# Isolation and Characterization of an Antioxidant Compound from Kayu Hitam Leaves (*Diospyros celebica* Bakh.F.)

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Keywords: Kayu Hitam (*Diospyros celebica* Bakh.F.), Isolation, Characterization, Methyl Gallate, Antioxidant.

Abstract: Isolation and characterization of an antioxidant compound from kayu hitam leaves (*Diospyros celebica* Bakh.F.) had been done by extraction and column chromatography method. Kayu hitam leaves powder was extracted with methanol and methanol extract reextracted with aquadest. Aqudest extract was partitioned with ethyl acetate and ethyl acetate extract repartitioned with n-hexane. The residues which are phenolic compounds were separated by column chromatography (SiO2, chloroform: methanol 90:10, 80:20, 70: 30,60: 40). The isolate obtained was purified with a preparative thin layer chromatography and obtained 9.5 mg of pure isolate in the form of yellow solid. characterization of pure isolate was determined by UV-Vis, FT-IR and 1H-NMR spectroscopic analysis. Based on the analysis carried out it can be characterized that the pure isolate obtained is methyl gallate. The antioxidant activity of methyl gallate was determined based on the DPPH free radical scavenging method. The activity of the methyl gallate was classified as strong with IC50 value of 4.41 µg / mL.

## 1 INTRODUCTION

Kayu hitam (*Diospyros celebica* Bakh.F.), classified as luxury wood species. Other names of kayu hitam in Indonesia including eboni, toetandu, sora, kayu lotong, kayu maitong, etc. (Prajadinata et al, 2011). Kayu hitam is endemic to Indonesia that distributes from Northern Sulawesi and Central Sulawesi to Southern (Larekeng, 2016). It is durable and strong wood, the heartwood with black and reddish brown stripes makes the texture very beautiful and widely used for luxury furniture, sculpture, carving, fan, statues, decorative tools, fancy veneer, musical instruments and ornaments (Prajadinata et al, 2011).

Sawdust from the processing of kayu hitam can function as a fungicide. At a concentration of 5% sawdust ethanol extract can cause a clear zone of 11 mm against the growth of Phytophthora palmivora Butler (Alwi et al., 2010) and Minimum Bactericidal Concentration (MBC) value of *S. aureus* and *E. coli* were 12% and 13% respectively (Wahyuni et al., 2018). This is due to, the sawdust extract contain chemical compounds such as tannins, saponins and terpenoids (Wahyuni et al., 2018). Ethanol extract of kayu hitam also had acute toxicity with LD50 value of 5.168 mg / kg against male mice (Mus musculus) (Syam, 2016. The toxicity of a plant depends on various factors, including quanti-consumed, time of exposure, different parts of the plant, individual chemistry, climate and soil, and genetic, species differences and strength of secondary metabolites (Mounanga et al, 2015).

Secondary metabolites are products of metabolism found in plants. Secondary metabolite compounds are divided into several parts, including phenolic compounds (Cheynier et al, 2013). Phenolics are characterized by having at least one aromatic ring with one or more hydroxyl groups attached (Crozier, et al., 2006). Phenolics are important components of the human diet due to their potential antioxidant activity and their capacity to diminish oxidative stress induced tissue damage resulted from chronic diseases (Khadem and Marles, 2010).

Antioxidants are compounds that neutralize chemically active products of metabolism, such as free radicals which damage the body. Sources of natural antioxidants are primarily phenolics that may occur in all products and parts of a plant such as fruits, vegetables, nuts, seeds, leaves, roots, and bark (Hajaji et al., 2010) and also in woody plants such as Toona sureni (Ekaprasada, et al., 2009 and in the Archidendron jiringa plants (Lubis, et al., 2018).



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Herein we report the isolation characterization of an antioxidant compound obtained from kayu hitam leaves and its antioxidant activity. Chemical structure was determined based on spectroscophy data interpretation and antioxidant activity based on scavenging activity of DPPH(1,1-diphenyl-2-picrylhydrazil) radical method and ascorbic acid was used as positive control. Isolation and characterization of an antioxidant compound obtained from kayu hitam leaves never been reported.

# 2 MATERIALS AND METHODS

## 2.1 Materials

Kayu hitam leaves were collected from the front yard of the Universitas Sumatera Utara, Medan, Sumatera Utara, Indonesia. Identification of plant was done at Herbarium Medanense (MEDA) Universitas Sumatera Utara. Silica (70 – 230 mesh, E-merck) for column chromatography, $FeCl_3$ 5%, chloroform (p.a E Merck), silica 60 F254 (E.Merck) for thin layer chromatography, TLC Preparative 60 F254, Benzene (p.a E Merck), Acetone (p.a Merck) methanol (p.a E Merck) and DPPH (Sigma Aldrich). Methanol as solvent was distilled before used.

