



## STUDY ACCLIMATIZED SEED SAMPLE SAPOSHNIKOVIA DIVARICATA (TURCZ) SCHISCHK IN VIETNAM

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### ABSTRACT

In this study, we evaluated several agronomic characteristics of *S. divaricata* when grown in various regions in Vietnam, including Northeast, Northwest, and Central Highlands. Among the three regions, *S. divaricata* grown in Northwestern Vietnam has the highest yield reaching 6.21 tons/ha, with a total active ingredient content of 0.655% (Prim-O-glucosylcimifugin 0.383% and 5-O-methylvisamminoside 0.272%). This is followed by the Central Highlands of which *S. divaricata* yielded 4.12 tons/ha, with a total active ingredient content is 0.543% (Prim-O-glucosylcimifugin 0.292% and 5-O-methylvisamminoside 0.251%). The lowest yield of *S. divaricata* was recorded in Northeast with 3.13 tons/ha, of which a total active ingredient content was 0.394% with 0.253% for Prim-O-glucosylcimifugin and 0.141% for 5-O-methylvisamminoside. With the applied analytical conditions, HPLC - DAD chromatograms are obtained with sharp peaks of prim-O-glucosylcimifugin ( $t_R = 7.51$  min) and 5-O-methylvisamminoside ( $t_R = 20.23$  min), balanced, clear on the background of the medicinal plants *Saposhnikovia divaricata* (Turcz.) Schischk. In particular, the applicable analytical conditions allow for simultaneous qualitative and quantitative analysis of both prim-O-glucosylcimifugin and 5-O-methylvisamminoside. The results of building the standard curve prim-O-glucosylcimifugin  $Y = (16146)X - 4020.3$ ,  $R^2 = 0.9992$ , standard curve 5-O-methylvisamminoside  $Y = (20490)X - 6921.8$ ,  $R^2 = 0.9999$ . The quantitative results of prim-O-glucosylcimifugin and 5-O-methylvisamminoside in all regions were slightly higher than that of the pharmacopoeias of Vietnam, China and Hong Kong with the total active ingredient content not less than 0.24%. Thus, The study concluded that Vietnam is a country that can develop medicinal plants *Saposhnikovia divaricata* (Turcz.) Schischk.

**KEYWORDS :** *Saposhnikovia divaricata* (Turcz.) Schischk; Yield; Prim-O-glucosylcimifugin and 5-O-methylvisamminoside; High Performance Liquid Chromatography (HPLC - DAD) (must be written in alphabetically order)

### 2. INTRODUCTION

Vietnam is one of the 10 richest biodiversity centers in the world and is ranked 16th in the world in terms of genetic diversity. In particular, many genetic resources are used as preventive and curative drugs. According the Institute of Medicinal Materials, up to now, there have been 5,117 species and subspecies, belonging to 1,823 genera, 360 families of 8 phyla of higher vascular plants, along with several taxa belonging to the group of Mosses, Algae, Fungi, accounting for about 36% of the 10,500 known vascular plant species. Compared with 35,000 species of medicinal plants worldwide, the number of known medicinal plant species in Vietnam accounts for about 11%. However, to develop new medicinal plant species, provide valuable medicinal materials, and enrich the source of medicinal plants in Vietnam.

The migration and introduction of medicinal plants will help diversify genetic resources, diversify plant structures and supplement good seed sources to provide raw materials for drug production. In addition, successful migration and introduction will help be more proactive in seed sources for domestic production of medicinal plants, no longer depend on imported raw materials, ensure the good and stable quality of medicinal herbs in the market and export direction. With a tropical climate to mostly mountainous terrain, accounting for 75% of the territory. The Northeast, Northwest and West of Vietnam are mountainous and midland regions including many mountain ranges, massifs, plateaus and hills. It is home

to many species of plants with medicinal value. The big mountain range here is Hoang Lien Son in the North and Central Highlands. Many mountains have an altitude of over 2,000 meters, of which the Fansipan is the highest, up to 3,143 meters above sea level. Temperature is low all year round, the annual average temperature is 15 – 210C. Many kinds of medicinal plants grow and develop.

