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Physiological Activities of *Rheum undulatum* and *Rheum palmatum* Extracts as Affected by Solvents

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Abstract *Rheum undulatum* and *Rheum palmatum* have been widely used as food material as well as medicinal ingredients for their therapeutic effects in oriental countries. Many kinds of herbs are being used in the manufacture of functional foods. The objective of this study was to determine polyphenol content, superoxide dismutase (SOD)-like activity, tyrosinase activity, and electron donating ability of *R. undulatum* and *R. palmatum*. Total polyphenol content were most effective in 50 and 100% ethanol extracts from *R. undulatum* and *R. palmatum*. SOD-like activities of *R. undulatum* extracts were higher than those of *R. palmatum* extracts, and water extracts of samples were highest. EDAs of *R. undulatum* extracts were higher (26.76-44.46%) than those of *R. palmatum* extracts, while those of both extracts were lower than 1.0 and 0.1% L-ascorbate. And these suggest that the extracts of *R. undulatum* and *R. palmatum* can be used as a material in functional food.

Keywords: physiological activity, *Rheum undulatum*, polyphenol content, electron donating ability

Introduction

Reactive free radicals have been postulated to contribute to the causes of chronic inflammatory proliferative diseases, especially arteriosclerosis and cancer, through oxidative damage of essential enzymes, cells, and tissues. There is therefore widespread interest in defining the possible role of diet in preventing and reversing reactive oxygen species (ROS)-induced chronic diseases (1). Although most organisms possess antioxidant defense and repair systems that can protect them against oxidative damage, these systems are unable to prevent all damages (2). Antioxidant supplements or foods containing antioxidants, can be used to help the human body in reducing oxidative damage. These protective effects have been attributed partly to the various antioxidant compounds present in fruits and vegetables, for example, vitamins C, E, β-carotene, and polyphenolics (3). In vegetables, quercetin glycosides are predominant, but the glycosides of kaempferol, luteolin, and apigenin are also present (4,5). The potential roles, including free radical scavenging, of antioxidant agents such as herb and phenolic compounds have been extensively studied for the prevention of numerous degenerative diseases (6,7) Thus, it might be beneficial to prevent diseases in which the ROS play a part.

The rhizome of the species is one of the most frequently prescribed Traditional Chinese Medicines used as a purgative or astringent (8). Many components from the rhizome of *Rheum undulatum* are well known to be anthraquinone derivatives and stilbenes such as rhein, emodin, aloe-emodin, chrysophanol, physcion, resveratrol, rhapontigenin, and rhaponticin (9,10). They have been reported to possess diverse biological activities, an anti-platelet aggregation activity (11), antioxidant activity (12), antiallergic activity (13,14), and antidiabetic activity (15).

The overall objectives of this study were to examine the potential of *R. undulatum* and *Rheum palmatum* as a functional food material by measuring its physiological activity, such as its antioxidative ability. Also, the intent of the study was to determine the optimum extraction conditions for the functional substances of *R. undulatum* and *R. palmatum*.

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Materials and Methods

Preparation of *R. undulatum* and *R. palmatum* extracts The *Rheum undulatum* and *Rheum palmatum* were purchased from the Kyungdong market in Seoul, Korea. After the *R. undulatum* and *R. palmatum* samples (10 g) were washed and crushed, they were extracted with 100 mL of water, 50, and 100% ethanol for 24 hr at 37°C shaking water bath. The process was repeated twice. The extracts were centrifuged at 13,000×g for 10 min and filtered with Whatman filter paper No. 2. Filtered extracts evaporated under reduced pressure and redissolved in 100 mL of distilled water for further experimentation.

Determination of superoxide dismutase (SOD)-like activity SOD-like activity was measured by a modified method of Kim *et al.* (16). Briefly, the pH of each sample was adjusted to 8.5 using a Tris-HCl buffer (50 mM of tris[hydroxymethyl]amino-methane+10 mM of ethylene diamine tetraacetic acid (EDTA), pH 8.5). And then, 3 mL of the Tris-HCl buffer and 0.2 mL of 7.2 mM pyrogallol were added to 0.2 mL of each sample. The mixtures were held at 25°C for 10 min before stopping the reaction by adding 1 mL of 1 N HCl. Absorbances were measured at 420 nm using a UV/VIS spectrometer (SSE-343; Jasco, Hachioji, Japan). SOD-like activity was expressed in a

percentage using the following equation:

$$\text{SOD-like activity (\%)} = 1 - \frac{A}{B} \times 100$$

where A is the absorbance difference between the treated sample and control, and B is the absorbance difference between the untreated sample and control.

