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Study on Extraction and Purification of Total Flavonoids from Lamiophlomis Rotata

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Abstract:

Lamiophlomis rotata (Duyiwei, DYW) is the dry aboveground part of Lamiophlomis rotata (Benth.) Kudo. The total flavonoids of DYW were extracted by ethanol heating and reflux. With rutin as the reference substance, an ultraviolet spectrophotometry was established to determine the content of total flavonoids of DYW, and the methodology was verified. Taking the yield and content of total flavonoids as the investigation index, the optimum extraction process conditions were optimized by single factor and orthogonal test. Finally, the optimum extraction conditions of total flavonoids of DYW were as follows: solid-liquid ratio 1:6 (g/mL), extraction temperature 60 ℃, extraction time 1.5h and ethanol concentration 85%. Under these conditions, the yield of total flavonoids of DYW could reach 30.24% and the purity could reach 22.78%. Different types of macroporous resins were investigated according to the static adsorption experiment, and AB-8 macroporous resin was selected. Then, the saturated adsorption capacity, pH value of loading solution and concentration of eluting solvent were investigated through the dynamic experiment to determine the method for efficient separation and purification of total flavonoids from ethanol extract. The results show that the optimum process conditions are pH = 5, dynamic saturated adsorption capacity of 3.2 g/g and elution with 80% ethanol. When AB-8 macroporous resin combined with polyamide resin was used to purify the total flavonoids of DYW, the purity was 92.86% and the yield was 69.74%.

Keywords*: Lamiophlomis rotata, Total flavonoids, Extraction, purification, Yield, AB-8 macroporous adsorption resin.*

I. INTRODUCTION

Lamiophlomis rotata (Benth.) Kudo (DYW) is the only plant of Lamiophlomis in Labiatae, also known as dubutong, also known as "Daba" and "dabuba" in Tibetan. It was first recorded in the famous Tibetan medical books “Jingzhu Bencao” and “Four Medical Classics”, with a history of 1200 years [1]. DYW is widely distributed in Tibet, Sichuan, Qinghai, Gansu and other regions in China. It is one of the commonly used medicinal materials of Tibetan, Mongolian and Naxi nationalities [2]. As a traditional

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national medicine, its roots and rhizomes or whole grass are used as medicine. The surface of the medicinal material is dry yellow or yellowish brown, bitter and flat. It has the effects of hemostasis and analgesia, promoting blood circulation and removing blood stasis, antibacterial and anti-inflammatory. It is mainly used to treat traumatic injury, traumatic bleeding, rheumatic arthralgia and Yellow water disease [3]. It is often used as oral agent to treat bone trauma, gynecological bleeding, epistaxis, ophthalmic diseases, oral ulcer, ulcerative conjunctivitis, herpes zoster, lumbar disc herniation and other diseases. It has clear anti-inflammatory, analgesic, hemostatic and other effects [4].

DYW mainly contains flavonoids, iridoids, phenylethanol glycosides, volatile oil and other components [5]. Flavonoids are mainly quercetin, luteolin, luteolin-7-O-glucoside, quercetin-3-O-arabinoside, and luteolin-7-O-β-D-glucopyranoside, forsythin B, apigenin, apigenin-7-O-β-D-glucopyranoside, apigenin-7-O-(6-O)-β-D-furan (celery sugar)-β-D-glucopyranoside et al.[5-7]. The 2020 edition of Chinese Pharmacopoeia states that the aboveground part of DYW is calculated as dry product, and the total amount of geniposide methyl ester ($C_{17}H_{26}O_{11}$) and 8-O-acetylgeniposide methyl ester ($C_{19}H_{28}O_{12}$) shall not be less than 0.50% [8].

The extraction process of total flavonoids from DYW was studied in this paper. The total flavonoids of DYW are difficult to dissolve in water and easy to dissolve in ethanol. In this study, ethanol was used as the solvent to extract the total flavonoids of DYW, rutin was used as the reference substance, the content of total flavonoids was determined by UV spectrophotometry, the standard curve of total flavonoids was established, and the methodology was investigated. The results showed that the method had high sensitivity, simple operation, accurate results and strong specificity. The ethanol extraction process was optimized by single factor influence experiment and orthogonal experiment. The yield and purity of total flavonoids of DYW were used as the indexes to optimize the extraction process conditions.

At present, the separation and purification of substances by macroporous adsorption resin is more and more widely used in the pharmaceutical field. We studied the purification process of total flavonoids of DYW by macroporous adsorption resin. The adsorption and desorption capacities of several different types of macroporous adsorption resins were studied, and AB-8 type with excellent performance was selected as the macroporous adsorption resin for the purification of total flavonoids of DYW. By studying the dynamic and static purification process of AB-8 macroporous adsorption resin and optimizing various parameters, the optimum purification process conditions of total flavonoids of DYW were determined [9]. The extraction and purification process of total flavonoids of DYW was studied to provide theoretical basis for subsequent activity research and valuable basis for product development.

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II. MATERIALS AND METHODS

2.1 Experimental Materials

D101 Macroporous adsorption resin (Shanghai mosu scientific equipment Co., Ltd), AB-8 Macroporous adsorption resin (Shanghai mosu scientific equipment Co., Ltd), HPD500 Macroporous adsorption resin (Cangzhou Baoen adsorption material technology Co., Ltd), HPD600 Macroporous adsorption resin (Cangzhou Baoen adsorption material technology Co., Ltd), Methanol (AR), (Shanghai Titan Chemical Co., Ltd, batch number: P1410953), lamiophlomiol standard (China Institute for the control of pharmaceutical and biological products, batch number: 200602), DYW, (Anguo Shenghui traditional Chinese medicine decoction pieces Co., Ltd, batch number: 1704075).