*Saposhnikovia divaricata* (Turcz.) Schischk is a perennial plant, about 0.3 - 0.8m tall, leaves are alternate, long petiole, below the stem develops into a sheath hugging the stem, the leaves are compound 2-3 times. split feathers look like wormwood leaves, self-shaped flowers double canopy, each double canopy has 5-7 small canopies, peduncles are small unevenly, each canopy has 4-9 small flowers, white.

**Distribution and Ecology:** The maple tree is a naturally growing tree and is widely grown in the provinces of Sichuan, Guizhou, and Yunnan, China. It is a moisture-loving and light-loving herb. Based on its origin and growing area, it is said that the maple room is adapted to climatic conditions in humid temperate or subtropical regions. The aboveground part of the plant can die out every year in cold winter. The tree produces a lot of fruit and can be grown from seeds (Medicinal plants and medicinal animals in Vietnam, Volume 2, Do Huy Bich et al.).

Currently, leprosy is mainly distributed in northern China such

as Heilongjiang, Liaoning, Shanxi, Gansu, ... with an altitude of 400 - 800m above sea level and is assessed to have active ingredient content. In Vietnam, there is no official report on the occurrence of this plant.

#### Parts Used:

Medicinal herbs are dried or dried roots, large, strong roots, thin, smooth skin, hairless root tips, brown ring in cross-section, light yellow centre in the centre is good. The outer shell is rough, hairy head with hard buds is a poor type.

#### Chemical composition:

Essential oil, Manit, a substance with bitter Phenola Glucoside, sugar, organic acid (Clinical Applied Chinese Medicine), Mannitol, Phenol (Chinese Medicine), Xanthotoxin (Journal of Biopharmaceutical [Japan] 1989, 43(3): 216), Anomalin, Scopolatin (Tieu Lam Hoang My, Biopharmaceutical Journal [Japan] 1983, 37(3): 276), Marmesin (Guo Dean et al., CA 1993, 118: 240548h), Panaxynol Falcariol, Falcariindiol, 8E-Heptadeca-1, 8-Dien-4, 6-Diyn-3, 10 - diol (baka K et al., Journal of Biopharmaceuticals [Japan] 1987, 37 ( 3): 276), Saposhnikovan (Shimizu N et al., Chem Pharm Bull 1989 37 (5): 1329).

In Vietnam, maple trees imported from China have not been focused on planting and developing but have only been planted scattered and small in some northern mountainous provinces such as Lai Chau, Lao Cai, and Lang. Son, Hoa Binh, Ha Giang and there have been no official studies on the adaptability of the tree in Vietnam. Besides, the demand for domestic medicinal herbs for leprosy is increasing with the price of medicinal plants for leprosy. up to 300,000 - 500,000 VND / 1 kg of dried herbs.

Therefore, the study and assessment of the adaptability of maple trees imported from China in ecological conditions in Vietnam serve as a premise for future studies towards improving the quality of medicinal plants for leprosy in Vietnam. Manufacturing on a large scale, proactively supplying medicinal plants to meet domestic demand is the top urgent issue.

In China, there have been a number of studies on potassium fertilizers affecting the yield and quality of medicinal plants against leprosy (Shandong, 2014).

Jiang Debin has researched and developed a technical process for planting maple trees in Gansu in April, sowing density at a distance of 15 x 20cm, fertilizer 2.5 - 3 tons of manure + 20 - 22kg NPK for one year. the hectare of China 667m<sup>2</sup>, for the highest yield.

### 3. MATERIALS AND METHODS

#### 3.1. Plant Materials

The maple room variety was planted by the Institute of Epigenetic Medicine from An Quoc District, Hebei Province, China and planted in three locations in the Northeast, Northwest and Central Highlands of Vietnam.

Fertilizers: use rotting manure, nitrogen fertilizers; superphosphate and potassium (Evi, 2012)

#### 3.2. Site Description And Soil Analyse

The experiment was conducted in the year 2019 - 2020 in 3 climate sub-regions of Vietnam: Northeast, Northwest and Central Highlands. Site located in a subtropical monsoon climate with mean daily temperature and solar irradiance during indoor growth of 21°C and 25.3 MJm<sup>-2</sup>d<sup>-1</sup>, respectively. The soil in the field rice field was taken from the study farm. Textured soil with pH 6.01 - 6.55 and soil properties of experimental sites are analyzed as in Table 1.

