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Four Flavan-3-ol Compounds from The Stem Bark of *Chisocheton balansae* C. DC. (Meliaceae)

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ABSTRACT. *Chisocheton balansae* C.DC., is one of the Meliaceae family plants which is the endemic plants from Sopotan Mountain, North Sulawesi, Indonesia. This study was aimed to determine the chemical structure of flavan-3-ol compounds from ethyl acetate extract of *C. balansae* C.DC stem bark. Dried powder of *C. balansae* C.DC stem bark was extracted consecutively with *n*-hexane, ethyl acetate, and methanol solvents. Four flavan-3-ol compounds, named catechin (1), epicatechin (2), epigallocatechin-3-O-gallate (3), and epicatechin-3-O-gallate (4) were successfully isolated from ethyl acetate extract. The chemical structure of these isolates was determined by spectroscopic methods (¹H-NMR, ¹³C-NMR, DEPT, and 2D-NMR) and comparison with previous reported spectral data. These compounds are first time reported from this plant.

Keywords: *Chisocheton balansae*, flavan-3-ol, epigallocatechin-3-O-gallate, epicatechin-3-O-gallate

INTRODUCTION

Chisocheton is one of the genera of Meliaceae family, which has 53 species mainly distributed in tropical and subtropical regions such as southern China, Thailand, Malaysia, Vietnam, Indonesia and northern Australia (Shilpi et al., 2016; Zhang, He, Wu, Chen, & Yue, 2012). Studies on the investigation of chemical constituents from *Chisocheton* plant have been undertaken extensively since 1979 leading to the isolation of various types of compounds, such as limonoids (Awang et al., 2007; Chong et al., 2019; Najmuldeen et al., 2012; Nugroho et al., 2017; Nurlelasari et al., 2017; Supriatno et al., 2018), apotirucallane-type triterpenoids (Xie, Yang, Zhang, & Yue, 2009; Yadav, Katakya, & Mathur, 1999; Yang, Wang, Wang, Kong, & Luo, 2012; Zhang et al., 2012), dammarane-type triterpenoids (Chan, Mohamad, Ooi, Imiyabir, & Chung, 2012; Katja, Harneti, Mayanti, Farabi, & Supratman, 2017; Phongmaykin, Kumamoto, Ishikawa, Suttisri, & Saifah, 2008), lanostane-type triterpenoids (Katja et al., 2016), tirucallane-type triterpenoids (Katja et al., 2017; M. H. Yang, Wang, Luo, Wang, & Kong, 2011), steroids (Huang, Jian, Li, Kong, & Yang, 2016; Najmuldeen et al., 2011), alkaloids (Tzouros et al., 2004), coumarins (Nurlelasari et al., 2014; Phongmaykin et al., 2008) and sesquiterpenoids

(Phongmaykin et al., 2008). Flavonoid compounds were also found in the *Chisocheton* species as minor components compared to limonoid and triterpenoid as the major constituents (Shilpi et al., 2016; Supriatno et al., 2017).

In our continuous effort to discover interesting molecules from the *Chisocheton* plant, this study revealed the presence of flavan-3-ol compounds from *C. balansae* C. DC. Although the compounds have been previously reported by Davis, Cai, Davies, & Lewis, (1996), these secondary metabolites in *C. balansae* C.DC., has not been reported yet.

EXPERIMENTAL SECTION**General Experiment Procedure**

Ultra-violet spectra were analyzed in methanol on Jasco UV-1575 spectrophotometer. IR spectra were recorded on a Perkin-Elmer spectrum-100 FT-IR (Waltham, MA, USA) KBr. Mass spectra were obtained by Synapt G2 mass spectrometer instrument (Waters, Milford, MA, USA). NMR spectral data were performed on a Bruker Topspin spectrometer at 500 MHz (Bruker BioSpin GmbH, Silberstreifen 4, D-76287 Rheinstetten, Germany), with CD₃OD and acetone-d₆ as a solvent, chemical shifts were given on a δ (ppm) scale and tetramethylsilane (TMS) as an

internal standard. Column chromatography was conducted on silica gel 60 (Merck, Darmstadt, Germany) and Octa Decyl Sylane DS, Fuji Sylisia, Japan). TLC plates were precoated with silica gel GF₂₅₄ (Merck, 0.25 mm) and detection was achieved by spraying with 10% H₂SO₄ in EtOH, followed by heating and analyzed under UV light at wavelength 254 and 365 nm.