## 2.2 Instrument

The $^1$H-NMR spectrum was recorded on a Agilent 2NMR 500MHz spectrometer instrument with CD3OD as a solvent and TMS as an internal standard and chemical shifts are given in δ (ppm). IR spectrum were recorded on FT-IR (Shimadzu), UV spectrum were recorded on Spectrophotometer UV-Vis (Hewlett Packard Agilent), solvent evaporation with rotary evaporator (Heidolph), monitoring sample spots with UV lights (254nm / 356nm, UVGL 58) and measuring antioxidant activity with a UV-Vis spectrophotometer (SP-300).

## 2.3 Procedure

### 2.3.1 Extraction and Isolation

This extraction and isolation were done based on Megawati, et al (2015) with a slight modification. The leaves powder of kayu hitam (1800 g) was macerated with 8L methanol for 2 x 24 hours. The macerate is collected, concentrated with a rotary evaporator and dried on a water bath. Methanol extract (209.63g) was dissolved with aquadest, the filtrate obtained were reextracted using ethyl acetate. The solvent in the ethyl acetate fraction is evaporated to obtained Ethyl acetate extract. Ethyl acetate extract (32.25 g) was dissolved with methanol and reextracted by using n-hexane. The methanol layer was dried using a rotary evaporator so that the dry methanol extract (12 g) was obtained. The phenolic compounds in the methanol extract were separated by using column chromatography using chloroform: methanol (100:0; 90:10, 80:20, 70:30, 60:40 (%v/%v). Isolates were collected in the vial every 10 mL and analyzed by TLC using chloroform: methanol 90:30. Each fraction with the same Rf value was c combined and evaporated. Fraction 38-92 (100 mg) at Rf 0.29 was purified by preparative TLC (Hostettmann et al., 1995) with chloroform: ethyl acetate 50:50 (% v /% v) and produced one band spot at the Rf 0.45. The band spot was crushed, eluted and tested with 5% $FeCl_3$, evaporated to obtain pure isolates 9.5 g in the form of yellow solid. The pure isolate was identification by UV-Vis, FT-IR and $^1$H-NMR analysis and antioxidant activity test.

### 2.3.2 Antioxidant Activity Test

Use Antioxidant activity test for pure isolate from kayu hitam leaves was done based on free radical scavenging method using DPPH (1,1-diphenyl-2-pikrylhydrazil) developed by Molyneux (2004) and Saranya et al., (2017). Samples and ascorbic acid were dissolved in methanol (p.a E Merck) with concentrations of 0.5, 10, 15 and 20 µg/mL. The inhibition percentage can be determined using equation formula (1) as follow:

$$\boldsymbol{inhibition\ percentage} = \frac{\boldsymbol{blank\ absorbance - sample\ absorbance}}{\boldsymbol{blank\ absorbance}} \boldsymbol{x100\%} \quad (1)$$

# 3 RESULTS AND DISCUSSION

## 3.1 Isolation and Characterization

Kayu hitam (Figure 1A) used in this study was the Ebenaceae family, a species of *Diospyros celebica* Bakh. F. with the local name kayu hitam. Pure isolate was isolated from kayu hitam leaves (Figure 1B) was phenolic compound, this was evidenced by the formation of black colloid on the addition of FeCl3 5%. The pure isolate is a yellow solid (Figure 1C). Identification of phenolic compounds was determined by UV-Vis, FT-IR and $^1$H-NMR spectroscopic analysis.

The UV-Visible (CH3OH) spectrum $\lambda_{max}$ 290 nm which is the length of the gallic acid group (Sujata, 2005) is shown in Figure 2.

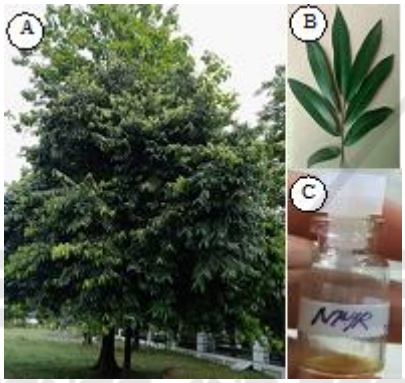


Figure 1: A Kayu hitam plant, 1B Kayu hitam leaf, 1C Pure isolate.

