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Formulation Optimization of the Loquat Leaf Hypoglycemic Solid Beverage

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Abstract [**Objectives**] To optimize the formulation of loquat leaf hypoglycemic solid beverage. [**Methods**] Using sensory evaluation scores based on product appearance (granularity), solution state, odor, and taste as the evaluation indicators, the formulation of the loquat Leaf hypoglycemic solid beverage was optimized by an $L_9(3^4)$ orthogonal design. [**Results**] The optimal formulation for the loquat leaf triterpenoid solid beverage was as follows: a 1 : 13 ratio of the main ingredient to the excipient blend (maltodextrin : mannitol = 2.2 : 12); and mogrosides content at 0.20%. [**Conclusions**] The optimal formulation for the loquat leaf triterpenoid solid beverage, obtained in this study, is scientifically sound and practical. It can serve as a valuable reference for the future product development and utilization of loquat leaf resources.

Key words Loquat leaf, Total triterpenes, Orthogonal design, Preparation technology, Hypoglycemic effect

1 Introduction

Loquat leaf (Eriobotryae Folium) is the dried leaf of *Eriobotrya japonica* (Thunb.) Lindl., a plant belonging to the Rosaceae family. As a commonly used traditional Chinese medicinal material, it is characterized by a bitter taste and a slightly cold nature. It possesses functions such as clearing lung heat to relieve cough and descending adverse energy to stop vomiting[1–2]. Loquat leaf was first documented in *Supplementary Records of Famous Physicians* (Ming Yi Bie Lu), which states: "It is bitter in taste, neutral in nature, and non-toxic, and it is used to treat persistent hiccups and to direct qi downward"[3]. Modern research has revealed that loquat leaf possesses hypoglycemic, anti-inflammatory, and hepatoprotective effects[4]. The triterpenoid acids in loquat leaf are the primary components responsible for its hypoglycemic activity. The corosolic acid exhibits significant blood glucose-lowering effects. As its physiological action resembles that of insulin, it is often referred to as "plant-based insulin"[5]. It has progressed to Phase II clinical trials by the U.S. FDA as a new drug for preventing and treating type 2 diabetes[6]. Given the remarkable hypoglycemic efficacy of triterpenoid acids in loquat leaf, they hold substantial promise for further development and utilization. This study focuses on optimizing the formulation of a hypoglycemic solid beverage utilizing the total triterpenoid acids from loquat leaves, so as to provide a valuable reference for the subsequent product development and resource utilization of loquat leaves.

2 Materials

2.1 Experimental materials and reagents Loquat leaf samples were purchased from Yulin medicinal materials market in Guangxi. They were identified as the dried leaves of *Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae) by Lu Hailin Senior Laboratory of School of Pharmacy of Guangxi University of Chinese Medicine; Metformin Hydrochloride Tablets (batch No.: 2301031), produced by Beijing Jingfeng Pharmaceutical Group, absolute ethyl alcohol, vanillin, glacial acetic acid, ethyl acetate and other reagents are analytically pure, produced by Chengdu Cologne Chemical Co., Ltd.

2.2 Instruments and equipment BP211D Electronic Analytical Balance, Sartorius, Germany; CG-16W High-Speed Micro Centrifuge, Beijing Medical Centrifuge Factory; DSH-50-1 Electronic Moisture Analyzer, Yueping Scientific Instruments (Shanghai) Co., Ltd.; UPC-11-20T ULUPURE Series Ultra-Pure Water System, Sichuan ULUPURE Technology Co., Ltd.; YT1030 Ultrasonic Cleaner, Yunyi Technology (Shenzhen) Co., Ltd.; N-1100 Rotary Evaporator, OSB-2100 Water Bath, Ailang Instruments (Shanghai) Co., Ltd.; SHZ-D(III) Circulating Water Multi-Use Vacuum Pump, Lichen Bangxi Instrument Technology (Shanghai) Co., Ltd.; DHG-9146A Electric Thermostatic Blow-Drying Oven, Jinghong Laboratory Equipment (Shanghai) Co., Ltd.

