Journal of Medicinal Plants Research Vol. 5(10), pp. 2064-2070, 18 May, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Response surface methodology for optimization of polysaccharides extraction by mild alkaline hydrolysis from fruiting body of medicinal mushroom, *Ganoderma lucidum*

Gao-Qiang Liu*, Yan Zhao, Xiao-Ling Wang and Chao-Yang Zhu

Hunan Provincial Key Laboratory of Forestry Biotechnology, College of Life Science and Technology, Central South University of Forestry and Technology, Changsha 410004, P. R. China.

Accepted 10 March, 2011

Polysaccharides (PLS) is a major source of biological activity and therapeutic use of medicinal basidiomycete, *Ganoderma lucidum*. The aim of this study was to systematically obtain a model of factors that would yield an optimized extraction condition of PLS from fruiting body of *G. lucidum*, and compared alkaline hydrolysis extraction and aqueous extraction methods in PLS extraction. Independent variables such as alkaline (NaOH) concentration (X_1), extraction temperature (X_2), and extraction time (X_3), were optimized using a 3-factor, 3-level Central Composite Design. The dependent variable was extraction rate (Y_1). A mathematical model obtained, $Y_1 = 3.76 + 0.94\ x_1 + 0.48\ x_2 + 0.03\ x_3 - 0.12\ x_1^2 + 0.20\ x_1x_2 - 0.54\ x_2^2 - 0.36\ x_3^2$ ($r^2 = 0.89$), explained the main and quadratic effects, and the interaction of factors that affected the extraction rate. Response surface methodology (RSM) predicted the levels of factors X_1, X_2, and X_3 (1.29 mol/l, 58.55°C and 2.16 h, respectively), for a maximized response of extraction rate. The observed and predicted values of Y_1 were in close agreement. Furthermore, the PLS content and the total extract rate from alkaline extraction method were obviously higher than those from the aqueous extraction method.

Key words: Medicinal mushroom, *Ganoderma lucidum*, polysaccharides extraction, response surface methodology, central composite design.

INTRODUCTION

Ganoderma lucidum (Fr.) Krast (Polyporaceae) is a basidiomycete, lamellaless fungus belonging to the family of polyporaceae. The use of this bracket fungus dates back more than 4000 years and is recorded in famous Chinese Pharmacopoeia, "Bencao Gangmu" written during the Ming Dynasty (Zhang et al., 2002; Lin, 2007). It has been used in both Chinese and Japanese traditional medicine for the prevention and treatment of various types of diseases, such as cancer, hepatopathy, arthritis, hypertension, neurasthenia, and chronic hepatitis (Liu and Zhang, 2005; Lin, 2007).

Modern chemistry studies shown that polysaccharides (PLS) and triterpenoids are the major source of biological activity and therapeutic use of *G. lucidum* (Lin, 2007; Sato et al., 2009; Liu et al., 2010a; Qiao et al., 2007; Xu et al., 2010). Recent advances demonstrate that the PLS from *G. lucidum* can promote the function of macrophages, B cells, T cells as well as dendritic cells (Wang et al., 1997; Bao et al., 2001; Cao and Lin, 2002; Zhang et al., 2002; Lin, 2007). Furthermore, the anticancer effects of *G. lucidum* have been demonstrated by many studies, and drawn considerable attention (Gao et al., 2002, 2003; Stanley et al., 2005; Wang et al., 2002;

*Corresponding author. E-mail: gaoliuedu@yahoo.com.cn. Tel/Fax: +86-731-85623498.

Table 1. Experimental range and levels of the independent variables of polysaccharides extraction from fruiting body of *G. lucidum.*

Independent variables	Range and levels				
	-1.682	-1	0	1	1.682
Alkaline concentration, X_1, (mol/l)	0.08	0.25	0.5	0.75	0.92
Extraction temperature, X_2, (°C)	33.2	40	50	60	66.8
Extraction time, X_3, (h)	0.32	1	2	3	3.68

Liu and Zhang, 2005; Lin, 2007). The production of *G. lucidum* PLS has become essential to meet the increasing demands in the international markets.

