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Studying on polysaccharide extraction prepared by ultrasonic-microwave from bulbil of *dioscorea opposita*

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Abstract
Bulbil of *Dioscorea opposita* is riched with nutrients, having extremely value of pharmacology research. In this study orthogonal test was used to choose the optimum conditions of polysaccharides extraction prepared by the ultrasonic-microwave from bulbil, including the different part of the bulbil, different microwave power, different solid-liquid ratio and different extract time. The results showed that the optimal extraction parameters were microwave power 500 W, extract time 30 min and solid-liquid ratio 1:25 (g/mL). Our experiment also showed that the yield rate of polysaccharide was 19.23%, which indicated that there was a good fit between the predicted value and the experimental value. These findings suggest that ultrasonic-microwave assisted extraction could greatly improve the extraction rate of the polysaccharides from bulbil.

Keywords: ultrasonic-microwave assisted extraction, bulbil, polysaccharides

1. Introduction

Dioscore opposita Thunb is a perigynous wing plant in the family dioscorea genus dioscorea. There are about 49 species in China, and most of them have medical, health and food functions [1, 2]. Polysaccharide is the main medicinal component of bulbil [3]. Bulbil is the bud between the axils of Dioscorea opposite Thunb, which contains saponins choline starch (polysaccharides), amino acids, glycoproteins, vitamin C and other effective components [4, 5, 6]. Bulbil can not only make waist and feet stronger but also enhance satiety. The function of bulbil is stronger than *Dioscorea opposita* Thunb. It can improve human immune function and disease resistance. The yield of bulbil per acre can reach 33-49 kg. However its yields were discarded as waste at present. The main components of bulbil are carbohydrates and proteins, among which the content of polysaccharides is high [7]. Crude polysaccharide refers to the complex hetero polysaccharide, containing muco polysaccharide, lipopolysaccharide, combined polysaccharide (glycoprotein and mucin) and other impurities. The rsearch showed that crude polysaccharides had the biological activities of lowering blood lipid [8], anticoagulation [9], antioxidation [10] and Antiathero sclerosis [11]. The common extraction methods of polysaccharides include hot water extraction [12], ultrasound-assisted extraction [13], microwave-assisted [14], enzymatic extraction[15]. However, ultrasonic-microwave assisted extraction is rarely used to extract plant polysaccharides [16]. Simultaneous ultrasonic-microwave assisted extraction (UMAE) coupled the advantage of microwave and ultrasonic, presenting many advantages, has been used for the extraction and separation of some natural plant compounds Microwaves provide rapid and convenient heating for natural plant compounds but have masstransfer limitations whereas the ultrasound produces intense physical mixing by cavitation but lacks the ability to generate high thermal energy for natural plant compounds. Consequently, the combination of ultrasound and microwave radiation is a complementary technique that may exhibit the advantages of both methods [17]. The purpose of this study was to explore the best conditions for extracting polysaccharides from the bulbil and provides scientific reference for bulbil's extraction, rational development, and utilization.

2. Materials and methods

2.1 Materials

2.1.1 Collection of Plant samples

The bulbil were supplied by Wenxian Juhuai Food Co. LTD and authenticated by Prof. Lin Ma in the Engineering Research Center for Biomass Resource Utilization and Modification of Sichuan Province(Mianyang, Sichuan).

2.1.2 Chemicals, reagents

Anhydrous ethanol (Chengdu Jinshan Chemical Reagent Co. LTD) Acetone (Chengdu Kelong Chemical Reagent Factory) Anhydrous ether (Chengdu Kelong Chemical Reagent Factory) Concentrated sulfuric acid (Chengdu Kelong Chemical Reagent Factory) Phenol (Chengdu Kelong Chemical Reagent Factory) Glucose (Xian Shui Water Gu Industrial Park, Jinnan District, Tianjin)

