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Development of an iron diagnostic method using Malaka fruit based on digital imaging

Pengembangan Metode Diagnostik Besi Menggunakan Buah Malaka Berbasis Pencitraan Digital

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ABSTRACT

Background: *Iron (Fe) is a geochemically active element that needs to be monitored because excessive levels in water can reduce water quality and pose health risks. WHO sets a safe limit of Fe in drinking water at 0.3 mg/L. This condition demands an accurate, simple, and applicable Fe detection method without complex instruments.*
Objective: *This study developed a digital imaging-based Fe(III) detection method using a natural reagent, malacca fruit extract, which is rich in gallic acid and forms a blue Fe-gallic acid complex.*
Methods: *The study design included laboratory experiments involving gallic acid extraction, optimization of the Fe(III): extract ratio, optimization of measurement time, and evaluation of linearity, precision, accuracy, LoD, LoQ, and t-test against the UV-Vis spectrophotometry method.*
Results: *The results showed an optimum Fe(III): extract ratio of 1:2, with reaction stability up to the 5th minute. A linear relationship was obtained in the range of 0.1–2 ppm (R^2 = 0.9969), with an LoD of 0.1377 ppm and a LoQ of 0.4589 ppm. The %RSD values of 2.34–3.82% indicate good precision, while the accuracy ranges from 92.76–111.2%. A t-test confirmed that the digital imaging results were not significantly different from those of UV-Vis spectrophotometry.*
Conclusion: *Overall, this method offers a portable, economical, and environmentally friendly analytical approach for Fe(III) detection and provides a basis for the development of digital application-based diagnostic systems using natural reagents.*

Keywords: *digital imaging, gallic acid, iron, malacca fruit, smartphone*

ABSTRAK

Latar belakang: Besi (Fe) merupakan unsur aktif secara geokimia yang perlu dipantau karena kadar berlebih dalam air dapat menurunkan kualitas dan menimbulkan risiko kesehatan. WHO menetapkan batas aman Fe dalam air minum sebesar 0,3 mg/L. Kondisi ini menuntut metode deteksi Fe yang akurat, sederhana, dan dapat diaplikasikan tanpa instrumen kompleks.
Tujuan: Penelitian ini mengembangkan metode deteksi Fe(III) berbasis pencitraan digital menggunakan reagen alami ekstrak buah malaka yang kaya asam galat dan membentuk kompleks Fe-asam galat berwarna biru.
Metode: Desain penelitian mencakup eksperimen laboratorium yang meliputi ekstraksi asam galat, optimasi rasio Fe(III):ekstrak, optimasi waktu pengukuran, serta evaluasi linearitas, presisi, akurasi, LoD, LoQ, dan uji-t terhadap metode spektrofotometri UV-Vis. Hasil menunjukkan rasio optimum Fe(III):ekstrak sebesar 1:2, dengan stabilitas reaksi hingga menit ke-5. Hubungan linier diperoleh pada rentang 0,1–2 ppm (R^2 = 0,9969), dengan LoD 0,1377 ppm dan LoQ 0,4589 ppm. Nilai %RSD 2,34–3,82% menunjukkan presisi yang baik, sedangkan akurasi berada pada rentang 92,76–

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111,2%. Uji-t mengonfirmasi bahwa hasil pencitraan digital tidak berbeda signifikan dibandingkan spektrofotometri UV-Vis.
Kesimpulan: Secara keseluruhan, metode ini menawarkan pendekatan analitis yang portabel, ekonomis, dan ramah lingkungan untuk deteksi Fe(III), serta memberikan dasar pengembangan sistem diagnostik berbasis aplikasi digital menggunakan reagen alami.
Kata kunci: asam galat, besi, buah malaka, pencitraan digital, smartphone

INTRODUCTION

Iron (Fe) is an abundant element in the environment and highly geochemically active, making its presence important to monitor in various biological and environmental systems. Excessive Fe levels in water can cause various problems, such as discoloration and odor, corrosion in piping systems, and a decline in overall water quality[1]. In addition, long-term exposure to high Fe levels can increase the risk of health problems, including gastrointestinal discomfort and chronic toxic effects. According to WHO standards, Fe concentrations in drinking water should not exceed 0.3 mg/L to maintain water safety and quality[2]. Given these impacts, an accurate, easy-to-implement Fe detection method is needed that can be used for various types of samples, including water.

