Investigation of the impact on the antioxidant capacity and Flavonoids components of different drying methods for *Sophora japonica* L.

Yandong Wang\(^{1, a}\), Jiaming Wang\(^{2, b}\), Yingli Wang\(^{3, c}\), Zemin Zhang\(^{4, d})*$

\(^{1}\)State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, Guangdong Provincial Key Laboratory of Plant Molecular Breeding, South China Agricultural University, Guangzhou 510642, China

\(^{2}\)School of Chinese Medicine and Food Engineering, Shanxi University of Traditional Chinese Medicine, 030619 Jinzhong, Shanxi, China

\(^{3}\)School of Chinese Medicine and Food Engineering, Shanxi University of Traditional Chinese Medicine, 030619 Jinzhong, Shanxi, China

\(^{4}\)State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, Guangdong Provincial Key Laboratory of Plant Molecular Breeding, South China Agricultural University, Guangzhou 510642, China

\(^{a}\)wyd112156074@163.com, \(^{b}\)jiaminwang1997@163.com, \(^{c}\)wyl@sxtcm.edu.cn, \(^{d}\)zmzhang@scau.edu.cn

**ABSTRACT :** To develop the optimal drying method for the processing of *Sophora japonica* L., three approaches including freeze-drying, air-drying and oven drying were investigated. The water contents in dried Sophora flower samples treated using different drying methods were compared. The total amounts of Flavonoids in methanol extracts of dried Sophora flower were measured using UV-vis spectrophotometer, and the amounts of Rutin were measured using High Performance Liquid Chromatography (HPLC). The antioxidant capacity of extract of oven dried Sophora flower was determined by three different antioxidant assays, including measurement of clearance ability for DPPH, clearance ability for free hydroxyl radical and Ferric reducing antioxidant power (FRAP) assay. In our research, freeze-drying method demonstrated a minimal impact on the morphology, color, smell and other features of Sophora flower, and the freeze-dried Sophora flower reserved the highest value of total Flavonoids component amount. However, Sophora flower dried in the oven had the lowest water content and highest Rutin amount. In addition, oven dried Sophora flower could be stored easily and had less loss of the active components. Sophora flower has been demonstrated to be antioxidant in chemical reactions. In the measurement of clearance ability of DPPH, the clearance activity was positively correlated to Sophora flower extract concentration. In terms of clearance ability for free hydroxyl radical, the antioxidant capacity of Sophora flower extract increased with the increase of its concentration in the low concentration range, while after exceeding a certain concentration, clearance activity reduced with the increase of concentration. In FRAP assay, the total antioxidant capacity of Sophora flower extract first decreased as concentration increased when concentration was below 0.2 mg/mL, but then increased with the concentration after exceeding 0.2 mg/mL. In conclusion, oven drying method could be applied in the processing of Sophora flower. Sophora flower possesses good antioxidant capacity and this could be the theoretical basis for using it in the production of health products.
1. Introduction

Sophora japonica L. contains various active components, and it has been reported that the flower of Sophora have several features including antioxidant capacity, the ability for vasodilation and antibacteria capacity. The flower of Sophora has been widely used in the food products. For the processing of Sophora flower, it is normally collected in the summertime and processed by several steps including drying, removing the branches, root and other contaminations.

The volatile oil of Sophora flower contains various components including 1-Hexanol, Linalool, Palmitic acid, Nerolidol, and 6,10,14-trimethyl-2-Pentadecanone. The volatile oil is reported to have significant clearance ability of the DPPH and ABTS free radicals [1,2]. Sophora flower contains large amounts of Flavonoids components and obtains significant pharmacological activity. The major components in the flower of Sophora are Rutin, Nicotiflorin, Narcissoside and Quercetin [3-7]. Reports demonstrated that the Flavonoids components in Sophora flower had a great therapeutic effect on the streptozotocin induced diabetic rat models. The level of glucose in serum and Leptin in rats decreased significantly while the level of insulin in serum and C-peptide increased significantly [8].

