JURNAL ILMU KEFARMASIAN INDONESIA, Oktober 2020, hlm. 198-201 Vol.18, No.2
ISSN:1693-1831, E-ISSN: 2614-6495

Isolation and Identification of Phenolic Compounds from *Macaranga hispida* Blume Mull.Arg Leaves

(Isolasi dan Identifikasi Senyawa Fenolik dari Daun *Macaranga hispida* Blume Mull.Arg)

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Submitted 19 Descmber 2019, Accepted 20 Agustus 2020

Abstract: Continuation of research on Macaranga plant has been conducted. In this study, the total phenol content (TPC) of *Macaranga hispida* Blume Mull. Arg leaves extracts were investigated. Gallic acid (1) and methyl gallate (2) have been isolated from the ethyl acetate fraction which highest phenolic content (8.41 mg GAE/g). Serial techniques of chromatographic and spectroscopic methods, namely extraction, fractionation, purification, FTIR, LCMS, and FT-NMR were used to isolate and identify the isolates. Cytotoxicity of compound 1 and 2 against P-388 cell line showed that compound 2 has higher anticancer activity compared to compound 1 with IC_{50} value of 75.53 µg/mL.

Kata kunci: *Macaranga hispida*, phenolic compounds, gallic acid, methyl gallate, cytotoxicity.

Abstrak: Kelanjutan penelitian pada tanaman Macaranga telah dilakukan. Pada penelitian ini dilakukan kandungan fenol total dari ekstrak daun Macaranga hispida Blume Mull.Arg. Asam galat (1) dan metil galat (2) telah diisolasi dari fraksi etil asetat yang memiliki kandungan fenolik tertinggi (8,41 mg GAE /g). Serangkaian teknik kromatografi dan spektroskopi yaitu ekstraksi, fraksinasi, pemurnian, FTIR, LCMS, dan FT-NMR digunakan untuk mengisolasi dan identifikasi isolat. Sitotoksisitas senyawa 1 dan 2 terhadap sel murine leukimia P-388 menunjukkan bahwa senyawa 2 memiliki aktivitas antikanker yang lebih tinggi dibandingkan dengan senyawa 1 dengan nilai IC_{50} 75,53 µg/mL.

Keywords: *Macaranga hispida*, senyawa fenolik, asam galat, metil galat, sitotoksisitas.

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INTRODUCTION

THE genus *Macaranga, Euphorbiaceae* is one of the Indonesian bioresources, consist more than 300 species, and widely found in Indonesia region[1]. More than 26 species of *Macaranga* have been studied phytochemically with more than 190 secondary metabolite compounds already identified, with phenolic compound as a major compound group[2]. Our previous phytochemical study on *Macaranga* genus collected from Mekongga forest, were succeed isolated and identified some phenolic compounds such as macarangin, apigenin, apigenin glycoside, and scopoletin from *M. Gigantifolia*[3-5], 5,7,3',4'-tetrahydroxy-3,6-diprenylflavone and kaempferol 7-O-β-glucose from *M. gigantifolia* and *M. hispida (Blume)* [3,6] and scopolotin compound from *M. hispida (Blume)*[7]. Previous studies reported that flavonoids and terpenoids are the main components of the genus *Macaranga*[8-11]. Apigenin and active compounds were isolated from Portulaca. oleracea L which has potential antibacterial activity and can be used for drug development as an antibacterial for the treatment of diseases associated with pathogenic bacteria[12].

A further study of the phytochemical content of the *Macaranga* genus needs to be done especially from *M. hispida (Blume) Mull. Arg*. The previous phytochemical study of *M. hispida* showed that the presence of phenolics of *M. hispida* extract (6). Therefore the study to isolate phenolic compounds from *M. hispida* needs to be conducted. In this study, the total phenol content (TPC) from methanol extract, n-hexane, ethyl acetate and buthanol fractions were investigated. After that, the isolation and identification of phenolic compounds from the fraction of *M. hispida* which has highest of TPC were carried out. Furthermore cytotoxicity of the isolates were examined against P-388 cell lines.

MATERIALS AND METHODS

General-FTIR was measured with Shimadzu IR Prestige-21. NMR spectra was recorded with JEOL JNM 500 operating at 500 (^{1}H) and 125 (^{13}C) MHz and LCMS spectra were obtained with Mariner Biospectrometry (ESI system). Column chromatography was carried out using Merk silica gel 60 GF_{254} and TLC analysis used precoated silica gel plates (Merck, Kieselgel 60 GF_{254}, 0,25 mm).

