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Chemical Composition and Antifungal Activity of Essential Oils from Different Tissues of Japanese Cedar (*Cryptomeria japonica*)

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In this study antifungal activities of essential oils from different tissues of Japanese cedar (*Cryptomeria japonica* D. Don) against four wood decay fungi and six tree pathogenic fungi were investigated. In addition, the yields of essential oils obtained by water distillation were compared and their constituents determined by GC-MS analyses. The yield of essential oils from four tissues of Japanese cedar is in the decreasing order of leaf (27.38 mL/kg) > bark (6.31 mL/kg) > heartwood (3.80 mL/kg) > sapwood (1.27 mL/kg). Results obtained from the antifungal tests demonstrate that the essential oil of Japanese cedar heartwood used against *Laetiporus sulphureus* and *Trametes versicolor* and sapwood essential oil used against *L. sulphureus* had strong antifungal activities at 500 μg/mL, with IC_{50} values of 39, 91, and 94 μg/mL, respectively. Besides, the essential oils of Japanese cedar heartwood used against *Rhizoctonia solani*, *Collectotrichum gloeosporioides*, *Fusarium solani*, and *Ganoderma australe* had strong antifungal activities at 500 μg/mL, with IC_{50} values of 65, 80, 80, and 110 μg/mL, respectively. GC-MS analyses showed that the sesquiterpene hydrocarbon compounds dominate in the essential oil from Japanese cedar heartwood, amounting to a total percentage of 82.56%, with the major compounds of δ-cadinene (18.60%), isoledene (12.41%), and γ-muurolene (11.82%). It is proposed that the excellent antifungal activities of Japanese cedar heartwood essential oils might correlate with the presence of these compounds.

KEYWORDS: ***Cryptomeria japonica*; heartwood; essential oil; GC-FID; GC-MS; wood decay fungi; tree pathogenic fungi; antifungal activity**

INTRODUCTION

The search for simple bioactive compounds derived from plants that can be used against fungi has been a research direction for ecologically safe products (*1*). For instance, essential oils are known to contain a natural mixture of monoterpenes, sesquiterpenes, diterpenes, and hydrocarbons, with a variety of functional groups, giving them antibacterial, antifungal, antimite, antitermite, and antimosquito activities (*2*−*9*).

The Japanese cedar, *Cryptomeria japonica* D. Don, is a widely distributed conifer called "sugi" in Japanese. *C. japonica* is well-known in Taiwan as one of the important plantation tree species because of its beautiful yellowish red to red heartwood (*10*). Among plantation trees, it has one of the highest values as a building material for Japanese-style houses and is also commonly used for ceiling board, wall paneling, and posts (*11*). Because of its industrial importance, the chemical components including terpenoids have been investigated by many researchers (*12*−*23*). These compounds show various bioactivities such as antifungal (*24*−*26*), termiticidal (*27*, *28*), antimite (*25*, *29*), antipathogenic (*30*, *31*), and antifeeding against snail species (*32*) and against the pill-bug (*33*) and develop resistance against the *Cryptomeria* bark borer (*34*). Our own results, reported in previous studies, have demonstrated that leaf essential oil from Japanese cedar has excellent antitermite (*35*) and antimosquito (*8*) activities. However, to the best of our knowledge there is no literature concerning the differences in antifungal activities of essential oils from different tissues of Japanese cedar against wood decay fungi and tree pathogenic fungi. Therefore, we studied the chemical composition of essential oils from four different tissues of the Japanese cedar by using gas chromatography−mass spectroscopy (GC-MS), and their antifungal activities against four wood decay fungi and six tree pathogenic fungi were also investigated herewith.

MATERIALS AND METHODS

Plant Material. Fifty-three aged Japanese cedar (*C. japonica* D. Don), in the Taxodiaceae class, were collected in July 2000 from the Experimental Forest of National Taiwan University located in Nantou County in central Taiwan. The species was identified, and the voucher

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specimens (CJHO01, CJSO01, CJBO01, and CJLO01) including heartwood, sapwood, bark, and leaf, were deposited at the Laboratory of Wood Chemistry (School of Forestry and Resource Conservation, National Taiwan University).

