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Original Article

HPLC ANALYSIS AND PHARMACOKINETIC STUDY OF MANGOSTIN AFTER ORALLY ADMINISTRATION IN RATS

Syamsudin[1], Faizatun[2] and Lestari Rahayu[1]

Affiliated to:

[1]Department of Pharmacology, Faculty of Pharmacy, Pancasila University. Jl. Srengseng Sawah, Jagakarsa, South Jakarta 12640, Indonesia
[2]Department of Pharmaceutic, Faculty of Pharmacy, Pancasila University. Jl. Srengseng Sawah, Jagakarsa, South Jakarta 12640, Indonesia

ABSTRACT

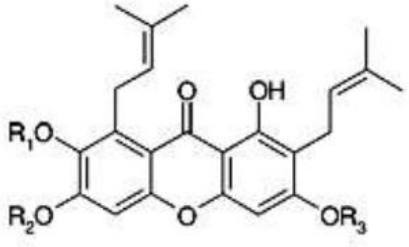
A simple sensitive isocratic method for the detection and quantification of mangostin in plasma has been developed. The assay consisted of reversed-phase HPLC with ultraviolet detection. Separation was achieved on a C_{18} reversed-phase column. The mobile phase consisting of methanol and water (95:5 %v/v) was delivered at a flow rate of 1.0 ml/min. The assay was shown to be linier over the range 4-100 µg/mL ($r \geq 0.9998$). The HPLC analysis has been successfully applied to pharmacokinetic studies of mangostin after oral administration at a dose 40 mg/kgbwt to rats. The main pharmacokinetic parameters were: $t_{½}$ 7.24 h ; K el 0.058/h ; t_{max} 62.99 min ; C_{max} 4.79 µg/mL; AUC 702.45 µg min/mL, respectively.

Key words: mangostin HPLC Pharmacokinetic studies, rat.

*Corresponding Author:
Syamsudin
Department of Pharmacology, Faculty of Pharmacy,
Pancasila University. Jl. Srengseng Sawah, Jagakarsa,
South Jakarta 12640, Indonesia
E. mail: syamsudin27@yahoo.com

1.0 INTRODUCTION

Garcinia parvifolia (Miq) Miq. has been widely used traditional medicine for the treatment of malaria and α-mangostin is one of an active compound (Syamsudin, 2009). Mangostin (Figure 1), which was isolated from *G. mangostana* Linn to, and it's was found to have a antiinflammatory (Gopalakrisnan *et al*., 1980), antioxidant (Williams *et al*., 1995), antimycobacterial (Suksamran *et al*., 2003), 5-hydroxytryptamine 2A receptor antagonist (Chadrungulired *et al*., 1998) and cytotoxic effect against patocelluler cell lines (Ho *et al*., 2002). It was also reported to inhibit alveolar duct formation in a mouse mammary organ culture model and to supress the carcinogen induced formation crypt foci in a short-term colon carcinogen model (Nabendth *et al*., 2004).



Alpha-mangostin : $R_1 = CH_3$, $R_2 = R_3 = H$

Beta-mangostin : $R_1 = R_3 = CH_3$, $R_2 = H$

Gamma-mangostin : $R_1 = R_2 = R_3 = H$

Figure 1. Chemical structure of mangostin

Various analytical methods to quantitative analysis α-mangostin have been reported such as gas chromatography (GC) and high performance liquid chromatography (HPLC) (Jefferson *et al*., 1971; Pothitirat and Gritsanapan, 2008). However, there was no data of the pharmacokinetic of α-mangostin in male Sprague-Dawley rats. This paper was reported for the determination of α-mangostin in biological fluids and urine. A sensitive, simple, fast and releable bioanalystical method is required in order to evaluate the pharmacokinetic disposition of α-mangostin.

2.0 EXPERIMENTAL

2.1 Materials and reagents

Mangostin used for this study was isolated from the stem barks of *G. parvifolia* (Miq)Miq as previously described (Syamsudin, 2009). α-mangostin (98.5% pure) for reference standard was ordered from the Chengdu Biopurify Phytochemicals Ltd. (Chengdu Sichuan China).

2.2 Apparatus

The HPLC-UV system consisted of HPLC Hewlett Packard ® model 1100, Germany was used for the determination of mangostin. An Alltima ® RP C-18 (5 µm, 4.6 x 250 mm) column and 5 % water in methanol were a stationary phase and mobile phase suitable for the separation. Peak and detection at the maximum UV absorption at 319 nm was performed over the range of 25-125 µg/mL of mangostin.