The polysaccharide in Sophora flower has been reported to have the ability to enhance the immune system as well as to improve the ability for anti-bacteria and antioxidant [9-12]. In addition, the polysaccharide in Sophora flower has also been proven to improve the immune response of rex rabbit against Bordetella bronchiseptica [13]. Sophora flower contains various proteins, and the proteins in the lyophilized Sophora flower show better performance in the foam stability, moisture retention ability and clearance of free hydroxyl radical, and the proteins in the flower of Sophora dried in the vacuum condition show better viscosity and inhibition rate of lipid peroxidation free radicals [14].

The major investigation of Sophora flower focuses on the extraction of Flavonoids components and its application in the hemostasis and treatment of cardiovascular disease [15]. In this work, the impact on the Flavonoids components of the different drying methods after collection was investigated. The antioxidant capacity of Sophora flower was examined to provide the direction for the selection of drying methods for Sophora flower.

2. Devices and reagents

2.1 Chemicals and reagents

Sophora japonica L. (from the campus of Shanxi University of Chinese Medicine), Rutin (100080-200707, Chinese National Institutes for Food and Drug Control). Methanol (HPLC grade, batch number: 20171201, Damao Chemical Reagent Factory). Aluminum Nitrate (batch number: 20160808, Tianjin Tianli Chemistry Reagent Co., Ltd.), Sodium Nitrite (batch number: 20160818, Tianjin Tianli Chemistry Reagent Co., Ltd.), Sodium Hydroxide (batch number: 20110104, Tianjin University Chemical Industry), Absolute Ethanol (batch number: 20160901, Tianjin Beichenfangzheng Chemical Reagent Factory), 1,1-diphenyl-2-picyrylhydrazyl (DPPH, TCI), 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine (TPTZ, Nanjing Duly Biotech Co., Ltd), Vitamin C (Vc, Shanxi Taiyuan Pharmaceutical Industry Co., Ltd.), Phenanthroline, ferrous sulfate, sodium dihydrogen phosphate, sodium hydroge phosphate (Tianjin Kemiou Chemical Reagent Co., Ltd.).

H$_2$O$_2$ (batch number: 20160607), Acetic Acid (batch number: 20090210), Ferrous Sulfate (batch number: 20160223), Sodium Acetate (batch number: 20130828), FeCl$_3$ (batch number: 20100910), Phenanthroline, ferrous sulfate, sodium dihydrogen phosphate, sodium hydrogen phosphate (Tianjin Kemiou Chemical Reagent Co., Ltd.).

2.2 Devices

Christ Alpha Alpha-4 LD Plus freeze dryer (Martin Christ, Germany), GZX-9140MBE Electric heating blast drying oven (Shanghai Boxun Industrial Co., Ltd. China), TU-1810 UV/vis spectrometer (Beijing Persee, China), EX125ZH scale (Ohaus Instrument, Shanghai, China), RIGOLL—3000 HPLC system (Guangzhou Evertech Instrument Technology Co., Ltd. China), SC-02 low speed centrifuge (Anhui
3. Methods

3.1 Different drying methods for Sophora flower

Fresh Sophora flowers (Figure 1) were collected and divided into 9 groups. The samples were dried using the three different methods as describe below, each method was repeated three time \cite{16-17}.

Air-drying: samples were put in the plate evenly and placed in the room avoiding the direct sunlight at room temperature (25-30 °C).

Freeze Drying: samples were placed in the freeze dyer at –80 °C for 24 h.

Oven drying: samples were placed in the plate evenly and put in the oven at 80 °C for 4 - 5 h.

3.2 Water content of dried Sophora flower

The water contents left in Sophora flower samples after being dried with different methods as mentioned previously were measured using direct drying methods.

3.3 Measurement of total Flavonoids components

50 mg Rutin standard sample was dissolved with methanol in the water bath. After Rutin standard sample was completely dissolved, the solution was cooled to room temperature. Methanol was added to top up the volume of dissolved Rutin to 25 mL. 10 mL dissolved Rutin sample was diluted to 100 mL using water for the following preparation of the Rutin reference samples.

