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Optimization of enzyme assisted extraction of polysaccharides from *Poria cocos*

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Water-soluble polysaccharide was isolated from *Poria cocus* by enzyme-assisted method using orthogonal methodology. By single factor test and orthogonal group, the extraction conditions of water-soluble polysaccharide were investigated, such as liquid-solid ratio, temperature, time and pH. The optimum conditions for single factor were as follows Enzyme concentration (%), Extraction temperature oC, Extraction time/h, Extraction pH. The result revealed that 2% complex enzyme, remained most important factor of polysaccharide extraction, followed by temperature and pH. The liquid-solid ratio was 1:50, temperature was 40°C, time was 3.0 h, and pH was 5. The highest extraction rate of crude polysaccharide remained 4.14%. Results indicated that the complex-enzyme assisted remained best technique for extracting polysaccharide from *P. cocus*. It proved to be as highly effective as well as energy and time saving extraction techniques.

Key words: *Poria cocus* enzyme assisted extraction, polysaccharides, alpha amylase, cellulase, Taka-diastase.

INTRODUCTION

Enzyme-assisted extraction (EAE) has been demonstrated to be effective in improving the yield of the model component (Li et al., 2006). Mushrooms have long been used as oriental medicine, as well as dietary supplements and in natural cosmetics (Kaneno et al., 2004). *Poria cocus* is a fungus that grows on the roots of pine trees, and used as most important traditional medicines in China and other Asian countries; moreover, it consists of culinary and medicinal properties such as anti-inflammatory, antitumor, complement activating, and immune stimulating activities (Kanayama et al., 1983; Lee and Jeon, 2003; Yasukawa et al., 1998; Yu and Tseng, 1996). *P. cocus* mainly contain polysaccharide (Jia et al., 2016; Wu et al., 2016). The major chemical constituents of *P. cocus* are polysaccharides, triterpenoids, ergosterol and proteins (Tai et al., 1992, 1995a, b; Wang and Wan, 1998; Yang and Bao, 2005).

Polysaccharide is a vital bioactive substance with some

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physiological functions, such as immune, regulating cell growth and senescence (Jin and Xu, 2002). It can be widely used in medicine, health products and functional foods. Many mushrooms revealed a great range of the healthy properties of polysaccharides, being the most significant modulation of the immune system. Bioactive carbohydrates, including β-glucans, are generally examined as biological response modifiers (BRM) (Wasser, 2002). *P. cocus* useful in health products it can be used as raw material to produce various kinds of health products, such as a *P. cocus* polysaccharide capsule, tablet, electuary and so on. Hot water extraction of several mushrooms used in traditional Chinese medicine believed to be effective in the treatment of different diseases including many forms of cancer.

The enzymatic extraction process is manipulated by several factors, including enzyme concentration, temperature, pH, activators and/or inhibitors (Dam, 2004). While an enzyme is a protein-based catalyst, its rate of reaction depends on its concentration and has an optimum temperature at which shows maximum. Stirring speed also facilitates contact and the reaction between the substrate and the catalyst. An optimization of reaction time is important to ensure maximum extraction without degrading product quality. In order to get the pharmacological composition of mushroom presently, the extraction methods of mushroom polysaccharides (MP), generally include hot water extraction, ultrasonic extraction, enzyme extraction, alkaline extraction, acid extraction and microwave extraction (Jiang et al., 2008; Ghosh et al., 2005; Huang and Ning, 2010; Yu et al., 2009). These methods are regularly associated with higher temperature, extended extraction time and excessive energy consumption and lower yield. Whereas the enzyme assisted extraction with lower temperature, less time, less power consumption and high extraction yield is arising technology in the food industry.

It is therefore necessary to evaluate on the extraction method of polysaccharides; the comparative analysis about extraction method of *P. cocus* polysaccharides has seldom been studied.

