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Effects of Carbohydrates on Rosmarinic Acid Production and In Vitro Biological Activities in Seedlings of *Perilla frutescens*

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Introduction

Dlggae (Perilla frutescens) belongs to the Lamiaceae family which is chiefly found in East Asia and has been used as curative plants and food [1]. In particular, dlggae seeds and leaves are commercially beneficial due to use of perilla oil, essential oil, seasoner, and garnish [2-4]. Since ancient times, dlggae has been considered a medicinal plant to treat various diseases (allergy, asthma, cough, depression, tumor, intoxication, fever, shivering, headache, and nasal stuffiness). It might be because of strong biological activities (antioxidant [5], anticancer [6], and antibacterial [7] effects) originated from bioactive compounds produced in dlggae.

Previous studies reported the presence of various secondary metabolites and its biological functions. For example, Dlggae has secondary metabolites, including alkaloids, phenolics, terpenes, and glycosides etc., in fruit, leaf, stem, and seed [2]. It has been confirmed that rosmarinic acid, ferulic acid, and caffeic acid were detected in this crop and that the accumulation of caffeic acid and rosmarinic acid were positively correlated with its antioxidant activity [5]. Particularly, rosmarinic acid have regarded as an efficient antioxidant agent [8]. Furthermore, antimicrobial activity against pathogens [9] by rosmarinic acid of dlggae leaf has been confirmed as well as apoptogenic activity in hepatocarcinoma (Hep-G2) cells has been observed due to secondary metabolites (caffeic acid, rosmarinic acid, triterpenes, and luteolin) in dlggae leaf extract [10].

Rosmarinic acid is a type of caffeic acid ester, which is present in herbs (Salvia Rosmarinus, Melissa officinalis, Ocimum basilicum, and origanum vulgare etc.) at the Labiatae family [11]. In plant rosmarinic acid biosynthesis, 4-coumaroyl-CoA and 4-hydroxyphenyllactic acid can be formed from amino acids (phenylalanine and tyrosine, respectively), followed by condensation into 4-coumaroyl-4′-hydroxyphenyllactic acid by rosmarinic acid synthase. This intermediate can be converted to rosmarinic acid by CYP98A14 [12]. Rosmarinic acid has been reported to have many biological functions,

seedlings, which are defined as plantlets between sprouts and baby greens, contains high amounts of useful phytochemicals. In addition, eatable seedlings have been used in food (seasoner and garnish etc.) and have been reported to possess often more amount of nutrients and phytochemicals than their seed or adult plant [15]. The previous study described that seedlings of p. frutescens var. frutescens and p. frutescens var. crispa are more volatile-dense than their four-week plants (four-week plants) [16]. Additionally, seedlings has been shown high levels of health-maintaining biological activity and phytochemical in the medicinal or herb plants, such as Trigonella foenum-graecum [15,17] and Panax ginseng [15,18]. Thus, seedlings derived from the medicinal or herb plants can be a fount of health-valuable functional foods.

⟐Exogenous supply of monosaccharide and disaccharide have been reported to affect metabolite production in plant materials. Increase in glucosinolate and phenolic compound production in radish sprouts [19] and increase in glucosinolate and anthocyanin production in broccoli sprouts [20] have been observed after treatment of different carbohydrates [19, 20]. In addition to sprouts, the enhancement of secondary metabolite production have been reported *Sccutellaria baicalensis* hairy roots [21], *Taxus chinensis* cell cultures [22] and *Morinda citrifolia* adventitious roots [23].

Results

Figure 1. *P. frutescens* seedlings grown different carbohydrate treatment.

Table 1. Production of rosmarinic acid and total phenolc contents in *P. frutescens* seedlings treated with sucrose, galactose, mannose, maltose, and glucose.

	Total phenolics	Rosmarinic acid
Control	53.50 ± 3.63	22.66 ± 3.63
Sucrose	48.87 ± 1.54	20.70 ± 1.54
Galatose	47.45 ± 1.83	20.10 ± 1.83
Mannose	61.40 ± 1.12	26.00 ± 1.12
Maltose	49.62 ± 3.83	21.02 ± 3.83
Glucose	51.00 ± 1.01	21.60 ± 1.01

