Preparation of Bamboo Leaf Hydrosols by the Steam-distillation Extraction Process

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The best extraction characteristics and optimal parameters for the steam-distillation extraction of bamboo leaf hydrosols were investigated to provide a data foundation for the subsequent study of bamboo leaf hydrosols and their industrial applications. The colligation score (CS) of the bamboo leaf hydrosols antioxidant activity (based on its DPPH free radical scavenging ability and total antioxidant capacity) was used as an evaluation index. The extraction time, crushing degree, and solid-to-liquid ratio were selected as the extraction parameters and response surface methodology was used to optimize the extraction process. The CS and sensory evaluation were used to determine the best distilled volume of bamboo leaf hydrosols. The results showed that the prepared bamboo leaf hydrosols had a high CS of 0.9967 (the DPPH free radical scavenging capacity was 0.052 mg/mL Vc equivalent, and the total antioxidant capacity was 0.2771 mg/mL Trolox equivalent) when the bamboo leaf crushing degree was 10 mesh, the solid-to-liquid ratio was 1:25, and the extraction time was 6 h. When the distilled volume was one-quarter of the total aqueous extract volume, the bamboo leaf hydrosols were highly active, colorless, and had a pleasantly aromatic odor.

Keywords: Bamboo leaf hydrosols; Steam-distillation extraction; Colligation score; Response surface methodology

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INTRODUCTION

With improvements to the living standards of people, the issue of food and cosmetic safety has drawn more attention. However, it has become an increasingly difficult challenge to improve the preservation of food and cosmetics because of the development of new food-borne diseases and production of pathogenic microorganisms (Tajkarimi *et al.* 2010). Synthetic chemical preservatives are widely used because of their effective preservative effects. However, synthetic chemical preservatives not only have some negative effects on the environment, but they also pose a certain threat to human health (Abdalla *et al.* 2007; Lis-Balchin *et al.* 2010). Therefore, it is necessary to produce safe, healthy, pollution-free, and natural preservatives.

Hydrosols, also known as flower water, distillate water, and aromatic water, are the by-product of plant essential oils. Hydrosols are complex mixtures that contain traces of essential oils and some water-soluble compounds. There have been many reports on

the activity and application of plant hydrosols. Boyraz and Özcan (2005) reported that rosemary, cumin, sater (savory), basil, and pickling herb hydrosols had relevant fungicidal activity for inhibition of *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *tulipae*, *Botrytis cinereal*, and *Alternaria citri*. Hussien *et al.* (2011) reported that basil, cardamom, clove, cinnamon, and thyme hydrosols are effective at eliciting inhibitory effects against *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli*. The antibacterial activities of thyme, black cumin, sage, rosemary, and bay leaf hydrosols against *Salmonella typhimurium* and *E. coli* O157:H7, as well as the application of plant hydrosols on fresh-cut apples and carrots, have been investigated in previous studies (Tornuk *et al.* 2011). There have also been many other reports on the antibacterial activity and antioxidant ability of plant hydrosols (Sağdıç and Özcan 2003; Boyraz and Özcan 2006; Meng *et al.* 2014; Shao *et al.* 2015). Although there have been numerous studies on the activity of plant hydrosols, few studies had been done on the optimization of the preparation process. If hydrosols are to be applied in industrial production, there is minimal specific data for guidance.

Bamboo, which contains important nutrients, is one of the most valuable natural plants in the world. Among its uses, bamboo leaves have been used to treat fever and for detoxication purposes for more than 1000 years, and they have the effect of clearing away heat, eliminating diuresis, and promoting fluids and diuresis (Zhang and Ding 1996; Lv *et al.* 2012). However, no one has studied bamboo leaf hydrosols.

In this study, bamboo leaves (*Phyllostachys heterocycla*) were used as the raw materials. Patented equipment for extracting essential oils was used to prepare bamboo leaf hydrosols *via* the steam-distillation method (Lv *et al.* 2009). The best process for extracting bamboo leaf hydrosols was determined with the colligation score (CS) indicator (based on its DPPH free radical scavenging ability and total antioxidant capacity). This study provides a theoretical basis and data support for the application of bamboo leaf hydrosols in preservatives and industrial production.

