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Improved bioactivity and composition of *Cordyceps militaris* cultured with *Panax ginseng*

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Abstract

Cordyceps militaris is a commonly used edible and medicinal fungus in East Asia. Preparation of the mycelium and fermentation liquid by submerged fermentation using additives such as *Panax ginseng* for skin whitening applications has attracted wide attention. In the present study, we screened traditional Chinese medicines as additives to the liquid fermentation medium for *C. militaris* and performed systematical research on the changes of bioactivity and ginsenoside levels during the fermentation process. Total polysaccharide contents, cordycepin content, and polysaccharide molecular weight in the fermentation products were measured by UV spectrophotometry, high-performance liquid chromatography-diode array detection (HPLC/DAD), and gel permeation chromatography-multi angle laser light scattering (GPC-MALLS), respectively. In addition, the ginsenosides Rb1, Re, Rd, Rg1, and pseudoginsenoside F11 in the fermentation products were quantitatively analyzed by ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS). *Panax ginseng* significantly increased the cordycepin and total sugar contents, and the average molecular weight of polysaccharides in the fermentation liquid and the mycelium of *C. militaris*. At the same time, *C. militaris* enhanced the production of ginsenoside pseudoginsenoside F11, which is a unique component of *Panax quinquefolium*. The research provides scientific evidence for the application of *C. militaris* and *P. ginseng* as raw materials for food and medicines.

Keywords: *Cordyceps militaris*; *Panax ginseng*; fermentation; melanin inhibition; table.

Practical Application: Improved pseudoginsenoside F11 upon fermentation.

1 Introduction

Skin pigmentation is influenced by various factors including melanin content, melanin metabolism, oxyhemoglobin content, carotenoid content, skin water content, and collagen in the dermis. Among these factors, the tyrosinase level and melanin content of B16F10 cells play an important role in skin pigment expression and skin whitening (Kim, 2015). *Cordyceps militaris* is a widely used fungal and food supplement in East Asia. Its anti-tumor and anti-fatigue properties have been extensively studied. Nucleosides, cordycepin, and polysaccharides are the primary active components of *C. militaris,* which perform a wide range of physiological functions (Aramwit et al., 2015; Zhang et al., 2015). Prior studies have reported that extracts from *C. militaris* fruiting bodies and cordycepin can significantly inhibit tyrosinase activity and reduce the melanin content of B16F10 cells stimulated by α-melanocyte-stimulating hormone (α-MSH) in a dose-dependent manner. The reduction in melanogenesis is believed to decrease the level of microphthalmia-associated transcription factor (MITF) of B16F10 (Dong et al., 2018). Fermentation of *C. militaris* with a liquid medium is a common method to extract the pharmaceutically active compounds of *C. militaris* including cordycepin. In recent years, it has been reported that traditional Chinese medicines, such as powders of *Portulaca oleracea*, *Houttuynia cordata*, *Phellodendron amurense*, *Atractylodes lancea*, *Forsythia suspensa*, and *Angelica sinensis*, have been added to the fermentation medium during the traditional fermentation process to improve the microbial transformation of active compounds and their relevant bioactivity levels (Lee et al., 2012).

Panax ginseng is a traditional medicine used to enhance immunity, which is therapeutically beneficial in terms of invigorating Qi, relieving fatigue, and strengthening the physique. The traditional functions of *P. ginseng* are similar to those of *C. militaris*. The fermentation of *C. militaris* along with *P. ginseng* may, therefore, exert a synergistic effect on the production of these medicines. According to prior literature, *P. ginseng* extracts, ginsenosides, and polysaccharides are capable of inhibiting melanin synthesis and metabolism. Ginsenosides Rb1, F1, Rd, Re, Rg1, and Rh4 are the key active constituents with melanin-inhibition activity (Kim, 2015).

To investigate the melanin-inhibition activity of the fermentation liquid and mycelium of fermented *C. militaris*, 13 kinds of traditional Chinese medicinal powders were added to the fermentation medium during the traditional fermentation process. The interaction with *P. ginseng* was further investigated with regard to its chemical composition and its utility in skin-whitening applications.

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2 Materials and methods

2.1 Fungal strain and raw materials

C. militaris (CICC14013) samples were purchased from the China Center of Industrial Culture Collection and stored at 4 °C in the Beijing Key Laboratory of Plant Resources Research and Development (Beijing Technology and Business University, Beijing, China). The fruiting bodies were obtained from the official flagship store of Baozhilin online.

