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Antioxidative and Free Radical Scavenging Activities of Some Plants Used in Thai Folk Medicine

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Abstract

Ten methanol extracts from various parts of seven medicinal plants, *Angiopteris evecta* Hoffm., (Marattiaceae) *Citrus hystrix* DC., (Rutaceae) *Laurentia longiflora* (L.) Peterm., (Campanulaceae) *Nelumbo nucifera* Gaertn., (Nelumbonaceae), *Piper sarmentosum* Roxb., (Piperaceae), *Portulaca oleracea* Linn., (Portulacaceae) and *Stachytarphera indica* (L.) Vahl (Verbenaceae), commonly used in Thai traditional medicine, were evaluated for their antioxidative and free radical scavenging activities. Among them, only that prepared from sacred lotus (*N. nucifera*) leaves exhibited a pronounced activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay with an IC_{50} of 90 μg/ml, compared with an IC_{50} of 30 μg/ml for the butylated hydroxytoluene (BHT) control. The same extract was also found to be the most potent in removing the superoxide anion ($O_2^{\bullet -}$) radical and in inhibiting the 2,2′-azo-*bis*-(2-amidinopropane) dihydrochloride (AAPH)-induced erythrocyte hemolysis and lipid peroxidation in a rat brain homogenate. The extract from leaves and peels of the Kaffir lime (*C. hystrix*) exerted the strongest effect on production of the hydroxyl radical ($OH^{\bullet}$). They conferred a twice greater protection of deoxyribose from $OH^{\bullet}$ than did tannin. However, none of the extracts examined in this study showed a significant pro-oxidant action in the bleomycin-dependent DNA oxidation system.

Keywords: *Angiopteris evecta*, antioxidants, *Citrus hystrix*, free radical scavenging activity, *Laurentia longiflora*, *Nelumbo nucifera*, *Piper sarmentosum*, *Portulaca oleracea*, *Stachytarphera indica*.

Introduction

Naturally generated reactive oxygen species (ROS) are molecules that can attack cell components and create several types of biological damage. They play important roles in the pathogenesis of various diseases ranging from carcinogenesis to aging (Ames et al., 1993). Thus, substances that scavenge ROS can protect against these diseases. Because the possibility has been raised that some synthetic antioxidants may be toxic (Yang et al., 2000), there have been numerous investigations into the possibility that some natural compounds may exist with potent antioxidative activity against free radicals but with low toxicity. So far a number of them, including polyphenolics, flavonoids, and various plants extracts, have been reported to be effective radical scavengers and inhibitors of lipid peroxidation (Abuja et al., 1998; Kerry & Abbey, 1998; Pearson et al., 1999; Zhang et al., 2001).

Thailand is located in the tropical region of Southeast Asia. Because innumerable varieties of tropical plants are found there, the Thais have a long ethnomedical knowledge, and have utilized alleged medicinal herbs and plants as cures for many ailments. In this study, various parts of seven plants namely, *Angiopteris evecta* Hoffm., (Marattiaceae) *Citrus hystrix* DC., (Rutaceae) *Laurentia longiflora* (L.) Peterm., (Campanulaceae) *Nelumbo nucifera* Gaertn., (Nelumbonaceae), *Piper sarmentosum* Roxb., (Piperaceae), *Portulaca oleracea* Linn., (Portulacaceae) and *Stachytarphera indica* (L.) Vahl (Verbenaceae), selected from those that have appeared most frequently in Thai pharmacopoeia recipes, were investigated. A wide range of pharmacological properties of these plants and some of their chemical constituents have been reported. For example, a unique group of flavonoids,

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apigenin di–C-glycosyl flavones, have been detected in *A. evecta* (Wallace et al., 1981). Glyceroglycolipids from *C. hystrix* leaves have been demonstrated to have antitumor activities both *in vivo* (Murakami et al., 1999) and *in vitro* (Tiwawech et al., 2000), and they also contain a variety of flavonoids (Berhow et al., 1996). An alcohol extract of *P. sarmentosum* leaves has neuromuscular blocking activity (Ridtitid et al., 1998) and antiplasmodic activities (Rahman, 1999). An aqueous extract of *P. oleracea* has antimicrobial activities (Ongsakul et al., 1992–1993) and contains omega-3 fatty acids along with several antioxidants in its leaves (Simopoulos et al., 1992). *S. indica* contains an anti-inflammatory agent, ipolamiide (Tantisewie & Sticher, 1975). In spite of these reports, none have yet mentioned any inhibitory effect on free radicals, that is the principal mechanism of antioxidants. Therefore, in this study we have screened these plant extracts for their free radical scavenging activities as an attempt to find potential new sources for natural antioxidants.

