Journal of Food and Drug Analysis, Vol. 18, No. 1, 2010, Pages 24-33 藥物食品分析 第十八卷 第一期

Comparative Study of the Antioxidant Activity of Forty-five Commonly Used Essential Oils and their Potential Active Components

HSIAO-FEN WANG[1], KUANG-HWAY YIH[2]* AND KEH-FENG HUANG[1]

[1.] Department of Applied Chemistry, Providence University, No. 200, Zhongqi Rd., Shalu Township, Taichung, Taiwan, R. O. C.
[2.] Department of Applied Cosmetology, Hungkuang University, No. 34, Zhongqi Rd., Shalu Township, Taichung, Taiwan, R. O. C.

(Received: April, 13, 2009; Accepted: December, 18, 2009)

ABSTRACT

This study was to examine the *in vitro* antioxidant activities of forty-five commonly used essential oils and their major components. The oils and major components were subjected to screening for their possible antioxidant activity by measuring the $ABTS^{+\bullet}$ radical scavenging ability, reducing power and metal chelating activity. The $ABTS^{+\bullet}$ radical scavenging ability and reducing power of cinnamon leaf and clove bud essential oils are the best two among these essential oils. At the concentration of 1 mg/mL, cinnamon leaf (96.45 ± 0.01%) and clove bud (96.33 ± 0.01%) essential oils showed the strongest $ABTS^{+\bullet}$ radical scavenging ability. The EC_{50} values of cinnamon leaf and clove bud essential oils are 12 μg/mL and 10 μg/mL, respectively. At the concentration of 10 mg/mL, cinnamon leaf and clove bud essential oils showed reducing power of 119.42 ± 0.68% and 112.92 ± 0.87% relative to butylated hydroxyanisole (BHA), respectively. Eugenol is the main component of cinnamon leaf and clove bud essential oils that contributes significantly to their $ABTS^{+\bullet}$ radical scavenging activity and reducing power. In the metal chelating activity test system, basil essential oil was determined to be 57.48 ± 0.25% and its EC_{50} value is 984 μg/mL. Methyl chavicol is the major component of the basil essential oil that attributes greatly to its metal chelating activity. The higher phenolic content may explain the higher $ABTS^{+\bullet}$ radical scavenging and reducing power activity of the forty-five kinds of commonly used essential oils. High electron density of the oxygen atom and low steric hindrance of the plane molecule of the methyl chavicol are the two possibilities that account for its higher metal chelating ability.

Key words: $ABTS^{+\bullet}$ (2, 2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical scavenging ability, metal chelating, reducing power, essential oil

INTRODUCTION

In living organisms, the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to cause damage to lipids, proteins, enzymes, and nucleic acids leading to aging as well as wide range of degenerative diseases including cancers, atherosclerosis and coronary heart pathologies(1). ROS and RNS include diverse entities namely superoxide, hydroxyl, peroxyl, peroxinitrite, and nitric oxide radicals(2).

On the other hand, the aerobic organisms develop antioxidant defense mechanisms that restore the damages caused by ROS and RNS entities. The defence mechanisms can be enzymatic and non-enzymatic. The enzymatic mechanisms comprised, for instance, superoxide dismutase, catalase, glutathione reductase and peroxidase among others. The non-enzymatic mechanisms are composed of endogenic and dietary antioxidants and trapping agents such as ascorbic acid, α-tocopherol, β-carotene, flavonoids and trace elements such as zinc and selenium(3).

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are allowed to use in certain food preparations because of their antioxidant potency, even though there are some experimental evidences indicating that they could induce DNA damage(4). Therefore, there is an increasing interest in searching antioxidants from natural origin to scavenge free radicals to prevent human body from oxidative stress produced by ROS and RNS(5).

Essential oils of herbs and their components have many applications in folk medicine, food flavoring and

* Author for correspondence. Tel: +886-4-26318652 ext. 5308; Fax: +886-4-26310579; E-mail: khyih@sunrise.hk.edu.tw

preservation as well as in the fragrance and pharmaceutical industries[6]. The antimicrobial[7] and antioxidant properties[8] of essential oils have been known for a long time, and a number of investigations have been conducted on their antimicrobial activities using various bacteria and viruses.

Many essential oils also have been confirmed to possess the antioxidant activity and were evaluated in terms of reducing power, activities of scavenging 2,2'-azinobis(3-ethylbenzo thiazoline-6-sulfonic acid) diammonium salt radical ($ABTS^{+\bullet}$) and ability of chelating ferrous ions[8-13]. In this study, the antioxidant capacity of forty-five commonly used essential oils was investigated using three *in vitro* antioxidant assays including $ABTS^{+\bullet}$ radical scavenging ability, metal chelating activity and reducing power. These essential oils were compared with those of the commercial antioxidants, and the major components showing antioxidant activity were further identified by GC-MS. The compositions of commonly and commercially used essential oils were different from their original extraction and were rarely investigated. The purpose of this study may provide an opportunity to find out new antioxidant ingredients[14] from herbs for functional foods and cosmetics uses.

MATERIALS AND METHODS

I. *Materials*

2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was purchased from Sigma (St. Louis, MO, USA). Ammonium thiocyanate, hydrogen peroxide and iron (II) chloride tetrahydrate were purchased from Showa (Tokyo, Japan). Potassium hexacyanoferrate (III), linalool, methyl chavicol, eugenol and *dl*-limonen were purchased from Merck (Darmstadt, Germany). L(+)-Ascorbic acid, *3*-carene and thymol were purchased from Acros Organics (Geel, Belgium). Butylated hydroxyanisole (BHA), eugenol acetate and β-caryophyllene were purchased from TCI (Shanghai, China). α-Pinene was purchased from Aldrich (St. Louis, MO, USA). *p*-Cymene was purchased from Fluka (Buchs, Switzerland). Menthol was purchased from MP (Eschwege, Germany). Methyl salicylate was purchased from Alfa Aesar (Karlsruhe, Germany). The forty-five kinds of essential oils of 100% purity were purchased from the Australian Botanical Products (Hallam Victoria, Australian) (TGA warrant by Australia government, USDA and ACO certification). All the other chemicals and solvents were of standard analytical grade and purchased from Echo Chemical Co. (Miaoli, Taiwan).