3 Methods and results

3.1 Extraction of total triterpenic acid from loquat leaves

Loquat leaves were placed in an oven and dried at 55 ℃ for 8 h until completely dry. They were then crushed, passed through a 10-mesh sieve, and packaged in sealed bags for storage. An appropriate amount of loquat leaves was taken and extracted three times with 20 times the volume of 80% ethanol at 45 ℃, with each extraction lasting 2 h. The extracts were combined and concentrated under reduced pressure until a significant amount of precipitate adhered to the wall. The concentrated solution was centrifuged at 5 000 r for 10 min, and the precipitate was collected. The precipitate was vacuum-dried at 45 ℃ to obtain the dry powder of

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total triterpenic acids extract from loquat leaves, which was then sealed and stored for subsequent use.

3.2 Study on formulation of loquat leaf solid beverage In this study, we designed a minimum packaging unit of 10 g per sachet for the loquat leaf total triterpenoid solid beverage. Using sensory evaluation as the assessment criterion, we employed $L_9(3^4)$ orthogonal design to screen the ratios between the main ingredient and excipients, as well as among different excipients and sweeteners. The product formulation was optimized based on parameters such as granulation effect, odor, and taste, thereby achieving optimization of the ratio of maltodextrin to mannitol, the ratio of the main ingredient to excipients, and the dosage of mogrosides in the solid beverage, so as to imrpvoe the product quality and user experience.

3.2.1 Establishment of sensory evaluation criteria. We selected 10 food science students trained in sensory evaluation as subjects to score the loquat leaf total triterpenoid solid beverage under study. The total score for all evaluation items was calculated on a 100-point basis. The comprehensive evaluation was calculated according to the product appearance score P (weight 20%), solution state score Z (weight 25%), odor score W (weight 25%), and taste score X (weight 30%), using the formula: Comprehensive evaluation = $(P \times 20\%) + (Z \times 25\%) + (W \times 25\%) + (X \times 30\%)$. The sensory evaluation criteria[7] for this solid beverage are shown in Table 1.

Table 1 Sensory evaluation criteria of granular solid beverage

Item	Scoring criteria	Score//points
Product appearance (full score: 20 points)	Granular size uniform	76 – 100
	Granular size not uniform	51 – 75
	Existence of small amount of big granules or powder	26 – 50
	Existence of many big granules or powder	0 – 25
Solution state (full score: 25 points)	Complete dissolution, no precipitation, no layered color, uniform color and luster	76 – 100
	Most dissolved, slightly precipitated, and uniform color	51 – 75
	Basically dissolved, partially precipitated, slightly suspended, and layered in color	26 – 50
	Most not dissolved, with a large amount of precipitation, and turbid with layered variegation	0 – 25
Odor (full score: 25 points)	Comfortable raw material fragrance and pleasant odor	76 – 100
	The fragrance of raw material slightly light or slightly strong without peculiar odor	51 – 75
	Too light or strong fragrance	26 – 50
	No special fragrance of raw material	0 – 25
Taste (full score: 30 points)	Moderate sweet	76 – 100
	Slightly sweet or too light	51 – 75
	Sweet	26 – 50
	Extremely sweet or not sweet, bitter and astringent taste	0 – 25

3.2.2 Single factor investigation test. In this study, the loquat leaf total triterpenoid extract was used as the fixed main ingredient, with maltodextrin and mannitol as excipients, collectively accounting for 99.8% of the total mass. Mogrosides accounted for 0.20% of the mass. The ratios of the main ingredient to excipients were set at 1 : 10, 1 : 11, 1 : 12, 1 : 13, and 1 : 14 for investigation and evaluation. The study revealed that the ratio of active pharmaceutical ingredient (API) to excipients during the granulation process directly determined the granule formability and the product's sensory characteristics. As shown in Fig. 1 and 2, at a 1 : 10 ratio, the insufficient amount of excipients resulted in loose granules with poor formability that failed the particle size test. Conversely, at a 1 : 14 ratio, the excessive excipients produced brittle granules that were prone to breakage, complicating packaging and transportation. Furthermore, the increasing proportion of excipients diluted the API's flavor, adversely affecting the taste. Based on these findings, the optimal ratios of API to excipients were preliminarily identified as 1 : 11, 1 : 12, and 1 : 13.

