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Phytochemicals from *Cunninghamia konishii* Hayata Act as Antifungal Agents

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ABSTRACT: The aims of the present study were to isolate and identify the antifungal compounds from the ethanolic extract of *Cunninghamia konishii* wood and to evaluate their antifungal activities against wood decay fungi. The results showed that the *n*-Hex soluble fraction of the ethanolic extract from *C. konishii* wood had an excellent inhibitory effect against *Lenzites betulina*, *Trametes versicolor*, *Laetiporus sulphureus*, and *Gloeophyllum trabeum*, with IC_{50} values of 33, 46, 62, and 49 μg/mL, respectively. By following the bioactivity-guided fractionation procedure, four sesquiterpenes, T-cadinol, cedrol, T-muurolol, and (−)-*epi*-cedrol, and three diterpenes, 13-*epi*-manool, *cis*-abienol, and isoabienol, were identified from the active subfractions. Among the main constituents of the ethanolic extract from *C. konishii*, T-cadinol, cedrol, and T-muurolol efficiently inhibited the growth of four wood-rot fungi at the concentration of 100 μg/mL, with antifungal indices of 51.4−100.0%, 68.3−100.0%, and 39.5−100.0%, respectively. Results of this study show that the ethanolic extract of *C. konishii* wood may be considered as a potent source of T-cadinol, cedrol, and T-muurolol as new natural antifungal agents.

KEYWORDS: *Cunninghamia konishii, wood, extract, fungi, antifungal activity*

INTRODUCTION

Wood, a naturally occurring polymer composite, is widely used for home furnishings and construction materials, but unprotected wood is susceptible to wood rotting fungi and termite, resulting in the reduction of mechanical strength.[1] To extend the service life, wood products are therefore often treated with wood preservatives such as creosote, alkaline copper quaternary (ACQ), and copper azole (CuAz).[2,3] However, these preservative agents will be phased out in the near future due to their potential adverse impact on human health and environmental pollution. Therefore, the search for safer phytochemicals with lower environmental and mammalian toxicity is of major concern and is imperative.[4] Our previous studies showed that *Taiwania cryptomerioides*,[5,6] *Cinnamomum osmophloeum*,[7,8] *Calocedrus macrolepis* var. *formosana*,[1,9] *Chamaecyparis formosensis*,[10] and *Cryptomeria japonica*[11] possessed significant antifungal activities.

Cunninghamia konishii Hayata (Taxodiaceae) is an endemic species widely distributed in northern and central Taiwan at altitudes of 1300−2700 m. The wood of this tree is one of the best building materials. It possesses excellent wood quality with fragrance and outstanding durability. Thus, *T. cryptomerioides*, *C. macrolepis* var. *formosana*, *C. formosensis*, and *Chamaecyparis obtusa* var. *formosana* as well as *C. konishii* are considered five precious woods of Taiwan. Prior studies on the chemical composition of wood, bark, and leaf of *C. konishii* have been reported.[12−20] Among these, Ikeda and Fujita[12,13] were first to study the volatile constituents of the wood from *C. konishii*, which were found to be several terpenoids, including pinene, limonene, sabinene, α-terpenol, *l*-borneol, and *d*-cedrol. In addition, He et al.[17] reported that konishiol from *C. konishii* showed strong bioactivities in brine shrimp (BST) and mosquito larvae (YFM) bioassays as well as cytotoxicities against three human solid tumor cell lines. Although essential oils of *C. konishii* wood have been shown to have strong antifungal activities against wood decay fungi and plant pathogenic fungi,[21] to our best knowledge there is no research investigating the antifungal properties of ethanolic extractives and the antifungal compounds from *C. konishii*. For this reason, the study aimed to isolate and identify the antifungal compounds from the ethanolic extracts of *C. konishii* wood and leaf, and to evaluate their antifungal activities against wood decay fungi.