2.2 Experimental Instruments

Bath pot (B-260 type): Shanghai Yarong Biochemical Instrument Factory; Electric heating mantle (DZTW type): Beijing Yongguangming Medical Instrument Factory; pH meter (PHSJ-3F type): Shanghai Precision Scientific Instrument Co., Ltd.; Rotary evaporator (RE52CS-1 type): Shanghai Yarong Biochemical Instrument Factory; Medicine sieve (GB6003.1-1997 type): Wusi Sieve Factory, Shangyu City, Zhejiang Province; Grinder (GY-FS-06 type): Jiangxi Ganyun Food Machinery Co., Ltd; Digital display thermostatic magnetic stirrer (85-2 type): Changzhou Jintan Kexing Instrument Factory; Ultraviolet-visible spectrophotometer (LIV-1800PC): Shanghai Mepuda Instrument Co., Ltd.

2.3 Extract

Crush the DYW herb and pass through 40-mesh sieve, accurately weigh 100g of the DYW powder, add a certain concentration of ethanol, heat at reflux and extract for a certain time, then filter.

2.4 Make UV Standard Curve

2.4.1 Establishment of standard curve

Preparation of the reference solution: accurately weigh out 5.0 mg of the rutin reference substance that has been dried under reduced pressure to a constant weight, dissolve it with 95% ethanol, shake it, and dilute to 25.0mL to make it a unique concentration of 200 μg/mL. Blindly taste the total flavonoid standard solution as a stock solution for later use.

Preparation of the test solution: take the DYW pieces, smash, pass through a No. 4 sieve, accurately weigh 0.2000 g, add 50 mL of methanol, weigh, heat and reflux in a water bath for 1 h, let cool, then

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weigh, and use methanol to make up the reduction. Lost weight, shake well, filter through 0.45 μm microporous membrane, take the filtrate, and get it.

Take a small amount of standard solution diluent and sample extract to scan the full wavelength with an ultraviolet spectrophotometer to find the maximum absorption wavelength.

Preparation of the standard curve: Precisely draw the standard solution 0, 1.0, 2.0, 3.0, 4.0, 5.0 mL of the DYW total flavonoids in a 10 mL colorimetric tube, add 95% ethanol to the mark, shake well, and set it at the maximum absorption wavelength of 505 nm Measure the absorbance, and the absorbance value is shown in Table I. The linear regression method is used to calculate the regression equation of the standard curve for the measured data, and the standard curve is drawn with absorbance as the abscissa and the concentration of the reference substance as the ordinate, as shown in Fig 1.

2.4.2 Stability test

Accurately weigh 1.0 mg of the sample in 1.1, dilute to 25 mL with 95% ethanol, store in the dark at room temperature, and sample at 361 at 0 h, 1 h, 2 h, 4 h, 6 h, 12 h, and 24 h. Measure the OD value of the sample solution at nm. Record the absorbance value and calculate the RSD value. The results are shown in Table II.

RSD%=Standard deviation of measurement results/arithmetic mean of measurement results x 100%.

2.4.3 Precision test

Take a sample to prepare according to the above-mentioned sample solution preparation method, and then divide it into 3 parts. According to the operation steps of 4.1, determine the OD value according to the law, and read the DYW total flavonoids in each test solution from the standard curve. Content, RSD is calculated according to the formula in 4.2, and the results are shown in Table III.

2.4.4 Reproducibility test

5 mL of each of the five treated samples was precisely drawn into five 10 mL volumetric flasks, and the volume was fixed to the scale, measure the absorbance value according to the method under the standard curve preparation item, and read the test solution from the standard curve. The total flavonoid content of DYW was calculated according to the formula RSD, and the results are shown in Table IV.

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2.4.5 Sample recovery test

Take the test substance of known content to make the same concentration solution, draw the same amount and add different amounts of the reference solution of known concentration, according to the operation steps of 4.1, measure the absorbance value, and read it from the standard curve The content of total flavonoids in each test solution was calculated according to the following formula, and the results are shown in Table V.

Recovery rate=content measured after mixing-total flavonoid content in the sample / standard addition amount of total flavonoids in DYW x100%.

2.4.6 Assay

Accurately weigh 0.2 g of three different batches of DYW powder, prepare the test solution according to the method under 4.1, measure the absorbance at the maximum absorption wavelength, and determine the total flavonoid content of each batch of DYW. The results are shown in Table VI.

2.5 Method for Determining the Yield and Purity of Total Flavonoids in DYW

Operate the test product according to the method under 4.1, measure the absorbance at the maximum absorption wavelength, and then calculate the total flavonoid mass concentration of DYW according to the regression equation.

Purity of total flavonoids of DYW (%) = content of total flavonoids(g)/quality of total flavonoids (g)×100%.

Total flavonoids yield of DYW (%) = mass of total flavonoids(g)/quality of DYW medicinal materials (g)×100%.

2.6 Effect of Ethanol Concentration on Extraction of Total Flavonoids from DYW

The ratio of fixed material to liquid was 1:10 (g/mL), the extraction time was 2 h, the extraction temperature was 90 ℃, and the extraction was conducted once. The different ethanol concentrations were investigated, 80%, 85%, 90% and 95%. The purity and yield of total flavonoids of DYW were taken as the investigation indexes, as shown in Table VII and Fig 2.

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2.7 The Effect of Material-liquid Ratio on the Extraction of Total Flavonoids from DYW

The ethanol concentration is fixed at 90%, the extraction time is 2 h, the extraction temperature is 90 ℃, and the extraction is performed once. The different material-to-liquid ratios (1:6, 1:8, 1:10, 1:12) (g/mL) are investigated. The effect of the purity and yield of total DYW flavonoids.

2.8 Effect of Extraction Time on the Extraction of Total Flavonoids from DYW

Fixed ethanol concentration of 90%, extraction time of 2 h, extraction temperature of 90 ℃, extraction once. The effects of different solid-liquid ratios (1∶6, 1∶8, 1∶10, 1∶12) (g/mL) on the purity and yield of total flavonoids of DYW were investigated.

2.9 The Effect of Extraction Temperature on the Extraction of Total Flavonoids from DYW

Fixed ethanol concentration 90%, solid-liquid ratio 1:6 (g/mL), extraction time 2h, extraction once. The effects of different extraction temperatures of 60℃, 70℃, 80℃, 90℃ and 100℃ on the purity and yield of total flavonoids of DYW were investigated.