EXPERIMENTAL

Materials

Fresh bamboo (*Phyllostachys heterocycla*) leaves were collected in June of 2017 in Anji City, Huzhou Province, China. The leaves were sent to Beijing Forestry University School of Biological Science and Biotechnology for final identification. After drying, the bamboo leaves were crushed with a high-speed pulverizer (FW100, Taisite, Tianjin, China) and sieved into particle sizes of less than 10 mesh, 10 mesh, 20 mesh, 40 mesh, and 60 mesh for use.

The chemicals DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate)), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and vitamin C were purchased from Sigma Chemical Co. (St. Louis, USA). All of the other chemicals used were of analytical grade.

Methods

Single factor experiment design for the preparation of the bamboo leaf hydrosols

The bamboo leaf hydrosols were prepared using the device shown in Fig. 1. Approximately 50 g of bamboo leaves (less than 10 mesh, 10 mesh, 20 mesh, 40 mesh, and 60 mesh) were placed into a flask (2 L) with double distilled water (1:10, 1:20, 1:30,

1:40, and 1:50 w/v) for different extracting time (2 h, 4 h, 6 h, 8 h, and 10 h) (Lv *et al.* 2009). After hydro-distillation, the water steam containing essential oils and hydrosols was condensed. Then, the water was passed through a n-hexane layer such that the essential oil was extracted by the n-hexane. The hydrosols were collected from a two-way valve, and it was ensured that the condensed liquid dripping speed was consistent with the hydrosol collection rate. The hydrosols, of which 50 mL were collected for subsequent determination, were kept in covered sterile bottles overnight at 4 °C until they were used. All of the tests were performed in triplicate.

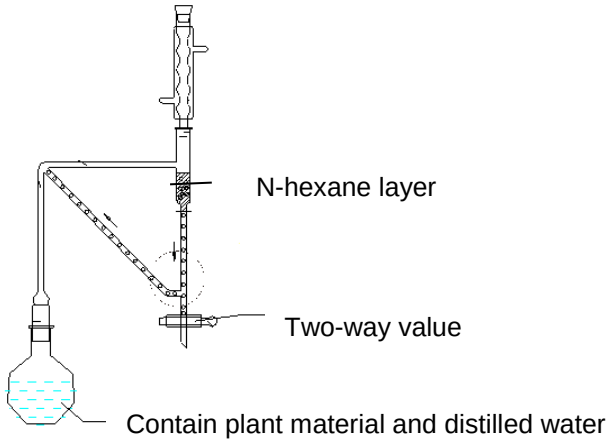

Fig. 1. Equipment used to extract the bamboo leaf hydrosols

Response surface test to optimize the bamboo leaf hydrosols extraction

A full second-order polynomial model of the design was used to evaluate the best extraction process for bamboo leaf hydrosols. The CS of the antioxidant activity (response variable Y) was a function of the independent variables (X), namely the extraction time (X_1), solid-to-liquid ratio (X_2), and crushing degree (X_3). Response surface methodology was used to generate the independent variable regression model of the response variables. The value range for each factor was selected based on the results from the single factor experimental data. The factors and their levels are shown in Table 1.

Table 1. Response Surface Test Design Factors and Levels

Factor	Level		
	-1	0	1
Extraction time (h)	4	6	8
Solid-to-liquid ratio	1:20	1:30	1:40
Crushing degree (mesh)	0	10	20
*0 mesh referred to as less than 10 mesh			

The data were analyzed using the Design-Expert software (Version 7.0, Stat-Ease Inc. Company, USA) to obtain 17 values for each response. Then the data were fitted to a second-order polynomial equation to optimize the extraction conditions. Based on the analysis, statistically significant effects (major and interacting) were included in the

developed model. A quadratic response surface, with the design variable inputs x_1 and x_2 and output variable y, was formulated as,

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_1^2 + a_4x_2^2 + a_5x_1x_2 \quad (1)$$

where y is the response function and a_i (i = 0,…,5) is the unknown coefficients of the least-squares fitting estimate of the model for the experimental results of the design point (Zheng *et al.* 2013).

When fitting any regression model, residual analysis of the fit model is necessary to determine the adequacy of the least-squares fit. The Design-Expert software was used to generate response surfaces and profiles and to obtain the best theoretical extraction process of the bamboo leaf hydrosols.

