**Original Research** 

# **Comparative Study on The Quality of Chuanwu from Four Representative Areas of Production**

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# Abstract

Objective: There are many differences among Chuanwu from different producing areas. Therefore, it is extremely urgent to make a comparative analysis of the quality of Chuanwu from different producing areas. It will provide a theoretical reference for the formulation of relevant quality standards for Chuanwu. Methodology: 48 batches of Chuanwu samples were collected from Sichuan Province, Shaanxi Province, and Yunnan Province, and the environmental investigation was carried out in the main production areas. Results: There are significant differences in the quality of Chuanwu among the four producing areas. The content and proportion of neoline and zongorine are different, which can effectively identify Chuanwu from different producing areas. Conclusion: The appearance characteristics and alkaloid content of Chuanwu from different producing areas are closely related to climatic conditions and planting management. In this study, the quality identification index of Chuanwu from each producing area was preliminarily established.

Keywords: Chuanwu, areas of production, quality evaluation, characteristic index, HPLC-ELSD

# Introduction

Chuanwu, the dry mother root of Aconitum carmichaelii Debx., which is mainly cultivated in Sichuan Province, Shaanxi Province, Yunnan Province, and some other places, has the efficacy of treating various rheumatic diseases and relieving pain as an anesthetic. In recent years, the output of the authentic production areas in Jiangyou City in Sichuan Province has decreased, while the output of other major producing areas has increased [1]. The mixed trading from different producing areas resulted in the unstable production quality of Chuanwu in the market. In order to standardize the various local markets, it is necessary to make a clear distinction among Chuanwu from different producing areas.

Alkaloids are the main active substances in Chuanwu, including diester alkaloids, monoester alkaloids, and water-soluble alkaloids [2]. Diester alkaloids, such as aconitine, hypaconitine, and mesaconitine (Fig. 1.), are

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Fig. 1. Structures of six representative alkaloids of Chuanwu.

the main toxic substances in Chuanwu, whose toxicity mainly affects the nervous system, muscle tissue, and heart [3]. Water-soluble alkaloids, such as neoline, fuziline, and zongorine (Fig. 1.), are also important active components of aconite, which have the effects of cardiotonic, antiarrhythmic, anti-inflammatory, and analgesic [4]. High performance liquid chromatography is an efficient and accurate method for the determination of alkaloids [5]. Solid phase microextraction combined with high performance liquid chromatography (SPE-HPLC) was used to determine the 6 alkaloids in Chuanwu preparation products [6]. The content of 6 alkaloids in processed aconite was determined by HPLC [7]. It was found that the content of monoester alkaloids increased significantly, and the content of diester alkaloids disappeared or decreased significantly. The UPLC/Q-TOF-MS/MS method was used by Ye [8] to analyze the chemical composition differences of raw and processed products of Chuanwu. It was found that different heating methods and accessories would affect the chemical composition of Chuanwu.

Due to the different ecological environments and cultivation conditions, the morphological habits of the original plants, the shape and structure, and the active components of traditional Chinese medicine may change. Ma [9] used HPLC to determine the contents of 6 alkaloids in 12 batches of commercially available Chuanwu. It was found that the contents of 6 alkaloids in 12 batches of samples were quite different. He speculated that this may be related to origin, harvest time, genetic basis, growth climate, and ecological environment. Luo [10] simultaneously determined the contents of five main alkaloids in the Fuzi water-soluble alkaloid extract and corresponding steamed aconite tablets by HPLC-ELSD. Subsequently, Luo [11] used the same method to determine the contents of three water-soluble alkaloids in Fuzi from different producing areas and found that the content of neoline can be used as an important evaluation index of Fuzi. Alkaloid content is one of the important indices in the quality evaluation of Chuanwu. In this study, HPLC-ELSD was applied to determine the contents of six representative alkaloids in Chuanwu from different producing areas, which were fuziline, neoline, zongorine, mesaconitine, hypaconitine, and aconitine. The characteristic indexes of different productions were explored. It will provide a theoretical basis for the quality evaluation of Chuanwu.

