

In Vitro the Hypoglycemic Activity of Polysaccharide Extracts from *Lethariella* Spp.

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Abstract—To investigate the hypoglycemic activity, the inhibitory effect of *Lethariella* spp. polysaccharide extracts (LPE) on α -amylase activity was studied *in vitro*. Controlling concentration, reaction time and solution pH as the single factor variable to evaluate α -amylase inhibition ratio of LPE, and compared with acarbose. The results showed that α -amylase inhibition ratio was positively correlated with the concentration of polysaccharide. The α -amylase inhibition ratio of LPE reached a maximum (about 73%) when the concentration of LPE was 0.50 mg/mL and reaction time was 20 minutes. Furthermore, in the pH 3~6 range, the α -amylase inhibition was stable. In addition, α -amylase inhibition ratio of LPE was compared with those of tea, fungi and algae polysaccharides. It showed LPE had a strong α -amylase inhibition. These results indicated *Lethariella* spp. was worthy to be a good material for hypoglycemic drinks and health care products.

Index terms—*Lethariella* spp., polysaccharide, α -amylase, inhibitory effect, hypoglycemic activity

I. INTRODUCTION

Lethariella spp. is a Tibetan traditional medicine herbs [1-2]. It belonged to Lichens from *Lethariella*, Parmeliaceae and mainly grows on the high mountains at an altitude of 4000~5000 meters in the western of China [3-4]. *Lethariella* spp. had long been used as a Chinese folk medicine to treat swollen and sore throats as well as dizziness and neurasthenia. It was often processed into a tea of daily life in Yunnan Province [5]. Modern medical research showed that *Lethariella* spp. had the effect of lowering cholesterol, liver protection, anti-fatigue, anti-radiation and anti-inflammatory, et al [6-9]. Many compounds had been isolated from *Lethariella* spp., such as phenolic acids, steroids, polysaccharides, volatile oil, inorganic elements, et al [10]. Polysaccharide was one of the main functional components of *Lethariella* spp., and it had important biological activity [11-13].

α -amylase (α -1,4-D-glucose-glucoside hydrolase) is an important starch hydrolytic enzyme, which plays an important role in the metabolism of glycolipids. [14-15]. α -amylase inhibitors were classic drugs used in various types of diabetes, it could effectively prevent the hydrolysis and digestion of carbohydrates in food, which hindered the conversion of food into sugar and reduced

body's intake of sugar to achieve the effect of reducing blood sugar and blood lipids [16-18].

At present, the inhibitory effect of LPE on α -amylase has not been reported in the literature. Our previous study showed that *in vitro* LPE had a good antioxidant capacity [19] and lipid-lowering effect (submitted). In this study, the hypoglycemic activity of *Lethariella* spp. was evaluated. The comprehensive study on LPE enhances *Lethariella* spp.'s value of development and utilization.

II. MATERIAL AND METHOD

A. The PREPARATION of LPE

Chemicals. *Lethariella* spp. (Shangri-La, Yunnan, China). α -amylase, soluble starch and acarbose were purchased from Shenzhen HangSeng Biological Technology Co., Ltd.(Guangdong, China). Glucose, chloroform, n-butanol, anhydrous ethanol, phenol, concentrated sulfuric acid, concentrated hydrochloric acid, iodine (granular), potassium iodide (flaky), citric acid and disodium hydrogen phosphate were purchased from Chongqing Chuandong Chemical Reagent Factory (Chongqing, China). All reagents were standard commercial products of analytical-reagent grade.

The methods of polysaccharide's extraction and separation are described previously [19] and the specific process was as followed: 30.00g sample was accurately weighed by BT25S analytical balance (Beijing Sartorius Balance Co., Ltd., China) and cut into small pieces, and mixed with distilled water according to the ratio of 1:20 (material to liquid). Then the mixture was heated at 82°C 2h by electronic universal furnace (Beijing Yong Guang Ming Medical Instrument Co., Ltd., China). After the tea residues were filtered off, added 3 times absolute ethanol and ethanol precipitation for 12 h in 4°C refrigerator. Then the solution was centrifugalized by 800B centrifuge (Shanghai Anting Scientific Instrument Factory, China) at 4000 r/min for 2 min and collected flocculent red polysaccharide precipitation. The precipitation was dissolved in 150.00 mL distilled water and purified using Sevage method to remove protein until the lower layer had no protein emulsion layer. At last, the purified polysaccharide was obtained by ethanol precipitation and freeze-dried.

