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Research Article

Development and validation of Thin-Layer Chromatographic method for determination of α-mangostin in young pericarp, ripe pericarp and bark extract of *Garcinia mangostana* L. using TLC-Densitometry

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ABSTRACT

A simple, fast and precise quantitative high performance thin-layer chromatographic method has been developed for quantitative estimation of α-mangostin in young pericarp, ripe pericarp and bark extract of *Garcinia mangostana* L. (Hypericaceae). Determination was performed by using TLC plates with F254 silica gel as stationary phase and chloroform-methanol (10: 0,1) as mobile phase. 2 µL of extract spotted on TLC plate, then eluted and analyzed by using densitometry. Concentration of α-mangostin was determined by using calibration curve from some standard concentrations of α-mangostin. This study performed that the highest concentration of α-mangostin was in ripe pericarp (15,85%). Meanwhile, concentration of α-mangostin in young pericarp was 4,19% and 3,88% in bark extract.

Keywords: *Garcinia mangostana* L; α-mangostin; TLC-Densitometry

INTRODUCTION

Mangosteen (Garcinia Mangostana L.) is a tropical tree of Guttiferae family. It is cultivated for centuries in tropical rain -forest of South- East Asia. Mangosteen (*Garcinia mangostana* L) has many pharmacological activities (Chaverri et al., 2008). It contains abundant xanthones, such as: a-mangostin, b-mangostin, g-mangostin, gartanin, 8-deoxygartanin, and mangostano (Nilar and Harrizon, 2002; Bennet and Lee, 1989; Suksamrarn et al., 2002). a-Mangostin is the major component. It has been used world wide as traditional medicine for anti-inflammatory (Gopalakrishnan et al., 1980) , antibacterial (Linuma et al., 1996), and anti-cancer effects Moongkarndi et al., 2004) . It can lower total cholesterol level, and triglycerides and LDL and also increase blood HDL level of white male mice (Dachriyanus et al., 2007).

a-Mangostin represents the majority of the clinical benefits of this herbal medicine. It was use as a chemical marker for the quality control of *G. mangostana* and its products. Several studies were carried out to determine the concentration of a-mangostin in mangosteen extract (Ji et al., 2007; Misra et al., 2009). In this study, concentration of α-mangostin in bark, young pericarp and ripe extract by using TLC densitometry were reported.

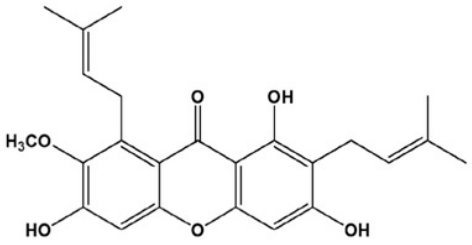

Figure 1: Chemical structure of a-mangostin

MATERIAL AND METHODS

Reagents and chemical

All reagents and solvents were analytical. Standard α-mangostin was purchased from Wuxi Gorunjie Natural-Pharma Co., Ltd. (Jiangsu, China; purity of 90,3 %). And used as the external standards.

Instrumentation

A computerized TLC scanner 3 with *win CATS* online *Planar Chromatography Manager version 1.4.2* (CAMAG, Switzerland) was used for quantitative chromatographic evaluation of test spots. Camag's *Nanomat 4* was used for application of sample spots on to TLC plate.

Plant materials

Young pericarp, ripe pericarp, and bark of mangostin were collected from Batu Busuk, West Sumatra. The

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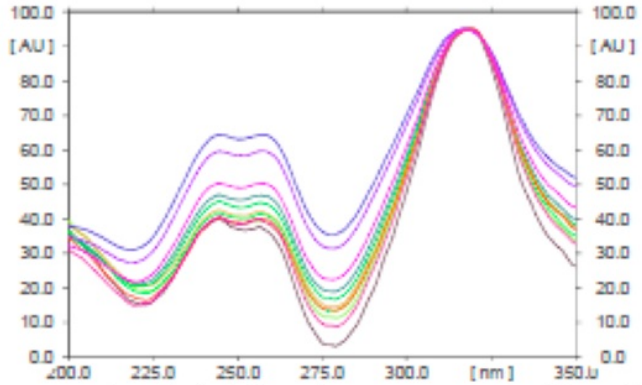

Figure 2: Absorption spectra of standard solution and extract mangosteen at 318 nm after chromatographic