For the preparation of Rutin reference samples with different concentrations, firstly, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL and 6 mL of diluted Rutin solutions were further diluted with water to a final volume of 6 mL. Secondly, 1 mL of 5 % sodium nitrite was added to all 6 Rutin samples and the mixtures were placed for 6 min. After that, 1 mL of 10 % aluminum nitrate was added to each sample, and the mixtures were placed for another 6 min after mixing. Finally, 10 mL sodium hydroxide was added, and the sample volumes were adjusted to 25 mL with water. The final Rutin mixtures (Rutin reference samples) were placed for 15 min before the absorbance measurement at 394 nm. The standard curve was plotted using absorbance at 394 nm for y-axis and the concentrations of Rutin for x-axis \cite{5,18}.

1 g of dried Sophora flower powder was taken and placed in the Soxhlet extractor. Ether was added and the mixture was heated until the ether extracted solution was clear. The mixture was cooled, and ether extract was discarded. Then 90 mL methanol was added to the Soxhlet extractor and was heated until the methanol extract was clear. The methanol extract was then transferred to the 100 mL volumetric measurement flask. Methanol used for the rinsing of the extractor was combined with the extract, and the final methanol extract volume was adjusted to 100 mL by adding methanol. 10 mL of the methanol...
extract was diluted 10 times using water. 3 mL of the diluted extract was further diluted to 25 mL with water for absorbance measurement. The absorbance at 394 nm was measured and the concentrations of Flavonoids components were calculated using the standard curve.

3.4 Measurement of Rutin in Sophora flower
Octadeclisilane bonded silica gel was used as the stationary phase of HPLC, and Methanol-1 % Acetic acid (v/v = 32/68) was used as the mobile phase. The detected wavelength was 360 nm. The theoretical plate number (TPN) of the peak for Rutin should not be lower than 2000 [19-20].

Rutin standard sample was dissolved by methanol to produce the 0.1 mg/mL standard solution. 0.2 mg of dried Sophora flower powder was taken and placed in the Erlenmeyer flask, and mixed with 50 mL of methanol. The mixture was weighted and processed using ultrasonicator (220 W power, frequency 25 kHz) for 30 min. The solution was cooled down and methanol was used to make up the weight loss. Solution was then mixed thoroughly and filtered. 2 mL of filtered solution was diluted with Methanol to 10 mL, and the final sample was mixed thoroughly for the testing.

10 μL of reference solution and sample solution were taken. The amount of Rutin in Sophora flower was examined using one-point method.

3.5 Investigation of antioxidant capacity

3.5.1 Measurement of DPPH free radical clearance ability. The methanol extract of oven dried Sophora flower was diluted to produce 1 mg/mL, 0.8 mg/mL, 0.6 mg/mL, 0.4 mg/mL, and 0.2 mg/mL Sophora flower solutions. Vitamin C (Vc) solutions of the same concentrations were also prepared. 0.5 mL of each Sophora flower solution and each Vc solution were mixed with 2.5 mL of 1.52x10⁻⁵ M DPPH methanol solution. The mixed samples were reacted for 30 min at room temperature in dark. Absorbance at 517 nm of samples (As) were measured using UV-vis spectrophotometer. The absorbance of absolute ethanol was used as blank control (Ac). Three parallel measurements were conducted. The clearance rate of DPPH free radical was calculated using the equation 1 as follows:

\[ \text{DPPH clearance rate (\%)} = \left(1 - \frac{\text{As}}{\text{Ac}}\right) \times 100\% \]  

(1)

