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DERIVATIZAÇÃO DE CLOROFILA DE FOLHAS DE PANDAN (*Pandanus amaryllifolius* Roxb.) E SUA ATIVIDADE ANTIOXIDANTE

DERIVATIZATION OF CHLOROPHYLL FROM PANDAN (*Pandanus amaryllifolius* Roxb.) LEAVES AND THEIR ANTIOXIDANT ACTIVITY

DERIVATISASI KLOROFIL DAUN PANDAN (*Pandanus amaryllifolius* Roxb.) DAN AKTIVITAS ANTIOKSIDANNYA

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RESUMO

A clorofila é um corante natural com atividade antioxidante. Durante o processo de extração e processamento de alimentos, a clorofila é facilmente degradada e derivatizada. O processo de derivatização resulta em mudanças na estrutura química da clorofila que podem resultar em uma mudança na cor e na sua atividade antioxidante. Os outros compostos secundários extraídos podem afetar sua atividade antioxidante. Este estudo teve como objetivo identificar as alterações na estrutura química da clorofila extraída das folhas de pandan (*Pandanus amaryllifolius* Roxb) afetadas pelo processo de derivatização e avaliar a atividade antioxidante dos extratos e frações de clorofila e seus derivados. A clorofila foi extraída das folhas de pandan com acetona, derivatizada e purificada por fracionamento por cromatografia em coluna. O extrato de clorofila e seus derivados foram analisados quanto aos teores de carotenóides totais, fenólicos totais e flavonóides. A atividade antioxidante do extrato e da fração foi medida pela inibição da peroxidação do ácido linoléico, atividade sequestrante de radical 2,2-difenil-1-picrilhidrazil (DPPH), capacidade de redução férrica / poder antioxidante (FRAP) e ensaio quelante de metais. Com base nos espectros de FTIR e MS/MS, foi observado que os primeiros derivados eram feofitina e clorofilida, enquanto o segundo derivado era feoforbida. Os outros compostos extraídos, incluindo compostos fenólicos, flavonóides e carotenóides, podem aumentar a atividade antioxidante dos derivados da clorofila. A taxa de inibição da peroxidação do ácido linoléico pela clorofila, clorofilida e extrato de feoforbida não foi significativamente diferente do BHT, onde o extrato de feofitina foi menor. A atividade de eliminação de radicais do ensaio DPPH e FRAP mostrou que os extratos de clorofila e clorofilida exibiram a maior atividade, seguidos por feofitina e feoforbídeo. Enquanto isso, o ensaio quelante de metal mostrou que a fração clorofilida exibiu a menor atividade. Portanto, a atividade antioxidante dos primeiros derivados da clorofila foi maior do que os segundos derivados. Em geral, o extrato de clorofila e seus derivados exibiram maior atividade antioxidante do que sua fração.

Palavras-chave: *Clorofila, Pandanus amaryllifolius Roxb, atividade antioxidante.*

ABSTRACT

Chlorophyll is a natural coloring agent that has antioxidant activity. During the extraction and food processing process, chlorophyll is easily degraded and derivatized. The derivatization process results in changes in the chemical structure of chlorophyll which can result in a change in color and its antioxidant activity. The other extracted minor compounds can affect its antioxidant activity. This study aimed to identify the chemical structure

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changes of chlorophyll extracted from pandan (*Pandanus amaryllifolius* Roxb) leaves as affected by the derivatization process and evaluate the antioxidant activity of the extracts and fractions of chlorophyll and its derivatives. Chlorophyll was extracted from pandan leaves with acetone, derivatized, and then purified by fractionation using column chromatography. Chlorophyll extract and its derivatives were analyzed for total carotenoid, total phenolic, and flavonoid contents. The antioxidant activity of extract and fraction was measured by the inhibition of peroxidation of linoleic acid, radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing/ antioxidant power (FRAP) ability, and metal chelating assay. Based on the FTIR and MS/MS spectra, it was observed that the first derivatives were pheophytin and chlorophyllide, while the second derivative was pheophorbide. The other extracted compounds, including phenolic compounds, flavonoids, and carotenoids, might enhance the antioxidant activity of the chlorophyll derivatives. The inhibition rate of linoleic acid peroxidation by chlorophyll, chlorophyllide, and pheophorbide extract was not significantly different from BHT, where pheophytin extract was lower. The radical scavenging activity of DPPH and FRAP assay showed that chlorophyll and chlorophyllide extracts exhibited higher activity, followed by pheophytin and pheophorbide. Meanwhile, the metal chelating assay showed that chlorophyllide fraction exhibited the lowest activity. Therefore, the antioxidant activity of the first derivatives of chlorophyll was higher than the second derivatives. In general, the extract of chlorophyll and its derivatives exhibited higher antioxidant activity than that of their fraction.

Keywords: *Chlorophyll, Pandanus amaryllifolius Roxb, antioxidant activity.*

ABSTRAK

Klorofil merupakan pewarna alami yang memiliki aktivitas antioksidan. Selama proses ekstraksi dan pengolahan makanan, klorofil mudah terdegradasi dan terderivatisasi. Proses derivatisasi mengakibatkan perubahan struktur kimiawi klorofil sehingga terjadi perubahan warna dan aktivitas antioksidannya. Keberadaan senyawa minor terekstrak juga dapat mempengaruhi aktivitas antioksidannya. Penelitian ini bertujuan untuk mengidentifikasi perubahan struktur kimia klorofil daun pandan (*Pandanus amaryllifolius* Roxb) akibat proses derivatisasi dan mengevaluasi aktivitas antioksidan ekstrak dan fraksi klorofil dan turunannya. Klorofil diekstraks dari daun pandan dengan aseton, diderivatisasi, kemudian dimurnikan dengan cara fraksinasi menggunakan kromatografi kolom. Ekstrak klorofil dan turunannya dianalisis kandungan karotenoid, fenolik dan flavonoid total. Aktivitas antioksidan diukur dengan menggunakan metode penghambatan peroksidasi asam linoleat, daya tangkap radikal 2,2-diphenyl-1-picryl hydrazyl (DPPH), *Ferric reducing/antioxidant power* (FRAP) *ability* dan uji aktivitas mengkhelat logam. Berdasarkan spektra FTIR dan MS/MS diketahui bahwa turunan pertama klorofil adalah feofitin dan klorofilid, sedangkan turunan kedua adalah feoforbid. Keberadaan bahan ikutan seperti fenolik, flavonoid dan karotenoid meningkatkan aktivitas antioksidan ekstrak klorofil dan turunannya. Kemampuan menghambat peroksidasi asam lenoleat ekstrak klorofil, klorofilid, dan feoforbid tidak berbeda dengan BHT, sedangkan ekstrak feofitin lebih rendah. Hasil uji daya tangkap radikal DPPH dan FRAP menunjukkan bahwa ekstrak klorofil dan klorofilid mempunyai aktivitas yang lebih tinggi diikuti dengan feofitin dan feoforbid. Sedangkan hasil uji mengkhelat logam menunjukkan bahwa fraksi klorofilid mempunyai aktivitas mengkhelat logam yang paling rendah. Oleh karena itu dapat disimpulkan bahwa aktivitas antioksidan turunan pertama klorofil lebih tinggi dibanding turunan kedua. Secara umum ekstrak klorofil dan turunannya mempunyai aktivitas antioksidan yang lebih tinggi dibanding masing-masing fraksinya.

Kata kunci : *Klorofil, Pandanus amaryllifolius Roxb., aktivitas antiokasidan*

1. INTRODUCTION:

Currently, the use of natural food colorants is increasing because synthetic colorants in food can cause negative health effects (El-Wahab and Moram, 2012; Dafallah *et al.*, 2015). One of the natural food colorants is chlorophyll. Chlorophylls are chlorin-type tetrapyrrole with a coplanar system conjugated double bonds that form an aromatic structure with the delocalization of electron density in π-orbitals. In general, chlorophylls bind magnesium as the central metal ion and a phytol (C20) esterified with the propionic acid moiety at C17, although there are exceptions to these characteristics. Structurally, they differ on the side-chain substituent at C7, a methyl group in the *a*-series and a formyl group in the *b*-series. Chlorophyll *a* ($C_{55}H_{72}O_5N_4Mg$) is found in all photosynthetic organisms, except some groups of bacteria. Chlorophyll *b* ($C_{55}H_{70}O_6N_4Mg$) is present in all higher plants and algae of the divisions (Roca *et al.,* 2016).

As a food coloring agent, chlorophyll has an advantage because it has antioxidant activity. Chlorophyll can prevent autoxidation of vegetable oils stored in the dark. Chlorophyll can donate hydrogen ions to break the chain reaction of radical formation during lipid oxidation (Endo *et al.,* 1985a; 1985b). Meanwhile, Cahyana *et al.* (1992) stated that π-cation radicals can donate electrons to break the chain reaction during the oxidation

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process. Chlorophyll can also capture free radicals (Lanfer-Marquez *et al.*, 2005; Ferruzzi *et al.*, 2002). Free radicals result from initial lipid oxidation, which has roles in the lipid oxidation chain reaction. If the numbers of free radicals are reducing, the process of lipid oxidation will be reduced too.

