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Extraction optimisation of total polyphenols from *Bletilla striata* by high-shear dispersing emulsification technology, and their anti-melanogenesis activity

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Abstract

Bletilla striata is a good source of plant polyphenols which have regulatory functions on melanogenesis. In the present work, to maximise the yields of total polyphenols extracted from *B. striata*, the effects of Tween-60 concentration, shear speed, shear time, ethanol concentration, temperature, and material-to-solvent ratio on extraction of polyphenols were assayed using the high-shear dispersing emulsification technology (HSDE). The obtained data were analysed using the response surface methodology, which showed that the optimal extraction conditions were as follows: Tween-60 concentration of 2.00%, ethanol concentration of 69%, shear speed of 17,200 rpm, and extraction temperature of 55°C. Under these conditions, the extraction of the total polyphenols from *B. striata* was 13.79 ± 0.34 mg/g. Furthermore, the anti-melanogenesis activity of the total polyphenols extracted from *B. striata* was evaluated using *α*-MSH-induced B16F10 cells. Results showed that the total polyphenols from *B. striata* could significantly inhibit melanin production. Overall, these results presented the optimal conditions for the extraction of total polyphenols from *B. striata*, and also revealed the potential anti-melanogenesis values of *B. striata* polyphenols.

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Introduction

Bletilla striata (Thunb.) Rchb.f. (Orchidaceae) is a perennial herbal bulbous plant (Xu *et al*., 2019a) that mainly grows in Sichuan, Yunnan, Hubei, and Zhejiang Provinces of China. Its tuber, locally known "*baiji*" in Chinese, has been used as traditional Chinese medicine (TCM) for more than 2,000 years. Previously, phytochemical investigation on the genus *Bletilla* revealed almost 100 compounds, including glucosides, bibenzyls, phenanthrenes, quinones, biphenanthrenes, dihydrophenanthrenes, anthocyanins, steroids, triterpenoids, and phenolic acids (Xu *et al.*, 2019b). These chemical components showed antibacterial and antitumor properties, promoted haematopoiesis in the bone marrow, inhibited tyrosinase activity, and facilitated wound healing, which demonstrate the medicinal values of *B. striata* (Li *et al.*, 2012).

Previous research mainly focused on the polysaccharides in *B. striata*. However, *B. striata* also contained high contents of phenolic compounds (Song and Cheng, 2019). Phenolic compounds exert various biological activities including antioxidant, anti-inflammatory, alleviation of skin disorder symptoms, and repair of skin lesions (Wittenauer *et al.*, 2015). To date, 12 phenolic acids have been obtained from *B. striata* including *p*-hydroxybenzoic acid, protocatechuic acid, cinnamic acid, caffeic acid, 2-hydroxysuccinic acid, palmitic acid, syringaresinol, pinoresinol, 3-methoxynyasol, *p*-hydroxybenzaldehyde, ferulic acid, and 3-hydroxycinnamic acid (Xu *et al*., 2019a). As important secondary metabolites in plants, these molecules help *B. striata* to defend insects, viruses, and bacteria (Heleno *et al.*, 2015). Reportedly, extracts of fibrous roots of *B. striata* showed high phenolic content and strong DPPH scavenging, ferric-reducing antioxidant, and tyrosinase inhibition activities (Jiang *et al*., 2013). Moreover, phenolic compounds in *B. formosana* extracts, a closely related species to *B. striata*, also displayed antioxidant,

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wound-healing, and antimicrobial activities (Dong *et al*., 2014). Intake of phenolic acid-rich foods could accelerate the elimination of oxygen free radicals, and protect the cells (Bae *et al*., 2017).