2.2 Method

2.2.1 Extraction and preparation of bulbil polysaccharides

Total bulbil polysaccharides in the extracts was based on Huang's [18] method with some modifications. Divide the bulbil into two parts, one part is peeled, the other part is not peeled, then respectively cut and dried in the constant oven at 60℃ for 8 hours. Then, the dried bulbil were crushed to powder (60 mesh) and collect thpowder in bottles. The powder of 3.00g bulbil was weighed and extracted with ultrasound-microwave assist at 60℃ for 20min. Besides, kept the ultrasonic power 50w during that time. Above conditions are respectively for peeled bulbil, not peeled bulbil and the bulbil peel; the microwave power at 400w, 500w, 600w, 700w, 800w; the solid-liquid ratio (w:v) 1:10, 1:15, 1:20, 1:25, 1:30. As for the extraction time, kept the microwave power at 600w, and separately extracted 10min, 15min, 20min, 25min and 30min. The four conditions were tested with a single factor. The extract was centrifuged at 4000r/min for 20min, concentrated in a water bath at 90℃ to 1/4 of the volume, and 4 times the volume of ethanol was added. Then deposited the solute overnight. After centrifuging at 4000r/min for 20min, the resulting product was extracted and filtered. The precipitate was washed with: ethanol, acetone and ethyl ether. Then dried and weighed the precipitate. Weighing 2.0mg crude polysaccharide, dissolve it in distilled water. Transfer this solution to a 50 ml volumetric flask, diluted with water to volume. It was used for sample to be measured [19].

2.2.2 Preparation of standard curve

Standard curve assay was performed according to the method of Xiao [20, 21]. The appropriate amount of anhydrous glucose after 105℃ of drying was precisely weighed and added to water to form a standard glucose solution with a concentration of 0.100 mg/ml. 0.2, 0.4, 0.6, 0.8, 1.0, 0.12 and 0.14ml of Glucose reference solution was precisely measured in 7 test tubes, and distilled water was added to achieve a total volume of 2ml. Besides, 2.0 mL distilled water was added in another test tube as blank control. Then added 1.0 mL of 5% phenol solution and sulfuric acid 5.0 mL to each tube with rapidly shaking. Leaving the tube at room temperature for 30 min, used spectrophotometer to measure the absorption at 490nm. The standard curve was drawn with the concentration of glucose solution C (mg· ml^{-1}) as the abscissa and the absorbance A as the ordinate. The regression equation is A=6.032C - 0.024, r=0.995.

2.2.3 Determination of polysaccharide yield

The 3g bulbil powder is processed according to the above operation, and the obtained precipitate is dried and weighed, and the precipitate is crude polysaccharide. The yield of crude polysaccharides was calculated according to formula (1).

$$p = \frac{m_1}{m_2} \times 100\% \quad (1)$$

In the formula:
Yield of crude polysaccharide, %
m_1-The quality of crude polysaccharides, g
m_2-The quality of bulbil powder, g.

2.2.4 Determination of polysaccharide content

Total determination of polysaccharide content method was based on He's [22] method with some modifications. The polysaccharide can be hydrolyzed into monosaccharides under the condition of concentrated sulfuric acid. The monosaccharides could dehydrate to form glycolaldehyde or glycolaldehyde derivatives, which could react with phenol to produce orange products, that is, the color reagent condenses to form colored complexes. The color depth is positively correlated with the content of polysaccharides. By using this principle, the absorbance was measured and the content of polysaccharide was calculated. Add 1.0 mL Sample to be measured, 1.0 mL of 5% phenol solution and ulfuric acid 5.0 mL to each tube with rapidly shaking. Leave the tube at room temperature for 30 min, then use spectrophotometer to measure the absorption at 490nm. The context of crude polysaccharides was calculated according to formula (2).

$$\text{The polysaccharide content (\%)} = \frac{\text{Quality of monosaccharide} \times \text{Diluted multiples}}{\text{The polysaccharides were weighed}} \times 0.9 \times 100\% \quad (2)$$

3. Results and Discussion

3.1 Single factor test results and analysis

3.1.1 The Effects of different microwave power on the content and yield of polysaccharides

Under the extraction time was 20min with 50w ultrasonic power at the solid-liquid ratio 1:15, researched the effects of microwave power on the content and yield of polysaccharides. The result was shown in figure 1. As you can see from figure 1, the raw materials in the process of microwave power from 400w to 800w, the content and yield of polysaccharides was increased first and then decreased. Among them, the content of bulbil polysaccharides reached the highest point at 600w. However, the yield of bulbil polysaccharides reached the highest point at 500w. Considering the change of polysaccharide content and yield of bulbil, the appropriate microwave power was 600w and 500W respectively.

