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Flavanoids fromthe Stembark of*Chisocheton pentandrus*(Meliaceae)

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Abstract

Two flavanoid compounds, catechin (**1**) and epicatechin (**2**), have been isolated from the stembark of *Chisocheton pentandrus*. The chemical structure of compounds**1**and**2**were identified byspectroscopic data including, UV, IR, NMR (^{1}H, ^{13}C, DEPT 135°, HMQC, HMBC, ^{1}H-^{1}H COSY) and MS and by comparing with previously reported spectral data. Compounds **1** and **2**, were isolated in this plant for first time and showed no cytotoxic activity against MCF-7 breast cancer cells.

Keywords:*Chisocheton pentandrus*, catechin, epicatechin, Meliaceae, MCF-7.

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1. INTRODUCTION

The tropical plant of *Chisocheton* belong to Meliaceae family is a higher plant that can grow up to 25m in height (Mabberly and Pannell, 1989).This plant is widespread in the tropical and subtropical countries including Indo-China, Papua New Guinea, Southern China, Thailand, Malaysia, Nepal, India, Bhutan and Myanmar (Vossen and Umali, 2002). Some of these plant species have been traditionally used as laxatives, medicinal and cosmetic ingredients as well as for toxins in fish (Lim, 2008). *Chisocheton* species were known to produce bioactive compounds with complex molecular structures such as erythrocarpine E and chisomecine A (Awang *et al*., 2007, Najmuldeen *et al*., 2011). Plant from this genus have been known to be a rich source of secondary metabolites including various sterols, terpenoids and alkaloids with biologically properties such as antifungal, antibacterial, antiviral, anti-inflammatory, and antiplasmodial agents (Mohamad *et al*., 2009, Joshi *et al*., 1987, Agbedahunsi *et al*., 2004).During the course of our continuing search for novel secondary metabolites from Indonesia *Chisocheton* plants, we isolated and described a new limonoid, dysobinol, from the bark of *C. macrophyllus* (Nurlelasari *et al*., 2017) and a new lanostane-type triterpenoid, 3β-hydroxy-25-ethyl-lanost-9(11),24(24′)-diene, from the stembark of *A. cumingianus (*Katja *et al*., 2016*)*. In the further search for novel compounds from Indonesia *Chisocheton* plants, recently we explore the phytochemistry of the stembark of*C. pentandrus*. The plantis a higher plant and mainly distributed in nothern part of Sulawesi in Indonesia (Inada *et al*., 1997; Mabberley *et al*., 1995). Its stembark have been used as an Indonesian folk medicine for reducing fever, moisturizing the lungs, and for treating contused wound (Heyne., 1982). Although secondary metabolite compounds of other *Chisocheton* plants have been investigated previously, the chemical constituents of *C. pentandrus* is yet to be reported. In this paper, we describe the isolation and structural elucidation of two flavanoids, **1** and **2**.

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2. MATERIAL AND METHODS

General Experimental Procedure

Melting points were measured on an electrothermal melting point apparatus and are uncorrected. UV spectra were measured by using a TECAN Infinite M200 pro,with MeOH. The IR spectra were recorded on a SHIMADZU IRPrestige-21 in KBr. The mass spectra were recorded with a Waters Xevo QTOF MS. NMR data were recorded on a Bruker Topspin spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C using TMS as an internal standard. Column chromatography was conducted on silica gel 60. TLC plates were precoated with silica gel GF_{254} (Merck, 0.25 mm) and detection was achieved by spraying with 10% H_2SO_4 in EtOH, followed by heating and under UV light at wave lenght at 254 and 367 nm.

Plant Material

The stem bark of *C. pentandrus* were collected in Bogor Botanical Garden, Bogor, West Java Province, Indonesia in June 2016. The plant was identified by the staff of the Bogoriense Herbarium, Bogor, Indonesia and a voucher specimen (No. Bo-104) was deposited at the herbarium.

Plant Extraction

Dried ground stembark (1.8 kg) of *C. pentandrus*was extracted with methanol exhaustively (14 L) at room temperature for 7 days. After removal of the solvent under vacuum, the viscous concentrated of MeOH extract (340.01 g) was first suspended in H_2O and then partitioned with *n*-hexane, EtOAc, and *n*-butanol, successively. Evaporation resulted in the crude extracts of *n*-hexane (10.90 g), EtOAc (25.18 g), and *n*-butanol (228.63 g), respectively. The EtOAc soluble fraction (25.18 g) was fractionated by column chromatography on silica gel using a gradient *n*-hexane, EtOAc and MeOH to give eight fractions (A–H), combined according to TLC results. Fraction B (1.73 g) was subjected to column chromatography over silica gel using a gradient mixture of CH_2Cl_2-EtOAc (10:0-1:1) as eluting solvents to afford sixsubfractions (B1-B6). Subfraction B3 (460 mg) was chromatographed on a column of silica gel, eluted with CH_2Cl_2:EtOAc (7;3), to give five subfractions (B3A–B3E). Subfraction B3B was separated on preparative TLC, eluted with CH_2Cl_2:EtOAc (6.5:3.5), to give **1** (18.5 mg). Subfraction B3C (100 mg) was chromatographed on a column chromatography of silica gel, eluted with CH_2Cl_2:EtOAc (6.5:3.5), to give **2** (37.9 mg).

