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감자(*Solanum tuberlosum*) 에탄올 추출물의 항산화 활성

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Antioxidant Activity of Ethanolic Extract of Potato(*Solanum tuberlosum*)

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Abstract

감자는 전세계적으로 섭취되는 주요 작물로서 우리나라에서는 감자의 생즙을 관절염 및 통증을 억제하는 민간요법으로 사용되어 왔다. 본 연구에서는 감자 에탄올 추출물의 항산화 작용을 검토하였다. 감자 에탄올 추출물(0.05~5 mg/mL)은 1,1-diphenyl-2-picrylhydrazyl radical에 대하여 강한 free radical scavenger 활성을 나타내었다. 또한 감자 추출물(0.5 mg/mL)은 쥐간 균질액에서 생성된 TBARS의 양을 유의적으로 억제하였으며 ($p<0.05$) Fe^{2+}/ascorbate induction system에서 감자 추출물(0.5~2 mg/mL)은 DNPH 반응으로 측정된 carbonyl 형성을 억제하였다. 항산화 활성과 마찬가지로 감자 추출물의 환원력은 0.5 mg/mL 농도에서 가장 높았고 이는 *a*-tocopherol의 경우와 비슷하였다. 이 결과는 감자 추출물이 항산화력을 통해 생체에서 작용할 수 있는 가능성을 제시한다.

Key words: *Solanum tuberosum*, scavenging effect, antioxidant activity.

Introduction

Oxidation in living organisms is essential for the acquirement of energy to precede biological processes. However, oxygen-centered free radicals and other reactive oxygen species(ROS) that are continuously produced *in vivo* result in cell death and tissue damage. ROS can easily initiate the peroxidation of the membrane lipids, leading to the accumulation of lipid peroxides, which may be related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis(Halliwell & Gutteridge 1999). Although most living species have an efficient defense system to protect themselves against the oxidative stress induced by ROS, these systems are insufficient to entirely prevent the damage (Simic 1988). Antioxidants can interfere with the oxidation process by reacting with free radicals and chelating free catalytic metals. Hence, antioxidant supplements or foods containing antioxidants may be used to help human body reduce oxidative damage. The antioxidants are also used to maintain food quality from oxidative deterioration of lipid and play a very important role in the food industry. Although many synthetic chemicals such as phenolic compounds are found to be strong radical scavengers and widely used in the food industry, they usually have side effects. Synthetic antioxidants, such as butylated hydroxytoluene(BHT) and butylated hydroxyanisole (BHA) have suspected of being responsible for liver damage and carcinogenesis(Grice 1986, Wichi 1988). Therefore, there is a preference for natural food and food ingredients that believed to be safer, healthier, and less subject to contamination than their artificial counterparts and the development and utilization of more effective antioxidants of natural origin are desired.

The aim of the present study was to investigate the antioxidative activity of potato ethanol extract in order to evaluate its medicinal value and to point out an easily accessible source of natural antioxidants that could be used as a possible food supplement or in the pharmaceutical industry.

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Materials and Methods

1. Preparation of Extract

Potatoes were purchased from a market in Seoul, Korea. The potatoes were peeled off to a depth of ~1mm with a vegetable peeler. The fresh blended potato tubers were immediately extracted in EtOH three times and the extract was filtered(110 mm) and concentrated with a rotary evaporator at temperature below 50℃ and then freeze dried (yield: 2.2% w/w).

2. Scavenging Effect on DPPH Radical

The effect on DPPH(1,1-diphenyl-2-picrylhydrazyl) radical was estimated according to the method of Shimada et al. (1992). Samples(0~1 mg/mL) in 4 mL of methanol were added to a solution of DPPH(10 mM, 1 mL) in methanol. The mixture was shaken and left to stand at room temperature for 10 min; the absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The percentage of scavenging effect (%) was calculated as follow: scavenging effect %(capacity to scavenge the DPPH radical) = (1 - absorbance of sample/absorbance of control)×100. α-Tocopherol, was used as a positive control.

