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A STUDY ON THE PROCESS OF POLYSACCHARIDE EXTRACTION FROM *MYXOPYRUM SMILACIFOLIUM* (WALL.) BLUME ROOTS

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Optimization, extraction, parameters, Myxopyrum smilacifolium (Wall.) Blume, polysaccharide.

Abstract:

Myxopyrum smilacifolium (Wall.) has been demonstrated to contain many bioactive compounds, including polysaccharides, saponin, and flavonoids. This study aimed to determine the effects of the single-factor model on the attraction process as a basis for optimal performance. The obtained extract will be evaluated for its antioxidant capacity. The results of the single-factor when extracting the total polysaccharide from *Myxopyrum smilacifolium* (Wall.) showed the effects of the solvent concentration, solvent/material ratio, extraction time, and temperature on the extraction process with the corresponding results: ethanol 60% (v/v), 20/1 (ml/g), 75 minutes and 70°C respectively. The optimal conditions have been found for the total polysaccharide extraction from *Myxopyrum smilacifolium* (Wall.): ethanol concentration 66.58%, solvent/material ratio 20.46/1 (ml/g), extraction time 76.77 min, extraction temperature 70°C, respectively. Under the conditions, the total polysaccharide content of the extract reached 3.34937 mg/g of the raw material of *Myxopyrum smilacifolium* (Wall.) leaves. The extract from *Myxopyrum smilacifolium* (Wall.) gave an IC_{50} value of 522.56 ± 13.67 (µg/ml) when evaluating its antioxidant activity.

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NGHIÊN CỨU QUY TRÌNH CHIẾT XUẤT POLYSACCHARIDE TỔNG SỐ TỪ LÁ SÂM XUYÊN ĐÁ

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Từ khóa:

Tối ưu, chiết xuất, thông số, sâm xuyên đá, polysaccharide.

Tóm tắt

Sâm Xuyên Đá (*Myxopyrum smilacifolium* (Wall.) Blume) đã được chứng minh chứa nhiều hợp chất có hoạt tính sinh học như polysaccharide, saponin, flavonoid. Nghiên cứu này xác định ảnh hưởng của đơn yếu tố đến quá trình chiết xuất, làm cơ sở thực hiện tối ưu. Dịch chiết thu nhận sẽ được đánh giá khả năng chống oxy hóa. Kết quả đơn yếu tố khi chiết xuất polysaccharide tổng số từ lá sâm xuyên các thông số nồng độ dung môi, tỉ lệ dung môi/nguyên liệu, thời gian, nhiệt độ chiết ảnh hưởng tới quá trình tách chiết polysaccharide tổng số từ lá sâm xuyên đá cho kết quả tương ứng: ethanol 60% (v/v), 20/1 (ml/g), 75 phút và 70°C. Đã tìm được điều kiện tối ưu quá trình tách chiết polysaccharide tổng số từ lá sâm xuyên đá: nồng độ ethanol 66,58%, tỉ lệ dung môi/nguyên liệu là 20,46/1 (ml/g), thời gian chiết 76,77 phút, nhiệt độ chiết 70°C. Trong điều kiện này hàm lượng polysaccharide tổng số chiết đạt 3,34937 mg/g nguyên liệu lá cây sâm xuyên đá. Dịch chiết từ lá sâm xuyên đá cho giá trị IC_{50} là 522,563,67 (µg/ml) khi đánh giá hoạt tính chống oxy hóa.

1. Introduction

*Myxopyrum smilacifolium (*Wall.) Blume is also known as Nhuong Le Kim Cang, Duong Le Kim Cang which is a plant capable of growing through rocks and precious herbs [5]. This plant is distributed in the following countries on the world such as: Hainan (China), Bangladesh, Cambodia, India, Laos, Myanmar, Thailand, and the Andaman and Nicobar Islands. In Vietnam only found in old forests in the Northwest region of Viet Nam for example Ha Giang, Lao Cai, Lai Chau., Thai Nguyen and Yen Bai.... Where have the appropriate soil. This plant lives mainly on the rock crevices of Limestone Mountains. The main biological activities of Myxopyrum smilacifolium Blume involving antioxidant, antibacterial, reducing blood sugar, oxidizing, antibacterial, reducing blood sugar, lowering blood fat, and against obesity [2].

