Distribution and Habitat of Danshen

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2.1 Geographic Distribution

Danshen is widely distributed throughout China, from Jiangxi, Hunan in the south, to Liaoning in the north, to Sichuan in the west. It grows in mountainous hill regions with elevations from 120 to 1,300 m.

Danshen can be either wild or domestic. Wild Danshen is mainly distributed in Liaoning, Hebei, Beijing, Shanxi, Shandong, Hubei, Hunan, Jiangsu, Jiangxi, Gansu, Guizhou, and Shaanxi. Domestic Danshen is mainly distributed in Hebei, Tianjin, Jiangsu, Shanghai, Zhejiang, Anhui, Sichuan, Shandong, Henan, and Shaanxi. The main producing areas are as follows: Hebei's Anguo, Funing, Qianxi, Lulong, Pingquan, Zanhuang, and Yixian; Liaoning's Dalian, Xinjin, Gaixian, Jinxi, and Xingcheng; Shanghai's Congming; Jiangsu's Sheyang, Xinghua, Gaoyou, and Jurong; Zhejiang's Shengzhou, Sanmen, and Ningbo; Anhui's Boxian and Taihe; Shandong's Juxian, Pingyi, Yishui, Qixia, Laiyang, and Rizhao; Henan's Songxian, Lushi, Luoning, and Songshan; Hubei's Yingshan, Luotian, Qichun, and Suizhou; Shaanxi's Luonan and Shangzhou; Gansu's Kangxian and Hezheng; Sichuan's Zhongjiang and Chengdu; Yunnan's Ninglang, Lijiang, Yongsheng, and Binzhou.

2.2 Ecological and Biological Characteristics

Danshen grows well in a sunny, mild, and wet environment. It is able to tolerate cold weather, but it is vulnerable to drought and excessive water. Suitable growing conditions for Danshen are found in locations where the annual average temperature is 17.1 °C, and the annual average relative humidity is 77 %. The plant starts growing when the soil temperature reaches 10 °C in spring. When the air temperature reaches 20–26 °C and the relative humidity is 80 %, it grows very well. In autumn, when temperatures fall below 10 °C, its aboveground parts begin to wither. Danshen's roots can overwinter safely even at a soil temperature of −15 °C. It takes the seeds about 15 days to germinate at 18–22 °C. Adventitious buds begin to develop on the root when soil temperatures reach 15–17 °C. Insufficient sunshine and low temperatures during its growing season can slow down the growth and cause maldevelopment [1].

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2.3 Environmental Condition

2.3.1 Climatic Conditions

Danshen's distribution area can be divided into several climatic regions:

2.3.1.1 Liaodong–Jiaodong Peninsula

This region belongs to the warm temperate zone. It is influenced by ocean climate and has plenty of rainfall, and deciduous broad-leaved forests grow well in its mountainous area. This region is appropriate for Danshen's growth. Geologically, this region is the extension of Tai and Changbai Mountains.

2.3.1.2 Shanxi, Liaoning, and Loess Plateau Distribution Region

The region is a narrow zone between Luliang Mountain and Taihang Mountain, ranging from the Yellow River to the south and Yanshan Mountain to the north. As a warm temperate broad-leafed zone, Danshen is widely distributed in this region, making it one of the major production areas.

2.3.1.3 South of Huaihe River and the Lower-middle Reaches of Yangtze River Region

The region refers to the hilly basins of the middle and lower Yangtze River, reaching north to Qinling Mountain, south to Huaihe River, including Qinling, Dabashan, Wudangshan, Shennongjia, Tongbaishan, Dabieshan, Tianmushan, Huangshan, and Lushan Mountains. The region has a varied topography. Its western mountains are 800–2,000 m high, in general, and its eastern mountains, such as Lushan, Huangshan, Tianmu Mountains, are relatively low and isolated. Climatically, the region is hot in summer and warm in winter and has plenty of rainfall in spring and summer. It belongs to the subtropical zone with deciduous and evergreen broadleaf forests.