# Experimental

#### **Experimental Materials**

From 2019 to 2020, 48 batches of Chuanwu samples were collected from Sichuan Province, Shaanxi Province, and Yunnan Province. (Table 1), they were identified as the dry mother root of Aconitum carmichaelii Debx. of Ranunculaceae by the researcher Yanli Xia of Chengdu University.

#### Instruments

The Agilent 1100 high performance liquid chromatograph, Agilent 1260 Infinity evaporative light scattering detector (Agilent Technology Co., Ltd., Chengdu, China), SB25-12YDTD ultrasonic cleaner (Xinzhi Biotechnology Co., Ltd., Ningbo,

NO.	Producing area
JY-Z01, JY-Z02, JY-Z03, JY-Z04, JY-Z05, JY-Z06, JY-Z07, JY-Z08, JY-Z09, JY-Z10, JY-Z11, JY-Z12	Jiangyou City, Sichuan Province
CG-Z01, CG-Z02, CG-Z03, CG-Z04, CG-Z05, CG-Z06 , NZ-Z01, NZ-Z02, NZ-Z03, NZ-Z04, NZ-Z05, NZ-Z06	Chengdu County and Nan Zheng County, Shaanxi Province
BT-Z01, BT-Z02, BT-Z03, BT-Z04, BT-Z05, BT-Z06, BT-Z07, BT-Z08, BT-Z09, BT-Z10, BT-Z11, BT-Z12	Butuo County, Sichuan Province
YZ-Z01, YZ-Z02, YZ-Z03, YZ-Z04, YZ-Z05, YZ-Z06, BC-Z01, BC-Z02, BC-Z03, BC-Z04, BC-Z05, BC-Z06	Yun long County and Binchuan County, Yunnan Province

Table 1. Chuanwu Sample Collection.

China), Tgl-1650 bench type refrigerated centrifuge (Shuke Instrument Co., Ltd., Sichuan, China), and WP-UFT-20 ultrapure water meter (Woter Water Treatment Equipment Co., Ltd., Sichuan, China) were provided by Chengdu University.

#### Reagents

Mesaconitine (MUST-17041010), aconitine (MUST-17022206), hypaconitine (MUST-17041011), neoline (MUST-17102710), zongorine (DST180116-073), and fuziline (DST171130-013) were purchased from Chengdu Dester Biotechnology Co., Ltd. (Chengdu, China). Chromatographic grade acetonitrile and methanol were purchased from Fisher Scientific International Inc. (Shanghai, China). Chromatographic grade triethylamine was purchased from the Kelong Chemical Reagent Factory (Chengdu, China).

# **Experimental Methods**

#### Investigation of the Main Producing Areas of Chuanwu

Researchers conducted field investigations in Sichuan Province, Shaanxi Province, and Yunnan Province, collected samples, and consulted literature. The climate, altitude, soil, harvest time, and cultivation management methods were investigated and recorded.

# Investigation of the Appearance Properties of Chuanwu

The appearance characteristics of Chuanwu such as shape, surface color, texture, section color, hollowness, and shrinkage, were investigated and identified.

#### Determination of Alkaloids in Chuanwu

1. Determination of Aconitine, Mesaconitine, and Hypaconitine

(1) Preparation of reference substance solution: The appropriate amount of each reference substance was weighed accurately. A 50% methanol solution was added to prepare a mixed reference stock solution containing 20 mg aconitine, 20 mg mesaconitine, and 20 mg hypaconitine per 1 mL. (2) Preparation of the test solution: About 3.0 g of Chuanwu medicinal powder was weighed and dissolved in a conical flask with a stopper containing 75 mL 0.05 mol/L hydrochloric acid-70% acidic methanol solution. The mixture was ultrasonically treated for 30 min at a power of 250 W and a frequency of 40 kHz, and then centrifuged at 8000 r·min-1 for 6 min after cooling. 50 mL supernatant was recovered to about  $1\sim2$  mL under reduced pressure at 60°C. The recovery solution was then fixed to the scale in a 5 mL measuring bottle and filtered by a 0.45 µm filter membrane.

