Validation of Analysis Method α-Mangostin Compound on Skin Fruit Extract Kandis Acid (*Garcinia iowar* Rob. Ex Choisy) Method HPLC

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ABSTRACT

This study uses a method of High-Performance Liquid Chromatography (HPLC) for the analysis of α-mangosteen in the extract Kandis. Analyses were performed with a flow rate of 1 ml/min using Phenomenal C18 column (150 × 4.6 mm) detector used was a UV detector at a wavelength of 240.5 nm. The mobile phase used was acetonitrile: 0.1% phosphoric acid in the ratio (85: 15) v/v. The regression equation of the calibration curve with the concentration of 1, 3, 5, 7 and 9 mg/ml, y=66474x-4456 validation of methods of analysis have been qualified parameter validation analysis, the value of accuracy of 98.62%, 97.90%, 95, 75% precision with 1.27%, 0.7%, 0.3% linearity with correlation Toe Fien 0.9998, the Limit of Detection (LOD) and Limit of Quantification (LOQ) limits respectively 0.22 mg/ml and 0.73 mg/ml. Levels of acid α-mangosteen in lands gained 0.05%.

Keywords: α-mangostin, Kandis, HPLC

INTRODUCTION

Indonesian society has long used medicinal plants for traditional medicine in various types of herbal preparations [1]. One plant that is widely used as a herbal remedy that is from the genus Garcinia, genus Garcinia contains compounds known to many xanthones, bioflavonoid and benzophenone. Xanthones are compounds consisting of cyclic aromatic ring are substituted with an assortment of phenolic methoxy groups and isoprene [2].

Plants of the genus *Garcinia* lately researched content and activities, xanthones compound is mainly known for its potential as a deterrent to the growth of cancer cells and tumors and other diseases. One xanthone derivative compound is α-mangostin which has the ability to suppress the formation of carcinogenic compounds in the colon [1]. One of the plants in the genus Garcinia containing α-mangostin is *Garcinia iowa* Rob. Ex Choisy, known as Landis acid [3].

This plant is a small tree or moderate, slender stems, reaching 30 m high, trunk circumference rarely reaches 90 cm, dark lemon yellow and gummy. Petiole slender with a length of 1 cm, while children petiole small or medium sized. Leaves a slick texture, parallel secondary bone, soft, weak and raised on both sides of the leaf surface with the lower leaf surface much smoother [4].

This plant likes the atmosphere of a shady and humid atmosphere. The flowering period is usually after a long dry period (minimum 3 months) and can bloom twice a year. Small flowers clustered section of the base of the leaves, sepals and petals 4, the width of the male flowers 10-13 mm, length of the flower stalk 4-8 mm, petal size (7 × 5)-(10 × 6) mm, yellow, pink or red, stamen much like a ball, anthers 4 pieces. The ripe fruit is yellow-orange to orange pale dull, dark black when dried, has a longitudinal groove is rough, round, diameter 3 cm, round stigma, fruit stalk length of 5 mm. Seed is located in a flesh-colored pale yellow [4].

Garcinia iowa Rob. Ex Choisy plants has many benefits. In everyday life, dried fruit of this plant is used in India to treat dysentery [5]. In addition, the plant is also used as a pesticide, mosquito repellent and larvicidal [6]. While in Indonesia, particularly West Sumatra, dried fruit Garcinia Iowa Rob Ex. Choisy used for seasoning.

In rind Landis acids are α-mangosteen, which is an amorphous yellow powder, insoluble in water, soluble in alcohol, ether, acetone, ethyl acetate and chloroform, a melting point of 180-182°C and have a maximum wavelength of 220-410 nm [7].

RESEARCH METHODS

Tools and materials

The tool used is a set of tools High Performance Liquid Chromatography (HPLC) (HITACHI Primaide), a silica gel column C18 (Phenomenal C18 4.6 × 150 mm), UV detector, rotary evaporator (IKA® HB 10 basic), analytical balance (Ohaus), plate TLC Silica gel 60 F254 (Merck), vessel chromatography, water bath, aluminum foil, oven (Memmert), cup vaporizer, filter paper, Erlenmeyer, spatula, stir bar, a pipette, funnels, measuring cups, flask, glass beaker, blender, tissue, handschoen, measuring pipette test tube rack, pipettes mumps, spray bottles, glass fiber and bottle maceration.

