

Determination of the Content of Flavonoids and Total Phenols in Film Strips of 70% Ethanol Extract of Asam Gelugur Leaves (*Garcinia atroviridis* Griff. ex T. Anderson) using the Colorimetric Method

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ABSTRACT: Asam gelugur leaves contain phenolic compounds or polyphenols that act as natural antioxidants with anti-hyperlipidaemic activity, such as flavonoids and phenols. The aim of this study was to produce 70% ethanol extract film strips from asam gelugur leaves that meet the criteria for evaluation of physical, chemical and microbiological quality parameters, and to establish an analytical method for determination flavonoid and phenol content. The research method employed a colorimetric approach using a microplate reader. The results of this study yielded thin-film strip preparations characterised by a dark brown colour, a distinctive odour, a minty and sour taste, a disintegration time of 285 ± 8.50 seconds, a moisture content of 1.62–2.42 per cent, a weight uniformity of 0.4565, film thickness of 0.13 mm, a pH range of 2.53–2.68, no microbial contamination detected in week 0 and a count of 0.2768×10^4 in week 4, the average total flavonoid content in the extract was 3.3954 mg QE/g extract and in the film strips 1.8936 mg QE/g extract, and the average total phenolic content in the extract was 23.4090 mg GAE/g extract and in the film strips 13.8111 mg GAE/g extract. The conclusion of this study is that the film strips did not meet the requirements for several quality evaluation parameters, and the flavonoid and phenolic content levels were higher in the extract than in the film strips.

KEYWORDS: *Garcinia atroviridis* Griff. ex T. Anderson; Film Strip; Total Flavonoid; Total Phenolic, Spektrophotometry

1. INTRODUCTION

The asam gelugur plant (*Garcinia atroviridis* Griff. ex T. Anderson) belongs to the Clusiaceae family and the genus *Garcinia*, which is widely distributed across Indonesia, Thailand, Myanmar, Malaysia and India. Asam gelugur is cultivated in almost all tropical regions. It is commonly used as a flavouring in dishes across Southeast Asia. Asam gelugur contains compounds such as alkaloids, flavonoids, saponins, tannins, steroids/terpenoids, essential oils, phenols, proteins, carbohydrates, minerals, and organic acids such as ascorbic acid, tartaric acid, malic acid, and hydroxycitric acid [1].

Phenolic compounds or polyphenols, such as flavonoids and phenols, constitute the largest and most abundant group of compounds found in plants, acting as natural antioxidants [2,3]. The antioxidants in the asam gelugur plant exhibit antidyslipidaemic activity [4]. The flavonoids and phenols present are capable of inhibiting the oxidation of LDL (low-density lipoprotein) and increasing HDL (high-density lipoprotein) levels in the blood by inhibiting cholesterol absorption in the gut, enhancing bile acid synthesis, and promoting bile acid excretion via the faeces [5].

Traditionally, asam gelugur leaves are consumed raw, cooked, juiced, or boiled in water [1]. These methods are impractical and inconvenient, as boiling results in a sour taste. Therefore, to improve practicality, a 70% ethanol extract of asam gelugur leaves has been formulated as a film strip preparation. The advantages of film strips include the avoidance of the risk of choking, good stability, no need for external water during use, rapid disintegration in the oral cavity, and ease of administration to paediatric and geriatric patients, as well as those with dysphagia and recurrent vomiting [6].

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According to research carried out to determine the total flavonoid and phenolic content in a 70% ethanol extract of asam gelugur leaves using a colorimetric method, the concentrations obtained were 27.31 mg QE/g and 18.04 mg GAE/g respectively, indicating antidyslipidaemic activity in the inhibition of lipase enzymes [7]. In vivo testing of a 70% ethanol extract of asam gelugur leaves in mice induced by a high-fat diet showed that a dose of 400 mg/kg body weight was not significantly different from atorvastatin at 20 mg/kg body weight (positive control), both of which were able to reduce total cholesterol, LDL and triglyceride levels, and increase HDL levels [8]. The results of the formulation and evaluation of film-strip preparations from basil leaf extract yielded a formulation consisting of 6% maize starch, 4% HPMC (hydroxypropyl methylcellulose) and 4% 70% sorbitol [9].

In this study, asam gelugur leaves were extracted using the ultrasonication method with 70% ethanol as the solvent. The ultrasonication method utilises wave energy to generate small bubbles, which accelerate the contact time between the sample and the solvent, speed up the mass transfer of bioactive compounds from within the plant cells to the solvent, increase the yield of bioactive compounds, and shorten the extraction time [10]. A 70% ethanol solvent is capable of extracting compounds across a wide range of polarities, from polar to non-polar compounds; it is non-toxic compared to other organic solvents; it evaporates more readily than water; it is not prone to microbial growth; and it is relatively inexpensive [11].

The novelty of this research lies in its role as the starting point for the development of a natural medicine of a 70% ethanol extract of asam gelugur leaves to help reduce blood lipid levels in the form of film strips, whilst simultaneously developing the analytical methods required for quality assurance to determine the levels of flavonoids and total phenols in the extract and film strip preparations using colorimetry. The colorimetric method utilises colour differences as a comparative parameter by forming stable complexes of compounds, resulting in the absorption of electromagnetic radiation by the complex compounds in the UV-Vis region through transition events, namely the excitation of metal ions, the excitation of ligand molecules, and charge transfer [12].

