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Research Article

Development of TLC and HPTLC Method for Determination α-Mangostin in Mangosteen Peels (Garcinia Mangostana L.,)

Liza Pratiwi[1*], Achmad Fudholi[2], Ronny Martien[2], Suwidjiyo Pramono[2]

[1]*Faculty of Medical, University of Tanjungpura, Pontianak, Indonesia*
[2]*Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia*

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ABSTRACT

The objective of this study is to investigate quantitative estimation of α-mangostin in the fruit pericarp of Garcinia mangostana L. Five samples from mangosteen peels are named ethyl acetate extract, ethanol extract, ethyl acetate fraction, n-hexane fraction, and residue. Thin layer chromatography (TLC) method was developed for quantitative estimation of α-mangostin in the five samples. Then, samples with the highest levels of α-mangostin were tested by High Performance Thin Layer Chromatography (HPTLC) method. Ethyl acetate fraction gave the highest content of α-mangostin. TLC method α-mangostin content for ethyl acetate fraction, ethyl acetate extract, ethanol extract, n-hexane fraction, and residue were 33,61; 10,25; 8,73; 7,72; 7,50 % respectively. α-mangostin content for α-mangostin standard and ethyl acetate fraction with HPTLC method were 97,59; 34,17 % respectively. The result of the present study indicates that screening of extract and fraction of different solvents toward α-mangostin shows ethyl acetate fraction as the choice for future development.

Keywords: Garcinia mangostana L; α-mangostin; TLC; HPTLC determination.

INTRODUCTION

Garcinia mangostana Linn is a traditional plant that can be found in Southern Asia such as Indonesia, Malaysia, Philippines, and Thailand. This plant is used both for medicine and for cosmetics. Ancient medication used mangosteen peels extract for abdominal pain, diarrhea, dysentery, infections, and chronic ulcers. Reported that phenolic compounds such as tannins, flavonoids, and xanthone are identified in mangosteen peels extract. Xanthones are major secondary metabolites of a plant[1]. Mangosteen tree has been cultivated in tropical areas around the world. This tree originated in Southeast Asia, especially in Indonesia, Myanmar, Thailand, Vietnam, and Malacca[2]. Mangosteen tree can grow in lowlands with altitudes below 541 000 m above sea level. The best growth is achieved in the area with an altitude of 500-600 m below sea level. Mangosteen tree planting center is East Kalimantan, Central Kalimantan, West Java (Jasinga, Ciamis, Wanayasa), West Sumatra, North Sumatra, Riau, East Java and North Sulawesi. Mangosteen production centers in Java, among others Bogor, Subang, Purwakarta, Sukabumi, Cilacap, Banjarnegara, Purworejo, Banyuwangi, Terri, and Blitar[3]. Garcinia mangostana is the scientific name of the fruit with diameter overall 2,4-7,5cm, skin thickness 0,6-1cm with purple pigment[4]. Garcinia mangostana is a tropical fruit known as Superfruits because of the characteristics of its taste, smell, appearance, and quality wealth of antioxidant nutrients[5]. The mangosteen peels has been used extensively in traditional medicine for many years[1]. Mangosteen peels produce yellow resin-rich xanthones. Priya et al, found the mangosteen peels extract content of 95% xanthones, isoflavones, tannins and flavonoids[4]. Xanthone, vitamin C, phenolic and anthocyanin contained in mangosteen peels are antioxidant that can prevent premature skin aging. Mangosteen peels extract found traces xanthone, isoflavones, tannins, and flavonoids. Xanthone is a group of yellow pigments found in some families of higher plants, fungi, and mosses[5]. Xanthone is polyphenolic compounds with a chemical structure containing tricyclic aromatic (fig.1). The structures have biological activities such as antioxidants, antinflamasi, antibacterial, and anticancer[6]. The mangosteen peels have been used in traditional medicine for treatment of skin infection, wounds, dysentery, and diarrhea[7]. TLC and HPTLC are two of the modern sophisticated techniques that can be used for wide diverse applications. They are simple and powerful tools for making high-resolution chromatography and trace quantitative analysis possible.

