

Antioxidant Activities of Vietnamese Medicinal Plants

Phuong Thien Thuong¹, MinKyun Na², Nguyen Hai Dang¹, Tran Manh Hung¹, Pham Thanh Ky³,
Tran Van Thanh³, Nguyen Hai Nam³, Nguyen Duy Thuan⁴, DaiEun Sok¹, and KiHwan Bae^{1,*}

¹College of Pharmacy, Chungnam National University, Daejeon 305-764, South Korea

²Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333, South Korea

³Hanoi College of Pharmacy, 13-15 Le Thanh Tong, Hanoi, Vietnam

⁴National Institute of Medicinal Materials, 3B Quang Trung, Hanoi, Vietnam

Abstract – One hundred and twenty six Vietnamese traditional herbals belonging to 59 families were screened for their free radical (DPPH) scavenging activity and inhibitory effect on lipid peroxidation. Of these, MeOH extracts of seven plants, including *Euphorbia thymifolia* (leaf), *Gnetum montanum* (stem), *Heterosmilax erythrantha* (root), *Morus alba* (leaf), *Syzygium formosum* (leaf), *Jussiaea repens* (aerial parts), and *Camellia sinensis* (leaf), exhibited significant antioxidant activities. All of these herbs showed remarkable free radical scavenging activities with IC₅₀ values of 11.0, 14.5, 17.0, 13.6, 10.8, 7.7, and 8.5 µg/ml, respectively, and significant inhibitory effects on lipid peroxidation with 79.7, 83.8, 78.9, 82.5, 88.8, 88.0, and 96.2% inhibitions, respectively, at the concentration of 50 µg/ml.

Keywords – Vietnamese medicinal plants, antioxidant activity, free radical scavenging, lipid peroxidation

Introduction

Reactive oxygen species (ROS) such as hydroxyl radical, hydrogen peroxide, superoxide anion, and singlet oxygen have been known to be capable of chemically altering all major classes of biomolecules including lipids, proteins, and nucleic acids, thus leading to change of their structures and functions (Simonian and Coyle, 1996; Waddington *et al.*, 2000; Kohen and Nyska, 2002). This lead to lipid peroxidation, DNA and protein damages which result in various diseases, including inflammation, cancer, Parkinson's disease, cardiovascular diseases, multiple sclerosis, lupus, and aging (Halliwell, 1994; Waddington *et al.*, 2000; Kohen and Nyska, 2002). Fortunately, ROS might be scavenged by antioxidants derived from natural source, mainly from plant kingdom (Halliwell *et al.*, 1995; Pietta, 2000).

Historically, plants have been responsible for thousands of years of Vietnamese peculiarly traditional medicine (Loi, 2001). Vietnam, a Southeast Asian tropical country, possesses abundant and diversified flora (Ho, 1997). Conservative estimate suggested the existence of approximately 12,000 species of Vietnamese terrestrial plants, and no less than 2,500 species have been used in ethno-

medicine (Chi, 1997). Although these have been used as folk medicines for a long time, it is still an untapped source for potential bioactive agents (Ueda *et al.*, 2002; Nam *et al.*, 2003; Nguyen *et al.*, 2004). Therefore, it is expected that Vietnamese medicinal plants not only continue to be used traditionally, but also be a useful source of drug discovery. In continuation of our search program to find new biological agents from natural sources, we have preliminarily screened 126 medicinal plants, which are widely used in Vietnamese oriental medicine, for in vitro antioxidant activities. In this paper, the medicinal plants, traditional uses in ethnomedicine and the results obtained from antioxidant screening are presented and discussed.

Experimental

Reagents – 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ferrous sulfate (FeSO₄ · nH₂O), thiobarbituric acid (TBA), α-tocopherol, ascorbic acid, and butylated hydroxyl toluene (BHT) were purchased from Sigma Chemical Co., USA. Potassium phosphate, potassium chloride, and trichloroacetic acid (TCA) were obtained from Samchun Chemical Co., Ltd, Korea.

Plant material – Most of the medicinal plants were collected in the North of Vietnam in spring, 2004. Some

*Author for correspondence

Fax: +82-42-823-6566; E-mail: baekh@cnu.ac.kr

of them were purchased at Dongxuan oriental herbarium in Hanoi, Vietnam. All of these plants were identified by one of the authors, Professor Pham Thanh Ky, Department of Pharmacognosy, Hanoi College of Pharmacy, Vietnam. Voucher specimens have been deposited at the herbarium of the College of Pharmacy, Chungnam National University, Daejeon, South Korea.

Preparation of extracts and assay samples – The collected plants were dried and powdered. Each material (50 g) was extracted with MeOH (100 ml \times 2 times) for 3 hours under reflux. Then the MeOH extracts were combined and exhaustively concentrated *in vacuo* to dryness. The powdery extracts were redissolved in MeOH to various concentrations for experiment.