Extraction method significantly affects the extraction of certain compounds from plants. Ultrasonic, bioenzymatic, and microwave extraction (Chemat *et al.*, 2011) can effectively extract polyphenols from plants. For example, the yield of total polyphenols from *Psidium guajava* leaves at optimal conditions (ultrasonic power, 390.68 W; extraction time, 38.38 min; and temperature 63.23°C) using ultrasound-assisted aqueous extraction was similar to the predicted value (59.82 mg GAE/g) (Hayta and Iscimen, 2017). However, these methods are time-consuming. The high-shear dispersing emulsifier (HSDE), also named centrifugal homogeniser, was invented by the German FLUKO Company in 1948, and used for the disintegration and emulsification. HSDE has been widely applied in a variety of fields such as foods, medicines, cosmetics, and adhesives (Lin *et al*., 2008). At appropriate pressure and temperature, the HSDE extraction technique could prevent the decomposition of biologically active substances caused by severe pressure and temperature, which generally happen during other extraction processes. For example, Dong *et al*. (2014) found that HSDE had a higher tangent rate (the tea leaves were totally ruptured) and stronger kinetic energy to extract polyphenolic compounds from green tea as compared to heating reflux extraction and ultrasonic extraction. Similarly, the extraction rate of oleuropein and total flavonoids from *Olea europaea* L. were compared between reflux, ultrasonic, and HSDE methods. Results showed that the extraction rates had no significant difference among the three methods; but the extraction time of HSDE was only 150 s, which was much shorter than the other two methods (20 min) (Zhang *et al*., 2013). Overall, HSDE is an effective extraction method with advantages of fast extraction speed, short extraction time, low solvent consumption, and high extraction rate. However, the efficacy of polyphenols extraction from *B. striata* using HSDE has not been reported thus far.

Melanin is a photo-protective skin pigment. The irradiation intensity of UV and melanin production are strongly correlated. Long-term exposure to UV could induce a large number of free radicals, and stimulate the production of melanin through the regulation of tyrosinase expression. Alesiani *et al*. (2008) demonstrated that specific inhibitors could inhibit melanoma growth by suppressing melanin synthesis. Similarly, kojic acid and arbutin also revealed inhibitory effects on tyrosinase and melanin syntheses. However, these inhibitors exhibited low efficacies and sometimes severe side effects (Solano *et al.*, 2006). Therefore, more efforts are required to investigate non-toxic compounds with inhibitory activity against melanin synthesis. To this end, plant polyphenols have been suggested as potential sources of these non-toxic melanin inhibitory compounds.

In the present work, to maximise the yields of total polyphenols from *B. striata*, single-factor experiment was carried out to optimise the HSDE technology, and the data were analysed using the response surface methodology (RSM) approach. The inhibitory effects of *B. striata* total polyphenols against melanin production were also determined. These data are important for industrial production of polyphenols from *B. striata*.

Materials and methods

Extraction of total polyphenols from B. striata

B. striata grown in Qimen County, Anhui Province, China were collected in August 2017. The samples were oven-dried at 60°C for 24 h, finely pulverised using a QE-300 g omnipotent disintegrator (Zhejiang Yili Garment Co., Ltd.) for 3 min, and sieved through a 30-mesh sieve. Next, 2.0 g of samples were homogenised, and extracted in 60% ethanol solution at 20°C using a HSDE (10,000 rpm). Finally, the supernatant was collected, filtered, and diluted to the proper concentration for further experiments.

Determination of total polyphenols from B. striata

Exactly 1 mL of each sample (1.5 g/mL) or standard (a stock solution of 1.0 mg/mL tannic acid, diluted to different concentrations) was mixed with 1 mL of Folin-Ciocalteu's reagent. After 10 min, 2 mL of 20% Na_2CO_3 solution were added. The mixtures were then incubated at room temperature for 40 min, and the absorbance was measured at 760 nm using a SpectraMax 190 Multiskan Spectrum (Molecular Devices Company). The blank control was prepared using distilled water instead of extracts. The total polyphenols were calculated using the regression equation $y = 0.0014x + 0.0068$ ($R^2 = 0.9953$), where Y = absorbance, and x = tannic acid concentration.

The total polyphenols were expressed as milligrams of tannic acid per gram of *B. striata* extract (mg TA/g).

Single-factor experiment

To optimise the extraction efficacy, single-factor experiment was conducted on six parameters, including Tween-60 concentrations (0, 0.5, 1, 1.5, 2, and 2.5%), shear speeds (10,600; 13,400; 16,200; 19,000; and 21,800 rpm), shear times (90, 120, 150, 180, and 210 s), ethanol concentrations (40, 50, 60, 70, and 80%), temperatures (30, 40, 50, 60, and 70°C), and material-to-solvent ratios (1:20, 1:30, 1:40, 1:50, and 1:60 g/mL). When one factor was tested, other factors were set to constant as described previously. Each test repeated three times.

