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Investigation of *n*-Butanol and Ethyl Acetate Extracts from Thermal Treatment Yam (*Dioscorea batatas* DECNE.) for their Antioxidant Activities

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Abstract : *n*-Butanol and ethyl acetate extracts of thermal treatment yam (*Dioscorea batatas* DECNE.) belonging to the family *Dioscoreaceae* were measured for their radical scavenging activity and lipid peroxidation inhibition ability. In this study, ethyl acetate extract showed the most potent antioxidant activity evaluated by ferrous ion chelating activity and NO radical scavenging activity. Nevertheless, *n*-butanol extract was more effective in inhibiting linoleic acid peroxidation. A significant difference between *n*-butanol extract and ethyl acetate extract in nitrite scavenging activity β-carotene bleaching assays could not be found. Also, the results of this study showed that thermal treatment yam could be used as easily accessible source of natural antioxidants and as a possible food supplement.

Keywords : thermal treatment yam (Dioscorea batatas DECNE.), nitrite, NO radical, metal chelating, lipid peroxidation inhibition

1. Introduction

Yam (*Dioscorea batatas* DECNE.) belongs to the *Dioscoreaceae* family and usually serves as the crucial staple food as well as traditional medicine ingredient in many parts of world [10,18]. 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 [19,25].

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There are several previous studies have conducted that yam is rich in functional compounds such as steroidal saponin, mucilage and so on. 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 [3,20]. 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 [11]. Steroidal saponins, furostanol and spirostanol glycosides are the remarkable

functional compounds in yams [21]. For the past few years, some relevant biological studies on the steroid saponins of *Dioscorea* species described in the literature are related to cytotoxic (anti-tumor) and antifungal properties that will be used as drug formulas to treat some diseases. And other biological functions about active steroidal saponins from *Dioscorea* species are summarized in the follows: immunomodulating, antimicrobial, hormonal, anti-osteoporotic, anti-inflammatory, and anti-allergic activities [26].

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. Reactive oxygen species 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 and usually generated by oxidation product of biological reactions or exogenous factors [24]. The overproduction of ROS can damage cellular lipids, proteins or DNA and further cause cancer, aging, atherosclerosis, coronary heart diseases and neurodegenerative diseases [13,23,29].

In this study, we evaluated the possible antioxidant effects of *n*-butanol and ethyl acetate extracts from thermal treatment yam in different *in vitro* methods including ferrous ion chelating activity, NO radical scavenging activity, nitrite scavenging activity, β-carotene bleaching assay and lipid peroxidation inhibition.

2. Materials and Methods

2.1. Materials

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 various extraction yields of TTY by *n*-butanol and ethyl acetate were 0.38% and 0.25%, respectively. The yam extracts were collected and sealed in brown reagent bottles and frozen at -80℃ until required for further analyses.

2.3. Ferrous ion chelating activity determination

The chelating of ferrous ion by yam extracts was estimated by the method of Hsu et al. [12]. 1 mL of yam extract at different concentrations, 0.05 mL of 2 mM $FeCl_2 \cdot 4H_2O$, 0.1 mL of 5 mM ferrozine and 3 mL of ethanol were mixed. After 10 min of incubation at room temperature, the absorbance of Fe^{2+}-ferrozine complex was measured at 562 nm. The chelating activity of yam extract for Fe^{2+} was calculated as follows:

Ferrous ion chelating activity (%)

$$= (1 - \frac{A_s}{A_c}) \times 100$$

Where A_s and A_c are the absorbance of sample and control.

