Optimization of Extraction Process and Preliminary Study on Hypoglycemic Activity of Fructus Swietenia Macrophylla Extract

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Abstract. This project studies the method of ultrasonic-assisted organic solvent extraction to extract hypoglycemic components from Fructus Swietenia Macrophylla. It explores the effects of different solvents, ultrasonic time, solid-liquid ratio, extraction temperature, and ultrasonic frequency on the extraction rate of FSM extract. Single-factor experiments and orthogonal experiments are used to optimize the extraction process, and the extract yield is used as an indicator to evaluate the advantages and disadvantages of the process. With the α-Amylase and β-The inhibition rate of amylase is used as an indicator to evaluate the hypoglycemic activity of the above-mentioned FSM extract. The results showed that using ethyl acetate as the extraction solvent, ultrasonic time of 30 mins, solid-liquid ratio of 1:25 (m/V), ultrasonic frequency of 40 kHz, and ultrasonic temperature of 50℃ were the optimal extraction process for FSM extract. When adding 10 mg of FSM extract α-The inhibition rate of amylase can reach 78.74% ± 1.8%, which is beneficial for β-The inhibition rate of amylase can reach 85.12% ± 2.38%, indicating a good inhibitory effect, indicating that FSM extract has certain hypoglycemic activity.

Keywords: Fructus Swietenia Macrophylla (FSM), Extraction process, Amylase, inhibition, Hypoglycemic effect.

1. Introduction

Fructus Swietenia Macrophylla (FSM) is the dry fruit of the Meliaceae plant *Swietenia Macrophylla.* King, which commonly known as "Xiangtianguo".It is widely distributed in tropical and subtropical regions of the Pacific Ocean. It has been introduced and cultivated as a green tree species in Guangdong, Fujian, and Hainan of China[1]. According to the Malaysian Herbal Medicine Catalogue, FSM is bitter, cold, antipyretic, and astringent, mainly used to treat diabetes, hypertension, malaria, etc. [2]. According to statistics, the incidence rate of diabetes and hypertension in local people is far lower than in other parts of the world, which may be closely related to long-term consumption of FSM.

At present, there are many studies on the preparation of hypoglycemic capsules from traditional Chinese medicine and food. Huang Xiaoqin's study on the hypoglycemic effect of Momordica charantia hypoglycemic capsule. Jia Huiyu's study on the prevention and treatment of diabetes nephropathy in rats. However, there is no relevant literature of preparation process in FSM as raw material to produce related products.The research of FSM mainly about chemical composition and extracts animal experimental.

There were few research on the extraction process of FSM. Zhu Lili et al. used petroleum ether cold immersion and GC-MS analysis of FSM fatty acid components [3]. Compared with metformin hydrochloride to study the hypoglycemic effect of FSM. Wu Weihong et al. used ethanol cold soak

and heated reflux extraction to extract FSM [4]. The ethanol extract was extracted with petroleum ether, and GC-MS technology was used to analyze the composition of the extract.

The study used ultrasonic technology to extract FSM. Using the effects of ultrasonic frequency, ultrasonic time, extraction temperature, solid-liquid ratio, and solvent on the extraction rate. Using FSM extract to treat α-Amylase and β- Preliminary evaluation of the hypoglycemic activity of FSM extract by the inhibition rate of amylase as an indicator [5].

2. Materials and Methods

2.1. Materials and reagents

FSM purchased and imported from Southeast Asia by Guangzhou Shi'an Technology Co., Ltd., naturally dried in the shade, and identified by Huang Yicheng, Deputy Director of the Guangdong Institute of Traditional Chinese Medicine, as the fruit of the Meliaceae mahogany plant *Swietenia macrophylla.* King. α- Amylase (46.6 U/mg, Hefei BioBomei, BN: D2110v264), β-Amylase (100 U/mg, Shanghai MACKLIN, BN: C12602680).

2.2. Instruments

UV-1800 ultraviolet visible spectrophotometer (Shanghai Pumida), SB-300DTY ultrasonic scanning frequency cleaning machine (Ningbo Xinzhi Biotechnology), RE-52AA Rotary evaporator (Shanghai Yarong Biochemical), AUY120 electronic balance (1/10000, Shimadzu, Japan), SHZ-D (III) circulating water vacuum pump (Gongyi Yuhua Instrument), HW-500ASB infrared drying oven (Shanghai Yinxi Instrument), HH-S6 constant temperature water bath pot (Gongyi Yuhua Instrument).

