

Full Length Research Paper

Ultrasound assisted extraction for analysis of total phenolic content and antioxidant activity of medicinal plants

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In the present study, *Bupleurum chinense*, *Isatis indigotica* Fort., *Kadsura coccinea*, *Notopterygium incisum* and *Rhizoma Anemarrhenae* were investigated for total phenolic contents (TPC) in water, ethanol and methanol solvent. The antioxidant activities of extracts were determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activities (DPPH%) and reducing power (RP) assay. The results showed that at 1.0 mg/ml, the methanol extract of *R. Anemarrhenae* has exhibited highest TPC, 9.36 mg GAE/g dw, followed by the ethanol extract, 8.14 mg GAE/g dw. Antioxidant activities were also analyzed. The highest DPPH scavenging capacity with the lowest IC₅₀ value, 0.21 mg dw /ml was found in methanol extract of *R. Anemarrhenae*. The reducing power value with 15.86 mg AAE/g in methanol extract of *R. Anemarrhenae* was observed. A high correlation was established between the RP value, DPPH% and the TPC. Consequently, *R. Anemarrhenae* could be selected as an excellent antioxidant for functional foods as well as pharmaceuticals.

Key words: Antioxidant ability, 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, reducing power; total phenolics

INTRODUCTION

Oxidative damage is thought to be a major mechanism involved in the development of chronic renal disease such as cancer, diabetes, aging, heart etc. (Ashokkumar et al., 2008; Young and Woodside, 2001). Antioxidant therapy might be useful in preventing or delaying the progression of these diseases since free radicals inducing oxidative damage are considerable recent evidence (Krishnaiah et al., 2007). Consequently, extensive studies for antioxidants are an inevitable trend. However, synthetic antioxidants have a trend to substitute them with naturally antioxidants because of suspicion to cause negative effects. A great interest has focused on the use of natural antioxidants (Krisnaiah et al., 2011). The dietary of many foods, rich in antioxidant substances such as phenolic compounds (Kitts et al., 2000; Zheng and Wang, 2001), is helpful to eliminate diseases.

Therefore, the importance of studying natural antioxidants has increased generally.

Bupleurum chinense DC., *Isatis indigotica* Fort., *Kadsura coccinea* (Lem.) A.C. Smith., *Notopterygium incisum* Ting ex H. T. Chang and *Rhizoma Anemarrhenae* are selected as samples for study. *B. chinense* is a well-known and very important traditional Chinese drug, and has anti-lipid peroxidation capacity and antiradical effects (Zhu et al., 2009), which are used in at least 66% of the formulations/prescriptions in traditional Chinese medicine. It is often used to treat common cold with fever, alternating chill and fever, the feeling of fullness and oppression in the chest (Liang et al., 1998). *I. indigotica* is used in traditional drug for treating seasonal febrile diseases, pestilence influenza, epidemic meningitis, mumps (You et al., 2009). *K. coccinea* is widely used as antibacterial and antiphlogistic drug for a common fever. *N. incisum* is effective in common cold, headache, generalized pain, possess anti-influenza effect (Wang et al., 2006). *R. Anemarrhenae*, the dried rhizome of *Anemarrhena asphodeloides* Bge., is a well-known

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Table 1. General information regarding plants studied.

Chinese name	Latin name	Family	Function category
Chai Hu	<i>Bupleurum chinense</i> DC. (<i>B. chinense</i>)	Apiaceae	Diaphoretic drugs
Da Qing Ye	<i>Isatis indigotica</i> Fort. (<i>I. indigotica</i>)	Brassicaceae	Diaphoretic drugs
Du Huo	<i>Kadsura coccinea</i> (Lem.) A.C. Smith (<i>K. coccinea</i>)	Annonaceae	Diaphoretic drugs
Qiang Huo	<i>Notopterygium incisum</i> Ting ex H. T. Chang (<i>N. incisum</i>)	Apiaceae	Diaphoretic drugs
Zhi Mu	<i>Rhizoma Anemarrhenae</i> (<i>R. Anemarrhenae</i>)	Liliaceae	Diaphoretic drugs

traditional Chinese medicinal herb and has been used clinically in China for centuries to cure various diseases (Ma et al., 2008).

