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ISOLATION AND CHARACTERIZATION OF FLAVONOID DERIVATIVE OF ETHYL ACETATE EXTRACT FROM *Bauhinia latisiliqua* STEM BARK AND ITS ACTIVITY AS ANTIOXIDANT

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Abstract

Various bioactivities on natural compounds, the antioxidant potency can provide a broad spectrum in their utilization and development into medicinal raw material. One of the plants that have potency for antioxidant activity is the Kemerakan sapenit (*Bauhinia latisilqua*) plant. The study aimed to isolate, characterize, and determine the antioxidant activity of a compound isolated in the ethyl acetate extract of the stem bark of *B. latisilqua*. This research includes extraction, fractionation, purification, and elucidation of secondary metabolite structure as well as testing its antioxidant activity. Extraction was carried out by maceration technique, fractionation, and purification using liquid vacuum chromatography and gravitation column chromatography. The elucidation of the structural compound was determined by analysis of the UV, IR, and NMR spectra. The antioxidant test was performed using the DPPH method. Based on the analysis of the spectral data, the isolated compound was catechin, a derivative of flavan-3-ol. Antioxidant test on catechin compound showed a strong antioxidant with an IC_{50} value of 35.01 µg /mL (IC_{50} for positive control, ascorbic acid, of 42.94 µg /mL).

INTRODUCTION

Flavonoids are found in almost all higher plants and are primarily known for their antioxidant properties [1-3]. In addition, various bioactivity that have been investigated on flavonoid compounds include anti-histamine, anti-inflammatory, antibacterial, and antiviral and are thought to have a role in the prevention of neurodegenerative disease and cancer [1,2,4,5]. Due to the biological activity of flavonoid derivatives, exploration of the chemical content of numerous higher plants was continuously carried out. One of the higher plants estimated to be able to produce flavonoid compounds is *Bauhinia latisiliqua*.

In general, the genus *Bauhinia* consists of a separate class of secondary metabolites including flavonoids, coumarins, tannins, terpenoids, steroids, quinones, saponins, and alkaloids [6-12]. Isolated compound derivatives from *Bauhinia* exhibit a strong antioxidant activity such as 5,7,3',5'- tetrahydroxyflavanone with IC_{50} value of 6.33 µg/mL and bauhinia statin 4 with IC_{50} value of 32.7 µM [13,14].

Regarding the chemotaxonomic approach in the genus *Bauhinia*, plants in the same tribe contain organic compounds with the same skeleton so that it is possible to have the same potency for biological activity [15-26].

In addition, literature has been not found related to the containing of compound and biological activity in *B. latisiliqua* yet. So, it is necessary to isolate and characterize secondary metabolite and to test antioxidant activity in the ethyl acetate extract of *B. latisiliqua* stem bark through extraction, fractionation, and purification stages. UV-Vis, FTIR, and NMR were used to make decisions on the structural compound based on the analysis of spectral data.

EXPERIMENT

Gravitation column chromatography (GCC) and vacuum liquid chromatography (VLC) were conducted with Merck Si gel 60 (700-200 mesh) and Si gel 60 PF254. Analysis of Thin Layer Chromatography (TLC) was done on Merck kieselgel 60 GF254, precoated Si gel plates, with a thickness of 0.25 mm. This research utilized solvents that were already distilled and of analytical and technical grade.

Material

The stem bark of the B. latisiliqua plant was collected from Bogor Botanical Garden, Bogor, West Java. The plant was identified by the staff of the Bogoriense Herbarium, Indonesia Science Institute, Bogor, Indonesia

Gravity column chromatography (GCC) and vacuum liquid chromatography (VLC) were performed using Merck Si-gel 60 (700-200 mesh) and Si-gel 60 PF254. Thin layer chromatography (TLC) analysis was performed on Merck Kieselgel 60 GF254, pre-coated Si-gel plates with a thickness of 0.25 mm. Already distilled analytical and technical solvents were used in this study

Instrumentation

UV-Vis Varian Cary 100 Conc and FTIR One Perkin-Elmer spectrometers were used to analyze the maximum absorption and specific functional groups of the isolated compound. Using TMS as an internal standard, ^{1}H and ^{13}C NMR spectra were recorded on a JEOL J-500 spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C).

Procedure

Extraction and Isolation

The *B. latisiliqua* bark powder (2.2 kg) was extracted three times in methanol at room temperature. The use of a rotary vacuum evaporator was intended to remove solvent from the methanol extract. The crude extract was partitioned successively with *n*-hexane and ethyl acetate. About 15 g of concentrated ethyl acetate extract was fractionated using vacuum liquid chromatography (VLC) with a mixture of *n*-hexane: ethyl acetate eluent which was gradually increased in polarity. Based on the thin layer chromatography (TLC) test, the result of fractionation was grouped into two main fractions, namely fraction A and fraction B. By using a gravitation column chromatographic technique with a gradient mixture (n-hexane: ethyl acetate) eluent, fraction B was separated and purified repeatedly until a single compound was obtained. The single compound collected from fraction B was 27 mg.

