Antioxidant Properties and Total Phenolic Content of Selected Traditional Thai Medicinal Plants

นิพนธ์ต้นฉบับ

Original Article

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บทคัดย่อ

วัตถุประสงค์: พืชสมุนไพรไทยตามภูมิปัญญาท้องถิ่นที่พบในภาคตะวันออกเฉียงเหนือถูกนำมาใช้รักษาบาดแผลทางผิวหนังและอักเสบ งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาฤทธิ์ต้านอนุมูลอิสระและปริมาณสารประกอบฟีนอลิกทั้งหมด (total phenolic content; TPC) ของสารสกัดจากเปลือกพืชสมุนไพรไทยทั้งหมด 13 ชนิด **วิธีการศึกษา:** กระท่อมเลือด (Stephania venosa (Blume) Spreng.) เขียงใหญ่ (Smilax perfoliata Lour.) แคหางค่าง (Dolichandrone stipulate) ตานาใหญ่ (Glochidion coccineum) นมสาว (Xantolis cambodiana) ประดงขาว (Dalbergia cultrata) ประดงแดง (Bauhinia sirindhorniae K.) ประดงเลือด (Knema angustifolia) ประดงเหลือง (Tristaniopsis burmanica) มะเดื่อหิน (Ficus hirta Vahl) แส้ม้าทลาย (Capparis zeylanica) ไส้ตัน (Amphineurion marginata (Roxb.) G. Don.) และหางนกกะลิง (Microsorum pteropus (Blume) Copel.) โดยนำสมุนไพรไทยที่สกัดด้วยน้ำตามภูมิปัญญาท้องถิ่นทดสอบฤทธิ์ต้านอนุมูลอิสระด้วยวิธี 1,1-diphenyl-1-princylhydrazyl (DPPH) radical scavenging assay, ferric-reducing antioxidant power (FRAP) assay และ 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay ที่ค่าความยาวคลื่น 517, 593 และ 734 นาโนเมตร ตามลำดับ และทดสอบปริมาณ TPC ด้วยวิธี Folin-Ciocalteu method ทดสอบความสัมพันธ์ระหว่างฤทธิ์ต้านอนุมูลอิสระแต่ละวิธีกับ TPC ด้วย Pearson's correlation coefficient (r) **ผลการศึกษา:** พบว่าพืชแต่ละชนิดมีฤทธิ์ต้านอนุมูลอิสระและปริมาณ ฟีนอลิกต่างกัน โดยกระท่อมเลือด นมสาว ประดงแดง ประดงเลือด และแส้ม้าทลาย มีฤทธิ์ต้านอนุมูลอิสระสูงสุด (ร้อยละ 70 – 100) ในการทดสอบทั้ง 3 วิธี และมีปริมาณฟีนอลิกในปริมาณสูง (38,113 - 59,989 mg GAE/g) และพบว่าฤทธิ์ต้านอนุมูลอิสระ (ABTS และ FRAP) กับ TPC สัมพันธ์อย่างมีนัยสำคัญ โดยค่า r เท่ากับ 0.987 และ 0.956 ตามลำดับ **สรุป:** พืชสมุนไพรที่มีฤทธิ์ต้านอนุมูลอิสระอาจเนื่องมาจากมีองค์ประกอบของสารประกอบฟีนอลิกในสารสกัด ดังนั้น สมุนไพรไทยตามภูมิปัญญาท้องถิ่นจึงเป็นแหล่งของสารต้านอนุมูลอิสระและสารฟีนอลิกที่ดี ในอนาคตควรศึกษาสารออกฤทธิ์ในสารสกัดสมุนไพรไทยเหล่านี้ รวมถึงฤทธิ์ทางชีวภาพอื่นๆ