Orthographic graph is a statistic method to make the complex work simple. In this investigation, (A) complex enzyme amount (B) extraction temperature (C) extraction time and (D) extraction pH were selected as four independent variables, combined by three levels which formed the orthographic graph (Table 1). Furthermore, all three Taka-diastase, alpha amylase and cellulose enzyme were separately investigated; also, four independent variables (A) enzyme amount (B) extraction temperature (C) extraction time and (D) extraction pH were selected (Tables 2, 3 and 4). This diagram was used to optimize the amount of extract and polysaccharides yield. The aim of this work is to examine the performance of a combination of cellulose, alpha amylase, Taka-diastase, and complex-enzyme-hydrolysis-assist) extraction from *P. cocus*. After a set of prospective tests, four parameters (enzyme ratio, extraction temperature, extraction time and extraction pH) were optimised in order to improve the yields of polysaccharides extraction.

MATERIALS AND METHODS

The samples of *P. cocus* and enzymes were obtained from Wuhan, Hubei Province, China. Other analytical grade reagents were obtained from the Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

Isolation of polysaccharides from *Poria cocos* using complex enzyme

The *P. cocus* were cut into pieces, ground by electric mill, and sieved through screens 60-80 mesh (LD-Y500A, Ding Shuai Hardware Products Co., Ltd. Zhejiang, China). Ten grams of the powder were mixed in 200 mL distilled water respectively, of 9 bottles in which the experiments were carried out. The amounts of complex enzyme were also added corresponding to Table 2. The bottles were labelled and pH for extraction were adjusted for all samples shown in Table 2. The bottles were placed in a water bath at the temperature of 40, 50 and 60°C at extraction time of 3 h, 2 h and 1.5 h, respectively.

The sample was filtered and concentrated to about one-fifth of the original volume in a rotary evaporator (Model RE-2000A Yarong Bio-instrument Shanghai, China) at 40°C. One part of the solution was dried into solid and powdered to calculate the amount of extract. Ethanol was added into the remaining part and kept at room temperature for 24 h. The precipitate was collected after centrifugation (4,000 r/min, 15 min), and then dried to obtain the desired crude polysaccharides (Thulasi et al., 2008). Furthermore, the same methods as mentioned above were applied for the Taka-diastase, alpha amylase and cellulose enzyme and their amount of enzyme and pH were computed in Tables 3, 4 and 5 respectively.

Determination of amount of extract and polysaccharides yield

Glucose was accurately weighted 0.0200 g and dried in 105°C prior to dissolving in 250 mL and the standard solution was diluted to different concentrations making up the final concentration with pure water. Following that, 1.0 mL phenol and 5 mL sulfuric acid were added in 1.0 mL of glucose solution for each concentration, mixed by vortex and reacting samples for 10 min in boiling water bath and cooled at room temperature. The absorbance of each tube was measured at 490 nm with an ultraviolet-visible spectrophotometer (1.0 mL); distilled water was used as blank; standard curve was obtained with x-coordinate for concentration of glucose and y-coordinate for absorption value, and finally, based on the equation of glucose standard curve, the glucose contents of the samples were determined. Considering the dilution ratio, the amount of extract and polysaccharides yield was calculated by the following equation.

$$\text{Amount of extract (\%)} = \frac{\text{Weight of extraction (g)}}{\text{Weight of } \textit{Poria} \text{ powder (g)}} \times 100$$

$$\text{Polysaccharides yield (\%)} = \frac{\text{Polysaccharides yield (g)}}{\text{Weight of } \textit{Poria} \text{ powder (g)}} \times 100$$

Table 1. L9 (3^4) Orthogonal table of complex enzyme method

Level	Complex enzyme amount (%) [A]	Extraction Temperature (°C) [B]	Extraction Time (h) [C]	Extraction pH [D]
1	1.5	40	3	5
2	2	50	2	6
3	2.5	60	1	7

Table 2. L9 (3^4) Orthogonal table of Taka Distances enzyme method.