In the preparation of chlorophyll as a coloring agent, the chlorophyll is extracted from its source. Other compounds, including flavor components, carotenoids, phenolic compounds, and flavonoids, are also extracted during the extraction process. Various research results show that besides containing chlorophyll, leaves also contain carotenoid, phenolic, and flavonoid compounds (Fasakin *et al.*, 2011; Aryal *et al.*, 2019). Fasakin et al. (2011) showed that the phenolic component was soluble in acetone, which is one of the chlorophyll solvents. These substances are expected to enhance the stability of chlorophyll and its activity as an antioxidant. Kim *et al.* (2012) stated that phenolic compounds are capable of inhibiting the photooxidation reaction due to the chlorophyll photosensitizer. Chlorophyll is a pigment that functions to capture light energy during the photosynthesis process. In the use of chlorophyll as food coloring, it is impossible to be free from light so that chlorophyll can act as a sensitizer so that singlet oxygen can be formed. B-carotene compounds can reduce the lipid oxidation process because it can capture singlet oxygen (Lee and Min, 1988) and scavenge free radicals (Lee *et al.*, 2003). Meanwhile, flavonoids should protect lipid from light-induced quality deterioration through deactivation of (triplet-) excited states of chlorophyll, quenching of singlet oxygen, and scavenging of radicals (Huvaere and Skibsted, 2014).

However, chlorophyll is easily damaged by heat, light, acid, and base, leading to its derivatives. Roca *et al.* (2016) explained that four chemical modifications could occur during the storage and cooking process, which resulted in the formation of chlorophyll derivative compounds. The first is the pheophytinization reaction, where the central atom of magnesium of tetrapyrrole replaces with two hydrogen atoms due to acidic conditions or heat treatment. This reaction causes a brownish color change. If this reaction starts with chlorophyll, it will form pheophytin. When starting from chlorophyllide, it will form pheophorbide reaction de-esterification of the phytol branched-chain, which produces components without changing color due to enzymatic reactions by the enzyme chlorophyllase or due to alkaline conditions. If the reaction starts with chlorophyll, a chlorophyllide compound will be formed, whereas if it starts with pheophytin, a pheophorbide will be formed. The third reaction is a reaction that occurs due to a very high temperature. It induces a loss of the carboxymethoxy C-13^{2}, which produces pyro derivatives, most of which are formed pyropheophytin and pyropheophorbide than pyrochlorophyll. The last reaction is under medium temperature conditions, which result in the formation of epimers at C-13^{2} atoms from natural chlorophyll.

The changes in the chemical properties of chlorophyll may affect its antioxidative properties. Lanfer-Marquez *et al.* (2005) examined the antioxidant activity of chlorophyll and its derivatives. The derivatization process was carried out on spinach leaf chlorophyll. The results showed that the ability to inhibit β-carotene bleaching from pheophorbide b was higher than pheophobide a, pheophytin, and chlorophyll, which led to a functional role of the aldehyde group. Meanwhile, the DPPH radical scavenging assay spinach leaf chlorophyll was higher than pheophytin (Kang *et al.,* 2018). Ferruzzi *et al.* (2002) stated that the chlorophyll a derivative has a more effective DPPH radical scavenging power than the chlorophyll b derivative, and pheophorbide derivative has the same ability as chlorophyll a. It was further stated that metal-free derivatives such as chlorine, pheophytins, and pyropheophytins showed lower antiradical capacity than metaloderivatives such as Mg-chlorophyll, Zn-pheophytins, Zn-pyropheophytins, Cu-pheophytin a, and Cu-chlorophyllins.

It can be seen that the results of the analysis of the antioxidant activity of chlorophyll and its derivatives differ between methods of measuring antioxidant activity. The mechanisms of the antioxidants are based on the fact they slow down the oxidation rates of foods by a combination of scavenging free radicals, chelating prooxidative metals, quenching singlet oxygen and photosensitizers, and inactivating lipoxygenase (Choe and Min, 2009). In this study, various methods of measuring antioxidant activity were used, including the ability to inhibit the oxidation of linoleic acid with the ferric thiocyanate (FTC) method, the measurement of the free radical scavenging capacity of 2,2-diphenyl-1-picryl hydrazyl (DPPH), the ability of antioxidants to reduce ferric ions by using The ferric reducing/antioxidant power (FRAP) method, the ability to capture metal ions which can act as prooxidants with the chelation of metal ion assay. In contrast, the ability to capture singlet oxygen

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and lipoxygenase inactivation will be discussed in the next publish.

Fragrant pandanus (pandan) from *Pandanus amaryllifolius* Roxb. plant (Figure 1) is a leaf that is commonly used as a food colorant in Indonesia. Pandan leaves have advantages than other leaves because it has a specific scent with a very low odor threshold of 0.1 µg/kg (Wakte *et al.*, 2016) and a relatively high phenolic and flavonoid content (Suryani *et al.*, 2017). It was expected that the existence of phenolic, flavonoids, and carotenoids can increase the total antioxidant activity of the chlorophyll extracted from pandan leaves.

Therefore, this study aimed to identify the chemical structure changes of chlorophyll extracted from pandan (*Pandanus amaryllifolius* Roxb) leaves as affected by the derivatization process and to evaluate the antioxidant activity of the extract and fractions of chlorophyll and its derivatives.

2. MATERIALS AND METHODS:

2.1. Materials

Pandanus leaves were obtained from plants grown in the district of Bantul, Indonesia. This study used mature leaves or leaves at the section numbers of 13-18. The solvents and chemicals used in the extraction of chlorophyll, derivatization, and fractionation by column chromatography were acetone, hexane, petroleum ether, HCl, NaOH and silica gel (silica gel 60, Merck, 0063-0200 mm), all were laboratory grade, butylated hydroxytoluene (BHT), ascorbic acid, and ethylene diamine tetraacetate (EDTA) were from Sigma-Aldrich (Germany). The process of extraction, derivatization and analysis was conducted in a room with controlled lighting.

2.2. Preparation of chlorophyll and its derivatives extract

2.2.1 Preparation of chlorophyll extract

The pandan leaves were taken from the tree in the morning and wrapped with black plastic, then put into a cooling box while being transported to the laboratory. The extraction of chlorophyll from pandan leaves followed the Lanfer-Marquez *et al.* (2005) method. Fresh pandan leaves were cut crosswise at 1-2 cm, weighed as much as 30 g, and dry-milled for 3 min. The slurry (25 g) was put in a 500 mL Erlenmeyer, mixed with acetone in the ratio 1:5 (w/v), and stirred with a magnetic stirrer for 15 min. A cloth filter filtered the slurry, and the pulp was squeezed with a mini screw press. The extract was re-filtered with Whatman filter paper number 1. The chlorophyll extract was separated using petroleum ether and cold distilled water with a volume ratio of extracts: petroleum ether: distilled water (80/60/30). The mixture was put into a separating funnel, then shaken and separated. The chlorophyll extract in petroleum ether was stored in dark bottles prior to analysis. The total chlorophyll (Chl) content of the extract was determined by the method described by Vernon (1960) using Eq. 1 with A_{645} and A_{663} showed absorbance values of extract at wavelengths of 645 and 663 nm, and DF dilution factor, respectively.

$$\text{Chl (mg/L)} = (20.8A_{645}+8.02A_{663}) \times \text{DF} \quad \text{(Eq.1)}$$

2.2.2 Preparation of pheophytin extract

Preparation of pheophytin extracts was performed according to the Ngo and Zhao (2007) method. Chlorophyll extract in petroleum ether (30 mL) was slowly added with 10 mL of acetone containing 15 µL of concentrated HCl and stirred to form a brownish yellow-olive oil-like color. Afterward, the HCl excess was removed by washing it with cold distilled water (extract: distilled water = 30:30) and transferred into a separating funnel. The water-acetone layer was separated from petroleum ether containing pheophytin.

The content of pheophytin (Pht) was determined by the Vernon (1960) method as in Eq. 2 with A_{666}, and A_{655} showed absorbance values of the extract at wavelengths of 666 and 655 nm, respectively.

$$\text{Pht(mg/L)} = (6.75A_{666}+26.03A_{655}) \times \text{DF} \quad \text{(Eq. 2)}$$

2.2.3 Preparation of chlorophyllide extracts

Chlorophyllide extract was prepared using the Van Breemen *et al.* (1991) method through an enzymatic process. A slurry (25 g) of fresh pandan leaves was stored in the Erlenmeyer and incubated at 40°C for 2 h in the dark to activate the enzyme chlorophyllase. Chlorophyllide was then extracted with acetone (1:6 w/v), stirred for 15 min, and filtered by a cloth filter. The pulp was squeezed with a mini screw press. The extract was re-filtered with Whatman filter paper number 1. Chlorophyllide extract was purified with hexane in a volume

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ratio of chlorophyllide extract: hexane = 150:30. After being stirring with a magnetic stirrer for 2 min, the mixture was separated using a separating funnel. The hexane layer was separated from the chlorophyllide extract. The chlorophyllide extract in acetone was stored in the dark bottles and at cold temperatures before being used. The content of chlorophyllide (Chd) was determined by using the method described by Yang *et al.* (1998) using Eq. 3 with A_{667}, and A_{650} showed absorbance values of extract at wavelengths of 667 and 650 nm, respectively.

$$Chd\ (mg/L) = (8.22A_{667}+13.05A_{650}) \times DF \quad (Eq.\ 3)$$

2.2.4 Preparation of pheophorbide extract

The preparation of the pheophorbide extract was in accordance with the Van Breemen *et al.* (1991) method. Chlorophyllide extract in 60 mL of acetone was added with 20 mL acetone containing 15 µL of concentrated HCl dropwise while being stirred with a magnetic stirrer for 10 min to form a blackish color. The pheophorbide extract was concentrated by being blow with nitrogen gas and stored in dark bottles and at a cold temperature before being used. The pheophorbide (Phb) was determined by the total pheopigment content (Arar, 1997) as in Eq. 4. A_{665a} showed the absorbance value at a wavelength of 665 nm from the resulting pheophorbide extract. In comparison, A_{665b} showed the absorbance value at a wavelength of 665 nm from the resulting chlorophyllide extract before conversion into pheophorbide derivatives.

$$Phb(mg/L) = 26.7[1.7x(A_{665a}-A_{665b})] \times DF \quad (Eq.\ 4)$$

The content of other extracted compounds from the chlorophyll extract and its derivatives was analyzed for the total carotenoids (Hendry and Grime, 1993), total phenolic by Folin-Ciocalteu method total flavonoid (Chandra *et al.*, 2014). Total phenolic contents in the pandan leaf extracts in terms of gallic acid equivalent (mg EGA/L of extract) and total flavonoid in terms of quercetin equivalent (mg QE/L of extract).

