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Study on Antioxidant Activity of Thermal Treatment Yam (*Dioscorea batatas* Decne.) by *n*-Butanol and Ethyl Acetate Extracts

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Abstract : In Korea, yam with thermal treatment is widely used to produce tea and beverage, which appeals to all age groups. To better understand the antioxidative activity of thermal treatment yam (*Dioscorea batatas* Decne.), *n*-butanol and ethyl acetate extracts were evaluated with different antioxidant testing system. Their antioxidant activities, including ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] radical scavenging activity, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, reducing power, and ferric reducing antioxidant power as well as total phenol contents were assessed *in vitro*. Results showed ethyl acetate extract (51.63±2.51 mg CAE/g) possessed similar total phenol content compared with *n*-butanol extract (53.93±1.00 mg CAE/g). Ethyl acetate extract also exhibited similarly stronger antioxidant activity compared with *n*-butanol extract apart from ABTS radical scavenging activity assay.

Keywords : yam (Dioscorea batatas Decne.), thermal treatment, total phenol, antioxidative activity

1. Introduction

Reactive oxygen species (ROS) including free radicals such as superoxide anion radicals, hydroxyl radicals, singlet oxygen and non-free radical species such as hydrogen peroxide, which are various forms of active oxygen. They usually generated in living organisms by oxidation product of biological reactions or exogenous factors [15]. In pathological condition, the overproduction of ROS could damage cellular lipids, proteins or DNA and further cause cancer, aging, atherosclerosis, coronary heart diseases and neurodegenerative diseases [16-19]. Fruit and vegetables contain not only essential nutrients needed for daily life but also a wide variety of bioactive compounds (antioxidant phytochemicals) for health promotion and disease prevention [21, 22]. There are several previous studies have conducted that people who have diets rich in

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natural water-soluble antioxidants such as fresh fruits and vegetables can improve the antioxidant activity and reduce the risk of chronic diseases [20]. Currently, a very promising way to overcome oxidative degradation is to increase the consumption of diets rich in fruit or vegetable.

Yam (*Dioscorea batatas* Decne.) is the perennial trailing herb and belongs to the *Dioscoreaceae* family [9]. As yam is mainly composed of starch (75.6-84.3%), it is usually served as the crucial staple food in the diets of many tropical countries [5]. Numerous studies have conducted that yam extracts can reduce blood sugar and blood lipid, inhibit microbe activity and show antioxidant activity [5]. Yam tubers secrete mucilage believed to protect the root tip from abrasion by soil particles [26]. Mucilage is also thought to play an important role in forming stable soil aggregates and nutrition for microorganism, such as rhizobia. The medical effect of yam for health promotion may be due to immunomodulating activity of yam mucilage on the immune system and the exhibition of angiotensin converting enzyme inhibitory activities [23, 26]. Mucilages found in yam contain a mannan associated firmly with protein, which are mainly composed of mannan-protein macromolecules [24]. The extracted and purified yam tuber mucilage exhibited antioxidant activities evaluated by DPPH radical and hydroxyl radical scavenging activities, reducing power test, and anti-lipid peroxidation assay [25].

The aim of this study was to investigate the antioxidant activities of the *n*-butanol extract and ethyl acetate extract from thermal treatment yam. Studies included total phenol content, ABTS radical scavenging activity, DPPH radical scavenging activity, reducing power and ferric reducing antioxidant power.

2. Materials and Methods

2.1. Materials and chemicals

Yam (*Dioscorea batatas* Decne.) was purchased from Andong (Korea), which was seeded in March or April and harvested in the end of October or December. The fresh yam was processed in a procedure of washing, slicing (thickness, 0.4-0.6 cm), steaming (80-90℃, 24 h), drying (hot air, 60-70℃, 18-24 h) and smashing (150-mesh) into thermal treatment yam meals (TTY, commonly called black yam).

2.2. Preparation of yam extracts

Yam meals and extraction solvents including *n*-butanol and ethyl acetate were mixed in a ratio of 1:10 and kept in the dark about 3 h, and then used the Advantec No. 1 filter paper (Tokyo, Japan) to filter. The process of extraction was repeated 3 times. The filtrate was evaporated by rotary vacuum evaporator (EYELA, N-N series, Tokyo, Japan) until the solvents were completely removed. The yam extracts were collected and sealed in brown reagent bottles and frozen at -80℃ until required for further analyses.

