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TheCharacterization of Active Compound of Pedada Magrove Plants (*Sonneratia caseolaris*)

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Abstract. The present research aimedto isolate the compound from the acetone fractions of pedada leaves (*Sonneratiacaseolaris*) and test its antioxidant activity. The extraction wasperformed usinga multi-stage maceration method. The separation and purification were done using a vacuum and gravitational column chromatography whereasthe antioxidant activity was tested with theDPPH method. The structural elucidation of the compound was performed by spectroscopy data of UV, IR, alsoone- and two-dimensional NMR. The appearance of the pure isolate was odorless yellow crystal. Based on the spectroscopy data, the compound wasstigmasta-5,22-dien-3-ol. The activity testing of the extract antioxidant and pure isolate withIC50showed a value of 166.2 ppm dan 134.4 ppm, respectively.

Keywords:pedada (*Sonneratia caseolaris*), isolation, antioxidant, DPPH

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1 Introduction

Antioxidant compounds are compounds which can protect the body from free radical effects. Free radicals are highly reactive molecules which can disrupt cell integrityand canreact with the cell structure components (e.g. membrane-constituent molecules) and functional components (e.g. enzymes, DNA). Free radicals in the body may cause various degenerative diseases, such as cancer, atherosclerosis, rheumatoid, arthritis, diabetes, decreased immune response system, and the aging process (Stojanovic et al., 2001). The sources of antioxidant compounds can be either synthetic or natural. One plant which has the potential as a source of natural antioxidant is pedada plants (*Soneratia caseolaris*).

Several previous studies about the content on the plants revealed that ethanol extract from pedada leaves contains tannin and phenolic compounds, has antinociceptive activity and acts as an antioxidant (Sariful et al., 2012). Minqing et al.(2009) in their study found that traditionally

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pedada fruit extracts can be used as an antiseptic,treat sprains, and prevent bleeding. Another research by Vinny (2015) successfully extracted the skin of pedada fruits using methanol solvent which acted as an antioxidant. Moreover, the results of previous studies on antioxidant testing with the DPPH method showed that the percentage of inhibition of ethyl acetate extract was 70.39% (Latief et al., 2014). However, information about secondary metabolite compounds from the acetone fractions of pedada leaves has not beenknown yet. In fact, acetone solvent is highly potential for extracting semipolar compounds. Moreover, this compoundalso potentially acts as an antioxidant.

Based on those previous studies, the possibility to obtain an active antioxidant compound from the acetone fractions is quite prospective. The testing of antioxidant activity in this study was conducted using free radical scavenging method named DPPH (1,1-diphenyl-2-picrylhydrazyl). The method was selected because it is simple, quick, and easy to perform screening radical trapping activities of some compounds. In addition, the method has also been proven to be accurate and practical.

2. Materials and Methods

2.1. General

The tools used in this research were glassware commonly used in a chemical laboratory, analytical scales, rotary evaporator, TLC chamber, aluminum foil, Cary 100 UV-Visible spectrophotometer.

The materials were technical methanol, technical n-hexane, technical ethyl acetate, chloroform, dichloromethane, TLC-Silica gel Merck Kieselgel 60 GF254plate, and 0.25 mm . The technical solvent used in the research was distilled beforehand. The materials for the testing of antioxidant activity weremethanol p.a, DPPH, 75% methanol.

2.2. Plant Materials

A total of 5 kg pedada leaves (*S. caseolaris*) was collected from the mangrove forest area in Tanjung Jabung Timur Regency, Jambi Province. The leaves were cleaned, cut into small pieces, dried untilits weight was constant, and finely ground.

2.3. Extraction

The 5 kg of pedada leaves were finely chopped and dried. After that, the dried leaves were macerated in several stages twice with n-hexane, acetone, and ethanol. Each maceration result was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator to obtain the condensed extract of n-hexane, acetone, and ethanol of pedada leaves.