2.3. Methods

2.3.1 Raw material pretreatment

Dry the FSM fruit in a 60℃oven for 6 hours, peel off the outer skin, crush the kernels to obtain FSM powder, pass a 30 mesh sieve, and store under dry conditions for later use.

2.3.2 Single factor experiment

Weigh 3 g of FSM powder and place it in a 100 mL conical flask with a lid. Add the same volume of solvent according to a certain ratio of material to liquid, extract for a certain time at different temperatures and ultrasonic frequencies, and filter under pressure while hot to obtain FSM extract. The extract is evaporated to get FSM extract by Rotary evaporator at reduced pressure and low temperature. The obtained extract is weighed and the extraction rate of the extract is calculated. Respectively inspect the material liquid ratio (m/V): 1:5, 1:10, 1:15, 1:20, 1:25, 1:30; Ultrasonic temperature: 30℃, 40℃, 50℃, 60℃, Ultrasonic frequency: 25 kHz, 33 kHz, 40 kHz, 59 kHz, Ultrasound time: 10 mins, 20 mins, 30 mins, 40 mins, 50 mins, 60 mins, The influence of five key factors, including petroleum ether, ethyl acetate, methanol, chloroform, and ether, on the extraction rate of FSM extract.

2.3.2.1 Experimental Plan for the Effect of Different Solvent Types on Extraction Rate

Weigh 3 g of FSM powder and place it in a 100 mL conical flask with a stopper. Under the conditions of a material liquid ratio (m/V) of 1:30, ultrasound temperature of 40℃, ultrasound frequency of 59 kHz, and extraction time of 60 mins, select solvents such as petroleum ether, ethyl acetate, methanol, chloroform, and ether to investigate the effect of different solvents on the extraction rate of FSM extract.

2.3.2.2 Experimental Plan for the Effect of Different Material Liquid Ratios on Extraction Rate

Weigh 3 g of FSM powder and place it in a 100 mL conical flask with a lid. Under the conditions of ultrasound temperature of 30 ℃, ultrasound frequency of 40 kHz, ultrasound time of 30 mins, and

solvent of ethyl acetate, select a material to liquid ratio (m/V) of 1:5, 1:10, 1:15, 1:20, 1:25, 1:30 to investigate the effect of the material to liquid ratio on the extraction rate of FSM extract.

2.3.2.3 Experimental Plan for the Effect of Different Ultrasound Times on Extraction Rate

Weigh 3 g of FSM powder and place it in a 100 mL conical flask with a lid. Under the conditions of a material liquid ratio of 1:30, ultrasonic temperature of 40℃, ultrasonic frequency of 59 kHz, and solvent of ethyl acetate, select ultrasonic time of 10 mins, 20 mins, 30 mins, 40 mins, 50 mins, and 60 mins to investigate the effect of ultrasonic time on the extraction rate of FSM extract.

2.3.2.4 Experimental Plan for the Effect of Different Ultrasonic Temperatures on Extraction Rate

Weigh 3 g of FSM powder and place it in a 100 mL conical flask with a stopper. Under the conditions of a material liquid ratio (m/V) of 1:30, ultrasonic frequency of 59 kHz, ultrasonic time of 30 mins, and solvent of ethyl acetate, select ultrasonic temperatures of 30℃, 40℃, 50℃, and 60℃ to investigate the effect of ultrasonic temperature on the extraction rate of FSM extract.

2.3.2.5 Experimental Plan for the Effect of Different Ultrasonic Frequencies on Extraction Rate

Weigh 3 g of FSM powder and place it in a 100 mL conical flask with a lid. Under the conditions of a material liquid ratio (m/V) of 1:30, ultrasound temperature of 30℃, ultrasound time of 30 mins, and solvent of ethyl acetate, select ultrasound frequencies of 25 kHz, 33 kHz, 40 kHz, and 59 kHz to investigate the effect of ultrasound frequency on the extraction rate of FSM extract.

2.3.3 Optimization Plan for Orthogonal Experiment of Extraction Process

In order to further optimize the extraction conditions of FSM extract, the effects of various factors on the extraction rate of the extract were analyzed based on the results of a single factor investigation. Four factors were selected as the examination factors, including the type of extraction solvent, material liquid ratio, ultrasonic extraction temperature, ultrasonic extraction time, and ultrasonic extraction frequency. Each factor was selected at three levels, and the experiment was conducted according to the L9 (34) table. Set up three duplicate samples for each experiment and take their average values. Use the extraction rate of the extract as an indicator to determine the optimal group and verify the optimal combination, in order to achieve the goal of optimizing the experimental conditions. The level and coding of factors are shown in Table 1.