2.10 The Effect of Extraction Times on the Extraction of Total Flavonoids from DYW

Fixed ethanol concentration 90%, solid-liquid ratio 1:6 (g/mL), extraction time 2H and extraction temperature 70 ℃. The effects of different extraction times of 1, 2 and 3 times on the purity and yield of total flavonoids of DYW were investigated.

2.11 Orthogonal Tests

L9 (3^4) orthogonal table was used to design the orthogonal test. On the basis of single factor experiment, four factors with great influence, such as ethanol concentration, solid-liquid ratio, extraction temperature and extraction time, were selected to optimize the combination. The investigation index was the yield and purity of total flavonoids of DYW. See table XII for the level of factors.

2.12 Verification Tests

Three parallel experiments were carried out according to the above optimized extraction process conditions of total flavonoids of DYW. Accurately weigh 100g of DYW 40 mesh sieve, and extract the total flavonoids of DYW under the conditions of solid-liquid ratio of 1:6 (g/mL), extraction temperature of 60 ℃, extraction time of 1.5h and ethanol concentration of 85%. The final results are shown in table XV.

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2.13 Purification Method of Total Flavonoids of DYW

2.13.1 Pretreatment of macroporous resin

During the production of macroporous adsorption resin, it is usually not purified, and some preservatives may be added, so pretreatment must be carried out before the resin is used. Heat and refluxe with ethanol 3 ~ 4 times the weight of AB-8, D101, hpd500 and HPD600 macroporous adsorption resins for 3 hours, wash with distilled water until there is no alcohol taste, and the washed liquid is clear without turbidity.

2.13.2 Preparation of loading solution

According to the optimal extraction process of total flavonoids of DYW optimized in previous experiments, accurately weigh 250g of DYW powder (passing 40 mesh sieve), extract twice with 85% ethanol for 1.5 h each time, the extraction temperature is 60 ℃, and the material liquid ratio is 1:6 (g/mL). Combine the filtrate twice, record the volume, spin until it is nearly dry without alcohol flavor, add 40% ethanol of the same volume to make the suspension, and then obtain the total flavonoids loading solution of DYW. The concentration of raw medicine is 41.67 mg/mL.

2.13.3 Calculation of adsorption rate and resolution rate

2.13.3.1 Adsorption rate

Accurately weigh 2.0 g of resin into a triangular flask with a stopper, add 100 mL of the sample solution under 3.8.2, and shake in a shaker for 4 h (25 ℃, 130 r/min). Make it fully adsorb, filter, calculate the concentration of the supernatant, and calculate the adsorption rate according to the following formula.

Adsorption rate (%)=(C0-C1)/C0×100%.

In the formula: C0 is the mass concentration of the sample solution before adsorption (mg/mL), C1 is the mass concentration of the filtrate after adsorption (mg/mL).

2.13.3.2 Resolution rate

Rinse the adsorbed resin with distilled water until it is colorless, add 100 mL of 95% ethanol, shake in a shaker for 4 hours (25 ℃, 130 r/min), filter, determine the concentration of the desorbed solution,

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and calculate the resolution rate by the following formula.

Resolution rate (%)=(C2×V2)/(C0-C1)V1×100%.

In the formula: C2 Mass concentration of desorption solution (mg/mL), V2 Desorption liquid volume (mL), V1 Sample volume (mL).

2.13.4 Screening of macroporous resin

2.13.4.1 Macroporous resin adsorption experiment

Accurately weigh 2.0 g of AB-8, D101, HPD500, HPD600 resin into a cork flask, and calculate the adsorption rate according to the method under 13.3.1.

2.13.4.2 Macroporous resin desorption test

Rinse the adsorbed AB-8, D101, HPD500, HPD600 resins with distilled water until they are colorless, and calculate the desorption rate according to the method under 13.3.2.

2.13.5 Determination of the pH value of the adsorption solution

Use sodium hydroxide and concentrated hydrochloric acid solution to adjust the pH of the loading solution in 1.13.2 to 4, 5, 6, 7, and 8, respectively. Take 100 mL of each into a cork with a stopper, and add 2.0 g of AB-8 resin. Placed on a constant temperature shaker and oscillated for 4h (25 ℃, 130 r/min) to make it fully adsorbed, suction filtered, determine the concentration of phenolic acid in the supernatant, and calculate the adsorption rate.

2.13.6 Determination of saturated adsorption capacity

Weigh 10g AB-8 macroporous resin and install the column by wet method. Take the total flavonoids loading solution of DYW, prepare a diluted loading solution with a concentration of 20.84 mg/mL with 40% ethanol, adsorb it through the resin column at a flow rate of 2BV / h, and collect the effluent by sections. Each 50mL is one part, a total of 50 parts are collected. The effluent is detected by thin-layer chromatography, and the absorbance of each effluent is measured. Taking the number of collected parts of the eluent as the abscissa and the mass concentration as the ordinate, the dynamic adsorption leakage curve of the resin is drawn, and the loading volume is determined.

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2.13.7 Determination of eluent concentration

Weigh 10 g of AB-8 macroporous resin, wet mount the column, add the loading solution according to the optimal loading volume to the resin bed, elute with 2 bv distilled water, 40%, 60%, 80% and 95% ethanol successively at a flow rate of 2BV / h, collect one part of distilled water separately, collect 35 parts of other eluents every 200ml, measure the absorbance respectively, draw a curve and determine the optimal elution concentration.

2.13.8 Verification test

In order to verify the feasibility of the optimized process, the test was carried out according to the optimized process conditions. The eluent was concentrated under reduced pressure, dried in vacuum, weighed, and the purity and yield of total flavonoids were calculated.

Purity of total flavonoids (%) = Total flavonoids content (g)/ Total flavone mass (g)×100%.

Yield of total flavonoids (%) = Total flavone mass (g)/ Loading quantity (g)×100%.

III. RESULTS

3.1 Standard Curve and its Regression Equation

According to the method in item 4.1, we determined that the maximum absorption wavelength of the standard solution and sample extract was 505 nm, and the blank controls did not interfere with each other. The measured absorbance results of standard solution are shown in Table I, and its standard curve is drawn, as shown in Fig 1.The linear regression equation is $Y=0.0284X+0.0324(R^2=0.9996)$.The results showed that when the mass concentration of the test sample was 0 -100 ug/mL, there was a good linear relationship with the absorbance.

