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Studies on the Extraction of Guarana Peel Polysaccharides and its Effect on Blood Glucose Lipids in Mice

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Abstract: *Objective:* To study the extraction of Guarana peel polysaccharides (GPP) by two different methods and to investigate the effect of extracted polysaccharides on haemoglycaemia and lipids in mice. *Methods:* The polysaccharides were extracted by aqueous alcoholic precipitation with two different solvents, methanol/ethanol mixed solvent or 95% ethanol and the mice were fed and tested for changes in endogenous related substances, respectively. *Results:* The concentration of polysaccharide extracted by methanol/ethanol mixed solvent was 116.367 μg/mL, while the concentration of polysaccharide extracted by 95% ethanol was 101.465 μg/mL. The polysaccharide extracted by the former method did not significantly affect the blood glucose level of the mice, while the latter method did not significantly affect the lipid level of the mice. *Conclusion:* Methanol/ethanol mixed solvent extraction of Guarana peel polysaccharides had higher extraction rate and more significant effect on blood glucose control in mice, while 95% ethanol extraction of Guarana peel polysaccharides had more significant effect on lipid control in mice.

Keywords: Guarana peel; Polysaccharides; Blood glucose; Blood lipids

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1. Introduction

Guarana peel has a long history of use as part of traditional Chinese medicine. It is rich in saponins, flavonoids, polysaccharides and other bioactive components [1], exhibiting multiple pharmacological effects such as anti-inflammatory, antioxidant, anti-tumour and regulation of blood glucose and lipids [2]. With the development of modern science and technology, the extraction techniques of Guarana peel continue to advance for better extraction and utilization of its active ingredients.

The extraction of Guarana peel mainly includes water extraction method, alcohol extraction method and extraction method by filtration membrane technology. Water extraction is the most traditional method, which is simple and direct but less efficient. The alcohol extraction method uses organic solvents such as ethanol for extraction, which can effectively enhance the concentration and purity of active ingredients [3]. However, all these methods face challenges such as high labor intensity, low efficiency and difficult industrialization. Du C

et al. (2016) used UF-100 membrane filtration to purify and separate crude polysaccharides from Guarana peel, which is innovative compared to the first two traditional methods. Still, the extraction efficiency needs to be improved [4].

In terms of pharmacological effects, Guarana peel extract is particularly notable for its significant effects on blood glucose and lipid regulation. Studies have shown that Guarana peel extract significantly reduced blood glucose levels and improved insulin resistance in hyperglycaemic model animals. Wang H et al. (2017) successfully isolated a low relative molecular mass polysaccharide from Guarana peel, possessing significant renin inhibitory effects, which provided a basis for basic renin inhibition studies of GPP [5]. Several studies have shown that the active ingredients in Guarana peel may regulate glucose metabolism through various pathways and thus exert hypoglycaemic effects.

In terms of lipid regulation, Guarana peel extract effectively ameliorated hyperlipidemia by lowering serum total cholesterol (TC), triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) levels in hyperlipidemia model animals [6], while increasing high-density lipoprotein cholesterol (HDL-C) levels [7,8]. The specific mechanism may be related to the saponins and flavonoids in Guarana peel [1], which can promote cholesterol excretion by inhibiting cholesterol synthase activity and then achieve lipid-lowering effects. Lei X et al. (2020) found that active polysaccharides have significant pharmacological effects in regulating blood lipid levels, and this effect is mainly achieved through three mechanisms: (1) to promote cholesterol excretion effectively, (2) to affect the reabsorption process of bile acids in the intestine, and (3) to regulate the activity of enzymes closely related to lipid metabolism [3].

Although a large number of animal experiments have supported the pharmacological effects of guarana peel extracts, the specific mechanism of action has not yet been fully elucidated, and the safety and efficacy of clinical application need to be further verified. Therefore, future studies should focus on optimizing the extraction method, improving the extraction efficiency and purity, and conducting more clinical trials to comprehensively evaluate the effects of Guarana peel extract on physiological parameters so as to lay a solid foundation for its wide application in traditional Chinese medicine (TCM) and modern medicine.