2. MATERIALS AND METHODS

2.1. Material

70% ethanol (Indo Classica, Indonesia), distilled water (Ratu Cempaka, Indonesia), food-grade HPMC (hydroxypropyl methylcellulose) (Benecel MX) (BEN Abadi Sejahtera, Indonesia), food-grade maize starch (Palapa, Indonesia), food-grade 70% sorbitol (Pharmapreneur store, Indonesia), food-grade stevia (Beeru Official Store), food-grade menthol (Pouyesh Farasoo Kimia, Iran), confectionery oil (Kimia Jaya Abadi, Indonesia), sodium benzoate (Gloria Interchem, India), equimolar phosphate buffer (pH 4) and potassium biftalate (pH 7), phosphate buffer pH 6.8, potato dextrose agar (Merck 1.10130.0500), magnesium powder or plates, concentrated HCl, amyl alcohol, 1% iron(III) chloride solution, AlCl_3 (Merck 801081, Germany), Folin-Ciocalteu reagent (Merck 109001, Germany), Na_2CO_3 (Merck 106392, Germany), quercetin (Sigma Aldrich Q4951, USA), and gallic acid (Sigma Aldrich G7384, USA).

2.2. Procedure

2.2.1. Plant identification

To ensure that the right species, Asam gelugur (*Garcinia atroviridis* Griff. ex T. Anderson), was used, plant identification was done. The University of Indonesia's Department of Biology, Faculty of Mathematics and Natural Sciences, carried out plant identification.

2.2.2. Preparation of a 70% ethanol extract of asam gelugur leaves

Approximately 100 g of powdered asam gelugur leaves was weighed out. It was extracted using an ultrasonicator for 30 minutes with a total volume of 1000 mL of 70% ethanol (ratio 1:10). The extraction was carried out three times with the following solvent volumes: the first extraction used 500 mL of solvent; the residue from the first extraction was re-extracted using 250 mL of solvent; and the residue from the previous extraction was extracted using 250 mL of solvent. The collected filtrate was concentrated using a vacuum rotary evaporator. The concentrated extract was weighed to calculate the yield and native DER.

2.2.3. The moisture content of the extract was determined using the gravimetric method.

Approximately 1 g of the 70% ethanol extract of asam gelugur leaves was placed in a tared container and weighed accurately. It was dried at 105°C for 5 hours, placed in a desiccator for 30 minutes, and then

weighed. Drying was continued, with weighing carried out at 1-hour intervals, until the difference between two consecutive weighings was no more than 0.25%

2.2.4. Determination of residual solvent in the extract

Gas-liquid chromatography was used to determine the amount of residual solvent in the extract. The gas chromatograph was equipped with a flame ionization detector and a 1.8 m × 4 mm glass column that held a TR-wax-coated stationary phase with a particle size of 100–120 mesh. The carrier gas was nitrogen P. Pass the carrier gas through the column at a modest flow rate to condition it overnight at 235°C before using. In order to elute the internal standard, acetonitrile, in five to ten minutes, adjust the carrier gas flow rate and temperature to ± 120°C. [20].

2.2.5. Testing the miscibility of the extract with solvents

The miscibility of the extract with solvents was tested by examining the miscibility of the 70% ethanol extract of asam gelugur leaves with distilled water, 70% ethanol, 70% sorbitol, and sweet oil [21].

2.2.6. pH measurement of the extract

A 1 per cent solution was prepared from the 70 per cent ethanol extract of asam gelugur leaves. The pH was measured using a pH meter that had been previously calibrated with an equimolar phosphate buffer solution (pH 4) and potassium biftalate (pH 7). The pH electrode was immersed in the test solution, and the pH meter needle was allowed to move until it stabilised [9].

2.3. Formulation of a film strip containing a 70% ethanol extract of asam gelugur leaves

Table 1. Formulation of film strip

Material	Formula (%)			
	F0	F1	F2	F3
Extract 70%				
Ethanol asam gelugur leaves	-	9	12	15
HPMC	4	4	4	4
Corn starch	6	6	6	6
Sorbitol 70%	4	4	4	4
Stevia	0.25	0.25	0.25	0.25
Mentol	0.1	0.1	0.1	0.1
Candy oil	1	1	1	1
Sodium benzoate	0.2	0.2	0.2	0.2
Aquadest	100	100	100	100

2.4. Determination of total flavonoid content

2.4.1. Determination of the maximum absorption wavelength.

A quercetin reference standard solution (100ppm) was prepared. Volumes of 0.5, 1.0 and 1.5 mL of the reference standard solution were pipetted into 10 mL volumetric flasks, made up to volume with 70% ethanol and homogenised. For each concentration series of the quercetin reference solution, 1.0 mL was pipetted. Add 1.0 mL of a 2% aluminium chloride solution and 1.0 mL of a 120 mM potassium acetate solution, then mix thoroughly. Incubate for 15 minutes at room temperature in the dark. Then measure the absorbance at wavelengths between 400–450 nm. Note the λ_{max} obtained [23]).