3. Scavenging Effect on Superoxide Radical

Inhibition of superoxide anion radical generation was estimated by the xanthine-xanthine oxidase(XOD) system (Fukuda & Nakata 1999). The sample solution(0.1 mL) was added to the mixture(1 mL), consisting of 0.4 mmol/L xanthine and 0.24 mmol/L NBT(nitro blue tetrazolium) in phosphate buffer(pH 8.0). Xanthine oxidase(from butter milk, 0.049 unit/mL) (1.0 mL) diluted in 0.1 mol/L phosphate buffer(pH 8.0) was added, followed by incubation in a shaking water bath at 37℃ for 20 min, and then the coloration of NBT was measured at 560 nm. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity.

4. Scavenging Effect on H_2O_2

H_2O_2 was measured by the formation of a brown color (recorded at 436 nm) in reaction mixtures containing, in a final volume of 1 mL, 0.15 M KH_2PO_4/KOH buffer, pH 7.4, 50 μL of guaiacol solution(100 μL of pure liquid in 50 mL of water) and 10 μL of type IV horseradish peroxidase (5 g/L in the same phosphate buffer). The rate of absorbance change at 436 nm is proportional to the concentration of H_2O_2 added. Samples to be tested for their reaction with H_2O_2 were incubated with 10 mM H_2O_2 for 30 min at 37℃ and assayed for remaining H_2O_2 by using the peroxidase system (Aruoma et al. 1988).

5. Inhibition of Lipid Peroxides in the Mouse Liver Homogenate

Inhibition of lipid peroxides was estimated by quantitating the amount of TBARS formed from liver homogenate in a Fe^{2+}/ascorbate free-radical-induction system(Bishayee and Balasubramanian 1971) in the presence and absence of various concentrations of the extract(mg/mL of the reaction mixture). The reaction mixture contained 0.1 mL of 25% rat liver homogenate(w/v) prepared in 40 mM tris-HCl buffer (pH 7.0), 30 mM KCl, 0.16 mM ferrous iron, and 0.06 mM ascorbic acid in a final volume of 0.5 mL. The reaction was initiated with the addition of Fe^{2+}. The mixture was incubated for 1h at 37℃. The reaction mixture contained 0.1 mL of 25% rat liver homogenate(w/v) prepared in 40 mM tris-HCl buffer(pH 7.0), 30 mM KCl, 0.16 mM ferrous iron, and 0.06 mM ascorbic acid in a final volume of 0.5 mL. The reaction was initiated with the addition of Fe^{2+}. The mixture was incubated for 1h at 37℃. The amount of lipid peroxides formed was determined by the TBA reaction method (Ohkawa et al. 1979). Briefly, the reaction was carried out using 0.4 mL of the above reaction mixture treated with 0.2 mL of 8.1% SDS and 3 mL of TBA reagent(equal volumes of 0.8% TBA and 20% acetic acid pH 3.5). Total volume was made up to 4 mL with distilled water and kept at 95℃ for 1h in a water bath. Color was extracted with n-butanol and pyridine (15:1 v/v) and the absorbance was measured. The absorbance was measured at 530 nm. Possible interference due to the extract with TBA reactions was ruled out by control experiments by the addition of the extract after incubation. Percent inhibition of lipid peroxidation was calculated by comparing the absorbance values of the experimental sample with that of the control sample, which was not treated with the test material.

6. Inhibition of Protein Oxidation

The reaction was carried out according to Gassen et al. (1996). 1 mL of the reaction mixture contained liver homogenate with protein concentration of 1 mg incubated at 37℃ for 1 h in the presence of 15 mM ascorbic acid and 250 μM $FeSO_4$ in 100 mM Tris-HCl (pH 7.4). The reaction was

initiated by the addition of Fe^{2+}. Liver homogenate was prepared as described by Cao and Cutler(1995). The assay was performed immediately, or the extracted protein was stored in liquid nitrogen until used. In our assay, we used a homogenate with the ratio of A280 nm/A260 nm higher than or equal to 1.1. The amount of protein carbonyls formed in the presence and absence of various concentrations of date fruit extract(mg/mL of the reaction mixture) was measured using 2,4-dinitrophenylhydrazine(Levine, 1990). The values were calculated as nmol carbonyl/mg protein, and percentage inhibition was calculated by comparing both the experimental and control(without extract) and expressed as percent control.

