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INHIBITORY EFFECT OF DPPH RADICAL SCAVENGING ACTIVITY AND HYDROXYL RADICALS (OH) ACTIVITY OF *SOLANUM TUBEROSUM*

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ABSTRACT

This study aimed to investigate possible antioxidant activity of various concentration extracts of *Solanum tuberosum*. The explored items were 1- diphenyl 2-picrylhyorazyl (DPPH) activity and hydroxyl radicals (OH). DPPH scavenging activity of leaf extracts of *S. tuberosum* was evaluated 48.1% at 4.0 mg/ml and that of stem was 39.6% at same concentration. DPPH scavenging activity of tuber extracts of *S. tuberosum* was evaluated at 4.0 mg/ml was only 10.1% at same concentration. When the L-Ascorbic acid used as a control, extract for leaves of *S. tuberosum* was 63.7% effects on the activation of DPPH and those of stems and flowers were 52.4% and 28.5%, respectively. The highest OH activity was recorded in the leaf extract among three vegetative and one reproductive organs. OH activity of matured stems was 55.5% at 4.0 mg/ml and leaves, tubers, and flowers were 52.1%, 16.2, and 23.26% at same concentration, respectively. When the H_2O_2 used as a control, extract for stems of *S. tuberosum* was 71.6% effects on the activation of OH and those of leaves, tubers, and flowers were 67.3%, 20.9%, and 29.9%, respectively. These findings suggested that activity of DPPH enzymes and OH by extract from *S. tuberosum* might contribute to their antioxidant activities in biological and phytochemical examination.

KEYWORDS: 1, 1- diphenyl 2-picrylhyorazyl (DPPH), hydroxyl radicals (OH), *Solanum tuberosum*.

INTRODUCTION

The term antioxidant originally was used to refer specifically to a chemical that prevented the consumption of oxygen.[1] The recent growth in the knowledge of free radicals and reactive oxygen species (ROS) in biology is producing a medical revolution that promises a new age of health and disease management.[2] Naturally occurring antioxidants in leafy vegetables and seeds, such as ascorbic acid, vitamin E and phenolic compounds, possesses the ability to reduce the oxidative damages associated with many diseases.[3] Many research works have also been done for antioxidant activity of vegetable tissues.[4-6]

Free radicals are reactive species that frequently occur as intermediates in chemical reactions. The 1, 1- diphenyl 2-picrylhyorazyl (DPPH) is rapid, simple, accurate and inexpensive assay for measuring the ability of different compounds to act as free radical scavengers or hydrogen donors, and to evaluate the antioxidant activity of plant-foods and beverages.[7-8] The method by DPPH is widely used for measurement of free radical scavenging ability of antioxidants in plants.[9]

Hydroxyl radical ('OH) is the neutral form of hydroxyl ion and the most reactive free radical in biological systems generated from free metal ions (copper or iron) catalyzed breakdown of H_20_2 (Fenton reaction) or superoxide ion reaction with H_20_2 (Haber-Weiss reaction). The radical reacts with every cell components in living organisms such as lipid, polypeptides, proteins, and DNA, especially thiamine and guanosine.[10]

Solanum tuberosum L. (potato) is a herbaceous of flowering plants in the family, Solanaceae. *S. tuberosum* is native to Central and South America, but is now widely naturalized beyond its native range in extra-tropical regions and is considered a weed in many places including Australia, Indonesia, Micronesia, India and Turkey.[11] The potato is among the world's most important crop plants. There are over 5000 cultivars of this species, due to its long and widespread cultivation throughout the world. The species name *tuberosum* refers to this global use of the tuber as a food.

Potato as a whole food contains high levels of vitamins and important antioxidants including phenolic acids, carotenoids and flavonoids.[12] The purpose of the present study is to evaluate plant extracts as sources of natural antioxidants for DPPH and OH activity and to examine whether *S. tuberosum* having significant radical scavenging activity beside food.

