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The Determination of A-Mangostin Concentration in the Dry Infusion of Mangos teen Pericarp (Garcinia Mangostana L.)

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Abstract
use of mangosteen pericarp (Garcinia mangostana L.) as traditional medicine can be manifested in the form of herbal such as infusion. The main compound in mangosteen pericarp is α-mangostin which supports the inhibition of the growth of cancer cells, antibacterial and antioxidant. This research aims to determine the concentration of α-mangostin contained in the dry infusion of mangosteen pericarp (Garcinia mangostana L.). This research was carried out by extracting simplicia mangosteen pericarp (Garcinia mangostana L.) using the infundation method. Qualitative analysis of α-mangostin in dry infusion was carried out by TLC in the stationary phase of silica GF_{254} and the mobile phase of chloroform: ethyl acetate: methanol (80: 10: 5), as well as 10% sulfuric acid spray reagents in ethanol. Quantitative analysis of α-mangostin in dry infusion was carried out by HPLC in stationary phase C_{18}, mobile phase methanol: formic acid 0.1% in water (75:25) with a flow rate of 1 ml/min at λ 245 nm. TLC chromatograms indicate the suitability of the spots from the samples with the α-mangostin standard (Rf 0.625). Calculation of α-mangostin concentration is done with a standard curve ((2.00; 1.50; 1.00; 0.50 and 0.25) x10-2%) with a correlation coefficient of 0.999. The dry infusion of mangosteen pericarp (Garcinia mangostana L.) contains a relatively small concentration of α-mangostin (0.410 ± 0.039)%.

Keywords*: pericarp, mangosteen (Garcinia mangostana L.), infusion, α-mangostin, HPLC.*

INTRODUCTION

Mangosteen (Garcinia mangostana L.) is one of the plants that is used as a traditional medicine in the form of herbal. Mangosteen pericarp is used to treat thrush, dysentery, rebound tenderness, and constipation (Sudarsono et al., 2002). The use of mangosteen pericarp in infusion dose forms is an easy and simple method (Depkes RI, 2000).

The main components contained in mangosteen pericarp are xanthone (Jung et al., 2006) such as 9-hydroxy calaba xanthone, 3-isomangostin, gartanin, 8-desoxygartanin

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(Walker, 2007), α-mangostin, γ-mangostin, β-mangostin and methoxy-β-mangostin (Akao et al., 2008). The α-mangostin compound which is the most xanthone compound is found in the mangosteen pericarp (Jung et al., 2006). α-mangostin and other xanthone compounds found in mangosteen pericarp tend to be non-polar (Walker, 2007). The α-mangostin compound is antiproliferative against HL60 leukemia cells by inducing apoptosis (Matsumoto et al. 2003). Besides, α-mangostin has antibacterial activity against Mycobacterium tuberculosis and has antioxidant activity (Jung et al., 2006)

The determination of α-mangostin concentration can be done through the TLC method (Pothitirat and Gritsanapan, 2008). Walker (2007) uses HPLC in mobile phase methanol acid: 0.1% formic acid to separate xanthone compounds found in mangosteen (Walker, 2007).

Based on several previous studies above, it is captivating to conduct further research to determine the concentration of α-mangostin compounds contained in the infusion of mangosteen pericarp.

MATERIALS AND METHOD

Materials

Mangosteen pericarp, α-mangostin standard, methanol, silica gel plate GF_{254} (Merck Germany), chloroform: ethyl acetate: methanol (80: 10: 5), 10% sulfuric acid in ethanol, methanol: formic acid 0.1 % in water (75:25) and column C_{18} (RP 5µm no 122184).

