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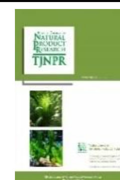

Optimisation of Extraction and TLC-Densitometric Analysis of *α*- and *γ*-Mangostin from the Rind of *Garcinia mangostana* L.

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ABSTRACT

Mangosteen (*Garcinia mangostana* L.) is a plant from Southeast Asia, including Indonesia, Malaysia, Thailand, and Myanmar, commonly used in traditional medicine. This study aims to determine the best solvent for the extraction of *α*-mangostin and *γ*-mangostin and develop a validated analytical method for simultaneously determining both compounds. The dried rind of *G. mangostana* (500 mg) was extracted with ethanol, ethyl acetate, and acetone by percolation. The percolates were evaporated to obtain viscous crude extracts. The TLC-densitometry technique was employed to determine the content of *α*-mangostin and *γ*-mangostin in the extracts. Chromatographic identification of the contents was carried out on a silica gel TLC plate (GF254) with a mobile phase of chloroform: ethyl acetate: hexane: formic acid (5:2:3:1) using standard *α*-mangostin and *γ*-mangostin as reference compounds. The method has satisfactory linearity and meets the criteria for precision and accuracy at concentrations of 100 – 500 µg/spot for *α*-mangostin and 200 – 500 µg/spot for *γ*-mangostin. The results showed that ethyl acetate extract had the highest concentration of *α*-mangostin and *γ*-mangostin at 3.05±0.22% and 0.22±0.15%, respectively. The TLC-densitometry technique is suitable and practical for the simultaneous routine analysis of *α*-mangostin and *γ*-mangostin in *G. mangostana* rind extract.

Keywords: mangosteen rind extract, mangostin, percolation, TLC-Densitometry

Introduction

Mangosteen (*Garcinia mangostana* L.) is a plant native to Southeast Asia, including Indonesia, Malaysia, Thailand, and Myanmar.[1] Mangosteens are a traditional medicine for treating and preventing disease and maintaining body wellnesss.[2,3] Mangosteen rind contained epicatechin flavonoids, anthocyanins, and xanthone derivatives, including *α*-mangosteen, *β*-mangosteen, *γ*-mangosteen, mangostanol, and gartanin.[2,4,5] The ripe mangosteen rind has about twice *α*-mangosteen content than young mangosteen fruit.[4] Xanthones are natural chemical compounds belonging to polyphenols or simple aromatic compounds characterised by a dibenzo γ– pyron core.[6] Several studies have reported that xanthone has pharmacological effects such as analgesia, antioxidant, anti-cancer, and anti-inflammation.[6,7] Secondary metabolites from a medicinal plant can be extracted in several ways, such as maceration, percolation, reflux, soxhlet, ultrasonication, pressure extraction, and microwave extraction.[8] The selection of the extraction methods are based on the characteristics of the material and metabolite compounds to be extracted, the extract yield, the extraction speed, and the extraction cost. Some of the compounds in the crude rind are relatively stable. In contrast, others are easily decomposed or damaged due to the extraction method leading to low extraction yield or loss of therapeutic effect.[9] Therefore, this study aims to determine the best solvent for extraction of *α*-mangostin and *γ*-mangostin and develop a validated analytical method for the determination of both compounds simultaneously by using the TLC-Densitometry.

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Materials and Method

Chemicals
Reference standards of *α*- mangostin and *γ*- mangostin were obtained from SIGMA. GF254 silica gel TLC plates, analytical grade chloroform, ethyl acetate, hexane, and formic acid, were obtained from Merck.

Plant Collection
Fresh mangosteen rind was collected from Solok Regency, West Sumatra, between November and December 2021. The plant sample was identified by Dr. Nurainas, a Botanist at the Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, where also a specimen with voucher No. DR-187 was deposited

Preparation of Rind Extract
Mangosteen rind (15 kg) was chopped in bits, dried at room temperature for 2 weeks, and then powdered using a grinder. The crude powdered rind was extracted by percolation using different solvents (ethanol, ethyl acetate, acetone) to obtain a thick viscous crude extract.

Determination of Ash Content
About 2 -3 g of rind powder was weighed into a porcelain crucible and incinerated in a furnace at 550 °C for at least 15 h. The ash content was calculated using the formula below:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} X\ 100$$

Determination of Water Content
About 3 g of rind powder was dried at 103°C for at least 4 h until constant weight. The moisture content was calculated using the formula below:

$$M_n = (W_w - W_d)/W_w) \times 100$$

M_n = moisture content (%) of material n
W_w = wet weight of the sample
W_d = weight of the sample after drying.