Determination of Antioxidant Activity

The antioxidant activity test was performed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method [9]. To prepare a 1000 µg/mL solution, 3 mg of the isolated compound was dissolved in 3 mL of methanol and then prepared at serial concentrations (10, 30, 50, 70, 90 µg/mL). To test antioxidant activity, 0.2 mL of each concentration was placed in a test tube and 3.8 mL of 50 µM DPPH solution was added. The mixture was homogenized and left to stand for 30 minutes in a dark room, the absorbance of the mixture was measured with a UV-Vis spectrometer at a wavelength of 517 nm (sample A). The preparation of ascorbic acid as a positive control was treated in the same way as the test sample. The antioxidant activity of the sample was determined by calculating the percentage inhibition of DPPH radical absorption using the formula:

$$\% \text{ inhibition} = \frac{\text{A Blank} - \text{A sample}}{\text{A Blank}} \text{x} 100\%$$

The IC_{50} value of the test sample was calculated using a linear equation. A linear equation was obtained from the curve of the relationship between sample concentration and percent inhibition.

RESULT AND DISCUSSION

The process of isolation worked out on *B. latisiliqua* stem bark resulted amorphous brown solid of as many as 27 mg. The UV spectra of the isolated compound gave absorption at a maximum wavelength of 281 nm and the addition of NaOH reagent showed a batochromic shift of 8 nm from 281 to 289 nm. Conforming to the spectral pattern marked the presence of phenolic chromophore. As shown in **Figure 1a** and **Figure 1b**.

Characterization of Compound

This was also confirmed by FTIR spectra showing several specific functional groups such as the wide bands at a wave number 3400-3200 cm^{-1}

indicating stretching vibration of OH group and absorption bands at 1627-1523 cm^{-1} expressing presence of aromatic C=C vibration. Furthermore, the existence of absorption bands at 2929-2582 cm^{-1} indicates aliphatic CH vibration as well as some absorptions on the fingerprint region. As shown in **Figure 2**.

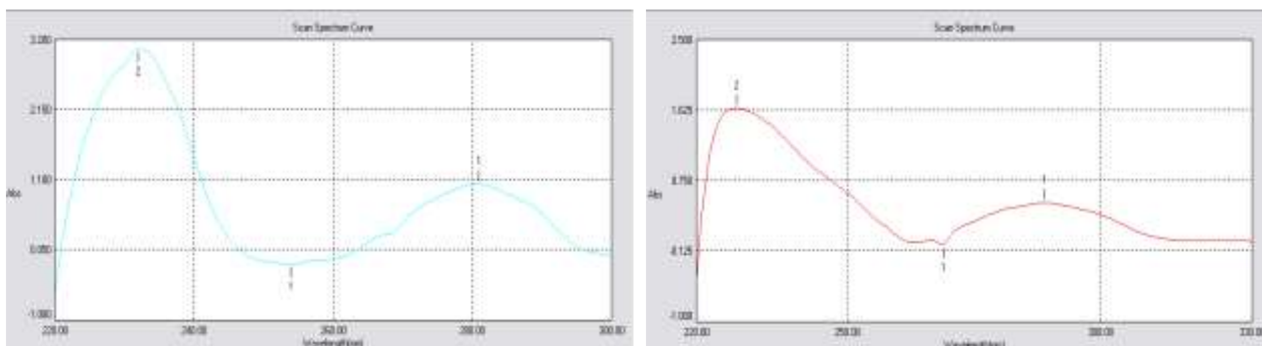

Figure 1. (a) UV-Vis (MeOH) spectra; (b) UV-Vis (MeOH+NaOH) spectra of the isolated compound.

