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Determination of the total composition in gallic residue

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Abstract. In this study, ten batches of gallic residue were used as raw materials. The contents of total protein, total polyphenol and total C were determined by Coomassie bright blue method, folin-phenol method and potassium dichromate oxidation method respectively, and the methodology was investigated. The results showed that gallic residue contained more protein, polyphenols and sugar. There were significant differences in total protein and total polyphenol content between different batches, while the difference in total C content was small. The experimental method was simple, quick and convenient.

1. Introduction

With the deepening of industrialization and scale of modern traditional Chinese medicine production, more and more plant-based medicinal residue wastes are generated in the production and processing process, which is bound to cause a sharp increase in the waste pharmaceutical residue. If not handled properly, it will have a great impact on the ecological environment. The discharge and treatment of pharmaceutical residue after extraction is a thorny problem in the extraction workshop. The residue contains a large number of beneficial components, such as crude protein, crude fiber, crude fat and a variety of metal elements. The effective components that can be extracted from plant medicinal materials account for about 5% of the original medicinal materials, and most of the rest will become pharmaceutical residues, so there is a large space for reuse of pharmaceutical residues. [1,2,3]

Gingival fluid is extracted from a single drug nutgall, which has the effects of strengthening teeth and fixing gingival, clearing blood and relieving pain. Gallic residue contains phenols, tannins, amino acids, crude fiber, starch, crude protein, crude fat and a variety of trace elements. The determination of total polyphenols and total protein in pharmaceutical residue can provide a theoretical basis for pharmaceutical residue as an organic fertilizer in the later stage, which is conducive to extending the industrial chain of enterprises, increasing the added value of products, improving the utilization rate of resources, and exploring the way of efficient, environmental protection, green and sustainable development of the industrial chain of traditional Chinese medicine.

2. Experimental materials and instruments

2.1 Specimen

The residue used in the experiment was all from Xinqi Kang Pharmaceutical Co., LTD., numbering S-1, S-2, S-3, S-4, S-5, S-6, S-7, S-8, S-9 and S-10 in 10 batches.

2.2 Instrument

Ultrasonic Cleaner (KQ-5200DE) was from Kunshan Ultrasonic Instruments Co., Ltd. Vacuum Drying Chamber (DZF-6021) was from Shanghai Precision Test Equipment Co., Ltd. Water Bath Kettle(B-260) was from Shanghai Yarong Biochemistry Instrument Factory. Digital Display Constant Temperature Water Bath (HH-S4) was from Jintan Medical Instrument Factory. One-thousandth part Electronic Balance (AL04) and One part in 100,000 Electronic Balance (XS105) were from Mettler-Toledo. Low Speed Bench Centrifuge (TDL-40BL) was from Shanghai

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Anting Scientific Instrument Factory. VIS Spectrophotometer (725) was from Shanghai Jinghua Technology Instrument Co. , Ltd..

2.3 Reagents

Bovine Serum Albumin was purchased from Shanghai LI Rui biotechnology limited company with batch number 0332-201304. Gallic acid was purchased from China Food and Drug Administration Research Institute with batch number 110831-201204. Glucose was purchased from Tianjin sheng 'ao chemical reagent Co., Ltd. with batch number 20140506. CBB G-250 was purchased from China Pharmaceutical Group Shanghai Chemical Reagent Company by Fluka import packing. Sodium tungstate (Na2WO4•2H2O), sodium molybdate, phosphoric acid, concentrated hydrochloric acid, lithium sulfate (Li2SO4), sodium carbonate, phosphoric acid, potassium dichromate, ethanol and so on were of analytical grade.

3. Methods

3.1 Preparation of Sample Solution

Preparation of total protein sample solution: Finely weigh 1.00g crude powder of pharmaceutical residue, add 25 mL distilled water, ultrasonic for 30 min, centrifuge, filter, and filtrate for later use.

Preparation of total polyphenol sample solution: Finely weigh 1.00g of coarse powder of pharmaceutical residue, add 25 mL 70% ethanol in water bath at 85℃ for reflux for 1 h, filter, centrifuge, rinse with a small amount of 70% ethanol, combine filtrate with constant volume to 25 mL, serve as mother liquor, dilute 1 mL mother liquor with 70% ethanol to 50 mL, and set aside.

Preparation of total carbon sample solution:0.10 g of residue was weighed, 2.5 mL of distilled water was added, ultrasonic was carried out for 30 min, centrifugation was carried out through 0.45 μm filter membrane, and test was carried out.

3.2 Preparation of chromogenic agent

Dye reagent CBB G-250: weigh CBB G-250 50.0 mg and add 95% ethanol 25 mL to dissolve, then add 50 mL 85% phosphoric acid and dilute to 500 mL with water.