MATERIALS AND METHODS

Plant Materials. Logs and mature leaves of a 30-year-old *Cunninghamia konishii* Hayata, in the Taxodiaceae class, were collected in January 2008 from the Experimental Forest of National Taiwan University located in Nantou County in central Taiwan. The species was identified by Dr. Nian-June Chung, and voucher specimens (CKW002 and CKL002) were deposited at the laboratory of wood chemistry (School of Forestry and Resource Conservation, National Taiwan University).

Preparation of Plant Extraction and Isolation. Wood chips (5.0 kg d.w.) and leaves (1.85 kg d.w.) of *C. konishii* were prepared from freshly cut trees. Air-dried samples were extracted twice and soaked in 95% ethanol at ambient temperature for 7 days and then filtered. The combined filtrate was concentrated by a rotary evaporator under vacuum at 40 °C to yield 3.42 ± 0.02% and 1.63 ± 0.08% (according to the weight of the dried samples), respectively. The ethanolic extract of wood (162.0 g) was sequentially partitioned into *n*-hexane (*n*-Hex, 70.5 g), ethyl acetate (EtOAc, 72.9 g), *n*-butanol (BuOH, 12.3 g), and water (3.4 g) soluble fractions, respectively.

The active *n*-Hex soluble fraction (50.0 g) was divided into 14 subfractions (H1−H14) using a silica gel column (Merck 70−230

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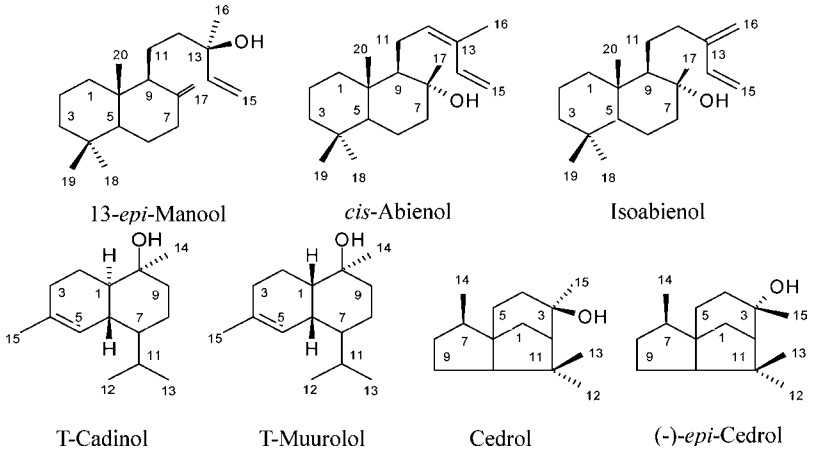

Figure 1. Chemical structures of compounds **1**−**7** isolated from *C. konishii* wood.

mesh, Darmstadt, Germany, 100.0 g) and successively eluted with a stepwise gradient of *n*-hexane/ethyl acetate (from 100/0 to 0/100 by volume). During this step, the active subfraction H5 (130.6 mg) of the *n*-Hex soluble fraction at the concentration of 100 μg/mL showed a strong antifungal activity (antifungal index = 77.3−100.0%). Compounds **1** (12.6 mg, retention time = 10.15 min), **2** (4.8 mg, retention time = 10.86 min), **3** (27.3 mg, retention time = 10.96 min), **4** (13.4 mg, retention time = 12.36 min), **5** (45.6 mg, retention time = 13.58 min), **6** (14.1 mg, retention time = 15.55 min), and **7** (8.6 mg, retention time = 16.92 min) (Figure 1) were isolated and purified from the active subfraction H5 by thin layer chromatography (TLC) with a silica gel 60 coating and high performance liquid chromatography (HPLC; Hitachi model L-7150 pump equipped with a L7490 refractive index detector) with a semipreparative Luna silica column (250 mm × 9.4 mm i.d., 5 μm; Phenomenex, Torrance, CA) (mobile phase, *n*-hexane/ethyl acetate = 98/2; flow rate = 4.0 mL/min; temperature, 25 °C). Structural determination of the active compound was made by spectral analysis. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DMX500 spectrometer at 500 and 125 MHz, and chemical shifts were given in ppm. Mass spectra (MS) were obtained on a Finnigan MAT-95S mass spectrometer. All compounds were identified by comparison with authentic standards.