Iron analysis methods commonly used today include UV-Vis spectrophotometry, atomic absorption spectrophotometry, and complexation methods such as Fe–thiocyanate and Fe–phenanthroline[3]. Although these methods offer high sensitivity and accuracy, their application requires expensive instrumentation and trained operators. These limitations make conventional methods impractical for field monitoring or use in resource-limited areas. Therefore, alternative approaches to iron diagnostics that are simpler, lower-cost, and easily implemented on-site are needed.

Research has been developed on the determination of iron using natural materials with Malacca fruit extract (Phyllanthus emblica or Indian gooseberry) using the UV-Vis spectrophotometry method and Flow Injection Spectrophotometer at wavelengths between 560 and 570 nm[4],[5]. The principle of this method is the formation of a light yellow to blue complex as a result of the reaction of iron (III) and gallic acid complexes from Malacca fruit extract at pH 5.6 with acetate buffer. The proposed research used gallic acid extract from Malacca fruit for iron diagnostics. By utilizing the compound extract, the use of chemical reagents for iron diagnostics can be minimized.

Digital imaging techniques have been developed as attractive tools for field analysis with good sensitivity, offering low cost, ease of use, and portability, encouraging their use in various diagnostic, health, and environmental fields. This technique has been developed for metal diagnostics using Whatman paper as a sensitive and selective device based on a colorimeter sensor that is dripped with samples and reagents[6], [7], [8].

Microfluidic Paper-Based Analytical Device(μPAD) is a paper-based analytical device as a cheap, portable, and easy-to-use testing method for the general public who do not have special expertise. By using PAD, a much smaller volume of reagents and samples is required, so the volume of waste produced is lower[9].

In this study, gallic acid was extracted from Malacca fruit using a maceration method with distilled water. Analysis was performed using Whatman paper prepared to form a hydrophobic boundary with wax crayons. The colorimetric analysis method was performed by photographing using an Android smartphone camera. The resulting photos were measured for color intensity using the ImageJ program[10]. The intensity data were converted to absorbance using the Lambert–Beer equation. This study

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aimed to develop a method for detecting Fe levels using gallic acid malacca fruit extract as a natural reagent.

METHODS

Study design

This research design was a laboratory experimental study aimed at developing and evaluating a Fe detection method using a digital imaging-based Microfluidic Paper-Based Analytical Device (μPAD). Method evaluation was carried out through analytical parameter testing, including reagent volume optimization, measurement time optimization, precision, accuracy, Limit of Detection (LoD), and Limit of Quantification (LoQ). In addition, the measurement results of the μPAD method were compared with the UV-Vis spectrophotometry method as a reference method to assess the suitability of the results. The research will be conducted at the Chemistry Laboratory of the Medical Laboratory Technology Department, Poltekkes Kemenkes Bandung in July-October 2025.

Data source and sampling procedure

The samples used in this study consisted of three water samples analyzed using the μPAD method and UV-Vis spectrophotometry. Each sample was measured in triplicate to ensure consistency and precision of the results.

Variables of the study

The variables in this study consisted of independent, dependent, and controlled variables. The independent variables included the reagent volume ratio (Fe(III) solution to gallic acid extract), pH conditions, measurement time, and Fe(III) concentration in the linearity test. The dependent variable was the analytical response in the form of RGB intensity values obtained from digital image analysis, which were further converted into absorbance values to represent Fe(III) levels. The controlled variables included the volume of Fe(III) solution (10 μL), the type of paper used (Whatman paper No. 42), the distance of image capture (25 cm), the imaging device (Android-based smartphone), and environmental conditions during the experiment.

Data Collection

Gallic Acid Extraction from Malacca Fruit

The dried Malacca fruit was then processed into a fine powder before extraction. A total of 30 g of Malacca fruit powder was weighed using an analytical balance and then extracted using a 1:50 (g/mL) maceration method using distilled water. The maceration process lasted for 48 hours at room temperature, protected from sunlight. The resulting extract was then used as a source of gallic acid in the μPAD analysis.

Making Microfluidic Paper-Based Analytical Device (μPAD)

The paper used to create the Microfluidic Paper-Based Analytical Device (μPAD) was Whatman paper No. 42. A hydrophobic pattern design was printed on the paper using a wax crayon. The pattern was heated using a hot plate at 120°C for 5 minutes. The resulting paper was used as a paper device for subsequent analysis procedures.