คำสำคัญ: พืชสมุนไพรไทย, ฤทธิ์ต้านอนุมูลอิสระ, ปริมาณฟีนอลิกทั้งหมด

Abstract

Objectives: In accordance with traditional local wisdom, medicinal plants from north-eastern Thailand are used for the treatment of dermatitis-related inflammations. This study aimed to investigate the antioxidant activity and total phenolic content (TPC) of the bark of thirteen medicinal plants. **Methods:** The study plants included Stephania venosa (Blume) Spreng, Smilax perfoliata Lour., Dolichandrone stipulata, Glochidion coccineum, Xantolis cambodiana, Dalbergia cultrata, Bauhinia sirindhorniae K., Knema angustifolia, Tristaniopsis burmanica, Ficus hirta Vahl, Capparis zeylanica, Amphineurion marginata (Roxb.) G. Don. and Microsorum pteropus (Blume) Copel. The antioxidant properties were determined by means of three approaches namely 1,1-diphenyl-1-princylhydrazyl (DPPH) radical scavenging assay at 517 nm, ferric-reducing antioxidant power (FRAP) assay at 593 nm and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay at 734 nm. The TPC of the plants was determined via the Folin-Ciocalteu method. The correlation between each of the antioxidant activity value with TPC was tested using the Pearson's correlation coefficient (r). **Results:** The antioxidant properties and TPC differed significantly among the plants. In each of the methods, S. venosa (Blume) Spreng., X. cambodiana, B. sirindhorniae, K. angustifolia and C. zeylanica consistently exhibited the most potent antioxidant property ranging from 70% to 100%. This further suggested their potential rich source of natural antioxidants. Coincidentally, these plants contained the highest TPC (38,113 - 9,989 mg GAE/g). Significant positive correlations were found between antioxidant activity (ABTS$^{\bullet}$+ and FRAP assays) and the TPC with r = 0.987 and 0.956, respectively. **Conclusion:** Thirteen medicinal plants contained antioxidant activity which was related to the phenolic content. The active ingredients and other biological activities of these herbal plants merit further investigations.

Keywords: Thai medicinal plants, antioxidant activity, total phenolic content

Introduction

Medicinal plants, used by both ancient and modern cultures, are much valued for their therapeutic properties, which may play a pivotal role in health care. Demand for medicinal plants is increasing because of their ready availability, relatively cheaper cost and non-toxic nature, in contrast to many comparable aspects of modern medicine.[1-3] There is a growing global interest in the exploration of the relatively untapped reservoir of the apparently benign properties of these plants. These plants contained various compounds considered phytochemical agents with antioxidant properties. Such antioxidants are known to protect somatic cells against the damaging effects of reactive

oxygen species, by a virtue of their anti-inflammatory and anti-microbial activity. Reactive oxygen species (ROS), also called free radicals, are active oxygen-containing molecules generated during metabolic and other activities within biological systems. Various forms of activated oxygen molecules exist as free radicals, such as superoxide ions (O_2^-) and hydroxyl radicals (OH^-) as well as non-free radical species such as hydrogen peroxide (H_2O_2).[4-6] Antioxidants interfere with these natural biological oxidative processes by scavenging free radicals, chelating free catalytic metals, and acting as electron donors.[1] In addition, compounds found in medicinal plants such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins which are commonly rich in phenolic substances are known to act by means of their free radical scavenging activities, or by inhibiting pro-inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenases (LOX) in the inflammatory cascade.[3,6-11] Plant-derived antioxidants can therefore potentially assist in protecting the body from various diseases by inhibiting the action of free radicals.[2,12]

It has been proposed that an imbalance in the relative concentrations of free radicals and antioxidants in the body may potentially contribute to pathological processes including ageing, various cancers, coronary disorders, atherosclerosis, and inflammation.[2,4,5] Several studies have investigated the potentially beneficial effects from the consumption of various herbs, vegetables, and fruits containing antioxidants.[6-9] Ipek et al. (2012) demonstrated that the natural wound healing processes of the body may be assisted by antioxidants which are components of the polyphenolic constituents of these plants.[4] However, as the quantities of antioxidants generated via natural metabolic mechanisms may be insufficient to effectively counter certain pathological conditions, enhanced dietary intake of antioxidant-containing compounds is considered desirable. Such considerations have stimulated much interest in the therapeutic effects of several medicinal plants and their potential roles in the provision of antioxidant-containing phytochemicals.[2,10,11]

Traditionally, these plant resources have been used widely throughout Thailand, especially in the north-east, owing to their multifarious characteristics and natural abundance. Accordingly, this present study aimed to investigate the antioxidant activities and polyphenolic constituents of the following thirteen medicinal plants namely *Stephania venosa* (Blume) Spreng., *Smilax perfoliata* Lour., *Dolichandrone stipulata*, *Glochidion coccineum*, *Xantolis cambodiana*, *Dalbergia cultrata*, *Bauhinia sirindhorniae* K., *Knema angustifolia*, *Tristaniopsis burmanica, Ficus hirta Vahl, Capparis zeylanica*, *Amphineurion marginata* (Roxb.) G. Don. and *Microsorum pteropus* (Blume) Copel. These specific plants were provided by a local wisdom authority named Mr. Somya Rattanapolthee, ensuring relevance and alignment, for study purposes, with the traditional wisdom of the north-eastern Thailand.