Materials used in the form of fruit acid peel Landis (*Garcinia iowar* Roxb. Ex Choisy), α-mangostin pure (Wuxi Gorunjie Natural-Pharma Co. Ltd.), methanol pro HPLC (Merck), aqua bidest (BRATACO), 70% ethanol (BRATACO), chloroform (Merck), ethyl acetate (Merck), pro HPLC 99.9% acetonitrile (Merck), and phosphoric acid 85% pro-HPLC (Merck).

Procedure

Sample preparation

The samples are candies sour fruit skin fresh ripe Sarika was taken from the river, Padang Pariaman, West Sumatra as much as 2 kg.

Acid plant identification kandis

Plant collections in Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Andalas, Padang.

Making simple

Landis sour fruit skin that has been separated from the fruit is washed with clean water, then peel the fruit is cut into small pieces, dried and then aired at room temperature. Dry samples blended into a powder, and then filtered through 20 mesh sieve. Samples were stored in an airtight container and protected from light until use [8].

Making extract

Kandis sour rind powder 400 g soaked with 1.5 L of ethanol 70% during the first 3 days, then filtered to obtain macerate I. Then the waste solvent were added 1.5 L 70% ethanol, soaked for the next 3 days, then filtered again in order to obtain Macerate II. The waste soaked again with 1.5 L 70% ethanol, soaked for 3 days and then filtered to obtain macerate III. All macerate combined and concentrated using a rotary evaporator, in order to obtain a thick extract [9].

Determining yield

Weighed thick rind extract Landis acid, then the yield is calculated by the formula:

$$\% \text{ Rendemen} = \frac{\text{Berat ekstrak kental}}{\text{Berat simplisia kering}} \times 100\%$$

Characterizations of skin fruit acid extract Landis

Characterization extract characterization consists of specific and non-specific characterization. For specific Characterization include identical extract, organoleptic, levels of water-soluble compounds, concentration of substances soluble in ethanol, non-specific include drying shrinkage, total ash content.

Test with TLC

Apply the sample solution, the reference solution, and the sample solution+comparative plate TLC Silica gel 60 F254 10 × 5 cm, in the manner, indicated on each monograph with a distance of 1 cm to 2 cm from the bottom edge of the plate TLC Silica gel 60 F254 and let dry up. Enter TLC plate silica gel 60 F254 on shelves buffer, the vessel chromatography, let the system until the mobile phase creeping page distance limit. Remove the TLC plate silica gels 60 F254 and dried in the air and watch the stains by using a 254 nm UV light. Determine Rf.

Conformance test system

Column: Phenomenek C18 4.6 × 150 (mm); Mobile phase: Acetonitrile: Asam fosfat 0.1% (85:15); Flow rate: 1 ml/min; Detector: UV λ240, 5 nm; Volume injection: 20 μl; Run time: 6 min; Retention time: ± 5 min. 0.1% phosphoric acid made by pro-HPLC phosphoric acid solution (85%) 0.6 ml pipette, put into a 500 ml measuring flask, dilute with aqua bidest, shake homogeneous and both ends meet to mark boundaries.

Preparation of α-mangostin parent 100 ml

Weighed 10 mg α-mangostin then dissolved with methanol pain 100 ml flask, shake homogeneous, and both ends meet to mark boundaries so that the mother liquor obtained a α-mangostin concentration of 100 ug/ml.

Preparation of standard solution for calibration curve α-mangostin

Each pipette 0.1, 0.3, 0.5, 0.7 and 0.9 ml of mother liquor of 100 pg/ml, put into a 10 ml volumetric flask, dilute with methanol, shake homogeneous, both ends meet to mark boundaries, to obtain a solution with a concentration of α-mangostin each 1 mg/ml, 3 mg/ml, 5 mg/ml, 7 ug/ml and 9 ug/ml. Then strain respectively with 0.45 μm membrane filter and then injected on HPLC as much as 20 ml. Create a calibration curve was made by plotting the area obtained from the analysis of the concentration of the standard solution. Determine the linear regression equation.

