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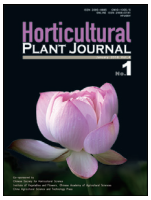

Effect of Different Cultivation Systems on the Accumulation of Nutrients and Phytochemicals in *Ligularia fischeri*

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A B S T R A C T

A hydroponic cultivation system was established to improve the nutraceutical properties of *Ligularia fischeri* (Ledeb.) Turcz, during which nutrient uptake by the plant from nutrient solution was measured using inductive coupled plasma-atomic emission spectroscopy (ICP-AES). Based on the obtained data, the uptake of macro and micro elements per gram of fresh weight was calculated. The uptake of macro-elements of NH_4^+-N, NO_3^--N, K, S, P, Ca, and Mg were 1.62, 4.27, 8.41, 1.19, 2.59, 2.79, and 0.84 mg·g^{-1} FW and micro-elements of B, Fe, Mn, Mo, Cu, and Zn were 9.91, 22.31, 25.73, 2.51, 2.91, and 5.07 μg·g^{-1} FW. Moreover, the effects of cultivation systems on growth and phytochemical composition of *L. fischeri* were compared. The greatest biomass was observed in the hydroponic cultivation system with continuous circulation nutrient solution compared to natural soil and Tosilee media based culture systems. The recirculated hydroponic system significantly increased the total phenol contents of the leaf, petiole, and root extracts by 17.6%, 30.6%, and 20.9% more compared to the soil grown. The recirculated hydroponic system treatment significantly increased the total antioxidant capacity of root extracts by 55.9% more compared to the soil treatment. Based on the contents of elements, total phenolic and flavonoid, it was concluded that hydroponic cultivation system is the optimal method to enhance medicinal value.

Keywords: *Ligularia fischeri*; hydroponic culture; micronutrient; macronutrient; phytochemical

1. Introduction

Ligularia fischeri (Ledeb.) Turcz is a perennial herbaceous plant which belongs to *Ligularia*, a class of composite family and distributed widely in South Korea, Japan, China, and Eastern Siberia. It usually grows on wood, water, or rocks at an elevation of 1 000 to 1 400 m. The roots and rhizomes of *L. fischeri* contain pharmaceutically important sesquiterpene compounds used in the treatment of cough, promoting blood circulation, relieving pain, etc. (Li et al., 2011). Further, the medicinal extracts prepared from the plant can be used to cure cough, sputum, sore throat, pulmonary tuberculosis, traumatic injury, etc. Owing to its numerous medicinal properties, the wild *L. fischeri* plant has been widely threatened by over exploitation by humans (Du and Li, 2005). However, the conventional cultivation of *L. fischeri* by seed propagation is difficult due to dormancy issues whereas the production of artificial planting of *L. fischeri* is low.

In order to develop and utilize the medical value of *L. fischeri* and to improve the yield of artificial cultivation, the application of hydroponic technology to the artificial cultivation has been widely utilized. Hydroponics is a convenient means for studying plants in the laboratory, for growing commercial crops and easily manipulating the plant's secondary metabolism for increased pharmacological activities of plant materials. The advantages of hydroponics include the cultivation of identical plant material around the year in a control environment, the possibility for

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enhanced phytochemicals, and protection from agrochemicals (pesticides, fungicides, etc.) to control different diseases in the open-air environment. Hence in the present endeavor, a simple, inexpensive, flexible, and robust hydroponics system for the cultivation of *L. fischeri* was established, which addresses the above-mentioned considerations.

2. Materials and methods

2.1. Plant materials and culture conditions

Seeds of *L. fischeri* were sown in 200-cell plug trays containing a commercial medium (Tosilee Medium, Shinan Grow Co., Korea) in 3rd April 2015 and maintained in a controlled-glasshouse (Gyeongsang National University, Korea) at 25 °C/18 °C day/night and 80% relative humidity. In the first experiment, 30-day-old plants were cultivated in magenta boxes wrapped by aluminum foil. The plants were supplied with a greenhouse multipurpose nutrient solution in $Ca(NO_3)_2{\cdot}4H_2O$ 467.6, KNO_3 232.3, KH_2PO_4 272.0, K_2SO_4 17.4, $MgSO_4{\cdot}H_2O_2$ 209.1, NH_4NO_3 80.0, Fe-EDTA 15.0, H_3BO_3 1.4, $NaMoO_4{\cdot}2H_2O$ 0.12, $MnSO_4{\cdot}4H_2O$ 2.10, $ZnSO_4{\cdot}7H_2O$ 0.80, $CuSO_4{\cdot}5H_2O$ 0.20 [$mg{\cdot}L^{-1}$]. One plant in each 350 mL box (GA_7 Magenta Box, Sigma Chemical Co., USA) filled with a 300 mL nutrient solution using a dispenser (Calibrex digital dispenser, Socorex 1SBA SA, Switzerland) was anchored and aerated with an air pump (DK-8000, Daekwang, Korea). Two holes were punched on the lid, one at the center and the other on the side, playing the role of holding the plant and providing aeration. The electrical conductivity (EC) and pH of the nutrient solution in each container was adjusted to 0.17 $S{\cdot}m^{-1}$ and 5.8, respectively (Fig. 1). Each treatment was repeated three times.