Fungal Strains. The wood decay fungi were obtained from the Bioresource Collection and Research Center (BCRC) of the Food Industry Research and Development Institute. There were a total of four fungi strains. They were white rot fungi including *Lenzites betulina* (BCRC 35296) and *Trametes versicolor* (BCRC 35253), and brown rot fungi including *Laetiporus sulphureus* (BCRC 35305) and *Gloeophyllum trabeum* (BCRC 31614). Cultures of each of the fungi were maintained on potato dextrose agar (PDA) medium and were stored at 4 °C.

Antifungal Assays. Antifungal assays were performed on the basis of the methods used in our previous studies.[11] Wood and leaf ethanolic extracts, dried fractions, and purified compounds were dissolved in 150 μL of ethanol and then added into 15 mL of PDA to obtain the different final concentrations. Mycelial plugs (5 mm in diameter) from the edges of each culture were incubated in the center of each PDA in 9-cm plates (Petri dish). After transfer of the mycelium of all four fungi strains, the testing Petri dishes were incubated in the dark at 26 ± 2 °C and 70% relative humidity. When the mycelium of fungi had reached the edges of the control Petri dishes (those without ethanolic extract, dried fractions, and purified compounds), the antifungal indices were calculated. Each test was repeated four times, and the data were averaged. The IC_{50} values (the concentration in μg/mL that inhibited 50% of fungi mycelium growth) were calculated by probit analysis. Didecyl dimethyl ammonium chloride (DDAC), a commercially available fungicide, was used as a reference compound. The formula for calculating antifungal indices is as follows:

$$\text{Antifungal index (\%)} = (1 - D_a/D_b) \times 100$$

where D_a is the diameter of the growth zone in the experimental dish (cm), and D_b is the diameter of the growth zone in the control dish (cm).

Statistical Analyses. All results were expressed as the mean ± SD (n = 4). To determine whether there was a statistically significant difference among wood and leaf ethanolic extracts and their soluble fractions from *C. konishii* against the fungi, Scheffe's method of SAS was employed to analyze the difference between antifungal indices calculated. Results with $P < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Antifungal Activity of Wood and Leaf Ethanolic Extracts. To evaluate the antifungal activities of wood and leaf ethanolic extracts from *C. konishii* against wood decay fungi, we first selected four typical fungi, two brown-rot fungi (*L. sulphureus* and *G. trabeum*) and two white-rot fungi (*L. betulina* and *T. versicolor*) as test strains. Figure 2 shows the antifungal activities of wood and leaf ethanolic extracts from *C. konishii* at a concentration of 500 μg/mL against wood decay fungi. Results showed that the antifungal indices of leaf ethanolic extract against the four wood decay fungi were 21.3, 16.5, 25.8, and 24.2% (Figure 2), respectively, with IC_{50} values exceeding 500 μg/mL (Table 1), indicating that it had no significant antifungal effect against wood decay fungi. However, wood ethanolic extract at the concentration of 500 μg/mL exhibited the stronger activity against *L. sulphureus*, *G. trabeum*, *L. betulina*, and *T. versicolor*, with antifungal indices of 97.4, 88.1, 100.0, and 89.6%, respectively (Figure 2). The IC_{50} values were