Instrumentation

Blender, filtering device, infusion pan, flannel cloths, glassware (Pyrex), thermometer, freeze-drying (Alpha 1-20 Lo Plus Wertheim Germany), UV lamps, elution vessels or chambers, micropipette (Socorex Swiss mode), analytical balance (Ohaus) and ultrasonicator (Cleaner Model no 28H), UV-Vis spectrophotometer (Shimadzu Spectrophotometer UV mini 1240), a set of HPLC tools (Hitachi detector UV-Vis L-7420), syringes (Hamilton co Reno Nevada), micropipette (Socorex) Swiss mode), glassware (Pyrex) and ependropes.

PROCEDURE

Collecting the materials needed

Mangosteen fruit used in this research was purchased in Pasar Gede, Surakarta in October 2009

Drying and Powder Making

Mangosteen pericarp was washed with clean running water and then thinly sliced, and dried in the sun with a black cloth covered. After half dry, it was grounded using a blender and then dried in the sun.

Making Infusion

A total of 10 g of mangosteen pericarp simplicia was boiled in 120 ml of water at 90 ° C for 15 minutes while it was stirred occasionally. Squeezed while it was hot with a flannel cloth, then thickened on a water bath. Drying was done through freeze-drying method for 3 days

Qualitative Test using TLC

Dissolved dried extract of mangosteen pericarp as much as 25.0 mg in methanol aided with ultrasonicator for 15 minutes. A total of 3 µl of the sample solution was bottled on the GF_{254} silica gel plate which is the stationary phase and eluted with chloroform: ethyl acetate: methanol (80: 10: 5) as the mobile phase. Then it was detected by spotting observation using UV light 254, 366 and 10% sulfuric acid spray reagents in ethanol by heating at 110 ° C for 5 minutes.

Quantitative Test using HPLC

Preparing Solutions: 1 mg of α-mangostin standard was dissolved in 5 ml of methanol so that a 0.02% stock solution concentration was obtained

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Making Standard Curve: A series of concentrations of the α-mangostin standard curve was made from a stock solution through the dilution process. A series of standard solution concentrations (2.00; 1.50; 1.00; 0.50; 0.25) x10-2% were made. Then, they were injected into an HPLC column with a volume of 10 µl. Methanol mobile phase: 0.1% formic acid in water (75:25) with a flow rate of 1 ml/min, a stationary phase C_{18} and detection at a wavelength of 245 nm. Later, a linear regression equation between area vs concentration was made.

Determining Sample Dose: A total of 25.0 mg of dry extract of mangosteen pericarp infusion was dissolved in 1 ml of methanol aided with ultrasonicator for 15 minutes. Then the sample solution was filtered with millipore using sartorius filter paper. A 10 µl sample solution was injected into the pump. The level of α-mangostin in the mangosteen pericarp infusion was calculated using a linear regression equation between area vs concentration of the standard curve of the α-mangostin standard solution.

RESULTS AND DISCUSSION

Production of Dried Infusion of Mangosteen Pericarp

A dried infusion was obtained from 10 g of mangosteen pericarp simplicia powder with 4 times replications. Extract drying was done through freeze-drying for 3 days. The yield obtained is (5,425 ± 0,330)%.

The low yield obtained shows that the extraction process cannot extract compounds contained in the mangosteen pericarp to its full potential. Xanthone compounds found in the mangosteen pericarp tend to be non-polar so that it will easily be extracted by non-polar solvents such as chloroform and hexane.

Qualitative Analysis of α-mangostin

The α-mangostin qualitative analysis was performed by the TLC method. The optimization results showed that with the GF_{254} silica stationary phase, a mobile phase of chloroform: ethyl acetate: methanol (80: 10: 5) can optimally separate compounds in the infusion. The TLC profile showed that there were patches of samples that had the same Rf as the standard Rf α-mangostin which was equal to 0.625 (Figure 1). The affirmation reaction was carried out by spraying 10% sulfuric acid in ethanol with the detection of UV light 366 nm. The result of the affirmation reaction showed that the standard α-mangostin and the spots from the mangosteen pericarp infusion had yellow fluorescence (Figure 1).