2.3. Chlorophyll and its derivatives fractionation

The fractionation of chlorophyll and its derivatives was carried out using the method of Milenković *et al.* (2012). The fractionation was conducted by column chromatography on silica gel with an eluent mixture of n-hexane/acetone in a ratio of 1:0 to 1:5. Preliminary experiments revealed that chlorophyll fractions were obtained in the eluent mixture ratio of 1:0.1, pheophytin fraction of 1:1, chlorophyllide fraction of 1:3.3, and pheophorbide fraction of 1:5. Chlorophyll and its derivative fractions were stored in dark bottles at a low temperature before being used. Analysis of the chlorophyll and its derivative content was carried out in Eq. 1-4.

2.4. Identification of chlorophyll and its derivatives

2.4.1 Fourier Transform Infrared (FTIR) spectral analysis

Spectra analysis of fractions of chlorophyll and its derivatives was carried out using FTIR iS 10 (Thermo Fisher Scientific). FTIR analysis was carried out at room temperature with a concentration of chlorophyll and its derivatives fraction of 200 mg/L with acetone and wavelength of 4000-400 cm^{-1}.

2.4.2 MS/MS spectral analysis

Chemical structure changes were identified by using mass spectrometry (MS/MS) Triple Q (quadrupole) (TSQ Quantum Access Max from Thermo Finnigan) with a source of ionization Electro Spray Ionization (ESI), controlled by TSQ Tune software, and operated by positive mode. The evaporation temperature was set at 50°C, the capillary temperature was 270°C, the spray voltage was 3000 volts, and 5 unit gas sheets were used. The operating condition of mass spectrometry was at a low flow with a flow rate of 5 µL/min.

2.5 Antioxidant activity of the chlorophyll and its derivatives

2.5.1 Determination of inhibition of linoleic acid oxidation

The determination of antioxidant activity in a linoleic acid emulsion system was measured by using the FTC method (Mathew and Abraham, 2006). A 4.0 mL extract or fraction of chlorophyll or its derivatives at a concentration of 100 ppm was mixed with linoleic acid emulsion. The linoleic acid emulsion was prepared as follows: 1.0 mL of 2.5% linoleic acid was mixed with 0.1 mL of Tween 20, sample 4.0 mL and potassium phosphate buffer (0.02 M) to reach a volume of 10.0 mL. The mixture was incubated at 37±1°C in a dark room. A 0.1 mL aliquot was taken daily during 6 days of incubation. The degree of oxidation was measured by adding 5.0 mL of ethanol (75% v/v), 0.1 mL of ammonium thiocyanate (30% w/v), and 0.1 mL of $FeCl_2$ (0.02 M in 3.5% HCl v/v), and then

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homogenized. The absorbance was measured at 500 nm with triplicate. Solution without extract was used as a blank solution, while BHT at 100 ppm concentration was used as a comparison. The percentage inhibition of lipid peroxidation was determined by following Eq. 5 with A_0 absorbance control and A_1 absorbance of the sample at a wavelength of 500 nm.

$$LPI (\%) = 100 - [(A_1 / A_0) \times 100] \quad \text{(Eq. 5)}$$

2.5.2 Determination of radical scavenging activity

DPPH free radical scavenging activity was determined by the Xu and Chang (2007) method. The sample and BHT concentrations were 100 ppm. A 0.2 mL of the extract or fraction of chlorophyll and its derivatives was added to 3.8 mL of 0.1 mM DPPH solution in methanol and mixed via vortex for 1 min. The filtrate was incubated in a dark room at room temperature for 30 min. The control was made by using methanol as a substitute sample and BHT. After incubation, the absorbance of the filtrate was read with UV-Vis spectrophotometry at λ 515 nm. The data obtained was A_0: absorbance at 515 λ of DPPH without sample, A_S: absorbance at λ 515 nm of samples with DPPH, and A_b: absorbance at λ 515 nm of the sample without DPPH. Radical Scavenging Activity (RSA) was expressed in percent (%). The RSA value showed the ability of the samples in DPPH discoloration and was calculated by the Eq. 6 (Kang *et al.,* 2018).

$$RSA(\%) = [(A_0 – (A_s - A_b))/A_0] \times 100 \quad \text{(Eq. 6)}$$

2.5.3 Ferric Reducing/Antioxidant Power (FRAP) ability

The determination of antioxidant activity with the FRAP method was carried out as described by Benzie and Devaki (2018). FRAP reagent was prepared by mixing 10 mL of acetate buffer (300 mM, pH 3,6), 1 mL of tripyridyltriazine (TPTZ) (10 mM in 40 mM HCl), and 1 mL of $FeCl_3.6H_2O$ (200 mM). A FRAP reagent (3 mL) was put in a test tube and heated at 37 °C for 10 min, then added with extracts or fractions of chlorophyll and its derivatives (100 µL) (extracts or fractions at concentrations of 50 ppm) and 300 µL of distilled water, mixed for 1 min and allowed to stand for 4 min. Then, the absorbance was measured at a λ 593 nm. Ascorbic acid was used as a comparison at the same concentration. The reducing ability (mol/L Fe^{2+}) was calculated based on the standard curve $FeSO_4.7H_2O$ (concentration range from 0 to 500 mol/L).

2.5.4 Chelation of metal ion assay

The chelating ability of cation Fe (II) was determined by Dinis *et al.* method (Mathew and Abraham, 2006). It used a sample with a concentration of extract or fractions of 5 ppm, aimed at reducing the interference color of the sample. Extracts or fractions of chlorophyll and its derivatives (3 mL) with 5 ppm concentration were added to 1 mM $FeCl_2$ (0.3 mL), then incubated for 30 min at room temperature. The reaction was initiated by the addition of 1 mM ferrozine (0.3 mL). Once the mixture reached equilibrium (10 min), the absorbance was measured at 562 nm. EDTA at the same concentration was used for comparison. The percentage inhibition of ferrozine-Fe^{2+} complex formation was determined using Eq. 7 with A_0 absorbance control and A_1 absorbance of the sample at a wavelength of 562 nm.

$$Chelating\ effect(\%) = \left[1 - \frac{A_1}{A_0}\right] X100 \quad \text{(Eq. 7)}$$

3. RESULTS AND DISCUSSION:

3.1. Structural analysis of chlorophyll and its derivatives

3.1.1 FTIR spectral analysis

The FTIR spectra of the chlorophyll and its derivatives fractions in acetone are shown in Figure 2. The FTIR spectra showed significant differences between chlorophyll compared to its derivatives. The spectra of chlorophyll were characterized primarily by the presence of phytol group at a wavelength of 2922 cm^{-1} and in pheophytin that detected the presence of the phytol group. Pheophytin was also detected in the spectra peak at a wavelength of 3392 cm^{-1}, indicating the presence of the N-H bond. The existence of the N-H bond on pheophytin and pheophorbide derivatives indicated that the derivatization with acid caused Mg^{2+} ions to be released and replaced by two H^+ ions.

Chlorophyllide and pheophorbide compound were characterized mainly by the loss of the phytol group on the spectra indicated by the loss of the peak at a wavelength of 2922 cm^{-1}, and the appearance of a new peak at a wavelength of 3318 cm^{-1}, indicating the presence of the O-H group due to the release of phytol. In the pheophorbide spectra, a peak was detected at a wavelength of 3392 cm^{-1}, wherein chlorophyllide was invisible. Pheophorbide fractions were detected on the peak at a wavelength of 3392 cm-

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[1], indicating the presence of ion H, which replaced Mg^{2+} ions. Pheophorbide has the simplest structure, shown by the lowest number of absorbance peaks that were related to the lowest number of the group. This was in accordance with the pheophorbide structure (Figure 3), which showed that all groups in R2 and R3 were the H ion and the R4 was still bonding $-CO_2CH_3$ group (Roca *et al*., 2016).