2. Materials and methods

2.1. Instruments and reagents

95% ethanol (AR) and methanol (AR) were purchased from Tianjin Damao Chemical Reagent Factory; 25% uracil was purchased from Aladdin Reagent Company; high sugar and high fat feed was purchased from Changsha Tianqin Biotechnology Co. Ltd; all other drugs were commercially available in analytical purity. Ultraviolet-visible spectrophotometer (UV9000, Shanghai Yuan Analytical Instruments); 723 visible spectrophotometer (Shanghai Analytical Instrument Factory); low-temperature centrifuge (JJ-6A, Beckman); rotary evaporator (YRE-301, Shanghai Yarong), automatic biochemistry analyzer (PUS-2018G, Beijing Prang).

2.2. GPP separation and extraction and content determination methods

2.2.1. Extraction method: Aqueous alcohol precipitation method

1 kg of Guarana peel was ground into powder and sieved through 20 mesh sieve, divided into two groups of 400 g each, in 5,000 mL round bottom flasks, using 95% ethanol or methanol/ethanol (1:1 by volume) two solvents for two days respectively. Water was added, heated to boiling, and kept at reflux for 4 h. The residue was repeated during this boiling process for 4 h. The residue was discarded, and the filtrates were combined after fine filtration. The filtrates were centrifuged at 3,000 r/min for 20 min and the supernatant was taken. The supernatant was concentrated to 1/4 of the original amount by rotary evaporator at 60 ℃ under moderate

speed and reduced pressure. The concentrate was also precipitated using either 95% ethanol or methanol/ethanol, two solvents (4 times the amount), and placed in a refrigerator at 4 °C for 24 h. The concentrate was dried under reduced pressure and dissolved in an appropriate amount of ethanol/methanol. It was dried under reduced pressure, dissolved in appropriate water, and the pH was adjusted to 3 with 2 mol/L hydrochloric acid and left overnight. The precipitated protein was removed by centrifugation at 3000 r/min for 5 min, centrifuged again under the same conditions for 20 min, and the above steps were repeated four times until there were no absorption peaks of proteins and nucleic acids at the wavelengths of 260 nm and 280 nm under the UV spectrum. The clarified liquid, after centrifugation, was put into a dialysis bag and dialyzed with distilled water, and then the dialysate was concentrated to 1/4 of the original amount by a rotary evaporator. A sufficient amount of 95% ethanol or methanol/ethanol was added to the concentrated solution for alcohol dialysis, so that the polysaccharides were precipitated out of solution to form a precipitate. The polysaccharide extract solid was obtained by drying under reduced pressure.

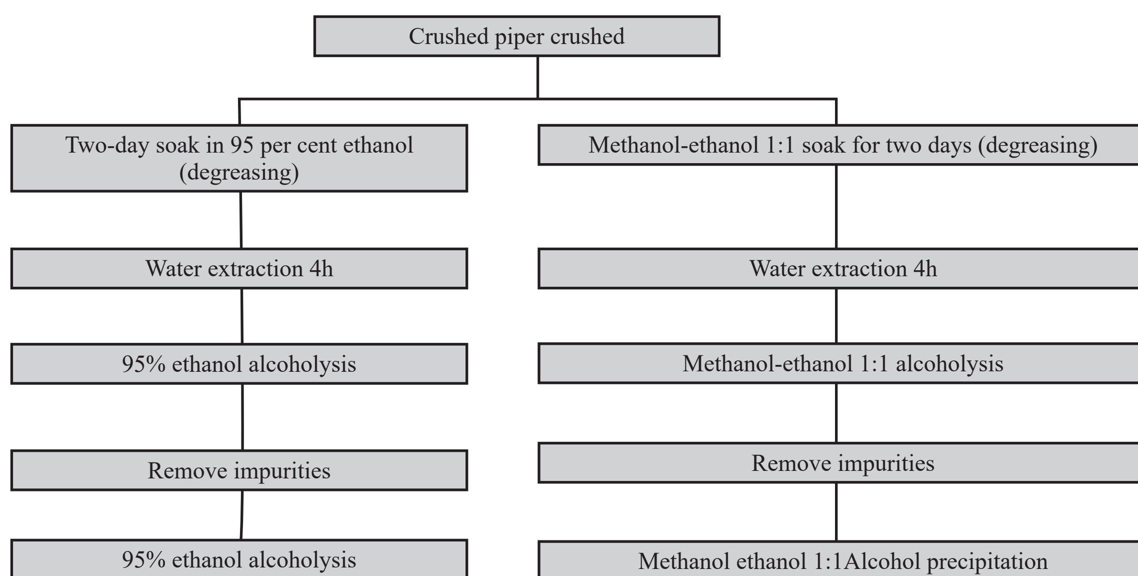

Figure 1. Flow chart of GPP extract preparation.