DPPH radical scavenging assay – DPPH radical scavenging activity was determined by the described method (Na *et al.*, 2003). Briefly, 5 μ l of each MeOH extract was added to 195 μ l of 150 μ M DPPH in methanol in 96 well plates. The solution was mixed for 1 min and incubated at room temperature for 30 min. Then the absorbance of the reaction mixture was measured at 520 nm on a microplate reader. The free radical scavenging activity was expressed as follow:

$$\text{DPPH scavenging activity (\%)} = 100 \times [(Ac - As)/(Ac - Ab)]$$

Where Ac was the absorbance of the control, As was the absorbance of the sample and Ab was the absorbance of the blank (MeOH). Each sample was assayed triple at five concentrations and four wells were used for each concentration. The IC₅₀ values were defined as the concentration that could scavenge 50% radical produced and were calculated by the reported method (Wu *et al.*, 1992).

Lipid peroxidation assay

Preparation of mitochondria – Mitochondria were prepared from liver of male Sprague-Dawley mice (200–250 g) by the method of Ham and Liebler (Ham and Liebler, 1995; Yen and Hsieh, 1998) with slight modifications. Briefly, rat livers were removed and washed with ice-cold 0.9% sodium chloride. The livers were minced, homogenized in 9 volumes of ice-cold buffer (0.25 M sucrose in 5 mM phosphate buffer, pH 7.4) and centrifuged at 600 rpm at 4 °C for 10 min. The supernatant was centrifuged at 9000 rpm at 4 °C for 15 min. The mitochondrial pellets were washed three times and finally suspended in the 0.15 M KCl in 20 mM phosphate buffer (pH 7.4) and stored at –70 °C until experiment. The protein concentration was determined by BCA method using bovine serum albumin as standard (Brenner and Harris, 1995).

Oxidation of mitochondria – Inhibitory effect on oxidation

of liver mitochondria assay was evaluated by thiobarbituric acid reactive substance method (Yen and Hsieh, 1998) with some modifications. The reaction mixture composed of 50 μ l of mitochondria (equivalent to 10 mg protein/mL), 390 μ l of 20 mM phosphate buffer (pH 7.4), 25 μ l of 2 mM FeSO₄, 25 μ l of 5 mM ascorbic acid and 10 μ l of tested sample. The mixture was incubated at 37 °C for 60 min and terminated by addition of 250 μ l of 20% TCA. Then the mixture reaction was reacted with 250 μ l of 1% TBA at 100 °C for 15 min. After being centrifuged at 5000 rpm for 10 min, the absorbance of the supernatant was read at 532 nm. Lipid peroxidation inhibitory was calculated as percentage of inhibitory effect by the equation below:

$$\text{Inhibition (\%)} = 100 \times [(Ac - As)/(Ac - Ab)]$$

Where Ac was the absorbance of the control, As was the absorbance of the sample and Ab was the absorbance of the blank (without sample, FeSO₄ and ascorbic acid).

Results and Discussion

As presented in Table 1, one hundred and twenty six medicinal plants belonging to 59 families are listed along with their local names. Among them, 52 plant materials showed strong to moderate free radical (DPPH) scavenging activity with IC₅₀ \leq 200 μ g/ml. Interestingly, 7 plants displayed remarkable scavenging activity, more potent than those of α -tocopherol (20 μ g/ml) and BHT (24.6 μ g/ml), which were used as positive controls. These plants include *Euphorbia thymifolia* (leaf), *Gnetum montanum* (stem), *Heterosmilax erythrantha* (root), *Morus alba* (leaf), *Syzygium formosum* (leaf), *Jussiaea repens* (aerial parts), and *Camellia sinensis* (leaf) with IC₅₀ values of 11.0, 14.5, 17.0, 13.6, 10.8, 7.7, and 8.5 μ g/ml, respectively. However, all of 126 MeOH extracts were less active than ascorbic acid (IC₅₀ = 4.5 μ g/ml). Similarly, all these samples showed significant inhibitory effects on lipid peroxidation. *Camellia sinensis*, well known to be rich in polyphenol compounds, exhibited the strongest inhibition, 96.2% at 50 μ g/ml, followed by *Syzygium formosum*, *Jussiaea repens*, *Gnetum montanum*, *Morus alba*, *Euphorbia thymifolia*, *Heterosmilax erythrantha* with inhibitions of 88.8, 88.0, 83.8, 82.5, 79.7 and 78.9% at 50 μ g/ml, respectively. Forty eight herbals, which showed the inhibitory effect on lipid peroxidation higher than 50.0% at the test concentration of 100 μ g/ml, were further evaluated at 50 μ g/ml (Table 1).

