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Verification of Methods for Determining The Antioxidant Capacity of Matoa (*Pomitea Pinnata*) Leaves Ethanol Extract By Uv-Vis Spectrofotometry

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Abstract
The testing laboratory of the Faculty of Agricultural Technology, Udayana University is a laboratory that conducts tests for various kinds of analysis, one of which is the antioxidant capacity test. Antioxidant capacity can be measured by UV-Vis spectroscopy method using DPPH that is determined spectrophotometrically through percent absorbance attenuation. The number of studies that focus on matoa leaf extract antioxidants is one reason to improve the quality of laboratory quality by conducting validation and verification tests of the methods used. The objectives of this study was to verify or validate the method of determining the antioxidant capacity content of matoa leaf ethanol extract by UV-VIS spectrophotometry used in the laboratory. The data analysis employed is non-statistical, utilizing descriptive analysis, where the data obtained from the research are presented and interpreted descriptively to provide an overview of the observed facts. The verification and validation parameters evaluated in this study included the Limit of Detection (LOD), Limit of Quantitation (LOQ), precision, accuracy, and linearity. Based on the study results, the linear regression equation obtained was y = 0.0042x - 0.0119 with a value of r = 0.9989, precision value with %RSD <2/3CvH, percent recovery in the range of 93.60 - 98.36%, detection limit of 1.73 ppm and limit of quantification of 5.76 ppm. Based on the verification results, the method satisfies the required standards and is suitable for application in the testing laboratory of the Faculty of Agricultural Technology at Udayana University.

Keywords:
antioxidant capacity, extraction, matoa leaf, UV-VIS spectrophotometer verification.

1. Introduction

Laboratories have a very important role for education, research, and community service activities. One of the duties of Educational Laboratory Staff is testing services. Standardization of sample testing as a form of service to the community requires recognition to improve testing.

Recognition of the laboratory's ability to test a parameter can be done through a series of verification and validation activities of a method.

Method verification is a key requirement in the implementation of the ISO 17025:2005 quality assurance system for testing laboratories, demonstrating that the laboratory meets competency standards in conducting sample testing [1]; [2]. Proper method verification ensures accurate analysis, thereby enhancing consumer confidence in the laboratory's performance [3]. According to [4], the quality, reliability, and consistency of analytical results can be evaluated using the results of method validation and verification. This is part of Good Laboratory Practice (GLP) [4].

Verification plays an important role in producing analytical results with accuracy and precision values that are under the requirements of the referenced test method. According to Rose and Oscroft (1993) in [5], thousands of analytical measurements have been made in the laboratory, but it is estimated that about 10-30% of all measurement data are invalid due to errors.

Data between laboratories or between analysts show variations in results on the same sample. Improper equipment, untrained personnel, and uncalibrated equipment are sources of these differences [5]. Method validation or verification activities need to be carried out so that a method has a high level of confidence and is in accordance with the requirements of a good method, so that it can be used for analysis. Testing laboratories need to verify methods routinely within a certain period to maintain the quality and accuracy of the data produced and maintain consistency and control their performance.

The testing laboratory of the Faculty of Agricultural Technology, Udayana University is a laboratory that conducts tests for various kinds of analysis, one of which is the antioxidant capacity test. Antioxidant capacity can be measured by UV-Vis spectroscopy method using DPPH, DPPH functions as a stable free radical compound that is determined spectrophotometrically through percent absorbance attenuation. Red purple color attenuation at a wavelength (λ) of 517 nm is associated with the ability of bioactive components as free radical antiradicals. One material that is often used as a sample in testing antioxidant capacity is matoa leaf. Several studies have been conducted on measuring antioxidants from matoa leaves. [6] reported that the antioxidant activity of matoa leaf herbal tea was 87.50%. Other research on matoa leaves that has been conducted in this laboratory, as reported by [7], antioxidant activity of matoa leaf extract ranged from 11.172 - 27.526 mg/100g. Research related to antioxidants began to be in great demand as evidenced by the number of test samples that carried out the antioxidant test process in the laboratory at the Faculty of Agricultural Technology, Udayana University as reported by [8] who developed a lemongrass-sugar palm sugar herbal drink with antioxidants of 69.64%. In addition, [9] also developed orange fruit leather and conducted antioxidant tests with 74.89%. The latest research reported by [10] on the potential of teter leaf herbal tea as a functional food also conducted antioxidant tests with a result of 85.55%.