The Chinese herbal medicines of *Portulaca oleracea*, *Houttuynia cordata*, *Atractylodes lancea*, *Forsythia suspensa*, *Angelica sinensis*, *Morus alba* (mulberry leaf), *Ligustrum lucidum*, *Glycyrrhiza uralensis*, and *Rehmannia glutinosa* were purchased from a Tong Ren Tang pharmacy in Beijing. *Fallopia multiflora*, *Clinopodium megalanthum*, and *P. ginseng* were purchased from a Chengdu Deyin traditional Chinese medicine supermarket in Sichuan. *Ginkgo biloba* was purchased from a Hebei Anguo wholesale market of traditional Chinese medicine. *C. militaris* fruiting bodies were purchased from Zhejiang Baozhilin Traditional Chinese Medicine Technology Co., Ltd.

Mushroom tyrosinase was purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD (China). Cell counting kit-8 (CCK-8) assay kit was purchased from Tongren Institute of Chemistry (China). L-Tyrosine and arbutin were purchased from Bailingwei Technology Co., Ltd (Beijing, China). Reference substances (ginsenosides Rg1, F11, Rd, Re, and Rb1) were procured from Nanjing Caobenyuan Biotechnology Co., Ltd. Cordycepin was procured from Saipu Ruisi Technology Co., Ltd (Beijing, China). Methanol used for high-performance liquid chromatography (HPLC) analysis was purchased from Merck (Germany).

2.2 Optimization of culture medium and culture conditions

C. militaris (4.0 g) was inoculated on a potato dextrose agar (PDA) slant. The slant was grown for 7 days at 25 °C, whereupon the 5-mm mycelium block was picked up from the PDA plates and moved to the seed culture medium. The seed culture was grown on a rotary shaker incubator (150 rpm) in a 250 mL flask with 100 mL seed culture medium. Subsequently, the following components were added to the seed culture medium: 20 g/L sucrose, 10 g/L yeast extract, 20 g/L peptone, and distilled water for 3 days at 25 °C. The experiments were performed in a 250 mL flask with 100 mL basal culture medium, inoculated with 4% (v/v) of the seed liquid. The following components were added to the basal culture medium: 20 g/L glucose, 40 g/L peptone, 0.5 g/L KH_2PO_4, 0.15 g/L MgSO4·7H2O, and distilled water. Traditional Chinese medicines (2.0 g) were added to the culture, and fermentation was carried out for 7 days. All experiments were performed in triplicate to ensure reproducibility.

2.3 Extraction of the mycelium, the fruiting bodies of C. **militaris** *and* **P. ginseng**

The products in the fermentation shaker were centrifuged (5000 g, 15 min) to obtain the supernatant (SF) and the mycelium (MF). The mycelium was freeze-dried and weighed, following which the soluble compounds were extracted with 1-h boiling water treatment (mycelium/water 1:20). The filtrate from the extraction was concentrated and dried to obtain the mycelium extract (MFE). The crude polysaccharide of the fruiting bodies was extracted with 1-h boiling water treatment (fruiting body/water 1:20). *P. ginseng* extract (R1) was also extracted with 1-h boiling water treatment (*P. ginseng*/water 1:20).

2.4 Tyrosinase assay

The effects produced by the MFs with *P. ginseng* (S1) and without *P. ginseng* (S2), and the SFs with *P. ginseng* (S3) and without *P. ginseng* (S4), as well as the effect of cordycepin on mushroom tyrosinase inhibition activity, were determined via spectrophotometry using a previously described method (Choudhary & Thomsen, 2001), with minor modifications, using L-Tyrosine as the substrate. Briefly, 400 μL L-Tyrosine, the samples at three concentrations (0.5, 1.0, or 2.0 mg/mL), and 0.1 M phosphate buffer (pH 6.8) were added to each well of a 96-well plate and mixed. The mixture was incubated at 37 °C for 10 min. Next, 400 μL of 1000 U/mL mushroom tyrosinase in 0.1 M phosphate buffer was added to each well and the reaction was continued at 37 °C for 25 min. The reaction of complex was determined against a blank solution at 475 nm on a microplate reader (Infinite M200PRO, Tecan) and compared with the positive control, 0.1% Arbutin.

2.5 Cell culture

B16F10 mouse melanoma cells (purchased from the Cell Bank of the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences) were cultured at 37 °C in DMEM supplemented with 10% FBS (GEMINI, Woodland, California, USA) in a humidified atmosphere of 5% carbon dioxide.