7. Determination of the Reducing Power

Reducing power: The reducing power of samples was determined according to the method of Oyaizu(1986). Sample in 1 mL methanol was mixed with phosphate buffer(5 mL, 0.2 M, pH 6.6) and potassium ferricyanide(5 mL, 1%) and the mixture was incubated at 50℃ for 20 min. Five milliliters of trichloroacetic acid(10%) were added to the reaction mixture, which was then centrifuged at 3000×g for 10 min. The upper layer of the solution(5 mL) was mixed with distilled water(5 mL) and ferric chloride(1 mL, 1%) and the absorbance was measured at 700 nm. Increased absorbance indicated increased reducing power.

Phosphomolybdenum method: The reducing power of sample having antioxidant capacity was also evaluated by the method of Prieto et al (1999). An aliquot of 0.1 mL of sample solution was combined with 1 mL phosphate and 4 mM ammonium molybdate. The tubes were capped and incubated in a boiling water bath at 95℃ for 90 min. After the samples had been cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm.

8. Determination of Total Polyphenols

The total phenolic content was determined with Folin-Ciocalteu reagent according to the method of Julkunen-Tiitto (1985), using tannic acid as a standard. The result was expressed as milligrammes of tannic acid equivalent per gramme of the sample.

9. Statistical Analysis

All experiments were performed three or four times and the results were expressed as mean±SD. Statistical significance was determined by analysis of variance and subsequent Duncan's multiple range test ($P<0.05$). The analysis was performed using SAS statistical software.

Results and Discussion

Antioxidants inhibit lipid(and protein) oxidation, either in the propagation phase(chain-braking mechanism) or by protecting the oxidation substrates against the first formed radicals in the initiation phase. Accordingly, evaluation of plant material for antioxiadtive activity should not depend only on a single method, but it should include measurement of reactions characteristic of both the initiation and the propagation phase(Schwarz et al 2001). In the present study, the antioxidative activities of the potato extract and its fractions were examined by several model systems.

1. Radical Scavenging Effect

The radical scavenging activity, using a DPPH radical, was tested with potato extract, along with *a*-tocopherol. As shown in Fig. 1, potato extract showed a high scavenging capacity for DPPH at 0.5 mg/mL. These results reveal that potato extract contain powerful inhibitor compounds, which may act as primary antioxidants that react with free radicals. Fig. 2 and 3 show the superoxide and H_2O_2 radical scavenging activity of the potato extract (0.05~5 mg/mL). Potato extract

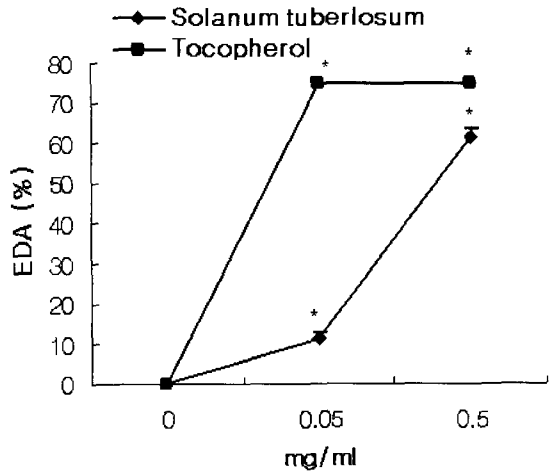

Fig. 1. **Effect of potato extract on the DPPH bleaching.** Each value is the mean±S.D. % of DPPH (1,1- diphenyl-2-picrylhydrazyl radical) bleaching = (1-absorbance of sample / absorbance of control)×100.

