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Original Article

Quantitative Analysis of Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin in the Crude Curcuminoid Extract from *Curcuma longa* in Thailand by TLC-Densitometry

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Abstract Curcuminoids, the principal natural yellow pigments comprising curcumin, demethoxycurcumin and bisdemethoxycurcumin, in *Curcuma longa* rhizome have been popularly used in drugs and cosmetics as potent antioxidants and coloring agents. This study, *C. longa* rhizomes were collected from 10 locations in the northern, northeastern, central and southern parts of Thailand. The powdered samples were continuously extracted using a soxhlet apparatus with hexane and 95% ethanol, respectively. Hexane was used to defat the sample. Curcuminoids, curcumin, demethoxycurcumin and bisdemethoxycurcumin, were isolated from the ethanol extract using column chromatography. Yields of crude curcuminoids in all samples were found in the range of 2.7-4.7% dry weight. Individual curcuminoid content was analyzed using a TLC-densitometry. The content of curcumin in all extracts was found in the range of 46.45 ± 3.21% to 67.31 ± 0.97% w/w while the contents of demethoxycurcumin and bisdemethoxycurcumin were found in the ranges of 11.47 ± 0.61% to 23.81 ± 0.28% w/w and 5.97 ± 0.41% to 13.88 ± 0.86% w/w, respectively. The highest average contents of curcumin (60.16 ± 3.23% w/w) and bisdemethoxycurcumin (12.84 ± 0.57% w/w) were found in the samples from the south while the highest average content of demethoxycurcumin (22.63 ± 1.33% w/w) was found in the samples from the north-east. In contrast, the lowest average contents of curcumin (53.65 ± 8.31% w/w) and demethoxycurcumin (16.23 ± 5.23% w/w) were found in the samples from the north, while the lowest content of bisdemethoxycurcumin (7.83 ± 0.28% w/w) was found in the samples from the central area. Total curcuminoid content in the crude curcuminoid extract was in the range of 67.13-96.25% w/w. This is the first report of each curcuminoid content in crude curcuminoid extract of *C. longa* from various locations of Thailand and will provide a useful guidance for further standardization of curcuminoid extracts used in pharmaceutical products and cosmetics.

Keywords: bisdemethoxycurcumin, *Curcuma longa*, curcumin, curcuminoid extract, demethoxycurcumin

INTRODUCTION

Thailand is a country endowed with a variety of medicinal plants with strong potential for therapeutic applications. *Curcuma longa* Linn. or turmeric is one of the most popular medicinal herbs[1], which is listed as one of the product champions of Thailand. It has been used for thousand years as a spice, coloring agent in foods, household medicine and insect repellent.[2] Recently, *C. longa* is widely used as a nutritional supplement and coloring agent in drugs and cosmetics. It has been found to be a rich source of polyphenolic curcuminoids, i.e. curcumin, demethoxycurcumin and bisdemethoxycurcumin[3] (Figure 1). Several biological activities of turmeric have been studied both *in vitro* and *in vivo*. Curcuminoids

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are well known for their antioxidant, anti-inflammatory, antitumour and cytotoxic properties.[4-7]

In Thailand, *C. longa* is cultivated throughout the country, mostly in the south.[8] Thai Herbal Pharmacopoeia and the Standard of ASEAN Herbal Medicine have recommended that dried turmeric should contain not less than 5% w/w of total curcuminoids.[8,9] It has also been reported that turmeric from various regions in Thailand had a high variation of curcuminoids and volatile oil contents,[10-12] but there is no report concerning the amount of each curcuminoid in the crude curcuminoid extracts.

