

# B29

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# Isolation of chlorophyll *a* from kirinyuh leaves *Chromolaena odorata* L.) as corrosion bioinhibitor in mild steel

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# Isolation of Chlorophyll *a* from Kirinyuh Leaves (*Chromolaena odorata* L.) as Corrosion Bioinhibitor in Mild Steel

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**Abstract.** Chlorophyll *a* (Chl<sub>a</sub>) is a pigment that is naturally occurring in many green plants. Chl<sub>a</sub> has conjugated double bonds and elements of nitrogen (N), Oxygen (O) that can form complex soluble compounds with metal ions capable of inhibiting corrosion in metal. In this research, chl<sub>a</sub> was isolated from kirinyuh leaves (*Chromolaena odorata* L.). Chl<sub>a</sub> extraction using acetone was selectively precipitated with dioxane and water with an overall yield of 1.43% from dried kirinyuh. The separation of chl<sub>a</sub> was conducted using various chromatographic techniques, such as sucrose column chromatography with petroleum ether: diethyl ether as eluent and gravitational column chromatography with *n*-hexane: ethyl acetate as eluent. Chl<sub>a</sub> was tested using thin layer chromatography to test the purity of the chl<sub>a</sub> that provides a red stain under UV 365 nm. The identity of chl<sub>a</sub> had been confirmed by UV-Vis at 400-700 nm. The UV-Vis absorption spectrum of chl<sub>a</sub> showed that a maximum Soret band is at 433 nm, while the Q band was at 655 nm. As observed from the functional groups of Chl, -CH<sub>2</sub> stretching of aliphatic groups at 2930 cm<sup>-1</sup>, C=O vibration at 1634 cm<sup>-1</sup>, C-O vibration at 1257 cm<sup>-1</sup>, and C-N vibration at 1444 cm<sup>-1</sup>. Chl<sub>a</sub> was tested as a corrosion inhibitor of mild steel (SAE/AISI Grade 1022) in a 3% brine solution and seawater. The inhibition tests were carried out using the methods of weight loss and microscope to see the surface of mild steel. The result showed that chl<sub>a</sub> decreased corrosion rate by 0.0036 mm/y with an inhibition efficiency of 60% in 3% brine solution and 0.0096 mm/y with an inhibition efficiency of 77.89% in seawater.

## INTRODUCTION

Nowadays, every aspect of our lives uses metals, especially mild steel. Mild steels are extensively used in the oil and gas industries because of their ideal characteristics as construction materials, in addition to their economic reasons [1]. However, the performance of mild steel can be decreased over time and result in corrosion. Corrosion has become a big issue of worldwide concern. Corrosion is the degradation of material properties, particularly metals, due to interactions with the surrounding environment [2]. Corrosion cannot be avoided but can be slowed down, so we need methods for controlling corrosion. An inhibitor is one solution that reduces the corrosion rate for control and protects the mild steel surface by the coating method [3].

In recent years, various methods to develop low-cost, eco-friendly, and renewable resources for controlling corrosion have become a subject for many researchers [4]. These problems have then prompted the search for bioinhibitor. Bioinhibitors are inhibitors derived from natural resources that are biodegradable and do not contain heavy metals. An effective corrosion bioinhibitor contains heteroatoms such as nitrogen, oxygen, sulfur, and phosphorus with lone electron pairs and moiety with  $\pi$ -electrons (aromatic rings and multiple bonds) that can interact with free orbital d metal, favoring the absorption process [5]. Among natural corrosion inhibitors are barbarine

(efficiency of 98%) [6], quinine (96%) [7], eugenol, and acetyl eugenol (91%) [8]. These compounds contain heteroatom that is required for inhibitory effect.