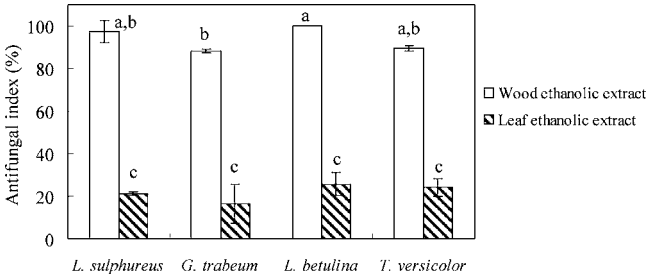

Figure 2. Antifungal activities of wood and leaf ethanolic extracts (500 μg/mL) from *C. konishii* against wood decay fungi. Each experiment was performed four times, and the data were averaged (n = 4). Different letters (a−c) are significantly different at the level of $P < 0.05$ according to Scheffe's test.

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Table 1. IC_{50} Values of Wood and Leaf Ethanolic Extracts from *C. konishii* against Wood Decay Fungi

	IC_{50} values (μg/mL)			
ethanolic extracts	*L. sulphureus*	*G. trabeum*	*L. betulina*	*T. versicolor*
wood	178	66	94	103
leaf	>500	>500	>500	>500

178, 66, 94, and 103 μg/mL, respectively (Table 1). As seen in the above results, wood ethanolic extract from *C. konishii* demonstrated higher activity than leaf ethanolic extract against the four decay fungi. Ho et al.[22] studied the antiwood-decay fungal activities of leaf essential oil from *Machilus philippinensis* and reported that fungi were completely inhibited at concentrations of 50−100 μg/mL. In addition, our previous studies on the antifungal performance of essential oil extracted from *C. macrolepis* var. *formosana* leaf (the antifungal indices were 10.9−67.7% at 1000.0 μg/mL) and heartwood (the antifungal index was 62.8% at 100.0 μg/mL),[1,9] *Cinnamomum osmophloeum* leaf (the IC_{50} values were 52−142 μg/mL),[7,8] and *Cryptomeria japonica* heartwood (the IC_{50} values were 65−110 μg/mL)[11] revealed that they had exhibited excellent antifungal activities. Comparing these values with that obtained in this study reveals that the ethanolic extract of *C. konishii* wood has an excellent antifungal activity.

Antifungal Activity of Different Fractions and Subfractions from Ethanolic Extract of Wood. Four wood decay fungi were employed to evaluate the antifungal potential of each partitioned fraction. As shown in Figure 3, the results of

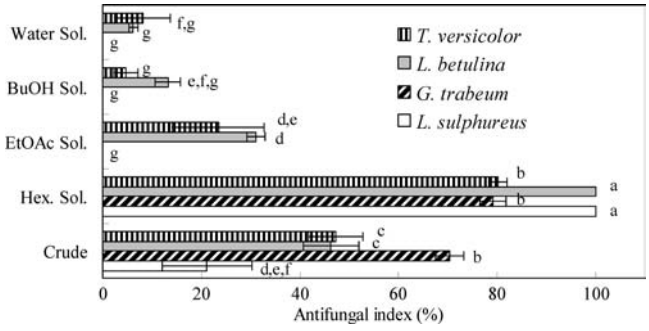

Figure 3. Antifungal activities of various soluble fractions of wood ethanolic extract (100 μg/mL) from *C. konishii* against wood decay fungi. Each experiment was performed four times, and the data were averaged (n = 4). Different letters (a−g) are significantly different at the level of $P < 0.05$ according to Scheffe's test.

antifungal tests showed that the *n*-Hex soluble fraction at the concentration of 100 μg/mL processed the most significant antifungal activity among four partitioned fractions against *L. sulphureus*, *G. trabeum*, *L. betulina*, and *T. versicolor*, with antifungal indices of 100.0, 79.1, 100.0, and 80.1%, respectively. As seen in the comparison of IC_{50} values of each partitioned fraction (Table 2), the *n*-Hex soluble fraction showed an excellent toxicity with IC_{50} values of 62, 49, 33, and 46 μg/mL, while the IC_{50} value of other fractions exceeded 100 μg/mL, indicating that the other fractions did not show antifungal activity. The IC_{50} values obtained from the ethanolic extract of *C. konishii* wood against the four wood decay fungi ranged from 66 to 178 μg/mL (Table 1), revealing clearly that the *n*-Hex soluble fraction is responsible for the excellent antifungal activity of the ethanolic extract from *C. konishii* wood.