B. The determination of α -amylase activity

The determination of α -amylase activity referred to Chinese national standard GB 8275-2009 [20], and absorbance was measured at a wavelength of 620 nm. The enzyme activity was calculated according to formula (1):

$$X=c \times n \quad (1)$$

X (U/mg) —sample enzyme activity; c (mg/mL) —sample solution concentration; n —sample dilution multiple.

C. Plotting of starch standard curve

The soluble starch standard solution of 0.10, 0.20, 0.30, 0.40, 0.50, 0.60 and 0.70 mg/mL was prepared respectively. Transferred 1mL above standard solution respectively to test tube and 37 °C water-bath heating for 30min and preheated for 15 minutes by DZKW-S-8 electric water bath thermostat (Beijing Yong Guang Ming Medical Instrument Co., Ltd., China). Used 0.30 mL hydrochloric acid solution (2.00mol/L) to terminate the reaction. After that, 4mL distilled water was used to dilute the solution. After shaken well, the solution was immediately mixed with 0.20 mL diluted iodine solution. Measured its absorbance at 620 nm by 722 grating spectrophotometer (Shandong Gao Mi Analytical Instrument Factory, China). Distilled water was used as blank control. At last, starch standard curve was drawn with starch mass concentration as abscissa and absorbance as vertical axis.

D. The inhibitory effect of LPE on α -amylase

The concentration of LPE, the reaction time and the pH value were used as single factor variable to investigate the relationship between LPE and α -amylase inhibition ratio. The effects of different LPE concentrations (0.10, 0.20, 0.30, 0.40, 0.50, 0.60 mg/mL), reaction time (5, 10, 15, 20, 25 min) and pH (3, 6, 9, 12) on α -amylase inhibition ratio were studied respectively. In addition, The effects of different concentrations of acarbose solution (0.05, 0.15, 0.25, 0.35, 0.45, 0.55 mg/mL), reaction time (5, 10, 15, 20, 25 min) and pH (3, 6, 9, and 12) on α -amylase inhibition ratio also were studied as the control group.

According to the method of determination of enzyme activity, α -amylase inhibition ratio of LPE and acarbose was calculated respectively by following the equation (2):

$$I= [(X_0-X_1)/X_0] \times 100\% \quad (2)$$

I (%) — α -amylase inhibition ratio; X_0 (U/mg) —the original α -amylase activity without inhibitor; X_1 (U/mg) —the α -amylase activity with inhibitor.

E. The inhibitory effects of different plants polysaccharides on α -amylase

The determination of α -amylase activity referred to Chinese national standard GB 8275-2009 [20], and absorbance was measured at a wavelength of 620 nm.

F. Statistical analysis

The experimental datas were processed and analyzed by Excel 2010 software.

III. RESULTS

A. Plotting of starch standard curve

“Fig. 1” shows there was excellent linear relationship between starch content and the absorbance in the range of 0.10-0.70 mg /mL. According to the regression equation of starch solution ($y = 0.0454x$), the formula of α -amylase activity was obtained as following:

$$X=(OD_1-OD_2)n/0.0454 \quad (3)$$

X (U/mg) — sample α -amylase activity; OD_1 — absorbance with inhibitor; OD_2 — absorbance without inhibitor; n — sample dilution multiple.

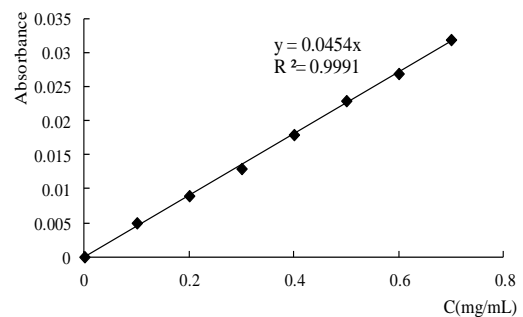


Figure 1. Standard curve of starch

B. Effect of different concentrations on α -amylase inhibition ratio

Different concentrations' LPE all had an inhibitory effect on α -amylase, but α -amylase inhibition ratio of LPE was less than acarbose (“Fig. 2”). In the concentration range of 0.10-0.50 mg/mL, the inhibitory effect of LPE on α -amylase was obvious concentration dependent. When the concentration of LPE was 0.50 mg/mL, α -amylase inhibition ratio reached a maximum, about 73.24%. When the concentration of acarbose solution was 0.35 mg/mL, α -amylase inhibition ratio reached 86.98% and tended to be stable.