2.3 Preparation of stock and calibration standard solutions

A stock solution of α-mangostin reference standard was prepared by dissolving an accurately

weighed 10 mg of α-mangostin in 100 mL methanol in a volumetric flask. From this solution various concentrations of the standard solution were prepared in 10 mL of methanol in a volumetric flask to obtain final concentrations at 20, 16, 8, 4 and 2 µg/mL.

2.4 Animal study

The developed HPLC method was used in a pharmacokinetic disposition study after orally of α-mangostin to male Spraque-Dawley rats (6-7 weeks, 250 ±12 g). Rats were anesthetized with single *i.p.* injection of sodium pentobarbital (60 $mgkg^{-1}$), cannulated via the right jugular vein one day prior to drug administration and fasted over-night. After a 1-day recovery period, α-mangostin dissolved in saline was orally at a dose of 40 mg/kg bwt. Venous blood samples were collected at 0, 5, 10, 15, 30, 80, 90, 120 and 180 min post dose and collected in heparinized tubes. Blood samples were immediately centrifuged at 3000 g for 5 min and harvested serum samples were stored as -20°C until analysis the volume of the serum samples used in the analysis was 50 µL.

2.5 Recovery

The recoveries of mangostin in rat plasma determined at the concentrations of 20.0 µg mL^{-1} was 58.45% (n=6).

2.6 Liniearity

Typical equation of the calibration curve were as follows Y=20.830 X + 1.3. 10^{-4} (R = 0.9998, n= 5) for rat plasma samples. The linier range of mangostin in rat plasma was from 4.00 to 100.00 µg mL^{-1}

2.7 Accuracy and Precision

The accuracy and precision were determined with five determinations per concentration. Within- and between-day accuracy and precision values are given in Table 2.

2.8 Application of the HPLC Analysis and Pharmacokinetic Study

In a previous study, we studied the effect of mangostin (at three dosages 10, 20, and 40 mg/kg) against *P. berghei* of mice. The results indicated that the dose 40 mg/kg yielded significant antiplasmodial activity. Therefore to study the pharmacokinetic profile of mangostin, the mangostin at the dosage of 40 mg/kg was administered to rats by oral gavage. Venous blood samples were collected at 0, 5, 10, 15, 30, 80, 90, 120 and 180 min post dose and collected in heparinized tubes. Blood samples were immediately centrifuged at 3000 g for 5 min and harvested plasma samples were stored as -20°C until analysis the volume of the plasma samples used in the analysis was 50 µL. Mean plasma concentration profile of mangostin in rats is presented in **Fig. 3.**

Table 1 summarizes the main pharmacokinetic parameters of mangostin in male SD rats after orally administration at dose of 40 mg/kg, the main pharmacokinetic parameters were: $t_{½}$ 7.24 h ; K el 0.058/h ; t_{max} 62.99 min ; C_{max} 4.79 µg/mL; AUC 702.45 µg min/mL, respectively

Table 1. Pharmacokinetic parameters of mangostin in male SD rats following an oral determination.

Parameters	Mangostin at dose 40 mg/kg
T max (min)	62.99
Cmax (μg mL^{-1})	4.79
AUC (μg min mL^{-1})	702.45
Half-life (h)	7.24

Male SD rats received 40 mg/kgbwt mangostin in corn oil orally

Table 2. Precision and accuracy of HPLC-UV method in determining mangostin in rat plasma (n=6).

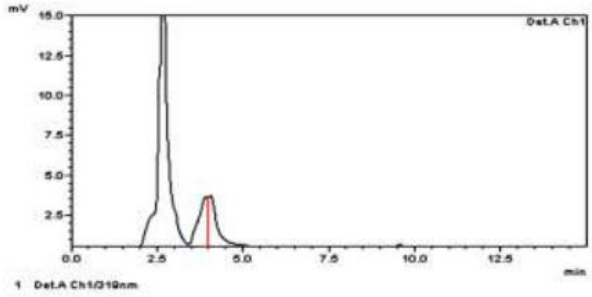
Concentration (μg mL^{-1})		**RSD (%)**		**RE (%)**
Added	**Found**	**Intra-day**	**Inter-day**	
80.78	80.06	2.8	7.3	0.0
85.37	85.57	4.9	4.9	2.4
81.03	80.64	3.6	8.2	-1.7

