



Optimization of dynamic microwave-assisted extraction of *Armillaria polysaccharides* using RSM, and their biological activity



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ABSTRACT

We used the Box–Behnken design to optimize polysaccharide extraction from *Armillaria luteo-virens* (Alb. et Schw. Fr.) Sacc. The independent factors included extraction time (X_1), microwave power (X_2) and water to raw material ratio (X_3). The experimental values were fitted to a second-order polynomial equation using multiple regression analysis and a statistical method. Analysis of Variance results indicated that all factors including $X_1 - X_3$ had an impact on *Armillaria luteo-virens* (Alb. et Schw. Fr.) Sacc. polysaccharide extraction. The optimal conditions for efficient yield of polysaccharide, giving a maximum yield of 8.43%, were: $X_1 = 30.24$ min, $X_2 = 600.6$ W and $X_3 = 40$ mL/g. The model was verified by modifying the optimal conditions ($X_1 = 30$ min, $X_2 = 601$ W and $X_3 = 40$ mL/g) for practical application. A pilot scale test was also carried out under optimal conditions. The obtained yields $8.40 \pm 0.12\%$ and $8.34 \pm 0.25\%$ were comparable with the optimized condition, which indicated that our model is accurate. Fourier transform infrared spectroscopy characterization revealed that the extracted polysaccharide produced typical absorption peaks. Oxygen radical absorbance capacity results showed the polysaccharides had good potential as an antioxidant. Moreover, the polysaccharide showed relatively strong inhibitory activity on the growth of NCI–H446 cells.

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1. Introduction

Since ancient times, a large variety of mushrooms have been used as food, medicine, and functional resources in many Asian countries because of their diverse biological activities (Hoshi et al., 2005). *Armillaria luteo-virens* (Alb. et Schw. Fr.) Sacc. is a special Chinese medicinal and edible mushroom. It is mainly distributed in the meadows and grasslands of Qinghai-Tibet plateau and is used as a traditional Tibetan medicine for the treatment of dizziness, headaches, neurasthenia, insomnia, numbness in limbs, and infantile convulsions (Yang et al., 1989). Previous phytochemical studies have demonstrated that proteins, polysaccharides, and alkaloids are the major bioactive constituents of this fungi. Among these constituents, polysaccharides have not been researched. To the best of our knowledge, there are no papers describing the optimization of polysaccharides extractions from *Armillaria luteo-virens* (Alb. et Schw. Fr.) Sacc.

Extraction is very important for further research into, and

development and application of *Armillaria luteo-virens* (Alb. et Schw. Fr.) Sacc polysaccharides named as ALSP. In recent years, dynamic microwave-assisted extraction (DMAE) has been successfully applied to numerous biologically active compounds from a wide variety of natural resources (Liu, Dang, Wang, 2013; Martins, Aguilar, Garza-Rodriguez, Mussatto, & Teixeira, 2010; Wang et al., 2010). This technique consists of the penetration of microwave energy into the material structure, which produces a volumetrically distributed heat source due to molecular friction resulting from dipolar rotation of polar solvents and from the conductive migration of dissolved ions, accelerating the mass transfer of target compounds. In general, the compounds are extracted more selectively and more quickly by this technique with similar or better yields compared with conventional extraction processes. Moreover, the technique, uses less energy and solvent, and is therefore more environmentally friendly (Eskilsson & Bjoklund, 2000; Bélanger & Paré, 2006; Srogi, 2006).

Response surface methodology (RSM) has been reported to be an effective tool for optimization of a process when the independent variables have a combined effect on the desired response. RSM is a collection of statistical and mathematical system that has been successfully used for developing, improving and optimizing such processes (Koocheki et al, 2008; Wu et al, 2007). The main idea

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behind RSM is the use of a sequence of designed experiments to obtain an optimal response. Using statistics, it explores the relationships between several explanatory variables and one or more response variable. The Box–Behnken design (BBD) is a type of response surface design: it is an independent quadratic design and there is no embedded factorial or fractional factorial design. In this design, the treatment combinations are at the midpoints of the edges of the process space and at the center. These designs are rotatable (or near rotatable) and require three levels of each factor. It is more efficient and easier to arrange and interpret experiments in comparison with others.

