Quality Standards for Mango Seeds in Tibetan Medicine

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Abstract [**Objectives**] To preliminarily investigate the morphological identification and content determination of mango seeds utilized in Tibetan medicine, thereby providing foundational data to support the further refinement of quality standards for mango seeds. [**Methods**] Powder microscopic examination, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC) were employed to identify mango seeds sourced from various regions in Sichuan Province. In accordance with the 2020 edition of the *Chinese Pharmacopoeia* (Volume IV), the extract content, total ash, acid-insoluble ash, and moisture content of the mango seeds were quantitatively determined. [**Results**] The morphological and powder microscopic characteristics of mango seeds in Tibetan medicine were described in detail. The methanol extract was qualitatively identified using TLC, and the content of gallic acid in the medicinal samples was determined by HPLC. The total ash content of mango seeds ranged from 1.82% to 2.73%, while the acid-insoluble ash content varied between 0.08% and 0.55%. The extract content ranged from 12.16% to 24.06%, and the moisture content was between 6.75% and 8.98%. [**Conclusions**] Specifications for mango seeds in Tibetan medicine have been established, indicating that the total ash content should not exceed 4.0%, the acid-insoluble ash content should not exceed 2%, the content of dilute ethanol extract should be no less than 15.0%, the moisture content should not exceed 12.0%, and the gallic acid content should be at least 1%. These parameters serve as a foundation for the development of quality standards for mango seeds in Tibetan medicine.

Key words Mango seed, Tibetan medicine, Quality standard

1 Introduction

Mangifera indica L., a species belonging to the Anacardiaceae family, is a well-known tropical fruit recognized for its rich nutritional content and diverse applications[1-3]. Mangiferin exhibits anti-inflammatory, antibacterial, and antioxidant properties; it can inhibit tumor cell proliferation, aid in the regulation of blood lipids, and protect hepatic function[4-8]. Additionally, polyphenols contribute to the elimination of free radicals and the retardation of aging processes[9-10]. *M. indica* is utilized in traditional medicine to reduce heat, promote the production of bodily fluids, alleviate thirst, induce diuresis, and ameliorate symptoms such as cough, dyspepsia, and skin inflammation. Contemporary research has demonstrated that extracts from *M. indica* leaves can lower blood glucose levels, while flavonoids present in the peel exhibit anti-allergic properties. These attributes position *M. indica* as a valuable resource with both medicinal and nutritional applications[11-12].

Mango seed is understood to be the dried seed of *M. indica*. During summer and autumn, when the fruits reach maturity, the seeds are harvested and subsequently dried. Mango seeds are abundant in various chemical constituents, including gallic acid tannins, flavonoids, organic acids, coumarin compounds, iridoid glycosides, and other bioactive components[12]. Mango seed is a commonly utilized medicinal substance in Tibetan medicine, recognized for its properties in eliminating phlegm, relieving cough, tonifying the kidney, dispelling kidney cold, strengthening the spleen, and harmonizing the stomach. Classical texts such as *Jingzhu Bencao* and the *Drug Standards of the Ministry of Health of the People's Republic of China: Tibetan Medicine Volume* document that the Tibetan name for mango seed is transliterated as "A Zhe". The preparation involves removing the inner peel to obtain the seed, which is then stir-fried in hot sand until it changes color and becomes loose. This processed form is attributed with functions including nourishing yin, tonifying the kidney, strengthening the stomach, and aiding digestion. Consequently, it is frequently employed in the treatment of conditions such as kidney deficiency and food accumulation[13-21]. However, the existing quality standards for mango seeds remain inadequate. The absence of systematic identification methods and standardized content determination indicators hinders the assurance of their safety and efficacy in clinical applications. Therefore, this study aims to develop a quality standard system for mango seeds that aligns with the characteristics of Tibetan medicine, thereby providing a scientific foundation for their resource development and clinical application.