Recently, Nguyen *et al.* have reported inhibitory effect on xanthine oxidase of 96 medicinal plants collected in

Table 1. Antioxidant activities of Vietnamese medicinal plants

medicinal plants			antioxidant activities ^a			
family name / scientific name	vernacular name	part used ^b	DSA	IC ₅₀	LI (1)	LI (2)
Amaranthaceae						
<i>Achyranthes aspera</i>	Coxuoc	Wh	6.4	>200	10.5	*
<i>A. bidentata</i>	Nguutat	R	15.4	>200	-	*
Amaryllidaceae						
<i>Crinum asiaticum</i>	Nanghoatrang	Ar	19.4	>200	13.1	*
<i>C. ensifolium</i>	Nanghoado	L	8.5	>200	8.6	*
<i>C. latifolium</i>	Hoangcung-trinhnu	L	56.4	199.0	78.5	53.7
Apiaceae						
<i>Bupleurum falcatum</i>	Saiho	R	21.1	>200	-	*
<i>Centella asiatica</i>	Rauma	Wh	38.3	>200	13.8	*
Apocynaceae						
<i>Catharanthus roseus</i>	Duacan	Ar	35.7	>200	57.2	33.7
Araceae						
<i>Alocasia odora</i>	Ray	R	23.6	>200	-	*
<i>Pistia stratiotes</i>	Beocai	Wh	12.1	>200	7.7	*
Araliaceae						
<i>Acanthopanax trifoliatum</i>	Ngugiabigai	C	92.5	69.5	28.7	*
<i>Aralia cordata</i>	Dochoat	R	43.6	>200	29.1	*
<i>Polyscias fruticosa</i>	Dinhlang	L	1.6	>200	-	*
<i>P. fruticosa</i>	Dinhlang	R	10.1	>200	-	*
Arecaceae						
<i>Areca catechu</i>	Cau	Se	29.7	>200	48.5	*
<i>Caryota urens</i>	Dungdinh	L	14.3	>200	-	*
Asteraceae						
<i>Ageratum conyzoides</i>	Hoacutlon	Ar	31.3	>200	27.7	*
<i>Astemisia vulgaris</i>	Ngaicuu	Ar	91.1	83.5	10.5	*
<i>Atractylodes macrocephala</i>	Bachtruat	Rh	22.1	>200	-	*
<i>Blumea lacera</i>	Caitroi	Ar	52.3	>200	51.2	25.6
<i>Chromolaena odorata</i>	Colao	Ar	91.1	69.8	92.6	74.0
<i>Eclipta alba</i>	Conhono	Ar	16.9	>200	13.2	*
<i>Elephantopus scaber</i>	Chithien	Ar	84.1	119.7	53.7	34.9
<i>Gynura crepidioides</i>	Rautaubay	Ar	40.2	>200	-	*
<i>G. pseudochina</i>	Thotamthat	Ar	32.5	>200	16.7	*
<i>Lactuca indica</i>	Bocongan	Ar	11.1	>200	33.3	*
<i>Saussurea lappa</i>	Vanmochuong	R	25.7	>200	59.7	21.6
<i>Siegesbeckia orientalis</i>	Hythiem	Ar	6.3	>200	18.1	*
<i>Wedelia calendulacea</i>	Saidat	Ar	13.5	>200	24.8	*
<i>Xanthium strumarium</i>	Kedaungua	Fr	93.2	84.6	55.4	11.2
Bignoniaceae						
<i>Oroxylum indicum</i>	Nucnac	C	94.0	30.1	96.5	75.5
Bombacaceae						
<i>Bombax ceiba</i>	Gao	C	19.4	>200	-	*
Campanulaceae						
<i>Platycodon grandiflorum</i>	Catcanh	R	13.1	>200	-	*
Caprifoliaceae						

Table 1. continued

<i>Sambucus javanica</i>	Comchay	L	91.1	69.8	78.3	37.1
Celastraceae						
<i>Celastrus hindsii</i>	Xaden	L	37.5	>200	7.0	*
<i>C. hindsii</i>	Xaden	St	93.7	32.3	88.4	55.5
Convolvulaceae						
<i>Argyrea acuta</i>	Bachau	Ar	52.6	>200	23.3	*
<i>Cuscuta sinensis</i>	Tohong	Wh	92.6	60.6	87.6	62.9
<i>Impomoea hederacea</i>	Bimbim	Ar	36.0	>200	-	*
Cucurbitaceae						
<i>Momordica charantia</i>	Muopdang	Fr	16.8	>200	11.4	*
<i>M. cochinchinensis</i>	Gac	R	6.9	>200	-	*
Cupressaceae						
<i>Thuja orientalis</i>	Trachbach	L	42.3	>200	80.7	41.2
Dilleniaceae						
<i>Tetracera scandens</i>	Daychacchiu	St	95.4	23.6	96.8	88.1
Dipsacaceae						
<i>Dipsacus japonicus</i>	Tucdoan	R	82.2	116.3	50.1	14.8
Elaeagnaceae						
<i>Elaeagnus latifolia</i>	Nhot	L	60.0	199.0	20.0	*
Ephedraceae						
<i>Ephedra sinica</i>	Mahoang	Wh	94.5	47.3	51.4	32.4
Equisetaceae						
<i>Equisetum arvense</i>	Comoctac	Wh	34.8	>200	14.0	*
Eucommiaceae						
<i>Eucommia ulmoides</i>	Dotrong	C	22.0	>200	-	*
Euphorbiaceae						
<i>Croton tonkinensis</i>	Khosamchola	L	43.8	>200	3.8	*
<i>Euphorbia thymifolia</i>	Cosuanhola	L	92.1	11.0	97.2	79.7
<i>Phyllanthus urinaria</i>	Choderangcua	Ar	92.3	29.7	86.9	63.4
Fabaceae						
<i>Abrus precatorius</i>	Camthaoday	L	34.9	>200	33.7	*
<i>Desmodium styracifolium</i>	Kimtienthao	L	48.5	>200	16.7	*
<i>Erythrina orientalis</i>	Vongnem	L	24.7	>200	17.0	*
<i>Glycyrrhiza glabra</i>	Camthao	R	15.1	>200	56.0	29.4
<i>Sophora flavescens</i>	Khosamchore	R	52.7	>200	83.6	59.5
Gnetaceae						
<i>Gnetum montanum</i>	Vuongton	St	95.3	14.5	93.1	83.8
Lamiaceae						
<i>Leonurus artemisia</i>	Ichmau	Ar	33.3	>200	-	*
<i>Mentha arvensis</i>	Bacha	Ar	50.8	200	30.2	*
<i>Ocimum sanctum</i>	Huongnhutia	Ar	89.1	63.5	38.7	*
<i>Perilla ocymoides</i>	Tiato	Ar	92.3	63.0	26.0	*
<i>Pogostemon cablin</i>	Hoachuong	Ar	67.3	108.0	24.0	*
<i>Prunella vulgaris</i>	Hakhothao	Ar	35.5	>200	-	*
<i>Schizonepeta tenuifolia</i>	Kinhgioi	Ar	39.8	>200	9.0	*
Lauraceae						
<i>Cinnamomum loureirii</i>	Que	C	73.8	112.5	75.5	54.4