2.2.2. Determination method

Anthrone-sulphuric acid method [9,10]: Using glucose as the standard reference, the absorbance of various standard solutions of different concentrations was measured at 620 nm, from which the standard curve was drawn.

(1) Preparation of glucose standard curve

The glucose standard solution was prepared at a concentration of 0.1 mg/mL. Take 0.0, 0.4, 0.6, 0.8, 1.0, 1.5, and 2.0 mL of the standard solution, transfer it to a 10 mL stoppered test tube and replenish it to a volume of 2 mL with deionized water. To each test tube, 0.5 mL of 0.2% anthrone-ethyl acetate solution and 5 mL of concentrated sulfuric acid were added sequentially and mixed well. The test tubes were kept in a boiling water bath for 10 min, removed and cooled naturally to room temperature. After cooling to room temperature, the absorbance value of each solution was determined at 620 nm. This procedure was repeated three times to ensure accuracy. The standard curve was plotted by taking the concentration of glucose X (Unit: μg/mL) as the horizontal coordinate and the corresponding absorbance value Y as the vertical coordinate.

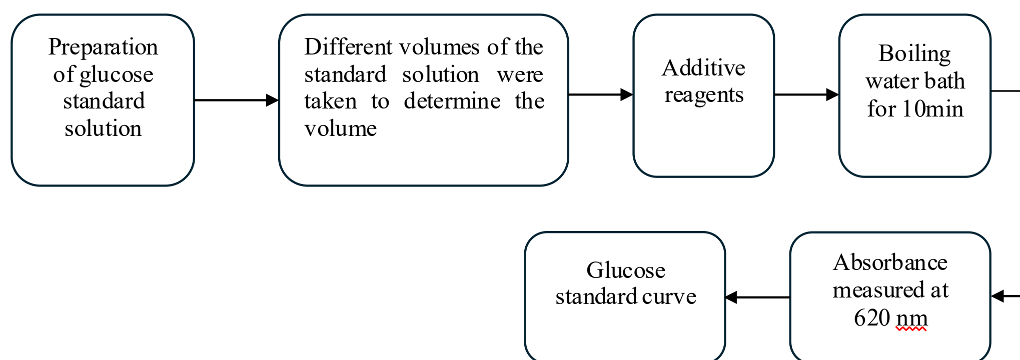

Figure 2. Flowchart for making glucose standard curve.

(2) Determination of polysaccharide content of samples

An appropriate amount of the GPP sample was accurately removed, dissolved in a 10 mL volumetric flask with distilled water, and replenished to the scale with distilled water. Then, 0.1 mL of the sample solution was transferred to another 10 mL volumetric flask, i.e., diluted to 100-fold by constant volume. The process was repeated, and the absorbance of the diluted solution was measured. Then, the specific content of polysaccharides was calculated using the regression equation.

2.3. Experimental animals and grouping

SD mice were purchased from Changsha Tianqin Biotechnology Co., Ltd, weighing 100 ± 10 g, half male and half female, a total of 32 mice. They were randomly divided into 4 groups, 8 mice in each group, and kept at room temperature of 25 ℃, with a normal diet and drinking water for 2 days then the following experiments were carried out and kept for 14 consecutive days:

(1) Blank control group (Group A): Normal feed, free drinking water, saline gavage at a dose of 4 mL/kg.

(2) High-sugar and high-fat control group (Group B): High-sugar and high-fat feed, free drinking water, saline gavage, the same dose as in Group A.

(3) Methanol-ethanol group (Group C): High-sugar and high-fat feed, free drinking water, methanol-ethanol GPP extract (30 mg/mL) by gavage at a dose of 4 mL/kg.

(4) 95% ethanol group (Group D): High sugar and high-fat feed, free drinking water, 95% ethanol GPP extract (30 mg/mL) by gavage, the dose is the same as that of Group C.

