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Original Article

QUANTITATIVE ANALYSIS OF A-MANGOSTIN IN MANGOSTEEN (*GARCINIA MANGOSTANA* L.) PERICARP EXTRACT FROM FOUR DISTRICT OF WEST JAVA BY HPLC METHOD

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ABSTRACT

Objective: The objective of this study was to determine the validity of analytical methods of α-mangostin using HPLC with UV detector and acidic methanol as mobile phase. Then, compare the level of α-mangostin and quality of mangosteen pericarp from four regions in West Java.

Methods: The determination was performed by HPLC with C-18 (octadecylsilane) as a column with methanol and 1% acetic acid in water (95:5) as mobile phase and a flow rate of 1 ml/min. UV detector was adjusted at 246 nm

Results: The calibration curves for the α-mangostin were linear over concentrations range from 5 to 200 µg/ml with a correlation coefficient (r) 0.9999. The coefficient of variation obtained from α-mangostin was less than 2 %. LOD and LOQ of α-mangostin were 2.122 and 7.072 µg/ml, respectively. The levels of α-mangostin from four regions were 12.39 % for Subang, 8.30 % for Purwakarta, 6.34 % for Bogor and 5.70 % for Tasikmalaya.

Conclusion: The modified HPLC method meets the validation criteria. The highest α-mangostin level and the best pericarp quality was mangosteen from Subang.

Keywords: Xanthone, α-mangostin, HPLC, Acidic methanol, Validation method

INTRODUCTION

Garcinia mangostana L. or mangosteen (family: Clusiaceace) is one of a tropical tree in the tropical rainforest, which found in Southeast Asia, such as Indonesia, Thailand and Malaysia [1]. Mangosteen pericarp has been used as a folk medicine in some countries, such as Myanmar, India, Sri Lanka and Thailand [2]. It is used for the treatment of skin and wound infection [3].

Mangosteen pericarp contains high antioxidant and dominated by xanthone. It was shown by Oxygen Radical Absorbance Capacity (ORAC) value is 17.000–20.000 per 100 gram [4]. Some compound has been isolated from mangosteen pericarps, such as α-, β-, and γ-mangostin, 8-deoxygartanin, mangostinone a and b, gartanin, garcinone b and mangostanol [5-8]. The major xanthone was α-mangostin[9]. It has been used as antioxidant[10], anti-inflammator [11, 12], antibacterial [13], anti-allergic [14], antifungal [15], and anticancer [16]. Due to its pharmacological activities, α-mangostin was applied for herbal cosmetics and pharmaceutical product [17].

Some analytical methods have been reported for the standardization of α-mangostin [18-20]. This study was expected to set up a routine of a validated method for quality control of α-mangostin from *Garcinia mangostana* L. using HPLC methods.

This research was conducted in order to develop and validate a new HPLC method for routine quantification α-mangostin and to compare a quality of mangosteen pericarp from 4 different regions/districts (Subang, Tasikmalaya, Purwakarta, and Bogor) in West Java, Indonesia.

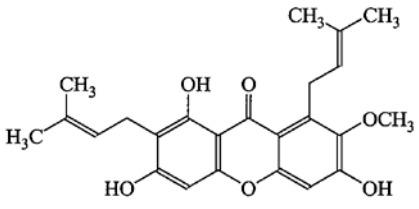

Fig. 1: Chemical structure of α-mangostin

MATERIALS AND METHODS

Plants

The hull fruits of mangosteen were collected from Tasikmalaya, Subang, Purwakarta, and Bogor, West Java province, Indonesia. The hull fruit was dried at 40 °C, powdered, and extracted by maceration with 70% ethanol. The plant was identified by Drs. Joko Kusmoro, MS., Scientist in Department of Biology of Faculty of Mathematic and Sciences, Universitas Padjadjaran and a voucher specimen of mangosteen from Tasikmalaya, Subang, Purwakarta, and Bogor (no. 009-/HB/11/2015, 010/HB/11/2015, 011/HB/ 11/2015, and 012/ HB/11/2015, respectively) has been deposited in the herbarium.

Chemicals

α-mangostin reference standard was purchased from Chengdu™ (China, purity 98%). All chemicals were used as received without further purification and all solvents were of reagent grade: Methanol HPLC grade (Merck) and acetic acid (Merck), aqua bidest (IPHA) Laboratories.