2.3.1.4 Other Regions

Danshen is also found on Luoxiao Mountain, which is located in the area between Hunan and Jiangxi, on Daloushan and Wulingshan mountains in Guizhou–Sichuan Plateau, and in hilly areas in Sichuan Basin. Sichuan's Zhongjiang and Pingwu counties are one of the China's main production areas for Danshen [2].

2.3.2 Soil Conditions

Danshen grows well in fertile sandy soil and is capable of adapting to soils with a wide range of pH values, including neural, acidulous, and alkalescent soil [3]. Domestic Danshen is mostly cultivated in thick layers of loose soil with good water drainage.

Guided by botanical nutritional ecology and cultivation and soil sciences, Zhang and Cheng [4] have studied systematically the comprehensive techniques for nuisanceless production of high-quality Danshen. The technique can be easy to implement and the effects in yield improvement are significant.

2.3.3 Community

Danshen has a strong adaptability and is often found in sparse forests and brushes, on hillsides and roadsides, and in fresh planted forests. Due to massive digging in recent years, it is no longer easy to find a pure community of wild Danshen. What can be found now is isolated and scattered. In its habitat, Danshen normally grows in the herbaceous layer under the trees. Common community types of Danshen include catalpa bungei forest community, fir-sandalwood community, raspberry-sedge community, etc., in metamorphic rock mountain areas.

2.3.4 Habitat of High-quality Danshen

Sichuan's Zhongjiang County is where high-quality Danshen is produced. After surveying the ecological and soil conditions of this region, Wu and Chen [5] found that most cultivated Danshen is grown in the sloping land to the northwest of the county, where the elevation is about

600–900 m. The climate of that region is warm and humid, and the soil types are medium loamy terra and neutral purple soil. The soil's organic matter, nitrogen, and potassium contents are mid- to low level, but rich in available phosphor. Among the trace elements, there is plenty of available iron, manganese, and copper, but available zinc and boron are insufficient.

2.4 Geographic Variation and Quality

In recent years, many researchers have carried out studies on Danshen's geographic variations across various production regions, but the results are not conclusive. Where is the high-quality Danshen produced? What are the characteristics of that habitat? These questions are discussed below.

2.4.1 Geographic Variation

The qualities of Chinese medicines are affected mainly by intra-species variation. As a result of long-time adaptation to different habitats, plants' variations can be detected at three different levels: individual, intra-community, and inter-community. For the purpose of quality evaluation and control, the geographic variation among communities is more important than the others, and it has received researchers' attention. The same is true for Danshen.

As mentioned above, Danshen is widely distributed on mountainous and hilly land of elevations between 120 and 1,300 m in China. Both wild and domestic Danshen are available in China. This section discusses geographic variations mainly in terms of phenotypic variation, genetic variation, chemical composition variation, and pharmacological and pharmacodynamic variation in different Danshen production regions.

2.4.1.1 Phenotypic Variation

There are many research reports on Danshen, and their descriptions of Danshen are generally the same: Danshen's rootstalk is short and thick, with residual caudex on the top; long cylindrical in shape; reddish or auburn rough skin with wrinkles; old root with a purple-brown loose skin that comes off in scale-shaped pieces; hard and brittle texture; slight smell and slight bitter taste.

Zhang et al. [6] classified Danshen into three types: big-leaf type (*Salvia miltiorrhiza* Bge. cv. *sativa*), wild type (*S. miltiorrhiza* Bge. cv. *foliolum*), and small-leaf type (*S. miltiorrhiza* Bge. cv. *silcestris*). The big-leaf-type Danshen is suitable for shallow and gentle sloping land; the small-leaf type is suitable for medium hills, and wild type is suitable for deep hills. After a continuous study over three years, they found that there are significant differences ($P < 0.001$) among the three types of Danshen in their biological properties, bio-productivities, disease resistance, and commercial yields. Also, a difference is also noted in ecologic adaptation, plant character, pollen grain, chromosome, isoenzyme, and quality character. These results suggest that different habitats could cause the changes in Danshen's quality. However, no such reports have been found to confirm this speculation.

