QUANTITATIVE ANALYSIS OF TOTAL MANGOSTINS IN *GARCINIA MANGOSTANA* FRUIT RIND

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ABSTRACT: The fruit of *Garcinia mangostana* Linn. (mangosteen) is very popular in Thailand. The fruit rind contains mangostins of which a major constituent is α-mangostin. The fruit rind extract and mangostin have been known to possess antibacterial causing acne. In Thailand, the extract is popularly used in herbal cosmetics for anti-acne effect. Thus quality assessment of this plant needs to be controlled for the limit of mangostin content. This study was undertaken to evaluate the content of total mangostins in the dried powder and the ethanolic extract of the fruit rinds of *G. mangostana* collected from 13 locations from the East and the South of Thailand. The UV-spectrophotometric method was validated for linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ). The linearity was found over the range of 2-20 μg/ml with regression coefficient (r^2) 0.9999. Intra- and interday precisions showed relative standard deviation (%RSD) less than 2 %. Accuracy of the method was determined by a recovery study conducted at 3 different levels, and the average recovery was found to be 100.68 %. The LOD and LOQ were 0.1622 and 0.4915 μg/ml, respectively. The total mangostin contents in all dried powder samples were in the range of 8.51 ± 0.05 to 11.50 ± 0.02 % w/w while in the crude ethanolic extracts were 30.19 ± 0.16 to 45.61 ± 0.09 % w/w. The average content of total mangostins (10.39 ± 1.04 % of the dried powder) was higher in samples from the South where it rains all year. The averages of total mangostin contents in all dried powder samples and in the ethanolic extracts were found to be 9.94 ± 0.88 and 36.25 ± 4.66 % w/w, respectively. The proposed UV-spectrophotometric method was found to be simple, rapid, and suitable for routine quality control of raw material of *G. mangostana* fruit rind and its extract. This information will be useful as a guidance for standardization of *G. mangostana* fruit rind and the extracts, and finding appropriate sources of high total mangostins content for good quality of *G. mangostana* raw materials in Thailand.

Key words: *Garcinia mangostana*, mangosteen, α-mangostin, Guttiferae, UV-spectrophotometry

INTRODUCTION: Mangosteen (*Garcinia mangostana* Linn.) is a tropical fruit tree of the family Guttiferae cultivated in Southeast Asian countries. In Thailand, the fruit of this plant is very popular and has been known as the "Queen of fruits". The main sources for growing mangosteen in Thailand are located in the eastern and southern parts of the country. The fruit rind of this plant has been used in Thai traditional medicine for treatment of diarrhea and skin infections[1]. It contains tannins and xanthones i.e. alpha-, beta- and gamma-mangostins (Fig. 1)[2-4]. Alpha-mangostin is a major component which possess anti-inflammatory[5] and antibacterial activities against methicillin-resistant *Staphylococcus aureus*, *S. epidermidis,* and *Propionibacterium acnes* which is the critical eticologic agent in acne[6-7]. It was also reported to inhibit alveolar duct formation in a mouse mammary organ culture model and to suppress the carcinogen induced formation of aberrant crypt foci in a short-term colon carcinogenesis model[8-9]. In Thailand, mangosteen fruit extract is popularly used as a food supplement while the fruit rind extract has been used in herbal cosmetics and pharmaceutical products. Thus, mangostin content in the fruit rind extract and its product is needed to be controlled. Various analytical methods for quantitative analysis of α-mangostin have been reported such as gas chromatography (GC) and high performance liquid chromatography (HPLC)[10-12]. However, these methods need expensive equipment, long time comsuming and complicate procedures for the preparation of sample and standard solutions.

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According to WHO guidelines, Thai Herbal Pharmacopoeia (THP) and the Standard of ASEAN Herbal Medicine, a UV-spectrophotometric method is the official method for determination of active compounds in many medicinal plants such as *Curcuma longa*, *Cassia alata*, *Aloe vera, Panax ginseng* and *Andrographis paniculata*[13-15]. In this study, we report a simple, rapid and inexpensive UV-spectrophotometric method for simultaneous quantification of total mangostins content in the dried powder and the ethanolic extracts of *G. mangostana* fruit rinds collected from 13 locations from the East and the South of Thailand. The results will be useful as a database of medicinal plant of Southeast Asian countries and also as a basis in further standardization of mangosteen fruit rind and its extract that have not been reported.