2.4.2. Stabilisation period.

A quercetin reference standard solution (100 ppm) was prepared. 0.5 mL of the reference standard solution was pipetted into a 10 mL volumetric flask, made up to volume with 70% ethanol and homogenised. 1.0 mL of a series of concentrations of the reference standard solution was pipetted. Add 1.0 mL of a 2% aluminium chloride solution and 1.0 mL of a 120 mM potassium acetate solution, then mix thoroughly. The

absorbance of the solution is then measured at a wavelength of 429.40 nm for 60 minutes at 5-minute intervals, using the solvent as a blank [23].

2.4.3. Determination of the calibration curve.

A quercetin reference standard solution (100 ppm) was prepared. Volumes of 0.0; 0.5; 1.0; 1.5; 2.0; and 2.5 mL of the reference standard solution were pipetted into a 10 mL volumetric flask, made up to volume with 70% ethanol, and homogenised. Add 50 μ L each of the 96-well series of reference standard solutions, 2% aluminium chloride solution and 120 mM potassium acetate solution to the wells, then mix thoroughly. Incubate for 15–35 minutes at room temperature in the dark. The absorbance of the solutions was then measured at a wavelength of 429.40 nm [24,25].

2.5. Method validation for quercetin.

2.5.1. Linearity test.

A quercetin reference standard solution (100 ppm) was prepared. Volumes of 0.0; 0.5; 1.0; 1.5; 2.0; 2.5 mL of the reference standard solution were pipetted into 10 mL volumetric flasks; 3.0 mL of the test solution (1000 ppm) was added to each volumetric flask, then the volumes were made up to the mark with 70% ethanol, and the mixtures were homogenised. Add 50 μ L each of the reference standard solution, the test solution, a 2% aluminium chloride solution and a 120 mM potassium acetate solution to a 96-well plate, then mix thoroughly. Incubate for 15–35 minutes at room temperature in the dark. The absorbance of the solutions was then measured at a wavelength of 429.40 nm. The absorbance values were plotted against sample concentration to obtain a linear regression [24,25].

2.5.2. Accuracy test.

A quercetin reference standard solution (100 ppm) was prepared. Volumes of 0.0; 0.5; 1.0; 1.5; 2.0; 2.5 mL of the reference standard solution were pipetted into 10 mL volumetric flasks; 3.0 mL of the test solution (1000 ppm) was added to each volumetric flask, then the volumes were made up to the mark with 70% ethanol, and the mixtures were homogenised. Add 50 μ L each of the 2% aluminium chloride solution and the 120 mM potassium acetate solution to the mixture of the reference standard solution and the test solution in a 96-well plate, then mix thoroughly. Incubate for 15–35 minutes at room temperature in the dark. The absorbance of the solution was then measured at a wavelength of 429.40 nm. The percentage recovery was then calculated [24,25].

2.5.3. Precision (accuracy) test.

A quercetin reference standard solution (100 ppm) was prepared. Volumes of 0.0; 0.5; 1.0; 1.5; 2.0; 2.5 mL of the standard reference solution were pipetted into 10 mL volumetric flasks; 3.0 mL of the test solution (1000 ppm) was added to each volumetric flask, then the volumes were made up to the mark with 70% ethanol, and the mixtures were homogenised. Add 50 μ L each of the mixture of the reference standard solution and the test solution, 2% aluminium chloride solution, and 120 mM potassium acetate solution to a 96-well plate, then mix thoroughly. Incubate for 15–35 minutes at room temperature in the dark. The absorbance of the solution was then measured at a wavelength of 429.40 nm. The standard deviation and coefficient of variation were subsequently obtained [24,25].

2.6. Method for determining total flavonoid content.

2.6.1. 70% ethanol extract of asam gelugur leaves.

A 50 μ L volume of the prepared 70% ethanol extract of asam gelugur leaves (1000 ppm) was pipetted and added to a 96-well plate. Add 50 μ L each of a 2% aluminium chloride solution and a 120 mM potassium acetate solution to the 96-well plate, then mix thoroughly. Incubate for 15–35 minutes at room temperature in the dark. The absorbance of the solution was then measured at a wavelength of 429.40 nm. The absorbance value of the test solution of the 70% ethanol extract of asam gelugur leaves was then plotted against the linear regression equation for quercetin. The total flavonoid content is expressed in mg quercetin equivalents (QE)/g extract [24,25].

2.6.2. Film strips of 70% ethanol extract of asam gelugur leaves.

The test solution of the 70% ethanol extract of asam gelugur leaves was prepared from a film strip of the 70% ethanol extract of asam gelugur leaves, weighed to be equivalent to 500 mg of the 70% ethanol extract

of asam gelugur leaves, and subjected to the same treatment as that used for the determination of the total flavonoid content of the test solution of the 70% ethanol extract of asam gelugur leaves [24,25].

Table 2. Determination of total Flavonoid film strip

	Extract/ film strip	Quersetin	AlCl ₃ 2%	KCH ₃ COOH 120 mM	70% Ethanol
Mixture of standard reference solution and test solution	50 µL	-	50 µL	50 µL	-
Extract test solution/film strip	50 µL	-	50 µL	50 µL	-
Quersetin	-	50 µL	50 µL	50 µL	-
Blank	-	-	50 µL	50 L	51 L

2.7. Determination of total phenol content

2.7.1. Preparation of the reference standard solution.

Weigh approximately 5 mg of gallic acid and place it in a 5 mL volumetric flask, then make up to volume with 70% ethanol. The mixture is then homogenised to produce a gallic acid reference standard solution with a concentration of 1000 ppm (reference standard solution I).

