Journal of Medicinal Plants Research Vol. 5(31), pp. 6822-6831, 23 December, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.1222

Full Length Research Paper

Antioxidant activity and bioactive phytochemical contents of traditional medicinal plants in northeast Thailand

Pornkamon Sakong, Tueanjit Khampitak, Ubon Cha'on, Chadamas Pinitsoontorn, Pote Sriboonlue, Puangrat Yongvanit and Patcharee Boonsiri*

Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.

Accepted 14 November, 2011

Traditional medicinal plants are efficacious for many ailments, but most of them still lacked the supportive scientific information for their therapeutic properties. We collected 31 medicinal plant species from 19 families commonly used as traditional medicine in northeast Thailand and classified into digestive tonic, diarrheal relief, anti-tussive and anti-inflammation groups. Their total antioxidant activity, free radical scavenging activity, total phenolic and ascorbic acid contents were determined. The results showed that these biological parameters were highly variable among plants examined. Stronger total antioxidant activity and higher amount of total phenolics and ascorbic acid were shown in anti-tussive than diarrheal relief and anti-inflammation plants, but digestive tonic group possessed strong free radical scavenging activity. When the top three plants with relatively high phenolics were selected from each group, alkaloids contents were found at high level in digestive tonic and anti-tussive plants, whereas tannins were high in digestive tonic and diarrheal relief plants. Our data provided significant scientific data on biological activities and phytochemical compositions of Thai medicinal plants consumed by local people in relation to their therapeutic activities.

Key words: Thai medicinal plants, 1,1-diphenyl-2-picryl hydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), antioxidant activity, phenolic compounds, alkaloids, ascorbic acid.

INTRODUCTION

Many plants containing phytochemicals have curative/protective properties against various diseases. Most phytochemicals, especially phenolics have health benefits by scavenging free radicals or quenching reactive oxygen species (Halliwell, 1997). Phenolics are, at least in part, plants responsible for antioxidant activity, and their contents in plants were associated with antioxidant activity (Tsai et al., 2008). Ascorbic acid also has antioxidant activity and is essential for the maintenance of normal function of living cells (Peckenpaugh, 2010). It has been reported that the majority of drugs come from natural resources and that approximately 60 to 80% of the world's population still believe in folk/traditional medicine (Kumar et al., 2006). Medicinal plants are therefore the main source of new pharmaceutical and health care products. According to Thai traditional medicine, some medicinal plants have been used for the treatment of many ailments. For instance, one of the popular Thai medicinal plants, a local name "Krachai dum" (*Kaempferia parviflora*) has been used for anti-inflammation (Tewtrakul et al., 2009), anti-allergy (Tewtrakul et al., 2008), anti-microbial (Chanwitheesuk et al., 2007) drugs and proven to have analgesic activity (Nualkaew et al., 2009). Similarly, Siamese neem tree (*Azadirachta indica*) has been used for the treatment of inflammation and skin diseases (Clayton et al., 1996), rheumatic and arthritic disorders including fever and diabetes (Van der Nat et al., 1991). However, comprehensive scientific investigations are required to access the usefulness of Thai medicinal plants in treating diseases.

The aim of this study is to determine total antioxidant activity, radical scavenging activity, total phenolic and

*Corresponding author. E-mail: patcha_b@kku.ac.th. Tel: +66 4334 8386; Fax: +66 4334 8386.

ascorbic acid contents of 31 local Thai medicinal plant species. In addition, several plants having high phenolic contents were selected and some important phytochemicals related to health benefits were determined semi-quantitatively. The relationship between biochemical activities and phytochemical compositions of the plants and the traditional medicinal wisdom in Thailand is discussed.

MATERIALS AND METHODS

Plant collection and extraction

Thai medicinal plants were collected from natural, but not cultivated, resources in three provinces, that is, Sakon Nakhon, Loei and Nong Bua Lamphu in northeast Thailand during September 2008 to February 2009. Taxonomic identification of the plants was performed using appropriate taxonomic keys.

Plants were cleaned, air-dried and their edible portions were homogenized in an electrical blender and subsequently with a mortar and pestle. The homogenized samples were extracted with 70% (v/v) ethanol in a ratio of 1:10 (w/v) by vigorous shaking with a vortex mixer for 5 min at 30°C. The supernatants were then separated from the residues by centrifugation at 2000 rpm for 10 min and filtered through Whatman No. 4 filter paper. The remaining residues were re-extracted and the two supernatants were combined. The crude extracts were stored at 4°C for further analyses.

Chemicals and reagents

Folin-Ciocalteau reagent, 1,1-diphenyl-2-picryl hydrazyl (DPPH), 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were all obtained from Fluka, Germany. Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) and ferric chloride hexahydrate ($FeCl_3.6H_2O$) were purchased from Scharlu, Spain. Gallic acid ($C_7H_6O_5$) was purchased from Sigma, Germany. The other chemicals and solvents used were analytical grade.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was modified from Benzie and Strain (1996) for the studies on serum. FRAP reagent was prepared by mixing 300 mM sodium acetate buffer pH 3.6 with 10 mM TPTZ solution in 40 mM hydrochloric acid and 20 mM ferric chloride in a proportion of 10:1:1 (v/v), respectively. Sample (500 µl of the plant extract) was mixed with 500 µl of FRAP reagent and incubated at 37°C for 4 min. The absorbance at 593 nm was measured (GENESYS^{TM}20, USA) against the reagent blank. Ferrous sulfate solution (100 to 2000 µM) was used as standard.

1,1-Diphenyl-2-picryl hydrazyl (DPPH) assay

The free radical scavenging activity by DPPH assay was measured using a modified version of the method (Gulcin et al., 2004). An aliquot of 20 µl of the plant extract was added to 1000 µl of 0.1 mM DPPH solution. The reaction mixture was incubated in the dark at 30°C for 10 min and then absorbance at 517 nm was recorded. The capacity of DPPH radical scavenging was expressed as mmol Trolox equivalent antioxidant capacity per 100 g dry weight of plant (TEAC/100 g DW).

