Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(16):52-57
(http://derpharmachemica.com/archive.html)

Development of Analytical Method of α-mangostin in Dichloromethane Extract of Green Fruit Latex *Garcinia mangostana* L. Using High Performance Liquid Chromatography

Roslinda Rasyid*, Meri Susanti and Indah Gusmiwalti

Faculty of Pharmacy, Andalas University, Kampus Limau Manis, Padang, West Sumatra, Indonesia, 25163

ABSTRACT

α-mangostin is a xanthon class that has been reported having antibacterial activity, antiproliferative, anti-inflammatory and antioxidant. α-mangostin is found in many plant of Clusiaceae family, one of them is Garcinia mangostana L. The aim of this study is to find analysis method of α-mangostin from dichloromethane extract of green fruit latex G. mangostana L. that is validated and to determine quantity of α-mangostin from this extract by High Performance Liquid Chromatography. Column that used was Shim-pack® VP- ODS 250 x 4.6 mm, UV-Vis detector SPD M-20A Diode Array with wavelength 244 nm, mobile phase methanol : formic acid 0,4 % (86: 14), a flow rate of 1 mL/min, isocratic system and injection volume 20 uL. The result showed LOD value is 0.345 mg/mL and the LOQ is 1.151 mg/mL with r = 0.999, intraday precision with RSD values 0.732%; 0.188%; 1.827%, interday precision with RSD value 1.228%; 1.359%; 1.472%, and the recovery of the value is 100.832%; 98.008% and 99.680%. The content of α-mangostin from dichloromethane extract of green fruit latex G.mangostana L. is 49.869 %.

Keywords: α-mangostin; Dichloromethane extract; *G. mangostana* L.; Green fruit latex; HPLC

INTRODUCTION

A source of α-mangostin most commonly found in mangosteen (*G. mangostana*)which has been widely used as a traditional medicine because almost all parts of the plant holds the potential for human life. This plant has been known to produce a wide variety of biologically active metabolites which α-mangostin, β-mangostin and γ-mangostin as its main component (5). α-mangostin was found in extracts from root, bark, green fruit latex (8), and fruit pulp (19) of *G. mangostana* L.

From previous studies it is known that α-mangostin is a major xanthone compounds that have anti-proliferative activity against human leukemia HL60 cells, human breast adenocarcinoma MCF-7 cells and human cervucal cancer HeLa cells. α-mangostin also showed activity can induce apoptosis in leukemia cells (Ahmat *et al*., 2010). α-Mangostin also showed significant activity against CEM-SS cell line with IC50 at a concentration of 5.5 µg/mL (9).

From research conducted by Dharmaratne *et al*. (8) using Preparative Thin Layer Chromatography, it is known that α-mangostin was contained in green fruit latex *G. mangostana* L., but so far there is no record about quantity of α-mangostin in green fruit latex of the fruit. To complete the data about quality and quantity of α-mangostin that accurate and precision from these plants that can be used as a source of raw materials of fitofarmaka, it is necessary to development of analytical methods of α-mangostin in dichloromethane extract of green fruit latex of mangosteen.
Quality assurance of herbal products using high performance liquid chromatography (HPLC) is a popular method because it is accurate, precise and not limited by the volatility or stability of the compound (12). Yodhnu *et al*.reported that compared with other analysis methods such as TLC-densitometry and spectrophotometry UV, HPLC

deliver precision, high accuracy and sensitivity. Therefore, in this study the authors chose to develop analytical methods of α-mangostin in green fruit latex extract of mangosteen by using HPLC method.

Based on this, the research aims to get HPLC method with a mobile phase of methanol-0.4% formic acid were validated as a method for analysis of α-mangostin in dichloromethane extract of green fruit latex *G. mangostana* L. and to determine quantity of α -mangostin in extract.