Plant material-*M. hispida* leaves was collected in March 2012 from Mekongga forest, Southeast Sulawesi, Indonesia. The plant was identified by the staff of Herbarium Bogoriense, Research Center for Biology, LIPI and a voucher specimen had been deposited at the herbarium.

METHODS. Extraction and Isolation. The dried leaves (2.15 kg) of *M. hispida* was macerated with methanol for 24 hours (3 times). The methanol extract (150 g) was partitionated with n-hexane, ethyl acetate and n-buthanol, successively, and all fractions were evaluated for their TPC using Follin Ciocalteu methods. Ethyl acetate fractions (14 g) which has highest of TPC further purified using column chromatography technique with gradient solvent system (n-hexane-ethyl acetate-methanol) as mobile phase to obtain compound 1 (12 mg) and compound 2 (10 mg).

Compound 1, yellowish white crystals, melting point 255°C; IR (KBr) v_{max} 3464, 1622, 1384, 1240, 1001, 773 cm^{-1}; NMR δH (500 MHz, in DMSO-d6): 6.90 (2H, s, H-2/6). ^{13}C-NMR (DMSO, 125 MHz) δ 120.41 (C-1), 108.69 (C-2/6), 145.41 (C-3/5), 137.99 (C-4), 167.49 (C=O). LC-ESI-MS (m/z) 170.30

Compound 2, white crystals, melting point 202oC; IR (KBr) v_{max}3437, 2924, 2872, 1716, 1375, 1236, 1062, 854 cm^{-1}; NMR δH (500 MHz, in CD3OD-d4): 7.05 (2H, s, H-2/6); 3.79 (3H, s, -OMe). ^{13}C-NMR (CD3OD-d4, 125 MHz) δ 121.43 (C-1), 110.08 (C-2/6), 146.39 (C-3/5), 139.68 (C-4), 169.07 (C=O), 52.38 (-OMe). LC-ESI-MS (m/z) 184.10.

Total Phenolic Content in Extract. The total phenolic content of the extract was determined by the Folin–Ciocalteu method[13]. Amount of 5, 10, 15 and 200 μL from 1000 ppm of gallic acid solution were pipetted and placed into test tubes. Each tubes were added with 1 mL of the sampel, and distilled water to 7.5 mL volume. Added with 0.5 mL of Folin–Ciocalteu reagent, and mixed thoroughly for 8 min, followed by the addition of 1.5 mL of 20% (w/v) of sodium carbonate. The mixture was allowed to stand for further 60 minutes in the dark, and absorbance was measured at 765 nm. The total phenolic content was calculated based on the calibration curve, and the results were expressed as mg of gallic acid equivalent per g of dry weight.

Cytotoxic Assay. The cytotoxicity assay was conducted according to the method described by modified Sofa et al., 2016[5] P-388 cells were seeded into 96-well plates at an initial cell density of approximately 3 x104 cells cm^{-3}. After 24 hrs of incubation for cell attachment and growth, varying concentration of samples were added. Sample firstly dissolved in DMSO at the required concentration. Subsequent of six desirable concentrations were prepared using PBS (phosphoric buffer solution, pH 7.30-7.65). Control wells received only DMSO. The

assay was terminated after an 48 hrs incubation period by adding MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; also named as thiazol blue] and the incubation was continued for another 4 hrs, in which the MTT-stop solution containing SDS (sodium dodecyl sulphate) was added and another 24 hrs of incubation was conducted. Optical density was read by using a microplate reader at 550 nm. IC_{50} values were taken from the plotted graph of percentage live cells compared to control (%) (receiving only PBS and DMSO) versus the tested concentration of the samples (µm). The IC_{50} value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged.

RESULT AND DISCUSSION

In this study, extraction of 2.15 kg of *M. hispida* leaves with methanol yielded 150 g viscous methanolic extract (6.98%) (w/w). The extract further partitionated using n-hexane, ethyl acetate and n-butanol, succesively to extract and separate the active compounds with a different polarity separated in the fractions. The yield of n-hexane, ethyl acetate, and n-butanol extracts were 21.19, 9.58, 19.39, and 20.34 % (w/w)[7], respectively. The highest yield of the extract was ethyl acetate fraction. It means the major compound contained of the extract from *M. hispida* leaves was semi polar compounds.