There are many factors affecting the extraction process such as microwave, solvent, temperature, time, the number of extraction... The purpose of this study is to determine the conditions for obtaining extracts with high total polysaccharide content and to evaluate the antioxidant capacity from the leaves of *Myxopyrum smilacifolium* (Wall.) Blume grown in Thai Nguyen.

Based on the published results, in this study, ethanol solvent was used in the range of 50 - 90%; extraction solvent/material ratio was used in 15/1-30/1 (ml/g), time for extraction from 45 - 90 minutes,

and extraction temperature at 60 - 90°C.

Reducing the parameter has little effect to perform the optimization problem, making the experimental process more convenient without significantly affecting the results of polysaccharide collection. Three factors significantly affecting the extraction efficiency were selected optimally according to the experimental design of Box-Behnken (Box et al., 1951) [1]. The extract after optimization was evaluated for antioxidant activity.

2. Material and methods

2.1. Material

Myxopyrum smilacifolium (Wall.) Blume- 2-year old was obtained in the early morning of October at La Hien commune, Vo Nhai district, Thai Nguyen province. The sample was identified by the comparative morphological method at Vietnam National University - Ho Chi Minh City, University Of Science. The leaves were used for the analysis.

Chemicals and media used in the experiment: ethanol (EtOH), phenol of Merck-Germany (pure form), concentrated H2SO4 (Vietnam); chemicals used in extraction and analysis met PA standards.

Equipment: Memmert UN110Plus drying oven - Germany, OHAUS analytical balance - USA, LV-VC1200 incubator - Vietnam, Memmert incubator - Germany, JSR JSAT-65 autoclave - Korea

2.2. Methods

2.2.1. Experimental layout method

Total polysaccharide was extracted from 10 gram of fresh leaves of *Myxopyrum smilacifolium* (Wall.) Blume and macerated with ethanol solvent at concentration of 50; 60 ; 70; 80 and 90% and extraction temperatures 60°C, 70°C, 80°C and 90°C for periods of 60, 70, 80, 90 minutes with a solvent:material ratio of 15:1; 20:1; 25:1 and 30:1 (ml/g) respectively. To detect the optimum solution for these circumstances, we use the Box-Behnken quadratic planning [1], which employs three components and 17 experiments, all determinations were performed five times at the best results for each unit element.

2.2.2. Determination of total polysaccharide content by using phenol - sulfuric method

Total polysaccharides were determine by the phenol-sulfuric acid method. The steps are briefly described as follows: 400 µl of sample solution containing total polysaccharide was reacted with 200 µl of 5% phenol solution, added 1 ml of concentrated H2SO4, and left for 30 min at room temperature.A spectrophotometer set at 490 nm measured the color of the reaction. The total polysaccharide content of the experimental sample was determined by comparing the resulting OD measurement to the glucose standard graph.

2.2.3. Evaluation of antioxidant activity

1,1 -diphenyl-2-picrylhydrazyl (DPPH) is a free radical used to screen for the antioxidant activity of the studied substances. The antioxidant activity was demonstrated by reducing the DPPH color of the reagent, as determined by measuring the absorbance at 517 nm on a spectrophotometer.

Prepare a DPPH solution in methanol with a concentration of 2mM. (MeOH). This solution is not light-stable and must be prepared prior to use.

Test solution: Take a sample mixed in water. Shake the tubes for 15 s, stabilize at room temperature for 30 min, and measure at 517 nm. Vitamin E was a positive control, ethanol was a negative control. The antioxidant activity was calculated according to the formula below.

$$\%\text{Reduction} = \frac{(OD_{blank} - OD_{test)} \times 100}{OD_{blank}}$$

Where: $OD_{blank,}$ OD_{test}:the absorbance values of the blank and of the test of sample respectively.

The value indicates the concentration of an extract that may decrease 50 % of DPPH free radicals under specific circumstances. The lower the value, the higher the DPPH free radical scavenging activity.

2.2.4. Analysis method

One factor analysis for the extraction process was analyzed for variance and compared the mean values with the level of $\alpha \leq 0.05$ using SPSS software (version 20). Analyze variance (ANOVA), compute coefficients of regression equations, and offer solutions for optimal models were all done with Design-Expert software (version 7.1.5, Stat-Ease Inc., USA). Optimization.