RGB Value Measurement

The Image J application was used to determine the RGB values of the color complexes formed on the μPAD paper. The scanned μPAD photo was entered into the software, then a homogeneous colored area was set as a measurement point to ensure signal uniformity. The RGB intensity values were extracted using the “RGB Measure” feature in the “Plugins” menu of the ImageJ application. The component

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with the maximum intensity was used to represent the color change due to the analyte reaction. This intensity value was then converted to absorbance using the Lambert–Beer equation.

$$A = -\log\left(\frac{I}{I_0}\right)$$

Description: A is absorbance; I is the intensity of the sample or control, and I_0 is the solvent intensity with a value of 255.

Optimization of Fe: Gallic acid Ratio of Malacca Fruit Extract

A 0.5 mg/L Fe(III) solution was used as the analyte and was dropped onto the detection zone of Whatman paper as much as 10 µL. Gallic acid extract from Malacca fruit was then added to the same area with varying volumes of 10, 20, 30, and 40 µL. The reaction was allowed to proceed for one minute before the paper was photographed using a smartphone camera at a fixed distance of 25 cm from the µPAD surface. The obtained photos were analyzed using the ImageJ application to obtain RGB intensity values, which were then calculated for absorbance. The Fe: extract ratio was determined based on the highest absorbance value, and this condition was used for the next stage of measurement.

pH optimization

pH optimization was performed by adjusting the solution conditions within the pH range of 3–9 using acetic acid (pH 3–4), acetate buffer (pH 5–6), and NaOH (pH 7–9). Each pH condition was analyzed using the same staining procedure as the previously determined optimum ratio conditions. The resulting color intensity at each pH was measured and compared, and the pH that produced the sharpest color change was determined as the optimum condition for further analysis.

Measurement Time Optimization

A 0.5 mg/L Fe (III) solution was used as the analyte and dripped onto the detection zone of Whatman paper as much as 10 µL. Gallic acid extract from Malacca fruit was then added to the same area based on the results of the Fe: extract ratio optimization. Time optimization was carried out by varying the measurement interval between 1 and 15 minutes. At each measurement time, the color formation was photographed using a smartphone camera at a fixed distance of 25 cm from the µPAD surface. The obtained photos were analyzed using the ImageJ application to obtain RGB intensity values, which were then calculated for absorbance. The time that produced the most stable and significant color intensity was selected as the optimum time and used in all subsequent measurement series.

Measurement and instruments

Tool

The tools used in this study include a 10 mL volumetric flask, a 1 mL measuring pipette, a 10 mL measuring pipette, a micropipette, a 50 mL beaker, an analytical balance, a hot plate, scissors, wax crayons, an Android-based smartphone, UV-Vis spectrophotometry, and the Image J application.

Material

The materials used in this study include Whatman paper No. 42, Malacca fruit, CH_3COOH (E-Merck), NaOH (E-Merck), FeCl3 (E-Merck), acetate buffer, distilled water, and water samples. The chemicals used are pro-analytical.

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Data Analysis

Measurement Linearity Test

The optimum conditions obtained from the optimization of the ratio, pH, and time were used to test the linearity of the μPAD method response. Fe(III) solutions with concentrations of 0.1, 0.5, 1, and 2 ppm were analyzed to determine the relationship between concentration and absorbance. The absorbance data from the RGB analysis were then processed to form a calibration curve, and a linear regression equation was obtained as the basis for determining the Fe content in the sample.

Validation Test

LoD and LoQ calculations

The lowest analyte concentration in a sample that can be detected by the analytical measurement mechanism of the method is defined as the Limit of Detection (LoD). Meanwhile, the Limit of Quantification (LoQ) is the smallest quantification value of the analyte in a sample that still meets accuracy and precision criteria.

$$\text{LoD} = \frac{3SD}{slope} \qquad \text{LoO} = \frac{10SD}{slope}$$

Information:
SD: Standard deviation
Slope: Slope of the calibration curve

Precision Calculation

Precision refers to the extent to which measurement results obtained from the same sample demonstrate consistency with each other when the analysis procedure is repeated. Precision is usually expressed through the standard deviation (SD) and relative standard deviation (%RSD). The standard deviation aims to obtain a value of the accuracy of the intensity values obtained.[13]. The formula for standard deviation and relative standard deviation is as follows:

$$\text{SD} = \sqrt{\frac{\sum_{i=1}^{n} (xi-\bar{x})^2}{n-1}} \qquad \%\text{RSD} = \frac{SD}{X} \text{ x } 100\%$$

Information :
Elementary School: Standard Deviation
% RSD : Relative Standard Deviation
$\bar{X}$: Average analyte content

Calculation of the acceptability of measurement precision values can be done using the Relative Standard Deviation (%RSD) value. A method is categorized as valid or acceptable if the %RSD obtained is below 5%.

