European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 09, 2020

Isolation, Identification, Validation of Determination Alpha Mangosteen on Mangosteen Rind (Garcinia mangostana L.)

Kurniawan[1], Azis Saifudin[2]

[1]Pharmacy Department, Darussalam Gontor University, Indonesia
[2]Faculty of Pharmacy, Muhammadiyah University of Surakarta, Indonesia

Abstract
Mangosteen rind is currently widely used by people as an ingredient in health drinks. Therefore, it is necessary to standardize the quality of raw materials, extract preparations and their compounds in order to meet the quality requirements as stated in the Indonesian Herbal Pharmacopoeia. The content of mangosteen rind (Garcinia mangostana L.) consists of various kinds of xanthones, especially α-mangosteen and γ-mangosteen. The presence of these two compounds can be a standard quality parameter of the mangosteen rind extract as an herbal medicinal ingredient. The method of determining the standard content in the methanol extract of mangosteen rind using the HPLC method. The extract was prepared by maceration method using methanol. Then fractionated and purified to obtain fractions into isolates using various chromatography techniques. The identification of isolated compounds by TLC used the mobile phase of hexane: ethyl acetate (2: 3), H-NMR and reverse-phase HPLC with the mobile phase of water: acetonitrile (80: 20). The assay results for the α-mangosteen standard compound had a mean value of 0.155 mg / mL. The validation of the α-mangosteen content determination method in methanol extract of mangosteen rind (Garcinia mangostana L.) was carried out by determining the values of linearity parameters, detection limits and quantitation limits. The α-mangosteen linearity study shows that the analysis carried out in the concentration range (0.4; 0.2; 0.1; 0.05; 0.025) mg / ml provides a measurement of the relationship coefficient (r) of 0.993 with a detection limit (LOD).) of 0.043 mg / ml and the limit of quantitation (LOQ) of 0.129 mg / ml. So that in this range α-mangosteen gives a linear response. The specificity value of the α-mangosteen spectrum purity test results obtained a correlation value above 0.99. The results of the α-mangosteen test met the validation criteria.

Keywords: Garcinia mangostana L, secondary metabolites, α-mangosteen, isolation, fractionation and purification

INTRODUCTION

Indonesia is a country rich in medicinal plants, the use of medicinal plants objectively by many pharmaceutical industries still does not meet the quality standards of raw materials. With the existence of standardization studies of natural ingredients from plants, both compounds can contribute to the government so that the quality of the traditional pharmaceutical industry is also in accordance with the standards. Secondary metabolite bioactive from Garcinia mangostana L. is xanthone, which is a compound consisting of a tricyclic aromatic ring substituted with various phenolic groups, methoxy, and isoprene (Walker, 2007). The xanthone compounds include 9-hydroxy calaba xanthone, 3-isomangosteen, gartanin, 8-desoxygartanin (Warker, 2007), α-mangosteen, γ-mangosteen, β-mangosteen and methoxy β-mangosteen (Akao et al., 2008). The α-mangosteen and γ-mangosteen

compounds are the most common compounds found in the skin of mangosteen (Jung, 2006). The α-mangosteen, γ-mangosteen and other xanton compounds found in mangosteen peel tend to be non-polar (Walker, 2007).

Xanthone compounds from mangosteen rind can inhibit the growth of colon cancer cells DLD-1 with a value of 1C50 Methoxy-β-mangosteen <β-mangosteen <α-mangosteen <γ-mangosteen (Akao et al., 2008). Research by Matsumoto et al (2003) states that α-mangosteen has antiploriferative activity against HL60 leukemia cells by inducing apoptosis. In addition, α-mangosteen has activity against Mycobacterium tuberculosis and also has antioxidant activity (Jung, 2006).

Isolation of α-mangosteen can be done by preparative chromatography, column and also by Sphadek. Identification with non-polar mobile phase TLC, HPLC uses a polar mobile phase with a stationary phase C18 (Aral et al., 2015), principally by using the HPLC method with a certain mobile phase to separate xanthone compounds contained in mangosteen (Walker, 2007).

Based on the explanation made, it is very interesting to conduct research whether α-mangosteen isolate can be obtained from the isolation of Garcinia mangostana L., in order to obtain a standard standard for the levels of α-mangosteen compounds found in mangosteen peel using the HPLC method and to know the validation of the determination of α- compound mangosteen.