Apart from the abovementioned studies, relatively few reports exist within the relevant literature regarding the antioxidant activity and total phenolic content (TPC) of these medicinal plants. It was therefore considered desirable, with respect to the pharmaceutical, medical and health food industries, to investigate these readily accessible sources of natural antioxidants for their potential therapeutic benefits. The present study investigated the antioxidant activity of the thirteen plant extracts, as determined by application and comparison of three different approaches, specifically DPPH, FRAP and ABTS assays. We also determined the TPC of these plant extracts by using Folin-Ciocateu test. Possible correlations between antioxidant activity and TPC of these plants were also examined.

Methods

This experimental research was conducted to evaluate the antioxidant activities of thirteen Thai medicinal plants by means of the following assays specifically 1,1-diphenyl-1-princylhydrazyl (DPPH) radical scavenging at 517 nm, ferric-reducing antioxidant power (FRAP) assay at 593 nm, and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay at 734 nm. The total phenolic content (TPC) was determined by using Folin-Ciocalteu test.

Reagents

All chemicals were purchased from Sigma-Aldrich (Germany). All reagents were of analytical grade and all absorbance values for determining antioxidant activity and TPC were investigated using a UV-visible spectrophotometer (Thermo Scientific, USA). All experiments were performed with three replications.

Procedures for plant material preparations and extractions

Thirteen Thai medicinal plants were collected from the Dongsaithong Learning Center, Tungfon district, Udon Thani province, Thailand. All plants were selected with the advice and assistance of an authority on local wisdom (Mr. Somya Rattanapolthee), who has used them as therapeutic agents for dermatitis-related inflammations, as shown in Table 1. The identities of all plants used in this study were elucidated by the Applied Taxonomic Research Center (ATRC), Department of Science, Khon Kaen University. Prior to conducting antioxidant and TPC evaluations, all plant materials were washed with water and dried at 50 – 60 °C for three days, then ground to a fine powder with a blender (Yason, China). Each sample was boiled in water in the ratio of plant to water of 1:15 w/v, and shaken for 2 hours. The extraction process was repeated three times. After boiling, the mixture was cooled at room temperature and centrifuged at 3,000 rpm for 15 minutes. The supernatant of the extract was filtered using No. 2 Whatman filter paper and concentrated using a rotary evaporator with the hot water bath set at 40 °C. The yield of each extract was calculated as a percentage of dry weight of the bark used and the quantity of dry mass obtained after extraction. The dried extracts were stored at -20 °C prior to analysis.

Determination of antioxidant activity

Determination of the free radical scavenging activity by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

In order to determine the DPPH radical-scavenging activity, based on the modified method of Cakmak et al.,[13] 100 µL of each sample (0.003 - 0.01 g/mL) was added to a solution of 2.9 mL of 100 µM DPPH in methanol and mixed well. After incubating for 30 minutes, the absorbance of the sample was determined at 517 nm relative to a control. The radical scavenging activity was measured as a decrease in absorbance of DPPH. The % inhibition was calculated as follows:

$$\% \text{ Inhibition} = (A_{control} - A_{sample}) / A_{control}$$

where A_{sample} and $A_{control}$ are the respective absorbances of DPPH in the resultant reaction mixture, i.e., test sample, and control. Ascorbic acid (AA) was used as a reference standard (Y = -0.0009x + 0.9783), R^2 = 0.9981. The results were represented as AAE (ascorbic acid equivalent / gm of dry mass).

Determination of the free radical scavenging activity by 2,2'-azino-bis(3-ethylbenthiazoline-6-sulfonic acid (ABTS) assay

An ABTS assay was conducted according to the method of Liu *et al.* (2001) with minor modifications.[14] The colorless ABTS molecule is converted into $ABTS^{\cdot+}$, a blue-green colored radical, by the loss of one electron (oxidation). $ABTS^{\cdot+}$exhibits maximal absorption at wavelengths 645 nm, 734 nm and 815 nm.[14] A 1500 µL solution of the $ABTS^{\cdot+}$ cation radical was prepared by mixing 7 mM of the ABTS reagent and 2.45 mM potassium persulfate in equal volumes, equilibrating the mixture for 16 hours and diluting with ethanol. The plant extract (100 µL) was mixed with 2.9 mL of the $ABTS^{\cdot+}$ solution. The mixture was incubated for 2 hr in the absence of light. The absorbance value was then recorded at 734 nm using the UV-visible spectrophotometer. Ascorbic acid (AA) was used as a reference standard (Y = -0.0009x + 0.7057, R^2 = 0.9984) and results were expressed as ascorbic acid equivalent/g dry mass (AAE). The percentage of scavenging activity in the test samples was calculated as per the DPPH procedure described above.

