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Antibacterial Activity of Methyl Gallate Isolated from the Leaves of *Toona sureni*

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***Abstract*— The phenolic compound has been isolated from the leaves of *Toona sureni* (Blume) Merr and evaluated against 3 microorganisms, including gram-positive and gram-negative bacteria. The structure was determined to be methyl 3,4,5-trihydroxybenzoate (methyl gallate), based on UV-vis, FTIR, NMR and MS spectra. The antibacterial activity of the compound was evaluated using an impregnated paper disk method and compared with that of chloramphenicol. It was effective on the inactivation of *Escherichia coli* , *Staphylococcus aureus* and *Bacillus subtilis.* The minimal inhibition concentration (MIC) of the compound was 7.5 mg/mL against all the test bacteria. These results may be an indication of at least one of the pharmacological actions of the leaves of *Toona sureni* (Blume) Merr.**

Keywords*— antibacterial activity, methyl gallate, *Toona sureni

I. Introduction

Toona sureni (Blume) Merr is one of the plant that includes family Meliaceae. In Indonesia it is found in Sumatra, Java and Sulawesi. Various parts of the plant, especially the bark and root, are used for medicinal purposes, e.g. to treat diarrhoea. Leaf extracts have antibiotic effect and repel the insect and kill the bedbug (*Cimex lectularius*), that live in the cracks of wooden floor or board-storey bed. In West Sumatra, the leaf *Toona sureni*, (Blume) Merr used also as a flavoring of traditional food [1]. The bark and fruits can be used for production of essential oils [2].

The leaves of the plant was previously studied and several compounds and biologycal activities have been reported due to the presence of tetranortriterpenoid (limonoid) [3]-[4], carotenoid [5]-[8], triterpenoid [9], methyl gallate [10], and essential oil [11]. Methyl gallate exhibited antioxidant activities [10]. Five triterpenes (cedrelone, piscidinol A, niloticin, bourjotinolone A, 3-episapelin A) showed antiplasmodial activity [9]. Ethanol extract of the leaves of Toona sureni showed antifiral acrivities [12].

In this paper, we report antibacterial activities of methyl gallate obtained from the leaves of the plant.

II. Materials and Methods

A. Materials

The plant materials were collected in Padang, West Sumatera in December 2008, and identified in the Herbarium of the Andalas University (ANDA), Padang, with specimen M.Taufik Ekaprasada, 0107 (ANDA.Fr).

Silica gels for column chromatography and thin layer chromatography were obtained from Merck. All solvents were redistilled before used.

B. Test organism

Microorganisms were obtained from the Department of Biology Faculty of Mathematic and Natural Science, University of Andalas, Padang, Indonesia. A strain of gram-negative bacteria (*Escherichia coli*) and two strains of gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) were used. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

C. *Extraction and isolation*

Air-dried and powdered the leaves (1.5 kg) of the plant were first macerated at room temperature with with *n*-hexane (12 L) then with acetone (12 L) to afford respectively 22.233 g, 119.73 g extracts. The acetone extract (8.641 g) was subjected to column chromatography of silica gel and 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 *n*-hexane and was further recrystalized from *n*-hexane to give white needle crystals (10 mg).

D. *Antibacterial Assay*

The disk diffusion susceptibility method was used in order to examine the sensitivity of the bacteria of interest toward the isolated compound from the leaves of *Toona sureni*. Antimicrobial assay was measured using the methods of Rojas *et al* [13] but with slight modifications. One loopful of the given test strain was inoculated into 2.5 ml of N-broth (Nutrient Broth) and incubated for 24 h in an incubator at 37 °C in order to activate the bacterial strain. The bacterial inoculum was diluted in the sterile saline solution (0.9 % NaCl) to obtain turbidity visually comparable to a McFarland No. 0.5 Standard (10^{6-8} CFU/mL). Nutrient Agar (NA), sterilized in a flash and cooled to 40-50 ^{o}C, was poured (15 mL) into sterilized Petri dishes (9 mm diameter) and allowed to harden under room temperature. This is followed homogenous distribution of 0,1 mL bacteria culture (10^{6-8} cfu/mL) onto medium in Petri dishes. The sterile paper disk (5 mm diameter) was impregnated with 20 µL of the compound. After incubation of thecultures at 37 °C for 48 h, the minimum inhibitory concentration (MIC) was determined as the lowest concentration of the test compound that demonstrated no visible growth. Chloramphenicol; 30 mg/mL (standard antibiotic) was used in order to provide a control for the sensitivity of the test organisms in the experiments.

