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FERTILIZER AND CULTIVAR METHODS EFFECT TO BAICALIN ACTIVE INGREFIENT OF *SCUTELLARIA BAICALENSIS GEORGI*

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Abstract

The field experiment with three factor (N, P, K) was conducted to evaluate the effects of the fertilization rates of N, Pand K on the root baicalin content of Scutellaria baicalensis Georgi at harvesting time by clustering of High Performance Liquid Chromatography (HPLC). This study was carried out to find the fertilizer formula that has active baicalin with the highest content and to investigate the effect of numerous fertilization to the active ingredients of baicalin in Scutellaria baicalensis Georgi. The standard regressive curve equation of baicalin content obtained by using HPLC was: Y = (25.235) X- 14.871, R^2 = 0.9999. The average reproducible experiment result of baicalin was 36.96, RSD (relative standard deviation) is 1.295%; recovery rate is 97.69% and RSD is 0.89%. The result showed that fertilizer combination P3 treatment (150 kg Nitrogen (N) + 100 kg P_20_5 phosphorus (P2O5) + 100 kg Kalidioxid (K_20)obtained the highest baicalin content in Scutellaria baicalensis Georgi. Here is the formula recommended for production process of high quality baicalin in the future.

Keywords: *Scutellaria baicalensis Georgi; Baicalin content; High Performance Liquid Chromatography (HPLC), Fertilizer treatment*

INTRODUCTION

Scutellaria baicalensis is a member of the Lamiaceae family and known with other name as Huang-Qin. Huang-Qin roots as medicine and has a cold and bitter taste. It has been used as a medicine in several East Asian countries for more than 2000 years, especially in China. The botanical characteristics plant and medicinal dry root of *Scutellaria baicalensis* was described as Fig 1A, B. Recent study indicated that *accumulated* Huang-Qin Clinical data was useful for treating colds and bacterial pneumonia [1], [2].According to modern medicine, the active of baicalin ingredients in the root *have been effective* to reduce activity of HIV virus and may be anti-cancer activity [3],*which* inhibits the development of prostate cancer cells [4] and human liver cancer strains [5]. Some Flavonoid was extracted from the *Scutellaria baicalensis* plant as baicalin, which removes free radicals (hydroxyl, DPPH radical, alkyl radical), antioxidant [6] and reduce inflammation in human [7]. Furthermore, the functions of baicalin content was useful in relieving fire and detoxifying, cooling blood, nosebleeds, bloody diarrhea, haemorrhage, jaundice, gestation and pacifying fetus [8].

In addition, the demand of Scutellaria baicalensis will increase in market but the wild resources sharply decrease. At the same time, Fertilizer and cultivar methods are problems *due*to low yield and quality. Recent, application of GACP in cultivation and production of medicinal herbs is to ensure the quality of Vietnam medicinal materials and *to* control various factors that affect

the quality of medicinal materials plant. Numerous researchers indicate that optimal amount of fertilization was obtained by establishing mathematical models may increase yield and quality of plant [9], [10], but there are not many studies on medicinal plants yet.

Although Fertilizer and cultivar play a major role to yield and quality of baicalin medicinal content, study on *Scutellaria baicalensis* Georgi plant are very limited and there are still no scientific reports yet. Huang – Qin medicinal plant mainly grows in the wild and is scattering cultivated in small scale in Northwest provinces in Viet Nam. Therefore, the expansion of *Scutellaria baicalensis* Georgi plant production area is necessary in order to has more research for completing the commercial cultivation process in the future. In this study, we study the effect of the fertilizer amount quality of medicinal herbs in Sa Pa - Lao Cai province and the formula level of fertilizer has selected 150 kg N + 100 kg P205 + 100 kg K20 to obtained the highest baicalin content and it was done with two main objectives such as: 1) to differentiate the compound of Baicalin in the *Scutellaria baicalensis* among different *Scutellaria baicalensis* cultivars using HPLC method, and 2) to determine the effect of N, P, K on Baicalin in the *Scutellaria baicalens is* using HPLC method.

