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Soil conditions during cultivation affect the total phenolic and flavonoid content of rosemary

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Abstract In this study, the effects of soil conditions on antioxidant activities of the aerial and underground parts of rosemary were assessed to determine the most effective soil conditions for cultivation. The antioxidant activity was the highest (51.58±2.93 µg/mL) when cultivated in the mixture of gardening soil and vermiculite using DPPH assay. The antioxidant activity of underground parts the highest (127.48±12.38 µg/mL) when cultivated in the mixture of soil, vermiculite, and perlite. ABTS assay showed that the antioxidant activity of aerial parts was 230.34±57.93 $\mu g \cdot mL^{-1}$ when cultivated in the mixture of gardening soil and vermiculite and that of underground parts was 320.98±16.04 $\mu g \cdot mL^{-1}$ when cultivated in the mixture of gardening soil, vermiculite, and perlite. The total phenolic content of aerial parts was the highest (155.25±2.96 mg GAE/g) when cultivated in the mixture of gardening soil. The total flavonoid content of aerial parts was the highest (67.32±5.27 mg QE/g) when cultivated in the mixture of gardening soil. Therefore, the mixture of gardening soil, vermiculite, and perlite is superior to gardening soil alone for cultivation of rosemary to increase its antioxidant activity as well as total phenolic and flavonoid content.

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Introduction

Herbal plants have long been widely used as food, medicine, medicinal herbs, and antibiotics, and are still being used in various forms and uses in various fields [1]. Recently, herbal plants have shown various uses in the field of urban agriculture, so for a stable supply of plants, production technology capable of mass production and management in a timely manner is required, and standardization for efficient distribution and field application is essential. However, the production technology and distribution standards of herb plants have not been standardized, so the industry has not been expanded. In particular, herb plants mainly used for urban greening and gardens have various types and characteristics, so detailed descriptions of containers and soil for the production of each plant are required [2].

The production technology of these plants for some plants reported in order to control the physical properties of the cultured soil, horticultural peat moss was mixed with massato, mushroom waste medium, compost, pine bark to determine the growth reaction according to the type and mixing ratio of the soil [3]. It was confirmed that the amount of nutrient absorption increased according to the amount of clay mineral illite treated in the soil, and there was a case that reported a difference in the growth of red pepper [4]. For successful planting and planting of plants, selection of high-quality seeds as well as selection of appropriate cultured soil and thorough environmental management must be preceded. In particular, the cultured soil should have little physical change during the culture period, be chemically stable, have good water holding capacity, and have excellent ventilation [5]. The horticultural soil used for sowing and planting of urban agriculture and garden plants is made by mixing peat moss, perlite, coco peat, vermiculite and organic substances, so that it is suitable for general crop growth and container characteristics, the soil considered the physical and chemical content is used. However, the soil considering the growth characteristics of each plant type has not been developed, and the study on the suitability and economic feasibility of products for each plant is insufficient.

Rosemary (*Rosmarinus officinalis*) is an evergreen herb belonging to the family Lamiaceae, and it was introduced in Korea around 1980 [6]. Rosemary has gained popularity as a bonsai, but upright or trailing varieties are also cultivated in greenhouses [7-8]. Rosemary is native to the Mediterranean coast. Although rosemary cuttings are the most common propagule for cultivation, it can also be propagated using seeds [9]. It is commonly used in food, medicines, spices, cosmetics, and preservatives [10].

The antioxidant activity of rosemary extracts is mostly attributed to polyphenols. Polyphenols are secondary metabolites that are widely distributed in plants, and phenolics are synthesized as defense compounds under stress or in the presence of external injury, such as damage caused by herbivory [11]. Polyphenols contain an aromatic ring substituted with one or more hydroxyl groups, which generate hydrogen atoms to scavenge and eliminate free radicals, thereby exhibiting antioxidant effects [12]. Rosemary has a unique aroma and also exhibits strong antioxidant potential; therefore, it can be used in various fields, such as food manufacture and storage.