A great deal of attention has been paid to polysaccharides in recent years owing their unique bioactivities and chemical structures. A large number of polysaccharides have been reported to exhibit varied biological activities, such as antioxidation, anti-inflammatory, antitumor, antiulcer, antiviral, and immunological activities (Zhong, Lin, Wang, & Zhou, 2012; Liao, Guo, & Lin, 2011; Zhu et al., 2011; Simas-Tosin et al., 2012; Simas-Tosin Thetsrimuang, Khammuang, Chiablaem, Srisomsap, Sarntthima, 2011). However, there are no papers describing the activity of polysaccharides from *Armillaria luteo-virens* (Alb. et Schw. Fr.) Sacc.

Therefore, the objectives of this study include: (I) optimizing the process of dynamic microwave-assisted extraction of ALSP using RSM; (II) a preliminary characterization of ALSP by Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM); (III) determining the antioxidant activity of ALSP; and, (IV) determining the antiproliferative activity of ALSP.

2. Materials and methods

2.1. Materials and chemicals

Armillaria luteo-virens (Alb. et Schw. Fr.) Sacc. fungi were collected from Qilian, Qinghai, China. All the other chemicals and reagents used were of analytical grade.

2.2. Extraction of ALSP using dynamic microwave-assisted extraction

Armillaria luteo-virens (Alb. et Schw. Fr.) Sacc. (500 g) was ground in a high speed disintegrator (SF-2000, Chinese Traditional Medicine Machine Works, Shanghai, China) to obtain a fine powder. To remove any substances that would affect the color, the powder was added to 80 mL/100 mL ethanol (2000 mL) in a water bath at 85 °C for 2 h. After incubation, the mixture was centrifuged at 3580 × g for 10 min, and the insoluble residue was treated again as mentioned above. The mixture was centrifuged again. After the insoluble residue had been dried in an oven at 50 °C, each pre-treated sample (1 g) was extracted using water for a designed extraction time, microwave power, and water-to-raw material ratio. The water extraction solutions were separated from insoluble residue by centrifugation (6000 rpm for 8 min), and then precipitated by the addition of ethanol to a final concentration of 85 mL/100 mL. Protein was removed using the Sevag method. The precipitate was separated by centrifugation (5000 × g for 8 min) and air-dried for 12 h. The content of total polysaccharides was measured using the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The yield of ALSP (%) was calculated as follows:

$$\text{The yield of ALSP (\%)} = \frac{\text{the polysaccharides content of extraction (g)}}{\text{weight of powder (g)}} \quad (1)$$

2.3. Experimental design and statistical analysis

RSM was used to estimate the effect of independent variables (extraction time, X_1 ; microwave power, X_2 and water to raw material ratio, X_3) on the extraction yield of ALSP (%). A BBD was employed for designing the experimental data. For statistical calculation, the variables were coded by the following equation:

$$X_i = \frac{X_i - X_0}{\Delta X_i} \quad (2)$$

where X_i is the independent variable coded value, X_i is the independent variable real value, X_0 is the independent variable real value on the center point. The range of independent variables, a BBD matrix, and the response value carried out for developing the model are listed in Table 1.

The whole designed experiment consisted of 17 trial points in random order. These trials were divided into 12 factorial points, and 5 replicates, which were used for the estimation of a pure error sum of squares at the center of the design. The response value in each trial was an average of duplicates. Based on the BBD experimental data, regression analysis was carried out and fitted into the empirical quadratic second-order polynomial model:

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_{12}X_1X_2 + A_{13}X_1X_3 + A_{23}X_2X_3 + A_{11}X_1^2 + A_{22}X_2^2 + A_{33}X_3^2 \quad (3)$$

where Y is the predicted response, and x_1 , x_2 and x_3 are input variables. A_0 is a constant and A_1 , A_2 , and A_3 are linear coefficients. A_{12} , A_{23} and A_{13} are cross-product coefficients and A_{11} , A_{22} , and A_{33} are quadratic coefficients. The model evaluated the effects of each independent variable on the response. The experimental design was analyzed and the predicted data were calculated using the Design-Expert software (v.8.0 trial, Stat-Ease, Inc., Minneapolis, USA) to estimate the response of the independent variables. Subsequently, three additional experiments were conducted to verify the validity of the statistical experimental strategies.