II. *Methods*

(I) *Gas Chromatography-Mass Spectrometry (GC-MS)*

The analyses of the volatile compounds were run on a Thermo GC-MS system (GC-MS Trace DSQ-Mass Spectrometer, MSD 201351, Thermo, Minneapolis, MN, USA). TR-5MS column was used (5% phenyl polysilphenylene siloxane, Thermo, USA) with a length of 30 m, an inside diameter of 0.25 mm and a film thickness of 0.25 μm.

Oven temperature was programmed as follows: isothermal at 40°C for 1 min, then increased to 250°C, at a rate of 10°C/min and subsequently held isothermally for 40 min; then increased to 300°C at a rate of 4°C/min. The carrier gas was helium (1 mL/min). The injection port temperature was 220°C and the detector temperature was 280°C. Ionization of the sample components was performed in the EI mode (70 eV). Injected volume was 1 μL. The linear retention indices for all the compounds were determined by co-injection of the samples with a solution containing a homologous series of C8-C22 *n*-alkanes[9]. The individual constituents were identified by their identical retention indices referring to the compounds known from the literature data[10], and also by comparing their mass spectra with spectra of either the known compounds or with the Trace DSQ-MASS spectral database (Thermo, USA).

(II) *$ABTS^{+\bullet}$ Radical Cation Decolourisation Assay*

The experiments were carried out using an improved $ABTS^{+\bullet}$ (decolourisation assay with slight modifications) and compared with that of BHA[11,12]. It's applicable for both lipophilic and hydrophilic compounds. The $ABTS^{+\bullet}$ was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark for 1 h at room temperature. The $ABTS^{+\bullet}$ solution (1.75 mL) was mixed with 0.05 mL of essential oils (1 mg/mL EtOH) in 1-cm path length disposable microcuvette and incubated in darkness at room temperature for 1 h. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 734 nm. All determinations were carried out in triplicate. The percentage of inhibition of $ABTS^{+\bullet}$ complex formation was calculated using the following equation:

Scavenging effect (%) = (1- absorbance of sample at 734 nm / absorbance of control at 734 nm) × 100%

(III) *Determination of Metal Chelating Activity*

The chelation of ferrous ions by the essential oils was estimated by the method of Oyaizu[13] with slight modifications and compared with that of EDTA-2Na.

The reaction mixture containing 0.05 mL of essential oils (1 mg/mL EtOH) was added to a solution of 2 mM $FeCl_2$ (0.75 mL). The reaction was initiated by the addition of 5 mM ferrozine (1.5 mL) and the mixture was

finally quantified to 0.25 mL with methanol by pipette, shaken vigorously and incubated in darkness at room temperature for 30 min. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562 nm. The lower the absorbance of the reaction mixture indicated the higher the Fe^{2+}-chelating ability. All tests and analyses were done in triplicate and average values were taken. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the following equation:

Scavenging effect (%) = (1- absorbance of sample at 562 nm / absorbance of control at 562 nm) × 100%

(IV) *Determination of Reducing Power*

The reductive potential of the essential oils was determined according to the method of Oyaizu[13] with slight modifications and compared with that of BHA.

The reaction mixture containing 0.05 mL of essential oils (10 mg/mL EtOH) was mixed with phosphate buffer (0.25 mL, 2 mM, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (0.25 mL, 1%, w/v). The mixture was incubated at 50°C for 20 min. A portion (0.25 mL) of trichloroacetic acid (10%, w/v) was added to the mixture, which was then centrifuged for 10 min. The upper layer of solution (0.1 mL) was mixed with distilled water (0.5 mL) and $FeCl_3$ (0.75 ml, 0.1% w/v), shaken vigorously and incubated in darkness at room temperature for 30 min and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. The percentage of reducing power complex formation was calculated using the following equation:

Reducing power effect (%) = (absorbance of sample at 700 nm / absorbance of 1 mg/mL BHA at 700 nm) × 100%

(V) *Statistical analysis*

Data were presented as mean ± standard deviation (S.D.) of three determinations. Statistical analyses were performed using a one-way analysis of variance. Differences were considered significant at $P < 0.05$. The EC_{50} values were calculated by linear regression analysis. It was defined as the effective concentration of sample to obtain 50% antioxidant or metal chelating activity. Data were calculated by employing the statistical software (SPSS, version 13.0, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

I. *Analysis of Chemical Composition by GC-MS*

Information of the forty-five essential oils was listed in Table 1. The GC-MS was employed to analyze the chemical composition of cinnamon leaf, clove bud, thyme red and basil essential oils.

The GC-MS studies on the cinnamon leaf essential oil resulted in the identification of thirteen components, which accounts for 98.64% of the total amount and the major component (82.87%) was eugenol (Table 2). The other twelve components were α-pinene, α-phellandrene, *p*-cymene, β-linalool, cinnamaldehyde, safrole, copaene, β-caryophyllene, cinnamyl acetate, α-caryophyllene, eugenol acetate and benzyl benzoate, respectively.

There are three major chemical compositions: eugenol (82.32%), eugenol acetate (11.17%) and β-caryophyllen (4.75%) in clove bud essential oil (representing 98.24% of the oil). Eight compounds: α-pinene, α-phellandrene, *d*-limonene, *p*-cymene, crithmene, β-linalool, thymol, and carvacrol in thyme red essential oil (representing 98.55% of the oil) and two major compounds of the essential oil are thymol (63.65%) and *p*-cymene (23.8%). GC-MS analysis of the basil essential oil identified three compounds, representing 98.96% of the oil. They are methyl chavicol (85.35%), β-linalool (12.53%), and menthol (1.08%). The major components of these essential oils those were purchased from pure chemicals and used to study the antioxidant activity with essential oils.

II. *$ABTS^{+\bullet}$ Radical Scavenging Activity*

Cinnamon leaf, clove bud, wintergreen and thyme red essential oils have apparently shown good $ABTS^{+\bullet}$ radical scavenging activity among forty-five commonly used essential oils. At the concentration of 1 mg/mL, cinnamon leaf (96.45 ± 0.01%), clove bud (96.33 ± 0.01%), wintergreen (96.15 ± 0.01%) and thyme red (95.98 ± 0.01%) displayed the strongest $ABTS^{+\bullet}$ radical scavenging activity. The $ABTS^{+\bullet}$ radical scavenging activities in Jasmine absolute, ylang ylang, yarrow and tea tree bush still essential oils were 94.63 ± 0.09%, 93.93 ± 0.04%, 91.27 ± 0.08% and 90.39 ± 0.19%, respectively. Other essential oils do not show apparent $ABTS^{+\bullet}$ radical scavenging activity up to 70% and the sandalwood eastindian essential oil was shown the lowest $ABTS^{+\bullet}$ radical scavenging activity (4.95 ± 0.42%). These results were listed in Table 1.