3. Result and discustion

3.1. Effect of solvent concentration

To evaluate the effect of solvent concentration on the extraction of total polysaccharides in *Myxopyrum smilacifolium* (Wall.) Blume leaves, experiments were carried out at solvent concentrations: 50, 60, 70, 80, and 90% respectively. With some of the fixed parameters such as mass sample: 10gram, extraction solvent/material ratio: 20/1 (ml/g), extraction temperature 70°C for 60 minutes. Based on the total polysaccharide content to opt for the appropriate solvent concentration, the research results are demonstrated in Table 1.

According to table 1, different ethanol concentrations will result in varied total polysaccharide content, and the quantity of ethanol concentration will grow from 50% to 60%. The greatest total polysaccharide content (3.13 mg/g) was found at a 60 % ethanol concentration, Futhermore when the ethanol concentration was increased, the total polysaccharide content declined. The amount of solvent to use for extraction is determined by the plant component and species [3]. In this study, 60 % ethanol was collected for use in future research.

Table 1: Effect of solvent concentration on total polysaccharide extraction efficiency from Myxopyrum smilacifolium (Wall.) Blume leaves

Ethanol concentration (%)	50	60	70	80	90
The amout of total polysaccharide (mg/g leaves)	1,89^{d}	3,13^{a}	2,73^{b}	2,72^{b}	2,40^{c}

Note: Values in the same row with different exponents represent the significantly different at the α= 0.05 level.

3.2. Effect of extraction solvent/material ratio

The amount of solvent used has a significant impact on the extraction of the substances in the raw materials. If the amount of solvent used is insufficient, it will simply moisten the material, resulting in low extraction efficiency. On the other hand, if the amount of solvent utilized is excessive, it will result in solvent waste, fuel consumptionduring the filtering process, and other expenditures. Experiment with the following solvent/extracting material ratios: 15/1, 20/1, 25/1, and 30/1, with an ethanol concentration of 60% and an extraction temperature of 70°C. The time limit is 60 minutes. Table 2 summarizes the findings.

Table 2. Effect of extraction solvent/material ratio on total polysaccharide extraction efficiency from Myxopyrum smilacifolium (Wall.) Blume leaves

Extraction solvent/material ratio	15/1	20/1	25/1	30/1
The amout of total polysaccharide (mg/g leaves)	2,21^{b}	3,13^{a}	3,14^{a}	3,14^{a}

Note: Values in the same row with different exponents represent the significantly different at the α= 0.05 level.

Table 2 shows that when the solvent/material ratio increases, the total polysaccharide content increases. The total polysaccharide content rose insignificantly when the solvent ratio was gradually raised from 20/1 to 30/1, and there was no significant difference at the 0.05 level. This is explained by a 20/1 ratio, which equalizes the concentration of the extract and the solvent. The extraction of total polysaccharides with a solvent/material ratio of 10/1 was examined by Vo Hoai Bac, and the study also revealed differences owing to different materials and extraction processes [8]. The solvent/material ratio of 20/1 was chosen as the foundation for subsequent research to assure extraction efficiency while minimizing production costs.

3.3. Effect of extraction time

Extraction efficiency, as well as energy and solvent costs, are affected by extraction time. The active components are released less when the extraction period is short, but when the extraction time is increased, energy is squandered and the production process is protracted.

Investigation of time levels 45, 60, 75, and 90 minutes, at 60% ethanol concentration, extraction solvent/material ratio: 20/1 (ml/g), extraction temperature 70°C, and results were obtained in Table 3

Bảng 3. Effect of extraction time on total polysaccharide extraction efficiency from Myxopyrum smilacifolium (Wall.) Blume leaves

Time (minutes)	45	60	75	90
The amout of total polysaccharide (mg/g leaves)	2,67^{c}	3,13^{b}	3,21^{a}	2,68^{c}

Note: Values in the same row with different exponents represent the significantly different at the α= 0.05 level.

