

Ethanol Extract of Areca Nut (*Areca catechu* L.) as a Complexing Agent to Determinate Fe(III) in Aqueous Medium

Ekstrak Etanol Buah Biji Pinang (*Areca catechu* L.) Sebagai Agen Pengompleks Untuk Penentuan Fe(III) Dalam Media Air

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ABSTRACT

A complexing agent is very critical in the analysis of metal ions with spectrophotometry UV-Vis method. The most widely used complexing agents are not eco-friendly and expensive industrial products. This work employed the ethanol extract of the areca nut as a complexing agent to determine the Fe(III) in water media, which is the most likely to pollute the environment. We found that the optimum reaction between areca nut extract with Fe(III) occurred at pH 5 and in the ratio of 21.6 mg/L Fe(III): 10 mg/L of extract. This mixture reached the highest absorbance at around 30 minutes. The spectrometric and digital image methods show the linear relation between Fe(III) concentration and absorbance with a correlation coefficient of 0.998 and 0.997 (for the green component), respectively. However, the spectrometric method shows more comparable performance parameters with the use of KSCN as a complexing agent than the digital image method using a camera (13MP) of a smartphone (Vivo V20S). This method was also applied to analyze Fe(III) in real water samples, with recovery rates ranging from 84.83% to 105.87%, all of which were deemed acceptable.

Keywords: Areca nut; complexon agent; Fe(III); Digital Image; UV-Vis Spectrophotometry; KSCN.

ABSTRAK

Agen pengompleks sangat penting dalam analisis ion logam dengan metode spektrofotometri UV-Vis. Pengompleks yang paling banyak digunakan adalah produk industri yang tidak ramah lingkungan dan mahal. Penelitian ini menggunakan ekstrak etanol biji pinang sebagai agen pengompleks untuk penentuan Fe(III) dalam media air yang merupakan pencemar lingkungan. Hasil penelitian menunjukkan bahwa kondisi optimum reaksi antara ekstrak dengan Fe(III) terjadi pada pH 5 dengan rasio 21,6 mg/L Fe(III) : 10 mg/L ekstrak. Campuran ini memiliki absorbansi paling tinggi pada pada menit ke ± 30 . Baik metode spektrofotometri maupun pencitraan digital memperlihatkan hubungan yang linear antara konsentrasi Fe(III) dengan absorbansi, dengan nilai koefisien korelasi berturut-turut 0.998 and 0.997. Namun demikian metode metode spektrofotometri memperlihatkan kinerja yang lebih baik dengan menggunakan KSCN sebagai pengompleks dibandingkan dengan metode pencitraan digital menggunakan kamera (13 MP) sebuah smartphone Vivo V20S. Metode ini juga diaplikasikan untuk analisis Fe(III) dalam sampel air alam, dengan nilai perolehan kembali yang berkisar antara 84,83% hingga 105,87%, yang semuanya dapat diterima.

Kata kunci: biji pinang; Agen pengompleks; Fe(III); Pencitraan digital; Spectrophotometri UV-Vis; KSCN.

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1. INTRODUCTION

Iron (Fe) is one of the most widely used metals in the world, causing this metal to most likely pollute the environment, especially water. Even though iron is an essential nutrient required for various biochemical and physiological functions, its excess existence in the human body can cause health problems [Jaishankar et al., 2014], such as upset stomach, constipation, nausea, abdominal pain, vomiting, and diarrhoea [Rahmana et al., 2020]. Therefore the efforts to routinely monitor the iron level in the aqueous media are of great importance. A spectrometric method based on forming a chelate complex between Fe(III) and a complexing agent is an established method for determining Fe(III). The well-known complexing agents for Fe(III) include thiocyanate, 1, 10-phenanthroline, 2, 2'-bipyridal, bathophenanthroline, sulfosalicylic acid, and thioglycolic acid [Chen and Deng, 2021]. However, all the complexing agents mentioned are industrial products that are expensive and not eco-friendly. Therefore it is critical to seek an inexpensive and environmentally friendly complexing agent to substitute the manufactured by the chemical industry. The candidate for this purpose should be from a natural product. In this work, ethanol extract of areca nut (*Areca catechu* L.) was applied as a complexing agent to analyse Fe(III) from aqueous media.

