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ORIGINAL ARTICLE

Systematic Estimation of Potential Risk Caused by the Replacement of Aconite's Cultivar

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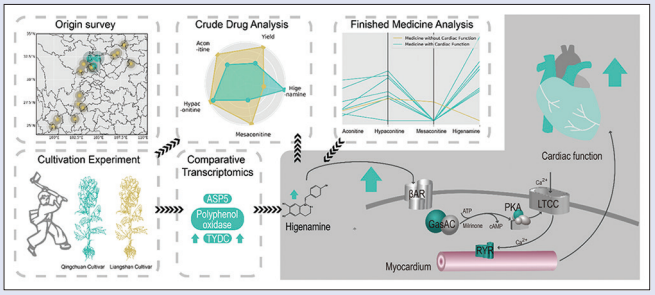
ABSTRACT

Background: Aconite is a famous toxic medicinal plant in the world. But in China, the aconite cultivar, Qingchuan cultivar, which has been stably used for 2000 years, are being rapidly replaced by the Liangshan cultivar, a new cultivar with better agronomic traits. However, the risk of the replacement is still unknown. **Materials and Methods:** We combine field research and cultivation experiments to clarify differences in provenance and differences in agronomic traits. We used high-performance liquid chromatography (HPLC) to analyze the content of key ingredients in commercially available medicines and semi-finished drugs. We then performed a transcriptome analysis of the two resources to study the key differential pathways of the two species and conducted a systematic discussion. **Statistical Analysis Used:** Statistical differences were assessed by analysis of variance or least significant difference. $P < 0.05$ was considered statistically significant. **Results:** The survey and cultivation experiments showed that the overwhelming superiority of agronomic traits had already made Liangshan cultivar occupy the vast majority of areas. HPLC showed that the content of diester alkaloids in the Liangshan cultivar was slightly higher and that of higenamine was significantly lower. Analysis of the patented drug and fuzi flake demonstrated a strong correlation between higenamine and cardiac activity. Transcriptome analysis revealed that the upregulation of multiple genes in the isoquinoline pathway in Qingchuan cultivar resulted in the large difference of higenamine.

Key words: Aconite, agronomic traits, germplasm, toxicity, transcriptome

SUMMARY

- In this study, we did field research and cultivation experiments and demonstrated that the superiority of Liangshan cultivar's agronomic traits had already made it occupy the vast majority of areas. We then used high-performance liquid chromatography to analyze the content of key ingredients in commercially available medicines and semi-finished drugs. The high-performance liquid chromatography results showed that the content of diester alkaloids in the Liangshan cultivar was slightly higher and that of higenamine was significantly lower, and higenamine was strongly correlated with cardiac activity. We then performed a transcriptome analysis of the two resources, which revealed that the upregulation of multiple genes in the isoquinoline pathway in Qingchuan cultivar resulted in the large difference of higenamine. Taken together, we concluded that there was a significant risk of unexpected medicinal effect caused by replacement of Qingchuan cultivar by Liangshan cultivar, which should be further investigated closely.



Abbreviations used: HPLC: High-performance liquid chromatography; ANOVA: Analysis of variance; LSD: Least significant difference; LD Lo: Lowest lethal dose; NMPA: National medical products administration; KEGG: Kyto encyclopedia of genes and genomes; GO: Gene ontology; ED: Erectile dysfunction; PCA: Principal component analysis; SD: Standard deviation; RSD: Relative standard deviation.

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INTRODUCTION

Aconitum is the most well-known poisonous plant genus around the world. Aconite, also named as *Aconitum carmichaelii* Debx., is a representative plant in the genus *Aconitum*.[1] Particularly, as the most important poisonous medicinal material in China, roots of aconite have been named tianxiong, wutou, and fuzi according to different growth sites (China Pharmacopoeia Committee, 2015),[2] among which fuzi is the radicle of aconite.[3] Although named distinctly, different parts of aconite root have similar vagal toxicity and efficacy in improvement of microcirculation and blocking of traumatic bleeding in cardiac disease. Benefitting from tiny size and thus convenience in quantification, fuzi, the oldest cardiotonic in human history, is recognized as a "No. 1 Reviving Medicine" and has been widely used in many prescriptions. Even now, there are over 4000 extensively used medicines which contain aconite.[4] Their vagal neurotoxicity can cause the heartbeat to succumb between acceleration and soothing, eventually enticing the poisoned person to die.[5]

As one of the most famous toxic economic crops in the world, there have been many hypotheses about the pharmacological activity of aconite. In recent years, the main focus has been on the secondary metabolites.[3]

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Some people believe that the sources of toxicity and activity are diester alkaloids,[6] namely aconitine, hypaconitine, mesaconitine, and its hydrolysates.[7,8] They believe that the strong heart function of aconite is due to the excitability of the vagus nerve by a diester alkaloid such as aconitine, hypaconitine, mesaconitine, and their hydrolysate.[9-12] On the basis of this theory, long-time boiling, which is the traditional way of aconite processing in China and India, can be considered as a procedure of aconitine degradation and aconitine reservation.[13] Another opinion on the active ingredients of aconitine came from Japanese scholar, who stated that instead of diester alkaloid, it is actually isoquinoline alkaloid, or more probably higenamine, that is responsible for the therapeutic effect of aconite.[14]

