Serican Journal of Medicine
2025, VOL. 2, NO. 3, 23863
https://doi.org/10.17161/sjm.v2i3.23863

Effects of different processing methods on the content of Rutin and Tannin in sophora japonica herb medicine

Shanwen You and Yingjie Fu*

Department of Chinese Materia Medica, Jining Medical College School of Pharmacy, Rizhao, Shandong 276800, China.
*Correspondence: pilipili@163.com; Tel: +86-15965001526.

ABSTRACT

Objective: To investigate the effects of different processing methods on the content of rutin and tannin in Sophora japonica herb medicine (Chinese herb medicine name: Huai Mi or Huai Hua).

Methods: Huai Mi was processed with different methods. Tannin content was determined using the potassium permanganate titration method. Rutin content was quantified using ultraviolet spectrophotometry.

Results: The tannin content was the highest in stir-fried-to-coke samples (1.87%), while the highest rutin content was observed in stir-fried-to-yellow samples (0.134%). The stir-fried-to-ash samples had the lowest contents of both rutin and tannin.

Conclusion: Processing methods critically impact the contents of bioactive compounds in Sophora japonica herb medicine. Optimizing these methods may enhance medicinal efficacy and utilization.

ARTICLE HISTORY
Received: April 19, 2025
Revised: April 30, 2025
Accepted: May 10, 2025

KEYWORDS
Sophora japonica, Huai Mi, processing methods, tannin, rutin, Chinese herb medicine

Introduction

Sophora japonica (currently known as Styphnolobium japonicum) is a medium-sized deciduous tree commonly found in China (also known as the Chinese scholar tree and pagoda tree or simply Huai Shu), Japan (also known as Japanese pagoda tree), Korea, Vietnam, and other countries. Dried flowers and buds of Sophora japonica have been used as a traditional Chinese medicinal herb (named Huai Mi or Huai Hua) for centuries. Huai Mi has been widely used for its hemostatic, anti-inflammatory, and antioxidant functions. It has been known that the primary bioactive components of Huai Mi are rutin and tannin, which contribute to its therapeutic effects. Rutin reduces capillary permeability and exhibits antiviral activity, while tannin enhances hemostasis [1]. Huai Mi needs to be processed prior to being administered orally to the patients. A few traditional processing methods have been widely used, such as stir-fried-to-yellow (Chinese term: Chao Huang), stir-fried-to-coke (Chinese term: Chao Jiao), stir-fried-to-charcoal (Chinse term: Chao Tan), and stir-fried-to-ash (Chinese term: Hui Hua). Excessive heating during processing may degrade rutin while increasing tannin content [2]. The decoction method is usually used to extract herbal medicine. Experiments have shown that this extraction method is simple to operate, has a short extraction time, and has a high yield and purity of flavonoids. It is an efficient, non-toxic, and environmentally friendly extraction technology [3]. Since rutin is dissolvable in methanol and methanol has preservative effects [4] and antibacterial effects [5], rutin is relatively stable during the storage process. However, due to the complex and unstable structure of tannin, which is prone to hydrolysis, its separation and purification are difficult, which restricts the development of tannin [6]. Therefore, extraction of rutin and tannin is performed using different processes. Tannin is determined immediately after extraction, while rutin extract is placed in methanol for seven days to fully extract the components. The objective of this study was to evaluate the effects of different processing methods on the rutin and tannin levels in Huai Mi, aiming to establish standardized protocols for maximizing the bioactive compounds.

Materials and Methods

Key Reagents

The key reagents were purchased from the following sources: Huai Mi (batch number 20230607) from Anhui Yuankang Chinese Herbal Decoction Pieces

Co., LTD. (Anhui, China); rutin reference standards (batch number 20230108) from Shanghai Ruji Biotechnology Co., LTD. (Shanghai, China.); gelatin (analytical reagent grade - AR grad; batch number 20230514) from Sinopharm Chemical Reagent Co., LTD. (Shanghai, China); sulfuric acid (AR grade; batch number 20230312), barium sulfate (AR grade; batch number 20220105), and potassium permanganate (AR grade; batch number 20220703) from Fine Chemical Factory (Laiyang Economic and Technological Development Zone, Laiyang, Shandong, China); indigo carmine (AR grade; catalogue number 6942101015556) from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China); sodium hydroxide (AR grade), aluminum sulfate (AR grade; batch number 20210816), sodium chloride (AR grade; batch number 20210803), sodium nitrite (AR grade), and methanol (guaranteed reagent - GR grade; batch number 20230803) from Tianjin Yongda Chemical Reagent Co., LTD. (Tianjin, China).

2. Experimental Procedures

2.1 Sample Processing Methods

Raw Huai Mi: 10 g of raw Huai Mi was crushed into fine powders in a mortar.

Stir-fried-to-yellow (Chao Huang): 20 g of raw Huai Mi was stir-fried over low heat until it became yellow.

Stir-fried-to-coke (Chao Jiao): 30 g of raw Huai Mi was stir-fried over low heat until it became charred.