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TLC Analysis

Preparation of Standard Reference Solutions

Standard solutions were prepared by weighing approximately 10 mg of each of the standard compounds *α* – and *γ*-mangostin and dissolving them in methanol up to 10.0 mL to obtain a stock solution of *α* – and *γ*-mangostin at a concentration of 1000 ppm.

Preparation of test samples solution

About 50 mg of rind extract was dissolved with methanol in a 10 mL volumetric flask to obtain a concentration of 5000 ppm.

Instrumentation and analysis condition

Analysis was carried out on standard and sample solutions. A standard solution of 1000 ppm and a sample solution of 5000 ppm was spotted on an F254 silica gel TLC plate with a 0.5 µL capillary pipette CAMAG nanomat 4 with a 1 cm distance between each spot. The plate was eluted to a height of 8 cm in a glass twin chamber that had previously been saturated with mobile phase vapour. The mobile phase was optimised using chloroform-ethyl acetate-hexane-formic acid solvents with various compositions to obtain the best separation conditions in the Rf 0.2-0.8. Densitometric scanning was carried out at the wavelength of 320 nm using TLC-densitometry "TLC-Scanner 4" equipped with CAMAG wiCATS software. The slit dimensions are 8 mm x 0.4 mm, and the scan speed was 10 mm per second.

Method validation

Method validation was carried out with reference to ICH guidelines, including linearity, the limit of detection (LOD), the limit of quantitation (LOQ), precision and accuracy[9].

Linearity

The concentrations of five series of standard solutions of 100 ppm, 200 ppm, 300 ppm,350 ppm, 400 ppm, 450 ppm, and 500 ppm were made from a 1000 ppm standard solution by dilution. Each concentration (0.5 µL) was spotted on the TLC plate. The plates were developed with a mobile phase of chloroform: ethyl acetate: hexane: formic acid (5:2:3:1). Analysis with a densitometer was performed at a wavelength of 320 nm. A calibration curve of the peak area values obtained from each concentration was made to determine the linearity[10,11].

Limit of Detection (LOD) and Quantitative Limit (LOQ)

The limit of detection value (LOD) and quantitative limit value (LOQ) measurements were calculated based on the residual standard deviation (SDR) and slope (b) of the calibration curve.[10,11]

Precision

Intra-day precision was carried out with a 100ppm standard solution with six repetitions in one day. Inter-day precision was carried out with standard solutions of 100 ppm, 300 ppm, and 500 ppm, with three repetitions for each concentration for three days. Each concentration (0.5µL) was spotted on a TLC plate. The plate was developed with the mobile phase of chloroform: ethyl acetate: hexane: formic acid (5:2:3:1), then analysed using a densitometer at a wavelength of 320 nm. The precision value was determined based on the calculation of standard deviation (SD) and relative standard deviation (RSD).[10,11]

Accuracy

The method's accuracy was determined by testing standard recovery using the standard addition method of increasing the concentration by 50%, 100% and 150 with three repetitions on the plate for each concentration.

Determination of the Content of α-mangostin and *γ*-mangostin

1 µL each of the test extract and the standard reference solutions were spotted on the TLC plate. The plate was developed in the chromatographic chamber saturated with the selected mobile phase until the mobile phase reached the upper boundary line on the plate. The spots on the TLC plate were observed under a UV lamp (320nm). The spots on the plate were further analysed using densitometry at the maximum absorption wavelength. The spot area was obtained, and the concentration of each compound in the extract was calculated to determine its concentration in the extract.

Results and Discussion

The extraction of *α* – and *γ*-mangostin from G. *mangostana* rind was carried out by percolation using several organic solvents (ethanol, ethyl acetate, and acetone). Ethanol gave the highest extract yield, followed by acetone and ethyl acetate, with yield percentages of 33.04 ± 4.21, 17.07 ± 3.79, and 13.78 ± 0.23, respectively (Table 1). The extract yield, ash and moisture contents obtained for each solvent are shown in Tables 1 and 2

The amounts of *α* – and *γ*-mangostin in each extract were determined by TLC-Densitometry. This study was carried out to develop and validate a method for the simultaneous analysis of *α* – and *γ*-mangostin in *G. mangostana* rind extract. The TLC analysis was done on GF254 silica gel aluminium plates as the stationary phase. The mobile phase was a mixture of chloroform, ethyl acetate, hexane and formic acid in the ratio of 5:2:3:1. The mobile phase optimisation was carried out using various mobile phase ratios. The results of TLC separation using different mobile phases showed that the mixture of chloroform: ethyl acetate: hexane: formic acid (5:2:3:1) gave the best separation of *α*-mangostin and *γ*-mangostin as it fulfilled established criteria including R_f value between 0.2 - 0.8 and a T_f = 1 value which indicated a perfectly symmetrical peak shape as well as a resolution value more than 1.25. Increasing the polarity of the mobile phase causes the R_f value of the two compounds to increase with a subsequent decrease in the resolution values (Figure 1, Table 3).