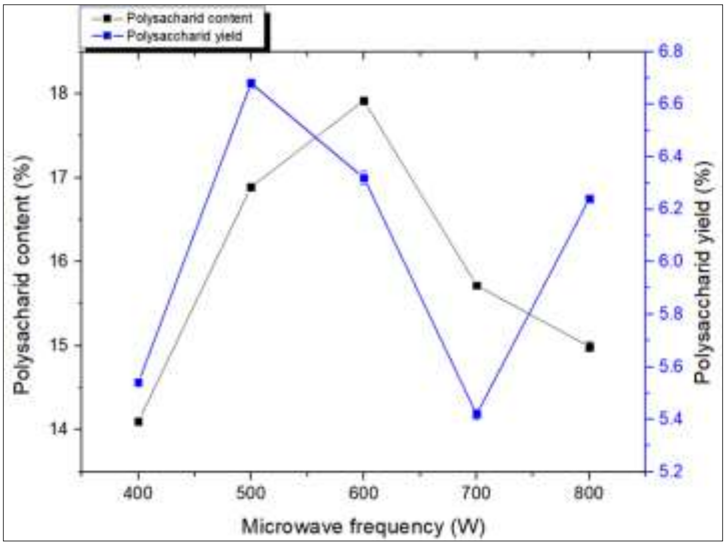

Fig 1: effect of microwave power on the content and yield of polysaccharides from bulbil

3.1.2 Effects of different solid-liquid ratio on the content and yield of polysaccharides from bulbil

The effect of the solid-liquid ratio on the extraction of polysaccharides was investigated under other conditions do not change. The result was shown in figure 2. From figure 2, with the increase of the solid-liquid radio, the content and yield of polysaccharides increases first. The content and yield of Polysaccharides increased slowly when the solid-liquid ratio was1:15 and 1:20. However, it increased significantly while the solid-liquid ratio reached 1:25. On this condition, the polysaccharide content reached 9.26%, and the yield of polysaccharides was up to 6.80%. The content and yield of polysaccharides began to decrease after the solid-liquid ratio reached 1:25. Therefore, 1:25 is the optimal solid-liquid ratio.