A B C

*Figure 1. Scutellaria baicalensis medicinal plant***A** *Scutellaria baicalensis* Georgi plant 25 DAP. B *Scutellaria baicalensis* Georgi plant180 DAP.**C** The dried root of *S. baicalensis* Georgi was used in traditional Vietnam medicine

MATERIALS AND METHODS

PLANT MATERIALS

Scutellaria baicalensis cultivars used in this study was *chosen* from China and planted in Vietnam as traditional Medicinal plant, the seeds were provided from resource division of National Institute of Medicinal Materials.

SITE DESCRIPTION AND SOIL ANALYSES

The experiment was conducted in 2018 at the farm yield of Sa Pa Research Center of Medicinal Materials.

Sa Pa Research Center of Medicinal Materials(22°22′ N, 103°52′E, 1200 - 1800 m asl), SaPa, town, Lao Cai province in Vietnam. The site *location*was in a subtropical monsoon climate zone with Daily mean temperature and solar radiation during the *Scutellaria baicalensis growth* were 20.4°C and 12.3 $MJm^{-2}d^{-1}$, respectively. The soil in field *Scutellaria baicalensis* paddy field was taken from the research farm. The soil was in texture with pH 6.62 and soil character of the experiment sites was analyzed as table 1.

Depth of Soil (cm)	Oganic matter (mg/100g)	Total N (N%)	Total P - (P2O5%)	Total K- (K2O%)	Available N (mg/100g)	Available P (mg/100g)	Available K (mg/100g)
0-40	13,9	0,15	0,17	0,79	0,63	1,93	4,18

Table 1. The soil of property analyse

EXPERIMENTAL TREATMENT AND DESIGN

Experiments with one experimental factor were used. 04the fertilizers with labels from P1-P4 were set as independent variable. Each treatment was replicated three times, placed in random complex blocks. Area of each plot was set 10 m^2. The total area of experiment was 120 m^2.

The fertilizers used formula P2:130 kg N + 87 kg P205 + 87 kg K20 as CK for each hectare. Size of cultivating places was specified 55 cm × 40 cm. In 2018, Scutellaria baicalensis seeds were cultivated at the farm of Sa Pa Research Center of Medicinal Materials. The fertilizers method included: (P1) 110 kg N + 73 kg P205 + 73 kg K20; (P3) 150 kg N + 100 kg P205 + 100 kg K20; (P4) 70 kg N + 113 kg P205 + 113 kg K20 respectively for P1, P3, P4 per ha -1. Three managements were set for each level and the formula application was adopted follow as: Basal fertilization: 50% N + 50% K20 + 100% P205; Top-dressing fertilizer: phase 1 (60 DAP): 25% N + 25% K20; phase 2 (180 DAP): 25% N + 25% K20.

INSTRUMENT AND REAGENT

In this experiment, Instrument and Reagent were used include: Waters Acquity UPC2 USA, HPLC, EmPower Color Spectrum Management System, PL203 electronic balance, KQ-5200DE numerical control ultrasonic cleaner, 5µl microliter syringe and 0.45 micro-perforated filter membrane. Acetonitrile and dichloromethane were both chromatographically purified. The Baicalin (98%) reference was provided by Chengdu Pufei De Biotech Co., Ltd, batch No21967-41-9.

CHROMATOGRAPHIC ANALYSIS CONDITION

Cosmosil C18 chromatographic column (UPC22-EP250 mm×4,6 mm, 5 µm), acetonitrile, monochloromethane and dichloromethane were the mobile phase, flow rate was 0.5 ml min^{-1}, column temperature was 28^0C; ELSD parameters: drift tube temperature 70^0C, spray tube temperature 25^0C, nitrogen gas flow rate was 1.5mL min^{-1}.

SOLUTION PREPARATION

Test article solution preparation: 0.6 g powder of *Scutellaria baicalensis* (filtered by Sieve 710) through precise weighing, and added 50 ml mobile phase through precise weighing, soaked the powder of *Scutellaria baicalensis* Georgi for 2 hours and placed it in ultrasonic treatment for 30 minutes and then taken out and cooled and weighed. After all, compensated the weight loss using mobile phase, filtered with 0.45 micro-perforated filter membranes, and finally took the subsequent filtrate as the finish solution.

