Food Sci. Biotechnol. Vol. 17, No. 6, pp. 1235 ~ 1239 (2008)

Food Science and Biotechnology

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Antioxidant and Antiproliferative Activities of Methanolic Extracts from Thirty Korean Medicinal Plants

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Abstract To study the health promoting effects of medicinal plants, 30 medicinal plants commonly available in Korea have been evaluated for their antioxidant compounds and antioxidant and antiproliferative activities. Total polyphenolics and flavonoids in the methanolic extracts were measured by spectrophotometric methods and 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activities and chelating effects have been determined for antioxidant activities. Moreover, the effects of medicinal plants on cell proliferation of intestinal (Caco-2) and pituitary (GH3) tumor cells were investigated using thiazolyl blue terazolium bromide (MTT) assay. The methanolic extracts of *Pueraria thunbergiana* and *Artemisiae asiatria* contained the highest total polyphenolic and flavonoid contents, respectively. *P. thunbergiana* exhibited the highest antioxidant activities. *A. asiatria* showed the strongest antiproliferative activity against Caco-2 and *Ponciruc trifoliata* Rafin and *Lophathrum gracile* Bronghiart exhibited the highest activities against GH3. Although there was positive correlation between ABTS radical scavenging activity and polyphenolic contents (R^2=8189), no relationship was found between antiproliferative and antioxidant activities.

Keywords: medicinal plant, antioxidant, antiproliferation, polyphenolic, flavonoid

Introduction

Free radicals produced by radiation, chemical reactions, and several redox reactions of various compounds may contribute to protein oxidation, DNA damage, lipid peroxidation in living tissues, and cells (1,2). This oxidative stress may be related to many disorders such as cancer, atherosclerosis, diabetes, and liver cirrhosis (3-5). Recent epidemiological studies have suggested that increased consumption of fruits and vegetables is associated with reduced risks of chronic diseases (6). This association may be attributed to the natural antioxidants from plant foods such as vitamin C, tocopherol, carotenoids, polyphenolics, and flavonoids which prevent free radical damage (7).

Medicinal plants have been used to treat various human diseases in Korea for centuries due to their good therapeutic effects and low toxicity. Moreover, the medicinal plants have been used as not only medicines but also foods, flavors, pigments, and cosmetics for thousands of years in many countries (8,9). The positive effect of medicinal plants suggests the presence of a wide variety of phytochemicals such as phenolics, flavonoids, and tannins, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (10,11). Recently, several medicinal plants have been shown to possess anti-inflammatory, anti-tumor, anti-allergic, anti-viral, and anti-bacterial effects (12-14). Even though a large number of medicinal plants have been studied, information on antioxidant and antiproliferative activities of medicinal plants is still requiring proper documentation.

The purposes of this study were aimed to determine polyphenolic and flavonoid contents, to measure the 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities and chelating effects, and to determine the antiproliferative activities of medicinal plant extracts on human and murine tumor cell lines *in vitro*, and to determine correlations between antioxidant or antiproliferative activities and total polyphenolic or flavonoid contents.

Materials and Methods

Chemicals Folin-Ciocalteu reagent, gallic acid, quercetin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and thiazolyl blue terazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Caco-2 human colon and GH3 murine pituitary cancer cells were from the Korean Cell Line Bank (KCLB).

Preparation of medicinal plants Total of 30 medicinal plants was collected from local medicine stores in Keomsan, Korea. The various information including scientific names, medicinal name, and used parts of the plants were shown Table 1. Approximately 10 g of finely ground samples were extracted into 200 mL of methanol in a shaker at room temperature for 24 hr. Subsequently, the extracts were centrifuged at 6,750×g for 15 min and the supernatants were filtered through a Whatman No. 2 filter paper. The filtrate was evaporated at 40°C. The dried residues were redissolved in methanol for the determination of antioxidant contents and antioxidant activities and in phosphate buffered saline (PBS) for antiproliferative activities to a concentration of 1 mg/mL and stored at −20°C until analysis.

