Indonesian Journal of Pure and Applied Chemistry
journal homepage: http://jurnal.untan.ac.id/index.php/IJoPAC

ISOLATION OF FLAVONOID FROM ANDONG LEAVES (*Cordyline fruticosa* (L.) A. Chev.) AND ITS ACTIVITY AS COMPLEXOR OF Fe^{2+}

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ARTICLE INFO

Article history:
Received
21 March 2019
Accepted
30 April 2019
Available online
30 April 2019

Keywords:
flavonoid, ethyl acetate fraction, andong leaves, complexor compound, Fe^{2+}

ABSTRACT

Flavonoid is one of the phenolic compounds that can complex metals. One of the native plants of Indonesia which has the potential as a metal complexor is the andong plant (*Cordyline fruticosa* (L.) A.Chev). The isolation process to obtain pure compound are extraction, fractionation, and separation by chromatography. Chromatography method using vacuum liquid chromatography (VLC), gravity column chromatography (GCC) and preparative thin layer chromatography (TLC). This research obtained relatively pure isolate F2.8. The IR (KBr) data on wave numbers of isolate F2.8 were 3430; 2925-2855; 1749; 1609-1512; 1171 and 1100 cm^{-1}.The result of the spectrum ^{1}H-NMR showed flavonoid compound has similar typical spectrum of apigenin compound which showed chemical shift (δ_H ppm) : 6.85 (1H, s, H-3); 6.29 (1H, s H-6); 6.57 (1H, H-8); 7.03 (2H, d, J = 11.96 Hz, H-3', H-5'); 8.03 (2H, d, J = 11.72 Hz, H-2', H-6'). The UV-Vis (CH_3OH) spectrum data of isolate F2.8 after the addition of Fe^{2+} showed a shift at λ_{max} towards a greater direction with a batochromic shift which was caused a decrease in absorbance after the addition of iron (Fe^{2+}) 20 ppm namely: 290 nm; 305 nm with respectively absorbances of 1.62 A; 0.42 A. Based on these data, isolate F2.8 is apigenin which is capable of complexing Fe^{2+}.

1. Pendahuluan

Blood transfusion aims to maintain hemoglobin levels in thalassemia patients. The side effect of transfusion is the increase in iron accumulation in the body [1]. The therapy given to patients who perform transfusions regularly is Fe metal chelation therapy [2] . Metal Fe chelation therapy can be done by giving the drug deferoxamine, deferiprone and deferasirox but giving side effects [3] . Phenolic compounds are compounds that are capable of plating metals.

Some plants which contain phenolic and flavonoid compounds have been successfully investigated have Fe chelating activity. According literature, extracts of *Leonurus cardiana* and *Grammosciadium platcarpum* contain phenolic and flavonoid compounds and have Fe chelating activity [4] . One of the native plants of Indonesia that has the potential as a metal chelating agent is

andong plant (*Cordyline fruticosa* (L.). *Cordyline fruticosa* (L.) contains a phenolic component in the form of flavonoids which have Ferro ion reduction activity [5]. The bark from andong contains flavonoid, too [6] . Based on these literature, andong is plant that contains flavonoid.

There has been no research on flavonoid compounds from andong plants as chelating metal Fe in thalassemia patients. Therefore, this study was conducted to determine the structure of phenolic compound in andong plant that is capable of chelating Fe metal and to determine the optimum concentration of metal Fe which is able to be absorbed by flavonoid compounds from andong plants.

2. Material and Methods

2.1 Collection of sample

Plant material was andong leaves that originating from the area of Pontianak City, West Kalimantan Province. The accuracy of plant species based on the results of determination in the Laboratory of Biology, Faculty of Mathematics and Natural Sciences, Tanjungpura University. The tools used are glassware, extraction tools, evaporation tools, vacuum liquid chromatography devices, chromatography equipment, 500 MHz NMR spectrometer agilent. The materials used are various types of organic solvents including: ethyl acetate, methanol, dichloromethane, *n*-hexane, reagents for phytochemical tests, 5% $CeSO_4$ and 1% $FeCl_3$ reagents, TLC silica gel 60 F_{254}, silica gel 60-70 mesh, silica gel 60 (230-400 mesh), and silica gel 60 G.

2.2 Extraction

Samples of 3 kg andong leaves powder were macerated with methanol solvent for 3x24 hours at room temperature. The filtrate was collected and the solvent evaporated using a rotary evaporator so that 110 g of methanol was obtained. Methanol maserate was multilevel partitioned using solvents with different polarity levels, *n*-hexane, dichloromethane and ethyl acetate. The ethyl acetate fraction was evaporated using a rotary evaporator to obtain 29.3171 g of ethyl acetate fraction.