Table 1. continued

<i>C. loureirii</i>	Que	L	94.0	55.4	67.5	25.4
Leeaceae						
<i>Leea rubra</i>	Goihac	R	94.5	36.6	51.6	34.3
Liliaceae						
<i>Anemarrhena aspheloides</i>	Trimau	Rh	34.6	>200	4.3	*
<i>Cordyline terminalis</i>	Huyetdu	L	26.0	>200	-	*
<i>Heterosmilax erythrantha</i>	Kimcang	R	87.3	17.0	94.2	78.9
<i>Ophiopogon japonicus</i>	Machmon	R	11.5	>200	16.9	*
<i>Smilax glabra</i>	Thophuclin	Rh	91.5	41.0	92.3	76.8
Loranthaceae						
<i>Loranthus parasiticus</i>	Tangkisinh	Wh	92.6	27.8	79.6	63.1
Malvaceae						
<i>Abutilon indicum</i>	Coixay	Ar	22.5	>200	15.8	*
Marsileaceae						
<i>Marsilea quadrifolia</i>	Cobo	Ar	6.5	>200	7.1	*
Menispermaceae						
<i>Cissampelos pareira</i>	Tietde	St	48.4	>200	-	*
<i>Stephania longa</i>	Loitien	Ar	45.5	>200	46.0	*
<i>S. tetrandra</i>	Phongky	R	54.2	195.8	77.3	44.6
Mimosaceae						
<i>Mimosa pudica</i>	Xauho	Ar	91.3	65.2	14.0	*
Moraceae						
<i>Ficus pumila</i>	Vayoc	Ar	22.2	>200	4.9	*
<i>Morus alba</i>	Dautam	L	93.5	13.6	98.8	82.5
<i>M. alba</i>	Dautam	St	86.6	82.1	91.7	77.9
Myrtaceae						
<i>Syzygium formosum</i>	Dontuongquan	L	95.0	10.8	98.4	88.8
Nelumbonaceae						
<i>Nelumbo nucifera</i>	Sen	L	73.1	143.2	57.3	31.0
Oenotheraceae						
<i>Jussiaea repens</i>	Duanuoc	Ar	93.0	7.7	93.0	88.0
Pandanaceae						
<i>Pandanus tectorius</i>	Duadai	R	24.0	>200	-	*
Passifloraceae						
<i>Passiflora foetida</i>	Lactien	Ar	38.9	>200	19.5	*
Philydraceae						
<i>Philydrum lanuginosum</i>	Coduoiluon	Ar	47.7	>200	31.5	*
Piperaceae						
<i>Piper lolot</i>	Lalot	L	13.2	>200	27.8	*
<i>P. nigrum</i>	Hotieu	Fr	25.1	>200	13.4	*
Plantaginaceae						
<i>Plantago major</i>	Made	Wh	22.5	>200	2.0	*
Poaceae						
<i>Eleusine indica</i>	Comantrau	Wh	4.64	>200	26.1	*
<i>Imperata cylindrica</i>	Cotranh	Rh	61.9	145.4	82.4	53.9
<i>Zea mays</i>	Ngo		7.0	>200	-	*