Level	Complex enzyme amount (%) [A]	Extraction Temperature (°C) [B]	Extraction Time (h) [C]	Extraction pH [D]
1	0.835(g)	40	3	5
2	0.01112(g)	50	2	6
3	0.03896(g)	60	1	7

Table 3. L9 (3^4) Orthogonal table of Alfa amylase enzyme method.

Level	Complex enzyme amount (%) [A]	Extraction Temperature (°C) [B]	Extraction Time (h) [C]	Extraction pH [D]
1	0.835(g)	40	3	5
2	0.01112(g)	50	2	6
3	0.03896(g)	60	1	7

Table 4. L9 (3^4) Orthogonal table of Cellulose enzyme method.

Level	Complex enzyme amount (%) [A]	Extraction Temperature (°C) [B]	Extraction Time (h) [C]	Extraction pH [D]
1	0.835(g)	40	3	5
2	0.01112(g)	50	2	6
3	0.03896(g)	60	1	7

RESULTS

Complex enzyme extraction method

Results of amount of extract and polysaccharides yield with complex enzyme are shown in Table 5. On the basis of single factor experiments, four factors and three levels of L9 (3^4) orthogonal test were selected. Conditions of pH and temperature were modified in each experiment to match the optimum conditions of the measured enzyme. The experiments were carried out for complex enzyme 1.5%, extraction temperature at 40°C, extraction time of 3 h and extraction pH at 5. After this condition, the amount of extract was 4.14% and polysaccharides yield was 5.3%. Furthermore, the results of 9 samples demonstrated in Table 5 and Sample 4 shows the highest amount of extract with Sample 1 showing the higher polysaccharides yield, that is, 7.6%. In order to get the consequence of different independent variables and their levels on polysaccharides yield and amount of extract, the K factor along with R factor were calculated are presented in Table 5.

Cellulose enzyme extraction method

The results of the polysaccharides yield and the amount of extract with cellulose enzyme are shown in Table 6. On the basis of single factor experiments, four factors and three levels of L9 (3^4) orthogonal test were selected. The factor levels are revealed in Table 2. The result showed that highest amount of extract was 8.4%, polysaccharides yield of 2.60% was observed at 50°C with the cellulose enzyme amount of 0.835. Furthermore, the results of 8 samples showed that sample 4 show lowest amount of extract 5.6% and sample 3 show lowest polysaccharides yield 1.76%. In addition, to get the result of different independent variables and their levels on polysaccharides yield and amount of extract, the K factor and R factor were calculated and presented in Table 6. Whereas, K is the standard value of related results of every independent variable. The factors which have an

Table 5. L9 (3^4) Orthogonal experiment results of complex enzyme extraction method (Cellulose, Amylase and Taka-diastase).

Level	Complex enzyme amount (g)	Extraction temperature	Extraction time	Extraction pH	Polysaccharide yield	Amount of extract
1	A1	B1	C1	D1	4.14	5.3
2	A1	B2	C2	D2	2.28	4.5
3	A1	B3	C3	D3	3.88	5.6
4	A2	B1	C2	D3	2.85	7.6
5	A2	B2	C3	D1	2.51	5.9
6	A2	B3	C1	D2	2.2	7.5
7	A3	B1	C3	D2	1.34	6.1
8	A3	B2	C1	D3	3.16	5.9
9	A3	B3	C2	D1	2.71	4.8
						Remarks
K1	5.1	2.77	2.77	5		
K2	7.0	2.85	2.65	5.5	A>D>C>B	
K3	5.6	2.93	2.93	6.0		
R	1.9	0.16	0.28	1.0		

R refers to the result of extreme analysis.

impact on the polysaccharides yield in appropriate order were extraction complex enzyme amount >pH>extraction time > extraction temperature.