Determination of nitrite scavenging activity (NSA) A procedure described by Gray *et al.* (17) was used to measure the NSA. One mL of a 1 mM $NaNO_2$ solution was added to 1 mL of each *R. undulatum* and *R. palmatum* extract, and the pH values of the resulting mixtures were adjusted to 1.2, 3.0, and 4.2 using 8 mL of buffer solutions: 0.1 N HCl for pH 1.2, and 0.2 N citric acid for pH 3.0, and 4.2. The final volume of each sample was adjusted to 10 mL. The samples were allowed to react at 37°C for 1 hr, and 1 mL of each the sample was taken from the solutions, mixed thoroughly with 5 mL of 2% acetic acid and 0.4 mL of Griess reagent, and kept at room temperature for 15 min. Prior to usage, the Griess reagent was prepared by mixing equal amounts of 1% sulfanilic acid and 1% naphthylamine, which were made with 30% acetic acid. The residual nitrite content was determined by measuring the absorbance at 520 nm. The NSA was also expressed in a % by using the following equation:

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

where A is the absorbance of the sample mixture and 1 mM of $NaNO_2$ after 1 hr reaction; B is the absorbance of the mixture of distilled water and 1 mM of $NaNO_2$ after 1 hr reaction; and C is the absorbance of *R. undulatum* and *R. palmatum* extracts.

Determination of electron donating ability (EDA) The EDA was determined in terms of reducing power of α,α-diphenyl-picrylhydrazyl (DPPH) in each extract according to a modified method by Kang *et al.* (18). One mL of each extract was mixed with 1 mL of 4×10^{-4} M DPPH dissolved in 99.9% ethanol to make a total volume of 2 mL. After vortexing the mixtures for 10 sec and holding them at room temperature for 30 min, the absorbance were measured at 525 nm using a UV/VIS spectrophotometer. The EDA was expressed in a % using the following equation:

$$\text{EDA (\%)} = 1 - \frac{A}{B} \times 100$$

where A is the absorbance of the sample treated with the extract and B is that of an untreated sample. All the data reported in this paper represent means of the 3 values measured separately.

Determination of the inhibitory effect on tyrosinase The inhibitory effect on tyrosinase was measured by a method reported by Wong *et al.* (19). A crude tyrosinase solution was prepared by dissolving mushroom tyrosinase (110 units/mL) in a 50 mM sodium phosphate buffer (pH 7.0). Subsequently, 0.2 mL of the crude tyrosinase solution and 0.1 mL of *R. undulatum* and *R. palmatum* extract were added to 2.8 mL of a 10 mM catechol solution. The absorbance of the resulting mixture was measured at 420 nm by a UV/VIS spectrometer in order to determine the tyrosinase activity. The inhibitory effect on tyrosinase was calculated by measuring changes in the absorbance per unit of time, as follows:

$$\text{Inhibitory effect (\%)} = \frac{1-(A-C)}{C} \times 100$$

where A is the difference in the absorbance of samples treated by the enzyme solution; B is the difference in the absorbance of samples treated by a buffer solution *in lieu* of an enzyme solution; and C is the difference in the absorbance of samples treated by distilled water *in lieu* of extracts.

Determination of total polyphenol content The total polyphenol content was measured by the Folin-Denis method (20). *R. undulatum* and *R. palmatum* extract 0.1, 8.4 mL of distilled water, and 0.5 mL of 2 N Folin reagent were set for 3 min before adding 1 mL of the 20% Na_2CO_3 solution. After holding the mixed solution for 1 hr, absorbance was measured at 765 nm using a UV/VIS spectrometer. The total polyphenol content was determined from the standard curve obtained using (+)-catechin.

Statistical analysis The analysis of variance (ANOVA) test and factorial analysis were carried out for all experiments using the statistical analysis system (SAS for Windows, ver. 8) program (21). Duncan's multiple-range tests were also used to examine the significance of the average of values among the experimental groups.