(3) Chromatographic conditions: Chromatographic column: Agilent Extend C18 column (250 mm×4.6 mm, 5µm). Mobile phase: (A) acetonitrile/tetrahydrofuran (v/v 25:15), (B) 0.1 mol/ L ammonium acetate solution (0.5 mL glacial acetic acid per 1000 mL). Gradient elution:  $0{\sim}48$  min,  $15\%{\rightarrow}26\%$  A.  $48{\sim}49$  min,  $26\%{\rightarrow}35\%$  A.  $49{\sim}58$  min, 35% A.  $48{\sim}49$  min,  $35\%{\rightarrow}15\%$  A. Detection wavelength: 235 nm. Flow rate:1.0 mL·min-1. Column temperature:  $35^{\circ}$ C. Detector: Diode array detector. Injection volume: 10 µL.

2. Determination of Fuziline, Neoline, and Zongorine

(1) Preparation of reference substance solution: The appropriate amount of each reference substance was weighed accurately. A 50% methanol solution was added to prepare a mixed reference stock solution containing 0.25 mg of neoline, 0.15 mg of fuziline, and 0.15 mg of zongorine per 1 mL.

(2) Preparation of test solution: The same as that of 1.

(3) Chromatographic conditions: Chromatographic column: Agilent Extend C18 column (250 mm×4.6 mm, 5 µm). Mobile phase: (A) acetonitrile, (B) 0.1% triethylamine. Gradient elution: 0~8 min,10% $\rightarrow$ 20% A. 8~15 min, 20% $\rightarrow$ 25% A. 15~25 min, 25% $\rightarrow$ 25% A. 25~30 min, 25% $\rightarrow$ 28% A. 30~35 min, 28% $\rightarrow$ 45% A. 3~42 min, 45% $\rightarrow$ 50% A. 42~48 min, 50% $\rightarrow$ 65% A. 48~60 min, 65% $\rightarrow$ 85% A. 60~70 min, 85% $\rightarrow$ 85% A. Flow rate: 1.0 mL·min-1. Column temperature: 35°C. Detector: Evaporative light scattering detector. Carrier gas: Air. An evaporation temperature: 50°C. Carrier gas flow rate: 1.3 L·min-1. Gain: 1. Injection volume: 20 µL.

# **Results and Discussion**

Investigations in Main Producing Areas

# Environmental Factors of Different Producing Areas

It can be seen from Table 2 that, compared with the other two provinces, Jiangyou City in Sichuan Province, Chengdu County, and Nan Zheng County in Shaanxi Province have a lower altitude, less sunshine, and a longer frost-free period. Located in Yunnan Guizhou Plateau, Chuanwu producing areas in Butuo County in Sichuan Province, Yun long County, and Binchuan County in Yunnan Province have the characteristics of a higher altitude, stronger light, and a shorter frost-free period.

#### Planting Mode, Field Management, and Harvest Time in Different Producing Areas

It can be seen from Table 3 that Jiangyou City in Sichuan Province, Chengdu County, and Nan Zheng County in Shaanxi Province are generally intercropped with corn and rotated with rice. The field management of removing redundant lateral roots and terminal buds was carried out. The harvest time is earlier, from late June to early August. Buckwheat is widely planted in rotation in Butuo County in Sichuan Province, Yun long County, and Binchuan County in Yunnan Province, with rough field management and a later harvest time from mid-September to early November. It is not difficult to find that with the increase in altitude, the harvest time of Chuanwu in different producing areas is delayed. The harvesting time of Chuanwu in Jiangyou City in Sichuan Province, Chengdu County, and Nan Zheng County in Shaanxi Province meets the provisions of Chinese Pharmacopoeia 2020.