Plant material

The stem bark of *C. balansae* C.DC. was collected in Gunung Sopotan, North Sulawesi, Indonesia on November 2017. The plant was identified by the staff of the Taxonomy Laboratory, Department of Biology, Faculty Mathematics and Natural Sciences, Universitas Padjadjaran (No. BO-1294551) has been deposited at the herbarium.

Extraction and isolation

The dried stem bark of *C. balansae* (C.DC.) (1.91 kg) was successively extracted with *n*-hexane, EtOAc, and MeOH. Each extract was evaporated under vacuum. After evaporation three crude were obtained: *n*-hexane (7.71 g), ethyl acetate (122.1 g) and methanol (287.1 g). Ethyl acetate extract (122.1 g) was separated by vacuum liquid chromatography (VLC) packed with silica gel G60 by gradient elution of CH₂Cl₂:EtOAc:MeOH (100:0:0 – 0:0:100) to give ten fractions (A-J). Fraction E (1.06 g) was subjected using fractionation chromatography (silica G₆₀ 230-400 mesh) with an *n*-hexane - EtOAc gradient system, yielding 8 fractions (E1-E8). Fraction E8 was further fractionated using *n*-hexane:CHCl₃:EtOAc (20:25:55) to mainly afford eight fractions (E8a-E8h). Purification of the fraction E8f on column eluted with *n*-hexane:chloroform: EtOAc (3:3:4) afforded compound 1 (26.1 mg). Fraction F (2.99 g) was separated using *n*-hexane: EtOAc (5:5) with 1% addition of concentrated formic acid afforded compound 1 (23.1 mg) and compound 2 (4.2 mg). Fraction G (4.49 g) was fractionated by column chromatography using CHCl₃:acetone (10:0:0:10) gradient system to mainly afford 10 fractions (G1-G10). Fraction G8 (358 mg) was separated with CH₂Cl₂: EtOAc:methanol (12:7:1) with 4% addition of concentrated formic acid until obtained eight fractions (G8a-G8h). Purification of the fraction G8c (56 mg) eluted with CHCl₃:acetone (8:2) with an addition of 4% of concentrated formic acid yielded compound 1 (4.3 mg). Fraction G8d (165 mg) was fractionated using CHCl₃: acetone (9:1) with 4% addition concentrated formic acid to mainly afforded compound 3 (24.3 mg) and other five fractions (G8d1-G8d5). Fraction G8d3 (87 mg) was purified by chloroform: acetone (8:2) with 4% addition concentrated formic acid afforded compound 4.

RESULTS AND DISCUSSIONS

The ethyl acetate extract of the stem bark of *C. balansae* C.DC was chromatographed over vacuum-

liquid chromatography (VLC) and silica gel column chromatography to afford compounds 1, 2, 3 and 4 (Figure 1)