Lin and Luo [7] showed that in thin and slender Danshen roots, which have a diameter of less than 1 cm, red to brown skin and are yellowish inside, the content of Tanshinone IIA could reach or exceed the standard set forth by the Pharmacopoeia. On the contrary, in the thick and stronger roots, which have a diameter of 1–2 cm, light red-brown skin and are yellowish white or purple brown inside, the content of Tanshinone IIA is lower than the standard. They suggested that Danshen's appearance is related to the content of tanshinone.

2.4.1.2 Genetic Variation

Guo et al. [8] did RAPD analysis of 44 individual plants of Danshen from nine communities in different regions. From more than 100 primers, they selected 11 primers with high polymorphism and good repeatability, and they amplified 129 bands. Their analysis showed that the polymorphic loci in different communities are 20.9–55.0 %, intracommunity genetic variation is 80.44 %, and intercommunity variation is

8.29 %. So, it is evident that Danshen's genetic variations are mainly intracommunity, and the variations among communities are relatively less common. Their cluster diagram also showed that, except for five individuals from Sichuan's Zhongjiang area, which belong to one group, all the individuals from the other eight communities do not cluster, suggesting that Zhingjiang's Danshen has similar genetic backgrounds, and the individuals of the other communities may have greater genetic variations among themselves than among communities. Generally speaking, in a cultivated species, the genetic backgrounds tend to be similar within one community and to be very different among communities due to human interference, such as breeding and vegetative reproduction. However, Guo Baolin's study shows that even Chinese atractylodes cultivated in the same community (e.g., No.6–No.10 samples from Henan's Lushi, No.22–No.25 samples from Shandong's Yinan, and No.30–No.32 samples from Shandong's Yishui) do not cluster to each other, rather, they are mixed with Danshen from other communities. This indicates that intercommunity genetic variation, even for cultivated Danshen, is not significant.

2.4.1.3 Chemical Composition Variation

The effective components of Danshen root include liposoluble phenanthraquinone compounds and hydrosoluble salvianolic acids. In addition, there are flavonoids, triterpenes, and sterols therein. Liposoluble constituents include: tanshinone I, IIA, II-B, V, VI, cryptotanshinone, isotanshinone I, II, isocryptotanshinone, hydroxy-tanshinone IIA, methyl-tanshinonate, methylenetanshiqunone, dihydrotanshinone, etc. Hydrosoluble salvianolic acids include: Tanshinol, salvianolic acid A, B, C, D, E, F, G, rosmarinic acid, methylrosmarinate, monomethyl lithospermate, dimethyllithospcrmate, ethyllithospermate, 1ithospermic acid B, protocatechuic acid, caffeic acid, and isoferulic acid, etc.

Many studies have shown that the chemical compositions of Danshen have geographical differences. Wu [9] used HPLC method to measure and compare contents of Tanshinone IIA and protocatechuic aldehyde of Danshen from different places. Chemical compositions of Danshen are different due to different origins, and their Tanshinone IIA and protocatechuic aldehyde contents are very different. Jin et al. [10] used HPLC method to analyze fingerprints of hydrosoluble and liposoluble constituents of Danshen from different production regions, and the results showed that there are differences and that the liposoluble constituent content is not related to the hydrosoluble constituent content. Huang et al. [11] used HPLC method to measure Tanshinol and protocatechuic aldehyde contents in Danshen from five different production places. Their results indicate that there are some differences in Tanshinol and protocatechuic aldehyde contents.