METHODS

The extraction method used is maceration, namely 400 grams of Garcinia simplicia powder plus 2000 mL of technical methanol left for three days, in Erlenmeyer and sonified for 30 minutes then the extract is filtered (3x) and evaporated at 70℃ with a rotary evaporator until the solvent is gone. and obtained a thick extract. The methanol extract obtained was fractionated with a separating funnel with a solvent. Then the separating funnel is taken from the separate extract. Entered into a gravity column with a diameter of 1-3 cm and a column length of 50cm, with the silica gel (merck Sie-gel 60 GF254) stationary phase weighs 150 g with a silica height of ± 10 cm when the column is inserted and the sample is mixed with 10 g of impregnated silica gel. (merck kieselgel 60 GF254 0.2-0.5 mm). The motion phase used is adjusted for each volume of 600 ml. Then the fractionation results are accommodated approximately 50 ml until all solvent is used up.

Purification was performed using sephadex chromatography and preparative TLC. The results of TLC profile checking showed that the fraction that was purified was fraction B with a weight of ± 3.26 g using Sephadex 20-LH (GE) exclusion chromatography. Column specifications are 50 cm long, 3 cm wide, 25 stationary height. The mobile phase used is methanol and column rinsing using methanol. Separation is done by observing the color separation on the column and accommodating each separation in a container with varying volumes. The results of the sub-fraction were checked for TLC profiles and seen in UV lamps of 254 nm and 365 nm. Solution fractions having the same stain profile or Rf are combined. The resulting fraction solution was evaporated and weighed. The sub-fraction of sephadex was further purified using preparative TLC with 0.25 mm GF_{254} silica gel as stationary phase and the optimized mobile phase. The results of the stain separation of the compound are scraped off and separated from the stationary phase using hexane: ethyl acetate (2: 3) solvent in the separating column. The purity of the isolates was checked using TLC normal phase silica gel GF_{254} 0.25 mm and TLC reverse-phase Silica gel 60 RP-18 F_{254}

RESULTS

In selecting the appropriate mobile phase compounds for normal phase TLC were carried out various variations including methanol-chloroform (1: 9), hexane-ethyl acetate (5: 5), chloroform-methanol (9,5: 0,5), hexane- ethyl acetate (2: 3). Whereas for the reverse phase, the stationary phase

C-18 with mobile phase methanol-water (1: 1) and acetonitrile-methanol-water (1: 1: 1). A number of samples were dissolved in methanol to make the standard alpha mangosteen standard. The results were seen with UV lamps of 254 nm and UV lamps of 366 nm. The 37g methanol extract was fractionated by adding 50 ml of water and 50 ml of ethyl acetate 3 times. Then the separating funnel is taken from the separate extract. The fraction was obtained as much as 16.49g. A total of 15g was taken into the KCV Column having a size specification of 15 x 4 cm with a stationary phase of silica gel (Merck Sie-gel 60 GF254) weighing 150 g with a silica height of ± 10 cm when entering the column and the sample was mixed with 10 g of impregnated silica gel (merck kieselgel 60 GF254 0.2-0.5 mm). The motion phases used are hexane sequences (100%); hexane: ethyl acetate (4: 1); hexane: ethyl acetate (3: 2), hexane: ethyl acetate (2: 3), hexane: ethyl acetate (1: 4) and ethyl acetate 100% each volume 600 ml.

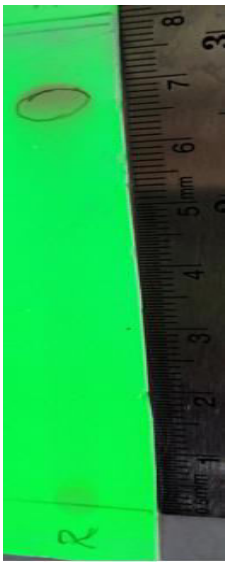


Figure 1. Identification results of TLC normal phase with hexane-ethyl acetate (2: 3) mobile phase

The TLC results from the isolation of the compound with Rf α-mangosteen were 0.8 with the hexane-ethyl acetate mobile phase (2: 3) compared to the Rf value in research journals (Muchtaridi, 2017; Andayani & Ismed, 2017). The identity of the isolated compound (isolate) was checked using HPLC with a mobile phase using water: ACN (8: 2) and the stationary phase using column C-18 (RP Cosmosil) with a size of 150 mm x 4.6 mm and scanning at 100-700 m / z. The elution results with the mobile phase showed that the alpha mangosteen separated at 2.982 minutes.