2.6 Cell viability assay

The cell viabilities were evaluated using a CCK-8 assay kit. B16F10 cells were pretreated with the S1-S4 extracts, and cordycepin at various concentrations. After incubation for 48 h, CCK-8 solution (10 μL) was added, followed by an additional round of incubation for 1.0 h at 37 °C. Finally, the absorbance of each sample was measured at 450 nm using a spectrophotometer to analyze the percentage of viable cells.

2.7 Analysis of melanin content

Melanin content was tested according to a previously described method with some modifications (Hosoi et al., 1985). B16F10 melanoma cells were plated (2.0×10^5 cells/well in 2.0 mL medium) in Costar 6-well culture plates (Corning, NY, USA) for 12 h. Thereafter, the cells were exposed to various concentrations of either the test extracts or arbutin (the control) for 48 h. The cells were then washed with phosphate buffer. The cells were lysed using 1 M NaOH containing 10% DMSO for 30 min at 80 °C. The absorbance of the mixer was measured spectrophotometrically at 475 nm. Melanin content was calculated according to the following Formula 1:

$$\text{melanin content } (\%) = OD475_{sample} / OD475_{control} \times 100 \quad (1)$$

2.8 Measurement of total polysaccharides

The aqueous extracts (S1-S4) were dissolved and mixed with four times the volume of 95% ethanol (*v/v*), stably maintained overnight at 4 °C, and then centrifuged at 5000 × *g* for 15 min to collect the precipitate. The crude polysaccharides were obtained via freeze-drying the precipitate. The total polysaccharide content of samples S1-S4 was determined using an improved phenol-sulfuric acid method (Fang et al., 2011). UV-vis spectrophotometric measurements were determined at 490 nm using a microplate reader (Infinite M200PRO, Tecan). The total polysaccharide content was determined as glucose/g using a calibration curve. Each sample was analyzed in triplicate.

2.9 Determination of the molecular weight of polysaccharides

Gel permeation chromatography-multi angle laser light scattering (GPC-MALLS) was used to determine the molecular weight of the polysaccharides (Mw) and polydispersity of the molecular weight (Mw/Mn) of the samples. The crude polysaccharides were further purified upon deproteinization using Sevage reagent (chloroform/*n*-butanol, 4:1 *v/v*). The resulting mixture was added to three volumes of 95% (*v/v*) ethanol, stirred overnight at 4 °C, and then centrifuged at 5000 × *g* for 15 min to collect the precipitate. The purified precipitate obtained from the crude polysaccharide of the fruiting bodies is referred to as P1 while the precipitates obtained from S1-S4 are termed P2-P5, respectively. Each sample was dissolved in the mobile phase (0.1 M $NaNO_3$ solution) at a concentration of 3.0 mg/mL for analysis via GPC-MALLS using a Waters HPLC system (Milford, MA, USA) equipped with Shodex SUGER KS-805/KS-803 columns and detected using a Waters e2695 refractive index detector and laser-scattering system at 50 °C. Samples (100 µL) were injected into the columns and eluted with deionized water at a flow rate of 0.8 mL/min. The molecular weight (Mw) was obtained from a β-glucan calibration curve (Wang et al., 2018).

2.10 The content of cordycepin determined by HPLC/DAD

Agilent 1260LC series system (Agilent Technologies, Palo Alto, CA, USA) equipped with an online vacuum degasser, quaternary pump, autosampler, column incubator, and diode array detector (DAD) was used to analyze the cordycepin content. Chromatographic separation was performed using an Agilent ZORBAX SB-C18 column (4.6 × 250 mm, 5 µm). The detection wavelength was set to 260 nm. The mobile phase consisted of water (A) and acetonitrile (B) (85:15, *v/v*). The flow rate was set to 1.0 mL/min while the column temperature was set to 30 °C. This established HPLC/DAD method was used to determine the cordycepin content in samples S1-S4.