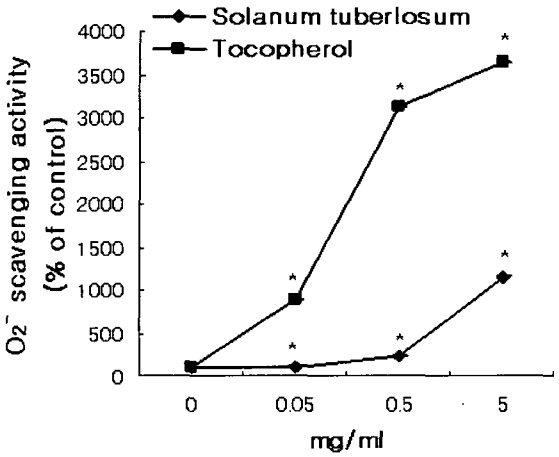

Fig. 2. **Scavenging effect of potato extract on superoxide radical.** Results are mean ± S.D. (n=7). * $p<0.05$ vs. control.

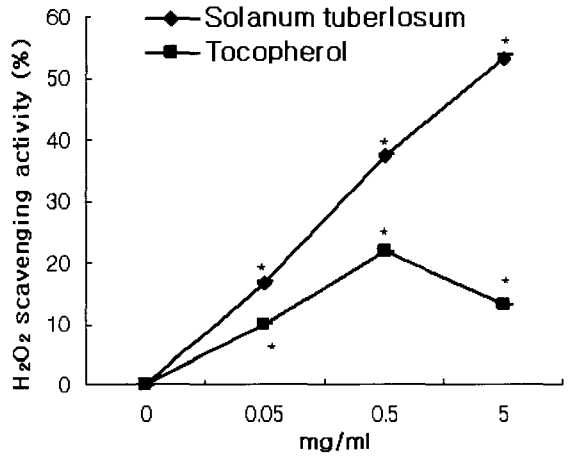

Fig. 3. **Scavenging effect of potato extract on hydroperoxide.** Results are mean ± S.D. (n=7). * $p<0.05$ vs. control.

appeared to have little scavenging activity on superoxide and H_2O_2 radicals in comparison with same dose of *a*-tocopherol.

There is increasing evidence that increased production of reactive oxygen species are involved in various disorders, and also are responsible for cellular damage (Johnson et al 1996). The peroxyl radicals is a key step in lipid peroxidation (Halliwell & Gutteridge 1990) and scavenging of non-lipid radicals is of important for protection against early events in oxidative damage, i.e. formation of first radicals by metal catalysis or light exposure(Skibsted 2000). Our experiments show that potato extract is a good scavenger of DPPH, O_2^-, and H_2O_2 *in vitro*, which results demonstrate that potato extract contains scavengers that may contribute to decompose peroxyls produced during inflammatory states(Dean et al 1993); hence potato extract consumption may afford cytoprotective effects. Of course, for potato extract to function as an antioxidant *in vivo* would require its presence, at sufficient concentration, at sites of oxidant generation. Whether this occurs remains to be established.

2. Inhibition of Lipid Peroxides

Virtually all cellular components appear to be sensitive to oxidative damage. Lipids, proteins, nucleic acids, and carbohydrates are all known to undergo oxidative modification (Pacifici & Davies 1991). Lipid peroxidation was the first type of oxidative damage to be studied in detail. Membrane phospholipids are continually subjected to oxidant challenges. During the oxidation process, peroxides are gradually decomposed to lower molecular weight compounds. One such compound is malonaldehyde, which is measured by the TBA method. The process of lipid peroxidation is initiated by the abstraction of a hydrogen atom in an unsaturated fatty acyl chain and propagated as a chain reaction(Halliwell & Gutteridge 1989). Therefore, inhibition of lipid peroxidation is of great importance in the disease processes involving free radicals. The production of lipid peroxides by ferrous/ascorbate systems in liver homogenates was inhibited by the extract of date fruit in a dose-dependent manner. A wide range of concentrations from 0.5 mg/mL to 4.0 mg/mL was used to assess the ability of the extract to inhibit lipid peroxide formation(Fig. 4). A concentration of 0.5 mg/mL or