Qualitative analysis shows that α-mangostin is present in the dry infusion of mangosteen pericarp, but the concentration is low. The α-mangostin compound in the infusion can be detected at a fairly large sample concentration (2.5%). Quantitative analysis is needed to determine the level of α-mangostin compounds found in the dry infusion of mangosteen pericarp.

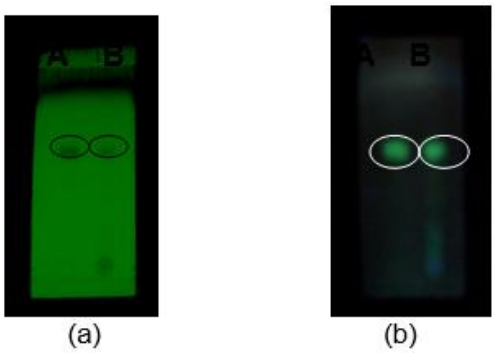

Figure 1. The 0.015% α-mangostin compound chromatogram bottled 4.5 µl (A) with dry mangosteen pericarp infusion sample 2.5% bottled 3 µl (B) with silica GF_{254} stationary phase, mobile phase of chloroform: ethyl acetate: methanol (80: 10: 5), with 254 nm UV

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light observations (a) and 366 nm UV light observations + 10% sulfuric acid in ethanol (b).

A. Quantitative Analysis of α-mangostin

Quantitative analysis of dried mangosteen pericarp infusion was performed using the HPLC method. The wavelength used for detection in the quantitative analysis by HPLC is 245 nm. The optimization results showed that in the stationary phase C_{18} (Octadesyl Silica or ODS), the mobile phase of methanol: 0.1% formic acid in water (75:25) can separate compounds optimally (Figure 2 and Figure 3). Peak at the retention time of 23,872 minutes showed α-mangostin (Figure 3).

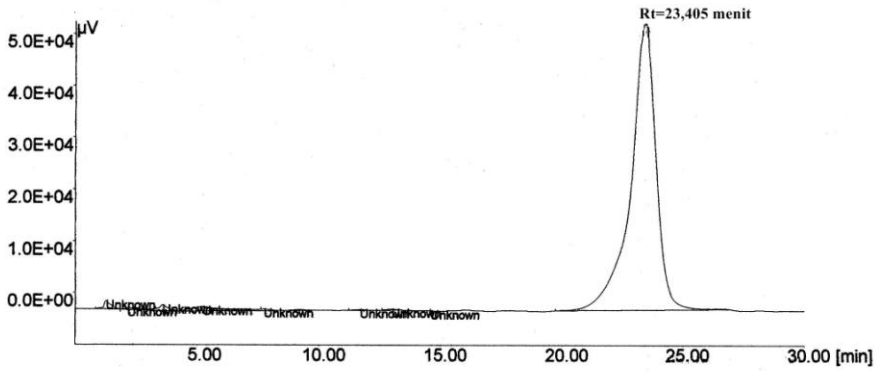

Figure 2. Standard chromatogram α-mangostin 0.01% injection volume of 10 µl, with a mobile phase of methanol: formic acid 0.1% in water (75:25), stationary phase C18 with detection of max λ 245 nm.\

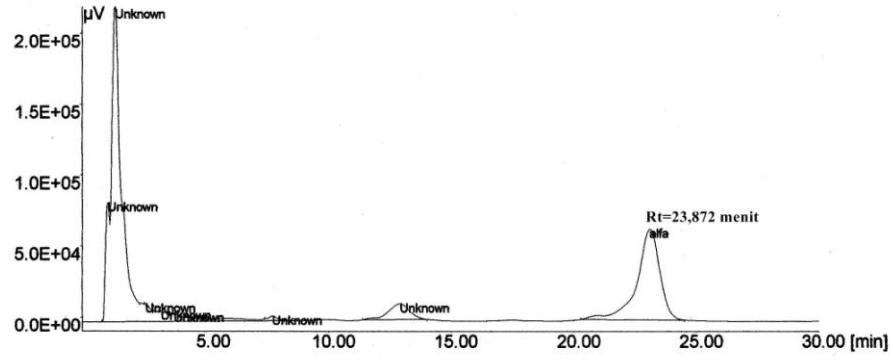

Figure 3. Infusion chromatogram 2.5% injection volume of 10 µl, with methanol mobile phase: formic acid 0.1% in water (75:25), stationary phase C18 with detection of max λ 245 nm.