Areca nut or betel nut is commonly referred to the seed of areca palm tree which is cultivated in South and Southeast Asian countries [Xiao et al., 2019]. The chemical composition of areca nuts, such as alkaloids, flavonoids, tannins, triterpenes, fatty acids, etc., with the tannin and flavonoids as the major components [Xiao et al., 2019]. This chemical composition benefits the medical

field that is employed as an anti-bacterial, antiviral and anti-tumour, anti-oxidation, anthelmintic action, effects on the nervous system and digestive systems [Xiao et al., 2019, Fu and Chen, 2019]. In addition, due to the tannin and flavonoids contained, the extract of areca nut can also be employed as a complexing agent for analysing Fe(III). It is because structurally, tannin and flavonoids abundantly pose -OH groups, which have two lone pair electrons that can be donated to the empty orbital of Fe(III). Several works have proven the formation of the complex between tannin [Ju et al., 2017, Kraal et al., 2006, Çakar and Özacara, 2016, Selvaraj et al., 2014] and flavonoids [Kasprzak, et al., 2015, Symonowicz and Kolanek, 2012, Iqbal et al., 2015]. Therefore, ethanol extract of areca nut hypothetically can form a complex with Fe(III), and consequently can be a basis for Fe(III) analysis. Among the various extraction methods reported, ethanol solvent extraction is the most commonly employed technique. This is partly because ethanol is comparatively less toxic than acetone and methanol [Chen et al., 2020]. Additionally, ethanol as a solvent has a greater ability to extract total phenols and total flavonoids (361.22 ± 0.26 mg GAE/g and 42.46 ± 0.15 mg QE/g, respectively) compared to water (267.61 ± 0.13 mg GAE/g and 16.22 ± 0.21 mg QE/g, respectively). Both of these compounds are targeted due to their ability to form complexes with metal ions [Prayitno and Rahim, 2020]. In this work, analysis of Fe(III) was conducted in two methods that were spectrophotometric and digital image methods. In addition, the spectrophotometric method with potassium thiocyanate as a complexing agent was used as a reference.

2. MATERIALS AND METHODS

2.1. Materials

The seed of the areca nut was obtained from Timor Tengah Selatan districts, Indonesia. The ethanol extract was obtained by mixing 50 g small pieces of areca nut with 250 mL of Ethanol (technical grade, $\pm 70\%$) at room temperature for 24 h. Then, the mixture was filtered, and the filtrate was evaporated

using a rotary evaporator at 70 °C for 5 h. The obtained thick extract was tested for its secondary metabolite content following the procedure reported by Iqbal [Iqbal *et al.*, 2015] such as shown in Table 1. Nitric acid (HNO_3), Sodium acetate (CH_3COONa), and acetic acid (CH_3COOH) used to prepare the buffer solution, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and potassium thiocyanate (KSCN) were purchased from Merck in pro analytic grade.

Table 1. Preliminary phytochemical screening of areca nut ethanol extract

Metabolites tested	Reagent used	Observation	Result
Tannin	FeCl_3	Blackish blue color	+
Flavonoids	Mg + concentrate HCl	Brown precipitate formed	+
Alkaloids	Wagner	Brick red color	+
Saponins	Aquades-HCl pekat	Not frothy	-
Terpenoids	Lieberman-Burchard	Brown-red	+

2.2. Determination of optimum condition

From the theoretical consideration, it is expected that a complex compound would be formed between tannin and flavonoid in the extract with Fe(III). Therefore, it is necessary to find the optimum conditions for forming a complex compound, including pH, ratio, and stable time. The determination of those parameters was conducted by modifying the procedure proposed by Liu [Liu *et al.*, 2011], as follows: 2.5 mL of tannin 100 mg/L (dissolved in methanol 50 %) and 21.5 mL of acetate buffer solution (prepared by mixing of a certain volume of nitric acid 0.1 mol/L, acetic acid 0.1 mol/L and sodium acetate 0.1 mol/L), were mixed. Into the mixture, 1.0 mL of Fe(III) salt of 1.0 mmol/L (dissolved in HCl 0.1 mol/L) was added and the absorbance of the mixture was measured.

2.3. Determination of analytical performance parameters

A series of Fe(III) standard solutions was prepared based on the obtained optimum conditions. Each standard solution absorbance was obtained using spectrophotometric and digital image methods. In the case of the digital image technique, the measurement

procedure was conducted by placing each standard solution in the bottom centre of the styrofoam box. This styrofoam box (20 x 20 x 20 cm) with a window (4 x 4 cm) was equipped with a 30-watt Visicom LED bulb lamp (**Fig. 1**) to maintain the same environmental light condition during image recording. Through the box window, the picture was captured using a camera (13 MP) of a smartphone (Vivo V20S) at a distance of 9 cm between the camera and the sample. The RGB viewer software installed in the computer was used to image the pictures as colour intensities of Red, Green and Blue (RGB), which were then converted to absorbance using the Lambert-Beer equation. A calibration curve was constructed using the obtained absorbance, where several analytical performance parameters can be evaluated. While the two other analytical performance parameters, viz precision and accuracy, can be estimated by repeated measurement of six standard solutions of Fe(III) with a certain concentration. As a reference method, a spectrophotometry UV-Vis with KSCN as a complexing agent has also been employed in calibration curve construction and precision and accuracy estimation.

