J. of Korean Oil Chemists' Soc.,
Vol. 32, No. 4. December, 2015. 599~606
ISSN 1225-9098 (Print)
ISSN 2288-1069 (Online)
http://dx.doi.org/10.12925/jkocs.2015.32.4.599

Antioxidant Activities of *n*-Butanol and Ethyl Acetate Extracts from Yam (*Dioscorea batatas* DECNE.)

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(Received August 23, 2015; Revised August 28, 2015; Accepted September 1, 2015)

Abstract : This study aimed at evaluating the antioxidant activity of raw yam (*Dioscorea batatas* DECNE.) extracted by *n*-butanol and ethyl acetate. The 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 studied *in vitro*. Results showed ethyl acetate extract (111.88±0.66 mg CAE/g) possessed higher total phenol content than *n*-butanol extract (78.68±0.58 mg CAE/g). Ethyl acetate extract exhibited stronger antioxidant activity compared with *n*-butanol extract apart from reducing power assay.

Keywords : yam (Dioscorea batatas DECNE.), total phenol, antioxidant activity, radical scavenging, reducing power

1. Introduction

An increasingly important health problem in the world is the rising incidence of some diseases such as age-related neurodegenerative diseases, cardiovascular disease and cancer. The overproduction of oxidative radicals such as reactive oxygen species (ROS) or reactive nitrogen species (RNS) results in oxidative stress, which is considered to contribute to these mentioned diseases [17].

Oxygen free radicals or, more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS) are products of normal cellular metabolism [28]. In order to deal with these reactive species, the body is equipped with an effective antioxidant defense system including various enzymes and some low or high molecular weight antioxidants [25]. There is a balance between generation of ROS and antioxidant defense system in organisms. In pathological condition, the overproduction of ROS could lead to lipid peroxidation and oxidative stress. The imbalance between ROS and antioxidant defense mechanisms brings about oxidative

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modification in cellular membrane or intracellular molecules. The excess ROS can damage cellular lipids, proteins or DNA and further cause cancer, aging, atherosclerosis, coronary heart diseases and neurodegenerative diseases [6, 12, 21, 28].

Yam (*Dioscorea batatas* Decne.) is the perennial trailing herb and belongs to the *Dioscoreaceae* family [18]. Yam is mainly composed of starch (75.6-84.3%) with small amounts of crude protein, crude fat, crude fiber and crude ash, whose contents are in the range of 6.7-7.9%, 1.0-1.2%, 1.2-1.8%, 2.8-3.8%, respectively. Due to its component characteristics, yam is usually served as the crucial staple food as well as traditional medicine ingredient to treat asthma, abscesses, chronic diarrhea and ulcers in many parts of world [13, 15]. Previous studies have shown that yam extracts can reduce blood sugar and blood lipid, inhibit microbe activity and show antioxidant activity [7]. Recently, many polysaccharides and polysaccharide protein complexes have been reported to be isolated from various natural sources, such as mushrooms, fungi, algae, lichens and plants [22]. The biological activities of these glycoproteins have attracted increasing attention in the biochemical and medical areas because of their immunomodulatory, anti-oxidative and antitumor activities [24, 8]. Glycoprotein isolated from yam (*Dioscorea batatas* Decne.) not only has been demonstrated to have an antioxidative potential as one of natural antioxidants, but also can serve as a potent anti-inflammatory agent [22, 23].

In the present study, the total phenol contents and antioxidant activities of the *n*-butanol extract and ethyl acetate extract from raw yam were determined and compared *in vitro*.

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 washed, sliced (thickness, 0.4-0.6 cm) and dried in the hot air for 18-24 h at 60-70℃. Then the dried chips were smashed (150-mesh) into raw yam meals (RY, commonly called white 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 [10]. 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 0.7 ± 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, 9]. Samples (1 mL) of yam extraction were mixed with 0.2 mM DPPH (1.5 mL) 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. [11]. 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, which was centrifuged at 3,000 rpm for 10 min. And then the supernatant (1 mL) was mixed with 3 mL of distilled water and 0.3 mL of ferric chloride (0.1%, w/v). BHA was used for the positive control. The absorbance was measured at 700 nm after 10 min of reaction at room temperature.

