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Mangosteen Compound Levels in the Decoction Preparation of Mangosteen Rind (Garcinia mangostanaL.)

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Abstract:

Mangosteenrind(Garcinia mangostana L.) can be used in herbal preparations in the form of decocta. The main content of the active compound in mangosteenrind is α-mangostin. This study aims to determine the levels of α-mangostin compounds in the decocta preparations of mangosteenrind by High-Performance Liquid Chromatography (HPLC) method.This research was carried out by making decocta preparationsof mangosteen rind (Gacinia mangostana L.) using a water solvent. The determination of levels used the HPLC method with the C18 stationary phase, methanol mobile phase: 0.1% formic acid in water (75:25), and UV detection. The determination of the standard curve was done with a standard compound curve to calculate the concentration of α-mangostin in the decocta preparations of mangosteen rind.The results showed that the levels of α-mangostin in the decocta preparations of mangosteen rind were (0.2644 ± 8.3067x10-2)%.

Keywords: *Garcinia mangostana L., α-mangostin, decocta, HPLC.*

I. INTRODUCTION

Mangosteen *(Garcinia mangostana L.)* is a plant that can be used in herbal medicine. Mangosteen rind has been used as a traditional medicine to treat skin infections and wounds in Southeast Asia. Mangosteen rind boiled water empirically is believed to be used for the treatment of tapeworms (Sudarsono, 2002). The use of mangosteen rind, in general, is for treatment in the community; many use boiling techniques with water, which is then filtered (Sudarsono, 2002). Decocta is one of the practical and inexpensive methods of extraction using a water extraction agent.

Compounds contained in mangosteen rind include flavonoids, anthocyanins, and cantonal derivatives. The dominant Cantonese derivatives found in the mangosteen rind are α-mangostin and γ-mangostin (Jung et al., 2006). The α-mangostin compound is potentially cytotoxic in breast cancer cells (Suksamrarn et al., 2006) and as an anti-plasmodium falciparum (Mahabu-

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sarakam et al., 2006). Biological activity as an anti-bacterial, anti-inflammatory, anti-cancer, and inhibition of prostaglandin E2 synthesis (Akao et al., 2008). Cantonese compounds are non-polar (Walker, 2007).

Methods for the analysis of α-mangostin that have been developed today include HPLC (high-performance liquid chromatography) (Walker, 2007), and TLC (thin layer chromatography) (Pothitirat et al., 2008). HPLC analysis method (high-performance liquid chromatography) was selected as a method of determining α-mangostin levels. It was because the HPLC method has the advantage of being able to separate molecules from a mixture, high speed of analysisand sensitivity, good resolution, can be used various detectors, and reusable columns (Putra, 2004).

Based on the description above, it is necessary to determine the levels of α-mangostin compounds contained in decocta preparations of mangosteen rind using the HPLC (high-performance liquid chromatography) analysis method.

II. RESEARCH METHODS

Materials and Tools

1. Materials used

Mangosteen rind *(Garcinia mangostana L.)* was obtained from PasarGede Surakarta in October 2009. Materials for the preparation of decocta preparations were water material for qualitative testing with TLC: Standard α-mangostin, methanol pa, silica gel plate GF_{254} (Germany), and chloroform: methanol (95.5: 0.5). Materials for the quantitative test with HPLC were: Standard α-mangostin, methanol: 0.1% formic acid in distilled water (75:25).

2. The tools used

The tools for making powder were blender, filter device, and glassware. The tools for making decocta were pans, flannel cloths, glassware, and thermometers. The equipment used in qualitative tests with TLC were UV lamp, vessel or elution chamber, micropipette (Socorex Swiss Mode), ependrope, glassware, and analytical balance (Ohaus). Equipment used in the quantitative test with HPLC was a set of HPLCtools (Hitachi L-7100), micropipette (Socorex Swiss Mode), analytical balance (Ohaus), and ependrope.