In addition, some researchers ranked Danshen from different regions based on their chemical composition contents. Zhang et al. [12] found that Danshen from four different regions differ in Tanshinone IIA contents; from high to low, the order is: Hebei's Pingshan, Xushui, Anguo, and Sichuan. Lin et al. [13] did qualitative and quantitative analysis of 13 Danshen samples from different regions or different growth modes with thin layer chromatography and HPLC methods and found that they differ in Tanshinone IIA contents: cultivated Danshen from Hebei's Chengde has the lowest content, while wild Danshen from Henan's Lushi County has the highest content. They also found that in Danshen samples from the same region, the wild ones contain more Tanshinone IIA than cultivated ones. Using ultrasonic extraction and HPLC analysis method, Li et al. [14] measured three liposoluble constituents, tanshinone IIA, cryptotanshinone, and Tanshinone I in Danshen from eight main production regions across the country. Their results showed that the contents of constituents in their Danshen samples are as follows: tanshinone IIA: 0.10–0.40 %; cryptotanshinone: 0.04–0.46 %; tanshinone I: 0.03–0.15 %. Danshen samples from Sichuan's Zhongjiang, Liaoning's Lingyuan, and Shandongs Pingyi have the highest contents of these chemicals, and those from Shanghai's Congming have the lowest contents. Chen et al. [15] compared chemical

compositions of Danshen cultivated in Sichuan's Zhongjiang, Shandong, and Henan and came to the conclusion that Tanshinone IIA contents in these samples are roughly the same.

From these studies, we can see that sorting Danshen from different regions is difficult, even though only the content of one component Tanshinone IIA is used as the parameter. For example, for the Tanshinone IIA content of Danshen from Sichuan, Zhang et al. [12] considered its content to be the lowest, while Lin et al. [13] considered its content to be moderate, and Li et al. [14] considered its content to be the highest. Chen et al. [15] showed that the Tanshinone IIA content in some samples was highest and in other samples was lowest. The study results from other places in China are basically similar. As these comparisons were performed based on the data from the same research group, the systematic error caused by study methods and operations factors could be eliminated, and these radically different results need to be re-evaluated.

2.4.1.4 Medical Effect Variation

Li et al. [16] compared the Danshen obtained from different regions and using Tanshinol and tanshinone components to conduct ADP-induced platelet aggregation tests, prothrombin (PT) time tests and MDA detection, performed cluster analysis on these results. They found that of the Danshen samples from eight regions, two extracts had the functions of resisting platelet aggregation, prolonging the PT time and decreasing MDA. The cluster analysis showed that as for the activity of these extracts, the wild and cultivated Danshen from Shanxi were similarly effective and they were superior to those from other places; cultivated Danshen from Sichuan's Zhongjiang, wild Danshen from Shandong, and cultivated Danshen from Henan were similarly effective and were moderate in quality by comparison; and the wild Danshen from Shanxi's Ankang and cultivated Danshen from Henan were relatively poor. The researchers noted the limitation of using limited indicators to evaluate the effectiveness of Danshen, and pointed out that more accumulated data is needed to perform a complete comparison and evaluation. From their EC_{50} data, it seems no clear relationships exist between Danshen samples from different regions and their medical effectiveness.

2.4.2 Genuineness

2.4.2.1 Indicators and Data Evaluation

A genuine medicinal material is a featured regional product, so the quality of an herb is associated closely with its producing region. From the above review, we can see that the Danshen samples from different regions have similar phenotypic characteristics with little genetic variations, but have differences in chemical constituency and pharmacological and medical effects. However, no generalizations could be made based on this data. This may be the reason why a genuine-producing region or regions of Danshen have not been identified yet.

Chemical constituents are the material basis of pharmacological effects, and they are also the key for any medicine's clinical effects. Thus, chemical constituents should be used to evaluate an herb's genuineness. The following analysis and discussion is based upon this assumption.

In consideration of its multiple chemical constituents and multiple targets among Chinese medicines, as well as the assurance of data quality, we choose the finger-printing data from Jin et al. [10] to make a further analysis. Our rationales are as follows: (1) Their data involved 33 Danshen samples from 18 regions of China, and a total of 7 chemical constituents, including hydrosoluble and liposoluble constituents, were monitored in each Danshen sample, and some of them were tested repeatedly, so it is one of the largest databases available so far. (2) This study was reported in Chinese Traditional and Herbal Drugs in October 2004, and their methods are reliable, so the quality of the data is assured. (3) The relationship between hydrosoluble and liposoluble constituents and geographical environments was one of the major topics of this study; the researchers even did cluster analysis using these constituents as the indicators. They concluded that the hydrosoluble constituents and liposoluble constituents would vary with different

production places of Danshen, so the analysis results of this research can be used directly.