Distillation of Essential Oils. Heartwood, sapwood, bark, and leaf of Japanese cedar were prepared from a green cut tree. The samples (200 g each), in triplicate, were subjected to water distillation in a Clevenger-type apparatus for 6 h (*9*), followed by determination of oil contents. Essential oils were stored in airtight containers prior to analysis by gas chromatography (GC) and GC-MS.

GC−Flame Ionization Detection (FID) Analysis. The essential oils from these different tissue types of Japanese cedar were analyzed using a Finnigan Trace GC with an FID and RTx-5MS (30 m × 0.25 mm; film thickness, 0.25 μm). For heartwood essential oil, the initial oven temperature was maintained at 120 °C for 1 min and programmed to increase at 2 °C/min to 140 °C (held for 2 min), then to 145 °C at a rate of 0.5 °C/min, maintained constant at 145 °C for 5 min, and then increased to 200 °C at a rate of 10 °C/min (held for 5 min). For sapwood, bark, and leaf essential oils, the oven temperature was held at 80 °C for 1 min, then programmed to increase from 80 to 200 °C at a rate of 2 °C/min, and held for 5 min. Helium was employed as the carrier gas at a 1 mL/min flow rate. The injector temperature was maintained at 220 °C. The samples (1 μL) were injected neat with a 1:10 split ratio.

GC-MS Analysis. The compositions of essential oils from these different tissue types of Japanese cedar were analyzed on a Finnigan Trace GC−Polaris Q mass instrument (Finnigan-Spectronex), equipped with a fused silica column (30 m × 0.25 mm i.d.), and coated with an RTx-5MS (df = 0.25 μm). Mass spectra were recorded over the 35−650 amu range at 1 scan/s, with an ionization energy of 70 eV and an ion source temperature of 200 °C. Helium was the carrier gas at a flow rate of 1 mL/min. The injector temperature was maintained at 220 °C. The oven temperatures were programmed as in the GC-FID analysis. The samples (1 μL) were injected neat with a 1:10 split ratio. Quantification was performed using percentage peak area calculations using the GC-FID, and the identification of individual components was done using their relative retention indices and the Wiley/NBS Registry of Mass Spectral Database and NIST MS Search between the mass spectrum and a few authentic reference compounds. The quantity of compounds was obtained by integrating the peak area of the spectrograms.

Fungal Strains. The wood decay fungi and tree pathogenic fungi were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute. The fungal strains used in experiments were as follows: four wood decay fungi [*Lenzites betulina* (CCRC 35296), *Trametes versicolor* (CCRC 35253), *Laetiporus sulphureus* (CCRC 35305), and *Gloeophyllum trabeum* (CCRC 31614)] and six tree pathogenic [*Fusarium oxysporum* (CCRC32121), *Rhizoctonia solani* (CCRC31626), *Ganoderma australe* (CCRC36246), *Fusarium solani* (CCRC32458), *Pestalotiopsis funereal* (CCRC35266), and *Collectotrichum gloeosporioides* (CCRC35003)]. 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 (*2*, *36*) with slight modifications. Briefly, 500, 200, 100, and 50 μg/mL of essential oils were added to sterilized PDA in 9 cm plates (Petri dish). After transfer of the mycelium of 10 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 essential oils), the antifungal indices were calculated. Each test was repeated three times, and the data were averaged. The formula of antifungal indices is shown as

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

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. To determine whether there was a statistically significant difference among different tissues of Japanese cedar essential oils against the fungi, Scheffe's method of SAS was used to analyze the difference of the antifungal index. Results with $P < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Yields and Chemical Compositions of Essential Oils from Different Tissues. The yields of essential oils from different tissues of Japanese cedar ranged from 1.27 to 27.38 mL/kg (**Table 1**). The yields of heartwood, sapwood, bark, and leaf essential oils of Japanese cedar were 3.80, 1.27, 6.31, and 27.38 mL/kg, respectively.

