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QUANTITATIVE DETERMINATION OF MANGIFERIN IN METHANOL EXTRACT OF BACANG MANGO (*MANGIFERA FOETIDA* L.) LEAVES BY THIN-LAYER CHROMATOGRAPHY DENSITOMETRY

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ABSTRACT

Bacang Mango (*Mangifera foetida* L.) is one of the mango species from the Anacardiaceae family. Mangiferin is an isolated compound from the genus Mangifera. Mangiferin has pharmacological effects such as antimicrobial, anti-inflammatory, antioxidant, and anticancer. The purpose of this research is to determine the levels of mangiferin in methanol extract of bacang mango leaves. Levels of mangiferin in methanol extract of bacang mango leaves are analyzed using validated Thin-Layer Chromatography (TLC) Densitometry method. Mangiferin levels are determined using TLC silica gel 60 GF_{254} plates as stationary phase and using a mobile phase of ethyl acetate: distilled water: formic acid (8.5: 1.5: 1 v/v). This analysis finds out that an R_f is 0.66. The analysis is done using densitometry at 257 nm wavelength. The validation of the analytical method shows linearity with correlation coefficient value 0.9997. Detection limits and quantification limits are 9.90 µg/mL and 32.98 µg/mL, respectively. Precision is indicated by a coefficient with a range of value 0.12 - 0.91%. Accuracy is obtained by percent recovery with a range of value 96.45 - 99.95%. Analysis mangiferin levels in methanol extract of bacang mango leaves show a percentage of levels around 0.28 ± 0.004%.

KEYWORDS: *Mangifera foetida* L., Mangiferin, Validation of Analytical Method, TLC-Densitometry.

INTRODUCTION

Genus Mangifera is one of the largest genera in the Anacardiaceae family. Around the world, there are around 69 species from the genus Mangifera that spread throughout Tropical Asia. *Mangifera foetida* L. is one of the mango species from the Anacardiaceae family. *Mangifera foetida* L., which also known as bacang mango, can be found in Java, Sumatera, Kalimantan, Malaysia to Thailand forest.[1]

Mangifera foetida L. has a huge tree that can grow up to 20 - 25 m tall with 10 m diameter and has a lot of leaves.[2] Its leaves have not been utilized even though it has great potential.[1] Whereas, many researchers have researched the pharmacology aspect, biochemical, medicine, and health of *Mangifera indica* L.[3]

The secondary metabolite compound which is found in bacang mango leaves is a flavonoid, alkaloid, steroid, polyphenol, tannin, and saponin.[4] *Mangifera foetida* L. fruit contains several volatile substances such as esters and monoterpene.[5] One of polyphenol that can be found in bacang mango is mangiferin.[6]

Mangiferin was originally isolated from *Mangifera indica* and was the main compound obtained from this species. Mangiferin has been isolated from various parts of plants such as leaves, fruits, stems, and roots from 16 families such as Anacardiaceae, Gentianaceae, and Iridaceae.[7] Mangiferin has several pharmacology effects such as antimicrobial,[8] anti-inflammatory,[9] antitumor,[10] antiviral,[11] antidiabetic,[12] antioxidant,[13] neuroprotection,[14] and anticancer.[15]

This research is using Thin-Layer Chromatography (TLC) - Densitometry methods. The advantage of this method is the use of fewer solvents, samples, and mobile phases, which makes this method more economical. TLC - Densitometry method also has high specificity, trusted, relatively easy, fast, and inexpensive.

The determination of levels of an active compound requires a sensitive and validated method. Therefore, the validation of the analytical method is needed. Validation is done to ensure that the analytical method used is accurate and specific.[17] Validation of the analytical method is an assessment of certain parameters to prove that the parameters meet the requirement for the researchers.[18]

This research is conducted to determine levels of mangiferin compound in methanol extract of bacang mango leaves using the TLC - Densitometry method and its validation of the analytical method.

MATERIALS AND METHODS

Equipment

The tools used for this research is a chamber (CAMAG®), capillary tubes, set of TLC - Densitometry analyzers (CAMAG®), spectrophotometers UV-vis (Shimadzu 7000 Pharmascpec), rotary evaporator (Buchi®), analytical scales (Kern®). Oven (Mammert®), measuring pipette (Pyrex®), micropipette (Glison®), volum

Chemicals

The material used for this research is pure mangiferin (Wuxi Gorunjie Natural Pharmaco), methanol extract of bacang mango leaves, TLC silica gel 60 GF_{254} plates (Merck®), the solvent used is methanol (Merck®), ethyl acetate (Merck®), and formic acid (Merck®).

Plant material

Bacang mango leaves with dark green color are taken from Rambatan, Kabupaten Tanah Datar, and identified in Herbarium of Andalas University, Indonesia.

Preparation of simplicia and extract

Bacang mango leaves are sorted and washed then dried at room temperature and ground to powder. Simplicia powder is macerated using methanol for 24 hours. After that, it is filtered using filter paper. The filtrate is concentrated using a rotary evaporator so that it can produce a dense extract.