MATERIALS AND METHODS

Reagents and chemicals

The main material used in this study was mangosteen peels obtained from Kaligesing, Purworejo District, Central Java province. The standard of α-mangostin (Sigma) HPTLC quality, purity 98%, methanol (Merck), ethyl acetate (Merck), Chloroform (Merck), n-hexane (Bratachem), silica gel F254 (Merck), HPTLC plate (Merck).

Instrumentation and Chromatographic condition

Instrument sample application using CAMAG Linomat 5. Detection with CAMAG TLC Scanner 3. Wavelength 254, lamp D2&W, measurement type remission, measurement

**Author for Correspondence: lyza_pratiwi@yahoo.com*

mode absorption, optical filter second order. Detector mode: automatic. PM high voltage: 301 V.

Mangosteens Peels Preparation

Fresh mangosteen fruits are grown in Central Java of Indonesia. The fully-ripe fruits (dark purple peel) were selected for the study. The pieces of fruit peels were ground and dried in hot air oven at 40±0,5 ºC[8].

Preparation of Plant Extract

500 g of powdered mangosteen peels was taken in five different extraction thimbles and extracted via maceration for 72 hrs using ethanol 70%, ethyl acetate. Extracted samples were evaporated with waterbath until they become thick extracts. These test solutions were spotted with standard α-mangosteen for assay.

Preparation of Plant Fraction

Some condensed ethanol extract were partitioned with n-hexane to obtain n-hexane soluble fractions and residues. Then, the residue were added with ethyl acetate to obtain the ethyl acetate fraction and residue. Furthermore, extracted soluble fraction of n-hexane, ethyl acetate fraction, and a residue were collected and concentrated by rotary evaporator and a waterbath at 40±0,5 ℃ to obtain a thick fraction[9].

Preparation of Standard α-mangostin and Calibration Curve

One mg of standard α-mangostin was dissolved in 10 ml of methanol in a volumetric flask and sonicated for 5 min for homogenizing it completely. A calibration curve was plotted between 1 µl to 5 µl spot -1 .

Preparation TLC and HPTLC plate

The TLC plate was activated by placing in an oven at the temperature of 110 ºC for 20 min. The plate was spotted with test and standard preparation, the mounting distance of 8 mm from the edge of a TLC plate. It was developed up to 75 mm in the twin trough chamber using mobile phase, dried in an oven and subjected for TLC scanning 200-400 nm[10].

Chromatographic analysis

TLC was performed on silica gel F254 (60 F254, E.Merck, Germany, 20.0 x 10.0 cm). 5µl of standard solution and samples were spotted on a TLC plate using linomat 5 (Camag). TLC plates were placed into the vessel that has been saturated with a mixture of chloroform: ethyl acetate (85: 15). The vessel was closed and allowed to mix the with mobile phase until reaching to the upper limit. TLC plate was stabilized by putting it to room temperature for 30 minutes and then was scanned using camag's TLC scanner with winCATS software.

Determination of the Active Compound Using TLC Densitometry Method

A stock solution of samples and α-mangosteen standard was prepared. α-mangostin standard with concentration 1 mg/10 ml. Prepared standard solution with a volume of 1 µl, 2 µl, 3 µl, 4 µl, and 5 µl of standard solution and samples were spotted on a TLC plate using linomat 5 (Camag).

Determination of the Active Compound Using the HPTLC Densitometry Method

HPTLC used aluminum plates (60 F254). A mobile phase of chloroform: methanol (85:15 v/v) was used. The plates were heated at 110 ℃ for 10 minutes. HPTLC plate was stabilized by at room temperature for 30 minutes and then was observed using camag TLC scanner at 200-400 nm[11].

Linearity

For the evaluation of linearity five different standard solutions of α-mangosteen. 1 mg of standard α-mangostin were dissolved in 10 ml methanol. From this stock solution, 1-5 µl was spotted as sharp bends on the precoated TLC plate. The plate was developed in the chamber, previously saturated for 10 minutes with mobile phase. The plate was removed from the chamber and dried in hot air.

Precision and Accuracy

The precision was determined by analyzing standard α-mangostin in the concentration of 5 µl/spot for 6 times. The precision was used to study the variability of the method. The accuracy of the method was studied using the standard addition method.

