Variation of Active Constituents of *Curcuma domestica* Rhizomes at Different Ages

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Abstract

Rhizomes of *Curcuma domestica* Valeton at different ages were collected from Nakorn Pathom and Prachuap Khiri Khan provinces. The quantities of the active constituents, curcuminoids and volatile oil, of the rhizomes were determined. It was found that rhizomes from five month-old plants yielded the highest content of the active constituents. Stability of the active constituents was also studied and the results were discussed.

Key word index: *Curcuma domestica* Valeton, different ages, rhizomes, curcuminoids, volatile oil, quantitative analysis.

Introduction

*Curcuma domestica* Valeton, commonly known in Thai as Khamin or Khamin Chan, is a herbaceous plant. Its rhizome is used as a major ingredient of curry powder and used in folk medicine to treat numerous conditions including flatulence, liver problems, menstrual difficulties, bruises, hemorrhage, diarrhea, stomachic, and colic\(^1\). The main constituents of the rhizomes are coloring matters and volatile oils. The coloring matters are composed of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin\(^2\). The volatile oils consist of turmerone, ar-turmerone, zingiberene, cineol, borneol, \(\alpha\)-phellandrene, sabinene, ar-curcumene, and curcumol\(^3\). Curcumin, desmethoxycurcumin, turmerene and ar-turmerene have been found to increase the bile secretion and curcumin could inhibit serotonin-induced damage of the stomach\(^2,4,5\). We previously reported the qualitative evaluation of these volatile oils and curcuminoids\(^6\). In this study, we further determined the age of the plant that yields the highest content of these active constituents. The results of this study will be beneficial for the industrial production of curcuminoids.
Materials and Methods

Materials

1. Plant material
   Fresh samples were collected from Nakorn Patom Province and Prachuap Khiri Khan Province and identified as Curcuma domestica Valeton (Zingiberaceae) by Botany Section, Division of Medicinal Plant Research and Development, Department of Medical Sciences, Nonthaburi. The rhizomes were washed, cleaned, cut into pieces about 0.5 cm thick and dried in the oven at the temperature about 40 °C. The dried rhizomes were pulverized and passed through sieve no. 180.

2. Chemicals
   Reagent grade of acetic anhydride, sulfuric acid, methanol, benzene, chloroform, absolute ethanol, phosphomolybdic acid, hydrochloric acid, toluene, xylene, tetrahydrofuran, N,N-dimethylformamide, acetone, and formic acid were used. The thin-layer chromatographic plate was silica gel G, precoated 20x20 cm, 0.25 mm thickness (Merck). Curcumin (Roth), desmethoxycurcumin and bisdesmethoxycurcumin (Organic Synthesis Section, Division of Medicinal Research and Development) were used as the reference substances.

3. Apparatus
   UV Visible Spectrophotometer JASCO, UVIDEC-650
   Volatile oil determination apparatus\(^{8}\)
   Water determination apparatus\(^{10}\).

Methods

The analyses were carried out in eight categories: chemical identification tests, determination of ash content, acid-insoluble ash content, water content, volatile oil content, curcuminoids and individual curcuminoid contents and stability.

1. Chemical identification tests:
   1.1 Preliminary test for curcuminoids\(^{7,8}\)
   Ten mg of the powdered drug were extracted by shaking with 2 ml acetic anhydride, a few drops of sulfuric acid were added and the color of the solution was observed under UV365.

   1.2 Confirmation test\(^{7}\) (Thin-layer chromatographic analysis)

   Test solution: One g of the powdered drug was added to 3 ml methanol in a stoppered test tube, shaken for a while and set aside for 1 hour. The mixture was filtered and the clear filtrate was used.

   Reference solution: One mg each of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin was dissolved separately in 1 ml methanol.
Adsorbent: Silica gel G

Developing solvent: Benzene:chloroform:absolute ethanol 49:49:2

Distance: 17 cm

Spotting amount: Test solution 5 μl
Reference solution 5 μl each

Spray reagent: 10% Ethanolic solution of phosphomolybdic acid

Detection:
1. Ultraviolet at 365 nm
2. Spray with spray reagent

The spots in the chromatogram obtained from the test solution were compared with those from the reference solutions.

2. Determination of ash content
The analytical procedure was carried out as described in the British Pharmacopoeia\textsuperscript{(9)}.