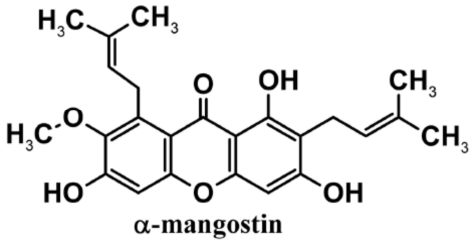

Figure 1. Chemical structure of α-mangostin

MATERIALS AND METHODS

Chemical and reagen

α-mangostin standard (Wuxi Gorunjie Natural-Pharma Co.Ltd, Jiangsu, China) ,chloroform p.a, ethyl acetate p.a, *n*-heksana, dichloromethane, formic acid, methanol p.a, metanol HPLC grade, aquabidest.

Plant materials

500 gram samples were collected from Pamuatan, Sijunjung, Sumatera Barat in the form of green fuit latex with greenish yellow color. The plants of samples was identified by taxonomist from Herbarium Anda, Andalas University.

The latexwere dried and powdered.100 gram of powder of dried sample were extracted in 200 mL of *n*-heksana for 3 daysat room temperature while stirring occasionally. After that filtering so obtained liquid extract and residue. Then, the residue was extracted again in dichloromethane. Maceration can be stopped when there are no more spots on the TLC plate when maserat spotted on the plate and viewed under a UV lamp. Maserat combined and concentrated by rotary evaporator to obtain a thick extract.

Instrumentation and chromatographic condition

HPLC method was performed on a Shimadzu LC-20 AD liquid chromatograph system model with UV-Vis SPD M-20A diode detector and column Shimadzu Shim-pack VP-ODS (250x4,6 mm). Separation was performed in reversed phase. The elution was carried out with isocratic solvent using methanol - 0.4% formic acid with a flow rate 1mL menit -1. The sample injection volume was 20µL while the wavelength of the UV-VIS detector was set at 244 nm.

Preparation of standard solutions

A stock solution of α-mangostin standard was prepared by dissolving 10,6mg of α-mangostin in 50mL of methanol in a volumetric flask.

Preparation of sample solutions

A stock solution of sample was prepared by dissolving 11,4mg of sample in 50mL of methanol in a volumetric flask.

Validation of the method

Linearity and calibration curve

Linearity was determined byusing α-mangostin standard solution in five different concentration (2,12 ; 4,24 ; 6,36 ; 8,48; 10,6 µg/mL). A volume of 20 µL of this solution was injected into HPLC system.Calibration curves were constructed by plotting peak area against the concentration of standards. A correlation coefficient above 0.99 was acceptable.

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

According to International Conference of Harmonization, the approach based on SD of theresponse and the slope were used for determining the detection and quantitation limits. LOD and LOQ were based on three times and ten times of signal-to-noise ratio.

Precision and Accuracy

The precision was determined by analyzing 2,12; 6,36; and 10,6 µg/mL of standard solution of α-mangostin on the same day for intraday precision and on 3 different days for interday precision. The precision was expressed as relative standard deviation (RSD). Accuracies were expressed as percentages of theoretical concentration, as accuracy (%) = (found concentration
/ theoretical concentration) x100%.

Recovery

The recovery of the method was tested by performing recovery studies at 3 levels of α-mangostin standard added to the samples. Three different concentrations (1,06; 3,18; and 5,3 µg/mL) of thestandard solution were added to the sample solution (4,56 µg/mL) and analyzed by the proposed HPLC method. The recoveries of α-mangostin were calculated as the following equation:

Recover (%) = $\frac{C_1 - C_2}{C_3}$ x 100%

where:

$C_1$is the observed concentration of α-mangostin detected in the sample solution (µg/mL).

$C_2$is the concentration of α-mangostin detected in green fruit latex extract sample solution without added standard α-mangostin solution (µg/mL).

$C_3$is the actual concentrations of standard α-mangostin solution (µg/mL).