MATERIALS AND METHODS

Sample extract

Solanum tuberosum was collected from cultivated populations in Korea. The plants were washed, shade dried and then milled into coarse powder by wind mill. The plants of *S. tuberosum* divided into four parts: leaves, stems, tuber, and flowers. Each sample (500 g) of plants was ground with pestles and liquid nitrogen at -70°C and homogenized prior to beginning extraction experiments for the fine powder. The ground powders were dissolved in 1000 ml ethanol and treated with ultrasound at room temperature for three hours. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA). The mixture was further stirred with a magnetic bar at 65°C for 12 hours. Extracted sample was filtered. The sample was evaporated to remove solvent under reduced pressure and controlled temperature by using rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan). To get dry powder, samples placed in a low temperature vacuum chamber.

DPPH free radical

The antioxidant activity of the *S. tuberosum* extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams et al.[4] with slight modifications. 1 ml of 0.1 mM DPPH solution in ethanol was mixed with 1 ml of plant extract solution of various concentrations (0.1, 1.0, 2.0 and 4.0 mg/ml). DPPH was added to the solutions prepared with plant extracts and standard antioxidant substances and stirred. A solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of ethanol, and the solution was kept in the dark at 4°C. A stock solution of the compounds was prepared at 1 mg/ml in DMSO. The stock solution was diluted to varying concentrations in 96-well microplates. Then, 5 ul of ethanol DPPH solution (final concentration 300 μm) was added to each well. The plate was shaken to ensure thorough mixing before being wrapped with aluminum foil and placed into the dark. After 30 min, the optical density (OD) of the solution was read using the UVmini-1240 Reader (Shimadzu, Kyoto, Japan) at the wavelength 517 nm. Absorbance changes are measured at 517 nm. Corresponding blank sample was prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard (positive control). The inhibition % was calculated using the following formula.

The inhibitory percentage of DPPH was calculated according to the following equation.

% DPPH radical scavenging activity = 100−{A517(Exp)/A517(control) ×100}

The 50% inhibition (IC_{50}) is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. A dose response curve was plotted to determine IC_{50} values.

To determine the IC_{50} value of the active component, the technique using 96-well microplates was employed.[13]

Hydroxyl radical (OH) assay

Hydroxyl radical scavenging activity of the examined compounds was measured based on the method of Halliwell et al.,[14] with a slight modification according to Jiang et al.[15] Briefly, 200 µL deoxyribose solution (2.8 mM), 200 µL H_2O_2 (1.4 mM) and 200 µL of the examined compound (5 mM) or oxygen free water (control), were placed in a test tube. The scavenging activity for hydroxyl radicals was measured with fenton reaction. All used solutions were oxygen free. Reaction mixture contained 60 µL of 1.0 mM $FeCl_2$, 90 µl of 1mM 1,10-phenanthroline, 2.4 mL of 0.2 M phosphate buffer (pH 7.8), 150 µL of 0.17 M H_2O_2 and 1.0 mL of extract at various concentrations. Adding H_2O_2 started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured with UV visible spectrometer (Shimadzu, UV-1800, Japan). The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula:

Inhibition % = (IA-As)/IA×100

Where IA is the absorbance of the 100% initial and As is the absorbance of the sample. IA and As were the values which were subtracted the average absorbance of the blank wells.

Statistical analysis

All the analysis were carried out in triplicate. The results were expressed as the mean values ± standard deviation (SD). Differences were tested with analysis of variance (ANOVA) followed by multiple comparison test. Correlation co-efficient (R) to determine the relationship between two or more variables among Radical Scavenging activity tests were calculated using the SPSS software (Release 21.0).