2.4. Phytochemical Screening (Harborne, 1987)

Alkaloid Test.A number of samples were dissolved in a few drops of 2N sulfuric acid, and then the samples were tested with three alkaloid reagents, namely Dragendorff reagent, Meyer reagent, and Wagner reagent. The test result was positive if yellowish white sediment was formed usingthe Meyer reagent, brown sediment was formed usingthe Wagner reagent, and red to orange sediment was formed usingthe Dragendorff reagent.

Flavonoid Test. Flavonoid test was conducted with two kinds of reagents, namely concentrated HCl with Mg powder and 10% NaOH. A few drops of concentrated HCl were added into thesamples, and then the Mg powder was also added. The positive results of HCl reagent and Mg powder were shown by the formation of foam and the changes in the color of the solution to orange. In the case of 10% NaOH reagents, a few drops of 10% NaOH reagent were added into the samples, and the positive indicator in the flavonoid test was shown bythe formation of yellow, red, brown, or green .

Saponin Test. Saponin can be detected by testing the foam in hot water. The presence of saponin was shown if there was stable foam for 30 minutes and it did not disappear if one drop of 2N HClwas added.

Tanin Test.FeCl3 was added to a number of samples, and then the mixture was homogenized. The positive reaction was indicated by the formation of red color in the mixture.

Steroid and Triterpenoid Test. Anhydrous acetic acid and concentrated sulfuric acid (Liebermann-Burchard reagent) were added to the samples. If purple or red color formed was transformed into purple-blue or green-blue color, it indicated the presence of steroid/triterpenoid (Harborne, 1987).

2.5. Antioxidant Activity Test

Antioxidant activity test was conducted using the DPPH method. In this test, a series of sample solutions from concentrated acetone extract was made with various concentrations, such as 50, 100, 200, 300, 400, and 500 ppm using methanol solvent. 10 mg of sample was weighed and dissolved in 10 mL of methanol p.a in a 10 mL of volumetric flask so that a concentration of 1 mg/mL was obtained. In order to determine the antioxidant activity, 0.2 mL of the sample solution was pipetted with a micropipette into the vial, and then 3.8 mL of 20 ppm DPPH solution was added to the sample. The solution mixture was homogenized and left for 30 minutes in a dark place. The absorption was measured by a UV-Vis spectrophotometer at 517 nm wavelength. For positive control, ascorbic acid was used with the same treatment as in the sample.

2.6. Isolation and Purification of Active Compounds

A total of 20 grams of acetone extract of pedada leaves were fractionated by column chromatography. Afterward, the acetone extract of pedada leaves was impregnated using 15 grams of silica gel until it became powder, inserted into a chromatographic column, and flowed with eluent. The phase of motion used was n-hexane-ethyl acetate solvent with various comparisons . The fractions obtained were stored in the vials. After dried, all fractions were analyzed using a thin layer chromatography (TLC). The fractions which had identical stain patterns were combined based on the similarity of Rf values in the chromatogram. The fractionation was performed again until the pure isolate was obtained.

2.7. The Characterization of Pure Compounds

Against the pure compound, the molecular structure was determined using the spectroscopy methods including IR, and NMR 1H, 13C, and two-dimensional NMR.

2.8. Data Analysis

Data analysis for the phytochemical screening test and the antioxidant test was conducted descriptively.

2.9. The calculation of IC50values.

Antioxidant activity expressed by Inhibition Concentration 50% (IC50) is the concentration of the sample that can reduce the DPPH radical by 50%. The value of IC50 is calculated based on the percentage of inhibition on DPPH radical from each concentration of sample solution with the following formula:

$$Inhibition\ (\%) = \frac{Absorbance\ of control - Absorbance\ of\ sample}{Absorbance\ of\ control}\ x\ 100\ \% \qquad (1)$$

In which:

A control = Absorbancedid not contain samples

A sample = Absorbance of samples

Furthermore, the calculation results were entered into a regression equation with the concentration of extract (ppm) as the abscissa (x-axis) and the value of inhibition in percentage (antioxidant) as the ordinate (y-axis). The IC50 value from the calculation when the percentage of inhibition was 50%. y = ax + b. The determination results of antioxidant activity were compared with the antioxidant activity of vitamin C that has been tested.