The number of studies that focus on antioxidants, especially extract samples such as matoa leaves and other leaf samples, raises the problem of differences in the methods used to test antioxidant capacity. This is one of the reasons for improving laboratory quality; it is necessary to conduct validation and verification tests of the antioxidant capacity testing methods used in testing antioxidant capacity in extract samples, especially matoa leaf extract. Based on this, to produce accurate data and maintain the quality of laboratory test results, verification of analytical methods needs to be carried out so that verification of analytical procedures is one step to improve research results in determining the content of antioxidant capacity, one of which is tested on ethanol extract of matoa leaves by UV-VIS spectrophotometry. Based on this, this research is expected to get the

correct method that can be used to measure antioxidant capacity in extract samples, which can later be applied to practicum and research activities in the Laboratory.

2. Material and Methods

Time and Place

This research was carried out at the Process Engineering and Quality Control Laboratory and Food Analysis Laboratory, Faculty of Agricultural Technology, Udayana University, from May to August 2024.

Materials

The raw material used is matoa leaf extract. Chemicals: sulfuric acid, sodium phosphate, ammonium molybdate, ethanol, and distilled water. Tools used Oven (Memmert), Whatman No. 42 filter paper, analytical balance (Shimadzu), micropipette (Socorex), weighing bottle (Pyrex), 60 mesh sieve (Retsch), UV-Vis spectrophotometer (Genesys 10S Uv-Vis), rotary vacuum evaporator, test tube (Pyrex), 1 ml volume pipette (Pyrex), 5 ml volume pipette (Pyrex), glass beaker (Pyrex) and volumetric flask (Pyrex), evaporation flask.

Procedure

Making Matoa Leaf Powder

The research began with the preparation of Matoa leaf powder. The leaves obtained were then washed under running water and dried using a clean cloth. Matoa leaves were dried using an oven for 24 hours at 40°C ± 5°C [11]. Once dried, the matoa leaves were ground using a blender and then sieved with a 60 mesh sieve to obtain the modified matoa leaf powder [12]; [10].

Preparation of Matoa Leaf Extract

The process of making matoa leaf extract was carried out using an extraction method with maceration, which refers to [10], which has been modified. Matoa leaf powder passed through a mesh 60 was weighed as much as 10 grams, and 96% ethanol solvent was added with a ratio of material to solvent of 1:10 b/v. Extraction was carried out for 48 hours. After that, the filtration process was continued using Whatman no.42 filter paper to separate the filtrate from the pulp. Then the extract from the solvent was thickened using a rotary vacuum evaporator at a temperature of ± 40°C for 2-3 hours.

Variables

The parameters observed in this study include determining the antioxidant capacity of matoa leaf extract using a UV-Vis spectrophotometer [13] and validating the antioxidant capacity determination method used by testing Accuracy (accuracy), Precision (precision), Limit of detection (LOD), limit of quantitation (LOQ), and linearity (standard curve) [14].

Antioxidant Capacity Procedure

Determination of the antioxidant capacity of the ethanol extract of matoa leaves was analyzed by the DPPH method. A total of 25 mg of sample was added to methanol until the volume was 10 mL. Then centrifuged at 3000 rpm for 15 minutes. The resulting filtrate was pipetted as much as 0.5 ml and placed in a recitation tube. Then, DPPH was added to as much as 3.5 mL, vortexed, and then incubated for 30 minutes. The absorbance was read by UV-Vis spectrophotometry at a wavelength of 517 nm. The readings were compared with the standard

curve using gallic acid concentrations of 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 ppm. Antioxidant capacity was calculated based on the equation:

$$\text{Antioxidant Capacity} = \frac{C \times V \times FP}{W} \times 100\%$$

Noted :
C = Sample Concentration (mg/L)
V = Sampel Volume (L)
FP = Dillution Factor
W = Sample Weight (mg)

3. Results and Discussion

Verification

Verification of analytical methods is a confirmation to ensure that the method of analysis or procedure used has met the requirements and proves that the laboratory concerned can conduct tests with these methods and produce valid results and data. In addition, the results of analytical method validation can be used to assess the quality, reliability, and consistency of analytical results [1]; [2]. Verification is carried out when the analytical method used comes from standardized methods such as methods derived from the Indonesian Pharmacopeia (FI), British Pharmacopeia (BP), United State Pharmacopeia (USP), and other compendials provided that the method has not undergone changes or newly developed methods, then what needs to be done is to validate the method of analysis[15].