RESULTS AND DISCUSSION

DPPH has frequently been used as a reactive hydrogen acceptor for the determination of radical scavenging activity of various natural and synthetic compounds.[16] Results listed in Table 1 indicate that the examined compounds of the *S. tuberosum* expressed anti DPPH radical activity ranged from 2.43% to 48.11%, leaves of them exhibited high scavenging activity (> 48%). Various concentrations of leaf and stem extracts were higher than those of tubers and flowers. The maximum high antioxidant activity found on leaf extracts. DPPH scavenging activity of edible tuber extracts of *S. tuberosum* was evaluated at 4.0 mg/ml was 10.1% and that of flowers was 21.6% at same concentration. When the L-Ascorbic acid used as a control, extract for leaves of *S. tuberosum* was 63.7% effects on the activation of DPPH and that of stems, tubers, and flowers were 52.4%, 13.4% and 28.5%, respectively (Fig. 1). The inhibitory activity of stem (IC_{50} = 133 ug/ml) was at the same levels as that of L-ascorbic acid (IC_{50} 28 ug/ml) (Fig. 3). The all groups for leaves, stems, tubers and flowers were

shown a statistically significant difference ($p > 0.05$).

Table 2 was shown the activity of hydroxyl radicals on *S. tuberosum* extracts. The highest OH activity was recorded in the stem extract among three vegetative and one reproductive organs. OH activity of matured stems was 55.5% at 4.0 mg/ml and leaves, tubers, and flowers were 52.1%, 16.2%, and 23.2% at same concentration, respectively. The overall values of OH activity of stem and leaves were higher than those of tubers and flowers and there were show a statistically significant difference ($p > 0.05$). When the H_2O_2 used as a control, extract for stems of *S. tuberosum* was 71.6% effects on the activation of OH and that of leaves, tubers and flowers were 67.3%, 20.9% and 29.9% (Fig. 2). The stem of *S. tuberosum* showed maximum inhibition of OH activity (IC_{50} = 45.0 ug/ml) (Fig. 3).

The amount of antioxidant activity of methanol extracts of potatoes in three common potato cultivars in Iran was between 92.89±1.23% and 94.10±1.89%.[12] Whereas, antioxidant activity of potato peel (Raja cultivar) in Iran was 31.60-61.91%.[17] Rumbaoa et al.[18] ranged antioxidant activity of Philippine potato varieties from 93.5±1.7 to 95.4±2.2%. Activity of ethanoic and aqueous potato extract at 40 mg is 62.3% and 62.5%, respectively.[19] Antioxidant activity values also depend strongly on the preparation of sample (leaching, extended steaming, lyophilisation) and the method used (ferric thiocyanate method).[20] In addition, genotype and growth conditions, such as water availability, light quality and temperature, could be affected the synthesis and accumulation of phenolic compounds in some parts of the plant, and consequently, antioxidant activity.[21]

Table 1: Free radical scavenging effects of *Solanum tuberosum* at different concentrations

Concentration (mg/ml)	Leaf	Stem	Root	Flower
0.1	17.66±1.32	14.20±1.81	2.43±0.81	6.14±0.97
0.5	27.09±1.47	20.44±0.75	5.63±0.98	11.02±0.23
1.0	33.67±0.78	29.81±1.23	6.78±0.92	14.83±1.64
2.0	39.32±1.69	34.18±2.21	8.00±1.02	18.85±1.67
4.0	48.11±3.65	39.57±1.91	10.13±1.80	21.55±2.02
F-test	F = 9.634, $p > 0.05$			

Data represent the mean ± SD from three replicates.

Table 2: Activity of hydroxyl radicals by *Solanum tuberosum* at different concentrations

Concentration (mg/ml)	Leaf	Stem	Root	Flower
0.1	12.12±2.95	19.75±0.96	5.08±0.74	3.86±0.65
0.5	21.69±2.23	28.06±1.14	9.72±2.16	10.79±0.90
1.0	28.54±2.55	33.20±3.07	11.97±2.26	13.60±1.39
2.0	43.93±3.53	41.43±3.82	13.64±2.53	18.27±2.26
4.0	52.12±3.58	55.50±4.47	16.19±2.88	23.15±0.60
F-test	F = 5.409, $p > 0.05$			

Data represent the mean ± SD from three replicates.