2.7. Ferric reducing antioxidant power (FRAP) assay

The working FRAP reagent was prepared by mixing 10 mL of 0.3 M sodium acetate buffer (pH 3.6), 1 mL of 10 mM TPTZ in 40 mM hydrochloride acid and 1 mL of 20 mM ferric chloride. The freshly prepared FRAP reagent (1.5 mL) 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 raw yam (RY) by *n*-butanol and ethyl acetate were shown in Table 1. The extraction yield of RY by *n*-butanol exhibited the maximum value of 0.66%, ethyl acetate was found to be 0.43%.

3.2. Total phenol contents (TPC)

Natural polyphenols include catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, rutin, caffeic acid, gallic acid and so on [27]. As the phenolic structure of hydroxyl substituent on the aromatic ring, phenolics can behave as antioxidants [26]. It was reported by Velioglu, Mazza, Gao, and Oomah [29] that there was a positively and highly significant relationship between total phenolics and antioxidant activity. Total phenol contents were 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 RY by different extraction solvents (*n*-butanol and ethyl acetate) were showed in Table 1. The total phenol contents in *n*-butanol and ethyl acetate extracts were determined to be 78.68±0.58 and 111.88±0.66 mg CAE/g extract, respectively. Obviously, ethyl acetate extract possessed significantly higher total phenol content than *n*-butanol extract. These results suggested that solvent type would influence the extraction of phenolics presented in raw yam [19].

3.3. ABTS radical scavenging activity

The radical-cation $ABTS^{\cdot+}$ is produced by the oxidation of ABTS. 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 [27]. Fig. 1 showed the inhibitory effect of various extracts (*n*-butanol and ethyl acetate extracts) on ABTS radical. The ABTS radical scavenging activity was marked and concentration-related (0.4 mg/mL, 0.7 mg/mL and 1.0 mg/mL). Significant scavenging of ABTS radical was evident at all the tested concentrations of various extracts. When at the concentration of 1.0 mg/mL, the ethyl acetate extract of RY displayed the maximum inhibition (82.94 ± 0.52%) among all extracts analyzed in this study, while BHA presented 99.99% within the concentration range. In other words, the antioxidant activities of various extracts against ABTS radical increased

Table 1. Extraction yields, total phenol contents and IC50 values in the antioxidant activity evaluation assays of raw yam (*Dioscorea batatas* Decne.)

	Extracts	
	n-Butanol	Ethyl acetate
Extraction yields (%)	0.66	0.43
Total phenol content (mg GAE/g)	$78.68\pm0.58^{a*}$	111.88 ± 0.66^{b}
ABTS (IC_{50}, mg/mL)	0.70 ± 0.01^{b}	0.45 ± 0.01^{a}
DPPH (IC_{50}, mg/mL)	0.50 ± 0.00^{b}	0.34 ± 0.01^{a}

*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.

in the following order: *n*-butanol extract (IC_{50}=0.70±0.01 mg/mL) and ethyl acetate extract (IC_{50}=0.45±0.01 mg/mL), respectively. In our anticipation, the extracts with higher total phenol content showed higher ABTS radical scavenging activity. There was a positive correlation between scavenging effects of extracts against ABTS radical and their amounts of total phenol contents. And our results were consistent with the finding of Velioglu, Mazza, Gao, and Oomah [29].

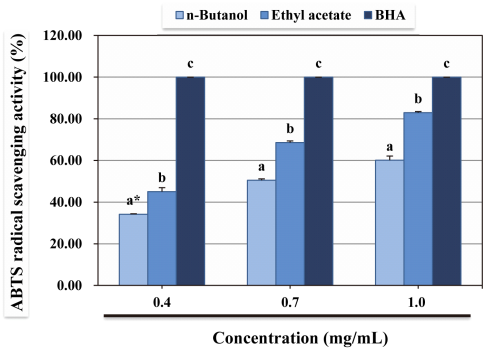

Fig. 1. ABTS radical scavenging activities of various extracts from raw yam (*Dioscorea batatas* Decne.).

*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 [20]. 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 ethyl acetate extract of RY (IC_{50}=0.34±0.00 mg/mL) possessed the higher activity upon the elimination of DPPH radical compared with *n*-butanol extract (IC_{50}=0.50±0.00 mg/mL). Remarkable correlation between total phenol contents and DPPH radical scavenging activities can be found clearly in all extracts, which were in keeping with the results of Hsu et al. [14]. Overall, extracts obtained by different extraction solvents differed in their DPPH radical scavenging activities, demonstrating that extraction solvents could significantly affect the antioxidant activity of the extracts. The strong scavenging capacities of the extracts on DPPH radical were most likely on account of the hydrogen donating ability of the phenolic compounds presented in the extracts.