Although the five herbs have performed well in clinical practice, the related information is scanty in available literatures regarding to total phenolic contents, antiradical capacity and reducing power of these four herbs extracts. The primary aim of this study was to optimize the extraction of antioxidant compounds from plants and compare the activity of different solvent extracts. This comparison will help to prioritize herbs for future investigating the efficacy of herbal therapies in the treatment of renal pathologies.

MATERIALS AND METHODS

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from TCI Co. Ltd (Japan). Gallic acid, ascorbic acid and Folin-Ciocalteu phenol reagent were obtained from Sinopharm Chemical Reagent Co. Ltd (Shang-Hai, China). Methanol and ethanol of analytic grade have been used as extraction solvents. All other chemicals used were of analytic grade.

Plant materials and plant extracts

Crude herbal materials listed in Table 1 were purchased and identified by a professional pharmacist from traditional Chinese medicine (TCM) shop in YanCheng city (Jiangsu, China). The dry herbs were ground to a fine powder using a domestic grinder, transferred to dark glass bottles until further analysis. The method of plant extracts were performing according to the paper (Wang and Dai, 2012). The final extracts of 20 mg/ml dw stock solution were obtained and stored in bottles at 4°C before analysis. The analysis results through the paper were expressed quantified as the dry weight (dw) of the assay solution.

Total phenolic content (TPC) assay

The TPC of extracts was assessed by the Folin-Ciocalteu method with gallic acid as standard by using ultraviolet-visible spectrophotometer (SPECORD-50, Jena, Germany). According to the method (Singleton and Ross, 1965), 0.5 ml of 20 mg/ml dw stock solution was mixed with 1 ml of Folin-Ciocalteu reagent, followed by the addition of 4 ml of 10% sodium carbonate and the volume was adjusted to 10 ml with distilled water. The mixtures were shaken thoroughly and placed for 1.5 h in the dark at room temperature. The absorbance at 760 nm was recorded. The TPC

was expressed as gallic acid equivalent (GAE) in mg GAE/g of sample.

DPPH scavenging ability

The potential antioxidant activity of the extracts was assessed on the basis of the scavenging activity of the stable DPPH free radical (Shun et al., 2008). Different volume (0.1-1 ml) of stock solution (20 mg/ml) were taken and mixed with 3 ml of 4.5×10^{-4} mol/ml of DPPH-ethanol solution followed by 1 ml of 50 mmol/l of Tris-HCl buffer (pH 7.4) and 1 ml of the sample extracts. The mixture was shaken and allowed to stand for 30 min at room temperature. The absorbance of the reaction mixture was measured at 520 nm against ethanol as blank in spectrophotometer. Ascorbic acid was used as a positive control.

When the purple DPPH radical molecule reacts with antioxidant molecule capable to donate a hydrogen atom, the disappearance of the violet color was undertaken. Therefore, the absorbance diminution depends on antioxidant molecule concentration. The scavenging rate (DPPH%) values were calculated according to the following equation.

$$\text{DPPH (\%)} = [(A_0 - A_s)/A_0] \times 100\%$$

Where A_0 is the absorbance of control solution, A_s is absorbance of sample. Inhibitory concentration (IC_{50}) was calculated from the plot of the scavenging activity against the extract concentration. Ascorbic acid (AA) was used as a standard.

Reducing power (RP) assay

The reducing power of all extract samples was determined according to the method of Oyaizu (1986). The reducing power method is based on a redox reaction in which an easily reduced ferric ion is used in excess and antioxidant acts as a reductant. Sample extracts 0.5 ml was mixed with phosphate buffer (1 ml, 0.2 mol/l, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (1 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 1000 rpm for 10 min. The upper layer of solution (1 ml) was mixed with distilled water (2 ml) and $FeCl_3$ (0.4 ml, 0.1%). The absorbance was measured at 700 nm in a spectrophotometer (SPECORD-50, Jena, Germany). Blank was prepared with all the reaction agents without extract. All determinations were performed in triplicate. Positive (ascorbic acid) control reactions were performed in order to plot the absorbance of ascorbic acid against concentration. Reducing power of the extracts was expressed as mg AAE/g of dw.

Statistical analysis

Experimental results were given as mean value $\pm$ SD of three

Table 2. TPC, IC₅₀ and reducing power of extracts from five herbs with different solvents.