Determination of levels of α-mangostin in skin extracts fruit acid Landis

Extract carefully weighed as much as 600 mg, then diluted with methanol in a 100 ml flask, shake homogeneous and both ends meet to mark boundaries and then the solution is filtered with a membrane filter of 0.45 lm. Inject a solution of 20 ml into the HPLC. The area under the peaks recorded. α-mangostin a concentration in the extract was calculated using the linear regression equation.

Percent accuracy test with achievements back

Percent recovery (% recovery) can be calculated with the following formula:

$$\% \text{ Recovery} = \frac{cf - cs}{cst} \times 100\%$$

Where, cf=Concentration obtained (extract+samples), cs=The concentration of α-mangostin sample, cst=Standard concentration of α-mangostin added

Precision test

Test precision (accuracy) is determined by the parameters of Relative Standard Deviation (RSD) with the formula:

$$\text{SBR} = \frac{SD}{X} \times 100\%$$

Where, SBR=Relative standard deviation, SD=Standard deviation, *X*=Levels of average

Linearity

Linearity is determined based on the correlation coefficient (r) of the calibration curve.

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

To determine the (LOD) and the (LOQ) can use the formula:

$$SD = \sqrt{\frac{\sum(y - yi)^2}{n - 2}}$$
$$BD = \frac{3 \times SD}{b}$$
$$BK = \frac{10 \times SD}{b}$$

Where, BD=Detection limit, BK=Quantitation limits.

RESULTS AND DISCUSSION

This study begins by preparing samples used in this study. The sample used in this study was obtained from Sarika River region, Padang Pariaman, West Sumatra. Samples were taken at one location for the purpose of uniformity of content of plant secondary metabolites. Because of the location may be external factors is the environment (soil and atmosphere) by which plants interact in the form of energy (weather, temperature, light) and matter (water, organic compounds and inorganic).

In this study, taken fresh Landis sour rind that has been cooked. The rind is obtained washed and sliced and then dried at room temperature in order to obtain simplistic rind Landis acid. Then the sample was extracted by maceration. This method is chosen because the process is easy and simple, does not require special equipment, can be used to sample in large quantities, and does not require heating processes that are safe for samples that have thermolabile active substances.

Maceration is done by immersing the samples in 70% ethanol for 3 days with 3 repetitions and occasional samples during immersion shaken so that the penetration of the solvent into the cell is optimized so that the active substance will dissolve faster. The content of extractable compounds has been perfectly marked in Maserati that has faded compared to the initial Maserati. Maceration process is done by using a brown glass bottle and place that is protected from light. It aims to prevent decomposition of the structure of the active substance, especially for substances that are less stable compounds to light.

Solvents in the manufacturing process is a good solvent extract (optimal) for the efficacious compounds or active content, thus the compound can be separated from the material and content of other compounds. Results maceration solvent is then evaporated with a rotary evaporator to obtain the thick extract. The viscous extract obtained weighing 41.90 g with a yield of 2.09%.

Then characterization consists of characterization extract specific and non-specific characterization (Table 1). For specific characterization include the identity of the extract, the organoleptic inspection, and the levels of water-soluble compounds and a concentration of substances soluble in ethanol. Identity extract soluble extract content of water, 0.0891% ± 51.80, levels of soluble extract ethanol, 0.0310% ± 68.32. Non-specific characterization includes drying shrinkage, ash content. Drying shrinkage bark extract Landis acid in the can was 0.76% ± 8.60, while the total ash content in the can was 0.44% ± 5.12. The purpose drying shrinkage is done to provide a maximum limit (range) of the amount of the compound is lost in the drying process while the ash content aims to provide an overview of internal and external mineral content originating from the beginning to the formation of the extract.