Evaluation index of the bamboo leaf hydrosols extraction

Some reports have shown that lavender, nutmeg, chestnut flower, Agilawood, and other plant hydrosols have antioxidant activities necessary for the ability to scavenge DPPH free radicals (Meng *et al.* 2014; Shao *et al.* 2015). Therefore, the CS of the scavenged DPPH free radicals (CS_1) and total antioxidant capacity (CS_2) was used as the evaluation index in this study. The CS_1 and CS_2 activities were both important indicators. Therefore, the weights of CS_1 and CS_2 were both set to 0.5. The CS was calculated as follows (Chen *et al.* 2012),

$$CS = (CS_1 / CS_{max} * 0.5 + CS_2 / CS_{max} * 0.5) \quad (2)$$

where CS_{max} is the maximum colligation score.

Evaluation of the DPPH scavenging activity

The DPPH free radical scavenging activity was measured using the methods by Yang *et al.* (2006) and Yang *et al.* (2008) with some modifications. Each hydrosol sample, obtained under different extraction conditions (0.6 mL), was mixed with 2 mL of ethanolic solution that contained 0.4 mM DPPH in a 10-mL colorimetric tube. The mixture was shaken homogenously and then left to react for 30 min under dark conditions. The absorbance at 517 nm was measured (A). The absorbance of the control group was obtained by replacing the sample with ethyl alcohol (A_1). The absorbance of the background interference was obtained by replacing the DPPH with ethyl alcohol (A_0). The DPPH free radical scavenging activity of the sample was calculated as follows:

$$DPPH\ radical\ scavenging\ activity\ (\%) = [1 - (A - A_0) / A_1] * 100\% \quad (3)$$

Vitamin C was used for the positive controls. All of the tests were performed in triplicate. The half maximal inhibitory concentration (IC_{50}) of the hydrosols under optimum extraction conditions was determined using the same method mentioned above *via* gradient dilution. The IC_{50} value for when 50% of the DPPH radicals were scavenged was obtained by interpolation of the linear regression analysis.

Evaluation of the total antioxidant capacity

The antioxidant activity was measured using the ABTS method, which was improved by Wei *et al.* (2009) and Wang *et al.* (2007). The ABTS dissolved in distilled water (7 mM final concentration) was oxidized using potassium persulfate (2.45 mM final concentration) in the dark at room temperature for 14 h. The reaction solution was diluted with phosphate-buffered saline (PBS) buffer (pH 7.4) to obtain an absorbance of

0.70 ± 0.02 at 734 nm. Each sample (1 mL) or Trolox standard (1 mL) was added to 2 mL of $ABTS^+$ solution and mixed evenly. The reaction mixture was allowed to stand at room temperature for 5 min and the absorbance at 734 nm was recorded immediately. A standard curve was obtained using a Trolox standard solution at various concentrations (ranging from 2 μg/mL to 20 μg/mL) in a PBS buffer. The antioxidant properties of the samples were expressed in terms of Trolox equivalent antioxidant capacity.

Optimum distilled volume of the bamboo leaf hydrosols

Because hydrosols can be collected continuously, the optimum distilled volume was determined according to the response surface tests to get a high activity and acceptable hydrosols. Sixty grams of bamboo leaves were used to extract hydrosols under the optimum extraction process. Each batch was 100 mL, and the batches were subsequently stored in 100-mL volumetric bottles for subsequent determination. The optimum distilled volume of the high-quality hydrosols was obtained by measuring the CS and conducting a sensory evaluation of the odor and color for each volume of hydrosols. The high-quality hydrosols should be a colorless and transparent liquid with a bamboo leaf fragrance. The sensory evaluation of the bamboo leaf hydrosols was conducted by a group of 20 experienced panelists (22-year-old to 25-year-old graduate students). The hydrosols were kept at room temperature and given to the panelists for odor and color evaluation on a 9-point hedonic scale. The scale was set with 1 being the lowest, 5 representing the medium, and 9 as the highest rating for the overall acceptance of the odor and color. The full scale was as follows: 1 was dislike extremely, 2 was dislike very much, 3 was dislike moderately, 4 was dislike slightly, 5 was neither like nor dislike, 6 was like slightly, 7 was like moderately, 8 was like very much, and 9 was like extremely (Stojković *et al.* 2011; Tabti *et al.* 2014). The sensory evaluation took a random sampling pattern and was completed in 1 d. The results were expressed as the average score given by the 20 panelists.