1 mL of reference standard solution I into a 10 mL volumetric flask, then make up to volume with 70% ethanol. It was then homogenised to produce a gallic acid reference standard solution with a concentration of 100 ppm [26].

2.7.2. Preparation of the test solution (70% ethanol extract of asam gelugur leaves).

a) Approximately 500 mg of the 70% ethanol extract of asam gelugur leaves (*Garcinia atroviridis* Griff. ex T. Anderson) was weighed accurately to approximately 500 mg and placed in a 50 mL volumetric flask, then made up to volume with 70% ethanol and vortexed for 30 seconds to obtain a test solution with a concentration of 10,000 ppm (test solution I).

b) A 2.5 mL pipette volume of test solution I was transferred to a 25 mL volumetric flask, then made up to volume with 70% ethanol and vortexed for 30 seconds to produce a test solution with a concentration of 1,000 ppm (test solution II).

2.7.3. Determination of the maximum absorption wavelength.

Prepare a standard reference solution of gallic acid (100 ppm). Pipette 0.5, 1.0 and 1.5 mL of the standard reference solution into 10 mL volumetric flasks, make up to volume with 70% ethanol and mix thoroughly. A series of concentrations of the standard reference solution was prepared by pipetting 300 µL of each. Add 1.5 mL of Folin-Ciocalteu reagent solution (1:10), mix thoroughly, and allow to stand for 3–4 minutes; then add 1.2 mL of 7.5% sodium carbonate solution and mix thoroughly. The mixture was incubated for 60 minutes at room temperature in the dark. The absorbance was then measured at wavelengths between 400–800 nm. The resulting λ_{max} was noted [26].

2.7.4. Determination of stability time.

A gallic acid reference standard solution (100 ppm) was prepared. 1.0 mL of the reference standard solution was pipetted into a 10 mL volumetric flask, made up to volume with 70% ethanol and homogenised. A 300 µL pipette volume of the reference standard solution was taken. 1.5 mL of Folin-Ciocalteu reagent solution (1:10) was added, followed by homogenisation and standing for 3–4 minutes; then 1.2 mL of 7.5% sodium carbonate solution was added, followed by homogenisation. The absorbance of the solution was then measured at a wavelength of 749.80 nm for 90 minutes at 5-minute intervals, using the solvent as a blank [26].

2.7.5. Determination of the calibration curve.

A gallic acid reference standard solution (100 ppm) was prepared. Volumes of 0.0; 0.5; 1.0; 1.5; 2.0; and 2.5 mL of the reference standard solution were pipetted into 10 mL volumetric flasks, made up to volume with 70% ethanol and homogenised. Add 10 µL of the concentration series of the standard reference solution to each well of a 96-well plate, followed by 50 µL of Folin-Ciocalteu reagent (1:10); mix thoroughly and allow to

stand for 3–4 minutes, then add 40 μ L of 7.5% sodium carbonate solution and mix thoroughly. Incubate for 65–75 minutes at room temperature in the dark. Then measure the absorbance at a wavelength of 749.80 nm [24,25].

2.8. Method validation.

2.8.1. Linearity test.

A standard reference solution of gallic acid (100 ppm) was prepared. Volumes of 0.0; 0.5; 1.0; 1.5; 2.0; 2.5 mL of the standard reference solution were pipetted into 10 mL volumetric flasks; 3.0 mL of the test solution (1000 ppm) was added to each volumetric flask, then the volumes were made up to the mark with 70% ethanol, and the mixtures were homogenised. Add 10 μ L of the mixture of the reference standard solution and the test solution to each 96-well plate, followed by 50 μ L of Folin-Ciocalteu reagent (1:10), then mix thoroughly and leave to stand for 3–4 minutes; add 40 μ L of 7.5% sodium carbonate solution, then mix thoroughly. The mixture was incubated for 65–75 minutes at room temperature in the dark. The absorbance was then measured at a wavelength of 749.80 nm. The absorbance values were plotted against sample concentration to obtain a linear regression [24,25].

2.8.2. Accuracy test.

Prepare a standard reference solution of gallic acid (100 ppm). Pipette 0.0; 0.5; 1.0; 1.5; 2.0; 2.5 mL of the standard reference solution were pipetted into 10 mL volumetric flasks; 3.0 mL of the test solution (1000 ppm) was added to each volumetric flask, then the volumes were made up to the mark with 70% ethanol, and the mixtures were homogenised. Add 10 μ L of the mixture of the reference standard solution and the test solution to each 96-well plate, followed by 50 μ L of Folin-Ciocalteu reagent (1:10), then mix thoroughly and leave to stand for 3–4 minutes; add 40 μ L of 7.5% sodium carbonate solution, then mix thoroughly. The mixture was incubated for 65–75 minutes at room temperature in the dark. The absorbance was then measured at a wavelength of 749.80 nm. The percentage recovery was then calculated [24,25].