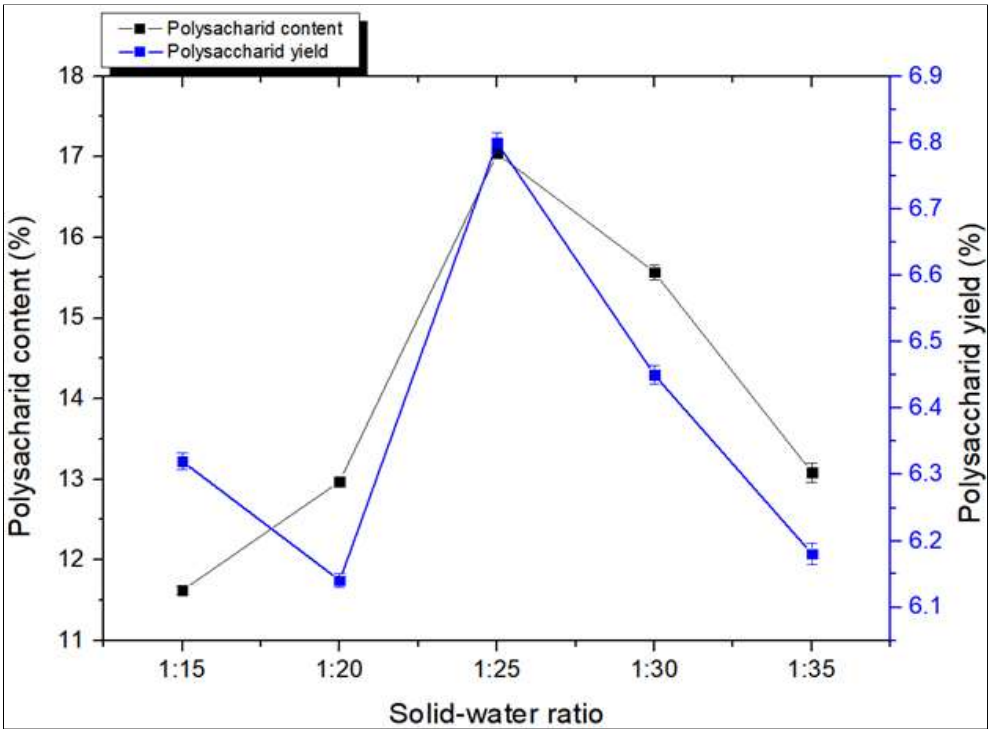

Fig 2: Effect of the Solid-Liquid Ratio on The Content And Yield Of Polysaccharides From Bulbil

3.1.3 Effects of different extraction sites on the content and yield of polysaccharides from bulbil

The effects of different extraction sites on the content and yield of polysaccharides were studied under other conditions do not change. The result is shown in figure 3. From figure 3, the content of polysaccharides in the bulbil peel was the largest while the yield of polysaccharides in the not-peeled bulbil is the highest. It was worth mentioning that there was still a small amount of suspended peel after bulbil peel centrifugation, resulting in the loss of yield from filtration.

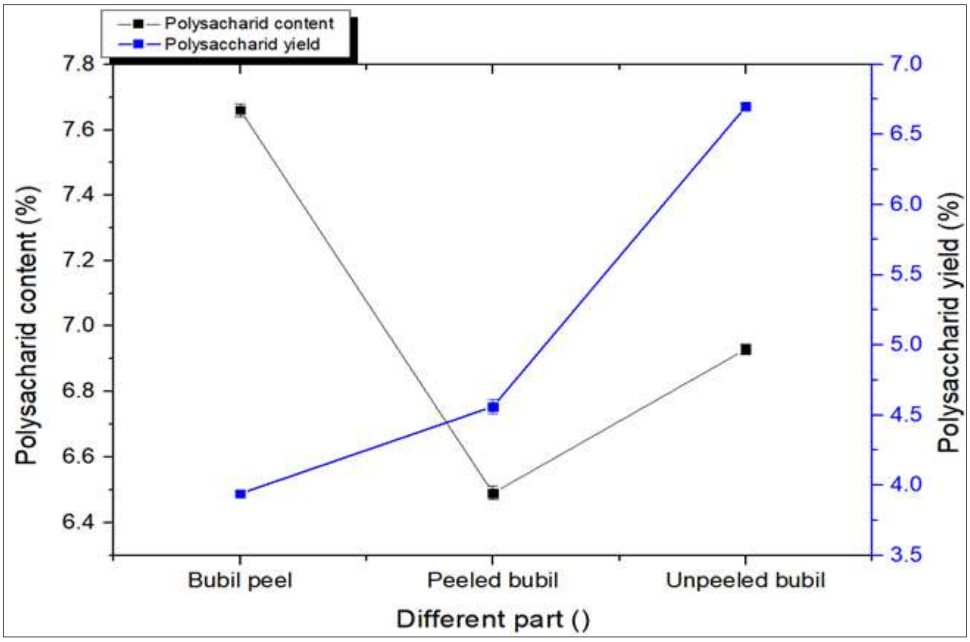

Fig 3: Effect of the extraction site on the content and yield of polysaccharides from bulbil

3.1.4 Effects of different extraction times on the content and yield of polysaccharides from bulbil

The effects of different time on the content of polysaccharides from bulbil were studied, under the other conditions do not change. The result is shown in figure 4. From the data we cuold see that as the time progresses, the content and yield of polysaccharides increased. It increased relatively slow at 10min to 15min. When the time was from 15min to 20min, the data grew fastest. And the maximum value was at 30min.