2.4. FTIR of ALSP

ALSP was mixed with spectroscopic grade potassium bromide powder, ground, and pressed into 1 mm pellets for FTIR measurement. FTIR spectra of the ALSP were determined using a Bruker TENSOR 27 FT-IR spectrometer (Bruker, Germany) in the frequency range of 4000–400 cm^{-1} .

2.5. SEM

The morphological features of the ALSP were studied with a Zesis EVO10 Field Emission Scanning Electron Microscope (Carl Zeiss AG, Germany). The dried sample was mounted on a metal stub and sputtered with gold to make the sample conductive.

Table 1

The levels of variables employed in the present study for the construction of Box–Behnken design (BBD).

Variables	Coded variables	Actual variables	Levels		
			−1	0	1
Extraction time (min)	x_1	X_1	20	30	40
Microwave power (W)	x_2	X_2	500	600	700
Water to solid (mL/g)	x_3	X_3	20	30	40

Table 2

Box–Behnken design (BBD) matrix of the three variables in coded units and response values for the extraction rate of the polysaccharides.

Run	Time (min) A	Microwave power (w) B	Ratio of water to raw material (mL/g) C	Polysaccharide yield (%)	
				Actual	Predicted
1	40	700	45	7.26	7.38
2	20	700	45	6.64	6.82
3	30	700	60	7.65	7.51
4	30	700	30	5.65	5.69
5	40	500	45	6.84	6.66
6	30	600	45	8.64	8.84
7	30	500	30	5.75	5.89
8	30	600	45	8.97	8.84
9	40	600	60	6.95	5.97
10	20	600	60	7.09	7.05
11	30	600	45	8.85	8.84
12	30	500	60	6.55	6.70
13	20	600	30	5.54	5.52
14	30	600	45	8.79	8.84
15	20	500	45	7.24	7.12
16	40	600	30	5.65	5.49
17	30	600	45	8.93	8.84

2.6. Measurement of oxygen radical absorbance capacity (ORAC)

The ORAC assay was based on that of Ou et al. (Ou, Hampsch-Woodill, & Prior, 2001). The polysaccharides were dissolved in 75 mmol/L (pH 7.4) phosphate buffer and tested at concentrations of 0.01–0.5 mg/mL in triplicate. Briefly, sample solution or Trolox was incubated with fluorescein and 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) in a total volume of 200 μ L. All ORAC analyses were performed at 37 °C using a fluorescence spectrometer (Fluoroskan Ascent, Thermo Labsystems, USA). The final ORAC values were expressed as micromole Trolox Equivalents per gram yam flour (μ mol TE/g). Each sample was tested at 10 concentrations in triplicate.

2.7. Assay of the antiproliferative activity on NCI–H446 cells

Antiproliferative activities *in vitro* of polysaccharides on the human lung cancer cell line NCI–H446 cells were determined by the MTT-based colorimetric method (MTT is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Mosmann, 1983). Briefly, The suspension of NCI–H446 cells was pipetted into a 96-well plate at a density of approximately 5000 cells/mL (100 μ L/well) and cultured for 24 h. Then test samples at different concentrations (0.02, 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, and 2.50 mg/mL) were added into each well separately. After 24 h incubation, MTT reagent was added to each well, and the plate was further incubated for 4 h. The media were then removed, and dimethyl

Table 3

Analysis of variance of the experimental result of the BBD.

Variables	Sum of squares	df	Mean square	F-value	p-value prob. > F
Model	24.00	9	2.67	73.85	<0.0001
A	0.020	1	0.020	0.55	0.4827
B	0.11	1	0.11	2.92	0.1314
C	13.65	1	13.65	378.05	<0.0001
AB	0.26	1	0.26	7.20	0.0314
AC	0.016	1	0.016	0.43	0.5317
BC	0.37	1	0.37	10.14	0.0154
A*A	3.93	1	3.93	108.69	<0.0001
BB	3.23	1	3.23	89.37	<0.0001
CC	10.29	1	10.29	284.83	<0.0001
Residual	0.25	7	0.036	–	–
Lack of fit	0.19	3	0.062	3.66	0.1212
Pure error	0.68	4	0.017	–	–
Correlation total	24.25	16	–	–	–

Table 4

Analysis of variance for the fitted quadratic polynomial model of extraction polysaccharides.