**Table 1. Soil properties of Study area**

Location	Depth of Soil (cm)	Organic matter (mg/100g)	Total N (N%)	Total P (P <sub>2</sub> O <sub>5</sub> ) (%)	Total K (K <sub>2</sub> O) (%)	Available N (mg/100g)	Available P (mg/100g)	Available K (mg/100g)
Tay Bac	0-40	14,5	0,14	0,15	0,65	0,55	1,56	3,78
Dong Bac	0-40	13,2	0,13	0,14	0,63	0,53	1,48	3,56
Tay Nguyen	0-40	14,3	0,15	0,16	0,67	0,58	1,52	3,60

#### 3.3. Experimental treatment and design

Using domestic imported Lepidoptera seed sources in China arranged in a completely randomized block with 3 repetitions, each repetition is 40m<sup>2</sup> with a total area of 120m<sup>2</sup> at each site, Planting and caring techniques for leprosy are carried out according to regulations. Technical process issued by the Institute of Medicinal Materials. Procedure.... the density of planting distance is 20 x20 cm.

Three managements were set for each level and the formula application was adopted follow as: Basal fertilization: 50% N + 50% K<sub>2</sub>O + 100% P<sub>2</sub>O<sub>5</sub>; Top-dressing fertilizer: phase 1 (60 DAP): 25% N + 25% K<sub>2</sub>O; phase 2 (180 DAP): 25% N + 25% K<sub>2</sub>O.

#### 3.4. Equipment's and Reagent

In this study, Instrument and Reagent were used include: Waters Acquity UPC2 USA, HPLC-DAD, EmPower Color Spectrum Management System, PL203 electronic balance, KQ-5200DE numerical control ultrasonic cleaner, 5μl microliter syringe and 0.45 micro-perforated filter membrane. HPLC-grade methanol (MeOH) was purchased from Merck (Germany). All other solvents were of analytical grade.

Prim-O-glucosylcimifugin (CAS: 80681-45-4, Lot: PRF9121704, 98%) and 5-O-methylvisamminosid (CAS: 84272-85-5; Lot: PRF10010701, 98%) were purchased from Biopurify Phytochemicals Ltd.

#### 3.5. Chromatographic Analysis Condition

The separation of compounds was performed on an Agilent C18 column (250 x 4.6 mm, 5 μm). The mobile phase was a mixture of methanol/water (40/60, v/v). The solvent flow rate was kept at 1 mL/min. The injection volume was 5 μL. Detection was set at a wavelength of 254 nm.

#### 3.6. Solution Preparation

**Test solution:** weigh accurately 0.25 g of the powdered sample and put it into a 50-mL round-bottomed flask, then add 10 mL of methanol 50% and weigh. Heat under reflux for 2 hours, cool and weigh again, replenish the loss of the solvent with methanol 50% and mix well, filter through a 0.45 μm filter, use the successive filtrate as the test solution.

**Standard Solution:** standard stock solutions of the two standards (prim-O-glucosylcimifugin and 5-O-methylvisamminosid) were prepared by dissolving them in methanol. The stock solutions were then diluted to establish calibration curves in the ranges of 13.9 – 278 μg/mL for prim-O-glucosylcimifugin and 29.2 – 292 μg/mL for 5-O-methylvisamminosid. The stock and working solutions were stored at 4°C.

#### 3.7. Calculation

Calculate the percentage of prim-O-glucosylcimifugin and 5-O-methylvisamminosid in the tested sample taken:

$$X(\%) = \frac{C_t \times V \times 10}{m \times (100 - \alpha)}$$

C<sub>t</sub>: the concentration of the analyzed compound in the sample solution from the calibration curve equation (μg/mL); V: volume of the sample solution (mL); m: weight of tested

sample taken to prepare the sample solution (mg);  $\alpha$ : the moisture of powder (%).