Since the poisoning amount of aconitine is only 0.028 mg/kg in lowest lethal dose test,[15] and the content of aconitine in aconite can be as high as 0.5 mg/g, in order to seek a balance between medicinal properties and toxicity, the ancient Chinese domesticated a kind of aconite germplasm, Qingchuan cultivar,[16] and developed a supporting cultivation and processing model for thousands of years.[17] However, this cultivation model is being destroyed and a new alternative cultivar, which was quickly domesticated from the wild aconite, named Liangshan cultivar, has been developed because of its better disease resistance and higher biomass. With assets such as higher yield, stronger disease resistance and a shorter growth period, the Liangshan cultivar has replaced the germplasm resources in almost all typical areas of aconite production within only 5–10 years, without any systematic assessment on safety and effectiveness.

To systematically assess the risks posed by the replacement of Qingchuan cultivar by Liangshan cultivar, we investigated the agronomic traits of the two cultivars together with transcriptomes. After conducting a cultivation experiment to investigate the difference in agronomic traits, we compared the concentration of higenamine in Chinese patent medicines and half-made medicines by high-performance liquid chromatography (HPLC) in order to better evaluate the difference between the two cultivars. Our results showed that the strong heart activity of Aconit is not derived from aconitine, but from higenamine. Subsequently, we examined the concentration of three diester alkaloids and higenamine in two cultivars by HPLC and analyzed their molecular mechanisms in combination with transcriptome. The results revealed that Liangshan cultivar is richer in diester aconitine than Qingchuan cultivar but is lower in higenamine and transcriptome results also support this. We found that although Liangshan species has better agronomic traits than Qingchuan cultivar, it is more toxic and less active than Qingchuan species for medical use.

MATERIALS AND METHODS

Plant

Wild plant material of fuzi was collected at Butuo County, Liangshan State, Sichuan Province and Qingchuan County, Guangyuan City, Sichuan Province in September, 2016. Professor Mengliang Tian from Sichuan Agricultural University has identified the material as *Aconitum carmichaelii* Debx. Qingchuan cultivar and aconite roots cultivated in Liangshan were planted in a plot with the same conditions as Qianjin Township, Qingchuan County, Guangyuan City, Sichuan Province in November 2016. Each cultivar was planted with 5 cells and each cell was planted with 1000 plants. According to the production habit, the row spacing was set to 15 cm × 15 cm. In the agronomic traits survey, samples were taken at each period and 10 individual plants with similar growth were extracted from each cell for agronomic traits survey. A total of nine periods of each species, five cells of the same condition, each cell repeated ten times [Table 1S].

Raw medicinal

Dried fuzi was collected from different plots in planting bases in Jiangyou, Ya'an, Qingchuan and Buto in Sichuan Province from August to October in 2017 [Table 1S]. Six samples were selected for each group for content determination and each sample was measured in parallel three times and the median was used for statistical analysis. Eight batches of processed products of fuzi from Shanxi, Butuo Sichuan, Anxian Sichuan, and Yunnan were purchased from the medicine market at Hehuachi, Chengdu, Sichuan [Table 1S].

Finished medicinal

All ten kinds of Chinese patent medicines containing root of aconite were purchased from major pharmacy stores in Chengdu in January 2018. All medicines are National Medical Products Administration certified and legally qualified.[2]

Standard material

Aconitine (purity >98%), hypaconitine (purity >98%), mesaconitine (purity >98%), and higenamine (purity >98%) were purchased from Mansite Bio-technology Co., Ltd, China.

Investigation on current status of cultivation of aconite (fuzi)

From November 2015 to May 2016, field investigation was conducted on the cultivars, yields and cultivation methods of 16 fuzi cultivation bases in different provinces. Each location surveyed more than 3 growers, obtained cultivar and yield information through the growers and each farmer identified more than 30 plants on-site in order to verification the obtained cultivar. The data for the production come from the survey of the various production areas, combined with the local government agricultural sector figures, grower records, andpurchaser invoices and our estimates based on field estimates, which are multiyear averages.

Dynamic record of growth and development of aconite (fuzi)

Since March 1, 2017, multiple indicators with regard to aconite growth and development have been recorded dynamically every 30 days by selecting ten plants taken from each plot of each recording, for a total of 50 plants from each of the two cultivars. Only the plants that have been growing well and have no pests were selected.

Transcriptomics study of aconite (fuzi)

On November 6, 2017, the two cultivars were harvested at the same time. For each cultivar, three well-grown roots of the same size were selected and numbered as Qingchuan cultivar Q 1–3 and Liangshan cultivar L 1–3. After cleaning and removing root hair, the roots were immediately frozen with liquid nitrogen and sent to Beijing Nuohe Zhiyuan Biotechnology Co., Ltd. for subsequent library construction and sequencing. The transcriptome sequence was assembled twice using Trinity on the Illumina Hiseq X platform.