Based on table 3: The total polysaccharide content increased as the extraction time increased. However, the amount of active components grows extremely slowly or tends to decrease up to a specific extraction period. The total polysaccharide content was low (2.67 mg/g) after 45 minutes of extraction. When extracted for 60 minutes, active compounds rose significantly, with a total polysaccharide content of 3.13 mg/g. However, the overall polysaccharide content tended to decrease after 75 minutes, which might explain why 75 minutes the maximum saturated polysaccharide dissolving time was. Increasing the extraction time may result in partial degradation of the total polysaccharide.

According to Damaso et al. (2020), the extraction time for the stem *Myxopyrum smilacifolium* (Wall.) Blume was 90 minutes [4], and the results indicated that total polysaccharide extraction time from the leaves was quicker than that from the stem. To extract the entire polysaccharide from *the Myxopyrum smilacifolium* (Wall.) Blume leaves, we set the extraction duration to 75 minutes in this investigation.

3.4. Effect of temperature extraction

Temperature is one of the most important elements in the extraction process. The higher the extraction temperature, the greater the material's porosity (due to swelling), the lower the viscosity, and the simpler it is for the active component to dissolve into the solvent.

On the other hand, Temperature is a limiting factor since it may produce unwanted reactions such as increasing the solubility of some impurities, making filtering harder, and encouraging chemical changes.

The quality of the extract becomes unprofitable as a result of the studies, which increases production costs. As a result, survey tests were done at 60°C, 70°C, 80°C, and 90°C, the ethanol concentration of 80 %, and extraction solvent/material ratio of 15/1 (ml/g), and time extraction were 75 minutes. Table 4 shows the findings that were achieved.

Table 4. Effect of time extraction on total polysaccharide extraction efficiency from Myxopyrum smilacifolium (Wall.) Blume leaves

Temperature (°C)	60	70	80	90
The amout of total polysaccharide (mg/g leaves)	3,09^{b}	3,21^{a}	3,08^{b}	2,57^{c}

Note: Values in the same row with different exponents represent the significantly different at the α= 0.05 level.

Table 4 shows that when the temperature rose, the total polysaccharide content rose proportionately. At 60°C, the total polysaccharide content was 3.09 mg/g, and when the temperature was raised to 70°C, the total polysaccharide content was 3.21 mg/g.

This may be explained by the fact that in the ethanol solvent extraction process when the temperature is gradually increased, the kinetics of the extraction process improves, and the compounds removed from the plant cells improve.

However, some compounds can disintegrate as the temperature rises; moreover, when the extraction temperature exceeds the boiling point of ethanol, it will interfere with the extraction process, lowering the extraction capacity. Research by Damaso et al. (2020) determined that the extraction temperature *Myxopyrum smilacifolium* (Wall.) Blume stem was 90°C [4]. According to research by Nguyen Van Binh et al. (2018), the extraction temperature of total polysaccharides in *Ganoderma lucidum* was 90°C [6]. The distinction is owing to the various materials, methods, and solvents employed. Therefore, the temperature of 70°C was chosen as the appropriate temperature for the extraction of active ingredients in *Myxopyrum smilacifolium* (Wall.) Blume

3.5. Optimization extraction process of total polysaccharides from *Myxopyrum smilacifolium* (Wall.) Blume leaves by quadratic planning method.

Solvent concentration, solvent/material ratio, and extraction duration are among the parameters that substantially influence the extraction process, according to research into factors impacting extraction conditions. The study employed the Box-Behnken experimental design response surface approach with three three-level variables. The data were processed on Design-Expert 7.0 software (Stat-Ease Inc, Minneapolis, USA) ANOVA was used to evaluate the statistical parameters..

The Box-Behnken model was used to construct an experiment with 17 experimental units and 3 replicates with specified variables to maximize the extraction of total polysaccharides from *Myxopyrum smilacifolium* (Wall.) Blume leaves. The factors to be optimized include ethanol concentration (X1) at (-1, 0, +1) respectively (50%, 60%, 70%); solvent/material ratio (X2) at (-1, 0, +1) is (15 ml/g, 20 ml/g, 25 ml/g) and extraction time (X3) at (-1), 0, +1) is (60 minutes, 75 minutes, 90 minutes).