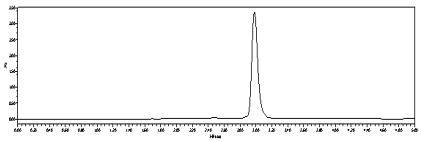


Figure 2. The alpha mangosteen standard

Analysis of the standard content is by comparing the retention time (Rt) of the sample compound with the concentration of the standard content obtained. The quantitative analysis conducted was the determination of mangosteen alpha levels

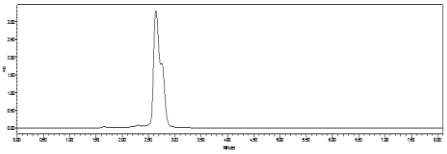
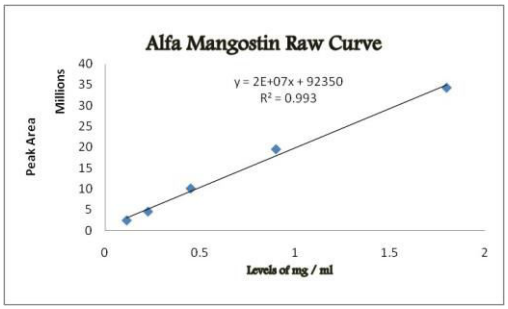


Figure 3. HPLC results for alpha mangosteen compounds



Regression equation: Y = bx + a, (r = 0.993; a = 92350; b = 85260071.8)

Figure 4. Raw Alfa mangosteen Curve

Concentration (mg/ml)	**Area (y)**	**Level (x) (mg/ml)**
0,4	34245780	0,39
0,2	19486138	0,218
0,1	10059574	0,107
0,05	4508168	0,042
0,025	2394386	0,17
		Rata2 = 0,155

Table 1. Calculation Results of alpha mangosteen levels

Concentration (mg/ml)	**Area (y)**	**ý**	**(y- ý)**	**(y- ý)^2**
0,4	34245780	35027528,64	-781748,64	61,11309361 x 10^{10}
0,2	19486138	2628714,403	168547423,6	28417,27304 x 10^{10}
0,1	10059574	1776107,285	8283466,715	6861,582082 x 10^{10}
0,05	4508168	1349803,274	3158364,274	997,5264887 x 10^{10}
0,025	2394386	125774,053	1136651,9	129,1977542 x 10^{10}
				$\sum$ = 36466,69246 x 10^{10}

$$Sy/x = \sqrt{\Sigma(y - \hat{y})^2} / n\text{-}2$$

$$= \sqrt{36466,69246^{10}} / (5 - 2)$$

$$= 11025227,5$$

$$LOD = 3,3 \times \frac{Sy/x}{b}$$

$$= 3,3 \times \frac{11025227,5}{852600711,8}$$

$$= 0,043 \text{ mg/mL}$$

$$LOQ = 10 \times \frac{Sy/x}{b}$$

$$= 10 \times 0,0129$$

$$= 0,129 \text{ mg/mL}$$

$Sy/x = 11025227,5$; LOD = 0,043 mg/mL; LOQ = 0,129 mg/mL

Analysis of Chemical Structure Identification with H-NMR

Isolates were reanalyzed using NMR (JEOL ECA 400) running at 400 MHz for H-NMR. NMR can be used to determine the status of a compound as a known or new compound through 1H NMR information. 1H NMR provides chemical shear data (δH) between 0-15 ppm. The type of carbon neighbor atoms of the H binding can be known, namely CH_3 at 0.5-1.5 ppm; CH_2 at 1-2 ppm; CH_2-C = there are 1.2-1.9 ppm; -CH-O at 3-4.5 ppm, CH_2 = at 4.5-5 ppm; - CH benzyl at 7-8 ppm and so on. The greater the amount of hydrogen, the higher the peak on the spectrum. The solvent used in H-NMR is acetone.