2.8.3. Precision (accuracy) test.

Prepare a standard reference solution of gallic acid (100 ppm). Pipette 0.0; 0.5; 1.0; 1.5; 2.0; 2.5 mL of the standard reference solution were pipetted into 10 mL volumetric flasks; 3.0 mL of the test solution (1000 ppm) was added to each volumetric flask, then the volumes were made up to the mark with 70% ethanol, and the mixtures were homogenised. Add 10 μ L of the mixture of the reference standard solution and the test solution to each 96-well plate, followed by 50 μ L of Folin-Ciocalteu reagent (1:10), then mix thoroughly and leave to stand for 3–4 minutes; add 40 μ L of 7.5% sodium carbonate solution, then mix thoroughly. The mixture was incubated for 65–75 minutes at room temperature in the dark. The absorbance was then measured at a wavelength of 749.80 nm. The standard deviation and coefficient of variation were subsequently calculated [24,25].

2.9. Method for determining total phenolic content.

2.9.1. 70% ethanol extract of asam gelugur leaves.

A 10 μ L volume of the prepared 70% ethanol extract of asam gelugur leaves (1000 ppm) was pipetted into a 96-well plate. Add 50 μ L of Folin-Ciocalteu reagent solution (1:10) to the 96-well plate, mix thoroughly, and leave to stand for 3–4 minutes; then add 40 μ L of 7.5% sodium carbonate solution and mix thoroughly. Incubate for 65–75 minutes at room temperature in the dark. The absorbance was then measured at a wavelength of 749.80 nm. The absorbance values obtained for the 70% ethanol extract samples were then plotted against the linear regression equation for gallic acid. Total phenolic content is expressed in mg gallic acid equivalents (GAE)/g extract [24,25].

2.9.2. Film strips of 70% ethanol extract of asam gelugur leaves.

The test solution for the 70% ethanol extract of asam gelugur leaves was prepared from a test strip of the 70% ethanol extract of asam gelugur leaves, weighed to be equivalent to 500 mg of the 70% ethanol extract of asam gelugur leaves, and subjected to the same treatment as in the determination of the total phenolic content of the 70% ethanol extract of asam gelugur leaves [24,25].

Table 3. Composition of the total phenol test solution

	Extract film strip	Galic acid	FCR (1:10)	sodium carbonate 7,5%	Ethanol 70%
Mixture of standard reference solution and test solution	10 µL	-	50 µL	40 µL	-
Extract test solution/film strip	10 µL	-	50 µL	40 µL	-
Reference standar galic acid	-	10 µL	50 µL	40 µL	-
Blanko	-	-	50 µL	40 µL	10 µL

3. RESULTS AND DISCUSSION

3.1. Plant Determination Results

The determination of the asam gelugur plant was conducted in the Laboratory of the Biology Department, Faculty of Mathematics and Natural Sciences, University of Indonesia, Depok, to ensure the authenticity of the plant used in this study. The results of the determination of the asam gelugur plant indicated that asam gelugur (*Garcinia atroviridis* Griff. ex T. Anderson) belongs to the Clusiaceae family.

3.2. 70% Ethanol Extract Of Gelugur Leaves

In this study, the extraction method used was ultrasonication. The purpose of ultrasonication is to prevent damage or loss of plant compounds and to increase cell permeability due to vibration. Based on the research conducted, the extract was sonicated for 30 minutes at a frequency of 50 kHz using 100 g of powdered simplicia and a total of 1000 mL of 70% ethanol solvent. The entire filtrate obtained was then collected and filtered through filter paper to prevent any residue from the filtrate being filtered.

After all the powdered simplicia leaves of asam gelugur were extracted using ultrasonication, the collected and filtered filtrate of the 70% ethanol extract of asam gelugur leaves was then thickened using a vacuum rotary evaporator at a temperature of 40°C, a pressure of 175 mbar, and a rotation speed of 45 rpm. The DER-native and yield values were calculated. DER-native is the ratio of the weight of the simplicia used to obtain one g of extract. Determining the yield value aims to determine how much extract is obtained, so the higher the yield, the more extract is obtained[8]. The results of the DER-native and the achieved yield can be seen in Table 4

Table 4. DER-native results and yield of 70% ethanol extract of asam gelugur leaves

Wight of simplisia (g)	Weight of extract (g)	DER-native	Rendemen (%)
2301.90	354.16	6.50	15.39

3.3. Determination Of Extract Water Rate

Determining the water content of a 70% ethanol extract of *asam* gelugur leaves aims to maintain and ensure the quality of the resulting extract over the long term during storage. The water content of the extract must be determined according to general requirements, namely $\leq 10\%$. Water is the fastest medium for microbial growth, so it can damage the resulting extract [10]. The results of determining the water content of the extract can be seen in Table 5.