2.4. Animal blood sampling and testing

The pretreatment of experimental animals consisted of fasting for 12 hours (without water) to simulate the natural fasting state and reduce food interference on endogenous substance levels. Subsequently, the animals were anesthetized by intraperitoneal injection of 25% urethane. After complete anesthesia, blood was collected by the ocular phlebotomy method, ensuring that the blood flowed naturally into the heparin-containing anticoagulant tubes, and the collection volume was about 1–2 mL. The blood samples were centrifuged by low-temperature centrifugation (2000 r/min for 10 min) to isolate serum, and the supernatant was transferred to EP tubes and stored frozen for testing.

An automatic biochemical analyzer detected the content of blood glucose, total cholesterol (TC), triglyceride (TG) and other key indicators in the serum.

2.5. Statistical analysis methods

In data processing and analysis, SPSS 20.0 was used to analyze the experimentally collected data statistically.

The experimental data were expressed in the form of mean ± standard deviation (SD), and the statistical method of independent sample *t*-test was used for the comparison of data between different groups, with $p < 0.05$ indicating that the difference was statistically significant.

3. Results

3.1. Glucose standard curve

Table 1. Absorbance values of different concentrations of glucose standard solution (A)

Concentration (μg/mL)	0	20	30	40	50	75	100
Absorbance value (A)	0	0.122	0.187	0.251	0.323	0.516	0.671

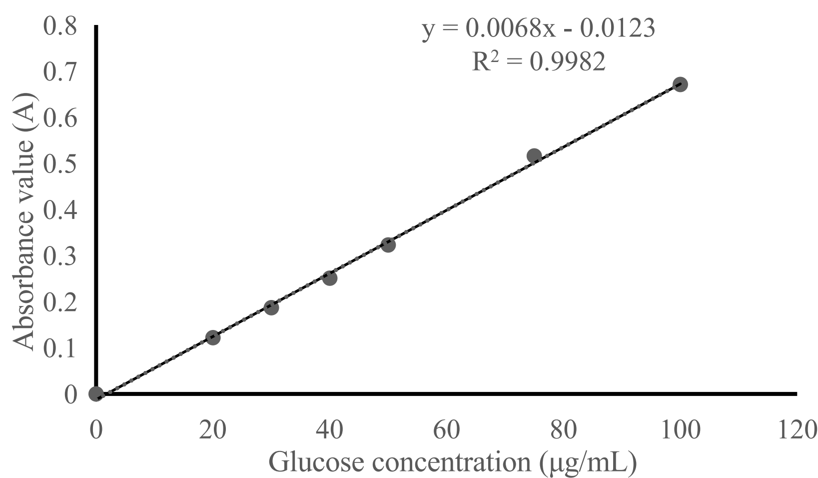

Figure 3. Glucose standard curve.

Table 1 shows the absorbance values of different concentrations of glucose standard solution measured at 620 nm. According to this data, the glucose standard regression equation curve is shown in **Figure 3**; the equation's expression is Y = 0.0068X - 0.0123, R2 = 0.9982. This indicates that the glucose concentration in the range of 20–100 μg/mL on the absorbance value presents a good linear relationship.

3.2. Determination of polysaccharide sample content

Table 2. Results of polysaccharides obtained by different extraction methods of Guarana peel

Groups	Methanol-ethanol mixed extraction group		95% ethanol extraction group	
	Absorbance (A)	Polysaccharide content (μg/mL)	Absorbance (A)	Polysaccharide content (μg/mL)
Results	0.779	116.367	0.681	101.955
	0.785	117.250	0.654	97.985
	0.773	115.485	0.698	104.455
Mean ± SD	0.779 ± 0.006	116.367 ± 0.882	0.677 ± 0.022*	101.465 ± 3.262*