3. Determination of acid-insoluble ash content
The analytical procedure for acid-insoluble ash content was carried out as described in the British Pharmacopoeia\textsuperscript{(9)}.

4. Determination of water content
Water content was determined by the method of Lou Zhi-cen\textsuperscript{(10)} using 25 g of powdered drug.

5. Determination of volatile oil content
Ten g of sample were placed in a 500 ml glass stoppered flask and 200 ml of water were added. Volatile oil content was determined by the method described in the Pharmacopoeia of Japan\textsuperscript{(11)}.

6. Determination of total curcuminoid content
A modified spectrophotometric method of L.R. Xu and S.Y. Sha et al.\textsuperscript{(7)} was used to determine total curcuminoid content of all the samples. About 0.3 g of the powdered drug was accurately weighed in a 10 ml volumetric flask and added with tetrahydrofuran to volume. The mixture was set aside at room temperature for 24 hrs. and shaken frequently. One ml of the clear supernatant liquid was accurately transferred into a 25 ml volumetric flask, diluted to volume with methanol and mixed well. One ml of this solution was transferred into a 50 ml volumetric flask, diluted to volume with methanol and mixed well. The absorbance of the test sample was measured at 420 nm. The concentration of curcumin in the test sample was read from the
standard curve and the percentage of total curcuminoids was calculated as curcumin in the sample. For constructing the standard curve, about two mg of curcumin were accurately weighed in five-ml volumetric flask and methanol was added to dissolve the standard substance. The solution was diluted to volume and shaken well. Different volumes of the solution (20, 40, 50, 60, 80 μl) were accurately transferred into five 10-ml volumetric flasks, diluted to volume with methanol and shaken well. The absorbance of the standard solution was measured at 420 nm using methanol as a blank. The standard curve was then plotted.

7. Determination of individual curcuminoid content

A modified TLC method of L.R. Xu and S.Y. Sha et al was used for the determination of individual curcuminoid content. (7)

**TLC condition**

**Adsorbent**: Six g of silica gel G were suspended in 12 ml of water to prepare each 20 x20 cm silica gel G plate of 0.25 mm thick. After coating, the plate was dried at room temperature overnight and impregnated by immersing in 1% N,N-dimethylformamide in acetone. The solvent was then evaporated before use.

**Sample determination**: Each sample was extracted with tetrahydrofuran. Appropriate amount of the tetrahydrofuran extract was applied on the prepared chromatoplate, and carried out TLC under the same condition as the standard solution. From the sample plate, scrape off three yellow zones at the same Rf value as standard curcumin, desmethoxycurcumin and bisdesmethoxycurcumin. Elute separately measure the absorbance of curcumin, desmethoxy-curcumin and bisdesmethoxycurcumin at 422, 416 and 414 nm, respectively. From the standard curve, calculate the percentage of curcumin, desmethoxycurcumin and bisdesmethoxy-curcumin in the sample.

**Standard curve**: 10, 15, 20, 30, 35 μl of curcumin, desmethoxycurcumin and bisdesmethoxy-curcumin standard solution 0.5 μg/μl methanol were applied separately on the prepared chromatoplates and TLC plate was developed in chloroform:methanol:formic acid 96:4:0.6. Rf values of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin were 0.73, 0.48 and 0.30, respectively. The chromatograms were allowed to dry in the air, each compound was scraped off and eluted with 5.0 ml of methanol. The absorbance of standard solutions of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin were separately measured at 422, 416 and 414 nm, respectively and methanol was used as a blank. Standard curve of each compound was then plotted.
8. Stability study

Six samples of *Curcuma domestica* from Prachuap Khiri Khan Province were determined for water content, volatile oil content and curcuminoids after stored at room temperature for 3, 6, 9, 12, 18 and 24 months, respectively.

Result

All samples showed blood red color in Preliminary test and showed six main substances (ar-curcumene, dl-turmerone, curcumol, curcumin, desmethoxycurcumin and bisdesmethoxy-curcumin) in the confirmation test by thin-layer chromatography as showed in figure 2 of the Thai Journal of Pharmaceutical Sciences Vol. 13 No.3 July-September 1988 P.322.

The quantitative analysis of samples are shown in Tables 1-2 and in Figures 1-2.