To compare with the $ABTS^{+\bullet}$ radical scavenging activity of BHA, cinnamon leaf, clove bud, wintergreen and thyme red, were diluted from 10 to 50 μg/mL. The experimental results were displayed in Figure 1(A). The $ABTS^{+\bullet}$ radical scavenging activity could be observed as follows: clove bud > cinnamon leaf > thyme red > wintergreen. The antioxidant capacities in series of concentrations of essential oils were used to calculate the 50% effective concentrations (EC_{50}). The amount of essential oil, necessary to decrease the absorbance of $ABTS^{+\bullet}$ by 50% (EC_{50}), was calculated graphically (% of inhibition was plotted against the antioxidant concentration in the reaction system). The data showed that clove bud possess the best radical scavenging capacity

Table 1. Forty-five essential oils' information and antioxidants

No.	Name	Scientific names	Department	Origin	Extraction Method	Extraction Part	$ABTS^{+\cdot}$ radical scavenging activity (%)*	Metal chelating activity (%)*	Reducing power (% of BHA)*
1	Basil	*Ocimum basilicum*	Lamiaceae	Madagascar	Distillation	Flower, Leaf	31.10 ± 0.34	57.48 ± 0.25	8.36 ± 0.01
2	Bergamot	*Citrus aurantium var. bergamia*	Rutaceae	Italy	Expression	Pericarp	23.89 ± 0.50	10.35 ± 0.15	7.97 ± 0.02
3	Cajeput ambon	*Melaleuca cajeputi*	Myrtaceae	Vietnam	Distillation	Leaf, Shoot	37.03 ± 0.41	24.27 ± 0.37	8.58 ± 0.18
4	Camphor	*Cinnamomum camphora*	Lauraceae	China	Distillation	Leaf	20.87 ± 0.52	10.76 ± 0.02	9.01 ± 0.06
5	Cardamom	*Elettaia cardamomum*	Zingiberaceae	Guatemala	Distillation	Seed	29.64 ± 0.33	9.46 ± 0.11	7.40 ± 0.07
6	Cedarwood atlas	*Cedrus atlantica*	Pinaceae	Morocco	Distillation	Wood	29.49 ± 0.49	19.91 ± 0.69	11.55 ± 0.35
7	Cinnamon leaf	*Cinnamomum zeylanicum*	Lauraceae	Madagascar	Distillation	Leaf, Bark	96.45 ± 0.01	4.63 ± 0.07	119.42 ± 0.68
8	Clary sage	*Salvia sclarea*	Lamiaceae	France	Distillation	Flower bud, Bud	28.89 ± 0.47	12.34 ± 0.29	7.77 ± 0.10
9	Clove bud	*Eugenia caryophyllata*	Myrtaceae	Madagascar	Distillation	Bract	96.33 ± 0.01	4.54 ± 0.16	112.92 ± 0.87
10	Coriander	*Coriandrum sativum*	Apiaceae	Australia	Distillation	Seed	19.70 ± 0.40	13.47 ± 0.07	6.85 ± 0.15
11	Cypress	*Cupressus sempervirens*	Cupressaceae	France	Distillation	Leaf, Strobile	47.65 ± 0.34	44.29 ± 0.54	10.05 ± 0.01
12	Everlasting	*Helichrysum italicum*	Asteraceae	France	Distillation	Flower	55.57 ± 0.20	1.82 ± 0.04	11.32 ± 0.31
13	Fennel	*Foeniculum vulgare*	Apiaceae	France	Distillation	Seed	19.49 ± 0.55	5.52 ± 0.13	68.82 ± 0.43
14	Frankincense	*Boswellia carterii*	Burseraceae	India	Distillation	Resin	32.37 ± 0.58	15.89 ± 0.88	15.49 ± 0.91
15	Geranium	*Pelargonium graveolens*	Geraniaceae	Morocco	Distillation	Flower, Leaf	20.92 ± 0.43	2.19 ± 0.04	9.43 ± 0.25
16	Ginger	*Zingiber officinale*	Zingiberaceae	Cochin	Distillation	Rhizome	21.72 ± 0.43	2.43 ± 0.19	12.08 ± 0.19
17	Grapefruit	*Citrus X paradisi*	Rutaceae	Australia	Distillation	Pericarp	35.91 ± 0.50	30.74 ± 0.28	7.70 ± 0.17
18	Jasmine absolute	*Jasminum grandiflorum*	Oleaceae	India	Distillation	Petal	94.63 ± 0.09	1.36 ± 0.10	23.61 ± 0.85
19	Juniperberry	*Juniperus communis*	Cupressaceae	France	Distillation	Fruit	26.77 ± 0.54	8.09 ± 0.09	8.73 ± 0.26
20	Lemon	*Citrus limon*	Rutaceae	Sicily	Expression	Pericarp	23.63 ± 0.21	20.56 ± 0.25	7.76 ± 0.2
21	Lemon eucalyptus	*Eucalyptus citriodorus*	Myrtaceae	Sicily	Distillation	Leaf, Withe	20.45 ± 0.48	11.56 ± 0.16	7.36 ± 0.14
22	Mandarin	*Citrus reticulata*	Rutaceae	Italy	Expression	Pericarp	22.55 ± 0.54	17.25 ± 0.17	8.06 ± 0.15
23	Myrrh	*Commiphora molmol*	Burseraceae	Ethiopia	Enfleurage	Resin	46.57 ± 0.38	3.88 ± 0.18	58.08 ± 0.85
24	Myrtle dalmatian	*Mytus communis*	Myrtaceae	Morocco	Distillation	Leaf	15.82 ± 0.59	3.61 ± 0.04	8.62 ± 0.18