GC-MS analyses of the oils identified most of the compounds, which are listed in **Table 1** along with their quantitative data. A total of 73 compounds were identified in the different tissues of Japanese cedar essential oils, constituting 91.94−100.00% of the oils. These compounds could be assigned to seven different classes: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, diterpene hydrocarbons, oxygenated diterpenes, and other. In the Japanese cedar heartwood essential oil, 26 compounds, representing 97.44% of the oil, were identified in total (**Table 1**). This oil was characterized by a high content of the sesquiterpene hydrocarbons (82.56%); δ-cadinene (18.60%), isoledene (12.41%), γ-muurolene (11.82%), humulene (9.43%), (−)-cubenene (9.27%), and 1,1,3a-trimethyl-7-methylenedecahydrocyclopropa[*a*]naphthalene (6.79%) were the main constituents. Oxygenated sesquiterpenes were represented by three components, accounting for 13.44% of the oil. The most abundant constituents of the fraction were β-eudesmol (5.69%) and δ-cadinol (5.24%). Diterpene hydrocarbons represented 1.03% of the oil, and the content of oxygenated diterpenes was even lower (0.41%). These results are in agreement with those of Nagahama et al. (*16*, *17*), who also found that the main constituent of wood essential oil was δ-cadinene.

Twenty-one components of the sapwood essential oil from Japanese cedar were identified, representing for 96.69% of the oil. Sesquiterpene hydrocarbons predominated in the oil (36.80%), with valencene (9.91%), 1,1,3a-trimethyl-7-methylenedecahydrocyclopropa[*a*]naphthalene (7.61%), δ-cadinene (6.11%), 2-methylene-5-(1-methylvinyl)-8-methylbicyclo[5.3.0]decane (5.57%), and isoledene (4.98%) as the main constituents. Four oxygenated sesquiterpenes constituted 12.81% of the oil, with β-eudesmol (9.34%) and δ-cadinol (2.35%) as the two main constituents of the fraction. This oil was characterized by a high content of the diterpene hydrocarbons (41.90%), and the main constituents were sclarene (27.56%) and cupressene (12.55%). Oxygenated diterpenes (two) and other (one) account for 4.94 and 0.24% of the oil, respectively.

In the bark essential oil of Japanese cedar, 38 compounds, representing 91.94% of the oil, were identified in total. The major fractions of the oil were monoterpene hydrocarbons and sesquiterpene hydrocarbons (37.63 and 33.31%, respectively). 3-Carene (18.62%) and limonene (9.74%) were the two main constituents of the monoterpene hydrocarbon fraction; the main constituents of the sesquiterpene hydrocarbon fraction were cadala-1,3,5-triene (10.51%), valencene (3.85%), and γ-muurolene (3.21%). Six oxygenated monoterpenes represent 7.53% of the oil; α-terpineol (3.71%) was the main constituent of the fraction. Oxygenated sesquiterpenes (two), diterpene hydrocarbons (four), oxygenated diterpenes (two), and other (one) constituted 3.02, 6.98, 3.04, and 0.43% of the oil, respectively. Yatagai et al. (*34*) found the major constituents of the essential oils of Japanese cedar inner barks were α-pinene (16.18−52.14%), 3-carene (6.57−13.16%), and limonene (7.42−13.35%).

Table 1. Constituents and Contents of Essential Oils from Different Tissues of Japanese Cedar