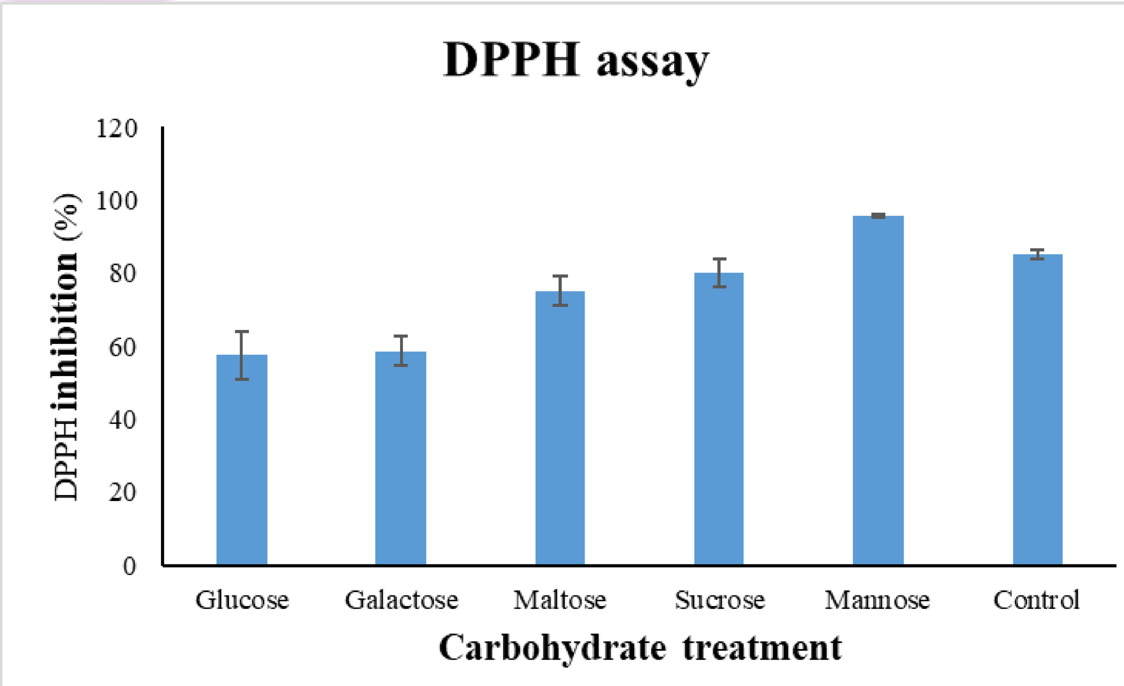

Figure 2. TPC and DPPH assay of *P. frutescens* seedlings treated with sucrose, galactose, mannose, maltose, and glucose.

Figure 3. Representative image showing antibacterial activity of *P. frutescens* seedlings treated with sucrose, galactose, mannose, maltose, and glucose.

Discussion

Rosmarinic Acid Production and Total Phenolic Contents in P. frutescens Seedlings

Even if the exogenously supply of different carbohydrates increase in secondary metabolite accumulation in plants has been confirmed, to date, there are little studies on the effect of different sugars on rosmarinic acid production in dlggae seedlings. Thus, this study is aimed to optimize seedling cultivation condition for improvement of biomass, rosmarinic acid production, and its biological activities in dlggae.

Table 1 shows the patterns of rosmarinic acid and total phenolic contents in *P. frutescens* seedlings grown under different carbohydrate treatments. The total phenolic contents in *P. frutescens* seedlings grown under carbohtydrate treatment ranged from 47.45 ± 1.83 mg/g DW (galactose treatment) to 61.40 ± 1.12 mg/g DW (mannose treatment). Furthermore, Rosmarinic acid values in *P. frutescens* seedlings grown under carbohtydrate treatment ranged from 20.10 ± 1.83 mg/g DW (galactose treatment) to 26.00 ± 1.12 mg/g DW (mannose treatment).

The DDPH free radical inhibition activity was assessed with methanol extracts of *P. frutescens* seedlings grown under mannose treatments (Figure 2). Bacterial growth was inhibited by methanol extracts of *P. frutescens* microgreens grown under both treatments on an agar medium containing *B. cereus* (KCTC 3624), *E. coli* (KCTC 1682), *P. aeruginosa* (KCCM 11803), *S. aureus* (KCTC 3881), and *K. pneumoniae* subsp. *pneumonia* (KCTC 2690).

In particular, extracts of P. frutescens microgreens grown mannose higher antibacterial effects against *B. cereus* (KCTC 3624), *E. coli* (KCTC 1682), *P. aeruginosa* (KCCM 11803), *S. aureus* (KCTC 3881), and *K. pneumoniae* subsp. *pneumonia* (KCTC 2690).

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