Journal of Medicinal Plants Research Vol. 5(10), pp. 1884-1891, 18 May, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

A comparative evaluation of the antioxidant activity of some medicinal plants popularly used in Nepal

Bimal Kumar Ghimire[1], Eun Soo Seong[2], Eun Hye Kim[1], Amal Kumar Ghimeray[3], Chang Yeon Yu[2], Bal Krishna Ghimire[2] and Ill Min Chung[1]*

[1]Department of Applied Life Science, Konkuk University, Seoul 143-701, South Korea.
[2]Bioherb Research Institute, Kangwon National University, Chuncheon 200-701, South Korea.
[3]Department of Bio-Health Technology, College of Bio-medical Science, Kangwon National University, Chuncheon 200-701, Korea.

Accepted 1 September, 2010

Recently, attention has focused on phytochemicals as new sources of natural antioxidants. Therefore, the methanolic crude extracts of 24 commonly used medicinal plants from Jhapa and Illam District, Nepal, were screened for total phenols, flavonoids, and free radical scavenging activity. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). Significant differences in DPPH scavenging activity were found between the species investigated, ranging from 14.67 ± 1.00% to 92.33 ± 1.53%. The highest radical scavenging activity was observed in *Artemisia vulgaris* (92.33 ± 1.53% inhibition), followed by *Ficus lacor* (92.00 ± 1.73%) and *Mallotus philippensis* (91.33 ± 1.53%). The total phenol content of the investigated species ranged from 28.87 ± 2.36 to 321.23 ± 1.06 mg GAE/g extract, while flavonoid content ranged from 13.53 ± 0.85 to 100.33 ± 1.53 mg QE/g extract. A weak linear correlation between total phenolic or flavonoid content and antioxidant activity was found (correlation coefficient, R^2 = 0.3004, R^2 = 0.4294, respectively), indicating that the major antioxidant components might not be phenolics. In particular, *A. vulgaris*, *F. lacor*, *M. philippensis*, *Trachyspermum ammi*, and *Amomum subulatum* showed strong activity against DPPH, and thus could be used as natural antioxidants in the food and/or pharmaceutical industry.

Key words: Phenolic content, flavonoids, medicinal plants, antioxidant activity, methanolic extraction.

INTRODUCTION

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl), and free radicals, such as the hydroxyl radical (·OH) and superoxide anion (O_2^-), are produced as normal products of cellular metabolism. Rapid production of free radicals can lead to oxidative damage to biomolecules and may cause disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular diseases, neurodegenerative diseases, and premature aging (Young and Woodside, 2001). Many medicinal plants contain large amounts of antioxidants, such as polyphenols, vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein, and other carotenoids, which play important roles in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Djeridane et al., 2006). Moreover, plant secondary metabolites such as flavonoids and terpenoids play an important role in defense against free radicals (Govindarajan et al., 2005).

Therefore, consumers should increase their intake of foods rich in antioxidant compounds that lower the risk of chronic health problems associated with the above diseases (Klipstein-Grobusch et al., 2000).

Data from various studies indicate that medicinal plants contain a wide variety of natural antioxidants, such as phenolics, flavonoids, and tannins, which possess more potent antioxidant activity than common dietary plants.

*Corresponding author. E-mail: imcim@konkuk.ac.kr. Tel: +82-2-450-3730. Fax: +82-2-446-7856.

Compounds responsible for such antioxidant activity can be isolated and used for prevention and treatment of free radical-related disorders (Middleton et al., 2000). Therefore, recent attention has increased to find naturally occurring antioxidants for use in food or medicine to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Velioglu et al., 1998).

Nepal is natural storehouse of medicinal plants (Manandhar, 2002). Approximately, 70 to 80% of the population of Nepal depends on traditional medicines (Sharma et al., 2004). Indigenous people residing in different belts depend on local plant and plant products to meet their daily requirements for food, fodder, medicines etc. (Acharya and Pokhrel, 2006). Local herbs and other plant resources found in rural area are the principle source of medicine for treating diseases since time immemorial.

Despite widespread use of wild plants as medicines in Nepal, little is known about the antioxidant potential and chemical composition of these plants.

The aim of the present study was to evaluate and compare 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and the phenolic and flavonoid content of traditional medicinal species from Jhapa and Illam District, Nepal. In addition, the study sought to determine the relationship between the DPPH radical scavenging activity and total phenolic content of 24 plant extracts that might be promising sources of natural antioxidants and functional foods.