Results and Discussion

SOD-like activity SOD-like activity is derived not from enzymes but from low molecular weight materials that play a role similar to the SOD. They are phytochemicals for the most part and can protect oxidative hindrance by suppressing the reactivity of superoxide. Nice *et al.* (22) purified SOD along with substances showing high thermal stability as well as SOD-like activity. They reported that these materials are phenolic compounds bound with SOD. Kim *et al.* (23) suggested that vitamin C has high SOD-like activity. With respect to the water extract *R. undulatum* and *R. palmatum*, the SOD-like activity was comparatively high, 46.46% of activity was retained in the *R. undulatum* extracts, whereas 27.97-30.01 and 16.31-18.49% of activity were observed in 50 and 100% ethanol extract, respectively (Fig. 1). On the other hand, the SOD-like activity of 0.1 and 1% L-ascorbic acid was 93.40 and 99.76%, respectively, which were similar to the results reported by Kim *et al.* (23). On the whole, the SOD-like activity of the *R. undulatum* extract was higher than that of the *R. palmatum* extract, and the water extract was more effective than ethanol extract. This result was in agreement with that of Kim *et al.* (23). Therefore, the antioxidative activity of substances associated with the inhibition of pyrogallol oxidation varies according to plant material and its habitat. Kim *et al.* (16) demonstrated that a specific substance could repress the reactivity of a superoxide throughout the oxidation or a radical reaction in a living body. Since the *R. undulatum* and *R. palmatum* extracts had

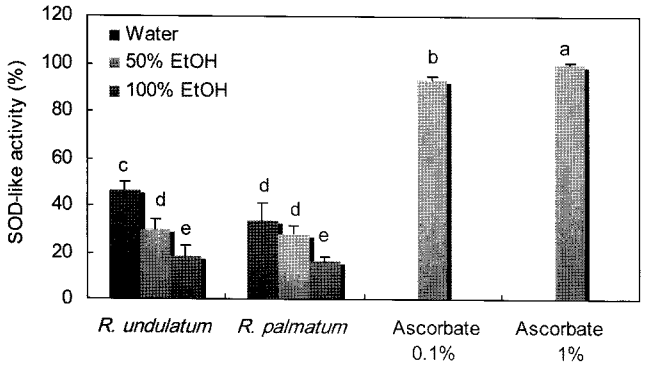

Fig. 1. Superoxide dismutase (SOD)-like activity of *R. undulatum* and *R. palmatum* extracts. Data are expressed as mean±SD. Significant differences within a set of experiment were analyzed by ANOVA test ($p<0.05$).

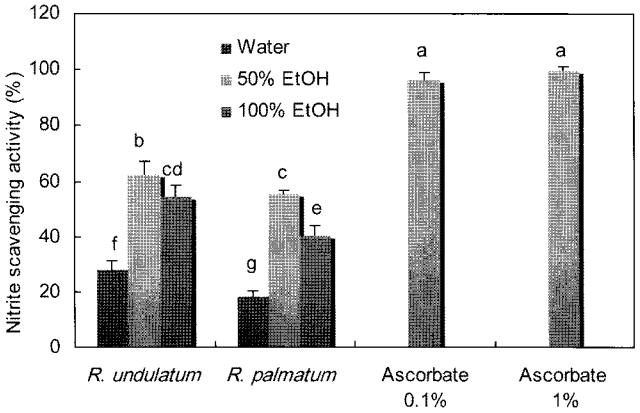

Fig. 2. Nitrite scavenging activities of *R. undulatum* and *R. palmatum* extracts (pH 1.2). Data are expressed as mean±SD. Significant differences within a set of experiment were analyzed by ANOVA test ($p<0.05$).

high inhibitory activity and eliminative activity against superoxides, the study of antioxidative materials capable of the repressing reactivity of superoxides, according to the type of active oxygens or reaction mechanism is necessary.

Effects of NSA The ingestion of large portions of nitrite-containing food leads to the development of toxic symptoms such as methemoglobin symptoms. Since a nitrosyl reaction between nitrite and second or third class amine takes place readily to form a carcinogen-like nitrosamine under low acidic conditions in the stomach, many attempts have been made to search for a natural substance that can remove these nitrites (17,18,24).