Analysis of the Appearance and Properties of Chuanwu

See Fig. 2, Fig. 3, Fig. 4, Fig. 5, and Table 4 for the investigation results of the appearance and shape of Chuanwu. The samples of Jiangyou have shrunk slightly, with a brown surface, a white section, and a firm and full texture. The samples of Shaanxi have serious shrinkage, a grayish brown to tan surface, a yellow section, a medium texture, and a slightly full texture. The samples of Butuo and Yunnan have serious shrinkage, a light weight, and obvious hollowness.

# Analysis of the Content Determination Results of Three Diester Alkaloids

The chromatograms of three diester alkaloids are shown in Fig. 6. The determination results are shown in Table 5.

It can be found that the content distributions of the three alkaloids vary from region to region.

The average content of three diester alkaloids in Chuanwu from Jiangyou is as high as 0.1015%, among which the content of aconitine is as high as 0.0520%, accounting for 50.73% of the three diester alkaloids.

Producing area	Altitude (m)	Annual sunshine time (h)	Annual precipitation (mm)	frost-free period (d)	Annual average temperature (°C)
Jiangyou City, Sichuan Province	450-600	1367	1100	280	16.2
Chengdu County and Nan Zheng County, Shaanxi Province	470-900	1532	554	240	14.5
Butuo County, Sichuan Province	2700-2800	1996	1115	201	17.3
Yun long County and Binchuan County, Yunnan Province	1700-3000	2000-2700	500-900	110-200	12-17

Table 2. Comparison of meteorological factors in different producing areas.

Table 3. Comparison of planting mode, field management, and harvest time of Chuanwu in different producing areas.

Draduaina anaa	Diantin a nottam	Field	Harve	est time
Producing area	Planting pattern	management	Date	Solar term
Jiangyou City, Sichuan Province	Intercropping with corn, rotating with rice	Very meticulous	Late June to early July	Summer solstice to heatstroke
Chengdu County and Nan Zheng County, Shaanxi Province	Intercropping with corn, rotating with rice	General meticulous	Late July to early August	Great heat to beginning of autumn
Butuo County, Sichuan Province	Rotating with buckwheat	Rough	Mid-September to early October	After the autumnal equinox
Yun long County and Binchuan County, Yunnan Province	Rotating with buckwheat	Rough	Late September to early November	After the autumnal equinox

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Produce Area	Appearance	Surface	Section	Texture
Jiangyou City, Sichuan Province	Slightly wrinkled	Dark brown	White	Solid and full
Chengdu County and Nan Zheng County, Shaanxi Province	Severely wrinkled	Grayish brown to dark brown	Yellow	Slightly solid and full
Butuo County, Sichuan Province	Severely wrinkled	Grayish brown to dark brown	Yellow	Light and obviously hollow
Yun long County and Binchuan County, Yunnan Province	Severely wrinkled	Grayish brown to dark brown	Yellow	Light and obviously hollow

Table 4. Appearance and shape of Chuanwu from different producing areas.



Fig. 2. Chuanwu (surface and section), Jiangyou City in Sichuan Province.



Fig. 3. Chuanwu (surface and section), Shaanxi Province.



Fig. 4. Chuanwu (surface and section), Butuo Country in Sichuan Province.



Fig. 5. Chuanwu (surface and section), Yunnan Province.

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Produce area	AC (%)	MAC (%)	HAC (%)	DA (%)	AC/DA (%)	MAC/DA (%)	HAC/DA (%)
Jiangyou City, Sichuan Province	0.0520 a	0.0330 b	0.0169 a	0.1015 a	0.5073 a	0.3604 c	0.1323 bc
Chengdu County and Nan Zheng County, Shaanxi Province	0.0472 a	0.0310 b	0.0161 a	0.0942 a	0.4953 a	0.3293 cd	0.1754 b
Butuo County, Sichuan Province	0.0034 b	0.0187 c	0.0073 ab	0.0294 c	0.1394 b	0.5462 b	0.3144 a
Yun long County and Binchuan County, Yunnan Province	0.0169 b	0.0496 a	0.0029 bc	0.0694 b	0.2262 b	0.7331 a	0.0407 d

Table 5. Determination results of three diester alkaloids in Chuanwu from different producing areas.

