



## Investigation on Influential Factors of Volatile Oil and Main Constituent Content from *Curcuma kzoangsiensis*

S. G. Lee C. F. Liang. In Guangxi Producing Areas

Xu Chen

Pharmacy College, Guilin Medical College, Guilin 541000, China

Jianhong Zeng

Pharmacy College, Guilin Medical College, Guilin 541000, China

Tel: 86-773-5893402 E-mail: zjhong0621@163.com

*Projects funded by Guangxi Natural Science Foundation (NO.0447101) and Guangxi Science and Technology Key Task and Trial Production.*

### Abstract

To quantitatively analyze volatile oil in *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. produced in different places and seasons, and to quantitatively analyze curcuminol by gas chromatographic (GC). The volatile oil was distilled by the steam distillation (XD) and the curcuminol was determined by GC on a HP-5 column (0.32 mm×30m, 0.25μm), Inlet temperature 200°C, FID 250°C, flow 1.0 ml·min<sup>-1</sup>, splitless. Temperature programming started at 60°C, holding for 4 min, then increased to 210°C at a rate of 3°C·min<sup>-1</sup>. The quantity of volatile oil in *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. in different places and collecting time were detected. The contents of volatile oil and curcuminol was the highest in Binyang place. The quantity of volatile oil was the richest in January and February, and the same result was obtained for curcuminol. As a result, January and February was the best time for the collection of *Curcuma kzoangsiensis* S. G. Lee C. F. Liang.. The contents of volatile oil and curcuminol should be taken as a standard for the evaluation of the quantity of *Curcuma phaeocaulis* val.. and the determination of its collecting time.

**Keywords:** *Curcuma kzoangsiensis* S. G. Lee C. F. Liang., Volatile oil, Curcuminol, Cultivation

Zedoary turmeric --- *Curcuma phaeocaulis* (Zingiberaceae) is the dry rhizome of *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. and common turmeric which is usually called *Curcuma Zedoary*. As a perennial herb, Zedoary turmeric grows mainly in provinces such as Guangxi, Sichuan and Yunnan, etc. And its main effective fractions are root and rhizome, whose active constituents mainly include volatile oil which is the effective components of anti-tumor and curcumin which is the main effective components of blood lipid reducing, antioxidation and antibacterial (China Pharmacopoeia I 2005, p.194). Studies have been revealed that the contents of effective components in medicinal plants are controlled by the genetic gene, while it is also influenced by ecological factors and the stages of growth and development. With a long cultivation history, the genuine medicinal material of *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. grows mainly in Binyang place, Luchuan place, etc. In the experiment, we extract and determine volatile oil in *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. collected from different producing places and in different collecting time by the steam distillation (XD), and determine the contents of the Curcuminol which is the effective components in volatile oil by gas chromatography (GC) to utilizing adequately germplasm resource and standardizing quality evaluation of Zedoary turmeric, and we study the effect of ecological factor in genuine producing area on the effective parts of *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. By determining the content of volatile oil, so that we can provide a scientific basis for the cultivation and harvesting and for the improvement on quality and output of *Curcuma kzoangsiensis* S. G. Lee C. F. Liang..

### 1. Experimental material and equipments

#### 1.1 Medicinal materials

The necessary materials for investigation on different producing areas factor: *Curcuma kzoangsiensis* S. G. Lee C. F. Liang collected from seven main producing areas in Guangxi province including Luchuan, Pingnan, Hengxian, Daxin, Yining, Shangsi, and Binyang in January, 2007; the necessary materials for investigation on different collecting times factor: *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. collected from Guilin YongFeng traditional Chinese medicinal materials cultivation bases on 15<sup>th</sup> in each month from October, 2006 to February, 2007 (They own identical in germplasm source and cultivation conditions); hoard the above materials for reserving after go through the following processing such as ablution, slicing, solar drying, smash, and sieving with a 20 mesh sieve. Our staff room make it clear

that all the medicinal materials are *Curcuma kzoangsiensis* S. G. Lee C. F. Liang., which is zingiberaceous plant of curcumol.

### 1.2 Equipments and reagents

Agilent 6890N gas chromatography. FA1004 Electronic balance (made in ShangHai JingKe Instrument Factory); tempering heater(made in SangHang Electrophysiological Instrument Factory); a set of volatile oil tester and reflux condensing works. Curcumol reference (National Institute for the Pharmaceutical and Biology Products, Batch number: 1018520104).All the reagent used in the experiment are analysis purity.