MATERIALS AND METHODS

Twenty-four traditional medicinal plants from Jhapa and Illam District, Nepal, were evaluated in this study. Plant material was gathered in May 2009, at different altitudes and identified by botanists from the Central Department of Botany, Tribhuwan University, Nepal. Data on the medicinal uses, plant parts used, preparation, and administration were collected from indigenous people (Table 1).

Flavonoid standards, quercetin and DPPH, gallic acid, BHA (tert-butyl-4-hydroxy-anisol), α-tocopherol, and Folin–Ciocalteu reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Graded methanol and ethanol was purchased from Daejung Chemical and Metals Co. Ltd., Korea.

Preparation of plant extracts

Plant material was washed, dried, and powdered at room temperature. The powdered samples (2 g each) were suspended and extracted in 25 ml of 80% methanol (v/v) and kept for 1 day on a shaker at room temperature.

The extracts were filtered through Advantec 4B (Tokyo Roshi Kaisha Ltd., Japan).

The extraction of the residue was repeated twice under the same conditions. The methanolic extract was first dried using a vacuum rotary evaporator (N-1000; EYLA, Tokyo, Japan) in a water bath at 40°C. Dried samples were weighed and kept at 4°C until use.

Free radical scavenging activity

Free radical scavenging activity was evaluated using α-tocopherol and BHA as standard antioxidants. The radical scavenging activity was measured using the stable radical DPPH according to the method described by Shimada et al. (1992) with some modifications. Various concentrations of the extracts were added to 4 ml of a 0.004% methanol solution of DPPH. The mixture was shaken and left for 30 min at room temperature in the dark, and the absorbance was then measured with a spectrophotometer at 517 nm. All determinations were performed in triplicate. The antioxidant activity was calculated as the percent inhibition caused by the hydrogen donor activity of each sample according to the following:

Inhibition (%) = (1 – absorbance of the sample/absorbance of the blank) × 100.

Total phenolic content (TPC)

The extraction of total phenolics was performed using the Folin–Ciocalteu assay, following the method of Kim et al. (2007) with some modifications. In total, 100 µl of each extract (1 mg/ml) was added to a test tube containing 50 µl of the phenol reagent (1 M). A further 1.85 ml of distilled deionized water was added to the solution and allowed to stand for 3 min after vortexing; then 300 µl Na_2CO_3 (20% in water, v/v) was added and vortexed, and the final volume (4 ml) was obtained by adding 1.7 ml of distilled deionized water. A reagent blank was prepared using distilled deionized water. The final mixture was vortexed, then incubated for 1 h in the dark at room temperature. The absorbance was measured at 725 nm using a UV-VIS spectrophotometer (V 530; Jasco, Tokyo, Japan). A standard curve was prepared using 0, 65.5, 125, and 250 mg/l gallic acid in methanol: water (50:50, v/v). Total phenolic values are expressed in terms of gallic acid equivalents (GAE) in milligrams per gram plant extract. All determinations were performed in triplicate.

Total flavonoid content (TFC)

The total flavonoid content in extracts was determined according to Moreno et al. (2000). A 0.5 ml sample (1 mg/ml) was mixed with 0.1 ml of 10% aluminum nitrate and 0.1 ml of potassium acetate (1 M), and 4.3 ml of 80% ethanol was added to make a total volume of 5 ml.

The mixture was vortexed and the solution was allowed to stand for 40 min for reaction at room temperature. The absorbance was measured spectrophotometrically at 415 nm. All determinations were performed in triplicate. Total flavonoid values are expressed in terms of quercetin equivalents (QE) per gram of plant extract. A standard curve was prepared using 0, 5, 10, and 100 mg/l solutions of quercetin.

Statistical analysis

Antioxidant activity, total phenolic content, and flavonoid content are reported as the mean ± standard deviation (SD). Significance differences for multiple comparisons were determined using one-way analysis of variance (ANOVA). Duncan's multiple range tests was used to assess the significant differences with the SPSS statistical analysis package (version 15.0; SPSS Inc., Chicago, IL, USA). Differences at $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

DPPH assay

The antioxidant activity of medicinal plants is mainly

Table 1. Indigenous use of 24 medicinal plants from Jhapa and Illam district, Nepal.