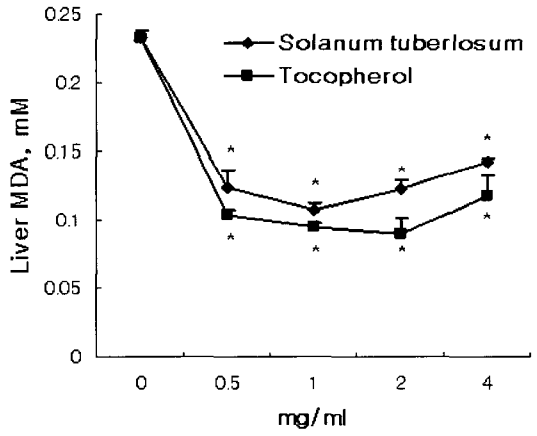

Fig. 4. **Antioxidant activity of potato extract in the linoleic acid peroxidation system.** The values obtained without antioxidants were used for 100% lipid peroxidation: (A) ferric thiocyanate method; (B) TBA method. Results are mean ± S.D. (n=7). * $p<0.05$ vs. control.

more was found to inhibit the lipid peroxide completely in this method. Part of the antioxidative activity may be due to flavonoids. In addition, antioxidative activities observed in potato extract could be the synergistic effect of more than two compounds that may present in the potato. It has been reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidative activities that creates an effective defense system against free radical attack(Lu & Foo 1995). Further studies on the identification and purification of components responsible for the antioxidative activities in different fractions of potato extract are now in progress.

3. Inhibition of Protein Oxidation

Free-radical-mediated oxidation of some amino acid residues of proteins such as lysine, arginine, and proline leads to the formation of carbonyl derivatives. Other oxidative mechanisms are also involved in the formation of carbonyl derivatives such as glycation and glycoxidation reactions (Dean et al 1997). In any case, the presence of carbonyl group has become a widely accepted measure of oxidative damage of proteins under conditions of oxidative stress, which react with DNPH to form stable hydrazone derivatives (Stadtman & Levine 2000). Fig. 5 shows that an extract of potato(0.5~2 mg/mL) could inhibit protein carbonyl formation in a dose-dependent manner. Generally, protein oxidation is less sensitive to antioxidants(Stadtman & Levine 2000). Protein oxidation takes place by binding metals to proteins in specific sites, and amino acid residues in the neighborhood of the metal-binding site are oxidized. Therefore, only a few compounds are available those are capable of inhibiting protein oxidation(Butterfield et al 1997). The present study, thus, demonstrates for the first time that a potato is able to inhibit protein oxidation *in vitro*.

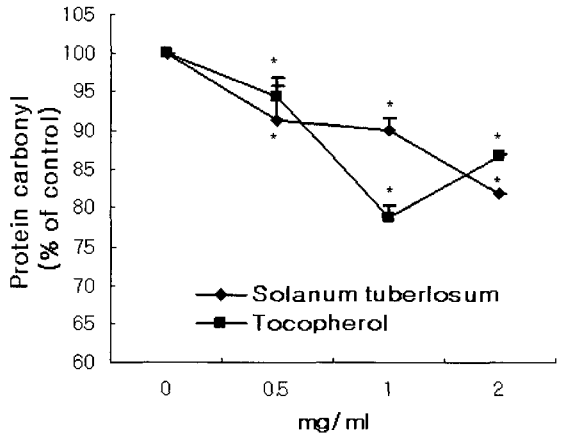

Fig. 5. **Inhibition of lipid peroxides and protein oxidation in the liver homogenate.** Results are mean ± S.D. (n=7). * $p<0.05$ vs. control.

4. Reducing Power

Fig. 6 shows the reductive capabilities of potato extract compared with *a*-tocopherol. For the measurements of the reductive activity, we investigated the Fe^{3+}~Fe^{2+} transformation in the presence of potato extract using the method of Oyaizu (1986). Potato extract showed high reducing power. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant components and the formation of a green Mo(V) complex with a maximal absorp-