RESULT AND DISCUSSION

Extract and fraction development

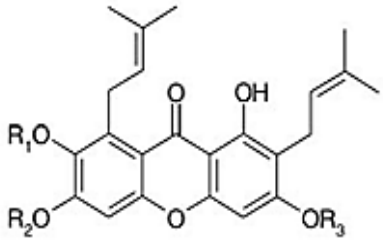
According to the study, to obtain the high antioxidative capacity of mangosteen peels extract, raw material preparation method, and extraction condition need to be considered. The study revealed that dried mangosteen peels yielded a higher antioxidative capacity of the extract than fresh peels. The suitable amount of ethanol for extraction was 6 times of mangosteen peel with 12 hours of contacting them[8]. In many studies, mangosteen extract was prepared from the dried peel with solvent extraction[12-15]. It has been reviewed[16], that drying process affected differently on polyphenolic content and antioxidant capacity of various plant materials. The solvent of material ratio and contacting time were also noted as important processing parameters for plant extraction[17]. Optimization of mobile phases like n-hexane-ethyl acetate, chloroform: methanol, chloroform: ethyl acetate was tried for separation and we opted for Chloroform: Ethyl acetate (85:15) (Figure. 2).

Verification of the Method

A quantitative TLC was performed using standard, extract, and fraction in the selected mobile phase and its spectrum was scanned, which clearly indicated its λmax.at 317 nm with satisfactory peak purity data. Chromatographies separation of α-mangostin (Rf= 0,55) is shown in Figure 3. The RSD was found to be less than 2%. A linear relationship between the peak area and the concentration of α-mangosteen was observed in determining the range of

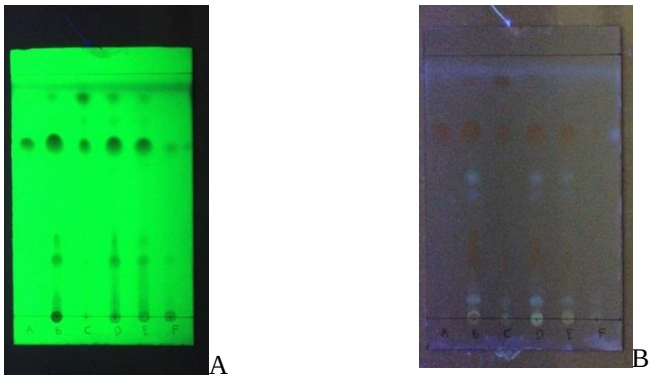
Table 1: Summary of validation parameters for α-mangostin standard.

Parameters	Result
Linearity	Linear
Range (µg/spot)	0,2-0,5
Linear equation	Y = m X + C
Slope (m)	27387
Intercept (C)	7970,2
Correlation coefficient (r)	0,9897
Precision (%RSD)	1,11 %
Recovery	91,95-105,3



Notes :
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 α-mangostin[6].



A B

Notes:
A: standard
B: Residue
C: Ethanol Extract
D: Ethyl acetate extract
E: Ethyl acetate fraction
F: n-heksan fraction

Figure 2: Elusion of samples and α-mangostin standard co-chromatography using chloroform: ethyl acetate (85:15) v/v on silica gel F_{254} (a) identification by UV_{254} (b) identification by UV_{366}.

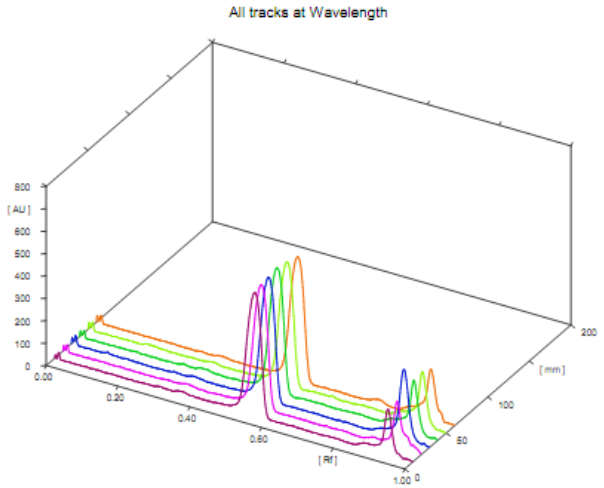


Figure 3: Three-dimensional densitogram profile derived from the wavelength of α-mangostin standard.