Reference solution Preparation: Took appropriate amount of baicalin reference through precise weighing dissolve it in methanol to obtain a stock standard solution of 208 µg / ml. quantity of the baicalin standard was diluted from the stock standard as a series of solutions accurate concentration 10.4; 20.8; 62.4; 124.8; 166.4 µg / ml baicalin standard respectively.

EXPLORATION ON LINEAR RELATIONSHIP

An appropriate amount of baicalin reference (208 μg / ml) was taken through precise weighing and mobile phase was added and diluted at 1, 2, 4, 6, 8 and 10 times, respectively and precisely drew 2 μL for sampling and then the peak area was measured.

PRECISION TEST

The same test article solution was taken through 2 μL and implemented samples repeated for 6 times, then the integral value of peak area were measured to analyses the precision.

STABILITY TEST

The same test article solution was taken and analyzed through sampling at 2, 4, 6, 8, 12 and 24 hours in accordance with above-mentioned chromatographic condition, then the integral value of peak area was measured to evaluate the stability of the test article solution.

REPRODUCIBILITY TEST

Six samples of the same test article were taken through precise weighing, and then the article solution test was prepared in accordance as required.

RECOVERY RATE TEST

Six samples of known contents were taken through precise weighing, then a precisely-weighed amount of baicalin reference was added in HPLC system, the article solution test was prepared as required by instructions and then the peak area and the contents were measured.

MEASUREMENT OF BAICALIN CONTENT (USP- HTTP://HMC.USP.ORG)

The logarithms of peak responses were plotted to compare with the logarithms of baicalin concentrations in mg mL^{-1} from the standard solutions and the regression line was determined using a least-squares analysis; or, a linear regression equation was established using a least-squares analysis according to the logarithms of the peak responses versus logarithms of baicalin concentrations in mg mL^{-1} from the standard solutions.

The concentration, C, in mg mL^{-1}, was determined by regression line of the relevant analyze in the sample solution or linear regression equation. The percentages of baicalin in *Scutellaria baicalensis* Georgi were calculated separately follow as:

$$X\ (\%)=\frac{CX\ 100\ X\ 10\ X\ 100}{wX\ 1000\ X\ (100-A)}\ X\ 100 = \frac{C\ X\ 10000}{w\ X\ (100-A)}$$

Explanation

X= Baicalin content (%)

C= Concentration of the relevant analyte in the sample solution as determined above (mg mL^{-1})

A= volume of the sample solution (oC)

W= weight of *Scutellaria baicalensis* Georgi seed taken to prepare the sample solution (mg)

STATISTICAL ANALYSIS

Statistical data analysis using IRRISTAT5.0 and Excel 2007 software, and then strain and variety fertilizing amount were included as experimental factor to calculate the significant difference of glyceryl trioleate content in *Scutellaria baicalensis* Georgi

RESULTS AND DISCUSSION

EVALUATE EXPLORATION BASE ON LINEAR RELATIONSHIP

The standard curve was drawn with baicalin reference (μg) as the horizontal axis and baicalin peak area as the vertical axis (y). The result was shown in Figure 2. The regression equation was calculated with least square method in accordance with the standard curve: Y=(25235)X-14871, R^2 = 0.9999 and Correlation coefficient R^2 than 0.9999 indicate that standard curve with high linearity total guaranteed to analyses quantitative baicalin content in *Scutellaria baicalensis* Georgi.

In addition, in the chromatogram (Figure 3), the peak of retention time of the solution *Scutellaria baicalensis* Georgi test article was 0.5 minutes which was consistent with baicalin content.