Determination of the Reducing Power by Ferric-Reducing Antioxidant Power (FRAP) Assay

The modified ferric ion reducing antioxidant activity of the extracts was measured according to Cai et al. (2004).[2] At a low pH, when a ferric-tripyridyltriazine (F^{III}-TPTZ) complex is reduced to the ferrous (Fe^{II}) form, an intense blue color with a maximal absorption at 593 nm develops.[2] 100 µL of plant extract and freshly prepared $FeSO_4$ standard solution was mixed with 2.9 mL of the working FRAP reagent. The fresh working solution was prepared by mixing 25 mL acetate buffer with 2.5 mL TPTZ solution and 2.5 mL $FeSO_4{}^{\cdot}7H_2O$ solution, and warmed at 37 ºC. The absorbance reading at 593 nm was taken after 10 minutes at 37 ºC. Ferrous sulfate ($FeSO_4$) was used as a reference standard (Y = -0.001x + 0.0462), R^2 = 0.9997. Half minimal inhibitory concentration (IC50) was conventionally determined in terms of dose dependency; however in practice no dose dependency was found in this case. The percentage inhibition was thus calculated as formally stated below:

$$\% I = [(A_O - A_S) / A_O] \times 100$$

where A_o is the absorption of control and A_s is the absorption of the test extract solution.

Determination of the Total Phenolic Content (TPC)

The total phenolic content was determined by means of the modified Folin-Ciocalteu colorimetric method.[15] Phenols react with phosphomolybdic acid in Folin-Ciocalteu reagent in an alkaline medium, producing a blue colored complex (molybdenum blue) that can be estimated colorimetrically at 650 nm. A solution of 7.3 mL distilled water and 200 µL of Folin-Ciocalteu reagent was added to 500 µL of each plant extract. The mixture was allowed to equilibrate at room temperature for 10 min before adding 600 µL of 10% sodium carbonate to the mixture. The resulting blue complex was then measured at 755 nm. Gallic acid was used as a reference standard for the calibration curve (Y = 0.0009x - 0.0168), R^2 = 0.9992). The TPC was expressed as gallic acid equivalent mg/g dry mass (GAE).

Data analysis

Data were presented as mean ± standard deviation (SD) of each triplicate. Duncan's test was applied to determine any significant differences on each measure among the thirteen selected plants. One-way ANOVA was used to test for significant differences of each measure between the three methods employed. Correlations between antioxidant activity and TPC were examined using Pearson's correlation coefficient (r). Values obtained at *P*-value < 0.05 were considered statistically significant.

Results

Extraction yields

Thai medicinal plants used for therapeutic purposes, as shown in Table 1, were selected with the advice of an authority on local wisdom (Mr. Somya Rattanapolthee). The yields of these plant extracts were in a range of 3.33% to 41.49% (Table 1). Of these, Bauhinia sirindhorniae K. produced the highest yield (41.49%), followed by Stephania venosa (Blume) Spreng. (17.57%), Ficus hirta Vahl (14.38%) and Knema angustifolia (12.76%).

Antioxidant activity

With respect to the DPPH assay, the antioxidant capacities of the medicinal plants ranged from 251.41 to 987.88 µmol AAE/g dry mass (Table 2). *Stephania venosa* (Blume) Spreng. possessed the highest antioxidant capacity (987.88 ± 5.41 µmol AAE/g dry mass), followed by *Knema angustifolia*, *Dalbergia cultrata*, *Bauhinia sirindhorniae* K., Glochidion coccineum and Amphineurion marginata (Roxb.) G. Don., with the antioxidant capacities of 950.58 ± 9.64, 940.94 ± 15.15, 921.02 ± 12.23, 902.12 ± 18.20 and 893.62 ± 54.24 µmol AAE/g dry mass, respectively (Figure 1A).

Table 1 List of the plants used in the study.