2.3 Isolation

Ethyl acetate fraction (29.3171 g) was fractionated by liquid vacuum chromatography (LVC). The sample was eluted with gradient solvent by combination of ethyl acetate: dichloromethane (3: 7), (1: 1), (7: 3), ethyl acetate: dichloromethane: methanol (8: 1: 1), 100% ethyl acetate and 100% methanol so that four factions are obtained (F1, F2, F3 and F4). The fraction F2 was chosen to carry out further purification with preparative TLC. TLC preparative was obtained 8 fractions (F2.1-F2.8). Fraction F2.8 (4,2 mg) purified by various solvent and purity tests by two-dimensional TLC.

Fraction F1 (0.1595 g) was fractionated by gravity column chromatography (GCC). The samples were eluted witth gradient solvents based on the TLC analysis by dichloromethane 100%, ethyl acetate: dichloromethane (9: 1), 100% ethyl acetate, ethyl acetate-methanol (9: 1), (8: 2), (7: 3), (6: 4), (3: 7) , 100% methanol so that four fractions were obtained (F1.1-F1.4). The F1.4 (4,8 mg) was the fraction selected for the preparative TLC analyzing. The purity test was carried out with one-dimensional TLC by using 100% ethyl acetate eluent. This fraction did not contain a target compound, so the fraction F2.8 was chosen for the ^{1}H-NMR, IR and spectrophotometer ultraviolet-visible analysis.

2.4 Testing of capability as complex agent

In the test solution contains no more than 150 μg Fe (III) and 45 mg Fe (II), added as follows: 1.2 mL of 1 x 10 10^{-3} moles of L^{-1}, 3 mL of methanol and hydrochloric acid in the amount so that the final 10 mL volume of 0.3 mol L^{-1} HCl is obtained. The sample was diluted to 10 mL with 0.01 mol of L^{-1} HCl and heated at 70 °C for 20 minutes. The solution is cooled and the volume is adjusted

to 10 mL with 0.3 mol of L^{-1} HCl in the case of evaporation. Fe (III) concentration was determined using absorbance at 291 nm and 293 nm and the corresponding regression equation.

3. Results and Discussion

3.1 Determination of compound classes

Phenolic test results of ethyl acetate fraction showed positive reaction of phenol compounds with greenish yellow color. Test results of flavonoid ethyl acetate fraction showed a positive reaction to the presence of flavonoid compounds. The intense color intensity indicates the quantity of the compound class [7]. Phytochemical test results of ethyl acetate fraction are shown in Fig 1.

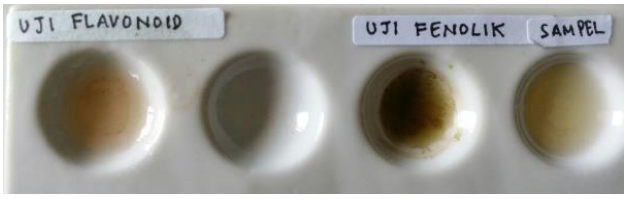

Figure 1. Phytochemical test results of flavonoid and phenolic groups on ethyl acetate fraction

Phytochemical test results showed a more intense of color intensity in the phenolic group compared to the flavonoid group. This indicates the quantity of phenolic compounds more than the flavonoid compounds.

3.2 Characterization of compound structure

The secondary metabolite compound (isolate F2.8) was analyzed using IR and UV-VIS spectrophotometer. The IR and UV-Vis spectrum data are shown in Table 1, Figure 2 and Figure 3.

Tabel 1. IR spectra data of isolate F2.8

Wavelength (cm^{-1})	Peak shape	Intensiy	Related group placement
3430	wide	Strong	OH stretch out
2925-2855	Sharp	Medium	C-H aliphatic, stretch out
1749	Sharp	Strong	C=O
1609-1512	Sharp	Weak	Aromatic ring
1171	Sharp	Strong	C-O-C stretch out
1100	Sharp	Medium	C-O-C