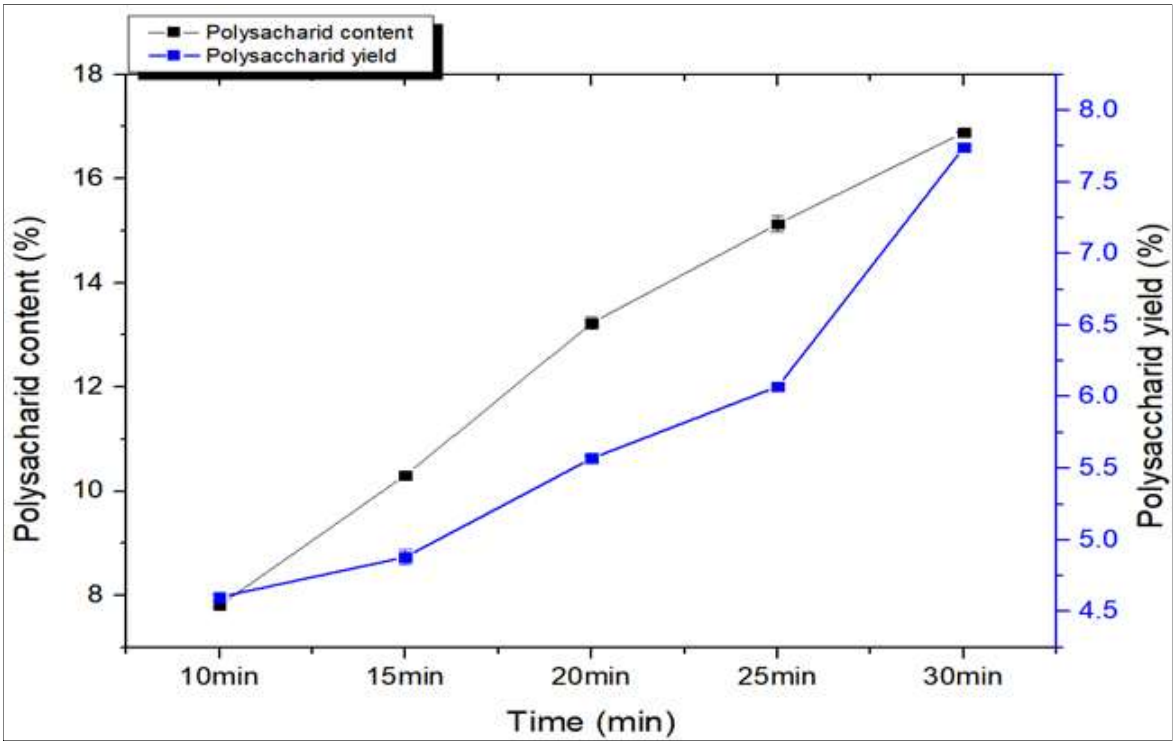

Fig 4: effects of different extraction times on the content and yield of polysaccharides from bulbil

3.2 The results and analysis of orthogonal test

3.2.1 Orthogonal experiment factor selection

Through statistical analysis, the three most significant factors were as follows: (A) microwave power; (B) extraction time; (C) solid-liquid ratio. They were selected to three-factor and three-level orthogonal test. The content and yield of polysaccharides were used as the indexes. The factors of orthogonal test was shown in table 1.

Table 1: the factors of orthogonal test

Level		Etract factor	
	Microwave power/W (A)	Time/min (B)	Solid-liquid ratio g/ml (C)
1	500	20min	1:20
2	600	25min	1:25
3	700	30min	1:30

3.2.2 The optimum extraction process of polysaccharide from bulbil

The content and yield of polysaccharides in the crude polysaccharides extracts of bulbil were determined, the results were shown in table 2. The results of intuitive analysis showed that $A_1B_3C_2$ combination was preferred, that is, the microwave power 00w, solid-liquid ratio 1:25, extraction time 30min. The conclusion could be drawn from table 2. The order of influence from the three factors was $R_A>R_B>R_C$.