Polygalaceae						
<i>Polygala tenuifolia</i>	Vienchi	R	38.7	>200	44.4	*
Polygonaceae						
<i>Polygonum cuspidatum</i>	Cotkhicu	Rh	96.2	28.7	91.2	73.3
<i>P. multiflorum</i>	Hathuodo	Rh	94.9	27.3	98.3	79.8
Polypodiaceae						
<i>Drynaria fortunei</i>	Cottoaibo	Rh	96.7	30.5	91.9	78.5
Ranunculaceae						
<i>Clematis sinensis</i>	Mochthong	R	33.0	>200	4.7	*
<i>Coptis teeta</i>	Hoanglien	R	52.9	197.4	72.2	38.6
Rhamnaceae						
<i>Berchemia lineata</i>	Rungruc	L	94.1	32.5	69.9	41.7
Rubiaceae						
<i>Ixora coccinea</i>	Dondo	L	93.7	22.7	57.1	21.8
<i>Morinda citrifolia</i>	Nhau	Fr	93.2	62.9	59.0	19.5
<i>M. longissima</i>	Nhodong	R	41.7	>200	21.8	*
<i>M. officinalis</i>	Bakich	R	9.2	>200	12.3	*
<i>M. umbellata</i>	Matqui	Ar	16.7	>200	34.8	*
<i>Oldenlandia capitellata</i>	Dacam	Ar	13.0	>200	29.1	*
Rutaceae						
<i>Glycosmis pentaphylla</i>	Buoibung	St	64.1	152.4	88.5	70.8
<i>Zanthoxylum nitidum</i>	Hoangluc	St	80.1	85.2	85.9	40.0
Saururaceae						
<i>Houttuynia cordata</i>	Diepca	Ar	70.3	159.4	21.6	*
Saxifragaceae						
<i>Dichroa febrifuga</i>	Thuongson	L	38.3	>200	38.5	*
Scrophulariaceae						
<i>Andenosma caeruleum</i>	Nhantran	Ar	90.5	73.5	91.1	62.0
<i>Seoparia dulcis</i>	Camthaodat	Ar	32.7	>200	24.5	*
Solanaceae						
<i>Datura metel</i>	Cadocduoc	Fl	45.0	>200	—	*
<i>D. metel</i>	Cadocduoc	L	15.5	>200	—	*
<i>Solanum lyratum</i>	Daytoan	Ar	42.9	>200	25.5	*
<i>S. mammosum</i>	Cavu	L	33.7	>200	5.3	*
<i>S. procumbens</i>	Cagaileo	Ar	24.0	>200	14.7	*
Theaceae						
<i>Camellia dormoyana</i>	Chehoabong	L	95.2	21.3	100	75.4
<i>C. sinensis</i>	Che	L	95.5	8.5	100	96.2
Urticaceae						
<i>Pouzolzia zeylanica</i>	Bomam	Ar	93.8	36.4	—	*
Verbenaceae						
<i>Clerodendron cyrtophyllum</i>	Bomay	Ar	81.3	119.0	64.2	31.9
<i>C. fragrans</i>	Bachdongnu	L	30.5	>200	14.8	*
<i>Verbena officinalis</i>	Coroingua	Ar	87.5	101.3	44.1	*
Zingiberaceae						

Table 1. continued

<i>Curcuma longa</i>	Nghe	Rh	33.1	>200	66.4	39.1
<i>Zingiber officinale</i>	Gung	Rh	76.5	81.9	97.5	70.2
α -tocopherol ^c			95.3	20.0	94.2	69.4
ascorbic acid ^c			96.1	4.5	*	*
BHT ^c			84.2	24.6	100	100

^a Values present are average of three independent experiments: DSA: DPPH scavenging activity (%) at 200 $\mu\text{g/ml}$; IC₅₀: the concentration ($\mu\text{g/ml}$) that could scavenge 50% DPPH radical produced; LI (1) and LI (2): lipid peroxidation inhibitory activity (%) at concentration of 100 and 50 $\mu\text{g/ml}$, respectively; ^b Wh: whole plant, R: root, Ar: aerial parts, L: leaf, C: cortex, Se: seed, Rh: rhizome, Fr: fruit, St: stem, Fl: flower; ^c positive controls; – inactive; * not determined.

the South of Vietnam (Nguyen *et al.*, 2004). Among them, five species were found to exhibit significant inhibitory activity. These species include *Artemisia vulgaris* (leaf), *Caesalpinia sappan* (wood), *Blumea balsamifera* (aerial parts), *Chrysanthemum sinense* (flower) and *Tetracera scandens* (root and stem). Two of these, *A. vulgaris* (aerial parts) and *T. scandens* (stem), were evaluated for their antioxidant activities in this study (Table 1). Despite having good free radical scavenging activity, herba of *A. vulgaris* showed a weak inhibitory effect on lipid peroxidation (10.5% at 100 $\mu\text{g/ml}$). The stem of *T. scandens* exhibited strong activities on all three assays, especially a remarkable inhibitory effect on lipid peroxidation (88.1% at 50 $\mu\text{g/ml}$). Thus, the traditional use of this plant as a remedy for rheumatism and hepatitis might be conceivable. However, no report on principles of *T. scandens* has been documented, suggesting further phytochemical and biological studies should be done.