3.5.2 Clearance ability for free hydroxyl radical. Oven dried Sophora flower methanol extract was diluted to produce 0.4 mg/mL, 0.2 mg/mL, 0.1 mg/mL, 0.04 mg/mL and 0.02 mg/mL Sophora flower sample solutions, and Vc solutions of the same concentrations were also prepared. 1 mL of each Sophora flower sample solution and Vc solution were mixed with 1 mL of 0.75 mM o-phenanthroline ethanol solution, 2 mL of PBS, pH 7.4, 1 mL of 0.75 mM FeSO₄, and 1 mL 0.01 % H₂O₂. The samples were mixed thoroughly and reacted in the water bath at 37 °C for 1 h, before the absorbance of each sample at 510 nm (Bs) was measured. Pure methanol was used as blank control and its absorbance was marked as Bc. Control sample was prepared using methanol replacing Sophora flower solution or Vc solution with the same volume and distilled replacing H₂O₂ with the same volume and its absorbance was marked as Bb. The clearance rate of hydroxyl radical was calculated using Equation 2:

\[ \text{Clearance rate of OH (\%)} = \left(\frac{\text{Bs} - \text{Bc}}{\text{Bb} - \text{Bc}}\right) \times 100\% \]  

(2)

3.5.3 Ferric reducing antioxidant power (FRAP) assay. 200 μL of Sophora flower sample solution and Vc solution prepared in section 3.5.2 was mixed with 6 mL FRAP working solution and 600 μL of distilled water. The mixed samples reacted in the 37 °C water bath for 10 min. The absorbance at 593 nm of each sample was then examined. Different concentrations of FeSO₄ were used for the standard curve. The antioxidant capacity of the sample is linear positively related to its absorbance at 593 nm. The values of the antioxidant capacity of the sample were illustrated using FRAP value (1 mM FeSO₄ =1 FRAP value). The higher the FRAP value is, the better antioxidant capacity of the sample is.

According to previous work, the FRAP standard curve is:

\[ A = -0.0248C + 0.664 \]  

\[ R^2=0.9924 \]  

(3)
4. Results

4.1 Determination of Sophora flower water content
Water contents of nine Sophora flower samples treated with three different drying approaches were measured through direct drying method, and the results are shown in Figure 2. The order of the water content of each group treated with different drying methods are as follows: oven drying group > air-drying group > freeze-drying group. Samples in oven drying group had significantly lower water content than those in groups treated with other two methods.

![Figure 2: Water contents of dried Sophora flower treated with different drying methods.](image)

4.2 Determination of total Flavonoids contents

4.2.1 Standard curve. Figure 3 shows the standard curve for total Flavonoids determination. The equation is \( Y = 17.195x + 0.0096, \) \( R^2 = 0.9938. \) It shows that Rutin concentration has a good linear relationship with absorbance in the range of 0.008 ~ 0.048 mg/mL.

![Figure 3: Standard curve of Rutin concentration vs absorbance at 394 nm.](image)

4.2.2 Determination results of total Flavonoids contents. Figure 4 shows the total Flavonoids contents of dried Sophora flower samples obtained from different drying methods. The contents of total Flavonoids from high to low were shown as the order: freeze-drying group > air-drying group > oven drying group. The total Flavonoids contents of dried Sophora flower after freeze-drying were the highest, and the total Flavonoids amounts of Sophora flower after air-drying and oven drying were similar to each other.
Figure 4: Total Flavonoids contents of dried Sophora flower treated with different drying methods, the data are presented with mean ± standard deviation, n = 3.

4.3 Determination results of Rutin contents
In HPLC, the retention time of Rutin was 21.973 min. When Rutin standard sample concentration was 0.125 mg/mL, peak area was 3012.121. According to HPLC analysis results, as shown in Figure 5, the order of Rutin contents in dried Sophora flower samples were as follows: oven drying group > freeze-drying group > air-drying group. Oven drying retained the highest amount of Rutin.

Figure 5: Rutin contents of dried Sophora flower treated with different drying methods, the data are presented with mean ± standard deviation, n = 3.

