ANTIOXIDANT ACTIVITY OF METHYL GALLATE ISOLATED FROM THE LEAVES OF *Toona sureni*

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ABSTRACT

An antioxidant compound has been isolated from the leaves of Toona sureni (Blume) Merr. The structure was determined to be methyl 3,4,5-trihydroxybenzoate (methyl gallate), based on UV-vis, FTIR, NMR and MS spectra. The isolated compound exhibited potent antioxidant activity in the α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging test, with IC_{50} value 1.02 µg/mL.

Keywords: *antioxidant activity, methyl gallate, Toona sureni*

INTRODUCTION

Methyl gallate occurs widely in the Aceraceae as well as other plant families [1]. Methyl gallate, a major component of *Galla Rhois* exhibits strong antimicrobial activity [2]. The compound also occurs in the methanol extract of the leaves Chinese toon (*Toona sinensis*) [3-4]. It was reported to have antioxidant activity [4].

Tona sureni (Blume) Merr is one of among five or six species of trees in the mahogany family Meliaceae [5]. In Indonesia it is found in Sumatra, Java and Sulawesi. Various parts of the tree, especially the bark and root, are used for medicinal purposes, e.g. to treat diarrhoea. Leaf extracts have antibiotic effect. The bark and fruits can be used for production of essential oils [6]. The literature search revealed that a number different compounds have previously been isolated from the leaves of the plant, including tetranortriterpenoid (surenin, surenone and surenolactone) [7-8], and carotenoids [9-10]. Another species of Toona genus i.e. *Toona ciliata* contains the essential oil from the leaves and the stems. Its leaves and stems are also contain limonoid [11] dan terpenoid [12].

Herein we report the identification and structural elucidation of an antioxidant compound and its antioxidant activity in the *α,α*-diphenyl-*β*-picrylhydrazyl (DPPH), obtained from the leaves of the plant.

EXPERIMENTAL SECTION

Material

Plant materials were collected in Padang, West Sumatera, Indonesia in December 2008, and identified in the Herbarium of the Andalas University (ANDA), Padang, with specimen M.Taufik Ekaprasada, 0107 (ANDA.Fr). Si gel 60 (E Merck 7733) for column chromatography and Si gel 60 (F254 Merck) for thin layer chromatography were obtained from Merck. DPPH (1,1-diphenyl-2-picrilhydrazyl), gallic acid were obtained from Sigma Chemical Co. All solvents were distilled and furified before used.

Instruments

NMR spectra were recorded on a 500 MHz JEOL JNM ECA- 500 NMR spectrometer (^{1}H NMR 500 MHz and ^{13}C NMR 125 MHz). Chemical shifts were referenced to Acetone-D_6 (δ_H 2.05) and Acetone- D_6 (δ_C 29.9 and 206.7). IR spectra were recorded on a JASCO FT-IR 460 plus spectrophotometer in KBr pellet. UV-vis spectra were recorded on UV-Vis Pharmaspect-1700 S S100 spectrophotometer, Shimadzu in methanol solution. MS were recorded on Agilent Technologies 6890 Gas Chromatograph with Auto Sampler and 5973 Mass Selective Detector and Chemstation Data System, ionization being induced by electron impact at 70 eV.

Procedure

Extraction and Isolation

Air-dried and powdered the leaves (1.5 kg) of the plant were first macerated at room temperature with *n*-hexane (12 L) then with acetone (12 L) and methanol (8 L) to afford respectively 22.233 g, 119.730 g and 121.820 extracts. The methanol extract (10.028 g) was subjected to column chromatography of silica gel and

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eluted with an increasing percentage of ethyl acetate in *n*-hexane (100:0; 90:0; 80:20; 70:30; 60:40; 50:50; 30:70; 0:100 v/v). Fractions with the same R_f on TLC were combined and rechromatographed on the silica gel column and eluted with an increasing percentage of ethyl acetate in methylene chloride and was further recrystalized from *n*-hexane to give white needle crystals (42 mg).

Antioxidant Activity

The measurement of antioxidant activity was performed according to a procedure described previously [13] with slight modifications. Gallic acid was used as the standard antioxidant sample. DPPH and MeOH were used as stable free radical reagent and blank, respectively. The sample was dissolved in MeOH in the ratio of 1:1 (w/v). It was diluted to achieve concentrations of 250, 125, 62.50, 31.25, 15.63, 7.81, 3.91, and 1.95 μg/mL, and 0.2 mL of each concentration was transferred to different vials. 3.8 mL DPPH (20 ppm) was then immediately added to these vials. The absorbance at 517 nm was measured after 30 min. The antioxidant activity was measured as the decrease in the absorbance of DPPH and expressed as percentage of the absorbance of a control DPPH solution without sample. The mean values were obtained from triplicate experiments. The IC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, was calculated from the results and used for comparison Total antioxidant activity (TAA) was expressed as the percentage inhibition of the DPPH radical and was determined by the following equation:

$$\%TAA = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} x100\%$$

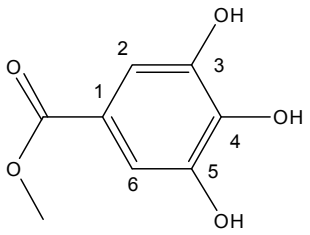
where TAA is the total antioxidant activity and Abs is the absorbance.

RESULT AND DISCUSSION

Isolation and identification of the compound

The methanol extract (10.028 g) was subjected to column chromatography of silica gel and eluted with an increasing percentage of ethyl acetate in *n*-hexane. Fractions with the same R_f on TLC were combined and rechromatographed on the silica gel column and eluted with an increasing percentage of ethyl acetate in methylene chloride and was further recrystalized from *n*-hexane to give white needle crystals (1) 42 mg.