In good agreement with phytochemical components that have been reported from *Gnetum montanum* (Li *et al.*, 2004; Xiang *et al.*, 2002; Yao *et al.*, 2004), *Morus alba* (Yen *et al.*, 1996; Kim *et al.*, 1999; Doi *et al.*, 2001), and *Euphorbia thymifolia* (Lee *et al.*, 1990; Lin *et al.*, 2002), three MeOH extracts of these herbs displayed significant antioxidant activities. The results might explain some applications of these herbs in traditional medicine for itchiness, impetigo, allergy, and fever.

To the present knowledge, the principles responsible for the antioxidant activities of *Heterosmilax erythrantha*, *Syzygium formosum*, and *Jussiaea repens* have not been identified. However, some *Syzygium* species were found to exhibit strong antioxidant activities (Lee and Shibamoto, 2001; Banerjee *et al.*, 2005). Therefore, it might be interesting and worthwhile to identify active components of these plants.

The radix of *Polygonum multiflorum* has long been used in China and Vietnam as an anti-aging. This is supported by our results, which indicate that the plant

possesses strong antioxidant activity, and previous phytochemical studies have revealed corresponding components of phenolic compounds, mainly stilbenes and flavonoids (Kimura *et al.*, 1983; Chen *et al.*, 1999; Nonaka *et al.*, 1982; Ryu *et al.*, 2002). The plant *P. cuspidatum* exerted a quite similar activity to that of *P. multiflorum*, suggesting they could have the similar phytochemical compositions.

Two herbals *Phyllanthus urinaria* and *Andenosma caeruleum* are well known in Vietnam for treatment of hepatitis. Of which, *P. urinaria* showed strong scavenging activity against free radical (IC₅₀ = 29.7 $\mu\text{g/ml}$) and anti-lipid peroxidation (63.4% at 50 $\mu\text{g/ml}$). Although *A. caeruleum* showed a weak scavenging effect (IC₅₀ = 73.5 $\mu\text{g/ml}$), it exhibited a strong inhibitory action against lipid peroxidation (62.0% at 50 $\mu\text{g/ml}$). The stem of *T. scandens*, which possesses significant activities, is also used as remedy for hepatitis. Nevertheless, some other herbs have been used to treat this disease, *Prunella vulgaris*, *Zea mays*, *Morinda longissima*, *Curcuma longa*, were all found to have very weak activities.

It is noteworthy that some medicinal plants in the list were prescribed as anticancer drugs. *Crinum latifolium* is one of many species from the genus *Crinum* growing in Vietnam (Ho, 2001). Nowadays, the leaves of this plant have been used as remedy for cancer or kidney disorder in the forms of decoction or tea (Loi, 2001; Nam *et al.*, 2004). The plant had not been used in traditional medicine until 1989 when it was reputed to have positive effects in some cases of cancer without scientific evidence (Loi, 2001). Recently, some anticancer compounds such as alkaloids (Nguyen *et al.*, 2001; Phan *et al.*, 2003), coumarin and flavonoids (Nam *et al.*, 2004), have been reported from the leaves of this plant. This plant showed moderate antioxidant activities, having the IC₅₀ value of 199.0 $\mu\text{g/ml}$ (DPPH radical scavenging activity) and 53.7% inhibitory effect on lipid peroxidation at 50 $\mu\text{g/ml}$. However, our results clearly showed that other plants from this genus, *C. asiaticum* and *C. ensifolium*, exhibited

very weak antioxidant activity. Similar to *C. latifolium*, although *Catharathus roseus* has been reported to have potent antitumor (Ueda *et al.*, 2002), the plant displayed moderate antioxidant activities. Its active principles are well known alkaloids such as vinblastin and vincristin, which are used recently to treat cancer. Interestingly, the stems of *Celastrus hindsii*, which also has been used lately for cancer treatment, showed a remarkable antioxidant activity on all three assays. Many studies have investigated antitumor principles from this plant, including triterpenoids (Kuo and Kuo, 1997) and alkaloids (Kuo *et al.*, 1995; Huang *et al.*, 2000). These suggest the presence of both antitumor and antioxidant agents in *C. hindsii*, even though no antioxidant ingredient has been reported. Interestingly, the activities of the leaf of this plant were much less active than its stem, indicating that the antioxidant principles might be mainly located in the stem.

In spite of the fact that some plants which have been documented to have cytotoxic or anticancer activities, e.g. *Siegesbeckia orientalis* (Nam, 2000; Nam *et al.*, 2003), *Mimosa pudica* (Nam *et al.*, 2003), *Ephedra sinica* (Nam *et al.*, 2003), *Bombax ceiba* (Nam *et al.*, 2003; You *et al.*, 2003), and *Drynaria fortunei* (Nam *et al.*, 2003), none of these herbs have traditionally been used to treat cancer. *E. sinica*, which is prescribed for diaphoretic agent and to allay coughing, showed moderate antioxidant activities. Meanwhile, rhizome of *D. fortunei* displayed remarkable free radical scavenging ($IC_{50} = 30.5 \mu\text{g/ml}$) and anti-lipid peroxidation ($LI = 81.9\%$ at $50 \mu\text{g/ml}$). Taken together, it seems likely that there is no link between antioxidant activities and antiangiogenic activity of these plants. Although further investigations are required to characterize their bioactivities as well as principle active compounds, it is expected that some additional therapeutic uses could be applied for these plants.