Calculation of yield

Yield is obtained by calculating percentage weight (w/w) between extract acquired and simplicia used.[19]

Preparation of standard solution

Pure mangiferin was carefully weighed as much as 50 mg, entered in a 50 mL volumetric flask and diluted to the mark with methanol. After that, it was diluted until it formed solution with 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL dan 500 µg/mL of concentrations.[18]

Determination of maximum absorption wavelength

Pure mangiferin solution with 100 µg/mL concentration is taken by pipette as much as 1 mL then placed into 10 mL volumetric flask and added methanol to the marks. The result of dilution is then measured for its maximum absorption wavelength using spectrophotometer UV-Vis on 200-400 nm wavelength.[20]

Optimization of mobile phase

Mobile phase optimization is done by making three variations of the ratio of the solvent between ethyl acetate: distilled water: formic acid, that is (9:1:0.5), (8.5:1.5:0.5), and (7:3:0.5). Pure mangiferin solvent is dripped with capillary tubes for 2 µL at the TLC plate. Then, the TLC plate is developed with the mobile phase, the chamber is closed and left open until the mobile phase is moving into the upper limit. Chamber is opened and the TLC plate is removed and dried. After that, it is observed below the UV 254 nm lamp for its patches. The mobile phase is selected by the one which forms round patches and fulfilled the requirements R_f with a range between 0.2-0.8. Also, it has great theoretical plates and a small value of the Height Equivalent of Theoretical Plate (HETP).[21]

Making the calibration curve

Pure mangiferin solution with concentration of 100 µg/mL 200 µg/mL, 300 µg/mL, 400 µg/mL dan 500 µg/mL were dripped to TLC plate for 2 µL. The plate was then developed with a selected mobile phase in the chamber. After that, the plate was analyzed using TLC-scanner on its maximum wavelength. Calibration curved is determined by plotting between area and concentration.

Validation of the analytical method

According to ICH, validation of the analytical method which determined were linearity, the limit of detection, the limit of quantitation, precision, and accuracy.[22]

Determination of mangiferin levels in extract

Bacang mango methanol extract is dissolved in methanol then dripped on to TLC plate for 5 µL. The plate is developed with a selected mobile phase by placed into the chamber. After that, patches are observed below the UV 254 nm lamp and analyzed with TLC-scanner on its maximum wavelength. Levels of mangiferin are calculated by plotting data to the linear regression equation which is obtained through the linearity test. From the densitometry analysis sample, area (y) is determined so that mangiferin levels can be obtained through

equation y = a + bx.[23] In this research, rinds of bacang mango leaves are used as a sample and made into simplicia and extract.

RESULTS AND DISCUSSIONS

Calculation of yield

The extract is made by maceration of simplicia powder of 1000 grams with methanol solvent. After going through the maceration process, it is evaporated under vacuum conditions using a rotary evaporator to reduce air pressure on the surface so that it can lower its boiling point. After that, an extract of 103.8 grams is obtained with a rendemen value of 10.38%.

Determination of maximum absorption wavelength

The maximum absorption wavelength is determined by using spectrophotometer UV-Vis (Figure 1). The result from this is mangiferin maximum absorption wavelength is 257 nm with the absorbance of 0.601.

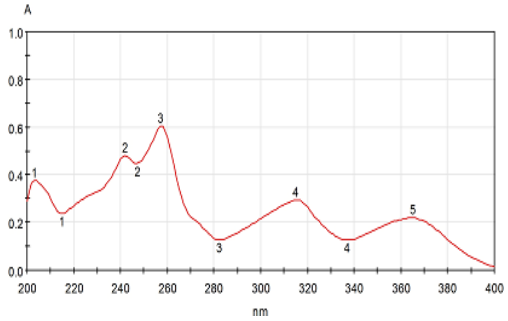

Figure 1: UV Spectrum of mangiferin 10 µg/mL in methanol.

Optimization of mobile phase

(A) Ethyl Acetate: Distilled Water: Formic Acid (8.5: 1.5: 1 v/v)

(B) Ethyl Acetate: Distilled Water: Formic Acid (10: 0.5: 0.5 v/v)

(C) Ethyl Acetate: Distilled Water: Formic Acid (7: 3: 1 v/v)

In selecting the mobile phase, R_f value lies between 0.2 - 0.8 to maximize separation.[16] The selection of the mobile phase is also needed to consider several parameters such as the number of theoretical plates and HETP.[21] In Table 1, it can be seen that all optimized mobile phase meets the requirements of the theoretical plate number, that is < 5000. But a plate that can provide a large number of theoretical plates (N) and a small number of HETP will be able to separate the components in a mixture better.[16] Therefore, the mobile phase selected is ethyl acetate: distilled water: formic acid (8.5: 1.5: 1 v/v).

Table 1: Mobile phase optimization data.

Mobile Phase	R_f Start	R_f End	R_f Max	N	HETP
A	0,57	0,70	0,64	387,79	0,13
B	0,27	0,39	0,34	128,44	0.39
C	0,62	0,77	0,70	348,44	0,14

Validation of the analytical method

From the data below, regression equation is obtained: y = 17,7845x + 194,030 (Figure 2). The correlation coefficient value (r) attained is 0.9997. This shows a significant linear correlation between the concentration variable and the mangiferin area.