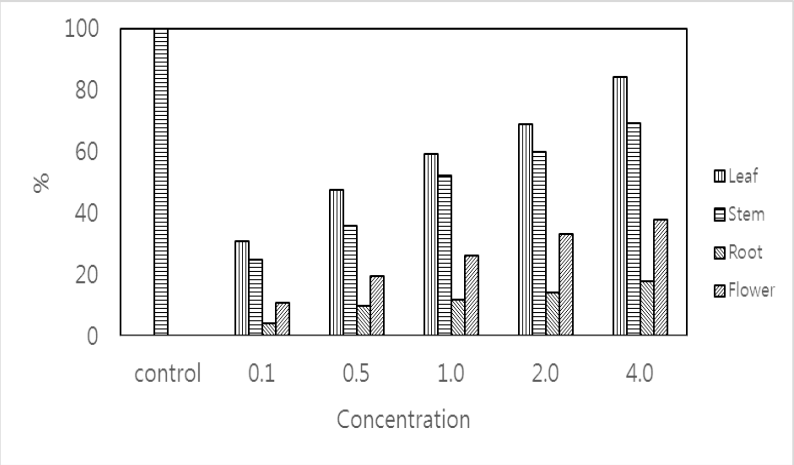

Figure 1: Relative antioxidant values of the *Solanum tuberosum* extracts for control group (L-Ascorbic acid).

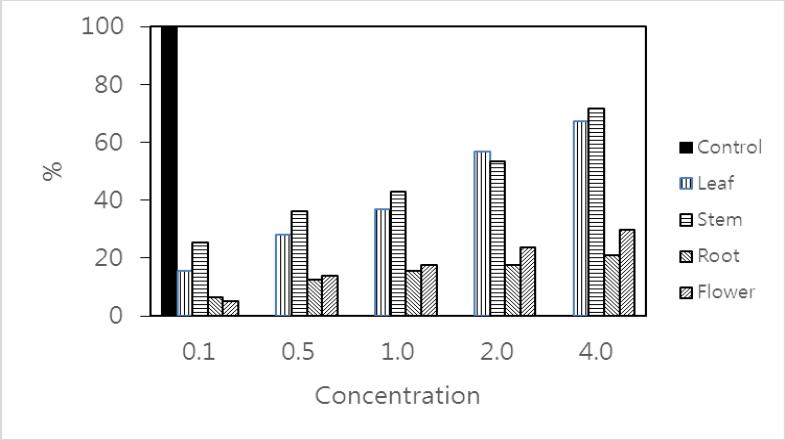

Figure 2: Relative activity of Hydroxyl Radicals of the *Solanum tuberosum* extracts for control group (H_2O_2).

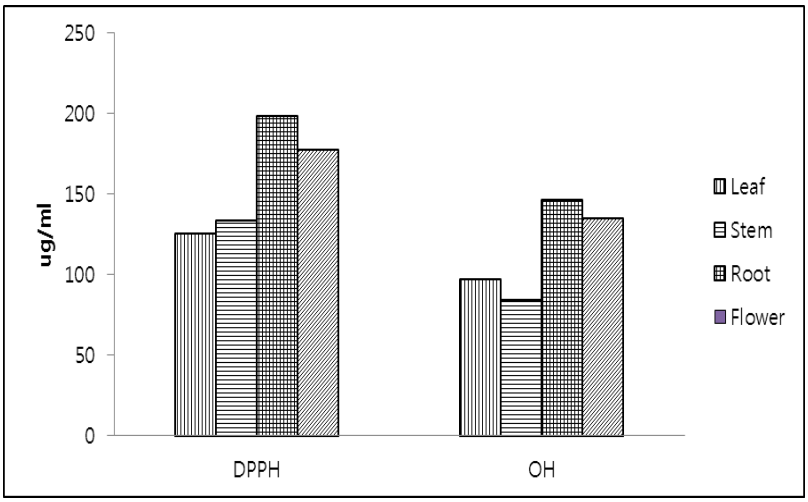

Figure 3: Inhibitory effects {IC_{50} (mg/ml)} on DPPH and OH activity by *Solanum tuberosum*.

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