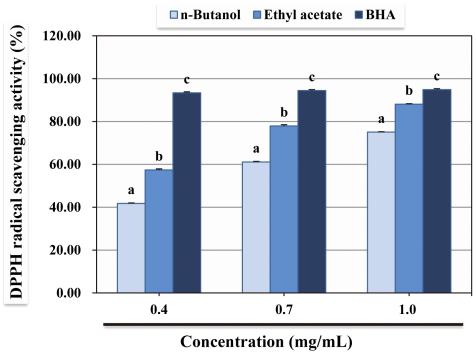

Fig. 2. DPPH radical scavenging activities of various extracts from raw yam (*Dioscorea batatas* Decne.).

*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. At a concentration of 1.0 mg/mL, ethyl acetate extract showed lower reducing power values than *n*-butanol extract. 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 contrast to our anticipation, the reducing power and total phenol content didn't exit a significant correlation. And our results were confirmed by the reports of Bhandari and Kawabata [5] and Kahkonen et al. [16], who found total phenol content had no significant correlation with antioxidant activity. We guessed not only total phenol content but also some nonphenolic compounds might affect the antioxidant activity of yam.

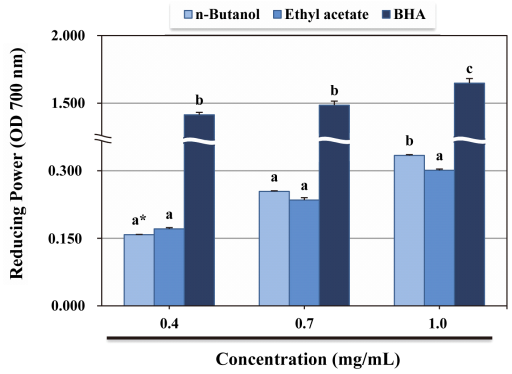

Fig. 3. Reducing power of various extracts from raw yam (*Dioscorea batatas* Decne.).
*The values are means±standard deviation (n=3). Bars with the different letters are significantly different (p<0.05) by Duncan's multiple range tests.

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. The ethyl acetate extract showed significantly higher ferric reducing antioxidant power values than n-butanol extract. Meanwhile, the positive control compound BHT always showed the greatest FRAP values at any concentrations compared with all tested extracts. Similar to the results obtained from DPPH radical and ABTS radical scavenging activity, correlation between FRAP values and total phenol contents could also be observed from our research. This results were in agreement with Wojdylo et al. [30], who found total phenol content strongly correlated with antioxidant activity evaluated by FRAP assay in herbs.

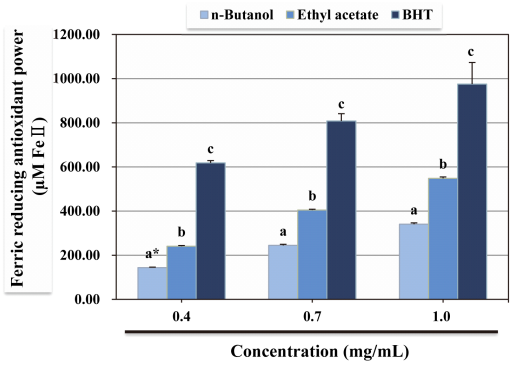

Fig. 4. Ferric reducing antioxidant power of various extracts from raw yam (*Dioscorea batatas* Decne.).
*The values are means±standard deviation (n=3). Bars with the different letters are significantly different (p<0.05) by Duncan's multiple range tests.

4. Conclusions

n-Butanol and ethyl acetate extracts obtained from raw yam (*Dioscorea batatas* Decne.) were analyzed their antioxidant activities. These *in vitro* systems 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.

Results showed ethyl acetate extract (111.88±0.66 mg CAE/g) possessed higher total phenol content than *n*-butanol extract (78.68±0.58 mg CAE/g). In our anticipation, ethyl acetate extract exhibited stronger antioxidant activity compared with *n*-butanol extract apart from reducing power assay.

Acknowledgement

This work was supported by a 2-Year Research Grant of Pusan National University.

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