Antibacterial activity of flavan-3-ol derivative compound from dichloromethane extract of *Artocarpus dasyphylla* tree bark

Indriani Indriani$^{1*}$, Wahyu Harso$^2$, and Husain Sosidi$^1$

$^1$Chemistry Department, Faculty of Mathematic and Science, Tadulako University, Jalan Soekarno Hatta, Tondo 94118, Palu, Indonesia.
$^2$Biology Department, Faculty of Mathematic and Science, Tadulako University, Jalan Soekarno Hatta, Tondo 94118, Palu, Indonesia.

*Corresponding author : indri.2707@gmail.com

**Abstract.** Infectious disease is one of sources of high morbidity and mortality caused by bacteria. *Artocarpus* plant is biosource to discover compounds with interesting bioactivities. The objective of this study is to find antibacterial compound from nature by extraction, fractionation, identification, and antibacterial test of secondary metabolite from dichloromethane extract of *Artocarpus dasyphylla* tree bark. The dichloromethane extract was fractionated repeatedly using various chromatographic techniques. The secondary metabolite was identified of its molecular structure based on UV-Vis, IR, NMR spectral data and confirmed by comparation with the reference. The secondary metabolite was known as catechin or 5,7,3'-4'-tetrahydroxyflavan-3β-ol. Antibacterial testing of catechin against *Escherichia coli* and *Staphylococcus aureus* showed a low antibacterial activity.

1. Introduction
Infectious disease is one of the main object of high morbidity and mortality caused by bacteria [1]. The method used to overcome infectious diseases is treatment using antibiotic. However, resistance of bacteria to antibiotic drugs has driven researchers to switch to plants as an option to get potential antibacterial compounds, among them come from *Artocarpus* genus. *Artocarpus* is widely distributed in tropic and subtropic areas. A certain part of the *Artocarpus* plant has been used as traditional medicine, since ancient times [2]. *Artocarpus* is a well known as source of flavonoid compounds which showed various activity such as anti-fungal [3], anti-platelet [4], cytotoxic against murine P388 leukemia cells [5], anti-inflammatory [6], anti-bacteria [7], and anti-malarial [8]. In continuation to discover chemical content of Indonesia’s plant, we have isolated flavan-3-ol derivative from *Artocarpus dasyphylla* called catechin. This article describes the structural determination of catechin and antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

2. Research Methodology
2.1 General
Melting point of secondary metabolite was determined on a micro-melting point apparatus. UV and IR spectra were measured with UV-Vis Varian Cary 100 Conc and One Perkin-Elmer spectrophotometers, respectively. $^1$H dan $^{13}$C NMR were recorded with a JEOL spectrometer, operating at 500.0 MHz ($^1$H)
and 125 MHz (13C) using TMS as a internal standard. The following adsorbents were used for fractination and purification: vacuum liquid chromatography (VLC) used Si gel Merck 60 GF254, gravitation column chromatography (GCC) used Si gel Merck 60 (700-200 mesh), and TLC analysis with Si gel plates (Merck Kieselgel 60 F254 0.25 mm).

2.2 Plant Material
The tree bark of *A. dasyphylla* was obtained from Botanical Garden Purwodadi, Pasuruan, Jawa Timur. The plant was identified by staff of Botanical Garden Purwodadi.

2.3 Extraction and Isolation
The dried powder of the tree bark *A. dasyphylla* (2.2 kg) was macerated with methanol three times at room temperature, and then concentrated by vacuum evaporatory. The methanol extract was partitioned sequentially with n-hexane and dichloromethane (30 g). The crude dichloromethan was fractionated using vacuum liquid chromatography eluting with mixtures dichloromethane-methanol (increasing polarity) to give four major fractions A-D. Fraction C (800 mg) was separated by gravitation column chromatography (GCC) eluting with mixtures of dichloromethane-methanol (silica gel, gradient system of CH2Cl2 – MeOH) to give subfractions C1 - C3. Subfraction C3, on repeated fractionation and purification using GCC, afforded cathecin (60 mg) (Figure 1). Cathecin, white solid, mp 130-131°C; UV (MeOH) λmax nm (log ε): 209 (2.42), 280 (1.49); IR (KBr) vmax (cm⁻¹): 3401, 2920, 1611, 1520, 1466, 1286, 1144; 1H-NMR (CD3OD, 500 Mhz) dan 13C-NMR (CD3OD, 125 MHz) see Table 1.