3.1.2 MS/MS spectral analysis

The result of MS/MS analysis of chlorophyll fragmentation is shown in Figure 4 and Table 1. Based on the chlorophyll structure (Figure 3) and the spectra of ions [M^+] of chlorophyll and its derivatives, it can be seen the possibility of compounds in each fraction and its results fragmentation. There were two types of chlorophyll compounds in the chlorophyll fraction of the pandan leaf: chlorophyll *a* and *b*. The chlorophyll *a* with a molecular weight (m/z) of 892.680 was fragmented with a loss of the phytol group (R3: $-C_{20}H_{38}$) and subsequent ester keto group (R4: $-CO_2CH_3$). Chlorophyll *a* could also be fragmented with the loss of Mg^{2+} ions (R2) and then bound two H^+ ions, which was the pheophytin *a*. The fraction of chlorophyll was also detected chlorophyll *b* having a molecular weight (m/z) of 906,908. Chlorophyll *b* could be fragmented, indicated by the loss of the phytol group or the keto ester group (R4: $-CO_2CH_3$).

The spectra of the pheophytin fraction indicated that pheophytin *a* with a molecular weight of 870.569 underwent fragmentation with the loss of the phytol group and keto ester group (R4:$-CO_2CH_3$) (Table 1). In this study, pheophytin *b* was undetectable, and this is consistent with the results of chlorophyll *b* fragmentation, which did not indicate the loss of Mg^{2+} ions. Similarly, in the chlorophyllide fraction, chlorophyllide *a* with a molecular weight of 614.559 was detected, but chlorophyllide *b* was undetectable. Unlike pheophytin and chlorophyllide, the pheophorbide fragmentation produced pheophorbide *a* and *b* with molecular weights of 592.865 and 606.670, respectively. This fragmentation is indicated by the loss of the keto ester group (R4:$-CO_2CH_3$). This is consistent with the research reported by Zvezdanovic *et al.* (2014), which showed that chlorophyll fragmentation from spinach leaves did not yield pheophytin *b*, although pheophorbide *b* was detected in pheophorbide derivative.

Based on the data in Table 1, it could be concluded that the derivatization of chlorophyll produced the first derivative of chlorophyll, which was pheophytin *a*, while enzymatic derivatization produced chlorophyllide *a,* but chlorophyllide *b* was undetectable. The derivatization of chlorophyllide with acidic reaction produced the second derivative, i.e. pheophorbide *a* and *b*. This was following the scheme of the chlorophyll derivatization explained by Simpson *et al.* (2012) and Roca *et al.* (2016).

3.2. The content of carotenoids, phenolics, and flavonoids

The results revealed that chlorophyll derivatization also affected the total carotenoid content, total phenolic content and total flavonoid content. Figure 5 shows that the total carotenoid content in chlorophyll extracts was the highest, followed by pheophorbide extract, and the lowest were in chlorophyllide and pheophytin extract. Carotenoid is soluble in non-polar solvents and, thus, is largely lost during refining chlorophyllide and pheophytin extract. In contrast, the total phenolic and flavonoid content was higher in chlorophyllide and pheophytin extract than in others, allegedly due to a decrease in the proportion of carotenoids and chlorophyll in both types of extracts as they were dissolved in hexane.

3.3. Antioxidant activity

3.3.1. The inhibitory activity of lipid peroxidation

The inhibitory activity of lipid peroxidation of chlorophyll extract and its derivatives from pandan leaves is presented in Figure 6. Based on the result of regression analysis, it is known that the slope of BHT was 7.32, chlorophyll extract was 7.67, pheophytin extract was 6.35, chlorophyllide extract was 7.66, and phephorbide extract was 7.65. The slope of the chlorophyll, chlorophyllide and phephorbide were not significantly different from the slope of BHT, whereas the slope of pheophytin is significantly different ($P < 0.05$). It shows that the inhibition rate of lipid peroxidation by extract of chlorophyll, chlorophyllide and pheophorbide was not significantly different from that BHT, whereas the pheophytin was lower. According to Endo *et al.* (1985b), chlorophyll is more effective to inhibit lipid peroxidation than pheophytin, because the presence of Mg may activate the antioxidant activity of chlorophyll.

Furthermore, Lanfer-Marquez *et al.* (2005) reported that the high antioxidant activity of pheophorbide was related to the lowest degree of decolorization. However, in this study, the effectiveness of linoleic acid peroxidation inhibition is allegedly associated with higher levels of total

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phenolic, flavonoid, and carotenoid content (Cervantes-Paz *et al.,* 2014; Sarker *et al.*, 2018; Zeb and Imran, 2019). This was supported by results of the correlation, which indicated that the inhibition of lipid peroxidation was highly correlated with the levels of total phenolic content (r = 0.96, P <0:01) and the levels of total carotenoid (r=0.98, P<0.01) but negatively correlated with total flavonoid (r=-0.533, P<0.05). Chlorophyllide and pheophorbide had a higher total phenolic and total flavonoid content than chlorophyll and pheophytin extract, while chlorophyll extract had the highest carotenoid content (Figure 5). The content of total phenolic and flavonoid was one of the advantages of pandan leaves as a source of chlorophyll over other types of leaves as a food colorant. These results agree with previous studies that showed that the phenolic and flavonoid component of pandan leaves was able to inhibit the formation of lipid peroxides (Suryani *et al.*, 2017).

3.3.2. DPPH radical scavenging activity

DPPH radical scavenging activity was one of the most widely used methods of measuring the antioxidant activity because it was relatively simple and rapid. The DPPH method described by Xu and Chang (2007) was chosen because the sample volume is much smaller than the DPPH solution; thus, the effect of the color of chlorophyll and its derivatives could be minimized. The evaluation of DPPH radical scavenging activity by chlorophyll extracts or fractions and its derivatives is presented in Figure 7. It is known that chlorophyll and its derivatives exhibited antioxidant activity that varied within a range between 36.98-96.78% compared to BHT. According to Endo *et al.* (1985b), the antioxidant activity of chlorophyll and its derivatives is mainly affected by the presence of porphyrin ring, not because of phytol, metal, and isocyclic rings. The formation of n-cation porphyrin compound during oxidation was able to reduce free radicals, including DPPH.

The results showed that the DPPH radical scavenging activity of extracts and fractions of chlorophyll and its derivatives was in the order as follows: chlorophyll extract = chlorophyllide extract > chlorophyll fraction > chlorophyllide fractions and pheophorbide extracts > pheophytin extract > pheophytin fraction > pheophorbide fraction. These results were consistent with the studies of Ferruzzi *et al.* (2002) and Kang *et al.* (2018). Furthermore, Ferruzzi *et a*l. (2002) reported that metal-free derivatives such as pheophytin had lower antiradical capacity than chlorophyll derivatives. The DPPH radical scavenging activity of chlorophyll, pheophytin, chlorophyllide, and pheophorbide extracts were higher than that of the fractions. This might be because of the presence of other antioxidant compounds, such as phenolic and carotenoids. Similar results were toward in the case of antioxidant activity by the FTC method.

3.3.3. Ferric Reducing/ Antioxidant Power (FRAP)

The FRAP method was chosen because it is a direct method for analyzing the combined total antioxidant activity and the ability to reduce (electron donor) antioxidants in the sample simultaneously (Benzie and Devaki, 2018). Figure 8 shows that the extract and fractions of chlorophyll and its derivatives could reduce between 7.26 to 100% compared to ascorbic acid. The FRAP value of ascorbic acid was 592.13 µmol/L Fe^{2+}. Similar to the result of DPPH radical scavenging activity, FRAP assay results indicated that the chlorophyll and chlorophyllide extract had a higher reducing ability than chlorophyll fractions. The lowest FRAP values were the extract and fraction of pheophytin and pheophorbide. The high FRAP value of chlorophyll and chlorophyllide extract can be associated with the high total phenolic content.

Chlorophyllide fraction had the highest reducing ability than any other fraction and was not different from ascorbic acid. Chlorophyllide is a derivative of chlorophyll with loss of the phytol group, chlorophyllide is more hydrophilic similar to ascorbic acid. Pheophorbide fraction had the lowest FRAP value. The reducing agent (electron donor) is part of a redox couple with a redox potential that is relatively lower under reaction conditions (Halliwell, 2012). Ohashi *et al.* (2008) stated that oxidation potential was influenced by the inductive effect of substituent groups on the π-electron conjugated system in the macrocycle. Furthermore, Kobayashi *et al.* (2007) stated that the oxidation potential rank was chlorophyll *b* > chlorophyll *d* > chlorophyll *a*, while pheophytin *a*, *b,* and *d* had a higher oxidation potential than chlorophyll. This is because derivatization decreased the electron density in the π-system due to the replacement of magnesium with hydrogen, which was more electronegative.