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Received April 14, 2008; Revised July 16, 2008;
Accepted July 18, 2008

Table 1. Korean medicinal plants used in this study

Sample No.	Scientific name	Medicinal name	Part used
1	*Agastache rugosa*	Agastachis herba	Aerial part
2	*Angelica gigantis* Radix	Angelica gigatis radix	Root
3	*Artemisia iwayomogi* Kitamura	Artemisia iwayomogii herba	Aerial part
4	*Artemisiae asiatria*	Artemisia herba	Leaf
5	*Astragalus membranaceus*	Astragali radix	Root
6	*Cassia obtusifolia*	Cassiae semen	Seed
7	*Chaenomeles sinensis*	Chinese quince	Fruit
8	*Coix lachrymajobi* var. *mayuen*	Coicis semen	Aerial part
9	*Cornus officinalis*	Corni fructus	Fruit
10	*Crataegus pinnatifida* Bunge	Crataegi fructus	Fruit
11	*Dioscorea batatas* Decaisne	Dioscoreae rhizome	Root
12	*Elsholtzia ciliata* (Thunb.) Hyl	Common Elsholtzia	Aerial part
13	*Epimedium koreanum* Nakai	Epimedii herba	Leaf
14	*Eucommia ulmoides* Oliver	Eucommiae cortex	Bark
15	*Ganoderma lucidum*	Reishi mushroom	Whole plant
16	*Gardenia jasminoides* Ellis	Gardeniae fructus	Fruit
17	*Gastrodia elata*	Gastrodiae rhizome	Aerial part
18	*Liriope platyphylla*	Liriopis tuber	Root
19	*Lonicera japonica*	Lonicorae flos	Flower
20	*Lophathrum gracile* Bronghiart	Lophatheri herba	Leaf
21	*Perilla frutescens* var. *acuta*	Perillae herba	Leaf
22	*Pinus densiflora*	Pine leaf	Leaf
23	*Plantago asiatica* L.	Asian plantain	Whole plant
24	*Platycodon grandiflorum* Palibin	Platycodi radix	Root
25	*Polygonatum sibiricum* Redoute	Polygonati rhizome	Root
26	*Polygonum multiflorum*	Polygoni mutiflori radix	Root
27	*Ponciruc trifoliata* Rafin	Ponciri fructus	Fruit
28	*Pueraria thunbergiana*	Puerariae radix	Root
29	*Schizandra chinensis*	Schizandrae fructus	Fruit
30	*Taraxacum platycarpum*	Taraxai herba	Root

Determination of total polyphenolics Polyphenolic contents in the methanolic extracts from medicinal plants were determined using the Folin-Ciocalteu method (15) and results were expressed as mg gallic acid equivalents/1 g of sample residue. Standard solutions or extracts (200 μL) was mixed with 2 mL of 2% sodium carbonate solution and 100 μL of a 50% Folin-Ciocalteu reagent. After incubation for 30 min at room temperature, the absorbance was measured at 750 nm. All extracts were analyzed in triplicate.

Determination of total flavonoids Flavonoid contents in the methanolic extracts were determined by a colorimetric method described by María *et al.* (16) and results were expressed as mg quercetin equivalents/1 g of sample residue. An aliquot of sample (50 μL) was added to an Eppenedorf tube containing 100 μL of 10% aluminium nitrate, 100 μL of 1 M aqueous acetate, and 1 mL of distilled water. After incubation for 40 min at room temperature, the absorbance was measured at 415 nm. All extracts were analyzed in triplicate.

DPPH radical scavenging activity The scavenging activity of the methanolic extracts on DPPH radical was measured according to the method of Cheung *et al.* (17). Aliquots of 0.8 mL of 0.2 mM DPPH ethanolic solution was mixed with 0.2 mL of the extracts. The mixture was vigorously shaken and left to stand for 10 min under subdued light. The absorbance was measured at 520 nm.

ABTS radical scavenging activity The scavenging activity of the methanolic extracts on ABTS radical cation was measured according to the method of Re *et al.* (18). ABTS radical cation was generated by adding 7 mM ABTS to 2.45 mM potassium persulphate solution and the mixture was left to stand for overnight in the dark at room temperature. The ABTS radical cation solution was diluted with distilled water to obtain an absorbance of 1.0 at 734 nm. Diluted ABTS radical cation solution (1 mL) was added to 50 μL of the extract or Trolox® standard solution. After 90 min, the absorbance was measured at 734 nm. The ABTS radical cation scavenging activity was expressed as Trolox® equivalent antioxidant activity (TEAC) and defined as mg of Trolox® equivalents/1 g of sample residues.