2 Materials and methods

2.1 Instruments and reagents The instruments employed in this study comprised a DGU-20A5R high-performance liquid chromatograph (Shimadzu Corporation), a KQ-250DE CNC ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.), an ME204E/02 electronic balance [Mettler Toledo Instrument (Shanghai) Co., Ltd.], and a WondaSil C_{18}-WR column (4.6 mm × 150 mm, 5 μm). Gallic acid reference substance (CAS No.: 149-91-7) was procured from Lemeitian Pharmaceutical Biotechnology Co., Ltd. Additional reagents used included deionized water, ethanol, and chromatographic-grade methanol.

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2.2 Sample information Nine batches of stir-fried mango seeds were obtained from Tibetan hospitals throughout Sichuan Province, and an additional batch was procured from the Hehuachi Chinese Herbal Medicine Market in Chengdu. Dr. Feng Jingqiu of Southwest Minzu University identified these samples as dried seeds of *M. indica* L., with the endocarp removed, belonging to the Anacardiaceae family (Table 1). The seeds were subsequently ground into powder, passed through a No. 3 mesh sieve, and stored for future use.

Table 1 Sample information of mango seeds

Batch No.	Sample supplier	Place of origin
MGH-1	Ganluhai Tibetan Hospital	Sichuan
MGH-2	Ganzi Tibetan Autonomous Prefecture Tibetan Hospital	Sichuan
MGH-3	Aba Tibetan Autonomous Prefecture Tibetan Hospital	Sichuan
MGH-4	Baiyu County Tibetan Hospital	Sichuan
MGH-5	Dege County Tibetan Hospital	Sichuan
MGH-6	Derge Dzongsar Tibetan Hospital	Sichuan
MGH-7	Shiqu County Tibetan Hospital	Sichuan
MGH-8	Seda County Tibetan Hospital	Sichuan
MGH-9	Derong County Tibetan and Chinese Hospital	Sichuan
MGH-10	Hehuachi Chinese Herbal Medicine Market	Sichuan

2.3 Examination of the characteristics of medicinal materials During summer and autumn, once the fruits had ripened, the seeds were collected, washed, and dried. The endocarp was then removed to extract the kernel, which was subsequently stir-fried in hot sand until it changed color and became loose. Using sensory observation, the detailed characteristics of the sample—including its shape, color, surface, and cross-section—were examined. Subsequently, its texture, odor, and other sensory attributes were described. Additionally, the powder morphology was observed under a light microscope.

2.4 Determination Moisture content was measured in accordance with the *Chinese Pharmacopoeia* (2020 Edition, Volume IV; General Rules 0832, Method 2)[17]. Total ash content and acid-insoluble ash content were determined using the method described in the *Chinese Pharmacopoeia* (2020 Edition, Volume IV; General Rules 2302)[17]. Extracts were quantified employing the thermal extraction method for alcohol-soluble extracts specified in the *Chinese Pharmacopoeia* (General Rules 2201), utilizing dilute ethanol as the solvent[17].

2.5 Thin-layer chromatography (TLC) In accordance with the TLC method for mango seeds in the *Drug Standards of the Ministry of Health of the People's Republic of China: Tibetan Medicine Volume*[19], gallic acid was chosen as the reference substance for the TLC identification of mango seed herbal materials.

2.5.1 Preparation of test solution. 1 g of each batch of medicinal material powder (passed through No. 3 sieve) was combined with 10 mL of methanol and subjected to ultrasonic extraction (power 200 W, frequency 40 kHz) for 15 min, followed by filtration to obtain the test solution.

2.5.2 Preparation of reference solution. An appropriate quantity of gallic acid reference was measured, and methanol was added to prepare a reference solution with a concentration of 1 mg/mL. Subsequently, 2 μL each of the test solution and the reference solution were applied onto the same silica gel GF_{254} TLC plate. The plate was developed using a mobile phase composed of cyclohexane, ethyl formate, and formic acid at a ratio of 5 ∶ 10 ∶ 1. After development, the plate was removed, dried, and examined under ultraviolet light at 254 nm. The chromatogram of the test solution exhibited a fluorescent spot (dark spot) at the same position as that of the reference solution.

2.6 Determination of mango seed content The content of mango seeds was determined using high-performance liquid chromatography (HPLC) in accordance with the *Chinese Pharmacopoeia* (General Rules 0512)[17].