Materials and Methods

Plant materials

All plant materials were collected locally. Their voucher specimens were identified at the Herbarium of Faculty of Science, Prince of Songkla University, and deposited in the authors' laboratories at the Department of Chemistry.

Preparation of plant extracts

Parts of plants (Table 1) were cut into small pieces and extracted by three successive macerations in methanol at room temperature. The filtrates were combined and evaporated to dryness under vacuum at 45–55°C. The final residues were then stored at room temperature in a closed container until used.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was a product of Merck (Darmstadt, Germany). Butylated hydroxytoluene (BHT), bleomycin sulfate, DNA (type XIV from Herring testis), nitrobluetetrazolium dihydrochloride (NBT), and tetramethyl murexide (TMM) were purchased from Sigma (St. Louis, MO, USA). 2,2′-Azo-*bis*-(2-amidinopropane) dihydrochloride (AAPH), 2-deoxy-D-ribose, and thiobarbituric acid (TBA) were obtained from Fluka (Switzerland). All other chemicals were of analytical grade.

Determination of total phenolics

The amount of total phenolics in extracts was determined using the AOAC (1990) spectrophotometric method. Each sample was dissolved in methanol to give a final concentration of 1 mg/ml. A 40-µl aliquot of sample solution was added to 5 ml of distilled water and then mixed with 0.5 ml of Folin-Danis reagent. After standing at room temperature for 3 min, 1 ml of 35% (w/v) Na_2CO_3 was added to the reaction mixture and the mixture was diluted to 10 ml with distilled water. After a 20 min incubation period at room temperature, the absorbance was read at 760 nm. The amount of total phenolics in each extract was determined from a standard curve prepared from different amounts of catechin and was expressed as catechin equivalents in µmole per mg of plant extract.

Table 1. The parts of seven medicinal plants used for preparing crude extracts in this study.

Botanical name	Common name	Part tested	Medical uses
1. *Angiopteris evecta*	King fern	Rhizome	Astringent for buccal ulcer, antidiarrhetic, analgesic, antemetic, diuretic
2. *Citrus hystrix*	Kiffir lime	Leaf	Antitussive, antihemorrhage
		Fruit peel	antiflatulent, antiscurvy, antidandruff
3. *Laurentia longiflora*	Star of Bethlehem	Whole plant	antivenereal diseases (VD), antiasthma, antibronchitis, antiepileptic
4. *Nelumbo nucifera*	Sacred lotus	Leaf	Antidiarrhetic
		Pollen	Cardiac tonic
		Seeds head	Antidiarrhetic
5. *Piper sarmentosum*	Wild betel leaf	Whole plant	Expectorant, antidiabetic
6. *Portulaca oleracea*	Pigweed	Whole plant	Antidysenteric, antiasthma, cardiac and hepatic tonic, laxative, antipruritic insect bite treatment
7. *Stachytarphera indica*	Common snake weed	Whole plant	Antipyretic, diaphoretic, diuretic, antiphlogistic, antigonorrhea, antidote

Iron chelation activity

According to the method of Shimada et al. (1992), various concentrations of extract made to 2 ml in 10 mM hexamine, pH 5.0, were mixed with an equal volume of 3 mM $FeSO_4$. An 0.2 ml aliquot of TMM (tetramethyl

murexide) was then added and left for 3 min at room temperature. The absorbance was read at 485 nm against the control containing no extract. The percentage of chelation was then calculated from

$$\% \text{ Chelation} = \left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] \times 100$$

where A_{sample} is the absorbance of the test sample and $A_{control}$ is the absorbance of the control.