3.0 RESULTS AND DISCUSION

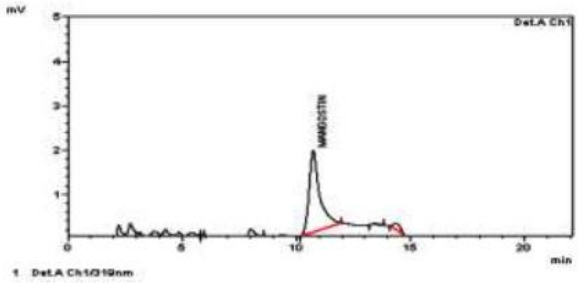
The retention time for mangostin was 10.84 min, respectively. No interference from endogenous components or mangostin metabolites was observed in plasma. The baseline was relatively free from drift. Validation of the method consisted of two distinct phases: *(a)* the development phase, in which the assay was defined, and *(b)* the application phase, in which the method was applied to the actual analysis of samples from a single 40 mg oral-dose mangostin pharmacokinetic study. Six concentrations (excluding blank values) defined the calibration curves. The linearity of the calibration curves was verified from 4 to 20 μg/L for mangostin in plasma. The correlation coefficients between the peak-area ratio of the drug to the IS and to concentration were >0.999.

Figure 2 shows the chromatogram of mangostin methanol and water (95:5) moving phase produced one peak. This shows the purity of the

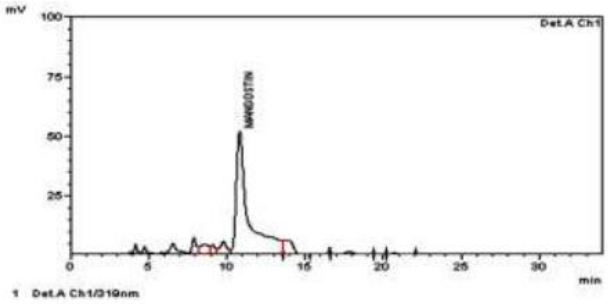
mangostin used in this research. Based on the mangostin chromatogram of the plasma sample, there are other peaks that could be from blood or metabolit of the mangostin. **Figure 3** shows the curve of mangostin content in the plasma based on time after the oral administration of 40 mg/kg. Table 1 presents the conclusions of pharmacokinetic parameters in the plasma when the mangostin doses of 40 mg/kg were administered orally. The pharmacokinetic parameters are: $t_{½}$ 7.24 h ; K el 0.058/h ; t_{max} 62.99 min ; C_{max} 4.79 µg/mL; AUC 702.45 µg min/mL



A. Blank rat plasma sample



B. Internal Standad



C. Plasma from rat 1 h after oral administration of mangostin 40 mg/kg

Figure 2. Chromatogram of mangostin of rat plasma blank (A) and rat

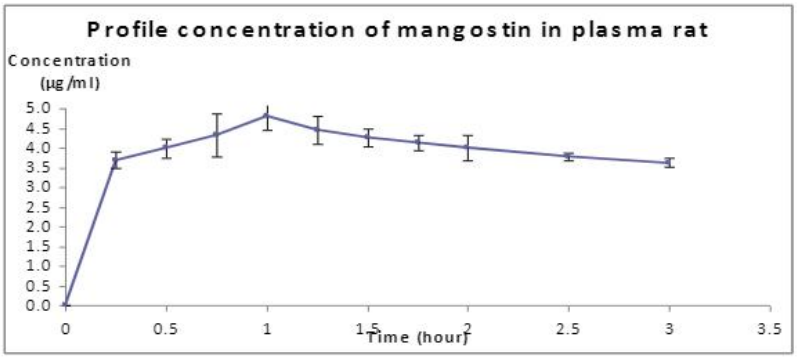


Figure 3. Mean plasma concentration of mangostin in male SD rats after oral administration of mangostin at a dose 40 mg/kg

4.0 CONCLUSION

HPLC analysis can be used to identify the pharmacokinetic parameters of mangostin. The oral administration of mangostin doses of 40 mg/kg produced significantly different pharmacokinetic parameters in t_{max}, C_{max}, half life, and AUC ($p < 0.05$).

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