Latin name		TPC (mg GAE/g)	IC ₅₀ of DPPH% (mg dw/ml)	Reducing power (mg AAE/g)
<i>B. chinense</i>	ME	2.63 ± 0.09	1.05 ± 0.08	7.57 ± 0.08
	WE	3.43 ± 0.11	0.63 ± 0.06	8.48 ± 0.07
	EE	2.46 ± 0.03	0.78 ± 0.06	6.78 ± 0.14
<i>I. Indigotica</i>	ME	2.34 ± 0.03	2.78 ± 0.17	6.93 ± 0.09
	WE	5.94 ± 0.17	0.38 ± 0.09	5.56 ± 0.07
	EE	1.82 ± 0.06	3.50 ± 0.25	6.15 ± 0.15
<i>K. coccinea</i>	ME	4.11 ± 0.12	0.97 ± 0.09	8.97 ± 0.07
	WE	6.82 ± 0.19	0.94 ± 0.11	9.23 ± 0.14
	EE	2.85 ± 0.08	1.65 ± 0.17	7.31 ± 0.16
<i>N. incisum</i>	ME	6.73 ± 0.06	0.52 ± 0.12	11.81 ± 0.06
	WE	4.57 ± 0.13	0.98 ± 0.18	8.77 ± 0.10
	EE	5.41 ± 0.04	0.91 ± 0.09	10.69 ± 0.13
<i>R. Anemarrhenae</i>	ME	9.36 ± 0.09	0.22 ± 0.05	15.89 ± 0.08
	WE	7.99 ± 0.16	0.29 ± 0.12	15.80 ± 0.12
	EE	8.14 ± 0.11	0.32 ± 0.07	12.72 ± 0.17
Ascorbic acid			2.44 ± 0.06 µg/ml	

Results showed mean ± SD. GAE—gallic acid equivalent; dw—dry weight; DPPH%—the percentage of DPPH scavenging ability; ME—methanol extract, EE—ethanol extract, WE—water extract.

separate experiments. Statistical analysis was conducted using Microsoft Excel software. Differences at $p < 0.05$, using t -test, were considered to be significant. Pearson's correlation test was carried out to calculation coefficients (r) between TPC and DPPH%, TPC and reducing power. All values are expressed as mean ± SD of three parallel results.

RESULTS AND DISCUSSION

TPC assay

Folin-Ciocalte's phenol reagent is selected for the TPC measurement in natural plants. TPC was determined as the GAE using an equation obtained from a calibration curve, $y = 0.0069x$, $R^2 = 0.9917$, where x was the absorbance and y was TPC (mg GAE /g dw). Results of TPC assay from the various extracts were showed in Table 2. The test result of five herbs showed that TPC varied from 1.82 to 9.36 mg GAE /g dw. The methanol extract of *R. Anemarrhenae* was identified to possess the highest amount of phenolic compounds at 9.36 mg GAE /g dw while ethanol extract of *I. Indigotica* showed lowest content at 1.82 mg GAE/g dw. The ethanol and water extract of *R. Anemarrhenae* also showed high TPC, 8.14 and 7.99 mg GAE/g dw, respectively.

DPPH scavenging activity

DPPH test method is used as a preliminary measurement

which posses the mechanism of a stable free radical quenched by the test compound. The DPPH solution with purple colour shows strong absorption at 520 nm, followed change transfer by yellow colour solution when it is reduced to a stable diamagnetic molecular by the test extract.

All extracts revealed a good DPPH scavenging activity in all herb extracts, which was showed in Figure 1. The percentage DPPH scavenging activities of the extracts were concentration dependent. The scavenging activities of all herbs extracts were from 34.85 to 89.34% at 2.0 mg dw/ml solution. *R. Anemarrhenae* has exhibited the highest DPPH scavenging activity (89.34%) in ME, followed by *R. Anemarrhenae* in WE (85.56%) and 85.01% in EE. The herb of lowest scavenging activity was *I. Indigotica* in water extract (only 34.85%). The IC₅₀ of all extracts calculated according to regression equation were listed in Table 2, although the antioxidant activities were lower than the standard (AA) (2.44 µg/ml). *R. Anemarrhenae* has the lowest IC₅₀ value (0.22 mg dw/ml), followed by IC₅₀ value in WE, 0.29 mg dw/ml and 0.32 mg dw/ml in EE. *I. Indigotica* have the poorest antioxidant activity (IC₅₀ value, 3.50 mg dw/ml in EE) with comparison to the other herbs extracts, which is closely related with the lower phenolic content.