The stability of samples when stored at various time are shown in Figures 3-4

Discussion and Conclusion

As we have reported the quality evaluation of *Curcuma domestica* Valeton rhizomes\(^6\), determination of the quantity of main substances are further studied. In this present work, *Curcuma domestica* Valeton rhizomes were collected at various ages from two provinces, Nakorn Pathom and Prachuap Khiri Khan. As the volatile oils were attributed to antidyspeptic properties\(^{12}\) and the curcuminoids were treated for anti-inflammatory\(^{13}\), the quality of *Curcuma domestica* Valeton rhizomes could be evaluated. It was found that *Curcuma domestica* Valeton rhizomes of five-month-old plant gave the highest percent of volatile oil, 9.79% and 11.98% for Nakorn Pathom and Prachuap Khiri Khan, respectively, and highest percent of curcuminoids in comparison with the others. The curcuminoids content of rhizomes from Nakorn Pathom was 10.12% where as that from Prachuap Khiri Khan was 10.62%.

The stability of volatile oil and curcuminoids was further studied in the rhizomes collected from Prachuap Khiri Khan province over two-year period it was found that the contents of both active constituents were gradually decreased.
Table 1 Quantitative analysis of various parameters of Curcuma domestica Valeton rhizomes collected at different ages from Nakorn Pathom Province

<table>
<thead>
<tr>
<th>Age of samples (month)</th>
<th>Water content (%)</th>
<th>Volatile oil (%)</th>
<th>Ash content (%)</th>
<th>Acid insoluble ash content (%)</th>
<th>Total Curcuminoinds (%)</th>
<th>Individual Curcumin</th>
<th>Curcuminoinds content (%)</th>
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<tr>
<td>5</td>
<td>9.91</td>
<td>9.79</td>
<td>7.94</td>
<td>0.76</td>
<td>10.12</td>
<td>4.24</td>
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<td>1.36</td>
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</tbody>
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* based on "Analysis Methods for Active Constituents in Chinese Herbal Medicine"(7)

Table 2 Quantitative analysis of various parameters Curcuma domestica Valeton collected at different ages Prachuap Khiri Khan Province

<table>
<thead>
<tr>
<th>Age of samples (month)</th>
<th>Water content (%)</th>
<th>Volatile oil (%)</th>
<th>Ash content (%)</th>
<th>Acid insoluble ash content (%)</th>
<th>Total Curcuminoinds (%)</th>
<th>Individual Curcumin</th>
<th>Curcuminoinds content (%)</th>
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* based on "Analysis Methods for Active Constituents in Chinese Herbal Medicine"(7)
The relationship between average
% volatile oil and rhizome age

 FIGURE 1

The relationship between average
% curcuminoids and rhizome age

 FIGURE 2
Stability of volatile oil after storage

FIGURE 3

Stability of curcuminoids after storage.

FIGURE 4
References

12. Manual of Medicinal Plant for Primary Health Care; Div. of Medicinal Plants Research and Development; Dep. of Medical Sciences; Ministry of Public Health, 1990 P.61.
การเปลี่ยนแปลงปริมาณสารสำคัญของขมิ้นชันในระยะเวลาเก็บเกี่ยวต่าง ๆ

ปราณี ชาติด้าวงศ์ และ วารุณี จิรวัฒนเกษร์

กองวิจัยและพัฒนาสมุนไพร กรมวิทยาศาสตร์การแพทย์ ซอยร.พ.บางนา-ศรีนครินทร์ หน่วยบังคับ 11000

บทคัดย่อ

จากการศึกษาค่าปริมาณน้ำมันมะพร้าวและปริมาณสารสำคัญของขมิ้นชันในระยะเวลาก่อนเก็บเกี่ยวต่าง ๆ ก็จากแปลงทดลองจังหวัดกระบี่และจังหวัดประจวบคีรีขันธ์ พบว่าระยะเวลาที่เหมาะสมในการเก็บเกี่ยวคือ 5 เดือน และจากการศึกษาความคงที่ของสาร พบว่าปริมาณของน้ำมันมะพร้าวและปริมาณของโคฟูมินอยอร์ดจะลดลงเมื่อนำมาเก็บไว้ในห้องเก็บเกี่ยวเป็นเวลานาน

ถุนแจงคำ : ขมิ้นชัน อาภูในการเก็บเกี่ยว น้ำมันมะพร้าว เโคฟูมินอยอร์ด