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A New Cinnamyl Acid Derivative from the Roots of *Willughbeia coriacea* Wall.

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Abstract – A new cinnamyl acid derivative, willughbein A (**1**) along with pinoresinol (**2**), alyterinate A (**3**), and scopoletin (**4**), were isolated from the roots of *Willughbeia coriacea* Wall. The structure of **1** has been determined based on HRESIMS, 1D, and 2D NMR spectral data. All of the isolates were evaluated for their cytotoxicity against three human cancer cells (HeLa, T47D, MCF-7, and P-388). Compound **3** showed moderate activity against P-388 cells with an IC_{50} value of 3.04 μg/mL.
Keywords – Willughbein A, Cinnamyl acid, *Willughbeia coriacea*, Cytotoxic

Introduction

Willughbeia coriacea Wall (Apocynaceae) is one of Kalimantan island endemic plants, and local people consume its fruit. The public knows *W. coriacea* as Dangu, and its stew used for diarrhea. Information on secondary metabolite compounds from *W. coriacea* has no scientific report.[1]

W. cochinchinensis from Vietnam is the only one reported about the content of secondary metabolites. *W. cochinchinensis* produces phenolic compounds, including cinnamic acids, lignans, coumarins, and diarylheptanoids. The phenolic compound of *W. cochinchinensis* shows activity as an inhibitor of acetylcholinesterase (AChE) and butylocholinesterase (BChE), which causes Alzheimer's disease.[2]

In summary, we reported the isolation of a new cinnamyl acid derivative, willughbein A (**1**), together with three known compounds from the roots of *W. coriacea*. The cytotoxic activity of compounds **1 - 4** against four human cancer cells (HeLa, T47D, MCF-7, and P-388) also reported.

Experimental

General experimental procedures – UV spectra measured with a Shimadzu UV-1800 recording spectrophotometer. IR spectra measured with a Shimadzu IR Tracer-100 spectrophotometer, respectively. 1D and 2D NMR run on a JEOL ECA 400 spectrometer in $CDCl_3$. HRESIMS measured on a Waters LCT Premier XE ESI-TOF mass spectrometer. Gravity column chromatography and planar radial chromatography were carried out using silica gel 60, Sephadex LH-20, and silica gel 60 F_{254}. The Spot of compounds on TLC visualized under a UV lamp and anisaldehyde reagent.

Plant materials – The roots of *W. coriacea* collected from Hajak Village, Muara Teweh, North Barito, Central Kalimantan, Indonesia, in September 2018. Mr. Ismail Rachman, a senior botanist from Herbarium Bogoriense, identified plant material. Voucher specimens (WC 20183) stored at the Bogoriense Herbarium, Center for Research and Development of Biology, National Science Institute, Bogor, Indonesia.

Extraction and isolation – The powdered and dried roots of *W. coriacea* (3.48 kg) were extracted with methanol for 24 hours at room temperature using the maceration method. The extraction of the material was carried out twice. The maceration results were filtered, and the solvent was evaporated with a rotary vacuum evaporator so that the methanol extract (400 g) was obtained. The MeOH extract redissolved in MeOH-water (9:1) and was partitioned with *n*-hexane (135 g) and EtOAc (12.4 g). The EtOAc extract (12 g) was further fractionated by gravity column chromatography on silica gel (150 g) eluted with *n*-hexane-EtOAc by increasing polarity (9:1, 4:1, and 7:3) to give three significant fractions A-C. Fraction B (2.15 g) separated by Sephadex LH-20 eluted with methanol to provide subfractions B_1-

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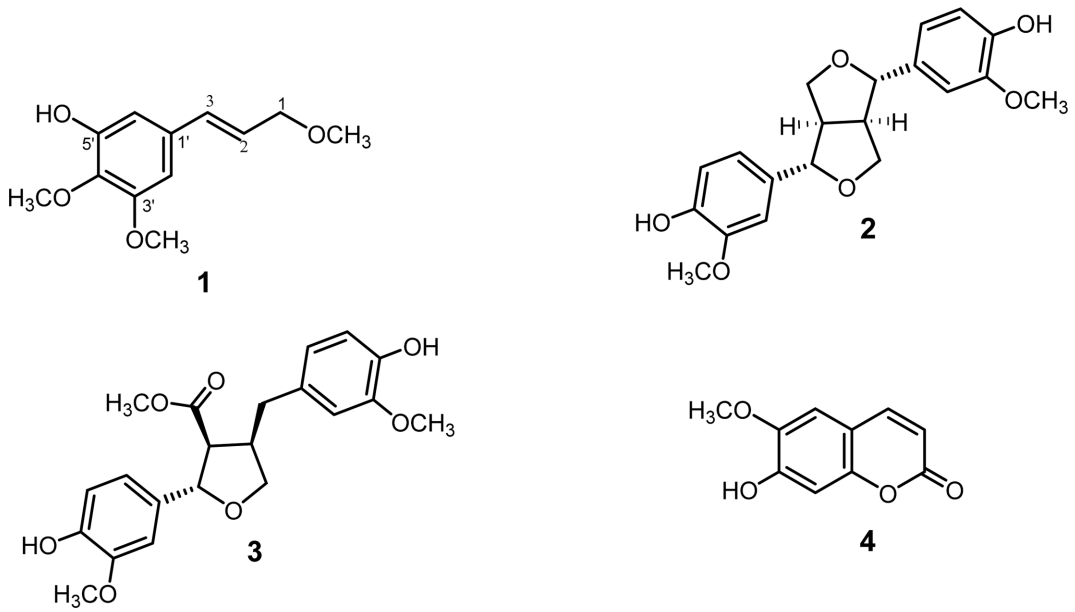