\*AC is the content ratio of Aconitine/total alkaloids. MAC is the content ratio of Mesaconitine/total alkaloids. HAC is the content ratio of Hypaconitine/total alkaloids. DA is the content ratio of three diester alkaloids/total alkaloids. AC/DA is the content ratio of Aconitine/three diester alkaloids. MAC/DA is the content ratio of Mesaconitine/total alkaloids. HAC/DA is the content ratio of Hypaconitine/total alkaloids. \*\*p<0.05

The proportions of aconitine, mesaconitine, and hypaconitine were 50.73%, 36.04%, and 13.23%, respectively.

The average content of the three diester alkaloids in Chuanwu from Shaanxi is as high as 0.0942%, among which the content of aconitine is as high as 0.0472%, accounting for 49.53% of the content of the three diester alkaloids. The proportions of aconitine, mesaconitine, and hypaconitine were 49.53%, 32.93%, and 17.54%, respectively. The distribution of alkaloids in Shaanxi is similar to that in Jiangyou City.

The sum of the three diester alkaloids in Butuo is the lowest, with an average value of only 0.0294%. The content of aconitine and mesaconitine was the lowest among the four producing areas. However, the ratio of the content of hypaconitine to the three diester alkaloids was as high as 31.44%, which was the highest among the four producing areas. The proportions of aconitine, mesaconitine, and hypaconitine were 13.94%, 54.62%, and 31.44%, respectively.

The total content of the three diester alkaloids in Yunnan was also low, with an average value of only 0.0694%, which was significantly lower than that in Jiangyou and Shaanxi. However, the content of mesaconitine was as high as 0.0496%, accounting for 73.31% of the three diester alkaloids, which was significantly highest among the four producing areas. The content of hypaconitine was only 0.0029%, accounting for 4.07% of the three diester alkaloids, which was significantly lower than that of other producing areas. The proportions of aconitine, mesaconitine, and hypaconitine were 22.62%, 73.31%, and 4.07%, respectively.

# Analysis of Content Determination Results of Three Water-Soluble Alkaloids

The chromatograms of three water-soluble alkaloids are shown in Fig. 7. The determination results are shown in Table 6.

It can be seen from Table 6 that the distribution content of the three water-soluble alkaloids in Chuanwu from different producing areas is obviously different. The content of the three water-soluble alkaloids in



Fig. 6. a) Reference Standards, b) Jiangyou City, Sichuan Province, c) Shaanxi Province, d) unnan Province, e) Butuo Country, Sichuan Province.

Chuanwu from Jiangyou City in Sichuan Province was as high as 0.1023%, which was the highest among the four producing areas. The content of fuziline was as high as 0.0145%, which was significantly higher than that of Butuo and Yunnan, accounting for 14.00% of the three water-soluble alkaloids. The content of neoline was as high as 0.0467%, which was the highest among the four producing areas, accounting for 45.50% of the three water-soluble alkaloids. The content of zongorine was as high as 0.0411%, accounting for 40.50% of the three water-soluble alkaloids. The ratio of the total content of the three water-soluble alkaloids to the total content of the three water-soluble alkaloids is about 1.2.

As for Chuanwu from Shaanxi Province, the sum of the three water-soluble alkaloids was as high as 0.0739%, which was significantly higher than that in Butuo and Yunnan. The content of fuziline was as high as 0.0146%, which was obviously higher than that in Butuo and Yunnan, accounting for 15.44% of the three water-soluble alkaloids. The content of neoline was as high as 0.0283%, which was significantly higher than that of Butuo and Yunnan, accounting for 37.40% of the three water-soluble alkaloids. The content of zongorine was 0.0309%, accounting for 47.17% of the three water-soluble alkaloids. The ratio of the total content of the three water-soluble alkaloids.