3.3.4. Metal chelating activity

The results of the evaluation of metal chelating activity are presented in Figure 9. Metal chelating activity, was in the order as follows: chlorophyll fraction extract = pheophytin extract > chlorophyll and chlorophyllide extract > pheophorbide extract > pheophytin and

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pheophorbide fraction > chlorophyllide fraction. Chlorophyll fraction had the highest metal chelating activity, while chlorophyllide fraction the lowest. Metal chelating activity of chlorophyll and its derivatives was affected by a lone pair electron from nitrogen and oxygen atoms and bound with empty orbitals of the metal cations. Nitrogen and oxygen atoms are very effective as sources of a free electron pair that played a role as a chelating agent (Hargreaves, 2003). In the chlorophyll molecule, the free electron pair was bound to Mg^{2+}, but Fe^{2+} had a higher reactivity than Mg^{2+} (Cheng *et al.*, 1992), such that Mg^{2+} was replaced by Fe^{2+} and was indicated to have a high metal chelating ability. Pheophytin, pheophorbide, and chlorophyllide extracts had also a metal chelating ability that was higher than its fractions. The metal chelating ability was also related to the flavonoid content of phenolic compounds in the extract. Phenolic and flavonoid compounds can chelation Fe^{2+} (Niciforovic *et al.*, 2010: Benjakul *et al.*, 2014; Taroreh et al., 2015) because they have the hydroxyl and carboxyl group (Cherrak *et al.*, 2016).

Contrary to the antioxidant activity evaluation by DPPH radical scavenging activity, FRAP and FTC methods showed that chlorophyllide had high antioxidant activity, the metal chelating ability chlorophyllide fraction was the lowest. Chlorophyllide is a chlorophyll derivative that loses the phytol group so that it becomes more polar. These results agreed to Shim (2012) that chlorophyll had a higher ability to chelate metal than its more polar derivatives. Besides, to chelate Fe^{2+} ions, chlorophyllide releases Mg^{2+} ions into pheophorbide (Suzuki *et al.*, 2014). This reaction required higher energy or heat (Roca *et al.*, 2016) or an acidic condition (Simpson *et al.*, 2012).

4. CONCLUSIONS:

Based on the FTIR and MS/MS spectra, it was revealed that the result of the derivatization process in the research was pheophytin *a,* pheophytin *b*, chlorophyllide *a*, pheophorbide *a,* and pheophorbide *b*. Other extracted compounds, including phenolics, flavonoids, and carotenoids, enhanced the antioxidant activity of chlorophyll extract and its derivatives. The chlorophyllide and pheophorbide extract exhibited the inhibition rate of lipid peroxidation, which is not significantly different from that of BHT, whereas pheophytin extract is lower. The assay of radical scavenging activity of DPPH and FRAP shows that chlorophyll and chlorophyllide extracts exhibited higher activity, followed by pheophytin and pheophorbide. The fraction of chlorophyllide showed the lowest metal chelating activity. Thus, it can be concluded that, in general, the first derivatives of chlorophyll had antioxidant activity higher than the second derivative. Chlorophyll and its derivatives extract were better in antioxidant activity than each fraction.

5. ACKNOWLEDGMENTS:

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6. REFERENCES:

1. Arar, E. J. (1997). In Vitro Determination of Chlorophylls a, b, c1 + c2 and Pheopigments in Marine And Freshwater Algae by Visible Spectrophotometry. National Exposure Research Laboratory Office of Research and Development U.S. Environmental Protection Agency, Cincinnati, Ohio 45268.

2. Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R. and Koirala, N. (2019). Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants. 8, 1-12*. DOI: 10.3390/plants 8040096

3. Benjakul, S.; Kittiphattanabawon, P; Sumpavapol, P; Maqsood, S. (2014). Antioxidant activities of lead (*Leucaena leucocephala*) seed as affected by extraction solvent, prior dechlorophyllisation and drying methods. *J. Food Sci. Technol. 51*, 3026–3037. DOI: 10.1007/s13197-012-0846-1.

4. Benzie, I.F.F. and Devaki, M. (2018). The ferric reducing/antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity: concepts, procedures, limitations and applications. In R. Apak, E. Capanoglu, and F. Shahidi, (Eds.). *Measurement of Antioxidant Activity and Capacity: Recent Trends and Applications.* (pp. 77–106). Hongkong: John Willey and son, Ltd.

5. Cahyana, A. H., Shuto, Y., and Kinoshita, Y. (1992). Pyropheophytin a as an Antioxidative Substance from the Marine Alga, Arame (*Eisenia bicyclis*). *Biosci.*

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Biotechnol. Biochem. 56, 1533–1535. https://doi.org/10.1271/bbb.56.1533.

6. Cervantes-Paz, B; Yahia, E. M., Ornelas-Paz, J. D. J., Victoria-Campos, C.I., Ibarra-Junquera, V., Pérez-Martínez, J.D. and Escalante-Minakata, P. (2014). Antioxidant activity and content of chlorophylls and carotenoids in raw and heat-processed Jalapeño peppers at intermediate stages of ripening. *Food Chem. 146*, 188–196. DOI: 10.1016/ j.foodchem.2013.09.060.

7. Chandra, S; Khan, S., Avula, B; Lata, H., Yang, M. H., Elsohly, M. A. and Khan, I. A. (2014). Assessment of Total Phenolic and Flavonoid Content, Antioxidant Properties, and Yield of Aeroponically and Conventionally Grown Leafy Vegetables and Fruit Crops: A Comparative Study. Evidence-Based Complement. Altern. Med. 2014, 1–9.

8. Cheng, K, L., Ueno, K. and Imamura, T. (1992). *Handbook of Organic Analytical Reagents* (2rd ed). Boca Raton: CRC Press.

9. Cherrak, S. A., Mokhtari-Soulimane, N., Berroukeche, F., Bensenane, B., Cherbonnel, A., Merzouk, H. and Elhabiri, M. (2016). In Vitro Antioxidant versus Metal Ion Chelating Properties of Flavonoids: A Structure-Activity Investigation. *PLoS One*. 11, 1–21. https://doi.org/10.1371/journal.pone.0165575.

10. Choe, E. and Min, D, B. (2009). Mechanisms of Antioxidants in the Oxidation of Foods. *Comprehensive Reviews In Foods Science and Food Safety.* 8, 345-358.

11. Dafallah, A. A., Adellah, A. M., Abdel-Rahim, E., M. and Ahmed, S., H. (2015). Physiologigal Effects of Some Artificial and Natural Food Coloring on Young Albino Rats. *Journal of Food Technology Research. 2(2). 21-32.* DOI: 10.18488/journal.58/2015.2.2/58.2.21.32.

12. Delgado-Vargas, F. and Paredes Lopez, O. (2003). *Natural Colorants for Food and Nutraceutical Uses*. CRC Press, Boca Raton.

13. El-Wahap, H. M.F.A. and Moram, G.S.E.D. (2012). Toxic effects of some synthetic food colorants and/or flavor additives on male rats. *Toxicology and Industrial Health.* 29(2). 224-232. DOI: 10.1177/0748233711433935

14. Endo, Y., Usuki, R. and Kaneda, T. (1985a). Antioxidant Effects of Chlorophyll and Pheophytin on the Autoxidation of Oils in the Dark. I. Comparison of the Inhibitory Effects. *J. Am. Oil Chem. Soc. 62*, 1375–1376.

15. Endo, Y., Usuki, R. and Kaneda, T. (1985b). Antioxidant Effects of Chlorophyll and Pheophytin on the Autoxidation of Oils in the Dark. II. The Mechanism of Antioxidative Action of Chlorophyll. *J. Am. Oil Chem. Soc. 62*, 1387–1390.

16. Fasakin, C. F., Udenigwe, C. and Aluko, R.E. (2011). Antioxidant properties of chlorophyll-enriched and chlorophyll-depleted polyphenolic fractions from leaves of Vernonia amygdalina and Gongronema latifolium. *Food Reseacrh International*. 44. 2435 - 2441. DOI: 10.1016/j.foodres. 2010.12.019

17. Ferruzzi, M.G., Bohm, V., Courtney, P.D. and Schwartz, S.J. (2002). Antioxidant and Antimutagenic Activity of Dietary Chlorophyll Derivatives Determined by Radical Scavenging and Bacterial Reverse Mutagenesis Assays. *J. Food Sci. 67*, 2589–2595.

18. Halliwell, B. (2012). Free radicals and antioxidants: updating a personal view. *Nutr. Rev. 70*, 257–265. DOI:10.1111/j.1753-4887.2012.00476.x.

19. Hargreaves, T. (2003). Chemical Formulation: An Overview of Surfactant-based Preparations Used in Everyday Life. Retrieved from https://www.globalspec.com/ reference/55565/203279/chelating-agents-sequestrants/.

20. Hendry. G, A, E. and Grime, J. (1993). Methods Comparatives Plant Ecology. Laboratory Manual (1st. ed.). Hongkong: Chapman and Hall.

21. Huvaere, K. and Skibsted, L. H.(2014). Flavonoids protecting food and beverages against light. *J Sci Food Agric,* 95: 20–35.

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DOI 10.1002/jsfa.6796.

22. Kang, Y.R., Park, J., Jung, S.K. and Chang, Y.H. (2018). Synthesis, characterization, and functional properties of chlorophylls, pheophytins, and Zn-pheophytins. *Food Chem. 245*, 943–950. https://doi.org/10.1016/j.foodchem.2017.11.079.

23. Kim, T.S., Decker, E.A. and Lee, J. (2012). Effects of chlorophyll photosensitisation on the oxidative stability in oil-in-water emulsions. *Food Chem. 133*, 1449–1455. https://doi.org/10.1016/j.foodchem.2012.02.033.

24. Kobayashi, M., Ohashi, S., Iwamoto, K., Shiraiwa, Y., Kato, Y. and Watanabe, T. (2007). Redox potential of chlorophyll d in vitro. Biochim. *Biophys. Acta 1767*, 596–602. DOI: 10.1016/j.bbabio.2007.02.015.