In summary, we have presented 126 medicinal plants from Vietnamese ethnomedicine and the results from our preliminary screening for their antioxidant activities. In addition, the traditional uses and activities of some considerable herbs were outlined and discussed. Further research is necessary to study on bioactive compounds and bioactivities from this untapped source in the future.

Acknowledgements

We are grateful to Pharmacist Can Tuyet Nga and Pharmacist Ta Thi Hoan for their kind help in collecting and providing plant materials.

References

- Banerjee, A., Dasgupta, N., and De, B., In vitro study of antioxidant activity of *Syzygium cumini* fruit. *Food Chem.* **90**, 727-733 (2005).
- Brenner, A.J. and Harris, E.D., A quantitative test for copper using bicinchoninic acid. *Anal. Biochem.* **226**, 80-84 (1995).
- Chen, Y., Wang, M.F., Rosen, R.T., and Ho, C.T., 2,2-Diphenyl-1-picrylhydrazyl radical-scavenging active components from *Polygonum multiflorum* Thunb. *J. Agr. Food Chem.* **47**, 2226-2228 (1999).
- Chi, V.V., Dictionary of Vietnamese medicinal plants. Medical Publishing House, Hanoi, Vietnam (1997).
- Doi, K., Kojima, T., Makino, M., Kimura, Y., and Fujimoto, Y., Studies on the constituents of the leaves of *Morus alba* L. *Chem. Pharm. Bull.* **49**, 151-153 (2001).
- Halliwell, B., Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet* **344**, 721-724 (1994).
- Halliwell, B., Aeschbach, R., Loliger, J., and Aruoma, O.I., The characterization of antioxidants. *Food Chem. Toxicol.* **33**, 601-617 (1995).
- Ham, A.J.L. and Liebler, D.C., Vitamin E oxidation in rat liver mitochondria. *Biochemistry* **34**, 5754-5761 (1995).
- Ho, P.H., 1997. Vietnamese Flora. Science and Technology Publishing House, Hanoi, Vietnam.
- Huang, H.C., Shen, C.C., Chen, C.F., Wu, Y.C., and Kuo, Y.H., A novel agarofuran sesquiterpene, celahin D from *Celastrus hindsii* Benth. *Chem. Pharm. Bull.* **48**, 1079-1080 (2000).
- Kim, S.Y., Gao, J.J., Lee, W.C., Ryu, K.S., Lee, K.R., and Kim, Y.C., Antioxidative flavonoids from the leaves of *Morus alba*. *Arch. Pharm. Res.* **22**, 81-85 (1999).
- Kimura, Y., Ohminami, H., Okuda, H., Baba, K., Kozawa, M., and Arichi, S., Effects of stilbene components of roots of *Polygonum* ssp. on liver injury in peroxidized oil-fed rats. *Planta Med.* **49**, 51-54 (1983).
- Kohen, R., and Nyska, A., Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol. Pathol.* **30**, 620-650 (2002).
- Kuo, Y.H., Chen, C.F., Kuo, L.M.Y., King, M.L., Chen, C.F., and Lee, K.H., Celahinine A, a new sesquiterpene pyridine alkaloid from *Celastrus hindsii*. *J. Nat. Prod.* **58**, 1735-1738 (1995).
- Kuo, Y.H., and Kuo, L.M.Y., Antitumor and anti-aids triterpenes from *Celastrus hindsii*. *Phytochemistry* **44**, 1275-1281 (1997).
- Lee, K.G., and Shibamoto, T., Antioxidant property of aroma extract isolated from clove buds (*Syzygium aromaticum*). *Food Chem.* **74**, 443-448 (2001).
- Lee, S.H., Tanaka, T., Nonaka, G., and Nishioka, I., Hydrolyzable tannins from *Euphorbia thymifolia*. *Phytochemistry* **29**, 3621-3625 (1990).
- Li, X.M., Lin, M., Wang, Y.H., and Liu, X., Four new stilbenoids from the lianas of *Gnetum montanum* f. *megaloacarpum*. *Planta Med.* **70**, 160-165 (2004).
- Lin, C.C., Cheng, H.Y., Yang, C.M., and Lin, T.C., Antioxidant