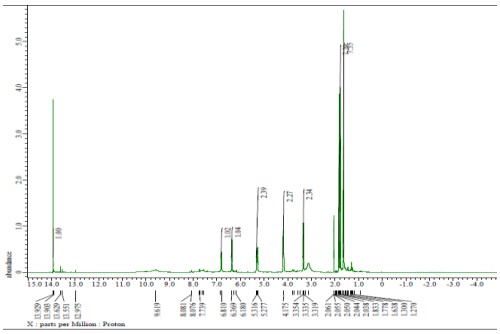


Figure 5.H-NMR results of α-mangosteen

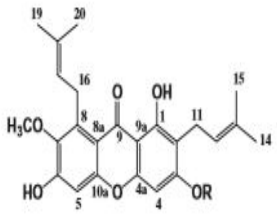


Figure 6. Chemical structure of α-mangosteen compound

α-mangosteen	Position
3.319	12
3.335	11
3.335	11
4.175	16
4.192	16

5.296	17
6.281	4
6.810	5

Table 2. Analysis of H-NMR spectra position

The validation of the α-mangosteen content determination method in methanol extract of mangosteen rind (Garcinia mangostana L.) was carried out by determining the values of linearity parameters, detection limits and quantitation limits. The α-mangosteen linearity study shows that the analysis carried out in the concentration range (0.4; 0.2; 0.1; 0.05; 0.025) mg / ml provides a measurement of the relationship coefficient (r) of 0.993 with a detection limit (LOD).) of 0.043 mg / ml and the limit of quantitation (LOQ) of 0.129 mg / ml. According to Lawson (1996) the acceptable minimum r value for the number of standard solutions of 5 solutions is 0.991; as much as 6 solutions is 0.974; as much as 7 solutions is 0.951 and as much as 8 solutions is 0.925. So that in this range α-mangosteen gives a linear response.

The specificity value of the α-mangosteen and γ-mangosteen spectrum purity test results obtained a correlation value above 0.99. According to Dhandhukia and Thakker (2011), the purity test meets the requirements if the correlation is> 0.95. From this research it can be seen that the peak and the resulting chromatogram can be said to be pure because the correlation value is above 0.99 and determines the peak purity using the cross-correlation function. The determination of LOD and LOQ was carried out using the signal to noise method (Chan et al., 2004; Ershadi & Shayanfar, 2018). Based on the values of these parameters, this analysis method can be declared valid for the determination of the α-mangosteen marker compound in the methanol extract of mangosteen rind (Garcinia mangostana L).

CONCLUSION

α-mangosteen can be obtained from the isolation of mangosteen (Garcinia mangostana L.) rind. The standard standard level of α-mangosteen is 0.155 mg / mL produced in methanol extract of mangosteen rind. The validation of the assay method has met the validation requirements.

REFERENCES

Akao, Y., Nakagawa, Y., & Nozawa, Y. (2008). Anti-cancer effects of xanthones from pericarps of mangosteen. International Journal of Molecular Sciences, 9(3), 355-370.

Andayani, R., & Ismed, F. (2017). Analisis α-Mangosteen dalam Minuman Herbal Kulit Buah Manggis (Garcinia mangostana L.) dengan Metode Kromatografi Lapis Tipis-Densitometri. Jurnal Sains Farmasi & Klinis, 4(1), 61-66.

Aral, T., Aral, H., Ziyadanoğulları, B., & Ziyadanoğulları, R. (2015). Synthesis of a mixed-model stationary phase derived from glutamine for HPLC separation of structurally different biologically active compounds: HILIC and reversed-phase applications. Talanta, 131, 64-73.

Chan, C. C., Lee, Y. C., Lam, H., & Zhang, X. M. (Eds.). (2004). Analytical method validation and instrument performance verification. John Wiley & Sons.

Dhandhukia, P. C., & Thakker, J. N. (2011). Quantitative analysis and validation of method using HPTLC. In High-Performance Thin-Layer Chromatography (HPTLC) (pp. 203-221). Springer, Berlin, Heidelberg.

Ershadi, S., & Shayanfar, A. (2018). Are LOD and LOQ reliable parameters for sensitivity evaluation of spectroscopic methods?. Journal of AOAC International, 101(4), 1212-1213.

Jung. (2006). Garcinia mangostana L, A Phytochemical and Pharmacological Review, 54, 6.
Lawson, L. (1996). Evaluation of Callibratiom Curve of Linearity. Guidance Memo, 96-007, 1-9.
Muchtaridi, M., Puteri, N. A., Milanda, T., & Musfiroh, I. (2017). Validation analysis methods of α-mangosteen, ϒ-mangosteen and gartanin mixture in mangosteen (Garcinia mangostana L.) fruit rind extract from west java with HPLC. Journal of Applied Pharmaceutical Science, 7(10), 125-130.
Ubaoji, K., Nwosu, O., Agu, K., Nwozor, K., Ifedilichukwu, N., & Okaka, A. (2020). Gas Chromatographic Analysis of the Phyto-Constituents and the Assessment of the Anti-Microbial Properties of the Leave Extracts of Nigeria-Grown Gingko biloba. Journal of Scientific Research in Medical and Biological Sciences, 1(2), 45-56. https://doi.org/10.47631/jsrmbs.v1i2.57
Uhrovčík, J. (2014). Strategy for determination of LOD and LOQ values–Some basic aspects. Talanta, 119, 178-180.
Walker, E. B. (2007). HPLC analysis of selected xanthones in mangosteen fruit. Journal of separation science, 30(9), 1229-1234.