Tabel 5. Results of determining the water content of 70% ethanol extract of asam gelugur leaves

Sampel	Result (%)
70% Ethanol extract (1)	8,27
70% Ethanol extract (2)	8,32
70% Ethanol extract (3)	8,25
Mean $\pm$ SD	8,28 $\pm$ 0,04

3.4. Results Of Determination Of Remaining Extract Solvent

Determination of residual solvent in 70% ethanol extract of asam gelugur leaves aims to determine the purity of the extract from the ethanol solvent content. The maximum limit requirement for residual solvent in ethanol is 1.0%. This indicates that the extract obtained can be used as a raw material for film strip preparations because it does not contain ethanol content [10]. The results of the determination of residual solvent in the extract can be seen in Table 6

Tabel 6. Results of determining the residual solvent of 70% ethanol extract of asam leaves

Sampel	Result
70% Ethanol extract (1)	ND
70% Ethanol extract (2)	ND
70% Ethanol extract (3)	ND
Mean $\pm$ SD	ND

*ND: Not Detected

3.5 Extract Solvent Mixture Test

The solvent miscibility test on the 70% ethanol extract of asam gelugur leaves aims to ensure that the additional ingredients (distilled water, 70% ethanol, sorbitol, and mint oil) used in the film strip formula can be mixed with the extract, thus facilitating the formulation. The results of the extract solvent miscibility can be seen in Table 7

Table 7 Results of the solvent miscibility test of 70% ethanol extract of asam leaves

Sampel	Result mixture
Extract : Aquadest (1 : 3)	Miscible
Extract : 70% Ethanol(1 : 5)	Miscible
Extract : Sorbitol (1 : 1)	Miscible
Extract : Candy oil (1 : 1)	Miscible

3.6 Results Of Extract pH Testing

The pH test on the 70% ethanol extract of *asam* gelugur leaves aims to determine the effect of the extract's pH on its development in the form of a film strip preparation [10]. The results of the extract's pH test can be seen in Table 8

Table 8 Results of pH examination of 70% ethanol extract of asam leaves

Sampelel	Result
70% Ethanol extract (1)	2.28
70% Ethanol extract (2)	2.24
70% Ethanol extract (3)	2.28
Mean $\pm$ SD	2.27 $\pm$ 0.02

3.7 Results Of Total Flavonoid

3.7.1. Result of calibration curve

The calibration curve was determined using quercetin as a reference standard at 5 concentrations, namely 5, 10, 15, 20, and 25 ppm. Based on the calibration curve determination, a regression equation of $y = a + bx$ will be obtained, where the x value indicates the flavonoid content in the test solution. The results of the calibration curve determination can be seen in Figure

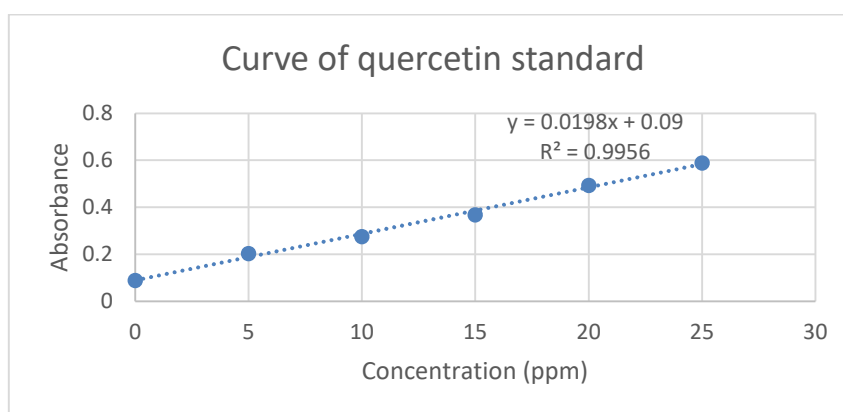


Figure 1. Curve of the relationship between absorption and concentration of quercetin standard reference solution

Based on the results of the calibration curve determination, the regression line equation is $y = 0.0196x + 0.09$ with a correlation coefficient (r) value of 0.9978. The correlation coefficient (r) value obtained is close to 1, this indicates that this method has a linear relationship between the instrument response and the analyte concentration [12].

3.7.2. Result of Validation Method

Partial validation parameters for the analytical method included linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ) tests. Validation of the analytical method was performed under optimum conditions, namely a maximum absorbance wavelength of 429.40 nm and a stability time of 15-35 minutes. This optimisation is carried out to obtain the best possible measurements; the method must be validated, as the response will vary depending on the time, location and equipment used.

3.7.3. Linearity test results.

The linearity test was conducted using a series of test solutions consisting of a mixture of 70% ethanol extract of asam gelugur leaves and/or film strips, supplemented with quercetin standard solutions at six different concentrations. The linearity test aimed to determine the effect of the matrix on the sample by observing the relationship between absorbance and the concentration of the standard solution added to the test solution. A matrix with a correlation coefficient (r) close to 1 is considered to have no effect [12]

The linearity test results for the extract test solution yielded a regression line equation: the first weighing $y = 0.019x + 0.1743$; the second weighing $y = 0.0188x + 0.1988$; and the third weighing $y = 0.0188x + 0.1871$. Each has a correlation coefficient (r) value of 0.9970; 0.9973; and 0.9978, respectively. The results of the linearity test on the film strip test solution obtained a regression line equation including the first weighing $y = 0.0191x + 0.1414$; the second weighing $y = 0.0195x + 0.1538$; and the third weighing $y = 0.0192x + 0.1549$.