Methyl 3,4,5-trihydroxybenzoate or methyl gallate (1) was obtained as white needle crystals, m.p 188-189 °C, UV-vis (CH_3OH) λ_{max} nm 225.40, 273.60, IR (KBr) v_{max} 3460.63 (OH), 2950.00 (C-H), 1698.02 (C=O), 1617.02 (C=C of benzene ring) cm^{-1}. ^1H NMR (Acetone-D6, 500 MHz) δ 3.78 (3H, s,OCH_3), δ 7.11 (2H, s, H-2, H-6); ^{13}C NMR (Acetone-D_6, 125 MHz) δ 51.96 (OCH_3), δ 109.82 (C-2, C-6), δ 121.79 (C-4), δ 138.79 (C-1), δ 146.11 (C-3, C-5), δ 167.24 (C=O), MS (70 eV) m/z 184 $[M]^+$ (55), 153 (100), 125 (25), 107 (8), 79 (20), 51 (8).



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The compound (1) was isolated as white needle crystals. The molecular formula was determined to be $C_8H_8O_5$ from the MS and 13C NMR data. The FTIR spectrum confirmed a carbon-carbon double bond (v 1617.02 cm-1) and C-H stretching (v 2950.00 cm^{-1}) and revealed the presence of OH (v 3460.63 cm-1 broad). 8 Carbons and 5 protons attached to carbon were observed in the ^{13}C and ^{1}HNMR spectra. Eights signals appeared in the ^{13}C NMR spectrum (C x 5, CH x 2, CH_3 x 1). Close examination of the ^1H and ^{13}C NMR spectrum showed a symmetrical molecule with two aromatic protons, δ 7.11 (2H, s, H-2, H-6), three hydroxyl δ_C 146.11 (C-3, C-5), and δ_C 121.79 (C-4), a methyl δ 3.78 (3H, s, OCH_3) and a ester carbonyl δ_C 167.24. It is consistent with NMR data have been reported from the literature [14-15]. The structure (**1**) revealed the methyl ester of 3,4,5-trihydroxybenzoate

A six-membered ring of the compound was established from analysis of the strong HMBC correlations. The structure of the side-chain was determined from the MS and HMBC data. The fragment ions were observed at *m*/*z* 153 due to the loss –OCH_3 from *m*/*z* 184 $[M^+]$ and m/z 125 due to the loss -CO from ion (m/z 153). They reveal the presence of ester group ($COOCH_3$). Besides that the fragment ions were observed at *m/z* 107, m/z 79 and m/z 51 due to the loss H_2O and –CO from benzene ring. They reveal the presence of three hydroxyls on the benzene ring.

Antioxidant Activity of the compound

Figure 1 shows the dose-response curve of DPPH radical scavenging activity of methyl gallate, compared with gallic acid. Methyl gallate and gallic acid exhibited the similar antioxidant activities. At a concentration of 125 μg/mL, the scavenging activity of methyl gallate reached 88.129%, while at the same concentration that of gallic acid was 93.621%. IC_{50} value of methyl gallate was compared with gallic acid in

Table 1. Antioxidant activities of methyl gallate and gallic acid using the (DPPH) free radical-scavenging assay [a]

Compounds	Concentrations (µg/mL)								IC_{50}[b]
	250	125	62.50	31.25	15.63	7.81	3.91	1.95	
Methyl gallate	86.32	88.13	88.44	76.22	37.18	17.72	7.39	0.90	1.02
Gallic acid	93.68	93.62	93.68	84.62	41.09	18.95	7.63	2.38	0.94

[a] Values represent percentage inhibition of the DPPH radical .
[b] Inhibitory activity was expressed as the mean of 50% inhibitory concentration of triplicate determinations and was obtained by interpolation of concentration-inhibition curves

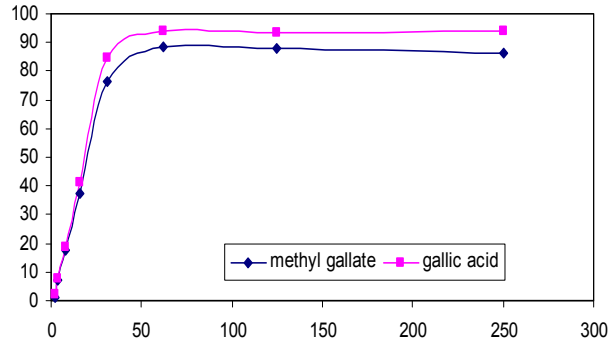

Figure 1. Free radical-scavenging activities of methyl gallate measured using gallic acid as DPPH assay reference compound

the system to access the antioxidant property of methyl gallate (Table 1). IC_{50} value of methyl gallate (1.02 µg/mL) had no a significantly different with reference gallic acid (IC_{50} 0.94 µg/mL) a known standard antioxidant used as positive control.

The literature search revealed that the phenyl ring with the same number of hydroxyl groups, such as 3,4-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid methyl ester, 3,4-dihydroxybenzoic acid ethyl ester, and 3,4-dihydroxybenzoic acid butyl ester or 3,4-dihydroxy-trans-cinnamic acid, 3,4-dihydroxy-trans-cinnamic acid ethyl ester, and 3,4-dihydroxy-trans-cinnamic acid pentyl ester exhibited the similar antioxidant activities [16].

This study showed that methyl gallate has protondonating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

CONCLUSION

In conclusion, methyl 3,4,5-trihydroxybenzoate (methyl gallate) has been isolated from the leaves of *Toona sureni* (Blume) Merr. This is the first report of the chemical constituent of this species. The results presented here for the antioxidant activity study demonstrate the activity of methyl gallate from *Toona sureni* (Blume) Merr and support the use of parts of this plant in used for medicinal purposes.

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