no.	compound[b]	RI[a]	relative % bark	leaf	sapwood	heartwood
1	*m*-cymene	1025	1.25	–[d]	–	–
2	α-pinene[c]	1031	–	3.08	–	–
3	sabinene	1056	3.30	–	–	–
4	β-pinene[c]	1058	–	0.70	–	–
5	3-carene[c]	1110	18.62	0.57	–	–
6	*p*-cymene[c]	1144	–	3.68	–	–
7	limonene[c]	1151	9.74	–	–	–
8	α-terpinene[c]	1220	–	0.35	–	–
9	artemiseole	1252	0.66	–	–	–
10	β-methylallylbenzene	1271	0.48	–	–	–
11	dehydro-*p*-cymene	1288	0.88	–	–	–
12	terpinene-4-ol[c]	1478	–	2.27	–	–
13	α-terpineol[c]	1503	3.71	–	–	–
14	(–)-bornyl acetate[c]	1674	1.41	0.97	–	–
15	nopol	1694	0.54	–	–	–
16	eucarvone	1721	0.44	–	–	–
17	bornylene	1751	0.93	–	–	–
18	α-terpinolene	1771	2.43	–	–	–
19	α-cubebene	1807	0.83	–	–	0.76
20	α-copaene	1828	–	–	–	0.22
21	germacrene-D	1837	–	–	–	0.62
22	β-caryophyllene[c]	1849	–	–	–	0.36
23	α-caryophyllene	1856	–	–	–	0.43
24	ylangene	1863	0.67	–	–	–
25	β-cubebene	1875	2.55	–	–	0.78
26	γ-muurolene	1931	3.21	0.29	–	11.82
27	α-guaiene	1940	0.60	–	–	–
28	(+)-cyclosativene	1950	1.39	–	–	–
29	α-muurolene	1957	2.21	–	0.51	3.98
30	aromadendrene	1961	–	–	–	0.28
31	γ-cadinene	1970	1.70	–	–	–
32	2,5-di-*tert*-butylphenol	1972	–	–	0.24	–
33	cadina-1,3,5-triene	1978	10.51	–	–	–
34	α-cadinene	1980	–	–	6.11	18.6
35	cadina-3,9-diene	1982	–	0.98	–	–
36	cadala-1(10),3,8-triene	1998	0.92	–	0.85	0.41
37	humulene	2002	–	–	–	9.43
38	2-methylene-5-(1-methylvinyl)-8-methylbicyclo[5.3.0]decane	2005	–	–	5.57	–
39	isopulegol acetate	2019	0.77	–	–	–
40	γ-elemene	2038	–	–	0.61	1.41
41	6-(2-butenyl)-1,5,5-trimethyl-cyclohexene	2044	0.43	–	–	–
42	*epi*-bicyclosesquiphellandrene	2046	–	–	–	0.77
43	caryophyllene oxide[c]	2051	–	–	–	0.26
44	(–)-cubenene	2054	–	–	–	9.27
45	β-maaiene	2061	–	–	–	2.67
46	1,1,3a-trimethyl-7-methylene-decahydrocyclopropa[*a*]-naphthalene	2063	–	0.33	7.61	6.79
47	α-gurjunene	2066	–	7.91	–	–
48	isoledene	2069	–	1.44	4.98	12.41
49	valencene	2074	3.85	19.91	9.91	–
50	α-cadinol[c]	2085	1.38	–	2.35	5.24
51	unidentified	2087	–	–	–	1.55
52	β-eudesmol	2091	1.64	5.90	9.34	5.69
53	eudesma-3,7(11)-diene	2092	2.33	8.40	–	–
54	4,5,9,10-dehydroisolongifolene	2098	0.72	–	–	–
55	cadalene	2109	0.83	–	–	–
56	azulene	2110	–	–	0.65	0.41
57	spathulenol	2136	–	–	0.49	–
58	longiverbenone	2148	–	–	0.63	2.25
59	4,4-dimethyl-3-(3-methylbut-3-enylidene)-2-methylene-bicyclo[4.1.0]heptane	2188	0.99	–	–	–
60	deoxyoblongifolion	2234	–	0.39	–	–
61	18-*nor*-isopimar-4(19),7,15-triene	2250	2.02	–	0.92	–
62	verticiol	2273	–	–	3.51	0.58
63	cupressene	2276	2.32	–	–	–
64	pimarinal	2286	–	0.47	1.43	–
65	sandaracopimaradiene	2289	0.91	–	–	–
66	ferruginol[c]	2302	–	–	–	0.45
67	*ent*-kaur-16-ene[c]	2310	–	40.62	0.24	–
68	abieta-8,11,13-triene	2319	1.73	–	0.63	–
69	sclareol	2346	–	1.41	–	–
70	kauran-16-ol	2374	2.61	–	–	–
71	cupressene	2376	–	–	12.55	–
72	totarol	2381	0.43	0.33	–	–
73	sclarene	2399	–	–	27.56	–
	identified components (%)		**91.94**	**100.00**	**96.69**	**97.44**
	monoterpene hydrocarbons (%)		37.63	8.38	0.00	0.00
	oxygenated monoterpenes (%)		7.53	3.24	0.00	0.00
	sesquiterpene hydrocarbons (%)		33.31	39.26	36.80	82.56
	oxygenated sesquiterpenes (%)		3.02	5.90	12.81	13.44
	diterpene hydrocarbons (%)		6.98	40.62	41.90	0.00
	oxygenated diterpenes (%)		3.04	2.60	4.94	1.03
	other (%)		0.43	0.00	0.24	0.41
	oil yield (mL/kg, v/dry wt)		**6.31**	**27.38**	**1.27**	**3.80**

[a] Retention index relative to *n*-alkanes on RTx-5MS column. [b] By comparison of the mass spectrum with those of the computer mass libraries. [c] By comparison with pure standard retention time. [d] Not detected.

