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Antioxidant activity of solvent extracts from Vietnamese medicinal plants

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The methanol, ethanol, acetone, and water extracts of six Vietnamese medicinal plants, *Premna integrifolia*, *Terminalia nigrovenulosa*, *Pseuderanthemum palatiferum*, *Streptocaulon juventas*, *Eclipta alba (L)*, and *Solanum hainanense* were investigated for their antioxidant and radical scavenging activities using reducing power, metal chelating activity, and 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) assays. Extraction yields, total phenolic and flavonoid contents of methanol extracts were higher than those of other solvent extracts. These results were also similar to reducing power and radical scavenging activities of extracts of *T. nigrovenulosa* and *P. integrifolia.* However, the highest metal chelating activity was found in methanol and water extracts of *S. juventas,* and methanol extract of *E. alba*. The methanol extracts of *T. nigrovenulosa* (bark and leaves) and *P. integrifolia* leaves, which were found to have high anti-radical capacities and reducing power, and those of *S. juventas* and *E. alba*, with their high metal chelating activity, have significant potential for use as natural antioxidants.

Key words: Medicinal plants, polyphenol, antioxidant activity, radical scavenging.

INTRODUCTION

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary hydroquinone (TBHQ) are commonly employed as preservatives by pharmaceutical, cosmetic, and food companies. However, they are also suspected of being responsible for liver damage and carcinogenesis (Whysner et al., 1994) and toxicity (Moure et al., 2001; Wanasundara and Shahidi, 1998). Therefore, there is an increasing need to replace synthetic antioxidants with natural, safer compounds. A great number of medicinal plants contain chemical compounds showing antioxidative properties. Natural antioxidants can protect the human body from radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods (Kinsella et al., 1993; Lai et al., 2001; Pryor, 1991). Numerous studies have been carried out on plants, including cinnamon, rosemary, sage, oregano, and costmary, and the results have resulted in the development of natural antioxidants for use in foods, cosmetics, and other applications. However, there are still many plants that remain unstudied regarding their antioxidative properties are. Therefore, assessment of such properties is an interesting and useful topic, especially in finding new sources of natural antioxidants, functional foods, and neutraceuticals.

The following plants from Vietnam were selected for investigation: *Premna integrifolia* Roxb, *Terminalia nigrovenulosa* Pierre ex Laness, *Pseuderanthemum palatiferum* (Nees) radlk, *Streptocaulon juventas* (Lour.) Merr, *Eclipta alba* (L) hassk, and *Solanum hainanense* Hance. To our knowledge, there have been few data on the antioxidative properties of these plants. *E. alba* (L) hassk belongs to the Astraceae family and is very common in tropical and subtropical regions. The herb is

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Abbreviations: EDTA, Ethylenediaminetetraacetic acid; **DPPH,** 2,2-diphenyl-2-picrylhydrazyl hydrate; **BHT,** butylated hydroxytoluene.

often used for the treatment of infective hepatitis in India (Wagner et al., 1986) and snake venom poisoning in Brazil (Melo et al., 1994). It has been reported that the leaves of this herb are useful in treating gastritis and respiratory disorders like cough and asthma (Kobari et al., 2004). In addition, the crude form of *E. alba* (L) hassk is reported to have anti-inflammatory, anti-fungal, and anti-hepatotoxic properties (Wong et al., 1995). However, there is no report yet concerning the antioxidant effects of this plant.

P. palatiferum (Nees) radlk of the Acanthacea family (Ho, 2000) is known as a new medicinal plant. It was first found in the Cuc Phuong forest in Northern Vietnam (Cuong and Quynh, 1999). After its discovery, the area of its cultivation as both a medicinal and an ornamental plant has expanded throughout the country. This plant can allegedly cure many types of human diseases, including wounds, trauma, stomachaches, colitis, high blood pressure, nephritis, and diarrhea (Cuong and Quynh, 1999; Khanh, 1998; Oanh, 1999). It has been used for the treatment and prevention of many types of diseases, including diarrhea not only in humans but also animals. However, any research on the antioxidant properties of this plant has not been conducted.

S. juventas (Lour.) Merr. is a member of the Asclepiadaceae family and is native to Indochina. In Vietnam*, S. juventas* is called “Ha thu o trang”, and it is used as a tonic for various conditions such as anemia, chronic malaria, rheumatism, menstrual disorders, neurasthenia, and dyspepsia.

T. nigrovenulosa Pierre ex Laness is a plant of the Combretaceae family and grows wild in deciduous forests in the southern part of Vietnam. *T. Pierre* ex Laness is called “Chieu lieu gan den” in Vietnamese and its trunk-bark and leaves are used as an anti-diarrhea in the treatment of chronic dysentery, sore throat, laryngitis, and hemorrhoids.

P. integrifolia Roxb. is known as a medicinal plant in Vietnam and is used in treating fever, colic, diarrhea, dysentery, urine retention, flatulence, dyspepsia, and rheumatism. Premnine (Basu and Dandiya, 1947) ganikarine, and premnazole alkaloids (Barik et al., 1992) have been reported in the roots of *P. integrifolia*, whereas the flavanoids luteolin, sterol, and triterpene (Debelas et al., 1973) are present in the leaves.

S. hainanense Hance is a traditional remedy that is grown in many areas of Vietnam. It is often used for the treatment of inflammation, histaminemia, and venomous snake bites.

The examination of the antioxidative properties of these plants is of interest primarily to find new promising sources of natural antioxidants, functional foods, and neutraceuticals. The aim of this study was to investigate the antioxidative activities of six Vietnamese medicinal plants extracted with methanol, ethanol, acetone, and water. Examinations were performed based on reducing power, DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) free radical scavenging activity, and ferrous ion chelating assays. The extraction yields, total phenolic and flavonoid contents of these extracts were also determined.

MATERIALS AND METHODS

Chemicals

The following chemicals were obtained from Sigma-Aldrich (St Louis, MO, USA): 2,2-diphenyl-2-picrylhydrazyl hydrate, ferrozine, gallic acid, quercetin, folin-ciocalteu, potassium ferricyanide, sodium nitrite, and aluminum chloride. Other chemicals and reagents were of analytical grade.

Plant materials

The medicinal plants were gathered from different places in Vietnam in August of 2009. Various data (local name, medicinal uses, used parts of the plant, method of preparation, and administration) were collected based on traditional use of the curative parts of these plants. *P. integrifolia* Roxb (leaves), *T. nigrovenulosa* Pierre ex Laness (leaves and trunk-barks), *P. palatiferum* (Nees) radlk (leaves), *S. juventas* (Lour.) Merr (whole plant), *E. alba* (L) hassk (whole plant), *S. hainanense* Hance (whole plant). Different parts of fresh plants were cut and dried at ambient temperature in a room with active ventilation. Afterward, they were packed in plastic bags and stored at -18°C before use.

Extraction

Dried plants were pulverized using a cutting mill (LM-M010W, LiHOM Inc., Seoul, Korea), followed by extraction using four solvents: methanol, acetone, ethanol, and water. Briefly, plant powder (10 g) was subjected to extraction for 24 h with 100 ml of solvent in a glass conical flask on a shaker at room temperature, followed by filtration through filter paper (No. 1, Whatman International LTD, Maidstone, England). The residue was then extracted twice more with 100 ml of solvent as described above. The combined solvent extracts were concentrated using a rotary evaporator (Heidolph VV 2011-Antrieb, Heidolph Instruments GmbH and Co. KG, Schwabach, Germany) at 40°C under a vacuum to obtain dry extracts. The extracts were stored at -20°C until use.

Evaluation of antioxidant activity

DPPH radical scavenging activity

Radical scavenging activity of the plant extracts against stable DPPH radical (was determined by spectrophotometry. When DPPH radical reacts with an antioxidant capable of donating hydrogen, it is reduced. Changes in colour (from deep-violet to light-yellow) were measured at 515 nm using an UV/visible light spectrophotometer. The free radical scavenging activities of the extracts were measured by a slightly modified method of Brand-Williams et al. (1995) and Miliauskas et al. (2004), as described below. Extract solutions were prepared by dissolving 25 mg of dry extract in 10 ml of methanol. The solution of DPPH radical in methanol (6×10^{-5} M) was prepared daily before the absorbance measurements. Three milliliters of this solution was then mixed with 77 µl (38 or 19 µl in additional assays) of extract solution (final mass ratios of extracts to DPPH radical were approximately 3:1, 1.5:1 and 0.75:1). The samples were kept in the dark for 15 min at room temperature, after which the decrease in absorption was measured. Absorption of a

blank sample containing the same amount of methanol and DPPH radical solution was measured daily. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

$$\% \text{ Inhibition} = [(A_B - A_A)/A_B] \times 100,$$

where A_B and A_A stand for absorption of the blank sample (t=0 min) and absorption of the tested extract solution (t=15 min), respectively. The extract that could scavenge 50% of the DPPH radicals (IC_{50}) was calculated from a plot of scavenging effect versus extract concentration. BHT dissolved in MeOH was also analyzed as a positive control.

