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Optimizing the Extraction Process of Polyphenols from the Kudzu(Pueraria Montana Var Lobata)

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A B S T R A C T

In today's modern life, pharmaceuticals are synthesized in an extraordinarily rich and diverse way, with fast effects, short treatment time, but if used for a long time, it will cause many harmful side effects to the body. the health of the patient. Therefore, the use of products derived from nature is increasingly widely used. And *Pueraria Montana var lobata* is an exceptionally good representative. It has appeared in many ancient folk remedies. However, at present, there has not been an in-depth study on the extraction process of the precious compounds inside *Pueraria Montana var lobata*. Therefore, performing a complete study on the extraction process of polyphenol compounds inside *Pueraria Montana var lobata* will have high scientific and practical significance, contributing to the exploitation and rational use of natural resources. nature of the country. From the above issues, we conducted the study: *"Optimizing the extraction process of polyphenols from the kudzu(Pueraria Montana var lobata)"*.

Keywords: Kudzu, Pueraria Montana var lobata, polyphenol, extraction process.

1. Introduction

The kudzu tree is scientifically known as Pueraria Montana var lobata, belonging to the genus Pueraria, legume family. In addition, the kudzu tree also has other names such as sand, white sand, khau sand, ... Forest tapioca is a plant native to East Asia (China, Japan, ...) and some Southeast Asian regions (Vietnam, Laos, Cambodia...)[1-3].

Kudzu tree is a perennial, fast-growing vines, after each season it can increase by 30m in length, corresponding to 30cm per day[4,5]. Plants can survive growing on a variety of soils, including sandy soils, lime soils, alum soils, and places where year-round heat drops below -32°[6,7]. Kudzu trees have soft, thriving roots, the length of which can be up to 2m. In the roots of the plant, there is a main root that develops into long, large tubers. The tuberous roots have scraped the outer cork, cylindrical, or semi-cylindrical, 12-15 cm long, 4 - 8 cm in diameter, sometimes longitudinal or beveled slices, thick, of different sizes. The outer surface is yellowish white, sometimes left in the slits a little brown cork[8]. The substance is hard, heavy and powdery. The cross section has many fibers forming light brown concentric rings; The longitudinal section has many longitudinal veins made up of fibers. The aroma is light, the taste is slightly sweet, cool. When mature, the leaves are spear-shaped, the maximum size there is to reach 18cm in length and 12cm in width. The leaves form bunches of 4 lobes, light purple, from 15 to 40cm long. The fruits of the kudzu tree are flat-shaped, about 10cm long, about 1cm wide and hairy[9-11].

Two parts of the tapioca plant have been used as herbal medicines: (1) roots, called Gegen or Pueraria lobate, and (2) flowers, named Gehu or Pueraria floss. The root of P. lobata is one of the oldest herbal medicines in traditional Chinese medicine. Although it has been used a lot in folk remedies, most of it is only used based on experience gained or left by predecessors without much scientific understanding. Therefore, the study, evaluation and extraction of potential biological activities from Pueraria Montana var lobata trees will have high scientific and practical significance, contributing to the rational exploitation and use of the country's natural resources. From the above issues, we conducted research: "Optimizing the process of extracting polyphenols from kudzu (Pueraria Montana var lobata)"[12-13].

2. Research Methodology

2.1 Determination of humidity by the method of drying to a constant mass

2.1.1. Principle

Use the heat to separate the moisture in the material, while retaining all the substances contained in the material. Therefore, the drying temperature should not be too high or too low.

2.1.2. How to proceed

Take the sample and crush it, use an analytical scale to weigh 2-5 g of the sample in a cup knowing the weight in advance, then place the cup containing the sample in the drying cabinet at a temperature of 105 ºC. Dry for about 4-5 hours, take the cup containing the sample and place it in a dehumidifier to cool. Then weigh and record the results. Continue until there is a constant weight.

2.1.3. Calculate results

Humidity in % (W) using the formula:

$$W = \frac{G_1 - G_2}{G_1 - G} \times 100\ (\%)$$

Inside:

W: Moisture content of food (%).

G: Volume of dried cups (g).

G_1: Volume of drying cups and test samples before drying (g).

G_2: Volume of drying cups and test samples after drying (g).

2.2 Determination of ash content according to TCVN 8124:2009

2.2.1. Principle

The test sample is heated until the organic matter burns completely, then weighs the remaining residue. This resulting residue takes the form of porous ash after firing at 550ºC.

