

Selection results of high-yield and high-quality *Saposhnikovia divaricata* (Turcz.) Schischk varieties

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**Abstract:** *Saposhnikovia divaricata* (Turcz.) Schischk, originating from China, was studied for acclimatization by the National Institute of Medicinal Materials from 2018 to 2020. Its productivity and quality were evaluated, showing a total content of prim-O-glucosylcimifugin and 5-O-methylvisamminoside at 0.65%. Based on the research and selection conducted at the Tam Dao Medicinal Plant Research Station, National Institute of Medicinal Materials, using the mass selection method and quality evaluation of *Saposhnikovia divaricata* by High-Performance Liquid Chromatography with UV Detection (HPLC-UV), a variety with the following characteristics was selected: Herbaceous perennial plant, height 50–70 cm, with 2–3 pinnately compound leaves, serrated leaf edges, and dark green color. Inflorescences consist of many compound umbels, each having 5–7 smaller umbels, and each small umbel containing 4–9 white flowers. Schizocarps, brown when mature, oval-shaped, with a thousand-seed weight of approximately 5.83 g. The medicinal part is the taproot, cylindrical in shape, averaging 23.8 cm in length, with a yellow-brown color. This variety was evaluated for resistance to soft rot disease, with a severity level of 5 points (CIP scale), average yield 966.7 kg/ha. The total content of prim-O-glucosylcimifugin and 5-O-methylvisamminoside reached 1.0%.

**Keywords:** The selected *Saposhnikovia divaricata* variety: morphological characteristics, agro-biological traits, and quality.

## 1. Introduction

*Saposhnikovia divaricata* (Turcz.) Schischk, commonly known as Fang Feng, is a perennial plant mainly distributed in northern China, including provinces such as Heilongjiang, Inner Mongolia, Jilin, Liaoning, Sichuan, Guizhou, Yunnan, Shaanxi, Gansu, and others, at elevations ranging from 800 to 1,800 meters above sea level. It is highly regarded for its high active ingredient content. The part used as a medicinal herb is the dried or sun-dried root. High-quality roots are large, robust, thin-skinned, smooth, hairless at the root tip, and have a cross-section with brown rings and a pale yellow core. Lower-quality roots have rough exteriors, hairy tips, and stiff buds. (Ministry of Health, 2017; Chinese Pharmacopoeia Commission, 2015).

In Traditional Chinese Medicine, the dried root of *Saposhnikovia divaricata* (Fang Feng) is used to treat diseases related to the immune system, nervous system, and respiratory system. Phytochemical and pharmacological studies have shown that the dried root of this herb contains various compounds such as chromones, coumarins, acid esters, polyacetylenes, volatile oils, polysaccharides, and inorganic elements. These compounds exhibit significant anti-inflammatory, analgesic, antioxidant, anti-proliferative, anti-tumor, antiviral, anticoagulant, anticonvulsant, and immunomodulatory activities. Among them, two compounds, prim-O-glucosylcimifugin and 5-O-methylvisamminoside, are designated as marker compounds by the pharmacopoeias of several countries to control the quality of *Saposhnikovia divaricata* herb. The Vietnamese Pharmacopoeia V 2017 and the Chinese Pharmacopoeia 2020 specify that the total content of these two compounds in the herb should not be less than 0.24% based on dried weight (Ministry of Health, 2017; Chinese Pharmacopoeia Commission, 2015; Chin, YW. et al., 2011).

In recent years, due to population growth, agricultural land expansion, and years of overexploitation, wild *Saposhnikovia divaricata* resources have been depleted. As a result, this medicinal plant has been listed as a Level III protected species in the national conservation list. The wild *Saposhnikovia divaricata* supply cannot meet the market demand of over 5,000 tons per year, making cultivated *Saposhnikovia divaricata* the primary source, accounting for more than 70% of the market share (Liu, Y. et al, 2016).

The transition from wild Fangfeng to cultivation also led to a significant decline in quality, with the active ingredient content halved (Cao, S. et al, 2022; Liu, Y. et al, 2016), and the price is only one-third to one-tenth compared to wild plants, improving the quality of cultivated Fangfeng medicinal herbs an urgent issue (Wang, S. et al, 2020).