Statistical analysis

All of the experiments were completed in triplicate, and the data were presented as the mean with the standard deviation. Significant differences between the means were verified using Duncan's multiple range test ($p < 0.05$).

RESULTS AND DISCUSSION

Single Factor Test of the Hydrosols Extraction

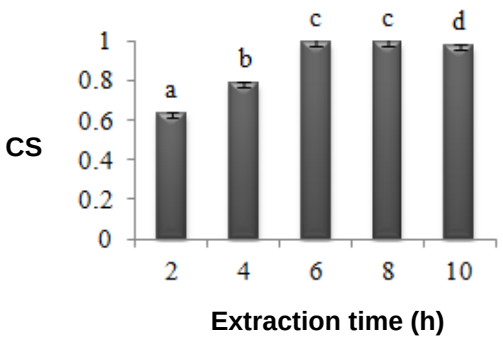

Fig. 2. Effect of the extraction time on the CS

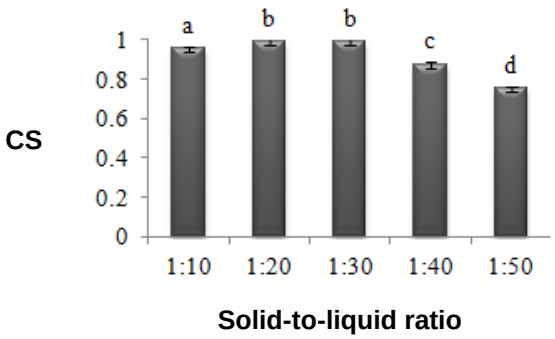

Fig. 3. Effect of the solid-to-liquid ratio on the CS

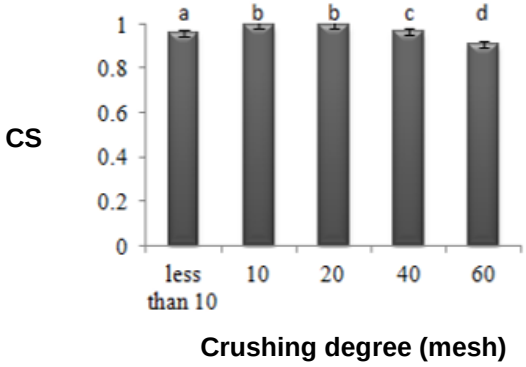

Fig. 4. Effect of the crushing degree on the CS

The data analysis (Figs. 2 to 4) showed that the CS of the antioxidant activity exhibited the trend where it first increased and then decreased as the extraction time, solid-to-liquid ratio, and crushing degree increased. The highest CS value was obtained when the extraction time was 6 h. There was no significant difference between the extraction times of 6 h and 8 h ($p < 0.05$). Therefore, 6 h was selected as the optimum extraction time because it shortened the time of the extraction process. Figure 4 shows that higher crushing degrees did not result in better hydrosol activities. The CS peaked at a 10-mesh crushing degree, which was significantly different from that of the other levels, excluding the 20-mesh samples ($p < 0.05$). The 10-mesh sample was chosen to be the best bamboo leaf crushing degree from the perspective of production savings. Figure 3 shows that the CS value obtained at a solid-to-liquid ratio of 1:30 was the largest, which was significantly different from that at the other levels ($p < 0.05$), excluding 1:20. Because the hydrosols were extracted *via* water-soluble methods, the researchers chose 1:30 as the best solid-to-liquid ratio to obtain more hydrosols.

Optimization of the bamboo leaf hydrosols extraction

The Response Surface Box-Behnken trial design and results are shown in Table 2. The effects of the parameters were analyzed using multivariate regression techniques. The results of the variance analysis and coefficient of variation are shown in Table 3.