Tools

Dionex-UltiMate® 3000, autosampler, column compartment, Ultimate 3000 pump, and UV detector. The chromatographic was carried out using a reverse phase Enduro C-18 column (250 mm × 4.6 mm, 5 µm) with C_{18} guard column. The pump system is isocratic with elution time 8.5 min, the flow rate was 1 ml/min, and the injection volume was 20 µl. The quantification data was set at 246 nm with a UV detector. UV-Vis spectrophotometer (Analytical Jena, specord 200), ultrasonic bath (Ney 1510), an analytical balance (Sartorius), filters vacuum with 0.45 µm membrane filter, and unusual glassware.

Methods

The mobile phase consisted of methanol and 1% acetic acid (95:5 v/v). The mixture was filtered using 0.45 p. m milipore with vacuum assistance and ultrasonic bath for 15-20 min.

Standard solution preparation

α-mangostin 10 mg dissolved in 10 ml measuring flask with methanol to achieve the final concentration of 2000 mg/ml, diluted with methanol to obtain concentrations of 20 mg/ml. The in-scanning solution with a UV-spectrophotometer at a wavelength of 200-400 nm, so the obtained spectrum maximum wavelength (λmax) of absorption.

Determination of molar extinction

α-mangostin standard solution with a concentration of 2.5, 5 and 10 μM measured at a wavelength of maximum absorbance α-mangostin, i. e 426 nm, and the calculated values of molar extinction.

Sample pre-treatment

Extract (50 mg) was dissolved in 60 ml methanol in a 100 ml volumetric flash, sonicated for 15 min, then added by methanol until 100 ml. The solution was filtered with 0.45 μm of the filter membrane, and 20 μl solution was injected to the HPLC.

Method validation analysis (ICH)

Selectivity was measured by looking at retention time in the chromatogram of α-mangostin standard and sample. 20 μl of analyte was injected into the HPLC equipment in optimum condition; the experiment was repeated six times and then calculated the coefficient of variation. The linearity was determined by making the standard curve of six serial concentrations of α-mangostin (5, 10, 25, 50, 100 and 200μg/ml). HPLC system embedded with UV (246 nm) to be used as followed column C-18 (octadecyl silane), length of 250 mm, diameter in 4.6 mm, and the particle size of 10 im, mobile phase methanol and 1% acetic acid in water with ratio of 95:5 v/v, and flow rate 1 ml/min.

Calibration curve equation with the best correlation coefficient was used to specify the sample. LOD and LOQ were determined statistically from the calibration curve equation using linear regression. Accuracy and precision were obtained by making the sample solution 50 μg/ml spiked by standar solution 5, 25, and 50 μg/ml. Twenty μl of analyte injected into the HPLC equipment in optimum condition; the experiment was repeated three times and then calculated percent accuracy (recovery) and precision (coefficient of variation).

Determination of α-mangostin in mangosteen pericarp extract

Twenty microliters of mangosteen pericarp extract 50 μg/ml was injected into the HPLC system (n=3). The concentration of α-mangostin in sample was calculated by applying the peak area to the linear regression equation

RESULTS AND DISCUSSION

The determination of maximum wavelength (λmax)

The result of scanning using UV at the wavelength of 200-400 nm of α-mangostin standard solutions in methanol showed maximum absorption at λmax of 246 nm. This result was in line with a previous study [21, 22].

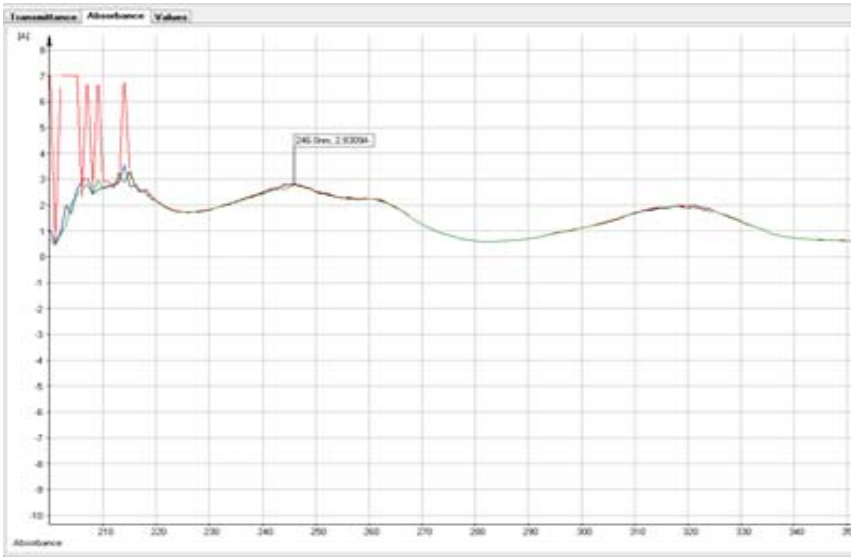

Fig. 2: Spectrum of α-mangotin standard was 246 nm

The λmax of α-mangostin was set as the wavelength used in the detection of the analysis result by HPLC, as mangosteen pericarp extract was the compound of target analysis. However, Zhao *et al.* (2010) adjusted the wavelength in ranged 317 nm [23]