TABLE I. Absorbance of total flavonoids standard solution

Mass concentration of total flavonoids (ug/ml)	**0**	**20**	**40**	**60**	**80**	**100**
Absorbance	0	0.626	1.191	1.732	2.294	2.863

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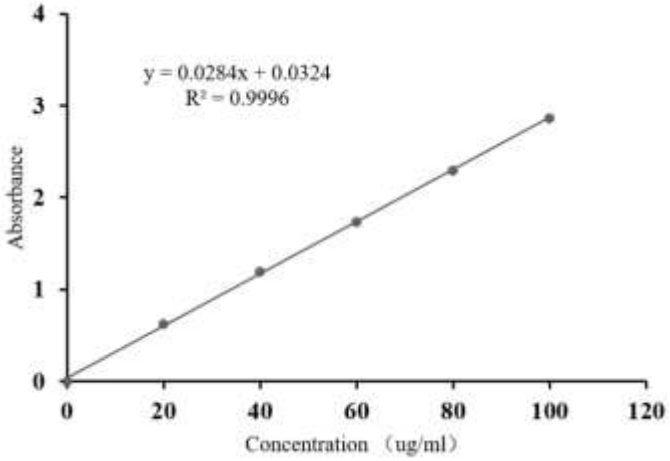

Fig 1: standard curve of total flavonoids

3.2 Stability Results

From the Table II, the absorbance value of the samples to be tested did not change significantly within 24 h, with an average RSD of 1.412%. The results showed that UV method was stable for the determination of total flavonoids from DYW.

TABLE II. Stability test

Measurement time (h)	Absorbance (A)	Average value	RSD (%)
0	1.144	1.116	1.412
1	1.109		
2	1.113		
4	1.126		
6	1.101		
12	1.098		
24	1.114		

3.3 Precision Results

UV method was used to detect the content of total flavonoids from DYW, we found that the relative standard deviation of the results was small in Table I, RSD = 0.408% (n = 3). This shows that the results have good reproducibility, high precision and accurate data.

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TABLE III. Precision test

Sample	Absorbance (A)	Average value	RSD (%)
1	1.128	1.124	0.408
2	1.119		
3	1.125		

3.4 Reproducibility Results

We detected the OD value of the samples to be tested for 5 times, with an average value of 0.3992 and RSD% of 0.875% in Table IV. The results showed that the UV method had good repeatability for the determination of the total flavonoids of DYW.

TABLE IV. Reproducibility test

Sample	Absorbance (A)	Average value	RSD (%)
1	1.157	1.165	0.881
2	1.171		
3	1.163		
4	1.179		
5	1.154		

3.5 Recovery Results

The average recovery was 103.33% and the relative standard deviation (RSD) was 1.149% in Table V. The results showed that UV method was used to determine the content of total flavonoids in DYW with good recovery.

TABLE V. Reproducibility test

Sample content (ml)	Content of added reference substance (ml)	Absorbance of sample (A)	Absorbance of added reference substance (A)	Measured absorbance (A)	Rate of recovery (%)	Average value	RSD (%)
5	1	1.125	1.191	2.347	102.60	103.33	1.149

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5	2	1.148	1.189	2.369	102.69		
5	3	1.169	1.191	2.416	104.70		

3.6 Content Determination

The results showed that the content of total flavonoids in three batches of DYW decoction pieces ranged from 0.726 to 0.933% in Table VI.

TABLE VI. Determination results of total flavonoids in DYW

Batch number	Quality of medicinal materials (g)	Absorbance (A)	Content (mg)	Percentage content (%)
1	0.2002	0.926	1.573	0.786
1	0.2002	0.917	1.557	0.778
1	0.2002	0.922	1.566	0.782
2	0.2001	0.865	1.466	0.733
2	0.2001	0.857	1.452	0.726
2	0.2001	0.861	1.459	0.729
3	0.1999	1.092	1.865	0.933
3	0.1999	1.046	1.785	0.893
3	0.1999	1.035	1.765	0.883

3.7 Effect of Ethanol Concentration on Extraction of Total Flavonoids from DYW

As shown in Table VII and Fig. 2, we found that with the increase of ethanol concentration, the yield and purity of total flavonoids of DYW first increased and then decreased, and the comprehensive value reached the peak when the ethanol concentration was 90%.When the alcohol concentration is low, a large number of water-soluble substances such as protein and sugar in the extract are dissolved, and the content of impurities is high. When the alcohol concentration is too high, the dissolution of fat-soluble substances is also increased, which affects the dissolution of total flavonoids from DYW.

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TABLE VII. Effects of different ethanol concentrations on the extraction of total flavonoids from DYW

Ethanol concentration	Purity of total flavonoids of DYW (%)	Yield of total flavonoids of DYW sinensis (%)	Comprehensive value
80	12.12	24.69	1.71
85	13.84	27.59	1.94
90	13.42	29.47	1.97
95	12.64	28.12	1.87

Note: comprehensive value = purity of total flavonoids / highest purity (13.84%) + yield of total flavonoids / highest yield (29.47%)

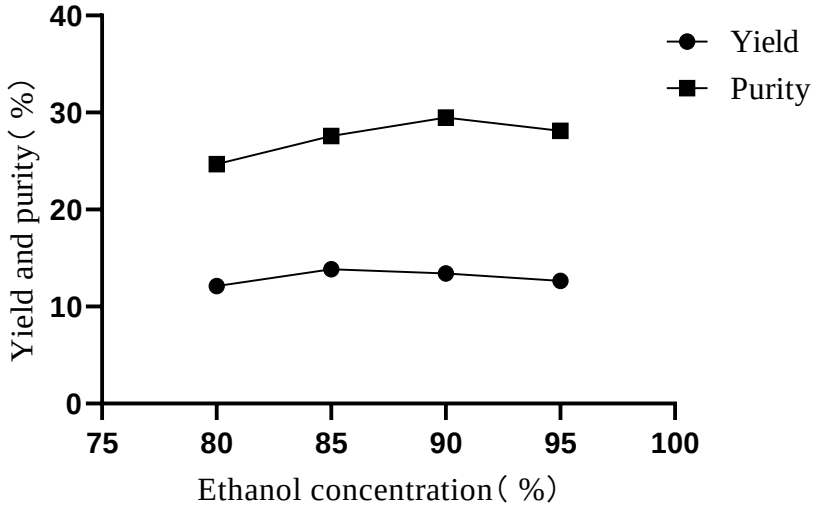

Fig 2: Effect of ethanol concentration on the yield and purity of total flavonoids from DYW