Alpha amylase enzyme extraction method

The results for the polysaccharides yield and amount of extract with alpha amylase are shown in Table 7. On the basis of single factor experiments, four factors and three levels of L9 (3^4) orthogonal test were selected (Table 3). The highest amount of the extract 7.6% was in Sample 2, moreover the higher polysaccharides yield 4.24% was found in sample 9 and extract were 6.5%. In order to get the effect of different independent variables and their levels on polysaccharides yield and amount of extract, the K factor along with an R factor were calculated as found in Table 7. The factors which have an impact on the polysaccharides yield in appropriate order were extraction enzyme amount >extraction temperature > extraction time > pH.

Taka-diastase enzyme extraction method

The results of the polysaccharides yield and the amount of extract with Taka-diastase are shown in Table 8. On the basis of single factor experiments, four factors and three levels of L9 (3^4) orthogonal test was selected. The factor levels were revealed in Table 4.The experiments were carried out, for example, condition of extraction Sample 1 was a complex enzyme amount of 1.5%, extraction temperature at 40°C extraction time of 3 h and extraction pH at 5. The result shows that the highest amounts was found in Sample 3 with extract of (7.4%), however, the high yield of polysaccharides (4.07%) was found in Sample 8. In order to get the significance of different independent variables and their levels on polysaccharides yield and amount of extract, the K and R factors were calculated as found in Table 8. The factors which have an impact on the polysaccharides yield in appropriate order were extraction temperature > extraction enzyme amount < extraction time > pH.

DISCUSSION

The use of medicinal mushroom extracts for the medicinal use is recognized and familiar in China, Japan, Korea, Russia and now increasingly in the USA (Mizuno et al., 1995). A number of methods have been developed to extract anti-cancer polysaccharides from mushroom fruit-bodies, mycelium and liquid media (Mizuno, 1999). In this study, extraction condition of total polysaccharides for effective yield enzyme amount, extraction temperature, extraction time and extraction pH is investigated. According to results of the orthogonal trial, these four methods were evaluated. Recently, several methods of extracting polysaccharides from mushrooms are reported, such as microwave assisted extraction, water extraction, ultrasonic assisted extraction and some enzymatic assisted methods (Boroushaki et al., 2010; Dkhil et al., 2011; Radheed et al., 2003). The literature reported that the function of enzyme is to degrade cell wall constituents and discharge intracellular contents. Generally, a plant cell wall comprises cellulose, hemicelluloses and pectin, while flesh is main content for pectin and proteins.

Cellulases and pectinases can therefore be used for

Table 6. L9 (3^4) Orthogonal experiment results of Cellulose enzyme extraction method.

Sample No.	Cellulose enzyme amount (g)	Extraction temperature	Extraction time	Extraction pH	Polysaccharide yield	Amount of extract
1	A1	B1	C1	D1	2.16	8.4
2	A1	B2	C2	D2	2.60	7.6
3	A1	B3	C3	D3	1.76	8.2
4	A2	B1	C2	D3	1.24	5.6
5	A2	B2	C3	D1	1.92	6.8
6	A2	B3	C1	D2	2.09	6.9
7	A3	B1	C3	D2	2.08	6.4
8	A3	B2	C1	D3	2.09	6.5
9	A3	B3	C2	D1	2.08	8.1
						Remarks
K1	7.76	2.17	1.82	4.84		
K2	6.53	1.75	2.20	4.74	A> B> C>D	
K3	7.20	1.96	1.85	4.91		
R	1.23	0.42	0.38	0.17		

R refers to the result of extreme analysis.

Table 7. L9 (3^4) Orthogonal experiment results of Alfa Amylase enzyme extraction method.