Table 1. Continued

No.	Name	Scientific names	Department	Origin	Extraction Method	Extraction Part	$ABTS^{+\bullet}$ radical scavenging activity (%)*	Metal chelating activity (%)*	Reducing power (% of BHA)*
25	Neroli bigarade	*Citrus aurantium*	Rutaceae	Tunisia	Distillation	Petal	27.82 ± 0.34	5.00 ± 0.33	10.44 ± 0.18
26	Nutmeg	*Myristica fragrans*	Myristicaceae	Indonesia	Distillation	Pericarp, Bark, Leaf	66.47 ± 0.41	10.99 ± 0.06	31.62 ± 0.42
27	Orange bitter	*Citrus aurantium*	Rutaceae	Egypt	Distillation	Leaf, Shoot	42.29 ± 0.43	37.87 ± 0.40	7.46 ± 0.13
28	Orange sweet	*Citrus sinensis*	Rutaceae	Sicily	Expression	Pericarp	33.78 ± 0.58	20.99 ± 0.49	7.55 ± 0.10
29	Papper black	*Piper nigrum*	Piperaceae	Sri Lanka	Distillation	Fruit	15.52 ± 0.50	5.34 ± 0.03	10.00 ± 0.25
30	Pennyroyal pulegone	*Mentha pulegium*	Lamiaceae	Australia	Distillation	Whole plant	18.42 ± 0.57	8.21 ± 0.30	9.54 ± 0.20
31	Peppermint arvensis whole	*Mentha arvensis*	Lamiaceae	Australia	Distillation	Whole plant	18.30 ± 0.60	7.73 ± 0.08	7.08 ± 0.28
32	Pine	*Pinus sylvestris*	Pinaceae	France	Distillation	Conifer	28.16 ± 0.48	24.16 ± 0.01	8.63 ± 0.14
33	Ravensara	*Ravensara aromatica*	Lauraceae	Madagascar	Distillation	Leaf, Shoot	30.57 ± 0.63	19.36 ± 0.51	8.98 ± 0.21
34	Rose geranium	*Pelargonium graveolens I'Her*	Geraniaceae	Morocco	Distillation	Flower, Leaf	20.67 ± 0.54	4.70 ± 0.08	9.09 ± 0.19
35	Rosemary moroccan	*Rosmarinus officinalis*	Rosaceae	Morocco	Distillation	Flower, Leaf	26.33 ± 0.87	22.10 ± 0.29	7.67 ± 0.26
36	Rosewood	*Aniba rosaeodora*	Lauraceae	Brazil	Distillation	Duramen	19.20 ± 0.61	9.53 ± 0.46	7.01 ± 0.20
37	Sage	*Salvia offcinalis*	Lamiaceae	Spain	Distillation	Whole plant	19.82 ± 0.52	7.78 ± 0.08	7.60 ± 0.24
38	Sandalwood eastindian	*Santalum album*	Santalaceae	India	Distillation	Duramen	4.95 ± 0.42	5.43 ± 0.46	8.22 ± 0.19
39	Smith eucalyptus	*Eucalyptus smithii*	Myrtaceae	South Africa	Distillation	Leaf	16.18 ± 0.62	5.64 ± 0.03	7.34 ± 0.17
40	Tea tree bush still	*Melaleuca alternifolia*	Myrtaceae	Australia	Distillation	Leaf, Twig	90.39 ± 0.19	11.58 ± 0.05	11.54 ± 0.40
41	Thyme red	*Thymus vulgaris*	Lamiaceae	France	Distillation	Flower, Leaf	95.98 ± 0.01	4.19 ± 0.16	92.11 ± 0.24
42	Vetiver	*Vetiveria zizanioides*	Poaceae	Madagascar	Distillation	Root	62.47 ± 0.38	7.23 ± 0.20	20.32 ± 0.30
43	Wintergreen	*Gaultheria procumbens*	Ericaceae	China	Distillation	Leaf	96.15 ± 0.01	4.33 ± 0.10	9.23 ± 0.14
44	Yarrow	*Achillea millefolium*	Asteraceae	France	Distillation	Whole plant	91.27 ± 0.08	9.76 ± 0.44	17.77 ± 0.79
45	Ylang Ylang	*Cananga odorata*	Anonaceae	Madagascar	Distillation	Flower	93.93 ± 0.04	9.18 ± 0.09	12.31 ± 0.46

* Values are mean ± SD (n = 3). The concentrations of each essential oil for $ABTS^{+\bullet}$ radical scavenging activity, metal chelating activity and reducing power testing are 1 mg/mL, 1 mg/mL and 10 mg/mL, respectively

Table 2. Composition of the essential oils from cinnamon leaf, clove bud, thyme red and basil

			Cinnamon leaf	Clove bud	Thyme red	Basil
R_t [a]	Compound [b]	M.f. [c]	Peak Area (%)			
9.04	α-Pinene	$C_{10}H_{16}$	2.05		1.26	
12.10	α-Phellandrene	$C_{10}H_{16}$	0.59		1.32	
12.18	*d*-Limonene	$C_{10}H_{16}$			2.82	
12.93	*p*-Cymene	$C_{10}H_{14}$	1.37		23.8	
13.24	Crithmene	$C_{10}H_{16}$			0.77	
16.41	β-Linalool	$C_{10}H_{18}O$	1.33		2.66	12.53
20.04	Menthol	$C_{10}H_{20}O$				1.08
21.00	Methyl chavicol	$C_{10}H_{12}O$				85.35
21.66	Thymol	$C_{10}H_{14}O$			63.65	
21.86	Carvacrol	$C_{10}H_{14}O$			2.27	
24.45	Cinnamaldehyde	C_9H_8O	0.19			
25.13	Safrole	$C_{10}H_{18}O_2$	0.96			
27.87	Eugenol	$C_{10}H_{12}O_2$	82.87	82.32		
28.75	Copaene	$C_{15}H_{24}$	0.78			
30.61	β-Caryophyllene	$C_{15}H_{24}$	3.40	4.75		
31.83	Cinnamyl acetate	$C_{11}H_{12}O_2$	0.74			
32.10	α-Caryophyllene	$C_{15}H_{24}$	0.50			
34.63	Eugenol acetate	$C_{12}H_{14}O_3$	2.00	11.17		
44.03	Benzyl benzoate	$C_{14}H_{12}O_2$	1.86			
	Unknown		1.36	1.76	1.45	1.04

[a] R_t: Retention time (min).
[b] The components were identified by their mass spectra and retention indicates (RIs) with that of the Wiley and NIST mass spectral databases and the previously published RIs.
[c] M.f.: Molecular formula.