3.8 Effect of Solid-liquid Ratio on Extraction of Total Flavonoids from DYW

As shown in Table VIII and Fig 3, we found that different feed liquid ratios had little effect on the purity of total flavonoids of DYW, with an increase of about 6.4%. When the solid-liquid ratio was 1:8–1:10, the purity of total flavonoids increased with the increase of the solid-liquid ratio and decreased when the solid-liquid ratio exceeded 1:10.The yield of total flavonoids decreased with the increase of solid- liquid ratio. When the material liquid ratio is small, the increase of solvent dosage will accelerate the mass transfer force, which is conducive to the extraction of total flavonoids. Based on these results, the extraction effect of total flavonoids was better than that of 1:8 (g/ml).

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TABLE VIII. Effects of different solid-liquid ratio on the extraction of total flavonoids from DYW

Feed liquid ratio (g/mL)	Purity of total flavonoids of DYW (%)	Yield of total flavonoids of DYW (%)	Comprehensive value
1:6	12.85	25.46	1.77
1:8	13.67	30.76	2.00
1:10	12.42	27.27	1.80
1:12	11.86	25.68	1.70

Note: comprehensive value = purity of total flavonoids / highest purity (13.67%) + yield of total flavonoids / highest yield (30.76%).

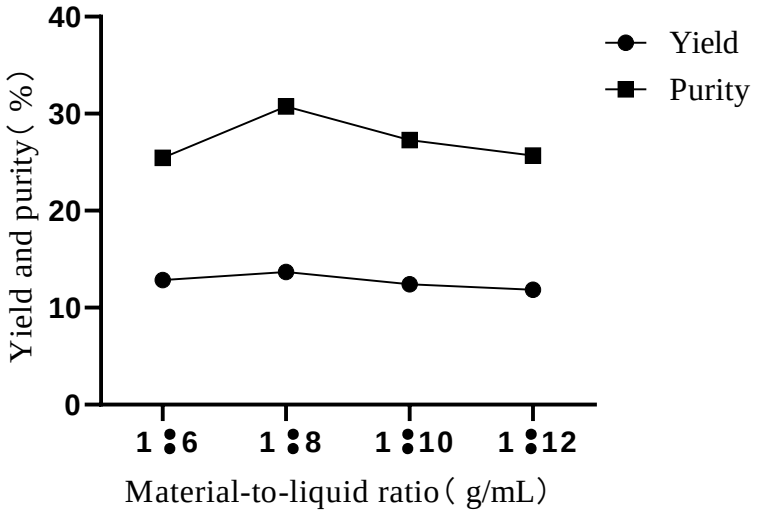

Fig 3: Effect of solid-liquid ratio on the yield and purity of flavonoids of DYW

3.9 Effect of Extraction Time on the Extraction of Total Flavonoids from DYW

As shown in Table IX and Fig 4, we found that the yield of total flavonoids increased slowly with the extension of time and began to decline after 2 h. The purity of total flavonoids of DYW was the highest at 2.5 h. This is because the increase of time can make the components of total flavonoids of DYW dissolve to the greatest extent, but too long time will make other components dissolve, reduce the content of total flavonoids of DYW, and the extension of cycle is not conducive to production.

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TABLE IX. Effects of different extraction time on the extraction of total flavonoids from DYW

Time of extraction (h)	Purity of total flavonoids of DYW (%)	Yield of total flavonoids of DYW (%)	Comprehensive value
1.0	12.34	21.33	1.61
1.5	12.53	25.89	1.78
2.0	13.47	29.52	1.97
2.5	13.93	28.23	1.96
3.0	12.84	24.62	1.76

Note: comprehensive value = total flavone purity / maximum purity (13.93%) + total flavone yield / maximum yield (29.52%).

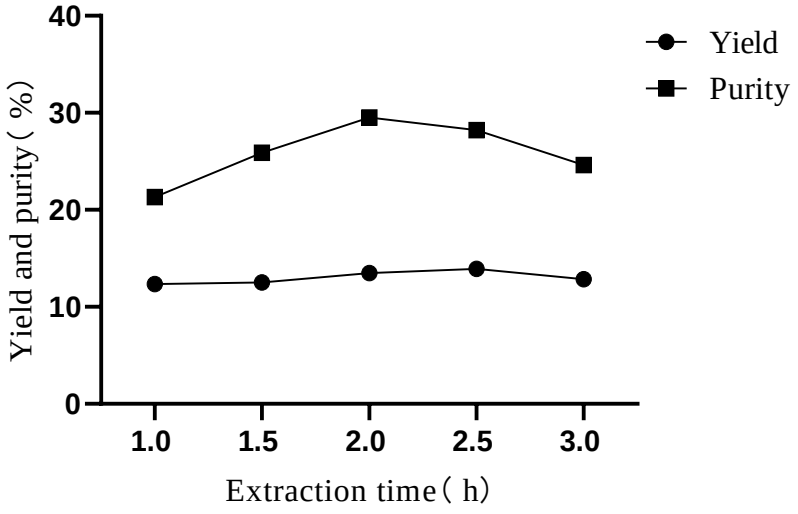

Fig 4: Effect of extraction time on the yield and purity of total flavonoids from DYW

3.10 Effect of Extraction Temperature on Extraction of Total Flavonoids from DYW

As shown in Table X and Fig 5, we found that with the increase of temperature, the yield and purity of total flavonoids of DYW increased, and then decreased. Because the increase of temperature will increase the solubility of total flavonoids in ethanol, and too high temperature will destroy the total flavonoids. In addition, if the temperature is too high, the dissolution of impurities will increase, which will bring difficulties to the subsequent separation and purification operation, so the temperature of 70 ℃ is more appropriate.