2,2′-Azinobis(3-ethylbenzoline-6-sulphonate) radical cation decolorization (ABTS) assay

The ABTS assay was measured with a slight modification from that of Re et al. (1999). The $ABTS^{\circ+}$ radical was generated by the oxidation of 8 mM ABTS with 3 mM potassium persulphate ($K_2S_2O_8$) in the dark at 30°C for 15 h. Before use, $ABTS^{\circ+}$ solution was diluted with 70% ethanol until the absorbance at 734 nm became 0.700 ± 0.030. Fifteen microlitre of the plant extract was added to 1485 µl $ABTS^{\circ+}$ solution, and the absorbance at 734 nm was recorded 1 min later. The capacity of $ABTS^{\circ+}$ radical scavenging was expressed as mmol TEAC/100 g DW.

Determination of total phenolic content

Total phenolic content was determined by the Folin-Ciocalteau method with a slight modification from that employed by Singleton and Rossi (1965). Sample (100 µl of the plant extract) was mixed with 500 µl of 0.2 M Folin-Ciocalteau reagent and kept at 30°C for 4 min, then 400 µl of 7% sodium carbonate solution was added. The reaction mixture was incubated at 30°C for 30 min in the dark and the absorbance was measured at 765 nm. Gallic acid was used as a standard and the total phenolic content was expressed as mg of gallic acid equivalents per 100 g dry weight of the plant (mg GAE/100 g DW).

Determination of ascorbic acid content

Ascorbic acid content was determined by an aluminium molybdate assay modified from Bajaj and Kaur (1981). Fresh plants were homogenized in an electrical blender and subsequently with a mortar and pestle. The homogenized sample (5 g each) was transferred into a test tube and 5 ml of the oxalic acid-EDTA solution (50 mM oxalic acid and 0.2 mM EDTA, ratio 1:1) was added. The mixture was shaken by a vortex mixer at 30°C for 5 min. The extract was filtered through Whatman No. 4 filter paper and then centrifuged at 2000 rpm for 10 min to separate the residues. The remaining residues were re-extracted and the two supernatants were combined and adjusted to a final volume of 10 ml with oxalic acid-EDTA, and kept at 4°C for further analysis.

A 500 µl crude extract was transferred into a test tube and added with 500 µl of oxalic acid-EDTA, 100 µl of metaphosphoric acid-acetic acid (3.75 M metaphosphoric acid in 170 mM acetic acid), 200 µl of 5% sulfuric acid, and 400 µl of aluminium molybdate. After 15 min the solutions were measured at 760 nm using ascorbic acid as a standard.

Tests for phytochemical compounds

The presence of some phytochemicals related to biological activity such as alkaloids, tannins, steroids and saponins, were tested in the plant extracts by the method of Kasolo et al. (2010). Three plants with high total phenolic contents in each therapeutic group were selected for testing the presence of phytochemicals. For this purpose, 25 g of the homogenized plants were extracted with 70% ethanol by the same procedure used for antioxidants measurement and then evaporated at 45°C. The solid crude extract was stored at 4°C until further analysis. For testing, the solid crude extracts were dissolved in deionized water to a final concentration of 10 mg/ml.

To detect the presence of alkaloids, 160 µl of 2% hydrochloric acid was added to 1 ml crude extract solution followed by the addition of 160 µl Dragendorff's reagent (12 ml of 1.33 M bismuth

Table 1. Medicinal plants examined including their Thai and scientific names, parts used, therapeutic purposes and medicinal groups.

Thai name	Scientific name	Family name	Parts used	Therapeutic purposes
Digestive tonic group (n = 11)				
Kun jong	*Limnocharis flava*	Alismataceae	Leaf	Digestive tonic, restorative, stimulate appetite
Hon han	*Pluchea eupatorioides*	Compositae	Leaf	Digestive tonic
Phak pae	*Hydrocharis dubia*	Hydrocharitaceae	Leaf, stem	Digestive tonic, expectorant, restorative, stops dysentery
Towa-yai	*Ottetia alismoides*	Hydrocharitaceae	Leaf	Digestive tonic
Towa-lek	*Blyxa echinosperma*	Hydrocharitaceae	leaf	Digestive tonic
Mang kang	*Ocimum gratissimum*	Labiatae	Leaf	Digestive tonic, carminative, restorative, relieves stomach ache and flatulence
Khi khom	*Glinus oppositifolius*	Molluginaceae	Leaf, stem	Digestive tonic, carminative, restorative, relieves stomach ache and flatulence
Nam tewlew	*Toddalia asiatica*	Rutaceae	Leaf	Digestive tonic
Ma sang	*Feroniella lucida*	Rutaceae	Stem	Digestive tonic, carminative, relieves flatulence
Sa ngae	*Trachyspermum* roxburghianum	Umbelliferae	Leaf, stem	Digestive tonic, relieves flatulence, nourishes the heart
Chi lao	*Anethum graveolens*	Umbelliferae	Leaf, stem	Digestive tonic, carminative, promotes diaphoresis, reduces swelling, relieves beriberi
			Root	Digestive tonic, carminative
Diarrheal relief group (n = 6)				
Ma kok	*Spondias pinnata*	Anacardiaceae	Leaf	Diarrheal relief, anti-tussive, anti-hyperglycemia, anti-rhinitis
Mok khruea	*Aganosma marginata*	Apocynaceae	Leaf	Diarrheal relief, treats hemorrhoids
Ma klam	*Adenanthera pavonina*	Leguminosae Mimosoideae	Leaf	Diarrheal relief, stops dysentery, restorative
Wai	*Calamus siamensis*	Palmae	Stem	Diarrheal relief
Ma tum	*Aegle marmelos*	Rutaceae	Leaf	Diarrheal relief, anti-pyretic, relieves asthma
Deplachon	*Tacca chantrieri*	Taccaceae	leaf	Diarrheal relief, digestive tonic
Anti-tussive group (n = 7)				
Som lom	*Aganonerion polymorphum*	Apocynaceae	Leaf	Anti-tussive, expectorant, use for muscle pain
Jamuk plalai	*Oxystelma esculentum*	Asclepiadaceae	Leaf	Anti-tussive, relieves throat pain
Mak som siao	*Bauhinia malabarica*	Leguminosae-Caesalpinioideae	Fruit	Anti-tussive, relieves throat pain
Phai	*Butomopsis latifolia*	Limnocharitaceae	Leaf	Anti-tussive
			Flower	Anti-tussive
Phia fan	*Clausena excavata*	Rutaceae	Leaf	Anti-tussive, anti- hyperglycemia, anti-rhinitis
Song fa	*Clausena harmandiana*	Rutaceae	Leaf	Anti-tussive, relieves throat pain
Ob-ab	*Cissus hastata*	Vitaceae	Leaf, stem	Anti-tussive, expectorant, relieves flatulence