At present, a variety of methods for quantitative analysis of curcuminoid content were reported. Most of them are spectrophotometric methods.[13-15] Disadvantages of this method are that it is not possible to analyze individual curcuminoids and the precision is not good due to some interferences by other pigments presented in the extract. A rapid and simple TLC-densitometric method has been developed for the simultaneous quantitation of curcumin, demethoxycurcumin and bisdemethoxycurcumin in *C. longa* powder. The accuracy and precision of this method were reported to be reliable.[10,11,16]

Thus, this study was undertaken to determine the amount of each curcuminoid in the crude curcuminoid extracts of *C. longa*, collected from different locations in Thailand by TLC-densitometric method.

MATERIALS AND METHODS

Chemicals and Reagents

Curcumin, demethoxycurcumin and bis-demethoxycurcumin were isolated in our laboratory from the ethanolic extract of *C. longa.* All chemicals and reagents used were analytical grade, except ethanol which was the commercial grade obtained from the Excise Department, Bangkok, Thailand.

Plant Material

The rhizomes of *C. longa* were collected from 10 different locations in the north, north-east, south and central area of Thailand (Figure 2) during January - April 2005. The samples were identified by comparison with the specimens at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok. The voucher specimens (WCL0105-WCL1005) were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

Fresh rhizomes were cleaned, cut into small pieces and air-dried for 2 days. The samples were then further dried in a hot air oven at 50°C for 24 hours, ground into powder and passed through a sieve (20 mesh).

Extraction and Isolation of Reference Standards

Dried powder of *C. longa* collected from Surat Thani Province (10.0 g) was extracted with 95% ethanol (600 ml) using a soxhlet apparatus for 56 hours.[17] The ethanol extract was dried using a rotary evaporator and yielded crude ethanol extract (2.56 g). The dried ethanolic extract (1 g) was further fractionated by silica gel 60 column chromatography (17.5 x 2.5 cm) eluted with hexane, hexane : dichloromethane and then dichloromethane : ethyl acetate with increasing polarity. Fractions containing curcumin (fractions 10-50, 200 ml) were refractionated by the column chromatography eluted with 100% dichloromethane to yield pure curcumin

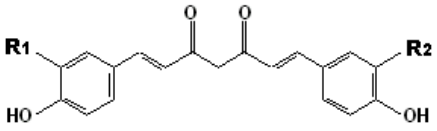
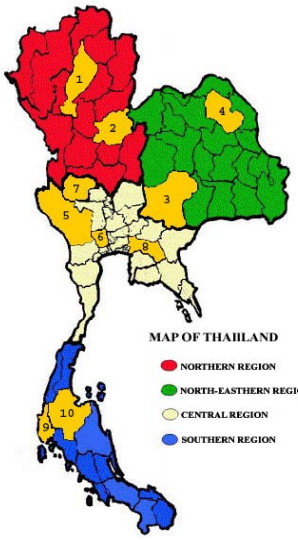


Figure 1. Chemical structure of curcuminoids.

Compounds	R_1	R_2
Curcumin	OMe	OMe
Demethoxycurcumin	H	OMe
Bisdemethoxycurcumin	H	H



1 =	Tambol Wang Tai, Amphoe Wang Nuea, Lampang	North
2 =	Tambol Noen Kum, Amphoe Bangkrathum, Phitsanulok	North
3 =	Tambol Wang Mi, Amphoe Wang Nam Khiao, Nakhon Ratchasima	North-East
4 =	Tambol Kut Bak, Amphoe Kut Bak, Sakon Nakhon	North-East
5 =	Tambol Tha Sao, Amphoe Sai Yok, Kanchanaburi	Central
6 =	Tambol Mungtarod, Amphoe Muang, Nakhon Pathom	Central
7 =	Tambol Ban-Tai, Amphoe Ban rai, Uthai Thani	Central
8 =	Tambol Khao Hin Son, Amphoe Phanom Sarakham, Chachoengsao	Central
9 =	Tambol Tham Thong Lang, Amphoe Thap Put, Phangnga	South
10 =	Tambol Khao Wong, Amphoe Ban Ta Khun, Surat Thani	South

Figure 2. Ten different locations of Thailand where *C. longa* was collected.