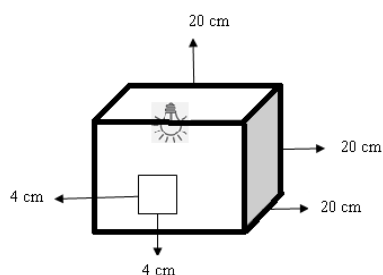


Figure 1. Styrofoam box for taking photos of samples

3. RESULTS AND DISCUSSION

3.1. Determination of optimum condition

Based on the preliminary phytochemical screening test, the ethanol extract of the areca nut contains both tannin and flavonoid (Table 1). These metabolites potentially react with metal ions due to their ability to donate their lone pair electron to the un occupation orbital of the metal ion. Therefore, the optimum condition for the reaction between extract and Fe(III), including pH, ratio, and stability time, should be found first. The result of optimisation is shown in the **Fig. 2**.

An amount of 0.5 mL Fe(III) 270 mg/L and 0.5 mL of extract 100 mg/L were mixed, and the mixture was then diluted with various pH of acetate buffer to figure the effect of pH on the reaction between Fe(III) with the extract. The absorbance of the mixture at 531 nm is shown in Figure 2(a). This result shows that the highest absorbance was observed at pH 5, which means that the highest concentration of Fe(III)-extract was formed at this pH. At the lower and the higher pH, the presence of H^+ and OH^- probably disturbed the stability of the complex. This optimum pH was then used to search the ratio between Fe(III) and the extract. The different volumes of Fe(III) 270 mg/L and 0.5 mL of extract 100 mg/L were mixed and diluted with acetate buffer pH 5. The absorbance of each mixture at 531 nm was recorded and displayed in Figure 2(b). Figure 2(b) shows that the highest concentration of Fe(III)-extract was achieved when 0.4 mL of Fe(III) 270 mg/L was added to 0.5 mL of extract 100 mg/L. The optimum pH and ratio were then applied to evaluate the

mixture's stable time, as indicated in Figure 2(c). This result implies that the best measurement was around 30 minutes after mixing.

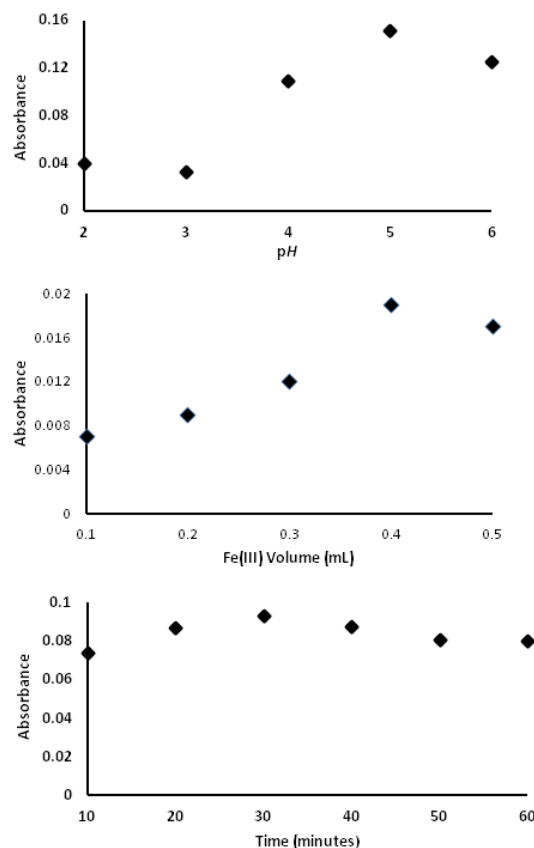


Figure 2. Optimum pH (a), ratio (b), and the stable time (c) for the reaction between Fe(III) and extract

3.2. Standard curve preparation and analytical performance parameters determination

Based on the result in **Fig. 2(b)**, 21.6 mg/L Fe(III) needed 10 mg/L of extract to form a mixture with the highest absorbance. Considering this result, the extract concentration needed to prepare the Fe(III) standard solution with concentrations of 2, 4, 6, 8, and 10 mg/L were around 5 mg/L. Fe(III) and extract concentrations mentioned were the concentration after diluting with buffer pH 5,

which also served as a blank solution. Those standard solutions were then recorded their

absorbance with a spectrophotometer UV-Vis, and the result is presented in **Fig. 3**.