3. Results and Discussion

3.1. Extraction of Sonneratia Caseolarisleaf powder

The sample extraction was performed by the maceration technique in stages using n-hexane, acetone, and ethanol solvents to separate nonpolar, semipolar, and polar components. After each extraction result was obtained, the percentage of its rendemen was calculated as presented in **Table 1**.

Table 1. The percentage of rendemen from the maceration results of n-hexane, acetone, and ethanol.

No.	Solvent	Part used	The weight of the extracted powder (Kg)	The weight of condensed extract (gram)	Rendemen %
1	*n*-hexane	Leaf	5	10.400	0.208 %
2	Acetone	Leaf	5	263.563	5.271 %
3	Ethanol	Leaf	5	63.240	1.265 %

Table 1 shows that the highest percentage of rendemen among the three solvents was obtained with acetone extracts with 5.271%. It indicates that pedada leaves is soluble in the acetone solvent and contain many semipolar chemical components rather than nonpolar or polar compounds.

3.2. Separation and purification of compounds from the acetone fraction of Pedada leaves (Sonneratia caseolaris)

The separation and purification of the acetone extract of Sonneratia caseolaris leaf were done by vacuum liquid chromatography method. Based on the VLC results, three combined fractions were obtained namely F1, F2, and F3. Fraction 3 with 1.54 grams was dikolom using eluent with a graded degree of polarity. Seven combined fractions were obtained (F 7.1. to F 7.2). The Combined Fraction 2 (F 7.2) shows a single stain pattern (one) with anRf value of 0.67. Moreover, F 7.2 in TLC returned with different eluent variations (100% n-hexane, n-hexane: dichloromethane (DCM) and ethyl acetate: dichloromethane (DCM)

3.3. Antioxidant Activity Test on Acetone Extract of Sonneratia CaseolarisLeaf

The results of antioxidant activity test of the crude acetone extract from Sonneratia caseolaris leaf and vitamin C are presented in Table 2. Based on Table 2, the acetone extract of Sonneratia caseolaris leaf had a higher IC50 value (166.2 ppm) than the IC50 value of vitamin C (20.5 ppm). On the other hand, the IC50 value of the pure isolate was 134.4 ppm, and its activity was classified as moderate. Molyneux (2004) states that if the IC50 value is more than 150 ppm, it is classified as a weak antioxidant.

The small antioxidant activity in Sonneratia caseolaris leaf,when compared with vitamin C, is because the acetone extract of Sonneratia caseolaris leaf is still a mixture of several compounds,

such as alkaloids, flavonoids, tannins, phenolics, and terpenoids/steroids. In contrast, vitamin C is a pure synthesis of compounds which have the potential as antioxidants. On the other hand, the activity of pure isolate is strongly related to the molecular structure of the compounds obtained.

Table 2. The results of antioxidant activity test on the crude acetone extract of sonneratia caseolaris leaf and ascorbic acid (Vitamin C)