2.3. Total phenol content (TPC) assay

In brief, samples (0.5 mL) were mixed with 2.4 mL of distilled water, 2 mL of 2% sodium carbonate (w/v) and 0.1 mL Folin-Ciocalteau's phenol reagent in the test tubes. Then the mixture was incubated in the temperature for 60 min. The absorbance of the reaction mixture was measured at 700 nm using uv/vis-spectrophotometer (Specord 200, Analytikjena, Jena, Germany). Caffeic acid was used as a standard for the calibration curve. Total phenol contents were expressed as mg of caffeic acid equivalents per g of extracts (mg CAE/ g) [1].

2.4. ABTS radical scavenging activity assay

ABTS radical scavenging activity was evaluated according to the method of Gülçin [7]. The mixture of 30 mL of 7 mM ABTS and 528 μL of 140 mM potassium persulfate

was stored at room temperature in the dark for 16 h to get the green-blue free radical $ABTS^{\bullet+}$. Then the solution was diluted with ethanol until the absorbance was 0.70±0.02 at 734 nm. Samples (0.1 mL) were mixed with 2.9 mL of ABTS working solution. After 6 min of reaction, the absorbance was taken at 734 nm. BHA was used as positive control. The percentage of ABTS radical scavenging effect was calculated as follow:

ABTS radical scavenging effect (%)

$$= [1 - (A_s/A_c)] \times 100$$

where A_s is the absorbance in the presence of sample or BHA, and A_c is the absorbance of control reaction.

2.5. DPPH radical scavenging activity assay

DPPH radical scavenging activity was measured according to the method of Blois and Duan [2, 6]. 1 mL of sample solution was mixed with 1.5 mL of 0.2 mM DPPH and then vigorously shaken. The mixture solution was stood in the dark for 30 min at 37℃ water bath. BHA was used as positive control. Then the absorbance of the reaction mixture was read with spectrophotometer at 517 nm. The percentage inhibition of DPPH radical scavenging activity was calculated based on the control reading using the following calculation:

DPPH radical scavenging activity (%)

$$= [1 - (A_s/A_c)] \times 100$$

where A_s is the absorbance in the presence of sample or BHA, and A_c is the absorbance of control reaction.

2.6. Reducing power assay

The reducing power of yam was determined according to the method of Zhao et al. [8]. 1.5 mL of phosphate buffer (0.2 M, pH 6.6), 1.5 mL of sample and 1.5 mL of potassium ferricyanide (1%, w/v) were mixed in test tubes, incubated at 50℃ water bath for 20 min. An aliquot of 1.5 mL trichloroacetic acid (10%, w/v) was added to the mixture. After centrifuged at 3,000 rpm for 10 min, 1 mL of supernatant was mixed with 3 mL of distilled water and 0.3 mL of ferric chloride (0.1%, w/v). The absorbance was measured at 700 nm after 10 min of reaction at room temperature. BHA was used for the positive control.

2.7. Ferric reducing antioxidant power (FRAP) assay

The working FRAP reagent: the FRAP reagent was prepared with 0.3 M sodium acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM hydrochloride acid and 20 mM ferric chloride in a ratio of 10:1:1 (v/v/v). 1.5 mL of freshly prepared FRAP reagent was incubated at 37℃ water bath for 10 min, at the same time, $A_{\text{reagent blank}}$ was read. Then sample (150 μL) was added to the FRAP reagent. The reaction mixture was incubated at 37℃ water bath for 4 min; the absorbance was read at 593 nm (A_{sample}). The mixture of sodium acetate buffer (1.5 mL) and sample (150 μL) was used as sample blank. BHT was used as the positive control. And the difference between A_{sample}, $A_{\text{sample blank}}$ and $A_{\text{reagent blank}}$ was used to calculate the FRAP values. Aqueous solution of $FeSO_4 \cdot 7H_2O$ were used for calibration curve and final results were expressed as μM Fe^{II} [3].