## 2. Experimental methods and results

### 2.1 Extraction of volatile oil

Both the extraction method of volatile oil and determination of the yield of oil base on that of referred on the appendix XD of China pharmacopoeia 2005. We weight 50g medicinal materials referred above (*Curcuma kzoangsiensis* S. G. Lee C. F. Liang. collected from different producing places and in different collecting times), respectively. Then put them into a round-bottom flask ( Limitation of volume should be 1000ml),and enter 400ml water with several glass drops ,and soak for 2h after swaging to make them homogeneous. Connect the volatile oil tester and reflux condensation tube, and enter waters from the top of the condensation tube until it submerged the calibration part of the volatile oil tester and flow into the flask. Put them into the heating sleeves (heating temperature 130-140°C), warm slowly until the liquid boil slightly, then cold it naturally for 10min, open the plug on the lower end of the volatile oil tester to let the water out slowly and close it when the top of the oil reaches 5mm above the calibration 0. After having been placed for 1.5h, we should open the plug again, just close it when the top of oil reaches the calibration 0, read the volume of the volatile oil and copy it.

### 2.2 Chromatogram conditions and system suitability method (Zhu, Y. E., 2006, p.389)

HP-5 column (0.32 mm×30m, 0.25 μm) Fused-Silica Capillary Column, Inlet temperature 200°C, FID 250°C, flow 1.0 ml·min<sup>-1</sup>, splitless. Temperature programming starts at 60°C, holding for 4 min, then make temperature reach 210°C at a rate of 3°C·min<sup>-1</sup>. In this chromatographic condition, the peak of the curcumol and that of the others' can be separated effectively, as Figure1.

### 2.3 Preparation of reference solution

Put 2.5 mg precisely weighted reference of curcumol into volumetric flask (Limitation volume 5ml), and enter ethyl acetate to make reference solution of 2000 μg·ml<sup>-1</sup> and 400 μg·ml<sup>-1</sup>.

### 2.4 Preparation of sample solution

Weight precisely 10g powder of the *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. collected from different producing places and in different collecting times, respectively, then put them into a round bottom flask (Limitation volume 500ml),enter 300ml water, the extraction method of volatile oil is same with "item 2.1".And enter ethyl acetate into the tube and extract twice,4h for each time, transfer separately the volatile oil into a measuring flask (Limitation volume 10ml) with ethyl acetate , ethyl acetate reaches the calibration, swag for making them homogeneous , then samples are gotten .

### 2.5 Test of linear relationship

Take precisely 2000 μg·ml<sup>-1</sup> solution of reference in high content 0.2, 0.4, 2, 4, 10 ml into volumetric flask of 10ml volume, enter ethyl acetate until it reaches the calibration, swag to make them homogeneous. take precise 1μl into the gas chromatography, ordinate the area of the peak and abscissa the content of curcumol, linear regression, get linear equations  $Y=2.09X-21.67$ ,  $r=0.9998$ , which indicates that the curcumol has a good linear relationship ranging from 40.0μg·ml<sup>-1</sup> to 2000 μg·ml<sup>-1</sup>.

### 2.6 Test of accuracy

Take separately identical solution of reference and samples are tested for continuously 6 times in the chromatographic condition of "item 2.2", then determine area of the peak, RSD=0.34%, which makes it clear that the accuracy is good.

### 2.7 Test of stability

Take one portion identical solution of reference. Then analyze it on the rule of 0, 1, 2, 4, 8, 16, 24h in the chromatographic condition of "item 2.2", separately. And the result is RSD=1.67%, the tested component is stable within 24h.

### 2.8 Test of reappearance

Weight precisely identical sample 10g, 6 portion, and prepare the solution of sample as "item 2.4",then determine it in the chromatographic condition referred above, result may be RSD=1.8%, whose repeatability is good.

### 2.9 Recovery rate of added sample

Weight 5g sample, whose content has been known, 12 portions. And put them into a round bottom flask(volume 500ml),

enter separately solution of reference in  $2000 \mu\text{g}\cdot\text{ml}^{-1}$  and  $400 \mu\text{g}\cdot\text{ml}^{-1}$  each 6 portions, enter 300ml water, then prepare sample solution as “item 2.3”, and determine as “item 2.2”. Then calculate contents together with recovery rate according to the area of peak, and recovery rate of the two different sample solutions are 98.0% (RSD=2.0%), 100.5% (RSD=1.9%).

### 2.10 Determination on the contents of volatile oil and curcuminol

#### 2.10.1 Determination on the contents of volatile oil and curcuminol collected in different producing places

Extract and determine volatile oil sample collected from different producing areas as “item 2.1”, and determine the contents of curcuminol in chromatographic condition of “item 2.2”. The statistics and analysis are treated by SPSS10.0, and the obtained results show in Table 1, Figure 2.