In the granulation process, maltodextrin and mannitol, as key excipients, play a critical role in granule formability, and were therefore included as one of the evaluation criteria. The maltodextrin-to-mannitol ratios in this solid beverage were set at 1.6 : 12, 1.8 : 12, 2.0 : 12, 2.2 : 12, and 2.4 : 12. The results are shown in Fig. 3. When the maltodextrin-to-mannitol ratio reached 2.0 : 12, the resulting granules exhibited a more uniform particle size distribution. However, as the proportion of maltodextrin increased further, the granule size demonstrated a decreasing trend. The product became more brittle, leading to an increase in fine particles. Therefore, the optimal ratios for the two excipients were

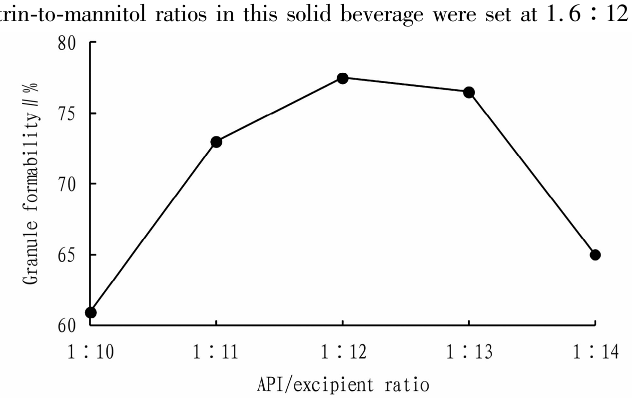

Fig. 1 Effects of API/excipient ratio on granule size

preliminarily identified as 1.8 : 12, 2.0 : 12, and 2.2 : 12.

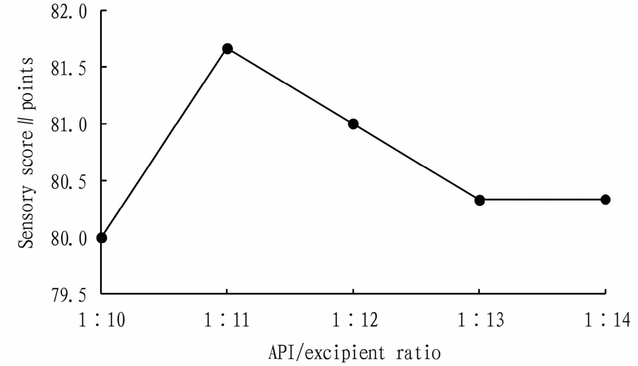

Fig.2 Effects of API/excipient ratio on sensory evaluation

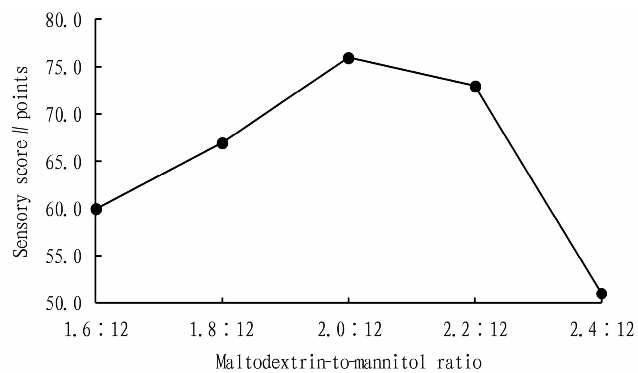

Fig.3 Effects of the ratio of maltodextrin to mannitol on granule size

Mogrosides, which have the sweetness approximately 300 times that of sucrose[8], significantly influence the product's sweetness even at very low concentrations. In this solid beverage, mogrosides were added at concentrations of 0.08%, 0.14%, 0.20%, 0.26%, and 0.32%. The results (Fig.4) show that the sensory score reached its peak at a concentration of 0.20%. At this level, the reconstituted beverage offered a moderate sweetness that aligned well with popular taste preferences. A lower concentration of 0.08% resulted in insufficient sweetness and a poor taste, leading to a low sensory score. By contrast, a higher concentration of 0.32% produced an excessively sweet profile that exceeded the acceptance threshold of the panelists, also resulting in a low score. Therefore, the optimal concentrations of mogrosides were preliminarily identified as 0.14%, 0.20%, and 0.26%.