Reducing power

The reducing power of the plant extracts was determined by a slightly modified method of Oyaizu (1986). One milliliter of each plant extract concentration (0.1, 0.5 and 1 mg/ml) was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixtures were then incubated at 50°C for 20 min. Aliquots (2.5 ml) of trichloroacetic acid (10%) were added to each mixture, which were then centrifuged for 10 min at 1036 x g. The upper layer of the solutions (2.5 ml) were mixed separately with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance levels were measured at 700 nm using a spectrophotometer. Increased absorbance indicates increased reducing power, and the IC_{50} value is the concentration at which the absorbance is 0.5. BHT was used as positive control.

Metal chelating activity

The chelation of ferrous ions by the plant extracts and standards was estimated by the method of Dinis et al. (1994). Aliquots (1 ml) of the plant extracts dissolved in the same solvents at concentrations of 0 (control), 1, 2.5 and 5 mg/ml were separately added to 2.8 ml of distilled water, followed by mixing with 50 µl of 2 mM $FeCl_2 \cdot 4H_2O$ and 150 µl of 5 mM ferrozine. The mixtures was then shaken vigorously and left standing at room temperature for 10 min. Absorbance levels of the solutions were measured using a spectrophotometer at 562 nm. All tests and analyses were run in triplicate and averaged. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was calculated using the formula given below:

$$\%\text{Inhibition} = [(A_0 – A_1)/A_0] \times 100,$$

where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the plant extracts or standards. EDTA was used as a reference compound.

Amount of phenolic and flavonoid compounds

Total phenolic content of the plant methanol extracts was determined by the Folin–Ciocalteu method (1927). To prepare a calibration curve, 1 ml aliquots of 0.024, 0.075, 0.105 and 0.3 mg/ml ethanol gallic acid solutions were separately mixed with 5 ml of Folin–Ciocalteu reagent (diluted ten-fold) and 4 ml (75 g/l) of sodium carbonate. The absorption levels were read after 30 min at 20°C at 765 nm, and the calibration curve was drawn. One milliliter of the plant extracts (1 mg/ml) was then mixed with the same reagents as described above, and absorption levels were measured after 30 min to determine the total phenolic contents. All determinations were performed in triplicate. Total content of phenolic compounds in the plant extracts in gallic acid equivalents (GAE) was calculated by the following formula:

$$C = c \times V/m$$

where C represents the total content of phenolic compounds, mg/g of plant extract, in GAE; c, the concentration of gallic acid established from the calibration curve, mg/ml; V, volume of the extract, ml; and m, weight of the pure plant extract, g.

Flavonoid contents were measured using a modified colorimetric method (Jia et al., 1999). Extract solution (0.25 ml, 1 mg/ml) was added to a test tube containing 1.25 ml of distilled water. Sodium nitrite solution (0.075 ml, 5%) was then added to the mixture, followed by incubation for 5 min. Then, 0.15 ml of 10% aluminum chloride was added. After 6 min, 0.5 ml of 1 M sodium hydroxide was finally added, after which the mixture was diluted with 0.275 ml of distilled water. The absorbance of the mixture at 510 nm was measured immediately in comparison to a standard curve prepared with quercetin. Flavonoid contents were expressed as mg of quecetin equivalent (QE)/g dry basis.

Data analysis

All experiments were performed in triplicate (n=3), and an ANOVA test (using STATGRAPHICS Centurion XV statistical software) was used to compare the mean values of each treatment. Significant differences between the means of parameters were determined by LSD test ($p<0.05$). The results are presented as mean ± standard deviation (STD) of three replicated determinations.

RESULTS AND DISCUSSION

Extraction yield

Extraction yield from the studied plant was dependent upon the solvents and plants given in Table 1. Among them, the extraction yields obtained using methanol were higher than those from plants extracted using other solvents. Methanol extract of *T. nigrovenulosa* Pierre ex Laness bark gave the highest extraction yield (30.59%) when compared to 27.98 and 27.57% in the ethanol and acetone extracts, respectively, followed by methanol extracts of *E. alba* (L) radlk (16.00%) and *P. integrifolia* Roxb (15.15%). The lowest extraction yield from the studied plants was found with the acetone extracts. Therefore, variation in the various extract yields was due to the polarities of different compounds present in the plants, and such differences have been reported in the literature concerning fruit seeds (Jayaprakasha et al., 2001), mulberry leaves (Saeedeh et al., 2007), and lotus rhizome (Dongmei et al., 2007). The highest yield in the sequential extractions was achieved with polar solvents.

Total phenolic and flavonoid contents

Phenolic compounds are associated with antioxidant activity or free radical scavenging and play an important role in lipid peroxidation (Yen et al., 1993). This led us to determine their total amounts in selected plant extracts. Flavonoids, as one of the most diverse and widespread

Table 1. Yields of medicinal plant extracts using different solvents.

Scientific name	Parts	Extraction yields (%)			
		MeOH extract	EtOH extract	Acetone extract	Water extract
T. nigrovenulosa Pierre ex Laness.	Bark	30.177 ± 0.467^{aA}	27.957 ± 0.068^{aB}	27.573 ± 0.055^{aB}	20.883 ± 0.258^{aC}
	Leaves	13.947 ± 0.067^{bA}	12.963 ± 0.580^{bB}	13.737 ± 0.050^{bC}	13.250 ± 0.240^{bC}
P. integrifolia Roxb	Leaves	15.390 ± 0.269^{cA}	13.537 ± 0.137^{cB}	11.630 ± 0.160^{cC}	15.290 ± 0.131^{cA}
P. palatiferum (Nees) radlk	Leaves	10.503 ± 1.106^{dA}	10.320 ± 0.581^{dA}	9.777 ± 0.300^{dA}	17.407 ± 0.038^{dB}
E. alba (L) hassk	Whole plant	15.933 ± 2.821^{bA}	8.593 ± 0.415^{eB}	7.637 ± 0.055^{eC}	13.170 ± 0.216^{bD}
S. juventas (Lour.) Merr	Whole plant	10.403 ± 0.680^{dA}	10.120 ± 0.173^{dA}	9.813 ± 0.300^{dB}	11.150 ± 3.615^{dA}
S. hainanense Hance	Whole plant	8.910 ± 0.770^{eA}	9.937 ± 0.127^{fB}	7.940 ± 0.082^{eC}	9.357 ± 0.035^{eB}

Results are means ± SD of triplicate measurements). In the same column different labels (a-e) and in the same raw different labels (A-D) indicate a significant difference at $p<0.05$.

Table 2. Total phenolic contents of medicinal plant extracts using different solvents.

Scientific name	Parts	Total phenolics content (mg GAE/g extract)			
		MeOH extract	EtOH extract	Acetone extract	Water extract
T. nigrovenulosa Pierre ex Laness.	Bark	833.802 ± 1.432^{aA}	598.251 ± 1.951^{aB}	785.067 ± 1.432^{aC}	439.050 ± 0.286^{aD}
	Leaves	453.608 ± 0.937^{bA}	416.120 ± 0.937^{bB}	412.059 ± 0.541^{bB}	181.131 ± 0.286^{bC}
P. integrifolia Roxb	Leaves	246.485 ± 0.000^{cA}	163.386 ± 1.432^{cB}	131.834 ± 0.541^{cC}	82.037 ± 0.216^{cD}
P. palatiferum (Nees) radlk	Leaves	91.409 ± 0.108^{dA}	62.855 ± 0.216^{dB}	139.394 ± 0.108^{dC}	33.115 ± 0.054^{dD}
E. alba (L) hassk	Whole plant	75.289 ±0.108^{eA}	48.485 ± 0.216^{eB}	140.581 ± 0.000^{dC}	48.204 ± 0.054^{eD}
S. juventas (Lour.) Merr	Whole plant	76.913 ± 0.108^{fA}	66.042 ± 0.108^{fB}	93.221 ± 0.108^{eC}	92.065 ± 0.143^{fD}
S. hainanense Hance	Whole plant	62.168 ± 0.108^{gD}	68.604 ± 0.000^{gB}	81.350 ± 0.000^{fC}	39.675 ± 0.108^{gD}

Results are means ± SD of triplicate measurements. In the same column different labels (a-g) and in the same raw different labels (A-D) indicate a significant difference at $p<0.05$.

groups of natural compounds, are the most important natural phenolic (Agraval, 1989). Therefore, the contents of flavonoids in the extracts were also determined. Total phenolic contents varied widely in the selected medicinal plant extracts, as shown in Table 2. Higher levels of total phenolics were found in the bark and leaf extracts of *T. nigrovenulosa* (439 to 833 GAE/g of extract (bark) and 181 to 453 GAE/g of extract (leaves)) and *P. integrifolia* Roxb (82 to 246 GAE/g of extract), depending on the extraction solvent. These results clearly show that the solvent influences the extractability of the phenolic compounds. Methanol was the most effective solvent for extraction of phenolic compounds from *T. nigrovenulosa* (bark and leaves) and *P. integrifolia* Roxb, followed by acetone, ethanol, and water in decreasing order. This is in agreement with previous studies that found that pure methanol is an effective solvent for antioxidant extraction of phenolic compounds (Moure et al., 1999; Siddhuraju et al., 2003). Water, the most polar solvent, extracted the lowest content of phenolics, which is similar to the results of Demiray et al. (2009). These results suggest that the content of moderately polar phenolic compounds in these plants was higher than that of

very polar or slightly polar compounds. In contrast, higher contents of total phenolics in the *P. palatiferum*, *S. juventas*, *E. alba*, and *S. hainanense* extracts were observed with acetone extraction (Table 2), which agrees with research that found that acetone extract of lotus rhizome contained highest content of phenolics (Dongmei et al., 2007). This suggests that phenolics content varied considerably as a function of solvent polarity and plant type, which is in accordance with previous studies that found that the solvent greatly influences the phenolic extraction capacity in many species (Akowuah et al., 2005; Turkmen et al., 2006).