The differences of volatile oil are very clear in *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. collected from different producing places, it is extremely remarkable according to variance analysis ( $F=31.95>F_{0.01}$ ,  $\text{Sig.}=0.000<0.01$ ) in Table 1 and Figure 2. The maximal result of the samples produced in Binyang is 2.776%, the minimal produced in Shangsi is 1.742%. The differences of contents of curcuminol are remarkable according to analysis of variance ( $F=110.59>F_{0.01}$ ,  $\text{Sig.}=0.000<0.01$ ). The maximal result of samples produced in Binyang is 0.044%, the minimal produced in Daxin is 0.023%, and no curcuminol can be obtained from samples of Hengxian place and Yining place.

#### 2.10.2 Determination on the contents of volatile oil and curcuminol collected in different collecting times

Extract and determine volatile oil sample collected in different collecting times as “item 2.1”, then determine the contents of curcuminol in chromatographic condition of “item 2.2”, The statistics and analysis are again treated by SPSS10.0, the result shows in Table 2 and Figure 3.

From Table 2 and Figure 3, the differences of volatile oil are known clearly in *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. collected in different collecting times, which is extremely remarkable according to variance analysis ( $F=84.65>F_{0.01}$ ,  $\text{Sig.}=0.000<0.01$ ). Order of the contents of volatile oil is January's > February's > December's > November's > October's. The differences of contents of curcuminol are remarkable according to analysis of variance ( $F=17.07>F_{0.01}$ ,  $\text{Sig.}=0.000<0.01$ ). The order of the contents of curcuminol is January > February > December > October > November.

### 3. Discussions

According to *Chinese Pharmacopoeia* in 2005, the result of extracted volatile oil by standard steam distillation should be 2.2%, while result obtained in the experiment is, the minimal contents of volatile oil is 1.742%, the maximal 2.776%, the average 2.284%. The volatile oil contents of *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. from Shangsi place and Pingnan place are minimal, however, other places are all accorded with the standard in *Chinese Pharmacopoeia*.

The contents of curcuma oil should be taken as a standard for the quantity evaluation, for curcuminol is one of the main effective components of volatile oil. According to the determination, the differences of volatile oil contents are very clear and samples produced in different producing places and collected in different collecting times, the author should take high in content of volatile oil and curcuminol as a reasonable basis, if one can confirm the clinical significance of these differences in the later research.

Of those genuine producing areas of *Curcuma kzoangsiensis* S. G. Lee C. F. Liang., Binyang has the best zedoary turmeric, which depends on examination of the producing area factor, the contents of curcuminol in Binyang is maximal compared to other places, and by examination of the volatile oil contents, that of Binyang's is also the highest.

January and February should be the best collecting time for *Curcuma kzoangsiensis* S. G. Lee C. F. Liang., for during this time contents of the volatile oil and the curcuminol is maximal and exceed the standard of pharmacopoeia. However, in December when usually zedoary turmeric is collected as it's above-ground part withered is 1.984% is lower than the standard of pharmacopoeia according to the determination in the factor of collecting time.

Determination of the medicinal materials' best collecting time bases on both its quality and quantity, which is to say the best collecting time is maximal peak of both accumulation dynamics of effective components and of dry-matter in the plants' growth and development stages (Liu, M.Y., 1995, p.209). So it has some reference value to confirm January and February are the best collecting time of *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. by the content determination of volatile oil and effective components. But if taking the output of rhizome as one of the factors, the determination of the best collecting time will be more reasonable and perfect.

### References

- Pharmacopoeia Committee of People's Republic of China. *China Pharmacopoeia(T)*. Beijing: Chemical Industry Press, 2005:194.
- Zhu, Y. E and Zhu, X.P. (2006). Determination of curcuminol in Rhizoma Curcunme by GC. *China Journal of Chinese Materia Medica*, 31(5):389-390.
- Liu, M.Y., Wang, Dong and Du, X.W. (1995). Medicinal herbs biology of root drugs. Beijing: Chinese Agricultural technological press, 209-210.

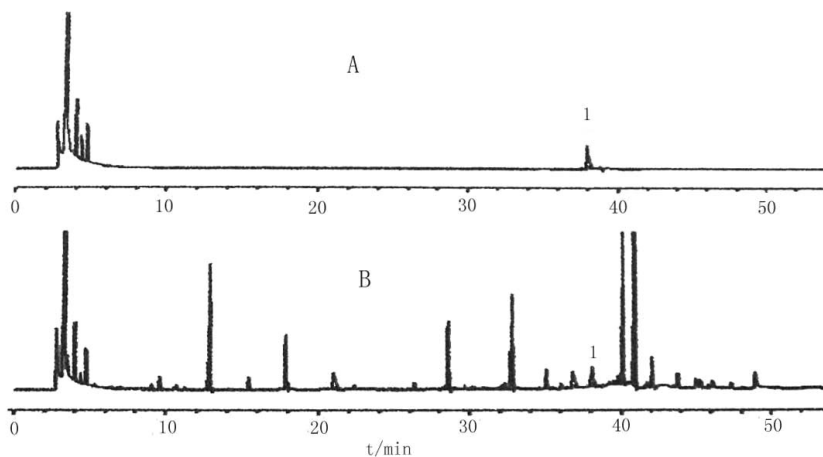


Figure 1. Gas Chromatogram of *Curcuma kzoangsiensis* S. G. Lee C. F. Liang.