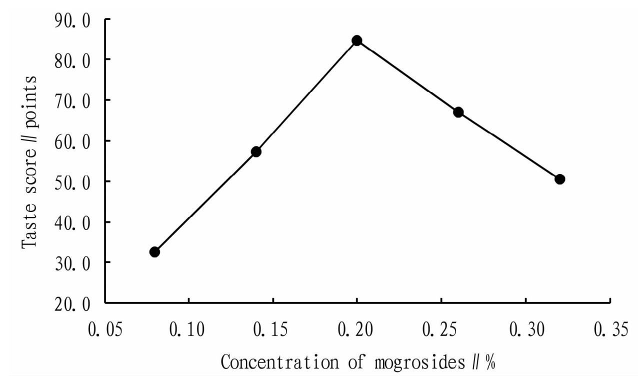

Fig.4 Effects of concentration of mogrosides on the taste

3.2.3 Orthogonal optimization test. After the single factor test, the orthogonal experimental factors and levels of the solid beverage are shown in Table 2.

Table 2 Orthogonal experimental factors and levels of loquat leaf hypoglycemic solid beverage

Level	Factor			
	A Maltodextrin-to-mannitol ratio // g/g	B API/excipient ratio // g/g	C Concentration of mogrosides // %	D Dissolution temperature // ℃
1	1.8 : 12	1 : 11	0.14	50
2	2.0 : 12	1 : 12	0.20	50
3	2.2 : 12	1 : 13	0.26	50

The orthogonal experimental results of the solid beverage are shown in Table 3; the variance analysis results are shown in Table 4.

Table 3 Orthogonal experimental results of loquat leaf hypoglycemic solid beverage (points)

Experiment No.		A	B	C	D	Product appearance	Solution state	Odor	Taste	Comprehensive evaluation
1		1	1	1	1	61.0	68.8	24.0	54.0	51.60
2		1	2	2	2	86.5	75.6	23.6	84.0	67.30
3		1	3	3	3	41.0	66.0	24.0	67.0	50.80
4		2	1	2	3	56.5	74.8	24.0	81.0	60.30
5		2	2	3	1	77.5	71.2	23.6	65.3	58.79
6		2	3	1	2	73.5	57.2	23.2	61.6	53.28
7		3	1	3	2	52.0	61.6	24.0	61.0	50.10
8		3	2	1	3	80.5	70.4	24.0	63.6	58.78
9		3	3	2	1	92.0	72.8	23.0	87.3	68.54
Comprehensive score points	K_1	56.567	54.000	54.553	59.643					
	K_2	57.457	61.623	65.380	56.893					
	K_3	59.140	57.540	53.230	56.627					
	R	2.573	7.623	12.150	3.016					

Table 4 Variance analysis of orthogonal experimental results of loquat leaf hypoglycemic solid beverage

Indicator	Factor	Sum of squares	Degree of freedom	*F* ratio
Comprehensive score	A	10.248	2	0.621
	B	87.320	2	5.218
	C	266.590	2	15.931
	Error	16.734	2	

According to the data in Table 3, formulation $A_3B_3C_2$ received the highest sensory evaluation score. The order of influence, based on the range analysis, was C > B > A, with the mogrosides concentration being the most significant factor. The optimal production process for the solid beverage was determined as a primary-to-auxiliary ingredient ratio of 1 : 13 (equivalent to a maltodextrin-to-mannitol ratio of 2.2 : 12) and a mogrosides concentration of 0.20%. This formulation achieved a high score, indicating reliable results. Further analysis of the data in Table 7 shows that the *p*-values for Factor A (maltodextrin : mannitol ratio), Factor B (primary-to-auxiliary ingredient ratio), and Factor C (mogrosides concentration) all exceed 0.05, suggesting that the effects of these individual factors are not statistically significant.

