P03-034

The Influence of Dark and Light Irradiation on the Accumulation of Rosmarinic Acid and In Vitro Antimicrobial Activities in *Perilla frutescens* Microgreens

Seom Lee[1)], Sang Yeob Lee[1)] , and Chang Ha Park[1),*]

1)Department of Biological Sciences, Keimyung University, Daegu 42601, Korea.

Introduction

Perilla frutescens, known as the Korean perilla or Dlggae, is a member of the Labiatae family distributed in China, Korea, and Japan. This plant is an annual herbaceous plant and has been used for medicinal plants and foods [1]. Its seeds are commercially used for perilla seed oil [2] as well as its leaves are used for perilla essential oil, a culinary garnish, and seasoning [3,4]. Traditionally, P. frutescens has been a medicinal herb for the treatment of allergy, asthma, cough, depressive disorders, tumor, intoxication, fever, shiver, headache, congested nose [4]. The use of P. frutescens as a herb medicine may be due to its strong antioxidant [5], anticancer [6], antiallergic [7], antibacterial [8] properties and these biological activities may be derived from secondary metabolites biosynthesized in P. frutescens. Hou et al., 2022 listed secondary metabolites (alkaloids, terpenes, phenolics, glycosides, and benzoxipen derivatives) found in seeds, leaves, stem, and fruits of P. frutescens [2] and reported its biological functions. For example, Lee et al., 2021 identified caffeic acid, ferulic acid, and rosmarinic acid in this plant and reported the positive correlation between its antioxidant ability and composition of phenolic acids (caffeic acid and rosmarinic acid) [5] and rosmarinic acid and caffeic acid has been considered effective antioxidants against free radicals [9]. Furthermore, rosmarinic acid, caffeic acid, luteolin, apigenin, methoxyflavanone, and alpha-linolenic acid present in P. frutescens was reported to have anti-allergic property [10], rosmarinic acid of P. frutescens leaves has been shown antimicrobial activity against bacterial and fungal pathogens [11], as well as the activity of P. frutescens leaf extract toward apoptosis of hepatic carcinoma (Hep-G2) cells was due to rosmarinic acid, caffeic acid, luteolin, and triterpenes [12].

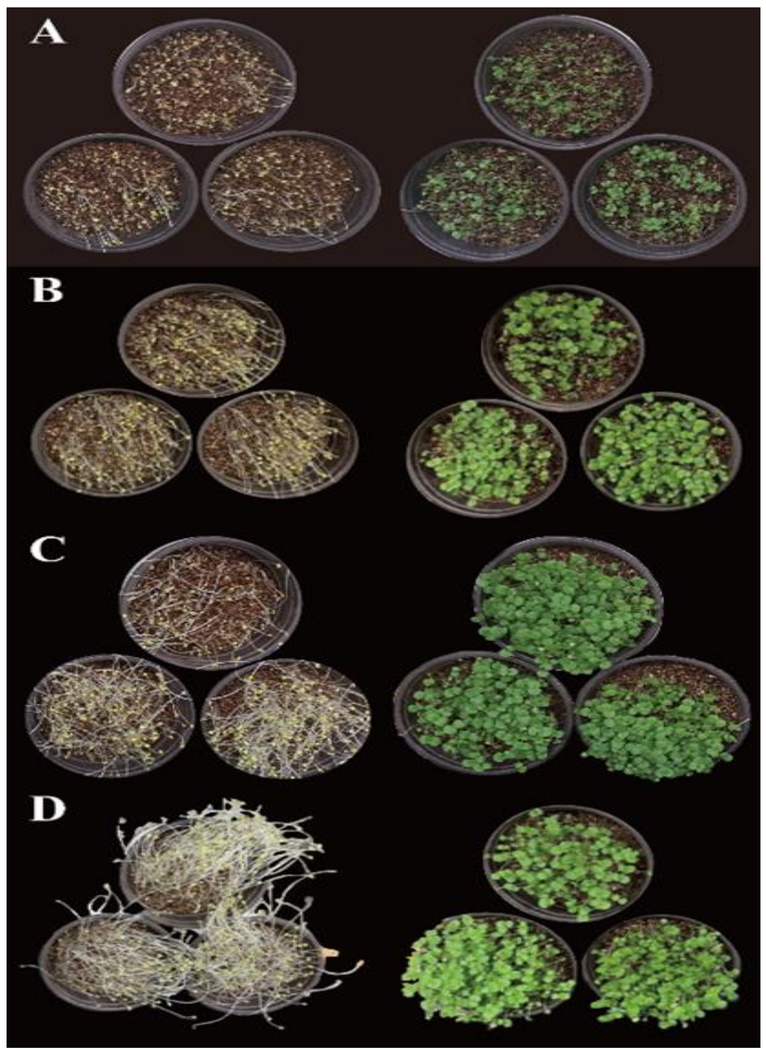

Figure 1. *P. frutescens* microgreens grown under long-day photoperiod or continuous darkness conditions. (A) Microgreens grown for 10 days under dark condition (left) and microgreens grown for 10 days under long-day photoperiod condition (right), (B) microgreens grown for 15 days under dark condition (left) and microgreens grown for 15 days under long-day photoperiod condition (right), (C) microgreens grown for 20 days under dark condition (left) and microgreens grown for 20 days under long-day photoperiod condition (right), and (D) microgreens grown for 25 days under dark condition (left) and microgreens grown for 25 days under long-day photoperiod condition (right).