Item	S.D.	Mean	C.V.%	Press	R ²	R ² _{Adj}	R ² _{pred}	Adeq. precision
value	0.19	7.23	2.63	3.07	0.9896	0.9762	0.8734	22.931

sulfoxide was added into each well for the dissolution of the formazan crystals. The absorbance of each well was read at 492 nm using the Varioskan Flash spectral scan multimode plate reader. The antiproliferative activity was calculated according to the formula below:

$$\text{Antiproliferative activity (\%)} = \frac{A_c - A_s}{A_c - A_b} * 100 \quad (4)$$

where A_s is the absorbance of samples, A_c is the absorbance of the control (without sample), and A_b is the absorbance of the blank (the absorbance of dimethyl sulfoxide).

3. Result and discussion

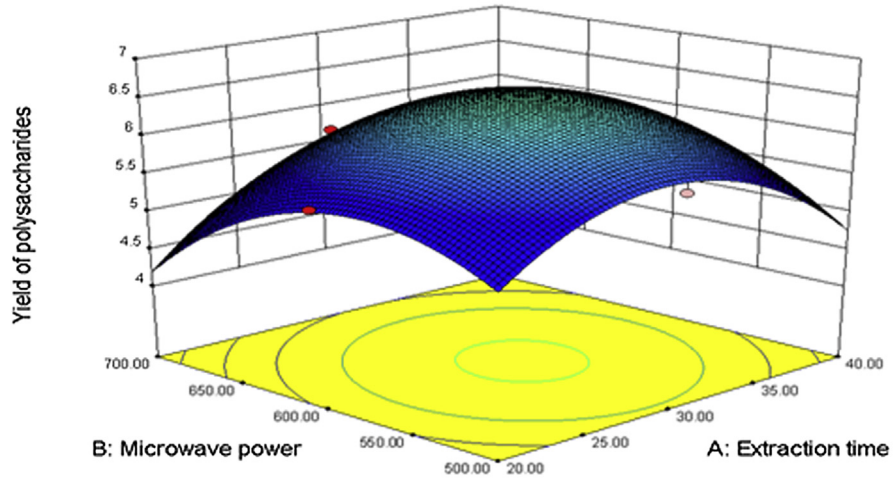
3.1. Model fitting and statistical analysis

To examine the combined effects of the variables extraction time (min), microwave power (W) and water to raw material ratio (mL/g) on the crude polysaccharide extraction by RSM, a BBD of 12 runs with five center points (for estimation of pure error) leading to a set of experiments was performed randomly. The experimental results are summarized in Table 2. By applying multiple regression analysis the experimental data, the predicted response Y (the yield of polysaccharides) was obtained using the following equation:

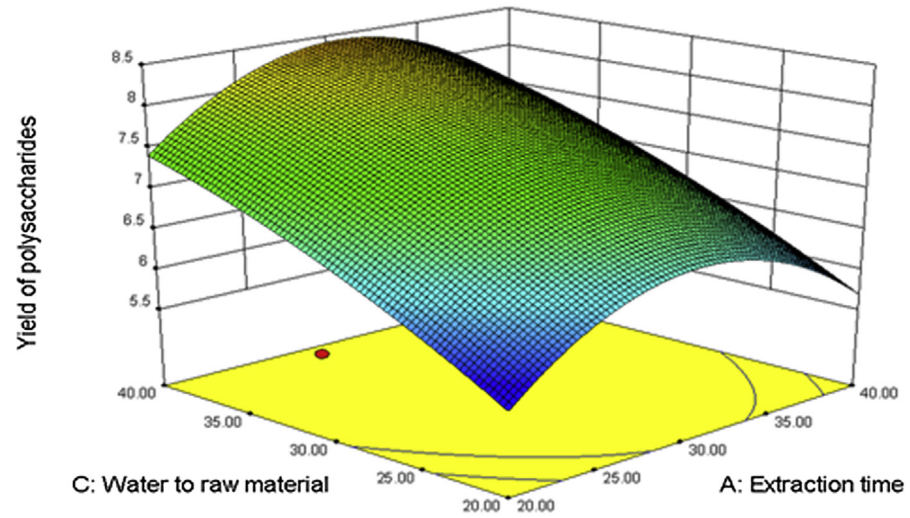
$$Y = 6.57 + 0.086 X_1 - 0.2 X_2 + 2.55 X_3 + 0.25 X_1 X_2 - 0.042 X_1 X_3 + 0.2 X_2 X_3 - 0.971 X_1^2 - 0.88 X_2^2 - 0.69 X_3^2 \quad (5)$$