To obtain *G. lucidum* PLS, the extraction of PLS from fruiting body of *G. lucidum* is the first step. The PLS from the fruiting body of *G. lucidum* is the neutral type. Conventional method for extracting neutral PLS from mushrooms mainly employs aqueous extraction (Hou et al., 2008; Dong et al., 2009). However, this process requires high temperature, 100°C, long time, and lower extraction rate due to the cell wall and cell membranes of the mushroom are not decomposed.

In our previous studies, we described a method employing mild alkaline (NaOH) hydrolysis for extracting PLS from mycelia of medicinal mushrooms, *Grifola frondosa* and *Agaricus blazei* (Liu et al., 2010b; Wang et al., 2010). Alkaline hydrolysis can decompose cell wall and cell membranes, so as to increase the extraction rate of PLS. Meanwhile, this method requires lower temperature than the traditional. However, data on efficient PLS extraction by mild alkaline hydrolysis from fruiting body of *G. lucidum* are scarce, and the mutual interactions between extraction conditions on extraction rate of PLS are not well understood.

In this work, the extraction process by mild alkaline hydrolysis was optimized using a central composite design (CCD), and response surface methodology (RSM) predicted the levels of the key factors for a maximized response of javascript:figwin()extraction rate HYPERLINK. In addition, mild alkaline hydrolysis and aqueous extraction methods were compared in PLS extraction from fruiting body of *G. lucidum*.

MATERIALS AND METHODS

Mushroom materials

Artificially grown *G. lucidum* (Fr.) Karst. was purchased from a local herbal drug store and uthenticated by Dr Guo-Ying Zhou, College of Forestry, Central South University of Forestry and Technology, China. The fruiting bodies was dried at 60°C for sufficient time to a constant mass, and then ground to powder (40 mesh) for PLS extraction.

Extraction of PLS

Aqueous extraction of PLS from fruiting body of *G. lucidum* was according to the traditional method (Liu and Wang, 2009). For the alkaline (NaOH) hydrolysis extraction, the powder fruiting bodies was hydrolyzed for 2 h at 55°C with 0.8 mol/l NaOH at liquid (ml): solid (g) ratio of 20. The retained powder fruiting bodies was removed by filtration. Then, pH value of filtrate was adjusted to 10 and 11 with 2 mol/L H_2SO_4 and left it for precipitation overnight. The supernatant was filtered. The retained solution was washed with pure water several times till its pH reached neutral, and all the supernatants were precipitated with addition of 95% (v/v) ethanol by four times of volume, then separated by centrifugation at 10,000 rpm, and washed with 95% (v/v) ethanol several times, and separated by centrifugation at 10,000 rpm again, to obtain the PLS crude extract. The total extract rate (%) was defined as the content of PLS of the fruiting body of *G. lucidum*.

Determination of total sugar, PLS, reducing sugar and protein

Determination of PLS was equal to total sugar content minus reducing sugar content. Reducing sugar in PLS samples was assayed by 3,5-dinitrosalicylic acid method (Liu and Wang, 2009). Total sugar concentration was determined by phenol–sulfuric acid method (Liu and Wang, 2009). Protein in PLS samples was assayed by Coomassie brilliant blue staining methods (Sedmak and Sedmak, 1977).

RSM experimental design and statistical analysis

Adoption of a CCD implied prior knowledge on the upper and lower limit of parameters and awareness of fermentation process and its factors (Gu et al., 2005). The preliminary trails indicated alkaline concentration, extraction temperature and extraction time were significant variables for extraction rate of PLS. Therefore, these three variables were chosen to obtain the optimum levels. The range and the levels of the variables investigated in this study are given in (Table 1). The lowest and the highest levels of variables were: alkaline concentration, 0.08 and 0.92 mol/l; extraction temperature 33.2°C and 66.8°C; extraction time, 0.32 and 3.68 h.