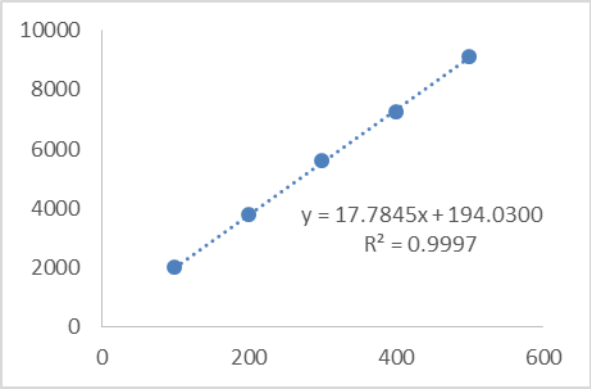

Figure 2: Mangiferin calibration curve.

Linearity

Table 2: Mangiferin linearity data.

Concentration (X) (μg/mL)	Area (Y) (AUC)
100	1957,2
200	3771,9
300	5569,5
400	7225,8
500	9122,5

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ of the calibration curve were obtained in the linear regression equation. LOD was the smallest number of substances in the sample that can be detected while LOQ was the smallest concentration that can be determined by the method with good accuracy and precision. LOD of mangiferin is 9.90 μg/mL and LOQ of mangiferin is 32.98 μg/mL.

Precision

The precision test aims to determine the proximity of analysis results from one to another. In this precision test, three different concentrates of pure mangiferin solutions are used. These concentrations are low concentration (100 µg/mL, medium concentration (300 µg/mL, and high concentration (500 µg/mL). The test used is an intraday precision test which is a precision test with repetition dripping in one day and is done thrice. The results of coefficient variation in percentage are 0.91 %, 0.59 %, and 0.21 %. Inter day precision is a precision test with repetition dripping in three days in a row. The results of coefficient variation in percentage for three days are 0.49 %, 0.17 %, and 0.12 %. Requirements for value of coefficient variation in percentage is < 2 %.[18] Based on the coefficient variation value obtained, the precision shows that the analysis of mangiferin has met the requirement for the precision test.

Accuracy

In this research, the accuracy test is using the standard addition method, in which adding some pure mangiferin solution to the analyzed sample then calculating the percentage of recovery of pure mangiferin solution added. The result of the accuracy test for adding 40% of pure mangiferin solution is 26.806 µg/mL or percent recovery is 96.45%. The result of the accuracy test for adding 80% of pure mangiferin solution is 53.612 µg/mL or percent recovery is 97.38%. The result of the accuracy test for adding 120 % of pure mangiferin solution is 80.418 µg/mL or percent recovery is 99.95 %. The requirement for percent recovery is 85 – 115%. [18]

Determination of mangiferin level in extract bacang mango

The result of TLC scanning of mangiferin with a concentration of 400 µg/mL dripped 2 µL, R_f attained is 0.66 and area 7313.4 (Figure 3). The result of TLC scanning of the extract is 161.221 mg which is dissolved in 10 mL methanol and dripped for 5 µl obtained area is 1010.2 at Rf 0.66 (Figure 4). From the mangiferin regression equation which has been done, the levels of mangiferin in methanol extract of bacang mango are 0.28 ± 0.004%.

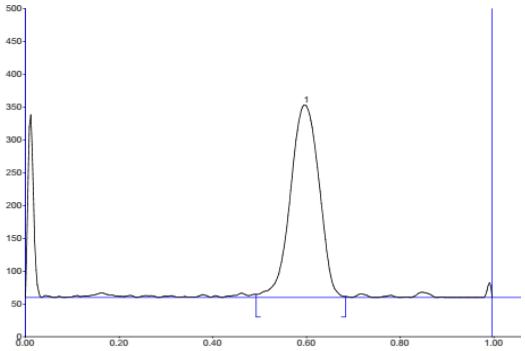

Figure 3: Mangiferin densitogram.

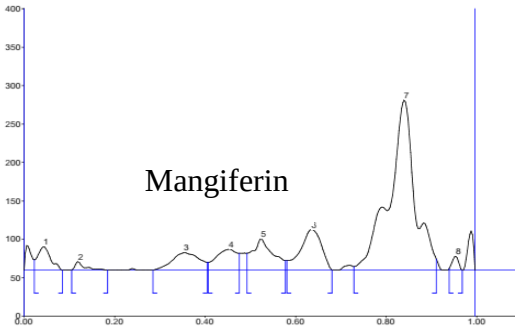

Figure 4: Extract of bacang mango densitogram.

CONCLUSION

Mangiferin levels in methanol extract of bacang mango (*Mangifera foetida* L.) leaves are 0.28 ±0.004%. Determination of mangiferin levels in methanol extract of bacang mango leaves with Thin-Layer Chromatography Densitometry has met the validation parameter.

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