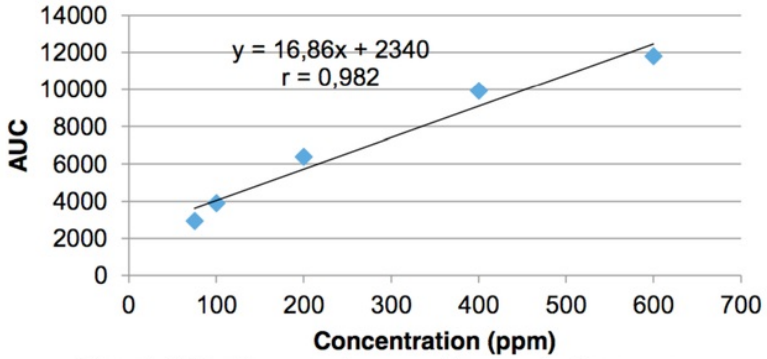

Figure 3: Calibration curve of α-mangostin concentration vs. area

Table 1: Results of densitograms of mobile phase optimization

Mobile phase	Rf	Tf	k'	R	N	HETP
9:01	0,99	1	0,01	1,47	27801,17	0,000359
10 : 0,005	0,37	1,06	1,703	1,53	970,81	0,0103
10 : 0,1	0,51	1	0,96	1,79	3653,53	0,00274

plant samples were identified by taxonomist from Herbarium ANDA Andalas University. The samples were cut into small pieces and dried in a hot oven at 50°C for 72h. The dried samples were ground into powder and passed through a sieve (20 meshes). The samples were stored separately in airtight containers and protected from light until use.

Preparation of sample solutions

Each of dry powder of samples was weighed 100 g and it was macerated with methanol (70%) in a place protected from direct lights. Macerate was combined and concentrated by rotary evaporator to obtain a viscous extract. The viscous extract was freeze-drying to form a highly viscous extract.

To determine the characteristics of the extract, the identification was done by visual observation including the shape, color, and flavor of the extracts. Non-specific extract test method was done by drying shrinkage and ash content.

Preparation of Standard Solution

10 mg of α-mangostin standard that has been dried at 25° C was weighed until constant weight, it was put in a 10 ml volumetric flask and methanol was added to mark boundaries. This standard was diluted with the same solvent until the concentration desired for testing.

Optimization Selection of Mobile Phase

Bark extract solution with a concentration of 10000 ppm spotted to the TLC plate and eluted with various mixtures of mobile phase. The ratio of chloroform and methanol, respectively were 9:1; 10: 0.005; and 10: 0.1.

Conformance Test

Standard solutions 1000 ppm of α-mangostin and rubraxanthon in methanol and standard mixture of α-mangostin and rubraxanton were spotted on TLC plates with a mobile phase of chloroform: methanol

Table 2: Data % recovery

The addition of standard µg/ml	AUC	Level µg/ml	Average levels µg/ml	Level shouldbe µg/ml	% Recovery
58,2	5651,2	1,963,938	1,753,835	174,794,925	101,12 %
	5098,7	1,636,239			
	5141,0	1,661,329			
116,6	6161,4	2,266,548	2,335,053	2,330,599	100,32 %
	6798,8	2,644,603			
	5870,5	2,094,009			
174,8	7250,3	2,912,396	2,877,857	291,324,875	97,97 %
	7163,5	2,860,913			
	7162,4	2,860,261			

Table 3: Data precision on the calculation of a-mangostin

Concentration	Day	AUC	SD	% RSD	SD	% RSD
200 ppm	1	6083,1	59,139	26,417	73,613	32,429
		6031				
		6229,1				
	2	6251,6	29,386	12,484		
		6336,9				
		6377,9				
	3	6060,3	1,889	0,8521		
		6114,7				
		6058,8				
400ppm	1	9320,5	147,164	35,426	252,838	59,916
		9108,1				
		9602,7				
	2	10074,2	87,321	19,084		
		9779,8				
		9922,5				
	3	9120,4	13,328	0,3327		
		9086,1				
		9078,1				
600 ppm	1	10965,4	36,164	0,7115	315,872	60,789
		10844,7				
		10920,1				
	2	11717,3	58,575	10,548		
		11596,9				
		11792,7				
	3	10715	17,922	0,3619		
		10656,2				
		10697,7				

(10: 0.1) with 2 mL spot volume. Then, it can be read on TLC scanner tool.

Validation Methods

1. Calibration Curves Linearity was conducted by series analysis of standard solution of α- mangostin (75 ppm, 100 ppm, 200 ppm, 400 ppm and 600 ppm) and spotted on TLC plate with 2µl volume. A calibration curve was made by plotting the area obtained from the analysis of a standard concentration using the linear regression equation (y = a + bx). Linearity is determined by the price of r (correlation coefficient).

2. Sensitivity The sensitivity is determined from the calculation of LOD and LOQ.

3. Standard solutions with various concentrations (200 ppm, 400 ppm and 600 ppm) with three times repetition were spotted the plate on the same day for intra-day variables, then three consecutive days for interday variables. Relative Standard Deviation (RSD) is used as the value of precision.

4. Accuracy of the method is determined by testing standard recovery using standard methods (standard addition method) by 50%, 100% and 150 with three times repetitions on the plate for each concentration.

