Research Progress on Authenticity Formation of *Scutellaria baicalensis* Georgi

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Abstract By analyzing the key factors affecting the authenticity of *Scutellaria baicalensis* Georgi, this paper focuses on summarizing the research progress of environmental factors (light, temperature, water content, soil), genetic factors and other factors, in order to ensure the quality of *S. baicalensis* Georgi, find potential suitable areas of *S. baicalensis* Georgi, and further promote the sustainable development of Chinese medicine resources.

Key words *Scutellaria baicalensis* Georgi, Authenticity, Environment

1 Introduction

Authenticity is a specific term to identify the quality of Chinese herbal medicines. Authentic medicinal materials refer to high-quality authentic medicinal materials with specific geographical producing areas, the advantages of "high quality, excellent shape and strong effect", standardized production and processing, and recognized by the world[1]. Clarifying the mechanism of authenticity formation of Chinese herbal medicines is the premise of sustainable development of Chinese herbal medicine resources. *Scutellaria baicalensis* Georgi is a medicinal plant in the Labiatae family[2]. In China, wild *S. baicalensis* Georgi resources are concentrated in North China, Northwest China and Northeast China, and they are most distributed in Hebei, Shanxi, Inner Mongolia, Liaoning, Heilongjiang and other provinces[3]. The authenticity of *S. baicalensis* Georgi is obvious, especially the *S. baicalensis* Georgi produced in Chengde, Hebei Province and its surrounding areas has the best quality. In modern times, Chen Renshan compiled *Drug Identification*, which recorded that *S. baicalensis* Georgi was produced in Shanxi, Zhili and Rehe (Yanshan Mountain and Hill, Chengde City, Hebei Province), which was the first to explain the existence of *S. baicalensis* Georgi in Rehe[4]. Throughout the distribution changes of *S. baicalensis* Georgi, the overall trend of moving northward may be related to the change of geographical environment. *S. baicalensis* Georgi is one of the most commonly used medicinal materials in ready-for-use traditional Chinese medicine. With the increasing demand for Chinese herbal medicines in the market and the depletion of wild *S. baicalensis* Georgi resources, the existing resources can no longer meet the demand. Artificially planted and cultivated *S. baicalensis* Georgi has become the main supply source. However, the quality of artificially planted *S. baicalensis* Georgi is uneven, which leads to poor medicinal efficacy[5]. By explaining the scientific connotation of "authenticity", this paper expounds the authenticity formation of *S. baicalensis* Georgi, and provides effective reference for ensuring the quality and therapeutic effect of Chinese herbal medicines and further development and utilization of *S. baicalensis* Georgi.

2 Characteristics of *S. baicalensis* Georgi

Chengde City (115°54′ - 119°15′ E, 40°11′ - 42°40′ N) is located in the northeast of Hebei Province, and in the area where the Greater Khingan Mountains connect the northern Yanshan Mountains to the southwest. It is an important node connecting Beijing, Tianjin, Hebei, Liaoning and Inner Mongolia, the former capital of Rehe Province, and a high-quality producing area of *S. baicalensis* Georgi[6-7]. The *S. baicalensis* Georgi produced in Rehe area is thick, long and solid, golden yellow after removing the outer skin, and the quality is the best. Chengde area is considered as the authentic producing area of *S. baicalensis* Georgi, so the *S. baicalensis* Georgi produced in Chengde is known as "Rehe Huangqin". *S. baicalensis* Georgi has two specifications, Ziqin and Kuqin, which can treat different symptoms. Kuqin "purges the fire of stomach and lung", while Ziqin "purges the fire of large and small intestines"[8]. Zhao Jiawen[9] proved that Ziqin was better than Kuqin in treating damp-heat syndrome of large intestine. The main effective components of *S. baicalensis* Georgi are baicalin, wogonin, baicalein, wogonin, oroxylin A, chrysin, scutellarin and other chemical components. The *Chinese Pharmacopoeia* stipulates that baicalin is the content detection standard of *S. baicalensis* Georgi, and the content of baicalin shall not be less than 9.0%[10]. Data show that the highest content of baicalin and wogonin is in Longhua County, Chengde City, Hebei Province; the highest content of oroxylin A is in Luanping County, Chengde City, Hebei Province; the highest content of chrysin is in Luanping County, Chengde City, Hebei Province[11]. To sum up, it shows that the quality of *S. baicalensis* Georgi produced in

Received: July 13, 2023 Accepted: September 18, 2023
Supported by Special Project of Traditional Chinese Medicine of Natural Science Foundation of Hebei Province (H2022406079).
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Chengde, Hebei Province is the best, which strongly proves that Chengde, Hebei Province is a producing area of authentic *S. baicalensis* Georgi.