The stationary phase C_{18} was chosen in determining the α-mangostin concentration because it can separate compounds with low, medium or high polarity. Polar solutes, especially those with bases, will provide a tailing peak during the use of the silica stationary phase. This is due to the adsorption interaction between the solut and the silanol residue found in silica. This problem can be overcome by using a slightly acidic mobile phase, namely 0.1% formic acid in water.

The α-mangostin retention time obtained in this quantitative analysis is 23-25 minutes. This retention time is longer than the one mentioned in research conducted by Jujun et al. (2009) and Walker (2007). Jujun et al. (2009) used the mobile phase of methanol: water (95: 5) and it gave a retention time of 6 minutes. Walker

(2007) used the mobile phase of methanol: 0.1% formic acid with a 65-90% gradient system yielded 21 minutes α-mangostin retention time. The selection of the mobile phase in this research was based on the study conducted by Walker (2007).

The determination of standard curves was done by using standard curves of standard compounds, with 5 series of concentrations (Table 1, Figure 4).

Table 1. Standard Curve of α-mangostin

The concentration of α mangostin (10^{-2} %)	RT (minute)	Area
2.00	24.335	7,205,263.70
	25.275	7,789,661.58
1.50	24.972	6,136,248.90
	25.427	5,538,474.29
1.00	24.815	3,926,564.90
	25.527	3,943,564.68
0.50	24.123	2,028,961.08
	25.743	1,931,325.47
0.25	24.315	892,653.76
	25.960	852,771.38

Figure 4. Standard curve graph of α-mangostin concentration (2.00; 1.50; 1.00; 0.50 and 0.25) x10-2% with a maximum wavelength of 245 nm

Tabel 2. The concentration of α-mangostin in the dry infusion of mangosteen pericarp

Replication	RT (minute)	Area	Concentration % (b/b)	Average concentration (%)
1	23.097	4,085,685.45	0.431	0.410 ± 0.039
2	23.243	4,376,480.80	0.434	
3	23.872	3,608,279.05	0.364	

The determination of the concentration of dry infusion of mangosteen pericarp was done with 3 replications (Table 2). The concentration of α-mangostin in the infusion is (0.410 ± 0.039)%.

The concentration of α-mangostin in dry infusion is relatively small (0.410 ± 0.039)% (Table 4) this is possibly due to the extraction process with water that cannot extract α-mangostin optimally. This is also shown in the relatively small yields. Extraction using polar water solvents produces a relatively small concentration of α-mangostin, this also occurs in steeping tea (7.740 ± 0.564) x10-2% (b /b) (Nurusyifah, 2010). Extraction with ethanol gives greater α-mangostin level which is (27.730 ± 2.130)% (b /b) (Yuniasri et al., 2010). α -mangostin compound tend to be non-polar so that the extraction is more optimal when it is done with non-polar solvents. Other compounds that may be extracted with water solvents other than xanthone are polyphenols, tannins, monoterpenes/sesquiterpenes, quinones, and saponins (Pradipta et al., 2008).

Ethanol extract which has a high α-mangostin level has cytotoxic activity in breast cancer cells (T47D) with an IC_{50} value of 10.986 µg / ml. The dry infusion of mangosteen pericarp has no cytotoxic activity (Melannisa, 2010). The antioxidant activity of the mangosteen pericarp may be influenced by active compounds other than xanthone which are extracted in extraction with polar solvents. Water extract and 50% ethanol extract have better antioxidant activity than ethanol extract 95% and ethyl acetate extract by the DPPH method (Weecharangsan et al., 2005).