2.8. Statistical analysis

The experimental data in triplicate were subjected to analysis of variance (ANOVA) and expressed as mean ± standard deviation (n=3). Analyses of variance were performed by using the one-way analysis of variance procedures. Duncan's multiple-range test was used to analysis the significant difference of means, and $p<0.05$ was considered to be statistically significant for all statistic procedures. IBM SPSS statistic 21 program was used for data analysis.

3. Results and discussion

3.1. Yields

The various extraction yields of thermal treatment yam (TTY) by *n*-butanol and ethyl acetate were shown in Table 1. The extraction yield of TTY by *n*-butanol exhibited the maximum value of 0.38%, ethyl acetate was found to be 0.25%.

3.2. Total phenol contents (TPC)

Natural polyphenols include catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, rutin, caffeic acid, gallic acid and so on [12]. A positively and highly significant relationship between total phenolics and antioxidant activity was reported by Velioglu, Mazza, Gao, and Oomah [13]. Due to the phenolic structure of hydroxyl substituent on the aromatic ring, phenolics can behave as antioxidants [11]. Total phenol content was determined by according to the colorimetric Folin-Ciocalteau method with caffeic acid as a stand compound ($y=2.4819x+0.0133$, $R^2=0.9997$). The total phenol contents of various extracts (*n*-butanol and ethyl acetate extracts) were showed in Table 1. The total phenol contents in *n*-butanol and ethyl acetate extracts were determined to be 53.93±1.00 and 51.63±2.51 mg CAE/g extract, respectively. Obviously, significant difference was not found between *n*-butanol extract and ethyl acetate extract in the total phenol content assay.

3.3. ABTS radical scavenging activity

The $ABTS^{\bullet+}$ is produced by the $ABTS/K_2S_2O_8$ system. In the absence of antioxidants, ABTS is rather stable, but it reacts actively with an H-atom donor (i.e. phenolics). Therefore, the blue/green chromophore would discolor gradually or be converted into a non-colored form of ABTS up to the antioxidant capacity of antioxidants [12]. Fig. 1 exhibited the ABTS radical scavenging effect of various extracts (*n*-butanol and ethyl acetate extracts). The ABTS radical scavenging activity increased with the increasing concentrations (0.4 mg/mL, 0.7 mg/mL and 1.0 mg/mL) of samples. When at the concentration of 1.0 mg/mL, the ABTS radical scavenging effect of various extracts and standard compound exhibited the following order: BHA, ethyl acetate extract and *n*-butanol extract, respectively. In addition, IC_{50} value of ethyl acetate extract was calculated to be 0.98±0.02 mg/mL, which was lower than that of *n*-butanol extract (IC_{50}=1.31±0.05 mg/mL). A positive correlation between scavenging effects of extracts against ABTS radical and their amounts of total phenol contents was not could be found.

Table 1. Extraction yields, total phenol contents and IC_{50} values in the antioxidant activity evaluation assays of thermal treatment yam (*Dioscorea batatas* Decne.)

	Extracts	
	n-Butanol	**Ethyl acetate**
Extraction yields (%)	0.38	0.25
Total phenol content (mg GAE/g)	53.93±1.00[a*]	51.63±2.53[a]
ABTS (IC_{50}, mg/mL)	1.31±0.05[b]	0.98±0.02[a]
DPPH (IC_{50}, mg/mL)	0.70±0.00[a]	0.74±0.01[b]

*The values are means±standard deviation (*n*=3). Values with the different letters in the same row are significantly different ($p<0.05$) by Duncan's multiple range tests.

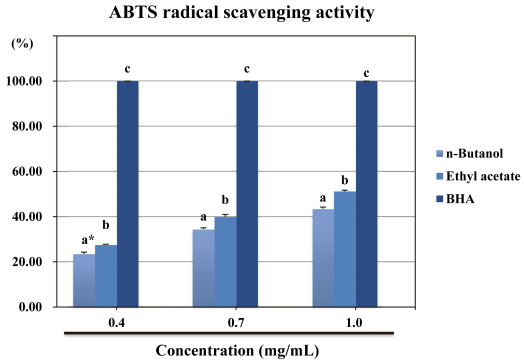

Fig. 1. ABTS radical scavenging activities of various extracts from thermal treatment yam (*Dioscorea batatas* Decne.).