(EC_{50} = 10 μg/mL). The EC_{50} values of the best four essential oils, clove bud, cinnamon leaf, thyme red, and wintergreen were determined to be 10 μg/mL, 12 μg/mL, 18 μg/mL and 21 μg/mL, respectively.

At the concentration of 30 μg/mL, cinnamon leaf essential oil and four of its main components (α-pinene, *p*-cymene, β-caryophyllene, eugenol) were designed to examine the $ABTS^{+\bullet}$ radical scavenging activity. In Figure 1(B), it is clear that the $ABTS^{+\bullet}$ radical scavenging activity is in the following order: eugenol > cinnamon leaf essential oil > *p*-cymene > β-caryophyllene > α-pinene.

The $ABTS^{+\bullet}$ radical scavenging activity and work-up of clove bud and thyme red essential oils were similar to that used in cinnamon leaf essential oil.

In Figure 1(B), $ABTS^{+\bullet}$ radical scavenging activity of clove bud essential oil and three of its main components are in the following order: eugenol > clove bud essential oil > eugenol acetate > β-caryophyllene. In Figure 1(B), the $ABTS^{+\bullet}$ radical scavenging activity of thyme red essential oil and four of its main components are in the following order: thymol > thyme red essential oil > β-linalool > *p*-cymene > *d*-limonene.

Eugenol and thymol are the major components that attribute the high $ABTS^{+\bullet}$ radical scavenging activity of cinnamon leaf, clove bud and thyme red essential oils. The close correlation between antioxidant activity and phenolic content of extracts obtained from various natural sources has been demonstrated by many researchers[15,16].

From the above description, it is clear that the phenolic compound is effective in the $ABTS^{+\bullet}$ scavenging ability. It is reported that phenolic compounds in plants possess strong antioxidant activity and may help to protect cells against the oxidative damage caused by free radicals[17].

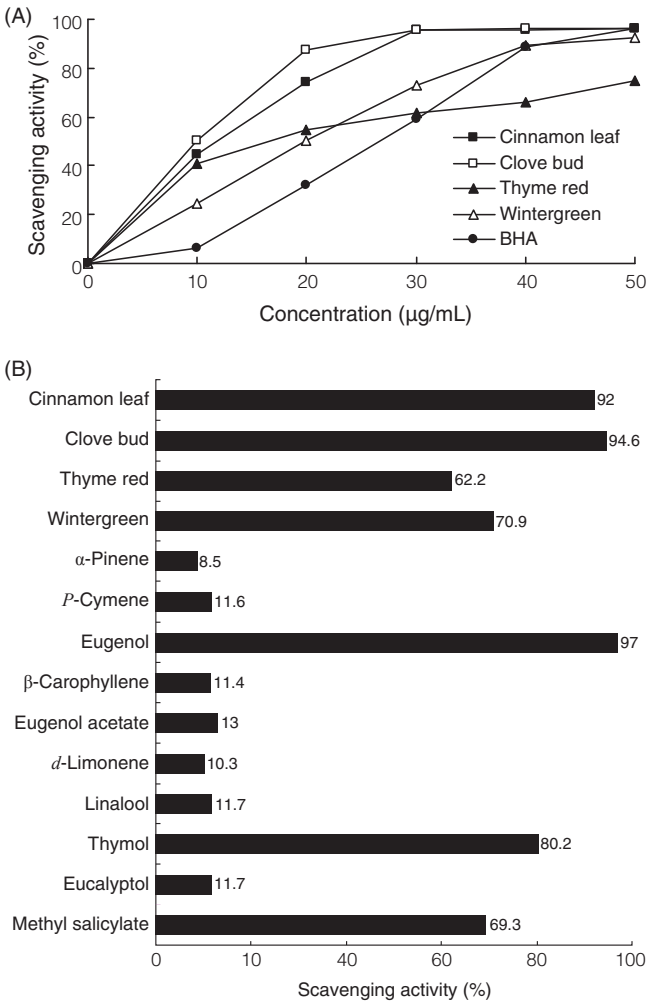

Figure 1. $ABTS^{+\bullet}$ radical scavenging activity of the top four essential oils with the highest activity and their major components.
(A) Concentration-dependent effect of $ABTS^{+\bullet}$ radical scavenging activity of cinnamon leaf, clove bud, thyme red, wintergreen essential oils and BHA.
(B) $ABTS^{+\bullet}$ radical scavenging activity of major chemical components of cinnamon leaf, clove bud, thyme red, wintergreen compared with its essential oils at the concentration of 30 μg/mL.