2.3.4 α-Amylase activity inhibition experiment

Take 1% of the FSM extract (0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1.0 mL) and add 0.25 mL at a concentration of 1.4 u/mL α- Mix the amylase evenly in a water bath at 37℃ for 10 mins, then add 0.4 mL of 1% starch phosphate buffer solution. After heating at 37 ℃ for 10 mins, immediately add 0.5 mL of DNS reagent, boil in water for 5 mins, cool, and dilute to 10 mL with distilled water. Measure the absorbance of the mixed solution at a wavelength of 540 nm. Among them, use the sample solution as the blank group and replace it with phosphate buffer solution α- Amylase is used as a control.

The experimental conditions for the α-amylase activity inhibition experiment are shown in the table, with a concentration of 10 mg/mL and a volume of (0.2, 0.4, 0.6, 0.8, 1.0) mL of FSM extract. All items were measured for absorbance at a wavelength of 540 nm. Calculate the absorbance of FSM extract based on the measured absorbance of each volume α Amylase activity inhibition rate (%). The calculation formula is as follows:

Inhibition rate/%=(1- (A-B)/(C-B)) × 100%

Among them, A: the absorbance reading of the extract of FSM at 540 nm,

B: Blank group reading at 540 nm

C: Background reference reading at 540 nm

2.3.5 α-Determination of amylase activity inhibition

Take a certain amount of FSM extract and a certain amount of α-Place the amylase at 37℃ for 10 mins, add a certain volume of 1% starch solution, and react at 37 ℃ for 10 mins. Add 0.5 mL DNS reagent, stir the glass rod evenly, take a boiling water bath for 5 min, cool it to room temperature, add 8 mL distilled water, and measure the absorbance at 540 nm. Among them, use the sample solution as the blank group and replace it with phosphate buffer solution α- Amylase is used as a control.

2.3.6 β-Amylase activity inhibition experiment

Take 1% of the FSM extract (0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1.0 mL) and add 0.30 mL at a concentration of 30 u/mL β- Mix the amylase evenly in a water bath at 37 ℃ for 10 mins, then add 0.4 mL of 1% starch phosphate buffer solution. After heating at 37 ℃ for 10 mins, immediately add 0.5 mL of DNS reagent, boil in water for 5 mins, cool, and dilute to 10 mL with distilled water. Measure the absorbance of the mixed solution at a wavelength of 540 nm. Among them, use the sample solvent as the blank group and replace it with phosphate buffer solution β- Amylase is used as a control.

The experimental conditions for the β-amylase activity inhibition experiment are shown in the table, with a concentration of 10 mg/mL and a volume of (0.2, 0.4, 0.6, 0.8, 1.0) mL of FSM extract. All items were measured for absorbance at a wavelength of 540 nm. Calculate the absorbance of FSM extract based on the measured absorbance of each volume α Amylase activity inhibition rate (%). The calculation formula is as follows:

Inhibition rate/%=(1- (A-B)/(C-B)) × 100%

Among them, A: the absorbance reading of the extract of FSM at 540 nm,

B: Blank group reading at 540 nm

C: Background reference reading at 540 nm

2.3.7 β- Determination of amylase activity inhibition

Take a certain amount of FSM extract and a certain amount of β-Place the amylase at 37℃ for 10 mins, add a certain volume of 1% starch solution, and react at 37 ℃ for 10 mins. Add 0.5 mL DNS reagent, stir the glass rod evenly, take a boiling water bath for 5 min, cool it to room temperature, add 8 mL distilled water, and measure the absorbance at 540 nm. Among them, use the sample solution as the blank group and replace it with phosphate buffer solution α- Amylase is used as a control.

3. Results

3.1. Single factor experiment

3.1.1 Determination of the type of extraction solvent

The experimental conditions and results are shown in Table 2.The extracting solvents include methanol, ether, ethyl acetate, and chloroform. The experimental results are shown in Fig. 1. It can be seen that the extraction rate of FSM extract varies with the types of extraction solvents. When ethyl acetate is used as the extraction solvent, FSM extract is the most obtained, with an extraction rate of 68.6%. When methanol is used as the extraction solvent, the extract obtained from FSM is the least, with an extraction rate of 30.1%. This may be due to the principle of similar solubility, where FSM contains more moderately polar substances. Therefore, choosing ethyl acetate as the solvent for extracting FSM is the most suitable.