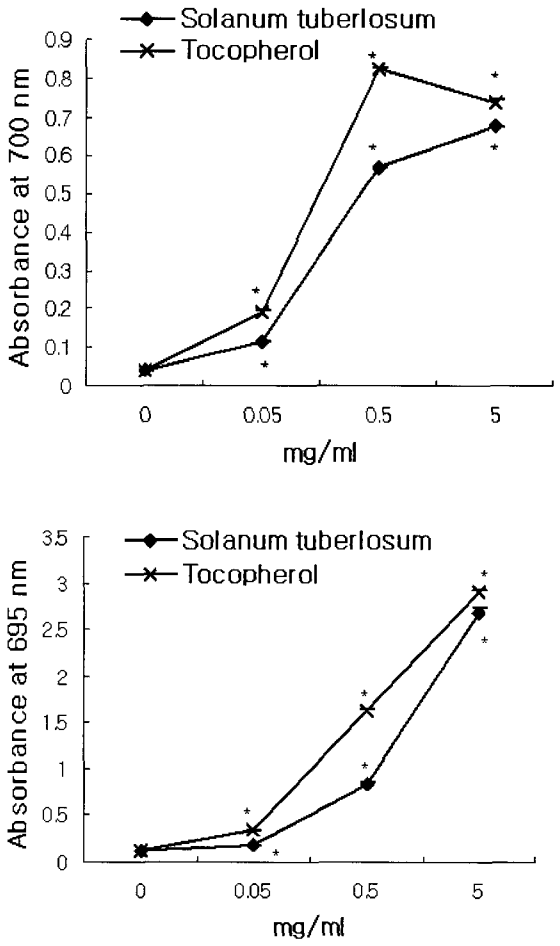

Fig. 6. **Reducing power of potato extract.** (A) Reducing power by the method of Oyaizu (1986); (B) Reducing power by phosphomolybdenum method. Results are mean ± S.D. (n=7). * $p<0.05$ vs. control.

tion at 695 nm(Fig. 6B). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity(Meir et al 1995). It has been cited in the reports(Yen & Duh 1993) that the reducing power was associated with the antioxidant activity and this relationship was also established with the compounds of some anthraquinones(Yen et al 2000). The reducing power of potato extract might be due to their hydrogen-donating abilities, as described by Shimada et al (1992). Accordingly, potato extract might contain a higher amount of reductone, which could react with free radicals to stabilize and terminate radical chain reactions. However, the antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain reaction, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging(Diplock 1997, Yildirim et al 2001).

5. Contents of Total Phenolics

The presence of polyphenolic compounds could be the reason for reasonably good activity in the potato extract. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups(Hatano et al 1989), and good correlation between the concentration of plant phenolics and the total antioxidant capacity has been reported(Madsen et al 1996). Moreover, these compounds have been found effective in many health-related properties, such as anticancer, antiviral, anti-inflammatory activities, effects on capillary fragility, and an ability to inhibit human platelet aggregation(Benavente-Garcia et al 1997). Fig. 7 shows the total phenolic content of potato extract. In the ethanolic extract(100 mg) of potato extract, 2.49 mg tannic acid equivalent of phenols was detected. The high correlation between the scavenging activities on DPPH radical and superoxide anion, and total phenolic contents of samples was reported, which infer that the phenolic compounds were effective hydrogen donors(Benavente-Garcia et al 1997). Simonetti et al (1997) found that the total antioxidative activity of wine investigated was correlated well with the phenol (r^2=0.990) and flavanol (r^2=0.927) contents.

Based on the active profile exposed through various assays, it can be concluded that potato extract shows antioxidant activity, reducing power, and free radical scavenging activities. The results of this study show that potato extract can be of use as an easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry.

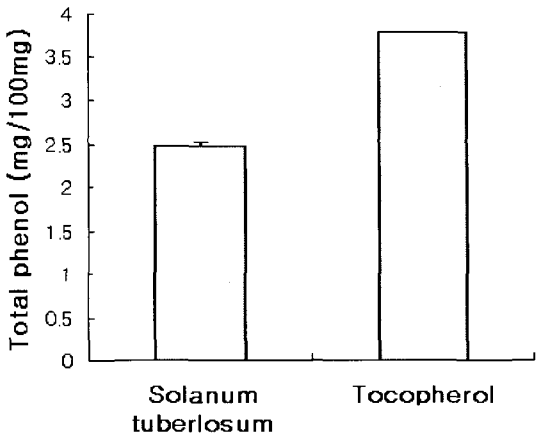

Fig. 7. The content of total polyphenols of potato extract.

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