Compound 1 was obtained as a white amorphous solid. The molecular formula was determined to be C₁₅H₁₄O₆ on the basis of the HR-TOFMS spectrum showing [M+H]⁺ *m/z* 290.0878, (calculated *m/z* 290.0787). The IR spectra of compound 1 showed absorption peaks for hydroxyl (3293 cm⁻¹), aliphatic (2936 cm⁻¹), C=C aromatic bond (1623 cm⁻¹) and ether (1147 and 1057 cm⁻¹) groups. Additionally, the UV spectrum showed absorption peaks at 210 and 281 nm, both peaks indicated the presence of conjugated π-π* transitions arising from the aromatic rings (Shiono et al., 2013; Shiono et al., 2016). ¹³C-NMR (CD₃OD, 125 MHz) and DEPT 135 data showed fifteen atom carbons, with one methylene, two oxygenated methines, five methine olefinic carbons and seven quaternary olefinic carbon (12 sp² carbons). Based on the molecular formula and NMR data (Table 1), nine-degree of unsaturation were identified, which described as six pairs of C sp² and tricyclic flavonoid. ¹H-NMR (CD₃OD, 500 MHz) spectrum showed signals proton were observed, which were two aromatic proton at δ_H 5.92 ppm (1H, d, *J* = 2.1 Hz) and 5.83 ppm (1H, d, *J* = 2.1 Hz) that were assigned to H-6 and H-8 of ring A, three aromatic protons at δ_H 6.83 ppm (1H, d, *J* = 8.1 Hz), 6.76 ppm (1H, d, *J* = 8.1 Hz) and 6.72 ppm (1H, dd, *J* = 8.1, 1.6 Hz) proven to be H-2', H-5' and H-6, respectively of ring B, two oxygenated methine signals proton at δ_H 4.56 ppm (1H, d, 7.5 Hz) and 3.97 ppm (1H, dd, *J* = 7.5, 5.5 Hz) also one ethylene proton at 2.50 ppm (1H, dd, 16.1, 8.1 Hz) and 2.83 ppm (1H, dd, *J* = 16.1, 5.5 Hz) assigned to H-2, H-3, H-4α and H-4β of ring C. Two meta-coupled proton between H-6 and H-8 (*J* = 2.1 Hz) with HMBC correlation between H-6 to C-5, C-9 and C-7 and H-8 to C-7, C-9, C-10 strongly suggest of ring A moiety. Ring B is benzene trisubstituted that has been identified as meta-coupling between H-2' to H-6' (*J* = 1.6 Hz), ortho-coupling between H-5' to H-6' (*J* = 8.1 Hz) and these data were supported by ¹H-¹H COSY cross peak between H-5'/H-6' and the presence of HMBC correlation between H-2' to C-1', C-3', C-4' and C-2. The flavan-3-ol skeleton was confirmed in ring C, after the presence of ¹H-¹H COSY cross peak between H-2/H-3/H-4 and also HMBC correlation between H-2 to C-4, C-3, C-9, and H-4 to C-2, C-3, and C-10 (Figure 2). Based on ¹H-NMR data, the coupling constant between H-2 and H-3 was ^{2,3}*J* = 7.5 Hz, referred to *trans* pseudoaxial-pseudoaxial relationships between C-2 and C-3 conformations. As a result, H-2 is β-orientation and H-3 is α-orientation. Consequently, the structure of compound 1 was deduced as *trans*-3,5,7,3',4'-pentahydroxyflavan. A detailed comparison of NMR spectra with literature (El-Razek, 2007) showed compound 1 is a (+)-catechin.

Compound 2 was obtained as a yellow amorphous solid. The molecular formula was determined to be $C_{15}H_{14}O_6$ on the basis of the HR-TOFMS spectrum showed $[M+H]^+$ m/z 290.0878, (calculated m/z 290.0787). UV spectrum of **2** (MeOH) λ_{max} nm (log ϵ) 276 (3.94), IR (KBr) V_{max} (cm^{-1}) 3330 (O-H stretch), 1550 (C=C aromatics stretch), 1140 (asymmetric C-O-C stretch), 1045 (symmetric C-O-C stretch), 830 (substituted benzene ring). The NMR data (Table 1.) for compound **2**, almost similar with compound **1**, except for the proton H-2 and H-3 which appeared as singlets and broad singlets, respectively. Based on the splitting pattern, compound **2** has a smaller coupling constant than compound **1**.