Japanese cedar leaf essential oil shows the presence of 20 identified components, accounting for 100.00% of the whole oil. Both sesquiterpene hydrocarbons (39.26%) and diterpene hydrocarbons (40.62%) were the main fractions of the whole, with valencene (19.91%), eudesma-3,7(11)-diene (8.40%), and α-gurjunene (7.91%) as the main sesquiterpene hydrocarbons, with *ent*-kaur-16-ene (40.62%) as the main diterpene hydrocarbons. Five monoterpenes represent 8.38% of the oil; *p*-cymene (3.68%) and α-pinene (3.08%) were the two main constituents of the fraction. Two oxygenated monoterpenes, one oxygenated sesquiterpene, and four oxygenated diterpenes constitute 3.24, 5.90, and 2.60% of the oil, respectively. Lee and Lin (*14*) also reported that the main constituents of leaf essential oil from Japanese cedar were (−)-kaurene (27.71%), elemol (26.90%), and sabinene (25.07%).

Antifungal Activity of Essential Oils from Different Tissues against Wood Decay Fungi. To evaluate the antifungal activities of essential oils from different tissues of Japanese cedar against wood decay fungi, we first selected four typical fungi, two white-rot fungi, *L. betulina* and *T. versicolor*, and two brown-rot fungi, *L. sulphureus* and *G. trabeum*, as test strains. **Figure 1** shows the antifungal activities of essential oils from different Japanese cedar tissues against wood decay fungi at a

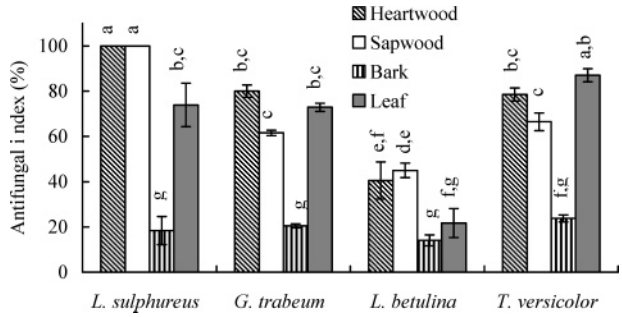

Figure 1. Antifungal activities of essential oils (500 μg/mL) extracted from four different tissues of Japanese cedar against four wood decay fungi. Each experiment was performed three times, and the data were averaged ($n = 3$). Numbers followed by different letters (a–g) are significantly different at the level of $P < 0.05$ according to the Scheffe test.

Table 2. IC_{50} Values of Essential Oils from Different Tissues of Japanese Cedar against Four Wood Decay Fungi

fungus	IC_{50} (μg/mL)			
	heartwood	sapwood	bark	leaf
T. versicolor	91	139	>500	118
L. betulina	>500	>500	>500	>500
L. sulphureus	39	94	>500	260
G. trabeum	157	253	>500	145

concentration of 500 μg/mL. On the basis of the results of the antifungal test, the antifungal indices of Japanese cedar heartwood essential oil were 40.5, 78.5, 100.0, and 80.0% against *L. betulina*, *T. versicolor*, *L. sulphureus*, and *G. trabeum*, respectively, and the IC_{50} values were >500, 91, 39, and 157 μg/mL, respectively (**Table 2**). In addition, the antifungal indices of sapwood essential oils against the four wood decay fungi were 45.0, 66.5, 100.0, and 61.6%, respectively, and the IC_{50} values were >500, 139, 94, and 253 μg/mL, respectively. The antifungal indices (the IC_{50} values) of leaf essential oils against four wood decay fungi were 21.7% (>500 μg/mL), 87.0% (118 μg/mL), 73.9% (260 μg/mL), and 72.9% (145 μg/mL), respectively. However, the antifungal indices of Japanese cedar bark essential oil against the four wood decay fungi did not exceed 24.0%, and the IC_{50} values were >500 μg/mL, indicating that none of the bark essential oil could inhibit the fungal growth of *L. betulina*, *T. versicolor*, *L. sulphureus*, and *G. trabeum*.