Fig. 1. The phenolic compounds **1** - **4** from the roots of *W. coriacea*.

B_2. The purification of subfraction B_2 by planar radial chromatography using *n*-hexane-diisopropyl ether (from 4:1 to 3:7) to yield compounds **2** (14 mg), and **3** (17 mg). Fraction C (13 g) was fractionated using Sephadex LH-20 and eluted with methanol to give subfractions C_1-C_2. Subfraction C_2 was purified by planar radial chromatography using *n*-hexane-acetone (from 9:1 to 1:1) to yield compounds **1** (17 mg), and **4** (32 mg).

Willughbein A (**1**) – Colorless oil, UV (MeOH) λ_{max} nm (log ε) : 220 (4.14), 244 (3.56), and 268 (3.79). IR (KBr) ν_{max} cm^{-1}: 3525, 1585, and 1126. ^1H and ^{13}C NMR see Table 1. HRESIMS: *m/z* $[M+H]^+$ calcd. for $C_{12}H_{17}O_4$ 225.1121, found 225.1127.

Pinoresinol (**2**) – Yellow solid, mp. 125 - 126 ºC. The ^1H and ^{13}C NMR spectral data in $CDCl_3$ of **2** very similar to published data.[3]

Alyterinate A (**3**) – White solid, mp. 114 - 115 ºC. The ^1H and ^{13}C NMR spectral data in $CDCl_3$ of **3** very similar to published data.[4]

Scopoletin (**4**) – White solid, mp. 203 - 204 ºC. The ^1H and ^{13}C NMR spectral data in $CDCl_3$ of **4** very similar to published data.[3]

Cytotoxic activity – All of the compounds (**1** - **4**) were assayed for their cytotoxicity against HeLa (human cervical cells), P-388 (murine leukemia cells), MCF-7, and T47D (human breast cells) according to the MTT method. Each cell-cultured RPMI-1640 medium was supplemented by 10% fetal bovine serum at 37 ºC for 48 h in a 5% CO_2 incubator. Briefly, before the active compounds were added, approximately 4×10^4 cells/well were seeded in 96-well and incubated at 37 ºC for 24 h in a CO_2 incubator. The death cells by each the active compounds **1** - **4** were measured using a microplate reader spectrometer at λ 540 nm. The IC_{50} values can be calculated through extrapolation 50% percentage cells vs. various concentrations of the active compounds using regression analysis. Doxorubicin was used as the positive control for HeLa, MCF-7, T47D, and artonin E for P-388 cells.[5-8]

Result and Discussion

Compound **1** or willughbein A isolated as a colorless oil, and showed a positive molecular ion peak $[M+H]^+$ at *m/z* 225.1127 (calculated 225.1121), indicating a chemical formula of $C_{12}H_{16}O_4$ by HRESIMS spectra. The UV spectra showed maximum absorption at λ_{max} (log ε): 220 (4.14), 244 (3.56), and 268 nm (3.79) characteristic for propenyl phenol derivative.[9] Compound (**1**) showed absorptions for hydroxyl (3456 cm^{-1}), aromatic (1601 - 1496 cm^{-1}), and ether (1178 cm^{-1}) groups, respectively by IR spectra.[10] The ^1H NMR spectra (Table 1) of **1** showed the presence of a *trans* 3-methoxy-1-propenyl signal at δ_H 6.53 (1H, d, J = 15.9 Hz, H-3), δ_H 6.20 (1H, dt, J = 15.9; 6.1 Hz, H-2), δ_H 4.09 (2H, dd, J = 6.1; 1.4 Hz, H-1), and δ_H 3.39 (3H, s, 1-OCH_3).[11] The ^1H NMR spectra of **1** also showed that the presence of a 1,3,4,5-tetrasubstituted benzene at δ_H 6.62 (2H, s, H-2′/H-6′), a hydroxyl signal at δ_H 5.95 (1H, s, 5′-OH), two methoxyl signals at δ_H 3.84