Reducing power assay

The total antioxidant potential of the extracts was further

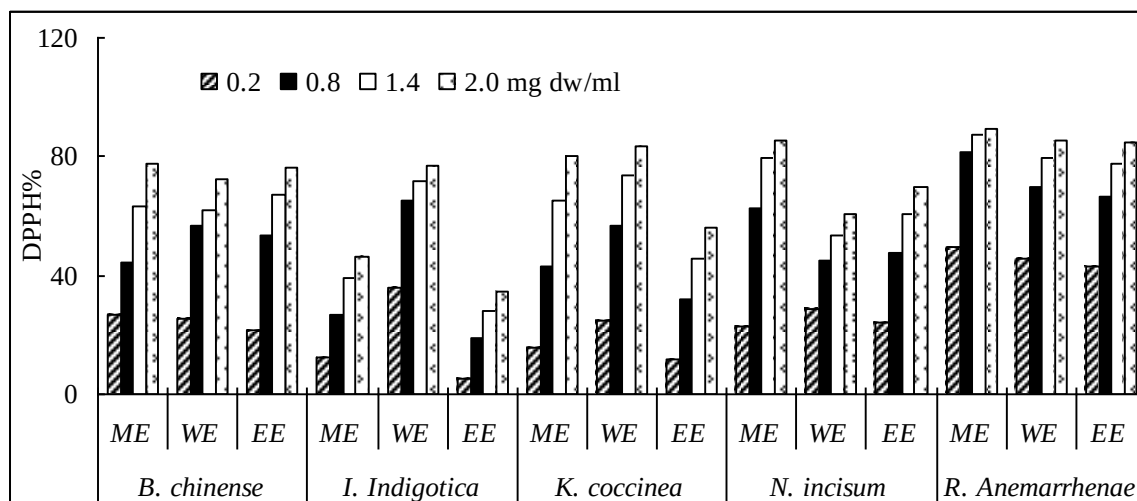


Figure 1. DPPH% of extracts from five herbs with different solvents. ME—methanol extract, EE—ethanol extract, WE—water extract; DPPH%—the percentage of DPPH scavenging ability; dw—dry weight.

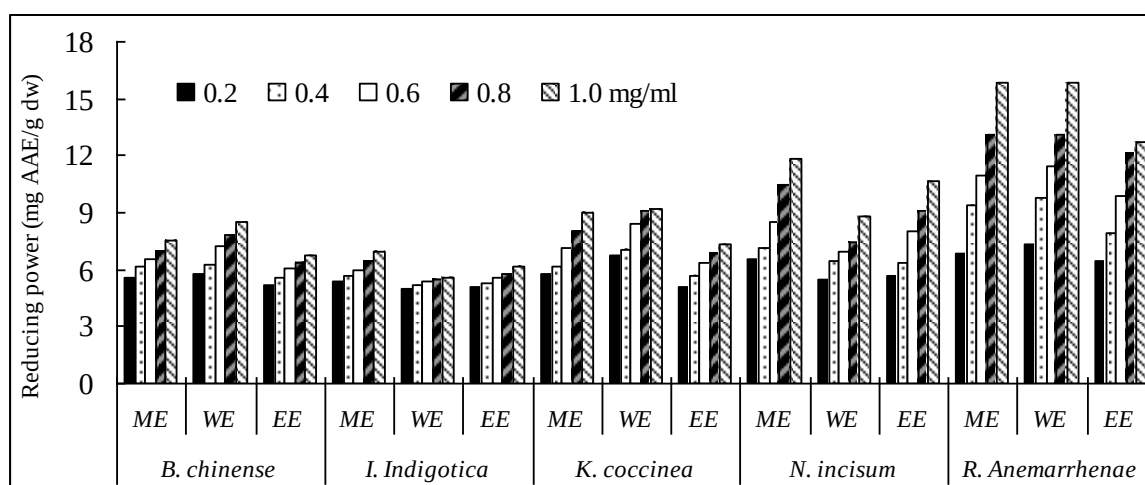


Figure 2. Reducing power of extracts from five herbs with different solvents. ME—methanol extract, EE—ethanol extract, WE—water extract; DPPH%—the percentage of DPPH scavenging ability; dw—dry weight.

evaluated by reducing power assay. The values of reducing power of all extracts were expressed as mg AAE per gram of dw according to the calibration curve: $y = 0.0116x$, $R^2 = 0.9786$, where x was the absorbance and y was reducing power, mg AAE/g dw. The tested results of the reducing power from all herbs in three solvents were proportional to their concentrations (Figure 2). The reducing power of *R. Anemarrhenae* was 15.89 mg AAE/g in ME and 15.80 mg AAE/g in WE at 1.0 mg/ml (Table 2), respectively, although the activities were lower than ascorbic acid. The greatest reducing power was also found for *R. Anemarrhenae*, and the smallest one was the extract of *I. Indigotica*. The reducing power of two herbs was moderate.