2.2.2. How to proceed

Burn washed porcelain dishes in furnaces 550 - 600ºC to a constant volume. Allow to cool in a dehumidifier and then balance the analytical balance. Weigh 5g of wild cassava tubers into a porcelain cup. Put in a kiln and raise the temperature slowly to 550 - 600ºC. Heat to white ash, that is, remove all organic matter, usually about 6-7 hours. In case of black ash, remove to cool, add a few drops of concentrated H_2O_2 or HNO_3 and reheat to white ash. Allow to cool in a dehumidifier and weigh. Continue heating for more than 30 minutes, then cool in a dehumidifier and weigh it. The process is repeated until the mass is constant.

2.2.3. Result

Ash content in % is calculated by the formula.

$$X = \frac{G_2 - G}{G_1 - G} \times 100\ (\%)$$

Inside:

G: cup weight (g)

G_1: in the number of cups and samples before firing (g)

G_2: in the number of cups and samples after firing (g)

2.3 Determination of total protein content by the Kjeldahl method

2.3.1. Principle

The specimen is digested with a mixture of concentrated sulfuric acid and potassium sulfate. Use copper (II) sulfate as a catalyst to convert the organic nitrogen present into ammonium sulfate. Using potassium sulfate is to increase the boiling point of sulfuric acid and create a stronger oxidation mixture for decomposition. The addition of excess sodium hydroxide to the cooled digestion causes the release of ammonia. The ammonia released is steam distilled in an excess solution of boric acid, and the solution is then titrated with a standard volumetric solution of hydrochloric acid. The nitrogen content is calculated from the amount of ammonia formed.

2.3.2. How to proceed

Sample inorganic:

Weigh 5g of kudzu root and 5g of catalytic mixture (put in a burning vessel). Add 10ml of concentrated H_2SO_4 to the jar. Heat on the stove (placed in a fume cabinet) until the solution is colorless or cyan of $CuSO_4$.

Protein storage:

Put the entire post-inorganic solution in a nitrogen storage tank. Rinse the jar with distilled water. Put 2-3 drops of phenolphthalein in a storage jar. Add 40% NaOH to the jar until the solution turns purple black. Insert the tank into the nitrogen storage kit. the NH_3 receiver side has a receiver tank with 10ml H_2SO_3 + a few drops of the tare's directive. Check the end point of the nitrogen distillation process by checking the condensate (use purple kneeling to try).

Delimitation:

Take out the catch tank and titration with H_2SO_4 0.1N. Titration until the solution turns purple, durable for 30 seconds.

2.3.3. Result

The total protein content is calculated by the formula:

$$Pr = \frac{n \times 0.00142 \times 100}{m(100 - W)} \times 5.95 \times 100\ (\%)$$

Inside:

n: volume H_2SO_4 0.1N consumed for titration (ml)

0.00142: grams of nitrogen corresponding to 1ml H_2SO_4 0.1N

m: volume of analytical samples (g)

W: sample humidity (%)

5.95: conversion factor from total nitrogen to protein.

2.4 Method for determining the total polyphenol content after the separation process according to the Folin- Coculture method

2.4.1. Principle

The total polyphenol content is determined by the Folin-Coculture method.

2.4.2. How to proceed

Gallic Acid Standard Formulation:

Dilute Gallic Acid solution into samples with corresponding concentrations of 5, 10, 15, 20, 25 µg/mL. Mix 2mL of Gallic Acid solution, 5mL of 10% Folin solution and 3mL of 7.5% Na_2CO_3 solution into test tubes. Place the test tubes into a Vortex shaker, then incubate the specimens in a thermostatic tank at 40°C for 60 minutes. Use a spectrophotometer to survey the optical absorption of each test sample at a wavelength of 765 nm. From the data obtained on the optical absorption and concentration of the samples, we obtain a data table.

Table 2.1 - ABS optical absorption of Gallic Acid test samples

Sample	Concentration (µg/mL)	ABS
1	0.00	0.000
2	5.00	0.164
3	10.00	0.406
4	15.00	0.620
5	20.00	0.789
6	25.00	0.953

Based on the data table, we obtain the standard line equation of Gallic Acid of the form y = ax + b.

Graph 1: Standard line graph of Gallic Acid

Gallic Acid Standard Line Equation:

$y = 0.0392x - 0.0009$

$R^2 = 0.9956$

Determination of polyphenol content:

Soak the test specimens in ethanol solution. Carry out filtration of impurities with a food filter, dilute the resulting extracted solution into different concentrations. Mix 2mL of test sample, 5mL of 10% Folin solution and 3mL of 7.5% Na_2CO_3 solution into test tubes. Proceed to place the test tubes into the Vortex shaker, then incubate the specimens in a thermostatic tank to perform polyphenol extraction. Use a spectrophotometer to survey the optical absorption capacity of each test sample. With the results of the optical absorption capacity of the samples (y), we instead change the standard line equation to calculate the concentration of polyphenols present in the sample (x).