Table 1. Contd.

Anti-inflammation group (n = 8)				
Khi nak	*Dregea volubilis*	Asclepiadaceae	Leaf	Poultice for wounds
Khlu	*Pluchea indica*	Compositae	Leaf	Treats hemorrhoids, diuretic
Kon kan	*Dracaena angustifolia*	Dracaenaceae	leaf	Relieves stomach ache
Niam huesea	*Plectranthus amboinicus*	Labiatae	leaf	Cure otorrhea, otitis
Ma fueang	*Averrhoa carambola*	Oxalidaceae	leaf	Relieves toothache, relieves irritation
Som kop	*Hymenodictyon orixense*	Rubiaceae	leaf	Relieves toothache, relieves irritation
Song fa	*Clausena harmandiana*	Rutaceae	root	Relieves headache, restrains lactation
Phet sangkhat	*Cissus quadrangularis*	Vitaceae	leaf, stem	Anti-hypertensive, poultice for swelling, digestive tonic

nitrate in 30% nitric acid and 50 ml of 3.26 M potassium iodide and then the volume was adjusted to 100 ml with distilled water). An orange or red precipitate indicated the presence of alkaloids. Caffeine (10 mg/ml) was used as a standard.

To test for tannins, 90 µl of 1% w/v ferric chloride solution was added into 1 ml of crude extract solution. A blackish blue color indicated the presence of tannins. Gallic acid (10 mg/ml) was used as a standard.

In the case of steroids test, 1 ml of the crude extract solution was added with 500 µl of chloroform. The mixture was pipetted into test tube and 1 ml of concentrated sulfuric acid was added. A red upper layer and a yellowish sulfuric acid layer with a green fluorescence indicated the presence of steroids. Cholesterol (10 mg/ml) was used as a standard.

To detect saponins, 90 µl of dimethylsulfoxide (DMSO) and 5 ml of distilled water were added into 1 ml of the crude extract solution. The mixture was shaken well and foam formation persisting for more than 15 min was taken, which indicated the presence of saponins. Saponins (10 mg/ml) was used as a standard.

Statistical analysis

Each measurement was carried out in triplicate, and the results were shown as mean ± SD. Correlation coefficients between the level of phenolics or ascorbic acids and antioxidant/radical scavenging activities were calculated by using SPSS, version 11.5. The probability of $P < 0.050$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Species of the plants studied and the specific parts examined and their therapeutic function as informed by the local dwellers in the three provinces are shown in Table 1. Based on their therapeutic purposes as suggested by the local dwellers with reference to Thai traditional medicine texts, a total of 31 plants were classified into four defined groups, that is, digestive tonic (n = 11), diarrheal relief (n = 6), anti-tussive (n = 7) and anti-inflammation (n = 8) groups. In the present study, to obtain a high antioxidant activity in the plant extracts, 70% (v/v) ethanol was used as an extraction solvent. The use of mixed ethanol and water solvent has been shown to obtain both high and low polarity antioxidants (Burkey et al., 2006). Total antioxidant and free radical scavenging activities, total phenolic and ascorbic acid contents of 31 studied plants are shown in Table 2.

Total antioxidant activity determined by FRAP assay, was highly variable ranging from as low as 0.43 ± 0.02 µmol Fe(II)/100 g DW in *Cissus hastata* to as high as 1865.19 ± 35.25 µmol Fe(II)/100 g DW in *Aganonerion polymorphum*. This is probably because the oxidative stress to plants depends on their growing environments, plants synthesize various antioxidant compounds including alkaloids, terpenes, phenolics and vitamins with varying proportions for protecting themselves (Diaz et al., 2001).

The free radical scavenging activity of the plant extracts were measured by two different methods, ABTS and DPPH assays. The results showed wide range of variation among the species of plants examined. In addition, the range of ABTS values (from 4.94 ± 0.10 to 291.60 ± 0.68 mmol TEAC/100 g DW) was far higher than that of DPPH values (from undetectable to 7.96 ±0.02 mmol TEAC/100 g DW). ABTS assay measures both hydrophilic and lipophilic antioxidants (Re et al., 1999), whereas DPPH assay determines only the hydrophilic one. Therefore, Thai medicinal plants generally contain higher proportion of lipophilic antioxidants. The results were supported by a good positive correlation ($r = 0.642$, $P = 0.000$) between ABTS and total antioxidant activities, suggesting that radical scavenging is a major mechanism of total antioxidant activity. Phenolic contents of the plant extracts were also highly variable, from the lowest in *Limnocharis flava* (2.39 ± 0.02 mg GAE/100 g DW) to the highest in *Clausena excavata* (1669.81 ± 6.33 mg

Table 2. Medicinal plants examined, scientific names and parts used, total antioxidant activity, free radical scavenging activity (ABTS and DPPH), total phenolic and ascorbic acid contents (mean ± SD).