A summary of ANOVA of the experimental results of the BBD is provided in Table 3. The F-test was used to check the statistical significance of the regression equation. The ANOVA was done using the software Design-Expert to determine whether or not the quadratic model was significant. P-values were used as a tool to check the significance of each coefficient, which in turn might indicate the pattern of the interactions between the variables. The F-test had a very high model F-value (73.85) and very low P-values ($P < 0.0001$). There is only a 0.01% chance that a “Model F-value” that large could be due to noise. The smaller the F-values, the bigger the significance of the corresponding coefficient, which implies the

Design-Expert?Software
 Factor Coding: Actual
 Yield of polysaccharides
 ● Design points above predicted value
 ○ Design points below predicted value
 8.97
 5.54
 X1 = A: Extraction time
 X2 = B: Microwave power
 Actual Factor
 C: Water to raw material = 30.00



Design-Expert?Software
 Factor Coding: Actual
 Yield of polysaccharides
 ● Design points above predicted value
 ○ Design points below predicted value
 8.97
 5.54
 X1 = A: Extraction time
 X2 = C: Water to raw material
 Actual Factor
 B: Microwave power = 600.00



Design-Expert?Software
 Factor Coding: Actual
 Yield of polysaccharides
 ● Design points above predicted value
 ○ Design points below predicted value
 8.97
 5.54
 X1 = B: Microwave power
 X2 = C: Water to raw material
 Actual Factor
 A: Extraction time = 30.00

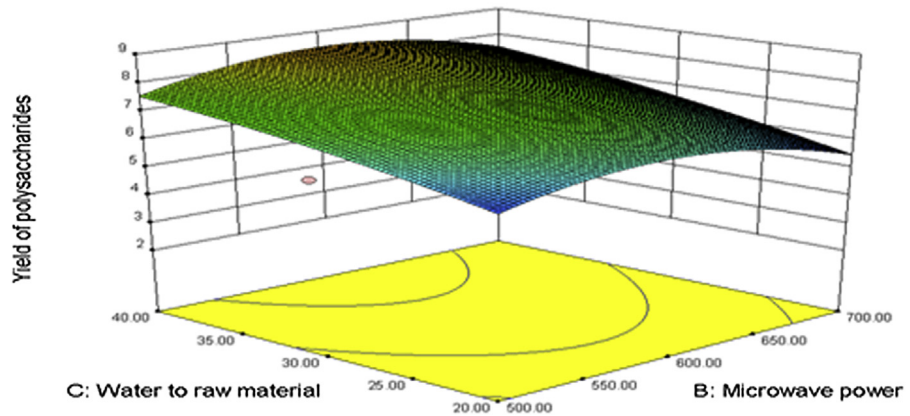


Fig. 1. Response surface (3D) showing the effect of the extraction time (X_1), microwave power (X_2), and water to raw material ratio (X_3). (A) The response surface plots of the effect of extraction time (X_1), microwave power (X_2) and their reciprocal interaction on polysaccharide yield (%). (B) The response surface plots of the effect of extraction time (X_1), water to raw material ratio (X_3) and their reciprocal interaction on polysaccharide yield (%). (C) The response surface plots of the effect of microwave power (X_2), water to raw material ratio (X_3) and their reciprocal interaction on polysaccharide yield (%).