Plant material	Local name	Indigenous uses
Achyranthes aspera L. (Amaranthaceae)	Dattiwan	To cure fever, swelling and rheumatism (Ghimire and Bastakoti, 2009).
Acorus calamus L. (Acoraceae)	Bojho	To treat dysentery, cough (Joshi, 1990).
Amomum subulatum Roxb. (Zingiberaceae)	Alaichi	Plant is used cure dysentery, skin diseases, anorexia, ulcers and wounds (Sharma et al., 2002).
Artemisia vulgaris L. (Asteraceae)	Titepati	Remedy for the Scabies, gastric, headache (Ghimire and Bastakoti, 2009).
Asparagus racemosus Willd. (Liliaceae)	Kurilo	To cure dyspepsia, diarrhea, inflammations, hyperdipsia, neuropathy, (Goyal et al., 2003).
Azadirachta indica A. Juss (Meliaceae)	Neem	Leprosy, skin infections (Biswas et al., (2002).
Berginia ciliata Sternb. (Saxifragaceae)	Pakhanbeth	Remedy for the Sprain, diarrhoea, ophthalmic (Manandhar, 1994).
Cassia fistula L. (Leguminosae)	Rajbrikshya	Plant extracts is used to cure insomnia, gastric and diarrhea (Ghimire and Bastakoti, 2009).
Chenopodium album L. (Chenopodiaceae)	Bethu	To cure constipation, joint pains (Ghimire and Bastakoti, 2009).
Cuscuta reflexa Roxb. (Convolvulaceae)	Mhasu lahara	Jaundice Cough, stomach disorders (Joshi 1990).
Drymaria cordata L. (Caryophyllacea)	Abhijalo	Leaves extract are useful in Sinusitis, diarrhoea, dysentery (Manandhar, 1994).
Ficus lacor Buch.-Ham. (Moraceae)	Kabro	To cure Gastric, ulcer, typhoid, dysentery and boils, stomach disorder (Rai et al., 2004).
Mallotus philippensis Lam. (Euphorbiaceae)	Sindhure	To cure tape gastric, diarrhea, bronchitis, typhoid and meningitis (Ghimire and Bastakoti, 2009).
Mentha arvensis L. (Labiatae)	Pudhina	Leaves extract are useful in internal heat, fever (Ghimire and Bastakoti, 2009).
Ocimum sanctum L. (Labiatae)	Tulasi	To cure bronchitis, dysentery, dyspepsia, skin diseases, chronic fever (Kirtikar K and Basu B 1993).
Persea odoratissima Nees (Lauraceae)	Kaulo	Plant extracts are considered as antiseptic and anti-inflammatory remedies (Giang et al., 2006).
Premna integrifolia L. (Verbenaceae)	Gineri	Treatment of cardiovascular diseases, rheumatism, anorexia and jaundice (Rajendran et al., 2009).
Rauvolfia serpentina L. (Apocynaceae)	Sarpagangha	Remedy for high blood pressure, insomnia, epilepsy, hypochondria, (Bhatara, 1997).
Santalum album L. (Santalaceae)	Shrikhanda	Remedy for dysentery, gonorrheal, urethral, bronchial disorders (Parekh et al., 2005).

Table 1. Contd.

Sapium insigne Royle. (Euphorbiaceae)	Khirro	To kills germs, quick healing (Manandhar, 1995).
Swertia chirayita Roxb. (Gentianaceae)	Chiraito	Remedy for the fever (Manandhar, 1994).
Tachyspermum ammi L. (Umbelliferae)	Jwano	Seed extract is useful as wound, pimples and rashes (Ghimire and Bastakoti, 2009).
Terminalia chebula Retz. (Combretaceae)	Harro	As an astringent, antitussive, antidiarrhea, and styptic agent (Juang et al., 2004).
Withania somnifera Dunal. (Solanaceae)	Ashogandha	Remedy for Cough, dropsy, rheumatism, ulcer, sore eyes (Mahesh and Satish, 2008).

related to their bioactive compounds, such as phenolics, flavonols, and flavonoids. In this study, the antioxidant capacity of 24 methanolic extracts of medicinal plants from Jhapa and Illam district, Nepal, was systematically evaluated. The DPPH inhibition of different plant extracts is summarized in Table 2. Significant differences ($P < 0.05$) in DPPH scavenging activity were found between the 24 plants investigated, ranging from 14.67 ± 1.00% to 92.33 ± 1.53%. This wide range of antioxidant activity may be attributable to the wide variety of bioactive compounds, such as phenolics, flavonols, carotenoids, and tannins, present in the selected medicinal plants. On the basis of antioxidant activity, a sample can be classified into one of four major groups, viz., very high, high, moderate, or low antioxidant content. Five plants (20.83%) possessed very high antioxidant activity (>90% inhibition of DPPH), eight (33.33%) had high antioxidant activity (80 to 90%), five (20.83%) had moderate antioxidant activity (<80%), and six (25%) had low (<50%) activity.