Scientific name	Parts used	Total antioxidant activity	ABTS	DPPH	Total phenolic content	Ascorbic acid content
		(μmol Fe(II)/100 g DW)	(mmol TEAC/100 g DW)		(mg GAE/100 g DW)	(mg/100 g DW)
Digestive tonic group						
L. flava	Leaf	3.17 ± 0.04	21.94 ± 0.29	1.27 ± 0.15	2.39 ± 0.02	3.53 ± 0.12
P. eupatorioides	Leaf	26.99 ± 0.57	8.77 ± 0.03	2.65 ± 0.10	23.20 ± 0.78	2.85 ± 0.09
H. dubia	Leaf, stem	21.99 ± 0.55	17.32 ± 0.02	3.26 ± 0.11	51.42 ± 0.88	5.93 ± 0.17
O. alismoides	Leaf	2.03 ± 0.07	41.73 ± 0.61	ND	28.97 ± 1.25	7.64 ± 0.15
B. echinosperma	Leaf	4.50 ± 0.20	12.65 ± 0.05	0.58 ± 0.14	21.08 ± 1.65	5.40 ± 0.14
O. gratissimum	Leaf	3.45 ± 0.15	13.61 ± 0.51	0.92 ± 0.04	38.47 ± 0.31	3.91 ± 0.09
G. oppositifolius	Leaf, stem	7.96 ± 0.22	13.04 ± 0.09	ND	40.14 ± 1.82	1.74 ± 0.00
T. asiatica	Leaf	1668.87 ± 26.32	109.77 ± 0.78	2.53 ± 0.01	285.36 ± 2.21	2.55 ± 0.03
F. lucida	Stem	1154.69 ± 30.29	226.76 ± 0.26	2.12 ± 0.03	98.67 ± 1.73	5.19 ± 0.18
T. roxburghianum	Leaf, stem	28.74 ± 0.21	23.01 ± 0.30	0.53 ± 0.06	70.32 ± 0.86	5.84 ± 0.09
A. graveolens	Leaf, stem*	12.91 ± 0.27	18.58 ± 0.58	2.31 ± 0.07	41.15 ± 0.41	3.89 ± 0.10
	Root*	7.30 ± 0.05	12.80 ± 0.23	ND	24.50 ± 0.13	1.76 ± 0.08
Diarrheal relief group						
S. pinnata	Leaf	22.84 ± 1.34	188.65 ± 6.66	3.71 ± 0.06	728.64 ± 15.01	12.68 ± 0.09
A. marginata	Leaf	1640.01 ± 7.73	144.86 ± 0.50	2.26 ± 0.05	396.51 ± 7.86	9.89 ± 0.19
A. pavonina	Leaf	14.69 ± 0.34	34.70 ± 0.26	3.71 ± 0.07	120.37 ± 7.01	3.19 ± 0.09
C. siamensis	Stem	5.56 ± 0.05	4.94 ± 0.10	0.32 ± 0.06	10.40 ± 0.17	2.88 ± 0.03
A. marmelos	Leaf	145.73 ± 0.38	103.87 ± 1.41	2.05 ± 0.06	220.96 ± 6.13	6.36 ± 0.14
T. chantrieri	Leaf	23.70 ± 0.09	37.39 ± 0.37	ND	66.82 ± 1.49	9.95 ± 0.24
Anti-tussive group						
A. polymorphum	Leaf	1865.19 ± 35.25	291.60 ± 0.68	3.24 ± 0.04	647.05 ± 5.87	6.92 ± 0.20
O. esculentum	Leaf	340.97 ± 2.76	35.84 ± 0.84	5.23 ± 0.08	169.18 ± 7.82	14.11 ± 0.18
B. malabarica	Fruit	119.94 ± 0.60	34.82 ± 0.26	2.51 ± 0.03	726.43 ± 11.95	3.05 ± 0.05
B. latifolia	Leaf*	25.89 ± 0.20	47.06 ± 0.57	0.38 ± 0.10	57.60 ± 1.16	5.59 ± 0.13
	Flower*	8.17 ± 0.04	23.87 ± 0.54	ND	33.64 ± 0.56	2.44 ± 0.06
C. excavata	Leaf	964.65 ± 7.37	19.62 ± 0.08	2.02 ± 0.02	1669.81 ± 6.33	1.76 ± 0.06
C. harmandiana	Leaf*	1633.81 ± 7.48	92.49 ± 1.73	2.34 ± 0.01	126.07 ± 2.44	22.81 ± 0.05
C. hastata	Leaf, stem	0.43 ± 0.02	53.13 ± 1.72	6.61 ± 0.00	136.25 ± 7.31	25.86 ± 0.54
Anti-inflammation group						
D. volubilis	Leaf	214.57 ± 1.95	45.42 ± 1.08	3.29 ± 0.04	125.76 ± 4.72	7.44 ± 0.08

Table 2. Contd.

P. indica	leaf	79.83 ± 1.33	71.28 ± 0.62	7.96 ± 0.02	250.00 ± 7.58	3.56 ± 0.08
D. angustifolia	leaf	15.13 ± 0.33	10.49 ± 0.32	1.19 ± 0.02	26.42 ± 0.52	2.41 ± 0.06
P. amboinicus	leaf	12.62 ± 0.09	33.05 ± 0.33	3.89 ± 0.02	463.30 ± 3.53	3.74 ± 0.11
A. carambola	leaf	1.23 ± 0.04	160.01 ± 1.20	2.40 ± 0.02	339.62 ± 2.06	9.58 ± 0.33
H. orixense	leaf	7.18 ± 0.10	43.50 ± 0.71	3.07 ± 0.01	99.25 ± 4.01	1.19 ± 0.04
C. harmandiana	root*	270.12 ± 4.43	17.57 ± 0.97	1.12 ± 0.04	372.06 ± 13.76	0.94 ± 0.02
C. quadrangularis.	leaf, stem	67.87 ± 1.30	11.42 ± 0.27	2.94 ± 0.10	49.22 ± 2.33	41.59 ± 0.28

Values were the mean ± SD (n=3) and calculated in dry weight, DW = dry weight , ND = not detected, * Different plant parts were determined.