Research Procedure

1. Making decocta preparations

A total of 10 g of mangosteen rind powder that has been dried, boiled in 110 ml of water at 90 ° C for 30 minutes while stirring occasionally. Squeezed it while hot with a flannel cloth, then thickened it on a waterbath. Next, dried it with freeze-drying for three days.

2. Qualitative Test with TLC

The 25 mg mangosteen rind extract was dissolved in methanol and assisted with an ultrasonicator for 15 minutes. Then, it was filtered with millipore. One µl of the sample solution was bottled on a GF_{254} silica gel plate and eluted with a mobile phase of methanol: 0.1% formic acid in water (75:25). The eluted plate was then dried by aerating it. It was detected by spotting observations using UV light 254 and UV 366 and 1% acetic acid spray reagents in chloroform.

3. Quantitative test with HPLC

a. Making Stock Solution

Standard α-mangostin as much as 1 mg was dissolved in 5 ml of methanol p.a and then

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shaken until dissolved so that a stock solution concentration of 0.02% was obtained.

b. Making the Standard Curve

A series of 0.0200% α-mangostin concentration was made; 0.0150%; 0.0100%; 0.0050%; and 0.0025% with a injection volume of 10 µl using a mobile phase of methanol: 0.1% formic acid in distilled water (75:25), a flow rate of 1 ml/min and read at a wavelength of 245 nm, the area was recorded.

c. Determination of Sample Content

As much as 25 mg carefully weighed, dry extract of decocta preparations of mangosteen rind dissolved in 1 ml of methanol p.a with the help of an ultrasonicator for 15 minutes. Then, the sample solution was filtered to remove particles that could pollute the column. Afterward, a 10 µl sample solution was injected into the pump.

Analysis Techniques

The quantitative test with TLC was calculated based on the value of Rf produced by decoctapreparations spots of mangosteen rind against markers spots of α-mangostin.

$$R_f = \frac{Distance\ \ travelled\ \ by\ substance}{Distance\ \ travelled\ \ by\ solvent} \quad (1)$$

Furthermore, quantitative tests were carried out using the HPLC method based on the area produced. Then a linear regression was calculated between the series of concentration vs. area produced by the equation y = bx + a, y is the area and x is concentration.

The levels of α-mangostin in the decocta preparations of mangosteen rind were calculated by entering the sample area in a linear regression equation between the concentration vs. area of the standard curve of the Standard α-mangostin solution.

III. RESULTS AND DISCUSSION

A. The Result of Making the Decocta Preparations of Mangosteen Rind

The results of the preparation of mangosteen rind decocta*(Garcinia mangostana L.)*, after preparation for three days using freeze-drying, obtained decocta extract of mangosteen rind with a yield (5.9 ± 0.62) % (Table 1). The low yield was possible because the extraction with water solvent could not take all the compounds in mangosteen rind extract. The α-mangostin compound in the decocta preparation was then determined qualitatively and quantitatively.

Table 1. Table of Extraction Results

Replication	Weight of thick extract (gr)	Weight of dry extract (gr)	Yield (%)	Average Yield ±SD (%)
1	43.20	0.52	5.2	5.9 ± 0.62
2	50.88	0.58	5.8	
3	58.23	0.59	5.9	
4	68.41	0.67	6.7	

B. Qualitative Analysis of α-mangostin

Qualitative analysis was performed by TLC (thin layer chromatography) with the silica

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gel GF_{254} stationary phase and the mobile phase of chloroform: ethyl acetate: methanol (80: 10: 5). The results showed the suitability of the chromatogram profile between the α-mangostin standard compound and the decocta preparation sample. There was one sample spot thatwas thought to bethe α-mangostin compound with the hRf of the sample was the same as the hRf of the α-mangostin comparison compound, which was 0.625. Before spraying, it was detected with UV 254, showing a blackout marked by the presence of dark zones, and the plates on UV irradiation with 366 nm spots were not visible (Table 2; Figure 1). Based on the results after being sprayed with 1% acetic acid in a bright yellow fluorescent chloroform spot, it can be concluded that in the decocta preparation of mangosteen rind, there is an α-mangostin compound.