Preparation of Folin reagent: Weigh 100g of sodium tungstate (Na2WO4•2H2O) and 25 g of sodium molybdatum (NaMoO4•2H2O) in a 1000 ml mill mouth reflux device, add 700 ml of water, 50 mL of 85% phosphoric acid, 100 ml of concentrated hydrochloric acid, gently reflux for 10 h, cool, add 150 g of lithium sulfate (Li2SO4), 50 ml distilled water, add a few drops of liquid bromine, and boil for 15 min to expel the residual bromine and remove the colour, the solution should be bright yellow. If the solution is green, add a few drops of bromine, boil it to remove, and filter it to 1000 ml after cooling.

Preparation of 15% sodium carbonate: weigh 75 g sodium carbonate (Na2CO3) and dissolve it in distilled water to 500 ml.

Preparation of potassium dichromate solution:0.98 g potassium dichromate was dissolved in 100 mL distilled water.

3.3 The establishment of standard curves

Bovine albumin protein standard curve:10.08 mg bovine serum albumin (BSA) was precisely weighed and placed in a 100 mL volumetric flask, dissolved in distilled water and diluted to scale, then shaken well to obtain 0.1008 mg/mL reference solution. Precisely take 0.0, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, and 2 mL of reference solution and put them into 10 mL corkscrew test tube, add 5 mL CBB G-250 dye, fill it with distilled water to 10 mL, slightly stir, mix well, and scan within the wavelength range of 400-700 nm, as shown in Fig. 4-1. The results showed that the maximum absorption was at 595 nm, so the total protein content in the residue was determined at 595 nm in

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this experiment. Linear regression was made with the content of reference solution (mg) as abscissa and absorbance value A as ordinate. [4]

Gallic acid standard curve:10.5 mg of gallic acid was precisely weighed and placed in a 100 mL volumetric flask, dissolved and diluted to scale with 70% ethanol, and shaken well. The concentration of gallic acid reference solution was 0.105 mg/mL. Carefully absorb 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 mL of the solution respectively into a 10 mL stopper tube, add Folin reagent 0.5 mL, shake well, then add 15% sodium carbonate 1.5 mL, shake well, supplement with distilled water to 10 mL, heat in 75℃ water bath for 15 min, remove, cool. Scanning in the wavelength range of 500-800 nm, as shown in Figure 4-3, the results showed that there was the maximum absorption peak at 760 nm, so the content of total polyphenols in the residue was determined at 760 nm in this experiment. The standard curve was drawn with the content of reference solution (mg) as abscess and absorbance A as ordinate.[5,6,7]

Glucose standard curve: Accurately weighing 5.0 g glucose in a 100 mL volumetric flask, adding distilled water to dissolve and dilute to scale, shaking well, the concentration of 50.0 mg/mL glucose reference solution (C content is 20 mg/mL) was obtained. Accurately absorb 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 mL of the above solution (C content is 6, 8, 10, 12, 14, 16, 18 mg, respectively) into a 25 mL volumetric flask, add 2.5 mL of potassium chromate reagent, fill with water to 3 mL, shake well, concentrated sulfuric acid 5 mL, Shake it well for 10 min, then fill it with distilled water to 25 mL, shake it well, cool it, and scan it in the wavelength range of 500-700 nm, as shown in Figure 4-5. The result shows that there is the maximum absorption peak at 580 nm, so the total C content in the residue is determined at 580 nm in this experiment. The standard curve was drawn with the content of C (mg) as the abscissa and the absorbance value A as the ordinate. [8]

3.4 Determination of sample content

A certain amount of extract in item 2.1 was accurately absorbed, and the absorbance was measured according to the measurement method in 2.3. The contents of total protein, total polyphenols and total carbon in the residue were calculated according to the corresponding linear regression equation.

4. Results

4.1 Standard curves of total protein, total polyphenols and total carbon of gallic residue

The standard curves of total protein, total polyphenols and total carbon were fitted, and the linear relationship was shown in Table 1.

Table 1. The linear relationship of standards.

Standards	Standard curve	Linear range (mg/mL)	R2
BSA	A=3.4101C+0.0975	0.081 ~ 0.202	0.9995
Gallic acid	A=13.524C+0.0044	0.0105 ~ 0.0525	0.9992
Glucose	A=0.048C-0.0939	6.0 ~ 18.0	0.9978

4.2 Methodological investigation

4.2.1 Accuracy test.

An appropriate amount of BAS control solution, gallic acid control solution and glucose control solution were precisely absorbed into a 10mL test tube with stopper, and the absorbance was measured according to the experimental method under 2.3. The RSD of the samples were 0.97%, 0.18% and 0.85%, respectively, indicating that the instrument used had good precision.

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4.2.2 Stability test.

An appropriate amount of BAS control solution, gallic acid control solution and glucose control solution were precisely absorbed into a 10mL test tube with stopper, and the absorbance was measured every 5min, 2h and 10min according to the experimental method described in 2.3. RSD of the samples were 2.02%, 0.33% and 1.29%, indicating good stability.