Free radical scavenging assays

DPPH Assay

This was carried out according to Yen and Hsieh (1997). To different concentrations of a sample in methanol (0.5 ml each) was added 1 ml of a methanol solution of 0.2 mM DPPH. After mixing thoroughly, the mixture was allowed to stand in the dark for 30 min and the absorbance at 523 nm was measured using methanol for the baseline correction. The results were then compared with that of the control prepared as above but without added sample. Radical scavenging activity was expressed as a percentage and was calculated using the following formula:

$$\% \text{ Scavenging} = \left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] \times 100$$

where A_{sample} is the absorbance of the test sample and $A_{control}$ is the absorbance of the control.

For each sample, the result was presented as an IC_{50} (sample concentration that produced 50% scavenging of the DDPH radical).

Hydroxyl radical scavenging assay

The method previously described by Murcia et al. (2001) was modified. Various amounts of test sample were mixed with 134 µl of 30 mM KH_2PO_4-KOH buffer (pH 7.4), 67 µl of 17 mM deoxyribose, 33 µl of 34 mM H_2O_2, 33 µl of 1.2 mM EDTA, and 67 µl of 300 µM $FeCl_3$. A 67 µl aliquot of 0.6 mM ascorbic acid was then added to start the reaction. After incubation at 37°C for 1 h, the products of the hydroxyl radical attack on deoxyribose were determined by adding 333 µl of 1% (w/v) TBA (thiobarbituric acid) in 50 mM NaOH, followed by 333 µl of 2.8% (w/v) TCA (trichloroacetic acid). After further incubation at 80°C for 20 min, the reaction mixtures were centrifuged. The absorption of the clear supernatants was then measured at 532 nm. A parallel assay omitting the test sample acted as a control, whereas the normal reaction mixture without deoxyribose was used as a sample blank. The results were expressed as percent inhibition of the deoxyribose attack using the following formula:

$$\% \text{ Inhibition} = \left[\frac{A_{control} - (A_{sample} - A_{sample\ blank})}{A_{control}}\right] \times 100$$

where A_{sample} is the absorbance of the test sample, $A_{control}$ is the absorbance of the control, and $A_{sample\ blank}$ is the absorbance level of the sample blank.

For each sample, the result was presented as an IC_{50} (sample concentration that produced 50% inhibition of hydroxyl radical).

Superoxide radical scavenging assay

The assay was carried out according to the method for estimating superoxide dismutase activity by Beyer and Fridovich (1987). Test samples were prepared in methanol, and 20 µl of each was added to 1 ml of the reagent composed of 9.9 mM L-methionine, 1.72 mM NBT (nitroblue tetrazolium), 1% (w/v) Triton X-100, and 117µM riboflavin in 50 mM K_2HPO_4, pH 7.8. After mixing, the reaction mixture was illuminated beneath a 40-W fluorescent light for 7 min. A control tube in which the test sample was replaced by methanol and a sample blank that contained no riboflavin were also run in parallel. The absorbance at 560 nm was then measured. The results were expressed as percent scavenging of the superoxide radical using the following formula:

$$\% \text{Scavenging} = \left[\frac{(A_{control} - (A_{sample} - A_{sample\,blank}))}{A_{control}}\right] \times 100$$

where A_{sample} is the absorbance of the test sample, $A_{control}$ is the absorbance of the control, and $A_{sample\ blank}$ is the absorbance level of the sample blank.

For each sample, the result was presented as an IC_{50} (sample concentration that produced 50% inhibition of the superoxide radical).

Antioxidation of biomolecules

Lipid peroxidation assay

Based on the Liu and Ng method (2000), the brains of normal female albino rats of the Wistar strain (age 3 months) were dissected and homogenized in chilled 20 mM Tris-HCl, pH 7.4, at a ratio of 1:10 (w/v). The homogenate was centrifuged at 20,000 × *g* for 15 min at 4°C. An 0.5-ml aliquot of the supernatant was then taken to incubate with 0.3 ml of the test sample, 0.1 ml of 10 µM $FeSO_4$, and 0.1 ml of 0.1 mM ascorbic acid at 37°C for 1 h. The reaction was then stopped by addition of 0.75 ml of 28% (w/v) trichloroacetic acid (TCA) and 0.5 ml of 1% (w/v) thiobarbituric acid (TBA), successively. The mixture was then heated at 100°C for 45 min. After centrifugation, all precipitated proteins were removed and the color of the malondialdehyde (MDA)-TBA complex in the supernatant was detected at 532 nm. The percent inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] \times 100$$

where A_{sample} is the absorbance of the test sample and $A_{control}$ is the absorbance of the control.