Method Accuracy Test

Accuracy is one of the parameters needed to determine whether a method is valid. Accuracy is a value that indicates how close the measured concentration in a sample is to the actual concentration. The % Recovery value can be used to indicate method accuracy. The % Recovery formula is as follows:

$$\%Recovery = \frac{[Fe(II)]\ terukur}{[Fe(II)]\ yang\ sebenarnya} \text{ x } 100\%$$

Comparison t-Test of Two Methods

A two-method comparison t-test was used to determine whether there was a significant difference in the data obtained from the two methods. When measuring the Fe(III) content in the sample, the μPAD method was compared with the standard UV-Vis spectrophotometry method. The hypothesis in the two-method comparison t-test was:

H_0: There is no real difference between the two methods

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H_1: There is a real difference between the two methods

H_0accepted if thitung < t table, this indicates that there is no real difference between the two methods. While H_0rejected if thitung > t table, this indicates a real difference between the two methods.

RESULT

Optimization of Fe: Gallic acid Ratio of Malacca Fruit Extract

Optimization of the Fe: gallic acid extract ratio was carried out to determine the optimum volume ratio between Fe and gallic acid extract that provided the highest absorbance value in forming a blue Fe-gallic acid complex. The results of determining the optimum conditions for the Fe: gallic acid extract ratio are presented in Figures 1 and 2.

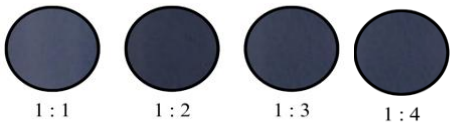

Figure 1. Digital imaging results on optimizing the concentration ratio of Fe: Gallic acid extract from Malacca fruit

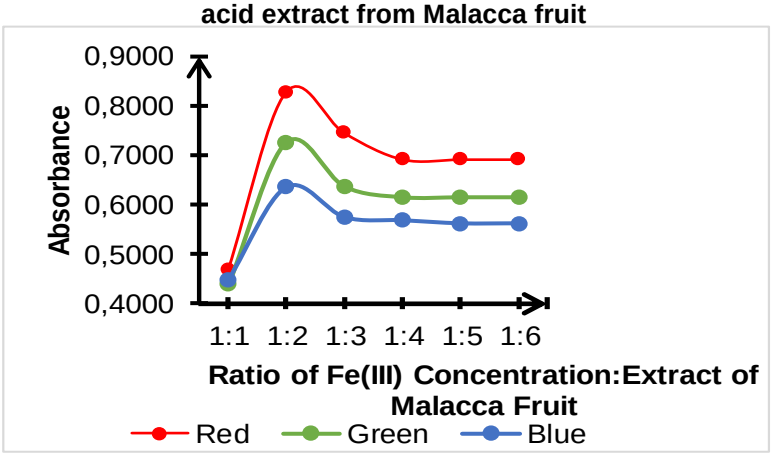

Figure 2. Absorbance curve at Ratio of Fe (III) concentration:Extract of Malacca Fruit

Based on Figure 1, it can be seen that at a ratio of 1:2 (Fe Concentration: Gallic Acid Extract) a more concentrated blue complex is formed compared to other ratios. Figure 2 shows an increase in absorbance values from a ratio of 1:1 to a ratio of 1:2. The absorbance tends to decrease and then stabilizes at a ratio of 1:3 to 1:6. This pattern indicates that the amount of extract added at a ratio of 1:2 provides optimum conditions for the formation of the Fe–gallic acid complex on µPAD paper. Therefore, a ratio of 1:2 was used as the optimum condition for the next stage of analysis.

Measurement Time Optimization

Measurement time optimization was performed to determine the optimum time required for the Fe-acid complex to form completely. The results of determining the optimum conditions for measurement time are presented in Figures 3 and 4.