Table 2. IC_{50} Values of Various Soluble Fractions of Wood Ethanolic Extract from *C. konishii* against Wood Decay Fungi

	IC_{50} values (μg/mL)			
fungi	Hex sol.	EtOAc sol.	BuOH sol.	water sol.
L. sulphureus	62	>100	>100	>100
G. trabeum	49	>100	>100	>100
L. betulina	33	>100	>100	>100
T. versicolor	46	>100	>100	>100

Accordingly, we continued to analyze the antifungal compounds in the *n*-Hex soluble fraction of ethanolic extract from *C. konishii* wood. The *n*-Hex soluble fraction was divided into 14 subfractions (H1 to H14) by liquid chromatography with silica gel 60 in an open column eluted with gradient *n*-C_6H_{14}/EtOAc from 100/0 to 0/100. Fourteen subfractions at the concentration of 100 μg/mL were screened for their antifungal activities, and the results are shown in Figure 4. Among the 14

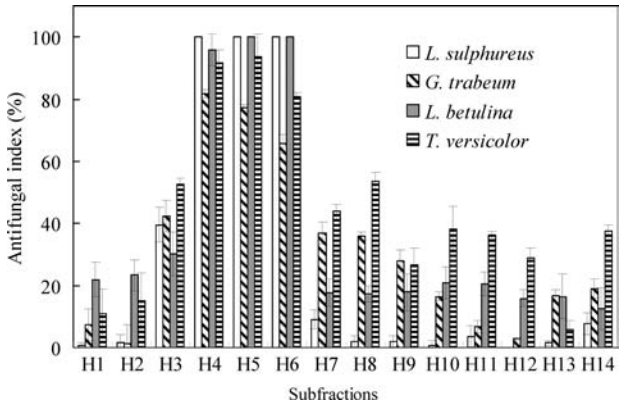

Figure 4. Antifungal activities of subfractions from Hex soluble fraction (100 μg/mL) of *C. konishii* wood against wood decay fungi.

subfractions, only subfractions H4−H6 exhibited an antifungal effect. The antifungal indices of subfractions H4, H5, and H6 at the concentration of 100 μg/mL against the four wood decay fungi were 81.9−100.0, 77.3−100.0, and 65.6−100.0%, respectively. However, subfractions H1−H3 and H7−H14 at the concentration of 100 μg/mL showed antifungal indices of less than 60.0% against the four wood decay fungi (Figure 4) and were consequently considered less active. Accordingly, we continued to isolate compounds from subfraction H5 using HPLC.

Identification of Active Compounds. Bioassay-guided fractionation of subfraction H5 yielded seven active constituents identified by instrumental analyses, including MS, IR, and NMR, and by comparisons with published data.[6,16,23−28] The constituents were characterized as 13-*epi*-manool (**1**), *cis*-abienol (**2**), isoabienol (**3**), T-cadinol (**4**), cedrol (**5**), T-muurolol (**6**), and (-)-*epi*-cedrol (**7**). They were identified on the basis of the following evidence.