2.6.1 Chromatographic conditions. The mobile phase consisted of methanol and 0.2% phosphoric acid solution at a ratio of 5 ∶ 95. Detection was performed at a wavelength of 272 nm. The number of theoretical plates, as determined by the gallic acid peak, was required to be no less than 2 000. Precisely 10 μL of each reference and test solution was injected into the liquid chromatograph for analysis.

2.6.2 Preparation of reference solution. An appropriate amount of gallic acid reference substance was accurately weighed and dissolved in 50% methanol to prepare a solution with a concentration of 0.2 mg/mL of gallic acid. This solution was subsequently utilized as the reference solution.

2.6.3 Preparation of test solution. Approximately 0.2 g of the sample was accurately weighed and placed into a stoppered conical flask. Precisely 50 mL of 50% methanol solution was added, and the flask was then sealed tightly. After weighing the flask, it was subjected to ultrasonic treatment at a power of 250 W and a frequency of 40 kHz for 30 min. Following cooling, the flask was weighed again, and additional 50% methanol solution was added to compensate for any weight loss. The mixture was then thoroughly shaken and filtered to obtain the test solution.

3 Results and analysis

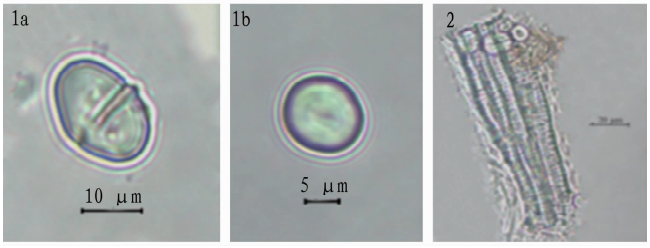
3.1 Characteristics of medicinal materials

3.1.1 Characteristic identification. The mango seed was kidney-shaped or elongated oval, slightly flattened, measuring 4 – 7 cm in length, 3.0 – 4.5 cm in width, and 1 – 2 cm in thickness. Its surface exhibited a yellow-white or earthy yellow coloration, characterized by several slightly curved shallow furrows and covered with hairy fibers approximately 2 – 5 mm in length. The texture was hard, producing a rattling sound when shaken (Fig. 1). Following crushing, the endocarp exhibited a fibrous texture, and its inner surface was smooth and light yellow in color. A single kernel was present, characterized by a light grayish-green testa and two cotyledons that were milky white in appearance. The taste was described as slightly sour, astringent, and mildly bitter.

3.1.2 Microscopic identification. The mango seeds were removed their shells and subsequently crushed. A small portion of the crushed material was stained using a dissecting needle and placed onto a glass slide. Following permeabilization with chloral hydrate, the slices were prepared with dilute glycerin. Microscopic

Fig. 1 Illustration of stir-fried mango seed medicinal materials

examination revealed a yellow-white to yellow-brown powder. Numerous starch granules were observed, exhibiting shapes that were oval, round, or triangular, with diameters ranging from 5 to 15 μm and distinct layered striations. The catheters were predominantly threaded, exhibiting diameters ranging from 5 to 30 μm (Fig. 2). These microscopic characteristics may serve as a basis for authenticating mango seeds.



NOTE 1a. Starch grain; 1b. Starch grain; 2. Catheter.