Table 2. Table of TLC Results of DecoctaMangosteen Rind Sample compared to Standard α-Mangostin

Compound	Rf	Before spraying		After spraying	
		UV 254 nm	UV 366nm	UV 254 nm	UV 366 nm
Standard α-mangostin	0.625	Blackout	-	-	Yellow light up
Decocta	0.625	Blackout	-	-	Yellow light up

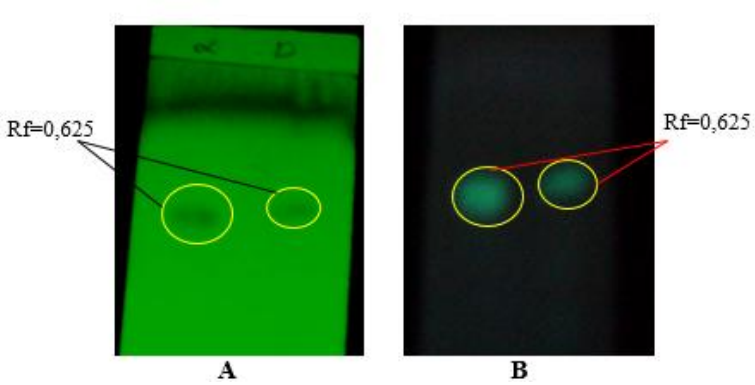

Figure 1 . Thin-layer chromatography of α-mangostin compound and decocta of mangosteen rind; (A) before spraying with 10% sulfuric acid in ethanol in 254 nm UV light (B) after spraying and seen under UV 366 nm. The concentration of 1% was bottled as much as 3 µl on a silica plate with a development distance of 4 cm with methanol solvent. Description:silica gel GF_{254}, stationary phase; Chloroform mobile phase: ethyl acetate: methanol (80: 10: 5)

C. Quantitative Analysis of α-mangostin in Decocta preparations by HPLC method

Quantitative analysis of α-mangostinin HPLC used a reverse-phase, which was a non-polar (C_{18}) stationary phase and a polar mobile phase (methanol: 0.1% formic acid in water (75:25)) at 245 nm UV.

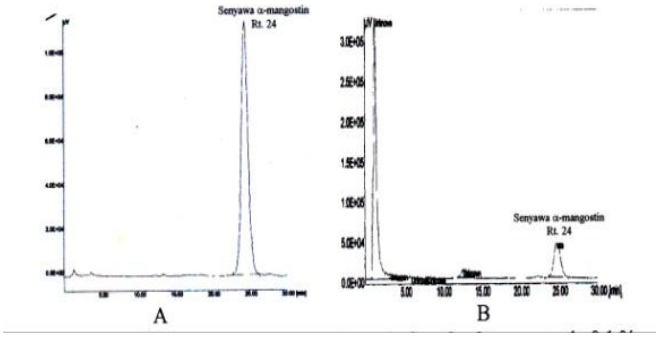

Figure 2. HPLC Chromatogram Profile (A) Standard α-mangostin of 0.1%, (B) Decocta 2.5% description: stationary phase of C_1; mobile phase of methanol: formic acid 0.1% in water (75:25); UV detection; fluorescence 1ml/minute.

Decocta preparations containedα-mangostin, which was characterized by the appearance of a peak at Rt that was the same as the α-mangostin standard (Figure 2.).

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The determination of the standard curve was done with a standard compound curve. Based on the equation of concentration and sample area of α-mangostin, it could be obtained the correlation coefficient value for the α-mangostin standard curve was quite good, which was 0.999. Then, the standard curve could be used to calculate the α-mangostin concentration in the dec 7 preparations of mangosteen rind (Table 3; Figure 3).