RESULTS AND DISCUSSION

HPLC method with isocratic elution was developed for the quantification of α-mangostin in green fruit latex of dichloromethane extracts of *G. mangostana*. Optimization of mobile phase was performed based on resolution (R), number of theoritical plate (N), high equivalent theoritical plate (HETP), *tailing factor* (Tf), capacity factor (k) and retention time (tR). Different mobile phases were used but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase methanol and 0.4% formic acid (86:14%v/v). The retention time of α-mangostin was found to be 18,067 min, the peaks showed a resolution value was at 1,827 (>1.5), which indicates a good separation (**Figure 3**). The number of theoretical plates was found to be 2664,624 (>2500), which indicates efficient performance of the column. The high equivalent theoritical plate was 0,093; *tailing factor* (Tf) 1,105 (0,95-1,15); and capacity factor (k) 8,375 (1-10). The UV spectra of α-mangostin showed the maximum absorption at 244nm. Thus, it was chosen as detection wave length in liquid chromatography.

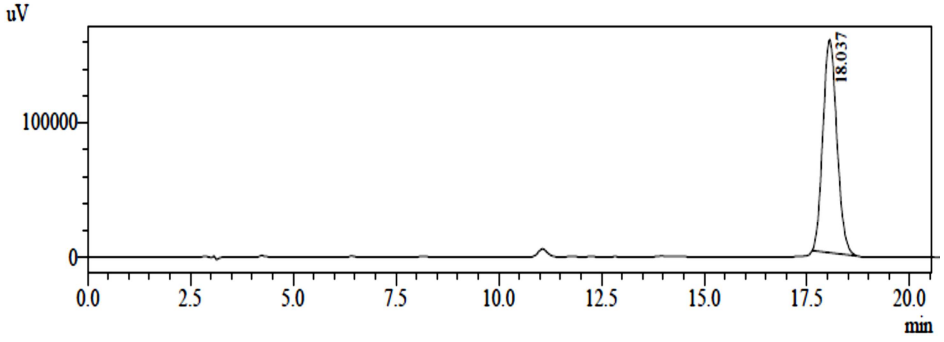

Figure 2. HPLC fingerprint of α-mangostin standard

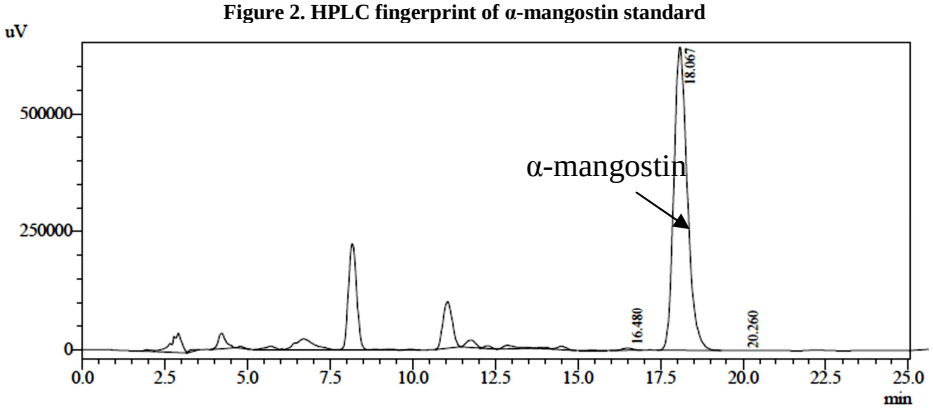

Figure 3. HPLC fingerprint ofdichloromethane extracts ofgreen fruit latex of *G.mangostana*

The method was validated for its linearity, precision, accuracy, LOD, and LOQ. The calibration curve for α-mangostin was within the concentration range of 2,12 – 10,6 µg/mL. The equation for the calibration curve was *y* = 361.005,200 x - 122.648,800, it showed a good correlation between peak area and concentration of α-mangostinon the analytical range with a regression coefficient of 0.999**(Figure 4)**.The results of LOD and LOQ were found to be 0,345 µg/mL and 1,151 µg/mL, which were lower than consentration of calibration curve that indicating the good sensitivity of this analytical method.