GAE/100 g DW). Thephenolic contents of plants are affected by both endogenous (for example, genetic) and environmental factors (for example, UV light, heavy metal and pathogens) (Achakza et al., 2009).

Ascorbic acid contents were also highly variable ranging from as low as 0.94 ± 0.02 mg/100 g DW in the root of *Clausena harmandiana* to as high as 41.59 ± 0.28 mg/100 g DW in the leaf and stem of *Cissus quadrangularis*.

Relationship between phenolics/ascorbic acid to antioxidant/scavenger activities

Since phenolics and ascorbic acid are both known to have antioxidant activity, we examined whether their contents in medicinal plants correlate to the total antioxidant activity or to the free radical scavenging activity. The results were shown in Figure 1. Total phenolic contents strongly correlated (r = 0.370, *P* = 0.031) to the total antioxidant activity (Figure 1A) and moderately to radical scavenging activity determined by ABTS. On the other hand, ascorbic acid content positively correlated (r = 0.291) to DPPH activity (Figure 1F) but not statistically significant probably due to small sample numbers. Close relationship of the scavenging properties of the plant extracts with the amount of ascorbic acid has been reported (Du et al., 2003; Burkey et al., 2006).

Relationship between biological activity and medicinal effects

Since antioxidant/radical scavenging activities and total phenolic/ascorbic acid contents were extremely variable among the medicinal plants examined, we analyzed further whether these activities or contents have any relations to the particular medicinal effects of the plants. For this purpose, total antioxidant activity, radical scavenging activity, total phenolics and ascorbic acid contents of each plant were scattered against the medicinal effects to visualize their correlation (Figure 2).

Among 11 digestive tonic plants, only 2 plants (*Toddalia asiatica* and *Feroniella lucida*) had high total antioxidant activity. These plants had also high ABTS scavenging activity, suggesting that their antioxidant activity is due to high scavenging activity (Table 2). In the digestive tonic group, total phenolic and ascorbic acid contents were very low compared with other medicinal plant groups (Figure 2). Therefore, whether phenolics have digestive tonic effects remained to be clarified. Among 6 diarrheal relief plants, 3 plants (*Spondias pinnata*, *Aganosma marginata* and *Aegle marmelos)* had high radicals scavenging activities by both ABTS and DPPH. In addition, these 3 plants had high total phenolic and ascorbic acid contents (Figure 2 and Table 2). Interestingly, *S. pinnata* possessed high radical scavenging activities and high phenolic and ascorbic acid contents, in contrast, its total antioxidant activity was at low level among all plants examined (Table 2*).* This plant might contain some inhibitors of antioxidants.

The anti-tussive plants had higher total antioxidant activity, free radical scavenging activities and higher total phenolic and ascorbic acid contents, comparing with other three medicinal plant groups (Figure 2). Intake of plants with high ascorbic acid content is known to prevent chronic cough (Grievink et al., 1998; Nosal et al., 2003). In addition, ascorbic acid can potentiate the immune system (Hemila, 2003). Moreover, in the anti- tussive effects described in the Ayurvedic (Scartezzini et al., 2006) and Chinese (Shu et al., 2010) traditional recipe, ascorbic acid, phenolic acids, flavonoids and alkaloids are the important ingredients to prevent against oxidative stress and decrease the activity