Previous studies on the simultaneous analysis of *α*-mangostin and *γ*-mangostin compounds in *G. mangostana* extracts have been carried out by HPLC with an Enduro C-18 reversed-phase column and acetonitrile: water containing 0.1% phosphoric acid as mobile phase[4].

Table 1: The average percentage yield of mangosteen rind extract from ethanol, ethyl acetate, and acetone

Ethanol	Ethyl acetate	Acetone
37.40%	13.98%	15.20%
32.74%	13.52%	21.44%
28.98%	13.84%	14.58%

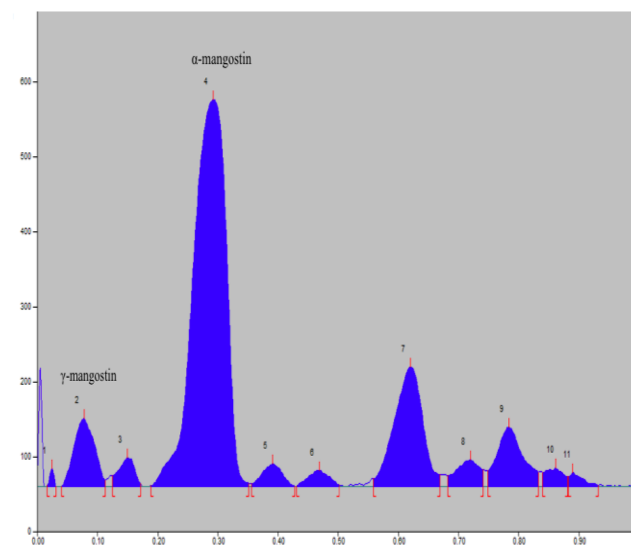

Figure 1: Chromatogram of TLC Densitometry of fruit rind extract of *Garcinia mangostana* using silica gel GF_{254} plate as stationary phase and chloroform: ethyl acetate: hexane: formic acid as mobile phase (5:2:3:1), Rf *γ*- mangostin = 0.10 and Rf *α*-mangostin = 0.31

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Table 2: Percentage of Ash and Water contents of mangosteen rind from the different solvents

Solvent	Repetition	Ash Content	Water Content
	1	0.58 %	0.42 %
		0.43 %	0.59 %
		0.0039 %	0.96 %
	2	0.09 %	0.60 %
		0.03 %	0.88 %
		0.08 %	0.74 %
	3	0.09 %	0.84 %
		0.16 %	0.65 %
Ethanol		0.04 %	0.72 %
	1	0.019 %	0.49 %
		0.15 %	0.84 %
		0.02 %	0.52 %
	2	0.64 %	0.50 %
		0.52 %	0.51 %
		0.58 %	0.42 %
	3	0.45 %	0.49 %
		0.48 %	0.40 %
Ethyl acetate		0.65 %	0.66 %
	1	0.13 %	0.74 %
		0.35 %	0.80 %
		0.45 %	0.54 %
	2	0.60 %	0.57 %
		0.17 %	0.42 %
		0.26 %	0.49 %
	3	0.13 %	0.31%
		0.39 %	0.47 %
Acetone		0.12 %	0.27 %

Table 3: Parameters of TLC Densitometry with the best solvent system

Mobile phase	Compound	R_f	K'	TF	N	HEPT	Rs
		0.2-0.8	>2	≤ 2	>2000	<<<<	>1,25
Chloroform: ethyl acetate: hexane: formic acid (5:2:3:1)	α-mangostin	0.31	2.20	1.80	5020	4.94×10^{-4}	0.9
	γ-mangostin	0.10	10.10	1.30	1692	4.25×10^{-5}	1.14

Keys: Rf = Retardation time; K' = Factor Capacity; TF = Tailing Factor; N = Theoretical plate number; HETP = Height of Packing Equivalent to a Theoretical Plate; Rs= Resolution

Under these conditions, the extract might damage the HPLC stationary phase because it contains complex compounds. Therefore, a simpler method that does not require maintenance of the stationary phase is needed. TLC- densitometry using silica gel GF_{254} could be an alternative method that offered simplicity and low cost compared to the previously published method.

The results of linear regression on the calibration curve of the relationship between the concentrations of the test substances and the peak areas of the chromatograms in the concentration range of 100-500 µg/spot for α-mangostin and 200-500 µg/spot for γ-mangostin showed good linearity (R^2> 0.999),[12] with correlation coefficients (R^2) for α-mangostin and γ-mangostin = 0.9993 and 0.9997, respectively (Figure 2).