A mathematical model, describing the relationships between the process indice (PLS extraction rate) and the extraction conditions in second-order equation, was developed. The PLS extraction rate was multiply regressed with respect to the extraction conditions by the least squares method as follows:

$$Y_i = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j \quad (1)$$

where Y_i is the predicted response variable; β_0, β_i, β_{ii}, β_{ij} are constant regression coefficients of the model, and x_i, x_j (i =1,3; j =1,3, $i \neq j$) represent the independent variables (extraction condtions) in the form of coded values. The accuracy and general ability of the above polynomial model could be evaluated by the coefficient of determination R^2. (Table 2) gave central composite design matrix and the response of PLS extraction rate. SAS statistical package (version 8.1, USA) was performed for regression and graphical

Table 2. The central composite design matrix and the responses of extraction rate of *G. lucidum* polysaccharides.

Runs	X_1	X_2	X_3	Y_1, extraction rate (%)
1	-1	-1	-1	1.7
2	-1	-1	1	1.9
3	-1	1	-1	2.1
4	-1	1	1	2.4
5	1	-1	-1	2.5
6	1	-1	1	2.8
7	1	1	-1	3.7
8	1	1	1	4.1
9	-1.682	0	0	1.2
10	1.682	0	0	5.9
11	0	-1.682	0	1.4
12	0	1.682	0	3.3
13	0	0	-1.682	3.1
14	0	0	1.682	2.6
15	0	0	0	3.9
16	0	0	0	3.8
17	0	0	0	3.8
18	0	0	0	3.5
19	0	0	0	3.6
20	0	0	0	3.9

Table 3. Analysis of variance (ANOVA) for full quadratic model for polysaccharides extraction from fruiting body of *G. lucidum*.

Regression	DF	Sum of squares	R-Square	F value	Pr > F
Linear	3	15.3880	0.6420	19.57	0.0002**
Quadratic	3	5.6288	0.2348	7.16	0.0075**
Crossproduct	3	0.3300	0.0138	0.42	0.7429
Total model	9	21.3469	0.8906	9.05	0.0010**

R^2 = 0.8906, Adj. R^2 = 0.8262, **Significant at 0.01 level.

analysis of data obtained. The optimum conditions of alkaline concentration, extraction temperature and extraction time were obtained by solving the regression equation.

RESULTS

The effects of three variables on PLS extraction from the fruiting body of *G. lucidum* were studied. The experimental design matrix is presented in (Tables 1 and 2). Twenty experiments were performed in triplicate. The (CCD) and the corresponding experimental data were shown in (Table 2).

Table 3 shows the analysis of variance for the experiment. The Fisher's F-test with a very low probability value (0.001) for total model indicated the model was highly significant, and the coefficient of determination (R^2) was shown as 0.8906, indicating that 89.06% of the variability in the response could be explained by the model.

The results of the regression analysis are shown in (Table 4). The significance of each coefficient was determined by Student's *t*-test and P-value, and the larger the magnitude of *t*-test and smaller the P-value, the more significant is the corresponding coefficient. The polynomial model for PLS extraction rate Y_1 ($Y_{extraction\ rate}$) was regressed by mainly considering the significant terms and was expressed by Equation (2).

$$Y_1 = 3.76 + 0.94\ x_1 + 0.48\ x_2 + 0.03\ x_3 - 0.12\ x_1^2 + 0.20\ x_1x_2 - 0.54\ x_2^2 - 0.36\ x_3^2 \quad (2)$$

The equation and (Table 4) reveal that alkaline concentration (x_1) had a strong positive linear effect on

Table 4. Results of regression analysis of a predictive polynomial model for polysaccharides extraction from fruiting body of *G. lucidum*.

Parameter	DF	Coefficients estimated	Standard error	t value	Pr > |t|
Intercept	1	3.7569	0.2088	17.99	<.0001**
X_1	1	0.9449	0.1385	6.82	<.0001**
X_2	1	0.4829	0.1385	3.49	0.0059**
X_3	1	0.0262	0.1385	0.19	0.8533
X_1X_1	1	-0.1163	0.1348	-0.86	0.4086
X_1X_2	1	0.2000	0.1810	1.10	0.2951
X_2X_2	1	-0.5405	0.1348	-4.01	0.0025**
X_3X_3	1	-0.363805	0.134863	-2.70	0.0224*

*Significant at 0.05 level; **Significant at 0.01 level.

