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Comparison analysis of different parts and geographical origins from southwestern China on artemisinin content of *Artemisia annua* L.

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Abstract – In the study we focus on determining the contents of artemisinin in *Artemisia annua* L. from different geographical origins of southwest district of China. The artemisinin contents of eleven samples are measured by the high performance liquid chromatography (HPLC). As a result, Artemisinin exists in different parts of *A. annua* plant and the content is as follow: inflorescences > leaves > stems. Except for the material from Qianjiang district, Artemisinin content of four materials in Chongqing area is higher than those of Sichuan and Guizhou by the average of 0.728%. We conclude that the artemisinin contents of *A. annua*, collected from Sichuan and Guizhou province, do not reach the value of industrial development, while the others can be exploited.

Key words - *Artemisia annua* L, artemisinin, content, HPLC

1. INTRODUCTION

Artemisia annua L. is an annual herb which comes from *Artemisia* (Asteraceae), whose dried aerial part is named sweet wormwood that is famous traditional medicinal plant of china. And it is widely distributed in Asia and East Europe. As one of the indigenous plants used in traditional Chinese remedies, its medicinal ingredients can be divided into two parts of volatile and non-volatile, which is used to be antimalarial drug, to clear away heat and toxic material, and so on [1], [2]. Artemisinin, a sesquiterpene lactone with an endoperoxide, was the secondary metabolite and volatile species which synthesized and accumulated by *A. annua* and isolated more than 30 years ago from the aerial parts of *A. annua* [3], [4]. As the main medicinal ingredients of sweet wormwood, it was highly effective antimalarial agent for *Plasmodium falciparum* and supplied sustaining resistance compared to other drugs [5].

Artemisinin source can be got in a variety of ways. At present taking low cost into consideration, people prefer extracting that from *A. annua* directly to achieving by cell engineering, chemical synthesis or others in the industrial. Cultivation of the plant is still the only cost effective source of artemisinin [6]-[9]. *A. annua* widely distributes in China, whose artemisinin content is in a range from 0.02%-1.09% affected by climate and geography, including temperature, precipitation, elevation, sunlight, soil, and so on. Meanwhile, the site and time sampling are the important influencing factors besides ecological factors [10]. The *A. annua*, which the artemisinin content is less than 0.6%, cannot reach the value of industrial development. And increasing content by 1% could lower the cost by 60%~70% [11]. Therefore it can be effective to alleviate the market demand of artemisinin to conduct the research of *A. annua* resources in the more areas. The southwest of China has abundant resources of *A. annua*, such as some areas of Sichuan, Guizhou and Chongqing, which has high content of artemisinin [11]-[13]. Ten different resources of Chongqing city had been found to have content by 0.671%-1.019% [12]. However, it had not been reported if some other *A. annua* resources had enough Artemisinin to utilize. In this study the artemisinin contents in different parts of *A. annua* from some geographical origins of southwest district of China were determined, aiming at studying the artemisinin situation of *A. annua* in these areas and providing theoretical basis for resources exploitation and industrial utilization areas.

2. Materials And Methods

2.1 Materials

2.1.1 Plant materials

The tested materials, shown in Table (1), were collected in parts of Guizhou, Sichuan and Chongqing and identified by Yong-Hong Zhou professor of Sichuan Agriculture University. And the different origin cultivated *A. annua* were planted in the medicinal botanical garden of College Life and Basic Sciences of Sichuan Agricultural University. The stems, inflorescences and leaves of every plant at harvest were collected, dried at 50 °C for 24 h in oven, ground using a grinder and sieved, passed through a 60-mesh sieve and stored in desiccators as sample.

2.1.2 Main instruments

LC-20A-type high performance liquid chromatography was purchased from Shimadzu Corporation; KQ-300GDV-type thermostatic NC ultrasonic cleaner was purchased from Kun Shan Ultrasonic Instruments Co., Ltd; RE-52AA-type rotary evaporator was purchased from Shanghai Ya Rong Biochemical Instrument Factory; SHZ-D-type vacuum filter was bought from Ying Yu Instrument Factory of Gongyi City.

2.1.3 Main reagents

Artemisinin, the purity more than 99.0%, was purchased from the National Institute for the Control of Pharmaceutical and Biological Products; methanol and acetonitrile was chromatographically pure and bought Fisher Scientific; petroleum ether (the boiling range is 60 °C to 90 °C); anhydrous ethanol, sodium hydroxide and others were analytical pure sold in the market; water used was ultrapure water.

