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VARIATION OF TOTAL CURCUMINOIDS AND VOLATILE OIL CONTENT IN TURMERIC RHIZOME IN INDONESIA

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INTRODUCTION

Turmeric rhizome (*Curcuma longa* L., Zingiberaceae) has been used as a coloring agent in food and cosmetics for a long time (1). The bioactive substances in the turmeric rhizome are curcuminoids and volatile oil. Turmeric has been reported to possess numerous pharmacological activities such as anti-inflammatory, hepatoprotective, antitumor and antiviral activies (2). Additionally, the main biological activities of the turmeric oil are carminative, antiflatulence and antifungal (3). The content of total curcuminoids and volatile oil in the turmeric rhizome has important role for some pharmacological activities and effectiveness of the products. The contents of these compounds are varied based on geographic distribution (4). The Standard of ASEAN Herbal Medicine recommends that dried turmeric rhizome should contain volatile oil and total curcuminoid not less than 6.0 % v/w and 5.0 % w/w, respectively, while World Health Organization (WHO) recommends that dried turmeric should contain not less than 4.0 % v/w of turmeric oil and 3.0 % w/w of total curcuminoids (5, 6). Therefore, this study was designed to evaluate the amounts of total curcuminoids and volatile oil of turmeric rhizome collected from different regions of Indonesia. TLC-fingerprints of all samples were also performed.

MATERIALS AND METHODS

Chemicals and reagents: Standard curcumin, demethoxycurcumin and bisdemethoxycurcumin were isolated in our laboratory. The identification of this compound was performed using ^{1}H and ^{13}C NMR spectroscopy and comparing with the reference (7). All solvents used in this experiment were analytical grade.

Plant materials: The rhizomes of turmeric were collected from different locations in Indonesia during August, 2012 (Table 1). The samples were identified by Assoc. Prof. Dr. Wandee Gritsanapan of the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Thailand. The voucher specimens (WPCL0112-WCL0512) were deposited at the herbarium of the Faculty of Pharmacy, Siam University, Bangkok, Thailand. Fresh rhizome was cut into small pieces and dried in a hot air oven at 50°C for 24 hours, then ground into powder and passed through a sieve (20 mesh). The samples were kept in air-tight container and protected from light until used.

Table 1 Sources of turmeric rhizomes

No.	Code	Source
1	Ind 1	Bering Harjo market, Yogyakarta, Indonesia
2	Ind 2	Solo market, Solo, Indonesia
3	Ind 3	Kulonprogo, Yogyakarta, Indonesia
4	Ind 4	Bantul, Yogyakarta, Indonesia
5	Ind 5	Gunung Kidul, Yogyakarta, Indonesia

Determination of volatile oil content in turmeric powder (5, 6)**:** Ten grams of turmeric powder were weighed and put in a 500 ml round bottom flask. One hundred milliliters of distilled water were added. The mixture was distilled using Clevenger apparatus at a rate of 2 - 3 ml per min for 5 hours. The content of volatile oil was calculated as percentage in dried powder.

Determination of total curcuminoid content in turmeric powder (5, 6)**:** The content of total curcuminoids of all samples was evaluated using ultraviolet spectrophotometric method as recommended by the Standard of ASEAN Herbal Medicine. Briefly, stock standard solution of curcumin was prepared to make a final concentration of 400 µg/ml using methanol as the solvent. From this stock solution, different dilutions at concentrations of 0.8, 1.6, 2.0, 2.4 and 3.2 µg/ml were prepared and used for the preparation of the calibration curve. Sample solutions were prepared by separately transferring three hundred milligrams of the turmeric powder of each sample into a 10-ml volumetric flask. Tetrahydrofuran was added to volume and mixed. The mixture was set aside at room temperature for 24 hours with

frequent shaking. One milliliter of the clear supernatant liquid was transferred and diluted with methanol to a final volume of 25 ml. This solution (1 ml) was then transferred to a 50-ml volumetric flask, and adjusted to volume with methanol. The quantitative analysis of total curcuminoid content in each sample of turmeric was performed using spectrophotometer. The total curcuminoid content was calculated using a standard curve of curcumin.

TLC-fingerprint study: TLC fingerprints of turmeric sample collected from 5 in Indonesia were performed on a precoated aluminum plate of silica gel GF_{254} (10×20 cm). Dichloromethane:benzene:ethanol (49:49:2) were used as mobile phase. The developing distance was 8.0 cm. After removing the plate from the chamber, the plate was dried. The plate was sprayed with 10 %w/v phosphomolibdic acid in ethanol, followed by heating at 105 °C for 10 min. The *hRf* values of the main components were determined comparing with the *hRf* values of curcumin, demethoxycurcumin and bisdemethoxycurcumin standards.

RESULTS

Table 2 Total curcuminoid content and volatile oil content in dried powder of turmeric rhizome collected from different locations in Indonesia.