2.11 Quantitation of ginsenosides by UHPLC/MS/MS

Chromatographic analysis for the detection and quantification of ginsenosides was performed on an ACQUITY UPLC system (Waters Corp., Milford, MA, USA) with a cooling autosampler and a column oven enabling temperature control. An ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm) was employed: the column temperature was set to 35 °C, and the flow rate was set to 0.25 mL/min. A water (0.1% formic acid, A)-methanol (B) gradient elution was used. The gradient program was 0-2.0 min of 10-90% B and then 2.0-2.5 min at 90% B. The autosampler was set at 10 °C, and the injection volume for analysis was 10 µL. An XEVO TQD triple quadruple mass spectrometer (Waters Corp.) equipped with an electrospray ionization (ESI) source was used for mass-spectrometric detection. Detection was operated in the multiple reaction monitoring (MRM) mode under unit mass resolution in the mass analyzers. The dwell time was set to 200 ms for each MRM transition. The MRM transitions were scanned from higher to lower *m/z* of 799.4-637.4 (ginsenoside Rg1, 1), 799.4-653.4 (ginsenoside F11, 2), 945.5-783.2 (ginsenoside Rd, 3), 945.5-475.5 (ginsenoside Re, 4), and 1107.4-179.1 (ginsenoside Rb1, 5).

After optimization with MS tone and IntelliStart, the source parameters were set with ion spray voltage of 3.0 kV at 450 °C; the cone voltage (V) and collision energy (eV) of each reference substance (Rg1, F11, Rd, Re, and Rb1). MassLynx 4.1 software (Waters Corp.) was used for data acquisition and instrument control. The calibration curve of the references was determined in duplicate with at least six concentrations. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on S/N ratios of 3:1 and 10:1, respectively. Sample stability was evaluated using continuous analysis of the same sample solution every 4 h for 24 h.

2.12 Data analysis

The data were analyzed using Excel 2010 and SPSS v22.0 software (IBM Corp., Armonk, NY, USA). The data were expressed as mean ± standard deviation. A value of $p < 0.05$ was considered statistically significant.

3 Results and discussion

3.1 Growth of C. militaris and tyrosinase inhibition activity

The molecular weights of the mycelium from *C. militaris* fermented with 13 species of traditional Chinese medicines are shown in Figure 1. All the medicines improved mycelium growth. The medicines with the highest mycelium yield were *P. ginseng* (34.9 ± 0.12 g/L), *P. oleracea* (33.3 ± 0.25 g/L), and *M. alba* leaves (30.2 ± 0.17 g/L), followed by *L. lucidum* (29.4 ± 0.10 g/L), *H. cordata* (27.8 ± 0.36 g/L), and *G. uralensis* (25.1 ± 0.31 g/L). All the SF and MFE from fungi fermented with the traditional Chinese medicines showed higher tyrosinase-inhibition activity level than that of the *C. militaris* fruiting body extract. For SF, the tyrosinase-inhibition rate of *R. glutinosa* (57.6%), *G. uralensis* (54.4%), *A. sinensis* (43.6%), and *P. ginseng* (33.2%) was relatively better than that of the other samples. For MFE, the tyrosinase-inhibition rates of *G. uralensis* (43.3%), *R. glutinosa* (35.9%), *A. sinensis* (30.4%), and *P. ginseng* (29.2%), were better than those of the other extracts. *P. ginseng*, which showed the greatest mycelium growth improvement and relatively good tyrosinase-inhibition activity, was chosen for further analysis.