Regarding the flavonoid content, the yield of flavonoids depended on not only upon plant type but also upon the extraction solvent, which is shown in Table 3. Similar to the total phenolic content, high levels of flavonoids were also observed in the extracts of *T. nigrovenulosa* (45 to 262 mg QE/g of bark and 28 to 139 mg QE/g of leaves) and *P. integrifolia* Roxb (60 to 188 mg QE/g). The methanol extracts of these plants contained higher flavonoid contents compared to the other solvents. This implies that neither the highest polar solvent (water) nor the lowest (acetone) were suitable for extraction of flavonoid compounds from these plants. Moreover, methanol was the most efficient solvent for the extraction of flavonoids, which is accordance with previous studies that found that higher yields of flavonoids were achieved using polar solvents (Yao et al., 2004; Velikovic et al., 2007). On the contrary, higher contents of flavonoids in the extracts of *T. nigrovenulosa* leaves (139 mg QE/g) and *E. alba* (77 mg QE/g) were obtained by acetone extraction. These results suggest that a higher content of slightly polar flavonoid compounds, as opposed to highly polar ones, existed in these plants.

DPPH radical scavenging activity

The results of the DPPH inhibition assay using different plant extracts and control (BHT) are shown in Table 4. DPPH radical scavenging activities of the extracts depended not only on plant type but also upon the extraction solvent. In general, DPPH scavenging activities increased with increasing phenolic content. Among the four extraction solvents, methanol was the most effective for extraction of DPPH scavengers, followed by acetone, ethanol, and water, which is in agreement with previous studies that found that extracts of methanol and acetone demonstrate higher scavenging activities compared to solvent extracts from lotus (Dongmei et al., 2007), medicinal plants (Miliauskas et al., 2004), and rockrose leaves (Amensour et al., 2010). Two of the plant extracts, which contained the highest contents of phenolics and flavonoids, almost completely inhibited DPPH absorption in *T. nigrovenulosa* (93.62 to 96.09%, bark; 93.567 to 94.837%, leaves) and *P. integrifolia* Roxb (59.171 to 93.083%), which were about two-fold higher than that of synthetic antioxidant (BHT, 41.715% DPPH inhibition). The methanol and acetone extracts of *E. alba* along with the water extract of *S. juventas* possessed high levels of DPPH scavenging activity, 40.10%, 43.815% and 44.072%, respectively, which were approximately the same as that of BHT. The DPPH radical scavenging activities of water extracts were the lowest compared to those of other solvents. This suggests that the use of water for extraction of radical scavenging compounds from selected medicinal plants was not effective, except for *S. juventas*, which is in accordance with previous research that demonstrated that the lowest antioxidant and radical scavenging activities were in water extracts of guarana seed (Majhenic et al., 2007) and mulberry leaves (Arabshahi-Delouee and Uroo, 2007).

These results indicate that the extracts with high total phenolic and flavonoid contents presented high radical scavenging activities, which could be related to the inherent nature of phenolic and flavonoid compounds, thus contributing to their electron transfer/hydrogen donating ability. High correlation between radical scavenging and phenolic content has been reported in cereal (Peterson et al., 2001), fruits (Gao et al., 2000; Jimenez-Escrig et al., 2001), beverages (Fogliano et al., 1999), and culinary herbs (Zheng and Wang, 2001). In our results, methanol was the best solvent for the extraction of radical scavenging compounds from plants.

To further assess DPPH scavenging, the most effective plant extracts were diluted 2- or 4-fold. The final mass ratios of the extracts containing DPPH radical were 1.5:1 and 0.75:1. The results in Table 4 show that the most active radical scavengers were in the methanol extracts of the bark and leaves of *T. nigrovenulosa.* Radical scavenging activities of these extracts after 4-fold dilution remained nearly the same as those of the initial extracts, whereas that of extract of *P. integrifolia* Roxb leaves as the same conditions reduced over two-fold. The ethanol and acetone extracts of the bark and leaves of *T. nigrovenulosa* also contained strong radical scavenging activities, indicating that active compounds of variable polarity could be present in this plant.

Reducing power

Antioxidant activity depends on the metallic catalyst used to generate the reactive species (Roedig-penman and Gordan, 1998). In this study, to measure reductive capability, we investigated the Fe^{3+}- Fe^{2+} transformation in the presence of medicinal plant extracts using the method of Oyaizu (1986). The reducing capacity of a compound may serve as an indicator of its potential antioxidant activity (Meir et al., 1995). The presence of reducing compounds causes reduction of the Fe^{3+}/ferricyanide complex to ferrous ion (Fe^{2+}) (Sousa et al., 2008). Figures 1, 2, 3 and 4 show the reducing power

Table 3. Effect of extraction solvent on flavonoid contents of medicinal plant extracts.

Scientific name	Parts	Flavonoids content (mg QE/g extract)			
		MeOH extract	EtOH extract	Acetone extract	Water extract
T. nigrovenulosa Pierre ex Laness.	Bark	262.890 ± 1.258[aA]	50.109 ± 0.000[aB]	45.752 ± 0.000[aC]	92.229 ± 1.258[aD]
	Leaves	103.123 ± 1.258[bA]	28.322 ± 0.000[bB]	139.434 ± 0.000[bC]	124.909 ± 0.629[bD]
P. integrifolia Roxb	Leaves	188.090 ± 0.629[cA]	156.500 ± 0.629[cB]	110.748 ± 4.912[cC]	60.907 ± 0.629[cD]
P. palatiferum (Nees) radlk	Leaves	90.414 ± 1.089[dA]	27.959 ± 1.258[bB]	82.426 ± 0.629[dC]	32.317 ± 0.629[dD]
E. alba (L) hassk	Whole plant	59.187 ± 0.629[eA]	26.144 ± 0.000[bB]	77.342 ± 0.000[eC]	45.752 ± 2.882[eD]
S. juventas (Lour.) Merr	Whole plant	33.043 ± 0.629[fA]	31.590 ± 0.000[dB]	38.126 ± 1.089[fC]	58.097 ± 1.664[fD]
S. hainanense Hance	Whole plant	35.221 ± 0.629[gA]	31.590 ± 0.000[dB]	30.864 ± 0.629[gC]	36.311 ± 1.664[gD]

Results are means ± SD of triplicate measurements. In the same column different labels (a-g) and in the same raw different labels (A-D) indicate a significant difference at $p<0.05$.

Table 4. DPPH radical scavenging activity (%) of medicinal plant extracts using various solvents at various ratios of $M_{extract}$: M_{DPPH}.

Scientific name	Parts	DPPH inhibition (%)			
		MeOH extract	EtOH extract	Acetone extract	Water extract
		$(M_{extract}):(M_{DPPH})$ = 3:1			
T. nigrovenulosa Pierre ex Laness.	Bark	96.090 ± 0.150[a]	94.337 ± 0.149[b]	94.834 ± 0.086[c]	93.620 ± 0.478[d]
	Leaves	94.837 ± 0.087[a]	94.486 ± 0.149[bc]	94.386 ± 0.172[c]	93.567 ± 0.878[d]
P. integrifolia Roxb	Leaves	93.083 ± 0.150[a]	91.058 ± 0.298[b]	92.548 ± 0.194[c]	59.171 ± 0.319[d]
P. palatiferum (Nees) radlk	Leaves	31.529 ± 0.608[a]	23.299 ± 0.455[b]	35.569 ± 1.014[c]	7.443 ± 0.560[d]
E. alba (L) hassk	Whole plant	40.100 ± 0.828[a]	32.439 ± 0.228[b]	43.815 ± 0.149[c]	15.577 ± 0.803[d]
S. juventas (Lour.) Merr	Whole plant	25.564 ± 0.542[a]	19.523 ± 0.298[b]	28.664 ± 0.228[c]	44.072± 0.645[d]
S. hainanense Hance	Whole plant	24.160 ± 0.528[ac]	15.450 ± 0.228[b]	24.441 ± 0.977[ac]	24.296 ± 0.719[acd]
BHT		41.715 ± 0.880			
		$(M_{extract}):(M_{DPPH})$ = 1.5:1			
T. nigrovenulosa Pierre ex Laness.	Bark	95.919 ± 0.085[a]	94.468 ± 0.083[bc]	94.661 ± 0.289[bc]	91.444 ± 0.328[d]
	Leaves	94.444 ± 0.085[a]	94.228 ± 0.289[a]	94.324 ± 0.083[a]	90.499 ± 0.241[d]
P. integrifolia Roxb	Leaves	92.970 ± 0.085[a]	49.591 ± 1.024[b]	62.867 ± 1.227[c]	NE
		$(M_{extract}):(M_{DPPH})$ = 0.75:1			
T. nigrovenulosa Pierre ex Laness.	Bark	95.370 ± 0.17[a]	92.635 ± 0.361[b]	93.639 ± 0.219[c]	87.972 ± 1.957[d]
	Leaves	94.737 ± 0.15[a]	77.905 ± 0.287[b]	90.770 ± 0.361[c]	77.023 ± 0.162[d]
P. integrifolia Roxb	Leaves	45.029 ± 0.44[a]	31.707 ± 1.174[b]	33.812 ± 0.299[c]	NE

Results are means ± SD of triplicate measurements. Different labels (a-d) indicate a significant difference at $p<0.05$. NE – values were not estimated.