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TABLE X. Yield and purity of total flavonoids of DYW at different extraction temperatures

Temperature of extraction (℃)	Purity of total flavonoids of DYW (%)	Yield of total flavonoids of DYW (%)	Comprehensive value
60.0	13.56	23.12	1.70
70.0	14.56	30.26	1.95
80.0	13.89	28.39	1.94
90.0	13.64	27.54	1.85
100.0	12.57	25.26	1.70

Note: comprehensive value = purity of total flavonoids / highest purity (14.56%) + yield of total flavonoids / highest yield (30.26%).

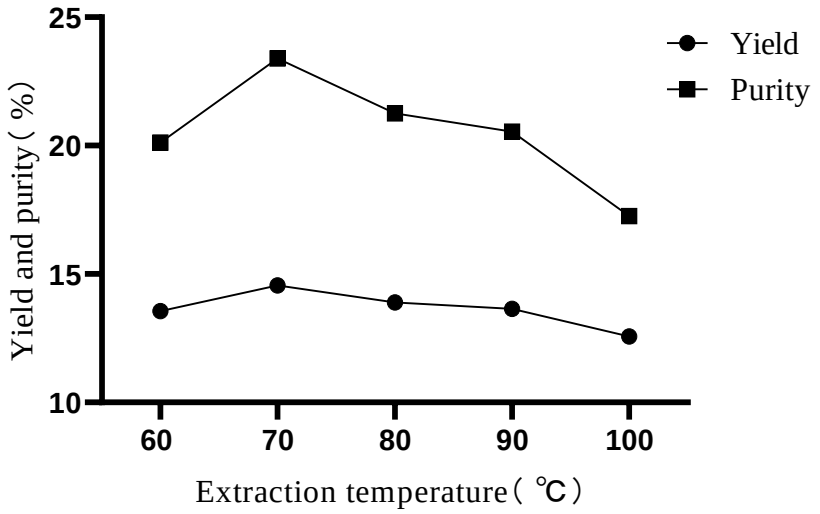

Fig 5: Effect of extraction temperature on purity and yield of total flavonoids from DYW

3.11 Effect of Extraction Times on the Extraction of Total Flavonoids from DYW

As shown in Table XI and Fig 6, we observed that the purity of total flavonoids of DYW decreased with the increase of extraction times, while the yield of total flavonoids was the opposite. This is because the longer the extraction time, the more fully the total flavonoids of DYW are extracted, but the more other components are dissolved.

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TABLE XI. Effect of extraction times on total flavonoids of DYW

Time of extraction	Purity of total flavonoids (%)	Yield of total flavonoids (%)	Comprehensive value
1	13.79	23.42	1.77
2	13.24	26.83	1.84
3	12.23	30.59	1.89

Note: comprehensive value = purity of total flavonoids / highest purity (13.79%) + yield of total flavonoids / highest yield (30.59%).

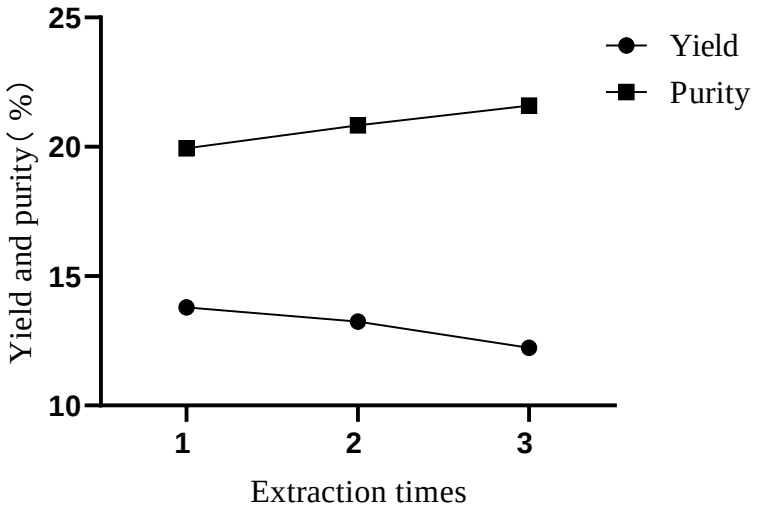

Fig. 6: Effect of extraction times on the purity and yield of total flavonoids of DYW

TABLE XII. Table of factor level

Le-vel	Factor			
	A Concentration of Ethanol (%)	B Feed liquid ratio (g/mL)	C Time of extraction (h)	D Temperature of extraction (℃)
1	85	1:6	1.5	60
2	90	1:10	2.0	70
3	95	1:12	2.5	80

3.12 Orthogonal Experiment on Extraction of Total Flavonoids from DYW

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Based on Table XII, we found that the primary and secondary order of the influence of various factors on the comprehensive value of total flavonoids of DYW is: extraction temperature > solid-liquid ratio > ethanol concentration > extraction time. Since the ethanol concentration had the least effect on the extraction of total flavonoids from DYW, it was set as the error column, and then SPSS 17.0 was used for analysis of variance. Table XIV shows that the effects of B, C and D are not significant. From economic considerations, B1, C1 and D2 should be selected. Therefore, the optimal level a1b1c1d2, that is, the ethanol concentration is 85%, the solid-liquid ratio is 1:6 (g/ml),the extraction time is 1.5 h, and the extraction temperature is 70 ℃. If time allows, we should also explore the changes of test results when the extraction temperature and extraction time are smaller and determine the most economical and reasonable extraction conditions.