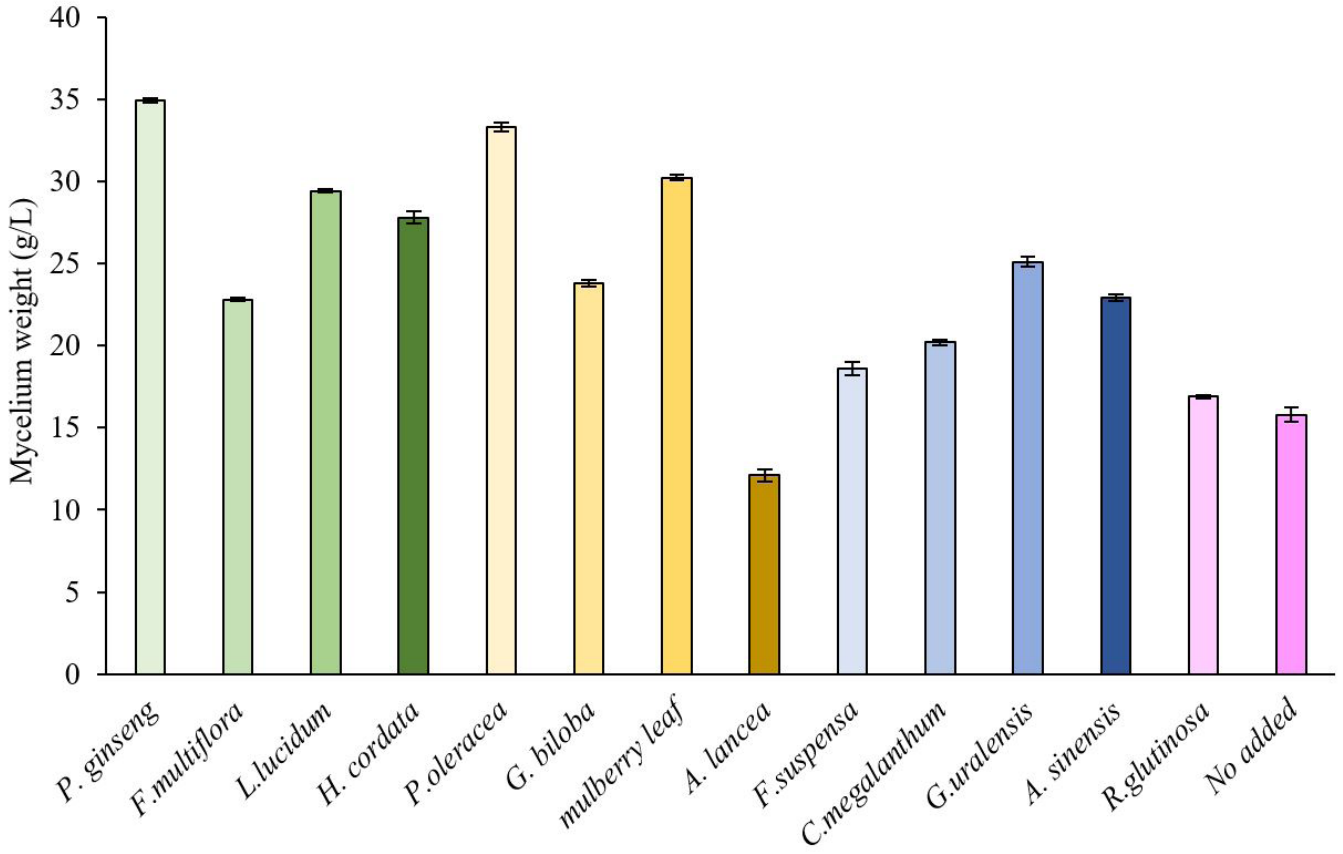

Figure 1. The weight of mycelium of 13 traditional Chinese medicine added into the culture medium of *C. militaris* (*n*=3).

3.2 Tyrosinase and cellular melanin synthesis inhibition activity and cordycepin content of C. militaris *fermented with* P. ginseng

At all three concentrations (0.5, 1.0, and 2.0 mg/mL), S1 showed higher tyrosinase-inhibition activity than S2, while S3 showed higher tyrosinase-inhibition activity than S4 (Table 1), which indicates that the addition of *P. ginseng* during the fermentation process promotes tyrosinase inhibition in the fermentation liquid and mycelium of *C. militaris*.

B16F10 mouse melanoma cell viabilities in the presence of different S1-S4 and cordycepin contents are shown in Table 2. At S1-S4 concentrations of 0.125 and 0.25 mg/mL, cell survival rates are > 80%. Concentrations of 0.125 and 0.25 mg/mL of S1-S4 and cordycepin were therefore further used to test intracellular melanin synthesis. The fermentation liquids (S3 and S4) showed more obvious melanin-inhibition activity than the mycelium extracts (S1 and S2). The concentration of *P. ginseng* added to the *C. militaris* culture during the fermentation process affected the melanin-inhibition activity of the fermentation liquid and mycelium of *C. militaris*. At a concentration of 0.25 mg/mL, the melanin-inhibition activity of S1 was higher than that of S2 ($p < 0.05$); at a concentration of 0.125 mg/mL, the melanin-inhibition activity of S3 was higher than that of S4 ($p < 0.05$, see Table 2).

Moreover, cordycepin (0.025 mg/mL) showed 26.49 ± 3.14% melanin-inhibition activity ($p < 0.01$), and tyrosinase-inhibition activity levels of 13.80 ± 1.20%, 37.60 ± 4.64%, and 51.40 ± 6.53% at concentrations of 0.025, 0.125, and 0.25 mg/mL, respectively. These results suggested that cordycepin may be one of the active constituents in the fermentation liquid.

Table 1. Effects of fermentation products and cordycepin on tyrosinase inhibition activity (*n*=3).