### 3.8. Statistical Analysis

Statistical analysis of data using IRRISTAT5.0 and Excel 2010 software was used to calculate the significant difference between the total content of prim-O-glucosylcimifugin and 5-O-methylvisamminoside in *Saposhnikovia divaricata* (Turcz.) Schischk acclimatized from China

## 4. RESULTS AND DISCUSSION

HPLC-DAD analysis of prim-O-glucosylctmifugin and 5-O-methylvisamminosid in *Saposhnikovia divaricata*

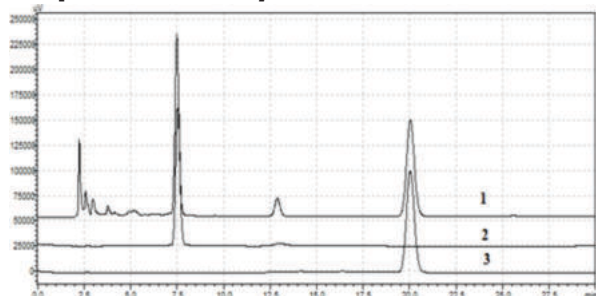


Figure 1. HPLC-DAD chromatograms analysis of prim-O-glucosylcimifugin and 5-O-methylvisamminosid in *Saposhnikovia divaricata*

(1: *Saposhnikovia divaricata* sample; 2: prim-O-glucosylcimifugin; 3: 5-O-methylvisamminosid)

### 4.1. Evaluate Exploration base on Linear Relationship

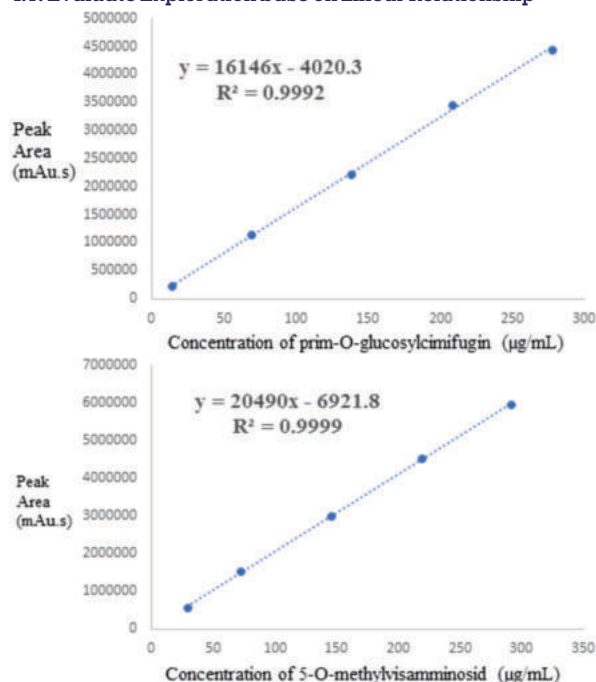


Figure 2. The calibration curves of prim-O-glucosylcimifugin (A) and 5-O-methylvisamminosid (B)

The standard curve was drawn with prim-O-glucosylcimifugin reference ( $\mu\text{g}$ ) as the horizontal axis and prim-O-glucosylcimifugin peak area as the vertical axis (y). The result was shown in Figure 2A. The regression equation was calculated with least square method following the standard curve:  $Y = (16146)X - 4020.3$ ,  $R^2 = 0.9992$  and Correlation coefficient  $R^2$  than 0.9999 indicate that standard curve with high linearity total guaranteed to analyse quantitative prim-O-glucosylcimifugin content in *Saposhnikovia divaricata* (Turcz.) Schischk.

The standard curve was drawn with 5-O-methylvisamminosid reference ( $\mu\text{g}$ ) as the horizontal axis and 5-O-methylvisamminosid peak area as the vertical axis (y). The result was shown in Figure 2B. The regression equation was calculated with the least square method following the standard curve:  $Y = (20490)X - 6921.8$ ,  $R^2 = 0.9999$  and Correlation coefficient  $R^2$  than 0.9999 indicate that standard curve with high linearity total guaranteed to analyse quantitative 5-O-methylvisamminosid content in *Saposhnikovia divaricata* (Turcz.) Schischk.

### 4.2. Validation HPLC-DAD method

#### Specificity

The results in Figure 1 showed that the retention times of prim-O-glucosylcimifugin and 5-O-methylvisamminosid were 7.51 min and 20.23 min, respectively. The obtained chromatograms showed clear separation peaks, low background noise. Additionally, when comparing the UV spectrum of three points of analyzed peaks in the sample, the matching ratio was approximately 100%. These demonstrated the specificity of this method.