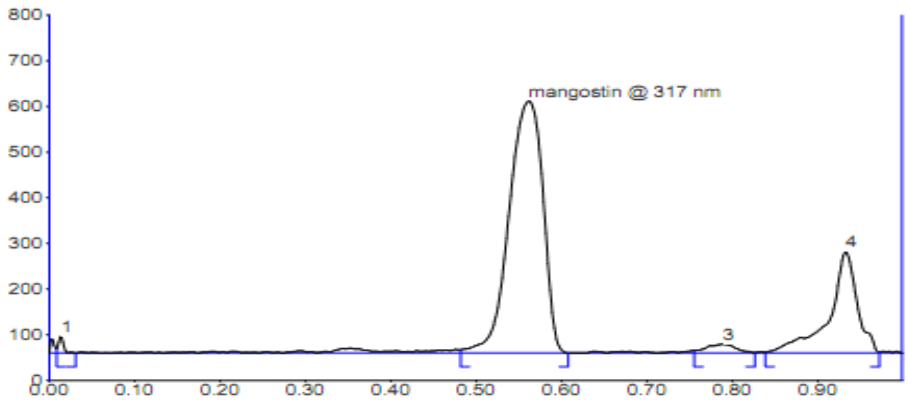


Figure 4: Densitogram profile derived from α-mangostin.

0,1-0,5 µg/mL but a good linearity obtained was from 0,2-0,5 µg/mL. The equation of the linear regression curve obtained was Y-mX+C, with a correlation coefficient (r) = 0.9897. The precision was used to study the variability of the method. The % RSD were 1,11 % respectively, and % recovery were 91,95-105,3% (table 1).

Determination of the Active Compound Using TLC Densitometry Method

Determination by using a combination of TLC densitometry methods was quite economical because it uses less mobile phase, consuming a relatively short time and be done multiple assays simultaneously[18]. TLC also could investigate sample, Rf, value and major spots[19]. The solution of samples and α-mangosteen standard were spotted 5 µl, respectively on the TLC plate with various concentrations. The results of elution TLC is in (Figure 5). The result of quantitative TLC analysis (table 2) revealed that ethyl acetate fraction reached highest α-mangostin. The decreasing order of α-mangostin recovery is: Ethyl acetate fraction > Ethyl acetate extract > Ethanol extract > n-hexane > Residue. The results of this study were shown in Figure 3. Based on the data of densitometry, the detected mean concentration of samples is 7,50-33,61 %.

Figure 5: Elution of α-mangostin standard and samples using using chloroform : ethyl acetate (85:15) v/v on silica gel F_{254} (a) identification by UV_{254} (b) identification by UV_{366}.

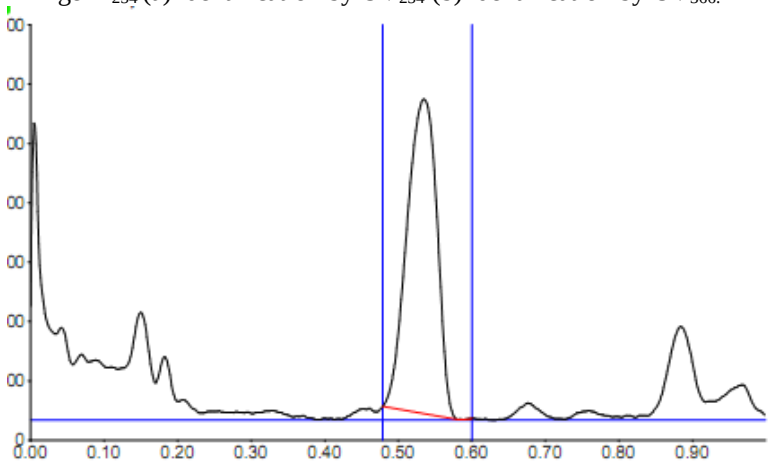


Figure 6: Densitogram profile derived from α-mangostin in ethyl acetate fraction.