4.2.3 Repeatability test.

Appropriate amount of S-1 sample was weighed, and the absorbance was measured according to the experimental method in 2.3. The RSD of appropriate amount of extract was 1.63%, 0.40% and 1.81%, respectively, indicating that the experimental method had good repeatability.

4.2.4 Recovery test.

Six extracts of the same gallic residue samples with known contents were taken, and an appropriate amount of reference solution was added respectively. The absorbance was measured according to the experimental method in Item 2.3, and the recovery rate was calculated. The results were shown in Table 2. The recoveries of total protein, total polyphenols and total carbon in gallic residue were 102.06%, 101.75% and 99.42%, respectively. Their RSD values are all less than 3%, indicating that the method is feasible.

Table 2. The recovery test results oftotal protein、total polyphenol and total C

Standards	Amount of standard taken(mg)	Amount of sample taken(mg)	Amount found (mg)	Recovery (%)	Average (%)	RSD (%)
total protein	0.081	0.061	0.145	103.70	102.06±1.01	0.99
	0.081	0.061	0.143	101.23		
	0.081	0.061	0.144	102.47		
	0.081	0.061	0.143	101.23		
	0.081	0.061	0.143	101.23		
	0.081	0.061	0.144	102.47		
total polyphenol	0.021	0.0156	0.0364	99.05	101.75±2.19	2.15
	0.021	0.0156	0.0364	99.05		
	0.021	0.0156	0.0372	102.86		
	0.021	0.0156	0.0371	102.38		
	0.021	0.0156	0.0375	104.29		
	0.021	0.0156	0.0372	102.86		
total C	6.0	5.4	11.12	95.33	99.42±2.76	2.77
	6.0	5.4	11.50	101.67		
	6.0	5.4	11.56	102.67		
	6.0	5.4	11.23	97.71		
	6.0	5.4	11.41	100.17		
	6.0	5.4	11.37	99.50		

4.3 Determination of sample content

Calculated according to the measured absorbance and the corresponding linear regression equation, as shown in Table 3.

Table 3. The results of determination of total polyphenols total protein and total C content

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Component	Sample	Content (mg/g)	RSD (%)
total protein	S-1	0.854	0.67
	S-2	1.156	0.79
	S-3	5.085	1.33
	S-4	1.027	0.53
	S-5	0.839	0.78
	S-6	1.867	1.76
	S-7	1.263	1.53
	S-8	1.983	1.22
	S-9	1.683	1.65
	S-10	1.453	0.15
total polyphenol	S-1	327.47	0.18
	S-2	383.95	0.41
	S-3	374.31	1.12
	S-4	499.80	0.62
	S-5	441.15	0.95
	S-6	416.88	0.39
	S-7	357.00	1.59
	S-8	411.00	0.30
	S-9	466.50	1.36
	S-10	481.25	1.79
total C	S-1	108.03	0.17
	S-2	127.96	0.21
	S-3	146.61	0.12
	S-4	143.79	0.42
	S-5	128.90	0.51
	S-6	128.72	0.12
	S-7	125.85	0.14
	S-8	133.85	0.22
	S-9	138.14	0.10
	S-10	131.24	0.25

5. Discussion

It can be preliminarily judged that gallic residue contains a lot of phenols and proteins by systematic preliminary experiment. There are many methods for the determination of total polyphenols and total protein, but each method has its own advantages and disadvantages, some are complicated and time-consuming, some are poor in sensitivity and large in error. This experiment, using gallic acid as folin-phend method standard by ethanol extract the polyphenols in the liquid ingredients, determine the content of total polyphenols, using the method of coomassie brilliant blue with BSA as the standard by water extraction liquid to the protein, determine the content of total protein, potassium dichromate oxidation method has been applied to the determination of the total C in liquid content, The method for the determination of total protein, total polyphenols and total C has good precision, stability and convenient operation. Therefore, in this experiment, the folin-phenol method was used to determine the content of total polyphenols, coomassie bright blue method to determine the content of total protein, potassium dichromate oxidation method to determine the content of total C.

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In the determination of total protein and total polyphenols in 10 batches of gallic residue, the contents of total protein and total polyphenols in different batches were different. The content of total protein in the third batch of residues was relatively high, and the content of total polyphenols in the fourth batch was relatively high.The C content difference is relatively small, and only the C content in the first batch is significantly different from other batches. Therefore, it can be judged that the content of ingredients in different batches of residue is different.

The determination of protein in the residue can provide a basis for the later calculation of the total N content in the residue. C and N in the residue are the nutrients in the organic fertilizer, C is the organic matter, and N is one of the total nutrients. The determination of N and C provides a theoretical basis for the residue as an organic fertilizer.

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