Stir-fried-to-charcoal (Chao Tan): 40 g of raw Huai Mi was stir-fried over high heat until it became carbonized.

Stir-fried-to-ash (Hui Hua): 100 g of raw Huai Mi was stir-fried over high heat until it became ashes.

2.2 Extraction of Bioactive Compounds

Ten grams of each processed sample was boiled in 500 mL distilled water for 1 hour, filtered, and stored in a 500 mL volumetric flask. After one week, the supernatant was filtered and collected.

2.3 Tannin Content Determination

2.3.1 Titration: 10 mL extract was mixed with 500 mL H_2O, 5 mL of 0.6% indigo carmine, and 20 mL of H_2SO_4. The solution was titrated with 0.02 mol/L $KMnO_4$ until it became yellow green. The volume of $KMnO_4$ used was recorded as volume A.

2.3.2 Blank Test: 100 mL extract was treated with 30 mL of 2.5% gelatin, saturated with NaCl, and then added with 10 mL of 10% H_2SO_4, and 10 g $BaSO_4$. After being mixed for 3 minutes and filtered, 10 mL filtrate was titrated with 0.02 mol/L KMnO4 until it became yellow green. The volume of $KMnO_4$ used here was recorded as volume B.

2.3.3 Tannin content (%) was calculated as (A-B) × 0.004157×100%.

2.4 Rutin Content Determination

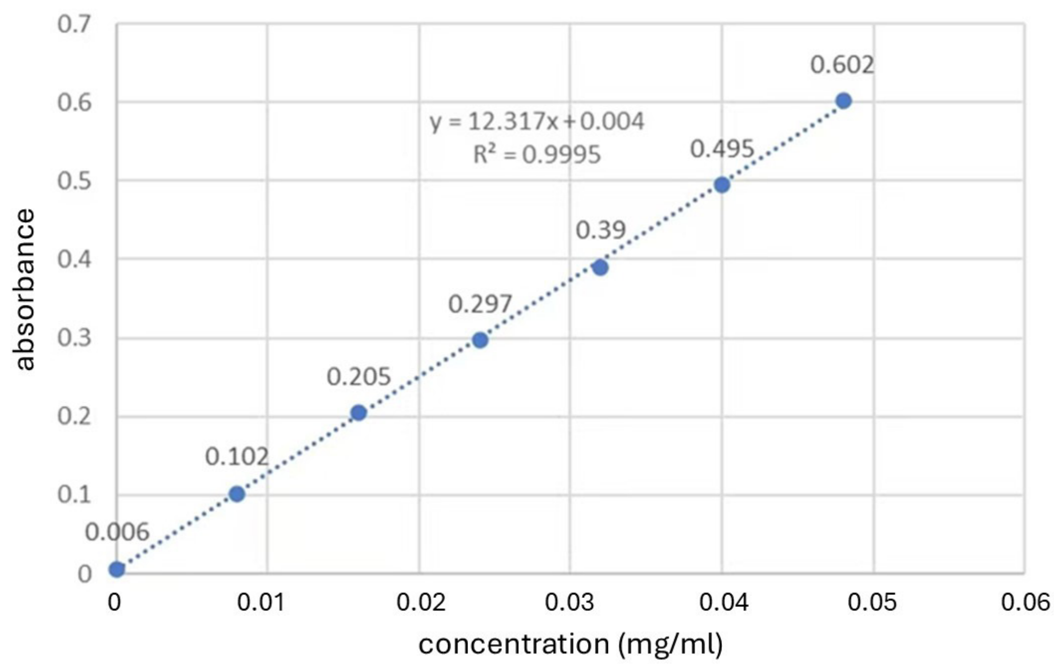
2.4.1 Standard Curve: A rutin standard solution (0.2 mg/mL) was prepared. A serial volume of the rutin standard solution was taken into 25 mL flasks, including 0, 1, 2, 3, 4, 5, and 6 mL. Water was added to a total volume of 6 mL for each standard solution. 1 mL of 5% sodium nitrite was added and mixed. Six minutes later, 1 mL of 10% aluminum sulfate was added and mixed. After another six minutes, 10 mL of 4% NaOH was added and mixed. Fifteen minutes later, absorbance at 500 nm was measured using a double-beam UV-Vis spectrophotometer (Beijing Puxi General Instrument Co., Ltd., Beijing, China). A linear calibration curve was plotted (Fig 1).

2.4.2 Rutin Content Determination in the Samples: 1 gram of each processed sample was added in 50 mL methanol and incubated for one week. The condition is to store it in a dry refrigerator in a sealed container (at 0 degrees Celsius, away from light). After filtration, 3 mL of the filtrate was taken into 25 mL flasks and added with 3 mL water to a total volume of 6 mL. 1 mL of 5% sodium nitrite was added and mixed. Six minutes later, 1 mL of 10% aluminum sulfate was added and mixed. After another six minutes, 10 mL of 4% NaOH was added and mixed. Fifteen minutes later, absorbance at 500 nm was measured using a double-beam UV-Vis spectrophotometer (Beijing Puxi General Instrument Co., Ltd., Beijing, China). The absorbance was plotted in the standard curve (Fig. 1) to obtain the rutin concentration (C). The rutin content in the Huai Mi sample was calculated as C/0.121%.