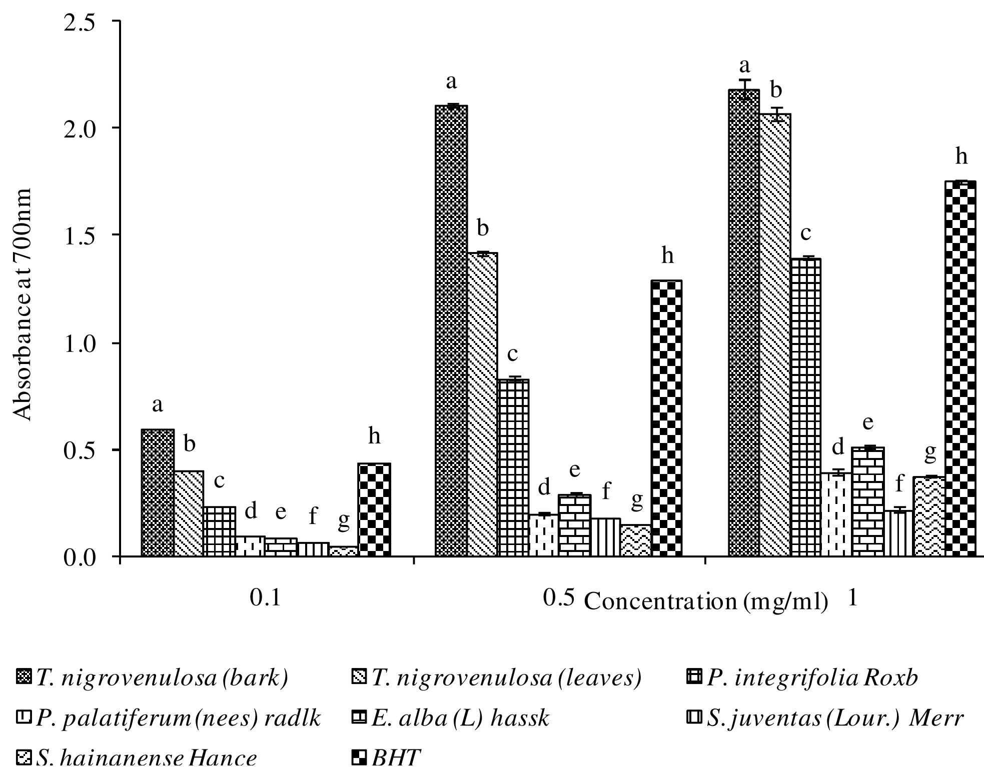

Figure 1. Reducing power of methanol extracts of medicinal plants. Results are means ± SD of triplicate measurements. Different labels (a-h) above the bars for the same concentration indicate a significant difference at $p<0.05$.

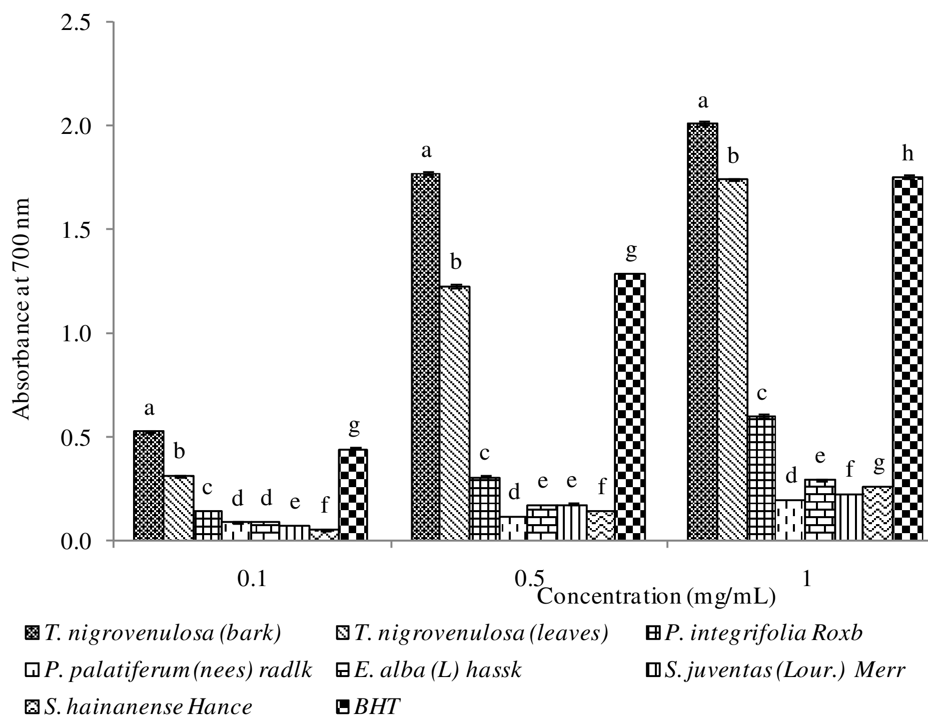

Figure 2. Reducing power of ethanol extracts of medicinal plants. Results are means ± SD of triplicate measurements. Different labels (a-h) above the bars for the same concentration indicate a significant difference at $p<0.05$.

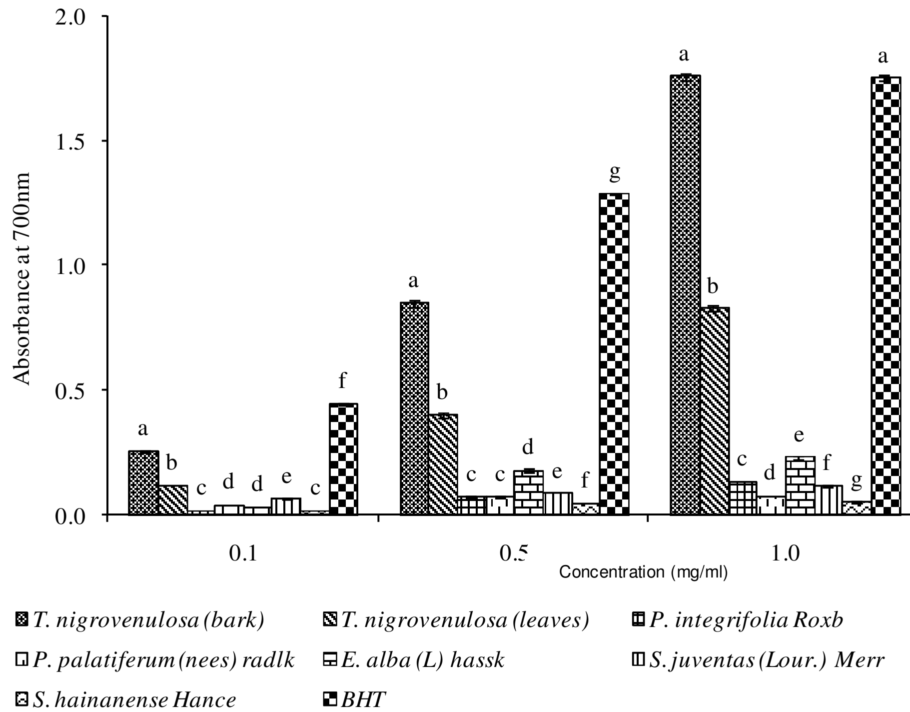

Figure 3. Reducing power of acetone extracts of medicinal plants. Results are means ± SD of triplicate measurements. Different labels (a-g) above the bars for the same concentration indicate a significant difference at $p<0.05$.