Erythrocyte hemolysis assay

Human blood was obtained from healthy volunteers by venepuncture and collected in heparinized tubes. Erythrocytes were separated from plasma and washed three-times with 10 volumes of PBS, pH 7.4. During the last wash, the erythrocytes were centrifuged at $1000 \times g$ for 10 min to obtain a constantly packed cell preparation.

In the test system, following the method of Liu and Ng (2000), erythrocyte hemolysis was mediated by peroxyl radicals. A 10% suspension of erythrocytes in PBS, pH 7.4, was added to the same volume of 0.2 M AAPH in PBS containing the test sample at different concentrations. The reaction mixture was shaken gently while being incubated at 37°C. After 2 h, the reaction mixture was removed, diluted with 8 volumes of PBS, and centrifuged at $1000 \times g$ for 10 min. The absorbance of the supernatant was then read at 540 nm. In a similar manner, the reaction mixture was diluted with 8 volumes of distilled water to achieve a complete hemolysis, and the absorbance was read at 540 nm. The percentage of hemolysis was calculated compared with that of the control from

$$\% \text{ Inhibition} = \left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] \times 100$$

where A_{sample} is the absorbance of the test sample and $A_{control}$ is the absorbance of the control.

Bleomycin-dependent DNA damage

The assay was performed according to Liu and Ng (2000). A 100 µl aliquot of sample to be tested at different concentrations was added to a mixture containing 50 µl of 0.5 mg/ml DNA, 50 µl of 0.05 mg/ml bleomycin sulfate, 200 µl of 5 mM $MgCl_2$, and 100 µl of 50 µM $FeCl_2$. After mixing, the reaction mixture was incubated at 37°C for 1 h. The reaction was then terminated by addition of 0.1 M EDTA (50 µl). The color was developed by adding 0.5 ml each of 1% (w/v) TBA and 25% (v/v) HCl followed by heating at 80°C for 10 min. After centrifugation, the extent of DNA damage was indicated by an increase in absorbance at 532 nm. L-Ascorbic acid was used as a positive control in this assay.

Statistical analysis

All experiments were performed in triplicate. The results are presented as the mean ± standard deviation (SD). The IC_{50} values were determined by non-linear regression analysis using Microsoft Excel 2002. Bivariate correlations were analyzed by Spearman's test using SPSS 10.0 on Windows. Statistically significant differences in radical scavenging activity among plant extracts were performed using unpaired one-way analysis of variance (ANOVA) followed by Scheffe's test; $p < 0.05$ was considered statistically significant.

Results and Discussion

Ten methanol extracts of the seven selected plants were screened for their free radical scavenging activities by using DPPH, hydroxyl radical ($OH^{\bullet}$), and superoxide anion ($O_2^{\bullet -}$) assays. Their IC_{50} values were calculated and compared with those of the standard compounds as shown in Figure 1. Of all the extracts examined, the one prepared from lotus leaves was shown to have the best antioxidant activity. It exhibited the highest potency in both DPPH and $O_2^{\bullet -}$ inhibition at concentrations comparable with those of the standards and also possessed strong scavenging activity for $OH^{\bullet}$.