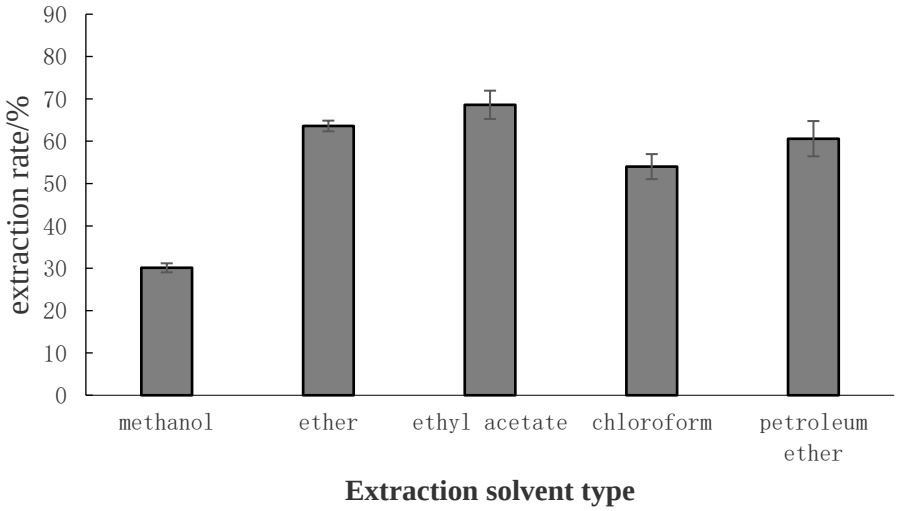

Fig. 1 Effect of Different Solvents on the Extraction of FSM

3.1.2 Determination of material liquid ratio

When the ratio of extract to liquid was 1:5, 1:10, 1:15, 1:20, 1:25, and 1:30, the experimental operating conditions and results are shown in Table 3. It can be seen that with the increase in the ratio of extract to liquid, the extraction rate of the Fruit of Tenacissima extract gradually increases. The specific trend results are shown in Figure 2.When the ratio of extract to liquid was 1:25, the most FSM extract was obtained, with an extraction rate of 78.1%. When the ratio of extract to liquid was 1:5, the least FSM extract was obtained, with an extraction rate of 62.9%. Therefore, it is appropriate to choose 1:15, 1:20, and 1:25 as the ratio of extract to liquid levels for subsequent orthogonal experiments.