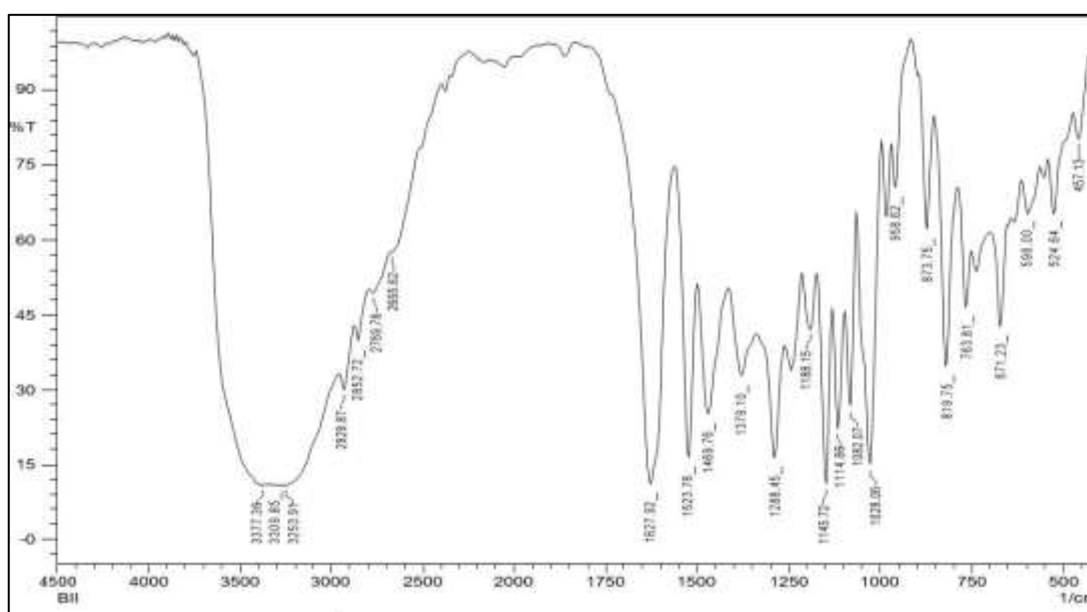

Figure 2. FTIR spectra of the isolated compound.

^{1}H-NMR (methanol-d4, 500 MHz) spectra showed aliphatic proton signals at δH ppm 2.50 (1H, dd, J = 16.35 & 8.3 Hz), 2.84 (1H, dd, J = 16.45 & 5.15 Hz), 3.97 (1H, m) and 4.56 (1H, d, J = 7.4 Hz) which were two protons H-4, H-3 and H-2 respectively, and these signals correlated to a flavan-3-ol derivate compound [28]. In addition, two aromatic protons oriented meta-coupling at δH ppm 5.86 (1H, d, J = 2.3 Hz) and 5.92 (1H, d, J = 2.3 Hz), for each protons H-8 and H-6 in aromatic ring A. These protons were a part of 1,2-disubstituted-3,5-dihydroxyphenyl system. The other three aromatic protons oriented orto-meta, orto and meta coupling at δH ppm 6.71 (1H, dd, J = 8.1 & 2.0 Hz), 6.76 (1H, d, J = 7.8 Hz), and 6.83 (1H, d, J = 2.0 Hz) represented H-6', H-5', and H-2' respectively in aromatic ring B. These hydrogen proton signals became section of 1-substituted-3,5-dihydroxyphenyl. Based on the ^{13}C-NMR (methanol-d4, 125 MHz) spectra, there were 15 carbon signals composed of five oxyaryl carbons at δC ppm 157.6 (C-5), 157.9 (C-7), 156.9 (C-8a), 146.2 (C-3'), and 146.3 (C-4'); two aromatic quarternaries at δ_C ppm 100.8 (C-4a) and 132.3 (C-1'); five aromatic methine at δ_C ppm 96.3 (C-6), 95.5 (C-8), 115.3 (C-2'), 116.1 (C-5'), and 120.0 (C-6'); two sp3 oxygenated methine at δ_C ppm 82.9 (C-2) and 68.8 (C-3); and one sp^3 methylene at δ_C ppm 28.5 (C-4). According to NMR spectral data and reference [28] the isolated compound was namely 5,7,3',4'-tetrahydroxyflavan-3-ol or catechin (Figure 3). On the other hand, the structural compound was also established by supporting Heteronuclear Multiple

Bond Correlation (HMBC) spectra observed via 2J-3J such as H-4a/C-4a, C-3; H-4b/C-4a, C-3, C-2; H-2/C-3, C-2’, C-6’. For further correlation was seen between H-6/C-5,C-7; H-8/C-4a and H- 2’/C-3’,C-4’; H-6’/C-2’,C-5’, H-5’/C-1’,C-4’,C-5’. Completely 1D and 2D NMR data of secondary metabolite was presented in **Table 1**.

Table 1. NMR spectroscopic data of isolated compound.