Methanolic extracts of leaves of *Artemisia vulgaris* possessed the highest DPPH scavenging activity (92.33 ± 1.53% inhibition of the DPPH radical), followed by *Ficus lacor* and *Mallotus philippensis* (92.00 ± 1.73% and 91.33 ± 1.53%, respectively), comparable to the commercial antioxidant BHA (93.03% inhibition of the DPPH radical) and α-tocopherol (92.00%). The higher antioxidant activity of *A. vulgaris* could be due to the high concentration of total phenolics and flavonoids in the plants. Leaf extracts of *A. vulgaris* were reported to contain phenolic acid and a complex mixture of 20 known flavonoids, predominantly eriodictyol and luteolin (Wojdylo et al., 2007). High antioxidant activity was also found in the leaves of *F. lacor*, which had comparatively low total phenolic content, indicating that the major antioxidant components might not be phenolics. Moreover, Dey et al. (2008) reported high tannin content in leaves of *F. lacor*, which may contribute to the high antioxidant properties. For *M. philippensis*, high antioxidant activity could be attributable to the presence of known bioactive compounds, such as tannins, dimeric chalcone derivatives, triterpenoids, and steroids (Tanaka et al., 1998; Arfan et al., 2009). The lowest levels of DPPH scavenging activity were in the leaves of *Drymaria cordata*, with an estimated 14.67 ± 1.00% of the DPPH radical quenched ($P < 0.05$).

Total phenolic content

Phenolics are well established to show antioxidant activity and contribute to human health. In this study, the total phenolic content was determined using the Folin–Ciocalteu method, with gallic acid as a standard. The content of phenolics was evaluated from the regression equation of the calibration curve ($R^2 = 0.993$, $y = 0.006x + 0.0437$), expressed in GAE as milligrams per gram of extract (mg GAE/g extract). The total phenolic content of the 24 plant samples showed large variations, between 23.80 ± 2.36 and 321.23 ± 1.06 mg GAE/g extract (Table 2). Many plant species have a remarkably high total phenolic content. The leaf methanolic extract of *A. vulgaris* contained the highest total phenol content (321.23 ± 1.06 mg GAE/g extract), followed by *Terminalia chebula* (156.00 ± 0.62 mg GAE/g extract) and *Premna integrifolia* (143.67 ± 2.37 mg GAE/g extract). The high level of phenolics in *A. vulgaris* could be due to known phenolic compounds, such as caffeic acid, neochlorogenic acid, and ferulic acid (Wojdylo et al., 2007). Leaf extracts of *Drymaria cordata* had the lowest total phenolics (23.80 ± 2.36 mg GAE/g extract), while *T. chebula* had the highest total phenolics (156.00 ± 0.62 mg GAE/g extract) and is reported to contain gallic acid, chebulic acid, 1,6-di-*O*-galloyl-β-D-glucose, punicalagin, 3,4,6-tri-*O*-galloyl-β-D-glucose, casuarinin, chebulanin, corilagin, neochebulinic acid, terchebulin, ellagic acid,

Table 2. Antioxidant activities, phenolic and flavonoid contents of 24 medicinal plants from Jhapa and Illam district, Nepal.