Applying regression analysis method of experimental data, obtained a quadratic polynomial model illustrated total polysaccharide content:

$$Y = + 3,17 + 0,61*A + 0,08325*B + 0,63*C + 0,052*A*B + 0,570*A*C - 0,14*B*C – 0,72*A^2 – 0,79*B^2 – 1,29*C^2$$

Where: The total polysaccharide content of the produced leaf extract is denoted by Y, while the factors of solvent concentration, solvent/material ratio, and extraction time are denoted by A, B, and C, respectively.

The model was evaluated using an ANOVA analysis, which revealed the interaction between parameters impacting total polysaccharide content. Table 5 displays the results of the ANOVA analysis.

Table 5. Matrix of a three-factor Box−Behnken design and total polysaccharide content from *Myxopyrum smilacifolium* (Wall.) Blume leaves under different extraction conditions

Experiment	Factors			The amout of total polysaccharide (mg/g leaves)
	A - ethanol	B -solvent/ material ratio	C-extraction time	
1	50	15	75	1,066
2	70	15	75	2,193
3	50	25	75	1,0281
4	70	25	75	2,3625
5	50	20	60	0,4543
6	70	20	60	0,675
7	50	20	90	0,6375
8	70	20	90	2,8575
9	60	15	60	0,3075
10	60	25	60	0,5475
11	60	15	90	1,905
12	60	25	90	1,6
13	60	20	75	3,12
14	60	20	75	3,195
15	60	20	75	3,165
16	60	20	75	3,157
17	60	20	75	3,2

Note: A: Ethanol concentration (%); B: solvent/ material ratio (ml/g); C:Extraction time (minutes).

Table 6. Analysis of variance ANOVA of the model extracting extracts from Myxopyrum smilacifolium (Wall.) Blume leaves

The source	F Standard	P Value
Model	823,00	<0,0001
Lack of Fit	4,78	0,0825
R^2	0,9991	

Note: F standard: Fisher Standard; "Lack of Fit": The standard for determining the model's incompatibility with the experiment. R^2 is the coefficient of regression.

By examining Table 8, we were able to determine the model's significance and compatibility. Using ANOVA, we discovered that the model's probability value P-value < 0.0001 < 0.05, indicating that a model is a viable option. R^2 = 0.9991 as a regression coefficient.

This result indicates that 9.91% of the experimental data are compatible with the model's predictions.

Design-Expert software was used to optimize the total polysaccharide content obtained from

Myxopyrum smilacifolium (Wall.) Blume leaves extract using the anticipated function technique (DX 7.1.5).

The optimal option to optimize the objective function is: ethanol 66,58%, extraction solvent/ material ratio 20.46 ml/g leaf, and extraction time 76.77 minutes. The total polysaccharide content attained under the circumstances mentioned was then estimated to be 3.34934 mg/g leaves (Figure 2). When compared to the experimental test findings, this result shows a high level of compatibility. The results are shown in figure 2.

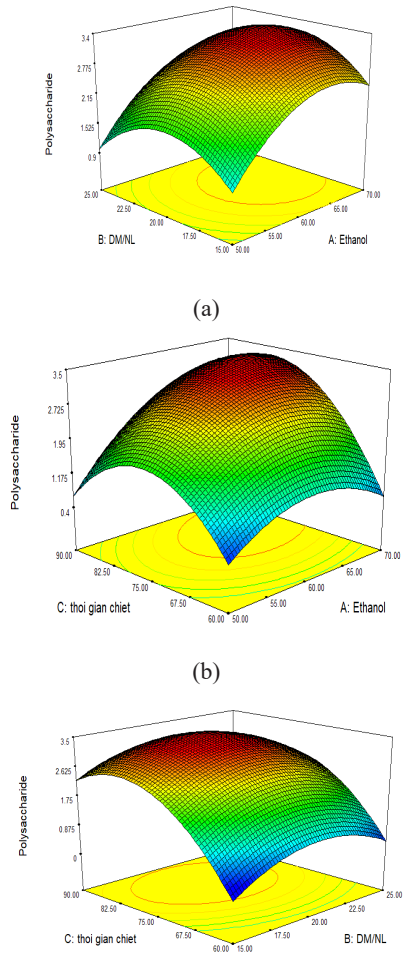

Figure 1. Shows how the surface reacts to total polysaccharide content.