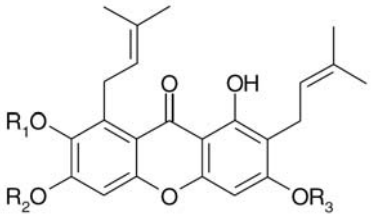

Fig. 1 Chemical structures of mangostins
Alpha-mangostin : $R_1 = CH_3$, $R_2 = R_3 = H$
Beta-mangostin : $R_1 = R_3 = CH_3$, $R_2 = H$
Gamma-mangostin : $R_1 = R_2 = R_3 = H$

MATERIALS AND METHODS:

Chemicals and Reagents

Alpha-mangostin (purity 97%) was purchased from Chroma Dex Inc. (Santa Ana, CA). The other chemicals and solvents used in this experiment were analytical grade, which were purchased from Labscan Asia (Bangkok, Thailand), except the 95 % ethanol which was obtained from the Excise Department, Bangkok, Thailand and was distilled before use.

Plant Materials

The ripe fruits of *G. mangostana* were collected from 13 different locations of Thailand (Table 1) during June - August 2006. The samples were identified by Dr. W. Gritsanapan, Faculty of Pharmacy, Mahidol University. The voucher specimens (WGM0106 - WGM1306) were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

The samples were cleaned and the edible aril parts were removed. The fruit rinds were cut into small pieces and dried in a hot oven at 50 °C for 72 hours. The dried samples were ground into powder, passed through a sieve (20 mesh). Each powdered sample was separately kept in an air tight container protected from light until used.

Extraction of *G. mangostana* Fruit Rind Extract

Ten grams of each sample was placed into a trimble and was extracted with 400 ml of 95 % ethanol in a soxhlet apparatus. Extraction was carried out for 15 h with approximate 5 cycles/h. Each extract was filtered through a Whatman no. 1 filter paper. The filtrate was concentrated under reduced pressured at 50 °C using a

Table 1 *Garcinia mangostana* fruit collected from different locations in Thailand

Sample No.	Code	Source	Region
1	GM01	Tambol Thung Khwai Kin, Amphoe Klaeng, Rayong	East
2	GM02	Tambol Nong Taphan, Amphoe Ban Khai, Rayong	East
3	GM03	Tambol Phlapphla, Amphoe Mueang, Chanthaburi	East
4	GM04	Tambol Song Phi Nong, Amphoe Tha Mai, Chanthaburi	East
5	GM05	Tambol Wang Krachae, Amphoe Mueang, Trat	East
6	GM06	Tambol Khao Saming, Amphoe Mueang, Trat	East
7	GM07	Tambol Thung Nonsi, Amphoe Khao Saming, Trat	East
8	GM08	Amphoe Lang Suan, Chumphon	South
9	GM09	Tambol Ratchakrut, Amphoe Mueang, Ranong	South
10	GM10	Tambol Khao Wong, Amphoe Ban Ta Khun, Surat Thani	South
11	GM11	Tambol Tha Di, Amphoe Lan Saka, Nakhon Si Thammarat	South
12	GM12	Amphoe Panare, Pattani	South
13	GM13	Amphoe Sukhirin, Narathiwat	South

rotary vacuum evaporator. The extraction of each sample was done in triplicate.

TLC Analysis of the Extracts of Mangosteen Fruit Rinds from 13 Locations

TLC fingerprints were performed on a precoated aluminium plate of silica gel $60F_{254}$ (10×10 cm) using chloroform : ethyl acetate : methanol (80:10:5) as a mobile phase. The developing distance was 8.0 cm. After removing the plate from the chamber, the plate was dried using an air dryer and sprayed with 10 % sulfuric acid in ethanol, followed by heating at 110°C for 10 min. The plate was examined under ultraviolet light (366 nm). The hR_f value of main component was determined comparing with the hR_f value of α-mangostin reference standard. Video densitometry of the chromatoplate was carried out using CAMAG Reprostar 3 with cabinet cover and mounted digital camera.

Instrumentation and Analytical Condition

The UV method was performed using Perkin-Elmer spectrophotometer at 320 nm using a 1.0 cm quartz cell. Software UV Winlab was used for all absorbance measurements.

Preparation of Standard Solutions

A stock solution of α-mangostin reference standard was prepared by dissolving an accurately weighed 10 mg of α-mangostin in 100 ml of methanol in a volumetric flask. From this solution, various concentrations of the standard solution were prepared in 10 ml of methanol in a volumetric flask to obtain final concentrations at 20, 16, 8, 4, and 2 µg/ml.

