Comparative Research on Alkaloid's

Quantity among Clone Plants of Pinellia Ternate

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Abstract

Determine the quantity of Alkaloid among 15 clone plants of *Pinellia ternate* (Thunb.) Breit so as to offer theoretical basis on boosting the enrichment of effective elements in Pinellia ternate in plants' bodies. According to the principle of acid-dye colorimetry, adopt the method of chloroform extraction to determine under 417nm wavelength and make analysis of variance. Obtain favorable linear relationship (r=0.9989) and recovery (100.1%). RSD is 2.3%. The quantity of Alkaloid among the 15 clone plants of Pinellia ternate reaches to a prominent level (P=0.002<0.01). The differences of the elements' quantity measured among clone plants are extremely obvious, making it possible to propagate asexually by high-content variation plants in the short term to boost the quantity of effective elements in Pinellia ternate, and then realize the objective to boost the quantity rapidly from the individual selection.

Keywords: Pinellia ternate, Clone plants, Alkaloid, Comparative research

Traditional Chinese medicine (TCM) Pinellia ternata, widely distributed in most regions in our country, Korea peninsula and Japan, is Perennial herb dry stem tubers, which belongs to Araceae. The Pinellia ternata adding to the TCM was firstly recorded in *ShenNongBenCaoJing,* and it has been collected as the only medicinal materials plant in the Pinellia ternata and its adulterants in the *Chinese Pharmacopoeia* in 2005. The tuberosity contains various chemical elements, including alkaloid, â-Sitosterd, polysaccharide, Pinellia ternate protein, amino acid , volatile oil and inorganic elements and so on, and it is been reported that it has the efficacies such as drying damp and resolving phlegm, debasing or stopping vomit, eliminating pains and dispersing kink. Almost all the compound preparation of TCM which sells in the current market contains the Pinellia ternate, and Alkaloid, which possesses obvious bioactivity and physiological functions among many chemical elements, is one of the main effective elements in TMC Pinellia ternata. At present, the determination on the quantity of Alkaloid in Pinellia ternate has been worked on a lot(Yu, C., 2002, p.73. Zeng, J. H., 2004, p.477. Zeng, J. H., 2004, p.109. Zhuang H. M., 2007, p.384. Lu, S. P., 2006, p.1027), and the research on the chemical elements of Pinellia ternate is fastened on nonclone plants, but the research that clone plants produce Alkaloid hasn't been reported by now. Pinellia ternate can do the clone growth by stem tuber and bulbil, giving birth to the offspring which are completely the same as the heredity of mother plants and its clone growth is the main way to regenerate itself. Therefore, by discussing whether there has been one's own variation on the genetic level of the clone individuality with the same genotype, it is possible to reveal whether it has the distinct differences on the quantity of measured elements among clone plants, and realize the objective to boost the quantity rapidly from the individual choice.

1. Experimental Materials

1.1 Sample

The materials are from No.6 population fine clone stem tuber of *Pinellia ternata* (Thunb.) Berit in the Pinellia ternata bases of *Life Sciences College in China West Normal University* on Nov.6th, 2006, and those taken materials are the stem tubers from the same plant by asexual propagation . After collecting the materials, peel off and clean them, dry 4 hours in the oven at 105°C and then dry in the oven at 60°C till the constant weight, finally lay them in the desiccators by 60 meshes as the standby.

1.2 Reagent

Reference substance Ephedrine Hydrochloride (NICPBP, B.N.714-9903). Ammonia, dichloromethane, sodium citrate buffer solution (pH = 6. 0) and 0.1% of bromothymol blue solution, etc. are all the analytical reagent in the market.

1.3 Apparatus

GT3C916 Ultraviolet-Spectrophotometer (Australian GBC Scientific Apparatus Co., Ltd.), and Rotary Evaporator (Shanghai Shen Ke Electronics Co., Ltd.).

2. Experimental Methods and Results

2.1 The Preparation of Reference Substance Solution

Weigh up precisely 16.4mg of Ephedrine Hydrochloride reference substance which is dried to constant weight at 105°C. Put it in 250ml volumetric flask. Dissolve and dilute it to calibration with distilled water, shake it up, and finally make the reference substance solution which contains 0.0656 mg /ml concentration of Ephedrine Hydrochloride.