Produce area	FL (%)	NL (%)	ZR (%)	WA (%)	FL/WA (%)	NL/WA (%)	ZR/WA (%)	WA/DA (%)
Jiangyou City, Sichuan Province	0.0145 a	0.0467 a	0.0411 a	0.1023 a	0.14	0.4550 a	0.4050 c	1.1956 b
Chengdu County and Nan Zheng County, Shaanxi Province	0.0146 a	0.0283 b	0.0309 b	0.0739 b	0.1544	0.3740 b	0.4717 c	0.8280 bc
Butuo County, Sichuan Province	0.0054 b	0.0039 c	0.0399 a	0.0492 c	0.1088	0.0770 c	0.8143 a	2.0680 a
Yun long County and Binchuan County, Yunnan Province	0.007 ab	0.0063 c	0.0331 b	0.0463 c	0.1495	0.1287 c	0.7218 b	0.7105 c

Table 6. Determination results of three water-soluble alkaloids in Chuanwu from different producing areas.

\*FL is the content ratio of fuziline/total alkaloids. NL is the content ratio of neoline/total alkaloids. ZR is the content ratio of zongorine/total alkaloids. WA is the content ratio of three water-soluble alkaloids/total alkaloids. FL/WA is the content ratio of fuziline/three water-soluble alkaloids. NL/WA is the content ratio of neoline/three water-soluble alkaloids. ZR/WA is the content ratio of zongorine/three water-soluble alkaloids. WA/DA is the content ratio of three water-soluble alkaloids/three diester alkaloids. \*\*p<0.05

to the total content of the three diester alkaloids is about 0.83.

However, the sum of the three water-soluble alkaloids in Chuanwu from Butuo Country in Sichuan Province was only 0.0492%, which is significantly lower than that in Jiangyou and Shaanxi. The content of fuziline was only 0.0054%, which was significantly lower than that of Jiangyou and Shaanxi, accounting for 10.88% of the three water-soluble alkaloids. The content of neoline was only 0.0039%, which was significantly lower than that of Jiangyou and Shaanxi, accounting for only 7.70% of the three water-soluble alkaloids. The content of zongorine was 0.0399%, accounting for 81.43% of the three watersoluble alkaloids. The ratio of the total content of the three water-soluble alkaloids to the total content of the three diester alkaloids was about 2.07.

As for Chuanwu from Yunnan Province, the sum of the three water-soluble alkaloids was the lowest, which was only 0.0463% and significantly lower than that in Jiangyou and Shaanxi. The content of fuziline was only 0.0070%, accounting for 14.95% of the three water-soluble alkaloids. The content of neoline was only 0.0063%, which was significantly lower than that of Jiangyou and Shaanxi, accounting for only 12.87% of the three water-soluble alkaloids. The content of zongorine was 0.0331%, accounting for 72.18% of the three water-soluble alkaloids. The ratio of the total content of the three water-soluble alkaloids to the total content of the three diester alkaloids is about 0.71.

By comparing the four producing areas, it can be found that the contents of the three water-soluble alkaloids, fuziline, and neoline, from Jiangyou and Shaanxi are significantly higher than those from Butuo and Yunnan. Particularly, the content of neoline in Jiangyou was significantly higher than that in the other three producing areas, with an average of 0.0467% accounting for about 45% of the three water-soluble alkaloids. It was about 1.65 times the average value of Shaanxi, 11 times that of Butuo, and 7 times that of Yunnan. Therefore, the content of neoline can be used as a characteristic difference component to distinguish Jiangyou from other producing areas.

> Analysis of Characteristic Indexes and Establishment of Quality Standards for Chuanwu from Four Producing Areas

Combined with the analysis of the content determination results of three diester alkaloids and three water-soluble alkaloids, the contents of three diester alkaloids and three water-soluble alkaloids in the four main producing areas were comprehensively analyzed, and the different components in each producing area were screened out. Based on the average value of each production area, the key characteristic indexes of each production area were selected, respectively, and then combined with the original sample data. Based on the data range that each sample can reach, the quality standards of the characteristic indexes of Chuanwu in four producing areas were established. The results are shown in Table 7.