Basically, the stages of work carried out in verification are the same as validation, except that the parameters that must be carried out are not as complete as when validating [1]. In the verification of analytical methods, the minimum parameters that must be tested are precision and accuracy. Then, other parameters that can be tested in the verification of analytical methods include linearity, Limit of Detection (LOD), Limit of Quantification (LOQ), specificity and robustness [16].

Linearity

Linearity testing is a test conducted to determine the ability of the analytical method to give a proportional or straight-line response to the concentration of the test substance in the specimen [2]. Linearity testing is carried out using a minimum of five concentrations of the standard solution. The response results of each concentration are then plotted on a calibration curve, with the X-axis being the standard concentration and the Y-axis being the response of the testing instrument. After the curve is formed, the correlation coefficient (r) is determined. A correlation coefficient value close to one is considered to be evidence that the method has a good linearity value [17].

Determination of the linearity of the standard curve with solvent and blank was carried out by making a series of standards with concentrations of 0; 0.2; 0.4; 0.6; 0.8; 1.0 mg/L and measured at a maximum wavelength of 517 nm, then obtained absorbance values and obtained concentration versus absorbance data as in Table 1.

Table 1. Linearity results

Concentration (mg/L)	Absorbance
0	0.589
0.2	0.496
0.4	0.408
0.6	0.321
0.8	0.221
1.0	0.140

Based on Table 1, a curve of the relationship between concentration and absorbance values was made as shown in Figure 1.

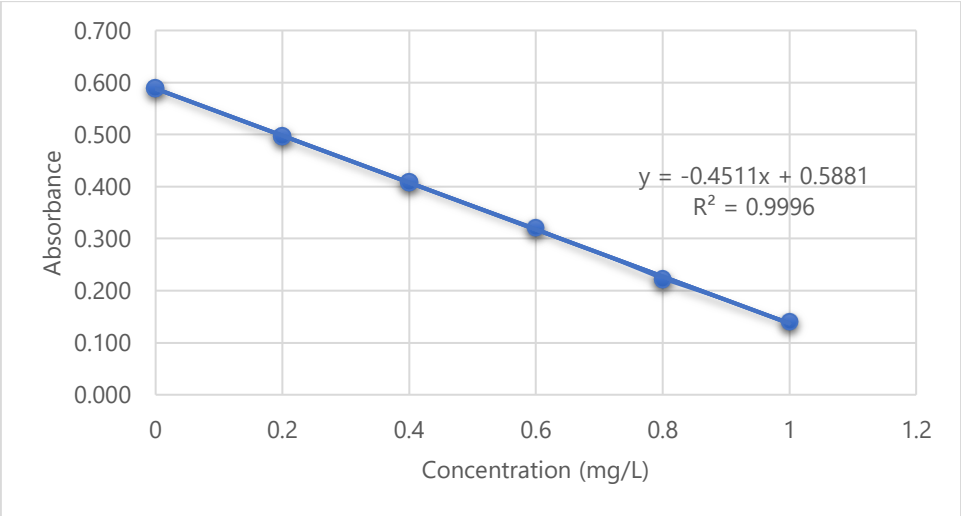

Figure 1. Graph of linearity measurement results based on gallic acid standard curve

Figure 1 shows that the calibration curve using Spectrophotometric method which relates the concentration with absorbance, obtained a linear equation y = -0.4511x + 0.5881 with the coefficient of determination (R2) on the determination of antioxidant capacity levels with gallic acid standard curve of 0.9995. The standard curve obtained has a similar graph to the research conducted by [18], with an R2 value of 0.9924 smaller when compared to the research conducted which is 0.9995. In addition, [19] also emphasized that the coefficient of determination obtained is in accordance with the requirements for acceptance of the linearity test, which is> 0.9970 [19]. This statement is also reinforced by [20] that the coefficient of determination value of more than 0.9 indicates a good relationship between the two parameters so that the concentration range can be used as a reference for determining the concentration of each analyte in other validation tests.

Accuracy

Accuracy testing is a test conducted to determine whether the analytical method used is able to produce a good recovery value. The recovery value obtained is an indication of the closeness of the analysis results to the actual analyte levels [15]; [16]. The results of the calculation of the average percent recovery can be seen in Table 2.

Table 2. Average recovery values at 0.6 mg/L and 0.8 mg/L levels

Actual Concentration (mg/L)	**Measured Levels (mg/L)**	**Recovery (%)**
0.6	0.5892 ± 0.0045	98.19
0.8	0.8119 ± 0.0013	101.49

The calculation results show that the recovery is between 98.19 - 101.49%, the results obtained are greater when compared to the results reported by [21] which amounted to 97.67%. According to [22]; [23] the higher recovery results indicate that the analysis method used is expected to provide data close to the actual. This statement is also supported by a report from [24] that the recovery is required in the range of 80-110% at each level so that the results obtained in this study are in accordance with the requirements.