Table 2. Response Surface Design and Results

Run	Factor			CS
	A	B	C	
1	8	0.0375	20	0.9152
2	6	0.0375	10	0.9915
3	4	0.0375	20	0.7754
4	6	0.0375	10	0.9899
5	6	0.0500	0	0.9465
6	8	0.0500	10	0.9489
7	6	0.0500	20	0.9587
8	8	0.0250	10	0.8214
9	4	0.0375	0	0.7491
10	6	0.0250	20	0.8689
11	4	0.0250	10	0.7092
12	4	0.0500	10	0.7761
13	6	0.0375	10	0.9898
14	6	0.0375	10	0.9915
15	6	0.0375	10	0.9915
16	6	0.0250	0	0.8457
17	8	0.0375	0	0.9032
A - Extraction time (h); B - Solid-to-liquid ratio; C - Crushing degree (mesh)				

Table 3. Variance Analysis and Coefficient of Variation

Source	Sum of Squares	df	Mean Square	F Value	*p* Value (Prob > F)
Model	1.487E-01	9	1.652E-02	7.214E+03	< 0.0001
A	4.189E-02	1	4.189E-02	1.829E+04	< 0.0001
B	1.853E-02	1	1.853E-02	8.091E+03	< 0.0001
C	6.790E-04	1	6.790E-04	2.965E+02	< 0.0001
AB	9.180E-04	1	9.180E-04	4.009E+02	< 0.0001
AC	5.110E-05	1	5.110E-05	2.232E+01	0.0021
BC	3.020E-05	1	3.020E-05	1.321E+01	0.0083
A^2	6.379E-02	1	6.379E-02	2.786E+04	< 0.0001
B^2	1.221E-02	1	1.221E-02	5.333E+03	< 0.0001
C^2	4.320E-03	1	4.320E-03	1.887E+03	< 0.0001
Residual	1.600E-05	7	2.290E-06		
Lack of Fit	1.280E-05	3	4.250E-06	5.199E+00	0.0726
Pure Error	3.270E-06	4	8.180E-07		
Cor Total	1.487E-01	16			
A - Extraction time (h); B - Solid-to-liquid ratio; C - Crushing degree (mesh)					

The analysis of variance showed that the F value of the model was 7214.026 and the *p* value (Prob > F) was less than 0.0001, which indicated that the quadratic model used in this study was statistically significant. The lack of fit was used to indicate the

degree of fit between the model and test. The P lack of fit (0.0726), which was greater than 0.05 for this test, was beneficial to the model, and there was no loss factor. Accordingly, researchers could use this model instead of experimental data for statistics and calculations. The calibration coefficient of the model was 0.9998 and the coefficient of variation was 0.17%, which both indicated that only 0.0002 of the model variance could not be explained by the model and the model was well fitted. The coded linear regression equation (Eq. 4) was obtained from the regression results and parameter selection of the factor experiments ($p < 0.05$):

$$Y = 0.99 + 0.072A + 0.048B + 0.0213E - 003C + 0.015AB - 3.575E - 0.003AC - 2.750BC - 0.12A^2 - 0.054B^2 - 0.032C^2 \quad (4)$$

where Y is the CS, A is the extraction time (h), B is the solid-to-liquid ratio, and C is the crushing degree (mesh).