Response surface methodology

Based on the preliminary results of single-factor experiment, RSM was conducted using Box-Behnken design (BBD) (Ferreira *et al*., 2007). As shown in Table 1, the four chosen factors were assigned as A (Tween-60 concentrations, %), B (shear speeds, rpm), C (ethanol concentrations, %) and D (temperatures, °C), and prescribed into three levels (-1, 0, and 1). The responses of total polyphenols to variation of each factor were then analysed.

Table 1. Independent variables and their levels used for Box-Behnken design.

Factor	Code	Level		
		-1	**0**	**1**
Tween-60 concentration (%)	A	1.5	2	2.5
Shear speed (rpm)	B	13,400	16,200	19,000
Ethanol concentration (%)	C	60	70	80
Temperature (°C)	D	40	50	60

Validation of the optimised conditions

To validate the optimised conditions, triplicate extraction experiments were performed under the optimal conditions. The average values of the experiments were compared with the predicted values under the optimised conditions in order to evaluate the accuracy and suitability of the optimised conditions.

Effects of B. striata polyphenols on B16F10 cells

Cell culture and determination of cell viability

Mouse melanoma cells B16F10 (American Model Culture Repository) were cultured in a humidified incubator at 5% CO_2 and 37°C. The high-glucose Dulbecco's Modified Eagle's Medium (DMEM, Gibco Company) containing 10% foetal bovine serum (FBS) and 100 U/mL penicillin was used as the culture medium. Next, 100 µL of 1×10^8 cells/L B16F10 cells were seeded in each well of 96-well plates. After incubation for 24 h, cells were treated with different concentrations of *B. striata* polyphenols (0.0025, 0.005, 0.01, and 0.02 mg/mL) for 24 h. Next, 5 mg/mL methylthiazoletrazolium (MTT) solution was added, and then incubated for 3 h. The supernatant was removed and replaced with 200 µL of dimethyl sulfoxide (DMSO). Finally, the absorbance was measured at 570 nm (Mustapha *et al.*, 2015). The relative cell viability (%) was calculated using this formula: $A_{treatment}/A_{control} \times 100\%$. Each assay repeated three times.

Determination of melanin content

Cells at the logarithmic growth stage were seeded into 12-well plates, and each well contained 1 mL of B16F10-cell suspension (5×10^4 cells/mL). Next, B16F10 cells were pre-treated with different concentrations of BSP for 1 h. Next, 0.2 µmol/L *α*-melanocyte stimulating hormone (*α*-MSH) was added for stimulation of melanin synthesis. In total, six groups were prepared, including the control group (no *α*-MSH, no arbutin, and no BSP), the *α*-MSH model group, the positive control group (*α*-MSH + 100 mg/L arbutin), and the BSP group (*α*-MSH + BSP at 5, 10, and 20 mg/L). After incubation for 48 h, the supernatants were discarded, and the cells were washed three times with phosphate-buffered saline (PBS), and dissolved in 200 µL of NaOH solution containing 10% DMSO. The cells were fully lysed via heating at 80°C for 30 min. The cell lysates were centrifuged for 10 min at 17,500 *g*, and the absorbance of the supernatant was measured spectrophotometrically at 475 nm (Huang *et al.*, 2016). The value of each measurement was expressed as the percentage of changes to the *α*-MSH model group.

The protein content in the cell lysates was determined using the Bradford method (Bradford, 1976). Melanin was used as the standard. The formula for the standard curve was $y = 0.009x + 0.00008$ ($R^2 = 0.9960$), where Y = absorbance, and x = content of melanin. Relative melanin contents (%) were calculated using this formula: (melanin content in the experimental group/melanin content in the α-MSH model group) × 100%. Each assay repeated three times.

Determination of tyrosinase activity

Tyrosinase activity was determined by measuring the rate of L-3,4 dihydroxyphenylalanine (L-DOPA) oxidation (Skandrani *et al*., 2010). Briefly, cells (1×10^5 cells/mL) were treated with BSP (0.0025, 0.005, 0.01, 0.02, and 0.1 mg/mL, respectively) for 48 h, and then resuspended in PBS (0.1 M; pH 6.8) containing 0.1% Triton 100. Lysate was clarified by centrifugation at 17,500 *g* for 10 min at 4°C. Next, 400 µL of supernatant was mixed with 400 µL of the substrate L-DOPA (0.15%), and absorbance was measured spectrophotometrically at 475 nm. The relative tyrosinase activity (%) was calculated using this formula: (tyrosinase activity per mg of protein in the experimental group/tyrosinase activity per mg of protein in the control group) × 100%.