Analysis by TLC-densitometry

About 50 mg ripe, young pericarp and bark extract of mangosteen were dissolved in 50 ml of methanol.

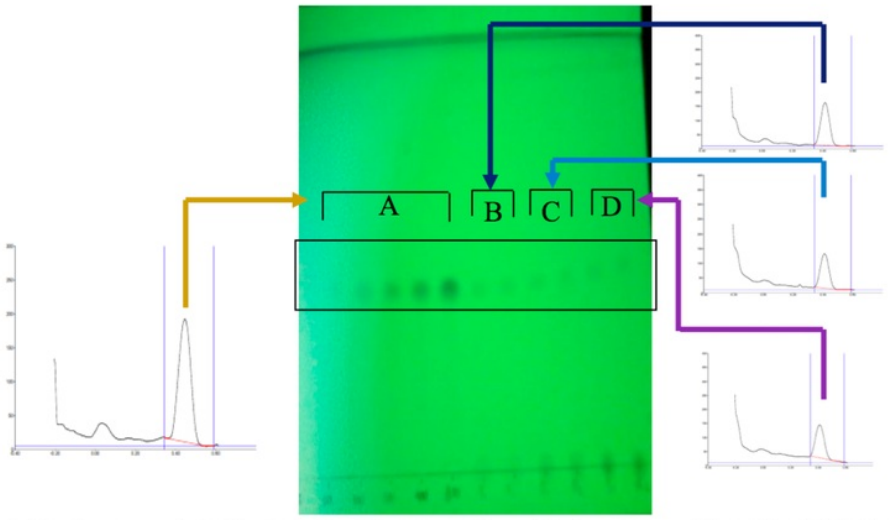

Figure 4: Densitograms of standard solution (A) young pericarp (B), ripe pericarp (C), and bark (D) of mangosteen

Spotting was scanned with a Camag TLC Scanner 4 with a wavelength of 318 nm and a broad histogram of the data obtained from the test compound.

RESULTS AND DISCUSSION

Determination of drying shrinkage of young pericarp, ripe pericarp, and mangosteen bark in a row was equal to 20.93%, 13:46%, and 22.98%. The ash content of young pericarp, old pericarp, and mangosteen bark obtained respectively were 2.70%; 2.65%; and 2.23%.

In this study, the optimizations of mobile phases were conducted on the stem bark extract of mangosteen by varying the ratio of mobile phase composition. The data showed in Table 1. Rf values which is good for analysis is more than 0.5, the value of Tf = 1 indicates a perfect symmetrical peak shape and resolution values that give good separation is> 1.5. From the above parameters, it can be seen that the mobile phases of chloroform: methanol in the ratio 10: 0.1 provided optimal results.

In suitability test of TLC-densitometry system, UV detector was at a wavelength of 318 nm. The mobile phase used was chloroform: methanol (10: 0.1) with a spot volume of 2 mL. From standardized testing of α-mangostin and rubraxanton obtained the value of Tailing factor (Tf) by 1; the value of the column capacity (k') by 0.923; the resolution value (R) by 3.08; number of theoretical plates (N) by 2022.568; and HETP value by 0.00494. Price R> 1 it can be said that the two peaks of the densitogram away completely, so it is longer need to do a system optimization and the value of Tf = 1 indicates perfect symmetry peaks form so that the system is ready to use for analysis.

Preparation of calibration curve of α-mangostin in this study was made with concentrations 75, 100, 200, 400, 600 ppm. The correlation coefficient showed linear result, because it met the acceptance criteria, namely the value of the correlation coefficient (r) approaches 1.

Determination of LOD and LOQ is one of the requirements to validate the analytical method. LOD and LOQ values of α- mangostin are 9.2397 mg / mL and 354.6423 mg / mL.

Accuracy is expressed as percent recovery (% recovery). From the test results (Table 2), α-mangostin recoveries were in the range allowed (85-115%). Thus, this prove that this method gives accurate results.

Precision test was measured by the spread of individual results from the average. % RSD of the value obtained in this study (Table 3) were obtained % RSD values were less than 16%, it can be said that this method has good repeatability value.

From the test results with TLC-densitometry method known levels of α-mangostin in each extract of young pericarp was 4.195%, old pericarp was 15.86% , and mangosteenbark was 3.88%. From this data it appears that the levels of α-mangostin in extracts> 1% so that it can be said that α-mangostin is one of the major compounds in mangosteen. From this study, it is also obtained information that the content of the highest of α-mangostin in plants is on the skin of the old pericarp of mangosteen.

CONCLUSION

Methods by TLC-densitometry using silica gel stationary phase plates F254 (Merck) and a mobile phase of chloroform: methanol (10: 0.1) has met the validation parameters that include precision, accuracy and recovery, linearity, and specificity. Levels of α-mangostin in

each old pericarp extract were 15.85%, young pericarp 4.19%, and bark 3.88%.

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