No	Isolated compound of *B. latisiliqua*		
	™C	™H (multiplisitas, *J* in Hz)	HMBC (^{1}H<=>^{13}C)
2	82.9	4.56 (*d*, 7.4)	C-3, C-2’, C-6’
3	68.8	3.97(*m*)	-
4	28.5	2.50 & 2.84 (*dd* & *dd*)	C-2, C-3, C-4a
4a	100.8	-	-
5	157.6	-	-
6	96.3	5.92 (*d*, 2.3)	C-5, C-7
7	157.8	-	-
8	95.5	5,85 (*d*, 2.3)	C-4a
8a	156.9	-	-
1’	132.3	-	-
2’	115.3	6,83 (*d*, 2.0)	C-3’, C-4’, C-6’
3’	146.2	-	-
4’	146.3	-	-
5’	116.1	6,76 (*d*, 8.0)	C-1’, C-3’, C-4’
6’	120.0	6,72 (*dd*, 8.3 & 2)	C-3’, C-4’,C-5’

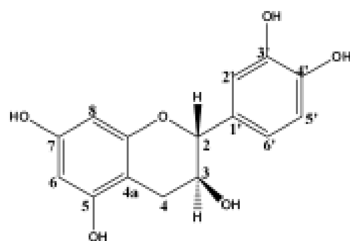

Figure 3. Catechin**,** the structural compound of *Bauhinia latisilqua* stem bark.

The NMR spectroscopy data of the isolated compound is also supported by NMR data for the same compound (catechin) which was previously isolated from *Artocapus reticulatus* which is shown in **Table 2** [28].

Table 2. NMR data for isolated compounds and reference compounds.

Isolated compound of *B. latisiliqua*			References (isolated compound of *Artocarpus reticulatus*)	
No	™C	™H (multiplicity, *J* in Hz)	™C	™H (multiplicity, *J* in Hz)
2	82.9	4.56 (*d*, 7.4)	82.88	4,56 (*d*, 7.5)
3	68.8	3.97(*m*)	68.83	3,97 (*m*)
4	28.5	2.50 & 2.84 (*dd* & *dd*)	28.53	2.49 & 2.48 (*dd* & *dd*)
4a	100.8	-	100.84	-
5	157.6	-	157.9	-
6	96.3	5.92 (*d*, 2.3)	96.31	5.91 (*d*, 2.4)
7	157.8	-	157.86	-
8	95.5	5,85 (*d*, 2.3)	95.52	5.84 (*d*. 2.4)
8a	156.9	-	156.93	-
1’	132.3	-	132.25	-
2’	115.3	6,83 (*d*, 2.0)	115.28	6.83 (*d*, 2.0)
3’	146.2	-	146.24	-
4’	146.3	-	146,17	-
5’	116.1	6,76 (*d*, 8.0)	116.10	6.75 (*d*, 8.0)
6’	120.0	6,72 (*dd*, 8.3 & 2)	120.04	6.71 (*dd*, 8.2 & 2.2)

Isolated compound, ^{1}H-NMR (methanol-d4, 500 MHz), ^{13}C-NMR (methanol-d4, 125 MHz)
References compound, ^{1}H-NMR(CD_3OD, 500 MHz), ^{13}C-NMR (CD_3OD, 125 MHz)

Antioxidant Activity

The antioxidant activity of the compound at different concentrations was evaluated using the DPPH method with ascorbic acid as a control positive. The antioxidant measurement of the test sample demonstrated the power of antioxidants which was indicated by decreasing DPPH radical absorption at each adding of concentration of the test sample. The curve of regression displayed a relation between the concentration of the sample and the percentage of inhibition can be shown in **Figure 4**.

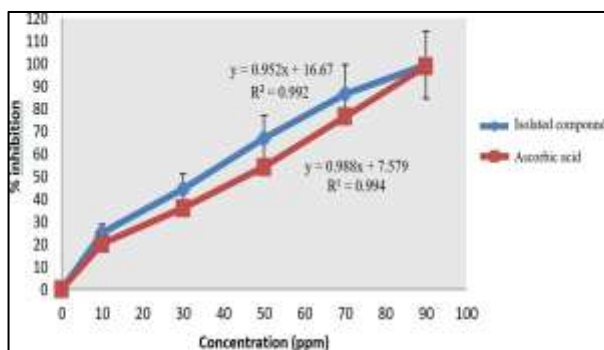

Figure 4. The curve of the relation between concentration and percentage of inhibition.

Refers to the regression equation, the IC_{50} value of the antioxidant activity of the isolated compound and ascorbic acid was 35.01 µg /ml and 42.94 µg /ml, respectively. The antioxidant power of the isolated compound showed a strong activity, it was proven that its IC_{50} value was better than ascorbic acid.

CONCLUSION

From ethyl acetate extract of *B. latisiliqua* stem bark had been isolated and identified a flavonoid derivative of the flavan group, namely 5,7,3',4'-tetrahydroxyflavan-3-ol or catechin. The antioxidant power of catechin showed a strong activity with an IC_{50} value of 35.01 µg /mL.

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