Statistical analysis

All experiments were performed in triplicate. The experimental data were expressed as mean and standard deviation (SD). Response surface analysis was performed using the Design-Expert 8.0.6.1 (Stat-Ease Inc., Minneapolis, MN, USA). One-way analysis of variance (ANOVA) was used to compare the effects of BSP on tested cell parameters using SPSS 18.0 software, followed by LSD tests, and $p < 0.05$ indicated that the results were significantly different.

Results

Single factor results

As shown in Figure 1a, total polyphenols had a positive correlation with Tween-60 (1 - 2.5%), and reached the maximum value (10.57 ± 0.13 mg TA/g) at 2% Tween-60, which was 38.17% higher than that of the control. Similarly, the maximum value of total polyphenols was 10.67 ± 0.60 mg TA/g at the shear speed of 16,200 rpm (Figure 1b). When the shear time increased from 90 to 150 s, total polyphenols slowly increased, and the maximum value (9.89 ± 0.35 mg TA/g) was obtained at 150 s (Figure 1c). After 150 s, there was no obvious increase in total polyphenols with further increase in shear time. As for the concentration of ethanol, total polyphenols was significantly higher at 70% (9.95 ± 0.34 mg TA/g) when compared with the control (Figure 1d). The effect of extraction temperature (30 - 70°C) on total polyphenols is shown in Figure 1e. When other conditions were kept stable, total polyphenols reached the highest value (10.53 ± 0.57 mg TA/g) at 50°C, and then decreased significantly, indicating that the diffusion and solubility of polyphenols in water increased with extraction temperature; whereas a higher temperature might lead to the degradation of phenolic compounds due to internal redox reactions, hydrolysis, and polymerisations (Toma *et al*., 2001). As shown in Figure 1f, total polyphenols kept increasing with the increase in the material-to-solvent ratio (1:20 - 1:50), and the maximum value of total polyphenols (10.56 ± 0.47 mg TA/g) was obtained at the ratio of 1:50. Based on these results, Tween-60 concentration (1.5, 2.0, and 2.5%), shear speed (13,400 - 19,000 rpm), ethanol concentration (60 - 80%), and temperature (40 - 60°C) were selected for further RSM analysis (Table 1), whereas shear time and material-to-solvent ratio were not included.

Optimisation of parameters by RSM

All 29 experiments were used for the ANOVA analysis except for five runs (13 - 17) with the factors set at the central levels to investigate the stability of the experiments (Table 2). The *p*-values were used to characterise the significance of the model and coefficients.

The adequacy and the fitness of the model were evaluated using ANOVA (Hayta and Iscimen, 2017). As shown in Table 3, the lack of fit was insignificant ($p > 0.05$), thus indicating that the regression equation fit well in the whole regression area (Li and Fu, 2005). Among these four factors, independent variables (A, B, C, and D) and four quadratic terms (A^2, B^2, C^2, and D^2) showed crucial influences on the TPC ($p < 0.05$). There were significant interactions between Tween-60 concentration and temperature (AD), and between shear speed and ethanol concentration (BC) ($p < 0.05$). Moreover, a higher *F*-value and a lower *p*-value of the model indicated a more significant effect on the respective response variables (Li and Fu,