Note: a): Model of interaction between ethanol concentration and solvent/material ratio ;

(b): Interaction model between ethanol concentration and extraction time,

(c): Extraction time interaction model and solvent/material ratio ;

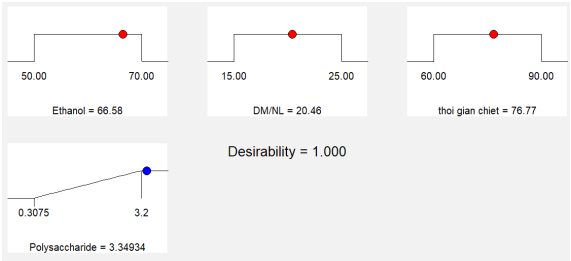

Figure 2. Expected function and optimal conditions for total polysaccharide content

3.6. Results antioxidant activity testing of extracts produced from Myxopyrum smilacifolium (Wall.) Blume leaves using a parameter-optimized total polysaccharide extraction technique

The antioxidant activity of polysaccharide extract derived from Myxopyrum smilacifolium (Wall.) Blume leaves, stems, and roots shows in Table 7

Table 7: The antioxidant activity of polysaccharide extract derived from *Myxopyrum smilacifolium* (Wall.) Blume

Sample	IC_{50} (µg/ml)
Vitamine E	61.177,52
The antioxidant activity of polysaccharide extract derived from *Myxopyrum smilacifolium* (Wall.) Blume leaves	522.563,67

Antioxidant-capable substances give electrons to DPPH free radicals, resulting in stable DPPH molecules that lose their initial purple hue.The IC50 value was used to assess the antioxidant properties of whole polysaccharide extracts. Table 7 shows that the *Myxopyrum smilacifolium* (Wall.) Blume polysaccharide extract possesses DPPH free radical scavenging activity, however, it is considerably lower than the control vitamin E.This suggests that it might be a good source of antioxidants. This finding is similar to Rajameena R. and Cs's (2013) [9] research to inhibit the advancement of oxidative stress.

4. Conclusion

The extraction of total polysaccharides from *Myxopyrum smilacifolium* (Wall.) Blume leaves is affected by solvent concentration, solvent/material ratio, extraction duration, and temperature in this study, with the following results: ethanol 60% (v/ v), 20/1 (ml/g), 75 minutes, and 70°C. The following parameters were determined to be optimal for extracting total polysaccharides from *Myxopyrum smilacifolium* (Wall.) Blume leaves: ethanol concentration 66.58%, solvent/material ratio 20.46/1 (ml/g), extraction time 76.77 min, extraction temperature 70°C, respectively. The total polysaccharide content of the extract in these circumstances was 3.34937 mg/g of *Myxopyrum smilacifolium* (Wall.) Blume leaves raw material. When it came to antioxidant activity, the ideal post-optimum extract from *Myxopyrum smilacifolium* (Wall.) Blume leaves (IC50 = 522.56 13.67 (g/ml)) was not as powerful as the stem extract (522.56 ± 13.67 (µg/ml))

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ASSESSMENT OF THE GROWTH AND YIELD OF *ANGELICA ACUTILOBA* KIT. AND *SALVIA MILTIORRHIZA* BUNGE GROWN IN HONG THAI COMMUNE, NA HANG DISTRICT, TUYEN QUANG PROVINCE

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Abstract:

Angelica acutiloba Kitagawa was immigrated to Vietnam in 1990 and *Salvia mitiorrhiza* Bunge was immigrated to Vietnam in the 1960s from China. Currently, both herbs are grown and developed in various places. They are precious medicinal plants, are important medicinal plants in many traditional medicines. Research results show that tuber diameter is 0.79cm, tuber length is 27.8cm, yield is 68.8g/plant or *Salvia mitiorrhiza* Bunge; reached 1.80cm in tuber diameter, 19.0cm in tuber length and yield is 16.0g/plant for the *Angelica acutiloba* Kitagawa. The main diseases on *Salvia mitiorrhiza* Bunge are root rot in an extremely common level; for *Angelica acutiloba* Kitagawa plants, root rot did not appear, but pests mainly included small snails and leaf folders, with a low degree of prevalence. In general, the growth and development ability of these two species are completely suitable with the soil and climate conditions in Na Hang district as well as other localities with similar conditions.