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Antileukemic activity of lignans and phenylpropanoids of *Cinnamomum parthenoxylon*

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ABSTRACT

In this study, we evaluated the in vitro cytotoxicity of fractions and isolated constituents from *Cinnamomum parthenoxylon* woods against human leukemia HL-60 and U937 cells. The *n*-Hex, EtOAc, and MeOH–H_2O fractions of the woods inhibited cell proliferation in both cell lines. Our phytochemical investigation of the *n*-Hex and EtOAc fractions led to the isolation of lignans and phenylpropanoids, whose chemical structures were confirmed by spectroscopic analyses. All isolated compounds were evaluated for their in vitro antileukemic activity; especially, hinokinin and cubebin exhibited strong inhibition toward U937 cell proliferation. Morphological observation indicated that these cytotoxic actions were mediated by apoptosis. Our findings suggested that an oxygenated functional group at the C-9 position in dibenzylfuran skeleton contributed their potency. In addition, these results enhanced the ethnopharmacological value of *C. parthenoxylon*.

Cinnamomum is one of the larger genera of *Lauraceae* family, with more than 250 species of wildlife worldwide. A number of these species are used as spices, especially in foods, fragrances, fumigants, and traditional medicines.[1,2] Cytotoxic and apoptotic activities of essential oils and several constituents of cinnamon species (Ceylon cinnamon *Cinnamomum verum*, Indonesian cinnamon *Cinnamomum burmannii*, Saigon cinnamon *Cinnamomum loureirii*, and Chinese cinnamon *Cinnamomum cassia*) have been shown in various cancer cell lines.[3–6] *Cinnamomum parthenoxylon* (synonym *Cinnamomum porrectum*) is distributed in South and East Asian regions. *C. parthenoxylon* has been reported to exhibit growth inhibition against strains of methicillin-resistant *Staphylococcus aureus*.[7] Given its significant and varied pharmacological efficacies including antifungal, antioxidant, and antidiabetic properties,[8,9] there has been increased interest in the research of *C. parthenoxylon*. Previous phytochemical investigations led to the isolation of several phenylpropanoids such as safrole, cinnamaldehydes, and alkyl *trans*-ferulates from the woods.[10]

Natural product frameworks are found in antineoplastic molecules (e.g., etoposide, teniposide, vinorelbine, and topotecane), making them ideal candidates for cancer chemoprevention or associated agents in clinical treatment. Within the scope of our ongoing program aimed at the systematic chemical study of Indonesian medicinal plants with a biologically intriguing profile,[11–13] we herein describe the utilization of chemical constituents as antileukemic agents from *C. parthenoxylon*.

Woods of *C. parthenoxylon* (1.15 kg) were grounded and macerated at room temperature with *n*-hexane and then methanol. Removal of solvents under reduced pressure afforded each extract (*n*-hexane; 77.9 g and methanol; 28.1 g). A portion of methanol extract (10 g) was suspended in water and partitioned with EtOAc, to yield EtOAc (5.2 g) and methanol–water (4.8 g) fractions. We evaluated the in vitro cytotoxicity of these fractions (*n*-Hex, EtOAc, and MeOH–H_2O) against human leukemia HL-60 and U937 cells using the CCK-8 assay.[13,14] Cells in the rapid phase of growth were exposed to tested fractions for 48 h. Treatment of the cells with each fraction indicated that they have the antileukemic potency in both cell lines. At a final concentration of 100 μg/ml, these fractions inhibited the cell proliferation, with cell survival rates of 47.1% (*n*-Hex), 26.6% (EtOAc), 50.7% (MeOH–H_2O) in HL-60 cells, and 28.4% (*n*-Hex), 38.1% (EtOAc), 52.2% (MeOH–H_2O) in U937 cells. Moreover, we observed morphological changes in HL-60 cells stained with Hoechst 33342.[11–15] These fractions induced apoptotic actions including fragmentation of the nuclei and condensation of the chromatin (Fig. 1).

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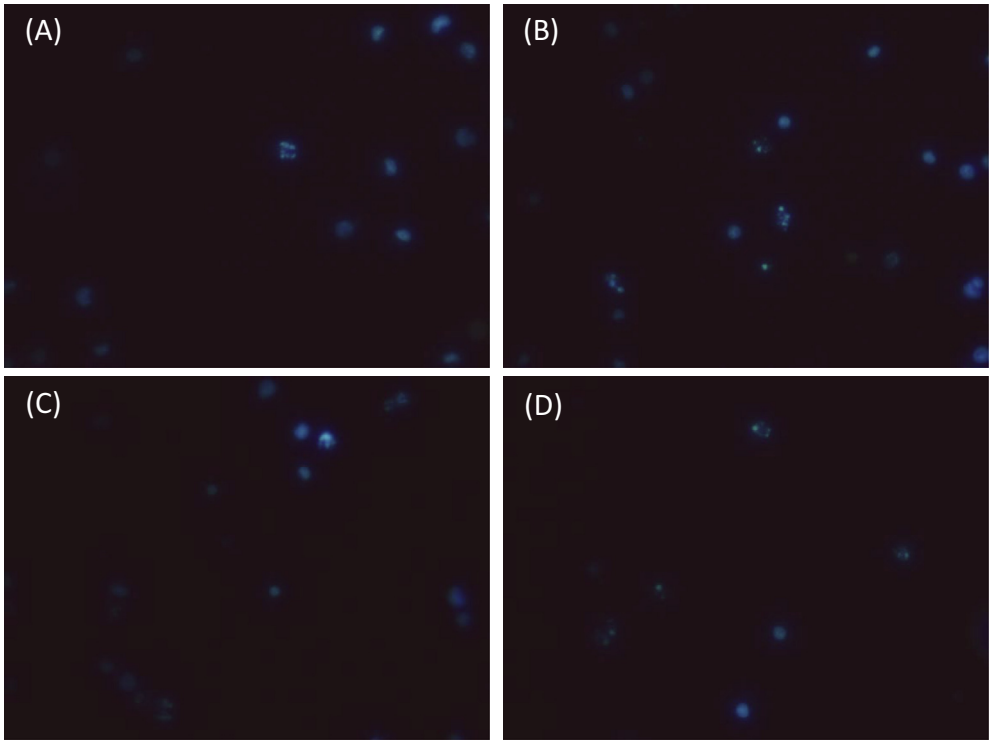