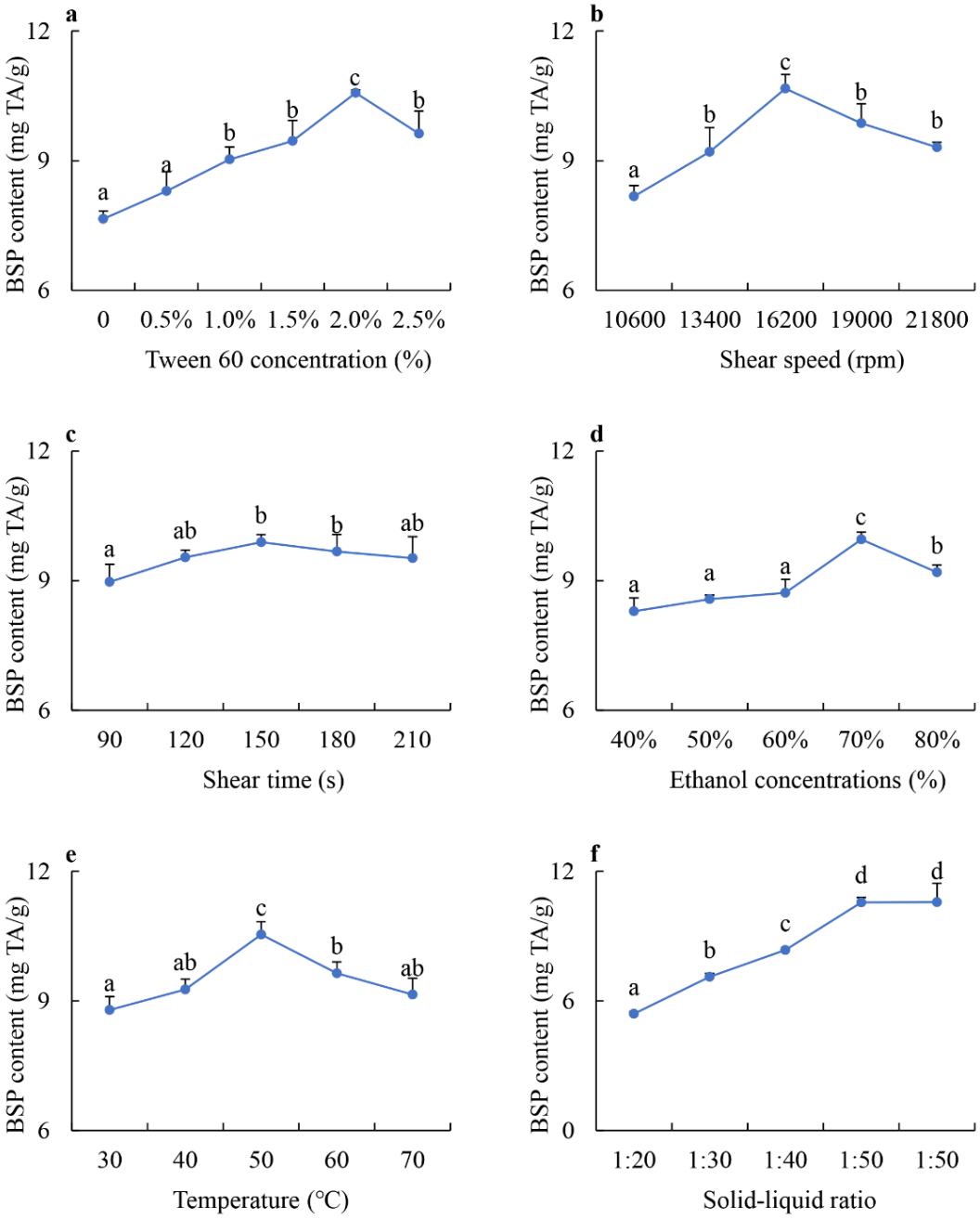

Figure 1. Effects of **(a)** Tween-60 concentration, **(b)** shear speed, **(c)** shear time, **(d)** ethanol concentration, **(e)** temperature, and **(f)** solid-liquid ratio on the extraction of total polyphenols from *B. striata*. Means with different lowercase letters indicate a significant difference at $p < 0.05$.

Table 2. Box-Behnken design (BBD) and corresponding actual response values for the total polyphenols of *B. striata* extracted using the HSDE method (mean ± SD).