*The values are means±standard deviation (n=3). Bars with the different letters are significantly different (p<0.05) by Duncan's multiple range tests.

3.4. DPPH radical scavenging activity

DPPH is a stable free radical and can be scavenged by antioxidants through donating hydrogen. The discoloration from purple to yellow induces the absorbance of reaction mixture decreases at 517 nm [10]. Various fractions obtained by using different extraction solvents indicated different DPPH scavenging capacity (Fig. 2 and Table 1). Various extracts had effective DPPH radical scavenging activity in a concentration-dependent manner. The *n*-butanol extract of TTY (IC_{50}=0.70±0.00 mg/mL) possessed higher activity upon the elimination of DPPH radical compared with ethyl acetate extract (IC_{50}=0.74±0.01 mg/mL). Remarkable correlation between total phenol contents and DPPH radical scavenging activities could not be found clearly in all extracts. And this result was not in accordance with the founding of Liu et al. [27], who found a direct correlation between DPPH radical scavenging activity and phenolic content of kudingcha. Overall, extracts obtained from different extraction solvents differed in their DPPH radical scavenging activities, demonstrating that extraction solvents could significantly affect the hydrogen donating ability of the bioactive compounds presented in the extracts.

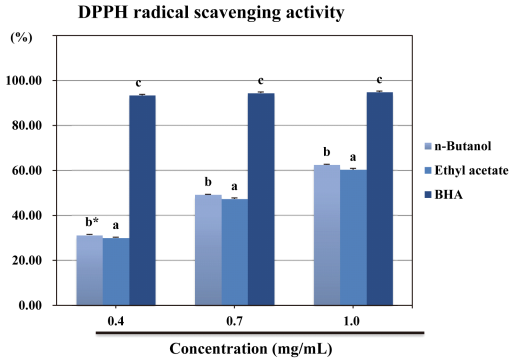

Fig. 2. DPPH radical scavenging activities of various extracts from thermal treatment yam (*Dioscorea batatas* Decne.).

*The values are means±standard deviation (n=3). Bars with the different letters are significantly different (p<0.05) by Duncan's multiple range tests.

3.5. Reducing power

In the presence of reductants (i.e. antioxidants), the Fe^{3+}/ferricyanide complex was reduced to its ferrous form. As a consequence, the color of the test solution changed from yellow to different shades of green and blue, up to the antioxidant ability. Hence, the reducing power can be indicated by the number of the Fe^{2+} complex, which was monitored through measuring the formation of Perl's Prussian blue at 700 nm [4]. The reducing power of various extracts at varying concentrations was measured and the results were depicted in Fig. 3. The reducing power of various extracts and reference compound BHA steadily increased with the increasing concentrations up to 1 mg/mL. BHA showed significantly higher reducing power than that of extracts. And there was no significant difference could found between ethyl acetate extract and *n*-butanol extract at concentrations of 0.4 mg/mL and 0.7 mg/mL.

In our anticipation, the reducing power correlated with total phenol contents of samples. It was reported that the reducing power of extracts might be account for their hydrogen-donating ability [28].

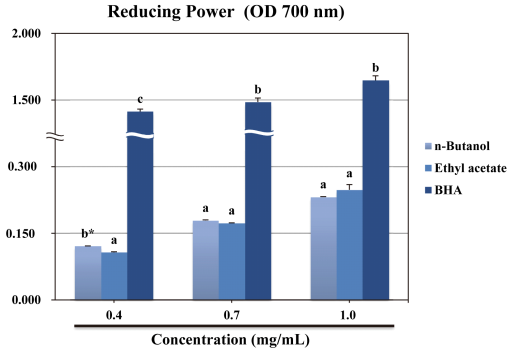

Fig. 3. Reducing power of various extracts from thermal treatment yam (*Dioscorea batatas* Decne.).

*The values are means±standard deviation (n=3). Bars with the different letters are significantly different (p<0.05) by Duncan's multiple range tests.