Besides flavonoids, *S. baicalensis* Georgi also contains phenylpropionate glycosides, iridoid glycosides, triterpenes, alkaloids and other components[12]. It has anti-tumor, antibacterial, antioxidant and cardiovascular malformation treatment effects[13]. And some studies have found that the active constituents in *S. baicalensis* Georgi have certain therapeutic effects on mental diseases[14]. *S. baicalensis* Georgi has anti-inflammatory, immunoregulatory, antibacterial and antiviral effects, and it plays an important role in preventing and treating Novel Coronavirus, so it is necessary to further explore the target and mechanism of *S. baicalensis* Georgi in preventing and treating Novel Coronavirus infection[15]. Studies have shown that *S. baicalensis* Georgi produced in Chengde, Hebei Province has better antipyretic, anti-inflammatory and antiviral effects than medicinal materials produced in other regions[16]. With the continuous development of modernization of traditional Chinese medicine, DNA molecular genetic markers, three-dimensional quantitative analysis of tissue morphology, chemical fingerprint and content determination of traditional Chinese medicine, bioavailability evaluation and other technologies have been widely used to evaluate the authenticity of medicinal materials and further reveal the essence of authenticity[17].

3 Key factors affecting the authenticity of *S. baicalensis* Georgi

3.1 Environmental factors "Authenticity" indicates a specific ecological environment, including temperature, light, water, soil, altitude and other ecological factors[18]. The authenticity of *S. baicalensis* Georgi is the result of the continuous adaptation of medicinal materials to the environment, which may be formed after countless times of environmental stress. Suitable natural conditions, as the preliminary conditions for the formation of traditional authentic medicinal materials, are conducive to the formation of good properties of medicinal materials and the aggregation of internal components[19-20]. Environmental factors are the primary factors affecting the authenticity of *S. baicalensis* Georgi. Orange change with their environment is a typical phenomenon of "homogeneity". The same plant bears fruits in different shape in different areas, which reflects the key influence of ecological factors on the growth and development of plants. Yuan Yuan *et al.*[21] put forward the model hypothesis that phenotypic characteristics, genotypic characteristics and environmental modification of medicinal materials affect the formation of authentic medicinal materials. Tian Tian[22] also found that ecological factors are significantly related to the quality of *S. baicalensis* Georgi. To sum up, ecological factors have certain influence on the content of effective components in medicinal plants, so it is very important to choose a suitable ecological environment for medicinal plants, in order to improve the quality of medicinal plants, promote the sustainable development of Chinese medicine resources and the innovative development of Chinese medicine industry.

3.1.1 Light. Light is the main environmental factor affecting seed germination, plant growth and physiological process of *S. baicalensis* Georgi, and it is also the main energy source for plant photosynthesis, through which plants carry out physiological metabolism and material accumulation. Through statistical analysis, Han Mei *et al.*[23] studied the diurnal variation characteristics of photosynthesis of *S. baicalensis* Georgi (diurnal variation of net photosynthetic rate of *S. baicalensis* Georgi leaves, diurnal variation of stomatal conductance of *S. baicalensis* Georgi, *etc.*) and its relationship with environmental factors under three different habitats (Linjiang, Changchun and Taonan, Jilin Province). It was found that the diurnal variation of Pn (net photosynthetic rate of *S. baicalensis* Georgi leaves) in three different habitats showed slight photosynthetic "midday depression", which was caused by stomatal limitation of plant leaves. Under different habitat conditions, the environmental factors affecting Pn of *S. baicalensis* Georgi are different. For example, PAR (photosynthetic active radiation) has the greatest effect on Pn of *S. baicalensis* Georgi, while TL (temperature of leaf) has the smallest effect in Linjiang area; in Changchun, Ta (atmospheric temperature) is the most important to Pn of *S. baicalensis* Georgi, and in Taonan area, PAR has the greatest influence on Pn of *S. baicalensis* Georgi, but Ta (atmospheric temperature) has the least influence. Wei Hao *et al.*[24] analyzed the effects of different altitudes and different light conditions (shady slope and sunny slope) on flavonoids in *S. baicalensis* Georgi, and found that with the increasing altitude, the content of other six flavonoids increased except wogonin. The sample size is not large enough, there is no significant difference in flavonoids content of *S. baicalensis* Georgi under different light conditions (shady slope and sunny slope), but the average value of sunny slope is higher than that of shady slope. *S. baicalensis* Georgi is suitable for planting on sunny slope.