Fig. 2 Microscopic image of stir-fried mango seed powder

3.2 Determination of moisture content, total ash, acid-insoluble ash, and extract content of stir-fried mango seeds

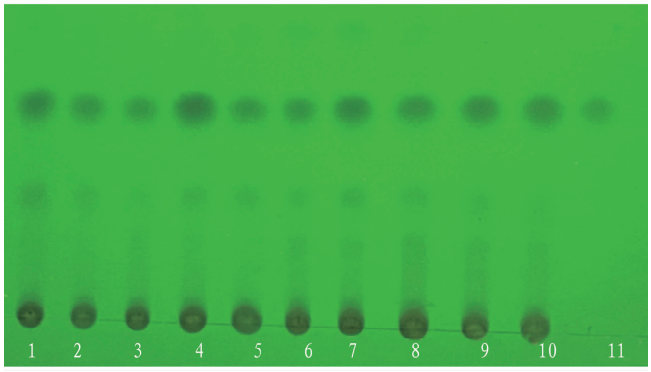
The moisture content of 10 sample batches ranged from 6.75% to 8.98%, with a mean value of 8.03%. Accordingly, the moisture limit should not exceed 12.0%. The total ash content varied between 1.82% and 2.73%, averaging 2.27%, with a specified maximum limit of 4%. The acid-insoluble ash content ranged from 0.08% to 0.55%, with an average of 0.23%, and the limit was set at 2%. Using dilute ethanol as the solvent, the extract content of the 10 sample batches ranged from 12.16% to 24.06%, with an average of 18.93% (Table 2).

Table 2 Determination of moisture content, total ash, acid-insoluble ash, and extract content of stir-fried mango seeds %

Batch No.	Moisture	Total ash	Acid-insoluble ash	Extract
MGH-1	7.62	2.73	0.44	20.45
MGH-2	7.64	2.66	0.52	15.55
MGH-3	8.35	2.16	0.18	10.00
MGH-4	8.86	1.94	0.18	18.51
MGH-5	8.05	2.36	0.19	21.37
MGH-6	6.78	2.12	0.31	17.17
MGH-7	8.96	1.83	0.14	16.48
MGH-8	7.76	2.35	0.18	19.24
MGH-9	7.90	2.54	0.09	22.47
MGH-10	8.41	2.03	0.15	17.05
Mean	8.03	2.27	0.24	17.83

3.3 TLC identification of mango seeds

The results demonstrated that the chromatographic spots of the test solution exhibited fluorescent spots of identical color at positions corresponding to those of the reference solution (Fig. 3), with an *Rf*(11) value of 0.47. Therefore, this method can be employed for the identification of mango seed medicinal materials.

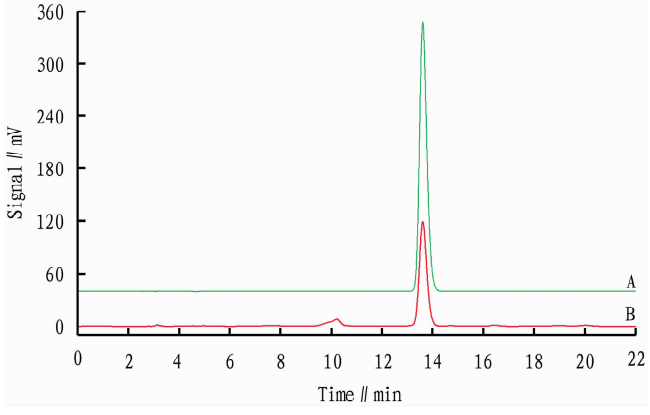


NOTE 1-10. Samples of stir-fried mango seed medicinal materials; 11. Gallic acid reference substance.

Fig. 3 TLC identification map of stir-fried mango seeds (254 nm)

3.4 Determination of mango seed content

10 μL of each reference and test solution were accurately pipetted, injected into the liquid chromatograph, and analyzed (Fig. 4).



NOTE A. Gallic acid reference substance; B. Sample of mango seeds.

Fig. 4 Chromatogram of mango seeds

3.4.1 Investigation of preparation method of test solution. (i) Extraction methods. Four samples of stir-fried mango seed powder from batch MGH-10, each weighing approximately 0.2 g, were placed into stoppered conical flasks. Precisely 50 mL of 50% methanol was added to each flask, which were then sealed tightly and weighed. Two samples were subjected to ultrasonic extraction, while the remaining two underwent reflux extraction, each for a duration of 30 min. After cooling, the flasks were weighed again, and any weight loss was compensated by adding 50% methanol. The solutions were then thoroughly mixed and filtered to obtain the test solutions. 10 μL of each filtrate was injected into the chromatograph to analyze and quantify the gallic acid content under various extraction methods. The results demonstrated that the gallic acid content obtained via reflux extraction and ultrasonic extraction

were both 1.50%, with no significant difference observed between the two methods (Table 3). Consequently, ultrasonic extraction, being simpler to perform, was selected as the preferred extraction technique.