Table (1) Experimental materials

A. annua (Code)	Original places	Acquisition time	Comment
1	Dejiang, Guizhou, China	October	Wild
2	Zunyi, Guizhou, China	October	Wild
3	Jinsha, Guizhou, China	October	Wild
4	Jinsha, Guizhou, China	October	Cultivated
5	Qianjiang, Chongqing, China	October	Wild
6	Youyang, Chongqing, China	October	Wild
7	Xiushan, Chongqing, China	October	Wild
8	Fuling, Chongqing, China	October	Wild
9	Fuling, Chongqing, China	October	Cultivated
10	Beichuan, Sichuan, China	October	Wild
11	Yaan, Sichuan, China	October	Wild

2.2 Methods

2.2.1 Preparation of tested solution

1.0 g (± 0.0001 g) *A. annua* powder and 43.00 mL petroleum ether were put in the 50 mL weighing bottle with stopper and extracted ultrasonically at the conditions of the temperature of 42 °C (± 2 °C), the power of 120 W for 30 minutes. After filtering the extract, petroleum ether was removed by vacuum distillation. The residue was dissolved by 95% ethanol and diluted to 25.00 mL. All of the experiments were repeated three times and the results are expressed by average.

2.2.2 Preparation of control solution

10.00 mg artemisinin were dissolved by 95% ethanol and diluted to 100.00 mL. Thus, solution with 100.0 μg artemisinin in every 1 mL was obtained.

2.2.3 Precolumn derivatization

1.00 mL solution (in “2.2.1” and in “2.2.2”) was transferred into 25.00 mL volumetric flask. After adding 7.00 mL of 0.2% NaOH solution, the mixed solution was put in water bath at 50 °C for 30 min. After cooling, 8.00 mL of 0.08 mol/L acetic acid solution was added to volumetric flask. Then the mixed solution was diluted to the scale mark by 95% ethanol. Thus, the samples were obtained.

2.2.4 Chromatographic conditions

Chromatographic column: Phenomenex C_{18} (250 mm × 4.6 mm, 5 μm, Torrance, USA); mobile phase: anhydrous methanol - acetonitrile - 0.9 mmol Na_2HPO_4 - 3.6 mmol NaH_2PO_4 buffer solution (pH=7.76) (45:10:45, V/V); flow rate: 0.5 mL/min; column temperature: 35 °C; injection volume: 10 μL.

2.2.5 Methodological study

2.2.5.1 Investigation on linear relationship, limit of detection (LOD) and limit of quantification (LOQ)

According to the chromatographic conditions in "2.2.4", 1, 2, 3, 4 and 5 μL control samples were put into HPLC, respectively. Taking the value of peak area as ordinate (X) and artemisinin concentration content as the abscissa (Y), linear regression equation was obtained.

LOD and LOQ were estimated by the three and ten times of signal-to-noise ratio (S/N) respectively.

2.2.5.2 Precision test

10 μL control sample was injected for 5 times, RSD of peak area was calculated.

2.2.5.3 Stability test

The same sample solution was injected at 0, 2, 4, 8 and 12 h; and RSD of peak area was calculated.

2.2.5.4 Repeatability test

According to the method in "2.2.1", 5 samples of 1.0 g (± 0.0001 g) *A. annua* powder were compared into tested samples. Based on the chromatographic conditions in "2.2.4", RSD of peak area was calculated.

2.2.5.5 Recovery test

5 samples of *A. annua* powder with known content were added into 1mL control solution (0.1 mg/mL). According to the method in "2.2.1", the tested solutions were prepared and injected into the HPLC. They were detected under the chromatographic conditions in "2.2.4". The

peak area was recorded and the average recovery rate and RSD were calculated.

2.2.6 Determination of samples content

The tested samples were put into the HPLC and detected under the chromatographic conditions in "2.2.4". The extraction ratio of artemisinin was calculated through the following equation:

$$Y(\%) = \frac{C \times N \times V}{m \times 10^6} \times 100\%$$

In the equation, Y is the artemisinin extraction ratio; C is the artemisinin concentration, μg/mL; N is the dilution ratio; V is the total volume, mL; m is the mass of sample, g.

2.3 Data analysis

The testing data were analyzed on the correlation and the least significant difference by SPSS software.

3. Results And Analyses

3.1 Methodological study.

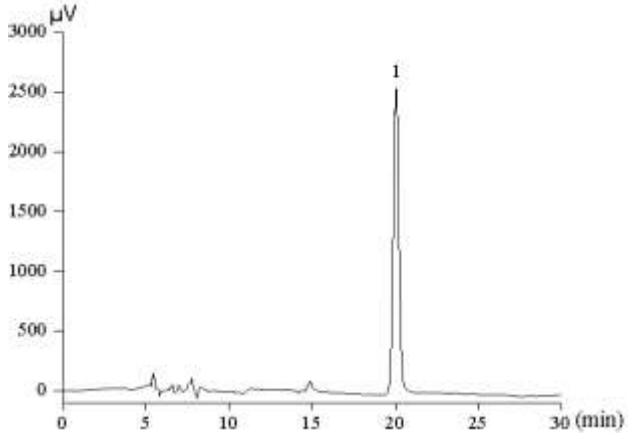
3.1.1 Investigation on linear relationship, limit of detection (LOD) and limit of quantification (LOQ)

Based on calculation of the control samples, the regression equation was as follow:

$$Y = 5.0016e^{-4}X - 5.330761$$

In the equation, Y is the peak area; X is the artemisinin concentration. R was 0.9999, which showed that the concentration content and peak area had good linear relationship within the range of 0.6072~2.429 mg/mL.