In Vietnam, the demand for domestic Fangfeng medicinal herbs is increasing, while the majority of this herbal material is imported from China, with prices being unstable, sometimes reaching 300,000 – 500,000 VND per kilogram of dried herbs, and the quality of the herbs is also difficult to control. Therefore, it is essential to proactively secure a supply of medicinal herbs to meet domestic demand and develop this valuable genetic resource.

Recognizing the production requirements, the Hanoi Institute of Medicinal Plants Research and Processing has focused on researching and developing high-yield, high-quality *Saposhnikovia divaricata* (Fangfeng) varieties to create superior seed products that meet the needs of medicinal plant cultivation in Vietnam. The selected variety originates from the Institute's task of importing foreign varieties during the period 2018-2020, with a yield of 4.48 tons/ha. The essential oil content ranges from 0.34% to 0.40%, and the total content of two compounds, prim-O-glucosylcimifugin and 5-O-methylvisamminoside, ranges from 0.39% to 0.65%, higher than the Chinese Pharmacopoeia standard of 0.24% (Nghiem Tien Chung et al., 2020).

## 2. Materials and methods

### 2.1. Research Materials

The Fangfeng seed samples originated from the introduction mission of the National Institute of Medicinal Materials between 2018 - 2020.

### 2.2. Research Methods

The mass selection method was used: initially, the seeds obtained from the introduction evaluation process between 2018 and 2020 were sown and grown, with growth characteristics being monitored. Superior plants, which met the selection criteria, were chosen, and their seeds were mixed. These seeds were then further sown, grown, and compared with the original variety.

### 2.3. Research Duration and Location

The study was conducted from December 2021 to December 2024 at the Tam Dao Medicinal Plant Research Station – National Institute of Medicinal Materials.

#### \* Monitoring Indicators

Description of the morphological characteristics of the selected Fangfeng variety.

Describe the morphological features of the stem, leaves, and roots at the seedling stage and when the plant grows with strong stems and leaves before harvest. Describe the flowers, fruits, and seeds in terms of shape and color.  
Individual yield (g): Weigh the dry weight of the plant's roots.

Actual medicinal herb yield (kg/ha): Harvest all medicinal plants and calculate the total weight obtained from each experimental plot.

#### \* Quality Evaluation Criteria for Medicinal Herbs:

Dried roots of Fangfeng plants (according to DĐVN V standards).

Quantification of total content of prim-O-glucosylcimifugin and 5-O-methylvisamminosid using High-Performance Liquid Chromatography (HPLC) connected with a UV absorbance detector (according to DĐVN V standards).

Mobile phase: Methanol – water (40:60)

Test solution: Accurately weigh approximately 0.25 g of medicinal herb powder (sieved through a 0.180 mm sieve) into a conical flask, add precisely 10 ml of 50% methanol, seal the flask, weigh, and reflux in a water bath for 2 hours. After cooling, reweigh, and add methanol to restore the original weight, shake well, and filter through a 0.45  $\mu\text{m}$  membrane filter.

Standard solution: Dissolve prim-O-glucosylcimifugin standard and 5-O-methylvisamminosid standard separately in methanol to obtain two standard solutions, each with a concentration of approximately 60 mg/ml.

Chromatographic conditions:

Column size (25 cm x 4.6 mm), stationary phase C (5  $\mu\text{m}$ )

UV detector set at 254 nm

Injection volume: 5  $\mu\text{l}$

Flow rate: 1.0 ml/min

Procedure:

Perform chromatography with the standard solution, calculate the theoretical plate number of the column based on the prim-O-glucosylcimifugin peak. The theoretical plate number should not be less than 2000.

Perform chromatography successively with the standard solution, test solution, and calculate the content of 5-O-methylvisamminosid and prim-O-glucosylcimifugin in the medicinal herb based on the peak area of the 5-O-methylvisamminosid and prim-O-glucosylcimifugin in the chromatogram of the test solution, the standard solution chromatogram, and the content of  $\text{C}_{22}\text{H}_{28}\text{O}_{10}$  in 5-O-methylvisamminosid and  $\text{C}_{22}\text{H}_{28}\text{O}_{11}$  in prim-O-glucosylcimifugin.

The medicinal herb must contain no less than 0.24% of the total content of prim-O-glucosylcimifugin ( $\text{C}_{22}\text{H}_{28}\text{O}_{11}$ ) and 5-O-methylvisamminosid ( $\text{C}_{22}\text{H}_{28}\text{O}_{10}$ ) based on dry weight.