Results

Table 1. The effect of light and dark treatment on dry weight of *P. frutescens* microgreens.

Exposure time (d)	Dry weight (g)	
	Light	Dark
10 days	0.51 ± 0.05c[1]	0.49 ± 0.18b
15 days	0.86 ± 0.08b	0.71 ± 0.05ab
20 days	1.01 ± 0.10a	0.90 ± 0.16a
25 days	0.99 ± 0.05ab	0.87 ± 0.12a

Table 2. Production of rosmarinic acid in *P. frutescens* microgreens grown under long-day photoperiod or continuous darkness conditions.

Treatment	Duration	Rosmarinic acid (mg/g dry weight)
Dark	10 days	9.01 ± 7.36 b[1]
	15 days	13.01 ± 2.82 b
	20 days	22.66 ± 0.55 a
	25 days	13.75 ± 20.68 b
Light	10 days	21.89 ± 0.80 a
	15 days	20.18 ± 1.40 a
	20 days	21.08 ± 1.20 a
	25 days	20.68 ± 2.93 a

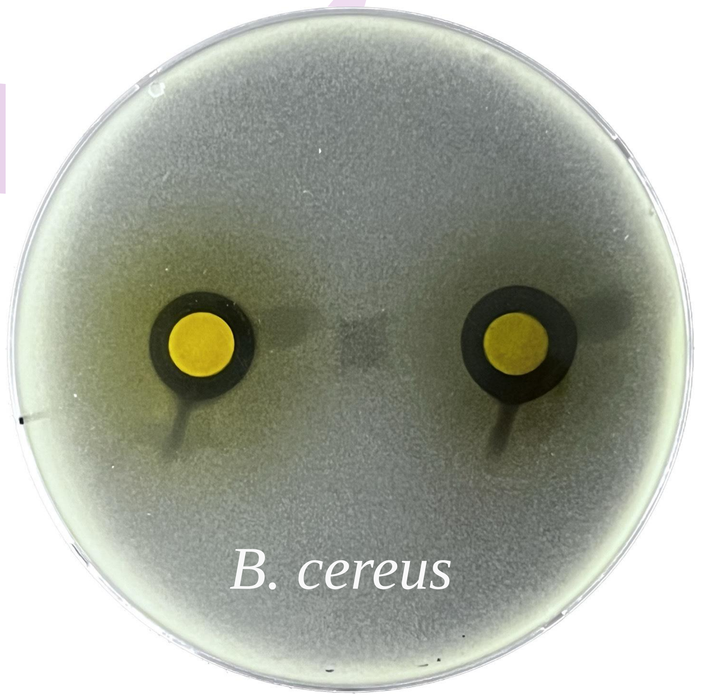

Figure 2. Representative images showing antibacterial activities of methanol extracts of *P. frutescens* microgreens for 20 days of long-day photoperiod and continuous darkness conditions.

Discussion

Perilla frutescens (known as Korean perilla) belongs to the Labiatae family and is annual herbaceous plant species, traditionally used as medicinal plant or food. P. frutescens has been known to possess high amount of phenolic compounds, such as rosmarinic acid and caffeic acid, and has various phenolics exhibiting various biological functions including antioxidant, anticancer, antidepressant, and antimicrobial activities. This study aimed to investigate the effect of light [a long-day photoperiod (16 h light/8 h dark cycle)] and dark treatment on the production of rosmarinic acid in P. frutescens microgreens and determine antioxidant and antibacterial activities. The microgreens of P. frutescens were grown under light treatment and darkness treatment and harvested after 10, 15, 20, and 25 days of each treatment. Though dry weight values of microgreens gradually increased from 10 days of 25 days of both treatment, the microgreens grown under light treatment possessed slightly higher levels of dry weight than those of microgreens under darkness conditions. Furthermore, rosmarinic acid and total phenolic contents (TPC) were analyzed. The accumulation patterns of rosmarinic acid and TPC gradually increased and decreased in in P. frutescens microgreens grown under the continuous darkness conditions. The highest accumulation was observed in the microgreens grown for 20 days. However, the values of rosmarinic acid and TPC were not significantly different in the microgreens grown under light conditions. According to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical inhibition assay, the extracts of P. frutescens microgreens were confirmed strong antioxidants and the abilities to scavenge DPPH radicals were positively correlated with the concentrations of total phenolic contents in the microgreens after 10, 15, 20, and 25 days of both treatment. Considering relatively higher values of dry weight, rosmarinic acid, TPC, and DPPH assay, P. frutescens microgreens after 20 days of darkness and 20 days of light treatment, respectively, were selected for screening antibacterial activity using nine pathogens. The extracts of both microgreens revealed strong antibacterial activity against B. cereus (KCTC 3624), E. coli (KCTC 1682), P. aeruginosa (KCCM 11803), S. aureus (KCTC 3881), V. parahaemolyticus (KCTC 2471), and P. aeruginosa (1113). In particular, the extracts of microgreens grown in 2- days of light treatment showed higher antimicrobial effects. Therefore, the light treatments for 20 days as well as the darkness treatment for 20 days were the best conditions for P. frutescens microgreens production due to their high levels of dry weight, phenolics, and biological activities.

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