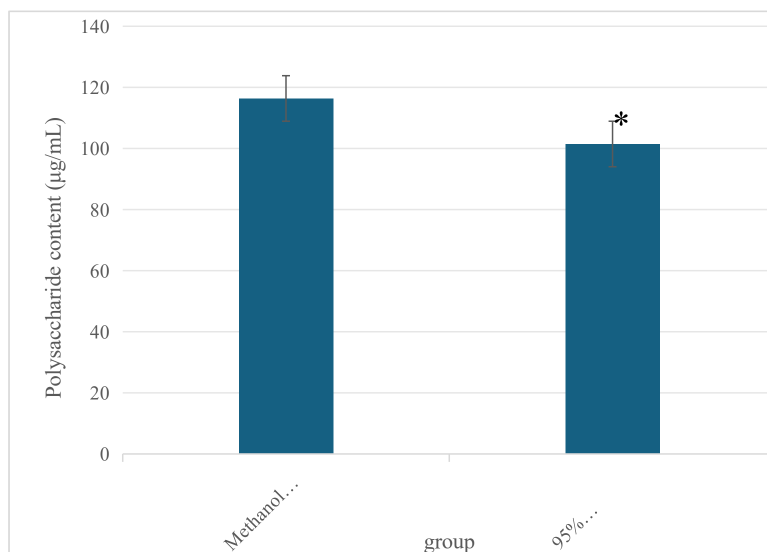

Figure 4. Effect of different solvent extraction on polysaccharide content. Mean ± standard error; compared with the methanol-ethanol mixed extraction group, the 95% ethanol extraction group *$p < 0.05$, which is statistically different.

The results show that the difference between the mean values of the two groups of data on polysaccharide content is significant and statistically significant, as evidenced by the fact that the extraction effect of one group of solvents is significantly better than the other group, according to **Figure 4**, it can be seen that the extracted polysaccharide content of the sample solution of the methanol-ethanol mixture group is higher than the polysaccharide content of the solution of the 95% ethanol group and the reproducibility is better, and the extraction of polysaccharides by the methanol-ethanol mixture group has a better effect.

3.3. Effects of different extraction methods on blood glucose and blood lipids

Table 3. Blood glucose content in mouse serum (mmol/L)

Blank control group	High fat and high sugar group	Methanol-ethanol group	95% ethanol group
5.57	6.52	4.21	4.32
5.46	6.43	4.94	6.28
4.57	6.54	5.93	5.36
4.98	7.51	5.72	6.14
4.57	6.23	4.56	5.08
5.58	6.54	5.63	6.19

Table 4. Effect of GPP extract on serum levels of glucose, TC and TG (mmol/L) in SD mice (mean ± SD)

	Blank control group (A group)	High fat and high sugar group (B group)	Methanol-ethanol group (C group)	95% ethanol group (D group)
Blood glucose	5.121 ± 0.480	6.628 ± 0.447†	5.165 ± 0.698**	5.561 ± 0.782*
TC	1.87 ± 0.34	2.60 ± 0.79†	2.39 ± 0.11*	2.27 ± 0.14**
TG	2.20 ± 0.10	2.58 ± 0.07†	2.41 ± 0.17*	2.25 ± 0.12**

Mean ± standard error; $^{\dagger}p < 0.01$ vs blank control group; *$p < 0.05$, **$p < 0.01$ vs high sugar and high-fat group.

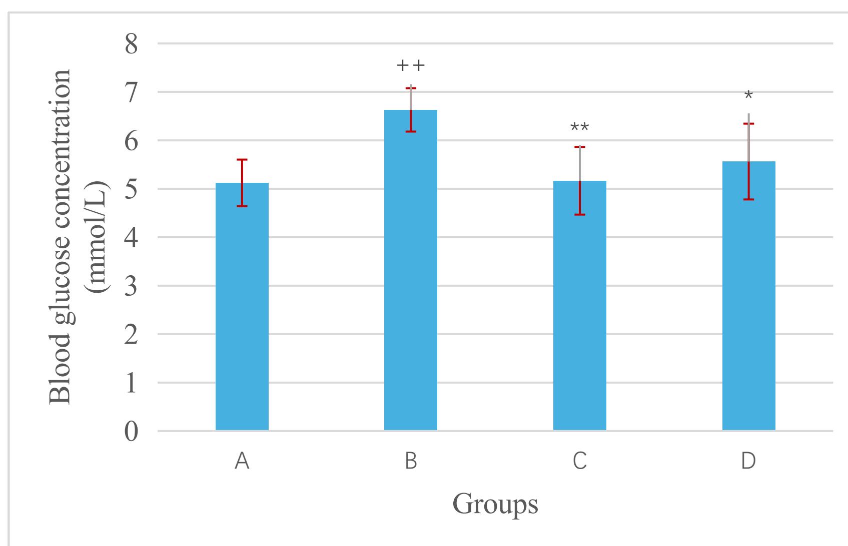

Figure 5. Effect of GPP extract on serum glucose level (mmol/L) in mice. Mean ± SD. $^{++}p < 0.01$ vs blank control group; $*p < 0.05$, $**p < 0.01$ vs high sugar and high-fat group.