#### **2.4. Data Processing Method:**

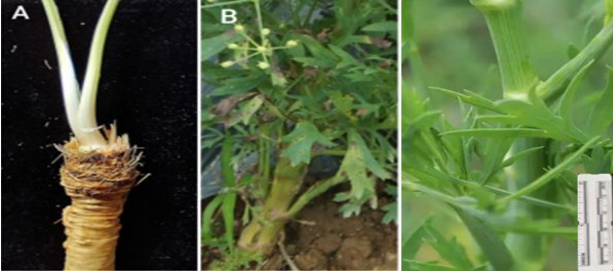



The experimental data were processed using Excel software.


### **3. Results and discussion**

#### **3.1. Morphological Characteristics of the Selected Fangfeng Variety**

The results of the study evaluating the morphological characteristics of the selected Fangfeng variety are presented in the following table:

Table 3.1: Some Morphological Characteristics of the Selected Fangfeng Variety

Trait	Description	Illustration
Stem	<p>The plant is herbaceous, perennial, about 50 – 70 cm tall, but can grow up to 90 cm. During the vegetative growth phase, the stem is short, and cylindrical, ranging from 1.0 – 2.5 cm in length. In the flowering phase, the stem grows quickly, with a deep green color and a cylindrical shape; the base diameter is approximately <math>2.3 \pm 0.4</math> cm, and the number of primary branches is about <math>13.7 \pm 1.6</math> branches. The branching is abundant, with a spreading branch pattern.</p>	 <p><b>Figure 3.1: Stem of Fangfeng</b>                      A. Stem in the vegetative growth phase.                      B. Stem in the reproductive growth phase.</p>
Leaves	<p>Compound leaves, with leaflets 2-3 times pinnately divided, serrated edges, dark green. The leaves are alternately arranged, with petioles approximately <math>13.4 \pm 3.0</math> cm long, the lower part of the petiole forms a sheath wrapping around the stem. The leaf blade is about <math>24.4 \pm 3.2</math> cm long and <math>23.75 \pm 3.3</math> cm wide.</p>	 <p><b>Figure 3.2: Leaves of Fangfeng</b></p>
Flowers	<p>The inflorescence is a compound umbel, with each compound umbel containing 5-7 smaller umbels, with unequal lengths of pedicels. Each smaller umbel has 4-9 small white flowers.</p>	 <p><b>Figure 3.3: Flowers of Fangfeng</b></p>
Fruits and Seeds	<p>The fruit is a paired schizocarp. When immature, the fruit is light green and rough, and when ripe, it turns brown. The seeds are oval, brown, and about <math>5.79 \pm 0.45</math> mm in length and <math>3.20 \pm 0.30</math> mm in width. The seeds are flat, with broad wings on both sides, about 0.5 – 1.0 mm; the upper surface is slightly convex, with three longitudinal ribs on the back, and the underside is flat. The weight of 1000 seeds is about 5.83g. Germination rate is 61%.</p>	 <p><b>Figure 3.4: Fruits of Fangfeng</b>                      A. Immature fruit; B. Ripe fruit</p>

<p><b>Roots</b></p>	<p>The roots are cone-shaped or cylindrical, gradually tapering towards the bottom, approximately <math>23.8 \pm 1.3</math> cm long and <math>1.8 \pm 0.3</math> cm in diameter. The outer surface is yellow-brown, rough with horizontal grooves, and the outer skin often peels off. The roots have many white lenticels and protrusions from the lateral roots.</p>	 <p><b>Figure 3.5: Roots of Fangfeng</b>  A. Dried roots of Fangfeng; B. Cross-section of root tuber; C. Fresh roots of Fangfeng</p>
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**3.2.2. Some Agronomic Characteristics of the Selected Fangfeng Variety**

Certain agro-biological indicators are among the key criteria for evaluating plant growth and development, reflecting their ability to synthesize and accumulate organic matter. Table 3.2 presents the results of monitoring the growth period and the increase in height, number of leaves, and canopy diameter of the two Fangfeng samples.