Table 3. Standard Curves for α-mangostin

Day	C (g/100ml) x10^{-2}%	RT	Area	Average	SD	CV (%)
I	0.25	24.315	892,653.76	872,712.57	28,201.1	3.23
II		25.960	852,771.38			
I	0.50	24.123	2,028,961.08	1,980,143.28	69,038.80	3.49
II		25.743	1,931,325.47			
I	1.00	24.815	3,926,564.9	3,935,064.79	12,020.66	0.31
II		25.527	3,943,564.68			
I	1.50	24.792	6,136,248.9	5,837,361.56	422,690.48	7.24
II		25.427	5,538,474.29			
I	2.00	24.335	7,205,263.7	7,497,562.64	413,373.13	5.51
II		25.273	7,789,861.58			

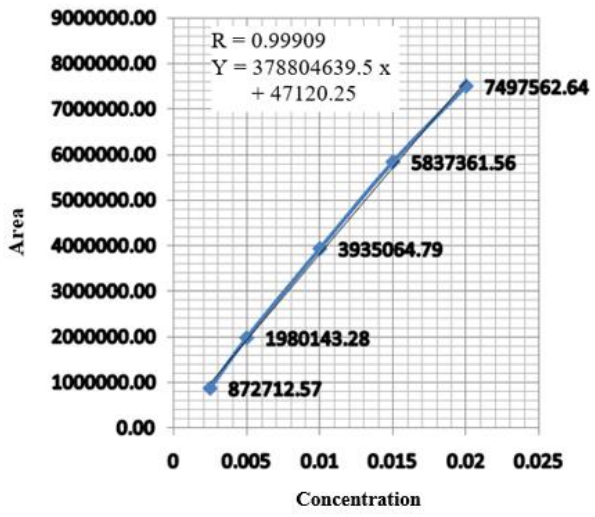

Figure 3. Graph of the α-mangostin standard curve.

The level of active compound α-mangostin contained in the decoctapreparation of mangosteen rind (Table 4) is (0.2644 ± 8.3067x10-2) %.According to Pradikta et al., 2008, compounds that can be exposed to water include flavonoids, polyphenols, tannins, saponins. These levels are smaller than the 70% ethanol extract, which is (27.729 ±2.126x10^{-2}) %. It is due to the nature of α-mangostin, which tends to be non-polar, so it should be used non-polar solvents (hexane, methanol) to extract α-mangostin from mangosteen rind.Relatively small levels of α-mangostinin decocta preparations may reduce the activity of the preparations. The higher levels of the α-mangostin compound in the ethanol extract of 70% gives greater cytotoxic effects (Yuni-asri

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et al., 2010).Other studies mention α-mangostinwith IC_{50} 7.5 μMcan inhibit the growth of DLD-1 colon cancer cells in humans (Akao et al., 2008) and have anti-proliferative activity against HL60 leukemia cells by inducing apoptosis from cancer cells (Matsumoto et al., 2003).

Table 4 Calculation of sample levels

R	Weight (mg)	g/100ml	RT	Area	%(b/v)	%(b/b)
RI	24.98	2.498	24.610	2533678.85	$6.56422x10^{-3}$	0.2627%
RII	24.59	2.459	24.838	2594442.00	$6.72463x10^{-3}$	0.2734%
RIII	24.00	2.400	25.642	2391576.55	$6.17117x10^{-3}$	0.2571%
				Average	$6.48667x10^{-3}$	0.2644%
				SD	$2.84762x10^{-4}$	$8.3067\ x10^{-2}$
				CV	4.3899	3.668

IV. CONCLUSIONS AND SUGGESTIONS

Conclusion

Based on the research results obtained, it can be concluded that the levels of α-mangostin compounds in the decocta preparations of mangosteen rind were (0.2644 ± 8.3067x10-2)%.

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