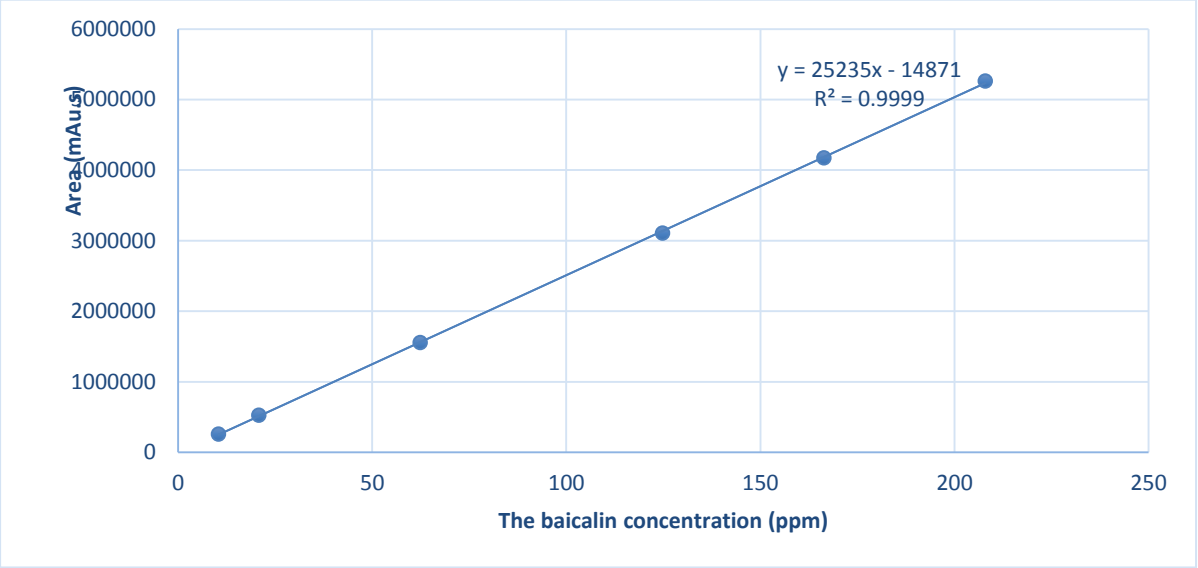

Figure 2. Standard curve for HPLC quantitative analysis of baicalin

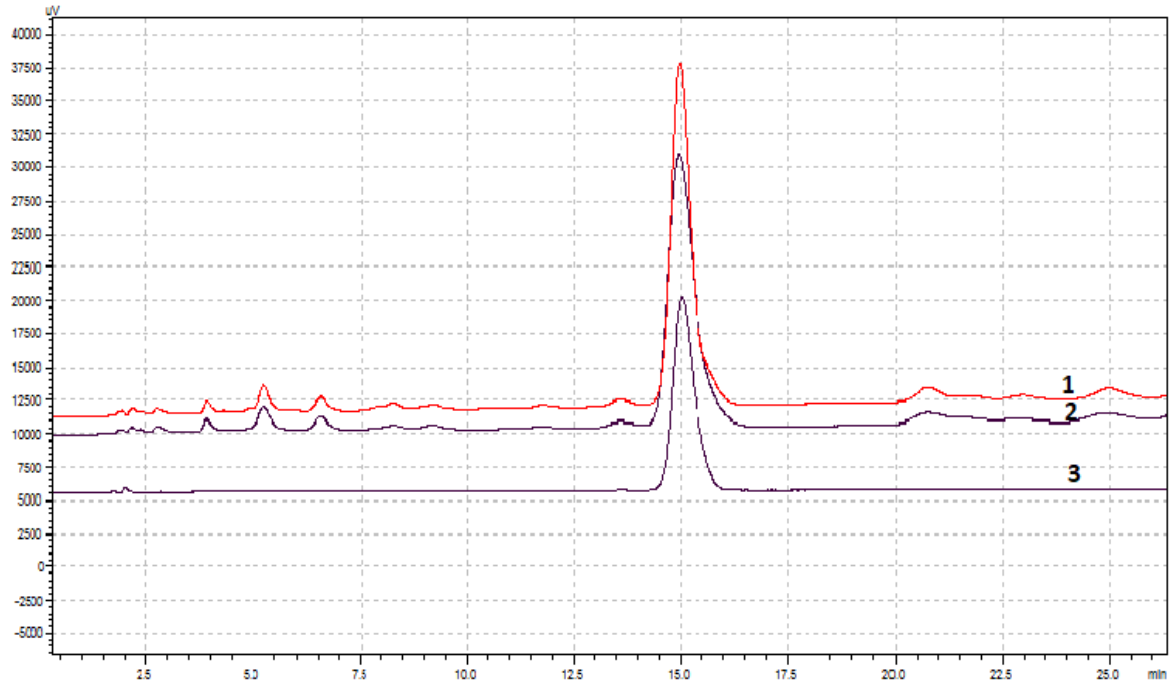

Figure 3. HPLC chromatograms of Baicalin in *Scutellaria baicalensis* Georgi (1. The curve of