**Table 3.2: Some Agronomic Characteristics of the Selected Fangfeng Variety**

Indicators	Selected Fangfeng Variety
Time from sowing to germination (days)	14 ± 0,9
Time to harvest medicinal material (days)	270 ± 17,7
Plant height (cm)	40,0 ± 2,6
Number of leaves per main stem	20,0 ± 1,3
Canopy diameter (cm)	46,5 + 14,2
Susceptibility to soft rot	+++
Field purity	quite

The results from Table 3.2 indicate that Fangfeng seeds take an average of 14 days to germinate, suggesting that the seeds do not exhibit deep dormancy. The harvesting time for medicinal materials is between 9 to 12 months after sowing. Growth indicators show that plant height ranges from 38–40 cm, with 18–20 leaves per plant. The canopy diameter of the selected variety is approximately 46.5 cm. During the monitoring period, the selected Fangfeng variety was primarily affected by soft rot disease at a severity level of +++ (score 5). These growth indicators for the selected Fangfeng variety align reasonably well with the descriptions provided by (Urgamal, M. et al., 2006).

**3.2.3. Yield Components and Productivity of the Selected Fangfeng Variety**

The results of monitoring the yield components and productivity of the selected Fangfeng variety are presented in Table 3.3.

**Table 3.3: Yield Components and Productivity of the Selected Fangfeng Variety**

Indicators	Root Length (cm)	Root Diameter (cm)	Root Weight (g/plant)	Yield per Experimental Plot (kg/plot)	Theoretical Yield (kg/ha)	Actual Yield (kg/ha)
Selected Fangfeng Variety	23,8	1,8	40,5	2,9	1.012,5	966,7

Yield components such as root length, diameter, and weight of the selected Fangfeng variety are key factors that determine the variety's medicinal yield. Whether these indicators are high or low, heavy or light, depends on the variety's genetic characteristics, cultivation conditions, and farming environment.

The research results show that the root length of the selected Fangfeng variety is quite large, reaching 23.8 cm, indicating that the variety has the characteristic of producing long roots. The root diameter is narrow, measuring 1.8 cm. The average root weight is 40.5 g, suggesting that the variety is relatively suitable for small, slender root crops. The yield per experimental plot is around 2.9 kg/plot. The theoretical yield (1,012.5 kg/ha) reflects the potential maximum yield based on theoretical factors, assuming all conditions are ideal. The actual yield of the selected Fangfeng variety is 966.7 kg/ha, which is 95.5% of the theoretical yield. This ratio indicates that the selected variety can maintain high productivity under real conditions.

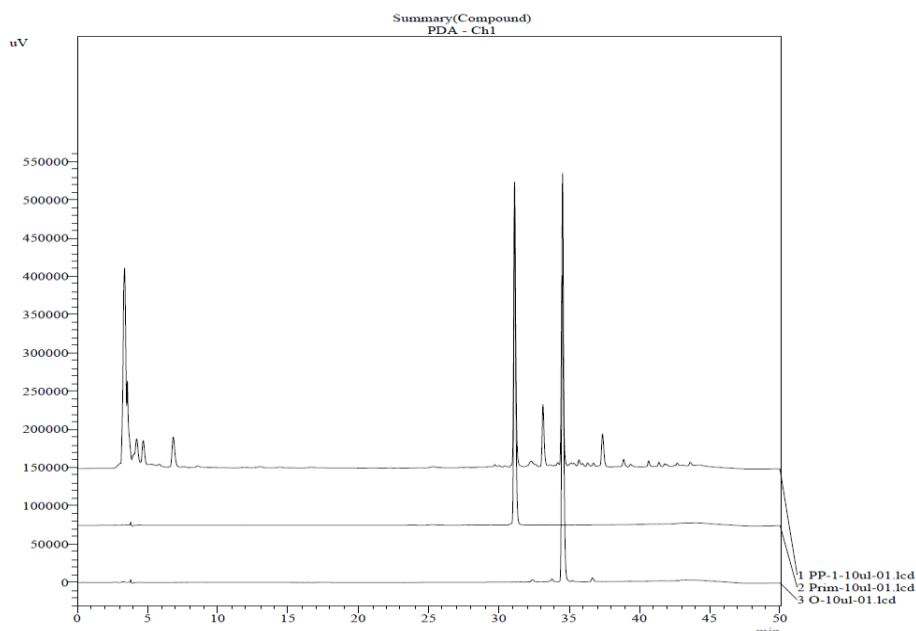
Although the actual yield is lower than the yield of the germplasm from 2018–2020, which exceeded 4,000 kg/ha (Chung et al., 2023), this reduction is due to the significant damage caused by Typhoon No. 3 (Yagi) that hit Vietnam in Se, which 2024. The storm caused severe flooding, leading to substantial root rot and plant death, which had a significant impact and significantly impacted the selected Fangfeng variety.