Another potential source of bioinhibitor is kirinyuh (*Chromolaena odorata* L.). Gerengia *et al.* reported that kirinyuh leaves in 100 ppm extract protected mild steel from corrosion effectively because the extract has inhibition efficiency up to 95% [9]. One of the major products of kirinyuh leaves is *chla*. The kirinyuh leaves typically contain 1.075 g/L *chla*. However, the *chla* from kirinyuh leaves has never been isolated. Sandiningtyas and Suendo [11] isolated *chla* from spinach leaves using a sucrose column without further purification. Therefore, the purpose of this research is to isolate *chla* using various chromatographic techniques with sucrose and silica as stationary phases and to explore the potency of *chla* as a corrosion bioinhibitor. There has been no report on *chla* as a bioinhibitor in mild steel retrieved from literature reviews. However, it has been acknowledged that *chla* containing heteroatoms such as nitrogen and oxygen as well  $\pi$ -electrons of multiple bonds and aromatic rings enhance adsorption phenomena and protect metal surfaces.

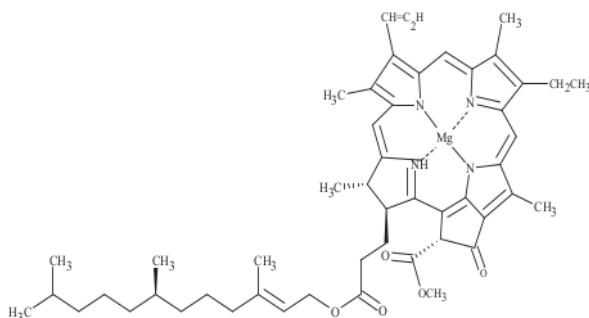


FIGURE 1. Structure of *chla*

## EXPERIMENTAL

### Materials and Equipment

Materials used were kirinyuh leaves from Lombok-Indonesia, sucrose, 1,4-dioxane from Merck, petroleum ether, diethyl ether from Merck, *n*-hexane, ethyl acetate, acetone, aqudest, NaHCO<sub>3</sub>, NaCl. The required laboratory instruments were rotary evaporator, filter flasks, vacuum pump, refractometer, pH meter, UV 356 nm, oven, freezer, digital hot plate, microscope binocular (Zeiss Primo Star iLED), and UV-Vis spectrophotometer (Shimadzu UV-1601PC).

### Preparation of Solutions

Seawater for corrosive media in this research was obtained from Kuta beach, Lombok-Indonesia. A 3% brine solution (w/v) was prepared by dissolving 30 g NaCl and 100 mg NaHCO<sub>3</sub> in a 1 L aquadest. The corrosive media were measured the salinity and pH.

### Preparation of Sample Testing

Mild steels used in this research were SAE/AISI Grade 1022 according to AS 1443. According to the spectrographic analysis, the sample contain 98.87% Fe and a series of minor components with varied amounts, include C (0.22%), Mn (0.74%), Si (0.02%), S (0.014 %), P (0.02%), Ni (0.05%), Cr (0.04%), Mo (<0.01%), Cu (<0.01%), Al (<0.01%). Mild steel was cut into dimensions of 2.7 x 2.7 x 0.3 cm.

## Isolation of Chla

The crude chla from kirinyuh leaves was prepared from 100 g dried kirinyuh leaves that were crushed in 500 mL cold acetone. The filtrate containing crude chla was separated using a Buchner funnel with Whatman filter paper. The solid residual was then washed with 100 mL of acetone. 1,4-dioxane and aquadest were added to the filtrate, each one-seventh of the filtrate volume, before stirring using a magnetic stirrer. The mixture was precipitated by storing the filtrate in the freezer at -20 °C for 10 days. After 10 days, the step was repeated twice. The precipitate was filtered using a Buchner funnel and diluted with acetone until the filter paper turned clear. The solution containing crude chla was evaporated to remove the solvent, leaving only the remaining solid green crude chla.