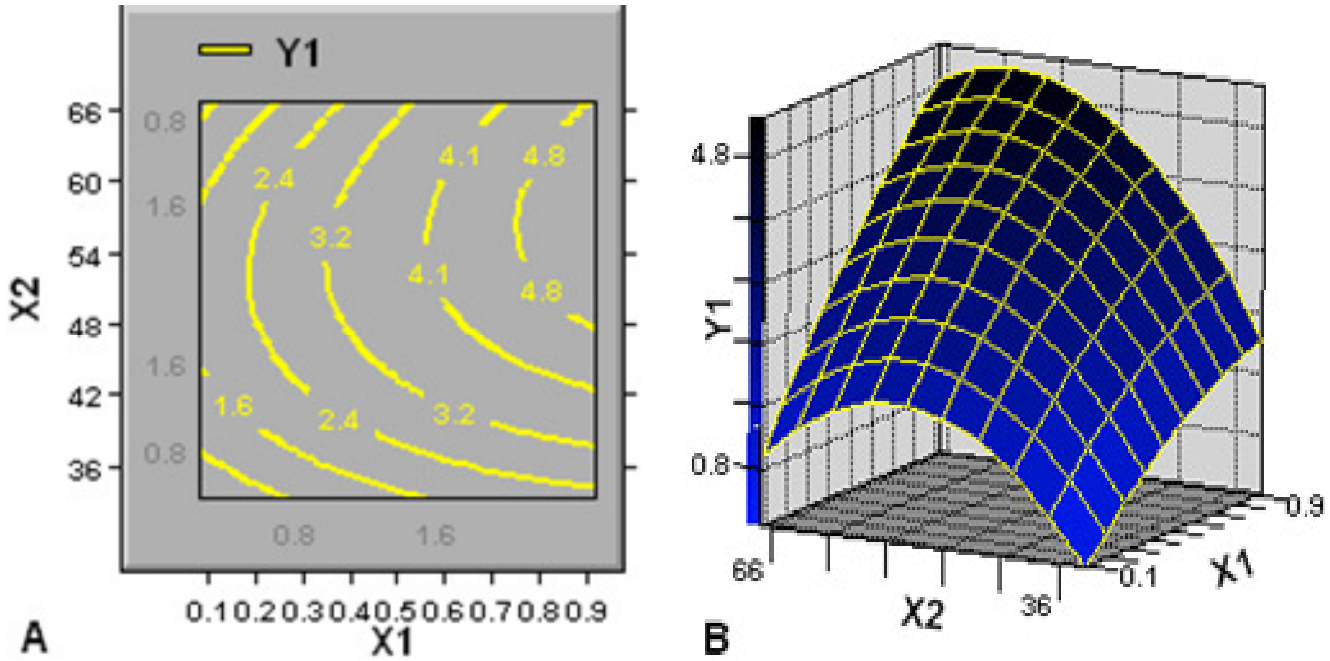

Figure 1. The contour (A) and surface (B) plots of the combined effects of alkaline (NaOH) concentration (X_1) and extraction temperature (X_2) on extraction rate (Y_1) of polysaccharides from fruiting body of *G. lucidum*. Fixed level: extraction time (X_3) = 2 h.

the response ($P < 0.01$) on Y_1 as it had the largest coefficient, followed by extraction temperature (x_2). However, extraction time (x_3) had no significant effect on the extraction rate ($P > 0.05$), and the x_2 and x_3 variables also indicated negative quadric effects on the extraction rate ($P < 0.05$). No significant interactions were noted between any two of the three variables ($P > 0.05$).

The 2D contour plot and 3D response surface are generally the graphical representation of the regression equation. Figures 1 to 3 represent the 2D contour plots (A) and 3D response surfaces (B) for the optimization of PLS extraction conditions. Each figure presented the effect of two variables on the extraction of PLS, while other one variable was held at the middle level.