2.2 The Preparation of Sample Test Solution

Weigh up precisely Pinellia ternata powder, mix round the material with 12% ammonia to be wet, add 18 times higher chloroform than the quantity of the sample and dip in for 25 hours, then refluence and extract in the 70°C water boiler for 5 hours, filtrate, divide the leavings into three parts and wash them with 10ml chloroform. Combine them, displace the liquid into the 50ml volumetric flask, dilute it to the calibration with chloroform, shake it up, extract 2ml extract solution accurately to the Rotary Evaporator, and concentrate till becoming dry. Then add 5ml buffer solution of which the pH is 6.0, add minutely 10ml chloroform and 1ml 0.1% of bromothymol blue solution, then surge them enough. Finally, put it into the 60ml tap funnel for 0.5 hours statically to extract the chloroform layer as the sample test solution.

2.3 The Choice of Experimental Conditions

2.3.1 The Choice of the Wavelength of Maximum Absorption Spectroscopy

Weigh up the sample precisely, prepare the sample test solution based on item 2.2 and make the spectrometer scanning of reference substance solution and sample test solution between the wavelengths 380~ 600 nm. The result is that both of them will be at the absorption maximum in the 417nm wavelength, and their absorption peak as well as absorption spectroscopy is completely the same. Therefore, 417 nm is chosen to be the determining wavelength, as shown below.

2.3.2 The Study of Stability

Weigh up the sample precisely, determine in the 417 nm wavelength according to prepared sample test solution and blank control solution in item 2.2, once every 20 min under the room temperature. The result indicates that the developing of test sample solution is stable with 80 min. RSD=0.3% (n=5). Because this experiment only studies the developing stability of the test solution with 80 min, it can complete the whole determination within 80 min.

2.3.3 The Drawing of the Standard Curve

Extract accurately the reference substance 0.1, 0.3, 0.5, 0.7, 0.9ml, add distilled water respectively to 1.0ml, then add 5ml pH6. 0 sodium citrate buffer solution . Add accurately 10ml chlorine & 1ml 0.1% bromothymol blue solution and surge it enough, displace it to the 60ml tap funnel, lay statically for 0.5 hour, separate the chloroform layer and get the reference substance test solution. In addition, get 1ml water to make blank control solution by the same method, and determine the absorption value in the 417 nm wavelength.

Regard the concentration as abscissa, the absorption value as the vertical coordinate that gets the regression equation— Y= 0.1576X+ 0.0245, r= 0.9989 (n= 5). It indicates that ephedrine hydrochloride has a favorable liner relationship with absorptivity, which is in accordance with Beer's law.

2.4 The Study of Precision and Recovery

2.4.1 Precision

Extract the reference substance solution precisely, determine the absorptivity five times continuously in the 417 nm wavelength according to the prepared sample test solution and blank control liquid in item 2.2. RSD=0.06%. That indicates the apparatus has a better precision.

2.4.2 Recovery

Get Pinellia ternata powder about 0.657g, weigh up five parts precisely, add moderate ephedrine hydrochloride, then determine its absorptivity in the 417 nm wavelength according to the preparation test solution in 2.2. The average recovery is 100.1%, and RSD=2. 3%.

2.5 The Determination of the Sample Quantity

Weigh up Pinellia ternata powder accurately, prepare the sample test solution and blank control solution according to the method in item 2.2, determine its absorptivity and calculating quantity respectively in the 417nm wavelength, and do the analysis of variance by SPSS10.0. The result is as the Table 1and Table 2.

From Table 1 and Table 2, the variation among the genotypes of 15 clone alkaloid's quantity is shown. And the analysis of variance of alkaloid' quantity indicates that the quantity of different clone alkaloid has reached to the extremely significant level (F=3.604>F0.01, its significance probability Sig. =0.002<0.01). In the 15 clone alkaloid quantity, No.8 plant is the highest

to 0.2074% and No.13 plant is the lowest to 0.0677%, and their difference is 3.06 times as much. Therefore, it can be indicated that the genetic variation of alkaloid's quantity in No.8 plant is bigger than the other clone plants.

From the above, it can be seen that Pinellia ternata plants which are of the same age, in the same growing period and on the same ground may have complex and various changes of the quantities of alkaloid in different individuality. This illustrates that the genetic gene of Pinellia ternata, which decides individual growth, is also the key factor of controlling the metabolite of alkaloid. Because of this important effective element—quantity of alkaloid in Pinellia ternata, it is possible to make use of this kind of obvious genetic differences in quantity to propagate asexually by the current variation plants in the short term and to boost the quantity.