Fractions containing demethoxycurcumin and bisdemethoxycurcumin were further eluted with dichloromethane : ethyl acetate (95:5) in a column chromatography. Curcumin, demethoxycurcumin and bisdemethoxycurcumin were recrystallized and yielded 103.2, 43.3 and 86.0 mg, respectively.

Identification of the compounds was characterized using ^{1}H NMR and ^{13}C NMR comparing to references.[18] Melting point of each curcuminoid was also investigated and compared with the reference.[19]

Extraction of Crude Curcuminoids[20]

The dried powder (15.0 g) of each sample was extracted with hexane (300 ml) using a soxhlet apparatus for 20 hours. The hexane extract was evaporated at a reduced pressure to separate volatile oil. The marc was further extracted with 95% ethanol using a soxhlet apparatus for 10 hours, two times, and the ethanol extracts were combined and filtered. The filtrate was concentrated under reduced pressure at 50°C using a rotary vacuum evaporator. The concentrated extract was evaporated on a boiling water bath to yield crude curcuminoids. The crude extract was further purified by dissolving in 95% ethanol, 1:6 w/v. The yellow curcuminoids were precipitated, filtered and dried. The extraction of each sample was done in triplicate and the yield was reported as mean ± S.D.

Preparation of Standard Solutions and the Calibration Curves

Stock solutions of individual curcuminoid standards were separately prepared in methanol at 1.0 mg/ml. One milliliter of the stock solution was transferred to a 10-ml volumetric flask and adjusted to volume with methanol.

Calibration curves of curcumin, demethoxycurcumin and bisdemethoxycurcumin were derived from separately applying five concentrations of each curcuminoid on the TLC plate to obtain final amounts of 100-1,200, 150-800 and 200-1,000 ng/spot, respectively.

The amount of each curcuminoid presented in the sample was calculated using peak area with linear regression. Linearity, reproducibility and accuracy of each curcuminoid were determined.

Preparation of Sample Solutions

For sample preparation, five milligrams of each crude curcuminoid extract were transferred to a 10 ml volumetric flask. The sample was dissolved in methanol and adjusted to a concentration of 0.5 mg/ml.

Instrumentation and Analytical Condition

Four microliters of each sample solution were spotted as a band width of 6.0 mm on a precoated silica gel aluminium plate $60F_{254}$ (20 × 10 cm; E. Merck, Germany) using a Camag Linomat 5 syringe. The following conditions were employed: application rate, 150 nl/s; space between each band, 13.0 mm; slit dimension, 5.00 mm × 0.45 mm; and scanning speed, 20 mm/s. The mobile phase consisting of chloroform : benzene : methanol (80:15:5) was used. Linear ascending development was carried out in 20 × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The length of each chromatogram run was 8 cm. After developing, the TLC plate was dried using an air dryer. Densitometric scanning was performed on Camag TLC Scanner 3 in the reflectance-absorbance mode at 420 nm, operated by CATS software (V 1.2.6, Camag). The source of radiation utilized was a tungsten lamp. Video densitometry of the TLC chromatogram was carried out with the help of Camag Reprostar 3 with cabinet cover and mounted digital camera.

Determination of Individual Curcuminoid Content

A volume of each sample solution was applied in a triplicate manner on TLC plate and analyzed by the proposed method. Each curcuminoid content was calculated using its calibration curve. The contents of curcumin, demethoxycurcumin and bisdemethoxycurcumin were expressed as the amount in gram per 100 grams of the extract.

RESULTS AND DISCUSSION

Purified curcumin, demethoxycurcumin and bisdemethoxycurcumin showed single spots on TLC (Si-$60GF_{254}$, chloroform : benzene : methanol = 80:15:5) after detecting by UV 366 (Figure 3). The Rf values of these compounds were investigated as shown in Table 1. By NMR, Rf values and melting points, the compounds were confirmed to be curcumin, demethoxycurcumin and bis-demethoxycurcumin.