Light can affect the growth and development of plants through three aspects: light intensity, photoperiod and light quality[25]. Light has no significant effect on the germination stage of *S. baicalensis* Georgi seeds, but has significant effect on the growth process of *S. baicalensis* Georgi. High intensity can accelerate the synthesis of photosynthetic pigment of *S. baicalensis* Georgi, and PAL (phenylalanine ammonia lyase), C_4H (cinnamate-4-hydroxylase) and CHS (chalcone synthase) will also increase significantly, and then the secondary metabolites of *S. baicalensis* Georgi will increase, and finally it will improve the quality of *S. baicalensis* Georgi[26-27]. This indicates that light can affect the gene expression of PAL and UBGAT and the role of UBGAT in the accumulation of baicalin. It shows that light can greatly promote the biosynthesis and accumulation of flavonoids in *S. baicalensis* Georgi, and these theoretical discoveries will further provide strong support for light to the quality formation of authentic medicinal materials.

3.1.2 Temperature. The content of effective components in the roots of *S. baicalensis* Georgi is mainly affected by temperature,

and the influence of high temperature is greater than that of low temperature[28]. Hou Yunliang *et al.*[29] studied the changes of baicalin content in *S. baicalensis* Georgi callus at different culture temperatures, and it was found that the content of baicalin was higher at 25 ℃ and lower at 5 ℃ and the high temperature of 40 ℃ was not conducive to the accumulation of baicalin in *S. baicalensis* Georgi callus, and the growth of *S. baicalensis* Georgi required suitable temperature.

The average annual temperature in Chengde is between 5 ℃ and 9 ℃, which is beneficial to seed germination and baicalin accumulation. Most chemical components in *S. baicalensis* Georgi are positively correlated with temperature. High temperature is beneficial to the accumulation of baicalin and ensures the quality of *S. baicalensis* Georgi.

3.1.3 Water content. Water content is one of the important ecological factors affecting the authenticity of *S. baicalensis* Georgi, and it is also an important condition for photosynthesis of medicinal plants. Water content directly affects the growth and development of medicinal plants, the accumulation of biomass and the accumulation of metabolites. SWC (soil water content) is an important environmental factor affecting baicalin content[30]. Zhou Guofu *et al.*[31] used Maxent model to predict the ecologically suitable area of *S. baicalensis* Georgi, analyzed the correlation between ecological factors and chemical components in *S. baicalensis* Georgi, and found that the variability of precipitation had a great influence on the chemical components of *S. baicalensis* Georgi.

S. baicalensis Georgi is drought-resistant but is not waterlogging-resistant. Excessive water content will cause the root of medicinal materials to rot. The response of medicinal plants to drought stress can effectively promote the biosynthesis of active components of traditional Chinese medicine[32]. Drought is a special water condition. Excessive water content is not good, different germplasm of *S. baicalensis* Georgi in different stages of growth and development requires different water content. When the water condition was 150 mm, short-term drought was not conducive to the accumulation of baicalin in Gansu and Jilin germplasm of *S. baicalensis* Georgi, but after 1 – 5 d drought, the baicalin content in Hebei germplasm of *S. baicalensis* Georgi increased greatly. With the aggravation of drought, the baicalin content of Hebei germplasm of *S. baicalensis* Georgi also decreased[33]. Moderate drought stress was beneficial to the transfer of *S. baicalensis* Georgi biomass to roots and increased the distribution ratio of root biomass[34].

Chengde City is rich in water resources, and the annual water output of rivers reaches 3.76 billion m^3, which ensures the water content needed for the growth of *S. baicalensis* Georgi[35]. Baicalin and wogonin in *S. baicalensis* Georgi were negatively correlated with annual precipitation[36]. Less rain in early spring is the key factor affecting the accumulation of active components in *S. baicalensis* Georgi.