The separation of chla used a sucrose column chromatography which was connected to a vacuum pump. The solid green crude chla was dissolved in a small volume of diethyl ether and introduced into the sucrose column that has been filled with petroleum ether. After that, the elution was performed by adding the petroleum ether until the yellow band appeared. Furthermore, the elution was continued with different eluent, diethyl ether in petroleum ether, until distinct yellow, gray, and green bands appeared. Then the green bands were taken using a spatula in order to obtain chla.

## Characterization of Chla

Chla was characterized using a UV-Vis spectrophotometer in a wavelength of 700-300 nm. The structure of chla was determined using Fourier Transform Infrared (FTIR), and the spectrum was recorded in a wavelength range of 4000-400 cm<sup>-1</sup>

## Weight Loss Analysis

Mild steel was prepared by coating using corrosion bioinhibitors, which are kirinyuh leaves extract and chla. After that, the experiment was carried out in a jar containing 15 mL of corrosive media with and without adding 100 ppm corrosion bioinhibitors at room temperature (27 °C) in the 7 days of immersion. Mild steel was weighed before and after immersion in corrosive media. The following equations were used to calculate weight loss (W), corrosion rate (CR), and inhibition efficiency (%IE) [10].

$$W = W_{(i)} - W_{(f)} \quad (1)$$

$$CR = \frac{W \times K}{D \times A \times T} \quad (2)$$

$$\%IE = 1 - \frac{CR_{inhibited}}{CR_{uninhibited}} \times 100\% \quad (3)$$

Where W=weight loss (g), W<sub>(i)</sub>= initial weight, W<sub>(f)</sub>=end weight, CR=corrosion rate (mm/y), A=area of coupon (cm<sup>2</sup>), D=density of metal (7.86 g/cm<sup>3</sup>), t=time of exposure (hours), %IE=inhibition efficiency percentage, CR<sub>inhibited</sub>=corrosion rate with inhibitor, CR<sub>uninhibited</sub>=corrosion rate without inhibitor.

## Surface Analysis

An optical microscope was used to study the microstructure of mild steel specimens before and after the weight loss test. The specimen of mild steel was immersed in 3% brine solution and sweated with and without inhibitors.

## RESULT AND DISCUSSION

### UV-Vis Absorption Analysis

The UV-Vis absorption transitions are usually present in the form of absorption bands. This is due to the contribution of vibration and rotation on the electronic transitions. Based on the absorption spectrum, chl *a* has two main absorption regions in the wavelength ranges of 400-450 nm and 650-700 nm, and it is referred to as the Soret and Q bands [10]. The absorption spectrum of chl *a* in Figure 2 shows that the maximum of the Soret band is at 433 nm, while the Q band is at 655 nm. The spectrum indicates the distinct absorption maximum match well with the light absorption characteristics of chl *a* reported elsewhere.

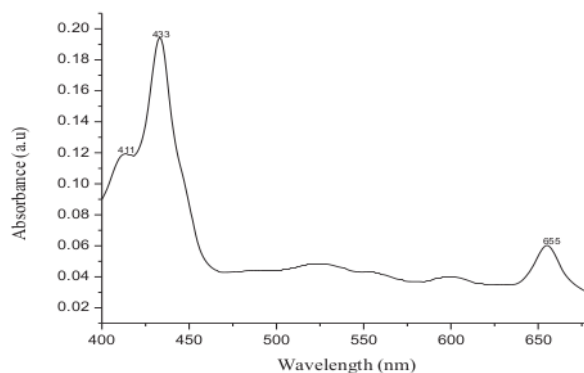


FIGURE 2. Absorption spectra of chl *a*

Furthermore, from Figure 2, it can be seen that chl *a* was pure because there is no widening in the absorption spectrum around 450-500 nm. This indicates that there are no pigments present in the chl *a* solution [11].