The model predicted the maximum PLS extraction rate of 6.62% appeared at alkaline concentration, extraction temperature, extraction time of 1.29 mol/l, 58.55°C, 2. 16 h, respectively.

Verification of the models

The triplicate experiments were carried out to verify the availability and accuracy of the model (Equation 2) for PLS extraction. Under the calculated optimal extraction condition, the extraction rate was 6.81%, which was in agreement with the predicted value (6.62%). The good correlation between predicted and measured values of

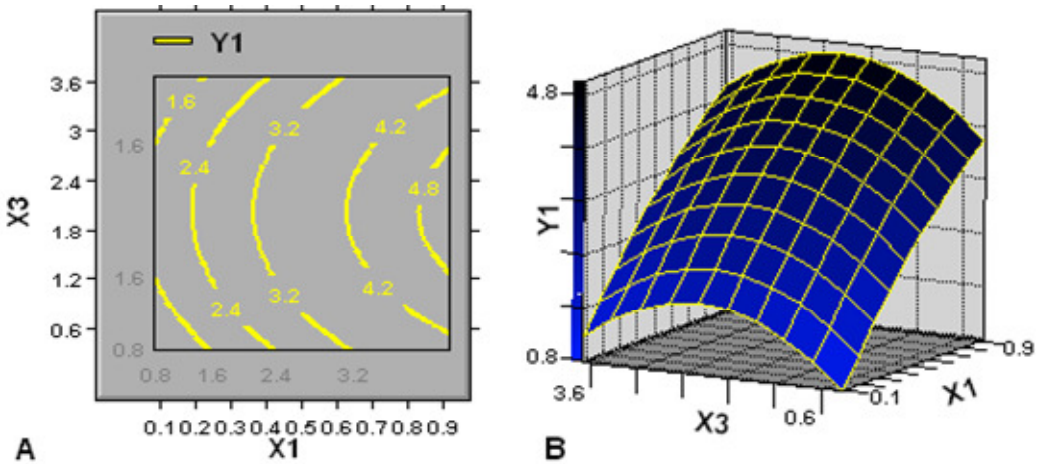

Figure 2. The contour (A) and surface (B) plots of the combined effects of alkaline (NaOH) concentration (X_1) and extraction time (X_3) on extraction rate (Y_1) of polysaccharides from fruiting body of *G. lucidum*. Fixed level: extraction temperature (X_2) = 50°C.

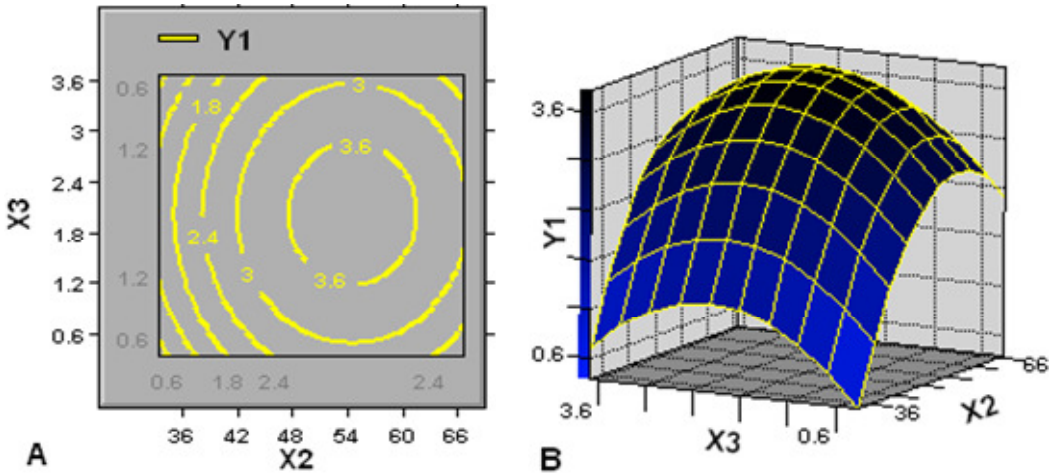

Figure 3. The contour (A) and surface (B) plots of the combined effects of extraction temperature (X_2) and extraction time (X_3) on extraction rate (Y_1) of polysaccharides from fruiting body of *G. lucidum*. Fixed level: alkaline (NaOH) concentration (X_1) = 0.5 mol/l.

these experiments justifies the validity of the response model and the existence of an optimum point.