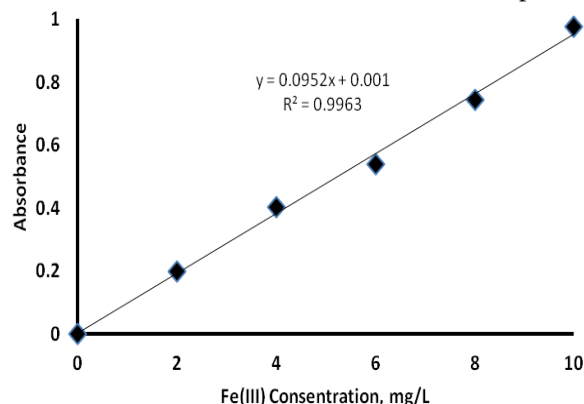


Figure 3. Standard curve of a spectrometric method with extract as a complexing agent

The correlation coefficient (r) of 0.998 indicated that the ethanol extract of the areca nut could be utilized as a complexing agent to determine the Fe(III) concentration from the aqueous media. In addition, the absorbance of

those standard solutions was also obtained from imaging pictures using RGB viewer obtained by digital image method using a styrofoam box (Figure 1), as shown in **Fig. 4**.

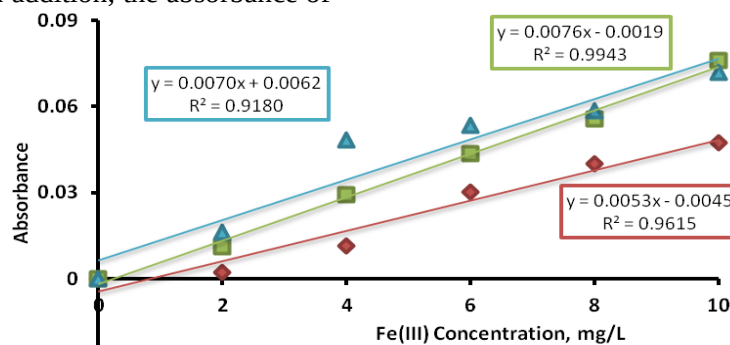


Figure 4. Standard curve of a digital image method with extract as a complexing agent

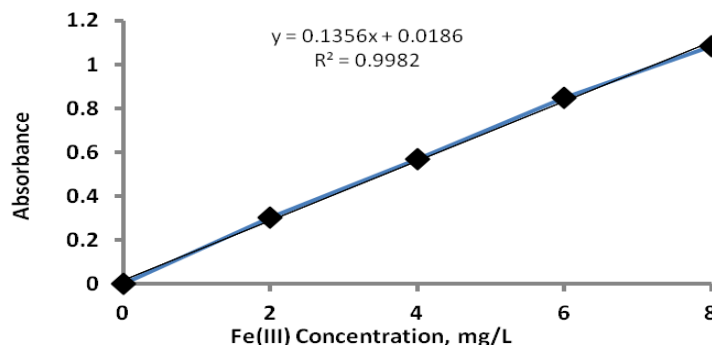


Figure 5. Standard curve of a spectrometric method with KSCN as a complexing agent

Fig. 4 shows that the ethanol extract of the areca nut can also be employed as a complexing agent using the digital image

method. The green component indicated the straightest line ($r = 0.997$), and the green colour was employed in the further experiment. As a

reference, Fe(III) standard curve was also constructed using spectrometric method with KSCN as a complexing agent as presented in Fig. 5.

The two other analytical performance parameters, viz precision and accuracy, were estimated by repeating measurement concentration of six standard solutions of Fe(III) 8 mg/L, as denoted in Table 2.

Data in Table 2 was employed to calculate the t and f , which was then used to estimate the method's accuracy and precision by comparing them with the t and f table at a certain confidence level. Assisted by Fig. 3, Fig. 4, Fig. 5, and Table 2, the analytical performance parameters could be evaluated as represented in Table 3.