Scientific name (Family)	%Yield [a]	Medicinal used	Part used
Stephania venosa (Blume) Spreng. (Menispermaceae)	17.57	Used against topical skin problems e.g. wounds, itching, or abscess	Bark
Smilax perfoliata Lour. (Smilacaceae)	4.91	Against abscesses, skin inflammation, purifying blood	Bark
Dolichandrone stipulata (Bignoniaceae)	3.33	Treatment of skin disorders such as ringworm, eczema	Bark
Glochidion coccineum (Euphorbiaceae)	5.24	Wound healing for fresh, chronic sores, swollen contusion	Bark
Xantolis cambodiana (Sapotaceae)	10.94	Anti-inflammatory, relieving hot pain symptoms, bruises	Bark
Dalbergia cultrata (Leguminosae-Papilionoideae)	11.05	Taken as tonic, promotes the flow of blood, externally for skin swelling, sores, and insect bite	Bark
Bauhinia sirindhorniae K. (Leguminosae-Caesalpiniaceae)	41.49	Taken as tonic, promotes the flow of blood, externally for skin swelling, sores, and insect bite	Bark
Knema angustifolia (Myristicaceae)	12.76	Taken as tonic, promotes the flow of blood, externally for skin swelling, sores, and insect bite	Bark
Tristaniopsis burmanica (Myrtaceae)	10.54	Taken as tonic, promotes the flow of blood, externally for skin swelling, sores, and skin burns	Bark
Ficus hirta Vahl (Moraceae)	14.38	Treatment for skin disorders such as ringworm, eczema	Bark
Capparis micracantha (Capparaceae)	7.79	Anti-inflammatory, relieving hot pain symptoms, fevers and bruises	Bark
Amphineurion marginata (Apocynaceae)	10.87	Used against topical skin problems e.g. wounds, itching, or abscess	Bark
Microsorum pteropus (Blume) Copel. (Polypodiaceae)	9.80	For infected skin/urticaria or skin burns, itching, or abscesses	Bark

[a] on dried weight basis.

Relatively high DPPH radical scavenging activities were found in *Stephania venosa* (Blume) Spreng., *Knema angustifolia*, *Dalbergia cultrata, Bauhinia sirindhorniae, Glochidion coccineum* and *Amphineurion marginata* (Roxb.) G. Don. with inhibitions of 88.76%, 85.52%, 84.68%, 82.95%, 81.30% and 80.56%, respectively (Table 2). The plant extracts showing weaker inhibition of the $DPPH^{\cdot+}$ radical were *Tristaniopsis burmanica* (44.82%) and *Microsorum pteropus* (Blume) Copel. (48.86%).

For the ABTS assay, the antioxidant capacities of the medicinal plants ranged from 52.95 to 744.91 µmol AAE/g dry mass (Table 2 and Figure 1A). *Bauhinia sirindhorniae* K. possessed the highest antioxidant capacity (744.91 ± 20.83 µmol AAE/g dry mass), followed by *Stephania venosa* (Blume) Spreng., *Xantolis cambodiana*, *Knema angustifolia* and *Capparis micracantha* with the antioxidant capacities of 732.18 ± 17.92, 720.07 ± 26.54, 697.11 ± 30.12 and 568.33 ± 33.32 µmol AAE/g dry mass, respectively.

Relatively high $ABTS^{\cdot+}$ radical scavenging activities were also found in *Bauhinia sirindhorniae, Stephania venosa* (Blume) Spreng, *Xantolis cambodiana, Knema angustifolia and Capparis micracantha* with inhibitions of 94.13%, 92.57%, 91.08%, 88.25% and 72.40%, respectively. Plant extracts typified by relatively weaker inhibition of the $ABTS^{\cdot+}$ radicals were *Ficus hirta* Vahl *(*8.96%) and *Microsorum pteropus* (Blume) Copel. (19.75%) (Figure 1B).

The reducing capacity of water extracts of the medicinal plants was determined by FRAP assay, with the highest value being obtained from *Bauhinia sirindhorniae* K. (912.69 ± 86.05 µmol $FeSO_4{}^{\cdot}7H_2O$/g dry mass), followed by *Stephania venosa* (Blume) Spreng., *Xantolis cambodiana*, *Knema angustifolia*, and *Capparis micracantha* with values of 790.02 ± 98.64, 720.07 ± 26.54, 697.11 ± 30.12 and 673.58 ± 21.15 µmol $FeSO_4{}^{\cdot}7H_2O$/g dry mass, respectively (Table 2 and Figure 1C). The lowest result was given by *Ficus hirta* Vahl (77.58 ± 12.54 µmol $FeSO_4{}^{\cdot}7H_2O$/g dry mass).