The LOD and LOQ of the artemisinin were 7.776 μg/mL and 25.92 μg/mL respectively. Fig. 1 showed the chromatogram of artemisinin control samples.



Note: 1- Artemisinin

Fig. 1. The chromatogram of artemisinin control samples

3.1.2 Precision test

10 μL artemisinin control sample was injected for 5 times, and the RSD of the peak area was 0.5%, indicating high precision.

3.1.3 Stability test

The RSD of 0.7% indicated that the sample was stable within 12 h.

3.1.4 Repeatability test

The RSD of repeatability tests of 5 samples was 1.4%, indicating good repeatability.

3.1.5 Recovery test

The average recovery rate of 5 samples was 96.7% and the RSD was 4.1%. Therefore, this method was accurate and reliable and could be used for the content determination of artemisinin in *A. annua*.

3.2 Comparative analysis on the artemisinin contents in different growth parts of *A. annua*

Table (2) showed the artemisinin contents in different growth parts of the eleven materials. According to Fig. 2, we found the significant difference in the content: the artemisinin content in stems was the least, which was almost below 0.1% and the average was 0.053%; leaves had higher content than stems by 0.489%~0.923% and the average was 0.652%, but there were five samples below 0.6%; the artemisinin content in inflorescences was the highest in a range from 0.892%~1.663%, which the average was 1.209%.

Table (2) The multiple comparison of artemisinin content from different parts in 11 materials

Code	Artemisinin content (%)		
	Stems	Inflorescences	Leaves
1	0.019 ± 0.40^{e}	0.987 ± 1.49^{de}	0.547 ± 0.80^{c}
2	0.021 ± 0.20^{e}	1.165 ± 2.31^{bcde}	0.589 ± 1.03^{c}
3	0.012 ± 0.36^{e}	0.892 ± 1.74^{e}	0.489 ± 1.86^{c}
4	0.101 ± 0.31^{a}	1.276 ± 1.78^{bcd}	0.581 ± 0.39^{c}
5	0.076 ± 1.05^{b}	1.069 ± 1.13^{cde}	0.604 ± 0.36^{bc}
6	0.107 ± 1.56^{a}	1.366 ± 2.53^{abc}	0.909 ± 1.00^{a}
7	0.022 ± 0.70^{e}	1.663 ± 2.07^{a}	0.923 ± 1.33^{a}
8	0.056 ± 0.89^{c}	1.298 ± 1.22^{bcd}	0.643 ± 0.80^{bc}
9	0.081 ± 1.21^{b}	1.475 ± 1.71^{ab}	0.779 ± 1.73^{ab}
10	0.048 ± 0.56^{cd}	1.064 ± 2.68^{cde}	0.611 ± 0.94^{bc}
11	0.039 ± 1.56^{d}	1.039 ± 0.06^{de}	0.502 ± 0.74^{c}

Note: The data with different letters of a, b, c, d or e indicated significant differences ($P<0.05$); and data with the same letters showed no significant differences ($P>0.05$).

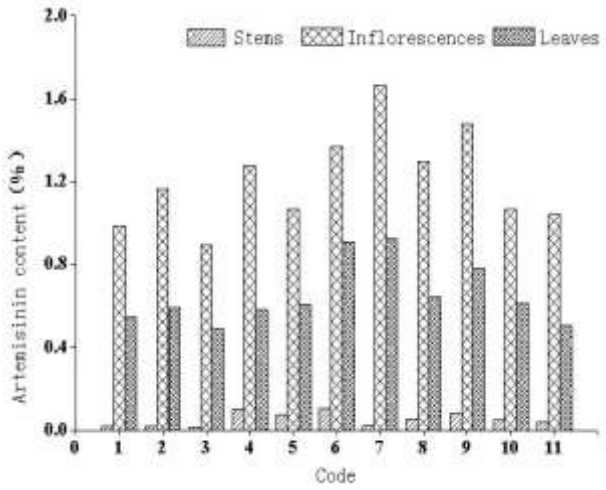


Fig. 2. The artemisinin content of different parts in 11 materials

3.3 Comparative analysis on the artemisinin content of *A. annua* from different geographical origins

The artemisinin contents of the eleven plant materials, determinated by calculating the average content of the entire plant (including stems, leaves and inflorescences), were shown in Fig. 3. The artemisinin content ranged from 0.464%~0.869%, in which the contents of six plant materials from Guizhou and Sichuan were below 0.6%. And, above 0.6%, except of one *A. annua* from Guizhou, the plants all came from Chongqing.

