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Research Article

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Isolation and characterization of antioxidative constituent from stem bark extract of *Ceiba pentandra* L.

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ABSTRACT

Compound 5, 3'- dihydroxy-7, 4 ', 5'- trimethoxyisoflavon or Vavain has been isolated from ethyl acetate fraction of kapok (Ceiba pentandra L.) stem bark was conducted using column chromatography method. Structure elucidation performed using spectroscopy method (UV-Vis, IR, 1D and 2D NMR). This compound has potential as an antioxidant with IC_{50} value is 81.66 µg/mL and vitamin C as a standard of comparison using radical scavenging method.

Keywords: *Ceiba pentandra* L., isoflavone, total phenolic, antioxidant activity

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INTRODUCTION

Ceiba pentandra L grows in low areas up to 400 meters above sea level, in the garden, on the edge of the road, and other place that sultry [1]. *Ceiba pentandra* L. is a tropical tree that widely grown in Asia and a tree that flowers abort with trees 8-30 m tall and has a trunk large enough to reach a diameter of 3 m. Another name of the kapok namely: Randu (Indonesia), silk cotton tree (England), kapokier (France), kapokbaum (Germany), and ceiba (Spain) [2].

This species is also widely used in traditional medicine. In Asia, Oceania, Africa, and Central America it is used for a variety of disorders including diarrhea, fever, gonorrhea, as a diuretic and an emmollient, parasitic infections, and wounds .In the Samoan Islands, a cold water infusion of the bark of *Ceiba pentandra L* (vernacular name *vavae*) is used for asthma. The studies have shown antiinflammatory properties of the bark extract, both in vitro and in vivo, which can be related to several reports of traditional use in the treatment of ailments of an inflammatory nature such as asthma and cough [4]. And also the appreciable antioxidant effects of diet formulated with *Ceiba pentandra L* in the management of oxidative stress in diabetic condition has been demonstrated. Antioxidant activity of the phenolic extract of *Ceiba pentandra L* has also been established in a number of *in vitro* assays which include, DPPH, ABTS and metal chelation. There is great interest in phenolic compounds due to their effective free radical scavenging activity, which is related to their antioxidant potential.[5]

Compounds were reported to have been isolated from the bark of *Ceiba pentandra* L. namely vavain; flavan-3'-ol and vavain 3'-O-beta-D-glucoside with inhibition effect on prostaglandin biosynthesis cyclooxgenase-catalyzed [3], and 5-hydroxy 7,4',5'- trimethoxyisoflavon 3'-O-alpha-L-arabinofuranosil with as antidiabetic activity [6]. In this study was to determine the total phenolic content and to find active compounds as an antioxidant from the stem bark of *Ceiba pentandra L* extract.

EXPERIMENTAL SECTION

Plant material

The stem bark of *Ceiba pentandra* L. was collected from Padang sibusuk village, Sijunjung, Padang, West Sumatera, Indonesia at November 2014. It was identified in Herbarium of Andalas University (ANDA).

Chemicals

Ethyl acetate, n-hexane, methanol, gallic acid , sodium carbonat, silica gel 60 (0,063-0,200 mm), DMSO (dimethyl sulfoxide), Aquades, Follin, dan DPPH (1,1-diphenyl-2-picrylhydrazyl). All other chemicals used in this study were of analytical grade.

Instrumentation

Thermoscientific FTIR, Gallenkamp apparatus, Column chromatography, TLC, UV light 254 and 365 nm, Shimadzu® UV1700 spectrophotometer, 1D dan 2D NMR spectra were recorded on a JEOL spectrometer (500 MHz).

Procedure

1. Extraction

The dry stem bark of *Ceiba pentandra* L (4000 g) were powdered and macerated with n-hexane, ethyl acetate, and methanol at room temperature. Each extracts was combined and evaporated to obtain n-hexane (17.453 g), ethyl acetate (50.521 g) and methanol (98 g) extracts. Keep in refrigerator till further use.

2. Determination of Total Phenolic Content with Folin-Ciocalteu Method

Gallic acid standard created with a concentration of 20, 40, 60, 80, and 100 then its absorbance measured at a wavelength of 765 nm, then made a curve to obtain the linear regression equation Y = 0.015X - 0037. The phenolic content from extracts was determined following the method described by Prasith Kekuda T.R. *et al* [7] with slight modifications.

3. Antioxidant Test of Fraction Using The Radical Scavenging Method

The antioxidant activity of extract was conducted based on the radical scavenging method described by Okawa. *et al* [8] with slight modifications.

4. Isolation and Characterization

A total of 25 g of ethyl acetate fraction was subjected to column chromatography with 350 g of silica gel as the stationary phase. Furthermore, it was eluted using the eluent polarity increase to give 8 fractions (fraction A to H). The fraction F was continued to re-chromatography using eluent DCM (dichloromethane): n-hexane: ethyl acetate (7: 3: 0.5) with isocratic system to obtain compound 1. The purify of compound 1 was tested by melting point test, and elucidation of structure was carried out using UV-Vis, IR, 1D and 2D NMR.

5. Antioxidant Test of Isolated Compound

The compound 1 was continued to antioxidant test at a concentration of 0.15625, 0.3125, 0.625, 1.25, 2.5, 5 and 10 µg/mL by the same procedure for the antioxidant test of fraction above and Vitamin C was used as positive control.

RESULT AND DISCUSSION

The antioxidant activity from plants is often associated with the total phenolic content. Therefore, determination of total phenolic content was used to target antioxidant compound from stem bark extract of *Ceiba pentandra* L. The results of determination of total phenolic content fraction can be seen in Table 1.