Overall, the study plants could be conveniently partitioned into three groups. The first group referred to those functioning as good antioxidant sources which included *Stephania venosa* (Blume) Spreng., *Xantolis cambodiana, Bauhinia sirindhorniae, Knema angustifolia* and *Capparis micracantha*, all of which gave high values in all three antioxidant tests. The second group was those with relatively weak antioxidant sources, with low values obtained from all tests. These plants were *Smilax perfoliata* Lour*., Ficus hirta Vahl*, *Amphineurion marginata* (Roxb.) G. Don and *Microsorum pteropus* (Blume) Copel. The last group included plants with antioxidant sources with high or at least moderate values in only one or two tests (*Dolichandrone stipulate, Glochidion coccineum, Dalbergia cultrata* and *Tristaniopsis burmanica*), as shown in Figure 1.

Total phenolic content (TPC)

The TPC, determined by the Folin-Ciocalteu method, was highest in the water extract of *Bauhinia sirindhorniae* K (59,989.39 ± 1,011.46 mg GAE/g dry mass). Other plants with high TPC were *Knema angustifolia*, *Xantolis cambodiana* and *Stephania venosa* (Blume) Spreng with the TPC values of 56,459.02 ± 5,117.76, 55,143.10 ± 3,907.09 and 52,546.61 ± 2,916.55 mg GAE/g dry mass, respectively (Table 2 and Figure 1D).

Correlations between antioxidant activity and TPC

The antioxidant activities based on the FRAP and ABTS assays were significantly highly correlated with TPC with the correlation coefficients ranging from 0.956 to 0.987. The highest correlation was found between ABTS and TPC ($r = 0.987$, $P < 0.001$), followed by FRAP and TPC ($r = 0.956$, $P < 0.001$). However, no significance was found between DPPH and TPC ($r = 0.431$, $P > 0.05$) (Table 3).

Discussions and Conclusion

In this *in vitro* study, three methods namely DPPH, ABTS and FRAP assays were used to investigate antioxidant activity. The findings of our study could be reasonably reliable since multiple methods are more accurate predictors of antioxidant activity. These three methods required the use of spectrophotometer, a relatively common apparatus in most laboratories. Proceeding from the antioxidant activity, the free radical scavenging capacity of a number of Thai medicinal plant extracts was determined using the ABTS and DPPH decolorization assay.[1] The ABTS assay uses ABTS radicals produced by the oxidation of ABTS with potassium persulphate. Thus, this assay becomes time-consuming since it takes about 12 – 16 hours to generate ABTS radicals. On the other hand, for the DPPH assay, one does not have to wait for the free radicals to be

Table 2 Antioxidant activity of water extracts of Thai medicinal plants as determined by the DPPH, ABTS and FRAP assays and the phenolic compound content determined by Folin-Ciocalteu assay.

Scientific name (Family)	DPPH assay		ABTS assay		FRAP assay	TPC
	% Inhibition	µmol AAE/g dry mass	% Inhibition	µmol AAE/g dry mass	µmol $FeSO_4 \cdot 7H_2O$)/g dry mass	(mg GAE/g dry mass)
Stephania venosa (Blume) Spreng. (Menispermaceae)	88.76	987.88 ± 5.41	92.57	732.18 ± 17.92	790.02 ± 98.64	52,546.61 ± 2,916.55
Smilax perfoliata Lour. (Smilacaceae)	71.03	784.01 ± 25.54	28.68	213.19 ± 18.58	189.13 ± 22.34	15,771.34 ± 1,128.17
Dolichandrone stipulata (Bignoniaceae)	79.49	881.31 ± 32.42	28.34	210.45 ± 8.01	233.13 ± 26.10	14,710.11 ± 1,127.17
Glochidion coccineum (Euphorbiaceae)	81.30	902.12 ± 18.20	31.21	233.78 ± 11.25	254.36 ± 7.68	15,799.64 ± 1,347.72
Xantolis cambodiana (Sapotaceae)	71.93	794.41 ± 61.50	91.08	720.07 ± 26.54	752.91 ± 70.67	55,143.10 ± 3,907.09
Dalbergia cultrata (Leguminosae-Papilionoideae)	84.68	940.94 ± 15.15	23.46	170.76 ± 12.75	157.36 ± 19.58	16,846.72 ± 8,113.72
Bauhinia sirindhorniae K. (Leguminosae-Caesalpiniaceae)	82.95	921.02 ± 12.23	94.13	744.91 ± 20.83	912.69 ± 86.05	59,989.39 ± 1,011.46
Knema angustifolia (Myristicaceae)	85.52	950.58 ± 9.64	88.25	697.11 ± 30.12	611.58 ± 85.19	56,459.02 ± 5,117.76
Tristaniopsis burmanica (Myrtaceae)	44.82	482.82 ± 20.28	57.56	447.79 ± 20.87	460.80 ± 121.00	36,981.85 ± 362.69
Ficus hirta Vahl (Moraceae)	24.69	251.41 ± 20.93	8.96	52.95 ± 10.55	77.58 ± 12.54	4,543.49 ± 372.69
Capparis micracantha (Capparaceae)	78.18	866.21 ± 8.60	72.40	568.33 ± 33.32	673.58 ± 21.15	38,113.84 ± 1,411.93
Amphineurion marginata (Apocynaceae)	80.56	893.62 ± 54.24	36.10	273.46 ± 23.60	323.58 ± 15.87	15,700.59 ± 518.74
Microsorum pteropus (Blume) Copel. (Polydiaceae)	48.86	529.27 ± 38.26	19.75	140.68 ± 17.41	165.13 ± 22.26	13,033.36 ± 397.27