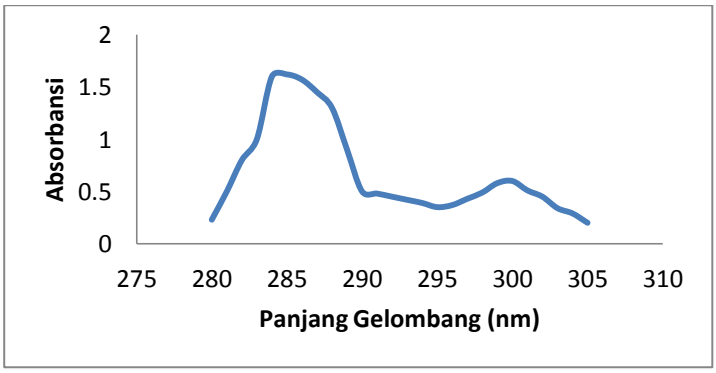

Figure 2. The wavelength curve of isolate F2.8

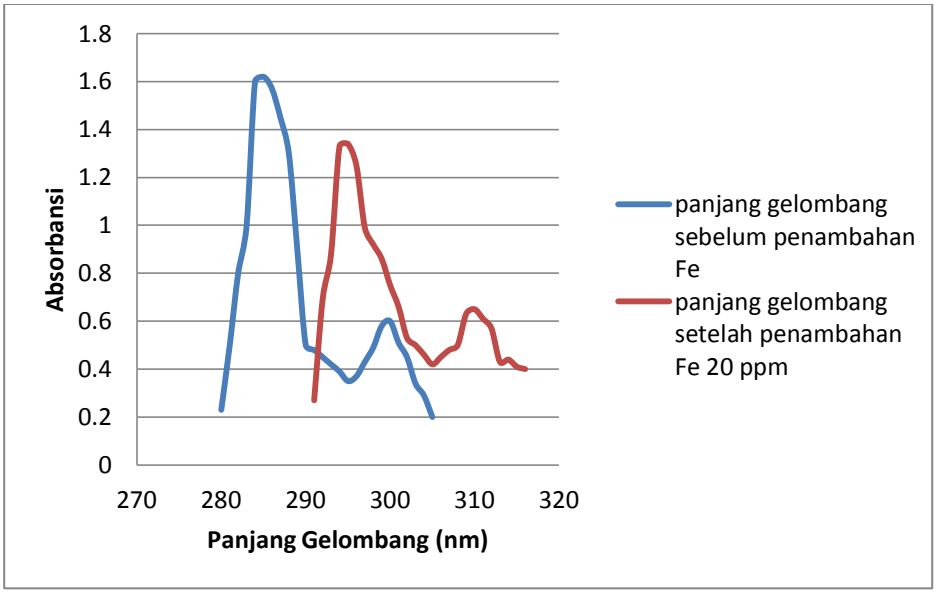

Figure 3. The wavelength curve of isolate F2.8 after addition of 20 ppm of $FeCl_2$

Based on infrared spectra data, it can be seen that the spectra pattern of isolate F2.8 compound showed a wide uptake at 3430 cm^{-1} which is thought to be the stretch absorption of the -OH group. This absorption indicates that the isolate F2.8 contains the -OH group. Uptake at wave number 2925-2855 cm^{-1} with sharp band shape and medium intensity is an aliphatic C-H stretch. Sharp uptake with strong intensity at wave number 1749cm^{-1} shows the presence of carbonyl. Uptake at wave number 1609-1512 cm^{-1} with sharp band shape and weak intensity shows the presence of C = C (aromatic ring). At wave number 1171 cm-1 shows absorption of stretched C-O-C groups. Meanwhile, the presence of C-O-C groups is shown by absorption at wave number 1100 cm^{-1}. Therefore, isolate F2.8 can be indicated as flavonoid compound.

The results of the spectrum in Figure 2 and Figure 3. show a shift at λ_{max} towards a larger direction with a batochromic shift which causes a decrease in absorbance after the addition of iron (Fe^{2+}) 20 ppm at λ_{max} 285nm and 305nm with absorbance respectively in sequence 1.62 A and 0.42 A. Batochromic shift is the maximum absorption shift to a larger wavelength. This is caused by the extension of conjugation (electron delocalisation π) from the structure of the compound caused by solvents, shifting and auxochrome reagents. larger wavelength shifts are caused by shifting reagents, namely Fe^{2+} metal, so that the transition energy will be smaller so the wavelength is greater which causes a decrease in absorbance [8].

Shifts in wavelength and absorbance indicate the formation of complex compounds by isolate F2.8. Complex compound is compound that is composed of central atoms or metals and the ligands that surround them form neutral molecules or ions with covalent coordination bonds [9] . Isolate F2.8 was allegedly a ligand in the process of neglect. According literature, flavonoids and phenolic compounds are bidentate ligands [10] . Bidentate ligand is a ligand coordinated with metal ions through two atoms. Phenolic components and flavonoids have hydroxyl and carboxyl groups that are capable of chelating metal ions [11] . The iron ion in this reaction acts as a central atom. Complex compound can be formed by ligands and central atoms, caused by the occurrence of coordination bonds between iron ions which have empty orbitals with ligands that have unpaired electrons [12] . Therefore, the easier of ligand donates electrons to the empty metal iron orbitals, the greater of value of the iron ion chelating.