LOD and LOQ were calculated to statistically analyse the amount of α-mangostin and γ-mangostin in mangosteen rind extract based on the equation of the calibration curve of the concentration to the area under the peak. In the method of validation, LOD and LOQ values are important parameters. The LOD and LOQ values of α-mangostin were 44.45 µg/spot and 148.14 µg/spot, respectively, while that for γ-mangostin was 38.52 µg/spot and 128.42 µg/spot (Table 4). These LOQ values were not in line with the precision test results. The precision of the developed TLC-densitometry method is expressed in percentage relative standard deviation (% RSD). The analytical method showed high precision due to the percentage of RSD < 2 (% RSD < 2.0) at concentrations of 200 and 500 µg/spot for α-mangostin; 200, 300 and 1500 µg/spot for γ-mangostin (Table 5).[10] The recovery test determines the accuracy of the method. The average percentage recovery from

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adding blank samples at three different concentrations for α-mangostin and γ-mangostin was obtained in the 85-115% range, showing that this method provides accurate results.
The analysis of α- and γ-mangostin content using TLC-densitometry showed that the amounts of α- and γ-mangostin in ethanol extract were 2.15 ± 0.51, ethyl acetate extract (3.06 ± 0.22) and acetone extract (2.15 ± 0.12), (Table 6).

Table 4: Results of LOD and LOQ

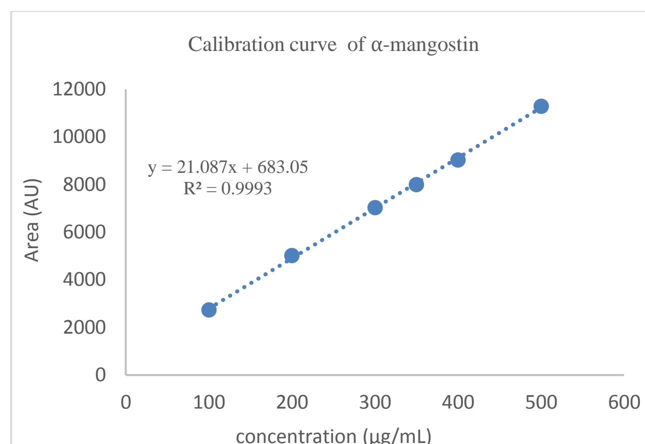
Compound	Concentration (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)
α-mangostin	100-500	44.45	148.14
γ-nangostin	200-500	38.52	128.42

Table 5: Results of Intraday and Interday Precision analysis

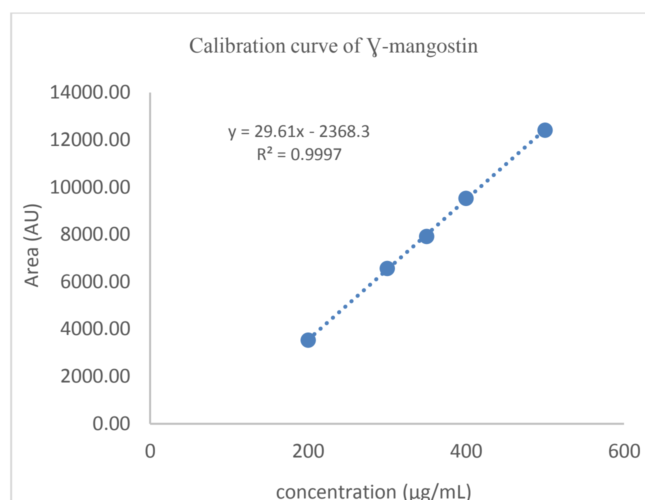
Compound	Concentration (µg/mL)	%RSD Intraday	Interday
α-mangostin	100	2.12	2.97
	200	1.55	1.47
	500	1.98	1.79
γ- mangostin	200	1.75	1.32
	300	1.02	1.28
	500	1.96	1.14

Table 6: Determination of α-mangostin and γ- mangostin in different extraction solvents

Solvent	Replicates	α-mangostin (%)	γ-mangostin (%)
Ethanol	1	0.41	0.05
	2	0.47	0.06
	3	0.55	0.07
	mean	0.47 ± 0.07	0.06 ± 0.01
Ethylacetate	1	1.45	0.39
	2	0.86	0.13
	3	0.53	0.14
	mean	0.95 ± 0.46	0.22 ± 0.15



(a)



(b)

Figure 2: Calibration curves of α-mangostin (**a**) and γ-mangostin (**b**)

Conclusion

The TLC-densitometry method using silica gel GF254 as the stationary phase and chloroform: ethyl acetate: hexane: formic acid as the mobile phase met the validation parameters, which include linearity, precision, accuracy, and recovery. The study concluded ethyl acetate was the best solvent for extracting α-mangostin and γ -mangostin

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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