3.6. Ferric reducing antioxidant power (FRAP)

Antioxidant activities of various fractions from TTY was estimated from their ability to reduce the ferric-tripyridyltriazine ($Fe^{Ⅲ}$-TPTZ) complex to ferrous-tripyridyltriazine ($Fe^{Ⅱ}$-TPTZ) at low pH, forming an intense blue color with an absorption maximum at 593 nm develops [3]. Therefore, we can use the FRAP to evaluate the ability of donating electron for yam. The antioxidant activities through the ferric reducing antioxidant power model system of various extracts at 0.4 to 1.0 mg/mL concentrations compared with BHT were presented in the Fig. 4. The results exhibited concentration-dependent ferric reducing antioxidant activities in all the tested concentrations. Ferric reducing antioxidant power values of n-butanol extract (237.86±4.07 μM $Fe^{Ⅱ}$) and ethyl acetate extract (244.05±9.37 μM $Fe^{Ⅱ}$) were not significantly different at a concentration of 1.0 mg/mL. However, the positive control compound BHT always showed the highest FRAP values at any concentrations compared with all tested extracts. Similar to the results obtained from DPPH scavenging activity and reducing powerassays, correlation between FRAP values and total phenol contents could also be observed from our research. This results were in agreement with Wojdylo et al. [14], who found total phenol content strongly correlated with antioxidant activity evaluated by FRAP assay in herbs.

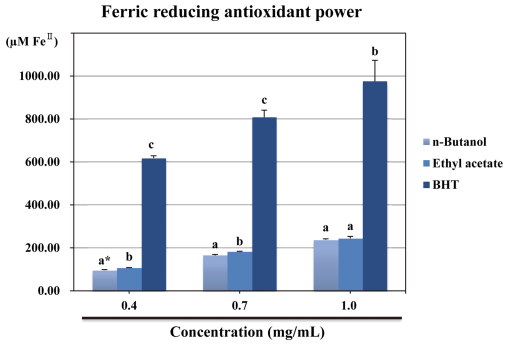

Fig. 4. Ferric reducing antioxidant power of various extracts from thermal treatment yam (*Dioscorea batatas* Decne.).

*The values are means±standard deviation (n=3). Bars with the different letters are significantly different (p<0.05) by Duncan's multiple range tests.

4. Conclusions

The antioxidative activity of thermal treatment yam (*Dioscorea batatas* Decne.) were studied using total phenol content, ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] radical scavenging activity, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, reducing power, and ferric reducing antioxidant power assays.

Various extracts including *n*-butanol and ethyl acetate extracts were prepared. Through these assays, we did not found a significant difference in total phenol content of ethyl acetate extract (51.63±2.51 mg CAE/g) and total phenol content of *n*-butanol extract (53.93±1.00 mg CAE/g). Ethyl acetate extract also exhibited similarly stronger antioxidant activity compared with *n*-butanol extract apart from ABTS radical scavenging activity assay.