# **Results and Discussion**

# Screaming of Characteristic Indexes and Cause Analysis of Chuanwu in Jiangyou City, Sichuan Province

Due to its low altitude, Jiangyou City in Sichuan Province has an annual frost-free period of nearly 280 days and abundant rainfall throughout the year. Due to such climatic conditions and man-made management, the surface of the local Chuanwu is brown, the cross section is white, and the texture is solid and full, which is obviously different from other producing areas. Through



Fig. 7. a) Reference Standards, b) Jiangyou City, Sichuan Province, c) Shaanxi Province, d) Yunnan Province, e) Butuo Country, Sichuan Province.

comprehensive evaluation, the characteristic index of Jiangyou Chuanwu was preliminarily established. The content of neoline is greater than 0.02%. The sum of the three water-soluble alkaloids is greater than 0.05%. The content of neoline accounted for more than 35% of the sum of three water-soluble alkaloids. The content of zongorine accounted for less than 50% of the sum of the three water-soluble alkaloids. The ratio of the sum of three water-soluble alkaloids to the sum of three diester alkaloids was greater than 0.6. Chuanwu which meets all the above conditions can be identified as being produced in Jiangyou City, Sichuan Province.

# Screaming of Characteristic Indexes and Cause Analysis of Chuanwu in Shaanxi Province

Hanzhong City in Shaanxi Province has a low altitude and an annual frost-free period of nearly 240 days. The field management there is relatively rough. Due to such climatic conditions and man-made management, the surface of the local Chuanwu is grayish brown to tan, the cross section is yellow, and the texture is medium and slightly plump. Through comprehensive quality evaluation, the Chuanwu from Hanzhong can be identified and distinguished by the

[able 7. Summary of Characteristic Differences i	in Each Producing Area.								
Producing area	Appearance	Texture	AC/DA (%)	MAC/DA (%)	HAC/DA (%)	FL (%)	(%) NL	NL/WA (%)	ZR/WA (%)
Jiangyou City, Sichuan Province	Slightly wrinkled	Solid and full					>0.02	>35	<50
Chengdu County and Nan Zheng County, Shaanxi Province	Severely wrinkled	Slightly solid and full					>0.01	>20	≤70
Butuo County, Sichuan Province	Severely wrinkled	Light and obviously hollow	<30		>10	<0.01	<0.01	<20	>70
Yun long County and Binchuan County, Yunnan Province	Severely wrinkled	Light and obviously hollow		>45	<10	<0.01		<30	>50
*AC/DC is the content ratio of aconitine/tf hypaconitine/three diester alkaloids. FL is a soluble alkaloids/total alkaloids. NL/WA is	rree diester alkaloids. N the content ratio of fuzi the content ratio of ne	AAC/DA is the content ratio of line/total alkaloids. NL is the coline/three water-soluble alkal	mesaconiti content ratic oids. ZR/W	ne/three diest of neoline/to A is the conte	er alkaloids. Ital alkaloids. Int ratio of zon	HAC/DA WA is th ngorine/tl	is the content in content hree wate	ntent ratio of t ratio of three r-soluble alka	: water- lloids.

WA/DA is the content ratio of three water-soluble alkaloids/three diester alkaloids

Yan-Li Xia, et al.

following indicators: The content of neoline was greater than 0.01%, accounting for more than 20% of the total content of the three water-soluble alkaloids. However, the content of zongorine accounted for less than 70% of the sum of the three water-soluble alkaloids. The Chuanwu that met all the above indicators could be identified as those that were produced in Hanzhong City, Shaanxi Province.