3. RESULT AND DISCUSSION

The methanol extract from the dried bark of *C. pentandrus* was concentrated and extracted successively with *n*-hexane, ethyl acetate and *n*-butanol The ethyl acetate extractsshowed rich of flavonoid compound by detecting under uv light and $AlCl_3$ reagent.. By using flavonoid test to guide separations, the ethyl acetate fraction was separated by combination of column chromatography on silica gelG60 and preparative TLC on silica gel GF_{254} to afford two flavanoid compounds 1 and 2.

Catechin (1). Yellow amorphous powder;m.p. 176-177°C;UV (MeOH) λ_{max} nm (log ε) 275 (3.93), IR (KBr) ν_{maks}(cm^{-1})3327, 1570, 1156, 1051 and 827. ^{1}H-NMR (DMSO-d_6, 500 MHz) and ^{13}C-NMR (DMSO-d_6, 125 MHz), see Tables 1; HR-TOFMS (positive ion mode)*m/z* 291.0878 $[M+H]^+$, (calcld. for$C_{15}H_{14}O_6$, *m/z*290.0787).

Epicatechin (2). Yellow amorphous powder; m.p. 176-177 °C; UV (MeOH) λ_{max} nm (log ε) 276 (3.94), IR (KBr) ν_{maks} (cm^{-1})3330, 1550, 1140, 1045 and 830. ^{1}H-NMR (DMSO-d_6, 500 MHz) and ^{13}C-NMR (DMSO-d_6, 125 MHz), see Tables 1; HR-TOFMS (positive ion mode) *m/z* 291.0878 $[M+H]^+$, (calcld. for $C_{15}H_{14}O_6$, *m/z*290.0787).

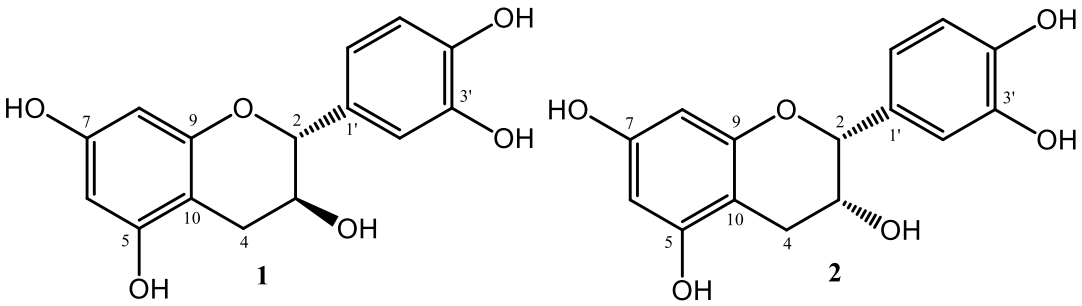

Figure 1. Structures of Compounds **1** and **2**.