Results

We found that tannin content was the highest (1.87%) in the stir-fried-to-coke (Chao Jiao) samples, followed by the stir-fried-to-yellow (Chao Huang)

Fig 1. The standard curve of rutin content determination.



samples (0.79%). The stir-fried-to-ash samples had almost no tannin content (Table 1). On the other hand, the stir-fried-to-yellow (Chao Huang) samples had the highest rutin content (0.134%), followed by the raw samples (0.104%). The stir-fried-to-ash samples had the lowest content of rutin (0.008%) (Table 1).

Table 1. Tannin and rutin contents in differently processed Huai Mi.

Processing Methods	Tannin Content (%)	Rutin Content (%)
Raw	0.17	0.104
Stir-fried-to-yellow	0.79	0.134
Stir-fried-to-coke	1.87	0.067
Stir-fried-to-charcoal	0.25	0.042
Stir-fried-to-ash	0	0.008

Discussion

It is worth noting that during the extraction and storage of rutin components, they should be kept in a cool and dark place, avoiding any contact with alkaline substances, Na_2SO_3, and iron containers. The addition of food additive sucrose and preservative sodium benzoate has almost no effect on the stability of flavonoids [7]. Our results showed that the tannin content in Huai Mi reached its maximum in the stir-fried-to-coke samples (1.87%), likely due to prolonged thermal exposure that facilitates tannin polymerization. The rutin content in Huai Mi peaked in the stir-fried-to-yellow samples (0.134%) but declined sharply in overcooked or ashed samples, indicating that rutin is liable to thermal degradation. These findings align with prior studies, suggesting that moderate heating enhances rutin stability, while excessive temperatures degrade it [8] We noted that the stir-fried-to-yellow samples also had relatively high tannin content (0.79%), though it was about half of the tannin content in the stir-fried-to-coke samples. Our results suggest that if both tannin and rutin are needed for the therapeutic purpose, the stir-fried-to-yellow (Chao Huang) processing method is optimal. In contrast, if only tannin is needed for the treatment, the stir-fried-to-coke (Chao Jiao) processing method should be used. Modernizing traditional methods with standardized temperature and time controls could optimize the retention of bioactive compounds. This study underscores the need for evidence-based protocols to ensure consistent quality in Sophora japonica herb preparations.

Conclusion

Processing methods critically impact the contents of bioactive compounds in Sophora japonica herb medicine. Optimizing these methods may enhance medicinal efficacy and utilization.

Acknowledgements:

This work was supported by grants from the Shandong Province Traditional Chinese Medicine Science & Technology Project (no. M-2023042) and the College Students' Innovation Training Program of Jining Medical University (cx2024123z).

Conflicts of interest:

The authors have no conflicts of interest to declare.

References:

1. Nian QB. **Determination of Tannin Content in Differently Processed Sophora japonica**. *Fujian Journal of Traditional Chinese Medicine*, 2004 (05): 48.
2. He DW, Zheng AL. **Comparison of Rutin Content in Sophora Japonica Processed by Different Methods**. *Gansu Journal of Traditional Chinese Medicine*, 2005 (07): 59-60.
3. Li SY, Peng YH, Zhang B, et al. **Research progress on extraction technology of total flavonoids from snow chrysanthemum**. *Guiding Journal of Traditional Chinese Medicine and Pharmacology*, 2017(5), 54-56. DOI:10.13862/j.cnki.cn43-1446/r.2017.05.018
4. Lin XZ, **Inspection and Treatment of Benzyl Alcohol Deterioration**. *Journal of Hospital Pharmacy,* 2017,23(05):54-56. DOI:10.13862/j.cnki.cn43-1446/r.2017.05.018.
5. Yan QY, Han XP, Zhang B, et al. **Quality Control Measures and Result Analysis of Proficiency Testing for the Preservative Benzyl alcohol in Cosmetics.** *China's Detergent Industry*, 2025, (03):91-96. DOI:10.16054/j.cnki.cci.2025.03.007.
6. Zhu XY, Bai L, Liu XQ, et al. **The research progress of traditional Chinese medicine in the recent ten years tannins**. *Chinese Journal of Traditional Chinese Medicine*, 2021, 46-48 (24): 6353-6365. DOI: 10.19540/j.carolcarrollnkiCJCMM.20210630.602.
7. Wang S, Wang XY, Lyu CN, et al. **The study of stability of apocynum leaves medium yellow ketone**. *Journal of Ginseng Research*, 2025 (01):47-51. DOI: 10.19403/j.carolcarrollnki.1671-1521.2025.01.008.
8. Yin YS, Chen RF. **Changes in Tannin Content of Sophora japonica Before and After Processing**. *Chinese Traditional Patent Medicine*, 1980 (06): 32-34.