Table 3 Investigation of various extraction methods

No.	Extraction method	Weight/g	Gallic acid content/%	Average content/%
1	Reflux	0.201 7	1.41	1.50
2		0.202 7	1.60	
3	Ultrasonic	0.201 2	1.55	1.50
4		0.202 6	1.46	

(ii) Extraction solvents. Eight samples of stir-fried mango seed powder from batch MGH-10, each weighing approximately 0.2 g, were extracted with 50 mL of methanol at concentrations of 30%, 50%, 75%, and 100%, respectively. Ultrasonic extraction was performed at a power of 250 W and a frequency of 40 kHz for 30 min. Following extraction, the mixtures were cooled, and any weight loss was compensated by adding the corresponding solvent. The solutions were then filtered, and 10 μL of each filtrate was injected into a chromatograph to analyze and quantify the gallic acid content extracted by different solvents. The results indicated that the gallic acid content extracted using 50% methanol was higher than that obtained with 30% methanol, 70% methanol, and pure methanol (Table 4). Therefore, 50% methanol was selected as the optimal solvent for extraction.

Table 4 Investigation of various extraction solvents

No.	Weight/g	Solvent	Gallic acid content/%	Average content/%
1	0.200 7	30% Methanol	1.37	1.39
2	0.201 9	30% Methanol	1.41	
3	0.200 1	50% Methanol	1.44	1.48
4	0.202 1	50% Methanol	1.52	
5	0.201 0	70% Methanol	1.40	1.39
6	0.202 6	70% Methanol	1.38	
7	0.201 7	Methanol	1.32	1.34
8	0.200 8	Methanol	1.36	

(iii) Extraction time. Six samples of stir-fried mango seed powder from batch MGH-10, each weighing approximately 0.2 g, were placed into stoppered conical flasks. Subsequently, 50 mL of methanol was added to each flask, which was then sealed tightly and weighed. Ultrasonic extraction was performed for 15, 30, and 60 min, respectively. After cooling, the samples were re-weighed, and any weight loss was compensated by adding 50% methanol. The mixtures were then thoroughly shaken and filtered to obtain the filtrate. 10 μL of each filtrate was injected into the chromatograph to analyze and quantify the gallic acid content under various extraction methods. The results demonstrated that the gallic acid content at an extraction time of 30 min was higher than that at 15 min, with no significant difference observed compared to 60 min (Table 5). Consequently, an extraction time of 30 min was selected.

Table 5 Investigation of varying extraction time

No.	Extraction time/min	Weight/g	Gallic acid content/%	Average content/%
1	15	0.201 4	1.26	1.29
2	15	0.200 5	1.32	
3	30	0.202 7	1.45	1.50
4	30	0.202 6	1.54	
5	60	0.201 5	1.45	1.48
6	60	0.200 3	1.51	

(iv) Solid-liquid ratio. Six samples of stir-fried mango seed powder from batch MGH-10, each weighing precisely 0.2 g, were placed in stoppered conical flasks. To these, 25, 50, and 100 mL of 50% methanol were added respectively. The flasks were then sealed tightly, weighed, and subjected to ultrasonic extraction for 30 min. After cooling, the flasks were weighed again, and any weight loss was compensated by adding 50% methanol. The solutions were thoroughly mixed, filtered, and the resulting filtrates were collected as the final products. 10 μL of each filtrate was injected into the chromatograph to analyze and quantify the gallic acid content under varying solid-to-liquid ratios. The results indicated that when 0.2 g of stir-fried mango seed powder was extracted with 50 mL of solvent, the gallic acid content was high and did not differ significantly from that obtained using 100 mL of solvent (Table 6). Consequently, a solid-to-liquid ratio of 1∶250 was determined to be optimal.