Compound 1 (13-epi-Manool). Colorless oil, $[\alpha]_D^{20}$ +46.2° (c = 0.7, $CHCl_3$), EIMS for $C_{20}H_{34}O$ (EIMS: 290), ^1H (500 MHz) NMR (in $CDCl_3$) δ(ppm) 0.65 (s, H-20), 0.77 (s, H-19), 0.84 (s, H-18), 1.25 (s, H-16), 4.45 (d, J = 1.5 Hz, H-17a), 4.78 (d, J = 1.5 Hz, H-17b), 5.03 (dd, J = 1.3, 10.7 Hz, H-15a), 5.18 (dd, J = 1.3, 17.4 Hz, H-15b), 5.89 (dd, J = 10.7, 17.4 Hz); ^{13}C (125 MHz) NMR (in $CDCl_3$) δ(ppm) 14.4 (C-20), 17.7

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Table 3. Antifungal Activities of Four Compounds (100 μg/mL) from *C. konishii* against Wood Decay Fungi[a]

fungi	isoabienol	T-cadinol	cedrol	T-muurolol	DDAC[b]
L. sulphureus	8.2 ± 2.5 f	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
G. trabeum	6.8 ± 1.1 f	100.0 ± 0.0 a	68.3 ± 2.4 b	100.0 ± 0.0 a	100.0 ± 0.0 a
L. betulina	25.6 ± 1.5 e	100.0 ± 0.0 a	95.5 ± 7.7 a	100.0 ± 0.0 a	100.0 ± 0.0 a
T. versicolor	27.8 ± 1.5 e	51.4 ± 2.8 c	74.8 ± 4.5 b	39.5 ± 3.1 d	100.0 ± 0.0 a

[a]Numbers followed by different letters (a–f) are significantly different at the level of $P < 0.05$ according to Scheffe's test. [b]Positive control.

(C-11), 19.4 (C-2), 21.7 (C-19), 24.5 (C-6), 28.0 (C-16), 33.5 (C-4), 33.6 (C-18), 38.4 (C-7), 39.1 (C-1), 39.9 (C-10), 41.4 (C-12), 42.2 (C-3), 55.6 (C-5), 57.2 (C-9), 73.7 (C-13), 106.3 (C-17), 111.6 (C-15), 145.2 (C-14), 148.8 (C-8).

Compound 2 (cis-Abienol). Colorless needle crystal, mp 63–65 °C, $[\alpha]_D^{20}$ +49.0° (*c* = 1.0, $CHCl_3$), EIMS for $C_{20}H_{34}O$ (EIMS: 290), ^1H (500 MHz) NMR (in CDCl3) δ(ppm) 0.78 (s, H-18), 0.82 (s, H-19), 0.85 (s, H-20), 1.17 (s, H-17), 1.78 (s, H-16), 5.10 (d, *J* = 10.9 Hz, H-15a), 5.18 (d, *J* = 17.1 Hz, H-15b), 5.48 (t, *J* = 7.4 Hz, H-12), 6.86 (dd, *J* = 10.9, 17.1 Hz, H-14); ^{13}C (500 MHz) NMR (in CDCl3) δ(ppm) 15.4 (C-20), 18.6 (C-2), 19.9 (C-16), 20.3 (C-6), 21.6 (C-19), 23.1 (C-11), 24.5 (C-17), 33.3 (C-4), 33.5 (C-18), 38.9 (C-10), 40.1 (C-1), 41.8 (C-3), 44.0 (C-7), 56.1 (C-5), 62.2 (C-9), 74.3 (C-8), 113.8 (C-15), 130.9 (C-13), 133.6 (C-14), 133.8 (C-12).

Compound 3 (Isoabienol). White solid, mp 65–66 °C, $[\alpha]_D^{20}$ −6.2° (*c* = 0.2, $CHCl_3$), EIMS for $C_{20}H_{34}O$ (EIMS: 290), ^1H (500 MHz) NMR (in $CDCl_3$) δ(ppm) 0.77 (s, H-19,20), 0.85 (s, H-18), 1.16 (s, H-17), 4.99 (br s, H-16), 5.03 (d, *J* = 10.6 Hz, Hb-15), 5.30 (d, *J* = 17.2 Hz, Ha-15), 6.33 (1H, dd, *J* = 17.2, 10.6 Hz); ^{13}C (125 MHz) NMR (in $CDCl_3$) δ(ppm) 15.5 (C-20), 18.4 (C-2), 20.6 (C-6), 21.5 (C-19), 24.0 (C-17), 24.5 (C-11), 33.2 (C-4), 33.4 (C-18), 35.1 (C-12), 39.1 (C-10), 39.7 (C-1), 42.0 (C-3), 44.6 (C-7), 56.1 (C-5), 61.8 (C-9), 74.2 (C-8), 113.5 (C-15), 115.6 (C-16), 138.8 (C-14), 147.4 (C-13).