2.4. NO radical scavenging activity determination

Nitric oxide scavenging activity was measured by the method of Duan et al. and

Sahoo et al. [4,28]. Nitric oxide was generated from sodium nitroferricyanide dihydrate and measured by the Griess reagent. 2 mL of sodium nitroferricyanide dihydrate (10 mmol/L) in 0.2 M PBS (phosphate buffered saline, pH 7.4) was mixed with 3 mL of different concentrations of extract and incubated at 25℃ for 150 min. After incubation period, 1mL of sulfanilamide (1% sulfanilamide in 2% H_3PO_4) was added to the 1 mL of reaction mixture. After 10 min of incubation, 1 mL of 0.1% N-(1-Naphthyl) ethylenediamine dihydrochloride was added, vortexed and incubated for 30 min at 25℃. The absorbance of the chromophore formed was read at 540 nm. In this assay, trolox was used as positive control compound. The scavenging activity was calculated using the following formula:

NO radical scavenging activity (%)

$$= (1-\frac{A_s}{A_c}) \times 100$$

Where A_c is the absorbance of the control (without sample extract), and A_s is the absorbance in the presence of sample extract.

2.5. Nitrite scavenging activity assay

The nitrite scavenging activity was determined according to a method using Griess reagent [1]. 2 mL of each sample solution was added to 1 mL of 1 mM $NaNO_2$ and mixed. Then the mixture was mixed with 6 mL of 0.2 M citrate buffer (pH 2.5). The reaction mixture was incubated in a water bath at 37 ℃ for 60 min. 3 mL of 2% acetic acid and 0.4 mL of Griess reagent (1% sulfanilic acid in 30% acetic acid:1% 1-Naphthylamine in 30% acetic acid, 1:1, v/v) were added to the 1 mL of reaction solution and then incubated at room temperature for 15 min. The absorbance of reaction solution was measured at 520 nm and nitrite scavenging activity was calculated by the following formula:

Nitrite scavenging activity (%)

$$= (1-\frac{A-B}{C}) \times 100$$

where A is the absorbance of the treated yam extract, B is the absorbance of the prepared sample solution, and C is the absorbance of 1 mM $NaNO_2$.

2.6. β-carotene bleaching assay

The antioxidant activity of different extract was evaluated according to the β-carotene bleaching method [6,16]. In brief, a solution of β-carotene was prepared by dissolving 1 mg of β-carotene in 10 mL of chloroform. One milliliter of this solution was then added to a round-bottomed flask containing a mixture of 20 mg linoleic acid and 200 mg Tween 40. After the chloroform was removed under vacuum using a rotary evaporator at 40℃, 100 mL of aerated distilled water were added to the flask with vigorous shaking. The emulsion obtained was freshly prepared before experiment. An aliquot (4.0 mL) of the β-carotene - linoleic acid emulsion was mixed with 0.4 mL of sample extracts, positive control standards (BHA). Then the mixture was incubated at 50℃ for 120 min. Absorbance readings were performed immediately (t=0 min) and after 120 min of incubation at 470 nm with. Antioxidant activity (AOA) was calculated using the following formula:

$$\text{AOA} = (1-\frac{A_0 - A_{120}}{A'_0 - A'_{120}}) \times 100$$

A_0 and A'_0 are the initial absorbance of sample and control, whereas A_{120} and A'_{120} are the absorbance of sample and control after 120 min.

2.7. Lipid peroxidation inhibition determination

The lipid peroxidation inhibition activity of the yam extracts was measured in a linoleic acid emulsion system according to the method of Je et al. [14]. Briefly, 1 mL of sample solution was added to a reaction mixture of in a screw cap vial. Each reaction mixture

consisted of 2 mL of 2.51% linoleic acid in ethanol and 10 mL of phosphate buffer (pH 7.0). Then the total volume was adjusted to 20 mL with distilled water. The mixture was incubated at 40℃ in the dark, and the degree of oxidation was evaluated by measuring the ferric thiocyanate (FTC) method. The mixture solution (100 μL) was mixed with 3.7 mL of 75% ethanol, 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 0.02 M of ferrous chloride solution in 3.5% HCl. After 3 min, the thiocyanate value was measured by reading the absorbance at 500 nm following color development with $FeCl_2$ and thiocyanate at different intervals during the incubation period at 40℃. The inhibition activity can be expressed by the following equation

Lipid peroxidation inhibition activity (%)

$$= (1-\frac{A_s}{A_c}) \times 100$$

Where A_s and A_c are the absorbance of sample and control.