The nitrite removal ability of the different types of *R. undulatum* and *R. palmatum* was compared at pH 1.2, 3.0, 4.5, and 6.0 using various extraction solvents (Fig. 2, Table. 1). As a whole nitrite removal ability of water and the ethanol extracts of *R. undulatum* and *R. palmatum* (pH 1.2, 18.42-62.3%) was lower than those of 0.1 and 1% L-ascorbic acid, 95.71 and 99.36% (Fig. 2). As nitrosamine is readily formed at low acidic pH in the stomach, high nitrite removal ability at low pH (e.g., pH 1.2) is considered to effectively suppress the formation of nitrosamine (16). All extracts had the higher level of ability to remove nitrites, as compared to the 1% L-ascorbic acid. This result was in good agreement with several reports in that some plant extracts, phenolic compounds or ascorbate can remove nitrites, thereby reducing hazards associated with them (18,24,25).

Table 1. Nitrite scavenging activities of *R. undulatum* and *R. palmatum* extracts

Habitat & Solvents	Nitrite scavenging ability[1)] (%)		
	pH 3.0	pH 4.5	pH 6.0
R. undulatum			
Water	21.5±1.4	15.2±2.9	8.49±2.0
50% Ethanol	60.4±2.4	54.4±3.8	14.15±5.1
100% Ethanol	61.7±5.2	55.5±8.2	40.42±4.4
R. palmatum			
Water	10.5±3.2	10.1±3.7	-
50% Ethanol	47.4±4.8	40.7±2.9	22.2±7.4
100% Ethanol	50.1±4.1	44.1±4.4	30.7±5.7

[1)]All values are expressed as mean±SD of triplicate determinations.

Effects of EDA The EDA measures hydrogen atom (or one electron) donating ability and hence provides a measure of freeradical scavenging antioxidant ability. The test used DPPH, a purple-colored stable free radical, which is reduced to the yellow-colored diphenylpicrylhydrazine when antioxidants are added (26). A higher EDA leads to a greater ability to eliminate active oxygen, which causes problems in the body (26). Kang *et al.* (18) determined the EDAs of phenolic acids, flavonoids, and other phenolic compounds as indices of antioxidative ability. They noted that compounds with a higher reducing ability have higher EDA.

In our result, the EDAs of 0.1 and 1% L-ascorbic acid, widely used as an antioxidant, were 97.65 and 97.73%, respectively. The EDAs of all extracts were lower than those of the L-ascorbic acid solutions. The EDAs of all sample extracts, except for the 50% ethanol extract of *R. undulatum* and *R. palmatum*, were between 22-45%. Although the EDAs of *R. undulatum* and *R. palmatum* were considerably not very high, the highest amount was no more than 30% (Fig. 3), they are presumed to have a certain degree of free radical binding ability. Thus, the ability to form a stable radical varies depending upon the antioxidative substances in the extract samples.

Effects of tyrosinase inhibition Tyrosinase (dihydroxy-L-phenylalanine oxygen oxidoreductase, EC 1.14.18.1) is known to be responsible for the browning reaction of phenolic substances during processing and storage because it utilizes a wide range of phenolic compounds, thus resulting in enzymatic coloration (27). The inhibitory activity of *R. undulatum* and *R. palmatum* extracts ranged between 30.52 and 66.22% ($p<0.05$) with the *R. undulatum* and being generally higher than the *R. palmatum* (Fig. 4). Tyrosinase was inhibited 74.97 and 99.11% by amounts of 0.1 and 1% L-ascorbic acid, respectively, which were used as a reference. Although the inhibition effects of the *R. undulatum* and *R. palmatum* extracts on tyrosinase activity were found to be somewhat lower than that of the 1% L-ascorbic acid, they

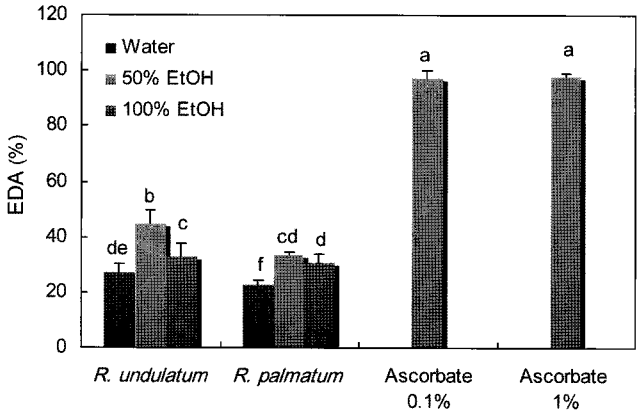

Fig. 3. Electron donating ability (EDA) of *R. undulatum* and *R. palmatum* extracts. Data are expressed as mean±SD. Significant differences within a set of experiment were analyzed by ANOVA test ($p<0.05$).