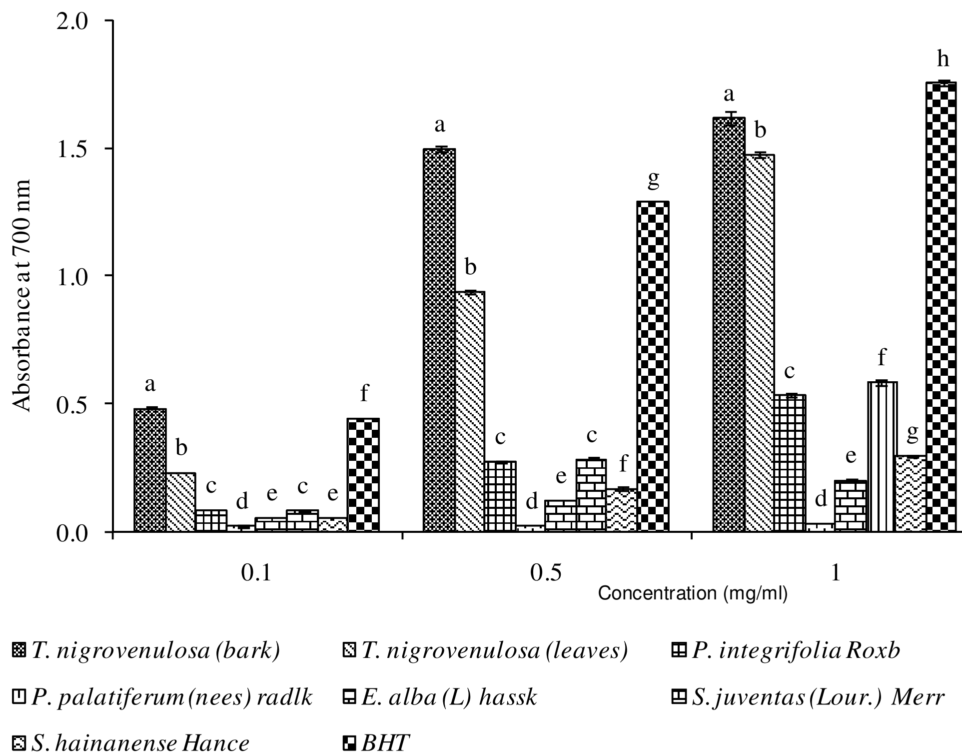

Figure 4. Reducing power of water extracts of medicinal plants. Results are means ± SD of triplicate measurements. Different labels (a-h) above the bars for the same concentration indicate a significant difference at $p<0.05$.

Table 6. Reducing power (IC_{50} values) of medicinal plant extracts obtained using various solvents.

Scientific name	Parts	IC_{50} (mg/ml)			
		MeOH extracts	EtOH extracts	Acetone extracts	Water extracts
T. nigrovenulosa Pierre ex Laness.	Bark	0.097 ± 0.000^{aA}	0.100 ± 0.002^{aB}	0.260 ± 0.005^{aC}	0.131 ± 0.001^{aD}
	Leaves	0.093 ± 0.005^{aA}	0.151 ± 0.001^{bB}	0.594 ± 0.006^{bC}	0.252 ± 0.002^{bD}
P. integrifolia Roxb	Leaves	0.280 ± 0.002^{bA}	0.821 ± 0.014^{cB}	3.859 ± 0.010^{cC}	0.932 ± 0.014^{cD}
P. palatiferum (Nees) radlk	Leaves	1.332 ± 0.036^{cA}	3.429 ± 0.022^{dB}	11.790 ± 0.350^{dC}	13.404 ± 0.181^{dD}
E. alba (L) hassk	Whole plant	0.963 ± 0.014^{dA}	1.912 ± 0.002^{eB}	2.155 ± 0.090^{eC}	2.773 ± 0.011^{eD}
S. juventas (Lour.) Merr	Whole plant	2.586 ± 0.170^{eA}	2.571 ± 0.013^{fB}	7.623 ± 0.096^{fC}	0.854 ± 0.013^{fD}
S. hainanense Hance	Whole plant	1.374 ± 0.029^{cA}	2.007 ± 0.009^{gB}	14.002 ± 0.332^{gC}	1.734 ± 0.009^{gD}
BHT		0.115 ± 0.002			

Values (mean ± SD, n=3). In the same column different labels (a-g) and in the same raw different labels (A-D) indicate a significant difference at $P<0.05$.

of the methanol, ethanol, acetone, and water extracts, respectively. Specifically, the results show that reducing power of the extracts depended on the extraction solvent, plant type, and extract concentration. The reducing power of all extracts increased with increasing extract concentration, indicating that reducing agents were present in these extracts. In general, methanol extracts exhibited the highest reducing capability, followed by ethanol, acetone, and water extracts. The reducing capacity of the methanol extracts of *T. nigrovenulosa* (leaves and bark) as shown in Figure 1 was higher than that of BHT at all concentrations, whereas the reducing capacity of the water extracts shown in Figure 4 was lower. This confirms that methanol was the most efficient solvent for the extraction of reducing agents from the selected plants, which is in agreement with previous studies (Amensour et al., 2010; Arabshahi-Delouee and Uroo, 2007). The methanol extracts containing high amounts of total phenolics displayed the highest reducing power, whereas water extracts containing the lowest amounts of total phenolics were the weakest. This is similar to the previously reported relationship between reducing power and total phenolic content (Benzeie et al., 1999; Gao et al., 2000; Zhu et al., 2002; Amarowicz et al., 2004; Arabshahi-Delouee et al., 2007).

Methanol extracts of the leaves and bark of *T. nigrovenulosa* and *P. integrifolia* Roxb, which had IC_{50} values of 0.097, 0.093 and 0.280 mg/ml, respectively, showed better reducing capacities compared to the other plants investigated as well as that of the synthetic antioxidant BHT (IC_{50} value of 0.115 mg/ml), as shown in Table 6. The higher reducing power in the extracts may have been due to the higher amount of total antioxidants, which is consistent with previous observations that the reducing power of a compound is dependent on its hydrogen donating ability (Shimada et al., 1992; Siddhuraju et al., 2002; Yen et al., 2001).

Ethanol, acetone, and water extracts were considerably less effective in reducing capability compared to the methanol extracts. However, the reducing capacities of the ethanol and water extracts of the leaves and bark of *T. nigrovenulosa*, as well as that of the acetone extract of the leaves of this plant, were also relatively high, which was similar to that of BHT.

Metal chelating activity

An important mechanism of antioxidant activity is the ability to chelate/deactivate transition metals, which possess the ability to catalyze hydroperoxide decomposition and Fenton-type reactions. Chelating agents may also serve as secondary antioxidants since they reduce redox potential, thereby stabilizing the oxidized forms of metal species. Therefore, the ion (II) chelating capacities of the extracts were screened. Ferrozine forms complexes with Fe^{2+}. In the presence of chelating agents, complex formation between ferrozine and Fe^{2+} is disrupted, resulting in reduction in the red colour of the complex. Measurement of the colour decrease therefore allows estimation of the chelating activity of the co-existing chelator (Yamaguchi et al., 2000). In this study, the solvent extracts of six medicinal plants and reference compounds (EDTA) were found to have interfered with the formation of the ferrous and ferrozine complex, indicating that they contained chelating activity and were able to capture ferrous before ferrozine.

The metal chelating capacities of the methanol, ethanol, acetone, and water extracts are shown in Figures 5, 6, 7 and 8, respectively. The metal inhibition capacities increased with increasing extract concentrations, indicating that chelating compounds were present in these plant extracts. The metal chelating activity of the extracts was strongly dependent on the solvent due to the various antioxidant potentials of the compounds, which possessed different polarities. Among these, methanol and water extracts of the selected plants possessed higher metal chelating activities compared to the other solvents. This suggests that the compounds

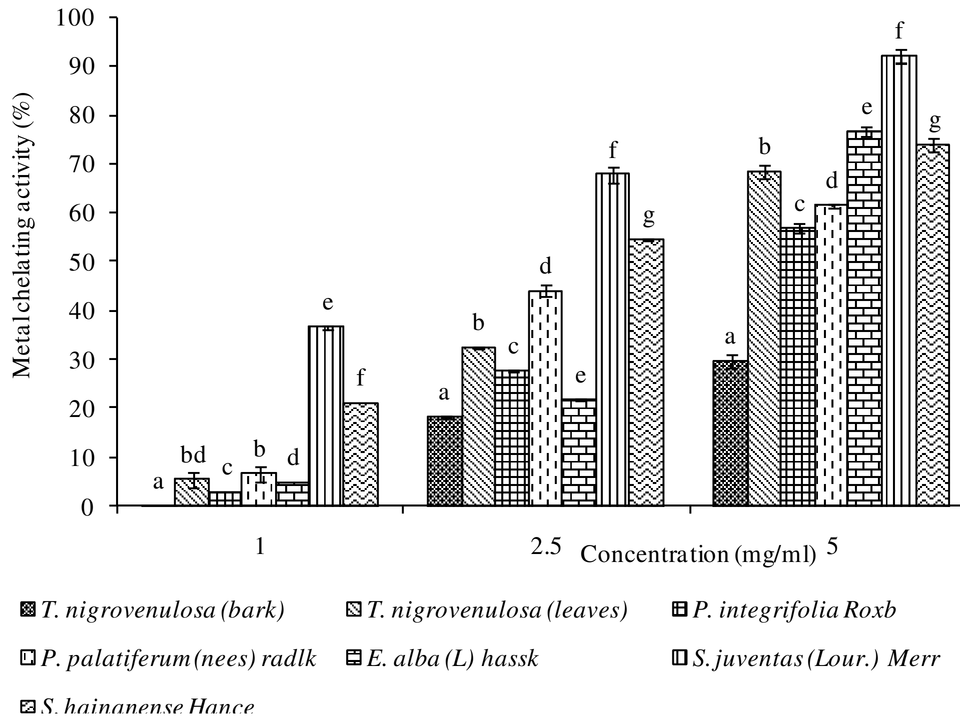

Figure 5. Metal chelating activity of methanol extracts of medicinal plants. Results are means ± SD of triplicate measurements. Different labels (a-g) above the bars for the same concentration indicate a significant difference at p<0.05.