This indicated that the factors of influencing the polysaccharide content and yield in the polysaccharide extraction of bulbil from large to small were: microwave power (A)>extraction time (B)> solid-liquid ratio (C).

4. Discussion

In the single factor experiment, comparing different part's affection of bulbil on the content and yield of polysaccharides, most content of the polysaccharide were low. This might because the screening particles of bulbil peel were too small, which resulted in centrifugal peel density less than water. Therefore, the powder of peel suspended on the surface of the liquid. During the filtration process, some of the polysaccharides remained in the gaps of the filter paper. In order to maintain the principle of a single variable, we filter both not-peeled and peeled bulbil, resulting in generally low levels.

Table 2: Results of orthogonal experiment

Test number		A	B	C	Content of polysaccharide/%	Yield of polysaccharide /%
1		1	1	1	15.86	5.15
2		1	2	2	19.23	6.13
3		1	3	3	17.18	5.66
4		2	1	2	14.69	5.59
5		2	2	3	12.20	6.77
6		2	3	1	17.18	6.81
7		3	1	3	16.74	5.71
8		3	2	1	14.54	6.10
9		3	3	2	16.16	6.73
Content of polysaccharide /%	K1	17.42	15.76	15.86		
	K2	14.69	15.32	16.69		
	K3	15.81	16.84	15.37		
	R	2.73	1.52	1.32		
Yield of polysaccharide /%	K1	5.64	5.48	6.02		
	K2	6.39	6.33	6.15		
	K3	6.18	6.40	6.05		
	R	0.75	0.92	0.13		

According to Jia's[1] combined enzyme extraction method the content of bulbil polysaccharides can reach to 15.80%, which compared with ultrasonic-microwave assisted is more. But it means more cost into it. In Gong's thesis [23], he use microwave-assisted method to extract astragalus polysaccharide,the peak of the content can reach 14.6%, while in Sun's ultrasonic assisted method[24], the content of astragalus polysaccharide can reach 4.96%. It has been proved that ultrasonic-microwave assisted method is an ideal method for extracting bulbil polysaccharides,which shorten the extract time and have high content and yield of polysaccharides.

5. Conclusion

On the basis of ultrasonic-microwave assisted extraction, the effects of different microwave power, different parts of bulbil, different solid-liquid ratio, and different extraction time on the content and yield of polysaccharides in bulbil were investigated. It was concluded that the optimal extracted conditions were as follows: extraction time 30min, microwave power 500w, solid-liquid ratio (w:v) 1:25. On this condition, the optimal yield of polysaccharides from bulbil was 19.23%. We can easily draw the conclusion that ultrasonic-microwave assisted extration method can enhance the efficiency of polysaccharid extraction. It may have wider application in the future.