On the basis of the above results, it is found that heartwood, sapwood, and leaf essential oils of Japanese cedar showed high activity against *T. versicolor*, *L. sulphureus*, and *G. trabeum*; the antifungal indices of these essential oils exceed 61.6%. Among these, heartwood and sapwood essential oils completely inhibited the growth of brown-rot fungus (*L. sulphureus*) at a concentration of 500 μg/mL. The IC_{50} values of three essential oils in terms of antifungal activity against *T. versicolor* and *G. trabeum* are ranked as heartwood (91 and 157 μg/mL) > leaf (118 and 145 μg/mL) > sapwood (139 and 253 μg/mL) (**Table 2**). On the other hand, the order of IC_{50} values against *L. sulphureus* is heartwood (39 μg/mL) > sapwood (94 μg/mL) > leaf (260 μg/mL). It is clear that, in comparison with IC_{50} values, heartwood essential oil has the strongest antifungal activities. Tellez et al. (*37*) studied the composition and biological activities of essential oil from *Callicarpa americana* and demonstrated that humulene (10.0%) was a major compound of the steam-distilled oil, which could inhibit the fungal growth. Other constituents, that is, α-copaene, germacrene-D, β-caryophyllene, caryophyllene oxide, and ferruginol, were also shown to have good antifungal activity elsewhere (*36*, *38*−*40*). Therefore, it could be suggested that the antifungal activity of heartwood essential oil against wood decay fungi is caused by these volatile compounds.

Antifungal Activity of Essential Oils from Different Tissues against Tree Pathogenic Fungi. The antifungal activities of essential oils from different Japanese cedar tissues against six tree pathogenic fungi at a concentration of 500 μg/mL are shown in **Figure 2**. For the activity studied, two seedling pathogens (*F. oxysporum* and *R. solani*), two root pathogens (*G. australe* and *F. solani*), and two leaf pathogens (*P. funereal* and *C. gloeosporioides*) were used. As shown in **Figure 2**, the antifungal indices of the essential oils from four different tissues of Japanese cedar against *F. oxysporum* and *P. funereal* did not exceed 45.7 and 34.0% at a concentration of 500 μg/mL, respectively, indicating that the essential oils from these four different tissues have no significant antifungal effects against

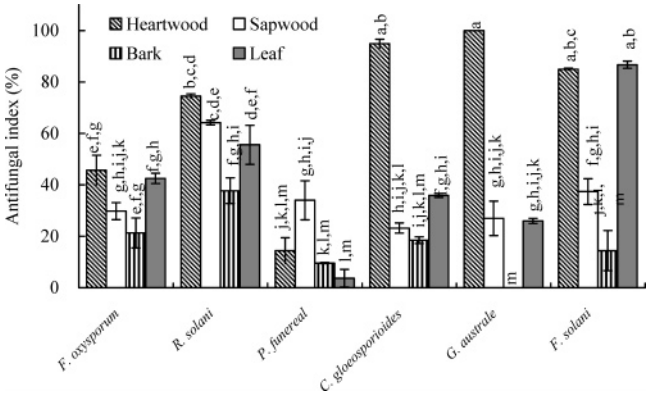

Figure 2. Antifungal activities of essential oils (500 μg/mL) extracted from four different tissues of Japanese cedar against six tree pathogenic fungi. Each experiment was performed three times, and the data were averaged ($n = 3$). Numbers followed by different letters (a–m) are significantly different at the level of $P < 0.05$ according to the Scheffe test.