In the DPPH assay, widely used as a screening test for the proton-donating ability of plant extracts (Zhu et al., 2002), most of the extracts in the current study were effective in inhibiting $DPPH^{\bullet}$. Lotus leaf extract had the strongest activity with an IC_{50} value of 90 µg/ml. The BHT control had an IC_{50} of 30 µg/ml (i.e., three-times more active). The extracts from lotus seed heads and *Laurentia longiflora* were not so effective in this system. The samples were also measured for their $OH^{\bullet}$ and $O_2^{\bullet -}$ free radical scavenging abilities. In cellular oxidation reactions, $O_2^{\bullet -}$ is normally formed first, and its effects can be magnified because it produces other kinds of cell-damaging free radicals including the most destructive radical, $OH^{\bullet}$ (Decker, 1998). The crude extracts that showed the best activity in the hydroxyl assay were those from lime leaves and lime peels with IC_{50} values of 155 µg/ml and 164 µg/ml, respectively, about half that of the standard tannin ($IC_{50} = 325$ µg/ml) (Fig. 1C). Lotus seeds head extract had very little activity in any of these scavenging tests (Fig. 1). When the relative inhibitory potency of the ten extracts on each free radical type was compared, some differences did exist. The rankings of the IC_{50} values in the DPPH assay (*N. nucifera* leaf > *N. nucifera* pollen > *A. evecta* > *P. oleracea* > *S. indica* > *P. sarmentosum* > *C. hystrix* leaf > *C. hystrix* peel > *N. nucifera* seeds head > *L. longiflora*) (Fig. 1A) and the $O_2^{\bullet -}$ assay (*N. nucifera* leaf > *S. indica* > *P. sarmentosum* > *A. evecta* > *N. nucifera* pollen > *C. hystrix* leaf > *C. hystrix* peel > *P. oleracea* > *N. nucifera* seeds head > *L. longiflora*) (Fig. 1B) were similar but considerably different from those for the $OH^{\bullet}$ assay (*C. hystrix* leaf > *C. hystrix* peel > *A. evecta* and *N. nucifera* leaf > *S. indica* > *N. nucifera* pollen > *L. longiflora* > *P. sarmentosum* > *P. oleracea* > *N. nucifera* seeds head) (Fig. 1C). With the exception of lime leaf, lime

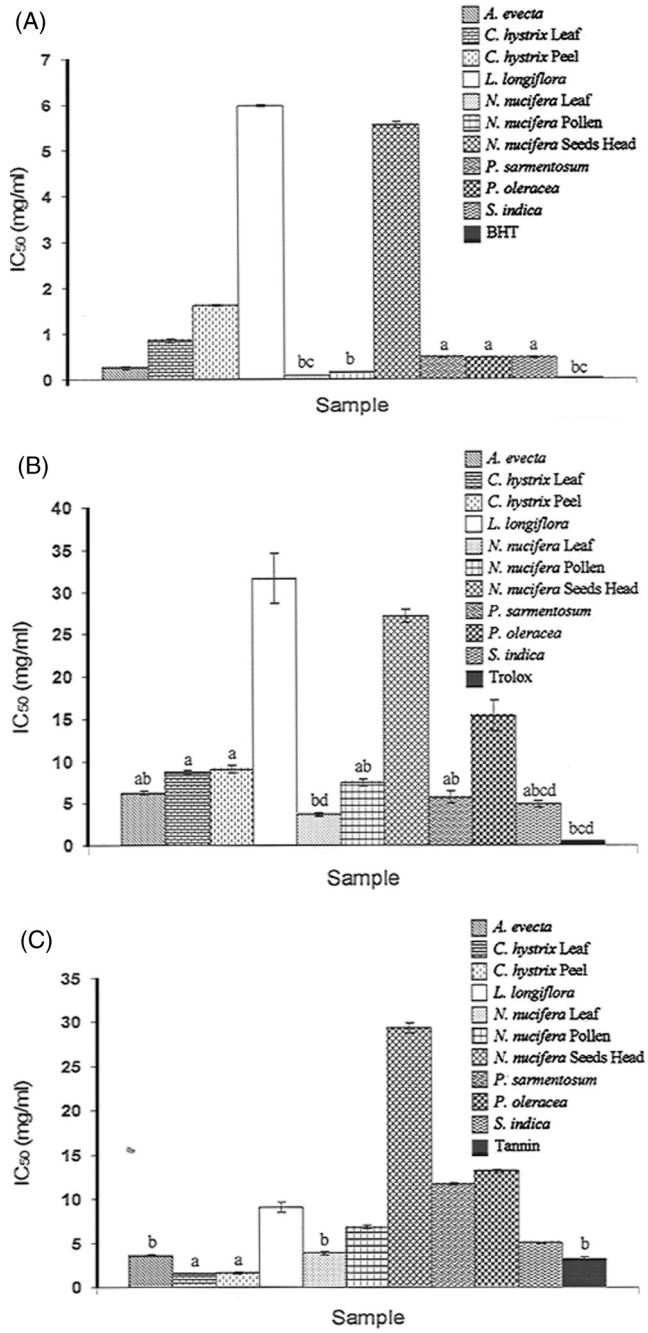

Figure 1. Comparison of IC_{50} values of the plant extracts against (A) DPPH, (B) $O_2^{\bullet -}$, and (C) $OH^{\bullet}$ free radicals. Each bar represents the mean $\pm$SD of triplicate values. Those bars with the same letter are not statistically different from each other ($p > 0.05$).

peel, and *L. longiflora* extracts, most of them became less effective at inhibiting $OH^{\bullet}$ than scavenging for $DPPH^{\bullet}$. The results imply that the $OH^{\bullet}$ scavenging activities of these plant extracts may not be solely attributable to their proton-donating abilities.