The determination result of molar extinction value of α-mangostin

The determination of molar extinction value was conducted to obtain the sensitivity value of α-mangostin. It could be calculated by comparing the absorptivity value or of molar α-mangostin absorptivity towards the thickness of cuvette (usually 1 cm), with the α-mangostin concentration measured. The molar extinction value of α-mangostin was 49987,333 M^{-1} cm^{-1}. This molar extinction value is greater than 10.000 M^{-1} cm^{-1}, indicating that α-mangostin is possible to detect the ultraviolet detector on the HPLC system [24]. The extinction value of α-mangostin could be seen in table 1.

Table 1: The extinction molar (ε) value of α-mangostin

No	Molarity (M)	Absorbance	Extinction molar (Ɛ) (M^{-1} cm^{-1})
1	0.00001	0.5179	51792
2	0.000005	0.2637	52743.333
3	0.0000025	0.1136	45426.667
Sum			149962
Ɛ Alfa-mangostin			49987.333

Selectivity

Selectivity test has been done by observing the retention time of standard and sample solution. Retention time for the standard was 6.66 min and sample 6.664 min and both of them in one range. According to the retention time, the HPLC method used had a good selectivity, and could be used to determine the α-mangostin levels by using external standard.

Linearity result

Linearity test was conducted to observe the capability of analytical method in giving a good response to various analyte concentrations on a calibration curve in order to produce a straight line. The parameter concerning linear relationship was expressed by the correlation coefficient and a valid analytical method which has a correlation coefficient more than 0.99 [24].

Alpha-mangostin urine was ranged from 5-200 µg/ml obtaining a linear calibration curve with the line equation y = 1.6395x+3.0077 and the correlation coefficient (R) = 0.9999 (fig. 3).

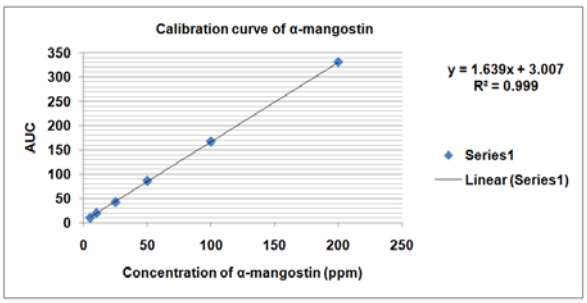

Fig. 3: Calibration curve of α-mangostin with ranged concentration (5-200 µg/ml)

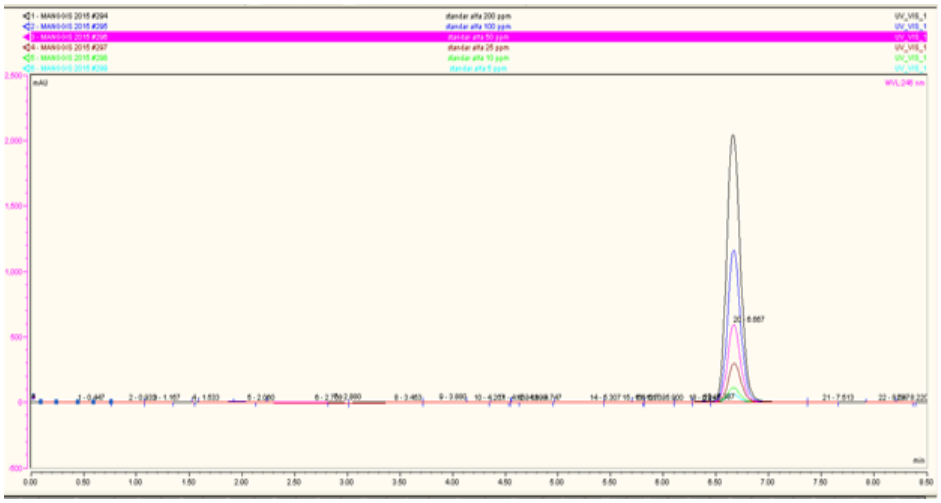

Fig. 4: Chromatogram of standar α-mangostin

Limit of detection (LOD) and limit of quantitation (LOQ)