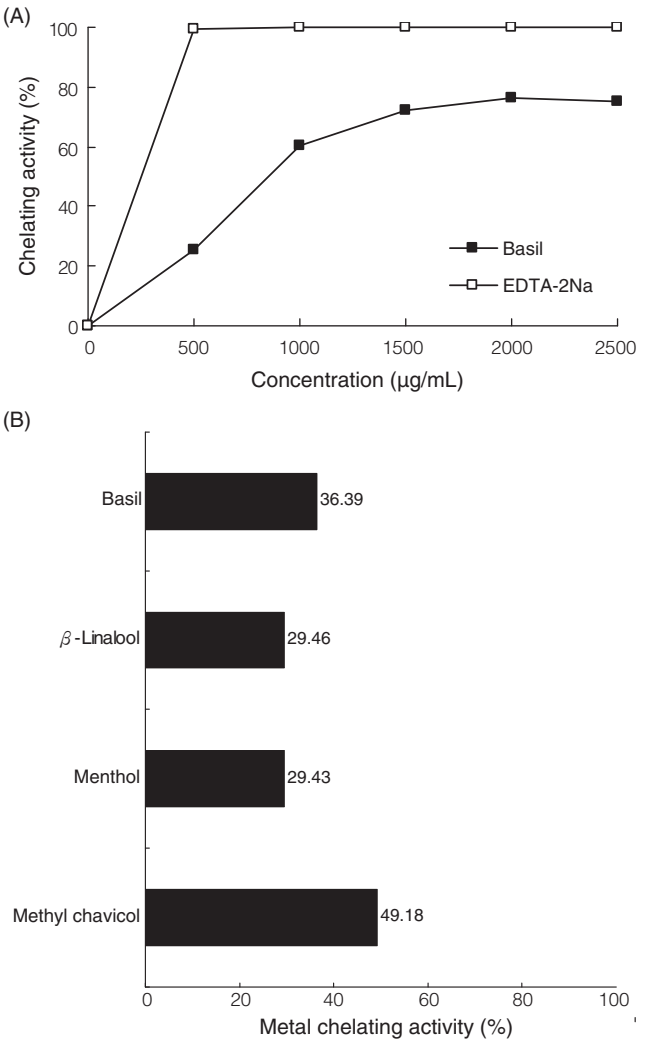

Figure 2. Metal chelating activity of basil essential oil and its major components.
(A) Concentration dependency of the metal chelating activity of basil essential oil and EDTA-2Na.
(B) Metal chelating activity of three major chemical components of basil compared with its essential oils at the concentration of 1 mg/mL.

III. *Determination of Metal Chelating Activity*

Basil essential oil has apparently shown the strongest metal chelating activity among forty-five commonly used essential oils. In the metal chelating activity test system, the basil essential oil was determined as 57.48 ± 0.25% at the concentration of 1 mg/mL, whereas the value of cypress was 44.29 ± 0.54%. At 5 mg/mL, chelating ability of *Satureja cuneifolia*[18] was 66.1%. *Agrocybe cylindracea* strain B[19] chelated ferrous ions by 90.6% at 5 mg/mL. *Agaricus bisporus*, *Pleurotus eryngii*, *Pleurotus ferulae* and *Pleurotus ostreatus*[20] chelated ferrous ions by 59.5%, 41.4%, 51.0%, and 64.0% at 5mg/mL and 76.4%, 79.4%, 73.1%, and 82.3% at 20 mg/mL, respectively. At 5-20 mg/mL, chelating abilities of *Coprinus comatus*[21] were 27.1-66.1%. However, *Pleurotus citrinopileatus*[22] showed chelating abilities of 58.4-75.7% at 5-20 mg/mL and *Hypsizigus marmoreus*[23] showed chelating abilities of 79.8-94.6% at 5-20 mg/mL. It can be seen that chelating abilities of basil and cypress essential oils were higher than those of *C. comatus*, *A. bisporus*, *P. eryngii*, *P. ferula*, *P. ostreatus*, *P. citrinopileatus*, and *H. marmoreus*.

Other essential oils did not show apparent metal chelating activity up to 40% and the jasmine essential oil has shown the lowest metal chelating activity 1.36 ± 0.10%. These results were listed in Table 1.

To compare the metal chelating activity of basil and EDTA-2Na, the set samples were diluted from 500 to 2500 μg/mL. The experimental results were displayed in

Journal of Food and Drug Analysis, Vol. 18, No. 1, 2010

Figure 2(A). The EC_{50} value of the basil essential oil was 984 μg/mL.

To investigate the metal chelating activity of the three major components of basil essential oil, the following experiments were undertaken. At the concentration of 1 mg/mL, β-linalool, menthol, methyl chavicol and basil essential oil were investigated in the experiment of the metal chelating activity. In Figure 2(B), the metal chelating activity is in the following order: basil essential oil > methyl chavicol > β-linalool > menthol.

Three major components of basil essential oil, β-linalool, menthol and methyl chavicol, are tertiary alcohol, secondary alcohol and ether, respectively. The electron pairs of the oxygen atom of the three components are responsible for the coordinating ability[24]. The methyl group of methyl chavicol increases the electronic density of oxygen atom that raises the coordinate ability. It is the possibility of the methyl chavicol representing the higher chelating ability than β-linalool and menthol. The order of the electron density of the three major components of the basil essential oil was shown in Figure 3. Steric effect is another possibility that influences the chelating ability. The methyl chavicol is a planar molecule with a sp^2 C-O bond. The β-linalool and menthol are two stereo-molecules with a sp^3 C-O bond, respectively. The more stability of the methyl chavicol-Fe metal complex than the other two proposed Fe complexes is due to the small steric hindrance and small repulsion of the chelating complex. The order of the steric effect of the proposed three metal complexes was shown in Figure 4.

IV. *Determination of Reducing Power*

Cinnamon leaf, clove bud and thyme red essential oils have apparently shown better reducing power among forty-five commonly used essential oils. At the concentration of 10 mg/mL, the three essential oils were determined to have 119.42 ± 0.68%, 112.92 ± 0.87% and 92.11 ± 0.24% reducing power relative to the BHA, respectively. These values are higher than that of *Agaricus blazei* (86% at 10 mg/mL) and lower than that of *Agrocybe cylindracea*[19] (99% at 5 mg/mL). With regard to ethanolic extracts, Lo[20] mentioned that at 20 mg/mL, reducing powers of *A. bisporus*, *P. eryngii*, *P. ferula*, and *P. ostreatus* were 76%, 75%, 70%, and 61%, respectively. Reducing powers of *C. comatus*[21] were 25% at 5 mg/mL and 56% at 20 mg/mL. Furthermore, at 20 mg/mL, *H. marmoreus*[22] showed a reducing power of 74%. However, *P. citrinopileatus*[23] showed a high reducing power of 103% at 5 mg/mL. It can be seen that reducing powers of cinnamon leaf, clove bud and thyme red essential oils were more effective than those of *A. bisporus*, *C. comatus*, *H. marmoreus*, *P. eryngii*, *P. ferula*, and *P. ostreatus* and less effective than the *P. citrinopileatus*.

Fennel essential oils showed 68.82 ± 0.43% reducing power. Other essential oils did not show apparent reducing power up to 70% and the coriander essential oil has the lowest reducing power (6.85 ± 0.15%). These results were listed in Table 1.