The sequencing results of the two cultivars were assembled in a mixed pool. The assembly results were used to calculate gene expression and facilitate the search for differentially expressed genes. After transcript splicing, the CORSET method was used to filter the splicing results and obtain Unigene in order to increase the detection rate of differentially expressed genes.[18] Diamond v0.8.22 was used to perform gene function annotations on Unigene using Nr, KOG/COG, Kyto encyclopedia of genes and genomes (KEGG), and gene ontology (GO) databases. DESeq was then used to analyze and calculate the difference in gene expression.

The sequencing results of the two cultivars were separately assembled and then used in next stage research, such as fluorescence quantification and gene cloning.

Simulation of fuzi processing

According to the relevant regulations of the China Pharmacopoeia, fuzi cultivar from Liangshan was immersed in biliary water (edible magnesium chloride solution) for 12 h, then cooked with black beans for 1, 2, 3, and 4 h and then peeled and dried.

Detection method for content of three diester aconitines

To prepare the testing solution, we precisely and accurately weighed 2.0 g powder, incubated with concentrated ammonia for 5 min, then added 90% methanol solution, executed ultrasonic extraction at 40°C for 30 min and extracted five times with chloroform (5, 5, 5, 10, and 10 mL). Afterward, we filtered the chloroform layer, washed the residue with 20 mL of chloroform, filtered again, combined the filtrate, evaporated to dryness in a 35°C water bath, and then re-dissolved the filtrate in a 10 mL volumetric flask with methanol. The sample was then adjusted for volume, shaken, and passed through a 0.22-μm microporous membrane. HPLC conditions: Agilent C_{18} cartridge, (5 μm, 4.6 × 250 cm), solvent system: A-triethylamine 0.1%, B-methanol, isocratic 35% A for 35 min, flow rate: 0.6 mL/min; injection volume: 20 μL; 35°C, UV: 235 nm. Six samples were taken from each group of dry roots and three samples were taken from each group of the fuzi flake and the finished medicine. Each sample was measured in parallel three times and the median was taken for subsequent statistical analysis. Robustness studies were performed on all HPLC methods [Table 2S].

Detection method for content of higenamine

To prepare the testing solution, we precisely and accurately weighed 2.0 g powder, infiltrated with concentrated ammonia for 5 min, then added 0.04% hydrochloric acid solution and executed ultrasonic extraction at 40°C for 30 min. Afterward, the sample was filtered, the filtrate was collected, shaken well, and passed through a 0.22 μm microporous membrane. HPLC conditions: Agilent C_{18} cartridge, (5 μm, 4.6 × 250 cm), solvent system: A-hydrochloric acid 0.01%, B-methanol, isocratic 10% A for 30 min, flow rate: 0.6 mL/min; injection volume: 20 μL; 35°C, UV: 230 nm. Six samples were taken from each group of dry roots and three samples were taken from each group of the fuzi flake and the finished medicine, each sample was measured in parallel three times, and the median was taken for subsequent statistical analysis.

Statistical analysis used

We use SPSS, r for data statistics and plotting. In the analysis of data such as content detection and agronomic traits, we used the analysis of variance method for the analysis of the difference between the two sets of data; for the analysis of the differences between the sets of data, we used the least significant difference-*t*-test to determine its significance. All data have more than three sets of biological replicates. In the statistical analysis of RNA-seq, we used the general NGS data processing flow for statistical analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Liangshan cultivar has better agronomic traits than Qingchuan cultivar

By a survey covering most of China's producing areas, we found that Qingchuan cultivar is gradually being replaced by Liangshan cultivar [Table 3s]. We further investigated several agronomic traits of these two species for a comprehensive comparison and the results showed that Liangshan cultivar has better agronomic traits in height, leaves number, roots weight, and so on than Qingchuan cultivar [Figure 1S]. The great advantage of agronomic traits explains the farmers' preference of Liangshan cultivar over Qingchuan cultivar.

Liangshan cultivar is richer in diester aconitine than Qingchuan cultivar

To assess the concentration of alkaloids in the local cultivars, we used HPLC to detect and quantify four alkaloids, aconitine, mesaconitine, hypaconitine, and higenamine, from dried fuzi which were collected from Qingchuan or Liangshan areas [Figure 1]. Of the four cultivars, Liangshan Butuo and Yaantian quan, which are from Liangshan area, contains higher concentration of diester aconitine than the other two cultivars which are from Qingchuan area and similar results were obtained from cultivation experiments conducted at the same location [Tables 4S-8S]. This supports the farmers' choice of Liangshan cultivar instead of Qingchuan cultivar if these three kinds of diester aconitine are the major substances causing the medicinal effects. However, at the same time, it is also notable that the concentration of higenamine in Liangshan cultivar is much lower than in Qingchuan cultivar.

Higenamine is more likely to be the major chemical with medicinal effects rather than diester aconitine

We quantified the concentration of the four alkaloids in both half-made medicines and patent medicines. The half-made medicines, fuzi flake, are classified into two types, black and white fuzi flake. The major difference with these two types of fuzi flake is that black fuzi flake is not peeled and is incorporated with black toner during cooking. From our results, the content of aconitine in black fuzi flake (0.009-0.021 mg/g) was generally higher than that in white fuzi flake ($P < 0.05$) and only one batch of white fuzi flake contained aconitine (0.002 mg/g) [Figure 2a]. This suggests that the major biologically active substance which grants the medicinal effect of these half-made medicines might not be diester aconitines. On the other hand, the content of higenamine in fuzi flake, 0.037–0.158 mg/g, was the highest among the four types of alkaloids, which indicates the potential biological activity of higenamine in these medicines [Tables 9S and 10S].