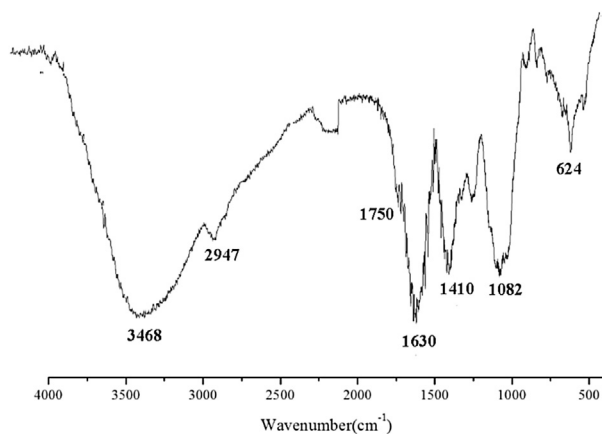


Fig. 2. FTIR characterization of ALSP.

model is suitable for use. The *F*-value (3.66) and *P*-values (0.12) of “lack-of-fit” indicate that the “lack-of-fit” was insignificant relative to the pure error. It indicates that the model equation is adequate for predicting the yield of polysaccharides under any combination of values of the variables. Values of “Prob. > *F*” less than 0.0500 indicate model terms are significant. In this case X_3 , X_1X_2 , X_2X_3 , X_3 , X_1^2 , X_2^2 , X_3^2 are significant model terms.

The results of ANOVA for the fitted quadratic polynomial model of extraction of polysaccharides are shown in Table 4. The “Pred *R*-Squared” of 0.8734 is in reasonable agreement with the “Adj *R*-Squared” of 0.9762. Both R^2 and Adj *R*-Squared values indicate that the accuracy and general availability of the polynomial model are adequate. The very low value of the coefficient of variation (CV%, 2.63) clearly suggests a very high degree of precision and a good deal of reliability of the experimental values. The “Adeq. Precision” was used to measure the signal to noise ratio, which is preferably greater than four. An “Adeq. Precision” of 22.931 indicates that this model could be used to navigate the design space.

3.2. Interaction between process variables

The response surface curves were plotted to investigate the interactions of the variables and to determine the optimal level of each variable for the maximum response. The optimal values of the selected variables were obtained by solving the regression equation using the Design-Expert software. The 3D response surface contour plots were provided as graphical representations of the regression equation. Fig. 1 show three independent response surface plots and

their respective contour plots, which reveal information about the interactions between two variables, while the third variable fixed. Fig. 1(A) shows the effect of extraction time (X_1), microwave power (X_2), and their reciprocal interaction on polysaccharide extraction yield (%), when water to raw material ratio (X_3) was fixed at 30 mL/g. The result revealed that the yield increased with the increase in extraction time and microwave power. With a further increase in extraction time and microwave power, the yield began to slight decrease. As shown in Fig. 1(B), the polysaccharide yield increased with increasing extraction time (X_1) and water to raw material ratio (X_3). With a further increase in extraction time, the yield showed slight decrease. As shown in Fig. 1(C), the polysaccharide yield increased with an increase in microwave power (X_2) and water to raw material ratio (X_3). With a further increase of microwave power, the yield showed a slight decrease.

3.3. Optimization of the variables

The optimum extraction conditions ($X_1 = 30.24$ min, $X_2 = 600.6$ W and $X_3 = 40.0$ mL/g) for maximum polysaccharides yield were estimated using the model equation by solving the regression equation and analyzing the response surface contour plots. The theoretical maximum polysaccharides extraction yield predicted under the above conditions was 8.43%.

3.4. Validation of the models

To validate the adequacy of the model equations, a verification experiment was carried out under optimal conditions: extraction time = 30 min; microwave power = 601 W; and water to raw material ratio = 40 mL/g. The mean yield value $8.40 \pm 0.12\%$ and ($n = 3$) obtained from real experiments demonstrated the validity of the RSM model, and indicated that the model was adequate for the extraction process. Also, a pilot scale test (100 g raw material) was carried out under optimal conditions, and the mean yield value was $8.34 \pm 0.25\%$ ($n = 3$). Therefore, the results yield values from the laboratory experiment and the pilot scale test are in good agreement with the predicted value.