The RSD of intraday precision for three levels of standard α-mangostin concentrations (2,12; 6,36; and 10,6 µg/mL) was 0,732 %; 0,188 % and 1,827 %, with accuracy was 99,488%; 97,639; and 99,807 %. The RSD of interday precision analysis was 1,228 %; 1,359 %; and1,472 % with an accuracy was100,479%; 98,845%; and 98,884%. All thesedata indicated good precision and accuracy. RSD values were within limits < 2 % (12). and accuracy values were 80-120 % (11).The accuracy andprecision data are shown in **Table 1 and Table 2**.

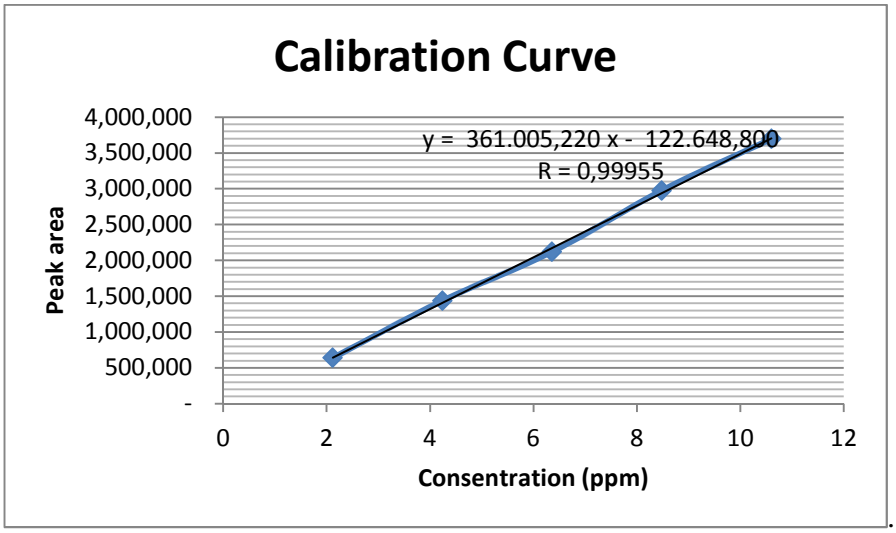

Figure 4. Calibration curve of standard α-mangostin solutions

Table 1. Intraday precision

Concentration (ppm)	Concentration found (ppm)	%RSD	%Accuracy
2,12	2,109	0,732	99,488
6,36	6,210	0,188	97,639
10,6	10,580	1,827	99,807

Table 2. Interday precision

Concentration (ppm)	Concentration found (ppm)	%RSD	%Accuracy
2,12	2,130	1,228	100,479
6,36	6,287	1,359	98,845
10,6	10,482	1,472	98,884

The recovery of the method was tested byspiking the α-mangostin standards at three differentlevels (1,06; 3,18; and 5,3 µg/mL) into dichloromethane extract of green fruit latex of mangosteensample, and then analyzing with HPLC. The resulting percentage recoveries were 100,832%; 98,008%; and 99,680%; with the %RSD range 0,1–1,9**(Table**

3). According (4),recovery values were within limit from 95% to 102 %. This results indicated that no different value within added standard concentration and recovered standard concentration.

α-Mangostin content in the samples of green fruit latex of *G.mangostana* which obtained fromSijunjung, West Sumatra determined by the developed HPLC method isgiven in **Table 4**. The contents of α-mangostin in dichloromethane extracts of sample was 49,869%.