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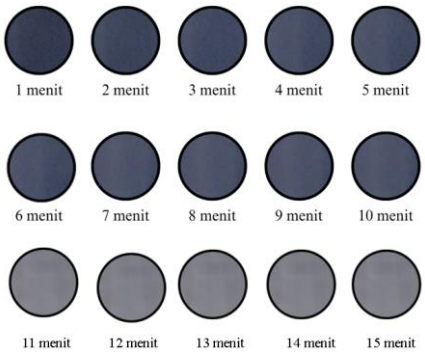

Figure 3. Digital imaging results of measurement time optimization

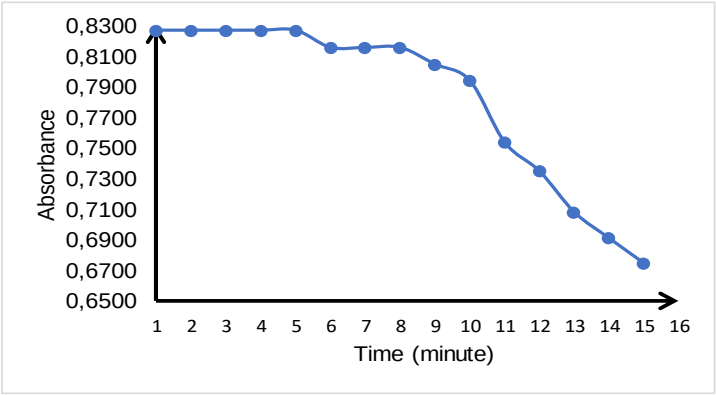

Figure 4. Absorbance curve at measurement time optimization

Based on Figure 3, the most concentrated color complex formed was at 1 minute. Figure 4 shows a stable absorbance value in the 1-5 minute range. At 6 minutes, absorbance began to decrease. This indicates that the optimum measurement time for color complex formation is 1-5 minutes.

pH optimization

Optimization of pH measurement is shown in Figure 5, while the results of absorbance data processing at pH variations can be seen in Figure 6.

Figure 5. Digital imaging results of pH optimization

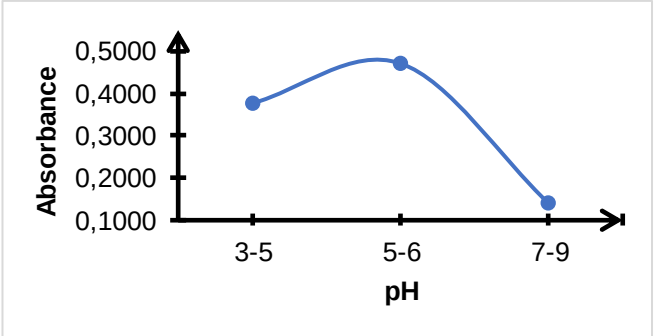

Figure 6. Absorbance curve at pH optimization

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Based on Figure 5, the color of the Fe–gallic acid complex appears most intense in the pH range of 5–6, while at pH 3–4 the color appears paler, and at pH 7–9 the color tends to fade. Figure 6 shows an increase in absorbance value from pH 3–4 to a maximum at pH 5–6, then decreases sharply at pH 7–9. Thus, the pH 5–6 condition produces the highest color intensity, which is used as the optimum condition for analysis in the next stage.

Measurement Linearity Test Using Digital Imaging Method

Fe(III) solution at concentrations of 0.1, 0.5, 1, and 2 ppm is shown in Figure 7 and there is a change in the color of the Fe(III) solution with gallic acid extract from Malacca fruit to blue which can be seen in Figure 8. The results of the digital imaging-based absorbance data are shown in Table 1.

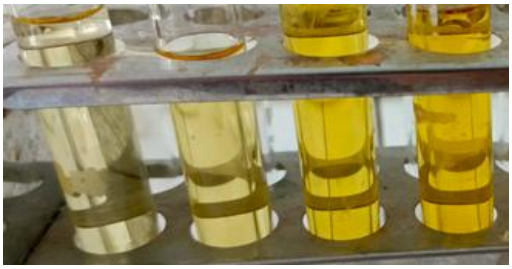

Figure 7. Fe(III) solution at a concentration

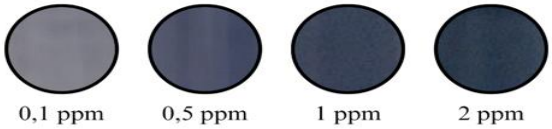

Figure 8. Digital imaging results of Fe-gallic acid from Malacca fruit

Table 1. Absorbance value of Fe(III)-gallic acid solution using the digital imaging method

Concentration	Absorbent
0.1	0.2079
0.5	0.3027
1	0.4670
2	0.7991

Based on Table 1, the Fe(III)-gallic acid calibration curve was obtained using the digital imaging method, which is shown in Figure 9.