Although higenamine is higher than other alkaloids in concentration, mesaconitine sometimes also appears to be of comparable concentration with higenamine in certain fuzi flakes (black fuzi flake from Yunnan and black fuzi flake from Jiangyou). To further clarify the biological activity of these alkaloids, we mimicked the process of making fuzi flakes into full medicine, by cooking for 1, 2, 3, and 4 h, respectively. The results showed nearly completely loss of aconitine, hypaconitine, and mesaconitine in 3 h of cooking, yet higenamine still remains a considerable amount [Figure 2b]. Given that the medicinal effects of the fuzi flake are retained after cooking into full medicines, we suspect that the major substance which grants fuzi flake the medicinal effects is higenamine rather than aconitine [Tables 11S and 12S].

To further confirm our assumption on higenamine, we quantified the concentration of the four alkaloids in patent medicines derived from different areas. These ten medicines are all capable of benefiting the heart function and improve blood circulation except Xiaojin Pill whose major activity is the elimination of the mass and reduce pain, which is the primary medicinal effect of fuzi. Our results showed that except for Xiaojin Pill, higenamine's concentration was much higher than the other three alkaloids, ranging from 4.411 to 546.078 ng/g [Figure 3 and Tables 13S-15S]. Xiaojin Pill shows a clear difference