Chelating effect on ferrous ion The chelating activity of the methanolic extracts was determined according to the method explained by Dinis *et al.* (19). The extract (1 mL)

was reacted with 100 μL of ferrous chloride (2 mM) and ferrozine (5 mM) for 10 min, and then the absorbance of the mixture was measured at 562 nm.

Antiproliferative activity Caco-2 and GH3 tumor cell lines were grown in Eagle's minimum essential medium (EMEM) and Dulbecco's modified Eagle's medium (DMEM), respectively, containing 10% fetal bovine serum (FBS), 2 mM glutamine, 100 unit/mL penicillin, and 50 μg/mL streptomycin. The cultures were maintained in a humidified incubator with 5% CO_2 and seeded onto 75 cm^2 area culture flask. Antiproliferative activities of the medicinal plants on tumor cells were measured by evaluating cell viability using the MTT assay. The cells were seeded at a density of 2×10^3 cell/well using a brief trypsinization and then the medicinal plant extracts (20 μL) were added in a 96-well plate. After 48 hr of incubation, 20 μL of MTT reagent (5 mg/mL) was added and incubated for a further 4 hr and the absorbance of formazan was determined at 550 nm. The cell viability (%) was obtained by comparing the absorbance between the samples and negative control (20).

Statistical analysis The results were reported as mean± standard deviation (SD). Correlations from regression analysis between the parameters were investigated using SAS version 8.1 (SAS Institute, Cary, NC, USA).

Results and Discussion

Antioxidant compounds It has been found that the major contribution on the antioxidant activities of plants was the amount of polyphenolic and flavonoid compounds in the plants (21,22). Therefore, it is important to quantify polyphenolic and flavonoid contents of medicinal plants and to assess their contribution to antioxidant and antiproliferative activities.

Total polyphenolic and flavonoid contents of methanolic extracts from medicinal plants are shown in Table 2. The amount of total polyphenolics varied in different plants and ranged from 3.2 to 203.1 mg/g residue. Among the 30 selected medicinal plants, the highest total polyphenolic level has been detected in *Peuraria thunbergiana* and the lowest in *Coix lachrymajobi* var. *mayuen.* Eight medicinal plants contained polyphenolics more than 100 mg/g residue: *Angelica gigas* Nakai (108.4), *Lophathrum gracile* Bronghiart (109.2), *Cassia obtusifolia* (110.4), *Pinus densiflora* (116.2), *Crataegus pinnatifida* Bunge (120.8), *Artemisia iwayomogi* Kitamura (175.7), *Chaenomeles sinensis* (182.7), *P. thunbergiana* (203.1). The amount of total flavonoids ranged from 6.6 to 62.7 mg/g residue. The relatively high total flavonoid levels (mg/g residue) have been detected in *C. lachrymajobi* (62.7) and *Artemisiae asiatria* (50.3) and the low levels (mg/g residue) in *Schizandra chinensis* (6.6), *Cornus officinalis* (6.7), *Liriope platyphylla* (6.9), *C. sinensis* (7.0), and *Polygonatum sibiricum* Redoute (7.8). In assessing 30 medicinal plants for the total polyphenolic and flavonoid contents, it was found that broad variability exists. A 67-fold difference was found in polyphenolic contents between *C. lachrymajobi* and *P. thunbergiana* and a 9-fold difference in flavonoid contents was measured between *S. chinensis* and *C. lachrymajobi.* Those differences could be due to different medicinal species or the parts used in this study.