Table 1: Test appearance skin fruit extract kandis acid (*Garcinia iowa* Roxb. Ex Choisy)

S. No.	Examination	Observation
1	Smell	Aromatic
2	Flavor	Bitter
3	Color	greenish brown
4	Form	extract viscous

In Thin Layer Chromatography (TLC) test staining obtained four samples, it is possible because there are his other substances contained in the extract, Rf value of the sample was 0.89, 0.64, 0.60, Rf value of 0.58, while the benchmark gained 0.61 Rf value. Approaching Rf value comparison and staining on penotolan on TLC plate and the value of Rf values obtained comparative sample+0.9, 0.65, 0.61, 0.58 (Table 2). From these results it can be stated that the extract Fruit Leather Asam Kandis are a-mangostin for Rf value on a sample of one approaching the value of Rf in comparison (Figure 1).
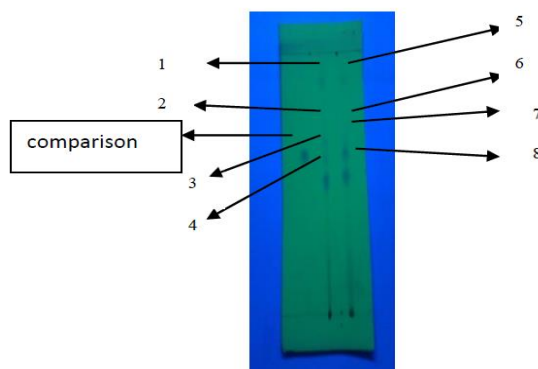
Figure 1: Profile KLT α-mangostin comparative sample extract skin and mixed fruit acid kandis comparators and samples

Table 2: Results identification data α-mangostin on fruit acid skin samples kandis by chromatography

	Distance spotting (cm)	The path length (cm)	Value Rf
Comparison	4.9		0.61
Samples	7.1		0.89
	5.1		0.64
	4.8	8	0.60
	4.6		0.58
Samples+comparison	7.2		0.90
	5.2		0.65
	4.9		0.61
	4.6		0.58

Determination of the wavelength of maximum absorption α-mangostin with UV-Vis spectrophotometric obtained the maximum wavelength of 240.5 nm. Then proceed with the analysis using HPLC. Before performing the analysis by HPLC first tested the suitability of the system namely the selection of the optimal mobile phase for analytical methods.

Selection of mobile phase for analysis tested on a standard solution of α-mangostin with a variation of the mobile phase composition of acetonitrile: 0.1% phosphoric acid, wherein the ratio of acetonitrile mobile phase: 0.1% phosphoric acid (85:15) is more suitable for the analysis of α-mangosteen.

To determine the feasibility of the method used the validated method of analysis. Validation of analytical methods performed to ensure that the analysis method is accurate, specific, reproducible, and hold at a range of analyte to be analyzed. Parameter validation of methods of analysis observed that the accuracy, precision, linearity, limits of detection and quantitation limits.

Linearity calculated based on the value of the correlation coefficient (r) of α-mangostin calibration curve calibration curve was made by plotting the curve between the area under the peaks with a concentration of a standard solution of α-mangostin. The α-mangostin standard solution used was a solution of α-mangostin concentrations of 1, 3, 5, 7 and 9 ug/ml.

The linearity of the correlation coefficient close to unity, where r=0.9998. From the acquisition of the correlation coefficient can be concluded that the analysis carried out valid and eligible linearity with a correlation coefficient (r) approaches 1. In addition, the calibration curve obtained from the linear regression equation y=66474x-4456 (Figure 2 and Table 3).

Table 3: The area of various concentrations of standard α-mangostin

S. No.	Concentrations (µg/ml)	Area
1	1	62705
2	3	189994
3	5	332166
4	7	464536
5	9	590167

The chart shows: $y = 66474x - 4456$, $r = 0,9998$, with Y-axis "Luas Area" (0 to 800000) and X-axis "Concentration (µg/ml)" (0 to 10).
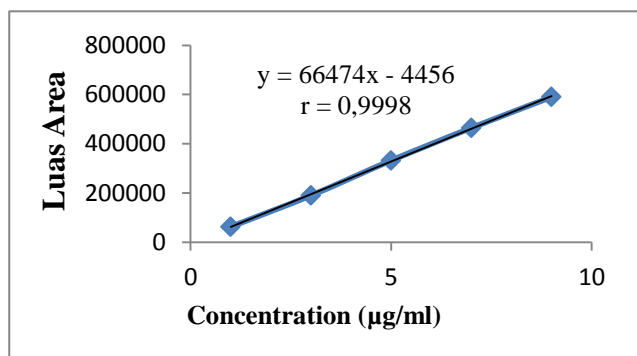
Figure 2: Calibration curves a-mangosten