No.	Tween-60 concentration (%)	High-shear speed (rpm)	Ethanol concentration (%)	Temperature (°C)	Total polyphenols (mg TA/g)
1	1.5	13,400	70	50	11.36 ± 0.24
2	1.5	16,200	60	50	11.22 ± 0.07
3	1.5	16,200	70	40	9.31 ± 0.41
4	1.5	16,200	70	60	12.35 ± 0.07
5	1.5	16,200	80	50	11.76 ± 0.29
6	1.5	19,000	70	50	11.97 ± 0.44
7	2	13,400	60	50	10.42 ± 0.37
8	2	13,400	70	40	9.83 ± 0.11
9	2	13,400	70	60	13.27 ± 0.34
10	2	13,400	80	50	12.71 ± 0.17
11	2	16,200	60	40	9.95 ± 0.36
12	2	16,200	60	60	12.62 ± 0.05
13	2	16,200	70	50	12.64 ± 0.24
14	2	16,200	70	50	13.19 ± 0.47
15	2	16200	70	50	13.34 ± 0.37
16	2	16,200	70	50	13.76 ± 0.12
17	2	16,200	70	50	13.07 ± 0.13
18	2	16,200	80	40	11.52 ± 0.36
19	2	16,200	80	60	12.21 ± 0.72
20	2	19,000	60	50	13.19 ± 0.27
21	2	19,000	70	40	10.15 ± 0.36
22	2	19,000	70	60	12.98 ± 0.17
23	2	19,000	80	50	12.74 ± 0.15
24	2.5	13,400	70	50	11.27 ± 0.06
25	2.5	16200	60	50	11.36 ± 0.15
26	2.5	16,200	70	40	12.41 ± 0.29
27	2.5	16,200	70	60	12.69 ± 0.35
28	2.5	16,200	80	50	12.72 ± 0.33
29	2.5	19,000	70	50	12.73 ± 0.42

Table 3. Analysis of variance (ANOVA) for the response surface model

Source	Sum of squares	df	Mean square	*F*-value	*p*-value
Model	34.66	14	2.48	7.23	0.0003
A	2.26	1	2.26	6.61	0.0222
B	2.00	1	2.00	5.85	0.0298
C	2.00	1	2.00	5.85	0.0298
D	13.98	1	13.98	40.83	< 0.0001
AB	0.18	1	0.18	0.53	0.4796
AC	0.17	1	0.17	0.49	0.4949
AD	1.90	1	1.90	5.56	0.0334
BC	1.88	1	1.88	5.48	0.0345
BD	0.093	1	0.093	0.27	0.6103
CD	0.98	1	0.98	2.86	0.1128
A^2	3.52	1	3.52	10.29	0.0063
B^2	1.99	1	1.99	5.80	0.0304
C^2	2.17	1	2.17	6.34	0.0246
D^2	6.10	1	6.10	17.81	0.0009
Residual	4.79	14	0.34		
Lack of fit	4.13	10	0.41	2.49	0.1969
Pure error	0.66	4	0.17		
Cor total	39.45	28			

2005). Analysis of the data showed robust parabolic relationships with these variables. Thus, the extraction rate of total polyphenols from *B. striata* might have reached the maximum value.

Verification of the model

Based on the RSM analysis, the optimum extraction condition for *B. striata* total polyphenolic extraction was as follows: Tween-60 concentration of 2.05%, shear speed of 17,186.47 rpm, ethanol concentration of 69.45%, and temperature of 55.07°C. The total polyphenolic extraction under this condition was predicted as 13.56 mg TA/g. Experimental determination under this condition showed that the actual total polyphenols was 13.79 ± 0.34 mg TA/g, which was close to the predicted value. This result suggested that the RSM model was precise.

Effects of B. striata total polyphenols on B16F10 cells

In response to treatments with 0.005 - 0.02 mg/mL *B. striata* total polyphenols, cell viability did not significantly change when compared with the control ($p > 0.05$, Table 4), which indicated that *B. striata* total polyphenols were not toxic to cells. When compared with the α-MSH model treatment, the tyrosinase activity and melanin content in B16F10 cells increased significantly in treatments with 0.005, 0.01, and 0.02 mg/mL *B. striata* total polyphenols; with the inhibition rates of intracellular tyrosinase activity of 8.44, 21.62, and 35.84%; and with the inhibition rates of intracellular melanin of 14.13, 31.15, and 51.95%, respectively. In the arbutin group, the inhibition rates of tyrosinase activity and melanin production were 29.06 and 39.33%, respectively. The results showed that the inhibitory effects of *B. striata* total polyphenols on tyrosinase activity and melanin content were stronger than those of arbutin. In addition, tyrosinase activity and melanin content were significantly negatively correlated with *B. striata* total polyphenols' concentration ($p < 0.01$; Table 5).

