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Inhibitory Effect of DPPH Radical Scavenging Activity and Hydroxyl Radicals (OH) Activity of *Chelidonium majus* var. *asiaticum*

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Keywords

1, 1- diphenyl 2-picrylhyorazyl (DPPH), *Chelidonium majus* var. *asiaticum*, hydroxyl radicals (OH).

Abstract

The present study was investigated plant extracts as sources of natural antioxidants and to examine whether *Chelidonium majus* var. *asiaticum* having significant1- diphenyl 2-picrylhyorazyl (DPPH) activity and hydroxyl radicals (OH) activity.DPPH scavenging activity of stem extracts of *C. majus* var. *asiaticum* was evaluated at 4.0 mg/ml was 55.8% and that of leaves was 23.3% at same concentration. DPPH scavenging activity of root extracts of *C. majus* var. *asiaticum* was evaluated at 4.0 mg/ml was only 19.6% at same concentration. When the L-Ascorbic acid used as a control, extract for stems of *C. majus* var. *asiaticum* was 50.3% effects on the activation of DPPH and that of leaves and roots were 31.3% and 23.2%. The highest OH activity was recorded in the stem extract among three vegetative organs. OH activity of matured leaves was 52.7% at 4.0 mg/ml and leaves and roots were 43.3% and 25.6% at same concentration, respectively. When the H_2O_2 used as a control, extract for stems of *C. majus* var. *asiaticum* was 38.3% effects on the activation of OH and that of leaves and roots were 36.2% and 23.5%, respectively. Strong activity of DPPH enzymes and OH by extract from *C. majus* var. *asiaticum* makes this pharmacopeial plant material an interesting topic for further biological and phytochemical examination.

Introduction

Antioxidant research is an important topic in the medical field. Oxidative is the condition as elevated levels of free radicals or other oxygen species which can direct either direct or indirect damage to the body (Chen et al., 2009). Free radicals have been implicated as playing a role in the etiology of cardiovascular disease, cancer, Alzheimer's disease and Parkinson's disease (Enujiugha et al., 2012). The antioxidant capacity of most plant food sources is usually associated with their phenolic contents (Kedare and Singh, 2011). The 1, 1-diphenyl 2-picrylhyorazyl (DPPH) is a well-known radical and a trap (scavenger) for other radicals (Brand-Williams et al., 1995). Many research works have also been done for antioxidant activity of leafy vegetables (Dasgupta and De, 2007; Sahu et al., 2013).

Hydroxyl radical ('OH) is a byproduct of normal metabolism and attacks biological molecules, leading to cell or tissue injury (Yen and Chen, 1995). Active oxygen and free radicals are produced by certain chemical carcinogens and play a role in the carcinogenic process (Cerutti, 1985).

Chelidonium majus var. *asiaticum* (Hara) Ohwi is a herbaceous of flowering plants in the family, Papaveraceae. The species is grows in moist rich soils on hillsides and in valleys over a narrow range which extends from eastern China to Korea, Japan, and the Russian Far East. The species has been known for its medicinal functions such as cytotoxic, virucide, and anti-inflammatory activities. The plant is used in the traditional medicine of Korea. The root has chelidonine which is the basis for chemical synthesis of chelidonicthiophosphoric acid triziridide, a potential immunostimulant drug for treatment of cancer and AIDS (Colombo et al., 1987; Kim et al., 1999).

The purpose of the present study is to evaluate plant extracts as sources of natural antioxidants for DPPH and to examine whether the herbal medicine (*C. majus* var. *asiaticum*) having significant OH activity.

Materials and Methods

Sample extract

Chelidonium majus var. *asiaticum* was collected from natural population in Korea. The plants were washed, shade dried and then milled into coarse powder by wind mill. The plants of *C. majus* var. *asiaticum* were divided into three parts: leaves, stems, and roots. Each sample (100 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 *C. majus* var. *asiaticum* 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[11]. 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 theUVmini-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.

Percentage inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = [1 - \text{OD (DPPH + sample)}/\text{OD (DPPH)}] \times 100\%.$$

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 (Lee, 1998).