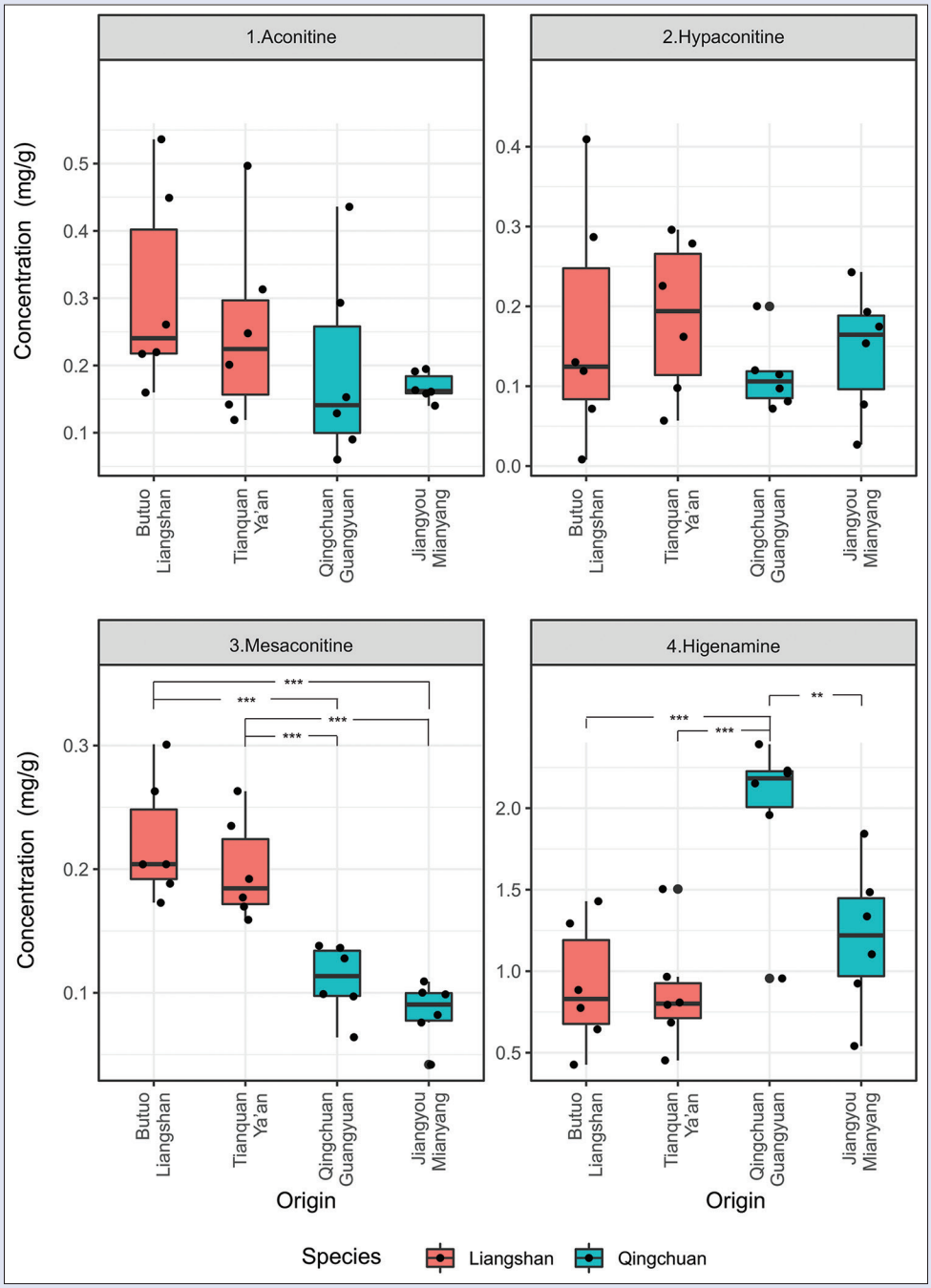

Figure 1: High-performance liquid chromatography results of four alkaloid contents in dried aconite from different producing areas. Red represents the Liangshan cultivar and blue represents the Qingchuan cultivar. The contents of aconitine, hypaconitine, and mesaconitine in Qingchuan cultivar were significantly lower than those in other producing areas, while the content of higenamine was significantly higher than that in Liangshan

from other drugs in principal component analysis analysis [Figure 2S]. Among the fuzi-made medicines, the concentration of the three aconitines is close to higenamine's (median fold change 1.34). However, in fuzi-roasted medicines, the concentration of higenamine is much higher than the sum of the three aconitines, with fold change 3.13 for Jinkuishenqiwan and 6.06 for Zhonghuadiedawan. These evidences also suggest that higenamine might be the primary substance which increase the heart function and improve blood circulation of fuzi in Chinese medicines.

Transcriptomic profiling revealed elevated higenamine biosynthesis pathway in Qingchuan cultivar

The replacement of Qingchuan cultivar by Liangshan cultivar can be risky since our evidences suggest that it might be higenamine that is responsible for the medicinal effect rather than diester aconitines and Liangshan cultivar showed no advantage in higenamine biosynthesis over Qingchuan cultivar. To further investigate the differences of aconitine