Samples	Concentration (mg/mL)		
	0.5	1.0	2.0
S1	13.63 ± 2.55%	21.88 ± 1.48%**	26.20 ± 0.20%*
S2	10.77 ± 1.24%	14.78 ± 1.86%	23.97 ± 2.95%
S3	17.43 ± 1.29%	20.63 ± 2.16%	22.50 ± 1.74%*
S4	15.66 ± 0.44%	17.38 ± 0.80%	17.98 ± 0.28%
Cordycepin	Concentration (μg/mL)		
	2.5	12.5	25.0
	13.80 ± 1.20%	37.60 ± 4.64%	51.40 ± 6.53%
0.1% Arbutin		56.69 ± 0.95%	

*$p<0.05$; **$p<0.01$.

3.3 Total polysaccharides and molecular weight distribution

The total polysaccharide content of S1 (0.221 ± 0.003 mg/mL) was higher than that of S2 (0.078 ± 0.003 mg/mL), and the total polysaccharide content of S3 (0.226 ± 0.006 mg/mL) was

Table 2. The effect of samples on the cell viability and melanin inhibition activity of B16F10 melanoma (n=3).

Samples	Concentration (mg/mL)	Cell viability (% of control)	Melanin inhibition (%)
Cell only	-	100.00 ± 3.52	-
Arbutin	0.1%	83.21 ± 6.82	43.71 ± 0.96**
S1	1.0	102.26 ± 13.60	-
	0.5	104.97 ± 10.98	-
	0.25	109.21 ± 4.63	12.29 ± 1.31
	0.125	106.51 ± 7.76	10.95 ± 1.13
S2	1.0	107.77 ± 6.88	-
	0.5	101.18 ± 1.36	-
	0.25	99.89 ± 9.86	2.34 ± 5.26
	0.125	102.12 ± 5.68	12.54 ± 14.62
S3	1.0	53.82 ± 1.41	-
	0.5	91.81 ± 7.75	-
	0.25	96.73 ± 7.11	26.89 ± 0.39*
	0.125	99.67 ± 2.69	39.05 ± 4.77**
S4	1.0	51.45 ± 6.47	-
	0.5	75.42 ± 8.01	-
	0.25	86.87 ± 5.74	26.74 ± 7.45*
	0.125	93.52 ± 5.08	17.43 ± 5.58
Cordycepin	0.1	48.11 ± 1.34	-
	0.05	57.78 ± 2.17	-
	0.025	83.59 ± 1.82	26.49 ± 3.14**
	0.0125	93.62 ± 3.41	-

*p<0.05; **p<0.01.

higher than that of S4 (0.05 ± 0.005 mg/mL), which indicates that the polysaccharides in *P. ginseng* are present during the fermentation process in addition to the total extracellular and intracellular polysaccharides of *C. militaris*. Furthermore, the changes in extracellular total polysaccharides were larger than those of the intracellular total polysaccharides.

The molecular weight of the polysaccharides from the fermentation liquid and mycelium of *C. militaris* was lower than that of the polysaccharides in the *C. militaris* fruiting body. Polysaccharides with small molecular weight may be easier for the human body to absorb. The order of relative Mw is: P1 > P2 > P3 > P4 > P5. The molecular weights of the intracellular polysaccharides (P2 and P3) were larger than those of the extracellular polysaccharides (P4 and P5). The amount of *P. ginseng* added to the culture of *C. militaris* during the fermentation process improved the Mw and Mw/Mn distribution of the intracellular and extracellular polysaccharides. Because the polysaccharides extracted from *P. ginseng* were of higher molecular weight than those of *C. militaris*. The trend in the Mw of the polysaccharides is similar to that of tyrosinase inhibition, suggesting that polysaccharides with higher Mw possess better tyrosinase-inhibition activity (Table 3).

Table 3. Determination of molecular weight and molecular weight distribution.

Samples	Mw (Da)	Mw/Mn
P1	6.393×10^7 (± 6.239%)	1.859 (± 6.585%)
	1.774×10^6 (± 0.692%)	1.487 (± 1.221%)
P2	2.744×10^6 (± 0.998%)	2.582 (± 1.246%)
P3	3.152×10^5 (± 0.841%)	1.489 (± 1.329%)
P4	2.003×10^5 (± 0.762%)	1.955 (± 1.542%)
P5	1.287×10^5 (± 0.694%)	1.716 (± 1.434%)