III. Results and Discussion

The Me2CO extract ((8.641 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 Rf on TLC were combined and rechromatographed on the silica gel column and eluted with an increasing percentage of ethyl acetate in n-hexane and was further recrystalized from n-hexane to give white needle crystals (1) 10 mg. Methyl 3,4,5-trihydroxybenzoate or methyl gallate (1) was obtained as white needle crystals, m.p 188-189 0C. The structural elucidation of the compound have been reported [10] while UV-vis (CH3OH) λmax nm 225.40, 273.60, IR (KBr) νmax 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,OCH3), δ 7.11 (2H, s, H-2, H-6); 13C NMR (Acetone-D6, 125 MHz) δ 51.96 (OCH3), 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).

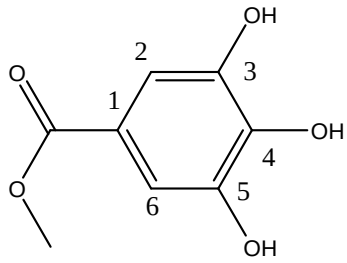

Fig.1 The molecular formula

The molecular formula was determined to be C8H8O5 from the EIMS and 13C NMR data. The FTIR spectrum confirmed a carbon-carbon double bond (ν 1617.02 cm-1) and C-H stretching (ν 2950.00 cm-1) and revealed the presence of OH (ν 3460.63 cm-1 broad). 8 Carbons and 5 protons attached to carbon were observed in the 13C and 1HNMR spectra. Eights signals appeared in the 13C NMR spectrum (C x 5, CH x 2, CH3 x 1). Close examination of the 1H and 13C NMR spectrum showed a symmetrical molecule with two aromatic protons, δ 7.11 (2H, s, H-2, H-6), three hydroxyl δC 146.11 (C-3, C-5), and δC 121.79 (C-4), a methyl δ 3.78 (3H, s, OCH3) and a ester carbonyl δC 167.24. 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 –OCH3 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 (COOCH3). Besides that the fragment ions were observed at m/z 107, m/z 79 and m/z 51 due to the loss H2O and –CO from benzene ring. They reveal the presence of three hydroxyls on the benzene ring.

A. *Antibacterial* activities of the compound

Fig 2 shows the Zones of Inhibition (ZoI) of growth of *E. coli* against the compound (1) around the paper disks impregnated with different concentrations. The results of the antibacterial assays for *E. coli*, *S. aureus*, and *B. subtilis* are reported in Table I. It shows the antibacterial activity the compound against Gram-positive (*S. aureus*, and *B. subtilis*) and Gram-negative (*E. coli*) bacteria. It was showed an antibacterial activity against all bacterial strains used in this study. Diameter values of the inhibitory zones of the compound at a concentration ≤ 7.5 mg/mL are lower or equal to 5 mm). Therefore minimal inhibition concentration (MIC) values the compound against all test bacteria were 7.5 mg/mL. In the other word the compound had an antibacterial activity to *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*.

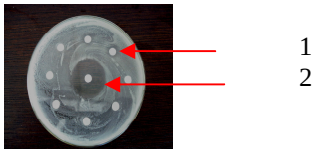

Fig 2. Zones of inhibition of *E. coli* against the compound at concentration 10 mg/mL (1) and chloramphenicol at concentration 30 mg/mL (2) on agar medium

TABLE I
ANTIBACTERIAL ACTIVITIES OF THE COMPOUND [a]

Bacterial species	Concentrations of the compound (mg/mL)							Chloramphenicol (30 mg/mL)	MIC (mg/mL)
	10	7.5	6.25	5.0	3.75	2.5	1.25		
B. subtilis	8.0	7.5	b	b	b	b	b	31	7.5
S. aureus	10.0	9.0	b	b	b	b	b	31	7.5
E. coli	8.0	7.5	b	b	b	b	b	29	7.5

[a] Values represent diameters of inhibitory zone (mm) at indicated dilutions (mg/mL).
[b] Not active (the inhibitory zone diameter is lower or equal to 5 mm)

IV. CONCLUSIONS

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 antibacterial 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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