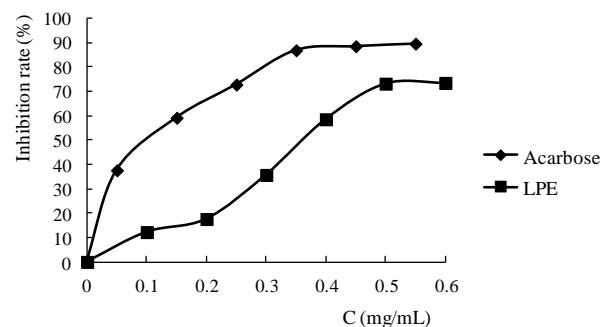


Figure 2. Effect of different concentrations on α -amylase inhibition ratio

C. Effect of different reaction time on α -amylase inhibition ratio

When concentration of LPE and acarbose was 0.35mg/ml and 0.50mg/ml, and pH value was 6, effect of

different reaction time on α -amylase inhibition ratio was presented in “Fig.3”. The α -amylase inhibition ratio of acarbose increased rapidly within 5 minutes, indicating that acarbose could quickly bind α -amylase in a short time. The binding capacity of LPE was lower than that of acarbose. However, when reaction time was 20 minutes, α -amylase inhibition ratio of LPE reached 72.55% and became stabilized. At that time, the difference of α -amylase inhibition ratio between acarbose and LPE was only about 8% (“Fig. 3”).

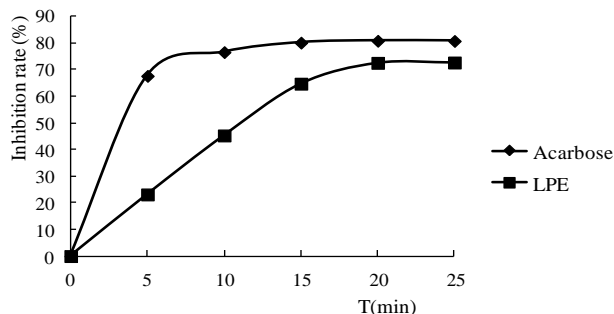


Figure 3. Effect of different reaction time on α -amylase inhibition ratio

D. Effect of different pH on α -amylase inhibition ratio

When concentration of LPE and acarbose was 0.35mg/ml and 0.50mg/ml, and reaction time was 25 min, effect of different pH on α -amylase inhibition ratio was showed in “Fig. 4”. The α -amylase inhibition ratio of LPE was relatively stable when pH value was 3~6. The α -amylase inhibition ratio curve showed a downward trend when pH was 6~12. In the range of pH 3~12, the α -amylase inhibition ratio of acarbose kept stable (“Fig. 4”).

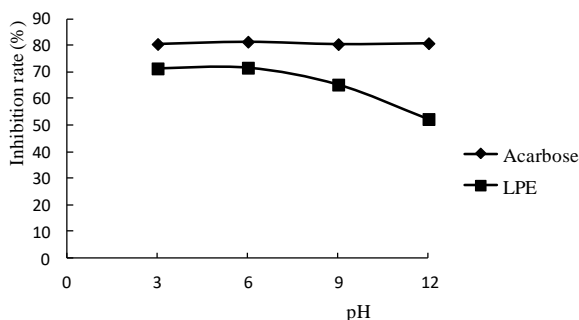


Figure 4. Effect of different pH on α -amylase inhibition ratio

E. The inhibitory effects of different plants’ polysaccharides on α -amylase

As shown in Tab. 1, α -amylase inhibition ratio of algal polysaccharides was more than 50%, while that of tea and fungi polysaccharides was less than 50% and the highest was only about 45% (*Camellia sinensis*). α -amylase inhibition ratio of *Chlorella* spp. and *Sargassum pallidum* polysaccharides was higher than that of LPE (73.55%). α -amylase inhibition ratios of the remaining 10 plants polysaccharides were all lower than LPE.

Table I.
The maximum α -amylase inhibition ratio of different plants’ polysaccharides

Plants polysaccharides		The maximum inhibition ratio *(%)	References
Tea	<i>Lethariella</i> spp.	73.55	our results
	<i>Camellia sinensis</i>	≈45	[21]
	Tea "Cha"	≈35.5	[22]
	Oolong tea	26.18	[23]
	Tea flower	29.5	[24]
Fungi	<i>Russula alutacea</i> fr.	23.55	[25]
	<i>Pleurotus eryngii</i>	≈20	[26]
	<i>Hericium erinaceus</i>	17.5	[27]
	<i>Cordyceps</i>	31.12	[28]
Algae	Seaweed (water extract)	59.38	[29]
	Seaweed (ethanol extract)	60.5	[30]
	<i>Chlorella</i> spp.	79.67	[31]
	<i>Sargassum pallidum</i>	85.02	[32]

* note: the maximum α -amylase inhibition ratio which obtained under the optimum process conditions of this literature.