Each has a correlation coefficient (r) value of 0.9975; 0.9975; and 0.9972, respectively. Seen from the curve of the relationship between concentration (x) and absorption (y) it provides a straight line with a correlation coefficient (r) value approaching 1, meaning that there is a correlation between the concentration presisvalue used and the absorption detected by the tool. The calculation results from the linearity test can be used to determine the limit of detection (LOD) and the limit of quantitation (LOQ). The limit of detection is carried out with the aim of determining the smallest amount of analyte in the sample that can still provide a significant response compared to the blank. The limit of quantitation (LOQ) is carried out with the aim of determining the sensitivity of the analytical method by measuring the analyte levels without any interference and still being able to provide a good instrument response as seen from the accuracy and precision tests based on the percentage error value of $\pm 20\%$ and the KV value $\leq 20\%$ [3,8,12]

3.7.4. Result of accuracy

The accuracy test was determined by the percent recovery value carried out by adding a number of standard reference solutions to the 70% ethanol extract test solution of asam gelugur leaves and/or film strips and then measured using a microplate reader according to the maximum absorption wavelength. The results of the accuracy test on the extract test solution obtained included 107.01; 88.94; 89.16; 98.24; and 96.92. The results of the accuracy test on the film strip test solution obtained included 104.60; 92.12; 91.01; 101.31; and 97.87. The results of the accuracy test obtained met the requirements, namely 80-120%, thus indicating that the colorimetric method in the microplate reader has an accuracy that can be used for determining total flavonoid levels [12].

3.7.5. Result of Precision

Precision tests were performed repeatedly on samples of the same size. Precision tests were conducted to determine the level of accuracy or closeness of the results of each process to repeated measurements of samples with the same concentration by calculating the standard deviation and relative standard deviation (SBR). The precision value is expressed as the relative standard deviation (SBR) obtained from the percent recovery. The results of the precision test on the extract test solution obtained included 6.43; 5.13; 3.86; 2.94; and 1.80. The results of the precision test on the film strip test solution obtained included 2.86; 5.65; 3.44; 3.80; and 1.95. The results of the precision test obtained met the requirements, because the permissible SBR value is less than or equal to 20% [12].

3.8. Result of the Total Flavonoid

The determination of total flavonoid levels was carried out to determine the amount of flavonoids contained in the 70% ethanol extract of asam gelugur leaves and/or film strips using a colorimetric method. Quercetin was used as a standard reference solution, a flavonoid compound of the flavonol group that has a keto group at the C-4 atom and a hydroxyl group at the neighboring C-4 or C-5 atom. Determination of total flavonoid levels using AlCl_3 reagents results in a wavelength shift towards the visible (visible) region, indicated by the formation of a more yellow complex compound. Potassium acetate was used as a reagent to maintain the wavelength in the visible region [12,13,14].

Based on previous research, the determination of total flavonoid levels in samples of 70% ethanol extract of asam gelugur leaves (*Garcinia atroviridis* Griff. ex T. Anderson) had a total flavonoid content of 27.31 mgQE/g sample, calculated against quercetin using a colorimetric method [7]. Meanwhile, based on this study, the total flavonoid content of 70% ethanol extract of asam gelugur leaves and 70% ethanol extract film strip of asam gelugur leaves were 3.3954 mgQE/g extract and 1.8936 mgQE/g extract, respectively. The difference in results may occur due to differences in drying time, growth location of asam gelugur leaves, and film strip matrix, which can affect the total flavonoid content [13,14]

3.9. Result of Total Phenol

3.9.1. Result of calibration curve

The calibration curve was determined using gallic acid as a reference standard at 5 concentration series, namely 5, 10, 15, 20, and 25 ppm. Based on the determination of the calibration curve, the regression equation $y = a + bx$ will be obtained, the value of x indicates the phenol content contained in the test solution.

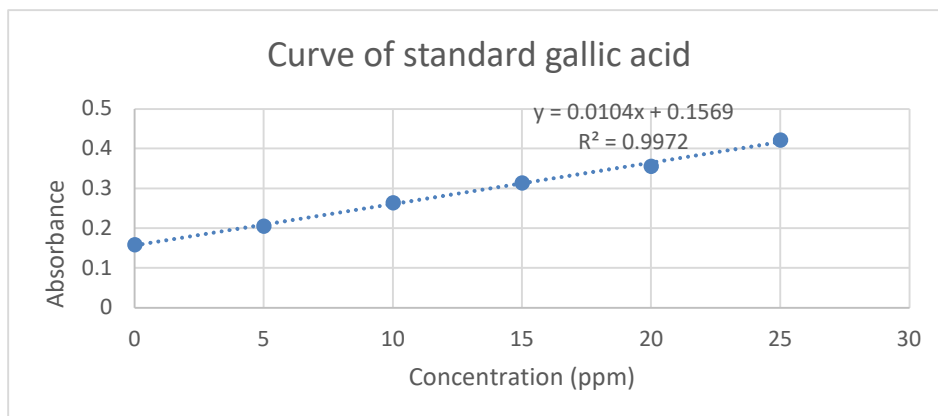


Figure 2. Curve of the relationship between absorption and concentration of standard gallic acid reference solution

Based on Figure 1. the results of the calibration curve determination, the regression line equation is $y = 0.0100x + 0.1569$ with a correlation coefficient (r) value of 0.9986. The correlation coefficient (r) value obtained is close to 1, this indicates that this method has a linear relationship between the instrument response and the analyte concentration [12].

Partial validation parameters for the analytical method included linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ) tests[17]. Validation of the analytical method was performed under optimum conditions, namely a maximum absorbance wavelength of 749.80 nm and a stability time of 65-75 minutes.