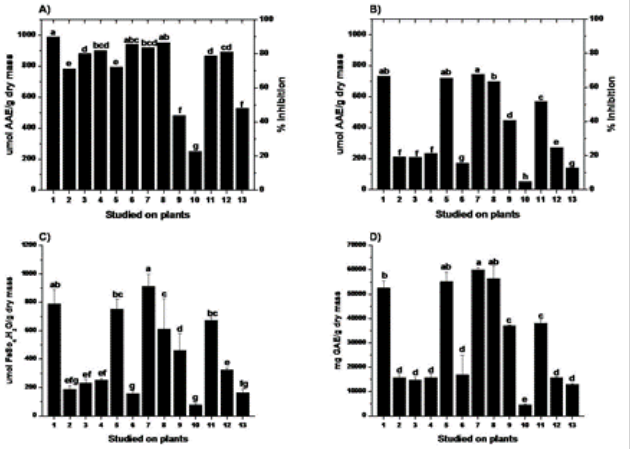

Figure 1 Antioxidant activity determined by A) DPPH assay, B) ABTS assay, and C) FRAP assay, and D) TPC determined by Folin-Ciocalteu method. Note: *Stephania* venosa (Blume) Spreng.(1), *Smilax perfoliata* Lour. (2), *Dolichandrone stipulata* (3), *Glochidion coccineum* (4), *Xantolis cambodiana* (5), *Dalbergia cultrata* (6), *Bauhinia sirindhorniae* K. *(7), Knema angustifolia* (8), *Tristaniopsis burmanica* (9), *Ficus hirta Vahl* (10), *Capparis zeylanica* (11), *Amphineurion marginata* (Roxb.) G. Don. (12), *Microsorum pteropus* (Blume) Copel. (13). Different letters above the bars indicate significant difference at $P < 0.05$ (Duncan's test).

Table 3 Pearson's correlation coefficients (r) of antioxidant activity and total phenolic compound content.

Method	FRAP assay	ABTS assay	TPC
DPPH assay	0.455	0.477	0.431
FRAP assay		0.979*	0.956*
ABTS assay			0.987*

* $P < 0.05$

generated.[1,6,16] For FRAP assay, this method is based on the reduction, at a low pH, of a colorless ferric complex (Fe^{III}-TPTZ) to a blue colored ferrous complex (Fe^{II}-TPTZ) by electron-donating antioxidants.[11]

Another advantage of the ABTS and FRAP approaches was the fact that the plant extracts reacted rapidly with ABTS (within 2 hr) and ferric ion (within 30 min). In contrast, the DPPH reaction took much longer (up to 24 hr). Working solutions of the DPPH and FRAP were used immediately after preparation; while that of ABTS had to be kept in the dark condition for 12 hr, to generate free radicals from the ABTS salt, then used within 4 hr.[2,17-19]

In the ABTS test, variability among the samples was distinctly greater than that of the other two, the value

obtained from *Bauhinia sirindhorniae* K. being over twelve times higher than that from *Ficus hirta* Vahl. The different water extraction values obtained from the assays may reflect differences in the relative abilities of the antioxidant fractions within the sample extracts to quench aqueous peroxyl radicals, and to reduce $ABTS^{\cdot+}$, DPPH free radicals and ferric irons in the *in vitro* systems. Although the interaction of medicinal plants and assays was significant in terms of the water extraction values, it explained only a small amount of the total variation as compared to one onther.[8,11,19]