2.8. Statistical analysis

The experimental data in triplicate were subjected to analysis of variance (ANOVA) and expressed as mean±SD (n=3). ANOVA was 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. Ferrous ion chelating activity

The ferrous state of iron is known as the most important lipid oxidation pro-oxidant, which can accelerate lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals via the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\bullet$). As ferrozine can quantitatively form complexes with Fe^{2+}, the metal chelating activity of sample was measured by a decrease in the red color of the ferrous-ferrozine complex [8]. Fig. 1 showed the chelating activity of various extracts (n-butanol and ethyl acetate extracts) on ferrous ion was marked and concentration related (0.4 mg/mL, 0.7 mg/mL and 1.0mg/mL). At a concentration of 1.0 mg/mL, ethyl acetate extract (60.86±0.83%) chelated more iron than n-butanol extract (43.13±0.18%), although both extracts were less efficient than commercial chelator EDTA (99.81±0.00%). IC_{50} values of n-butanol and ethyl acetate extracts were calculated to be 1.11±0.02 and 0.81±0.01 mg/mL, respectively (Table 1). Metal chelating capacity was significant because they reduced the concentration of the catalysing transition metal in lipid peroxidation [5]. It was reported that chelating agents, which form σ-bonds with a metal, are effective as secondary antioxidants science they reduce the redox potential thereby stabilising the oxidised form of the metal ion [9]. The data obtained from Fig. 2 revealed that the all extracts exhibited an effective

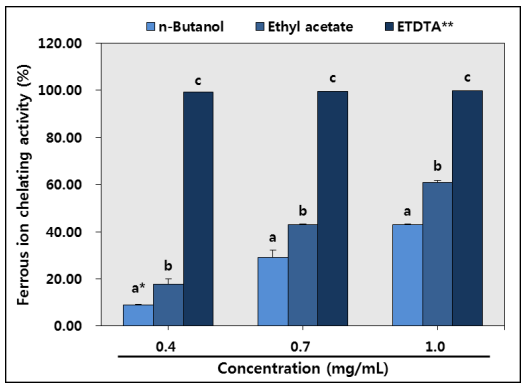

Fig. 1. Ferrous ion chelating activity of various extracts from thermal treatment yam (*Dioscorea batatas* Decne.)

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

**EDTA: ethylenediaminetetraacetic acid disodium salt dihydrate.

capacity for iron binding, suggesting that its action as peroxidation protector may be related to its iron binding capacity.

Table 1. IC_{50} values of various extracts from thermal treatment yam (*Dioscorea batatas* Decne.) in different antioxidant activity assays

IC_{50} (mg/mL)	Extracts	
	n-Butanol	Ethyl acatate
FICA*	.11±0.02^{b}**	0.81±0.01^{a}
NOSA	0.73±0.01^{b}	0.45±0.02^{a}
NO_2SA	3.78±1.24^{a}	3.87±0.4^{a}
βBM	0.11±0.01^{a}	0.11±0.00^{a}
LPI	0.02±0.00^{a}	0.05±0.01^{b}

*Ferrous ion chelating activity (FICA), nitric oxide radical scavenging activity (NOSA), nitrite scavenging activity (NO_2 SA), β-carotene bleaching assay (βBA) and lipid peroxidation inhibition (LPI).

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

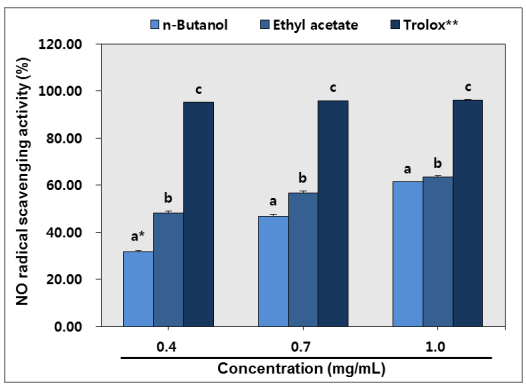

Fig. 2. NO radical scavenging activity of various extracts from thermal treatment yam (*Dioscorea batatas* Decne.).