Table 1. NMR data (400 MHz, $CDCl_3$) of willughbein A (**1**)

No.C	δ_H (mult, *J* in Hz)	δ_C	HMBC
1	4.09 (*dd*, 6.1; 1.4)	73.1	C-2; C-3; 1-OCH_3
2	6.20 (*dt*, 15.9; 6.1)	125.6	C-1; C-1′
3	6.53 (*d*, 15.9)	132.4	C-1; C-2′/6′
1′	-	132.5	-
2′/6′	6.62 (*s*)	103.5	C-1′; C-3′/5′, C-2′/6′; C-4′
3′	-	153.4	-
4′	-	149.2	
5′	-	137.9	-
5′-OH	5.95 (*s*)	-	C-5′
1-OCH_3	3.39 (*s*)	58.1	C-1
3′-OCH_3	3.87 (*s*)	56.1	C-3′
4′-OCH_3	3.84 (*s*)	61.0	C-4′

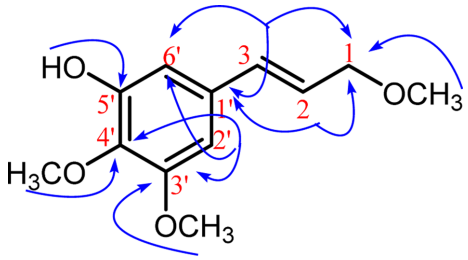

Fig. 2. Selected HMBC correlations of willughbein A (**1**).

(3H, s, 4′-OCH_3), and δ_H 3.87 (3H, s, 3′-OCH_3). The ^{13}C NMR spectra of compound **1** exhibited 12 carbon signals, consisting of three methoxyl carbons, one methylene carbon, four methine carbons, and four quaternary carbons. The 2D NMR (HMQC and HMBC) explained the position of hydroxyl and methoxyl groups (Fig. 2). The *trans* ethene signal at δ_H 6.20 (H-2) correlated with a quaternary carbon at δ_C 132.5 (C-1′), and a methylene carbon at δ_C 73.1 (C-1). The *trans* ethene signal at δ_H 6.53 (H-3) correlated with a methine carbons of aromatic at δ_C 103.5 (C-2′/C-6′) and a methylene carbon at δ_C 73.1 (C-1). The methylene signal at δ_H 4.09 (H-1) correlated to δ_C 125.6 (C-2), δ_C 132.4 (C-3), and δ_C 58.1 (1-OCH_3). The methoxyl group at δ_H 3.39 (1-OCH_3) showed correlations with a methylene carbon at δ_C 73.1. The signal of aromatic at δ_H 6.62 (H-2′/H-6′) showed correlations with three oxyaryl carbons at δ_C 153.4 (C-3′), δ_C 149.2 (C-4′), δ_C 137.9 (C-5′), a methine carbon at 103.5 (C-2′/C-6′), and a quaternary carbon at δ_C 132.5 (C-1′). Furthermore, the signal of the hydroxyl group at δ_H 5.95 (5′-OH) correlated to δ_C 137.9 (C-5′). The signal of the methoxyl at δ_H 3.87 (3′-OCH_3) correlated to δ_C 153.4 (C-3′), and δ_H 3.84 (4′-OCH_3) showed correlations with an oxyaryl carbon at δ_C 149.2 (C-4′).

From the HRESIMS, and NMR spectrum, the structure of **1** was assigned as willughbein A. Other HMBC correlations were supporting the structure of **1**, shown in Table 1 and Fig. 2. All of the compounds (**1** - **4**) were analyzed for their cytotoxicity against HeLa (human cervical cells), P-388 (murine leukemia cells), MCF-7, and T47D (human breast cells) by MTT method. Artonin E and doxorubicin were used as a positive control.[12] All of the compounds were inactive against three human cells, except compound **3** showed moderate activity against P-388 cells.

Table 2. Cytotoxicity data of compounds **1** - **4**

Compounds	IC_{50} (μg/mL)			
	HeLa	T47D	MCF-7	P-388
Willughbein A (**1**)	> 100	13.33 ± 1.42	> 100	11.95 ± 0.89
Pinoresinol (**2**)	90.21 ± 1.35	5.09 ± 0.37	> 100	17.38 ± 0.95
Alyterinate A (**3**)	83.04 ± 1.82	34.41 ± 1.42	> 100	3.04 ± 0.27
Scopoletin (**4**)	> 100	> 100	> 100	> 100
Doxorubicin	46.11 ± 0.45	23.18 ± 0.45	57.70 ± 0.51	-
Artonin E	-	-	-	1.33 ± 0.02

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