25. Lanfer-Marquez, U. M., Barros, R. M. C. and Sinnecker, P. (2005). Antioxidant activity of chlorophylls and their derivatives. *Food Res. Int. 38*, 885–891. https://doi.org/10.1016/j.foodres.2005.02.012.

26. Lee, E.C.and Min, B. (1988). Quenching Mechanism of p-Carotene on the Chlorophyll Sensitized Photooxidation of Soybean Oil. *Journal of Food Science*, 53(6), 1894-1895.

27. Lee, J.H., Ozcelik, B. and Min, D.M. (2003). Electron Donation Mechanisms of Carotene as a Free Radical Scavenger. *Journal of Food Science*, 69(3), 861-865.

28. Mathew, S. and Abraham, T.E. (2006). In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food Chem. Toxicol. 44*, 198–206. DOI: 10.1016/ j.fct.2005. 06.013.

29. Milenković, S.M., Zvezdanović, J.B. and Anđelković, T.D. (2012). The Identification of Chlorophyll and Its Derivatis in The Pigment Mixtures : HPLC-Chromatography, Visible and Mass Spectrofotometry Studies. *Adv. Technol. 1*, 16–24.

30. Ngo, T. and Zhao, Y. (2007). Formation of zinc-chlorophyll-derivative complexes in thermally processed green pears (*Pyrus communis* L.). *J. Food Sci. 72*, 397–404. https://doi.org/10.1111/j.1750-3841.2007.00465.x.

31. Niciforovic, N., Mihailovic, V., Maškovic, P., Solujic, S., Stojkovic, A. and Pavlovic, D. (2010). Antioxidant activity of selected plant species; potential new sources of natural antioxidants. *Food Chem. Toxicol. 48*, 3125–3130. DOI:10.1016/j.fct.2010.08.007.

32. Ohashi, S., Kasahara, M., Fukuyo, S., Nakazato, M., Iwamoto, K., Shiraiwa, Y., Kato, Y., Watanabe, T. and Kobayashi, M. (2008). Redox Potential of Chlorophyll d. In J. Allen, E. Gantt, J.H, Golbeck, and B. Osmond (Eds.). *Photosynthesis. Energy from the Sun: 14th International Congress on Photosynthesis.* (pp. 105–106). Springer.

33. Roca, M., Chen, K. and Pérez-Gálvez, A., 2016. Chlorophylls. In R. Carle, and R. M., Schweiggert (Eds.). *Handbook on Natural Pigments in Food and Beverages*. (p. 508) Cambridge: Elsevier Ltd. https://doi.org/10.1016/B978-0-08-100371-8.00006-3.

34. Sarker, U., Islam, T., Rabbani, G. and Oba, S. (2018). Variability in total antioxidant capacity, antioxidant leaf pigments and foliage yield of vegetable amaranth. *J. Integr. Agric. 17*, 1145–1153. https://doi.org/10.1016/S2095-3119(17)61778-7.

35. Shim, S. (2012). Chelating Effect of Leek (*Allium tuberosum* Rottler ex Sprengel) Containing Chlorophyll on Cd, Pb, and As. *J. Korean Soc. Appl. Biol. Chem. 55*, 311–315. DOI: 10.1007/s13765-012-1151-4.

36. Simpson, B.K., Benjakul, S. and Klomklao, S. (2012). Natural Food Pigments. In B.K, Simpson (Ed.). *Food Biochemistry and Food Processing.* (p.901). Ames, Iowa: Wiley-VCH Pubhliser.

37. Suryani, C.L., Tamaroh, S., Ardiyan, A. and Setyowati, A. (2017). Antioxidant activity of pandan (*Pandanus amaryllifolius*) leaf ethanol extract and it's fractions. *Agritech 37*, 271–279. DOI: 10.22146/ agritech. 11312.

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38. Suzuki, T., Inoue, M. and Shioi, Y. (2014). Purification and properties of metal-chelating substance in chlorophyll degradation. *J. Trop. Plant Physiol. 6*, 35–49.

39. Taroreh, M., Raharjo, S., Hastuti, P. and Murdiati, A. (2015). Antioxidant Activities of Sequentially Extracted Gedi (*Abelmoschus manihot* L) Leaves. *Agritech 35*, 280–287.

40. Van Breemen, R.B., Canjura, F.L. and Schwartzg, S.J. (1991). Identification of Chlorophyll Derivatives by Mass Spectrometry. *J. Agric. Food Chem. 39*, 1452–1456.

41. Vernon, L.P., 1960. Spectrophotometric Determination of Chlorophylls and Pheophytins in Plant Extracts. *Anal. Chem. 32*, 1144–1150.

42. Wakte, K., Zanan, R., Hinge, V., Khandagale, K., Nadaf, A. and Henry, R. (2016). Thirty-three years of 2-acetyl-1-pyrroline, a principal basmati aroma compound in scented rice (*Oryza sativa* L.): a status review. *J. Sci Food Agric. July*,1-14. DOI: 10.1002/jsfa.7875.

43. Xu, B.J. and Chang, S.K.C. (2007). A Comparative Study on Phenolic Profiles and Antioxidant Activities of Legumes as Affected by Extraction Solvents. *J. Food Sci. 72*, S159–S166. DOI: 10.1111/j.1750-3841.2006.00260.x.

44. Yang, C.M., Chang, K.W., Yin, M.H. and Huang, H.M. (1998). Methods for the Determination of the Chlorophylls and their Derivatives. *Taiwania 43*, 116–122.

45. Zeb, A. and Imran, M. (2019). Carotenoids, pigments, phenolic composition and antioxidant activity of *Oxalis corniculata* leaves. *Food Biosci. 32*, 1–9. DOI: 10.1016/j.fbio.2019. 100472.

46. Zvezdanovic, J.B., Petrovic, S.M., Markovic, D.Z., Andjelkovic, T.D. and Andjejkovic, D.H. (2014). Electrospray ionization mass spectrometry combined with ultra high-performance liquid chromatography in the analysis of in vitro formation of chlorophyll complexes with copper and zinc. *J. Serbian Chem. Soc. 79*, 689–706.

Figure 1. *Pandanus amaryllifolius Roxb. plant*

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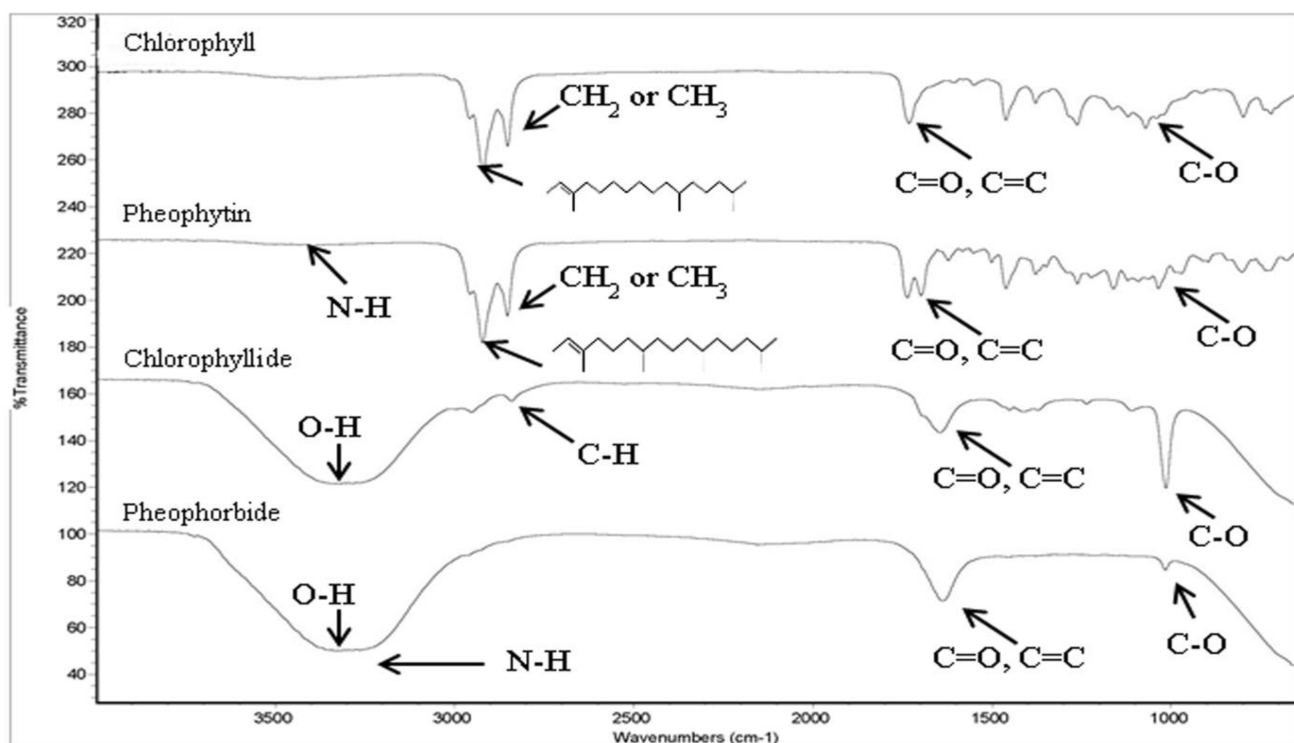
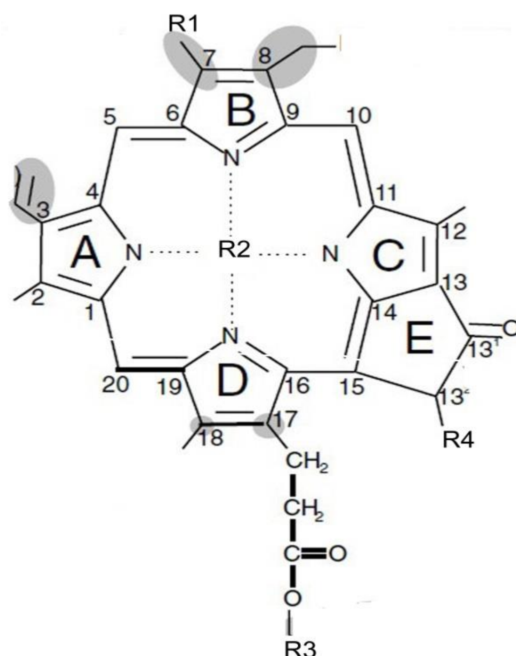