Precision

The precision test results are shown in Table 3. Based on Table 3, it shows that the %RSD value obtained is smaller than the CV Horwitz, which is 5.444 < 9.320, this proves that the test method used in the determination of antioxidant capacity using UV-visible spectrophotometry has met the requirements of the accepted %RSD value.

Table 3. Measurement results Precision

Concentration (mg/L)	Replica						
	1	2	3	4	5	6	7
0	0.633	0.587	0.587	0.601	0.611	0.567	0.538
0.2	0.526	0.485	0.487	0.522	0.501	0.468	0.485
0.4	0.425	0.411	0.398	0.422	0.412	0.384	0.402
0.6	0.328	0.324	0.324	0.324	0.325	0.298	0.325
0.8	0.252	0.207	0.207	0.222	0.207	0.207	0.246
1.0	0.175	0.124	0.122	0.132	0.125	0.134	0.168
average							0.363
SD							0.020
%RSD							5.444
(Cv Horwitz)							18.640
0,5 CV Horwitz				0,5 * CV Horwitz			9.320
Condition of acceptability Repeatability				%RSD < 0,5 CV Horwitz 5.444 < 9.320			ACCEPTED

The precision of an analytical method indicates the closeness of the results of a series of measurements obtained from repeated tests under certain conditions [16]. Precision is expressed in the value of the relative standard deviation (SBR) with the condition of acceptance is SBR < 2%11. Precision testing of analytical methods can be divided into three categories, namely repeatability, reproducibility and intermediate precision [16].

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of an analytical method is typically expressed through the Limit of Detection (LOD) and the Limit of Quantification (LOQ). The LOD represents the lowest concentration of an

analyte that can be detected and yields a signal distinguishable from background noise or a blank sample. In contrast, the LOQ indicates the minimum concentration at which the analyte can be quantified with acceptable accuracy and precision. When using the calibration curve method, the LOD and LOQ values represent the smallest detectable and quantifiable concentrations of the analyte measurable by the instrument.

Table 4. Limit of Detection and Limit of Quantitation values

Parameters	**Value**
Limit of Detection	0.053 ppm
Limit of Quantitation	0.175 ppm

Based on Table 4. It shows that the LOD and LOQ values in antioxidant capacity testing using the standard curve method still read with a detection limit of 0.0053 ppm while the limit of quantitation is 0.175 ppm. This means that at that concentration, the measurement can still provide accuracy.

Antioxidant Capacity Levels

Determination of levels was carried out by making 3 replicates and measuring the absorbance at a wavelength of 517 nm. The antioxidant capacity level was calculated with the equation y = -0.4511x + 0.5881 so that the results are shown in Table 5.

Table 5. Antioxidant capacity values

Replica	Absorbance	Antioxidant Capacity Levels (% GAEC)
1	0.293	5.84
2	0.321	5.29
3	0.311	5.48
	Average	5.56
	SD	0.28

Based on Table 5. It shows that the results of antioxidant capacity levels with the DPPH method using a spectrophotometer amounted to 5.56% GAEC. The antioxidant activity results indicate that the antioxidant capacity of matoa leaf extract is in the range of 5.56% which is the capacity of gallic acid as a standard comparison. By using the same test method conducted by [25] obtained the results of antioxidant capacity levels ranging from 3.15 - 5.54% were obtained. This indicates that the antioxidant capacity testing method in this study has a good level of observability.

4. Conclusion

Verification of the antioxidant capacity testing method of matoa leaf ethanol extract using Atomic Absorption Spectrophotometer (AAS) UV-VIS has been carried out in the laboratory, and obtained LOQ value of 0.053 ppm and LOD of 0.175 ppm. The precision test results met the acceptance criteria, with a Relative Standard Deviation (RSD) of 5.444%, which is lower than the Horwitz coefficient of variation (CV) of 9.320%. Recovery (%Recovery) ranged from 98.19 - 101.49%. The calibration curve demonstrated excellent linearity, with a correlation coefficient (r) of 0.9996. The antioxidant capacity, determined using the DPPH method with a spectrophotometer, was

5.56% GAEC. Based on the verification results, the method satisfies the required standards and is suitable for application in the testing laboratory of the Faculty of Agricultural Technology at Udayana University.

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