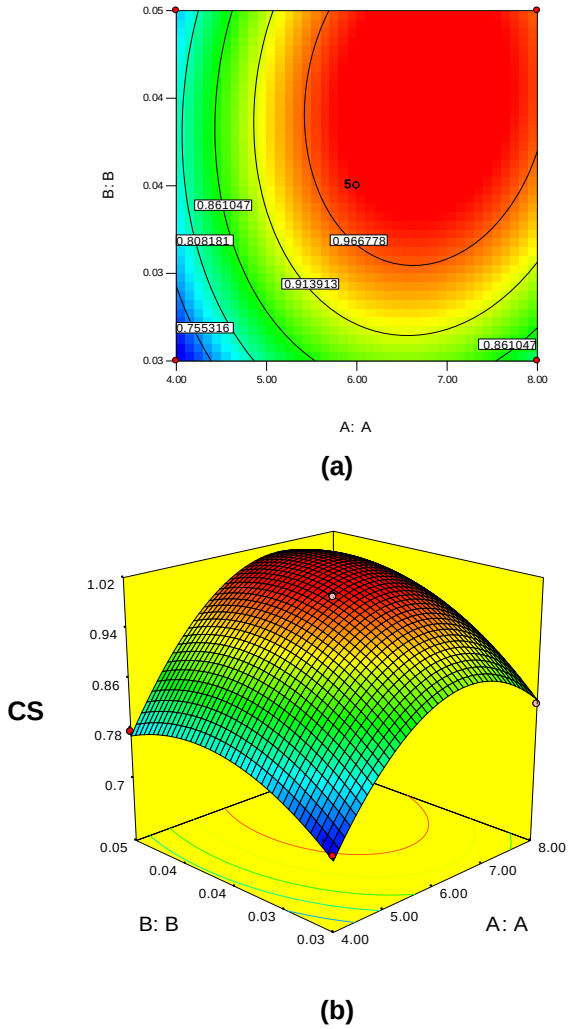

Fig. 5. Effect of the interaction of the extraction time and solid-to-liquid ratio on the antioxidant activity CS of the hydrosols at a 10-mesh crushing degree: (a) surface plot and (b) 3D plot

The contour lines on the 3D response surface curve represented the constant response curves on the two variable planes. The response variables at different variable levels were obtained through experimental factor fixing. The response surface was steep,

which indicated that the extraction time and solid-to-liquid ratio had a significant effect on the CS of the bamboo leaves. The contour had an oval shape, which indicated that the interaction between the two factors was relatively strong. The response surface plot (Fig. 5a) showed that the extraction time and solid-to-liquid ratio had a significant effect on the CS of the bamboo leaf hydrosols antioxidant activity. Figure 5b shows that the CS tended to increase first and then decreased with an increase in the solid-to-liquid ratio, while the extraction time was constant. Meanwhile, the CS initially increased and then decreased with an increase in the extraction time when the solid-to-liquid ratio remained unchanged. The contour line trend showed that the CS value peaked when the extraction time was 5 h to 7 h and the material ratio ranged from 0.031 to 0.044. The CS decreased outside of this range. The reason for this decrease was that when the extraction time was short, the active substances in the bamboo leaves were not completely extracted. Moreover, when the extraction rate of the active substances of the hydrosols reached a higher level, the CS value did not increase and some of the active substances in the bamboo leaves were degraded and oxidized because of the high temperature. The bamboo leaves adsorbed some active substances when the material ratio was relatively small, which was contrary to the improvement of the hydrosols activity. The energy for keeping the water in a boiling state increased and the active ingredient concentration in the steam was reduced when the solid-to-liquid ratio was too high.

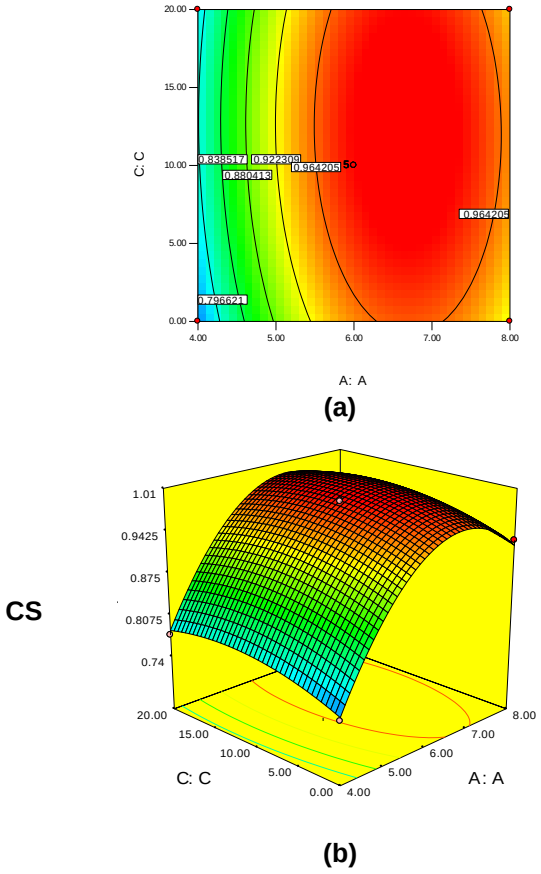

Fig. 6. Effect of the interaction of the extraction time and crushing degree on the antioxidant activity CS of the hydrosols at a solid-to-liquid ratio of 1:30: (a) surface plot and (b) 3D plot