Preparation of Sample Solutions

Each sample dried extract (10 mg) was accurately weighed and transferred to a 10 ml volumetric flask. Methanol was added to volume (final concentration 1,000 µg/ml). Aliquot of the solution (500 µl) was diluted with methanol in a 10 ml volumetric flask to make a concentration of 50 µg/ml.

Validation Method

Validation of the analytical method was done according to the International Conference on Harmonization (ICH) guidelines[16]. The method was validated for linearity, precision, accuracy, LOD and LOQ.

Linearity

Linearity was determined by using five concentrations of the standard solution (2 - 20 µg/ml). The calibration curve was obtained by plotting the absorbances versus the concentrations of the standard solution.

Precision

The precision of the method was determined by analyzing 4, 12, and 20 µg/ml concentrations of α-mangostin standard solution (n = 3) on the same day for intraday precision and on 3 different days for interday precision by the propose method. The precision was expressed as percent relative standard deviation (% RSD).

Accuracy

The accuracy of the method was tested by performing recovery studies at 3 levels of α-mangostin reference standard added to the sample. Three different volumes (0.4, 0.6, and 1 ml) of the standard solution (containing 100 µg/ml of α-mangostin in methanol) were added to the sample solution (10 µg/ml) and analyzed by the UV spectrophotometric method. The percentage recovery as well as the average percentage recovery was calculated. Three determinations were carried out for each level of concentration.

Limit of Detection and Limit of Quantitation

According to the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) recommendations, the approach based on the S.D. of the response and the slope were used for determining the limit of detection and limit of quantitation.

RESULTS AND DISCUSSION: TLC-chromatograms of the extracts of mangosteen fruit rinds from 13 locations showed similar fingerprints. They were found to contain six fluorescent bands of which hR_f values were found to be 18.07, 27.71, 39.76, 50.00, 61.45 and 67.47. The major component of all samples of *G. mangostana* fruit rind extracts was found to be α-mangostin at hRf value 50.00 (Fig. 2).

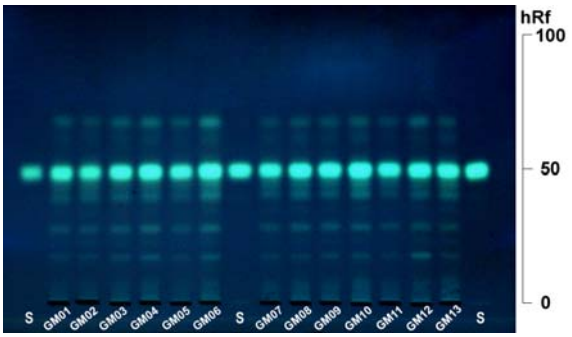

Fig. 2 TLC chromatograms of *G. mangostana* fruit rind extracts

Absorbent: silica gel 60 GF_{254}; Solvent system: chloroform / ethyl acetate / methanol (80:10:5, v/v); Detector: spray with 10% sulfuric acid in ethanol, heat at 110 °C for 10 min and observed under UV 366, S = α-mangostin standard

A UV spectrum of α-mangostin solution in methanol showed absorption peaks at 206, 243, and 320 nm. The wavelength at 320 nm was used for all measurements due to no interference from solvent absorbance. The method was validated for its linearity, accuracy, precision, limit of detection and limit of quantitation. A good linear relationship was obtained within the concentration range of 2-20 μg/ml of α -mangostin with a correlation coefficient (r^2) of 0.9999. The representative linear equation was y = 0.0512x + 0.0017. The LOD and LOQ of α -mangostin were 0.1622 and 0.4915 μg/ml, respectively (Table 2). The intraday and interday precisions of α -mangostin are given in Table 3. The results showed acceptable precision with the method as revealed by % RSD less than 2 %. The percentage recovery at 3 different levels of α-mangostin was found to be 101.17, 100.18, and 101.10 with an average of 100.82 % (Table 4), which indicates good accuracy of the method.