Discussion

Polyphenols are important secondary metabolites in plants. The solubility of polyphenols is governed by the chemical nature of the plant sample and the polarity of the solvent used. Since the extraction mechanism of HSDE is to break the cell

Table 4. Effects of the total polyphenols of *B. striata* on the viability, tyrosinase activity, and melanin production in B16F10 cells (mean ± SD).

Concentration (mg/mL)	Cell viability (%)	Tyrosinase activity (%)	Melanin content (%)
Control group			
0	100.00 ± 1.76	61.99 ± 2.89^{e}	45.79 ± 2.93^{e}
***α*-MSH model group**			
-	-	100.00 ± 1.80^{a}	100.00 ± 2.30^{a}
Treatment group			
0.005	101.47 ± 1.30	91.56 ± 3.81^{b}	85.87 ± 3.10^{b}
0.01	100.38 ± 2.03	78.38 ± 6.07^{c}	68.85 ± 9.97^{c}
0.02	98.55 ± 0.77	64.16 ± 5.83^{e}	48.05 ± 2.23^{e}
Arbutin group			
0.1	-	70.94 ± 3.84^{d}	60.67 ± 3.58^{d}

Means with different lowercase superscripts indicate a significant difference at $p < 0.05$.

Table 5. Correlation analysis of total polyphenols, B16F10 cell viability, tyrosinase activity, and melanin synthesis.

Parameter	Total polyphenols	Cell viability	Tyrosinase activity	Melanin content
Total polyphenols	1			
Cell viability	-0.74*	1		
Tyrosinase activity	-0.924*	0.768*	1	
Melanin content	-0.949*	0.83*	0.925*	1

*Significant correlation ($p < 0.01$).

wall and release the active compounds as much as possible, the key point of improving extraction efficiency is to increase the solubility of target compounds in the extraction solvents (Dong *et al*., 2014). The surfactant Tween 60 is amphiphilic which could reduce the interfacial tension of the solid-liquid phase, and increase the dissolution and exudation capacity of macromolecules (Chen *et al*., 2011). Reportedly, the combination of Tween 60 with cellulase not only improved the yield of total flavonoids from *Sargentodoxa*, but also retained the antioxidant property of the extract (Han *et al.*, 2012). Ethanol is another solvent for polyphenolic extraction, and safe for human consumption (Shi *et al.*, 2005). Ethanol with a certain volume fraction can easily destroy the hydrogen and hydrophobic bonds existing between polyphenols and polysaccharide proteins. The extraction production of polyphenols from pear peel showed the tendency of 70% ethanol > 50% ethanol > 95% ethanol > water (Song and Cheng, 2019). The diffusivity of total polyphenols from the untreated samples was in the range of 1.1 - 1.8 × 10^{9} m^2/s (without ethanol) and 3.2 - 5.9 × 10^{10} m^2/s (with ethanol), thus indicating that the addition of ethanol promoted the extraction efficiency (Boussetta *et al*., 2013).

The recovery of phenolic compounds from plant materials is influenced by the extraction time and temperature, which reflects the conflicting actions of solubilisation and analyte degradation by oxidation (Robards, 2003). An increase in the extraction temperature could promote higher analyte solubility by increasing both solubility and mass transfer rate. In addition, the viscosity and the surface tension of the solvents decrease at higher temperature, which helps the solvents reach the sample matrices and improve the extraction rate. However, phenolic compounds are easily hydrolysed and oxidised. Long extraction time and high temperature would increase oxidation of phenolics, and decrease their yields in the extracts. For example, conventional extraction of anthocyanins is typically conducted at temperatures ranging from 20 to 50°C (Jackman *et al*., 1987), and temperatures higher than 70°C cause rapid anthocyanin degradation (Havlikova and Mikova, 1985). Liu *et al*. (2015) confirmed that HSDE-assisted extraction could promote the release of intracellular metabolites, and

the production was clearly enhanced when the shearing time increased from 0 to 3 min.