Linearity of curcumin was found in the concentration range of 100-1,200 ng/spot while linearity of demethoxycurcumin and bis-demethoxycurcumin were found in the concentration ranges of 150-800 and 200-1,000 ng/spot, respectively with high reproducibility and accuracy.

The linear regression equations with correlation coefficient (r^2) of curcumin, demethoxy-curcumin and bisdemethoxycurcumin were given in Table 1.

From TLC-densitometric chromatogram (Figure 4), it was found that a major peak in the crude curcuminoid extracts of all samples was curcumin. The other two minor peaks were demethoxycurcumin and bisdemethoxy-curcumin. The identification was done by spiking with their standards and by determination of the Rf values. Chloroform : benzene : methanol (80:15:5) was found to be a suitable mobile phase for separation of curcumin, demethoxycurcumin and bis-demethoxycurcumin (Figure 3). Wavelength at λ max 420 nm which was used to analyze

Table 1. Linear regression equation, correlation coefficient (r^2), Rf value and melting point (m.p.) of each curcuminoid

Curcuminoids	Equation	r^2	Rf (cm)	m.p. (°C)
Curcumin	Y = 31.233X-1910.30	0.9959	0.69 ± 0.02	180-181
Demethoxycurcumin	Y = 32.716X+3204.10	0.9962	0.51 ± 0.02	160-161
Bisdemethoxycurcumin	Y = 14.386X+7340.50	0.9953	0.39 ± 0.02	220-221

each curcuminoid content in the samples is the same wavelength recommended by Thai Herbal Pharmacopoeia [9] and a previous study.[10]

Curcumin content in all extracts was found in the range of 46.45 ± 3.21% to 67.31 ± 0.97% w/w while the content of demethoxycurcumin and bisdemethoxycurcumin were found in the ranges of 11.47 ± 0.61% to 23.81 ± 0.28% w/w and 5.97 ± 0.41% to 13.88 ± 0.86% w/w, respectively. The average contents of these curcuminoids in the extracts were found to be 58.12 ± 6.46%, 18.48 ± 3.94% and 10.29 ± 2.72% w/w for curcumin, demethoxycurcumin and bisdemethoxycurcumin, respectively. The highest average contents of curcumin (60.16 ± 3.23% w/w) and bisdemethoxycurcumin (12.84 ± 0.57% w/w) were found in the samples from the south while the highest average content of demethoxycurcumin (22.63 ± 1.33% w/w) was found in the samples from the north-east. In contrast, the lowest average contents of curcumin (53.65 ± 8.31% w/w) and demethoxycurcumin (16.23 ± 5.23% w/w) were found in samples from the north, while the lowest average content of bisdemethoxy-curcumin (7.83 ± 0.28% w/w) was found in samples from the central area (Table 2).

From the results, *C. longa* samples from the south of Thailand where it rains the whole year and from the north-east where the weather is warm in summer and cool in winter, should be selected for the extraction of curcuminoids due to high total curcuminoid contents (average 89.72 ± 4.81% and 93.02 ± 2.55% w/w, respectively). These results support the former reports that *C. longa* grown in the southern part of Thailand contains high content of curcuminoids.[12,21,22]

CONCLUSION

C. longa grown in different parts of Thailand contains different amounts of various curcuminoids. Therefore, for obtaining the high yield of certain curcuminoid, the area for plant collection should be considered. This is the first report of each curcuminoid content in crude curcuminoid extract of *C. longa* from various locations of Thailand and it will provide a useful guidance for further standardization of curcuminoid extracts used in pharmaceutical products and cosmetics.