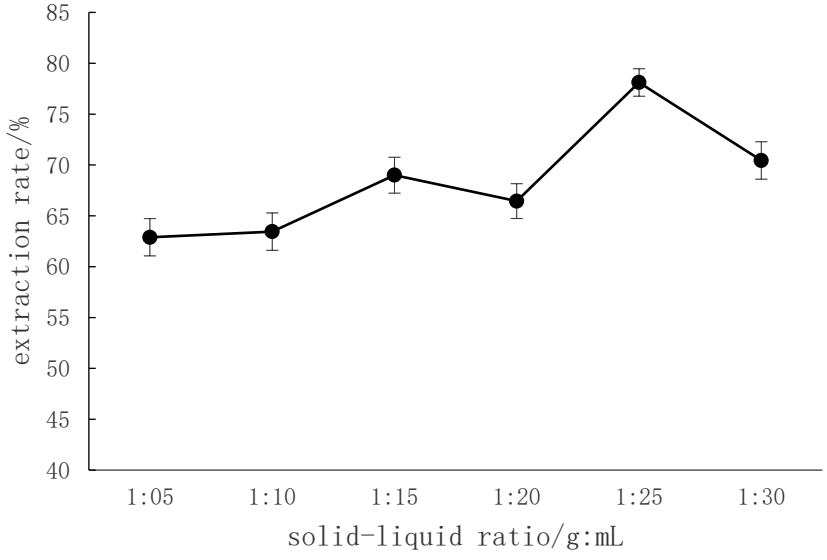

Fig. 2 Effect of Different Material Liquid Ratios on the Extraction of FSM

3.1.3 Determination of ultrasound extraction time

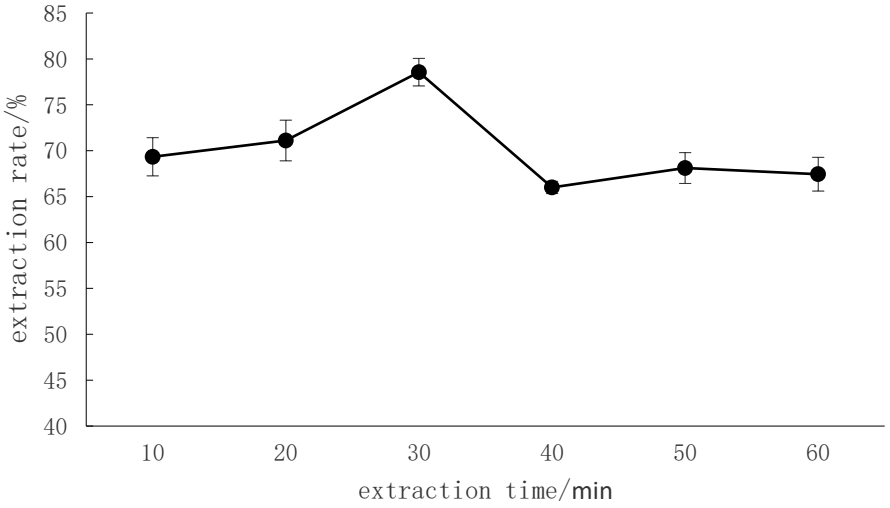

Fig. 3 Effect of Different Ultrasound Times on the Extraction of FSM

Table 4 shows ultrasonic extraction conditions and results with 10 min, 20 min, 30 min, 40 min, 50 min, and 60 min. It can be seen that with the increase of extraction time, the extraction rate of FSM extract first increases and then decreases. The specific trend results are shown in Figure 3. When the ultrasonic extraction time was 30 min, the obtained FSM extract was the most, with an extraction rate of 78.6%. The obtained FSM extract was the least, with an extraction rate of 66.0% with 40 min. This may be due to the gradual dissolution of the extract as the heating time increases, and the volatilization of the dissolved extract as the time further increases.Therefore, 30 min, 40 min, and 50 min were selected as the ultrasonic extraction time levels for subsequent orthogonal experiments.

3.1.4 Determination of ultrasonic extraction temperature

The experimental operating conditions and results are shown in Table 5 with the ultrasonic temperature is 30°C, 40°C, 50°C, and 60°C. Figure 4 shows with the temperature increases, the extraction rate decreases. When the ultrasonic temperature is 30°C, the highest extraction rate of 75.0%. When the ultrasonic temperature is 60°C, the lowest extraction rate of 61.4%. This may be due to the low boiling point of the components in the extracted fruit extract, which is easily evaporated and dispersed by heating.Therefore, 20°C, 30°C, and 40°C are selected as the ultrasonic extraction temperature levels for subsequent orthogonal experiments.