3.9.2. Linearity Test Results.

The linearity test was conducted using a series of test solutions consisting of a mixture of 70% ethanol extract of asam gelugur leaves and/or film strips, supplemented with gallic acid standard solutions at six different concentrations. The linearity test aimed to determine the effect of the matrix on the sample by observing the relationship between absorbance and the concentration of the standard solution added to the test solution. A matrix with a correlation coefficient (r) close to 1 is considered to have no effect [12].

The linearity test results for the extract test solution yielded a regression line equation consisting of the first weighing: $y = 0.0116x + 0.2328$; second weighing $y = 0.0113x + 0.2258$; and third weighing $y = 0.0115x + 0.2452$. Each has a correlation coefficient (r) value of 0.9978; 0.9979; and 0.9982, respectively. The results of the linearity test on the film strip test solution obtained the regression line equation includes the first weighing $y = 0.0112x + 0.209$; second weighing $y = 0.0113x + 0.225$; and third weighing $y = 0.0113x + 0.2213$. Each has a correlation coefficient (r) value of 0.9973; 0.9970; and 0.9976, respectively. The relationship between concentration (x) and absorbance (y) curve shows a straight line with a correlation coefficient (r) approaching 1, indicating a correlation between the concentration used and the absorbance detected by the instrument.

The results of the linearity test can be used to determine the limit of detection (LOD) and the limit of quantitation (LOQ). The limit of detection (LOQ) is used to determine the smallest amount of analyte in a sample that still produces a significant response compared to the blank. The limit of quantitation (LOQ) is used to determine the sensitivity of the analytical method by measuring the analyte concentration without interference and still providing a good instrument response, as determined by accuracy and precision tests based on a percentage error of $\pm 20\%$ and a CV value of $\leq 20\%$ [7].

3.9.3. Result of Accuracy

The accuracy test was determined by the percent recovery value, which was performed by adding a certain amount of standard reference solution to the 70% ethanol extract of asam leaves and/or film strip, then measuring it using a microplate reader according to the maximum absorption wavelength. The accuracy test results for the extract test solution were 106.41; 105.52; 119.72; 115.85; and 113.03. The accuracy test results for the film strip test solution were 93.10; 93.61; 111.66; 112.23; and 108.95. The accuracy test results met the

requirements of 80-120%, indicating that the colorimetric method used in the microplate reader has sufficient accuracy to determine total phenol content [23,29]

3.9.4. Precision test results.

The precision test was performed repeatedly on samples of the same size. Precision testing was conducted to determine the accuracy or closeness of the results of each work to repeated measurements of samples with the same concentration by calculating the standard deviation and relative standard deviation (SBR). The precision value is expressed as the relative standard deviation (SBR) obtained from the percent recovery. The results of the precision test on the extract test solution obtained include 11.44; 5.46; 3.81; 5.86; and 3.61. The results of the precision test on the film strip test solution obtained include 7.51; 6.43; 5.41; 3.65; and 2.62. The results of the precision test obtained meet the requirements, because the permitted SBR value is less than or equal to 20% [7].

3.9.5. Results of the Total Phenol Determination Method

The total phenol determination was carried out to determine the amount of phenol contained in the 70% ethanol extract of asam gelugur leaves and/or film strips. Gallic acid, a natural and stable phenolic compound, was used as a standard reference solution. Gallic acid is a hydroxybenzoic derivative of phenolic compounds and is classified as a simple phenolic acid. Total phenol determination uses the Folin-Ciocalteu reagent, and phenolic compounds can only occur in alkaline conditions. Therefore, sodium carbonate is added to provide an alkaline environment that can dissociate the protons in the phenolic compounds into phenol ions [29].

Based on previous research, the total phenol determination in samples of 70% ethanol extract of asam gelugur leaves (*Garcinia atroviridis* Griff. ex T. Anderson) showed a total phenol content of 18.04 mgGAE/g sample (7). Meanwhile, based on this study, the total phenol content of 70% ethanol extract of asam gelugur leaves and 70% ethanol extract film strip of asam gelugur leaves were 23.4090 mgGAE/g extract and 13.5956 mgGAE/g extract, respectively. The difference in results can occur due to differences in drying time, growth location of asam gelugur leaves, and film strip matrix, which can affect the total phenol content contained.

4. CONCLUSION

The colorimetric method using a microplate reader is a valid method for determining the levels of flavonoids and total phenols in 70% ethanol extract of asam gelugur leaves and film strips of 70% ethanol extract of asam gelugur leaves. The total flavonoid levels in 70% ethanol extract of asam gelugur leaves are 3.7542 mgQE/g extract; 3.0803 mgQE/g extract; and 3.3517 mgQE/g extract. While the total flavonoid levels in the film strips of 70% ethanol extract of asam gelugur leaves are 1.8954 mgQE/g extract; 1.9626 mgQE/g extract; and 1.8229 mgQE/g extract. The total phenol levels in 70% ethanol extract of asam gelugur leaves are 23.4143 mgGAE/g extract; 23.3506 mgGAE/g extract; and 23.4621 mgGAE/g extract. Meanwhile, the total phenol content contained in the film strip of 70% ethanol extract of asam gelugur leaves was 13.7225 mgGAE/g extract; 14.1153 mgGAE/g extract; and 13.5956 mgGAE/g extract.

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