Antioxidant activities The results of three *in vitro* assays (DPPH and ABTS radical scavenging activities and chelating effect) for antioxidant properties of the 30 medicinal plants are given in Table 2. The stable DPPH radical, which has a maximum absorption at 515 nm, is widely used to evaluate the free radical scavenging activity of hydrogen donating antioxidants in many plant extracts (23). The DPPH radical scavenging activities of the medicinal plants exhibited a large variation from 8.1 (*L. platyphylla*) to 93.9% (*P. densiflora*). Only 3 medicinal plants including *P. densiflora* (93.9%), *A. iwayomogi* Kitamura (91.7%), and *A. asiatria* (85.4%) exhibited relatively high DPPH radical scavenging activities indicating good potential as free radical scavengers. The ABTS assay is widely employed for measuring the relative radical scavenging activity of hydrogen donating and chain breaking antioxidants in many plant extracts (23,18). The TEAC values of ABTS assay also showed a wide variation from 134.1 (*Platycodon grandiflorum* Palibin) to 1432.9 (*P. thunbergiana*) mg Trolox® equivalents/1 g residue. The methanolic extracts of *P. thunbergiana, P. densiflora, C. sinensis, C. pinnatifida* Bunge, and *A. iwayomogi* Kitamura showed more than 1,000 TEAC values among the samples. Although iron is essential for oxygen transport, respiration, and activities of various enzymes in body system, it is a reactive metal that catalyzes oxidative damages in living tissues and cells (24). The chelating effects (%) of the medicinal plants on ferrous ion are presented in Table 2. The methanolic extracts of *Agastache rugosa* (96.3%) and *Ganoderma lucidum* (95.3%) showed excellent chelating ability even if their effects were lower than that of 4 mM ethylenediamine tetraacetic acid (EDTA, 100%). Moreover, the chelating effects of *Perilla frutescens* var. *acuta, A. asiatria,* and *A. iwayomogi* Kitamura were higher than 80% and *C. pinnatifida* Bunge (2.3%) showed the lowest value.

Antoproliferative activities Cell proliferation was analyzed at 48 hr after incubation of Caco-2 (colon cancer) and GH3 (pituitary tumor) cells with media containing extracts (100 μg/mL) from medicinal plants using MTT assay. Among 30 medicinal plants, the methanolic extracts of *A. asiatria* (90.7%), *Eucommia ulmoides* Oliver (87.6%), *A. iwayomogi* Kitamura (84.4%), *Ponciruc trifoliata* Rafin (79.0%), *P. grandiflorum* Palibin (78.3%) and showed relatively high antiproliferative activities against Caco-2 cells. The antiproliferative activities of medicinal extracts toward GH3 cells were slightly different from those toward Caco-2 cells. The methanolic extracts of *P. trifoliata* Rafin (99.1%), *Lophathrum gracile* Bronghiart (98.4%), *A. asiatria* (94.4%), *A. iwayomogi* Kitamura (90.0%), and *P. densiflora* (81.6%) exhibited excellent antiproliferative activities against GH3 tumor cells. In this study, *Artemisia* spp. (*A. asiatria* and *A. iwayomogii* Kitamura) exhibited relatively high antioxidant antiproliferative activities among the samples. Those antiproliferative activities have been attributed to artemisinin which is the major anticancer ingredient obtained from *Artemisia* spp. (25). Djeridane *et al.* (8) evaluated the antioxidant activity of some Algerian medicinal plants extracts and they found that *Artemisia*

Table 2. Contents of antioxidant compounds and antioxidant and antiproliferative activities of the methanolic extracts from medicinal plants