### 3.2.4. Quality of the Selected Fangfeng Variety

For medicinal herbs, quality is just as important as yield. Without proper quality, medicinal herbs fail to deliver effective results in diagnosis and treatment. Therefore, quality analysis must be performed according to the standards outlined in the Vietnamese Pharmacopoeia V (Ministry of Health, 2017) or the pharmacopeias of other countries worldwide.

In the case of Fangfeng, it is essential to determine the total content of *prim-O-glucosylcimifugin* and *5-O-methylvisamminosid* using High-Performance Liquid Chromatography (HPLC). The results obtained for this analysis are as follows:

#### \* HPLC Analysis Chromatogram



**Figure 3.6: HPLC chromatogram analyzing *prim-O-glucosylcimifugin* and *5-O-methylvisamminosid* of the selected Fangfeng sample**

Note: 1 - Test sample of selected Fangfeng medicinal herb;  
2 - Standard substance *prim-O-glucosylcimifugin*;  
3 - Standard substance *5-O-methylvisamminosid*.

**Observations:**

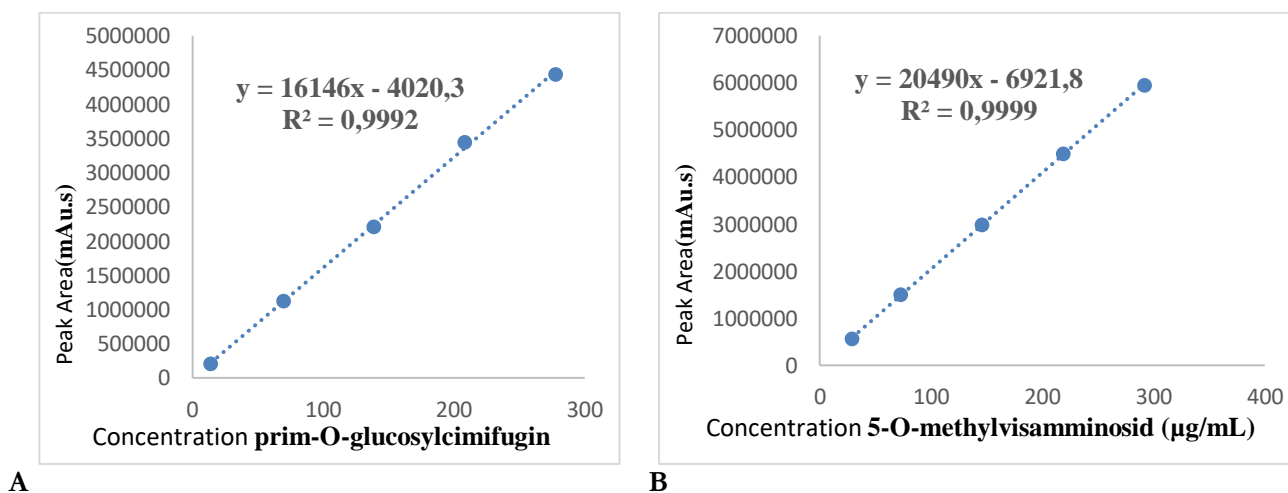
The HPLC chromatogram analysis under the applied conditions reveals sharp, balanced, and clear peaks for prim-O-glucosylcimifugin (tR = 31.3 minutes) and 5-O-methylvisamminosid (tR = 34.8 minutes) against the background of the herbal sample of Radix Saposhnikoviae. Notably, the analytical conditions allow for the simultaneous qualitative and quantitative analysis of both compounds, making it suitable for evaluating the quality of Radix Saposhnikoviae samples.