To this end, the purpose of the present study was to determine the optimum soil conditions for increasing the antioxidant activity and total phenolic and flavonoid content of rosemary during cultivation.

Materials and Methods

Plant cultivation

Rosemary plants measuring as 8 cm were cultivated under the following soil conditions: soil alone, soil + vermiculite (1:1), and soil + vermiculite + perlite (1:1:1). The soil used in this experiment was horticultural soil with a pH of 6.5 and electrical conductivity of 1.2 ds/m, vermiculite with a pH of 8, and perlite with a pH of 7.0. They were transplanted into pots and grown in growth chamber for 4 weeks under a 16/8 hour light/dark cycle at 25 ºC (Fig. 1). For the accuracy of the experiment, 5 plants were grown repeatedly per one condition. Water was irrigated twice a week.

Preparation of sample extracts

Rosemary herbs were collected and divided into the aerial and underground parts. The parts were dried at 60 ºC for 3 days and ground to powder with a blender. Extracts were prepared in 70% methanol and concentrated by filtration using filter papers (MN020250, HYUNDAI Micro Co., Seoul, Korea). The 10,000 ppm concentrated extracts were further dissolved in 70% methanol to prepare the samples for subsequent measurements.

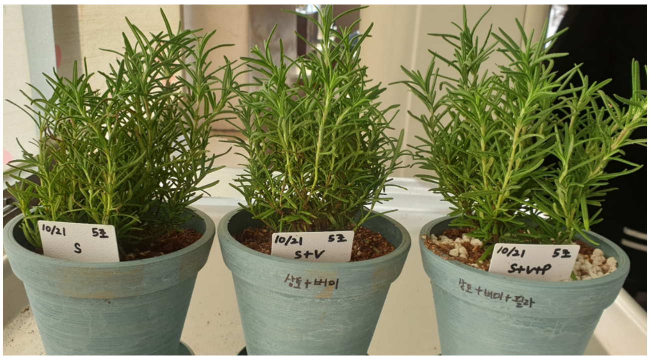

Fig. 1 Growth of rosemary in conditions of Soil, Soil+Vermiculite and Soil+Vermiculite+Perlite

Measurement of antioxidant activities

Antioxidant activity was measured using DPPH assay according to the experimental method described by Yoo et al. (2017) [13]. Briefly, 100 μL of 0.15 mM DPPH (Sigma-Aldrich Co., St. Louis, MO, USA) was added to 100 μL of 50 ppm sample and allowed to react for 30 min at room temperature in the dark. Next, absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Multiskan FC Microplate Photometer, Thermo Fisher Scientific Instruments Ltd., Waltham, MA, USA). DPPH radical scavenging activity was expressed as the RC_{50} value, and 10 $\mu g \cdot mL^{-1}$ ascorbic acid (Amresco LLC., Solon, OH, USA) was used as a control. Antioxidant activity was measured using ABTS assay according to the experimental method described by Jeon et al. (2016) [14]. Briefly, a 1:1 ABTS mixture [7.4 mM ABTS (Sigma-Aldrich Co., St. Louis, MO, USA) + 2.6 mM potassium persulfate (Daejung Chemicals and Metals Co., Ltd., Siheung, Korea)] was allowed to react with the samples for 24 hours at room temperature in the dark. Absorbance was measured at 732 nm using the UV-VIS spectrophotometer [Absorbance = 0.70 (±0.03)]. Next, 10 μL of the prepared solution was diluted with 990 μL of phosphate-buffered saline (pH 7.4) and allowed to react for 10 min. Absorbance was measured at 740 nm using the UV-VIS spectrophotometer. Absorbance of each sample at a concentration of 50 ppm was expressed as the RC_{50} values. As the control, 10 $\mu g \cdot mL^{-1}$ ascorbic acid was used.