Sample No.	Antioxidant compounds		Antioxidant activities			Antiproliferative activities	
	Polyphenolics[1)]	Flavonoids[2)]	DPPH[3)]	ABTS[4)]	Chelating[5)]	Caco-2[6)]	GH3[7)]
1	95.5	45.3	35.2	700.7	96.3	15.4	2.0
2	108.4	23.5	27.9	271.9	76.8	27.5	20.0
3	175.7	43.1	91.7	1060.8	85.6	84.4	90.0
4	83.2	50.3	85.4	637.0	88.0	90.7	94.4
5	39.5	15.3	10.4	190.2	60.7	21.0	3.1
6	110.4	47.2	46.5	764.9	57.9	13.5	1.0
7	182.7	7.0	77.6	1342.2	4.4	3.0	42.3
8	3.2	62.7	23.3	181.2	6.4	18.0	0.5
9	42.4	6.7	67.0	505.8	7.2	17.5	17.4
10	120.8	9.1	62.1	1085.6	2.3	9.4	15.5
11	25.8	11.1	17.2	176.8	51.9	69.1	51.7
12	55.3	33.9	40.5	547.3	4.7	56.7	37.4
13	62.8	34.4	40.2	432.9	66.0	45.8	43.4
14	58.2	44.8	27.3	431.7	16.3	87.6	58.5
15	56.8	41.8	49.3	369.0	95.3	51.3	38.7
16	58.2	15.0	54.7	461.7	4.5	8.0	2.9
17	42.4	9.7	11.7	245.6	43.2	68.0	43.4
18	18.8	6.9	8.1	68.9	4.2	20.6	20.6
19	36.8	14.9	38.1	225.0	39.5	32.9	57.9
20	109.2	43.8	47.5	728.8	74.3	62.1	98.4
21	49.6	19.2	55.3	363.8	88.1	24.2	12.8
22	116.2	29.9	93.9	1431.3	4.0	41.7	81.6
23	41.0	37.4	29.2	301.0	72.4	58.5	41.9
24	25.6	9.3	13.2	134.1	14.3	78.3	68.4
25	32.8	7.8	22.8	183.2	21.9	19.6	7.7
26	30.1	18.2	12.7	153.9	50.8	28.8	36.9
27	87.8	42.0	17.1	496.8	37.8	79.0	99.1
28	203.1	10.8	51.4	1432.9	68.1	2.4	19.3
29	30.9	6.6	18.9	180.9	5.0	13.5	23.9
30	41.5	30.9	39.1	245.6	34.1	5.0	2.0

[1)]Mean of triplicate determinations expressed as mg gallic acid equivalents/1 g of residue.
[2)]Mean of triplicate determinations expressed as mg quercetin equivalents/1 g of residue.
[3)]Scavenging effect (%) of methanol extracts from the medicinal plants on DPPH radical.
[4)]Scavenging effect (TEAC) of methanol extracts from the medicinal plants on ABTS radical.
[5)]Chelating effect (%) of methanol extracts from the medicinal plants.
[6)]Antiproliferative activity (%) of methanol extracts from the medicinal plants against Caco-2.
[7)]Antiproliferative activity (%) of methanol extracts from the medicinal plants against GH3.

Table 3. Correlation analysis between antioxidant contents and antioxidant/antiproliferactive activities

	Antioxidant activities			Antiproliferative activities	
	DPPH	ABTS	Chelating	Caco-2	GH3
Polyphenolics	0.4182[1)]	0.8198	0.0443	0.0060	0.0580
Flavonoids	0.0369	0.0083	0.1611	0.1470	0.0690

[1)]Correlation coefficient R^2.

spp. exhibited high polyphenolics and TEAC values. However, working on Indian medicinal plants, *Artemisia* spp. showed relatively low antioxidant and polyphenolic contents (10). The differences in antioxidant levels of *Artemisia* spp. are apparently attributable to different medicinal species.

Correlation analysis The correlations between antioxidant contents and antioxidant or antiproliferative activities are summarized in Table 3. Although there was positive correlation between ABTS radical scavenging activity and polyphenolic contents (R^2=0.8189), no relationship was found between polyphenolic contents and DPPH radical

scavenging activity (R^2=0.4182) or chelating effects (R^2= 0.0443). Additionally, there was no correlation between flavonoid contents and antioxidant activities. The unclear relationship between the antioxidant compounds and antioxidant activities may be explained by the fact that particular antioxidant compounds may act synergistically with some compounds or antagonistically with others (8). There was no obvious relationship between polyphenolic content and inhibition of cell proliferation (R^2=0.0060 for Caco-2, R^2=0.0580 for GH3) or flavonoid content and the inhibition of cell proliferation (R^2=0.1470 for Caco-2, R^2=0.0690 for GH3). Additionally, there was no correlation between antioxidant activities and inhibition of cell proliferation. Similar results were found in a recent study of fruits and may suggest that phytochemicals other than those tested in this study are responsible for inhibiting Caco-2 and CH3 cell proliferation (26).

Acknowledgments

This work has been supported by the Research Center for Bioresource and Health (RCBH) of ITEP&MOCIE, Korea.

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