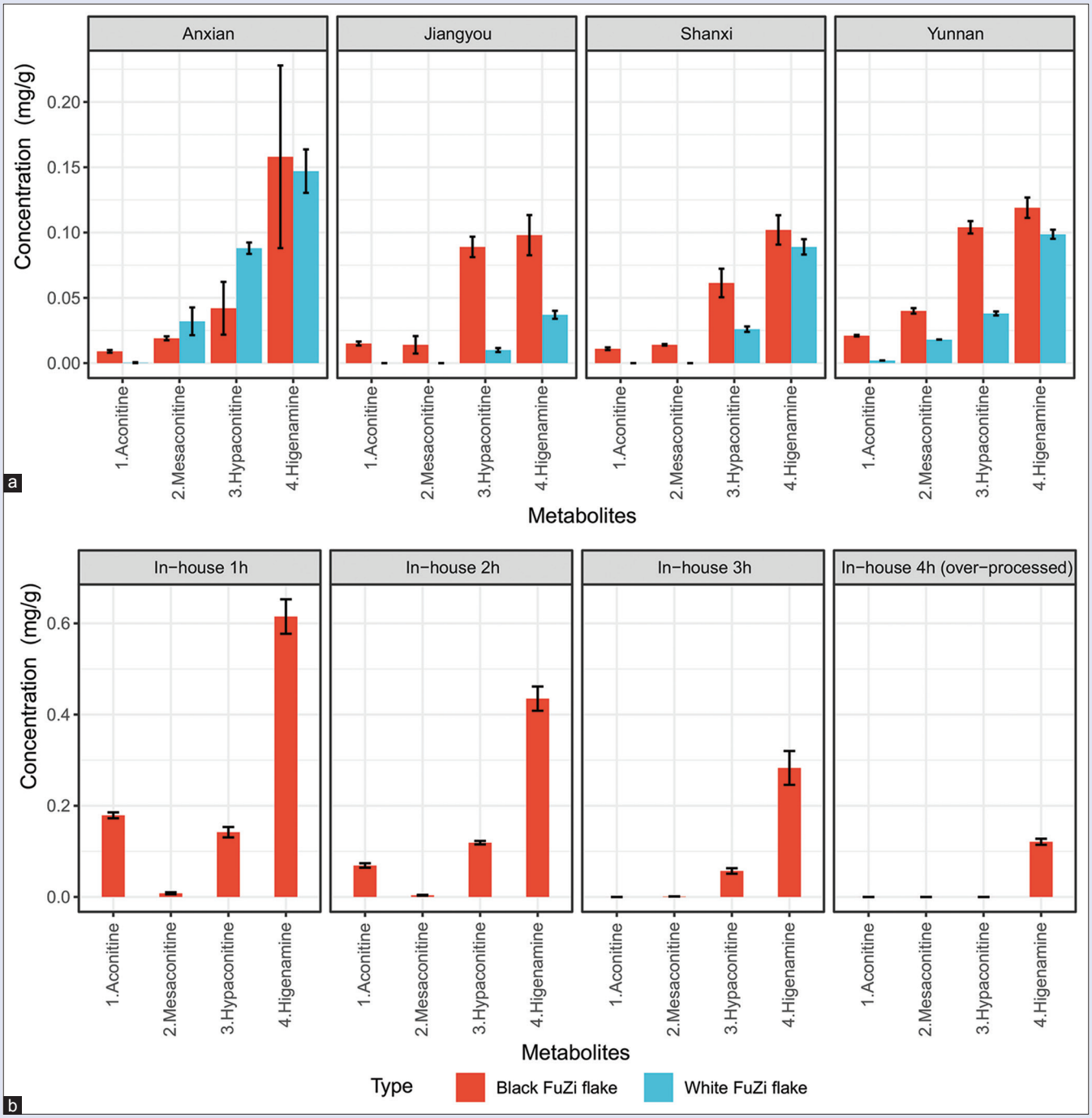

Figure 2: (a) Content of four alkaloids in the fuzi flake of different origins. Red represents the black attached piece and blue represents the White fuzi flake. The figure shows that no matter what the origin of the attachment, the content of the higenamine is significantly higher than the diester aconitine. (b) Dynamic changes in the content of four alkaloids during processing. With the increase of processing time, the degradation rate of three diester alkaloids is faster than that of higenamine and the contents of four alkaloids are significantly different with various processing time

and higenamine biosynthesis in Qingchuan cultivar and Liangshan cultivar, we performed RNA-seq using the roots of the two cultivars in the harvest stage. Through RNA-seq, a total of 311,654 Unigenes for the transcriptome assembly of the two cultivars were characterized by gene function, including: Nr (47.66%), KOG/COG (9.94%), KEGG (17.94%), and GO (32.82%), among which 175,338 (56.26%) Unigenes received at least one annotation. The enrichment results of the differential genes on the KEGG pathway mainly accumulate in the tryptophan metabolism, lysine degradation, photosynthesis-antenna proteins, and isoquinoline alkaloid biosynthesis pathways [Table 16S]. We pulled out the annotated genes which are known to be involved in diterpenoid (for aconitine) and isoquinoline (for higenamine) biosynthesis [Figure 2]. The results showed that few genes in diterpenoid biosynthesis has significant changes in Qingchuan versus Liangshan cultivar; however, several genes' expression in isoquinoline biosynthesis has changed significantly. We further mapped the gene expression changes on the aconitine and higenamine biosynthesis pathways and the results showed that higenamine biosynthesis pathway in Qingchuan cultivar has been