- and antiviral activities of *Euphorbia thymifolia* L. *J. Biomed. Sci.* **9**, 656-664 (2002).
- Loi, D.T., Vietnamese medicinal plants and ingredients. Medical Publishing House, Hanoi, Vietnam (2001).
- Na, M.K., An, R.B., Jin, W.Y., Min, B.S., Yoo, J.K., Kim, Y.H., and Bae, K.H., Antioxidant effects of plant extracts on free radicals and lipid peroxidation. *Nat. Prod. Sci.* **9**, 226-231 (2003).
- Nam, N.H., Cytotoxic principle of *Siegesbeckia orientalis* growing in Vietnam. *Tap Chi Hoa Hoc* **38**, 84-86 (2000).
- Nam, N.H., Kim, H.M., Bae, K.H., and Ahn, B.Z., Inhibitory effects of Vietnamese medicinal plants on tube-like formation of human umbilical venous cells. *Phytother. Res.* **17**, 107-111 (2003).
- Nam, N.H., Kim, Y., You, Y.J., Hong, D.H., Kim, H.M., and Ahn, B.Z., New constituents from *Crinum latifolium* with inhibitory effects against tube-like formation of human umbilical venous endothelial cells. *Nat. Prod. Res.* **18**, 485-491 (2004).
- Nam, N.H., Lee, C.W., Hong, D.H., Kim, H.M., Bae, K.H., and Ahn, B.Z., Antiinvasive, antiangiogenic and antitumour activity of *Ephedra sinica* extract. *Phytother. Res.* **17**, 70-76 (2003).
- Nguyen, M.T.T., Awale, S., Tezuka, Y., Tran, Q.L., Watanabe, H., and Kadota, S., Xanthine oxidase inhibitory activity of Vietnamese medicinal plants. *Biol. Pharm. Bull.* **27**, 1414-1421 (2004).
- Nguyen, T.N.T., Kamenarska, Z., Bankova, V., Popov, S., Zvetkova, E., Katzarovo, E., and Le, M.H., Cytotoxic activities of alkaloid fractions from *Crinum latifolium* L., Amaryllidaceae. *Tap Chi Duoc Hoc* **11**, 21-23 (2001).
- Nonaka, G., Miwa, N., and Nishioka, I., Tannins and related compounds. Part 2. Stilbene glycoside gallates and proanthocyanidins from *Polygonum multiflorum*. *Phytochemistry* **21**, 429-432 (1982).
- Phan, T.S., Tran, B.D., Phan, M.G., Nguyen, T.M., Hoang, T.H., and Le, M.H., Investigation of antimicrobial and cytotoxic properties of alkaloids from some species of *Crinum* in Vietnam. *Tap Chi Duoc Hoc* **4**, 18-21 (2003).
- Pietta, P.G., 2000. Flavonoids as antioxidants. *J. Nat. Prod.* **63**, 1035-1042.
- Ryu, G.S., Ju, J.H., Park, Y.J., Ryu, S.Y., Choi, B.W., and Lee, B.H., The radical scavenging effects of stilbene glucosides from *Polygonum multiflorum*. *Arch. Pharm. Res.* **25**, 636-639 (2002).
- Simonian, N.A., and Coyle, J.T., Oxidative stress in neurodegenerative diseases. *Ann. rev. Pharmacol. Toxicol.* **36**, 83-106 (1996).
- Xiang, W., Jiang, B., Li, X.M., Zhang, H.J., Zhao, Q.S., Li, S.H., and Sun, H.D., Constituents of *Gnetum montanum*. *Fitoterapia* **73**, 40-42 (2002).
- Yao, C.S., Zhou, L.X., and Lin, M., Preparation on oligostilbenes of isorhapontigenin by oxidative coupling reaction. *Chem. Pharm. Bull.* **52**, 238-243 (2004).
- Yen, G.C., and Hsieh, C.L., Antioxidant activity of extracts from Du-zhong (*Eucommia ulmoides*) toward various lipid peroxidation models in vitro. *J. Agr. Food Chem.* **46**, 3952-3957 (1998).
- Yen, G.C., Wu, S.C., and Duh, P.D., Extraction and identification of antioxidant components from the leaves of Mulberry (*Morus alba* L.). *J. Agr. Food Chem.* **44**, 1687-1690 (1996).
- You, Y.J., Nam, N.H., Kim, Y., Bae, K.H., and Ahn, B.Z., Antiangiogenic activity of lupeol from *Bombax ceiba*. *Phytother. Res.* **17**, 341-344 (2003).
- Ueda, J.Y., Tezuka, Y., Banskota, A.H., Tran, Q.L., Tran, Q.K., Harimaya, Y., Saiki, I., and Kadota, S., Antiproliferative activity of Vietnamese medicinal plants. *Biol. Pharm. Bull.* **25**, 753-760 (2002).
- Waddington, R.J., Moseley, R., and Embery, G., Reactive oxygen species: a potential role in the pathogenesis of periodontal diseases. *Oral Dis.* **6**, 138-151 (2000).
- Wu, L., Smythe, A.M., Stinson, S.F., Mullendore, L.A., Monks, A., Scudiero, D.A., Paull, K.D., Koutsoukos, A.D., Rubinstein, L.V., Boyd, M.R., and Shoemaker, R.H., Multidrug-resistant phenotype of disease-oriented panels of human tumor cell lines used for anticancer drug screening. *Cancer Res.* **52**, 3029-3034 (1992).

(Accepted March 1, 2006)