Plant material	Material used	% inhibition of DPPH	Total flavonoids (mg QE/g extract) *	Total phenols (mg GAE/g extract) **
BHA	-	93.03 ± 0.36 ^{j,k}	-	-
α-tocopherol	-	92.00 ± 0.58 ^{j,k}	-	-
Achyranthes aspera	Leaf	73.00 ± 4.36^{e}	16.83 ± 0.31^{a,d}	74.73 ± 0.76$^{e\sim h}$
Acorus calamus	Root	87.33 ± 2.08^{i}	23.90 ± 2.42^{d}	67.93 ± 0.15 $^{d\sim g}$
Amomum subulatum	Fruit	90.00 ± 1.00^{j}	66.00 ± 1.00^{i}	94.51 ± 2.50^{i,j}
Artemisia vulgaris	Leaf	92.33 ± 1.53^{j,k}	100.33 ± 1.53^{m}	321.23 ± 1.06^{m}
Asparagus racemosus	Root	30.00 ± 3.61^{c}	21.50 ± 1.99^{c,d}	45.37 ± 1.07^{b,c}
Azadirachta indica	Leaf	73.67 ± 2.52^{e}	39.17 ± 4.91^{e}	66.37 ± 1.27$^{d\sim f}$
Berginia ciliata	Leaf	80.34 ± 1.12^{f}	37.43 ± 2.76^{e}	80.50 ± 3.11$^{f\sim i}$
Cassia fistula	Leaf	52.33 ± 1.53^{d}	22.33 ± 1.62^{c,d}	36.43 ± 1.55^{a,b}
Chenopodium album	Leaf	80.67 ± 1.00^{f}	60.47 ± 2.57^{h}	77.03 ± 1.53$^{e\sim h}$
Cuscuta reflexa	Stem	84.67 ± 1.53^{g,h}	37.87 ± 3.24^{e}	123.33 ± 2.32^{k}
Drymaria cordata	Leaf	14.67 ± 1.00^{a}	15.50 ± 2.26^{a}	23.80 ± 1.11^{a}
Ficus lacor	Leaf	92.00 ± 1.73^{j,k}	69.83 ± 2.86^{j}	82.37 ± 1.12$^{g\sim i}$
Mallotus philippensis	Leaf	91.33 ± 1.53^{j,k}	89.00 ± 1.00^{l}	62.57 ± 1.06^{d,e}
Mentha arvensis	Leaf	81.33 ± 1.16^{f}	96.43 ± 2.08^{l}	99.43 ± 1.99^{j}
Ocimum sanctum	Leaf	74.00 ± 3.00^{e}	36.83 ± 3.76^{e}	73.50 ± 0.85$^{e\sim h}$
Persea odoratissima	Bark	30.33 ± 0.58^{c}	21.10 ± 2.02^{c,d}	54.00 ± 1.00^{c,d}
Premna integrifolia	Bark	85.67 ± 0.58^{h,i}	22.07 ± 2.04^{c,d}	143.67 ± 2.37^{l}
Rauvolfia serpentine	Leaf	16.33 ± 0.58^{a}	15.63 ± 0.91^{a}	29.43 ± 0.76^{a}
Santalum album	Leaf	50.33 ± 3.22^{d}	46.50 ± 2.72^{f}	85.63 ± 2.21$^{h\sim j}$
Sapium insigne	Fruit	22.00 ± 3.46^{b}	20.07 ± 1.62^{b,c}	34.30 ± 0.40^{a,b}
Swertia chirayita	Leaf	85.33 ± 0.58^{h,i}	60.30 ± 2.46^{h}	67.53 ± 2.08$^{d\sim g}$
Tachyspermum ammi	Seed	90.67 ± 0.58^{j,k}	51.90 ± 1.31^{g}	87.48 ± 1.36$^{h\sim j}$
Terminalia chebula	Fruit	82.67 ± 1.53^{f,g}	81.00 ± 1.00^{k}	156.00 ± 0.62^{l}
Withania somnifera	Leaf	46.00 ± 3.60^{d}	13.53 ± 0.85^{a}	28.97 ± 2.36^{a}

Values are mean (n = 3) ± SD (standard deviation). Values in the same column followed by the same letter are not significantly different at $P < 0.05$. *Values are expressed as quercitine equivalents in mg/g extract. **Values are expressed as gallic acid equivalent in mg/g extract.

chebulagic acid, chebulinic acid, and 1, 2,3,4,6-penta-*O*-galloyl-β-D-glucosein, which might be responsible for its high antioxidant activity (Juang et al., 2004).

Total flavonoid content

Flavonoids are well-known antioxidant constituents of plants and possess a broad spectrum of chemical and biological activity, including radical scavenging properties (Miliauskas et al., 2004). Therefore, the total content of flavonoids was evaluated from the regression equation of the calibration curve ($R^2 = 0.991$, $y = 0.0058x - 0.0048$), expressed in QE as milligrams per gram of extract (mg QE/g extract). The content of flavonoids varied from 13.53 ± 0.85 to 100.33 ± 1.53 mg QE/g extract (Table 2). The highest amount of flavonoids was found in the leaf extracts of *A. vulgaris* (100.33 ± 1.53 mg QE/g extract), followed by *Mentha arvensis* (96.43 ± 2.08 mg QE/g extract), *M. philippensis* (89.00 ± 1.00 mg QE/g extract), and *T. chebula* (81.00 ± 1.00 mg QE/g extract), indicating that these phytochemicals are likely to be responsible for the free radical scavenging activity. Flavonoids are reportedly responsible for the antioxidant activities of plants (Das and Pereira, 1990) through their scavenging or chelating activity (Kessler et al., 2003). However, a relatively low amount of flavonoids was detected in *Acorus calamus*, *Cuscuta reflexa*, and *P. integrifolia*, which had higher antioxidant activity.