Thirteen samples of *G. mangostana* fruit rind were collected during the harvesting period, June - August 2006. The contents of total mangostins, calculated as α-mangostin, in all of the ethanolic extracts were ranged from 30.19 ± 0.16 to 45.61 ± 0.09 % w/w, while in the dried powder were 8.51 ± 0.05 to 11.50 ± 0.02 % w/w. The average content of total mangostins in all samples in the crude extracts and the dried powder were found to be 36.25 ± 4.66 % w/w and 9.94 ± 0.88 % w/w, respectively. The results showed that the samples from the South contained a higher yield of total mangostins (36.92 ± 5.55 % w/w in the extract and 10.39 ± 1.04 % w/w in the dried powder) than the samples from the East (35.68 ± 3.79 % w/w in the extract and 9.55 ± 0.45 % w/w in the dried powder) (Table 5).

The quantitative results determined by the UV-spectrophotometric method showed high amount of α-mangostin. This might be because the absorbance measured by this method was interfered from other compounds in the extract at wavelength 320 nm. The UV-spectrophotometric method has some advantages over the other analytical procedures such as high performance liquid chromatography (HPLC) and TLC-

Table 2 Method validation parameters for quantitative analysis of total mangostins in term of α-mangostin by the proposed UV-spectrophotometric method

Parameters	Results
Range of linearity	2-20 μg/ml
Regression equation [a]	y = 0.0512x + 0.0017
Correlation coefficient (r^2)	0.9999
LOQ	0.4915 μg/ml
LOD	0.1622 μg/ml

[a] x is the concentration of α-mangostin in μg/ml, y is the absorbance at 320 nm

Table 3 Intraday and interday precisions of α-mangostin by the proposed UV-spectrophotometric method (n = 3)

Concentration (μg/ml)	Intraday precision (% RSD)	Interday precision (% RSD)
4	0.07	0.60
12	0.01	0.47
20	0.03	0.85

Table 4 Recovery study of α-mangostin in the proposed UV-spectrophotometric method (n = 3)

Serial no.	Amount present in the extract, (μg/ml)	Amount added, (μg/ml)	Amount found[a], (μg/ml)	Recovery[a] (%)
1	3.33	4.00	7.38 ± 0.02	101.17 ± 0.68
2	3.33	6.00	9.34 ± 0.04	100.18 ± 0.60
3	3.33	10.00	13.44 ± 0.05	101.10 ± 0.55
		Average		100.82

[a] expressed as mean ± SD (n = 3)

Table 5 Content of total mangostins in *G. mangostana* fruit rinds collected from different locations in Thailand

Sample No.	Region	Yield of crude extract (% dry weight) [a]	Total mangostin content (% w/w) [a]			
			In extract	Average	In dried powder	Average
1	East	27.34 ± 0.29	34.05 ± 0.14	35.68 ± 3.79	9.23 ± 0.04	9.55 ± 0.45
2		34.13 ± 1.58	30.19 ± 0.16		9.96 ± 0.05	
3		25.94 ± 2.16	37.83 ± 0.19		9.23 ± 0.05	
4		27.08 ± 0.97	38.96 ± 0.05		10.29 ± 0.01	
5		26.03 ± 1.41	36.36 ± 0.13		9.82 ± 0.03	
6		23.69 ± 1.32	40.99 ± 0.05		9.35 ± 0.01	
7		28.84 ± 0.23	31.36 ± 0.17		9.00 ± 0.05	
8	South	24.00 ± 0.08	45.61 ± 0.09	36.92 ± 5.55	10.99 ± 0.02	10.39 ± 1.04
9		26.83 ± 0.45	31.39 ± 0.20		8.51 ± 0.05	
10		32.47 ± 0.80	34.87 ± 0.18		11.12 ± 0.06	
11		28.11 ± 1.25	35.63 ± 0.05		9.69 ± 0.01	
12		25.08 ± 0.50	42.58 ± 0.22		10.52 ± 0.05	
13		36.00 ± 0.80	31.42 ± 0.07		11.50 ± 0.02	
Average		**28.12 ± 3.83**	**36.25 ± 4.66**		**9.94 ± 0.88**	

[a] expressed as mean ± SD (n = 3)

densitometry in terms of simple instrumentation, simple pretreatment of samples, low cost and less time consuming. However, some disadvantages were found such as lower precision and sensitivity of the method compared to HPLC, and disability in analysis of individual component in the samples.

CONCLUSION: The proposed UV-spectrophotometric method was found to be simple, rapid, sensitive and suitable for routine quality control of raw material of *G. mangostana* fruit rind and its extract. The information of total mangostin content will be useful as a guidance for further standardization of *G. mangostana* fruit rind powder and the extracts for pharmaceutical and cosmetic uses, and finding sources of good quality of *G. mangostana* fruit rind raw material in Thailand.