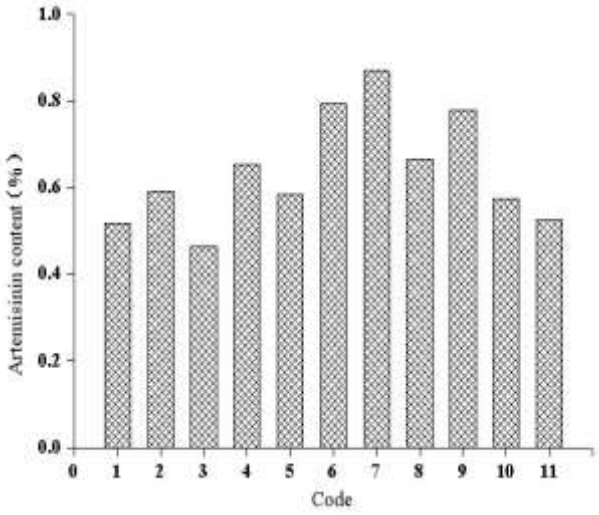


Fig. 3. The medial content of artemisinin in 11 materials

Fig. 4 showed the difference in the content of *A. annua* from Sichuan, Chongqing and Guizhou intuitively by calculating the average contents of the eleven geographical origins. According to the comparative analysis, whether in stems, leaves or inflorescences, the average amount of artemisinin in *A. annua* from Chongqing was higher than Sichuan and Guizhou, in which the result was statistically significant for that in leaves, not in stems or inflorescences. The average artemisinin content of the entire plant from Chongqing was the highest by 0.728%, while that 0.551% in Sichuan and 0.525% in Guizhou.

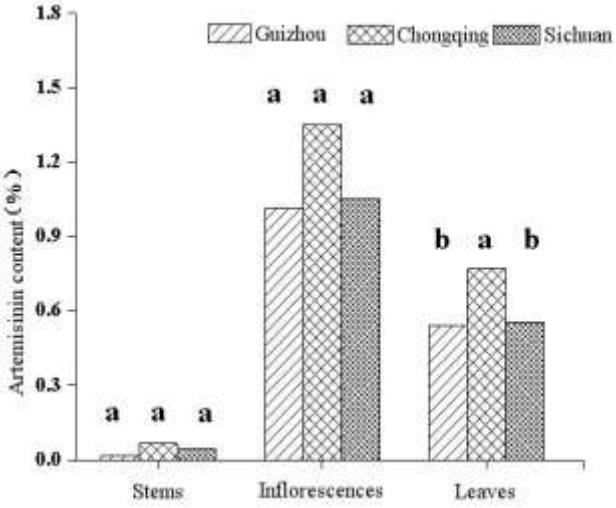


Fig. 4. The medial content of artemisinin in 3 provinces

4. Conclusion And Discussion

This research showed that artemisinin content was the highest in inflorescences, then in leaves and stems. Some of previous reported data showed similarity with present results: Zhao thought that the content in inflorescences was higher than leaves and Ferreira reported that the artemisinin content in inflorescences was 4~11 times higher than in leaves [14], [15]. But some others had a few difference. For example, Elhag found that the content was as follow: leaves >flowers >inflorescences and Wei reported that the artemisinin content in leaves was higher than in alabastrum by 10% and 2.13 times than in mature branches[16], [17]. The research on gene amorpha-4, 11-diene synthase(ADS) about artemisinin biosynthesis indicated that the relative expression of ADS had a positive relationship with artemisinin content and ADS had a higher expression in leaves and flowers [18]. So it was the same as our study in that it indicated that arteimisinin were mostly in leaves and flowers, not in stems.

In this study the significant difference of artemisinin content in *A. annua* from different geographical origins of southwest district of China indicated the big impact of the climate and geography [11]. The *A. annua* can reach the value of industrial development by 0.6% of content. The plants from Chongqing had enough content of artemisinin to exploit, especially Chongqing Youyang. At present, Chongqing has been one of the most important origins of Artemisinin in China for the abundant high-quality *A.annuna* germplasm resources. But the others, collected from Sichuan and Guizhou province, whose content was less than 0.6%, did not reach the value of industrial development. Therefore more advanced extraction technology or others need to

develop to take advantage of these abundant resources from Sichuan and Guizhou.

Furthermore, based on the artemisinin contents of *A. annua* from Chongqing Fuling and Jinsha, the cultivated had higher content than the wild. Similarly, the data of artemisinin contents in Guangxi showed higher content in the cultivated [19]. So the low artemisinin content could be increased by plants introduction and acclimatization to alleviate the market demand of artemisinin, too.

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