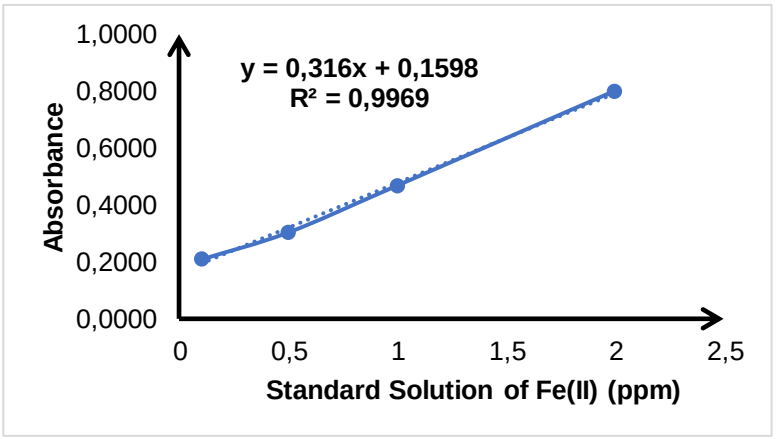

Figure 9. Calibration curve of FeIII)-gallic acid using the digital imaging method

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Based on Figure 9, the results of the linear regression equation are obtained, namely, y = 0.316x + 0.1598 with R^2 = 0.9969. After the curve is created, the LoD and LoQ calculations are carried out. The LoD obtained is 0.1377 ppm, and the LoQ is 0.4589 ppm.

Precision and Accuracy

In this study, the precision values are shown in Table 2.

Table 2. Results of the precision test for measuring Fe(I) levelsII-gallic acid

Sample	Fe(III) concentration(ppm)	%RSD
I	0.1	3.82
II	0.5	3.52
III	1	2.34

Accuracy measurement in this study used % recovery analysis. The % recovery value was obtained by recovering the Fe content using an Fe solution in the test sample at Fe(III) concentrations of 0.1, 0.5, and 1 ppm. Precision data are shown in Table 3.

Table 3. Results of the accuracy test of the content measurement of Fe (III)-gallic acid

Sample	Measured Fe(III) concentration(ppm)	Actual Fe(III) concentration (ppm)	%Recovery
I	0.1112	0.1	111.2
II	0.4638	0.5	92.76
III	0.9405	1	94.05

T-Test of Digital Imaging Method with UV-Vis Spectrophotometry Method

Determination of Fe(III) using the UV-Vis spectrophotometry method was carried out at a wavelength of 560-570 nm, which formed a blue color as shown in Figure 10 with variations in Fe(III) concentration of 0.1; 0.5; 1 and 2 ppm. The results of the Fe(III) calibration curve using this method are shown in Figure 11.

Figure 10. Fe (III)-gallic acid solution at Fe(III) concentrations of 0.1; 0.5; 1 and 2 ppm

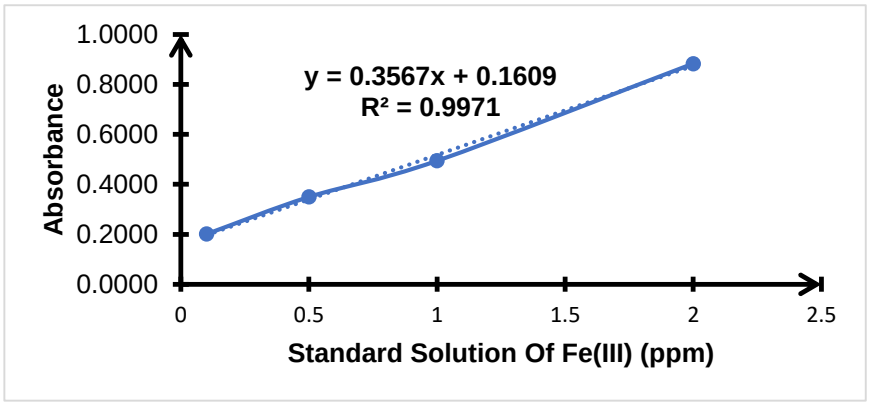

Figure 11. Calibration curve of FeIII)-gallic acid using the UV-Vis spectrophotometry method

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Samples containing Fe (III) were subjected to UV-Vis spectrophotometric measurements, which were compared with the results from the digital imaging method; the data are presented in Table 4.