3.4 Cordycepin content

The cordycepin content was calculated from the standard curve (y = 20.46x - 1.0767, r^2 = 0.999). It was lower in the mycelium extract with *P. ginseng* (S1, 2.92 ± 0.08 μg/mL) than it was in the extract without *P. ginseng* (S2, 4.04 ± 0.11 μg/mL). In contrast, the fermentation liquid with *P. ginseng* (S3, 1.62 ± 0.02 μg/mL) showed higher cordycepin content than that without *P. ginseng* (S4, not detected). This indicated that *P. ginseng* promotes the extracellular secretion of cordycepin from the mycelium to the fermentation liquid. Because cordycepin can inhibit the production of melanin and exerts tyrosinase-inhibition activity, it is speculated that the improved activity level of the fermentation liquid with *P. ginseng* (S3) at least partly results from the production of cordycepin.

3.5 Validation of the UHPLC/MS/MS method

The linearity of the calibration curves for each compound was established by plotting the peak area (y) and the concentration (x) of each analyte, as demonstrated by the equations listed in Table 4. All the calibration curves showed good linearity (r^2 > 0.998) within the ranges tested. The results (RSD < 2.0%) showed that the stability of the sample solutions was good during analysis. To assess repeatability, six different solutions of the same sample were analyzed. The RSDs were all < 3.0%, which indicated that the method demonstrates good repeatability. The LOD (ng), LOQ (ng), precision, and repeatability for all the analytes are summarized in Table 4. Therefore, the UPLC/MS/MS method was identified as a fast, accurate, and optimal method for quantitative analysis of the samples.

3.6 Biotransformation of content of ginsenosides in the fermentation process

P. ginseng is a widely used medicinal plant. The extracts of *P. ginseng* were orally and externally used to improve the overall skin condition, as well as to treat a wide variety of diseases, in addition to regulating immunity and relieving fatigue (Kim, 2015). Previous research has reported that crude extract and total ginsenosides showed anti-melanogenic activity and anti-aging efficacy (He et al., 2012; Lu & Yin, 2015; Wang et al., 2014). In the present study, the composition of the ginsenosides Rb1, Re, Rd, Rg1, and pseudoginsenoside F11 in the *P. ginseng* extract (R1), mycelium extract with *P. ginseng* (S1), and fermentation liquid with *P. ginseng* (S3) were determined according to the verified standard curves for these ginsenosides (Figure 2).

Table 4. Linear regression equation, precision, stability and repeatability of ginsenosides (n=3).

Samples	Linear regression	r^2	LOD (μg/mL)	LOQ (μg/mL)	Precision RSD (%)	Stability RSD (%)	Repeatability RSD (%)
Ginsenoside Rb1	y = 96.73x - 31.934	0.999	0.5	1	1.25	0.36	1.87
Ginsenoside Re	y = 345.2x - 85.102	0.993	0.05	0.1	0.73	0.47	1.58
Ginsenoside Rd	y = 285.2x - 76.486	0.995	0.05	0.1	1.64	0.14	1.05
Pseudoginsenoside F11	y = 287.2x+ 23.636	0.999	0.05	0.1	0.84	1.23	0.98
Ginsenoside Rg1	y = 618.7x - 71.779	0.995	0.005	0.01	0.27	0.14	0.54