Hydroxyl radicals (OH) activity

The scavenging activity for hydroxyl radicals was measured with fenton reaction. 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:

$$\text{Inhibition \%} = (\text{IA-As})/\text{IA} \times 100$$

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 and the results were expressed as the mean ±SD. 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

Table 1 was shown the antioxidant activities of the *C. majus* var. *asiaticum.* Various concentrations of stem extracts were higher than those of leaves and roots. The maximum high antioxidant activity found on stem extracts. DPPH scavenging activity of stem extracts of *C. majus* var. *asiaticum* was evaluated at 4.0 mg/ml was 55.8% and that of leaves was 23.3% at same concentration. DPPH scavenging activity of root extracts of *C. majus* var. *asiaticum* was evaluated at 4.0 mg/ml was only 19.6% at same concentration. When the L-Ascorbic acid used as a control, extract for stems of *C. majus* var. *asiaticum* was 50.3% effects on the activation of DPPH and that of leaves and roots were 31.3% and 23.2% (Fig. 2). The inhibitory activity of stem (IC_{50} = 59.3ug/ml) was at the same levels as that of L-ascorbic acid (IC_{50} = 25.7ug/ml) (Fig. 3). The all groups for leaves, stems, and roots were shown a statistically significant difference (p>0.05).

Table 1: Free radical scavenging effects of *Chelidonium majus* var. *asiaticum* at different concentrations

Concentration (mg/ml)	Leaf	Stem	Root
0.1	12.63±1.64	28.33±2.89	9.29±2.59
0.5	16.85±2.61	34.33±2.35	12.48±1.12
1.0	19.53±1.96	39.03±3.58	15.43±1.44
2.0	22.77±3.01	48.43±5.25	17.13±1.15
4.0	23.33±1.78	55.77±7.07	19.60±1.15
F-test	F = 4.115, p> 0.05		

Data represent the mean ± SD from three replicates.

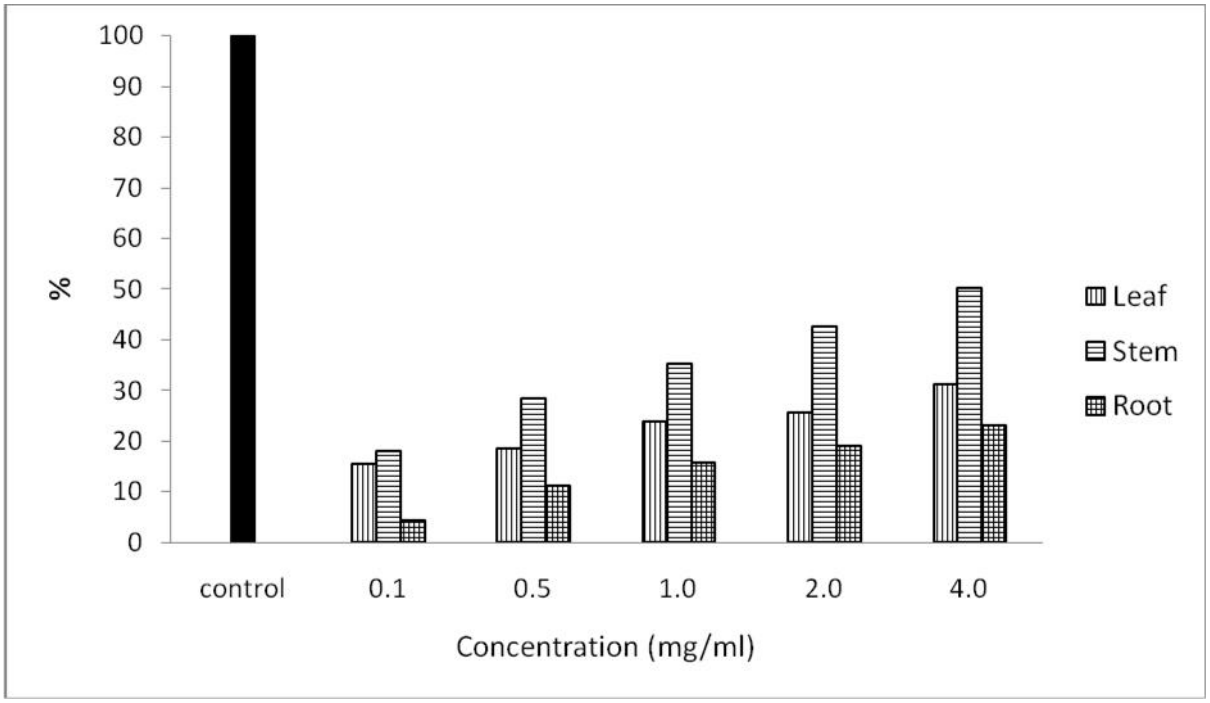

Figure 1: Relative antioxidant values of the *Chelidonium majus* var.*asiaticum* extracts for control group (L-Ascorbic acid).