3. Antibacterial activity test
In this research, antibacterial activity assay was done by disc diffusion method. The bacteria was cultivated in sterilized nutrient agar (NA) medium. The bacterial suspension was obtained by growth of each type of *Escherichia coli* and *Staphylococcus aureus* in NA medium at 37°C for 24 hours. 25 ml NA medium and 25 ml bacterial suspension were mixed and homogenized and then was poured into sterilized petri dish and allowed to solidify. On the petri bath was made three wells/holes (5 mm in diameter) using a sterilized hole tool. Each wells/holes was filled by isolated compound, chloramphenicol as positive control, DMSO as negative control and incubated at 37°C for 24 hours. Isolated compound and chloramphenicol were made in a series concentration. Antibacterial activity tests were duplicated in all this experiment [9]. Antibacterial activity of chemical compound was evaluated by measuring of the diameters of zones of inhibitory (mm) against test bacteria.

4. Result and discussion
The secondary metabolite from *A. dasyphylla* tree bark was obtained as white solid. UV spectra of secondary metabolite exhibited absorption at 209 and 280 nm was specific for phenolic chromophore. The IR spectrum showed a strong broad band at 3401 cm⁻¹ for a hydroxyl moiety and the strong IR absorption bands at 1611 cm⁻¹ and 1520 cm⁻¹ indicated the existence of aromatic ring. The 1H-NMR spectra showed characteristic signals for flavan-3-ol skeleton at δ1H ppm 4.56 (1H, d, J = 7.5 Hz, H-2), 3.97 (1H, m, H-3), 2.84 (1H, dd, J = 15.7& 4.9 Hz, H-4) and 2.50 (1H, dd, J = 15.7& 8.5 Hz, H -4). In addition, three aromatic proton signals at δ1H ppm 6.83 (1H, d, J = 2.0 Hz, H-2‘), 6.76 (1H, d, J = 8.1 Hz, H – 5’), 6.72 (1H, dd, J = 8.1 & 2.0 Hz, H – 6’) showed hydrogen protons in the B ring. Two aromatic proton signals at δ1H ppm 5.93 (1H, d, J =2.3 Hz, H-6) and 5.86 (1H, d, J = 2.3 Hz, H-8) were in the A ring.

The 13C-NMR spectra displayed 15 signals of carbon that included two quaternary aromatic carbons at δC ppm 100.9 (C-4a) and 132.3 (C-1’); five oxygenated aromatic 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’), five sp² aromatic methine at δC 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 δC ppm 82.9 (C-2) and 68.8 (C-3); and sp³ methylene at δC ppm 28.5 (C-4). There is no carbonyl carbon in 13C-NMR spectra. According to analysis of UV-Vis, IR, 1H-NMR, and 13C-NMR spectral data as well
as comparison with previously reported data [10], the secondary metabolite was identified as 5,7,3’,4’-tetrahydroxyflavan-3β-ol or catechin.

![Molecular structure of catechin](image)

**Figure 1.** Molecular structure of catechin

**Table 1.** NMR Spectrum of Secondary Metabolite from *A. dasyphylla* and Catechin Reference

| No | Secondary metabolite from *A. dasyphylla* | Catechin (Achmad, 1998) |
|----|------------------------------------------|-------------------------|
|    | δC | δH (mult., J in Hz) | δC | δH (mult., J in Hz) |
| 2  | 82.9 | 4.56 (d, 7.5) | 82.9 | 4.57 (d, 7.5) |
| 3  | 68.8 | 3.98 (m) | 68.8 | 3.97 (m) |
| 4  | 28.5 | 2.50 & 2.84 (dd & dd) | 28.5 | 2.50 & 2.84 (dd & dd) |
| 4a | 100.9 | - | 100.8 | - |
| 5  | 157.6 | - | 157.6 | - |
| 6  | 96.3 | 5.93 (d, 2.3) | 96.3 | 5.91(d, 2.4) |
| 7  | 157.9 | - | 157.9 | - |
| 8  | 95.5 | 5.86 (d, 2.3) | 95.5 | 5.86 (d2.4) |
| 8a | 156.9 | - | 156.9 | - |
| 1’ | 132.3 | - | 132.2 | - |
| 2’ | 115.3 | 6.84 (d, 2.0) | 115.3 | 6.84 (d, 2.0) |
| 3’ | 146.2 | - | 146.2 | - |
| 4’ | 146.3 | - | 146.2 | - |
| 5’ | 116.1 | 6.76 (d, 8.0) | 116.1 | 6.75 (d, 8.1) |
| 6’ | 120.0 | 6.72 (dd, 8.3 & 2) | 120.0 | 6.71 (dd, 8.2) |

Antibacterial activity of catechin from *A. dasyphylla* was tested against *Escherichia coli* and *Staphylococcus aureus* by disc diffusion method.