Compounds **1** was obtained as a yellow powder. HR-TOFMS spectrum of **1**showed $[M+H]^+$ *m/z* 291.0878 (calcd *m/z* 290.0787), which corresponded to the molecular formula of $C_{15}H_{14}O_6$ thus requiring nine degrees of unsaturations. UV spectra in MeOH showed λ_{max} nm (log ε) 275 (3.93), indicated the presence of flavan-3-ol skeleton (Andersen and Markham, 2006). The IR spectra showed absorption peaks at 3327, 1570, 1156, 1051, 827 cm^{-1}due to the presence of hydroxyl groups, C=C aromatic rings, symmetric and asymmetric C-O-C and substituted benzene ring, respectively. The ^{1}H-NMR spectrum of the compound showed the present of five olefinic methine protons, consist of twoprotons resonating at δ_H 5.77 and 5.76 (each 1H, d, *J* = 2.1 Hz) were assigned for H-6 and H-8 in A ring, three protons resonating at δ_H 6.83 (1H, d, *J* = 1.7, H-2′), 6.61 (1H, d, *J* = 8.6 Hz, H-5′), and 6.65 (1H, dd, *J* = 1.7, 8.6 Hz, H-6′) were assigned to ABC proton-type in C ring and two oxygenated methine protons at δ_H 4.41 (1H, d, *J* = 7.8 Hz, H-2) and 3.83 (1H, dd,*J*=7.8, 5.5, H-3), and one methylene proton at δ_H 2.70 (1H, dd, *J* = 8.3, 16.3 Hz, H-4) and 2.37 (1H, dd, *J* = 5.5, 16.3 Hz, H-4). Two *meta*-protons at ring A, evidenced by *J* constant coupling of H-6 and H-8 (2.1 Hz) and HMBC correlations between H-6 to C-5, C-7, and H-7 to C-7, C-9 (Figure 2). A trisubstituted benzene was observed at δ_H 6.83 (1H, d, *J* = 1.7, H-2′), 6.61 (1H, d, *J* = 8.6 Hz, H-5′), and 6.65 (1H, dd, *J* = 1.7, 8.6 Hz, H-6′) and ^{1}H-^{1}H COSY cross peak H-5′/H-6′ (Figure 2). The flavan-3-ol skeleton in ring C was evidenced by ^{1}H-^{1}H COSY cross peak H-2/H-3/H-4 also from HMBC correlation between H-2 to C-9 and C-1′, H-3 to C-2 and C-4, and H-4 to C-5, C-9, and C-10. The ^{13}C NMR and DEPT 135^0 spectra of the compound showed the presence of five olefinic methines and seven quartenary olefinic carbon (12 sp^2 carbons), two oxymethine, and one methylene. These functionalities accounted for six of the total nine degrees of unsaturation, and the remaining three degrees of unsaturation were consistent with the flavan-3-ol structure. Based on the signals of the ^{1}H-NMR spectra the compound, the coupling constant between H-2/H-3 (^3J) was 7.8 Hz, indicated that conformation of C-2 and C-3 were axial-axial, respectively. A detail comparison of NMR spectra of **1** to those of catechin (Davis *et al*., 1996), revealed that the structure were very similar, consequently compound **1** was identified as a catechin.

Compounds **2** was obtained as a yellow powder. HR-TOFMS spectrum of **2** showed $[M+H]^+$ *m/z* 291.0878 (calcd *m/z* 290.0787), which corresponded to the molecular formula of $C_{15}H_{14}O_6$ thus requiring nine degrees of unsaturations. UV spectrum of **2**(MeOH) λ_{max} nm (log ε) 276 (3.94), IR (KBr) ν_{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 spectra of **2** was very similar to those of 1, except ^3J of H-2/H-3 was 1.6 Hz, indicate that the conformation of C-2 and C-3 were axial-equatorial, respectively. A detail comparison of NMR spectra of **2** to those of epicatechin (Davis *et al*., 1996) was very similar, consequently compound **2** was identified as a epicatechin.

This flavonoid compounds, catechin (**1**) and epicatechin (**2**), was isolated in this plant for the firat time and support also the occurance of flavonoid compound in *Chisocheton* genus besides the limonoid as a chemical marker.

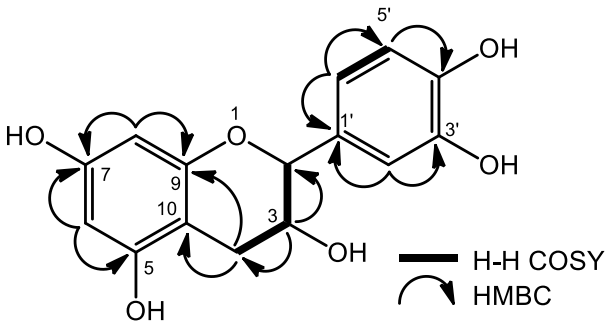

Figure.2. Selected HMBC and ^{1}H-^{1}H COSY correlations for **1** and **2**.

Table 1. NMR data (500 MHz for ^{1}H and 125 MHz for ^{13}C, in DMSO-d_6) for **1** and **2**