3.2.4 Verification test. The orthogonal experiments identified the $A_3B_3C_2$ combination as the optimal formulation, which consists of a 1 : 13 ratio of loquat leaf total triterpenoid extract (primary ingredient) to excipients, with the excipients being maltodextrin and mannitol at a ratio of 2.2 : 12, and a mogrosides concentration of 0.20%. Validation experiments conducted using this formulation are presented in Table 5. The solid beverage prepared with this optimal combination achieved a comprehensive score higher than all other groups, with a relative standard deviation (*RSD*) below 2%, confirming the high reliability of the results.

Table 5 Verification test results of loquat leaf hypoglycemic solid beverage (points)

Batch	Product appearance	Odor	Taste	Comprehensive evaluation
1	90.30	25.00	88.40	69.38
2	92.60	24.80	85.70	68.93
3	89.50	24.90	87.70	69.29
Mean	90.80	24.90	87.27	69.20
RSD//%	1.77	0.40	1.61	0.34

3.2.5 Mouse test. A subsequent investigation evaluated its effect on the oral glucose tolerance in normal mice. As presented in Table 6, the results demonstrate that the solid beverage, developed from the loquat leaf triterpenoid extract, produced a marked hypoglycemic effect. These findings indicate a considerable market potential for this product.

Table 6 Changes of blood glucose after oral administration of glucose ($\bar{x} \pm s$, $n = 10$)

Group	Dosage mg/kg	0 min mmol/L	30 min mmol/L	60 min mmol/L	120 min mmol/L	180 min mmol/L
Blank control	–	5.53 ± 0.51	20.69 ± 0.91	17.58 ± 2.66	8.50 ± 1.13	6.61 ± 0.69
Metformin hydrochloride	100	5.13 ± 0.91	12.59 ± 0.85**	10.76 ± 1.06**	7.11 ± 0.82	6.84 ± 0.52
Solid beverage	5 600	5.76 ± 0.95	11.08 ± 1.99**	8.26 ± 1.07**	7.90 ± 0.95	7.08 ± 0.85
Total triterpenic acid of loquat leaf	400	4.99 ± 0.87	13.40 ± 3.15**	9.66 ± 2.10**	7.20 ± 0.73	6.08 ± 0.71

NOTE ** denotes $P < 0.01$ when compared with the blank control group.

4 Conclusions

Loquat leaves are widely distributed and abundant resources, with production spanning multiple regions including Guangxi, Guangdong, Jiangxi, Hunan, and Gansu of China[9]. A solid beverage is defined as a solid, granular or powdered product intended for consumption after reconstitution. It is typically formulated with food extracts, excipients, and additives[10], and is characterized by its unique flavor, portability, and long shelf life[11]. The loquat leaf solid beverage developed in this study subsequently passed key quality control tests for granular preparations: the particle size inspection (wherein the combined weight passing through Sieve No.1 and Sieve No.5 was ≤15% of the total granule weight), moisture content (≤8%), and solubility (10 g of the product evenly dispersed and dissolved completely in 200 mL of hot water). The optimized formulation for the loquat leaf triterpenoid solid beverage is scientifically sound and provides a valuable reference for the future product development and utilization of loquat leaf resources.

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index was lower in hot drying because although airflow increases oxygen supply, it also accelerates the drying process, resulting in a smaller *BI*.

In summary, drying methods significantly influence the appearance characteristic of *F. cirrhosa*. In this study, we analyzed the appearance of *F. cirrhosa* bulbs and powder under different diameters and drying processing methods. Our results suggested that hot air drying and vacuum drying could improve appearance. Hot air drying at 35 – 40 ℃ as a processing method for *F. cirrhosa* production regions could maintain the color of *F. cirrhosa* bulbs and powder. Vacuum drying for bulbs > 8 mm in diameter dried at 40 ℃ maintained the colour and could making it suitable for functional food development in future.

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