Sample	Concentration	Absorbance	%Inhibition	Regression	IC_{50}
Control		0.8146			
Acetone	50 ppm	0.6024	26.05	y = 0.0016x	166.2
extract	100 ppm	0.5082	37.61	+ 0.2341	
DPPH of	200 ppm	0.3666	55.00		
Sonneratia	300 ppm	0.1552	80.95		
caseolaris	400 ppm	0.0681	91.64		
leaves	500 ppm	0.0752	90.77	y = 0.0019x	171
				+ 0.1746	
Ethanol	50 ppm				
extract of	100 ppm	0.5923	27.29%		
Sonneratia	200 ppm	0.4897	39.88%		
caseolaris	300 ppm	0.2395	70.60%		
leaves	400 ppm	0.1367	83.22%		
	500 ppm	0.0557	93.16%		
		0.0331	95.94%		
Vitamin C	5 ppm	0.6376	21.70	y = 0.0171x	20.5
	10 ppm	0.5517	32.27	+ 0.1491	
	20 ppm	0.4001	50.88		
	30 ppm	0.2888	64.54		
	40 ppm	0.0950	88.33		
	50 ppm	0.0254	96.88		

3.4. Determination of Molecular Structure of Pure Isolate

The pure isolate was yellow-shaped crystals obtained from the isolation process. The phytochemical test results showed positive for steroids and phenolic. The characterization using spectroscopy UV-Vis, FTIR, ^{1}H and ^{13}C NMR, and two-dimensional NMR.

3.5. Analysis with FTIR Spectrophotometry

The FTIR spectrum of the isolate can be seen in **Fig. 3**.

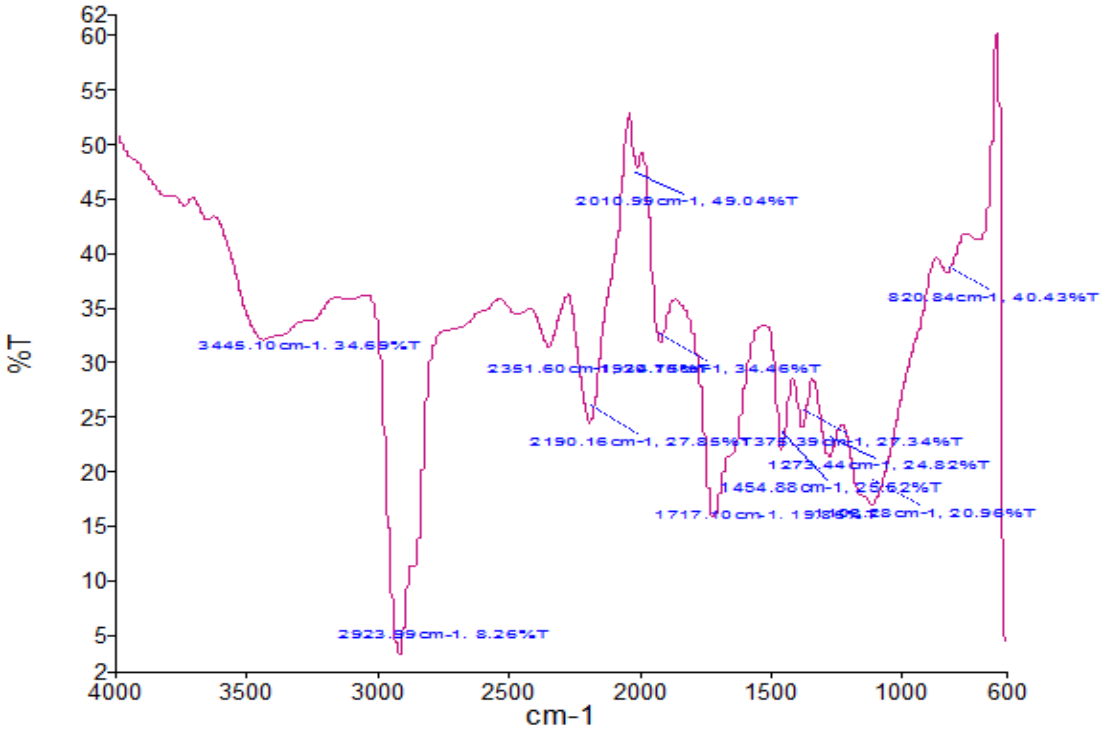

Figure 3.The FTIRSpectrum of The Isolate of *Sonneratiacaseolaris* Leaf

Based on the FTIR analysis shown by the spectrum in **Fig. 3**, the pure isolate of sonneratia caseolarisleaf may show the presence of hydroxyl (OH) groups. This is shown by the presence of widespread absorption at the 3445.10 cm-1wavenumber region. The presence of sharp bands with moderate intensity at the 2923.88 cm-1wavenumber region represents an aliphatic C-H cluster.