2.4.3. Result

The total polyphenol content is calculated by the formula:

$P = \frac{aV}{m}\ (mg)$

Inside:

F: Total polyphenol content (mg GA/g dry medicinal herbs)

a: X-value from gallic acid benchmark (µg/mL)

V: Extract volume (mL)

m: The volume of medicinal herbs presents in volume (g).

2.5 DPPH free radical capture method

2.5.1. Principle

The DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical detection method was developed by Blois. This analysis is based on the color change of the DPPH solution. DPPH is a purple stable free radical with an unpaired electron on a nitrogen atom to form an electron conjugate system. The free electron of the nitrogen molecule in D P PH, when combined with a hydrogen radical from an antioxidant, loses the characteristic purple color of the free radical DPPH in methanol solution. The antioxidant's ability to root D P PH is expressed in the degree of color reduction of the D P PH solution, which is determined by measuring optical absorption at λmax = 517 nm. Based on the standard curve to determine the value of IC50.

2.5.2. How to proceed

Dilution of DPPH solution: Weighing 0.004g DPPH dissolved in 100mL pure Methanol → obtains 2% DPPH solution, stored at cold temperature, covered with foil.

For highly extracted samples

+ Determination of sample dissolved solvents. Take a few samples, dissolve the sample with solvents of different polarity (water, methanol, ethanol, acetone, DMSO...). Observe the choice of solvents capable of completely dissolving the sample to be analyzed.

+ Dissolve 1000 µ g of sample in 1L of solvent to obtain a solution with a concentration of 1000 ppm. Double serial dilution to different concentrations and stop at a concentration range of 5 - 10 ppm. When performing the experiment, dilute the stock 10X →1X (3mM).

Control phase control: The positive control agent in this experiment was vitamin C, the mixture concentration ranged from 0 → 30 ppm, using water as a solvent (Vitamin C is well soluble in water).

In a well of plate 96 add the following components:

-100 µl sample analysis /control/ solution

-100 µl DPPH 3 mM/methanol

Cough reacts 30 minutes in the dark, measuring optical density at 517 nm wave bucks.

2.5.3. Results

The percentage of DDPH free radicals is calculated by the formula:

$SC_{DPPH} = (1 - \frac{As - Ab}{An}) \times 100$

Inside:

SC_{DPPH}: DDPH ability to capture free radicals (%)

A_s: photoreceptor degree of the analyzed sample

A_b: optical absorption \of white sample

A_n: negative control sample

Calculate the IC50 value of the test sample and control sample based on the linear equation between their concentration and % free radical scavenger activity, using the following formula:

$IC_{50} = \frac{50 - b}{a}$

Comprise:

IC_{50}: Sample concentration, standard can capture 50% of DDPH free radicals.

a, b are the slope and interception of the linear equation holding the concentration and % of free catch, respectively.

The sample is repeated three times, and the results are expressed as an average ±SD.

2.6 Data processing methods

Experimental results processed on SPSS 20.0 software.

3. Results and discussion

3.1 Results of analysis of the chemical composition of kudzu root (Pueraria Montana var lobata).

The results of moisture, ash and protein content of forest tapioca root (Pueraria Montana var lobata) is all shown in Table 3.1.

Table 3. 1 - Ash, moisture and protein content of Pueraria Montana var lobata

Number	Content	Results (%)
1	Humid	8 %
2	Ashes	9 %
3	Protein	6 %

Table 3.1 shows the percentage of ash, moisture as well as protein content of the starting material Pueraria Montana var lobata that has been put into storage and pre-dried.

3.2 Results of the study to select the concentration of solvents extracted polyphenol from kudzu root.

Ethanol is a solvent with quite good solubility. Using ethanol at different concentrations will have the effect of different extraction processes. The experiment to determine the effect of ethanol solvent concentration on the efficiency of polyphenol extraction was conducted and the results obtained as shown in the table below:

Table 3. 2 - Effect of ethanol solvent concentration on polyphenol extraction efficiency

Ethanol concentration(%)	50	60	70	80	90
Polyphenol content (µg/ml)	185.15^{d}	202.18^{e}	158.25^{c}	147.02^{b}	132.21^{a}
(Note: Values in the same column with different exponents differ in meaning $\alpha = 0.05$)					

In table 3.2, from there, we selected the most optimal concentration of extracted ethanol solvent, 60% ethanol, and used the results for the following studies.

3.3 Results of the study on the selection of the ratio of kudzu root material / solvent extract.

The amount of solvent affects the process of extracting substances in the raw materials. If the amount of solvent is too small, it is only enough to wet the material, so the extraction performance will be low. On the contrary, if the amount of solvent used is too much, it causes waste of solvents, fuel

during filtration and other costs. Therefore, finding out the ratio of raw materials / solvents is necessary for the process of extracting and collecting products.