Correlation between antioxidants and total phenolics and total flavonoids

The total phenolic content of the investigated plants extracts showed a weak correlation with antiradical activity (Figure 1; $R^2 = 0.3004$, $y = 0.2395x + 46.836$). This low correlation between total phenols and DPPH scavenging activity suggests that the major antioxidant components might not be phenolics, and could be sterols, tocopherols, ascorbic acid, and/or carotenoids. The correlation between total flavonoids and DPPH scavenging ($R^2 = 0.4294$, $y = 0.6327x + 38.869$) was very weak (Figure 2), consistent with the findings of Imeh and

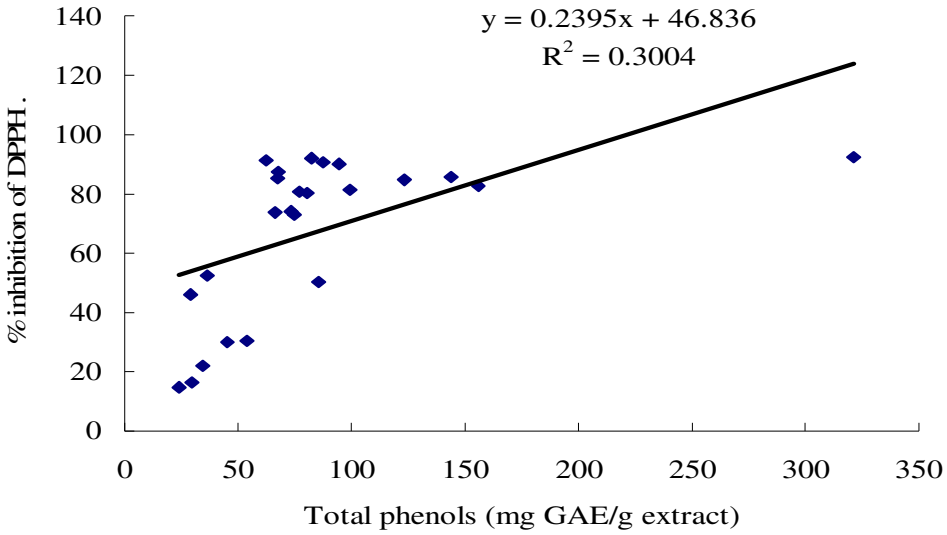

Figure 1. Linear correlation between the antioxidant activity and total phenolic content of the methanol extracts of 24 medicinal plants from Jhapa and Illam district, Nepal. GAE: gallic acid equivalents.

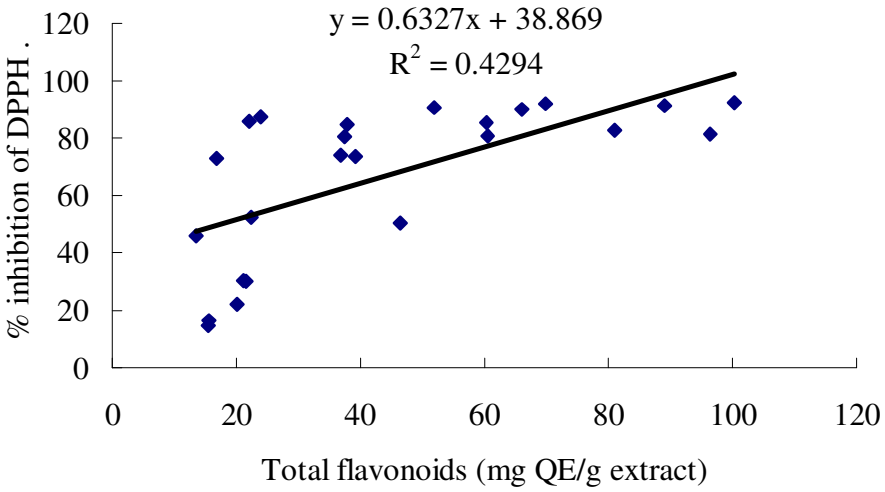

Figure 2. Linear correlation between antioxidant activity and the total flavonoid content of methanol extracts of 24 medicinal plants from Jhapa and Illam district, Nepal. QE: quercitin equivalents.

Khokhar (2002), who reported a weak correlation in fruits, but unlike the results of Kahkonen et al. (1999), who did not find such a weak correlation. The weak relationship between antioxidant activity and total phenolic compounds in this report may be caused by other factors; for example, flavonoids with a certain structure and hydroxyl position in the molecule can only act as proton donors and show radical scavenging activity (Hou et al., 2003). Moreover, measurement of phenolics using the Folin–Ciocalteu method might not be a good indicator of antioxidant capacity because this assay estimates total phenolics present in the extract, but is subject to interference, giving rise to elevated apparent phenolic concentrations (Prior et al., 2005). In addition, the antioxidant activity of plant extracts is not limited to phenolics, but also includes vitamins C and E, carotenoids, and chlorophylls (Rice-Evans et al., 1995), and synergism between antioxidants (Djeridane et al., 2006).