Position Carbon	**1**		**2**	
	^{1}H NMR δ_H (Int., mult, *J*=Hz)	^{13}C NMR δc (mult.)	^{1}H NMR δ_H (Int., mult, *J*=Hz)	^{13}C NMR δc (mult.)
2	4.41 (1H, d, 7.8)	81.5 (d)	4.66 (1H, d, 1.6)	78.5 (d)
3	3.83 (1H, dd, *J*=7.8, 5.5,	67.5 (d)	4.03 (1H, dd, 1.6, 4.6)	66.1 (d)
4	2.70 (1H, dd, 8.3, 16.3)	27.2 (t)	2.68 (1H, dd, 4.5, 16.1)	27.9 (t)
	2.37 (1H, dd, 5.5, 16.3)		2.61 (1H, dd, 2.8, 16.1)	
5		156.7 (s)		156.5 (s)
6	5.77 (1H, d, 2.1)	94.9 (d)	5.78 (1H, d, 2.1)	95.0 (d)
7		156.3 (s)		156.1 (s)
8	5.76 (1H, d, 2.1)	94.2 (d)	5.77 (1H, d, 2.1)	94.6 (d)
9		156.0 (s)		156.0 (s)
10		99.5 (s)		98.7 (s)
1′		131.0 (s)		130.9 (s)
2′	6.83 (1H, d, 1.7)	114.8 (d)	6.82 (1H, d, 1.8)	114.6 (d)
3′		144.9 (s)		144.9 (s)
4′		144.4 (s)		144.6 (s)
5′	6.61 (2H, d, 8.6)	113.9 (d)	6.60 (2H, d, 8.4)	114.0 (d)
6′	6.65 (1H, dd, 1.7, 8.6)	118.7 (d)	6.63 (1H, dd, 1.8, 8.4)	118.1 (d)

4. CONCLUSIONS

Two known flavanoid componds catechin (**1**) and epicatechin (**2**) have been isolated from the stembark of *Chisocheton pentandrus*. This compound was isolated from this plant for the first time.

5. ACKNOWLEDGEMENTS

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REFERENCE

Agbedahunsi JM, Fakoya FA, Adesanya SA. 2004. Studies on the anti-inflammatory and toxic effects of the stem bark of *Khaya ivorensis* (Meliaceae) on rats.*Phytomedicine*. 11(6): 504-508.

Andersen OM, Markham KR. 2006. Flavonoids: Chemistry, Biochemistry, and Applications. New York (US): Taylor and Francis.

Awang K, Lim CS, Mohamad K, Morita H, Hirasawa Y, Takeya K, Thoison O, Hadi AHA. 2007. Erythrocarpines A-E, new cytotoxic limonoids from *Chisochetonerytrocarpus.Bioorg. Med. Chem.*15(17): 5997- 6002.

Davis AL, Cai Y, Davies AP, Lewis JR. 1996. ^{1}H and ^{13}C NMR assignments of some green tea polyphenols. *Mag. Res. Chem*. 34(11): 887-890.

Heyne K. 1982. The Useful Indonesian Plants. Jakarta (ID): Research and Development Agency, Ministry of Forestry.

Inada A, Sukemawa M, Murata H, Nakanishi T, Tokuda H, Nishino H, Iwashima A, Darnaedi D, Murata J.1993. Phytochemical studies on Maleaceous Plant. Part VIII. Structures and Inhibitory Effects on EpsteinBarr Virus Activation of Triterpenoids from Leaves of *Chisocheton macrophyllus* King. *Chem. Pharm. Bull*. 41: 617–619.

Joshi MN, Chowdhury BL, Vishnoi SP, Shoeb A, Kapil RS. 1987. Antiviral activity of (+)-odorinol.*Planta Medica*. 53: 254-255.

Katja DG, Farabi K, Nurlelasari, Harneti D, Mayanti M, Supratman U, Awang K,Hayashi H. 2016. Cytototoxic constituents from the bark of *Chisocheton cumingianus* (Meliaceae).*Journal of Asian Natural Products Research*. 19(2): 194-200.

Lim CS. 2008. Master Science Thesis, Department of Chemistry, University Malaya, Malaysia.

Mabberly DJ, Pannel CM. 1989. Meliaceae in Tree Flora of Malaya, vol 4, Forest Research Institute Malaysia, Kuala Lumpur, Malaysia.

Mohamad K, HirasawaY, Lim CS, Awang K, Hamid A, Hadi A, Takeya K, Morita H. 2008. Ceramicines a and walsogyne a, novel limonoids from two species of Meliaceae. *Tetrahedron Letter*.49: 4276–4278.

Mohamad K, HirasawaY, Litaudon M, Awang K, Hamid A, Hadi A, Takeya K, Ekasari W, Widyawaruyanti A, ZainiNC, Morita H. 2009. Ceramicines B–D, new antiplasmodial limonoids from *Chisocheton ceramicus*. *Bioorg. Med. Chem*.17(2): 727– 730.

Najmuldeen IA, Hadi AHA, Awang K. 2011. Chisomicines A-C, limonoids from *Chisocheton ceramicus. Journal of Natural Products*. 74(5): 1313-1317.

Nurlelasari, Katja DG, Harneti D, Wardayo MM, Supratman U, Awang K. 2017. Limonoids from the seeds of *chisocheton macrophyllus. Chemistry of Natural Compounds*.53(1): 83-87.

Vossen VD, HAM, Umali BE. (Editors). 2002. *Plant resources of south-east Asia* No. 14, vegetables oils and fats. Bogor (ID): Prosea Foundation.