The result of the limit of detection (LOD) test was calculated based on the calibration curve from an equation with the best correlation coefficient (r). The LOD value was depending on the calibration curve of α-mangostin towards the peak area. The LOD value was 2.121 µg/ml. LOQ value of the area ratio is 7.072µg/ml. In the previous study, validation of α-mangostin analysis using the isocratic mobile phase consisted of 0.2% formic acid–acetonitrile (30:70, v/v), produced LOD and LOQ 0.02 and 0.08 µg/ml, respectively, It indicated that this method less sensitifity than its methods [22]

Precision and accuracy

The % RSD of the standard solution of α-mangostin was obtained peak area 0.310% and the retention time 0.123%. It indicated a good repeatability value, whereas the required value is<2% for sample analysis [24]**.** The results were presented in table 3. The data proved that the method used has a good repeatability, with CV value <2% for the analysis. In the other methods, Zhao *et al.* employed RP-HPLC method with an ultra-violet detector (UVD) was performed on a 5 µm Diamonsil™ C18 column (250.0 mm × 4.6 mm) at 25 °C; the mobile phase composition was methanol-water (83:17) produced RSD 1.87 %. It is mean that this method have more precision than Zhao's methods.

Table 2: Precision analysis of α-mangostin standard

Concentration (µg/ml)	Retention time (minutes)	Peak area (mAUC)
50	6.65	89.015
50	6.66	89.267
50	6.67	89.379
50	6.67	89,083
50	6.66	89.624
50	6.67	88.856
Sum	**39.98**	**535.224**
Average	**6.663**	**89204**
DS	**0.008**	**0.277**
%CV	**0.310**	**0.123**

The percentage recovery for 5, 25, and 50 µg/ml of α-mangostin in spike solution of standard and sample were 90.609%, 106.550% and 99.102%, respectively (table 3). These data indicated the result has good accuracy because of percentage of recovery in the range 80-120% [25].

Table 3: Accuracy

Concentration (ppm)	Average of peak area (AUC)	Recovery (ppm)	% Recovery
5	24.037	12.827	90.609
25	60.279	34.932	106.545
50	97.848	57.847	99.102

Content of α-mangostin in mangosteen pericarp extract

Four samples from a different region of mangosteen extract of ethanol 95 % were analyzed by HPLC method, and the level of α-mangostin were 12.39 % for Subang, 8.30 % for Purwakarta, 6.34 % for Bogor and 5.70 % for Tasikmalaya. The level of α-mangostin from 4 regions different it cause of temperature, humidity, landfill, rainfall and geographical of place [26, 27]. The chromatogram of samples can be seen in fig. 4. Zhao *et al.* (2012) reported that the optimum condition of extraction produced α-mangostin is 5.53% [28]. It means that the α-mangostin levels of mangosteen in West Java greater than that reported by Zhao *et al.* (2012).

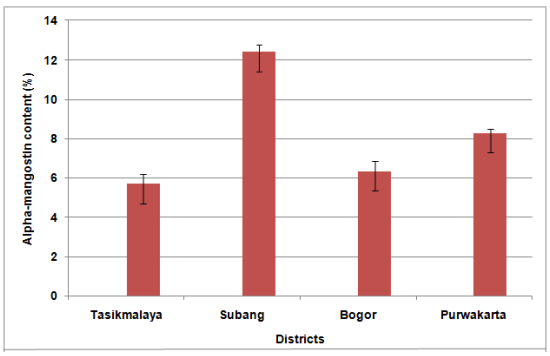

Fig. 5: Comparison level of α-mangostin

CONCLUSION

The modified HPLC method meet the validation criteria. The highest α-mangostin level and the best pericarp quality was mangosteen from Subang. The validation methods that including parameters: selectivity, linearity, detection limit, quantification limit, precision and accuracy, the methods used were valid according to the requirements that might be used to analyze α-mangostin in mangosteen pericarp extract.

ACKNOWLEDGMENT

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ABBREVIATION

HPLC/UV: High-Performance Liquid Chromatography-Ultra Violet, LOD: Limit of Detection, LOQ: Limit of Quantification

CONFLICT OF INTERESTS

Declared none

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