Measurement of total phenolic content

The total phenolic content was measured using the Folin-Ciocalteu reagent, as described by Taga et al. (1984)[15]. Briefly, 50 μL of the Folin-Ciocalteu reagent was added to 100 μL of 1,000 ppm sample and allowed to react for 3-5 min. Next, 300 μL of 20% Na_2CO_3 (Junsei Chemical Co., Ltd., Tokyo, Japan) was added and allowed to react for 15 min. Then, 1 mL of distilled water was added, and the sample was centrifuged for 2 min. Finally, 200 μL aliquots of the supernatant were added to a 96-well plate, and absorbance was measured at 725 nm using the UV-VIS spectrophotometer.

Measurement of total flavonoid content

The total flavonoid content was measured using the method described by Kim et al. (2016)[16]. Briefly, 100 μL of 10% aluminum nitrate (Junsei Chemical Co., Ltd.) and 1 M potassium acetate (Junsei Chemical Co., Ltd.) were added into an E-tube containing 500 μL of 1,000 ppm sample and allowed to react for 40 min. Next, 150 μL aliquots of the sample were added to a 96-well plate, and absorbance was measured at 415 nm using the UV-VIS spectrophotometer.

Statistical analysis

All data are expressed as mean±standard of at least three replicates. Data were analyzed with Duncan's multiple range test using IBM SPSS Statistics v24 (SPSS, Chicago, IL, USA). Statistical significance was analyzed at a 5% probability level.

Results and Discussion

As a result of examining the growth of the above-ground part by type of mixed soil, the biomass was the largest at 14.8±0.4 cm in the condition in which the soil and vermiculite were combined (Table 1). Differences in growth according to soil types when other growing environments were the same have already been reported for medicinal plants such as *Bupleurum falcatum* L. [17].

DPPH and ABTS assays revealed that when cultivated in soil alone, aerial parts showed higher antioxidant activity than underground parts (Tables 2 and 3). When cultivated in soil alone, the antioxidant activities of aerial and underground parts were respectively 129.43±35.12 and 820.50±22.08 μg/mL in DPPH assay and respectively 483.91±17.46 and 1,178.20±85.28 μg/mL in ABTS assay. When cultivated in the mixture of soil and vermiculite, the antioxidant activities of aerial and underground parts were respectively 51.58±2.93 and 920.30±94.15 μg/mL in DPPH assay. When cultivated in the mixture of soil, vermiculite, and perlite, the antioxidant activities of aerial and underground parts were respectively 66.52±13.92 and 127.48±12.38 μg/mL in DPPH assay and respectively 424.71±34.57 and 320.98±16.04 μg/mL in ABTS assay. Based on the results of free radical scavenging ability, the aerial parts of rosemary exhibited a higher antioxidant activity than the underground parts. Moreover, cultivation in the mixture of soil and vermiculite or in the mixture of soil, vermiculite, and perlite increased the antioxidant activity of aerial parts.

To the best of our knowledge, no study has analyzed the antioxidant activity of rosemary cultivated under different soil conditions. In rosemary, cultivation through cuttings is difficult. Moreover, in the absence of relevant studies, research on the effects of soil is warranted. In previous ORAC and DPPH assays, only a slight difference in antioxidant activity under various soil conditions was observed in Tudla strawberries [18]. Similarly, there was little difference in antioxidant activity of *Salicornia herbacea* under various salt and soil treatments [12]. Moreover, there was no significant difference in the growth of *Salicornia herbacea* according to the type of artificial soil; however, simultaneous salt and complex fertilizer (Hyponex) treatments to soil increased fresh weight, dry weight, plant height, and branch number [19]. Based on previous reports, there were slight differences in growth depending on fertilization, whereas based on our results, there were differences in antioxidant activity, rather