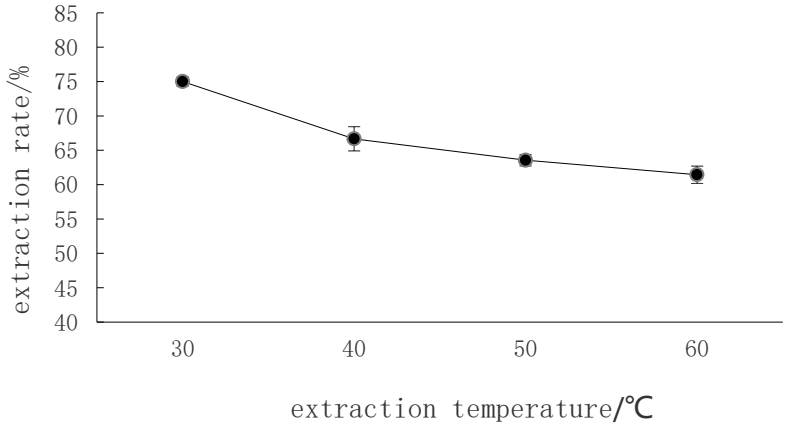

Fig. 4 Effect of Different Ultrasonic Temperatures on the Extraction of FSM

3.1.5 Determination of ultrasonic extraction frequency

Table 6 shows ultrasonic frequency and results with 25 kHz, 33 kHz, 40 kHz, and 59 kHz. When the material-to-liquid ratio, ultrasonic temperature, and ultrasonic time are constant, with the increase of ultrasonic frequency, the extraction rate of FSM increases steadily and then suddenly decreases. The specific trend results are shown in Figure 5. When the ultrasonic frequency is 40 kHz, the extraction rate of FSM is the highest with 73.3%. When the ultrasonic frequency is 25 kHz, the extraction rate of FSM is the lowest with 60.8%. This may be because as the ultrasonic frequency increases, the distribution of cavitation bubbles becomes more uniform, and more energy is transferred to the surrounding sample, accelerating the breakage of cell walls and causing a large amount of extract to precipitate. The extraction rate of the extract tends to be smooth or even slowly decrease with ultrasonic frequency exceeds 40 kHz, mainly due to the long-term thermal effect accelerating the evaporation of the ethyl acetate in the extraction solution. Therefore, the suitable ultrasonic frequency for extracting FSM is 33 kHz, 40 kHz, and 59 kHz.

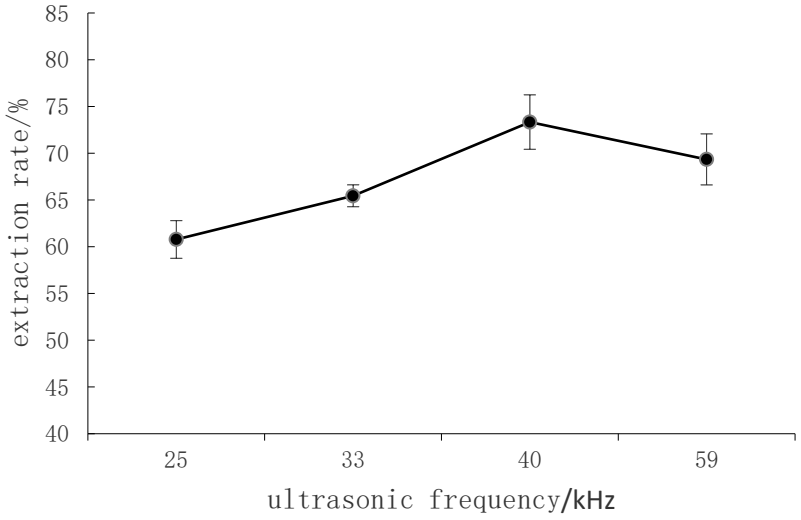

Fig. 5 Effect of Different Ultrasonic Frequencies on the Extraction of FSM

3.2. Orthogonal experiment for optimizing the extraction process of FSM

According to the results of the single factor experiment, four factors and three levels of L9 (34) orthogonal experiment were selected. The ultrasound time, 20 mins, 30 mins, 40 mins. The material liquid ratio (m/V),1:15, 1:20, 1:25. The ultrasound frequency,33 kHz, 40kHz, 59 kHz. The ultrasound temperature,30℃, 40℃, 50℃.

Table 1 Orthogonal experimental factors and levels of FSM extraction process Horizontal factor

Level	Factor			
	Extraction time	Material liquid ratio	Ultrasonic frequency	Extraction temperature
1	20min	1:15	33kHz	30℃
2	30min	1:20	40kHz	40℃
3	40min	1:25	59kHz	50℃

The orthogonal experimental results of the L_9 (3^4) extraction process of FSM are shown in Table 2. Table 2 shows primary and secondary factors affecting the extraction of FSM are: D>B>C>A. This means ultrasonic temperature > material liquid ratio > ultrasonic frequency > ultrasonic time. The optimal extraction process for FSM is A_2, B_3, C_2, D_3. With the ultrasound time is 30 mins, the material liquid ratio is 1:25, the ultrasound frequency is 40 kHz, and the ultrasound temperature is 50℃, the extraction rate of FSM extract is the highest.