The *in vitro* antioxidant activities of water extracts of thirteen selected Thai medicinal plants were investigated by DPPH, ABTS and FRAP assays. While the highest average antioxidant activity was observed by the DPPH method, all samples showed moderate antioxidant activity by the FRAP and ABTS methods. Overall, the selected medicinal plants could be partitioned into three groups. The first group were those with good antioxidant sources including *Stephania venosa* (Blume) Spreng., *Xantolis cambodiana, Bauhinia sirindhorniae*, *Knema angustifolia* and *Capparis micracantha* . This first group of plant extracts gave high values of all antioxidant tests (DPPH, ABTS and FRAP). The second group of plant extracts included those having weak antioxidant sources, with low values obtained from all tests (*Ficus hirta* Vahl and *Microsorum pteropus* (Blume) Copel.). The last group consisted of two plant extracts, *Amphineurion marginata* (Roxb.) G. Don. and *Tristaniopsis burmanica*, which had variable antioxidant capacities, with high or at least moderate values in only one or two tests. Although these thirteen plants generally possess antioxidant activity, scant information is currently available regarding their *in vivo* and *ex vivo* antioxidant behaviors, together with their possible toxicity.

The study confirms that results obtained from applying three distinct procedural methods to each sample can differ significantly. Such differences may be due to the presence of varieties of antioxidants within the samples, each reacting uniquely with the introduced radicals. Every method has its own advantage and limitations in terms of cost, availability of chemical reagents, procedural routines, preparation time, reproducibility, and so on.[11] In this present study, DPPH was found to be the most suitable method for determination of antioxidant activity of 13 Thai medicinal plants because it could be rapidly performed with a high reproducibility. In the DPPH method, the radicals did not have to be generated before the assay. On the other hand, the need to generate the radicals before the procedure was an advantage of the ABTS method. In contrast, the sole disadvantage of the FRAP method was that it was time-consuming for reagent preparation.[2,17-20] An attempt was made in this study to compare the different antioxidant assays. However, before selecting any particular assay, it is therefore important to weigh carefully the advantages and limitations of each method in order to discern the most appropriate choice for specific experimental purposes.

The antioxidant activity of plant extracts is generally associated with their phenolic compound content. Plant phenols constitute the major group of compounds acting as primary antioxidants.[11,15,17,18] Measurements of TPC in these extracts may relate to their antioxidant properties, as reported by Jimenez et al. (2001).[21] Phenolics are also known to play an important role in stabilizing lipids against peroxidation and inhibiting various types of oxidizing enzymes,[15,21] This phenomenon could be tentatively attributed to the different structures of these radicals and especially to their differing charges. Specifically, the DPPH molecule has no charge and the $ABTS^{\cdot+}$ radical is a cation. This may engender different reaction mechanisms both for the active compounds within medicinal plants and for standard antioxidants. As reported by Cai *et al* (2004), the presence of different categories of phenolics resulted in differences in radical scavenging activity.[2] For example, the activity of phenolic acids apparently depends upon the number and position of hydroxyl (-OH) groups and the methoxy ($-OCH_3$) substituent for the inhibition of lipid peroxidation at an earlier stage.[12]

In this study, no significant correlation was found between the antioxidant activity by the DPPH assay and the TPC. This result was similar to that of Hajimahmoodi et al (2009) where there was a positive correlation between the TPC and antioxidant capacity as measured by the FRAP, but not that measured by the DPPH assays.[16] Both the FRAP assay and Folin-Ciocalteu method were operated on the basis of metallic reduction. Therefore phenolic compounds were major contributors to the antioxidant capacities of cell mass extracts and the extracellular water fractions for the FRAP and Folin-Ciocalteu assays. In the DPPH assay, radical scavenging activity was evaluated and possibly another functional component would be effective. It should be noted that other antioxidant compounds, in the form of

carotenoids, polyunsaturated fatty acids and polysaccharides, may also play an important role.[22,23]

In conclusion, the results from these *in vitro* experiments suggest that certain plants showed promise as sources of natural antioxidants. Antioxidant properties and TPC differed significantly within the collection of thirteen plant extracts studied. Of these, *Stephania* venosa (Blume) Spreng., *Xantolis cambodiana, Bauhinia sirindhorniae* K., *Knema angustifolia* and *Capparis zeylanica* exhibited very strong antioxidant properties and high TPC levels. As a significant correlation was found between antioxidant activity and TPC, this suggests that phenolic compounds are major contributors to the antioxidant properties of the plant extracts. Clearly additional work is required to isolate and characterize the antioxidative components and biological activities of these active plant extracts.

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