Sample No	Alfa Amylase enzyme amount (g)	Extraction temperature	Extraction time	Extraction pH	Polysaccharide yield	Amount of extract
1	A1	B1	C1	D1	3.04	6.5
2	A1	B2	C2	D2	1.59	7.6
3	A1	B3	C3	D3	2.2	7.3
4	A2	B1	C2	D3	3.24	6.7
5	A2	B2	C3	D1	3.1	6.4
6	A2	B3	C1	D2	2.65	7.1
7	A3	B1	C3	D2	4.24	6.6
8	A3	B2	C1	D3	2.04	7.4
9	A3	B3	C2	D1	1.48	6.5
						Remarks
K1	7.13	2.27	3.56	4.84		
K2	6.73	2.99	2.24	4.74	C> B>A>D	
K3	6.83	2.64	2.11	4.91		
R	0.40	0.72	1.45	0.17		

R refers to the result of extreme analysis.

degradation of cell structure in the extraction process (Fernandes, 2010). It is observed from the results, that the extraction yield was decreased with the increasing of temperature from 40 to 50°C whereas there was no significant change found amongst all samples. According to Chunhua et al. (2014), enzyme assistant extraction method in polysaccharide extraction is an innovative research in recent years and has advantages over the conventional hot water extraction. Furthermore, complex enzyme hydrolysis method can be performed under low bath temperature (47°C) and the yield of the polysaccharide significantly improved to microwave-assist extraction and hot water extraction method (Chunhua et al., 2014). Enzyme assisted extraction is an advanced and efficient extraction method (Jiang et al., 2010).

Moreover, the study was carried out by the single enzyme assist extraction method, the results of the cellulose, alpha amylase and Taka-diastase enzymes

Table 8. L9 (3^4) Orthogonal experiment results of Taka-diastaseenzyme extraction method.

Sample No	Taka Distance enzyme amount (g)	Extraction temperature	Extraction time	Extraction pH	Polysaccharide yield	Amount of extract
1	A1	B1	C1	D1	1.37	6.4
2	A1	B2	C2	D2	2.53	7.1
3	A1	B3	C3	D3	1.82	7.4
4	A2	B1	C2	D3	3.93	6.1
5	A2	B2	C3	D1	3.63	6.0
6	A2	B3	C1	D2	3.19	5.8
7	A3	B1	C3	D2	3.31	5.8
8	A3	B2	C1	D3	4.07	7.0
9	A3	B3	C2	D1	3.27	5.7
					Remarks	
K1	6.96	1.90	2.87	4.87	B>A>C>D	
K2	5.96	3.58	3.26	4.88		
K3	6.16	3.55	2.76	4.84		
R	1.0	1.68	0.50	0.04		

R refers to the result of extreme analysis.

experiment as were shown in Tables 6, 7 and 8. However, in present study, the highest extraction yield amongst the three enzymes was 4.24% in the optimal conditions (50°C, 120 min,) with alpha amylase. In complex enzyme assay, extraction method and pH are the main factors which manipulated the effective extraction both for amount of extract and polysaccharides yield. pH can affect enzyme activity because every enzyme possesses their optimal pH, while the change of pH affects the structure of enzyme and enzymatic activity (Yin et al., 2011). Further study of Yin et al. (2011) explained the pH can affect enzyme activity as different enzymes have their own optimal pH. Study further revealed that extraction time remained a significant factor to affect the polysaccharides yield; similar findings are reported by Chunhua et al. (2014) in the extraction of polysaccharide from ganoderma lucidum.

The study further demonstrated that yield of polysaccharide increased with increase in temperature and amount of the extract enhanced with different enzymes. The correlated result was reported that due to a higher temperature, the polysaccharides matter leaved out from the mushroom particles into the solution (Li et al., 2006). Long extraction time observed plays a positive role in the production of polysaccharides (Ros et al., 2004). Multi enzymatic hydrolysis helps to split linkage and assists in releasing the polysaccharide, increasing the rate of extraction. Furthermore, the enzyme-hydrolysis-assist extraction showed its advantages in extraction conditions and great specificity for application.