3.1.4 Soil. Soil inorganic elements and soil microorganisms are one of the essential conditions for the formation of effective components of authentic medicinal materials. The content of effective components in Chinese herbal medicines is correlated with soil factors, and the quality of Chinese herbal medicines is affected by soil inorganic elements and soil fertility. Clarifying the correlation between soil factors and the content of effective components of Chinese herbal medicines can help improve the soil and ensure the quality of Chinese herbal medicines[37]. Xie Lixia[38] determined mineral elements, pH, particle composition, aggregate structure, organic matter, humus and cation exchange capacity in rhizosphere soil of Ephedra sinica in Gansu, Ningxia and Inner Mongolia. Through screening of soil factors affecting the quality of Ephedra sinica, the results showed that ephedrine was significantly or extremely significantly correlated with soil pH, aggregate structure, humus, mineral elements P, K, Mg, Mn, Zn and Sr, and pseudoephedrine was significantly correlated with humus and cation exchange capacity. This proved that the quality formation of medicinal plants was related to soil factors.

Most of Chengde area has mountainous landform, and a small part of plain. The main types of soil-forming parent materials in Chengde area are river and lake alluvium, aeolian loess and so on. The alluvial land of rivers and lakes has loose and fertile soil, which is very beneficial to planting *S. baicalensis* Georgi. Sandy loam soil with slightly alkaline pH also provides necessary conditions for the formation of authentic *S. baicalensis* Georgi[39].

S. baicalensis Georgi needs nutrient soil as its growth condition, and most of the nutrients in soil are provided by nitrogen, phosphorus and potassium. The total absorption and accumulation of nitrogen, phosphorus and potassium by *S. baicalensis* Georgi plants is as follows: nitrogen is the highest, followed by potassium and the lowest is phosphorus. Fertilization makes *S. baicalensis* Georgi tall and upright, and helps to improve the flavonoids content and the yield of medicinal materials[40].

Not only inorganic elements in soil are beneficial to the growth of *S. baicalensis* Georgi plants, but also microorganisms contained in soil are beneficial to the accumulation of effective components of *S. baicalensis* Georgi. Soil microorganisms include rhizosphere soil microorganisms and non-rhizosphere soil microorganisms. The quantity of these two microorganisms is quite different, and the quantity of rhizosphere microorganisms is much larger than that of non-rhizosphere soil microorganisms. Rhizosphere microorganisms can be divided into three types: growth-promoting bacteria, pathogenic microorganisms and human opportunistic pathogens, and the number of growth-promoting bacteria is the largest[41]. Not all rhizosphere microorganisms are beneficial to the growth and development of medicinal plants, among which the bacteria beneficial to the growth of medicinal plants are called growth-promoting bacteria, including nitrogen-fixing bacteria, phosphorus bacteria, *etc.* These bacteria form a symbiotic relationship with medicinal plants and promote the metabolism of medicinal plants[42]. Continuous cropping of wheat and *S. baicalensis* Georgi can increase the quantity of soil microorganisms, ensure soil fertility and promote the growth of *S. baicalensis* Georgi

plants. It was found that the total quantity of soil fungi and bacteria in wheat – *S. baicalensis* Georgi rotation land was the largest[43]. Quantitative characteristics of soil microorganisms are important indicators to indicate soil quality and measure soil fertility[44]. Soil microorganism is an important part of soil ecosystem, and it is an essential biological factor to regulate soil ecological processes such as litter decomposition, carbon and nitrogen mineralization, soil nutrient transformation and circulation[45]. Rotation and continuous cropping have great influence on the quantity of soil bacteria, fungi and actinomycetes, and rotation is beneficial to the diversity and stability of soil microbial community and the improvement of soil ecological environment[46]. In Chengde area, *S. baicalensis* Georgi is generally planted in rotation mode, which can not only ensure soil fertility, but also increase the economic benefits of growers.