TABLE XIII. L9 (34) Results of orthogonal test

Test number	Concentration of Ethanol (%)	Feed liquid ratio (g/mL)	Time of extraction (h)	Temperature of extraction (℃)	Purity of total flavonoids (%)	Yield of total flavonoids (%)	Comprehensive value
1	85	1:6	1.5	60	13.69	24.56	1.80
2	85	1:8	2.0	70	13.65	26.59	1.86
3	85	1:12	2.5	80	14.47	28.85	2.00
4	90	1:6	2.0	80	14.33	28.42	1.98
5	90	1:8	2.5	60	13.27	23.79	1.74
6	90	1:12	1.5	70	13.42	26.75	1.85
7	95	1:6	2.5	70	13.67	25.65	1.83
8	95	1:8	1.5	80	14.23	26.35	1.90
9	95	1:12	2.0	60	13.95	20.95	1.69
k1	1.84	1.77	1.78	1.85			
k2	1.77	1.77	1.88	1.80			
k3	1.78	1.85	1.73	1.74			
R	0.07	0.08	0.15	0.11			

Note: comprehensive value = purity / highest purity (14.47%) + yield / highest yield (23.95%).

TABLE XIV. Analysis of variance of Flavonoids Extraction

Variance source	SS	df	MS	F	P
B	0.014	2	0.008	1.856	0.421
C	0.036	2	0.019	5.234	0.274

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D	0.023	2	0.012	3.265	0.362
A(Error)	0.005	2	0.003	—	—

3.13 Validation Tests on Extraction of Total Flavonoids from DYW

We carried out three parallel experiments according to the above optimized extraction conditions of total flavonoids of DYW. Accurately weigh 100g of 40 mesh powder of DYW, and extract the total flavonoids of DYW under the conditions of solid-liquid ratio of 1:6 (g/ml),extraction temperature of 60 ℃, extraction time of 1.5 h and ethanol concentration of 85%.The final results are shown in Table XV. The comprehensive values of the yield and purity of total flavonoids of DYW are significantly higher than those of the above orthogonal experiments, and the RSD is less than 3%,which proves that the extraction of total flavonoids by this process is reasonable, feasible and stable.

TABLE XV. Validation test results of extraction of total flavonoids from DYW

Test number	Sampling quantity	Yield (%)	Purity (%)	Comprehensive value	Compre-he nsive value	RSD (%)
1	100.01	29.45	22.75	2.99	3.05	0.35
2	99.98	32.41	22.96	3.21		
3	99.99	28.86	22.63	2.94		

3.14 Macroporous Resin Adsorption Experiment

According to Table XVI, the order of adsorption capacity of different types of macroporous adsorption resins for total flavonoids of DYW is AB-8 > HPD600 > hpd500 > D101, and AB-8 macroporous adsorption resin has the best adsorption capacity.

TABLE XVI. Adsorption results of different types of macroporous adsorption resins on total flavonoids of DYW

Model of macroporous resin	D101	HPD500	HPD600	AB-8
Rate of adsorption (%)	67.82	73.62	77.42	85.62

3.15 Macroporous Resin Desorption Test

As shown in Table XVII, the desorption capacity of different types of macroporous adsorption resins for DYM total flavonoids was ranked as follows: AB-8>HPD600>D101>HPD500, and AB-8

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macroporous adsorption resin had the best desorption capacity.

TABLE XVII. Desorption results of different types of macroporous adsorption resins for total flavonoids of DYW

Model of macroporous resin	D101	HPD500	HPD600	AB-8
Rate of analytical (%)	59.27	58.42	63.75	78.42

3.16 Determination of pH Value of Adsorption Solution

Based on Table XVIII, we found that the adsorption rate was the highest when pH = 5. Since the initial pH of the total flavonoids loading solution of DYW is 4.9, which is very close to the optimal pH value, the pH value cannot be adjusted for the convenience of subsequent experiments.

TABLE XVIII. Effects of different pH on AB-8 resin adsorption of total flavonoids of DYW

Value of pH	4	5	6	7	8
Rate of adsorption (%)	85.42	89.46	87.25	85.45	83.26

3.17 Determination of Saturated Adsorption Capacity

As shown in Fig 7, the AB-8 resin adsorbed DYW total flavonoid solution, and there was leakage at the 24th adsorbent, and the concentration of total flavonoid adsorbed in the column decreased in a gradient from large to small until saturation was reached. By the 34th eluate, yellow fluorescence appeared in the TLC measurement, indicating that the resin was saturated with adsorption, and the concentration of total flavonoids in the effluent solution increased rapidly, indicating that the adsorption of AB-8 resins basically reached saturation, yielding a dynamic saturation adsorption amount of 3.2 g/g.

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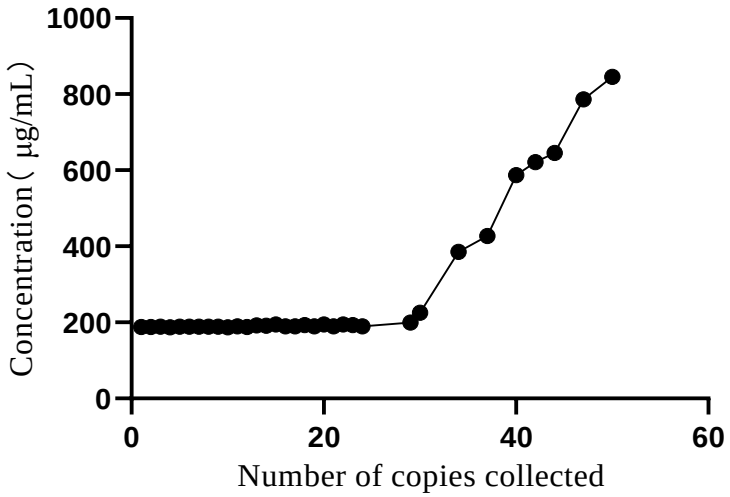

Fig. 7: Leakage curve of total flavonoids of DYW

3.18 Determination of Eluent Concentration

As shown in Fig 8, the 80% ethanol eluent can elute the total DYW flavonoids rapidly with less solvent usage. Therefore, the 80% ethanol solution was chosen as the elution solvent.