The extraction of phenolic compounds from plant materials could also be influenced by the material-to-solvent ratio and the particle size of the sample. The increase in the material-to-solvent ratio was found to positively enhance polyphenolic yields (Pinelo *et al.*, 2005a). However, treatments at high material-to-solvent ratios use more solvent and produce more waste. Therefore, we must find the optimised material-to-solvent ratio for extraction (Pinelo *et al*., 2006). Particles in small size could enhance the yield of polyphenolic compounds (Pinelo *et al.*, 2005b). HSDE has excellent shearing and grinding functions, and displays a high capacity to break cells and pre-treat samples (Hua *et al.*, 2012). Therefore, it is of critical importance to optimise an efficient extraction procedure/method and maintain the stability of the polyphenolic compounds. In the present work, the Tween-60 concentration, ethanol concentration, extraction temperature, shear time, and speed were optimised to extract polyphenols from *B. striata* using HSDE. Box-Behnken design was adopted to regulate the conditions of the extraction process based on single-factor tests.

Response surface methodology has been successfully applied to evaluate multiple process variables at a time, as well as their interactive effects, focusing on searching optimum condition of factors in a statistical way (Bas and Boyaci, 2007). Based on single-factor experiments, RSM results indicated that the optimal extraction of total polyphenols from *B. striata* was achieved at the Tween-60 concentration of 2.00%, shear speed of 17,200 rpm, ethanol concentration of 69%, and temperature of 55°C. Under these conditions, the quantity of total polyphenols extracted from *B. striata* was 13.7865 ± 0.34 mg TA/g. These data were similar to Di *et al.* (2013) in which the optimal conditions of polyphenolic extraction were as follows: 20% alcohol solution, 1:50 solid to liquid ratio, 10,000 rpm speed, 180 s extraction time, and 313.15 K extraction temperature. Under the optimum conditions, the extraction efficiency of active ingredients from tea leaves could reach up to 95%, thus indicating that HSDE is a promising technology for extraction.

The initial step of melanin biosynthesis is that tyrosinase enzyme catalyses the hydroxylation of L-tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA), and further to dopaquinone through a radical coupling pathway. After several steps, melanin is produced as a complex mixture of pigments (Chang, 2009). Compounds interfering with this biosynthetic pathway may be potential candidates to treat pigmentary disorders. Dysregulation of this mechanism may be responsible for pigmentation-related disorders (Lehraiki *et al*., 2014). Two studies have reported the strong antioxidant activity and free radical scavenging ability of phenolic extracts in *B. striata* (Song *et al.*, 2017; Lu *et al.*, 2021), thus suggesting that *B. striata* extracts potentially suppressed melanin production by inhibiting free radical pathways. In the present work, total polyphenols from *B. striata* significantly inhibited the tyrosinase activity and melanin synthesis in B16F10 cells after being induced by *α*-MSH. More importantly, the inhibitory effect of melanin was stronger than that of arbutin. The downregulation of tyrosinase enzyme expression is an important approach to inhibit melanogenesis (Azam *et al.*, 2017), thus indicating that total polyphenols from *B. striata* could inhibit the formation of melanin by regulating the activity of tyrosinase. Moreover, as potent antioxidants, polyphenols might reduce the *o*-dopaquinone in the reaction mixture to L-DOPA at high concentrations, thus limiting the formation of dopachrome and melanin (Chang, 2009). Based on this context, the exposure of B16F10 cells to higher concentrations of total polyphenols from *B. striata* might trigger several processes related to oxidation by acting as a negative modulator of melanogenesis. However, in the present work, total polyphenols from *B. striata* were in crude form which should contain various phenolic compounds as well as compounds other than phenols. More investigations are therefore required to identify the exact compounds responsible for the inhibition of melanogenesis. The present work demonstrated that *B. striata* is a good source of tyrosinase and melanogenesis inhibitors.

Conclusion

In the present work, total polyphenols were extracted from *B. striata* using the HSDE technology. Based on single-factor tests, RSM analysis was conducted to optimise the extraction conditions, which were as follows: Tween-60 concentration of 2.05%, shear speed of 17186.47 rpm, ethanol concentration of 69.45%, and temperature of 55.07°C. Under these conditions, the extraction of total polyphenols reached 13.79 ± 0.34 mg TA/g. Moreover, exposure to the obtained total polyphenols

decreased tyrosinase activity and melanin production in mouse melanoma cells B16F10. These results provided a theoretical basis and technical reference for improving the extraction and use of *B. striata* polyphenols.

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