4.4 Antioxidant capacity
4.4.1 DPPH free radicals clearance activity. As shown in Figure 6, Sophora flower extract was demonstrated to obtain the DPPH free radical scavenging ability. When concentration was 0.2 mg/mL, clearance rate of Vc was 89.71 %, which was higher than Sophora flower extract of the same concentration. This suggests that Sophora flower extract has a relatively weak DPPH scavenging ability compared with Vc. In addition, the DPPH clearance rate was positively correlated with the concentration of Sophora flower extract.
4.4.2 Clearance ability for free hydroxyl radical. It is illustrated in Figure 7 that the clearance activity of Sophora flowers with different concentrations on hydroxyl free radicals increased initially and then decreased with the increase of concentration. The turning point was at 0.1 mg/mL. The clearance rate of the hydroxyl free radicals of VC at 0.2 mg/mL was 72.73 %, which was significantly higher than that of Sophora flower extract at the same concentration.

4.4.3 FRAP antioxidant capacity. Based on the results shown in Figure 8, in the range of 0.02- 0.2 mg/mL, the antioxidant capacity of the Sophora flower extract sample decreased as concentration of Sophora flower extract increased. However, after exceeding 0.2 mg/mL, its antioxidant activity increased as concentration increased. It confirms that Sophora flower contains antioxidant active components, and the contents of antioxidant components is related to its antioxidant capacity to some extent.
5. **Discussion**

Through investigation of the effects of different drying methods on the total Flavonoids and Rutin contents in the Sophora flower, the freeze-drying method had the least influence on the morphology and water content. Air-drying method took a longer time, but did not require any equipment and was economical and practical. Oven drying method had the greatest impact on the morphology and odor of Sophora flower samples. After oven drying, the sample turned to slightly yellow in color and had a burnt fragrance. All three drying methods could reach the water content criterion of the "2015 Chinese Pharmacopoeia", and the water contents were less than 11%. Among three approaches, oven drying achieved the lowest water content, and the dried samples were more convenient for storage.

In this study, UV-vis spectrophotometry and HPLC were used to determine the total Flavonoids and Rutin contents of dried Sophora flowers obtained from different drying methods. The results showed that for the total Flavonoids, freeze-dried Sophora flowers had the highest contents, whilst air-dried samples and oven-dried samples had similar contents. As for the effective component, Rutin, oven drying reserved the highest contents, freeze-drying method had the second highest contents, and air-drying method has the least contents. Therefore, the drying method can be selected according to the changes in the contents of the effective components. Overall, oven drying method is simple and effective. The impact on effective components contents is also minor, which ensure it to be a promising drying method for the processing of Sophora flower.

Antioxidant studies of Sophora flower extract show that Sophora flower has antioxidant activity. In the experiment of measuring DPPH free radical scavenging ability, clearance rate increased with the increase of the concentration of Sophora flower extract. For hydroxyl free radical scavenging ability, when the concentration was low, the antioxidant capacity increased with the increase of the concentration of the extract. Above a certain concentration, the antioxidant capacity reduced with the increase of the concentration. In FRAP experiment, there was a negative correlation between the antioxidant activity of the extract and its content when below 0.2 mg/mL, and the correlation reversed when exceeding 0.2 mg/mL.

Additionally, the comparison of the antioxidant activities of Sophora flower and Vc shows that the antioxidant activity of Sophora flower was lower than that of Vc. However, because of the enhancement in the immunological activity, antibacterial activity, antioxidant capacity and other functions, Sophora flower is still of great value in the production of health products.

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**REFERENCES**

[1] G. Q. Zhu, F. Chen, Y. Feng, J. Y. Wang, H. F. Ren, X. M. Ma, and S. F. Bi, (2015). GC-MS analysis of essential oil from fresh Sophora flower and its antioxidant activity. China Condiment, 40(6), 115–118.

[2] Y. He, (2020). Response surface optimization of microwave-assisted extraction process of Sophora flower essential oil. The Food Industry, 41(06):28-31.

[3] W. J. Li and Z. H. Gao, (2020). Comparison of nutrient composition and Rutin content between Sophora flower and Sophora japonicus. The Food Industry, 41(03):337-339.

[4] W. X. Pi, W. W. Zhao, B. C. Cai, T. L. Lu and H. L. Yu, (2018). Preparation of Sophora flower control extract and determination of four Flavonoids in Sophora flower. China Pharmacy, 29(19):2652-2656.