3. Discussion

The quantity of effective elements is controlled by genetic gene of plants and the polymorphism of genetic factors influences the metabolizing function of plants, and this variation in the same kind not only represents the differences in the individual description, but also represents the differences in the respects such as physiology and biochemistry and so on (Lu, S. P., 2006, p.1027). In this experiment, the clone plants of Pinellia ternata which are of the same age, in the same growing period and on the same ground represent the differences of alkaloid's quantity because of the differences of the individual growth characteristics. It is indicated that, the genetic gene of Pinellia ternata , which decides individual growth, is also the key factor of controlling the alkaloid's quantity . The quality variation in one kind is the result of apparent variation which is brought by the phenotypic variation decided by heredity.

Due to the apparent differences of alkaloid's quantity among different clone plants of Pinellia ternata , in order to make the quantity of alkaloid in Pinellia ternata stable, it is necessary to choose the clone plants with the homozygous genotype as the provenance in the course of artificial cultivation , advance the quantity of the effective elements in Pinellia ternata through the asexual propagation of variation plants with high content, and realize the aim of advancing the quantity rapidly by individual choice.

Not all the variation in the same plant will bring big external configuration, while in most cases, it represents on the physiological and biochemical property. Therefore, in order to understand the essential reason which influences the quality of Pinellia ternata, not only is it necessary to undertake research on its physiological characteristics and chemical elements, but also on its morphological, genetics and bionomics features synthetically and generally from the species biology.

In the aspect of the quality control of Pinellia ternata, attention should be focused on reaching the formation and accumulation of its chemical elements on the cells level and molecules level by the method of modern biology. For example, at the aim of boosting the effective bioactive elements, filter directional plants with high productivity by the assistant means of gene marker and gene mapping, and clone, express and control the key gene of the main effective elements in Pinellia ternata by genetic engineering technology so as to obtain the transgenic Pinellia ternata plants in the end.

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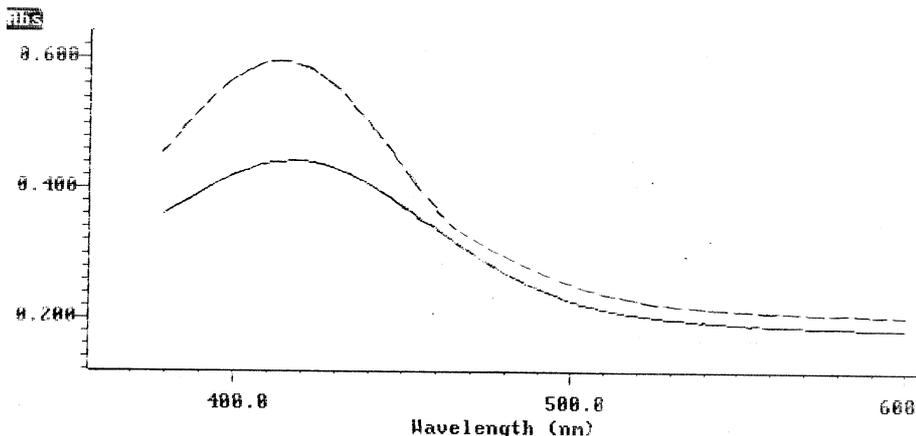
Figure 1. Macroscopic-ultraviolet Absorption Spectroscopy of Sample and Reference Substance
(the upper one is the sample curve, and the below one is the reference substance curve.)

Table 1. Determination Result of Alkaloid's Quantity in Different Clone Plants

No.	Average weight of the sample(g)	Average quantity of Alkaloid± standard deviation(%)
1	0.461	0.1478±0.025
2	0.336	0.1146±0.089
3	0.3801	0.1568±0.041
4	0.214	0.1347±0.043
5	0.2733	0.1803±0.039
6	0.738	0.0828±0.021
7	0.6243	0.0842±0.043
8	0.3313	0.2074±0.041
9	0.6523	0.1213±0.008
10	0.4017	0.1893±0.038
11	0.4837	0.1432±0.056
12	0.258	0.1229±0.022
13	0.434	0.0677±0.018
14	0.3073	0.1194±0.055
15	0.4843	0.0690±0.015

Table 2. One-way ANOVA Analysis of Alkaloid's Quantity in Different Clone Plants

	Sum of Squares	df	Mean Square	F	Sig.
Between	.077	14	.005	3.604	.002
Within Groups	.043	28	.002		
Total	.119	42			

$F_{0.05,14,28}$=2.12 $F_{0.01,14,28}$=2.9