6. Acknowledgement

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7. References

1. Wenhong Long, Huachun Guo. Research progress of Bulbil of Dioscore L. Journal of yunnan agricultural university. 2006; 21(4):76-77.
2. Chinese botanical journal editorial committee, Chinese academy of sciences. Chinese flora. Science Press, Beijing, China, 1985, 33.
3. Liyan Jia, Shengcai Ren. Optimization of combined enzymatic extraction on bulbil polysaccharide [J]. Journal of shanxi agricultural university (natural science edition). 2013; 33(2):130-135.
4. Bing Zhao. New techniques of Chinese Yam cultivation. Jindun Press, Beijing, 2010, 12.
5. Mingjun Li, Jiabao Zhang, Haibo Zhang. Study on the callus induction and plantlet regeneration from the bulbil of *Dioscorea opposita*. Acta botanica sinensis. 2000; 20(5):772-777.
6. Biao Zhang, Ke Ge, Lingshang. Comparison of structuraland functional properties of starches from the rhizome and bulbil of Chinese yam. Molecules. 2018; 23(2):427-438.
7. Wei Sheng, Jianping Xue, Bijun Xie. Chemical constituents in the bulbil of dioscoree opposite and its nutrition evaluation. Food Science and Technology. 2009; 34(8):76-79.
8. Minghui Xia, Daozong Xia, Ming Xia. Research of Lentina's Ettect on Blood Lipid and Its Mechanisms. Zhejiang Traditional Chinese Medicine College, Hangzhou (310053) China. 2005; 15(10):599-602.
9. Jiansu Cao, Yajing Yang, Ling qin. Structural characterization, anticoagulant and Thrombolytic activities of the seaweed polysaccharide UH3[J]. Chinese Marine medicine. 2019; 38(04):26-31.
10. Fenglong Cai, Xiaoming Wang, Chuanyao Tian. Extraction and antioxidant activity of polysaccharide from Chinese yam. Agricultural Technology and Equipment. 2017; 331(7):15-18.
11. hoi KW, Um SH, Kwak JH. Suppression of adhesion molecule expression by phenanthrene-containing extract of bulbils of Chinese Yam in vascular smooth muscle cells through inhibition of MAPK, Akt and NF-κB[J]. Food Chem Toxicol. 2012; 50(8):2792-2804.
12. Zhongyong Ceng, Jiang Su, Guanxing Liang. Hot water extraction technology for dendrobium polysaccharides from protocormsof candidum. Hubei Agric Science, 2011; 50(18):3807-3810.
13. Huaide XU, Shenghua Xu. Ultrasonic-assisted Extraction and *in vitro* Antioxidant Activity Evaluation of Polysaccharides from Chaenomeles sinensis (Thouin) Koehne. Food Scienc. 2010; 31(10):115-120.
14. Shengzhao Gong, zhuoru Yang. Study on microwave-assisted extraction of astragalus polysaccharides. Journal of South China University of Technology (Natural Science Edition). 2004; 32(8):93-96.
15. Lirong Teng, Qingfan Meng, Peiyuan Liu. Enzymatic extraction and *in vitro* antioxidant activity of polysaccharides from lily. Journal of Jilin University (Science Edition), 2003; 41(4):133-137.

16. Di Jin, Ying Liang, Gongbing Sun. Research progress of extraction techniques of plant polysaccharide. Journal of Heilongjiang Bayi Agricultural University. 2011; 23(5):76-79.
17. Sun H, Li C, Ni Y. Ultrasonic/microwave-assisted extraction of polysaccharides from Camptotheca acuminata fruits and its antitumor activity [J]. Carbohydr Polym. 2019; 2(06):557-564.
18. Jialin Ren, Rengui Huang, Zhonghai Li. Optimization on ultrasonic assisted extraction process of crude polysaccharides from bulbil. Food and Machinery. 2016; 32(9):149-153.
19. Rengui Huang. Study on isolation, identification and biological activity of polysaccharides derived from bulbil. Hunan:Central South University of Forestry and Technology, 2017, 13-15.
20. Kazuko, Shimada Kuniko, FujikawaKeiko, Yahara Takashi. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrinemulsion. Journal of Agricultural and Food Chemistr. 1992; 40(6):945-948.
21. Xiaolei Guo, Sichao Zhu, Xufeng Zhai. Anthracone sulfate method and phenol sulfate method for determination of polysaccharide content in ganoderma lucidum [J]. Chinese journal of traditional Chinese medicine. 2010; 28(09):2000-2002.
22. Jiaqi He, Zhensheng Yao, Yaokang Xionng. Content de termination of polysaccharide in radix and rootlet ophio pogonk by the method of phenol-sulfuric acid method [J]. Chinese Journal of Information on Traditio nal Chinese Medicine. 2006; 13(10):51-52.
23. Shengzhao Gong, Zhuoru Yang. Study on microwave-assisted extraction of astragalus polysaccharides [J]. Journal of south China university of technology (natural science edition). 2004; 32(8):93-96.
24. Yinghua Sun, Zongbing Liu, Lijuan Xu. Study on ultrasonic extraction technology of astragalus polysaccharides [J]. Journal of baicheng normal university. 2015; 29(2):5-8.