Table 3. Recovery

C_3	C_2	C_1	% *Recovery*	% RSD
1,06	2,274	3,343	100,832	1,998
3,18		5,391	98,008	0,112
5,3		7,557	99,680	0,443

Table 4. Content α-mangostin in sample

Peak area	Concentration of α-mangostin in 9,12 ppm sample solution (ppm)	% α-mangostin
1.519.448,333	4,548	49,869

CONCLUSION

Analytical method of α-mangostin in dichloromethane extract of green fruit latex of *G. mangostana* L. using High Performance Liquid Chromatography with reverse phase, Shim-pack® VP-ODS 250 x 4,6 mm coloumn, UV-Vis SPD M-20A *Diode Array* detector with the wavelength 244 nm, mobile phase methanol : 0,4% formic acid (86 : 14), flow rate 1 mL/menit, isocratic system and volume of injection 20 µL was showed specificity, good linearity, high precision and accuracy, and good recovery of the compounds, so this method can use for analytical research and for routine quality control analysisof α-mangostin in dichloromethane extract of green fruit latex of mangosteen. α-mangostin in dichloromethane extracts ofgreen fruit latex of *G.mangostana*was 49,869 %.

Acknowledgments
The authors are grateful for the support from Faculty of Pharmacy, Andalas University.

REFERENCES

[1] Ahmat, N., Azmin, N F., Ghani, Nurunajah Ab., Aris, SRS.,Sidek, NJ., Abdullah, Shamsiah., Jasmani, H. **2010**. *Middle-East Journal of Scientific Research*, 6(2);123-127.
[2] Aisha, AFA., Abu-S, KM., Siddiqui, MJ., Ismail, Z., Majid, AMSA. *Journal of Medicinal Plant Research*, 6(29):4526-4534.
[3] Andayani, R., Wahyuni, FS.,Yanwirasti, Dachriyanus. **2015**. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(2):174-178.
[4] AOAC. **2002**. AOAC International.
[5] Chaverri, JP., Rodriguez, NC., Ibarra, MO., Rojas, Jazmin MP. **2008**. *Food Chem*, Toxicol, 46 (10): 3227-3239.
[6] Chen, LG., Yang, LL., Wang, CC. **2008**., 46(2):688-693.
[7] Chin, YW., H.A, Jung., H, Chai.,WJ, Kelker., AD, Kinghorn. **2008**.*Phytochemistry*, 69:754-758.
[8] Dharmaratne, H.R.W., Piyasena, K.G.N.P., Tennakoon, S.B. **2005**. *Natural Product Research*, 19:239-243.
[9] Ee, GC.,Daud, S., Taufiq-Yap, YH., Ismail, NH., Rahmani, M. **2006**. *Nat. Prod. Res*. 20(12):1067-1073.
[10] Gopalakrishnan, G., Banumathi, B., Suresh, G. **1997**. *Journal of Natural Product*, 60(5): 519-524.
[11] International Conference on Harmonisation (ICH). **1996**. *Text andMethodologyQ2(R1).*
[12] Pedraza-Chaverri, J., Cardenas-Rodriguez, N., Orozco-Ibarra, M., Perez-Roja. *Food Chem, Toxicol*, 46 (10): 3227-3239.
[13] Pothitirat, W., Gritsanapan, W. **2009**.*Thai Journal of Agricultural Science*, 42(1):7-12.
[14] Rasyid, R., Wahyuni, FS.,Yanwirasti, Dachriyanus. **2014**. *International Journal of Research in Pharmaceutical Sciences*, 5 (4): 282-285.
[15] Walker, EB. **2007**. HPLC analysis of selected xanthones in mangosteenfruit . *J Sep Sci*, 30(9):1229-1234.
[16] Watson, DG. **1999**..Churcill, Livingstone.

[17] Yates, P., Stout, GH. **1958**.*Journal of American Chemical Society, Vol. 80 : 7*.
[18] Yodhnu, S., AS., CW. **2009**.*J. Chromatogr.Sci*. 47: 185-189.
[19] Zarena. **2009**.*Journal of Natural Product*, Vol 2 : 23-30.