2.4.2.2 Correlation Between Geographical Condition and Chemical Constituents

From the dendrograms provided by Jin et al., we can see that Danshen from all regions are randomly clustered, and the Danshen samples from the same production region are clustered into different groups. For example, with Danshen samples #2–5 and #7–9 from Shiquan, and samples #6 and #10 from Jifeng, both located in Sichuan's Zhongjiang, the difference in chemical constituents among these samples is much larger than those from other provinces.

However, considering that the authors did cluster analysis with data of hydrosoluble constituents and liposoluble constituents separately, which might not be a good way to find the actual relationship between geographical location and chemical constituents; also, from the dendrograms in the report, we can see that the differences between many samples are not embodied in these dendrograms. For example, in the liposoluble constituent dendrograms, a total of 17 samples, more than one half of total samples were regarded as identical, and the same problem is present in the hydrosoluble constituent dendrograms. As these researchers did not provide specific cluster distance formulas and cluster methods nor the standard data process methods, we cannot conjecture about it, but it gives us the impression that these data were not standardized, which might cause the oversight of some relatively low content variations.

Therefore, we adopted the raw data provided by Jin et al. to carry out the cluster analysis again, and our method as follows: The hierarchical cluster analysis in SPSS software is used to make the cluster analysis on the seven constituents of Danshen from 18 production regions, the data was standardized (0–1), the cluster method is average linkage (Within groups) and the distance formula is Squared Euclidean distance. The results are shown in Fig. 2.1.

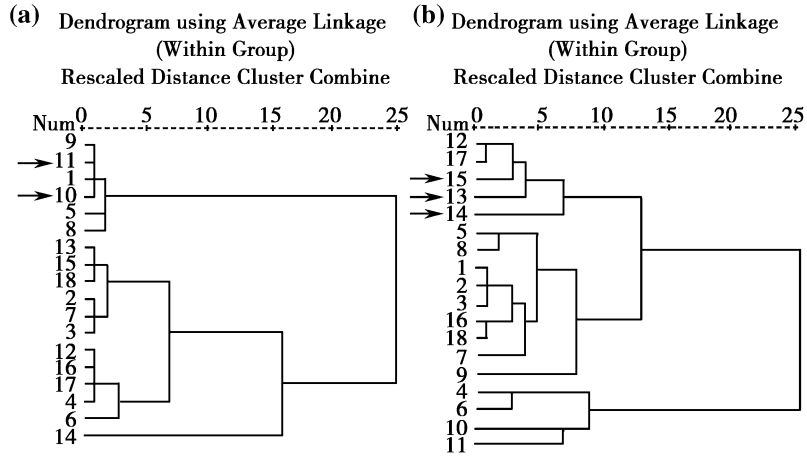

Fig. 2.1 Geographical dendrograms of Danshen's chemical constituents. *Note* **a** Samples #1–14 are from Hebei's Anguo, Hebei's Tangshan, Shandong, Shandong, Shandong's Jijing, Shandong's Laiwu, Laiwu's Liushan, Shanxi, Shanxi's Ruicheng, Sichuan Zhongjiang's Jifeng, Sichuan Zhongjiang's Shiquan, Zhejiang Qing'an's Shenze, Zhejiang Qing'an's Xinwo, and Zhejiang Shengzhou's Wangyuan, respectively. **b** The blank item was given the value of zero. Samples #1–18 are from Hebei's Anguo, Hebei's Tangshan, Henan's Tongbai, Shandong, Shandong, Shandong's Jijing, Shandong's Laiwu, Shandong's Laiwu, Laiwu's Liushan, Shanxi, Shanxi's Ruicheng, Sichuan, Sichuan Zhongjiang's Jifeng, Sichuan Zhongjiang's Shiquan, Zhejiang Qing'an's Shenze, Zhejiang Qing'an's Xinwo, and Zhejiang Shengzhou's Wangyuan, respectively. The *arrows* indicate Danshen samples from Sichuan Province