Figure 2. *FTIR spectra of chlorophyll, pheophytin, chlorophyllide, and pheophorbide fractions from pandan leaves.*



Name	R1*	R2	R3	R4
Chlorophyll *a*	CH_3	Mg	Phytol	CO_2CH_3
Pheophytin *a*	CH_3	H	Phytol	CO_2CH_3
Chlorophyllide *a*	CH_3	Mg	H	CO_2CH_3
Pheophorbide *a*	CH_3	H	H	CO_2CH_3

*R1 is CHO in series *b*

Figure 3. *Chemical structure of chlorophyll a and its derivatives (Adapted from Delgado-Vargas and Paredes Lopez (2003)).*

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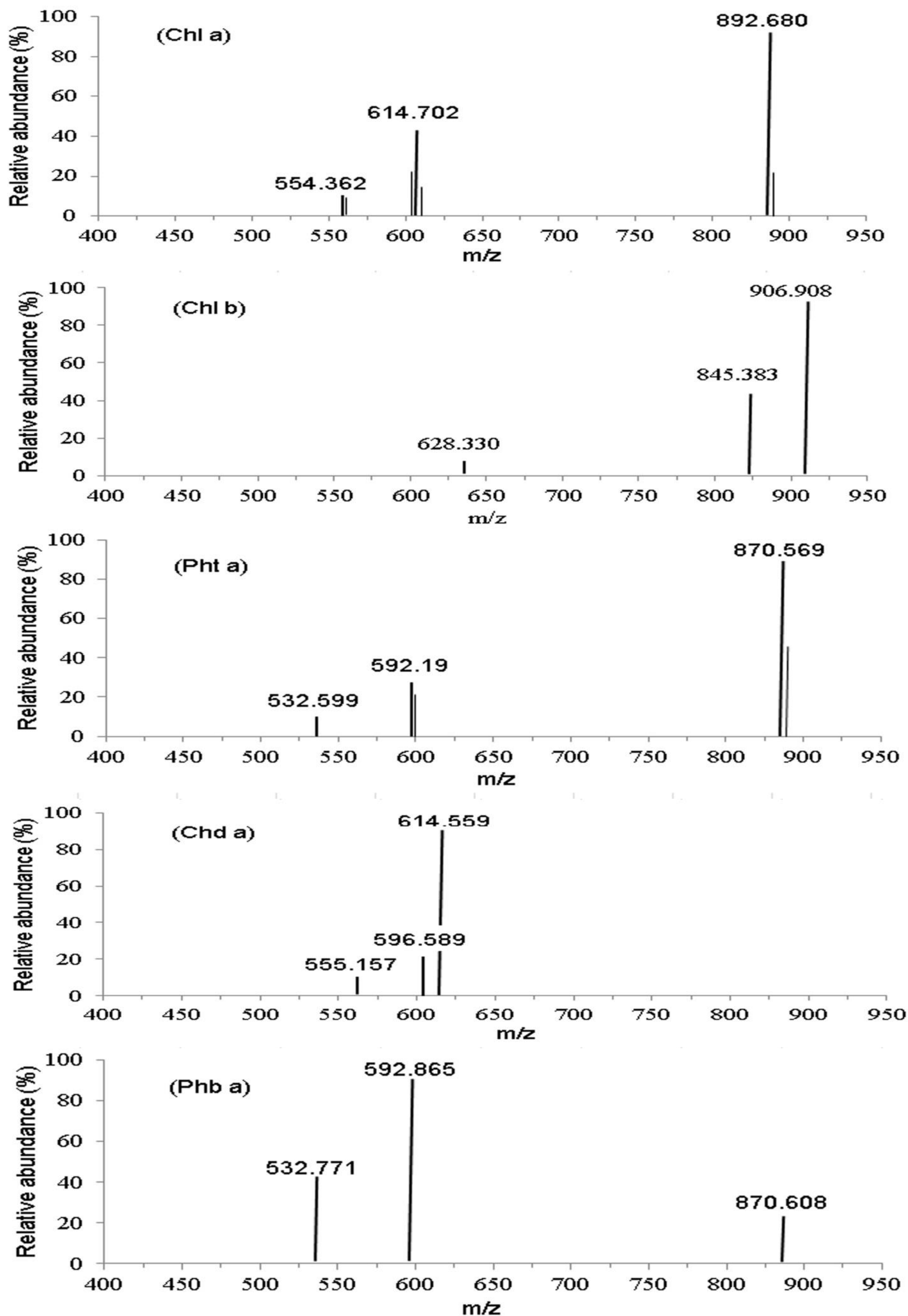

Figure 4. *Full scan MS/MS spectra of [M +] ion of chlorophyll a (Chl a), chlorophyll b (Chl b), pheophytin a (Pht a), chlorophyllide a (Chd a), and pheophorbide a (Phb a) taken from their MS spectra of chlorophyll and its derivatives fraction from pandan leaves.*

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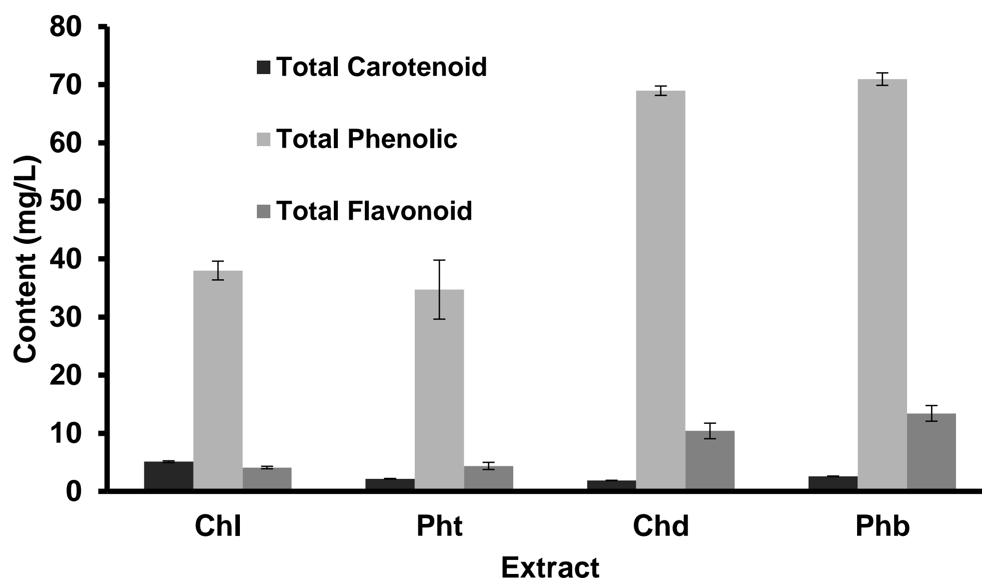
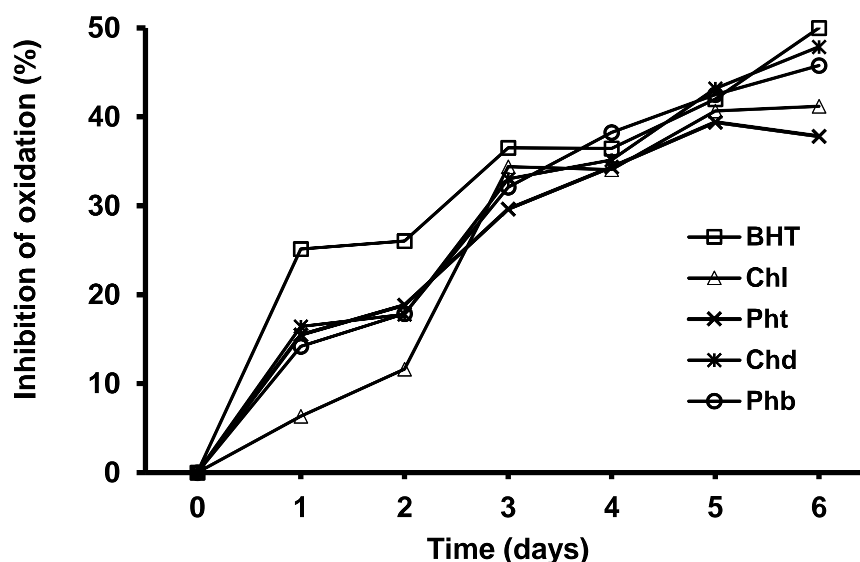