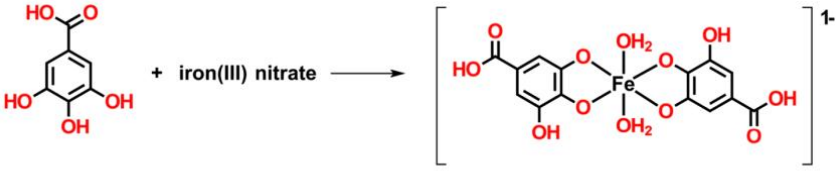
Table 4. t-test results

Sample	Fe(II) concentration (ppm)		t count	t table
	Digital imaging	Spectrophotometry UV-Vis		
I	0.1112	0.1034	0.15	2.36
II	0.4638	0.5554		
III	0.9405	1.0087		

DISCUSSION

This study developed a digital imaging-based Fe(III) detection method using natural reagents from Malacca fruit as a simple, fast, and portable alternative method compared to conventional methods that require large laboratory instruments. Detection of Fe levels using the digital imaging method is based on the principle of the formation of a blue Fe-gallic acid complex, which indicates the presence of Fe(III) ions in the sample. This complex is formed from the reaction between Fe(III) ions and gallic acid extract from Malacca fruit. Method validation was carried out through evaluation of accuracy, precision, LoD, LoQ, and t-test to ensure the feasibility of this method as an alternative for Fe(III) analysis.

The first stage was to optimize the Fe(III): gallic acid ratio to determine the optimum conditions that resulted in the formation of the Fe-gallic acid complex, analyzed in red, green, and blue. Based on the data in Figure 1, it can be seen that the blue color is more intense at a Fe: gallic acid ratio of 1:2. Based on Figure 2, the highest absorbance curve is shown in the results from Red, so measurements with Red were used for the next parameter. The highest absorbance with a sample ratio of the Malacca fruit reagent was 1:2. This is in line with research[5], which developed the Malacca fruit reagent for Fe(III) detection by UV-Vis spectrophotometry, found that the optimal ratio of Fe(III) sample and Malacca reagent was 1:2, as shown in Figure 12.



Gallic acid — Gallic acid:iron(III) complex (2:1)

Figure 12. Formation of Fe(III) complex with gallic acid[5]

Determining the sample ratio is crucial for producing a linear and stable optical signal. Mismatched ratios can reduce the sensitivity of the method due to changes in color density or excess reagents not involved in the reaction[12]. Measurement time optimization was also performed to ensure the complex formation reaction proceeds to completion and is stable. Another goal of this optimization was to determine the appropriate scanning time. If the scanning time is too short, the Fe-gallic acid complex formation reaction will not be complete or perfect. Meanwhile, if it is too long, the resulting color complex will fade due to light. The results show that the absorbance value is stable from the 1st to the 5th minute and experiences a decrease in absorbance at the 6th minute. The stability of absorbance up to a certain time reflects the kinetic process of complex formation that reaches its maximum point of stability. The stability of gallic acid depends on environmental conditions, although it is a relatively stable natural phenolic compound. The storage time of phenolic compounds affects the quantity of phenolic compounds. High temperatures, light, and the presence

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of oxygen will oxidize phenolic compounds due to the presence of unsaturated bonds in their molecular structure[13].

Optimization of pH measurements was carried out at acidic and basic pH levels. Figure 6 shows that the optimal pH stability of Fe-gallic acid is at pH 5-6. This is in line with research conducted in 2019.[5]that the color change to blue in the Fe-gallic acid complex occurs at pH 5.6. In basic conditions (high pH), gallic acid undergoes deprotonation and auto-oxidation, forming quinones that change color to yellow and brown, reducing the concentration of gallic acid due to the loss of hydroxyl groups[14]. This is in accordance with Figure 6, which shows the color change of Fe-gallic acid that occurs at pH 7-9 to brownish yellow.