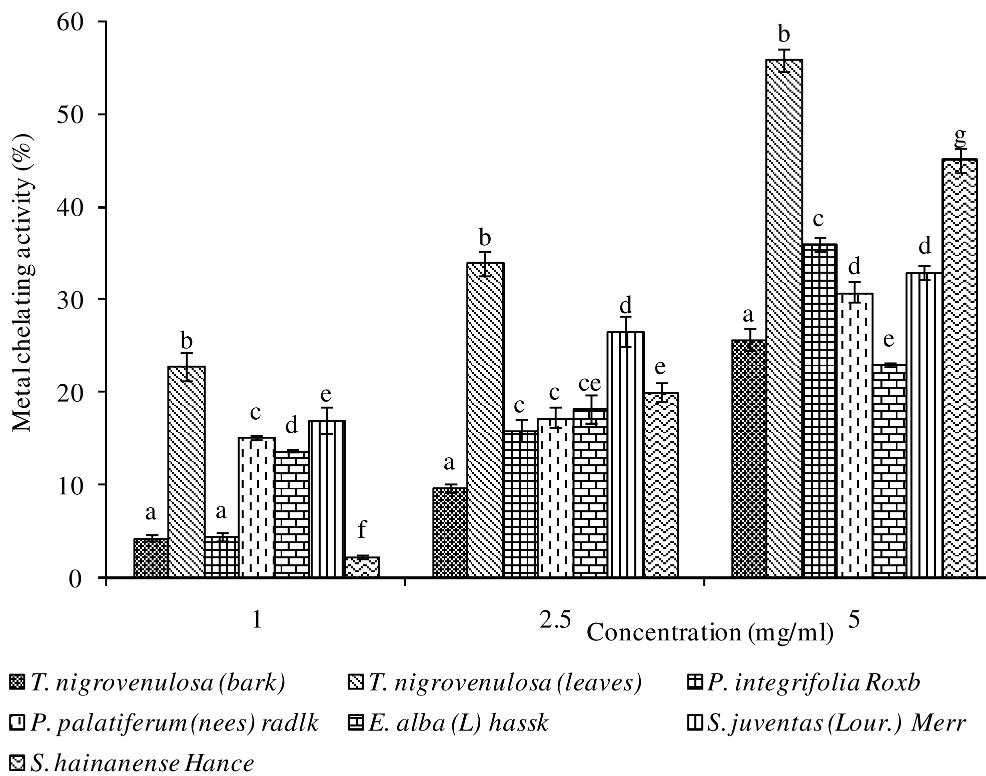

Figure 6. Metal chelating activity of ethanol extracts of medicinal plants. Results are means ± SD of triplicate measurements. Different labels (a-g) above the bars for the same concentration indicate a significant difference at p<0.05.

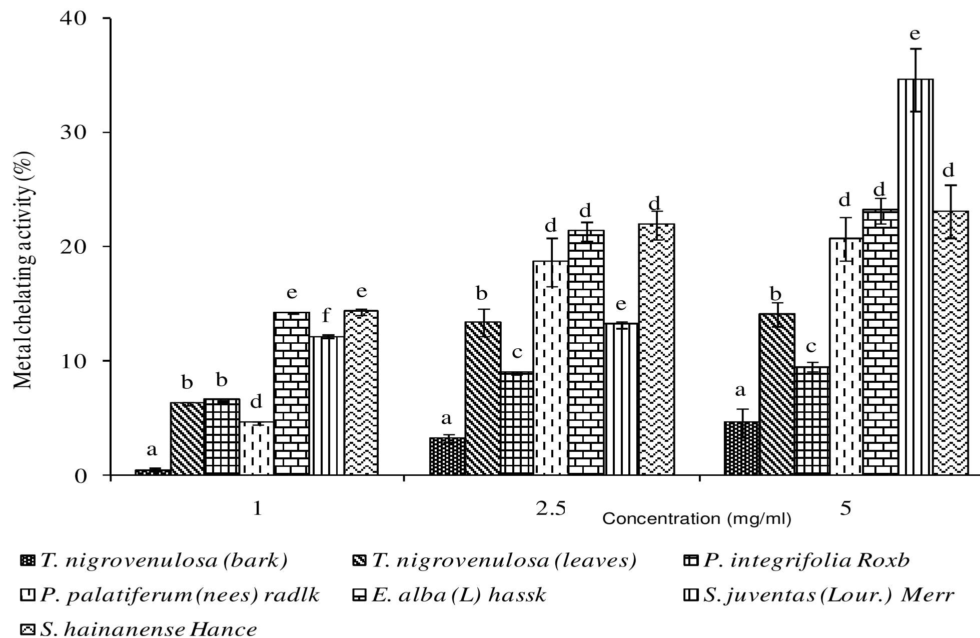

Figure 7. Metal chelating activity of acetone extracts of medicinal plants. Results are means ± SD of triplicate measurements. Different labels (a-f) above the bars for the same concentration indicate a significant difference at $p<0.05$.

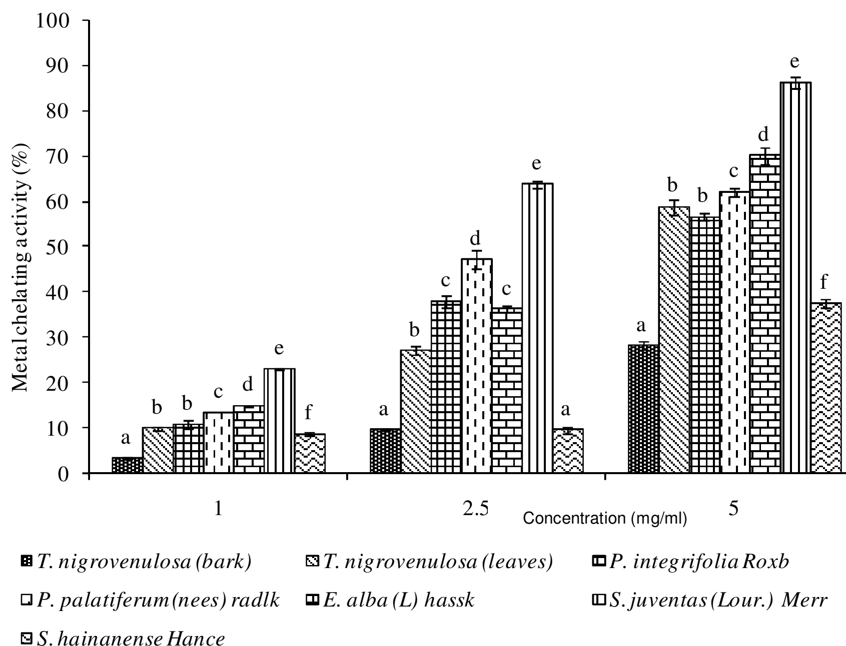

Figure 8. Metal chelating activity of water extracts of medicinal plants. Results are means ± SD of triplicate measurements. Different labels (a-f) above the bars for the same concentration indicate a significant difference at $p<0.05$.

with high polarity were responsible for metal chelating activity. The results are in agreement with previous studies that found that higher metal chelating activity was observed in water extracts of anise seed (Gulcin et al., 2003), *Thymus praecox subsp. skorpilii var. skorpilii* (Ozen et al., 2011), as well as in the methanol extracts of *Kappaphycus alvarezii* (Kumar et al., 2008), *Terminalia catappa* leaves (Chyau et al., 2002). Higher metal chelating activity was found in the extracts containing low contents of total phenolic and flavonoid

Table 7. IC_{50} values of metal chelating activity of medicinal plant extracts obtained using various solvents.