Table 6 Investigation of varying solid-liquid ratios

No.	Solvent volume/mL	Weight/g	Gallic acid content/%	Average content/%
1	25	0.200 3	1.38	1.39
2	25	0.201 2	1.40	
3	50	0.203 2	1.47	1.51
4	50	0.202 1	1.54	
5	100	0.203 1	1.49	1.50
6	100	0.200 4	1.51	

In summary, the preparation of the test solution involved the following steps: approximately 0.2 g of the powder was accurately weighed and placed into a stoppered conical flask. Subsequently, 50 mL of 50% methanol was precisely added, and the flask was then sealed tightly. The flask was then weighed and subjected to ultrasonic treatment at a power of 250 W and a frequency of 40 kHz for 30 min. After cooling, the flask was weighed again, and any weight loss was compensated by adding 50% methanol. The solution was thoroughly mixed, filtered, and the filtrate was collected for further analysis.

3.4.2 Methodological investigation. (i) Precision. The identical reference solution was injected continuously six times. The peak area of gallic acid was recorded for each injection, and the relative standard deviation (*RSD*) was calculated. The results indicated that the *RSD* of the gallic acid peak area was 0.09%, demonstrating good instrument precision (Table 7).

Table 7 Investigation results of precision

Number of injections	Peak area of gallic acid	*RSD*//%
1	6 294.003	0.09
2	6 309.211	
3	6 303.293	
4	6 297.290	
5	6 298.482	
6	6 304.806	

(ii) Linear relationship. An appropriate amount of gallic acid reference substance was weighed and transferred into a 10 mL volumetric flask, and diluted with 50% methanol to prepare a 1.0 mg/mL reference solution. Subsequently, 1 mL of this solution were precisely measured and transferred into a 5 mL volumetric flasks, and diluted to the mark with 50% methanol to yield the solution with a concentration of 0.2 mg/mL. Similarly, the solutions were diluted to concentrations of 0.08, 0.05, 0.025, and 0.012 5 mg/mL, respectively. Furthermore, 10 μL of each solution was injected into the liquid chromatograph for analysis to obtain the corresponding peak areas. The response curve was constructed by plotting concentration (X, mg/mL) on the abscissa against peak area (Y) on the ordinate.

The results demonstrated that within the concentration range of 0.012 5 to 0.200 0 mg/mL, the peak area of mango seed extracts exhibited strong linearity with concentration. The linear regression equation was $Y = 39\ 136X - 78.236$, with a correlation coefficient of $r = 0.999\ 8$ (Fig. 5).

(iii) Stability test. The peak area of gallic acid in the identical test solution (MGH-10) was measured at 0, 2, 4, 8, 16, and 24 h. The *RSD* was 0.53%, demonstrating that the test solution remained stable over 24 h.

(iv) Repeatability. The content of the same batch of samples (MGH-10) was repeatedly measured by the same operator to assess the method's repeatability. The results demonstrated that, across six replicate test solutions, the peak area of gallic acid was recorded, yielding a *RSD* of 0.35%, which indicates good repeatability of the method.

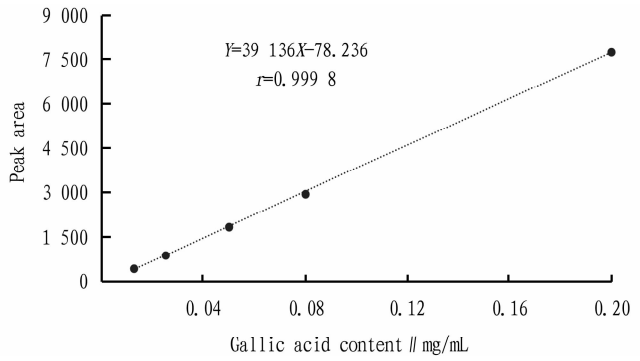


Fig. 5 Linear relationship of gallic acid

(v) Sample recovery test. Six samples of stir-fried mango seed powder (MGH-10), each weighing approximately 0.1 g with a known content, were accurately measured, and added with precise amount of gallic acid reference substance. The resulting sample solutions were prepared following the established procedure for test solution preparation, then injected for analysis. The gallic acid content was determined, and the recovery rate was subsequently calculated.