Comparison of aqueous extraction and alkaline hydrolysis extraction results

Gross sugar, PLS, reducing sugar and protein contents in crude PLS extracts obtained with alkaline and aqueous methods were comparatively analyzed. The results were shown in (Table 5). The contents of reducing sugars in PLS extracts were very low, and the contents of PLS were equal to total sugar content minus reducing sugar content, so the gross sugar and PLS contents were nearly the same in each sample, however, the PLS content from mild alkaline extraction method was obviously higher than that from the aqueous extraction method. The protein contents in mild alkaline extracts were little higher than that from the aqueous extraction method, but they were all very low in each sample. The PLS contents of the fruiting body of *G. lucidum* determined by the optimized alkaline extraction and aqueous extraction methods were 68.1 and 43.6 mg/g, respectively. That is, the total extract rates of the two methods were 6.81 and 4.36%, respectively. In addition, after optimization of alkaline extraction conditions by RSM, the extraction rate (6.81%) represented a 33.5% increase in titre compared to the non-optimized condition

Table 5. Comparison of aqueous extraction and alkaline hydrolysis extraction results.

Items	Extraction methods		
	Alkaline extraction (non-optimized)	Alkaline extraction (optimized)	Aqueous extraction
Gross sugar content of extract (%)	56.12	55.73	51.77
Reducing sugar content of extract (%)	1.67	1.52	1. 98
Protein content of extract (%)	2.13	2.21	1.92
PLS content of extract (%)	54.45	54.21	49.79
PLS content of fruiting body (mg/g)	51.0	68.1	43.6
Total extract rate (%)	5.10	6.81	4.36

PLS, polysaccharides, the content of PLS of extract was equal to total sugar content minus reducing sugar content.

(5.10%).

DISCUSSION

Mushrooms have recently become attractive as healthy foods (physiologically functional) and as a source material for the development of drugs (Liu and Zhang, 2007; Liu et al., 2010a). Many studies have been focused on the pharmacological effects of medicinal mushrooms and/or their key active compounds (Bao et al., 2001; Cao and Lin, 2002; Zhang et al., 2002; Gao et al., 2002, 2003; Stanley et al., 2005; Chen et al., 2010), and to quickly obtain the related products, submerged cultivation of medicinal mushrooms has received great interest as a promising alternative for efficient production of mycelial biomass and metabolites (Fang and Zhong, 2002; Liu and Zhang, 2007; Liu and Wang, 2009; Tang et al., 2009; Zhang and Zhong, 2010). However, fewer studies were paid attention to the improvement technologies of PLS extraction from the fruiting bodies of mushrooms, and currently the major method for the PLS extraction is aqueous extraction (Hou et al., 2008; Dong et al., 2009; Bao et al., 2002). Dong et al. (2009) optimized the hot water extraction of PLS from cultured mycelium of *Cordyceps sinensis* using a Box-Behnken design. Bao et al. (2002) extracted polysaccharides from *G. lucidum* by using hot water extraction, and the extraction rate was 3%. Studies have shown that aqueous extraction is associated with lower yields, high temperatures and long extraction times, so it is necessary to find a novel extraction technology for PLS that avoid the disadvantages of aqueous extraction.

Mild alkaline treatment is useful for extraction of PLS from the cell walls, and alkaline extraction requires lower temperature than the traditional aqueous extraction. Kim et al. (1980) has reported that the PLS extracted by alkaline solution were composed of four kinds of monosaccharide and 18 kinds of amino acid and displayed significant anti-tumour activity (Kim et al., 1980). Our previous work suggested that mild alkaline extraction is an effective method for PLS extraction from mycelia of the medicinal mushrooms, *G. frondosa* and *A. blazei* (Liu et al., 2010b; Wang et al., 2010).