3.5. FTIR spectroscopy

FT-IR spectroscopy is a powerful technique for the identification of characteristic organic groups in the polysaccharides. In order to confirm the identity of ALSP, it was analyzed by FT-IR. As shown in Fig. 2, the FTIR spectra of ALSP shows a broad peak at around 3500 cm^{-1} , the band between 3600 and 3200 cm^{-1} represents the

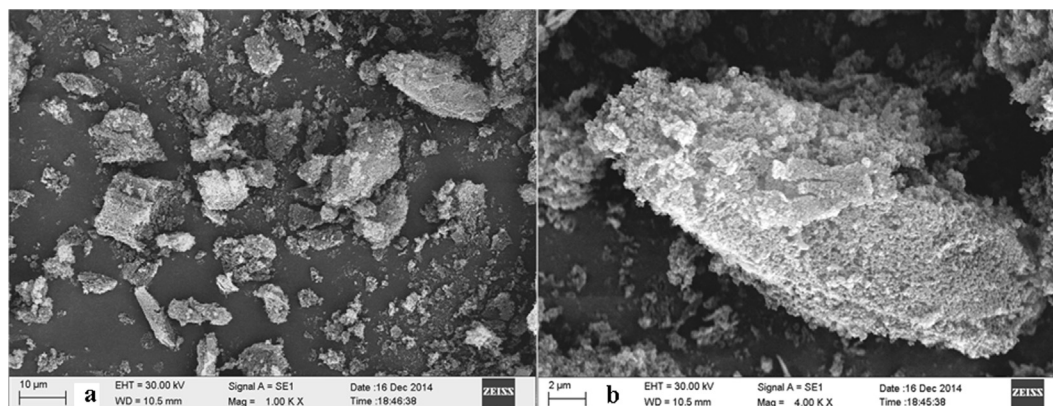


Fig. 3. Scanning electron microscope (SEM) photographs of ALSP.

stretching vibration of O–H, which produces glycosidic structures of the sugar residues. The band at 2947 cm^{-1} was associated with stretching vibration of C–H in the sugar ring (Lai, Wen, Li, Wu, & Li, 2010). The band at 1630 cm^{-1} arises from absorption owing to carbon–oxygen double-bond asymmetric stretching vibration (Cerna et al., 2003). FT-IR spectra in the wave number between 850 and 1200 cm^{-1} is considered as the “finger print” region for carbohydrates, which is unique to a compound. The strong extensive absorption in the region of 1000 – 1200 cm^{-1} is due to C–O–C stretching vibration and stretching vibrations of C–OH side groups (Barros et al., 2002). These results indicated that ALSP possesses typical absorption peak of polysaccharides.

3.6. SEM analysis

To study the surface characteristics of ALSP, we subjected the powder to SEM. The scanning electron micrographs of ALSP are shown in Fig. 3. The micrographs of ALSP shows distinct particles of fairly defined shape. Fig. 3(b) shows some irregular-shaped particles which point to the amorphous nature of the powder. The shape and structure, or the surface topology of the polysaccharide may be affected by the method of extraction and purification or preparation of the product (Nep & Conway, 2010). In summary, the polysaccharides from *Armillaria luteo-virens* (Alb. et Schw. Fr.) Sacc. extracted by DMAE were qualitatively identified by comparing their micrographs with those of the standards.

3.7. ORAC

The ORAC assay can be used to quantify the antioxidant capacity of foods by measuring the peroxy radical scavenging activity of the compounds present (Cao & Prior, 1999). Generally, the ORAC assay is highly regarded owing to its use of biologically relevant free radicals and also its integration of both degree and time of inhibition in to a single quantity (Awika, Rooney, Prior, & Cisneros-Zevallos, 2003).