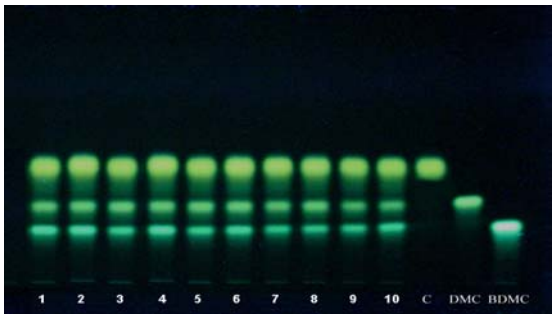


Figure 3. TLC fingerprints of curcuminoids in the extracts of *C. longa* collected from various locations. Stationary phase: Si-60GF_{254}; solvent system: $CHCl_3$: C_6H_6 : MeOH (80:15:5); detection: UV 366.

1 = Tambol Wang Tai, Amphoe Wang Nuea, Lampang
2 = Tambol Noen Kum, Amphoe Bangkrathum, Phitsanulok
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10 = Tambol Khao Wong, Amphoe Ban Ta Khun, Surat Thani
C = curcumin, DMC = demethoxycurcumin and BDMC = bisdemethoxycurcumin

Table 2. The content of each curcuminoid in crude curcuminoid extracts of *C. longa* from different locations of Thailand analyzed by TLC-densitometry

Location	Code	Yield of crude curcuminoids in dried powder (%)*	Each curcuminoid content (% w/w in extract)*						Total curcuminoid (% w/w in extract)	
			C	Average	DMC	Average	BDMC	Average	Content	Average
North	1	4.03 ± 0.25	60.84 ± 2.70	53.65 ± 8.31[4]	20.98 ± 0.33	16.23 ± 5.23[4]	13.18 ± 0.06	11.20 ± 0.35[3]	95.00 ± 3.07	81.07 ± 15.54[4]
(N)	2	3.05 ± 0.41	46.45 ± 3.21		11.47 ± 0.61		9.21 ± 0.64		67.13 ± 3.40	
North-East	3	4.59 ± 0.57	55.46 ± 0.51	58.60 ± 3.47[3]	21.45 ± 0.39	22.63 ± 1.33[1]	13.88 ± 0.86	11.79 ± 0.67[2]	90.79 ± 0.78	93.02 ± 2.55[1]
(NE)	4	2.98 ± 0.42	61.74 ± 0.52		23.81 ± 0.28		9.69 ± 0.48		95.24 ± 0.86	
	5	2.74 ± 0.11	64.42 ± 0.73		21.66 ± 0.35		10.17 ± 0.04		96.25 ± 0.72	
Central	6	3.10 ± 0.11	67.31 ± 0.97	59.10 ± 7.19[2]	21.51 ± 0.29	18.40 ± 3.64[2]	5.97 ± 0.41	7.83 ± 0.28[4]	94.78 ± 1.57	85.32 ± 10.81[3]
(C)	7	4.10 ± 0.10	52.27 ± 1.30		13.38 ± 1.25		7.76 ± 0.07		73.40 ± 2.30	
	8	3.17 ± 0.42	52.41 ± 0.65		17.06 ± 0.46		7.40 ± 0.59		76.86 ± 1.06	
South	9	4.72 ± 0.88	57.63 ± 2.58	60.16 ± 3.23[1]	15.89 ± 0.75	16.73 ± 1.08[3]	12.23 ± 0.51	12.84 ± 0.57[1]	85.75 ± 3.16	89.72 ± 4.81[2]
(S)	10	4.13 ± 0.06	62.69 ± 0.38		17.57 ± 0.45		13.44 ± 0.63		93.70 ± 0.60	
Average		3.66 ± 0.73	58.12 ± 6.46		18.48 ± 3.94		10.29 ± 2.72		86.89 ± 10.42	

1 = Tambol Wang Tai, Amphoe Wang Nuea, Lampang

2 = Tambol Noen Kum, Amphoe Bangkrathum, Phitsanulok

3 = Tambol Wang Mi, Amphoe Wang Nam Khiao, Nakhon Ratchasima

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8 = Tambol Khao Hin Son, Amphoe Phanom Sarakham, Chachoengsao

9 = Tambol Tham Thong Lang, Amphoe Thap Put, Phangnga

10 = Tambol Khao Wong, Amphoe Ban Ta Khun, Surat Thani

C = curcumin, DMC = demethoxycurcumin and BDMC = bisdemethoxycurcumin

[1….4] = ordering, maximum to minimum content

*Extraction and analysis of each sample were done in triplicate and % contents were expressed as mean ± S.D.