IV. CONCLUSIONS and DISCUSSION

In the concentration range of 0.10-0.50mg/mL, α -amylase inhibition ratio of LPE showed a certain dose-effect relationship. When the solution concentration was 0.50 mg/mL, α -amylase inhibition ratio of the polysaccharides reached a maximum (73.24%). With the increased of reaction time, the inhibition ratio increased gradually and was stable at 20 minutes. In addition, in acidic to neutral solution (pH=3~6), the α -amylase inhibition ratio of LPE remained stable. In addition, comparing with other plants’ polysaccharides, LPE showed a strong α -amylase inhibition.

Nowadays, acarbose is used in clinical medicine for treatment of the type 2 diabetes. However, there are some obvious side effects when people used acarbose, such as abdominal distension, abdominal pain, diarrhea and so on. Some studies had shown these side effects were mainly due to excessive inhibition of acarbose on α -amylase in the intestine [33~35]. This study showed that the inhibitory effect of LPE on α -amylase was weaker than that of acarbose. It indicated that LPE could reduce some side effects caused by excessive α -amylase inhibition in later applications. The specific mechanism of the inhibitory effect of LPE on α -amylase and hypoglycemic activity *in vivo* need further investigate.

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REFERENCES

[1] D. L. Niu, C. R. Yang. “Original species identification and geographic distribution of hongxuecha”. *Plant Diversity and Resources*, vol. 34, pp. 76-80, 2012.
 [2] B. Jiang, Q. S. Zhao, L. Y. Peng, et al. “Constituents from