Scientific name	Parts	IC_{50} (mg/ml)			
		MeOH extracts	EtOH extracts	Acetone extracts	Water extracts
T. nigrovenulosa Pierre ex Laness.	Bark	7.596 ± 0.377^{aA}	9.590 ± 0.459^{aB}	38.429 ± 0.426^{aC}	8.573 ± 0.257^{aD}
	Leaves	3.766 ± 0.051^{bA}	4.337 ± 0.101^{bB}	25.087 ± 3.149^{bC}	$4.308 \pm .117^{bB}$
P. integrifolia Roxb	Leaves	4.362 ± 0.057^{cA}	6.798 ± 0.148^{cB}	62.186 ± 6.764^{cC}	$4.184 \pm .024^{cD}$
P. palatiferum (nees) radlk	Leaves	3.799 ± 0.041^{dA}	9.974 ± 0.602^{dB}	12.574 ± 1.587^{dC}	3.226 ± 0.440^{d}
E. alba (L) hassk	Whole plant	3.685 ± 0.031^{eA}	16.717 ± 0.406^{eB}	17.760 ± 2.631^{eB}	3.534 ± 0.106^{dC}
S. juventas (Lour.) Merr	Whole plant	1.675 ± 0.048^{fA}	9.202 ± 0.034^{fB}	7.988 ± 0.802^{fC}	2.325 ± 0.020^{fD}
S. hainanense Hance	Whole plant	2.840 ± 0.051^{gA}	5.436 ± 0.115^{gB}	15.716 ± 0.697^{gC}	$6.967 \pm .224^{gD}$
EDTA		0.961 ± 0.018			

Values (mean ± SD, n=3). In the same column different labels (a-g) and in the same raw different labels (A-D) indicate a significant difference at $P<0.05$.

compounds, demonstrating that there were many phenolic compounds in the extracts that possessed different activities. This was due to the different reaction mechanisms as well as the fact that polyphenols play a minor role in the overall antioxidant activity of metal chelation (Rice et al., 1996). Further, the chelating capacity of phenolic compounds mainly depends on the accessibility of properly oriented functional groups (Van et al., 1996), which is similar to previous research that found that antioxidant constituents possess significant antioxidant activity (Thuong et al., 2007). Generally, low molecular weight phenolics possess less chelating capacity than that of high molecular weight phenolics.

Stronger metal chelating capacity was observed in the methanol and water extracts of *S. juventas* (Lour.) Merr as well as the methanol extracts of *E. alba* (L) hassk. The metal scavenging capacities of these extracts at a concentration of 5 mg/ml were found to be 92.006, 86.143 and 76.502%, respectively as shown in Figures 5 and 8. Furthermore, relatively high metal scavenging activities were also observed in the methanol extracts of *S. hainanense* Hance and *T. nigrovenulosa* leaves (73.983 and 68.411%) as well as the water extracts of *E. alba* (L) (70.010%).

IC_{50} value is a widely used parameter for measuring antioxidant activity. A lower IC_{50} indicates higher antioxidant activity. Table 7 shows the IC_{50} values of medicinal plant extracts obtained using various solvents as well as that of control. The lowest value was observed in methanol extract of *S. juventas* (Lour.) Merr (IC_{50} value of 1.675 mg/ml), followed by methanol extract of *S. hainanense* Hance (IC_{50} value of 2.840 mg/ml). For comparison, EDTA showed an IC_{50} value of 0.961 mg/ml, which was much lower compared to extracts. Chelating agents that form σ-bonds with metal ion are effective as secondary antioxidants since they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gordon, 1990). Our results show that all extracts had effective capacity for iron binding, and therefore their action as peroxidation inhibitors may be related to their iron-binding capacity.

Correlation between free radical scavenging activity and antioxidant and phenolic content

Plant extracts with high radical scavenging activities and/or antioxidant capabilities generally have higher phenolic content. In this study, regression analyses indicates a correlation between the IC_{50} of free radical scavenging activity, antioxidant activity, and phenolic and flavonoid contents of various extracts of medicinal plants (Table 8). In our research, correlation analysis of the methanol extracts found a relatively strong relationship between the IC_{50} values for radical scavenging activity, reducing power, and metal chelating activity and the contents of total phenolics (0.8075, 0.6984 and 0.8714, respectively) and total flavonoids (0.7804, 0.7370 and 0.9225, respectively). Moreover, the ethanol and acetone extracts also showed high correlation between the IC_{50} values for DPPH scavenging activity and reducing power and total phenolic content. However, a poor correlation between the IC_{50} values for DPPH radical scavenging activity, reducing power, and metal chelating activity and total flavonoid content was found in the ethanol, acetone, and water extracts. These results indicate that methanol extraction produced a better correlation between free radical scavenging, reducing power, and metal chelating activity and total flavonoid and phenolic contents compared to those of other solvent extracts. This might have been due to the lower contents of total phenolics and flavonoids as well as the type of phenolics and flavonoids present. Several studies have shown a correlation between total phenolic and flavonoid contents and antioxidant activity. Bucic-Kojic et al. (2009) demonstrated a strong correlation between total antioxidant activity and total phenolic content in grape seed. Andarwulan et al. (1999) also found a parallel increase between total phenol content and antioxidant activity during germination of *Pangium edule*. Park et al.

Table 8. Correlation coefficients (r) between IC_{50} values of antioxidant properties and total phenolic and flavonoid contents using various solvent extracts.

Antioxidant properties	Correlation coefficients (r)							
	Total phenolics				Flavonoids			
	MeOH extract	EtOH extract	Acetone extract	Water extract	MeOH extract	EtOH extract	Acetone extract	Water extract
DPPH	0.8075	0.8515	0.7421	0.5743	0.7804	0.1237	0.6362	0.7174
Reducing power	0.6984	0.8304	0.6441	0.4180	0.7370	0.3478	0.4808	0.5491
Metal chelating	0.8714	0.2911	0.3122	0.6844	0.9225	0.2377	0.4112	0.5682

(2006) observed a very good correlation between the percentages of inhibition of DPPH and ABTS radicals and total phenolic content in fresh and dried persimmon fruit. Tsaliki et al. (1999) observed an increase in the antioxidant activity of lupin seed flour containing phenolic compounds, peptides/amino acids, and phospholipids. Zhou et al. (2009) have demonstrated a high correlation between total phenolic content and antioxidant capacity. On the other hand, some authors have reported no direct correlation between phenolic content and antioxidant activity. No relationship between antioxidant activity and phenolic content was found in citrus residues (Bocco et al., 1998), fruit berry, fruit wines (Heininen et al., 1998), or plant extracts (Kähkönen et al., 1999).

Conclusion

The current work demonstrates that the type of extraction solvent strongly influenced the total phenolic and flavonoid contents as well as the antioxidant properties of the obtained extracts. The results show that methanol extraction displayed the highest total phenolic and flavonoid contents and also possessed the strongest free radical scavenging and antioxidant activities. Especially, regarding the extracts of *T. nigrovenulosa* (leaves and bark) and *P. integrifolia* Roxb leaves, their DPPH scavenging and reducing capacities were higher than that of BHT. Moreover, the highest metal chelating activity was found in the methanol and water extracts of *T. nigrovenulosa* leaves and *S. juventas* as well as in the methanol extract of *E alba* (L) hassk. Further studies are required to determine the types of bioactive compounds present in the most effective plant extracts as well as those responsible for antioxidant activity.

REFERENCES

Akowuah GA, Ismail Z, Norhayati I, Sadikum A (2005). The effect of different extraction solvents of varying polarities on polyphenols of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity. Food Chem., 93: 311-317.

Agrawal PK (1989). Carbon-13 NMR of flavonoids. New York:Elsevier.

Amensour M, Sendra E, Pérez-Alvarez JA, Skali-Senhaji N, Abrini J, Fernández-López J (2010). Antioxidant Activity and Chemical Content of Methanol and Ethanol Extracts from Leaves of Rockrose (*Cistus ladaniferus*) in Plant. Foods Hum. Nutr., 65: 170-178.

Andarwulan N, Fardiaz D, Wattimena GA, Shetty K (1999). Antioxidant activity associated with lipid and phenolic mobilization during seed germination of *Pangium edule Reinw*. J. Agric. Food Chem., 47: 3158-3163.

Arabshahi-Delouee S, Urooj A (2007). Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L) leaves. Food Chem., 102: 1233-1240.

Barik BR, Bhowmik T, Dey AK, Patra A, Chatterjee A, Joy SS (1992). Premnazole an isoxazole alkaloid of *Premna integrifolia* and *Gmelina arborea* with anti-inflammatory activity. Fitoterapia, 63/4: 295–299.

Basu NK, Dandiya PC (1947). Chemical investigation of *Premna integrifolia linn*. J. Am. Pharm. Assoc. Sci. Educ., 36: 389-391.

Bocco A, Cuvelier ME, Richard H, Berset C (1998). Anti-oxidant activity and phenolic composition of citrus peel and seed extracts. J. Agric. Food Chem., 46: 2123-2129.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of free radical method to evaluate antioxidant activity. LWT– Food Sci. Technol., 28: 25-30.

Bucic-Kojic A, Planinic M, Tomas S, Jakobek L, Seruga M (2009). Influence of solvent and temperature on extraction of phenolic compounds from grape seed, antioxidant activity and colour of extract. Intl. J. Food Sci. Technol., 44: 2394-2401.

Cuong ND, Quynh NH (1999). *Pseuderanthemum palatiferum (Nees) Radlk*. Pharmaceutical encyclopedia. Encyclopedia Publisher, Hanoi, 714 [In Vietnamese].

Debelas AM, Dobremez JF, Michel S, Benarroche L (1973). Medicinal Plants of Nepal. Plantes Medicinales et Phytotherapie, 7: 104-113.

Demiray S, Pintado ME, Castro PML (2009). Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: *Tilia argentea, Crataegi folium* leaves and *Polygonum bistorta* roots. World Acad. Sci. Eng. Technol., 54: 312-317.