Table 2 Orthogonal experimental results of FSM extraction process L_9 (3^4)

Serial number	Factor				Extraction rate/ (%)
	A (extraction time)	B (material liquid ratio)	C (ultrasonic frequency)	D (extraction temperature)	
1	1	1	1	1	65.0
2	1	2	2	2	59.7
3	1	3	3	3	67.3
4	2	1	2	3	72.0
5	2	2	3	1	63.0
6	2	3	1	2	67.7
7	3	1	3	2	59.7
8	3	2	1	3	67.7
9	3	3	2	1	70.0
K1	192.0	196.7	200.3	198.0	
K2	202.7	190.3	201.7	187.0	
K3	197.3	205.0	190.0	207.0	
$\bar{K}1$	64.0	65.6	66.8	66.0	
$\bar{K}2$	67.6	63.4	67.2	62.3	
$\bar{K}3$	65.8	68.3	63.3	69.0	
R	3.6	4.9	3.9	6.7	
Primary and secondary factors			D>B>C>A		
Best combination	A2	B3	C2	D3	

3.3. α-Amylase activity inhibition experiment

The experimental results of the α-amylase activity inhibition experiment are shown in Figure 6. The inhibition rate of the sample on α-amylase activity increases with the increase of the sample concentration. The lowest inhibition rate of the 0.2 mL sample group 1 is 2.71%. The highest inhibition rate of the 1.0 mL sample group 1 is 78.74%. The experimental results show that the extracts of FSM have a good inhibitory effect on α-amylase. With the higher the concentration of the extract, the better the inhibitory effect. It can inhibit the conversion of starch into glucose, reducing the absorption of glucose by the human body.

Table 3 Effects of FSM Extract on α-Table of results of amylase activity inhibition experiment

Group	Enzyme solution (mL)	Sample (mg)	Starch (mL)	DNS (mL)	Abs	Inhibition rate(%)	RD(%)
blank group	0.25	0	0.4	0.5	0.000	0.00	0.00
Blank control group	0.00	0	0.4	0.5	0.000	0.00	0.00
Sample Group 1	0.25	2	0.4	0.5	0.179	2.71%	1.32%
Sample control group 1	0.00	2	0.4	0.5			
Sample Group 2	0.25	4	0.4	0.5	0.208	24.06%	1.25%
Sample control group 2	0.00	4	0.4	0.5			
Sample Group 3	0.25	6	0.4	0.5	0.286	38.13%	2.12%
Sample control group 3	0.00	6	0.4	0.5			
Sample Group 4	0.25	8	0.4	0.5	0.310	64.56%	1.88%
Sample control group 4	0.00	8	0.4	0.5			
Sample Group 5	0.25	10	0.4	0.5	0.288	78.74%	1.90%
Sample control group 5	0.00	10	0.4	0.5			

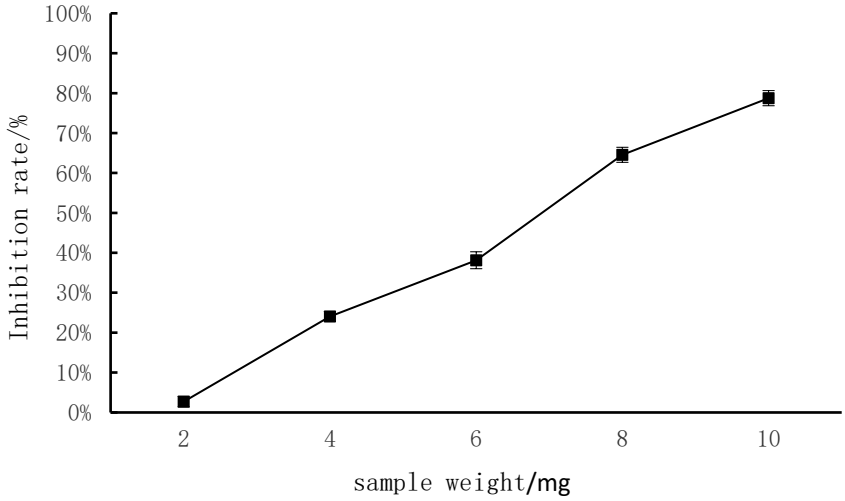

Fig. 6 Effect of FSM Extract on α- Result chart of amylase activity inhibition experiment

3.4. β-Amylase activity inhibition experiment

The experimental results of the β-amylase activity inhibition experiment are shown in Figure 7.The inhibition rate of the sample on β-amylase activity increases with the concentration of the sample. The lowest inhibition rate of 35.91% in the 0.1 mL sample group 1.The highest inhibition rate of 85.12% in the 1.0 mL sample group 1.The experiment shows that the extracts of FSM also have a good inhibitory effect on β-amylase, and the higher the concentration of the extract, the better the inhibitory effect.The overall inhibitory effect is consistent with the ability of α-amylase, and it can inhibit the conversion of starch into glucose, reducing the absorption of glucose by the human body.