Methanol extract and all fractions (n-hexane, ethyl acetate, n-buthanol) were evaluated for their TPC using Folin-Ciocalteu method. TPC of methanol extract was 6.225 ± 0.11% (w/w). It means that every 100 g of dry weight of methanol extract contains total phenolics which is equivalent to gallic acid of 6.225 ± 0.11 g. The TPC of the extract and all fractions are presented in the Table 1. It showed that the highest TPC was ethyl acetate fraction with TPC of 8.411 ± 0.02%. Based on the TPC, the ethyl acetate fraction was further purified to isolate phenolic compounds using various chromatography techniques.

The Ethyl acetate fraction (14 g) was purified by using column chromatography on silica gel, eluted successively with a gradient of n-hexane-EtOAc-MeOH to obtain10 fraction (F1-F10). F8 and F10 were further purified using sephadex LH-20 with eluent dichloromethane-MeOH (1:1) to produce compound 1 (12 mg) and compound 2 (10 mg).

The ESI-MS spectrum of compound 1 revealed a molecular ion peaks M^+ at m/z 170.30 corresponding to the molecular formula C7H6O5. The ^{1}H-NMR spectrum of compound 1 showed a A2 spin coupling system of two protons at δH 6.90 ppm (2H, s)

Table 1. Total phenolics content (TPC) of methanolic extract, n-hexane, ethyl acetate and n-buthanol fractions from *M. hispida leaves*.

Extract/Fraction	% TPC (GAE (b/b))
Methanol	6.225 ± 0.11
n-Hexane	1.183 ± 0.07
Ethyl Acetate	8.411 ± 0.02
n-Butanol	4.387 ± 0.32

GAE = Gallic Acid Equivalent

attributed to H-2 and H-6. The 13^C-NMR spectrum of compound 2 showed two methine carbon at δC 108.69 (2C) and five carbon quartenary at δC 120.41; 137.99; 145.41 (2C); and 167.49 (C=O). The presence of an hydroxyl group was deduced from a sharp peak in the FTIR spectrum at v 3464 cm^{-1}. Based on spectroscopic data and the comparison result with previous study[9] compound 1 suggested as gallic acid (Fig. 1).

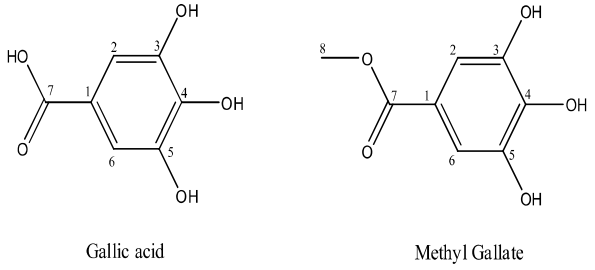

Figure 1. Chemical structures of gallic acid (1) and methyl gallate (2).

The spectroscopic data of compound 2 similar with compound 1. From NMR data, Compound 2 had a methoxy group at δH 3.69 (3H, s) and δC 55.67, with molecular weight 184.10. This methoxy group also showed in the FTIR spectrum at v 2924 and 2872 cm^{-1}. Therefore, based on spectroscopic data and reference (Darmawan et al., 2016), compound 2 suggested as methyl gallate, a gallic acid derivative (Fig. 1). Compound 1 and 2 were examined for their cytotoxicity against the leukemia murine P-388 cells line, and showed anticancer activity with IC_{50}>100 and 75.53 µg/mL, respectively. In other study, these compounds also exhibits as antioxidant and alpha-glucosidase inhibitor on *M. Allorobinsonii*[14]. Compounds gallic acid (1) and methyl gallate (2) are the first report from *M. hispida* leaves.

CONCLUSIONS

The ethyl acetate fraction of *M. hispida* leaves which highest TPC is a potential source of phenolic compounds. Further isolation of this fraction produced two phenolic compounds, namely gallic acid (1) and

methyl gallate (2). Compounds 2 exhibit cytotoxic activity against P-388 cell lines with IC_{50} of 75.53 µg/mL, while compound 1 less active with IC_{50} value more than 100 µg/mL

ACKNOWLEDGMENTS

Authors were grateful to thanks to Prof. Dr. Muhammad Hanafi and Mrs. Puspa Dewi NL.,M.Eng, for an interesting and useful discussion especially about chemical structure elucidation.

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