In the present work, we studied the optimization of PLS extraction using RSM from the fruiting body of *G. lucidum* by mild alkaline hydrolysis, and compared aqueous extraction and mild alkaline hydrolysis methods in PLS extraction. The results indicate that the extraction rate reached 6.81% after optimization of extraction conditions by RSM, which represented a 33.5% increase in titre compared to the non-optimized condition (5.10%), and the PLS content and the total extract rate from alkaline extraction method were obviously higher than those from the aqueous extraction method. In addition, the results reveal that all the extraction rates were higher than that (3%) of extraction of fruiting body of *G. lucidum* reported by Bao et al. (2002), but lower than those of extraction of mycelia of *G. frondosa* and *Agaricus blazei* in our previous works (Liu et al., 2010b; Wang et al., 2010).

In conclusion, the present study indicated that alkaline extraction is a effective method for PLS extraction from the fruiting body of *G. lucidum* and RSM is useful to maximum extraction of the PLS.

ACKNOWLEDGEMENTS

G. Q. Liu thank the financially support by the National Natural Science Foundation of China (Project No. 30700552), a Project Supported by Scientific Research Fund of Hunan Provincial Education Department, China (No. 09K054), and a Project Supported by Hunan Provincial Fund of Science and Technology Innovation for Graduate Students (No. CX2010B315).

REFERENCES

Bao XF, Liu CP, Fang JN, Li XY (2001). Structural and immunological studies of a major polysaccharide from spores of *Ganoderma lucidum* (Fr.) Karst. Carboohydr. Res., 322: 67-74.

Bao XF, Wang XS, Dong Q, Fang JN, Li XY (2002). Structural features of immunologically active polysaccharides from *Ganoderma lucidum*. Phytochem., 59: 175-181.

Cao LZ, Lin ZB (2002). Regulation on maturation and function of dendritic cells by *Ganoderma lucidum* polysaccharides. Immunol. Lett., 83: 163-169.

Chen NH, Liu JW, Zhong JJ (2010). Ganoderic acid T inhibits tumor invasion *in vitro* and *in vivo* through inhibition of MMP expression. Pharmacol. Rep., 62: 150-163.

Dong CH, Xie XQ, Wang XL, Zhan Y, Yao YJ (2009). Application of Box-Behnken design in optimization for polysaccharides extraction from cultured mycelium of *Cordyceps sinensis*. Food Bioprod. Process, 87: 139-144.

Fang QH, Zhong JJ (2002). Two-stage culture process for improved production of ganoderic acid by liquid fermentation of higher fungus *Ganoderman lucidum*. Biotechnol. Prog., 18: 51-54.

Gao YH, Zhou SF, Chen GL, Dai XH, Ye JX (2002). A phase I/II study of a *Ganoderma lucidum* extract (Ganopoly) in patients with advanced cancer. Int. J. Med. Mushroom, 4: 207-214.

Gao YH, Zhou SF (2003). Cancer prevention and treatment by *Ganoderma*, a mushroom with medicinal properties. Food Rev. Int., 19: 275-323

Gu XB, Zheng ZM, Yu HQ, Wang J, Liang FL, Liu RL (2005). Optimization of medium constituents for a novel lipopeptide production by *Bacillus subtilis* MO-01 by a response surface method. Process Bioechem., 40: 3196-3201.

Hou XJ, Chen W (2008). Optimization of extraction process of crude polysaccharides from wild edible BaChu mushroom by response surface methodology. Carbohydr. Polym., 72: 67-74.

Kim BK, Chung HS, Chung KS, Yang MS (1980). Studies on atineoplastic components of Korean basidiomycetes. Korean J. Mycol., 8: 107-113.

Lin ZB (2007). *Ganoderma*. Modern research of *Ganoderma* (third edition). Beijing Medical University Press, Beijing, China, pp. 1-359.

Liu GQ, Zhang KC (2005). Mechanisms of the anticancer action of *Ganoderma lucidum* (Leyss. ex. Fr.) Karst.: A new understanding. J. Integr. Plant Biol., 47: 129-135.