### Fourier Transform Infrared (FTIR) Analysis

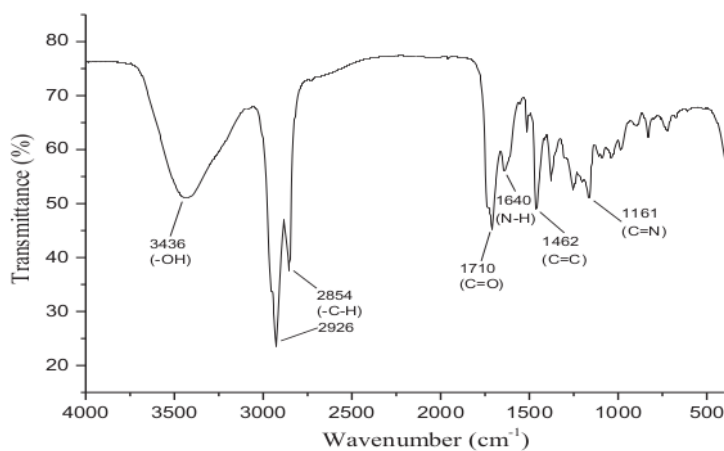


FIGURE 3. FTIR spectra of chl *a*

Figure 3 shows the FTIR spectrum of chl $a$  in spectral range within the wavenumber of 4000-400  $\text{cm}^{-1}$ . The characteristic peaks of chl $a$  are present in Table 1. The broad peak at 3436  $\text{cm}^{-1}$  is on -OH group from water molecule that interacts with C=O ketone group. The ketone oxygen coordinates to magnesium, and it becomes chl $a$  aggregate [16]. The bending vibration of CN and NH represents the tetrapyrrole ring in porphyrin.

**TABLE 1.** FTIR spectra of chl $a$  sample

Functional Grup	Wavenumber of the sample ( $\text{cm}^{-1}$ )	Theoretical Wavenumber ( $\text{cm}^{-1}$ ) [12]	Theoretical Wavenumber( $\text{cm}^{-1}$ ) [13]
-OH	3436	3379.75	3800-2700
C-H	2926; 2854	2923.97	3000-2850
C=O	1710	1722.63	1850-1600
N-H	1640	1627.22	1640-1550
C=N	1161	1225.06	1250-1000
C=C	1462	1450.50	1900-1400

### Weight-Loss Analysis

The weight-loss method was carried out for monitoring the effectiveness of inhibition. The weight-loss, also known as wheel test, was used as a screening method or quantitative analysis to evaluate the potential of corrosion inhibitors. This experiment measures the weight reduction after each specific day, and this reduced weight can be used to calculate corrosion rate.

**TABLE 2.** Weight loss using bioinhibitor (extract & chl $a$ ) at room temperature for 7 days

No	Composition	Dimension (cm)			A ( $\text{cm}^2$ )	Weight (g)			CR (mm/y)	EI (%)
		L	Wd	T		$W_{(i)}$	$W_{(f)}$	W		
1	B	2.8	2.7	0.3	18.42	14.3921	14.3896	0.0025	0.0090	
2	B + E	2.6	2.7	0.3	17.22	13.4772	13.4761	0.0011	0.0042	52.93%
3	B + C	2.8	2.7	0.3	18.42	14.4461	14.4451	0.001	0.0036	60%
4	S	2.7	2.7	0.3	17.82	13.6379	13.6262	0.0117	0.0435	
5	S + E	2.8	2.7	0.3	18.42	14.3892	14.3852	0.004	0.0144	66.92%
6	S + C	2.6	2.7	0.3	17.22	13.2782	13.2757	0.0025	0.0096	77.89%

**Note:** B=brine solution, S=seawater, E=extract, C=chlorophyll  $a$ , L=length, Wd=width, T=thickness, A=coupon area,  $W_{(i)}$ =initial weight,  $W_{(f)}$ = final weight, W=weight loss, CR=corrosion rate, EI=inhibition efficiency

The corrosive media in this weight loss test was 3% brine solution (pH: 13 and salinity: 34) and seawater (pH: 8 and salinity: 35). The concentration of the inhibitor for weight loss experiments was fixed at 100 ppm. Before weight loss is tested, the mild steel as a sample should be coated by inhibitors using an immersion technique. The experimental data in Table 2 represent the weight loss of samples using extract and chl $a$  as bioinhibitor. The weight loss values, corrosion rate, and inhibition efficiency were calculated using equation 1, equation 2, and equation 3, respectively. Table 2 shows that the use of inhibitors significantly decreased the corrosion rates. The corrosion rate of chl $a$  is lower than that when the extract is used as an inhibitor. Hence, chl $a$  is more effective as an inhibitor than the extract.