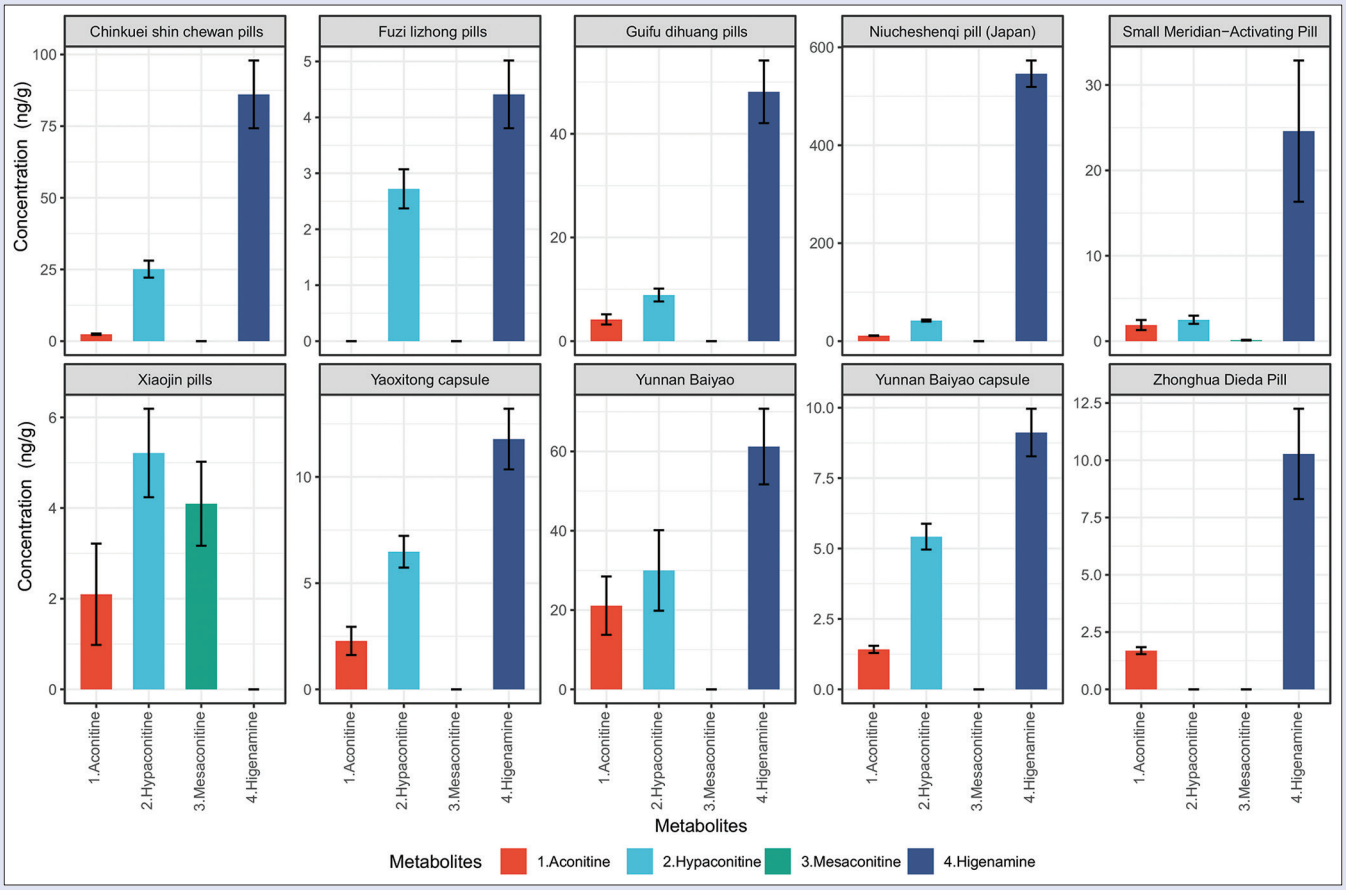

Figure 3: The content of four alkaloids in the finished medicine. Red stands for aconitine, light blue stands for hypaconitine, green stands for mesaconitine, and dark blue stands for higenamine. In Xiaojinwan, higenamine was barely detected, but three diester-type aconitines including mesaconitine were detected. The figure shows that except for Xiaojin Pill, all of the other nine medicines contain higenamine with high content

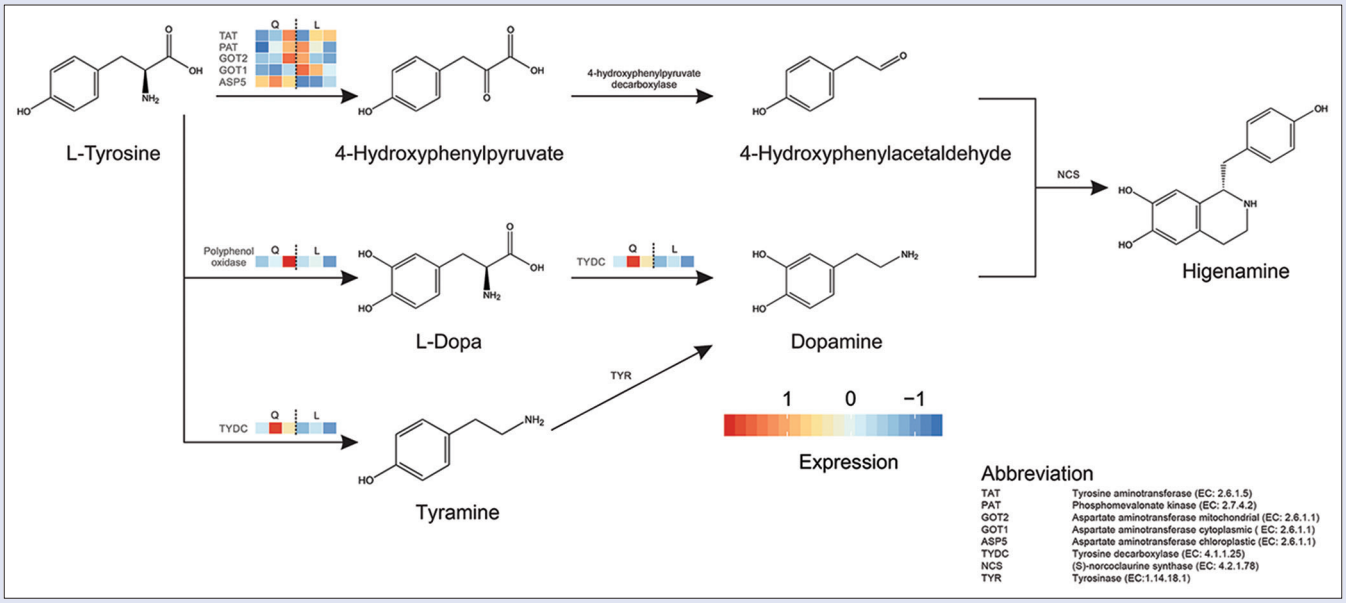

Figure 4: Enrichment results of differentially expressed genes in the higenamine synthesis pathway. Q stands for Qingchuan cultivar, L stands for Liangshan cultivar. Red represents upregulation and blue represents downregulation. Qingchuan cultivars showed significant upregulation of multiple genes in the dopamine synthesis pathway. ASP5 is located downstream of the higenamine and its overall significant upregulation in Qingchuan cultivar may be due to a significant increase in the content of sulfate

elevated compared with Liangshan cultivar [Figure 4]. Our results also suggest that in Qingchuan cultivar, the extra higenamine biosynthesis compared with Liangshan cultivars is due to the higher expression of genes involved in dopamine biosynthesis.

DISCUSSION

Based on the experimental results, we are able to explain the differences between the two cultivars of aconite. Perhaps due to the stronger light conditions and the harsher climate in Liangshan, the pathways of photosynthetic and cell wall synthesis in Liangshan have changed, which makes Liangshan cultivars have higher yield, greater biomass accumulation, and higher growth rate. This is also the main reason why Liangshan cultivars are being used by farmers to replace Qingchuan cultivars.