- Thamnomia vermicularis*". *Acta Botanica Yunnanica*, vol. 24, pp. 525-530, 2002.
- [3] L. Tang, L. Yang, Y. Ma, et al. "Ethnobotanical study of tibetan medicine icetea". *Journal of the CUN (Natural Sciences Edition)*, vol.18, pp. 83-85, 2009.
- [4] H. Fu, L. S. Wang, Y. H. Chen, et al. "A study on nutritional components of two different lichen teas from Yunnan". *Natural Product Research and Development*, vol. 17, pp. 340-343, 2005.
- [5] X. L. Gao, R. P. Zhang. "Studies on chemical constituents of *Lethariella cladonioides*". *Chinese Traditional and Herbal Drugs*, vol. 35, pp. 370-371, 2004.
- [6] J. H. Sung, J. W. Chon, M. A. Lee, et al. "The anti-obesity effect of *Lethariella cladonioides* in 3T3-L1 cells and obese mice". *Nutrition Research and Practice*, vol. 5, pp. 503-510, 2011.
- [7] C. Zhao, X. H. Zhang. "Study on the effects of Hongxue Tea mixture on small rats'anti-fatigue". *Yunnan Journal of Traditionnal Medicine and Material Medicine*, vol. 26, pp. 27-29, 21, 2005.
- [8] C. Zhao, X. H. Zhang, Q. Wang. "Protective effect of Hong-Xue Tea mixture against radiation injury in mice". *Chinese Journal of Radiological Health*, vol. 14, pp. 175-177, 2005.
- [9] G. Y. Mao, X. W. Zhuang, G. M. Cai, et al. "Study of prevention and treatment effects of *Lethariella cladonioides* polysaccharide on hyperlipidemic fatty liver in rats". *Chinese Pharmacological Bulletin*, vol. 28, pp. 1479-1480, 2012.
- [10] Y. B. Luo. *Studies on the chemical constituents of Lethariella cladonioides*. Shenyang: Shenyang Pharmaceutical University, 2005.
- [11] X. W. Zhuang, X. F. Liu, G. Y. Mao, et al. "Determination of *Lethariella cladonioides* polysaccharide from Tibet". *Chinese Journal of Experimental Traditional Medical Formulae*, vol. 18, pp. 103-106, 2012.
- [12] M. Y. Xie, S. P. Nie. "A Review about the research of structure and function of polysaccharides from natural products". *Journal of Chinese Institute of Food Science and Technology*, vol. 10, pp. 1-11, 2010.
- [13] J. N. Fang, K. Ding. "Bioactivities, isolation and purification methods of polysaccharide". *Chinese Journal of Natural Medicines*, vol. 5, pp. 338-347, 2007.
- [14] S. Y. Zhang, J. Q. Zhou, S. H. Li, et al. "Effect of okra polysaccharide on α -amylase inhibition and its spectral analysis". *Science and Technology of Food Industry*, vol. 36, pp. 101-104, 2015.
- [15] Z. G. Luo, J. F. Yang, F. X. Luo. "Properties and Applications of α -amylase". *Food Research and Development*, 2007, 28(8): 163-166.
- [16] X. N. Zhao, Q. Y. Jia, J. J. Shan. "Optimization of the bioassay method for screening of α -amylase inhibitors". *Journal of International Pharmaceutical Research*, vol. 35, pp. 321-324, 2008.
- [17] W. M. Wang. *Study on isolation and purification of α -amylase inhibitors and its characteristics*. Tianjing: Tianjing University of Commerce, 2010.
- [18] G. F. Jia. "Preparation and determination of alpha-amylase inhibitor". *Food and Drug*, vol. 9, pp. 34-36, 2007.
- [19] Q. S. Yang, Y. Zhao, Y. Wang, et al. "Optimization extraction conditions of polysaccharide from tibetan medicine *Lethariella spp.* by response surface method". *The Food Industry*, vol. 34, pp. 44-48, 2013.
- [20] Standardization Administration of the People's Republic of China, SAC. *Food additive-Alpha-amylase preparation*[S]. GB 8275-2009.
- [21] L. L. Jia, W. Xi, G. L. Peng, et al. "Influence of different extraction technologies on hypoglycemic activity of Hubei green tea polysaccharide *in Vitro*". *Herald of Medicine*, vol. 34, pp. 7-10, 2015.
- [22] X. B. He, C. K. Xue, S. R. Wei, et al. "Effects of cha polysaccharides(cp) on α -amylase suppression and blood glucose in diabetic rats". *Herald of Medicine*, vol. 26, pp. 1284-1286, 2007.
- [23] X. Q. Wang, L. L. Yu. "Ultrasonic Extraction of Oloong Tea Polysaccharide and its Hpyerglycemic Activity". *Chinese Agricultural Science Bulletin*, vol. 26, pp. 102-105, 2010.
- [24] Q. Han. *Isolation, purification, structure, characterization and bioactivity of tea flower polysaccharide*. Zhejiang: Zhejiang University, 2011.
- [25] L. Zhao. *Research on the extraction and antioxidant/antimicrobial activities of polysaccharide from Russula alutacea fr.* Yunnan: Kunming University of Science and Technology, 2012.
- [26] H. P. Zhang. *Study on structure characterization and biological activity of polysaccharides from Pleurotus eryngii*. Shaanxi: Shaanxi Normal University, 2013.
- [27] X. H. Hu, F. Xie, Y. W. Fang, et al. "Preparation of a functional beverage with α - amylase inhibitory activity". *Farm Products Processing*, pp. 11-14, 2016.
- [28] R. H. Peng. *Studies on active and nutritional components in artificial fermentation Cordyceps*. Jiangxi: Nanchang University, 2005.
- [29] W. W. Wang, Y. T. Min, J. Tao, et al. "Extraction of seaweed polysaccharide by water and inhibitory activity of α -amylase". *Journal of Zhongzhou University*, vol. 31, pp. 120-121, 2014.
- [30] Y. X. Song, Y. T. Min, J. Tao, et al. "Extraction of seaweed polysaccharide by ethanol and inhibitory activity of α -amylase". *Food Science and Technology*, vol. 39, pp. 232-234, 2014.
- [31] Y. Zhang, D. Y. Su, Y. J. Zhang, et al. "Optimization of chlorella polysaccharides extraction technology based on inhibition of α -amylase enzyme activity". *Food & Machinery*, vol. 29, pp. 128-131, 2013.
- [32] X. S. Li. *Isolation and identification of Sargassum pallidum Polysaccharide and their biological activities*. Guangzhou: South China University of Technology, 2015.
- [33] C. X. Yang, J. Zhou, B. L. Gong. "Reparation of weak cation exchange packing based on macroporous monodisperse hydrophilic resins and their application for the separation and purification of protamine". *Se Pu*, vol. 27, pp. 191-196, 2009.
- [34] M. K. Pugsley, V. Kalra, W. S. Froebel. "Protamine is a low molecular weight polycationic amine that produces actions on cardiac muscle". *Life Sciences*, vol. 72, pp. 293-305, 2002.
- [35] L. Feng, R. Gao, "Gopalakrishnakone P. Isolation and characterization of a hyaluronidase from the venom of Chinese red scorpion *Buthus martensi*". *Comparative Biochemistry and Physiology*, vol. 148, pp. 250-257, 2008.

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