### Surface Analysis

The mild steel specimens were observed under magnification of 10x or 100  $\mu\text{m}$  by using a binocular microscope. The microscopic image of mild steel specimens before and after the weight loss test is shown in Figure 4 and Table 3.

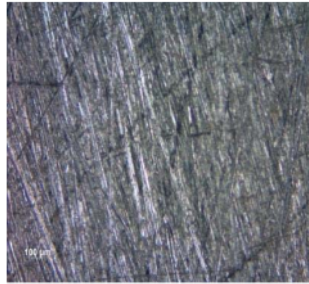
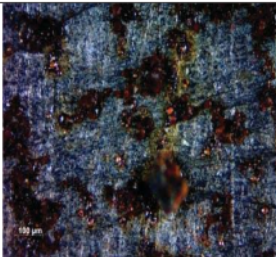
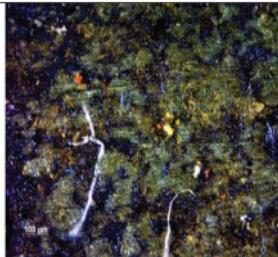
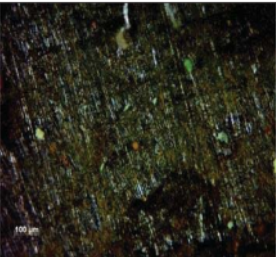
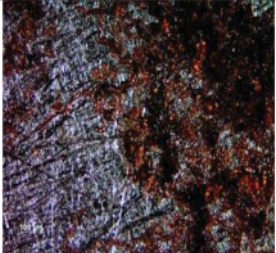

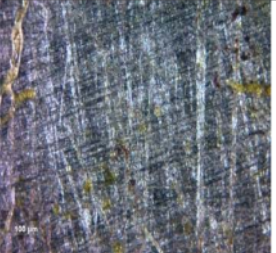


FIGURE 4. Microscopic image of mild steel specimens before weight loss test

Microscopic image shows that the surface of mild steel specimens before and after the weight loss test was significantly changed.

TABLE 3. Microscopic image of mild steel after weight loss test for 7 days

Corrosive Media	Inhibitor		
	Without Inhibitor	Extract	Chia
3% Brine solution			
Seawater			

After being immersed in corrosive media for 7 days, rust was formed onto the surface of the mild steel specimen. The mild steel specimen coated with extract had more rust in the surface than the mild steel specimen that was coated with *chl a*. Furthermore, Table 3 shows that mild steel specimen in brine solution was highly corroded than in seawater. This result is attributed to various factors, such as pH and salinity, that were controlled [14]. The pH of corrosive media is an essential factor in corrosion resistance because hydrogen ions can interact and modify the surface and alter a material's corrosion resistance. The decrease of pH can increase the rust or corrosion rate [15]. Moreover, higher salinity means lower oxygen solubility, while increased dissolved oxygen can increase corrosion risk [14].

## CONCLUSION

*Chla* has been isolated from kirinyuh leaves, and *chl a* was successfully separated by various chromatographic techniques. The pigment has been confirmed by UV-Vis absorption and FTIR spectra. The protection afforded by *chl a* was shown to be more active as a bioinhibitor than the extract. The weight loss test results showed that inhibition efficiency of chlorophyll *a* in a 3% brine solution was 60% and that in seawater was 77.89%.



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