### 4.3. Effect of acclimatisation on productivity and quality *Saposhnikovia divaricata* (Turcz.) Schischk.

The ultimate aim of the research is to determine the ability to record growth, development, and yield in terms of both active ingredients and medicinal ingredients in Vietnam, for production. The most important criterion to evaluate the influence of external factors, soil on the adaptability of plants...

In this study, as shown in Figure 3 *Saposhnikovia divaricata* (Turcz.) Schick tree planted in 3 locations in the Northeast, Northwest and Central Highlands of Vietnam, the results for all regions had a higher total activity content than the Vietnamese Pharmacopoeia V, China was 0.24%. about the rate of from 3.13 - 6.21 tons/ha. The North has a higher aggregate yield and quality than other regions at the 95% confidence level. It proves that in different regions, there is an effect on the energy and quality of *Saposhnikovia divaricata* (Turcz.) Schischk, for the northwest, is a region with a cool year-round climate with an average temperature lower than 10 - 15°C, altitude of 1000 - 3500m with sea level favorable for the accumulation of organic matter, Northeast region with an average temperature of 20 - 25°C, altitude 300 - 1000 m above sea level, Central Highlands is The region has 2 distinct seasons: the rainy season from May to October and the dry season from November to April next year, with the average temperature of 15 - 20°C, at an altitude of 500 - 1500m above sea level.

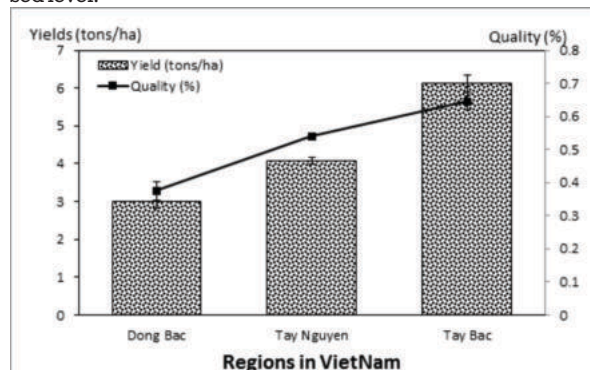


Figure 3. The effect of acclimatisation on the prim-O-glucosylcimifugin and 5-O-methylvisamminoside content and yield of *Saposhnikovia divaricata* (Turcz.) Schischk

## 5. CONCLUSION AND SUGGESTION

The study concluded that plants imported from China and planted in Vietnamese climatic condition give the following results in terms of yield and quantity the following results in

terms of yield and quality: yield and quality in the northeast reached 3.01 tons/ha, the content was aggregate yield is 0.375%, the Central Highlands yield is 4.08 tons/ha, the aggregate quality is 0.54%, the Northwest yield is 6.12 tons/ha, the aggregate quality is 0.645%, all of which are high. than Vietnamese Pharmacopoeia V (language definition 0.24%).

Two original active ingredients-O-glucosylcimifugin and 5-O-methylvisamminoside are both used by the Vietnam Pharmacopoeia, the Chinese Pharmacopoeia and the Hong Kong Pharmacopoeia as criteria for assessing office quality. the total content of two substances prim-O-glucosylcimifugin ( $C_{22}H_{28}O_{11}$ ) and 5-O-methylvisamminoside ( $C_{22}H_{28}O_{10}$ ) in the medicinal plants is not less than 0.24% by HPLC-DAD method, Reference systems is the database for the group of the appliers and method rating of the quality of the room.

The obtained results are quite consistent with several published studies on the quality of activities in medicinal plants [7,8], Lijie Ma et al [7] have published the content of prim-o-glucosylcimifugin in leprosy samples. Wild and cultivated samples were 0.496 and 0.361 (%) of 5-O-methylvisamnisoid in wild leprosy and cultivated samples, respectively, 0.391 and 0.437 (%) respectively.

The results shows that the characteristics, yield and quality in Tay Bac, Tay Nguyen and Dong Bac in VietNam all may be suitable for the development of *Saposhnikovia divaricata* (Turcz.) Schischk., however. this new grow only the same sample at the small sample is not a grown big tea at the area on all water, so further research is needed to find the most suitable state of the spawning area for seedlings in Vietnam.

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