The GPP-like components significantly reduced blood glucose, total cholesterol (TC) and triglyceride (TG) components in the serum of mice under high-sugar and high-fat dietary conditions. As seen from **Table 4**, comparing the high-fat and high-sugar diet group with the blank control group, the mean values of the experimentally measured blood glucose, TC, and TG contents were significantly higher, and the statistical results showed a significant difference at $p < 0.01$. From the results of blood glucose reduction, the methanol/ethanol group compared to the 95% ethanol group reduced more significantly. **Figure 6** shows that the 95% ethanol extraction group reduced TC and TG more significantly than the high-glucose and high-fat group.

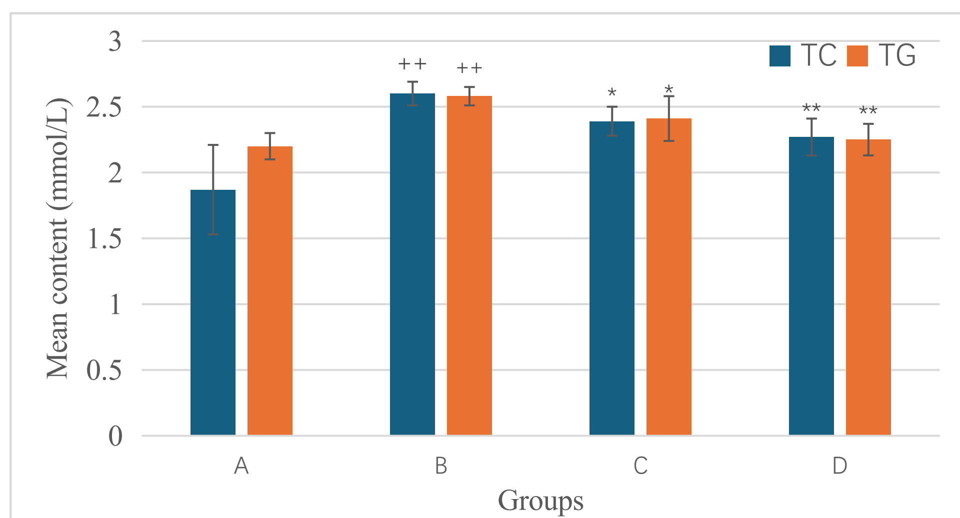

Figure 6. Effect of GPP extract on TC and TG levels in mouse serum (Mean ± SD).$^{++}p < 0.01$ vs blank control group; $*p < 0.05$, $**p < 0.01$vs high-sugar and high-fat group.

4. Discussion

The present study compared the effect of a methanol-ethanol solvent mixture with 95% ethanol in the extraction of GPP and found that the solvent mixture significantly increased the extraction rate of polysaccharides. This advantage was attributed to the unique interaction between the complex structure of polysaccharides (e.g.,

molecular weight, branched chain, and charge) and the solvent, and the mixed solvents were more effective in facilitating the precipitation and sedimentation of polysaccharides during the precipitation process.

In biological experiments, the GPP extract demonstrated significant glucose and lipid-modulating effects on mice fed high-fat and high-sugar diets. Particularly, the polysaccharide extracted from the methanol-ethanol solvent mixture performed better in hypoglycemia, offering new possibilities for diabetes treatment. In contrast, the 95% ethanol-extracted group performed better in hypolipidemia, confirming the positive effect of GPP on lipid regulation.

However, the specific mechanism of action of GPP and its potential for clinical application need to be explored in depth. Future studies should focus on the detailed mechanisms of GPP on glucose-lipid metabolism, including key processes such as insulin secretion, resistance, glycogen synthesis and catabolism. Meanwhile, the differences in the effects of different extraction methods on blood glucose and lipids in mice must also be analyzed from the levels of extract components and molecular structure.

In short, the present study reveals GPP's potential to lower blood glucose and blood lipids, which provides a scientific basis for developing novel therapeutic drugs for diabetes mellitus and the "Three Highs." However, further detailed and systematic studies are needed to fully understand GPP's mechanism and promote its clinical application.

5. Conclusion

Methanol/ethanol mixed solvent extraction of GPP has a higher extraction rate and a more obvious effect on blood glucose control in mice, while 95% ethanol extraction of GPP has a better effect on lipid control in mice.

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Disclosure statement

The author declares no conflict of interest.

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