Table 4 Effects of FSM Extract on β-Table of results of amylase activity inhibition experiment

Group	Enzyme solution(mL)	sample (mg)	Starch (mL)	DNS (mL)	Abs	Inhibition rate (%)	RD (%)
blank group	0.25	0	0.4	0.5	0.000	0.00	0.00
Blank control group	0.00	0	0.4	0.5	0.000	0.00	0.00
Sample Group 1	0.25	2	0.4	0.5	0.257	35.91%	3.82%
Sample control group 1	0.00	2	0.4	0.5			
Sample Group 2	0.25	4	0.4	0.5	0.271	41.87%	12.38%
Sample control group 2	0.00	4	0.4	0.5			
Sample Group 3	0.25	6	0.4	0.5	0.310	74.82%	8.21%
Sample control group 3	0.00	6	0.4	0.5			
Sample Group 4	0.25	8	0.4	0.5	0.352	82.91%	6.67%
Sample control group 4	0.00	8	0.4	0.5			
Sample Group 5	0.25	10	0.4	0.5	0.357	85.12%	2.18%
Sample control group 5	0.00	10	0.4	0.5			

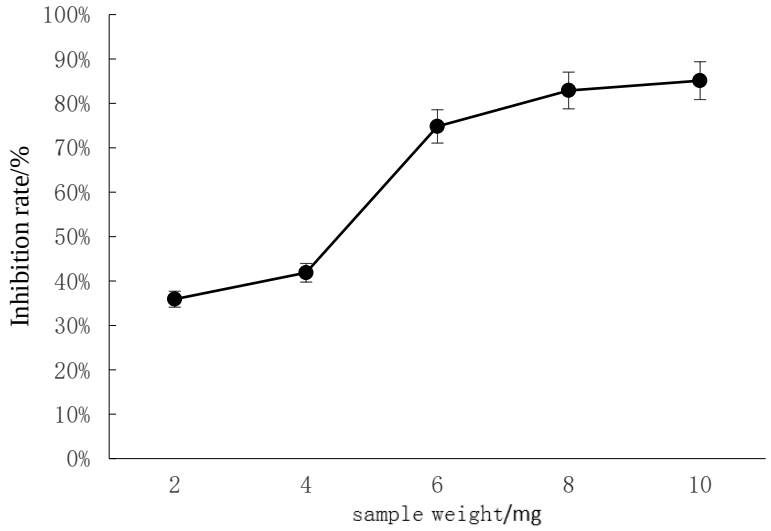

Fig. 7 Effect of FSM Extract on β- Result chart of amylase activity inhibition experiment

4. Discusstion

The project uses FSM as raw materials and uses an ultrasonic-assisted organic solvent extraction method. Through single-factor experiments, the optimal extraction solvent for FSM was determined to be ethyl acetate[11]. The more suitable ultrasonic extraction material liquid ratio (m/V) was selected as 1:15, 1:20, and 1:25, and the more suitable ultrasonic extraction time was selected as 20 mins, 30 mins, and 40 mins. The more suitable ultrasonic extraction temperature was selected as 30 ℃, 40 ℃, and 50 ℃, The more suitable ultrasound extraction frequencies were selected as 33kHz, 40kHz, 59kHz. Based on the results of the single-factor experiment mentioned above, a 4-factor and 3-level orthogonal experiment was designed and implemented, and the optimal extraction process for FSM extract was determined as follows. Using ethyl acetate as the extraction solvent, setting the solid-liquid ratio at 1:25 (g/mL), ultrasonic extraction for 30 mins, ultrasonic temperature at 50 ℃, and ultrasonic frequency at 40 kHz.

Using the previously extracted FSM extract, design experiments to α-Amylase and β-Amylase. The activity of amylase was tested for inhibition. When adding 10 mg of FSM extract, the inhibition rate of α-amylase can reach 78.74% ± 1.8%, which is beneficial for β-Amylase. The inhibition rate of amylase can reach 85.12% ± 2.38%. The experimental results show that FSM extract has a good inhibitory effect on the above two enzymes, preliminarily confirming that FSM has certain hypoglycemic activity.

The implementation of this project can provide a certain research basis for the follow-up research on the development and utilization of FSM. The FSM can inhibit amylase activity, reduce the glucose intake of diabetes patients. The FSM may be a further develop new food raw materials.

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