Table 3. 3 - Effect of ethanol solvent concentration on polyphenol extraction efficiency

ProportionMaterial	1/10	1/15	1/20	1/25	1/30
Polyphenol content (µg/ml)	106.08^{e}	115.26^{d}	141.28^{a}	131.58^{b}	120.32^{c}
(Note: Values in the same column with different exponents differ in meaning α = 0.05)					

In table 3.3, we choose a raw material/solvent ratio of 1/20 as the basis for further experiments.

3.4 Results of the study on the selection of the time to extract polyphenols from kudzu root

Extraction time has a strong influence on extraction efficiency and energy and solvent costs. If the extraction time is short, then the active ingredients release little, but when the extraction time is increased, it consumes energy, the production process is prolonged. Therefore, we conduct a survey of the time levels and results recorded in the following table:

Table 3. 4 - Effect of time on the effectiveness of polyphenol extraction

Time (day)	1	2	3	4	5
Polyphenol content (µg/ml)	112.70^{e}	152.50^{d}	198.93^{a}	185.15^{b}	177.50^{c}
(Note: Values in the same column with different exponents differ in meaning α = 0.05)					

Through Table 3.4, we choose the extraction time at 3 days as the time of separation.

3.5 Results of the study on the effect of ultrasonic tank treatment time on the efficiency of polyphenol extraction in kudzu root

Ultrasonic waves have the effect of breaking chemical bonds, breaking cells and supporting the ability to extract substances contained in the material, so ultrasonic processing will improve the extraction efficiency of the compounds contained in the raw materials.

Table 3. 5 - Effect of the duration of the use of the ultrasonic tank on the polyphenol extraction process

Time (minutes)	0	2	4	6	8
Polyphenol content (µg/ml)	90.26^{e}	115.77^{b}	142.81^{a}	105.05^{c}	92.81^{d}
(Note: Values in the same column with different exponents differ in meaning α = 0.05)					

Table 3.5 shows the total number of polyphenols extracted with different ultrasonic treatment times and the process of ultrasonic impact in 4 minutes was selected for further experiments.

3.6 Results of the study on the effect of treatment time with thermostatic tanks on the effectiveness of polyphenol extraction in kudzu root

Thermostatic tanks have the effect of breaking chemical bonds, breaking cells, supporting the ability to extract substances in raw materials. Polyphenols are important bioactive substances in kudzu root, so treatment with a thermostatic tank will improve the efficiency of extracting the polyphenol content in kudzu root.

Table 3. 6 - Effect of thermostatic tank treatment time on polyphenol extraction efficiency

Time (minutes)	30	60	90	120	150
Polyphenol content (µg/ml)	126.99^{e}	140.26^{b}	148.42^{a}	136.68^{c}	129.54^{d}
(Note: Values in the same column with different exponents differ in meaning α = 0.05)					

Though table 3.6, we decided to use a thermostat tank for 90 minutes before extracting the glass for further experiments.

3.7 Results of the study on the effect of the temperature of the thermostatic tank on the effectiveness of polyphenol extraction in kudzu root.

Temperature is one of the factors that greatly affects the extraction process. The higher the extraction temperature, the porosity of the material will increase (due to the expanding material), and the active ingredient will dissolve more easily into the solvent. However, temperature is a limiting factor because too high temperatures can cause unnecessary reactions such as increasing the solubility of some impurities, difficulties for the filtration

process, promoting chemical transformations that make the quality of extracts unprofitable and increase production costs. Therefore, we conduct survey experiments at temperatures of 60℃, 70℃, 80℃ and 90℃. Results are presented through the following table:

Table 3. 6 - Effect of temperature on the effectiveness of polyphenol extraction in forest tapioca root

Time (℃)	40	50	60	70	80
Polyphenol content (µg/ml)	89.23^{d}	101.99^{b}	112.19^{a}	95.36^{c}	84.64^{e}
(Note: Values in the same column with different exponents differ in meaning α = 0.05)					

Therefore, we chose a temperature of 60℃ as the appropriate temperature for extracting the active ingredients in kudzu root.

4. Summary

Through the research results obtained when conducting experiments at the Laboratory of the Institute of Agriculture and Life – Thai Nguyen University of Agriculture and Forestry, we came to the following conclusions: Determining the optimal conditions for extracting polyphenols from kudzu root is: ethanol concentration 60%, raw material/solvent ratio 1/20, extraction time is 3 days, ultrasonic tank treatment time is 4 hours, temperature of thermostatic tank is 60℃, thermostatic tank treatment time is 90 minutes.

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