Dinis TCP, Madeira VMC, Almeida LM (1994). Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys., 315: 161-169.

Folin O, Ciocalteu V (1927). On tyrosine and tryptophane determination in proteins. J. Biol. Chem., 27: 627-650.

Fogliano V, Verde V, Randazzo G, Ritieni A (1999). Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. J. Agric. Food Chem., 47: 1035-1040.

Gao X, Bjork L, Trajkovski V, Uggla M (2000). Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. J. Agric. Food Chem., 80: 2021-2027.

Gokani RH, Lahiri SK, Santani DD, Shah MB (2007). Evaluation of Immunomodulatory activity of *Clerodendrum phlomidis* and *Premna Integrifolia* Root. Int. J. Pharmacol., 3(4): 352-356.

Gülçin I, Oktay M, Kireçci M, İrfan OK (2003). Sreening of antioxidant and antimicrobial activities anise (*Pimpinella anisum L*) seed extracts. Food Chem., 83: 371-382.

Heinonen M, Lehtonen PJ, Hopia AL (1998). Antioxidant activity of berry and fruit wines and liquors. J. Agric. Food Chem., 46: 25-31.

Ho PH (2000). Vietnamese vegetation. Young Publishing House, 3: 69 [In Vietnamese].

Jayaprakasha GK, Singh RP, Sakariah KK (2001). Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chem., 73: 285-290.

Jayathirtha MG, Mishra SH (2004). Preliminary immunomodulatory activities of methanol extracts of *Eclipta alba* and *Centella asiatica*. Phytomed., 11: 361-365.

Jia Z, Tang M, Wu J (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 64: 555-599.

Jimenez-Escrig A, Rincon M, Pulido R, Saura-Calixto F (2001). Guava fruit (*Psidium guajava L.*) as a new source of antioxidant dietary fiber. J. Agric. Food Chem., 49: 5489-5493.

Karthikumar S, Vigneswari K, Jegatheesan K (2007). Screening of antibacterial and antioxidant activities of leaves of *Eclipta prostrata* (L). Sci. Res. Essay, 2(4): 101-104.

Khanh TC (1998). Contribution to the studies on botany, chemical composition and biological activities of *Pseuderanthemum palatiferum (Nees) Radlk*. J. Mater. Med., 3(2): 37-41 [In Vietnamese].

Kinsella JE, Frankel E, German B, Kanner J (1993). Possible mechanism for the protective role of the antioxidant in wine and plant foods. Food Technol. 47: 85-89.

Kobari M, Yang Z, Gong D, Heissmeyer V, Zhu H, Jung YK, Angelica M, Gakidis M, Rao A, Sekine T, Ikegami F, Yuan C, Yuan J (2004). Wedelolactone suppress LPS- induced caspase-11 expression by directly inhibiting the IKK complex. J. Cell Death Differentiation, 11(1): 123-130.

Lai LS, Chou ST, Chao WW (2001). Studies on the antioxidative activites of Hsian-tsao (*Mesona procumbens Hemsl*) leaf gum. J. Agric. Food Chem., 49: 963-968.

Majhenič L, Škerget M, Knez Z (2007). Antioxidant and microbial activity of guarana seed extracts. Food Chem., 104: 1258-1268.

Manian R, Anusuya N, Siddhuraju P, Manian S (2008). The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis (L.) O. Kuntz*, *Ficus bengalensis L.* and *Ficus racemosa L.* Food Chem., 107: 1000-1007.

Mathew S, Abraham TE (2006). Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various *in vitro* models. Food Chem., 94: 520-528.

Meir S, Kanner J, Akiri B, Philosoph-Hadas S (1995). Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J. Agric. Food Chem., 43: 1813-1819.

Melo PA, Nascimento MC, Mors WB, Surez-Kurtz G (1994). Inhibition of the mytotoxic and hemorrhagic activities of crotalid venoms *by Eclipta prostrata* (Astraceae) extracts and constituents. Toxicon., 32: 595-603.

Miliauskas G, Venskutonis PR, Van Beek TA (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem., 85: 231-237.

Moure A, Jose MC, Daniel F, Manuel DJ, Jorge S, Herminia D, María José N (2001). Natural antioxidants from residual sources. Food Chem., 72: 145-171.

Nguyen PT, Le Van T, Nguyen KH, Huynh Le (1998). Protective Efficacy of *Solanum Hainanense Hance* during Hepatotoxicity in Male Mice with Prolonged and Small Oral Doses of Trinitrotoluene. J. Occup. Health, 40: 276-278.

Oanh LT (1999). Investigation of some biochemical characters of proteolytic activity of *Pseuderanthemum palatiferum*. J. Mater. Med., 4(1): 13-17 [In Vietnamese].

Oliveira I, Sousa A, Ferreira CFRI, Bento A, Estevinho L, Pereira JA (2008). Total phenolics, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. Food Chem. Toxicol., 46: 2326-2331.

Oyaizu M (1986). Studies on product of browning reaction prepared from glucose amine. Jpn. J. Nutr., 44: 307-315.

Park Y, Jung S, Kang S, Delgado-Licon E, Ayala A, Tapia MS, Martin-Belloso O, Trakhtenberg S, Gorinstein S (2006). Drying of persimmons (*Diospyros kaki* L.) and following changes in the studied bioactive compounds and the total radical scavenging activities. LWT - Food Sci. Technol., 39: 748-755.

Peterson DM, Emmons CL, Hibbs AH (2001). Phenolic antioxidants and antioxidant activity in pearling fractions of oat groats. J. Cereal Sci., 33: 97-103.

Pryor WA (1991). The antioxidant nutrient and disease prevention—what do we know and what do we need to find out? Am. J. Clin. Nutr., 53: 391-393.

Rice-Evans CA, Miller NJ, Paganga G (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med., 20: 933-956.

Roedig-Penman A, Gordon MH (1998). Antioxidant properties of myricetin and quercetin in oil and emulsions. J. Am. Oil Chem. Soc., 75: 169-180.

Saeedeh AD, Urooj A (2007). Antioxidant properties of various solvent extracts of mulberry (*Morus indica L.*) leaves. Food Chem., 102: 1233-1240

Shiddhuraju P, Mohan PS, Becker K (2002). Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. Food Chem., 79: 61-67.

Shimada K, Fujikawa K, Yahara K, Nakamura T (1992). Antioxidative properties of xanthan on the auto-oxidation of xanthan on auto-oxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem., 40: 945-948.

Sousa A, Ferreira ICFR, Barros L, Bento A, Pereira JA (2008). Antioxidant potential of traditional stoned table olives "*Alcaparras*": influence of the solvent and temperature extraction conditions. LWT - Food Sci. Technol., 41: 739-745.

Thuong PT, Kang HJ, Na MK (2007). Anti-oxidant constituents from Sedum takesimense. Phytochem., 68: 2432-2438

Tsaliki E, Lagouri V, Doxastakis G (1999). Evaluation of the antioxidant activity of lupin seed flour and derivatives (*Lupinus albus* ssp. *Graecus*). Food Chem., 65: 71-75.

Turkmen N, Sari F, Velioglu YS (2006). Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. Food Chem., 99: 835-841.

Xiaoli L, Mouming Z, Jinshui W, Bao Y, Yueming J (2008). Antioxidant activity of methanol extracts of emblica fruit (*Phyllanthus emblica L*) from six regions in china. J. Food Compos. Anal., 21: 219-228.

Van Acker SA, Van Den Berg DJ, Tromp MN (1996). Structural aspects of antioxidant activity of flavanoids. Free Radic. Biol. Med., 20: 331-342.

Wanasundara UN, Shahidi F (1998). Antioxidant and pro-oxidant activity of green tea extracts in marine oils. Food Chem., 63: 335-342.

Wagner H, Geyer B, Kiso Y, Hikino H, Roa GS (1986). Coumestans as the main active principles of the liver drugs *Eclipta alba* and *Wedelia calendulacea*. Planta Med., 52: 370-374.

Whysner J, Wang CX, Zang E, Iatropoulos MJ, Williams GM (1994). Dose response of promotion by butylated hydroxyanisole in chemically initiated tumours of the rat fore stomach. Food Chem. Toxicol., 32: 215-222.

Wong SM, Antus S, Gottengen A, Fessler B, Roa GS, Sonnenbichler J, Wagner H (1995). Wedelolactone and coumestan derivatives a new antihepatotoxic and antiphlogestic principles. Arzeneim- Fortschritte derarzneimittel forschung progress in drug research, 40: 97.

Yen GC, Duh PD, Tsai CL (1993). Relationship between antioxidant activity and maturity of peanut hulls. J. Agric. Food Chem., 41: 67-70.

Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa K (2000). Antioxidative and antiglycation activity of garcinol from Garcinia indica fruit rind. J. Agric. Food Chem., 48: 180-185.

Zheng W, Wang SY (2001). Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem., 49: 5165-5170.

Zhou S, Fang Z, Lu Y, Chen J, Liu D, Ye X (2009). Phenolics and antioxidant properties of bayberry (*Myrica rubra Sieb. et Zucc.*) pomace. Food Chem., 112(2): 394-399.