At1375.39 wavenumbershows the presence of CH_3 group whereas the absorption at 1717.10cm-1wavenumbershows the presence of carbonyl group in the isolate. After that, the absorption at the 1454.88 cm-1wavenumber regionwas the absorption caused by the vibration of C=C bond which indicates that the isolated compound was an aromatic compound. The absorption at the wavenumber 1273.44 – 1108.88 indicates a C-O stretch. This is also strengthened by the absorption in820.84 cm-1wavenumberindicating the presence of the C=C-H bending group.

3.6. Analysis with 1H-NMR Spectrophotometry

Analysis with 1H NMR (CDCl3; 500 MHz; δ ppm) as in Fig. 1 shows the presence of six signals of methyl groups in which each signal appears at δH: 0.72, 0.83, 0.84, 0.86, 0.96,and 1.02 ppm.

Figure4. The spectrum expansion of[1]H-NMR pedada isolate pedadafor proton at chemistry0.7-1.4 ppm ($(CD_3)_2CO$, 500 MHz)

3.7. Analysis with 13C-NMR 1 andtwo-dimensionalspectrophotometry

The analysis with carbon atom resonance spectroscopy ^{13}C-NMR ($(CD_3)_2CO$, 125 MHz, □ ppm) as seen in **Fig. 2** shows 29 carbon signals comprising of 6 methyl carbon, 11 methylene carbon, 9 methyl carbon, and 3 quarternary carbon atoms.

The signals in ^{13}C: 12.3 and 19.9 ppm were for methyl groups bound to the steroid base order whereas the signals at ^{13}C: 12.4, 19.3, 19.4, and 20.2 ppm were signals for methyl groups bound to the side chain. Furthermore, a chemical shift of 71.8 ppm was the signal for C which was bound to the OH group whereas signals at a chemical shift of 121.7 and 142.5 ppm were for C sp2.

The two-dimensional NMR spectrum is important to see the correlation of proton with proton and carbon, such as HSQC technique (Heteronuclear Single Quantum Correlation) and HMBC technique (Heteronuclear Multiple Bond Connectivity). The HSQC spectrum in Fig.5 strengthens the analysis of the relationship between protons at a chemical shift of 0.72 ppm with carbon to carbon at ^{13}C: 12.3 ppm. Furthermore, the proton relationship at ^{1}H: 5.31 ppm and ^{13}C: 12.7 ppm is shown in **Fig.6.**

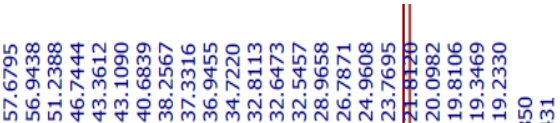

Figure5.The spectrum of ^{13}C NMR pedada isolate($(CD_3)_2CO$, 125 MHz)