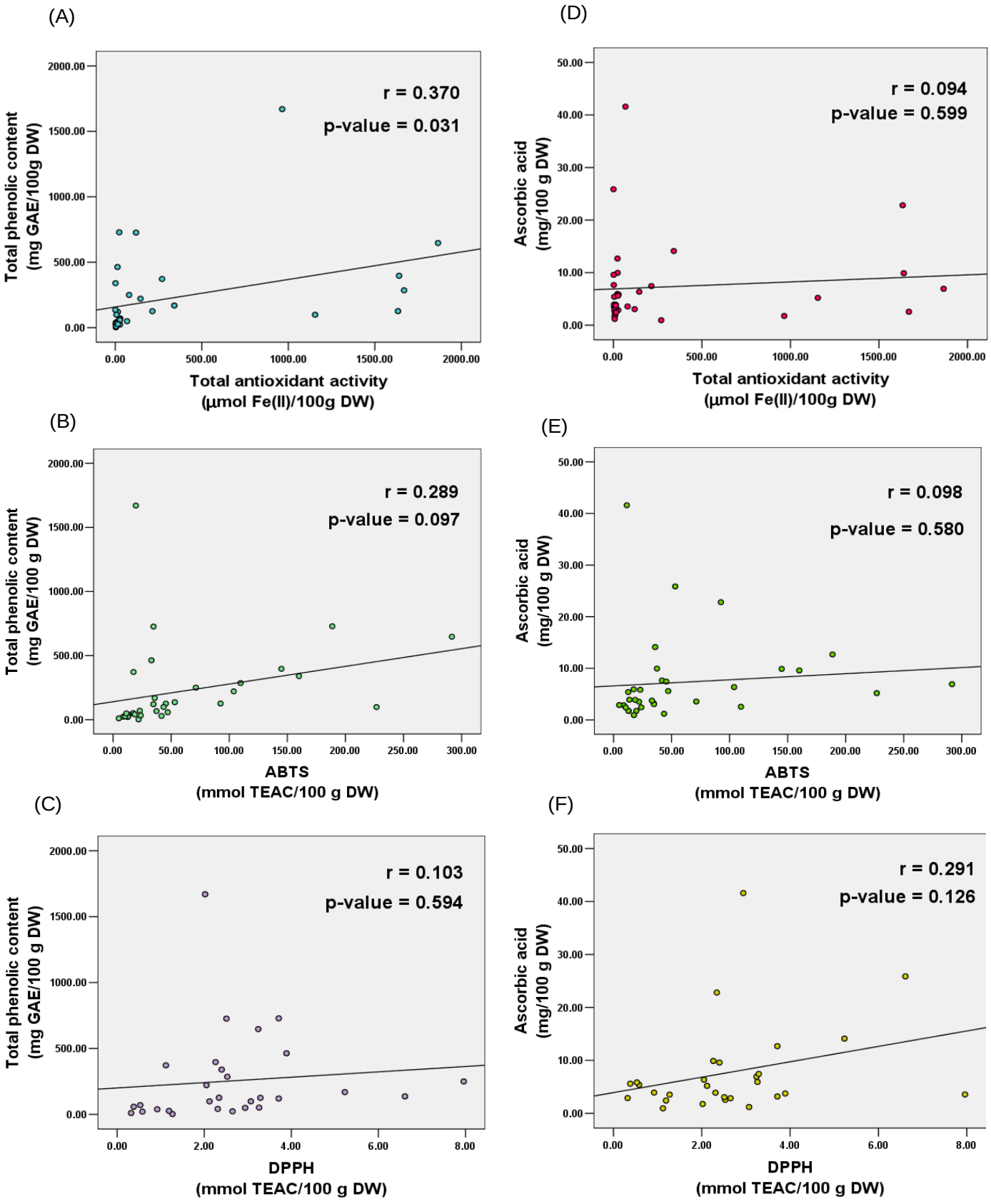

Figure 1. Relationship between total phenolic content and (A) total antioxidant, (B) ABTS, and (C) DPPH activities in the study plants. Relationship between ascorbic acid content and (D) total antioxidant, (E) ABTS and (F) DPPH activities.

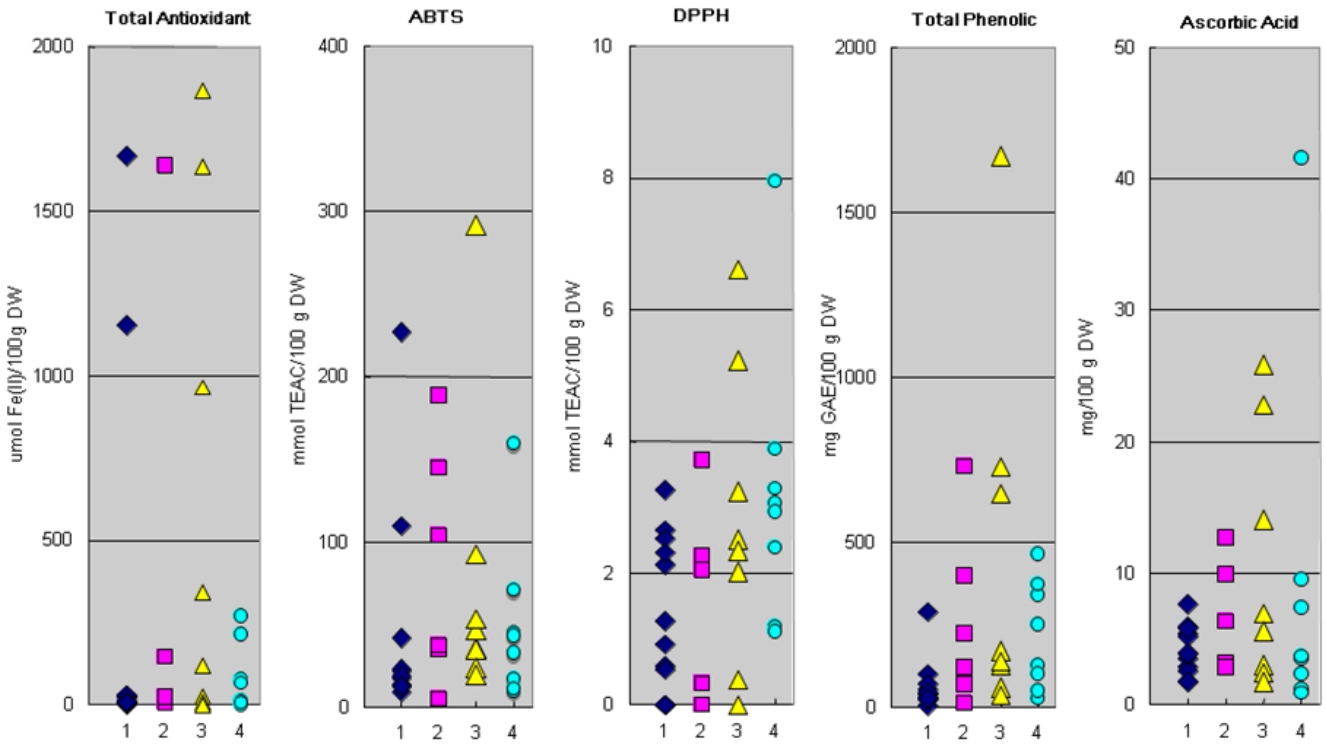

Figure 2. Relationship between the biological activities of plants (total antioxidant, ABTS and DPPH activities, total phenolic and ascorbic acid contents) and their medicinal effects (1: digestive tonic, 2: diarrheal relief, 3: anti-tussive, and 4: anti-inflammation).

of cholinesterase and xanthine oxidase and also increase the mucus secretion in the airway glands (Franova et al., 2006). Anti-inflammation plants have high DPPH but low ABTS and total antioxidant activity. Since DPPH technique measures only hydrophilic antioxidant activity, hydrophilic antioxidants may play an important role for anti-inflammatory effects. In this group, one plant, *C. quadrangularis* had extremely high ascorbic acid content. Supplement of vitamin C together with astaxanthin of *Ginkgo biloba* extract reduced respiratory inflammation in asthmatic guinea pig with the efficacy comparable to that of ibuprofen, a non-steroidal anti-inflammatory drug (Haines et al., 2011). Whether ascorbic acid has anti-inflammatory activity should be explored further. Although, polyphenolics such as flavonoids and tannins suppress inflammatory cytokines/chemokines (Aquila et al., 2009), scavenge free radical (Re et al., 1999) and inhibit xanthine oxidase activity (De las Heras et al., 1998), total phenolic contents in anti-inflammatory plants were comparable with other medicinal plants. Further compositional analyses will be necessary to elucidate anti-inflammatory substances.