Figure 1. Morphological changes of HL-60 and U937 cells induced by *n*-hexane and EtOAc fractions at a final concentration of 100 μg/mL. Cells were treated with the fractions for 48 h, and stained with Hoechst 33342. (A) *n*-Hex in HL-60; (B) *n*-hex in U937; (C) EtOAc in HL-60; (D) EtOAc in U937.

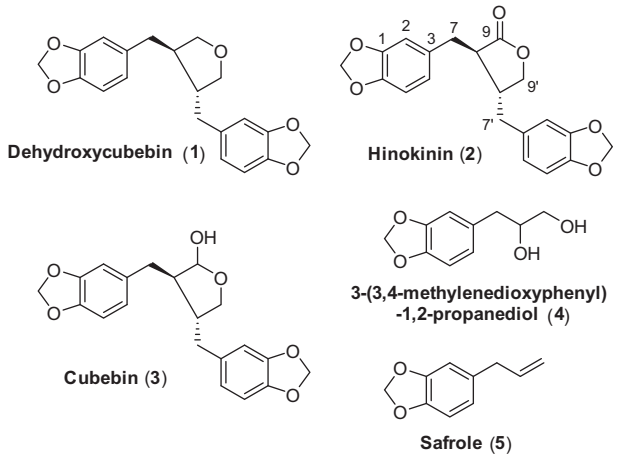

Figure 2. Chemical structures of lignans and phenylpropanoids isolated from *Cinnamomum parthenoxylon*.

Next step was accelerated in the direction of identification of the potential compounds responsible for antileukemic properties. The EtOAc fraction was separated by SiO_2 gel CC and PTLC to give four constituents (compounds **1**–**4**). Although the molecular formula of compound **1** was established as $C_{20}H_{20}O_5$ from HREIMS of the peak at *m*/*z* 340.1305 $[M]^+$ (calcd 340.1311), only ten carbon signals were observed in ^{13}C NMR spectrum, indicating compound **1** as a symmetric analog of two $C_{10}H_{10}O_2$ units. A remaining oxygen atom was presumed to have a place between them. The occurrence of a 1,3,4-trisubstituted aromatic ring was deduced from two doublet at δ_H 6.72 (*J* = 7.8 Hz) and 6.64 (*J* = 1.4 Hz) and a double doublet at δ_H 6.61 (*J* = 7.8 and 1.4 Hz) in ^1H NMR spectrum. A singlet at δ_H 5.93 revealed a methylenedioxy group attached to the benzene ring.[16] A methylene proton at δ_H 3.79 (dd, *J* = 11.5 and 0.8 Hz) and 3.50 (dd, *J* = 11.5 and 4.1 Hz), a methine proton at δ_H 1.84 (br s), and a methylene proton at δ_H 2.76 (dd, *J* = 13.8 and 8.7 Hz) and 2.62 (dd, *J* = 13.8 and 5.5 Hz) and the COSY suggested a link of Ph—CH_2—CH—CH_2—O moiety. On the basis of the evidences, the structure of compound **1** was identified as a dibenzylfuran type lignan, dehydroxycubebin.[17,18] The HREIMS analyses of compounds **2** and **3** gave their molecular formula $C_{20}H_{18}O_6$ and $C_{20}H_{20}O_6$, respectively. These compounds had the same substructures, however, did not express the symmetry in their skeletons. The presence of a carbonyl group appeared at δ_C 178.5 clarified compound **2** as a dibenzylbutyrolactone type lignan, hinokinin.[19] The downshifted proton of H-9 confirmed compound **3** as a dibenzylbutyrolactol type lignan, cubebin.[20] The isolation of these lignans **1**–**3** from *C. parthenoxylon* is reported in this article for the first time. The HRESIMS of compound **4** showed a $[M+Na]^+$ peak at *m*/*z* 219.0622 (calcd 219.0633), giving the molecular formula $C_{10}H_{12}O_4$. The spectroscopic studies resulted in the structural determination of compound **4** as a monomeric analog, 3-(3,4-methylenedioxyphenyl)-1,2-propanediol.[21] Purification of the *n*-hexane extract on column chromatography on SiO_2 gel isolated safrole (**5**).[22] The chemical structures of the isolated compounds were depicted in Figure 2.

Table 1
IC_{50} values of constituents isolated from *Cinnamomum parthenoxylon* (means ± SEMs, *n* = 3)

Compound	IC_{50} (μM)	
	HL-60	U937
Dehydroxycubebin (**1**)	51.1 ± 0.9	40.2 ± 2.4
Hinokinin (**2**)	31.3 ± 0.5	10.4 ± 0.3
Cubebin (**3**)	38.2 ± 0.7	15.7 ± 1.1
3-(3,4-Methylenedioxyphenyl)-1,2-propanediol (**4**)	60.0 ± 0.4	50.8 ± 1.6
Safrole (**5**)	53.2 ± 0.7	52.3 ± 1.6

All isolated compounds were evaluated for their in vitro antileukemic activity[13,14] and the results are shown in Table 1. Their IC_{50} values were less than 60 μM in all cases, as compared with