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# Variation of Active Constituents of Curcuma domestica Rhizomes at Different Ages(การเปลี่ยนแปลงปริมาณสารสำคัญของขมิ้นชัน ในระยะเวลาเก็บเกี่...

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# 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 volotile 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, α-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[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[9].

4. Determination of water content

Water content was determined by the method of Lou Zhi-cen[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[11].

6. Determination of total curcuminoid content

A modified spectrophotometric method of L.R. Xu and S.Y. Sha *et al.*[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. Appropiate 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, desmethoxycurcumin and bisdesmethoxycurcumin at 422, 416 and 414 nm, respectively. From the standard curve, calculate the percentage of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin in the sample.

**Standard curve** : 10, 15, 20, 30, 35 µl of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin standard solution 0.5 µq/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

| Age of samples (month) | Water content (%) | Volatile oil (%) | Ash content (%) | Acid insoluble -ash content (%) | Total Curcuminoids (%)* | Individual Curcumin | Curcuminoids content (%) Desmethoxy-curcumin | Bisdesmethoxycurcumin |
|---|---|---|---|---|---|---|---|---|
| 5 | 9.91 | 9.79 | 7.94 | 0.76 | 10.12 | 4.24 | 2.98 | 3.04 |
| 6.5 | 5.63 | 8.42 | 6.66 | 0.86 | 8.40 | 2.85 | 2.21 | 2.64 |
| 7.25 | 7.32 | 8.38 | 6.64 | 0.38 | 7.94 | 2.76 | 2.13 | 2.49 |
| 8 | 7.89 | 6.75 | 7.11 | 0.75 | 7.66 | 3.25 | 2.20 | 2.35 |
| 9 | 10.04 | 8.34 | 5.97 | 0.33 | 7.87 | 3.54 | 2.43 | 2.42 |
| 10 | 9.96 | 9.26 | 7.12 | 1.04 | 7.68 | 3.58 | 2.10 | 2.40 |
| 11 | 9.79 | 8.68 | 6.78 | 1.36 | 8.27 | 3.96 | 2.94 | 2.62 |

* based on "Analysis Methods for Active Contituents in Chinese Herbal Medicine"[7]

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

| Age of samples (month) | Water content (%) | Volatile oil (%) | Ash content (%) | Acid insoluble -ash content (%) | Total Curcuminoids (%)* | Individual Curcumin | Curcuminoids content (%) Desmethoxy-curcumin | Bisdesmethoxycurcumin |
|---|---|---|---|---|---|---|---|---|
| 5 | 8.35 | 11.98 | 7.34 | 1.12 | 10.62 | 5.20 | 2.57 | 2.86 |
| 6 | 3.62 | 11.32 | 7.26 | 0.96 | 10.21 | 4.84 | 2.36 | 2.94 |
| 7 | 5.95 | 6.38 | 6.68 | 1.09 | 8.37 | 3.78 | 2.02 | 2.40 |
| 8 | 10.54 | 10.86 | 7.42 | 1.11 | 8.94 | 3.94 | 2.17 | 3.00 |
| 9 | 6.07 | 8.99 | 6.81 | 0.97 | 7.82 | 3.20 | 1.42 | 2.10 |
| 10 | 5.24 | 6.70 | 8.22 | 1.90 | 7.05 | 2.71 | 1.25 | 1.74 |

* based on "Analysis Methods for Active Constituents in Chinese Herbal Medicine"[7]