Baicalin in sample; 2. The curve of Baicalin in sample and Baicalin reference 3. Standard of Baicalin reference)

The results show that the test sample has relatively clear and sharp separation peaks and the baicalin peak in the sample has a retention time similar with the standard of baicalin reference. Therefore, the method is accurate to analysis quanlity of baicalin in the *Scutellaria baicalensis* Georgi

PRECISION TEST

The measured RSD of baicalin peak area was 0.399%, indicated a good precision (Table 2). The results of the standard curve of peak areas are less than 2%, it's indicate that the selected chromatographic conditions and the HPLC system used are suitable and guaranteed for stability of the quantitative baicalin.

STABILITY TEST

The measured RSD of glyceryl trioleate peak area within 24 hours was 0.739, indicated that the test article solution was basically stable within 24 hours (Table 3).

REPRODUCIBILITY TEST

The average content of baicalin content was measured at 36.96 ppm, RSD was 1.295% (Table 4) less than 2%. Thus the method has good reproducibility.

Table 2. Precision of baicalin in *Scutellaria baicalensis* Georgi (n=6)

Repeat times	1	2	3	4	5	6	Average	RSD%
Peak area	4172313	4168557	4173270	4180828	4177382	4213994	4181057	0.399

Table 3. Stability of of baicalin in *Scutellaria baicalensis* Georgi (n=6)

Repeat times	2h	4h	6h	8h	12h	24h	Average	RSD%
Peak area	899736	896546	899044	901078	902640	915370	902402	0.739

Table 4. Reproducibility of baicalin in *Scutellaria baicalensis* Georgi (n=6)

Repeat times	1	2	3	4	5	6	Average	RSD%
Glyceryl strioleate content（ppm）	36.24	37.57	37.09	37.29	36.96	36.60	36.96	1.295

RECOVERY RATE TEST

The recovery rate of baicalin was measured at 95% to 105%, the average recovery rate was 97.69% and RSD was 0.89% (Table 5).

Table 5. The results of recovery test (n=4)

Repeat times	1	2	3	4	Average	RSD%
Baicalin recovery	0	103.0	97.00	98.30	97.69	0.89

IMPACT OF FERTILIZER MANAGEMENT TOBAICALIN CONTENT IN*SCUTELLARIA BAICALENSIS* GEORGI

In this study, as shown in Figure 4*Scutellaria baicalensis* Georgi plants under four fertilization treatment. After treatment, *Scutellaria baicalensis* Georgi leaves were collected for measurements of baicalin content. All data are means ± SD calculated from three replicates. Symbols * and ** indicate significant difference between lines at 0.05 and 0.01 levels, respectively. Three biological experiments were performed, which produced similar results.

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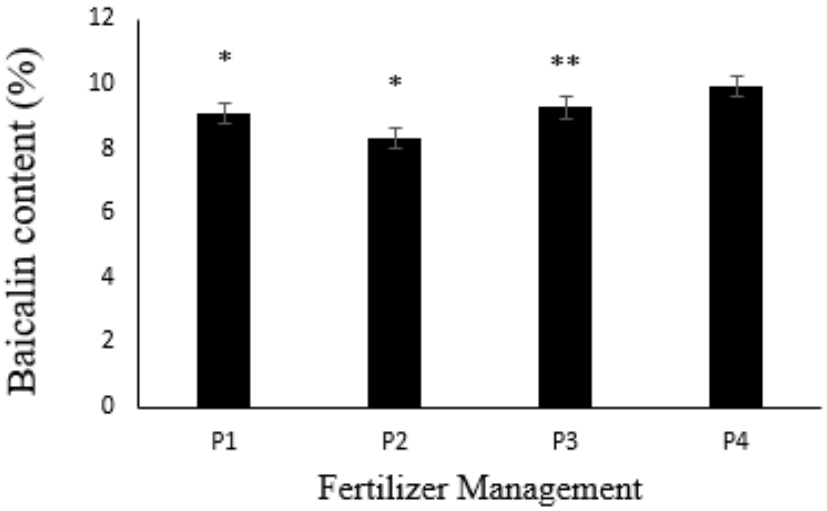