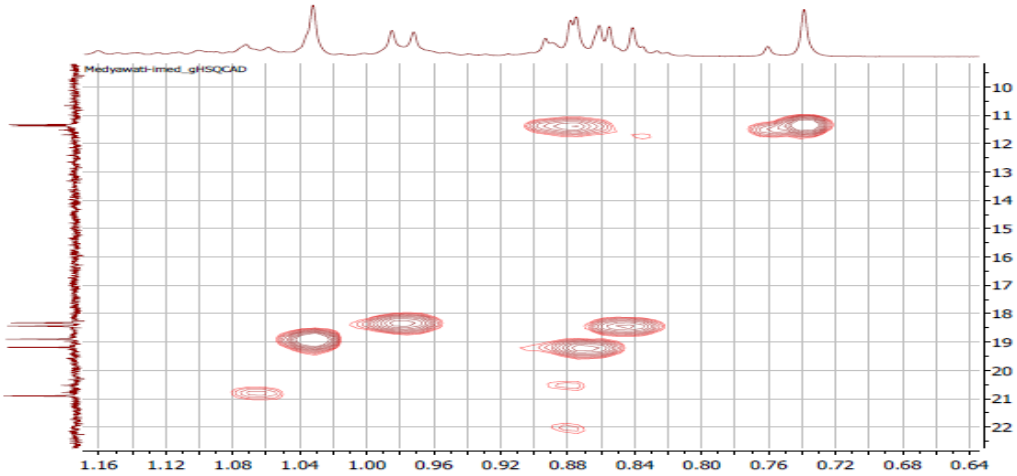

Figure6.The HSQC spectrum of pedada isolate for proton at the chemical shift ofδH0.72 – 1.08 ppm ($(CD_3)_2CO$, ^{1}H-500 MHz, ^{13}C-125 MHz)

The two-dimensional spectrum of HMBC shown in Fig.4 and Fig. 5 shows the relationship of proton and carbon with a distance of 2 to 3 bonds. At -^{13}C: 19.4 ppm, it had a correlation with HC: 0.86 ppm.

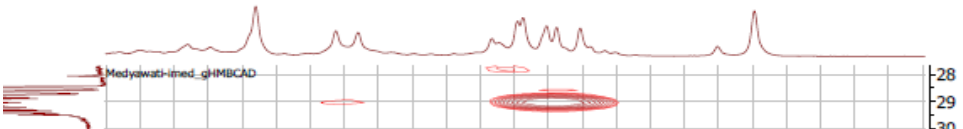

Figure7.The HMBC spectrum of pedada isolatefor the correlation of proton at the chemical shiftof 0.72-1.08 ppm with a carbon at thechemical shiftof 28 – 47 ppm ((CD3)2CO, 1H-500 MHz, 13C-125 MHz)

Based on infrared, ultraviolet, and NMR spectroscopy data, and after being compared with the physical properties data and ^{1}H dan ^{13}C NMR data of the same compound from the literature, the isolated compound from the acetone extract of pedada was stigmasta-5.22-dien-3-ol.The image of the molecular structure of the pure pedada isolate is presented in **Fig. 8**.

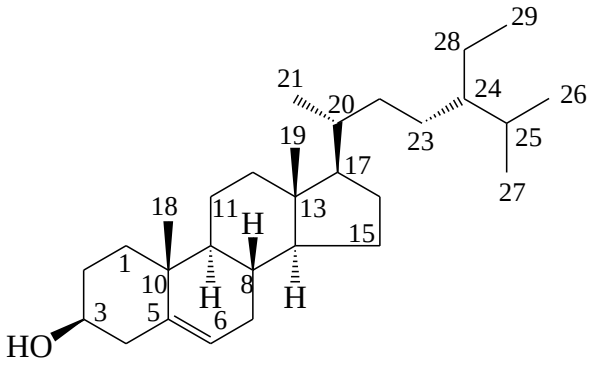

Figure8.The molecular structure of pedada isolate compounds (stigmasta-5-ene--3-ol)

4. Conclusion

Based on the present research, it can be concluded that:

1. The group of chemical compounds contained in the extract of the acetone fractions of pedada leaves include alkaloid, flavonoid, tannins, phenolic, steroid, and triterpenoid compounds.

2. Based on the characterization results, the chemical compound isolated from the acetone fractions was stigmasta-5-ene--3-ol.

3. The IC50value of the extract of the acetone fractions was 166.2 ppm which was classified as a weak activity whereas the IC50value of the pure isolate was 134.4 ppmwhich was classified as a moderate activity for isolates.

Acknowledgement

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