The effect of the bamboo leaf hydrosols extraction time and crushing degree of the bamboo leaves on the CS is shown in Fig. 6. The 3D surface (Fig. 6b) showed that the CS first increased and then decreased with an increase in the crushing degree and the constant extraction time of the bamboo leaves. When the crushing degree of the bamboo leaves was constant, the CS value increased and then decreased with an increase in the extraction time. The surface plot showed that the CS value had a maximum value when the extraction time of the bamboo leaf hydrosols was 5 h to 7 h and the crushing degree of the bamboo leaves was 5 mesh to 15 mesh. The CS value was reduced outside of this range because the active substances in the bamboo leaves were not completely extracted when the crushing degree of the bamboo leaves was relatively small, which led to a weak overall activity of the bamboo leaf hydrosols. The total surface area of the bamboo leaves increased, which adsorbed active substances of the bamboo leaves when the bamboo leaf crushing degree was too high. Then, the overall activity of the bamboo leaf hydrosols was reduced.

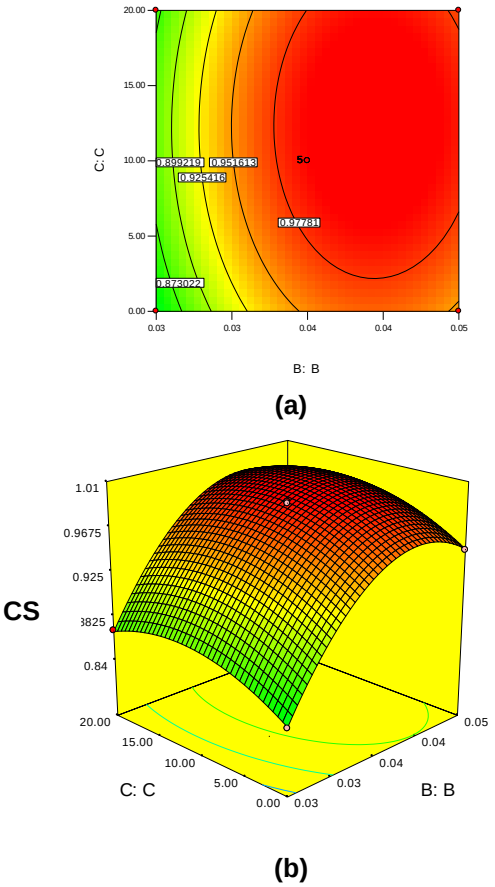

Fig. 7. Effect of the interaction of the crushing degree and solid-to-liquid ratio on the antioxidant activity CS of the hydrosols at an extraction time of 6 h: (a) surface plot and (b) 3D plot

The effect of the solid-to-liquid ratio and crushing degree of the bamboo leaves on the CS value of the hydrosols is shown in Fig. 7. The 3D surface (Fig. 7b) showed that the CS value tended to increase first and then decreased with an increase in the crushing degree of the bamboo leaves when the solid-to-liquid ratio was constant. Meanwhile, the CS value showed a trend of first increasing and then decreasing with an increase in the solid-to-liquid ratio and when the crushing degree of bamboo leaves was fixed. The

surface plot showed that the CS peaked when the solid-to-liquid ratio ranged from 0.031 to 0.044 and the crushing degree was between 5 mesh and 15 mesh. The CS value decreased outside of this range.

From the response surface analysis, the optimum extraction process was obtained and had an extraction time of 6.6 h, solid-to-liquid ratio of 1:23, and crushing degree of 11.04 mesh. At these conditions, the CS was 1.015. Subsequently, the extraction process was adjusted according to the practical conditions and were an extraction time of 6 h, solid-to-liquid ratio of 1:25, and crushing degree of 10 mesh. The CS value obtained at these conditions was 0.9967. The difference in the CS values from the adjusted experiment and response surface prediction extraction process was small, which meant these conditions could be used as the optimum extraction process.

The determination of the following conclusions was because of the oxidation resistance of the optimal extraction process of the bamboo leaf hydrosols. The curve of the scavenging ability (V_c) for the DPPH radical was,

$$y = 0.3142\ln(x) + 1.7574 \ (R^2 = 0.9909) \quad (5)$$

where y is DPPH free radical scavenging rate (%) and x is the concentration corresponding to V_c (mg/mL).