Recovery rate (%) = (Measured weight − Background weight)/Added weight × 100%

The results indicated that, among the six added test solutions, the recovery rate of gallic acid ranged from 97.39% to 103.28%, with a *RSD* of 2.2%. These findings demonstrate that the method exhibits good accuracy (Table 8).

Table 8 Accuracy investigation results of gallic acid

No.	Weight//g	Background weight//mg	Added weight//mg	Measured weight//mg	Recovery rate//%	Average recovery rate//%	*RSD*//%
1	0.100 8	1.493 6	1.136 6	2.600 5	97.39	101.41	2.2
2	0.100 4	1.487 6	1.136 6	2.661 5	103.28		
3	0.100 7	1.492 1	1.136 6	2.648 5	101.75		
4	0.100 7	1.492 1	1.136 6	2.658 3	102.60		
5	0.100 3	1.486 1	1.136 6	2.654 9	102.83		
6	0.100 5	1.489 1	1.136 6	2.632 7	100.62		

3.4.3 Determination of gallic acid content. The gallic acid content of the test solution was determined by preparing it from 10 batches of collected mango seed samples.

The results indicated that the gallic acid content in stir-fried mango seeds from various origins and batches ranged from 1.05% to 1.88%, with an average content of 1.52% (Table 9). On a dry weight basis, this product contained no less than 1.0% gallic acid ($C_7H_6O_5$).

Table 9 Determination results of gallic acid content ($n = 3$) %

No.	Batch No.	Gallic acid content	Average content
1	MGH-1	1.65	1.52
2	MGH-2	1.61	
3	MGH-3	1.23	
4	MGH-4	1.63	
5	MGH-5	1.37	
6	MGH-6	1.80	
7	MGH-7	1.48	
8	MGH-8	1.05	
9	MGH-9	1.88	
10	MGH-10	1.48	

4 Discussion

M. indica, recognized as one of the world's most significant economic crops, is not only abundant in various nutrients but also

holds considerable economic, ecological, and medicinal value. Extracts derived from different parts of *M. indica* plant can fulfill the production requirements of diverse industries[2-3]. Previous studies have demonstrated that the therapeutic effects of *M. indica* vary according to the differing contents and components of its active constituents in various plant parts, as well as the specific sites of application. The leaves of *M. indica* are particularly rich in compounds such as mangiferin. Among the Zhuang ethnic group in China, *M. indica* leaves are traditionally utilized and are characterized by a cool nature and a sour-sweet taste. They are attributed with properties including heat clearance, phlegm resolution, cough and asthma relief, promotion of body fluid production and thirst quenching, as well as enhancement of qi circulation and alleviation of stagnation. Clinically, these leaves are primarily employed to treat conditions such as lung heat cough with excessive phlegm, diabetes, abdominal pain resulting from heat stagnation, flatulence, and malnutrition in children[22-23]. Mango seeds are abundant in compounds such as gallic acid, which have been documented in both Tibetan and Mongolian traditional medicine. When stir-fried, mango seeds demonstrate therapeutic effects including nourishing yin, tonifying the kidneys, strengthening the stomach, and promoting digestion. They are commonly employed in the treatment of conditions such as kidney deficiency and food stagnation[13,20]. In contrast to prior studies that primarily concentrated on the chemical composition and general pharmacological properties of mango seeds, the quality standard system for mango seeds in Tibetan medicine developed in this study is more specific and distinctive.

This study investigates the medicinal properties and therapeutic applications of mango seeds used in Tibetan medicine. Through comprehensive research involving morphological and microscopic identification, TLC identification, and assessments of extract content, acid-insoluble ash, total ash, and moisture content, a quality standard system for mango seeds consistent with the characteristics of Tibetan medicine has been developed. This standard includes multidimensional indicators such as morphological characteristics, microscopic features, and the content of major components, thereby providing a scientific foundation for the quality control of mango seeds. Future research could further investigate the pharmacological mechanisms of mango seeds, optimize extraction methods, develop high-value-added products, and promote the modernization and internationalization of Tibetan medicinal mango seeds.

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