Secondary metabolite compound (isolate F2.8) was analyzed by using a proton core magnetic resonance spectrometer (^{1}H-NMR). The ^{1}H-NMR spectrum data of isolate F2.8 are shown in Table 2.

Table 2. ^{1}H-NMR spectrum of isolate F2.8

Position	^{1}H-NMR δ (ppm), *J* (Hz)
3	6,85 (1H, s)
6	6,29 (IH, s)
8	6,57 (1H, s)
3'/5'	7,03 (2H, d, *J* = 11,96)
2'/6'	8,03 (2H, d, *J* = 11,72)

The results of the ^{1}H-NMR spectrum analysis in Table 2 show chemical shift (δ_H) 6.85; 6,29; 6.57 (ppm) with singlet signal respectively, δH 7.03; 8.03 ppm with a doublet signal respectively which is characteristic of the aromatic group. The chemical shift are a feature of the framework of flavonoid [13]. A pair signal of ortho δ_H 7.03 and 8.03 ppm signals are a feature of the benzene proton signal commonly found in flavonoid B ring. The singlet signal δ_H 6.29 and 6.57 ppm are aromatic proton signal of the ring A. The chemical shift 6.85 ppm is the proton located close to the carbonyl group on the C ring.

^{1}H-NMR spectrum data isolate F2.8 at δ_H 7.03 ppm and 8.03 ppm with doublet signals show the presence of protons interacting in an aromatic group. This is supported by the magnitude of the coupling at 7.03 ppm as big as *J* 11.96 Hz which is the ortho coupling, at the chemical shift 7.14 ppm as *J* 11.72 Hz which is the ortho coupling. The signals that appear on the ^{1}H-NMR spectrum data in isolate F2.8 is predicted to be a characteristic of flavonoid compound which is located in chemical shift sp^2. Based on this arguments, the compound of isolate F2.8 is thought to be an apigenin compound.

The predicted signal which is characteristic of apigenin compound is that there are seven chemical shifts for the position of the aromatic proton. When compared with the structure of apigenin compound from *Lawsonia inermis* [14], *Chrysanthemum morifolim* [13] and *Desmostachya bipinnata* Stapf [15] , there is a chemical shift similar to compound from isolate F2.8. Following is the comparison of the proton chemical shift (δ_H) of apigenin compound from *Lawsonia inermis*, *Chrysanthemum morifolim* and *Desmostachya bipinnata* Stapf with isolate F2.8..

Table 3. Spectra ^{1}H-NMR of Apigenin from *Lawsonia inermis*, *Chrysanthemum morifolim*, *Desmostachya bipinnata* Stapf and isolate F2.8

H	Chemical Shifts (δ ppm)			
	apigenin (I)	apigenin (II)	apigenin (III)	Isolate F2.8
3	6,79	6,72	6,79	6,85
6	6,19	6,12	6,20	6,29
8	6,46	6,41	6,49	6,57
3'/5'	6,91	6,95	6,90	7,03
2'/6'	7,90	7,96	7,90	8,03

Note:

(I) = ^{1}H NMR (400 MHz, DMSO-d_6) of *Lawsonia inermis* [14]

(II) = ^{1}H NMR (400 MHz, DMSO-d_6) of *Chrysanthemum morifolim* [13]

(III) = ^{1}H NMR (400 MHz, DMSO-d_6) of *Desmostachya bipinnata* Stapf [15]

Based on the data in Table 3, the chemical shift value possessed by isolate F2.8 is relatively the same as the chemical shift of apigenin compound from *Lawsonia inermis*, *Chrysanthemum morifolim* and *Desmostachya bipinnata* Stapf. The similarity of the chemical shift value of isolate F2.8 with chemical friction which is characteristic of apigenin compound is at the position of protons 3 '/ 5' and 2 '/ 6'. Then also seen the chemical shifts at the position of the aromatic protons which are relatively the same as the position of the aromatic proton isolate F2.8

4. Conclusions

Based on the results of the research that has been done, it can be concluded that the isolate F2.8 is relatively pure. Data on wave number isolates of isolate F2.8 were 3430; 2925-2855; 1749; 1609-1512; 1171 and 1100 cm^{-1}. UV-Vis spectrum data of isolate F2.8 after addition of Fe^{2+} showed a shift at λ_{max} towards a greater direction with a batochromic shift which caused a decrease in absorbance after the addition of iron (Fe^{2+}) 20 ppm at λmax 285 nm and 305 nm with absorbancerespectively 1.62 A and 0.42 A. Based on the comparison of the chemical shifts of protons with the spectrum of apigenin compounds shows that the compound isolate F2.8 is an apigenin compound capable of complexing Fe^{2+} metal.

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