\* Calibration Curves for Prim-O-Glucosylcimifugin and 5-O-Methylvisamminosid

Based on the obtained results, calibration equations were established to represent the relationship between the peak area values and the concentrations of prim-O-glucosylcimifugin and 5-O-methylvisamminosid in the analytical solution. The results are presented in Table 3.4 and Figure 3.7

**Table 3.4: Calibration Results for Prim-O-Glucosylcimifugin and 5-O-Methylvisamminosid**

prim-O-glucosylcimifugin		5-O-methylvisamminosid	
Concentration (µg/ml)	Peak Area (mAu.s)	Concentration (µg/ml)	Peak Area (mAu.s)
13,9	210832	29,2	565215
69,5	1124539	73	1508063
139	2208281	146	2992963
208,5	3443713	219	4500188
278	4438576	292	5954895



**Figure 3.7: Calibration Curve for Prim-O-Glucosylcimifugin (A) and 5-O-Methylvisamminosid (B)**

It was observed that the obtained calibration curve equations had correlation coefficient ( $R^2$ ) values greater than 0.999, demonstrating a high linear correlation between the analyte concentration in the solution and the peak area value. This indicates strong linearity, making the method suitable for the quantitative analysis of prim-O-glucosylcimifugin and 5-O-methylvisamminoside.

Table 3.5: Quality Evaluation Results of the Selected *Saposhnikovia divaricata* Fangfeng variety

Selected Fangfeng Variety	5-O-Methylvisamminoside Content (%)	Prim-O-Glucosylcimifugin Content (%)	Total Prim-O-Glucosylcimifugin and 5-O-Methylvisamminoside Content (%)
PP1	0,58 ± 0,01	0,42 ± 0,02	1,00

Note: The results are calculated based on dry weight; PP1: Selected *Saposhnikovia divaricata* variety.

Both active ingredients, prim-O-glucosylcimifugin and 5-O-methylvisamminosid, are used in the Pharmacopoeia of Vietnam, the Pharmacopoeia of China, and the Pharmacopoeia of Hong Kong as criteria for evaluating the quality of Schisandra. These monographs all specify that the total content of prim-O-glucosylcimifugin and 5-O-methylvisamminosid in Schisandra should not be less than 0.24% by the HPLC-UV method. These official references serve as the basis for the research team to apply the criteria and procedures for evaluating the quality of the selected Schisandra seed samples.

Based on the analysis results of the selected Schisandra seed samples, it is observed that the sample meets the standards specified in the Pharmacopoeia of Vietnam V, China, Hong Kong, etc., with the total content of the two active ingredients exceeding 0.24%. The results show that the total active ingredient content of the selected Schisandra seed sample is 1.00%, higher than the initial imported sample from 2018–2020 (0.35 – 0.65%), further confirming that the selected Schisandra medicinal plant sample is quite suitable for expanding cultivation areas in Vietnam (Chen, B. et al., 2012). The results obtained are consistent with, and even higher than, some studies that have published the active ingredient content in Schisandra medicinal plants (Tôn Tích Ôn, 1956; Cao, S. et al., 2022; Chung, et al., 2023), which reported the prim-O-glucosylcimifugin content in wild and cultivated Schisandra samples as 0.496% and 0.361%, respectively, and the 5-O-methylvisamminosid content in wild and cultivated Schisandra samples as 0.391% and 0.437%, respectively.

#### 4. Conclusion

The selected Fangfeng variety, derived from the germplasm introduced in China during 2018–2020, exhibits morphological characteristics of a perennial herb with a 50–70 cm height. Its leaves are 2–3 times pinnately compound, with serrated edges and a dark green color. The inflorescence consists of multiple compound umbels, each with 5–7 smaller umbels containing 4–9 white flowers. The fruit is a schizocarp, usually with two seeds, turning brown and oval-shaped when mature, with a weight of 5.83 g per 1,000 seeds. The medicinal root is cylindrical, averaging 23.8 cm in length, and yellowish-brown in color. The variety is susceptible to soft rot disease at a severity level of +++ (score 5). These characteristics are consistent with descriptions by Li, W. et al. (2006) and Liu, Y. et al. (2016). Despite the significant impact of Typhoon No. 3 (Yagi) during the pre-harvest stage, the variety still achieved a yield of 966.7 kg/ha. The total content of prim-O-glucosylcimifugin and 5-O-methylvisamminosid reached 1.0%, surpassing the pharmacopoeial standards of China, Hong Kong, and Vietnam, which set the threshold at 0.24%.

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