Figure 4 the effect of fertilization on the baicalin content of *Scutellaria baicalensis* Georgi

The result indicated that under different fertilization application level, at 95%, 99% confidence level, all the treatments have significant differences in baicalin content compared with P2 treatment (without fertilizer application) and except for the P4 treatment.
Thus, it found that the fertilizer levels difference had significant effect on baicalin content in *Scutellaria baicalensis* Georgi plant in this experiment which P3, P4 had the highest content and P2 was preferred for the fertilization amount that has lowest fertilizer level. In order to increase the yield of baicalin and for the content of baicalin more than 14%, the formula of fertilization P3 150 kg Nitrogen (N) + 100 kg P_20_5 phosphorus (P2O5) + 100 kg K_20 Nitrogen (N) 90. 5 ~ 104. 7 kg and phosphorus (P2O5) 163. 9 Kalidioxid (K2O) ~ per hectare was chosen for experiment.

CONCLUSION AND SUGGESTION

In recent decades, many studies indicated that fertilization involved in *improving* the yield of root crops [9], [11] such as enhancing the yield and the content of herbal. However, the effects of N, P and K on the yield of different plants were not the same.
Li Jiaohong et al. 2009 found that the effect of fertilizer on the yield of Bletilla striata depends on the fertilization methods [12].Similarly, the effects of nitrogen, phosphorus and potassium on the yield of Scutellaria baicalensis Gewerp were analysed as *strong* effect nitrogen fertilizer, > potash fertilizer, > phosphate fertilizer, respectively. *This* research indicated that fertilizer was beneficial to astragalus membranaceus plant.

In the other hand, fertilization plays an important role in plant energy systems, specially under suitable phosphorus application level, it was also effective to store in the form of ATP and the energy is activated and released as needed to reverse the chemical should be carried out stably [13].Fertilizer factor can also promote the synthesis and transformation of photosynthetic products and transport, allowing more organic matter to be distributed to the root system, so that phosphorus is applied that It is beneficial to increase the yield of Scutellaria baicalensis. In this experiment, the yield of potash fertilizer to Scutellaria baicalensis[14], [15]. Otherwise,

several studies have suggested that S. baicalensis can effectively inhibit fibrosis and lipid peroxidation in rat liver [16], [17]. Moreover, baicalin can prevent the entry of HIV-1 into animal cells by perturbing the interaction between HIV-1 Env and HIV-1 co-receptors on the cell surface [18] Baicalin has been adopted as one of the popular lead natural products for preventing HIV infection [19].

In this experiment, the single factor effect of scutellaria baicalensis Geoli yield was analyzed. The results show that, the low range of fertilizer level may decreases the yield of *Scutellaria baicalensis* Georgi, but over fertilizing can even lead to disease resulting in severe crop loss [20]. The main factor of fertilizer effect analysis of baicalin content showed that it is beneficial to the synthesis of baicalin, which is similar to the research conclusion of Su Shuxin et al. 1996 [15].The fertilization management experiment showed that treatment P3 (150 kg Nitrogen (N) + 100 kg P_20_5 phosphorus (P2O5) + 100 kg Kalidioxid K_20 had the highest baicalin content in *Scutellaria baicalensis* Georgi. Thus, this formula was recommended to increase yield and quality of baicalin content. By optimizing *Scutellaria baicalensis* Georgi and its cultivar treatment, it can be targeted for the development to enhance the baicalin content as functional in the traditional medicine. The other compounds that contained in *Scutellaria baicalensis* Georgi seed should also further investigated. In addition, their ability in the medical treatment need further studies in the future.

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