# Screaming of Characteristic Indexes and Cause Analysis of Chuanwu in Butuo Country, Sichuan Province

Butuo Country in Sichuan Province has high altitude as well as strong sunshine, and the frostfree period is 200 days throughout the year. The field management there is rather rough. Due to such climatic conditions and man-made management, the local Chuanwu shrinks seriously, with a gravish brown to tan surface, a yellow section, a light texture, and an obvious hollow. The local ebony shrank seriously, with a grayish brown to yellowish brown surface, a yellow section, a light texture, and an obvious hollowness. Through comprehensive quality evaluation, Chuanwu from Butuo can be distinguished by the following indicators: The content of aconitine accounted for less than 30% of the total of the three diester alkaloids. The contents of fuziline and neoline were both less than 0.01%. The contents of neoline and zongorine accounted for less than 20% and 70% of the sum of the three water-soluble alkaloids, respectively. The content ratio of the three water-soluble alkaloids to the three diester alkaloids was greater than 0.8. The Chuanwu that met all the above indicators can be identified as Chuanwu produced in Butuo Country, Sichuan Province.

# Screaming of Characteristic Indexes and Cause Analysis of Chuanwu in Lijiang City, Yunnan Province

The climatic conditions in Lijiang City, Yunnan Province, are similar to those in Butuo County, Sichuan Province, with an annual frost-free period of about 100-200 days. On-site management is also relatively rough. Due to such climatic conditions and manmade management, Chuanwu from this produce area shrank seriously, with a grayish brown to tan surface, a yellow section, a light texture, and an obvious hollow. The following indicators can be used to distinguish Chuanwu from Yunnan: The content of mesaconitine accounted for more than 45% of the sum of the three diester alkaloids. The content of hypaconitine was less than 10% of the sum of the three diester alkaloids. The content of fuziline was less than 0.01%. The content of neoline was less than 30% of the sum of the three water-soluble alkaloids. The content of zongorine accounted for more than 50% of the total content of three water-soluble alkaloids. The Chuanwu produced in

accordance with all the above indexes could be identified as Chuanwu produced in Yunnan Province.

# Reason Analysis of Quality Formation of Chuanwu from Different Producing Areas

There were few studies on the quality of Chuanwu from different producing areas, but there were some studies on the quality differences of Fuzi from different producing areas. Luo [12] collected 42 batches of Fuzi samples from four producing areas. It was reported that the geographical origin might be the main factor affecting the geographical authenticity of Fuzi. Neoline might be the key component to identifying the geographical authenticity of Fuzi. Yue Conghui [13] analyzed and evaluated Fuzi in different cultivation areas and compared the cultivation system, yield, alkaloid content, polysaccharide, and protein content of Fuzi cultivated in Butuo County and Jiangyou City in Sichuan Province and Mianxian County and Chengdu County in Shaanxi Province. It was reported that the Jiangyou cultivation area was the best, Butuo and Mianxian were the second, and the Chengdu cultivation area was the worst. Jiang [14] determined the content of fuziline and zongorine in Fuzi, which was cultivated in Butuo County in Sichuan Province, Mianxian County, and Chengdu County in Shanxi Province, by HPLC-MS. It was found that the sum of the two alkaloids in Jiangyou was the highest, while that in Chengdu was the lowest. The contents of aconite alkaloids in different cultivation areas were compared by Shu [15]. It was found that there were great differences in the contents of aconite extract, total alkaloids, and diester alkaloids, which were speculated to be related to the growth environment and cultivation mode of Fuzi.

# Conclusions

In conclusion, it can be speculated that the growth environment, cultivation management modes, and harvesting time of producing Chuanwu might jointly contribute to the difference in product quality. Jiangyou City in Sichuan Province and Hanzhong City in Shaanxi Province both have low altitudes, weak sunshine, and early harvesting times, showing a similar law that the content of neoline is relatively high and the content of zongorine is relatively low. Butuo Country in Sichuan Province and Lijiang City in Yunnan Province both have high altitudes, strong sunshine, and later harvesting times, showing a similar law that the content of neoline is relatively low and the content of zongorine is relatively high. Therefore, the proportion of neoline content and zongorine content can be used as a characteristic identification index for the quality difference of Chuanwu in different producing areas, which provides a reference for establishing scientific quality evaluation methods and quality control standards for Chuanwu.

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#### **Conflict of Interest**

The authors declare no conflict of interest.

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