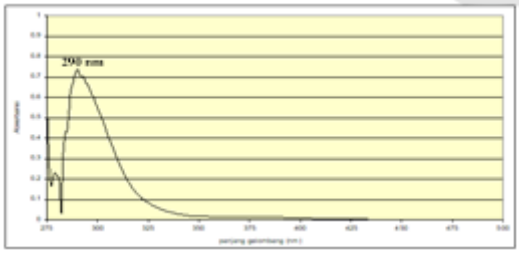


Figure 2: Spectrum UV-Visible of pure isolate.

This can be supported by the calculation of the wavelength for UV-Visibel in theory. Main Chromophore (246 nm), m-OH (2 X 7 nm = 14 nm), p-OH (25 nm), so that it is obtained $\lambda_{max}$ 285 nm. Based on the calculation results $\lambda_{max}$ pure isolate corresponds to $\lambda_{max}$ comparative compound that is gallic acid (Pavia, 2001).

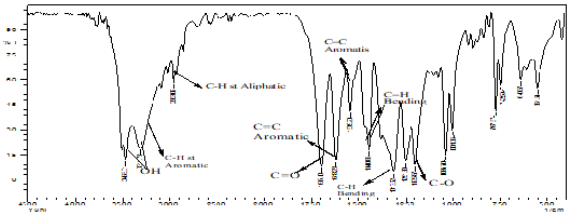


Figure 3: Spectrum FT-IR of pure isolate.

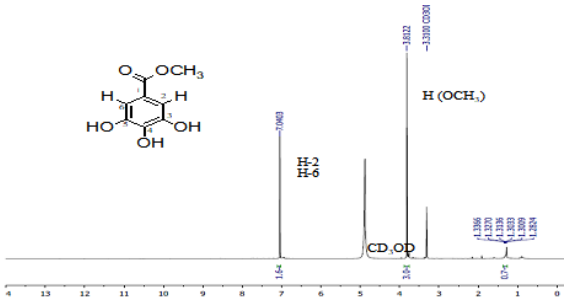


Figure 4: $^1$H NMR spectrum of pure isolate.

FT-IR spectrum of pure isolated was shown in Figure 3. The FT-IR spectrum for pure isolates showed (KBr, ν max, cm$^{-1}$) 3468.01 (O-H), 3311.78 (C-H), 2955.02 (C=H), 1618.29 (C = O), 1313.52 (C-H), 1251.80 (C-O), indicated that the pure isolate has a group commonly found in phenolic compound (Andersen and Markham, 2006). $^1$H NMR spectrum of pure isolate shown in Figure 4. Based on $^1$H NMR spectrum (Methanol-D6, 500 MHz, (ppm)) δ 7.04 (2H, s, H-2, H-6), δ 3.81 (3H, s, $OCH_3$), indicated that pure isolate had two aromatic protons and three methyl protons. The data in FT-IR and $^1$H NMR spectrum are similar to FT-IR and $^1$H NMR data reported by Ekaprasada, et al. (2009). Based on data analysis and interpretation carried out on the UV-Visible, FT-IR and $^1$H-NMR spectrum and comparative

Spectrum reported by Hisham, et al. (2011) it was stated that pure isolates obtained from the leaves of kayu hitam plant was simple phenolic compound, methyl gallate with the structure shown in Figure 5.

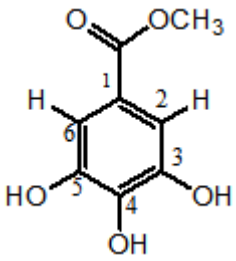


Figure 5: Structure of Methyl Gallate.

### 3.2 Antioxidant Activity of Pure Isolate

Table 1 showed the percentage of inhibition and IC50 values of methyl gallate and ascorbic acid as positive control.

Table 1: IC50 of ascorbic acid and methyl gallate.

| Sample | Concentration | Inhibition (%) | $IC_{50}$ (ug/mL) |
|---|---|---|---|
| Methyl gallate | 0 | 0 | 4.41 |
| | 5 | 84.77 | |
| | 10 | 92.38 | |
| | 15 | 95.43 | |
| | 20 | 97.71 | |
| Ascorbic acid | 0 | 0 | 4.09 |
| | 5 | 85.53 | |
| | 10 | 92.38 | |
| | 15 | 96.19 | |
| | 20 | 97.72 | |

IC50 value of methyl gallate had no significantly different with ascorbic acid. It showed that methyl gallate has proton donating ability and could scavenge the free radical of DPPH.

## 4 CONCLUSIONS

Pure compound had been isolated from kayu hitam leaves. Based on the data spectrum UV Vis, FT-IR and 1H NMR the pure compound was methyl gallate. Methyl gallate is an antioxidant compound with IC50 value 4.41 μg/mL.

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