In the meantime, enzyme hydrolysis assists extraction demonstrating its advantages in consideration for extraction conditions and extremely specific application. As it seldom damages the molecular three-dimensional structures, it consequently maintains the bioactivity of mushroom polysaccharide. Furthermore, it also helps to progress the purity of polysaccharide. This result will be consistent with that previous experiment in which the polysaccharide was extracted from the fruit body of mushroom *C. maxima* (Yang et al., 2011). Multi enzymatic hydrolysis, help to break down the linkage and assist in discharging the polysaccharide and raise the extraction rate as reported by Chen et al. (2013).

Conclusions

Extraction pH is the most important factor which influenced the extraction efficiency both for amount of extract and polysaccharides yield. Enzyme-hydrolysis-assist extraction remained effective. It can hydrolyze the cellulose, pectin and break down the cell walls. This study reports an effective extraction method for poly-saccharide, the best optimal conditions were determined: complex enzyme amount of 2%, extraction temperature at 40°C, extraction time is 3 h and extraction pH is 5. While the best optimal conditions for the amount of extract were as follow: complex enzyme amount of 2%, extraction temperature at 60°C, extraction time 1 hr and extraction pH is 6. Furthermore best optimal conditions with alpha amylase assist extraction as follows for enzyme amount of 0.03896%, extraction temperature at 50°C, extraction time of 2 h, and extraction pH is 6. Further studies on the complex enzyme hydrolysis assist extraction and single enzyme necessitates is suggested for finding out the best extraction condition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Boroushaki MT, Boskabady MH, Malek F (2010). Antitussive effect of Portulaca oleracea L. in guinea pigs. Iran. J. Pharm. Res. 3:187-190.

Chen J, Pufu L, Hengsheng SHEN, Hengguang Z, Rutao F (2013). Effect of extraction methods on polysaccharide of clitocybe maxima stipe. Adv. J. Food Sci. Technol. 5(3):370-373.

Chunhua X, Zhenle T, Chenju Z, Xiaobing Y, Huihua Z (2014). Optimization of enzyme assisted extraction of polysaccharides from Ganoderma lucidum. Eng. Sci. 12(1):17.

Dam S (2004). Food Biochemistry, National University of Ho Chi Minh City, Ho Chi Minh City, Vietnam (in Vietnamese).

Dkhil MA, Abdel Moniem AE, Al-Quraishy S, Saleh RA (2011). Antioxidant effect of purslane (Portulaca oleracea) and its mechanism of action. J. Med. Plants Res. 5:1563-1589.

Fernandes P (2010). Enzymes in food processing: a condensed overview on strategies for better biocatalysts. Enzyme Res. 2010:862537.

Ghosh P, Ghosal P, Thakur S, Lerouge P, Loutelier- Bourhis C, Driouich A (2005). Polysaccharide from Se- samum indicurnMeal: Isolation and Structural Features, Food Chem. 90(4):719-726.

Huang SQ, Ning ZX (2010). Extraction of Polysaccharide from Ganoderma lucidum and Its Immune Enhancement Activity. Int. J. Biol. Macromol 47(3):336-341.

Jia X, Ma L, Li P, Chen M, He C (2016). Prospects of Poria cocos polysaccharides: Isolation process, structural features and bioactivities. Trends Food Sci. Technol. 54:52-62.

Jiang YH, Jiang XL, Wang P, Mou HJ, Hu XK, Liu SQ (2008). The antitumor and antioxidative activities of polysaccharides isolated from Isaria farinosa B05. Microbiol. Res. 163(4):424-30.

Jiang ZH, Yin XL, You QH, Zheng SY (2010). Studies on Extraction of Tricholoma matsutake Polysaccharides by Complex Enzyme and Molecular Weight Distribution. Acta Edulis Fung. pp. 109-112.

Jin HL, Xu ZR (2002). Antiviral and Immune Research Progress of Polysaccharide. Feed Expo 1:28-30.

Kanayama H, Adachi N, Togami M (1983). A new antitumor polysaccharide from the mycelia of Poria cocos wolf. Chem. Pharm. Bull. 31:1115-1118.