Liu GQ, Wang XL (2007). Optimization of critical medium components using response surface methodology for biomass and extracellular polysaccharide production by *Agaricus blazei*. Appl. Microbiol. Biotechnol., 74: 78-83.

Liu GQ, Zhang KC (2007). Enhancement of polysaccharides production in *Ganoderma lucidum* by the addition of ethyl acetate extracts from *Eupolyphaga sinensis* and *Catharsius molossus*. Appl. Microbiol. Biotechnol., 74: 572-577.

Liu GQ, Wang XL (2009). Selection of a culture medium for reducing costs and enhancing both biomass and intracellular polysaccharide production by *Agaricus blazei* AB2003. Food Tech. Biotech., 47: 210-214.

Liu GQ, Wang XL, Wu YH, Han WJ, Zhang HY (2010a). Promotion of ganoderic acid production in *Ganoderma sinense* by the addtion of an ether extract from *Eupolyphaga sinensis*, a medicinal insect. Afr. J. Biotechnol., 9: 6129-6134.

Liu GQ, Han WJ, Zhang HY, Jin XC (2010b). Polysaccharides extraction from submerged-cultured mycelium of *Grifola frondosa*. 2010 International Conference on Bioinformatics and Biomedical Technology (ICBBT) Held at Chengdu, China, pp. 11-114.

Qiao Y, Zhang XM, Qiu MH (2007). Two novel lanostane triterpenoids from *Ganoderma sinense*. Mole., 12: 2038-2046.

Sato N, Ma CM, Komatsu K, Hattori M (2009a). Triterpene-Farnesyl hydroquinone conjugates from *Ganoderma sinense*. J. Nat. Prod., 72: 958-961.

Sedmak JJ, Grossberg SE (1977). A rapid, sensitive and versatile assay for protein using Coomassie Brilliant Blue G-250, Anal. Biochem., 79: 44-552

Stanley G, Harvey K, Slivova V, Jiang JH, Sliva D (2005). *Ganoderma lucidum* suppresses angiogenesis through the inhibition of secretion of VEGF and TGF-β1 from prostate cancer cells. Biochem. Biophys. Res. Commun., 330: 46-52

Tang YJ, Zhang W, Zhong JJ (2009). Performance analyses of a pH-shift and DOT-shift integrated fed-batch fermentation process for the production of ganoderic acid and *Ganoderma* polysaccharides by medicinal mushroom *Ganoderma lucidum*. Bioresource Technol., 100: 1852-1859.

Wang SY, Hsu ML, Hsu HC, Tzeng CH, Lee SS, Shiao MS, Ho CK (1997). The anti-tumor effect of *Ganoderma lucidum* is mediated by cytokines released from activated macrophages and T lymphocytes. Int. J. Cancer, 70: 699-705.

Wang XL, Liu GQ, Zhang HY, Zhou GY (2010). Extraction of Mycelial Polysaccharides from Submerged-Cultured *Agaricus blazei*. In: Proceedings of 4th International Conference on Bioinformatics and Biomedical Engineering (iCBBE 2010) Held at Chengdu, China, pp. 1-4.

Wang YY, Khoo KH, Chen ST, Lin CC, Wong CH, Lin CH (2002). Studies on immuno-modulating and antitumor activities of *Ganoderma lucidum* (Reishi) polysaccharides: functional and proteomic analyses of a fucose-containing glycoprotein fraction responsible for the activities. Bioorgan. Med. Chem., 10: 1057-1062

Xu JW, Zhao W, Zhong JJ (2010). Biotechnological production and application of ganoderic acids. Appl. Microbiol. Biotechnol., 87: 457-466.

Zhang JS, Tang QJ, Zimmerman KM, Reutter W, Fan H (2002). Activation of B lymphocytes by GLIS, a bioactive proteoglycan from *Ganoderma lucidum*, Life Sci., 71: 623-638.

Zhang WX, Zhong JJ (2010). Effect of oxygen concentration in gas phase on sporulation and individual ganoderic acids accumulation in liquid static culture of *Ganoderma lucidum*. Biosci. Bioeng., 109: 37-40.