This feature (2J value less than 1 Hz) is the characteristic of the flavan structure with *cis*-2,3 stereochemistry (Clark-Lewis, Jackman, & Spotswood, 1964; Usman, Thoss, & Nur-e-alam, 2016). In 1H - 1H COSY and HMBC analysis, compound **2** showed similar relation with compound **1**. In NOESY experiment, it showed the cross peak between H-2/H-3/H-4 β /H-2' to confirmed the conformation of H-2 and H-3 were *ax*-*ax* pseudoaxial-pseudoaxial relationships (*cis*-2,3) (Figure 3.). On the basis of data, the structure compound **2** was determined as *cis*-3,5,7,3',4'-pentahydroxyflavan and identified as (-)-epicatechin (**2**), by comparison with spectral data in the literature (El-Razek, 2007).

Compound 3 was obtained as a brown amorphous solid. ^{13}C -NMR spectra (acetone- d_6 , 125 MHz) compared with the DEPT 135° spectra were showed twenty-two atom carbons, with one quaternary carbon ester, one methylene, two oxygenated methines, then six methines and twelve quaternary methine carbons. 1H -NMR (acetone- d_6 , 500 MHz) spectrum showed the presence of seven signal proton region. Two oxygenated methines at 5.46 ppm (1H, s) and 4.93 ppm (1H, s) together with two signals of gem-protons at 2.83 ppm (1H, dd, $J = 17.3, 1.7$ Hz) and 2.94 ppm (1H, dd, $J = 17.3, 4.4$ Hz) that were supported with 1H -COSY, HSQC and HMBC correlations, revealed H-3, H-2, H-4 β and H-4 β in ring C of flavan-3-ol skeleton. Three methine singlet protons in the aromatic region at 5.93 ppm (2H, s), 6.53 ppm (2H, s) and 6.94 ppm (2H, s) indicated three aryl moieties in the structure compound **3**. Proton H-3 in compound **3** has a greater chemical shift than compounds **1** and **2** (up to 1.5 ppm), indicating the presence of galloyl ester groups in C-3 (Davis et al., 1996). The presence of galloyl moiety was confirmed by HMBC correlation between proton at 6.94 (2H, s) with carbon at 121.5 ppm (C-1''), 146.1 ppm (C-3'') and 139.4 ppm (C-4''). Other HMBC correlations showed that protons at 5.93 ppm (2H, s) assigned to H-6 and H-8 have correlation with carbon

at 99.0 ppm (C-10), 157.8 ppm (C-5 & C-7) and 157.7 ppm (C-9) for confirmation of ring A. Protons at 6.53 ppm (2H, s) have correlation with 130.6 ppm (C-1''), 146.4 ppm (C-3'') and 133.4 ppm (C-4'') have revealed moiety of ring B. In the coupling constant analysis, compound **3** has the same conformation as compound **2**, since the splitting pattern for H-2 and H-3 were singlet. NOESY showed correlation between H-2 to H-3. Compound **3** was characterized as (-)-epigallocatechin-3-O-gallate by the analysis of 1D and 2D NMR data and by comparison with their reported spectroscopic data (Choi et al., 2015).

Compound 4 was obtained as dark purple amorphous solid. The NMR data of compound **4** (Table 1) had fairly identical pattern to those of compound **2** with small distinction of H-2 and H-3 protons chemical shift. Compound **4** has greater chemical shift, since it has galloyl moiety that was confirmed by HMBC correlation of 5.52 ppm (H-3) to 167.7 ppm (gallic ester) in C-3 linkage. Thus, compound **4** was identified as (-)-epicatechin-3-O-gallate (Choi et al., 2015).

CONCLUSIONS

Four flavan-3-ol flavonoids, (+)-catechin (**1**), (-)-epicatechin (**2**), (-)-epigallocatechin-3-O-gallate (**3**) and (-)-epicatechin-3-O-gallate (**4**), have been isolated from ethyl acetate extract of *C. balansae* C.DC., stem bark for the first time.

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