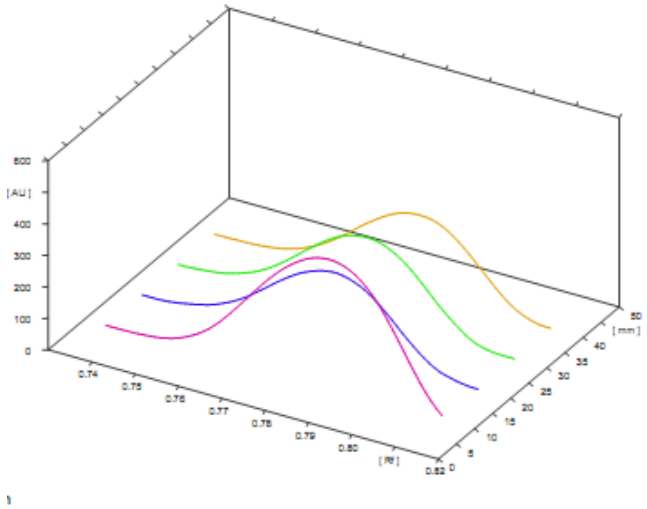


Figure 7: Three-dimensional densitogram profile derived from α-mangostin standard and ethyl acetate fraction.

Table 2: Screening of samples content of α-mangostin.

Samples	Area ± SD	Measurable level (µg) ± SD	Mean content of α-mangosteen (%) ± SD
Ethyl acetate extract	14985,6± 0,1414	0,2562±0,000071	10,25±0,00707
Ethanol extract	10958,7 ± 0,0707	0,1091± 0	8,73 ± 0
Ethyl acetate Fraction	19476,6± 0,0707	0,4201± 0,00014	33,61 ± 0,00707
n-hexana fraction	18537± 0	0,3813± 0,00962	7,72 ± 0,00707
Residue	18245,2± 0,0707	0,3752± 0,00007	7,50 ± 0,00707

Table 3: Screening of ethyl acetate fraction and α-mangostin.

Samples	Area ± SD	Measurable level (µg) ± SD	Mean content of α-mangostin (%) ± SD
α-Mangostin	10642,8 ± 0	0,0976 ± 0,0071	97,59 ± 0
Ethyl acetate Fraction 1	8438,67 ±1,5132	0,0200± 0,000141	34,14 ± 0,0283

Determination of the Active Compound Using the HPTLC Densitometry Method

The study conducted by Misra et al in 2009 determined the levels of α-mangostin in mangosteen peels using HPTLC method. The results indicated that the average content of α-mangosteen is mostly used in the extraction with methanol solvent> chloroform> ethanol> acetone> ethyl acetate [11]. Similarly, in another study quantitative analysis of α-mangostin in mangosteen rind extract and their microparticle preparations using HPLC method, it was found that the amount of α-mangostin were 49,60% for dichloromethane, 14,13% for 95% ethanol, 13,17% for 50% ethanol extracts, respectively, and dichloromethane extract contained significantly the highest amount of α-mangostin[20].

HPTLC is one of the modern sophisticated technique that can be used for wide diverse applications. It is a simple and powerful tool for high-resolution chromatography and makes trace quantitative analysis possible. It is most widely used for quick and easy determination of quality, authenticity and purity of the crude drug and market formulations[21]. HPTLC is a simple, rapid and reliable method which has been developed for simultaneous estimation of sample [22-24]. Based on α-mangostin assay by TLC method it is showed that the ethyl acetate fraction has the highest concentration of α-mangostin than all other samples that test followed by HPTLC (Table 4)(Fig.7). The result of high performance thin layer chromatography (HPTLC) analysis (table 3) indicated that standard α-mangostin has higher active compounds than ethyl acetate fraction (Fig.7). Based on the data, the detected mean concentration of standard α-mangostin and ethyl acetate fraction is 97,59; 34,17 % w/w.

CONCLUSION

The developed TLC method was precise and accurate based on verification parameters determined. Screening of extraction and fraction of different solvents toward α-mangostin shows the ethyl acetate fraction as the solvent choice for future development of analytical as well as industrial plant based development. The results of analysis by HPTLC indicated that standard α-mangostin has higher active compounds than ethyl acetate fraction in mangosteen peels.

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