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

**Trolox: (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2carboxylic acid.

3.2. NO radical scavenging activity

Nitrite oxide (NO) is very reactive which implicated in inflammation, cancer and other pathological conditions. NO interacts with oxygen to produce nitric ions that can be estimated by using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide [28]. NO radical scavenging activities of various fractions obtained from RY and TTY by using different extraction solvents were presented in Fig. 2 and Table 1. NO radical scavenging activity increased with the increasing concentrations. Similar to the results obtained for the ferrous ion chelating activity assay, ethyl acetate extract (IC_{50}=0.45±0.03 mg/mL) exhibited stronger scavenging activity than *n*-butanol extract (IC_{50}=0.73±0.01 mg/mL). But they all significantly lower than that of positive compound trolox at any concentrations. Yam extracts might possess the property to counteract the effect of NO formation and in turn might be of considerable interest in preventing the ill effects of excessive NO generation in the human body. And the scavenging activity was likely to contribute to retard the chain of reactions initiated by excess generation of NO that were detrimental to the human health [17].

3.3. Nitrite scavenging activity

Nitrite is toxic and is usually presented in large quantities in meat, leafy and root vegetables. The excessive consumption of nitrite could lead to oxidization of hemoglobin, which can arouse methemoglobinemia [2]. In the present study, we investigate the effect of different extracts from RY on nitrite scavenging activity varied from 0.4 mg/mL to 1.0 mg/mL at pH 2.5. Fig. 3 revealed all extracts exhibited a concentration-dependent anti-radical activity by inhibiting nitrite. The nitrite scavenging activities of extracts increased with the increasing concentration, but lower than those of the positive control compounds ascorbic acid at the same

concentration. In addition, *n*-butanol extract and ethyl acetate extract achieved 24.30±1.78% and 22.33±1.31% inhibition at a concentration of 1.0 mg/mL. Lee et al. [22] reported that the maximum scavenging percentage of aqueous extract from yam on nitrite was about 29.20% at 0.2 mg/mL. However, a significant difference between *n*-butanol extract (IC_{50}=3.78±1.24 mg/mL) and ethyl acetate extract (IC_{50}=3.87±0.40 mg/mL) was not found. According to the results in the present study, it is suggested that various extracts had a potency to scavenge nitrite.

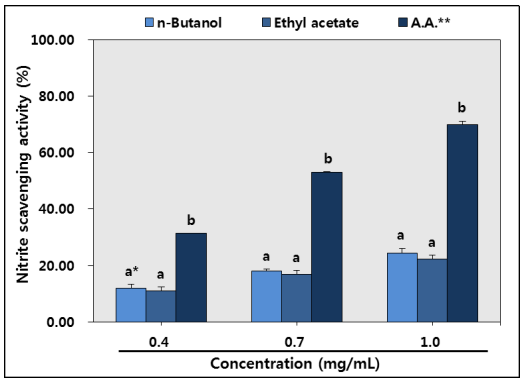

Fig. 3. Nitrite scavenging activity of various extracts from thermal treatment yam (*Dioscorea batatas* Decne.).
*The values are means±SD (*n*=3). Bars with the different letters are significantly different ($p<0.05$) by Duncan's multiple range tests.

3.4. β-carotene bleaching assay

The antioxidant activities of various extracts at 0.4 mg/mL to 1.0 mg/mL concentrations were compared with BHA measured by the bleaching of β-carotene were presented in Fig. 4. The highly unsaturated β-carotene molecules in this system can be attacked by free radicals generating from the oxidation of linoleic acid, and as a consequence, the characteristic orange color disappears. The presence of antioxidant can avoid the destruction of the β-carotene by neutralizing the free radicals formed in the system to keep the orange color [6]. As depicted in Fig. 4, the results exhibited concentration-dependent antioxidant activity by β-carotene bleaching method in all the tested concentrations of various extracts. In this assay, there was no significant difference between *n*-butanol extract (IC_{50}=0.11±0.01 mg/mL) and ethyl acetate extract (IC_{50}=0.11±0.00 mg/mL). BHA could significantly inhibit lipid peroxidation in linoleic acid emulsion system and the activity was higher than extracts. It was probable that the antioxidative components in extracts can reduce the extent of β-carotene destruction by neutralizing the linoleate free radical and other free radicals in this system. And our results were in accordance with Farombi, Britton and Emerole [7], who also found the yam showed antioxidant activity by using β-carotene bleaching method.