Compound 4 (T-Cadinol). Colorless oil, mp 64–65 °C, $[\alpha]_D^{20}$ +5.4° (*c* = 1.2, $CHCl_3$). IR (KBr) ν_{max}: 3454, 1660 cm^{-1}; EIMS for $C_{15}H_{26}O$ (EIMS: 222), ^1H (500 MHz) NMR (in $CDCl_3$) δ(ppm) 0.77 (d, *J* = 7.0 Hz, H-12), 0.89 (d, *J* = 7.0 Hz, H-13), 1.19 (s, H-14), 1.65 (s, H-15), 2.16 (m, H-11), 5.52 (br s, H-5); ^{13}C (125 MHz) NMR (in $CDCl_3$) δ(ppm) 15.2 (C-12), 19.8 (C-8), 21.4 (C-13), 22.6 (C-2), 23.8 (C-15), 26.2 (C-11), 28.5 (C-14), 26.2 (C-11), 30.9 (C-3), 37.7 (C-6), 40.3 (C-9), 46.7 (C-7), 47.9 (C-1), 70.7 (C-10), 122.6 (C-5), 134.4 (C-4). EIMS *m/z* (%): 222 (M+, 35), 204 (90), 189 (20), 161 (100), 134 (22), 121 (22), 105 (25).

Compound 5 (Cedrol). Colorless needle crystal, mp 87 °C, $[\alpha]_D^{20}$ +9.9° (*c* = 1.2, $CHCl_3$), EI-MS for $C_{15}H_{26}O$ found 222. IR (KBr) ν_{max}: 3342, 1373 cm^{-1}. ^1H (500 MHz) NMR (in $CDCl_3$): δ(ppm) 0.81 (d, *J* = 7.2, H-14), 0.97 (s, H-12), 1.24 (s, H-15), 1.30 (s, H-13). ^{13}C (125 MHz) NMR (in $CDCl_3$): δ(ppm) 15.6 (C-14), 25.4 (C-9), 27.6 (C-13), 28.9 (C-12), 30.2 (C-15), 31.6 (C-5), 35.4 (C-4), 37.0 (C-8), 41.5 (C-7), 42.0 (C-1), 43.4 (C-11), 54.1 (C-6), 56.5 (C-10), 61.0 (C-2), 75.1 (C-3).

Compound 6 (T-Muurolol). Colorless crystal, mp 80–81 °C, $[\alpha]_D^{20}$ −42.8° (*c* = 1.0, $CHCl_3$). IR (KBr) ν_{max}: 3454, 1660 cm^{-1}; EIMS for $C_{15}H_{26}O$ (EIMS: 222), ^1H (500 MHz) NMR (in $CDCl_3$) δ(ppm) 0.80 (d, *J* = 7.0 Hz, H-12), 0.86 (d, *J* = 7.0 Hz, H-13), 1.17 (s, H-14), 1.63 (s, H-15), 2.24 (m, H-11), 5.53 (br s, H-5); ^{13}C (125 MHz) NMR (in $CDCl_3$) δ(ppm) 15.4 (C-12), 19.4 (C-8), 20.9 (C-13), 21.6 (C-2), 23.6 (C-15), 26.7 (C-11), 29.3 (C-14), 31.3 (C-3), 34.5 (C-6), 34.6 (C-9), 43.9 (C-7), 46.1 (C-1), 72.4 (C-10), 124.8 (C-5), 133.5 (C-4). EIMS *m/z* (%): 222 (M+, 35), 204 (90), 189 (20), 161 (100), 134 (22), 121 (22), 105 (25).