3.2 Harvest time The harvest time is also "regional". The harvest time of *S. baicalensis* Georgi in different regions is different, which is caused by different geographical and climatic conditions. The content of effective components of *S. baicalensis* Georgi will change with seasons. The 2020 edition of *Chinese Pharmacopoeia* stipulates that the harvest time of *S. baicalensis* Georgi is spring and autumn. The proportion of chemical components of *S. baicalensis* Georgi harvested in autumn is obviously in a dynamic process with the increase of years, while that of *S. baicalensis* Georgi harvested in spring is in a relatively static state[47]. By comparing the content of baicalin in the roots of *S. baicalensis* Georgi in different harvesting periods, it was concluded that the content of baicalin in the roots of *S. baicalensis* Georgi harvested in June in Chengde area was the highest, and the content of baicalin generally showed a downward trend from June to October, which proved that the best time to harvest *S. baicalensis* Georgi in Chengde area was June[48]. Jin Weirong *et al.*[49] found through field investigation that the harvest time of *S. baicalensis* Georgi in Shandong is autumn. At the same time, it was proved by experiments that the content of baicalin, wogonin, scutellarin and wogonin in *S. baicalensis* Georgi harvested in September was the highest, which proved that the best harvest time of *S. baicalensis* Georgi in Shandong was autumn. The content of flavonoids in *S. baicalensis* Georgi in different harvesting seasons in Chengde area of Hebei Province was determined. The results showed that the quality of *S. baicalensis* Georgi harvested in spring was obviously better than that harvested in autumn[50]. Determining the harvest season of *S. baicalensis* Georgi can ensure the high quality of *S. baicalensis* Georgi harvested in a suitable time.

3.3 Genetic factors Genetic factors are the internal factors for the authenticity formation of *S. baicalensis* Georgi. There is obvious genetic variation in the population of *S. baicalensis* Georgi, and there are great differences in flower color, stem shape, stem color and other features for the varied *S. baicalensis* Georgi. The important factor affecting the geographical distribution and genetic variation of plant population is the gene flow of plants, and the gene is determined by planting seeds and spreading pollen. Different plants have different gene flow patterns[51]. Chai Junwen *et al.*[52] used ISSR molecular marker technology to analyze the genetic variation relationship between *S. baicalensis* Georgi plants at DNA molecular level, revealing that there is great variation between diploid *S. baicalensis* Georgi and autotetraploid. The shorter the genetic distance, the more similar the species are. Popularizing the extensive planting of tetraploid *S. baicalensis* Georgi is of great significance for maintaining the sustainable development of traditional Chinese medicine resources.

4 Conclusion

Studying the authenticity of *S. baicalensis* Georgi is a complex and comprehensive process. According to the connotation of authenticity of *S. baicalensis* Georgi, combined with textual research of herbs, the influencing factors were analyzed from internal factors (genetic factors) and external factors (environmental factors), and the two major factors cooperated with each other to form a complete system. The study on the influencing factors of authenticity of *S. baicalensis* Georgi is of great significance for discovering the potential suitable growth area of *S. baicalensis* Georgi, studying the development and utilization of related species of *S. baicalensis* Georgi, providing resource guarantee for the protection of *S. baicalensis* Georgi resources and the development of new drug sources, thus forming authenticity with obvious regional characteristics. In the long-term introduction and domestication process, people have summed up planting experience of *S. baicalensis* Georgi belonging to their own areas, thus forming the authenticity of *S. baicalensis* Georgi with obvious regional characteristics.

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related to the pathogenic factors in the category of traditional Chinese medicine and the pathogenesis of modern medicine. In case of the imbalance of "three factors" in Tibetan medicine, namely, "Long (basically equivalent to wind)", "Chiba (basically equivalent to fire)" and "Peigen (basically equivalent to earth and water)", yellow water comes from the essence of bile pathological changes, which travels inside and outside the viscera of flesh and bone, mostly between skin and muscle, viscera and joints, affecting the circulation of qi and blood wherever it goes, followed by external diseases. In traditional Chinese medicine, water metabolism is abnormal, and it is believed that the main culprit of yellow water is dampness, and that dampness and heat formed by the combination of dampness and heat are the most typical, both of which attack the vital points, leading to adverse flexion and extension, papules and other diseases. Finally, we discussed the defense function in modern medicine, pointing out the formation of yellow water due to bacterial infection, immune system dysfunction and other reasons, and finally coming down to the disease caused. Through the intersection of different medical theoretical systems and the exploration from multiple perspectives, we can have a clearer understanding of Yellow Water Disease, integrate the guidance of various medical theories, and provide a more solid guiding basis for the treatment of Yellow Water Disease based on syndrome differentiation and treatment.

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