[5] Y. Q. Ma, S. S. Guo, J. Ma and Y. L. Liu, (2018). Comparative study on the content of stotal Flavonoids in different Sophora flower processed products. Lishizhen Medicine and MateriaMedica Research, 29(01):76-78.
[6] H. Xia and M. M. Peng, (2014). Simultaneous determination of Rutin, quercetin and kaempferol in Sophora flower by HPLC. Applied Chemical Industry, 43(10):1919-1921.

[7] X. N. Zhu, W. W. Cao and M. J. Li, (2012). Ionic liquid optimized High Speed Countercurrent Chromatography for separation of Flavonoids from Sophora flower. Natural Product Research and Development, 24(12):1833-1836.

[8] M. S. Miao, C. R. Li, and Y. M. Chen, (2011). Effects of Sophora flower total flavonoids on serum insulin, leptin and C-peptide levels in diabetic rats. Chinese Journal of Modern Applied Pharmacy, 28(10):896-898.

[9] X. L. Hu, Q. Jiang, F. J. Ying and Z. Y. Song, (2012). Orthogonal experiment to optimize the optimal extraction technology and antibacterial activity of Sophora flower polysaccharide. Food Science and Technology, 37(04):164-167.

[10] X. Y. Cao and H. T. Yang, (2016). Optimization of extraction process and free radical scavenging activity of Sophora flower. Northern Horticulture, (13):141-146.

[11] R. S. Li, D. S. Jia, C. X. Wen, S. Z. Cui, L. D. Liu and X. L. Xie, (2016). Study on the effect of Sophora flower polysaccharide on immune function of immunosuppressed mice. Food Research and Development, 37(24):155-159.

[12] Z. J. Chen, L. H. Li, C. F. Li and Y. Hu, (2016). The study of the impact of Sophora flower polysaccharide on immune regulation in mice. Chinese Journal of Veterinary Medicine, 52(03):115-117.

[13] Q. Y. Zhao, M. F. Liang, X. Z. Gao, Z. M. Wang, C. L. Zhao and R. L. Zhu, (2013). The immune enhancement effect of Sophora flower polysaccharide on Bordetella bronchiseptica. Chinese Journal of Veterinary Medicine, 33(02):268-271.

[14] L.H. Ma, W. D. Qin, X. H. Chen and W. W. Cao, (2014). Effects of different drying methods on the processing characteristics and antioxidant properties of Sophora flower protein. Food Science and Technology, 39(09):104-108.

[15] J. J. Jia and M. S. Miao, (2014). The chemistry, pharmacology and clinical application of Sophora flower. Acta Chines Medicine, 29(05):716-717+745.

[16] X. Li, C. Y. Li, Z. Z. Qiang, J. G. He, J. H. Zhu, B. Li, S. Li, Y. Wang and G. S. Zheng, (2019). Determination of index components in Chinese Angelica processed by different drying methods. Modern Chinese Medicine, 21(08):1110-1113.

[17] C. Y. Qiu, H. M. Kong and F. Z. Zheng, (2020). Effects of different drying methods on Flavonoids content and antioxidant activity of Dendrobium officinale flour. Lishizhen Medicine and Materia Medica Research, 31(03):598-600.

[18] J. L. K, Z. F. Cen, R. B. He, F. P. Yang, S. J. Zheng and G. R. Luo, (2015). Optimization of the extraction process of total flavonoids from Sophora flower. Foshan University (Natural Science Edition), 33(05):48-51.

[19] H. T. Chu, J. Wang, H. Y. Qi, L. D. Gao and L. Q. Su, (2017). Rapid extraction of flavonoids in Sophora japonica and liquid chromatography analysis of Rutin and quercetin. Heilongjiang Animal Science and Veterinary Medicine, (17):223-225.

[20] Z. C. Li, Z. Y. Liang and Y. Z. Xu, (2016). Determination of Rutin in Jindan Sophora flower granules by High Performance Liquid Chromatography. Journal of North Pharmacy, 13(03):5-6.