Phytochemicals and medicinal effects

With respect to the therapeutic effects of medicinal plants, phenolics play the major role as antioxidant (Tsai et al., 2008). In addition, some phytochemicals such as alkaloids, tannins, steroids and saponins also play roles in exerting clinical effects. Therefore, we tested the presence of some phytochemicals in the top three of high total phenolic contents in each plant group (Table 3).

As shown in Figure 2, the plants in digestive tonic group have relatively low phenolic contents, although phenolics have protective effects on the digestive tract from mouth to colon (Tawaha et al., 2007). Interestingly, three plants selected with relatively high phenolic contents in this group have high contents of alkaloids (Table 3), which might be one of the important ingredients to exert digestive tonic effects, such as stimulation of bile secretion (Chan, 1977).

In case of diarrheal relief plants, flavonoids, tannins, saponins, steroids and alkaloids are known to inhibit bacterial infection and gastrointestinal diseases by modulating several enzymes or cell receptors (Palombo, 2006). Our results are in agreement with previous studies (Mukherjee et al., 1998; Palombo, 2006), in that various plants contain those phytochemicals with various degrees. As shown in Table 3, two of three selected plants in this group have high contents of gallic tannins. Tannins denature proteins to form protein tannate complex, which makes the intestinal mucosa more resistant and reduces electrolyte secretion (Mukherjee et al., 1998).

In anti-tussive group, all three selected plants having

Table 3. Phytochemicals tested in the ethanolic extracts of the top three medicinal plants of each plant group with high total phenolic content.

Medicinal plants	Parts used	Phytochemical tests			
		Alkaloids	Gallic tannins	Steroids	Saponins
Digestive tonic group					
T. asiatica	Leaf	+++	+++	++	++
F. lucida	Leaf	+++	++	+	+
T. roxburghianum	Leaf, stem	+++	-	++	-
Diarrheal relief group					
S. pinnata	Leaf	+	+++	+	++
A. marginata	Leaf	+	+++	++	+
A. marmelos	Leaf	+++	+	+++	+++
Anti-tussive group					
C. excavata	Leaf	+++	-	+	-
B. malabarica	Fruit	+++	++	++	-
A. polymorphum	Leaf	+++	-	+	+
Anti-inflammation group					
P. amboinicus	Leaf	+	+	++	+
C. harmandiana	Root	++	-	+++	-
A. carambola	Leaf	++	++	+	++

-: not detected, +: present in low, ++: present in moderate, and +++: present in high concentration. Values were duplicated.

high phenolics were also strongly positive for alkaloids (Table 3). Our results are in agreement with previous studies that total phenolic, ascorbic acid and alkaloids are mainly distributed in the anti-tussive plants (Grievink et al., 1998; Scartezzini et al., 2006). They might all contribute in scavenging of the free radicals in the respiratory tract thereby protecting from diseases and irritants (Gurbuz et al., 2009).

Distribution of biological activities in the plants

In the present study, only three plants were examined for biological activities (that is, total antioxidant activity, free radical scavenging activities) of different parts separately; leaf and stem combined and root separately from *Anethum graveolens*, leaf and flower separately from *Butomopsis latifolia* and leaf and root separately from *C. harmandiana*. For those three plants, different parts are used in different ways and/or different purposes in Thai traditional medicine (Tables 1 and 2). As reported previously, antioxidant activities or other parameters varied in different parts of the same plants (McCune and Johns, 2007; Achakza et al., 2009).

In conclusion, total antioxidant activity, free radical scavenging activity, total phenolic, ascorbic acid and some phytochemicals contents in studied plants were highly variable. We have demonstrated a significant correlation between total antioxidant activity and phenolic content, which showed that phenolics play major roles as antioxidants. In addition, the relationship between total antioxidant activity and ABTS or DPPH values showed that plant antioxidants had free radical scavenging activity, which might play a critical role in the medicinal properties against oxidative stress diseases. Furthermore, alkaloids may play important roles in the therapeutic properties such as digestive tonic and anti-tussive and also tannins for diarrhea relief. Our data provide evidences that support the potential of natural antioxidants in plants from Thailand to combat diseases and the usefulness of Thai traditional medicine wisdom regarding these plants.

ACKNOWLEDGEMENTS

The authors would like to thank the Commission on Higher Education (CHE-PhD-THA), the Office of the Higher Education Commission, Ministry of Education, Thailand and Khon Kaen University for funding and research grant. We would like to thank plant taxonomists of the Department of Biology, Faculty of Science and of the Walai Rukhavej Botanical Research Institute, Mahasarakham University for assistance in identification. We appreciate Prof. Yukifumi Nawa for his kind suggestion in preparation of the manuscript. We also

wish to acknowledge the support of research grant and the Visiting International Professor Program, Faculty of Medicine, Khon Kaen University.

REFERENCES

Achakza A, Achakzai P, Masood A, Alikayani S, Tareen R (2009). Response of plant part and age on the distribution of secondary metabolites on plants found in Quetta. Pak. J. Bot., 41: 2129-2135.

Aquila S, Giner RM, Recio MC, Spegazzini ED, Rios JL (2009). Anti-inflammatory activity of flavonoids from *Cayaponia tayuya* roots. J. Ethnopharmacol., 121: 333-337.

Bajaj K, Kaur G (1981). Spectrophotometric determination of L-ascorbic acid in vegetables and fruits. Analyst., 106: 117-120.

Benzie IF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal. Biochem., 239: 70-76.

Burkey KO, Neufeld HS, Souza L, Chappelka AH, Davison AW (2006). Seasonal profiles of leaf ascorbic acid content and redox state in ozone-sensitive wildflowers. Environ. Pollut., 143: 427-434.

Chan MY (1977). The effect of berberine on bilirubin excretion in the rat. Comp. Med. East West. 5: 161-168.