**Table 2.** Antibacterial Activity of Catechin

| Bacteria            | Diameter of Zone of Inhibitory (mm) |
|---------------------|-------------------------------------|
|                     | Sample Concentration                |
|                     | 2.5%  | 5%    | 10%   | 20%   |
| *Escherichia coli*  | 0.70  | 1.20  | 2.83  | 8.15  |
| *Staphylococcus aureus* | 1.39 | 2.77  | 2.82  | 3.02  |
Based on the test result of antibacterial activity of catechin against *Escherichia coli* and *Staphylococcus aureus* indicated a low antibacterial activity [11]. Antibacterial mechanisms of action of catechin may inhibit both of nucleic acid synthesis and cytoplasmic membrane function. Hydroxyl groups of the B ring may play a part in forming hydrogen bonding with the heaping of nucleic acid bases and causes inhibition of DNA and RNA synthesis [12]. Besides, catechin may annoy the lipid bilayers by damaging them and perturb the barrier function [13]. Study structure-activity relationship for antibacterial activity of flavonoids indicated that 5,7-dihydroxyl groups in the A ring of the flavonoid structure is significant for antibacterial activity [14]. Although this activity is not sufficient to compete with chloramphenicol as antibiotic drug (zone of inhibitory at a concentration of 20% is 22.35 mm to *E. coli* and 21.17 to *S. Aureus*), our results may provide a good start for improvement by chemical modification of catechin.

**Acknowledgement**

We thank to Ministry of Research, Technology and Higher Education Indonesia for financial support.

**References**

[1] Kadarsih R, Ningsih, Karuniawati, A, Kiranasari, A. 2007. Emerging Resistance Phatogen. Situasi Terkini di Asia, Eropa, Amerika Serikat, Timur Tengah, dan Indonesia. *Majalah Kedokteran Indonesia*. 57(3):75-79

[2] Heyne. 1987. Tumbuhan Berguna Indonesia. Badan Litbang Kehutanan, Jakarta.Jayasinghe L, Balasooriya B, Padmini WC, Hara N, Fujimoto Y. 2004. Geranyl chalcone derivatives with antifungal and radical scavenging. Phytochemistry. 65, 1287-1290.

[3] Weng JR, Chan SC, Lu YH, Lin HC, Ko HH, Lin CN. 2006. Antiplatelet prenylflavonoids from Artocarpus communis. Phytochemistry. 67, 824-829.

[4] Suhartati T, Achmad SA, Norio Aimi, Hakim EH, Mariko Kitajima, Hiromitsu Takayama, Koichi Takeya. 2001. Artoidonesianien L, A new Prenylatedflavon with cytotoxic activity from Artocarpus rotunda. Fitoterapia. 72(8), 912-918.

[5] Chen, C-C., Yu-Lin Huang and Jun-Chih Ou. (1993). Three New Prenylflavons from Artocarpus altilis. Journal of Natural Products. 56(9), 1594-1597.

[6] Khan MR, Omoloso AD, Kihara M. 2003. Antibacterial activity of Artocarpus heterophyllus. Fitoterapia. 74, 501-505.

[7] Widyawaruwanti A, Subehan, Kalauni SK, Awale S, Nindatu M, Zaini NC, Syafruddin D, Asih PBS, Tezuka Y, Kadota S. 2007. New prenylated flavones from Artocarpus champedan, and their antimalarial activity in vitro. Journal of Natural Medicines, 61, 410-413.

[8] Faoziyat AS, Muinak OT, Ahmed MW, Simisade OT, Idris OA, Faridat I Z, Temitope AA, Azido N, Oloyomi Sadeyem. 2014. Antimicrobial and toxic potential of aqueous extract of Allium sativum, Hibiscus sabdariffa and Zingiber officinale in Wistar Rats. Journal of Taibah University for Science, 8, 315-322.

[9] Achmad SA, Muriana, Udjiana SS, Aini M, Hakim EH, Makmur L. 1998. Tiga Senyawa Flavan-3-ol dari Tumbuhan Artocarpus reticulatus. Proceeding ITB, 30(2), 1-7.

[10] Davis WW and Stout TR. 1971. Plate Method of Microbiological Antibiotic Assay. Applied Microbiology, 22(4), 659-665.

[11] Mori A, Nishino C, Enoki N, Tawata S. 1987. Antibacterial activity and mode of action of plant flavonoids against Proteus vulgaris and Staphylococcus aureus. Phytochemistry, 26, 2231-4.

[12] Ikigai H, Nakae T, Hara Y, Shimamura T. 1993. Bactericidal catechins damage the lipid bilayer. Biochim Biophys Acts, 132-6.

[13] Tsuchiya H, Sato M, Miyazaki T. 1996. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant Staphylococcus aureus. Journal Ethnopharmacol, 50, 27-34.