Detection of Fe levels using a digital imaging method was carried out by varying the concentration, and in Figure 8, it can be seen that the higher the Fe concentration, the more intense the blue color of the Fe-gallic acid complex, which is directly proportional to the absorbance. Standard Fe(III) solutions with concentrations of 0.1, 0.5, 1, and 2 ppm were reacted with gallic acid extract from Malacca fruit at pH 5-6, then scanned directly according to the optimum conditions obtained.

The results of the digital imaging method calibration curve show a linear relationship between Fe(III) concentration and absorbance, with R^2 results approaching 1. LoD is the smallest acceptable measurement concentration of the measurement method. LoQ is the smallest value of the analyte in the sample that can still meet the accuracy and precision criteria. Based on the calculation results, the LoQ value is 0.4589, so the analyte level that can be said to meet accuracy is more than 0.4589 ppm. The LoD and LoQ results show that the digital imaging method can detect and quantify low Fe levels. The LoD and LoQ obtained are still within an adequate range for the detection of low Fe levels with the digital imaging method.

Validation tests were conducted to ensure that the developed µPAD method has adequate analytical capabilities and can be used as an alternative to the UV-Vis spectrophotometry method in detecting Fe(II) levels. Validation was conducted by comparing the results of Fe level measurements from both methods using external standard solutions in the concentration range of 0.1–2 ppm. The parameters tested included accuracy, precision, and a t-test to observe the significance of the differences between the digital imaging method and the UV-Vis spectrophotometer method.

The precision value can be seen from the %RSD value, which shows results <5% at 3 Fe(III) sample concentrations. Based on these results, the µPAD method has consistent measurement results between replications. Accuracy is expressed as the percent recovery (%recovery) of the measured analyte concentration compared to the actual analyte concentration. The accuracy test results show a %recovery value of 92.76 – 111.2%, respectively. This data is also in line with research conducted in 2019 [5]. Based on the requirements of the test method, the acceptable range of %recovery values is in the range of 85% - 115% (SNI 6989.4:2009)[15], and SNI ISO/IEC 17025:2017[16]. These results show that the digital imaging method has a good level of accuracy.

A t-test was then performed based on the results of Fe content measurements using digital imaging and UV-Vis spectrophotometry. The purpose of this comparison was to determine whether the results obtained from the digital imaging method were statistically equivalent to those from the standard laboratory method. In the UV-Vis spectrophotometry method, measurements were performed at a maximum wavelength of 560-570 nm.

Based on the t-test conducted, the results obtained were *t*-calculated 0.15 and *t*-*t*able 2.36. If If t_calculated < t_table then H_0accepted, which shows that there is no significant difference between the two methods. If t_calculated < t_table, then

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H_0rejected, which shows that there is a real difference between the two methods. The results of the t-test that has been carried out show that if t_calculated < t_table, which means there is no difference in the results of Fe level detection between the digital imaging method and the UV-Vis spectrophotometry method.

Overall, this study demonstrates that the Malacca fruit extract-based digital imaging method is a simple, environmentally friendly, and effective alternative for the detection of Fe(III), with results comparable to UV-Vis spectrophotometry. The main advantages of this method lie in the use of natural reagents, the small sample and reagent volume requirements, and the ease of operation without sophisticated laboratory instruments, making it applicable to users without special expertise and generating minimal waste. However, the stability of natural reagents such as gallic acid remains a limitation due to their sensitivity to light, oxidation, and long-term storage. This study contributes by integrating optimization of the ratio, pH, and measurement time into a validated digital imaging-based μPAD system, resulting in an analytical approach that is economical, portable, and feasible for rapid field monitoring. These findings also open up opportunities for broader applications, including the development of web-based applications that utilize digital color processing for practical and sustainable Fe detection.

CONCLUSION

This study showed that a digital imaging method based on Malacca fruit extract can be used as a simple, economical, and environmentally friendly alternative for quantitative Fe(III) detection without the need for specialized laboratory instruments. Through optimization of reagent ratio, pH, and measurement time, this method is able to produce stable color readings and provides a good linear relationship between Fe(III) concentration and color intensity. Analytical validation shows that this method has acceptable accuracy and precision and results equivalent to the UV-Vis spectrophotometry method, making it suitable as a portable and practical Fe(III) detection approach. The update of this study lies in the utilization of natural reagents from Malacca fruit in a digital imaging-based μPAD, resulting in a more sustainable metal detection method. Further research is recommended to evaluate the influence of interfering ions in the sample and develop a web-based application system to improve the implementation of the method in the field.

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