Table 2. Measured concentration of Fe(III) 8 mg/L

Repetition	Measured Fe(III) concentration, mg/L		
	Spectrophotometric, KSCN as complexing	Spectrophotometric, extract as complexing	Digital image, extract as complexing
1	7.881	7.839	7.907
2	7.859	7.844	7.578
3	7.874	7.857	7.907
4	7.866	7.849	7.907
5	7.903	7.895	7.578
6	7.888	7.891	7.907
Average	7.8785	7.8625	7.7973
SD	0.016	0.024	0.170

Table 3. Determination of several analytical performance parameters in comparison with the spectrometric method with KSCN as a complexing agent

Parameters	Result			
	S-KSCN	S-Extract	DI-Extract	
Error (%)	1.513	1.719	2.538	
Precision (%RSD)	0.203	0.305	2.180	
LoD, (mg/L)	0.354	0.756	67.105	
LoQ, (mg/L)	1.180	2.321	223.684	
Sensitivity	0.1356	0.0952	0.0076	
t_{count}	1.358	$t_{\text{count}} < t_{\text{table}}$	1.165	$t_{\text{count}} < t_{\text{table}}$
$t_{\text{table}} (\alpha = 0,05)$	2.228	Not significantly different	2.228	Not significantly different
f_{count}	2.250	$f_{\text{count}} < f_{\text{table}}$	112.891	$f_{\text{count}} > f_{\text{table}}$
		Not significantly different		Significantly different

S-KSCN: spectrometric method with KSCN as a complexing agent; **S-Extract:** spectrometric method with ethanol extract of areca nut as a complexing agent; **DI-Extract:** digital image method with ethanol extract of areca nut as a complexing agent.

Data on the Tabel 3 indicate that the precision of the digital image method with extract of areca nut as a complexing agent was significantly different with a reference method, namely the spectrometric method with KSCN as a complexing agent. It is exacerbated by a very high detection limit (LoD). Therefore, this method is not suitable

for analyzing samples of water that generally have low Fe(III) concentrations, typically measured in part per million or even part per billion units. On the other hand, the spectrometric method with an extract of areca nut as a complexing agent denotes better performance. The accuracy and precision of this method were like the reference method.

The LoD of this method was also far lower than the digital image method. Therefore the spectrometric method with areca nut extract as

a complexing agent was applied to analyse Fe(III) from the real sample of water.

3.3. Application of the method to the real sample of water

The applicability of this method to analyze Fe(III) has been verified to two kinds of samples, namely well water and tap water. A little hydrogen peroxide was added to each sample to oxidize Fe(II), which may be present in the sample to Fe(III). Firstly, the concentration of Fe(III) in the sample was determined using the spectrometric method

with KSCN as a complexing agent. Due to the very low Fe(III) concentration in the real water sample, an amount standard of Fe(III) was added so that its concentration became higher than LoD. Adding the ethanol extract of the areca nut to the sample was carried out according to the optimum ratio. The mixture was then diluted with an acetate buffer of pH 5. The absorbance was recorded, and the recovery is shown in Table 4.

Table 4. Recovery of Fe(III) in the real sample of water using the spectrometric method with areca nut extract as a complexing agent

Samples	pH	Fe(III) Concentration (mg/L)			Recovery (%)
		Initial*	added	measured	
Well water	6.15	0.006	0.6	0.528	87.60
			0.8	0.681	84.83
Tap water	6.97	0.007	0.6	0.637	105.70
			0.8	0.801	99.78

* Initial concentrations of 0.006 and 0.007 mg/L were in 4 mL of samples. Because of diluting to 10 mL, the initial concentration changed to 0.0024 and 0.0028 mg/L, respectively.

The result in Table 4 indicates that the measurement recoveries are in the range of acceptable. According to Latimer et al. (2023), the acceptable %R for an analyte concentration of 0.1% ranges from 80% to 110%. It means that the ethanol extract of areca nut could be a complexing agent candidate for Fe(III) analysing using the spectrometry method.

4. CONCLUSIONS

In this work, ethanol extract of areca nut was employed as a complexing agent in analysing Fe(III) from water media. The optimum reaction condition between extract and Fe(III) occurred at pH 5 and in the ratio of 21.6 mg/L Fe(III): 10 mg/L of extract. The highest absorbance was observed at around 30 minutes. The spectrometric method with extract as a complexing agent shows comparable analytical performance parameters (%E: 1.719; %RSD: 0.305; LoD: 0.756; LoQ: 2.321; and Sensitivity: 0.0952) with using KSCN as a complexing agent (%E:

1.513; %RSD: 0.203; LoD: 0.354; LoQ: 1.180; and Sensitivity: 0.1356). Applying this method to determinate Fe(III) in the real water sample indicates that the recovery of all samples experimented were in range of acceptable.

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