Compound 7 ((−)-epi-Cedrol). Colorless oil, mp 37–38 °C, $[\alpha]_D^{20}$ −8.0° (*c* = 1.2, $CHCl_3$), EI-MS for $C_{15}H_{26}O$ found 222. IR (KBr) ν_{max}: 3390, 3384, 2952, 2937, 1459, 1376, 1150 cm^{-1}. ^1H (500 MHz) NMR (in $CDCl_3$): δ(ppm) 0.83 (d, *J* = 7.5, H-15), 0.99 (s, H-12), 1.11 (s, H-13), 1.30 (s, H-15). ^{13}C (125 MHz) NMR (in $CDCl_3$): δ(ppm) 15.5 (C-14), 25.4 (C-9), 28.2 (C-13), 29.0 (C-12), 30.5 (C-5), 30.6 (C-15), 34.4 (C-4), 36.9 (C-8), 39.3 (C-1), 41.8 (C-7), 41.9 (C-11), 53.4 (C-6), 56.2 (C-10), 61.5 (C-2), 73.2 (C-3).

Antifungal Activity of Pure Compounds. The antifungal indices of isoabienol (**3**), T-cadinol (**4**), cedrol (**5**), and T-muurolol (**6**) at a concentration of 100 μg/mL against *L. sulphureus*, *G. trabeum*, *L. betulina*, and *T. versicolor* are presented in Table 3. All compounds were active, except compound **3** (antifungal index = 6.8–27.8% for the four wood decay fungi). Compounds **4**, **5**, and **6** at the concentration of 100 μg/mL revealed significant inhibitory effects against the four wood decay fungi, with antifungal indices of 51.4–100.0, 68.3–100.0, and 39.5–100.0%, respectively. Furthermore, compounds **4** and **6** at the concentration of 100 μg/mL completely inhibited the growth of *L. sulphuerus, G. trabeum*, and *L. betulina*. Similarly, compound **5** at the same concentration also exhibited strong antifungal action with antifungal indices of 100.0% against *L. sulphuerus*. However, these compounds isolated were slightly less active than DDAC (antifungal index = 100.0% for the four wood decay fungi).

A similar observation was also noted in Shieh and Sumimoto's[29] study on the antifungal activity of *Cunninghamia lanceolata* wood, and it was reported that cedrol at a concentration of 700 μg/mL inhibited the growth of six wood decay fungi. In our previous studies on the antifungal performance of *T. cryptomerioides* extractives, T-cadinol and T-muurolol exhibited antifungal activity for both *T. versicolor* and *L. sulphureus*.[6] The antifungal activity of leaf essential oil isolated from *C. formosana* was attributed to α-cadinol and T-muurolol, or their synergistic effect.[9] We also found that cedrol from the essential oil of *C. konishii* wood at 100 μg/mL exhibited the highest antifungal activity against wood decay fungi and plant pathogenic fungi.[21]

In the study, we investigated the antifungal activities of leaf and wood ethanolic extracts from *C. konishii* against four wood decay fungi, which had not been previously reported. Results obtained from antifungal tests demonstrate that the wood ethanolic extract from *C. konishii* had strong antifungal activities against wood decay fungi. Furthermore, we isolated and identified 13-*epi*-manool, *cis*-abienol, isoabienol, T-cadinol, cedrol, T-muurolol, and (−)-*epi*-cedrol from the active subfractions, among which T-cadinol, cedrol, and T-muurolol at 100 μg/mL efficiently inhibited the growth of wood decay fungi. In conclusion, *C. konishii* wood ethanolic extract, T-

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cadinol, T-muurolol, and cedrol could be used as potential antifungal agents for the control of wood decay fungi.

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