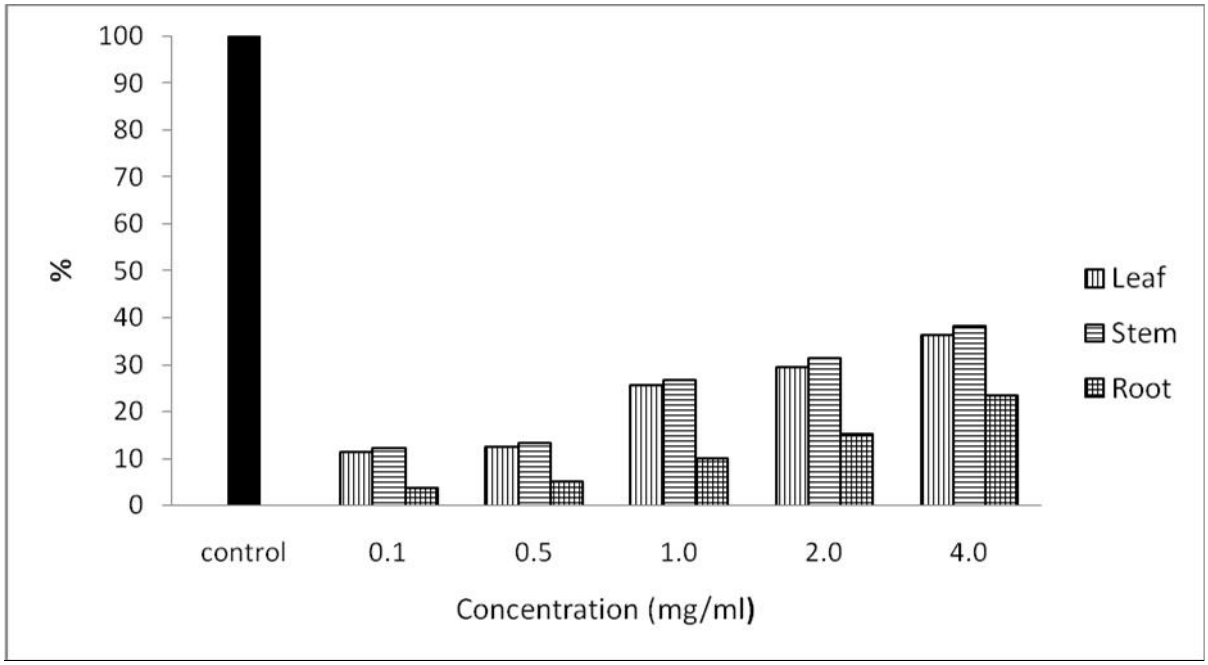

Figure 2: Relative activity of Hydroxyl Radicals of the *Chelidonium majus* var. *asiaticum* extracts for control group (H_2O_2).

Table 2 was shown the activity of hydroxyl radicals on *C. majus* var. *asiaticum* extracts. The highest OH activity was recorded in the stem extract among three vegetative parts. OH activity of matured leaves was 52.7% at 4.0 mg/ml and leaves and roots were 43.3% and 25.6% at same concentration, respectively. The overall values of OH activity of stem were higher than those of leaves and roots and there were show a statistically significant difference (p>0.05).

Table 2: Activity of hydroxyl radicals by *Chelidonium majus* var. *asiaticum* at different concentrations

Concentration (mg/ml)	Leaf	Stem	Root
0.1	20.18±5.42	22.53±1.93	8.50±1.39
0.5	26.37±4.08	29.63±0.97	13.20±1.56
1.0	30.19±3.89	36.90±1.44	18.17±0.63
2.0	37.38±1.70	43.10±2.60	22.83±2.58
4.0	43.29±1.07	52.67±4.15	25.57±1.69
F-test	F = 3.635, p> 0.05		

Data represent the mean ± SD from three replicates.

When the H_2O_2 used as a control, extract for stems of *C. majus* var. *asiaticum* was 38.3% effects on the activation of OH and that of leaves and roots were 36.2% and 23.5% (Fig. 2). The stem of *C. majus* var .*asiaticum* showed maximum inhibition of OH activity (IC_{50} = 42.0 ug/ml) (Fig. 3).