Moreover, significant differences in total phenolics can be attributable to extraction methods, time of collecting samples, environment, and genetic differences between

tested samples (Shan et al., 2005).

Several studies have reported a high correlation between phenolic content and antioxidant activity (Qusti et al., 2010; Kratchanova et al., 2010). In this study, a good relationship between antioxidant activity and phenolic compounds was only found for a few species, for example, the leaves of *A. vulgaris*, *M. arvensis*, and *T. chebula* with an estimated coefficient of determination of $R^2 = 0.912$ at $P < 0.05$, thus indicating that the high DPPH activity may be related to the phenolic compounds in these plants.

In contrast, the leaf extracts of *M. philippensis*, which exhibited high antioxidant activity, did not contain concomitantly high phenolic compounds. Very high antioxidant activity with low phenolics or flavonoids can be attributable to individual phenolic or non-phenolic compounds with specific high antioxidant activity. Similarly, *A. calamus*, which has low phenolics and flavonoids, had higher antioxidant activity, indicating that active compounds of different polarity could be present in this plant. Weber et al. (2007) reported on the bioactive compounds lignans, epieudesmin, terpenes, and quinine in extracts of *A. calamus*, which may contribute to the high antioxidant properties.

In conclusion, Jhapa and Illam District, Nepal medicinal plant extracts in this research exhibited different degrees of antioxidant activity. In particular, *A. vulgaris*, *F. lacor*, *M. philippensis*, *Amomum subulatum*, and *Trachyspermum ammi* can be considered as promising sources of natural antioxidants and as possible preventative agents of some common human health disorders. However, the total phenolic and flavonoid content showed a weak correlation with the antioxidant activity of the investigated plant. Hence, detailed studies on the role of individual phytochemicals involved in the antioxidant activity of specific plants are required for their use as functional foods and in the pharmaceutical industry.

ACKNOWLEDGMENTS

The authors express their gratitude to the Brain Pool (Konkuk University, Korea) for supporting this project.

REFERENCES

Acharya E, Pokhrel B (2006). Ethno-medicinal plants used by Bantar of Bhaudaha, Morang, Nepal. Our nat., 4: 96-103.

Arfan M, Amin H, Karamac M, Kosinska A, Wiczkowski W, Amarowicz, R (2009). Antioxidant activity of phenolic fractions of *Mallotus philippinensis* bark extract. Czech J. Food Sci., 27(2): 109-117.

Biswas KI, chattopadhyay R, Banerjee K, Bandyopadhyay U (2002). Biological activities and medicinal properties of Neem (*Azadirachta indica*). Curr. Sci., 82(11): 1336-1345.

Das NP, Pereira TA (1990). Effects of flavonoids on thermal autooxidation of Palm oil: Structure-activity relationship. J. Am. Oil Chem. Soc., 67: 255-258.

Dey A, Dutta N, Sharma K, Pattanaik AK (2008). Effect of dietary inclusion of *Ficus infectoria* leaves as a protectant of proteins on the performance of lambs. Small Ruminant Res., 75: 105-114.

Elujoba AA, Odelleye OM, Ogunyemi CM (2005). Review: Traditional medicine development for medical and dental primary health care delivery system in Africa. Afr. J. Trad. C.A.M., 2: 46-61.

Ghimire K, Bastakoti RR (2009). Ethnomedicinal knowledge and healthcare practices among the Tharus of Nawalparasi district in central Nepal. For. Ecol. Manage., 257: 2066-2072.

Giang PM, Son PT, Matsunami K, Otsuka H (2006). New neolignans and lignans from Vietnamese medicinal plant *Machilus odoratissima* NEES. Chem. Pharmaceut.l Bull., 54 (3): 380-383.

Govindarajan R, Vijayakumar M, Pushpangadan P (2005). Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. J. Ethnopharmacol., 99: 165-178.

Hou WC, Lin RD, Cheng KT, Hung YT, Cho CH, Chen CH, Hwang SY, Lee MH (2003). Free radical scavenging activity of Taiwanese native plants. Phytomed., 10: 170-175.

Imeh U, Khokhar S (2002). Distribution of conjugated and free phenols in fruits: Antioxidant activity and cultivar variations. J. Agric. Food Chem., 50 (22): 6301-6306.

Joshi AR, Edington JM (1990). The use of medicinal plants by two village communities in the Central Development Region of Nepal. Economic Bot., 44: 71–83.

Juang LJ, Sheu SJ, Lin TC (2004). Determination of hydrolyzable tannins in the fruit of *Terminalia chebula* Retz. by high-performance liquid chromatography and capillary electrophoresis. J. Separation Sci., 27: 718-724.

Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem., 47: 3954-3962.

Kessler M, Ubeaud G, Jung L (2003). Anti- and pro-oxidant activity of rutin and quercetin derivatives. J. Pharm.Pharmacol., 55: 131-142.

Kim KT, Yoo KM, Lee JW, Eom SH, Hwang IK, Lee CY (2007). Protective effect of steamed american ginseng (*Panax quinquefolius* L.) on V79-4 cells induced by oxidative stress. J. Ethnopharm., 111: 443-445.

Kirtikar K, Basu B (1993). In Indian Med. Plants, Pp. 3–6.

Klipstein-Grobusch K, Launer LJ, Geleijnse JM, Boeing H, Hofman A, Witterman JC (2000). Serum carotenoids and atherosclerosis: the Rotterdam study. Atherosclerosis, 148 (1): 49-56.

Kratchanova M, Denev P, Ciz M, Lojek A, Mihailov A (2010). Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds. Comparison of two extraction systems. Acta Biochemica Polonica, 57(2): 229–234.

Mahesh B, Satish S (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agric. Sci4 (S): 839-843.

Manandhar NP (2002). Plants and people of Nepal Oregon: Timber Press.

Manandhar NP (1994). An ethnobotanical survey of herbal drugs of Kaski District, Nepal. Fitoterapia, 65: 7–13.

Manandhar NP (1995). A survey of medicinal plants of Jajarkot district, Nepal. J. Ethnopharmacol., 48: 1-6.

Middleton EJ, Kandaswami C, Theoharides TC (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev., 52: 673-751.

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.

Miyakado M, Nakayam I, Yoshioka H, Nakatani N (1979). The piperaceae amides I: structure of pipercide, a new insecticidal amide from *Piper nigrum* L. Agric. Biol. Chem., 43: 1609–1611.

Moreno MIN, Isla MI, Sampietro AR, Vattuone MA (2000). Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. J. Ethnopharmacol., 71: 109-114.

Parekh J, Jadeja D, Chanda S (2005). Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. Turkish J. Biol., 29: 203-210.

Prior RL, Wu X, Schaich K (2005). Standardized methods for the determination of Antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem., 53(10): 4290-4302.

Rai SK, Subedi S, Mishra S (2004). Utilization pattern of medicinal plants in Thumpakhar, Sindhupalchok, Nepal. Botanica Orientalis, 4: 75-78.

Rached W, Benamar H, Bennaceur M, Marouf A (2010). Screening of the antioxidant potential of some Algerian indigenous plants. J. Biol. Sci., 10(4): 316-324.
Ravindran PN, Madhusoodanan KJ (2002). Cardamom: the genus *Elettaria*. Pp. 276, Taylor and Francis Inc., New York.
Rice-Evans CA, Miller NJ, Bolwell PG., Bramley PM, Pridham JB (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radical Res., 22: 375-383.
Siddhuraju P, Becker K (2007). The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. Food Chem., 101: 10–19.
Shan B, Cai YZ, Sun M, Corke H (2005). Antioxidant capacity of 26 species extracts and characterization of their phenolic constituents. J. Agric. Food Chem., 53: 7749-7759.
Sharma UR, Malla KJ, Uprety RK (2004). Conservation and management efforts of medicinal and aromatic plants in Nepal. Banko Janakari, Pp.14:3-11.
Sharma PC, Yelne MB, Dennis TJ, (2002). Database on medicinal plants used in ayurveda. 1st ed. Vol. 2. New Delhi: CCRAS, pp. 454-461.
Shimada K, Fujikawa K, Yahara K, Nakamura T (1992). Antioxidative properties of xanthan on the anti-oxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem., 40: 945-948.
Singh KA, Rai RN, Patiram, Bhutia DT (1989). Large cardamom (*Amomum subulatum* Roxb.) plantation-an age old agroforestry system in Eastern Himalayas. Agroforestry Systems., 9: 241-257.
Tanaka T, Ito T, Iinuma M, Takahashi Y, Naganawa H (1998). Dimeric chalcone derivatives from *Mallotus philippensis*. Phytochem., 48(8): 1423-1427.
Wojdyło A, Oszmiański J, Czemerys R (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem., 105(3): 940-949.
Weber M, Knoy C, Kindscher K, Brown RCD, Niemann S, Chapman J (2007). Identification of medicinally active compounds in Prairie plants by HPLC coupled to electron impact-mass spectrometry. Am. Lab., 39(12): 9-11.
Young IS, Woodside JV (€2001). Antioxidants in health and disease. J. Clin. Pathol., 54: 176-186.