The reducing power from methanol extracts decreased

in the following order: *R. Anemarrhenae* > *N. incisum* > *K. coccinea* > *B. chinense* > *I. Indigotica*, and ethanol extracts were the same order above. The decreasing order in water extracts was *R. Anemarrhenae* > *K. coccinea* > *N. incisum* > *B. chinense* > *I. Indigotica*. The results showed that the polarity of solvent is the main factors of influencing on assay. Musa et al. (2011) reported that TPC, DPPH and RP values were sensitive to extraction solvents whereby in pure solvents based on polarity index calculated. In this study, it is difficult to simply analyze the influence degree of the solvents used, while ignoring other factors (solvents purity, plant source etc.).

The reducing power is generally associated with the reductions in plants, which is capable to donate a hydrogen

Table 3. Correlation coefficient among TPC, RP and DPPH of various solvents.

Correlation coefficient (R^2)		TPC–RP	TPC–DPPH%	RP–DPPH%
<i>B. chinense</i>	ME	0.9951	0.9543	0.9269
	EE	0.9851	0.9981	0.9852
	WE	0.9835	0.9784	0.9522
<i>I. Indigotica</i>	ME	0.9456	0.9775	0.9776
	EE	0.9878	0.9334	0.9768
	WE	0.9870	0.8943	0.9459
<i>K. coccinea</i>	ME	0.9432	0.9672	0.9791
	EE	0.9951	0.9859	0.9953
	WE	0.8961	0.9352	0.9529
<i>N. incisum</i>	ME	0.9752	0.9478	0.8584
	EE	0.9919	0.9831	0.968
	WE	0.9751	0.9554	0.9417
<i>R. Anemarrhenae</i>	ME	0.9970	0.9970	0.9882
	EE	0.929	0.8602	0.9548
	WE	0.9865	0.9994	0.982

DPPH%—percentage of DPPH scavenging ability; RP—reducing power; ME—methanol extract, EE—ethanol extract, WE—water extract.

atom with free radical to terminate the reactive chain. *R. Anemarrhenae* possessing the strong reducing power in three solvents showed that the amount of reductions in *R. Anemarrhenae* was higher than other herbs, which is agreed with the results of DPPH scavenging activity assay and the TPC assay. It can be roughly showed that the closely relationship is exist between TPC and antioxidant activity.

Correlation between TPC and DPPH%, reducing power

These herbs in this study have multiple bioactive effects including antiradical, ferric reducing power due to high TPC. In some extracts of herb plants, fruits and vegetables were rich in phenolics (Luo et al., 2002), which are believed to be an important component and adsorbing free radicals, quenching triplet oxygen and decomposing peroxides. Numerous literatures indicated that a linear correlation exists between high TPC and antioxidant activity (Kim et al., 2010; Zhao, 2007). Sun et al. (2009) reported the relationship of the contents of total phenolics of *K. coccinea* and antioxidant activities of extracts. Reducing power assay is widely used in the evaluation of the antioxidant component in dietary polyphenols, which measures the reduction of ferric iron to ferrous iron by antioxidants. Reducing power assay confirmed further that antioxidant activity of *R. Anemarrhenae* was related to the highest TPC. Other

extracts of herbs were not remarkably different from reducing power values. Correlations between TPC and DPPH%, reducing power were established in order to research the relationship between the active components and antioxidant activities. The results were showed in Table 3. The correlation coefficients were approximately 0.9. The correlation between TPC and DPPH% was the highest (0.9994) in *R. Anemarrhenae* extracts. The higher DPPH% and reducing power were consistent with the higher TPC. This suggested that the active component of antioxidant activities in the extract is the possible phenolic compounds. The research report (Musa et al., 2011) found that the solvent polarity have remarkably impact on antioxidant activity. The aqueous organic mixing increased the effectiveness of the extraction. The solvent polarity appears to be no obvious composition for evaluating extract efficient in this study. A fact is not ignored that the extract effect is related to other factors such as environment condition, material source and preparing process.