Dubost et al. analyzed a wide variety of mushrooms using the ORAC assay and found a range between $39\text{ }\mu\text{mol TE/g}$ and $138\text{ }\mu\text{mol TE/g}$ (Dubost, Ou, & Beelman, 2007). As shown Table 5, the ORAC value of ALSP was $94\text{ }\mu\text{mol TE/g}$. The antioxidant activities of the polysaccharides were related to their compositions and structural features, including content of proteins and uronic acid, molecular weights, monosaccharide composition and types of glycosidic (Li, Fu, Huang, Luo, & You, 2015; Yang et al., 2009). In summary, the results of the *in vitro* antioxidant assays indicate that ALSP has potential antioxidant activity, and could be utilized as an antioxidant in the pharmaceutical and food industries.

3.8. Anticancer activity

It has been reported that polysaccharides play a certain role in anti-tumor activity. However, there is little information regarding

the anticancer potentials of polysaccharides from *Armillaria luteo-virens* (Alb. et Schw. Fr.) Sacc. In the present study, therefore, the anticancer activity of ALSP, *in vitro*, was evaluated. The viability of NCI–H446 cells treated with ALSP for 24 h was determined using a colorimetric MTT-based assay.

As shown in Table 5, the polysaccharides exhibited a dose-dependent activity within the concentration range 0.02 – 0.16 mg/mL , and the inhibitory effects of ALSP on NCI–H446 cells increased significantly ($P < 0.05$) with the increase of sample concentration. The highest inhibition rate on NCI–H446 cells was 61.5%, at a concentration of 0.16 mg/mL . However, when the concentration of ALSP was increased, the cancer cells inhibition rate decreased. We speculate that this effect might arise because the cells induce resistance at high drug concentrations. It has been reported that the antitumor effects of polysaccharides depend on their molecular weight, chemical composition, structure of the polymeric backbone, and degree of branching (Gan, Ma, Jiang, Xu, & Zeng, 2011). Jiang reported that if compounds can enhance the level of anti-oxidation and remove the reactive oxygen species in cancer cells, they may inhibit the cell growth, which might explain its high antitumor activity (Jiang, Wang, Liu, Gan, & Zeng, 2011). The mechanism for the inhibitory activity of cancer cell growth of polysaccharide is complex. So the mechanism for the inhibitory activity of cancer cell growth of polysaccharide from *Armillaria luteo-virens* (Alb. et Schw. Fr.) Sacc. needs further study.

4. Conclusion

In the past decade, it has been found that the polysaccharides in plants are not only valuable as an energy resource but play a very important role in biological processes. RSM was used to determine the optimum process parameters that produce the maximum extraction yield. ANOVA showed that all three parameters extraction time, microwave power and water to raw material ratio, were significant and quadratic models were obtained for predicting the responses. In the present study, we obtained optimum conditions for the extraction of ALSP. The optimum conditions were found to be: extraction time (X_1) = 30.24 min ; microwave power (X_2) = 600.6 W ; and water to raw material ratio (X_3) = 40 mL/g . For practical applications, the modified parameters were chosen as $X_1 = 30\text{ min}$, $X_2 = 601\text{ W}$, $X_3 = 40\text{ mL/g}$. These conditions produced a real experiment yield of $8.40 \pm 0.12\%$ and a pilot scale test yield of $8.34 \pm 0.25\%$, which were in a good agreement with the predicted value. FTIR characterization of the extracted polysaccharide revealed typical absorption peaks. As expected, antioxidant tests *in vitro* indicated that the polysaccharides showed rational ORAC activities. We found that ALSP can directly inhibit the proliferation of lung cancer cells. Further work on purification, function evaluation, and application is in progress to improve the effectiveness of ALSP.

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Table 5
In vitro antioxidant anti-tumor activities of ALSP.

Sample	ORAC value ($\mu\text{mol TE/g}$)	Concentration (mg/ml)	Antiproliferative activity on NCI–H446 cells (%)
ALSP	94	0.02	5.02 ± 1.06
		0.04	16.85 ± 1.55
		0.08	47.97 ± 2.62
		0.16	61.5 ± 2.08
		0.31	57.49 ± 2.73
		0.63	54.93 ± 0.78
		1.25	50.64 ± 1.49
		2.5	48.75 ± 2.62

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