For the comparison of phytochemical contents, 30-day-old plants were transplanted into a recirculated hydroponic system (T1), Tosilee medium (T2), or natural soil (T3). During the 4-week culture period, 35 L of a greenhouse multipurpose nutrient solution was given to each treatment. Each treatment was repeated three times. For uniform culture conditions, all plants were maintained in Gyeongsang National University (GNU) glasshouse at 25 °C /18 °C and 80% relative humidity. At the end of the experiment, plant height, length of the longest root, leaf length, leaf width, and fresh weight of shoot and root were recorded. Content of chlorophyll was measured on mature leaves with a chlorophyll meter (SPDA-502, Konica Minolata Sensing Inc., Japan).

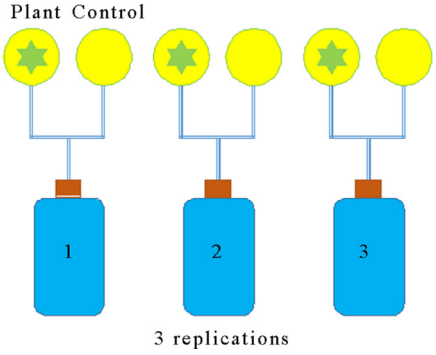

Fig. 1 Graphical representation of culture condition

2.2. Analysis of macro-and micro-elements in nutrient solution

At the end of each week, nutrient solution from hydroponics was sampled and passed through inductively coupled plasma-atomic emission spectroscopy (ICP-AES; Optima 4300DV/5300DV, Perkin Elmer, USA) system to quantify different macro and micro nutrients of nutrient solution. Based on obtained data, the uptake of macro and micro elements per gram of fresh weight in each week was calculated.

2.3. Analysis of macro-and micro-elements in plant samples

Plant leaf samples were put in a dry oven and dried at 70 °C for 72 h. Dried leaf samples were ground with a stainless mill (Cytcloec, model 1093, Tector, Sweden) and used for analysis. The powdered plant sample (0.5 g) in a porcelain crucible was ashed in the Nabertherm muffle furnace (Model LV 5/11/B180, Lilienthal, Germany) for 4 h at 525 °C. The obtained ash was dissolved in 25% HCl (5 mL) solution, followed by 20 mL of hot ddH_2O, and the final volume was adjusted to 100 mL with ddH_2O. Calcium (Ca), magnesium (Mg), potassium (K), phosphors (P), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) in leaf samples were measured by using an inductively coupled plasma atomic-emission spectroscopy (ICP-AES) (Optima 4300DV/5300DV, Perkin Elmer, USA).

2.4. Analysis of phytochemicals

For phytochemical and antioxidant assays, the leaf, petiole, and root were harvested after a 4-week culture and extracted with methanol according to Gracia-Perez et al. (2012) with slight modifications. Briefly, one gram of fresh plant tissue was homogenized using liquid nitrogen and extracted with 10 mL of 80% methanol upon continuous shaking (Shaking Incubator, Model 200FL, Koencon, Korea) for 5 h at 200 $r{\cdot}min^{-1}$. The extract was subsequently centrifuged at 10 000 $r{\cdot}min^{-1}$ for 10 min and the supernatant was used for analysis right after centrifugation or saved at 4 °C for afterword analysis.

The total phenol content (expressed as gallic acid equivalent) of the extract was estimated by the Folin-Ciocalteu (FC) principle according to Kumaran and Karuna Karan (2007). The aliquot of the extracts (0.1 mL) made up to 1 mL with ddH_2O was mixed with 0.5 mL of Folin-Ciocalteu reagent (1:1 with ddH_2O) and 2.5 mL of sodium carbonate solution (7.5%). The reaction mixture was incubated in the dark for 40 min and then absorbance was recorded at 725 nm. The total phenol content was estimated using standard gallic acid calibration curve.

The total flavonoid content (expressed in quercetin equivalent) was determined by aluminum chloride method outlined by Gracia-Perez et al. (2012). Samples (0.1 mL) made up to 1 mL with 80% methanol and were used for analysis by adding 1 mL of 2% aluminum chloride solution. The absorbance of the reaction mixture was measured at 415 nm after 30 min incubation and the total flavonoids were determined from the standard quercetin calibration curve.

2.5. Analysis of antioxidant properties

The total antioxidant activity of the extracts was analyzed using the phosphormolybdenum method according to