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ความหลากหลายของปริมาณรวมของสารแมงโกสตินในเปลือกผลมังคุด

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บทคัดย่อ: มังคุดเป็นผลไม้ที่นิยมบริโภคในประเทศไทยกันอย่างแพร่หลาย เปลือกผลมังคุดประกอบด้วยสารจำพวกแมงโกสติน โดยมีสารอัลฟา-แมงโกสติน เป็นสารหลัก ซึ่งสารดังกล่าว และสารสกัดเปลือกผลมังคุด มีฤทธิ์ยับยั้งแบคทีเรียที่ทำให้เกิดสิว ทำให้ในปัจจุบันสารสกัดเปลือกผลมังคุดถูกนำมาใช้ผลิตเป็นยารักษาสิว ดังนั้นจึงควรมีการควบคุมคุณภาพของสกัดเปลือกผลมังคุด โดยการกำหนดปริมาณขั้นต่ำของสารอัลฟา-แมงโกสตินในสารสกัด งานวิจัยนี้ได้ทำการวิเคราะห์หาปริมาณสารอัลฟา-แมงโกสติน ในเปลือกผลมังคุดแห้ง และในสารสกัดเอทานอล ของเปลือกผลมังคุด ที่เก็บมาจากภาคตะวันออกและภาคใต้ของประเทศไทยจำนวน 13 แหล่ง ด้วยวิธี UV - spectrophotometry ที่ได้ตรวจสอบความถูกต้องของวิธีการวิเคราะห์โดยการศึกษา linearity, precision, accuracy, limit of detection (LOD) และ limit of quantitation (LOQ) โดยพบว่าความสัมพันธ์ระหว่างปริมาณของสารอัลฟา-แมงโกสติน กับการดูดกลืนแสงเป็นเส้นตรงในช่วง 2-20 ไมโครกรัม/มิลลิลิตร มีค่าสัมประสิทธิ์สหสัมพันธ์ เป็น 0.9999 วิธีวิเคราะห์ดังกล่าวมีความแม่นยำ โดยมีค่า RSD น้อยกว่า 2 % และมีค่าเฉลี่ย % recovery เท่ากับ 100.68 ส่วน LOD และ LOQ มีค่า 0.1622 และ 0.4915 ไมโครกรัม/มิลลิลิตร ตามลำดับ ผลการวิเคราะห์ปริมาณรวมของสารแมงโกสตินในตัวอย่างเปลือกผลมังคุดแห้งทั้ง 13 ตัวอย่าง พบว่ามีปริมาณอยู่ระหว่าง 8.51 ± 0.05 ถึง 11.50 ± 0.02 % โดยน้ำหนัก ในขณะที่ในสารสกัด พบในปริมาณ 30.19 ± 0.16 ถึง 45.61 ± 0.09 % โดยน้ำหนัก โดยตัวอย่างที่เก็บจากภาคใต้จะมีปริมาณเฉลี่ยของสารแมงโกสติน (10.39 ± 1.04 % ในเปลือกผลมังคุดแห้ง) มากกว่าตัวอย่างที่เก็บจากภาคตะวันออก ค่าเฉลี่ยของปริมาณรวมของสารแมงโกสตินในเปลือกผลมังคุดแห้ง และในสารสกัดของตัวอย่างทั้งหมด เท่ากับ 9.94 ± 0.88 % และ 36.25 ± 4.66 % โดยน้ำหนัก ตามลำดับ จากงานวิจัยนี้พบว่า วิธี UV – spectrophotometry เป็นวิธีวิเคราะห์ที่ง่าย สะดวก รวดเร็ว และเหมาะสมที่จะใช้เป็นวิธีในการควบคุมคุณภาพของเปลือกผลมังคุดและของสารสกัดจากปลือกผลมังคุด เพื่อใช้เป็นวัตถุดิบ ข้อมูลจากงานวิจัยนี้จะสามารถนำไปใช้ประโยชน์ในการกำหนดมาตรฐานของเปลือกผลมังคุด และสารสกัดเปลือกผลมังคุด รวมทั้งทำให้ทราบถึงแหล่งปลูกมังคุดที่มีปริมาณรวมของสารแมงโกสตินสูง เพื่อใช้เป็นวัตถุดิบที่ดีอีกด้วย

คำสำคัญ: *Garcinia mangostana* มังคุด อัลฟา-แมงโกสติน Guttiferae สเปคโตรโฟโตเมตรี

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