxidants are reducing agents. Antioxidants are compounds capable of donating a single electron or hydrogen atom for reduction, but reducing agents may not express antioxidants [14].

However, a weak correlation was found between $1/EC_{50}$ and TEAC (r = 0.4930) which means the antioxidants act differently with two different free radicals ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$). Both DPPH and $ABTS^{\bullet+}$ assays have been widely used to measure the antioxidant activities of natural extracts based on their abilities to scavenge free radicals. The reactions of DPPH assays are determined in the absence of added DPPH-H, while the reduced form ABTS is usually present in test systems containing $ABTS^{\bullet+}$ [18]. In addition, the reaction time of discolouration for $ABTS^{\bullet+}$ assay is only

Table 3. Correlation coefficient (*r*) between studied parameters (TPC, $1/EC_{50}$, TEAC and FRAP) of the twenty selected Thai wild plant extracts ($P < 0.05$).

	TPC	FRAP	TEAC
$1/EC_{50}$	0.7672	0.9071	0.6988
TEAC	0.2973	0.8783	
FRAP	0.4930		

6 min, much shorter than that for the DPPH assay (30 min for this study). Therefore, it may affect the antioxidant activities of each assay. The antioxidant activity largely depends on the composition of the extracts and the assay methods. The same antioxidants may yield significantly different activity when assessed using different methods. It is thus necessary to perform more than one type of antioxidant activity measurement to take into account the various mechanisms of antioxidant action [17].

Interestingly, the correlations between TPC and all three antioxidant assays ranged from 0.2973 – 0.7672. These results indicated that the ability for phenolic compounds to scavenge DPPH free radicals, reduce $ABTS^{\bullet+}$ radical cation or reduce ferric ion depends on the availability of properly oriented functional groups. While the Folin-Ciocalteu assays estimate the sum of phenolic compounds present in plant extracts, Singleton and Rossi [10], stated that various phenolic compounds respond differently in each assay, depending on the number of phenolic groups they have. This indicates that, in this research, the polyphenols in the ethanolic extracts were partly responsible for the antioxidant activities. In addition, TPC does not incorporate necessarily all the antioxidants that may be present in an extract and plants contain a variety of effective antioxidant substances [19].

Conclusions

The antioxidant activities and total phenolic content of twenty selected Thai wild plants were examined. These plants showed statistical difference amongst their antioxidant activities and total phenolic content. In particular, the flower of *C. cochinchinense* and the leaves of *S. wallichii* possessed the highest antioxidant activities and thus could be potential rich sources of natural antioxidants and total phenolic contents. A strong correlation between FRAP and $1/EC_{50}$, and FRAP and TEAC, implied that antioxidants in these plants were capable of scavenging free radicals and reducing oxidants. However, a satisfactory correlation between TPC and all three antioxidant assays indicated that polyphenols were partly responsible for the antioxidant activities of these plants. For further study, safety and *in vivo* efficacy research on these potential plants should be conducted. In addition, identification and characterization of the active components from plant species should be examined, to gain more understanding of their antioxidant activity.

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