Table 1 Plant growth characteristics of rosemary in different soils

Soil types	Plant length (cm)
SVP	12.7±0.3^{b}
SV	14.8±0.4^{a}
S	10.1±0.1^{c}

Table 2 Comparison of antioxidant activity of rosemary in different soils through DPPH assay

Soil types	Parts of plant	RC_{50} (μg/mL)
SVP	Ground part	66.52±13.92^{a}
	Underground part	127.48±12.38^{a}
SV	Ground part	51.58±2.93^{a}
	Underground part	920.30±94.15^{c}
S	Ground part	129.43±35.12^{a}
	Underground part	820.50±22.08^{b}

*Concentration required for 50% reduction of DPPH at 30 min after starting the reaction

Table 3 Comparison of antioxidant activity of rosemary grown in different soils through ABTS assay

Soil types	Parts of plant	RC_{50} (μg/mL)
SVP	Ground part	424.71±34.57^{b}
	Underground part	320.98±16.04^{ab}
SV	Ground part	230.34±57.93^{a}
	Underground part	1120.61±184.67^{c}
S	Ground part	483.91±17.46^{b}
	Underground part	1178.20±85.28^{c}

*Concentration required for 50% reduction of ABTS at 10 min after starting the reaction

Table 4 Content of total phenol and flavonoid of rosemary grown in different soils

Soil types	Parts of plant	mg GAE[1)]/g	mg QE[2)]/g
SVP	Ground part	52.32±1.41^{d}	20.92±0.96^{d}
	Underground part	88.95±2.60^{b}	24.91±0.69^{b}
SV	Ground part	155.25±2.96^{a}	67.32±5.27^{a}
	Underground part	27.42±2.05^{e}	8.97±0.10^{e}
S	Ground part	76.71±0.50^{c}	14.94±1.56^{c}
	Underground part	23.44±0.26^{f}	11.18±1.99^{f}

[1)]GAE: Gallic acid equivalent
[2)]QE: Quercetin equivalent

than simply growth, depending on soil conditions.

The total phenolic content of aerial parts of rosemary was the highest (155.25±2.96 mg GAE/g) when cultivated in the mixture of soil and vermiculite. The total phenol content of underground parts of rosemary was the highest (88.95±2.60 mg GAE/g) when cultivated in the mixture of soil, vermiculite, and perlite (Table 4). Similar results were observed for the total flavonoid content. The total flavonoid content of aerial parts of rosemary was the highest (67.32±5.27 mg QE/g) when cultivated in the mixture of soil and vermiculite. The total flavonoid content of underground parts of rosemary was the highest (24.91±0.69 mg QE/g) when cultivated in the mixture of soil, vermiculite, and perlite (Table 4). The total phenolic content of Maletto strawberries was the highest when grown in volcanic soil, whereas the total phenolic content of Tudla strawberries was reduced or remained unaffected depending on soil composition [11]. Flavonoid content of strawberries is strongly influenced by environmental conditions, such as soil composition, temperature, and light intensity [20].

In our study, antioxidant activity and total phenolic content were higher in plants cultivated in soil mixed with vermiculite and/or perlite than in plants cultivated in soil alone. Therefore, such differences in total phenolic content may be closely linked to soil conditions. Plants grown in three soils with different properties under conditions have dissimilar water retention capacity and photosynthesis, so the amount of phytochemicals in the plant will vary. The correlation between the ground and the underground part did not show a consistent trend, but the sample with a high phenol content showed a correlation with an increased flavonoid content. Phytochemicals in plants are targeted to the necessary places in plant cells and it is thought that the appropriate soil type for each plant plays an important role in order to exert its efficacy. This is because the proper combination of organic raw material soil and inorganic vermiculite not only increases the antioxidant activity of plants, but also increases the total phenol and flavonoid content as a high-quality plant material. Basic data of standard cultivation methods for soil use is expected to be provided. In conclusion, to enhance the antioxidant properties of rosemary for use as a raw material for functional foods, soil selection appears to be the key step during cultivation.

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