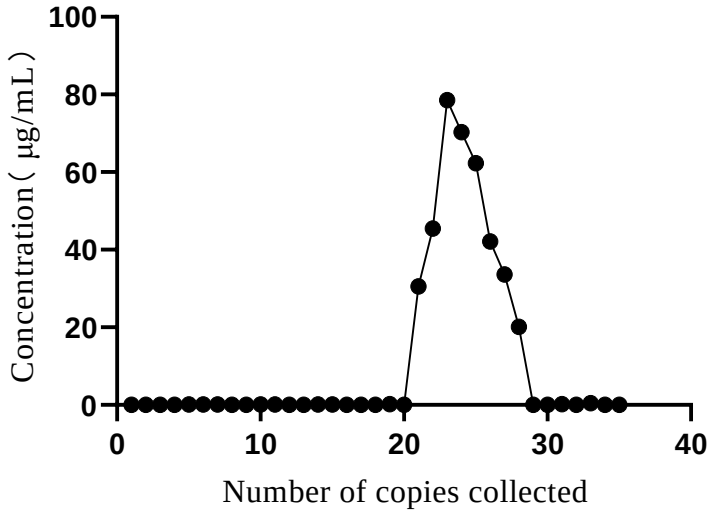

Fig. 8: Effect of eluent concentration on total flavonoids of DYW

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3.19 Best Process Validation Test

As shown in Table XIX, three validation tests showed that the purity of total DYW flavonoids could be as high as 55.38% with a maximum yield of 60.34% by the above optimal purification conditions. Further purification by polyamide resin was performed by removing impurities with distilled water and 40% ethanol, followed by gradient elution with 60%, 80% and 95% ethanol and combining the fractions to obtain total DYW flavones with a purity of up to 92.86% and a yield of 69.74%.

TABLE XIX. Validation test results of purification of total flavonoids from DYW

Test number	Sampling quantity (g)	Yield of total flavonoids (%)	Purity of total flavonoids (%)	Comprehensive value	average value	RSD (%)
1	49.99	59.92	55.25	1.98	1.95	0.78%
2	50.02	60.23	55.38	1.96		
3	49.96	60.34	55.16	1.95		

IV. CONCLUSION

In this study, we selected ethanol as the solvent to extract the total flavonoids of DYW, which has the characteristics of low polarity, insoluble in water and soluble in ethanol. The standard curve of total flavonoids of DYW was established with rutin as the reference substance. This experiment combines single factor and orthogonal test, the purity and yield of DYW total flavonoids were used as the indexes. Finally, we took the solid-liquid ratio of 1:6 (g / ml), extraction temperature of 60 ℃, extraction time of 1.5 h and ethanol concentration of 85% as the extraction conditions of total flavonoids. Under these conditions, the yield and purity of total flavonoids of DYW can reach 32.41% and 22.96%.According to the above conditions, we carried out confirmatory experiments, and the yield of total flavonoids was 30.24%.The results show that the process conditions obtained by orthogonal test are reliable, and the experimental method is relatively simple, which can be applied to industrial production. According to the above conditions, we carried out confirmatory experiments, and the yield of total flavonoids was 30.24%.The results show that the process conditions obtained by orthogonal test are reliable, and the experimental method is relatively simple, which can be applied to industrial production.

In this study, we selected four kinds of macroporous adsorption resins to study the adsorption and desorption of total flavonoids of DYW, and selected AB-8 type with excellent performance as the macroporous adsorption resin for the purification of total flavonoids of DYW. By UV spectrophotometry, we take the purity and yield of total flavonoids of DYE as the investigation index, and through the

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dynamic and static purification process of AB-8 macroporous adsorption resin, the optimal process conditions were determined: pH = 5,saturated adsorption capacity of 3.2 g/g and elution with 80% ethanol. The results showed that AB-8 macroporous adsorption resin combined with polyamide resin had a good purification effect on the total flavonoids of DYW, and most flavonoids could be eluted. Therefore, it was finally selected as the filling material, and the elution ability was the strongest when the ethanol concentration was 80%.

The experimental process steps are simple, the purification time is short, and the total flavonoids of DYW with high purity and yield can be obtained, which can provide high-quality resources for the further activity test and product development of the total flavonoids of Duyiwei, and provide a material basis for the development of new products, such as the development of new hemostatic, analgesic and other pure traditional Chinese medicine products for the elimination of ulcer bleeding and cancer pain.

Through the research on the extraction and purification process of total flavonoids from the effective part of DYW, we have greatly enriched and improved the content of its basic research and application development, which provides a theoretical basis and scientific reference for the further development and application of DYW.

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REFERENCES

[1] Li X M, Yang S S, Du W Q (2007) Research progress of Tibetan medicine Duyiwei. Journal of Practical Traditional Chinese Internal Medicine, 021 (007): 12-3.
[2] Yuan T, Wang S, Dun Z, et al. (2014) Research progress of Duyiwei in Tibetan Medicine.Chinese Traditional Patent Medicine, 36 (9): 4.
[3] Meng B H, Meng X L (2009) Research Progress on pharmacological action of Tibetan medicine Duyiwei. China Pharmacy, 20 (3): 2.
[4] Ma W Y, Xie H C, Tian Liping, et al. (2020) Research Progress on pharmacological activity of Duyiwei.Acta Chinese Medicine, 35 (10): 5.
[5] Zhang J H, Xv L T, Wang R, et al. (2015) Advances in pharmacognosy and chemical constituents of Tibetan medicine Duyiwei.Journal of Lanzhou University(Medical Sciences), 41 (5): 6.
[6] Wang R D, Sun L N, Tao C Y, et al. (2005) Chemical constituens of Lamiophlomis rotata. Academic Journal of Second Military Medical University, 26 (010): 1171-3.
[7] Zhang A J, Ren F X, Zhao Y M (2011) Study on chemical constituents of Tibetan medicine Lamiophlomis

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www.forestchemicalsreview.com
ISSN: 1520-0191
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Article History: Received: 22 July 2021 Revised: 16 August 2021 Accepted: 05 September 2021 Publication: 31 October 2021

rotata.Chinese Pharmaceutical Journal, 046 (002): 102-4.

[8] (2020) Chinese Pharmacopoeia Commission.Chinese Pharmacopoeia. Chinese Pharmacopoeia.

[9] Wang W F (2007) Studies onExtraction and Purification Technology and Chemical Constituents of *Lamiophlomis rotata(Benth.) Kudo*. Tianjin University.