It is generally known that the $OH^{\bullet}$ generated from an Fe^{2+}-catalyzed Fenton's reaction can be effectively suppressed not only by trapping the radicals but also by chelating free iron and thereby preventing the redox cycle of this transition metal ion. Based on such a concept, the crude extracts were then evaluated for their Fe^{2+} chelating abilities. The results are presented in Table 2. The ten extracts displayed a concentration-dependent effect. Among them, lotus leaf and *Angiopteris evecta* rhizome extracts were by far the most effective chelators in this assay producing 99.6% and 71.2% binding, respectively, at 2 mg/ml. Binding by extracts from other plants at 2 mg/ml ranged from 11% to 38%. Comparison of these results with the data in Figure 1 C also reveals a high correlation between the metal binding capacity of the tested extracts and their $OH^{\bullet}$ scavenging activities [Spearman's correlation coefficient (ρ_S) = 0.624]. With the exception of lotus leaf extract, they depend on each other.

Phenolics are the most widespread secondary metabolites in the plant kingdom. This diverse group of compounds has received much attention as potential natural antioxidants in terms of their abilities to act as both efficient radical scavengers and metal chelators (Sanchez-Moreno et al., 2000). We have therefore determined the total phenolics present in these extracts using the modified Folin-Ciocalteau method. Values are expressed as catechin equivalents per mg of dried plant extract (Table 3). The results obtained seem to follow those in the metal binding assay ($\rho_S = 0.745$). The findings that the lotus leaf and *A. evecta* extracts contained the most abundant phenolics while those exhibiting rather weak metal binding activity such as the lotus pollen, *L. longiflora*, and *Piper sarmentosum* were not so rich in these compounds, also confirm the contribution of phenolics in the tested extracts to their metal chelation activities. According to the above results, it is likely that the $OH^{\bullet}$ scavenging capability of the plant extracts could be predicted on the basis of their total phenolic contents. However, both radical scavenging and metal binding properties of phenolics are also related to their structural features (Arora et al., 1998; Brown et al., 1998; Croft, 1998; Morel et al., 1998). Hence, in addition to the amounts, the structural variations of phenolic constituents in extracts from different plants would have some effects on their antioxidant activities.

In addition to their free radical scavenging activities, we also evaluated the plant extracts for their abilities to protect biomolecules from oxidative damage. In the lipid peroxidation system, all extracts were able to decrease the oxidation of rat brain homogenate initiated by $OH^{\bullet}$. Oxidation of unsaturated fatty acids in biological membranes leads to the formation and propagation of lipid radicals, uptake of oxygen, rearrangement of the double bonds in unsaturated lipids, and eventual destruction of membrane lipids, with production of breakdown products such as malondialdehydes (Slater et al., 1987). At the testing concentrations of 250 and 500 μg/ml, all were inhibitory (Table 4). The extracts of *Portulaca*

Table 2. The Fe^{2+}-chelating activities of the plant extracts.

Sample	Concentration		
	0.5 mg/ml	1 mg/ml	2 mg/ml
A. evecta rhizome extract	4.31 ± 1.70	29.6 ± 1.78	71.2 ± 0.51
C. hystrix leaf extract	6.89 ± 1.05	10.9 ± 0.68	23.7 ± 1.31
C. hystrix peel extract	10.1 ± 1.02	16.8 ± 0.34	33.1 ± 1.21
L. longiflora extract	9.31 ± 2.04	15.6 ± 0.79	18.5 ± 1.95
N. nucifera leaf extract	10.8 ± 0.25	61.1 ± 1.01	99.6 ± 0.19
N. nucifera pollen extract	3.25 ± 0.10	7.10 ± 0.51	18.0 ± 1.56
N. nucifera seeds head extract	3.86 ± 0.44	10.9 ± 0.61	20.1 ± 0.70
P. sarmentosum extract	9.30 ± 0.59	12.0 ± 0.26	20.0 ± 1.69
P. oleracea extract	5.21 ± 0.44	8.63 ± 0.10	11.2 ± 0.35
S. indica extract	16.8 ± 0.68	28.8 ± 0.79	38.0 ± 3.75
0.2 M citric acid (positive control)		96.9 ± 0.26	