Figure 5. *Total carotenoid (mg/L), phenolics (GAE mg/L), and flavonoid (QE mg/L) content in chlorophyll and its derivatives extract from pandan leaves. Chl: chlorophyll extract, Pht: pheophytin extract, Chd: chlorophyllide extract, Phb: pheophorbide extract.*



Linear regression equation:

$Y_{BHT} = 7.32\,X + 9.75$ $\quad Y_{Chd} = 7.66\,X + 4.65$

$Y_{Chl} = 7.67\,X + 1.90$ $\quad Y_{Phb} = 7.65\,X + 4.28$

$Y_{Pht} = 6.35\,X + 6.18$

Figure 6. *Antioxidant activity of chlorophyll and its derivatives extract by FTC method. Chl: chlorophyll extract, Pht: pheophytin extract, Chl: chlorophyllide extract, and Phb: pheophorbide extract. The concentration of extract and BHT was 100 ppm.*

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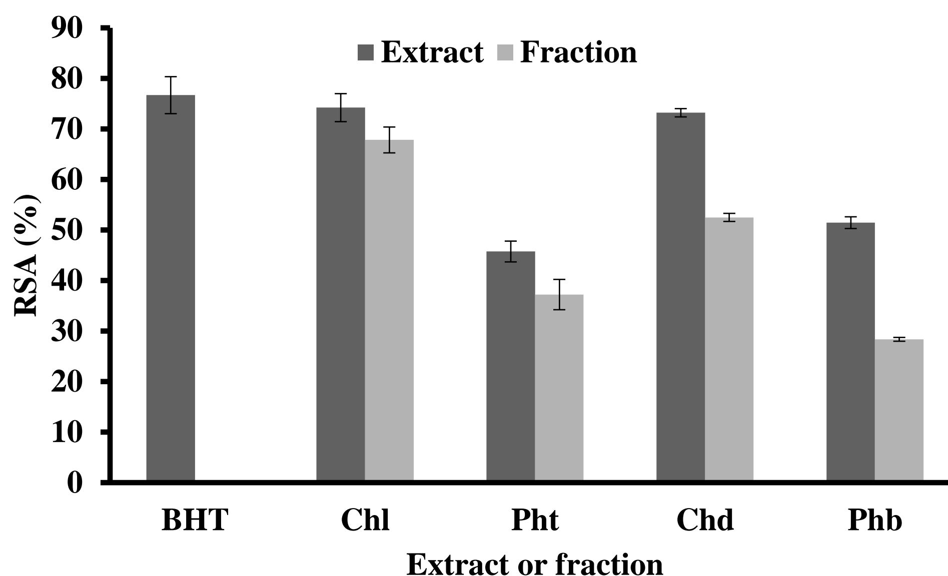

Figure 7. *Radical scavenging activity of extract or fraction of chlorophyll and its derivatives from pandan leaves on DPPH radicals. Chl: chlorophyll, Pht: pheophytin, Chl: chlorophyllide, and Phb: pheophorbide at a concentration of extract, fraction, and BHT was 100 ppm.*

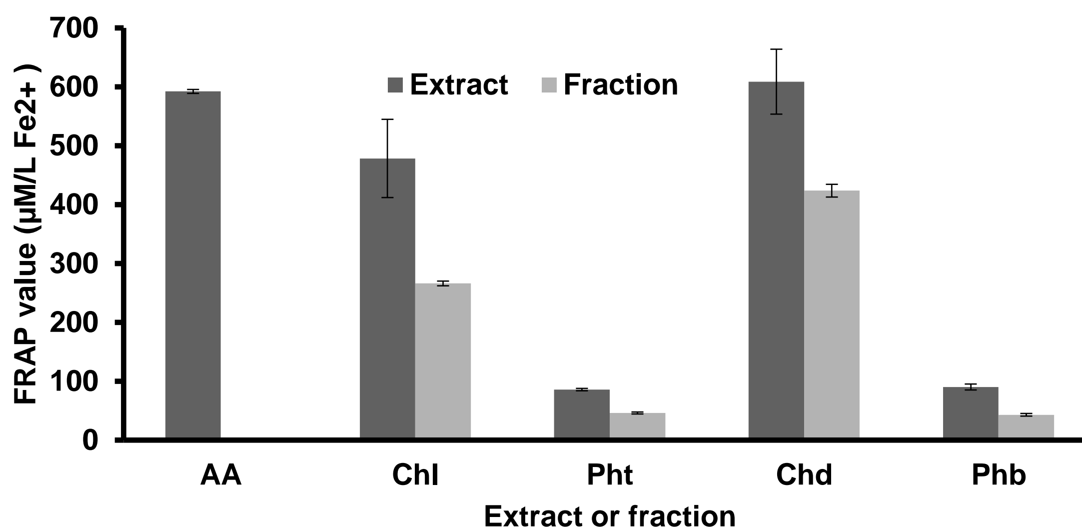

Figure 8. *FRAP value of chlorophyll and its derivatives extract or fraction from pandan leaves. Chl: chlorophyll, Pht: pheophytin, Chl: chlorophyllide, Phb: pheophorbide and AA: ascorbic acid at a concentration of extract, fraction, and ascorbic acid 50 was ppm.*

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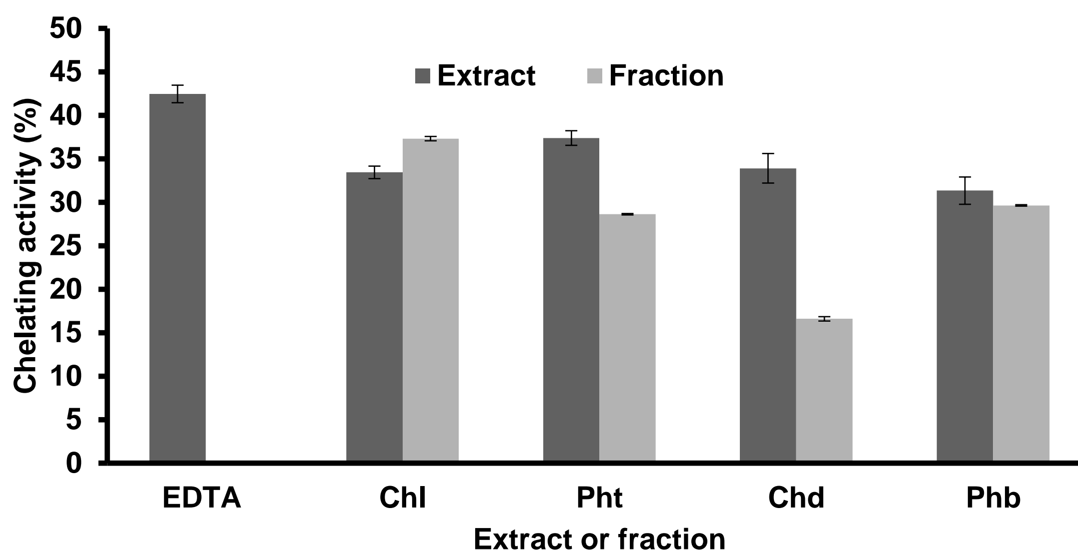

Figure 9. *Metal chelating activity of extract or fraction of chlorophyll and its derivatives from pandan leaves. Chl: chlorophyll, Pht: pheophytin, Chl: chlorophyllide and Phb: pheophorbide at a concentration of extract, fraction, and EDTA was 5 ppm.*

Table 1. *MS/MS fragmentation pattern of chlorophyll and its derivatives from pandan leaves*

Main Compound (M)	Founds MW/(m/z)	Average calculated MW/(m/z)[1]	Main observed fragment ions (m/z)	Structure of the fragment ion[1,2]
Chlorophyll a	892.680 (100%)	893.50	615[M-278]$^+$ 555[M-338]$^+$	$[M-C_{20}H_{38}]^+$ $[M-C_{20}H_{39}CO_2CH_3]^+$
	892.135* (25%)	893.50	874[M-24+2]$^+$	$[M-Mg+2H]^+$
Chlorophyll b	906.908 (100%)	907.52	846[M-60]$^+$ 628[M-278]$^+$	$[M-CO_2CH_3]^+$ $[M-C_{20}H_{38}]^+$
Pheophytin a	870.569 (100%)	871.21	593[M-278]$^+$ 533[M-278-60]$^+$	$[M-C_{20}H_{38}]^+$ $[M-C_{20}H_{39}CO_2CH_3]^+$
Chlorophyllide a	614.559 (100%)	614.30	596[M-17]$^+$ 555[M-59]$^+$	$[M-CH_3]^+$ $[M-CO_2CH_3]^+$
Pheophorbide a	592.865 (100%)	592.10	533[M-59]$^+$	$[M-CO_2CH_3]^+$
Pheophorbide b	606.670* (100%)	606.70	547[M-59]$^+$	$[M-CO_2CH_3]^+$

Reference: [1]Van Breemen *et al.* (1991) and [2]Roca *et al.* (2016).
*MS/MS fragmentation pattern not shown.

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