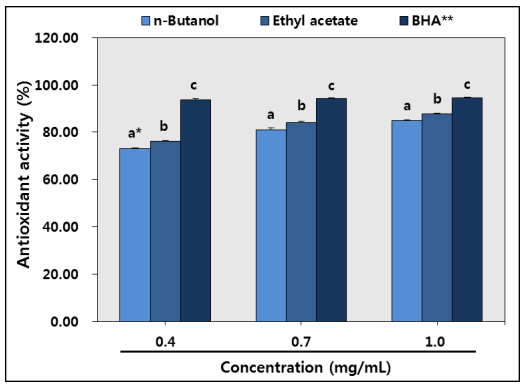

Fig. 4. Antioxidant activity of various extracts from thermal treatment yam (*Dioscorea batatas* Decne.) by using β-carotene bleaching method.
*The values are means±SD (*n*=3). Bars with the different letters are significantly different ($p<0.05$) by Duncan's multiple range tests.
**BHA: butylated hydroxyanisole.

3.5. Lipid peroxidation inhibition

Linoleic acid is a polyunsaturated fatty acid, which is vulnerable to attack by reactive oxygen species. As a result, lipid peroxides

such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) are generated. In this model system, these peroxides can oxidize Fe^{2+} to Fe^{3+}, then forms complexes with thiocyanate ion which have maximum of absorption at 500 nm [27]. Fig. 5 described the inhibition of linoleic acid peroxidation by various extracts from TTY. All extracts effectively inhibited the linoleic peroxidation in a concentration-dependent manner. BHA significantly inhibited lipid peroxidation in linoleic acid emulsion system and the activity was higher than that of TTY extracts. IC_{50} value of *n*-butanol extract was found to be 0.02±0.00 mg/mL, which was more effective in inhibition of linoleic acid peroxidation than ethyl acetate extract (IC_{50}=0.05±0.01 mg/mL). These extracts from TTY exhibited stronger antioxidant activity than the reported value in EtOAc extract (IC_{50}=423.1 μg/mL) from yam by Kwon et al. [15]. Moreover, these results implied those antioxidants from TTY were probable to be effective as chain breaking molecules.

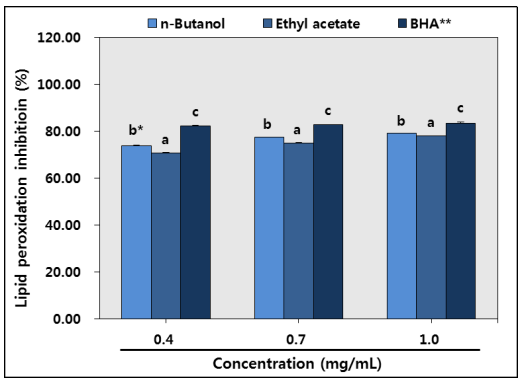

Fig. 5. Antioxidant activity of various extracts from thermal treatment yam (*Dioscorea batatas* Decne.) determined as inhibition of linoleic acid oxidation.
*The values are means±SD (*n*=3). Bars with the different letters are significantly different ($p<0.05$) by Duncan's multiple range tests.
**BHA: butylated hydroxyanisole.

4. Conclusions

In conclusion, *n*-butanol and ethyl acetate extracts of thermal treatment yam exhibited potent antioxidant activity. Various extracts might provide different antioxidants, which in general demonstrated strong activities within *n*-butanol and ethyl acetate fractions. As can be seen from the results, ethyl acetate extract was most effective in chelating ferrous ion and scavenging NO radical. Nevertheless, *n*-butanol extract and ethyl acetate extract showed similar antioxidant activity in nitrite scavenging activity and β-carotene bleaching assays. It is also suggested that the thermal treatment yam might be viewed as a potential source of natural antioxidants which can provide precious functional ingredients useful for the prevention of diseases related to oxidative stress.

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