Data are presented as the mean ± SD of each triplicate test. All values are expressed as percentage of chelation calculated from the equation stated in the "Materials and Methods" section.

oleracea, lime leaf, lime peel, and *L. longiflora* that produced less than 50% inhibition at 250 μg/ml produced more inhibition at 500 μg/ml. The maximum inhibition achieved was just less than 80% so those extracts that produced more than 60% inhibition gave only a small or no increase at 500 μg/ml. The most active extracts at 250 μg/ml from *A. evecta* and lotus leaf and pollen were those that gave the best activity in the DPPH assay. Therefore, those active materials in the tested extracts that can donate protons to peroxyl radicals and terminate the radical chain reaction may play a major role in this case. Surprisingly, the anti-lipid peroxidation activities of the plant extracts were not dependent on their $OH^{\bullet}$ scavenging activities. Lime leaf and lime peel extracts that previously demonstrated the highest and the next highest potency with respect to their $OH^{\bullet}$ scavenging assay (Fig. 1C) were less effective in this system, whereas those from the lotus seeds head and lotus pollen were among the most active ones. The reasons for such differences are not clear. In contrast with the inhibition of $OH^{\bullet}$ in the deoxyribose system, the protection of lipids from metal ion–mediated oxidation is also dependent on the solubility characteristics of the antioxidants. The more lipid-soluble they are, the better they may reach the site of oxidation and act on the reactive radicals.

In a study employing RBC hemolysis mediated by the peroxyl radicals from APPH thermolysis, the abilities of the ten extracts to inhibit non-metal-dependent lipid peroxidation on the RBC membrane were investigated (Table 5). The results that differ from those using the brain homogenate assay may arise from the different mechanisms involved in the two assays. The inhibitory process in the Fenton-type lipid peroxidation system is dependent on a number of factors such as metal chelation and proton or electron donation to the affecting radicals, whereas the hemolysis assay is less complex.

Table 3. Total amounts of phenolics in the plant extracts.

Extract	Total phenolics (μ mole of cathechin-equivalent/mg of dried extract)
A. evecta rhizome	9.31 ± 0.40
C. hystrix leaf	8.19 ± 0.40
C. hystrix peel	6.58 ± 0.14
L. longiflora	1.01 ± 0.38
N. nucifera leaf	15.98 ± 0.16
N. nucifera pollen	5.28 ± 0.26
N. nucifera seeds head	0.23 ± 0.14
P. sarmentosum	6.41 ± 0.14
P. oleracea	4.34 ± 0.16
S. indica	5.97 ± 0.14

Data are presented as the mean ± SD of each triplicate test.

Table 4. Effect of the plant extracts on lipid peroxidation in a rat brain homogenate.

Sample	Concentration	
	250 μg/ml	500 μg/ml
A. evecta rhizome extract	74.58 ± 0.26	77.58 ± 0.14
C. hystrix leaf extract	45.26 ± 0.41	70.18 ± 0.46
C. hystrix peel extract	26.06 ± 0.15	45.30 ± 0.27
L. longiflora extract	27.20 ± 0.14	32.36 ± 0.13
N. nucifera leaf extract	74.32 ± 0.39	75.46 ± 0.14
N. nucifera pollen extract	70.73 ± 0.41	76.16 ± 0.38
N. nucifera seeds head extract	64.76 ± 0.45	75.08 ± 0.51
P. sarmentosum extract	69.29 ± 0.33	72.80 ± 0.32
P. oleracea extract	49.19 ± 0.60	70.64 ± 0.19
S. indica extract	69.08 ± 0.27	70.30 ± 0.26
BHT	77.96 ± 0.08	79.36 ± 0.19

Data are presented as the mean ± S.D of each triplicate test. All values are expressed as percent inhibition of lipid peroxidation calculated from the equation stated in the "Materials and Methods" section.

Table 5. Effects of the plant extracts on hemolysis of human erythrocytes.