A. Reference B. Sample of *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. C. Curcumol

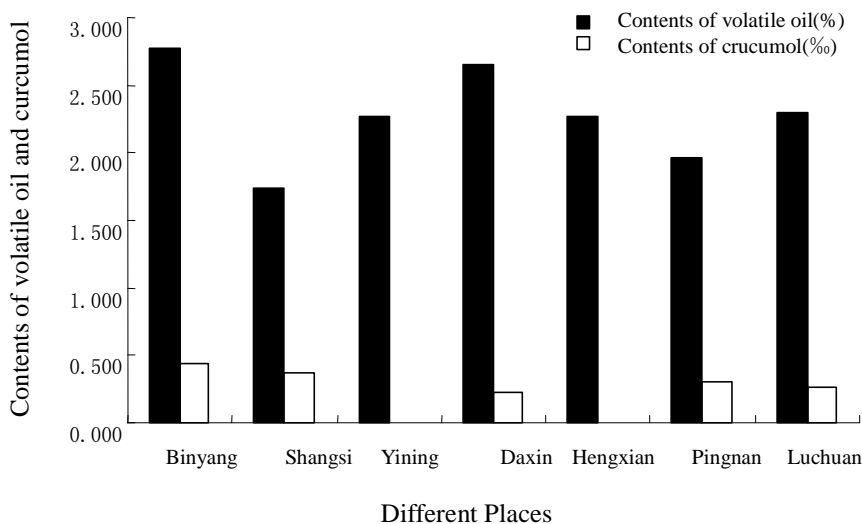


Figure 2. Contents of volatile oil and curcumol in *Curcuma kzoangsiensis* S. G. Lee C. F. Liang collected from different producing places

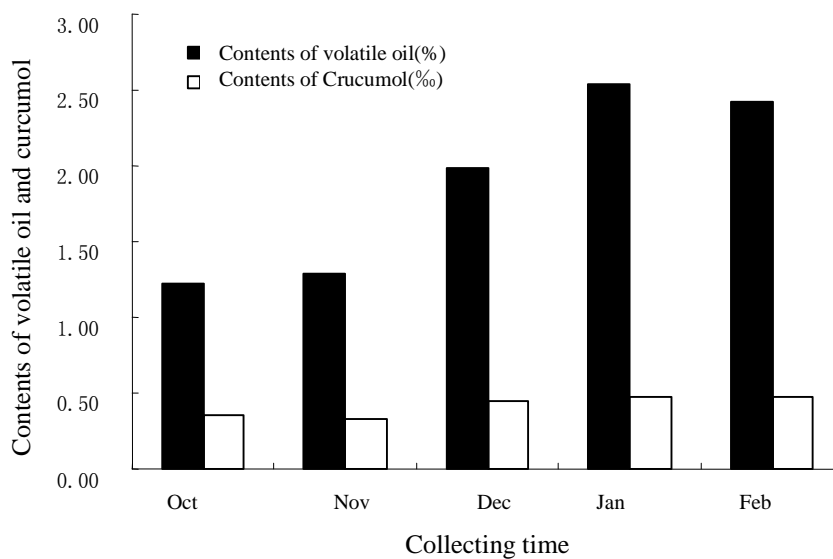


Figure 3. Contents of volatile oil and Curcumol in *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. collected in different collecting times

Table 1. Contents of volatile oil and curcumol in *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. collected from different producing places (n=3)

NO.	Producing places	Contents of volatile oil(%)	Contents of curcumol (%)
1	Luchuan	2.292	0.027
2	Pingnan	1.968	0.031
3	Hengxian	2.275	0.000
4	Daxin	2.658	0.023
5	Yining	2.275	0.000
6	Shangsi	1.742	0.037
7	Binyang	2.776	0.044

Table 2. Contents of volatile oil and curcumol in *Curcuma kzoangsiensis* S. G. Lee C. F. Liang. collected in different collecting times(n=3)

NO.	Collecting time	Contents of volatile oil(%)	Contents of curcumol (%)
1	October	1.225	0.035
2	November	1.290	0.033
3	December	1.984	0.044
4	January	2.546	0.048
5	February	2.426	0.047