Thus, IC_{50} was 0.01828 mg/mL of V_c equivalent. The curve of the scavenging ability of the hydrosols was,

$$y = 0.3519\ln(x) + 0.8679 \ (R^2 = 0.9976) \quad (6)$$

where y is DPPH free radical scavenging rate (%) and x is the dilution factor of bamboo leaf hydrosols.

This resulted in an IC_{50} of 0.3515 hydrosols content (v/v). Thus, the scavenging capacity of the hydrosols to the DPPH corresponded to 0.052 mg/mL of V_c equivalent. The following curve was obtained by measuring the total antioxidant capacity of the Trolox standard at different concentrations,

$$y = -0.0151x + 0.4037 \ (R^2 = 0.9972) \quad (7)$$

where y is the absorbance at 734 nm and x is the concentration of Trolox (μg/mL).

By measuring the total antioxidant activity of the bamboo leaf hydrosols under the same conditions, it was concluded that the total antioxidant activity of the hydrosols was 0.2771 mg/mL Trolox equivalent. It was concluded from the aforementioned data that the bamboo leaf hydrosols had the higher ability to scavenge DPPH free radicals and total antioxidant capacity, which provides a data basis for subsequent use with natural antioxidants and bacteriostats.

Optimum distilled volume of the bamboo leaf hydrosols

With an increase in the time and a decrease in the volume, the total activity CS of the bamboo leaf hydrosols showed a trend of first decreasing and then increasing. Additionally, there was a sharp decrease in the total activity CS at 500 mL. The reason for this result was that some volatile active substances were adsorbed by bamboo leaf. Even with an increase in the time, some active substances were oxidized, decomposed, or underwent other chemical reactions, which resulted in a gradual decrease in the activity. However, some adsorbed or even non-volatile active ingredients were extracted after this period of time. The ingredients were over-lengthened and the aqueous extract volume was reduced, which led to an increasing tendency for the total activity CS of the

hydrosols. The sensory score differed from the CS, in that it exhibited a downward trend. Even after 800 mL, the bamboo leaf hydrosol color had changed. The reason for this trend might have been the excessive prolongation of hydrosols production, which resulted in the original fragrance of the bamboo leaf hydrosols fading into faint smells. The colored substances were removed by the water vapor. Through the overall analysis of the bamboo leaf hydrosols active CS and sensory evaluation, it was concluded that it is best to obtain bamboo leaf hydrosols before 400 mL. When the distilled volume was one-quarter of the volume of the total aqueous extract, bamboo leaf hydrosols that were highly active, colorless, and pleasantly aromatic were obtained.

Table 4. Volume Experimental Results

Volume (mL)	Color	Sensory Score	CS
100	Colorless	8.75 ± 0.067^{a}	0.9967 ± 0.005^{a}
200	Colorless	8.25 ± 0.085^{b}	0.9548 ± 0.012^{b}
300	Colorless	7.85 ± 0.054^{c}	0.9027 ± 0.008^{c}
400	Colorless	7.25 ± 0.025^{d}	0.8795 ± 0.011^{d}
500	Colorless	6.05 ± 0.075^{e}	0.7254 ± 0.006^{e}
600	Colorless	5.45 ± 0.067^{f}	0.7865 ± 0.009^{f}
700	Colorless	5.05 ± 0.052^{g}	0.8061 ± 0.013^{g}
800	Colorless	4.55 ± 0.087^{h}	0.8247 ± 0.015^{h}
900	Micro-green	3.85 ± 0.052^{i}	0.8547 ± 0.007^{i}
1000	Light green	2.55 ± 0.043^{j}	0.8954 ± 0.012^{j}
Values represent the mean ± standard deviation of duplicate assays for each group; In each column, different superscripts indicate significant differences (p<0.05)			

CONCLUSIONS

1. The response surface test was performed on a single factor basis, and the optimum extraction process of the bamboo leaf hydrosols was obtained when the bamboo leaf crushing degree was 10 mesh, the solid-to-liquid ratio was 1:25, and the extraction time was 6 h.
2. When the distilled volume was one-quarter of the volume of the total aqueous extract, bamboo leaf hydrosols that were highly active, colorless, and pleasantly aromatic were obtained.
3. The extraction parameters for the bamboo leaf hydrosols was obtained through the analysis of this experiment. This experiment provided the data support for the production of bamboo leaf hydrosols.

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