The limit of detection (BD) is a small amount of analyte in a sample that can be detected and give a significant response to the blank, but the limit of quantitation (BK) is the smallest amount of analyte that can still affect the criteria carefully and thoroughly. The limits of detection and quantitation limits can be calculated statistically by linear regression equation of the calibration curve obtained where the detection limit of 0.22 mg/ml and quantitation limit of 0.73 mg/ml. Determining the value of the limit of detection (LOD) and limit of quantitation (LOQ) depends on the value of b (slope, where the ideal linear relationship is achieved if the value of a=0 and r+1 or -1 depending on the direction of the line). Sensitivity analysis method is said to be less if the value of b<0 so as to provide the limits of detection and quantitation were great. From the data obtained is said to be sensitive because it gives value for b>0, which gives the value of b=66474.

Furthermore, a precision test is done by knowing the value of relative standard deviation or coefficient of variance. After testing the precision seen this method simply provide repeatability (repeatability) or ketertiruan (reproducibility) were quite good at the intraday precision test concentration of 3 mg/ml where the value of the standard deviation is 0.015 and the relative standard deviation of 0.48%. Precision test at 5 mg/ml value of standard deviations is 0.004% and the relative standard deviation is 0.07%. Precision test at a concentration of 7 mg/ml value of the standard deviation is 0.04 and coefficient of variation is 0.51% and the precision test interday concentration of 3 mg/ml where the value of the standard deviation was 0.04 and the relative standard deviation of 1.27%. Precision test at 5 mg/ml value standard deviations are 0.04% and relative standard deviation of 0.7%. Precision test at a concentration of 7 mg/ml value of the standard deviation is 0.03 and coefficient of variation are 0.33% as the coefficient of variation <2%.

Accuracy is the closeness between the measured value (the average value of the results of the analysis) with the value received as a true value, good conversion value, actual value, or value of referrals. Rated accuracy can also be used as a guide systematic error. Usually, the accuracy is shown by percent recovery value. Reacquisition measured using an addition method. Shows the percent recovery of standard additions actually capable identified back to a method. Standard addition method selected for the test sample be extracted, so that the carrier component is very complex and cannot be known with certainty it is not possible to use the method of placebo. The percentage of recovery of α-mangostin in the fruit skin Landis acid respectively 98.62%, 97.90%, 95.74%. Price recoveries obtained in this method meet terms ranged between 80-120%.

Quantitative analysis of α-mangostin skin sour fruit Landis did HPLC. The most common method to define the concentration of unknown compound concentration in a sample is by plotting the concentration area. α-mangostin standard solution is referred to as an external standard for prepared and analyzed separately from the chromatogram. From the analysis of the sample chromatogram, Landis sour fruit skin extracts by HPLC showed a retention time (tR) is equal to tR α-mangostin is 5.04. From the test results are known HPLC levels of α-mangostin in the extract were 0.05%.

Differences in levels of α-mangostin can be affected by some common factors, such as how different harvest, post-harvest processing incautious which includes the sorting, washing, processing results (stripping the skin as well as erosion) and drying. While special factors affecting consisting of factors inside and outside factors. Factor in the covers things is genetically inherited from the mother plant. Types and causes of plant varieties are also differences in the properties, such as taste, odor, chemical content, and production quantities are produced. While external factors are influenced by the environment and the habitats where the plant life such as light, temperature, season, soil properties and nutrient available.

CONCLUSION

From the research that has been done can be concluded as follows: Validate analytical methods meet the test criteria set forth which includes linearity, LOD, LOQ, precision and accuracy. Kadar α-mangostin in the fruit skin extracts Landis acid is 0.05%.

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