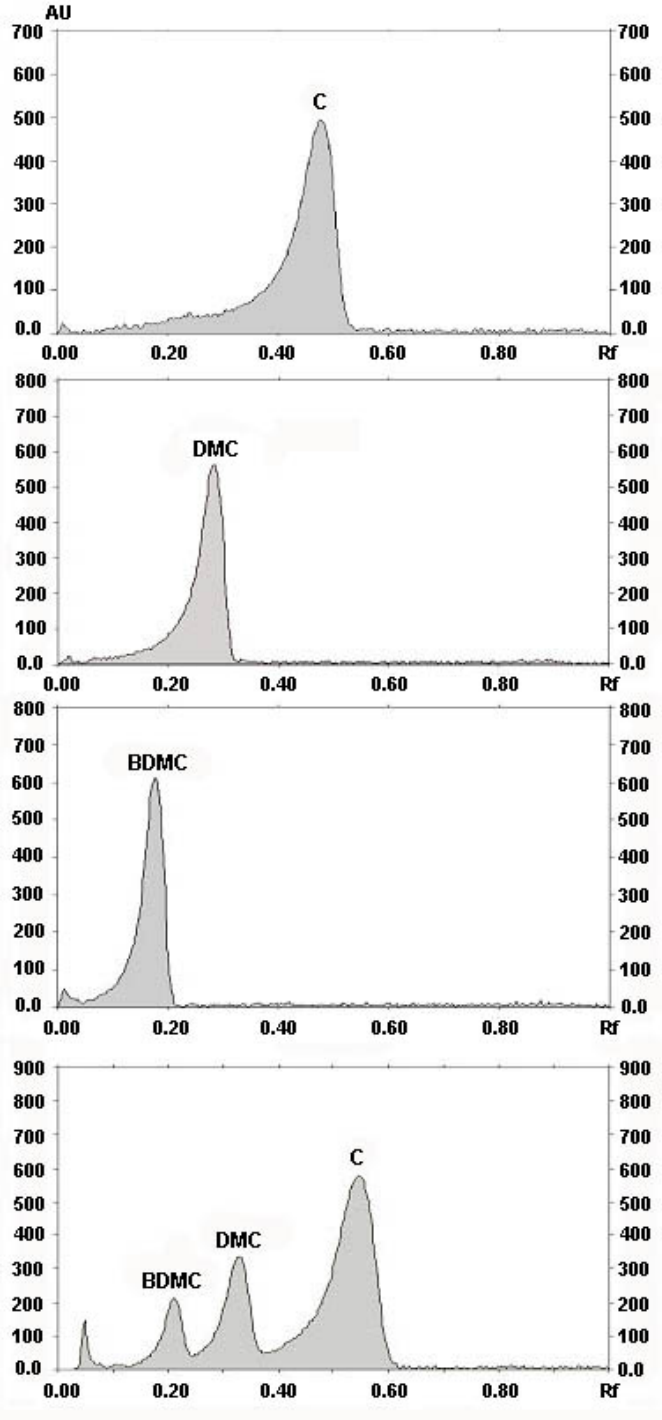

Figure 4. Densitometric thin layer chromatograms of crude curcuminoid extract of *C. longa* from various locations (at λ = 420 nm).
C = curcumin, DMC = demethoxycurcumin and BDMC = bisdemethoxycurcumin

ACKNOWLEDGEMENTS

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