Liangshan cultivars are more toxic than Qingchuan but may be less medicinal. The concentration of the three diester-type alkaloids indicates that Liangshan cultivars generally accumulate more toxic components than Qingchuan cultivars, which may lead to new drug safety problems.

In fact, even if drug manufacturers perform sampling tests during the production process, limited by the sampling density, the slight fluctuations in the normal processing process may still be neglected and the high toxicity of the diester aconitine may magnify it into a major drug safety problem. In addition, in many parts of East Asia, simply processed aconite is still used as a regular drug. This can lead to even worse consequences due to the lack of further processing. Even in places like Yunnan and other places in China, as a means of folk food supplements, local villagers use australis without any processing of cooking to improve the circulatory system and to protect against the cold winter. Although there is no detailed data for correlation analysis to determine whether it is related to germplasm replacement, in recent years, there have been many reports of eating aconite by traditional means which led to poisoning.

Compared with Qingchuan cultivars, Liangshan cultivars showed a downward trend in multiple genes on the isoquinoline synthesis pathway. Combined with our HPLC data, it is known that its norepinephrine content is low. Considering our detection of finished drugs and processed products, higenamine most likely is the material basis of the real aconite pharmacological activity.

As the most controversial ingredient in aconite, aconitine has extremely strong activity and toxicity. When applied externally, it can stimulate the nerve endings of local skin mucosa and cause temporarily loss of local sensation, which is also the reason for the high content of diester alkaloids in topical Yunnan Baiyao. When Yunnan Baiyao is applied externally, the diester alkaloids can exert its local anesthetic effect on the injured part and reduce the pain;[19] when taken orally, a safe dose of diester alkaloids can excite the vagus nerve to bring a systemic mild anesthetic effect and relieve pain. Diester alkaloids have been added to Chinese patent medicines, such as Xiaohuoluo Pill and Yaoxitong Capsule, for the same reason. However, when the dosage is too large, the excess diester alkaloid may cause symptoms such as numbness of the tongue, loss of feeling in the limbs, and disturbance of heart rhythm. The extremely low concentration of aconitine in commercially available Chinese patent medicines can actually protect the cardiomyocytes and lower blood pressure to a certain extent.

The ubiquitously present higenamine is a low-toxic,[20] water-soluble alkaloid that can act on the cardiac system. It is a type of β-adrenergic receptor agonist and its effect is mainly distributed in the skeletal muscle, the vascular smooth muscle of the liver and the heart in the human body due to β-adrenergic receptors.[21] Therefore, higenamine can effectively increase the heart rate by stimulating β-adrenergic receptors, increase cardiac output, inhibit thrombosis, and at the same time, shrink blood vessels to some extent, and increase hypertension. Although the monoester alkaloids (benzoylaconitine, benzoylmesaconine, etc.) formed by the degradation of diester alkaloids during usage of aconite also has a certain activity, the basis of cardiac function of aconite is higenamine. Long-term usage of higenamine can cause activity similar to aconite, and this activity is exclusively cardiac. In addition, other fuzi-based and aconite-based Chinese patent medicines, such as Jinguishenqi Pill and Guifulizhong Pill, have the effect of "treating impotence." It has been proved that higenamine has an upregulating effect on the β-ajdrenergic receptor cAMP system, which is helpful for the treatment of impotence; meanwhile, because higenamine is a β-adrenergic receptor agonist, it can only stimulate β-adrenergic receptors and since there is no β-adrenergic receptor in male genitalia, when taking higenamine (or Jinqishenqi Pill, Niucheshenqi Pill, etc.), the blood supply capacity of the heart is enhanced, some blood vessels in the human body are contracted, blood pressure is elevated, and organs without β-adrenergic receptors will be congested by vasodilatation, which, with the anesthetic effect of a small amount of diester alkaloids, can effectively improve erectile dysfunction.

The concentration of diester alkaloids (aconitine, hypaconitine, and mesaconitine) and higenamine in aconites varies in different cultivars, different fuzi flakes and different Chinese patent medicines. The contradiction caused by "toxicity-effect" in aconite makes it very crucial to establish a reasonable "monarch" and "regent" relationship in the process of concocting and medicating.[22] The content of each component in aconite directly determines the effectiveness and safety of its later use. Therefore, the merits of cultivars cannot be evaluated only by yield. Under the trend of economic benefits in recent years, people's blind introduction has destroyed the harmonious unity of traditional medicine sources-climate-cultivation mode-processing technology that has gradually formed over a long historical period, and it is very likely to cause new medication safety issues.

As a typical example of rapid germplasm replacement, research on aconite has just started. As one of the world's leading exporters of aconite, the rapid evolution of China's producing areas may also affect the world. After all, it is challenging to expect growers to return to low-yield and susceptible Qingchuan cultivars, and no scientific researcher can cause widespread change independently. Perhaps, based on the key differential genes in Liangshan and Qingchuan species, a promising direction for future research would be to design and breed new safe, effective, and high-yielding crop varieties to replace Liangshan species again.

CONCLUSION

Taken together, our results suggest that the risk of replacing Qingchuan cultivars by Liangshan cultivars has been underestimated and the influences on the related medicines should be closely investigated.

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Conflicts of interest

There are no conflicts of interest.

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