Sample	Concentration	
	250 μg/ml	500 μg/ml
A. evecta rhizome extract	56.08 ± 0.55	63.80 ± 0.69
C. hystrix leaf extract	90.02 ± 0.85	98.14 ± 0.19
C. hystrix peel extract	87.47 ± 0.73	98.06 ± 0.13
L. longiflora extract	69.77 ± 2.03	78.84 ± 0.25
N. nucifera leaf extract	93.82 ± 0.13	98.87 ± 0.07
N. nucifera pollen extract	64.60 ± 1.02	67.14 ± 0.43
N. nucifera seeds head extract	52.50 ± 0.27	57.17 ± 0.65
P. sarmentosum extract	80.08 ± 0.30	97.41 ± 0.07
P. oleracea extract	67.35 ± 0.49	81.21 ± 0.25
S. indica extract	85.57 ± 0.12	93.77 ± 0.26
Ascorbic acid	94.03 ± 0.30	96.47 ± 0.61

Data are presented as the mean ±SD of each triplicate test. All values are expressed as percent inhibition of hemolysis calculated from the equation stated in the "Materials and Methods" section.

Table 6. Pro-oxidant effects of the plant extracts on ferric bleomycin induced DNA damage.

Sample	Concentration	
	100 μg/ml	200 μg/ml
Blank	0.006 ± 0.001	
A. evecta rhizome extract	0.031 ± 0.001	0.059 ± 0.001
C. hystrix leaf extract	0.037 ± 0.001	0.066 ± 0.001
C. hystrix peel extract	0.022 ± 0.001	0.030 ± 0.002
L. longiflora extract	0.019 ± 0.001	0.029 ± 0.001
N. nucifera leaf extract	0.064 ± 0.002	0.123 ± 0.003
N. nucifera pollen extract	0.018 ± 0.001	0.023 ± 0.001
N. nucifera seeds head extract	0.016 ± 0.001	0.021 ± 0.001
P. sarmentosum extract	0.039 ± 0.001	0.079 ± 0.003
P. oleracea extract	0.087 ± 0.002	0.031 ± 0.001
S. indica extract	0.033 ± 0.001	0.061 ± 0.001
BHT	0.066 ± 0.002	0.013 ± 0.001
Ascorbic acid	0.631 ± 0.002	1.231 ± 0.002

Data are presented as the mean ±SD of each triplicate test. All values represent the extent of DNA damage and are expressed as the absorbance (O.D.) at 532 nm.

While the same mode of inhibition by donating protons to the peroxyl radicals was expected in both the DPPH and AAPH-induced RBC hemolysis assays, the potencies of these extracts to protect RBC membrane lipids from the AAPH-induced oxidation still varied from their $DPPH^{\bullet}$ scavenging activities (Fig. 1A). This could be due to the antioxidant materials of the plant extracts possessing differences in their lipid solubilities.

Although vitamin C is a strong antioxidant, a high intake of this vitamin may, in some situations, lead to a pro-oxidant activity in the body especially when free transition metals are available at the same time (Herbert et al., 1996). In this study, the pro-oxidant activity of vitamin C was more than ten times higher than that of all plant extracts when tested using the bleomycin-dependent DNA damage as a parameter. Bleomycin is an antibiotic that is also known for its capability to degrade DNA upon reacting with Fe^{2+} in the presence of O_2 (Gutteridge et al., 1981). In the method used, the resulting DNA fragments were detected as a pink chromogen of base propenals upon heating with thiobarbiturate at low pH. Increased absorbance at 532 nm indicating a stimulation of DNA degradation was then adopted as the pro-oxidant properties of test materials. At both concentrations tested, only lotus leaf extract displayed a slight pro-oxidant effect while the rest were very low (Table 6). All extracts also displayed a concentration-dependent pro-oxidation, except that of *P. oleraceae* and BHT, which showed inhibition in response to their increased concentrations under the same test system.

In the current study, we have demonstrated that methanol extracts of seven Thai medicinal plants contained constituents that could serve as natural sources to develop antioxidants. Among these plants, which are common and abundant in Thailand, the sacred lotus leaf seems to be most worthy of further studies because it showed remarkable activities in comparison with the others. Currently, efforts are underway to isolate and identify the active components responsible for its relatively high antioxidative activity.

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