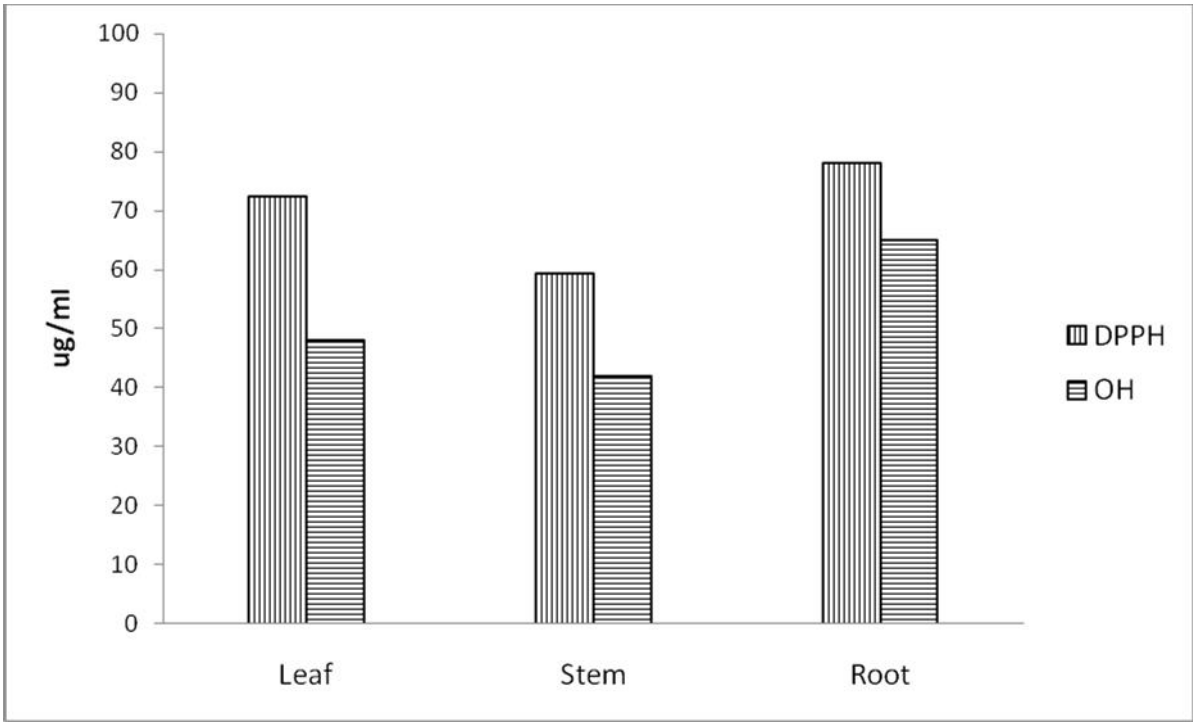

Figure 3: Inhibitory effects {IC_{50} (mg/ml)} on DPPH and OH activity by *Chelidonium majus* var. *asiaticum*.

Traditional use of herbal medicines implies substantial historical use and this is certainly true for many products that are available as widely acknowledged to be safe and effective (Oyetayo, 2008). Although modern medicine may exist side-by-side with such traditional practice, in many under developed countries or developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet healthcare needs. In the USA, by contrast, most herbal products in the marketplace are marketed and regulated as dietary supplements, a product category that does not require pre-approval of products on the basis of any of these criteria.

The many traditional herbal species in Korea exhibited DPPH-free radical scavenging activity (Choi et al., 2003; Huh and Han, 2015). Out of 40 species, four species (*Emcommia cortex*, *Moutan radices*, *Paeonine radix*, and *Rubus coreanus*) were shown above 90% inhibition of DPPH radical scavenging activity (Choi et al., 2003). Sahuet al. (2013) reported that *Leucas aspera* in India showed relatively high antioxidant activities (96.2%).

Akular and Odhav[14] reported antioxidants from 18 species in South Africa. *Portulaca oleracea* was high radical scavenging activity (96.5%) (Akular and Odhav, 2008). In this study, DPPH values of *C. majus* var. *asiaticum* were also relatively high (Table 1).

We have shown that 4.0 mg/ml weight of ethanol *C. majus* var. *asiaticum* extract has inhibitory effect of lipoxygenase and antioxidants for DPPH. In addition, strong activation of OH enzymes by extract from *C. majus* var. *asiaticum* makes this pharmacopeial plant material an interesting topic for further biological and phytochemical examination (Oyetayo, 2008).

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