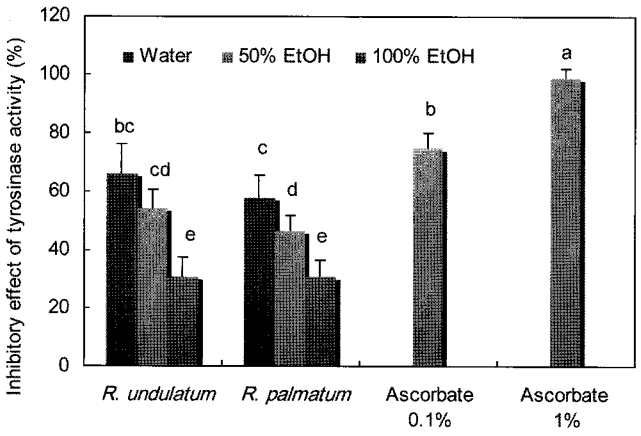

Fig. 4. Tyrosinase inhibition effects of *R. undulatum* and *R. palmatum* extract. Data are expressed as mean±SD. Significant differences within a set of experiment were analyzed by ANOVA test ($p<0.05$).

were considerably higher than that of the 0.1% L-ascorbic acid. Due to some safety and efficiency problems associated with various compounds in functioning as tyrosinase inhibitors, Jung *et al.* (27) searched for plant materials capable of inhibiting tyrosinase activity. They suggested the high tyrosinase activity of green and black teas were linked with the phenolic components in tea. They also reported that radishes, radish sprouts, and red peppers showed relatively high tyrosinase inhibition activity.

Total polyphenol content Many researchers have reported that the plant polyphenols contained in fruits and vegetables play important roles in preventing degenerative diseases if, they are consumed as part of a daily diet (28,29). The total polyphenol content was determined using the standard curve (R^2=0.9901) for catechins (Fig. 5). The total polyphenol content varied depending upon the habitat and solvents. The total polyphenol content of the water extracts ranged between 9.46 and 10.97 mg%, while those of the 50 and 100% ethanol extracts showed 34.16-48.53 and 60.1-67.49 mg%, respectively. The results showed that the total polyphenol content of the *R. undulatum* extracts were relatively higher than the *R. palmatum* extracts. Polyphenol

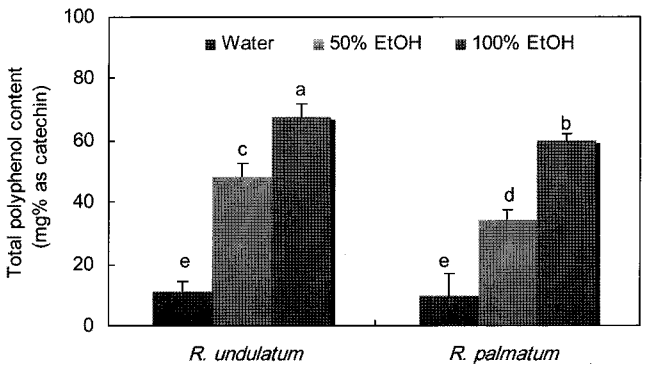

Fig. 5. Total polyphenol content of *R. undulatum* and *R. palmatum* extracts. Data are expressed as mean±SD. Significant differences within a set of experiment were analyzed by ANOVA test ($p<0.05$).

compounds contained such as caffeic, chlorogenic, ferulic, and ρ-coumaric acids showed antioxidant activity (29). Similar results were also reported by Kahkonen *et al.* (30), who examined the total phenolic content, of the following fruits, 1,100.0-1,000.8, regetables, 60-740, cereals, 20-130, and medicinal plants, 80-4,200.1 mg%.

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