Table 3. IC_{50} Values of Essential Oils from Different Tissues of Japanese Cedar against Six Tree Pathogenic Fungi

fungus	IC_{50} (μg/mL)			
	heartwood	sapwood	bark	leaf
F. oxysporum	>500	>500	>500	>500
R. solani	65	240	>500	430
P. funereal	>500	>500	>500	>500
C. gloeosporioides	80	>500	>500	>500
G. australe	110	>500	>500	>500
F. solani	80	>500	>500	235

seedling pathogenic fungus *F. oxysporum* and leaf pathogenic fungus *P. funereal*. On the other hand, heartwood essential oil of Japanese cedar against root pathogenic fungi *G. australe* and *F. solani*, seedling pathogenic fungus *R. solani*, and leaf pathogenic fungus *C. gloeosporioides* had strong antifungal activities at 500 μg/mL, with antifungal indices of 100.0, 85.0, 74.5, and 94.9%, respectively. In addition, the leaf essential oil exhibited an inhibitory effect against the root pathogenic fungus *F. solani* with an antifungal index of 86.7%, and sapwood essential oil had also an excellent inhibitory effect against the seedling pathogenic fungus *R. solani*, with an antifungal index of 64.2%. However, the antifungal indices of Japanese cedar bark essential oil against another four tree pathogenic fungi did not exceed 38.0%, indicating that none of the bark essential oils could inhibit the fungal growth of *G. australe*, *F. solani*, *R. solani*, and *C. gloeosporioides*.

From a comparison of the IC_{50} values of heartwood, sapwood, and leaf essential oils against *G. australe*, *F. solani*, *R. solani*, and *C. gloeosporioides*, heartwood essential oil exhibited the highest antifungal index for four tree pathogenic fungi, followed by leaf and sapwood essential oils (**Table 3**). The IC_{50} values of heartwood essential oil against *G. australe*, *F. solani*, *R. solani*, and *C. gloeosporioides* were 110, 80, 65, and 80 μg/mL, respectively. In addition, the IC_{50} values of leaf essential oil were 235 and 430 μg/mL against *F. solani* and *R. solani*, and the IC_{50} value of sapwood essential oil was 240 μg/mL for *R. solani*. Kofujita et al. (*31*) have studied antifungal activities of the bark of *C. japonica* and demonstrated that ferruginol was the major compound of hexane extracts, which had a good antifungal activity against *Alternaria alternate*, *Payricularia oryzae*, *R. solani*, and *F. oxysporum*. Furthermore, β-caryophyllene oxide and α-terpineol from *Hypericum* species essential oils were inhibitory to the growth of 10 agricultural pathogenic fungi (5 *Fusarium* species and 5 anastomosis groups of *R.*

solani) at a concentration of 1 mg/mL (*41*). These results suggest that the antifungal activity of heartwood essential oil against tree pathogenic fungi is caused by these volatile compounds.

In conclusion, we investigated the antifungal activities of essential oils from different tissues of Japanese cedar against four wood decay fungi and six tree pathogenic fungi, which had not been previously reported. In addition, the yields of essential oils obtained by water distillation were compared and their constituents were determined by GC-MS analyses. The yield of essential oils from four tissues of Japanese cedar was in the decreasing order of leaf (27.38 mL/kg) > bark (6.31 mL/kg) > heartwood (3.80 mL/kg) > sapwood (1.27 mL/kg). Antifungal tests demonstrated that the essential oils of Japanese cedar heartwood used against *L. sulphureus* and *T. versicolor* and sapwood essential oil used against *L. sulphureus* had strong antifungal activities at 500 μg/mL, whereas the IC_{50} values were 39, 91, and 94 μg/mL, respectively. Moreover, the essential oils of Japanese cedar heartwood had strong antifungal activities against *R. solani*, *C. gloeosporioides*, *F. solani*, and *G. australe* at 500 μg/mL; the IC_{50} values were 65, 80, 80, and 110 μg/mL, respectively. Using GC-MS analyses, the sesquiterpene hydrocarbon compounds dominate in the essential oil from Japanese cedar heartwood, amounting to a total percentage of 82.56%, with the major compounds being δ-cadinene (18.60%), isoledene (12.41%), and γ-muurolene (11.82%). It is proposed that the excellent antifungal activities of Japanese cedar heartwood essential oils might correlate with the presence of these compounds. Thus, the mechanisms of these compounds against fungal growth are worthy of further investigation.

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