References

1. T. Bahorun, A. Luximon-Ramma, A. Crozier, O. I. Aruoma, Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables, *J. Sci. Food Agric.*, **84**, 1553 (2004).
2. M. S. Blois, Antioxidant determinations by the use of a stable free radical, *Nature*, **181**, 1199 (1958).
3. I. F. F. Benzie, J. J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, *Anal. Biochem.*, **239**, 70 (1996).
4. L. Barros, M. J. Ferreira, B. Queirós, Isabel C. F. R. Ferreira, P. Baptista, Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities, *Food Chem.*, **103**, 413 (2007).
5. S. T. Chou, B. H. Chiang, Y. C. Chung, P. C. Chen, C. K. Hsu, Effects of storage temperatures on the antioxidative activity and composition of yam, *Food Chem.*, **98**, 618 (2006).
6. Y. S. Duan, M. A. Kim, J. H. Seong, H. S. Chung, H. S. Kim, Antioxidative activities of various solvent extracts from haw (*Crataegus pinnatifida* Bunge), *Korean J. Food Preserv.*, **21**, 246 (2014).
7. İ. Gülçin, Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid), *Toxicology*, **217**, 213 (2006).
8. G. R. Zhao, Z. J. Xiang, T. X. Ye, Y. J. Yuan, Z. X. Guo, Antioxidant activities of *Salvia miltiorrhiza* and *Panax notoginseng*, *Food Chem.*, **99**, 767 (2006).
9. J. T. Lin, D. J. Yang, Determination of steroidal saponins in different organs of yam (*Dioscorea pseudojaponica* Yamamoto), *Food Chem.*, **108**, 1068 (2008).
10. L. S. Lai, S. T. Chou, W. W. Chao, Studies on the antioxidative activities of hsian-tsao (*Mesona procumbens* Hemsl) leaf gum, *J Agric. Food Chem.*, **49**, 963 (2001).
11. R. J. Robbins, Phenolic acids in foods: an overview of analytical methodology, *J. Agricul. Food Chem.*, **51**, 2866 (2003).
12. V. Roginsky, E. A. Lissi, Review of methods to determine chain-breaking antioxidant activity in food, *Food Chem.*, **92**, 235 (2005).
13. Y. S. Velioglu, G. Mazza, L. Gao, B. D. Oomah, Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products, *J. Agric. Food Chem.*, **46**, 4113 (1998).
14. A. Wojdylo, J. Oszmiański, R. Czemerys, Antioxidant activity and phenolic compounds in 32 selected herbs, *Food Chem.*, **105**, 940 (2007).
15. M. Oktay, İ. Gülçin, Ö. İ. Küfrevioğlu, Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts, *LWT-Food Sci. Technol.*, **36**, 263 (2003).
16. A. A. Adelusi, A. O. Lawanson, Disease-induced changes in carotenoid content of edible yams (*Dioscorea* spp.) infected by *Botryodiplodia theobrornae* and *Aspergillus niger*, *Mycopathologia*, **98**, 49 (1987).
17. T. Agbor-Egbe, S. Treche, Evaluation of the chemical composition of Cameroonian yam germplasm, *J. Food Com. Anal.*, **8**, 274 (1995).

18. L. Barros, M. J. Ferreira, B. Queirós, I. C. F. R. Ferreira, P. Baptista, Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities, *Food Chem.*, **103**, 413 (2007).
19. J. S. Choi, S. H. Park, J. H. Choi, Nitrite scavenging effect by flavonoids and its structure-effect relationship, *Archive. Pharm. Res.*, **12**, 26 (1989).
20. J. Chen, W. M. Da, D. W. Zhang, Q. Liu, J. H. Kang, Water-soluble antioxidants improve the antioxidant and anticancer activity of low concentrations of curcumin in human leukemia cells, *Pharmazie.*, **60**, 57 (2005).
21. R. H. Liu, Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals, *Amer. Soc. Clin. Nutr.*, **78**, 517S (2003).
22. R. Prior, G. H. Cao, Antioxidant phytochemicals in fruits and vegetables: diet and health implications. *Hort Sci.*, **35**, 588 (2000).
23. M. H. Lee, Y. S. Lin, Y. H. Lin, F. L. Hsu, W. C. Hou, The mucilage of yam (*Dioscorea batatas* Decne) tuber exhibited angiotensin converting enzyme inhibitory activities, *Bot. Bull. Acad. Sin.*, **44**, 267 (2003).
24. A. Misaki, T. Ito, T. Harada, Constitutional studies on the mucilage of yamanoimo, *Dioscorea batats* Decen, *forma Tsukune*. Isolation and structure of a mannan, *Agric. Biol. Chem.*, **36**, 761 (1972).
25. W. C. Hou, F. L. Hsu, M. H. Lee, Yam (*Dioscorea batatas*) tuber mucilage exhibited antioxidant activities *in vitro*, *Plan. Med.*, **68**, 1072 (2002).
26. E. M. Choi, S. J. Koo, J. K. Hwang, Immune cell stimulating activity of mucopolysaccharide isolated from yam (*Dioscorea batatas*), *J. Ethno.*, **91**, 1 (2004).
27. L. X. Liu, Y. Sun, T. Laura, X. F. Liang, H. Ye, X. X. Zeng, Determination of polyphenolic content and antioxidant activity of kudingcha made from *Ilex kudingcha* C.J. Tseng, *Food Chem.*, **112**, 35 (2009).
28. I. C. F. R. Ferreira, P. Baptista, M. Vilas-Boas, L. Barros, Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity, *Food Chem.*, **100**, 1511 (2007).