Code	Total curcuminoid content in dried powder (% w/w)	Volatile oil content in dried powder (% v/w)
Ind 1	1.30 ± 0.01	2.05 ± 0.08
Ind 2	3.63 ± 0.19	4.98 ± 0.00
Ind 3	4.13 ± 0.25	4.98 ± 0.00
Ind 4	4.79 ± 0.96	3.98 ± 1.40
Ind 5	4.09 ± 0.19	4.99 ± 0.01
Average	3.59 ± 6.29	4.20 ±3.45
Standard of ASEAN Herbal Medicine	5	6
WHO	3	4

Ind 1-Bering Harjo market, Yogyakarta, Indonesia
Ind 2-Solo market, Solo, Indonesia
Ind 3-Kulonprogo, Yogyakarta, Indonesia
Ind 4-Bantul, Yogyakarta, Indonesia
Ind 5-Gunung Kidul, Yogyakarta, Indonesia

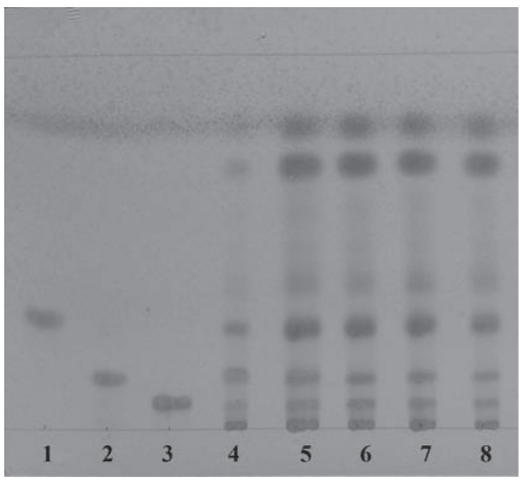

Figure 1 TLC fingerprints of the extracts of turmeric rhizomes collected from several locations of Indonesia
Absorbent : Silica gel 60 GF_{254}
Solvent system : Dichloromethane:benzene:ethanol (49:49:2)
Detector : Sprayed with 10 %w/v phosphomolibdic acid in ethanol, followed by heating at 105 °C for 10 min
Track 1 – Curcumin
Track 2 – Demethoxycurcumin
Track 3 - Bisdemethoxycurcumin
Track 4 - Bering Harjo market, Yogyakarta, Indonesia
Track 5 - Solo market, Solo, Indonesia
Track 6 - Kulonprogo, Yogyakarta, Indonesia
Track 7 - Bantul, Yogyakarta, Indonesia
Track 8 - Gunung Kidul, Yogyakarta, Indonesia

DISCUSSION

Identification of the isolated curcumin, demethoxycurcumin and bisdemethoxycurcumin were performed using ^{1}H NMR and ^{13}C NMR spectroscopy and comparison with the references (7). In this study, the limit of volatile oil in all turmeric samples was in the range of 2.05- 4.99 %v/w and the limit of total curcuminoids was 1.30-4.79 % dry weight. From Table 2, the average contents of total curcuminoids and volatile oil in dried powder of turmeric rhizome collected from different locations in Indonesia were found to be 3.59 ± 6.29 % w/w and 4.20 ±3.45 % v/w, respectively. High volatile oil contents were found in the samples from Solo market (4.98 %v/w), Kulonprogo (4.98 %v/w) and Gunung Kidul (4.99 %v/w), while low volatile oil content was found in the sample from Bering Harjo market (2.05 %v/w). High total curcuminoid content was found in the sample from Bantul (4.79 %v/w), while low curcuminoid content was found in the sample from Bering Harjo market (1.30 %v/w). The volatile oil contents in three out of five samples are within the limits recommended by WHO (not less than 4%v/w in dried powder). For total curcuminoid content, only one out of five samples was less than 3 %w/w of dried powder, which is lower than the amount recommended by WHO. In contrast, none of these samples passed the limits of volatile oil and total curcuminoids set by the Standard of ASEAN Herbal Medicine. TLC technique was used in fingerprint analysis of turmeric rhizome extract. TLC fingerprint of all samples from 5 locations were showed 3 main compounds, curcumin (Track 1), demethoxycurcumin (Track 2), and bisdemethoxycurcumin (Track 3). The system provided a good resolution of the curcumin, demethoxycurcumin and bisdemethoxycurcumin at hRf = 30, 15 and 6, respectively. TLC profiles of all extracts of turmeric rhizomes showed the same pattern (Figure 1).

CONCLUSION

These results indicate that turmeric rhizomes collected in Indonesia contain varying yields of bioactive constituents including the curcuminoids and volatile oil. These data are useful as basic information for the search for appropriate locations of high quality turmeric in Indonesia. It can also be used as a guideline for further standardization procedure of turmeric extracts for pharmaceutical productions and cosmetics.

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