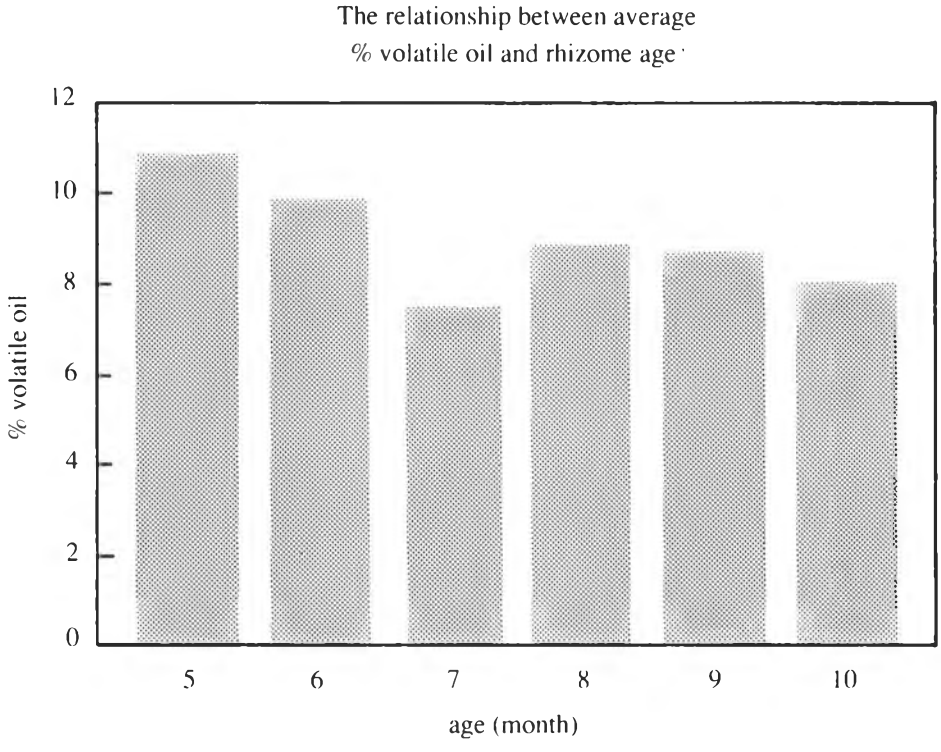


**FIGURE 1**


**FIGURE 2**

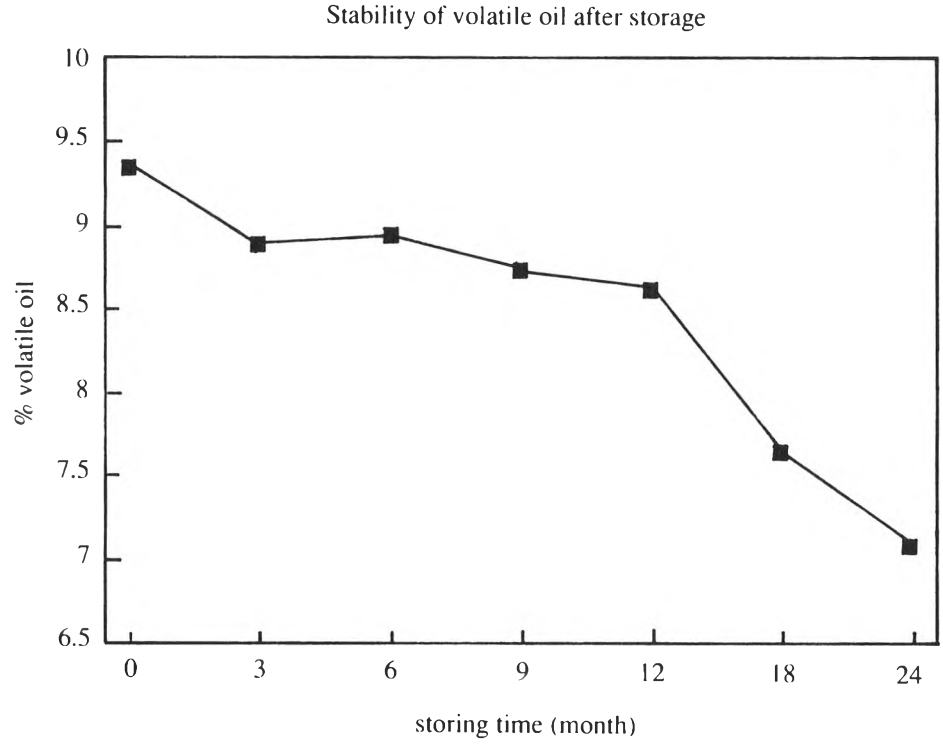


**FIGURE 3**

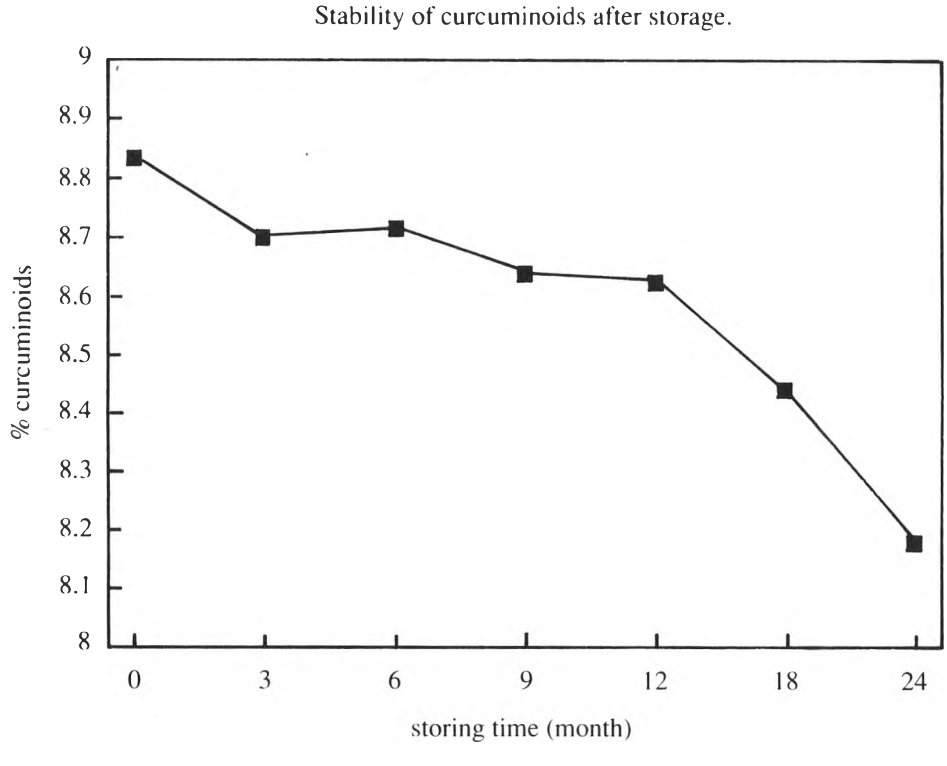


**FIGURE 4**

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# การเปลี่ยนแปลงปริมาณสารสำคัญของขมิ้นชัน ในระยะเวลาเก็บเกี่ยวต่าง ๆ

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**บทคัดย่อ**

*จากการศึกษาหาค่าปริมาณน้ำมันระเหยง่ายและปริมาณสารสำคัญของขมิ้นชันในระยะเวลาเก็บเกี่ยวที่ต่าง ๆ กันจากแปลงทดลองจังหวัดนครปฐมและจังหวัดประจวบคีรีขันธ์ พบว่าระยะเวลาที่เหมาะสมในการเก็บเกี่ยวคือ 5 เดือน และจากการศึกษาความคงตัวของสาร พบว่าปริมาณของน้ำมันระเหยง่ายและปริมาณของเตอร์คูมินอยด์จะลดลงเมื่อนำมาเก็บไว้ในห้องทดลองเป็นเวลานาน*

**กุญแจคำ** : ขมิ้นชัน อายุในการเก็บเกี่ยว น้ำมันระเหยง่าย เคอร์คูมินอยด์