CONCLUSION

Based on the results of this research it is concluded that the dried infusion of mangosteen (Garcinia mangostana L.) pericarp contains α-mangostin compounds with relatively low concentration (0.410 ± 0.039) %. This research suggests that further researcher do antioxidant test and screen the compounds available on the dried infusion of mangosteen pericarp (*Garcinia mangostana* L.).

REFERENCES

1. Akao, Y., Yoshihito, N., Munekazu, I., and Yoshinori, N., 2008, Anti-Cancer Effects of Xanthone from Pericarps of Mangosteen, *Int. J. Mol. Sci.*, 9: 355-370
2. Jujun, P., Krisana, P., Yanee, P., Prasit, T., and Chadarat A., 2009, HPLC Determination of Mangostin and Its Application to Storage Stability Study, *CMU. J. Nat. Sci.*, Vol. 8(1)
3. Jung, H.A., Su, B.N., Keller, W.J., Mehta, R.G., and Kinghorn, A.D., 2006, Antioxidant Xanthone from the Pericarp of Gacinia mangostana (Mangosteen), *J. Agric Chem*, 54: 2077-2082
4. Matsumoto, K., Akao, Y., and Kobayashi, E., 2003, Induction of Apoptosis by Xanthones from Mangosteen in Human Leukemia Cell Lines, Abstract, *J. Nat Prod*., 66: 11224-1127
5. Melannisa, R., Da'i, M., dan Indharini,

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ISSN: 0193-4120 Page No. 2157 – 2163

U., 2010, Uji Aktivitas Sitotoksik Sediaan Infusa Kulit Buah Manggis (*Garcinia mangostana* L.) terhadap Sel Kanker Payudara (T47D) dan Penetapan Kadar α-mangostinnya, *Log Book Penelitian Kolaboratif*, Fakultas Farmasi, Universitas Muhammadiyah Surakrta

6. Nurusyifah, 2010, Penetapan Kadar α-mangostin pada Sediaan Teh Kulit Buah Manggis (*Garcinia mangostana* L.), *Log Book Penelitian*, Fakultas Farmasi, Universitas Muhammadiyah Surakarta
7. Pothitirat, W., and Gritsanapan, W., 2008, Quantitative Analysis of Total Mangostins in Gracinia mangostana Fruit Rind, *J Health Res*, 22 (4): 161-166
8. Pradipta, I.S., Nikodemus, T.W., dan Susilawati, Y., 2008, Isolasi dan Identifikasi Senyawa Golongan Xanton dari Kulit Buah Manggis (*Garcinia mangostana* L.), [1]Fakultas MIPA, Jurusan Farmasi, Universitas Islam Indonesia, Yogyakarta dan Fakultas Farmasi, Universitas Padjajaran, Bandung
9. Sudarsono, Didik, G., Subagus, W., Imono, A.D., dan Purnomo, 2002, *Hasil Penelitian, Sifat-sifat dan Penggunaan*, Pusat Studi Obat Tradisional UGM, Yogyakarta
10. Walker, E.B., 2007, HPLC Analysis of Selected Xanthone in Mangosteen Fruit, *J. Sep. Sci.*, 30, 1229-1234
11. Weecharangsan, W., Praneet, O., Monrudee, S., Tanasait, N., Uthai, S., and Pongpan, S., 2006, Antioxidative and Neuroprotective Activities of Extracs from the Fruit Hull of Mangosteen (*Garcinia mangostana* Linn.), *Med Princ Pract*, 15, 281-287
12. Yuniasri, R., Purwati, A., dan Suroso, T.A., Pengaruh Sediaan Teh Celup dan Ekstrak Etanol Kulit Buah Manggis (*Garcinia mangostana* L.) dan Efek Sitotoksiknya pada Sel Kanker Payudara (T47D), *Log Book Penelitian*, Fakultas Farmasi, Universitas Muhammadiyah Surakarta