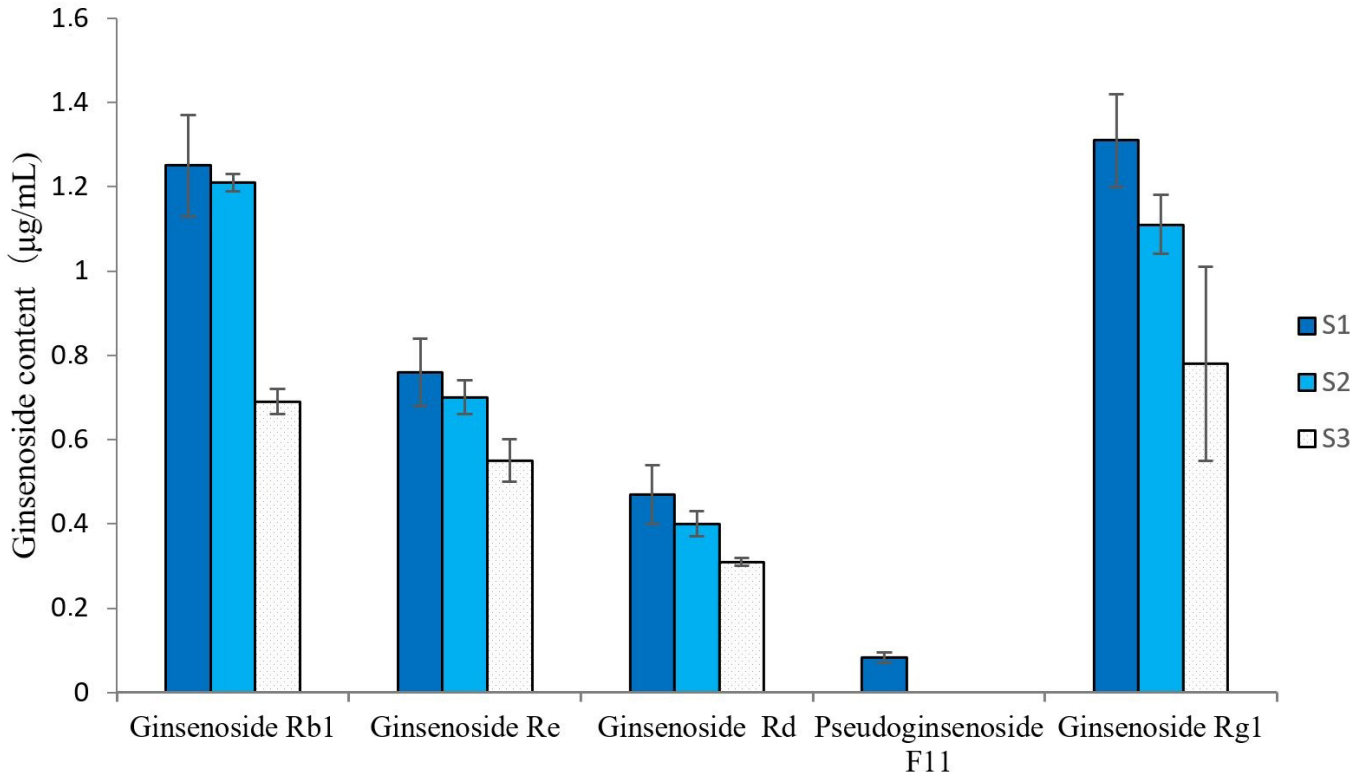

Figure 2. Determination of the five ginsenosides in samples (n=3).

Ginsenosides Rb1, Re, Rd, and Rg1 in samples S1 and S3 were present at an elevated level than those in sample R1, indicating that *C. militaris* promotes the synthesis of ginsenosides in *P. ginseng*. It is noteworthy that a variety of biological enzymes of *C. militaris* both created and transformed some ginsenosides, thereby promoting the production of certain novel ginsenosides during the fermentation process. For instance, pseudoginsenoside F11, a unique component of *Panax quinquefolium*, was detected in the S1 sample. This is because the C-20 position of ginsenoside Re was hydrolyzed and cyclized through a glycosidic bond during fermentation, which transformed ginsenoside Re into pseudoginsenoside F11.

Generally, ginsenosides are transformed and hydrolyzed under the action of intestinal flora (Bae et al., 2002). Ginsenoside after hydrolysis and metabolism is more easily absorbed and utilized by the human body. In the present study, the skin-whitening activity of the fermented *P. ginseng* extract was more potent than that of the *P. ginseng* extract. Some small molecular substances may be produced during the process of microbial fermentation, which are more likely to play a role in skin-whitening applications. Moreover, the increased content of ginsenosides may contribute to the tyrosinase- and melanin synthesis-inhibitory activity. Pseudoginsenoside F11 may prove to be a ginsenoside metabolite more absorbable for human skin utilization. As a result, fermentation techniques should be developed as an important traditional medicine preparation process for future research and application.

4 Conclusions

The present study demonstrates that certain traditional medicines can promote the growth of the mycelium of *C. militaris*. Among them, *P. ginseng* powder added to *C. militaris* fermentation medium not only improved the inhibition activity of tyrosinase and melanin synthesis but also promoted the growth of mycelium.

In addition, cordycepin, total polysaccharide, and ginsenoside are the main factors to consider for the enhancement of tyrosinase-inhibition activity and melanin synthesis. Furthermore, the fermentation of *C. militaris* enhanced the production of ginsenosides Rb1, Re, Rd, and Rg1 by *P. ginseng*, and promoted the transformation of ginsenoside Re into pseudoginsenoside F11. It was ultimately determined that *C. militaris* fermented with *P. ginseng* can be used as a more effective whitening agent than *C. militaris* and *P. ginseng* not being employed together in the fermentation process.

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