Chanwitheesuk A, Teerawutgulrag A, Kilburn JD, Rakariyatham N (2007). Antimicrobial gallic acid from *Caesalpinia mimosoides* Lamk. Food Chem., 100: 1044-1048.

Clayton T, Soralump P, Chaukul W, Temsiririrkkul R (1996). Medicinal plants in Thailand. Amarin Printing, Bangkok, Thailand, p. 44.

De las Heras B, Slowing K, Benedí J, Carretero E, Ortega T, Toledo C, Bermejo P, Iglesias I, Abad MJ, Gomez-Serranillos P, Liso PA, Villar A, Chiriboga X (1998). Antiinflammatory and antioxidant activity of plants used in traditional medicine in Ecuador. J. Ethnopharmacol., 61: 161-166.

Diaz J, Bernal A, Pomar F, Merino F (2001). Induction of shikimate dehydrogenase and peroxidase in pepper (*Capsicum annuum* L.) seedlings in response to copper stress and its relation to lignification. Plant Sci., 161: 179-188.

Du CB, Liu JW, Su W, Ren YH, Wei DZ (2003). The protective effect of ascorbic acid derivative on PC12 cells: Involvement of its ROS scavenging ability. Life Sci., 74: 771-780.

Franova S, Nosalova G, Mokry J, Mahmud THK, Ather A (2006). Phytotherapy of cough. In Iwu M (ed.) Advances in phytomedicine, Elsevier, U.S.A., pp. 111-131.

Grievink L, Jansen SM, Van't V, Brunekreef B (1998). Acute effects of ozone on pulmonary function of cyclists receiving antioxidant supplements. Occup. Environ. Med., 55: 13-17.

Gulcin I, Sat IG, Beydemir S, Elmastas M, Kufrevioglu OI (2004). Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L.). Food Chem., 87: 393-400.

Gurbuz I, Yesilada E, Ito S (2009). An anti-ulcerogenic flavonol diglucoside from *Equisetum palustre* L. J. Ethnopharmacol., 121: 360-365.

Haines D, Varga B, Bak I, Juhasz B, Mahmoud F, Kalantari H, Gesztelyi R, Lekli I, Czompa A, Tosaki A (2011). Summative interaction between astaxanthin, *Ginkgo biloba* extract (EGb761) and vitamin C in suppression of respiratory Inflammation: a comparison with Ibuprofen. Phytother. Res., 25: 128-136.

Halliwell B (1997). Antioxidants and human disease: a general introduction. Nutr. Rev., 55: 44-49.

Hemila H (2003). Vitamin C, respiratory infections and the immune system. Trends Immunol., 24: 579-580.

Kasolo JN, Bimenya GS, Ojok L, Ogwal-Okeng JW (2010). Phytochemicals and used of *Moringa oleifera* leaves in Ugandan rural communities. J. Med. Plant. Res., 4: 753-757.

Kumar S, Suresh PK, Vijayababu MR, Arunkumar A, Arunakaran J (2006). Anticancer effects of ethanolic neem leaf extract on prostate cancer cell line (PC-3). J. Ethnopharmacol., 105: 246-250.

McCune LM, Johns T (2007). Antioxidant activity relates to plant part, life form and growing condition in some diabetes remedies. J. Ethnopharmacol., 112: 461-469.

Mukherjee PK, Saha K, Murugesan T, Mandal SC, Pal M, Saha BP (1998). Screening of anti-diarrhoeal profile of some plant extracts of a specific region of West Bengal, India. J. Ethnopharmacol., 60: 85-89.

Nosal G, Mokry J, Hassan KMT (2003). Antitussive activity of the fruit extract of *Emblica officinalis* Gaertn. (Euphorbiaceae). Phytomed., 10: 583-589.

Nualkaew S, Rattanamanee K, Thongpraditchote S, Wongkrajang Y, Nahrstedt A (2009). Anti-inflammatory, analgesic and wound healing activities of the leaves of *Memecylon edule* Roxb. J. Ethnopharmacol., 121: 278-281.

Palombo EA (2006). Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. Phytother. Res., 20: 717-724.

Peckenpaugh N (2010). Nutrition essentials and diet therapy. Saunders Elsevier, St.Louis, U.S.A., pp. 106-108.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med., 26: 1231-1237.

Scartezzini P, Antognoni F, Raggi MA, Poli F, Sabbioni C (2006). Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation of *Emblica officinalis* Gaertn. J. Ethnopharmacol., 104: 113-118.

Shu P, Hong JL, Wu G, Yu BY, Qin MJ (2010). Analysis of flavonoids and phenolic acids in *Iris tectorum* by HPLC-DAD-ESI-MS^n. Chin. J. Nat. Med., 8: 202-207.

Singleton VL, Rossi JA (1965). A colorimetry of total phenolics with phosphomolybdic-phostungstic acid reagents. Am. J. Enol. Viticult., 16: 144-158.

Tawaha K, Alali FQ, Gharaibeh M, Mohammad M, El-Elimat T (2007). Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem., 104: 1372-1378.

Tewtrakul S, Subhadhirasakul S, Kummee S (2008). Anti-allergic activity of compounds from *Kaempferia parviflora*. J. Ethnopharmacol., 116: 191-193.

Tewtrakul S, Subhadhirasakul S, Karalai C, Ponglimanont C, Cheenpracha S (2009). Anti-inflammatory effects of compounds from *Kaempferia parviflora* and *Boesenbergia pandurata*. Food Chem., 115: 534-538.

Tsai PJ, Wu SC, Cheng YK (2008). Role of polyphenols in antioxidant capacity of napiergrass from different growing seasons. Food Chem., 106: 27-32.

Van der Nat JM, Van der Sluis WG, Hart LA, Van Dijk H, De Silva KT, Labadie RP (1991). Activity-guided isolation and identification of *Azadirachta indica* bark extract constituents which specifically inhibit chemiluminescence production by activated human polymorphonuclear leukocytes. Planta Med., 57: 65-68.