Table 1. Results of determination of total phenolic content fraction of n-hexane, ethyl acetate, and methanol

No.	Fraction	Absorbance	(µgGAE/mL)
1.	n-Hexane	0.091	8.53
2.	Ethyl acetate	0.708	49.7
3.	Methanol	1.326	90.87

It can be seen that in each of fraction having different content of phenolic compounds. Wherein the phenolic compounds are believed to be scavengers of free radicals. Their antioxidant activity depends on their chemical structure; specifically, it depends on their ability to donate hydrogen or electron from the aromatic structure. The result of radical scavenging efficacy of fractions can be seen in Table 2. It was shown to exhibit dose dependent scavenging of DPPH free radicals as indicated by bleaching of radical solution colour (purple to yellow) on

increasing the concentration of its. However, the radical scavenging effect of reference antioxidant i.e., vitamin C was higher than of all.

Table 2. The results of IC_{50} fraction n-hexane, ethyl acetate, methanol, and Vitamin C

No.	Fraction	IC_{50} (µg/mL)
1.	n-Hexane	14732.67
2.	Ethyl acetate	2845.27
3.	Methanol	155.43
4.	Vitamin C	2.86

The relationship between total phenolic content and antioxidant activity of the fraction have relevance. It was expressed in a linier curve, where the determination coefficient is $r^2 = 0.882$ and the regression equation is y = -177.0x + 14710 can be seen from Figure 1. These results showed that 88.2 % of the antioxidant activity is the contribution of phenolic compounds [9].

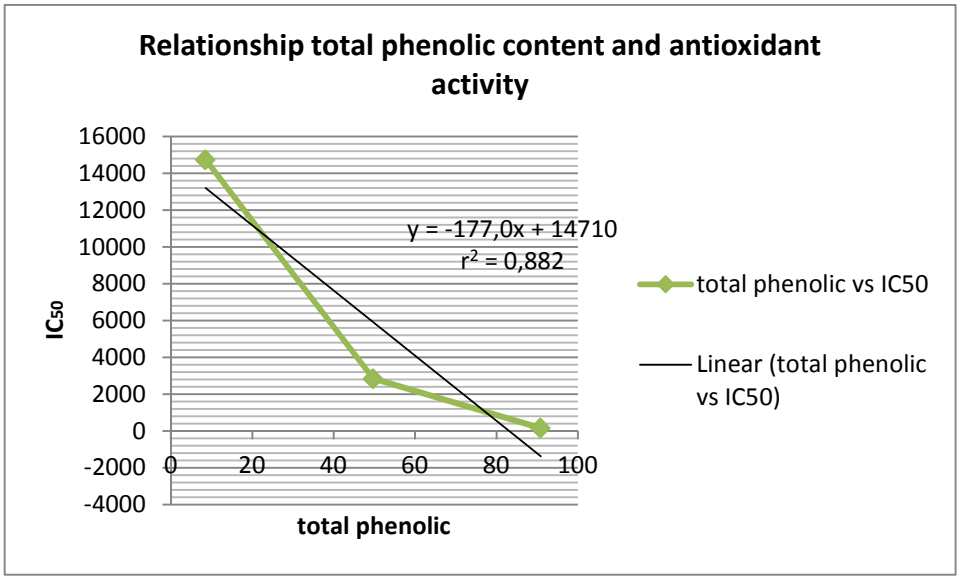

Figure 1. Relationship between total phenolic content and IC_{50} of fractions

The content of phenolic compound in ethyl acetate fraction gives opportunity to obtained active compound as an antioxidant. The purify of compound was tested by melting point test, and elucidation of structure was carried out using UV-Vis, IR, 1D and 2D NMR.

Isolated compound was obtained in the form of pale yellow amorphous powder as much as 12 mg; 162-163^o C; IR ν_{max} cm^{-1}: 3421.43 (OH), 1657.85 (C=O), 1197.65 (C-O ether), 1583.32 (C=C), 2935.04 (CH); UV (MeOH) λ_{max} nm: 262 nm, 330 nm. ^{1}H NMR (500 MHz, DMSO) 8.4643 (1H, s), 6.7314 to 6.7275 (1H, d), 6.6808 to 6.6730 (2H, t), 6.4266 to 6.4227 (1H, d), 12.9406 (1H, s, OH), 9.2871 (1H, s, OH), then 3.8700 (3H, s), 3.7909 (3H, s), and 3.6949 (3H, s) are a proton signal characteristic for a methoxy group [10]. ^{13}C NMR (500 MHz, DMSO) 155.2098, 122.4839, 180.1907, 161.7722, 98.1802, 165.3205, 92.5240, 157.4513, 105.4103, 125.9272, 110.3511, 150.2967, 136.4003, 152.9111, 104.4660, 56.1639, 55,8110, 59.9506. NMR data were comparison to those reported in the literature [3][6]. Compound 1 was identified as 5, 3'- dihydroxy-7, 4 ', 5'- trimethoxyisoflavon or Vavain (can be seen in Figure 2) and its an active compound as an antioxidant where the IC_{50} value is 81.66 µg/mL.

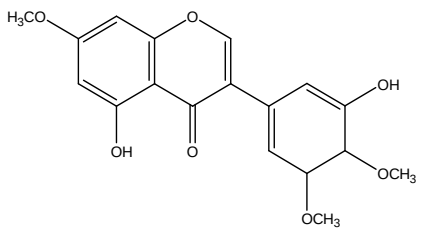

Figure 2. Structure of the compound 1

CONCLUSION

Compound 5,3'-dihydroxy-7,4',5'-trimethoxyisoflavon or Vavain was isolated by column chromatography and it was further confirmed by spectral characterization (UV-Vis, IR, 1D and 2D NMR). The isolated compound has potential as an antioxidant with the IC_{50} value is 81.66 µg/mL in DPPH assay.

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