Conclusion

Our experimental results suggested that the extracts of *R. Anemarrhenae* from three solvents have strong antioxidant activity, DPPH% due to high TPC. In five herbs tested, *R. Anemarrhenae* is a good source of bioactive compounds and is recommended for application of antioxidants and drug development.

REFERENCES

- Ashokkumar D, Mazumder UK, Gupta M, Senthilkumar GP, Selvan VT (2008). Evaluation of antioxidant and free radical scavenging activities of *Oxystelma esculentum* in vitro models. J. Comp. Integr. Med., 5(1)Article 9.
- Kim SI, Sim KH, Choi HY (2010). A comparative study of antioxidant activity in some Korean medical plant used as food materials. Mol. Cell Toxicol. 6:279-285.
- Kitts DD, Yuan YV, Wijewickreme AN, Hu C (2000). Antioxidant properties of a North American ginseng extract. Mol. Cell. Biochem. 203:1-10.
- Krishnaiah D, Bono AB, Rosalam S (2007). Phytochemical antioxidants for health and medicine – A move towards nature. Biotechnol. Mol. Biol. Rev. 1(4):97-104.
- Krisnaiah D, Nityanandam R, Sarbatly R (2011). A review on antioxidant potential of some species. Food Bioprod. Process. 3:217-233.
- Liang H, Zhao Y, Qiu H, Huang J, Zhang R (1998). A new saikosaponin from *Bupleurum chinense* DC. Yao Xue Xue Bao 33:37-41.
- Luo XD, Basile MJ, Kennelly EJ (2002). Polyphenolic antioxidants from the fruits of *Chrysophyllum cainito* L. (star apple). J. Agric. Food Chem. 50:1379-1382.
- Ma CH, Wang LX, Tang YH, Fan MS, Xiao HB, Huang CG (2008). Identification of major xanthenes and steroidal saponins in rat urine by liquid chromatography–atmospheric pressure chemical ionization mass spectrometry technology following oral administration of *Rhizoma Anemarrhenae* decoction. Biomed. Chromatogr., 22:1066-1083.
- Musa KH, Abdullah A, Jusoh K, Subramaniam V (2011). Antioxidant activity of Pink-flesh Guava (*Psidium guajava* L.): Effect of extraction techniques and solvents. Food Anal. Methods, 4:100-107.
- Oyaizu M (1986). Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44:307-315.
- Shun WC, Li S, Ching YK (2008). Antioxidant Activity of Chinese Medicinal Herbs. Pharm. Biol. 46:587-595.
- Singleton VL, Ross JA (1965). Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagent. Am. J. Enol. Vitic. 16:144-158.
- Sun J, Yao J, Huang S, Long X, Wang J, Garcia-Garcia E (2009). Antioxidant activity of polyphenol and anthocyanin extracts from fruits of *Kadsura coccinea* (Lem.) A.C. Smith. Food Chem. 117:276-281.
- Wang XH, Dai JT (2012). A comparative study on antioxidant activity of water and ethanol extracts of ten Chinese herbs. J. Med. Plants Res. 6:2210-2215.
- Wang XY, Jia W, Zhao AH, Wang XR (2006). Anti-influenza agents from plants and traditional Chinese medicine. Phytother. Res. 20:335-341.
- You WC, Hsieh CC, Huang JT (2009). Effect of extracts from indigowood root (*Isatis indigotica* Fort.) on immune responses in radiation-induced mucositis. J. Altern. Complement. Med. 15:771-778.
- Young IS, Woodside JV (2001). Antioxidants in health and disease. J. Clin. Pathol. 54:176-186.
- Zhao GR (2007). Antioxidant activities of *Salvia iltiorrhiza* and *Panax notoginseng*. Food Chem. 99:767-774.
- Zheng W, Wang SY (2001). Antioxidant activity and phenolic compounds in selected herbs. Agric. Food Chem. 49:5165-5170.
- Zhu Z, Liang Z, Han R (2009). Saikosaponin accumulation and antioxidative protection in drought-stressed *Bupleurum chinense* DC. plants. Environ. Exp. Bot. 66:326-333.