To compare the reducing power relative to BHA,

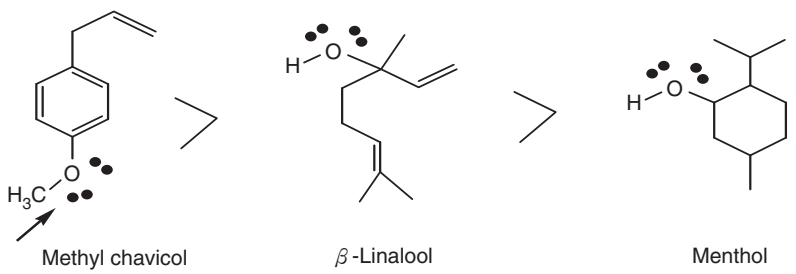

Figure 3. The order of the electronic effect of the three major components of the basil essential oil.

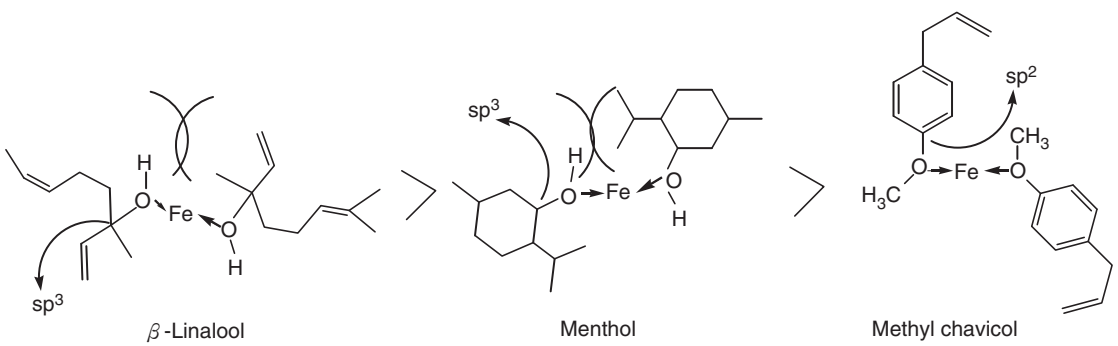

Figure 4. The order of the steric effect of the proposed three metal complexes.

essential oils of cinnamon leaf and clove bud were diluted from 10 to 200 μg/mL. The experimental results were displayed in Figure 5(A). The reducing power of essential oils of cinnamon leaf is better than that of clove bud essential oil. Cinnamon leaf essential oil and five of its major components α-pinene, *p*-cymene, β-caryophyllene, eugenol acetate and eugenol were employed to compare their reducing power. In Figure 5(B), the reducing power is in the following order: eugenol > cinnamon leaf essential oil > β-caryophyllene > eugenol acetate > α-pinene > *p*-cymene. Eugenol is the major component that accounts for the high reducing power of cinnamon leaf essential oil.

Clove bud essential oil and three of its major components eugenol, β-caryophyllene and eugenol acetate were used to compare the reducing power. In Figure 5(B), the reducing power of eugenol is also better than that of clove bud essential oil. From the above experiments, eugenol is the major component that contributes high reducing power activity of cinnamon leaf and clove bud essential oils.

In three test systems, all of the major components and cinnamon leaf, clove bud, thymol red, and basil essential oils exhibited remarkable antioxidant activities. In general, the phenolic compounds showed greater activity than its essential oils in $ABTS^{+\bullet}$ radical scavenging activity and reducing power systems. Our research shows that cinnamon leaf, clove bud, thyme red, and basil essential oils have the potential to develop into antioxidant ingredients for functional foods and cosmetic products.

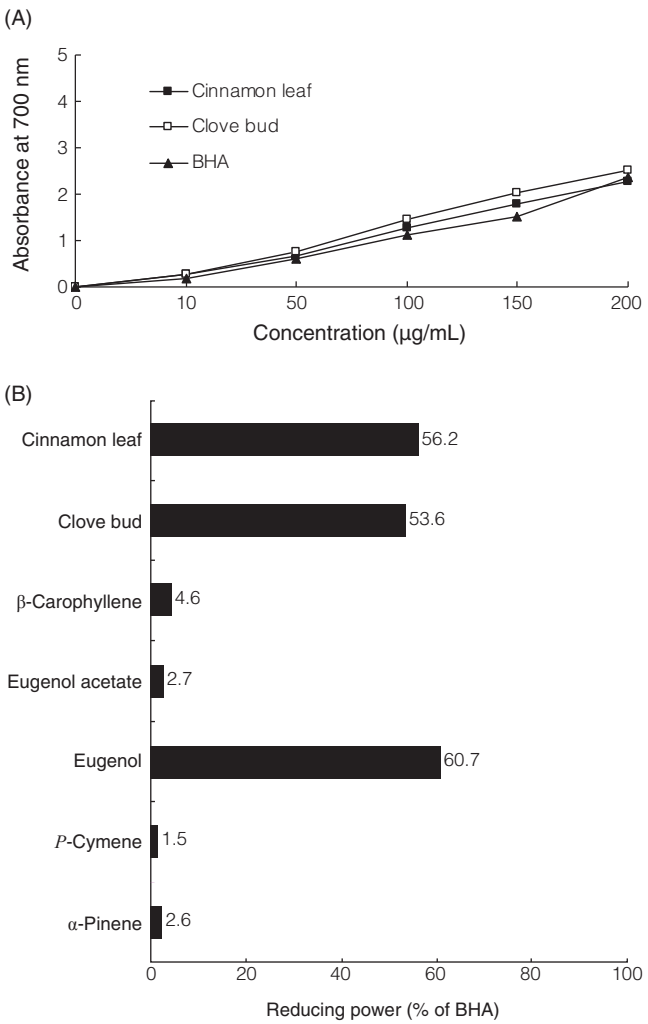

Figure 5. Reducing power of essential oils made from cinnamon leaf and clove bud and their major components.
(A) Concentration dependency of the reducing power of cinnamon leaf, clove bud and BHA. (B) Reducing power of the major chemical components compared with its essential oils at the concentration of 1 mg/mL cinnamon leaf and clove bud oils.

ACKNOWLEDGMENTS

We thank the National Science Council of Taiwan, the Republic of China for support (NSC96-2113-M-214-001-MY2).