Because the original report provided only data on the chemical constituents of Danshen from all production regions, we performed the cluster analysis on Danshen by using the production region as the parameter. From the figure, we can see that the effect of this cluster analysis is very good, and sufficiently presents the differences among the samples. However, the results are basically in accordance with the original report. Except for several Danshen samples from Sichuan being grouped together with samples from other production places, such as Shanxi, Hebei, and Zhejiang, all of the other samples, including the samples from the same province, even from very close production places (e.g., Qing'an County's Shenze, and Qing'an County's Xinwo, Zhejiang Province), were grouped in different clusters. The variations of their chemical constituents among these samples were far larger than those of Danshen samples from other provinces. Thus, the quality variation of Danshen samples from the same province or region may be larger than those from two or more provinces separated by a long distance, which shows that the chemical constituents of Danshen present no geographical correlation; the chemical constituents of Danshen from Sichuan Province have no difference with the chemical constituents of Danshen from other production places (at least including some Danshen samples from Shanxi, Hebei, and Zhejiang provinces), but these chemical constituents have high consistencies in their own species group.

2.4.2.3 About the Genuineness of Danshen

Based upon the above analysis, we have reached the following understandings:

First, the genetic background and small scale ecological factors have a relatively large influence on the accumulation of secondary metabolites in Danshen.

Danshen from all production areas shows no obvious differences, which is the very basic reason why the genuineness of Danshen is unclear, and why the genuine Danshen production region is uncertain. Our study indicates that it would be fruitless to try to establish the genuineness of Danshen by looking for the relationship between geography and chemical constituents, because on the one hand, Danshen roots from different regions have different constituents, and on the other, there are some overlaps in these components. Overall, these differences have no geographical characters, which is the reason why such studies gave varying results. The fact that Danshen's chemical composition has noting to do with geographic locations indicates that the large scale ecological factors, such as climate and soil type, do not alter the accumulation of Danshen's secondary metabolites significantly. On the other hand, the fact that Danshen samples from the same province have a greater chemical composition variation between each other than between samples from other provinces suggests that genetic background as well as small scale ecofactors, such as regional soils, microclimates, and human interference, might play a bigger role.

Second, the reason that Zhongjia's Danshen is regarded as genuine might have something to do with its homogeneous quality.

With the modernization and internationalization of Traditional Chinese Medicines, special attention has been paid to the standardization of Chinese medicinal materials. Presently, it has been realized that in addition to its special therapeutic effects, Chinese medicine's homogeneity of quality should be one of the important indicators for its evaluation and quality control. We find an interesting phenomenon in our analysis, i.e., the chemical constituents of Danshen from Sichuan (mainly from Sichuan's Zhongjiang area) have relatively high consistencies in the community group, which is consistent with the conclusion made in the genetic analysis on Danshen by Guo et al. [8]; it means that Danshen from Zhongjiang has relatively high genetic similarity at the community level, which may be the reason why Zhongjiang's Danshen is highly regarded.

The propagation of Danshen is usually done by dividing roots or basal stems, or by sowing the seeds. Among the three methods, the first one is most common. Whether it is intended or not, the process of cultivation and planting is the process of breeding. Recently, Zhongjiang has become the main production place of cultivated

Danshen, characterized by its large cultivation area and matured cultivation techniques. Therefore, the homogeneity of genetic background leads to the consistency of quality of Danshen, which is the reason that Zhongjiang's Danshen has been regarded as a well-known area for medicinal material. Consequently, it can be said that the genuineness of Danshen is associated closely with the planting and processing tradition of a certain location and its cultivation techniques and breeding during the cultivation.

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