Kaneno R, Fontanari LM, Santos SA, Di Stasi LC, Rodrigues Filho E, Eira AF (2004). Effects of extracts from Brazilian sun-mushroom (*Agaricus blazei*) on the NK activity and lympholiferative responsiveness of Ehrlich tumor-bearing mice. Food Chem. Toxicol. 42:909-916.

Lee KY, Jeon YJ (2003). Polysaccharide isolated from Poria cocos sclertium induces NF-kB/Rel activation and ions expression in murine macrophages. Int. J. Immunopharmacol. 3:1353-1362.

Li W, Cui SW, Kakuda Y (2006). Extraction, fractionation, structural and physical characterization of wheat β-D-glucans. Carbohydr. Polym. 63(3):408-416.

Mizuno T (1999). The extraction and development of antitumour-active polysaccharides from medicinal mushrooms in Japan. Int. J. Med. Mushrooms 1:9-29.

Mizuno T, Sakai T, Chihara G (1995). Health foods and medicinal usages of mushrooms. Food Rev. Int. 11(1):69-81.

Rashed AN, Afifi FU, Disi AM (2003). Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L. (growing in Jordan) in Mus musculus JVI-1. J. Ethnopharmacol. 88(2-3):131-6.

Ros JM, Laencina J, Hellí P, Jordan MJ, Vila R, Rumpunen K (2004). Characterization of juice in fruits of different Chaenomeles species. LWT-Food Sci. Technol. 37(3):301-307.

Tai T, Akahori A, Shingu T (1992). Triterpenes of *Poria cocos*. Phytochemistry 32:1239-1244.

Tai T, Shingu T, Kikuchi T, Tezuka Y, Akahori A (1995a). Isolation of lanostane-type triterpene acids having an acetoxyl group from sclerotia ofPoria cocos. Phytochemistry 40:225-230.

Tai T, Shingu T, Kikuchi T, Tezuka Y, Akahori A (1995b). Tritepenes from the surface layer of *Poria cocos*. Phytochemistry 39:1165-1169.

Thulasi PG, Nair CKK, Janardhanan KK (2008). Polysaccharides isolated from Ganoderma lucidum occurring in Southern parts of India protects radiation induced damages bothin vitro andin vivo. Environ. Toxicol. Pharmacol. 26(1):80-85.

Wang LY, Wan HJ (1998). Studies on the chemical constituents of Fuling (Poria cocos). Zhong Cao Yao 29:145-148.

Wasser S (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl. Microbiol. Biotechnol. 60(3):258-274.

Wu Y, Li S, Li H, Zhao C, Ma H, Zhao X, Wu J, Liu K, Shan J, Wang Y (2016). Effect of a polysaccharide from Poria cocos on humoral response in mice immunized by H1N1 influenza and HBsAg vaccines. Int. J. Biol. Macromol. 91:248-257.

Yang SD, Bao HY (2005). Progress of research on triterpenes and polysaccharides of WolfiPoria cocos. J. Yeast Fungal Res. 3:1672-3538.

Yang XY, Chen G, Li Z (2011). Compare study of the ultrasonic extraction and the enzymolysis method of polysaccharide in Clitocybe maxima. J. PuTian Univ. 18:28-32 (in Chinese).

Yasukawa K, Kaminaga T, Kitanaka S, Tai T, Nunoura Y, Natori S (1998). 3bp-Hydroxybenzoyl-dehydrotumulosic acid from Poria cocos, and its anti-inflammatory effect. Phytochemistry 48:1357-1360.

Yu R, Yin Y, Yang W, Ma W, Yang L, Chen X, Zhang Z, Ye B, Song L (2009). Structural elucidation and biological activity of a novel polysaccharide by alkaline extraction from cultured Cordyceps militaris. Carbohydr. Polym. 75(1):166-171.

Yu SJ, Tseng J (1996). Fu-Ling, a Chinese herbal drug, modulates cytokine secretion by human peripheral blood monocytes. Int. J. Immunopharmacol. 18(1):37-44.