REFERENCES

1. Duan, X. J., Zhang, W. W., Li, X. M. and Wang, B. G. 2006. Evaluation of antioxidant property of extract and fractions obtained from a red alga. Food Chem. 95: 37-43.
2. Mavi, A., Terzi, Z., Ozoen, U., Yildirim, A. and Coskun, M. 2003. Antioxidant properties of some medicinal plants: *Prangos ferulacea* (Apiaceae), *Sedum sempervivoides* (Crassulaceae), *Malva neglecta* (Malvaceae), *Cruciata taurica* (Rubiaceae), *Rosa pimpinellifolia* (Rosaceae), *Galium verum subsp. Verum* (Rubiaceae), *Urtica dioica* (Urticaceae). Biol. Pharm. Bull. 27: 702-705.
3. Chae, S. S., Kim, J. S., Kang, K. A., Bu, H. D., Lee, Y., Hyun, J. W. and Kang, S. S. 2004. Antioxidant activity of Jionoside D from *Clerodendron trichotomun*. Biol. Pharm. Bull. 27: 1504-1508.
4. Sasaki, Y. F., Kawaguchi, S., Kamaya, A., Ohshita, M., Kabasawa, K., Iwama, K., Taniguchi, K. and Tsuda, S. 2002. The comet assay with 8 mouse organs: Results with 39 currently used food additives. Mutat. Res. 519: 103-119.
5. Gonçalves, C., Dinis, T. and Batista, M. T. 2005. Antioxidant properties of proanthocyanidins of *Uncaria tomentosa* bark decoction: a mechanism for anti-inflammatory activity. Phytochemistry 66: 89-98.
6. Baratta, M. T., Dorman, H. J. D., Deans, S. G., Figueiredo, A. C., Barroso, J. G. and Ruberto, G. 1998. Antimicrobial and antioxidant properties of some commercial essential oils. Flav. Fragr. J. 13: 235-244.
7. Baratta, M. T., Dorman, H. J. D. and Deans, S. G. 1998. Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oil. J. Essent. Oil Res. 10: 618-627.

8. (a) Kim, H. J., Chen, F., Wu, C. Q., Wang, X., Chung, H. Y. and Jin, Z. Y. 2004. Evaluation of antioxidant activity of Australian tea tree (*Melaleuca alternifolia*) oil and its components. J. Agric. Food Chem. 52: 2849-2854.
   (b) Miguel, G., Simoes, M., Figueiredo, A. C., Barroso, J. G., Prdro, L. G. and Carvalho, L. 2004. Componsition and antioxidant activities of the essential oils of *Thymus caespititius*, *Thymus camphorates* and *Thymus mastichina*. Food Chem. 86: 183-188.
   (c) Sokmen, M., Angelova, M., Krumova, E., Pashova, S., Ivancheva, S. and Sokmen, A. 2005. *In vitro* antioxidant activity of polyphenol extracts with antiviral properties from *Geranium sanguineum* L. Life Sci. 76: 2981-2993.
   (d) Elzaawely, A. A., Xuan, T. D., Koyama, H. and Tawata, S. 2007. Antioxidant activity and contents of essential oil and phenolic compounds in flowers and seeds of *Alpinia zerumbet* (Pers.) Food Chem. 104: 1648-1653.
   (e) Erkan, N., Ayranci, G. and Ayranci, E. 2008. Antioxidant activities of rosemary (*Rosmarinus Officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chem. 110: 76-82.
   (f) Wang, W., Wu, N., Zu, Y. G. and Fu, Y. J. 2008. Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. Food Chem. 108: 1019-1022.
9. Dool, H. V. D. and Kratz, P. D. 1963. A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. J. Chromatogr. 11: 463-471.
10. Adams, R. P. 1995. Identification of essential oil components by gas chromatography/mass spectroscopy. pp. 57-332. Allured Publishing Corporation. IL, U.S.A.
11. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Evans, C. R. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26: 1231-1237.
12. Dinis, T. C. P., Madeira, V. M. C. and Almeida, L. M. 1994. Almeida, action of phenolic derivates (acetoaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315: 161-169.
13. Oyaizu, M. 1986. Antioxidative activities of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. Nippon Shokuhin Kogyo Gakkaishi. 35: 771-775
14. Lin, C. W., Yih, K. H., Yu, C. W. and Wu, S. C. 2009. DPPH free-radical scavenging activity, total phenolic contents and chemical composition analysis of forty-two kinds of essential oils. J. Food Drug Anal. 17: 386-395.
15. Liu, X., Dong, M., Chen, X., Jiang, M., Lv, X. and Yan, G. 2007. Antioxidant activity and phenolics of an endophytic *Xylaria sp.* from Gingko biloba. Food Chem. 105: 548-554.
16. Verzelloni, E., Tagliazucchi, D. and Conte, A. 2007. Relationship between the antioxidant properties and the phenolic and flavonoid content in traditional balsam vinegar. Food Chem. 105: 564-571.
17. Kahkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S. and Heinonen, M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47: 3954-3962.
18. Feyza, O., Belma, A., Sahlan, O. and Senol, A. 2009. Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. Food Chem. 112: 874-879.
19. Tsai, S. Y., Huang, S. J. and Mau, J. L. 2006. Antioxidant properties of hot water extracts from *Agrocybe cylindracea*. Food Chem. 98: 670-677.
20. Lo, S. H. 2005. Quality evaluation of *Agaricus bisporus*, *Pleurotus eryngii*, *Pleurotus ferulae* and *Pleurotus ostreatus* and their antioxidant properties during postharvest storage. Master's Thesis, National Chung-Hsing University, Taichung, Taiwan.
21. Tsai, H. L. 2004. Taste quality, antioxidant properties of *Agaricus blazei, Agrocybe cylindracdea*, *Boletus edulis* and *Coprinus comatus*. Master's Thesis, National Chung-Hsing University, Taichung, Taiwan.
22. Lee, Y. L., Huang, G. W., Liang, Z. C. and Mau, J. L. 2007. Antioxidant properties of three extracts from *Pleurotus citrinopileatus*. Lebensm. Wiss. Technol. 40: 823-833.
23. Lee, Y. L., Yen, M. T. and Mau, J. L. 2006. Antioxidant properties of various extracts from *Hypsizigus marmoreus*. Food Chem. 104: 1-9.
24. Borowski, T., Bassan, A. and Siegbahn, P. E. M. 2004. A hybrid density functional study of O-O bond cleavage and phenyl ring hydroxylation for a biomimetic non-heme iron complex. Inorg. Chem. 43: 3277-3291.