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Composition, Antioxidant, Antimicrobial and Anti-wood-decay Fungal Activities of the Twig Essential Oil of *Taiwania cryptomerioides* from Taiwan

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This study investigated the chemical composition, antioxidant, antimicrobial and anti-wood-decay fungal activities of the essential oil isolated from the twigs of *Taiwania cryptomerioides* from Taiwan. The essential oil was isolated using hydrodistillation in a Clevenger-type apparatus, and characterized by GC–FID and GC–MS. A total of 35 compounds were identified, representing 100% of the oil. The main components identified were α -cadinol (45.9%), ferruginol (18.9%) and β -eudesmol (10.8%). The antioxidant activity of the oil was tested by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capability test. The results showed an IC₅₀ of 90.8 ± 0.2 µg/mL. The active source compound was ferruginol. The antimicrobial activity of the oil was tested by the disc diffusion and micro-broth dilution methods against ten microbial species. The oil exhibited strong growth suppression against Gram-positive bacteria and yeast with inhibition zones of 45~52 mm and MIC values of 31.25~62.5 µg/mL, respectively. The anti-wood-decay fungal activity of the oil was also evaluated. The oil demonstrated excellent activity against four wood-decay-fungal species. For the antimicrobial and anti-wood-decay fungal activities of the oil, the active source compounds were determined to be α -cadinol, β -eudesmol and ferruginol.

Keywords: Taiwania cryptomerioides, Cupressaceae, Essential oil, Antioxidant activity, Antimicrobial activity, Anti-wood-decay fungal activity, α-Cadinol, β-Eudesmol, Ferruginol.

Taiwania cryptomerioides Hayata (Cupressaceae) is one of the five most valuable conifers in Taiwan [1]. Many previous studies have demonstrated that the leaf and heartwood essential oils and extractives of *T. cryptomerioides* have inhibitory effects against mites and fungi, as well as having antioxidant and anticancer properties [2-6]. However, no prior study has investigated the chemical composition and biological activity of the twig essential oil. Thus, this was obtained by hydrodistillation, analyzed for its chemical composition, and evaluated for its antioxidant, antimicrobial and anti-wood-decay fungal activities.

A dark-yellow oil was obtained from the twigs in a yield of $0.98 \pm 0.03\%$. Thirty-five compounds were identified (Table 1), of which oxygenated sesquiterpenes were predominant (75.3%), followed by diterpenes (19.8%), sesquiterpene hydrocarbons (4.3%), and oxygenated monoterpenes (0.5%). Among the oxygenated sesquiterpenes, α -cadinol (45.9%) and β -eudesmol (10.8%) were the major compounds, and of the diterpenes, ferruginol (18.9%) was the chief component.

The essential oil was tested for its DPPH free radical scavenging capability. Ascorbic acid was used as a positive control. The IC_{50} of the DPPH free radical scavenging capability of the twig essential oil was 90.8 ± 0.2 µg/mL. The individual main components of the twig essential oil, α -cadinol, β -eudesmol and ferruginol, were also compared for their DPPH free radical scavenging capability. The results in decreasing order were ferruginol ($IC_{50} = 48.0 \ \mu g/mL$), α -cadinol and β -eudesmol ($IC_{50} > 2000 \ \mu g/mL$). Hence, we deduced that ferruginol was mainly responsible for the radical scavenging. The results are also in congruency with the conclusions

of several other reports [3,7,8]. The antioxidant activity (IC₅₀ value) of *T. cryptomerioides* twig oil was superior to those of the leaf, heartwood, sapwood, bark and twig essential oils of sugi (*Cryptomeria japonica*) [8], which have IC₅₀ values of >2000, 327, 113, 580, and 124 µg/mL, respectively. Furthermore, the IC₅₀ values of the twig oil and those of the leaf oils of different *Cinnamomum osmophloeum* clone strains, which ranged from 33.4 to 708.5 µg/mL [9], were within the same range.

The twig essential oil of T. cryptomerioides was tested against three Gram-positive and five Gram-negative bacteria, as well as two fungi. The results, presented in Table 2, show strong growth suppression against all ten microbes studied. The most sensitive were Bacillus cereus, Staphylococcus aureus, S. epidermidis, and Candida albicans, with inhibition zones of 45~52 mm and MIC values of 31.25~62.5 µg/mL, respectively. The essential oil showed superior suppressive activity toward the Gram-positive bacteria than either the Gram-negative bacteria or the fungi. The probable cause of the susceptibility of Gram-positive bacteria and relative tolerance of Gram-negative bacteria to essential oils has been correlated with the presence of a hydrophilic outer layer [11]. It is presumed that penetration of hydrophobic components in Gramnegative microorganisms is more difficult due to the presence of a second physical barrier formed by the outer membrane [12]. In comparison with the antimicrobial activity of the essential oils from Metasequioa glyptostroboides [13], Litsea kostermansii [14], L. akoensis [15], Machilus pseudolongifolia [16], and M. kusanoi [17] the antimicrobial activity of the twig essential oil of T. cryptomerioides was superior. The results validated the excellent antimicrobial activity of T. cryptomerioides twig essential oil.

Table 1: Chemical composition of the twig essential oil of Taiwania cryptomerioides.

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Consituents	RI ^{a)}	Conc.(%)	Identification b)
Camphor	1146	0.2	MS, KI, ST
Citronellyl acetate	1353	0.4	MS, KI, ST
α-Cedrene	1412	0.2	MS, KI, ST
γ-Muurolene	1480	0.2	MS, KI
α-Muurolene	1500	0.4	MS, KI
γ-Cadinene	1514	0.9	MS, KI
δ-Cadinene	1523	0.3	MS, KI
trans-Calamenene	1529	0.4	MS, KI
α-Cadinene	1539	0.2	MS, KI
α-Calacorene	1546	0.4	MS, KI, ST
Elemol	1550	4.4	MS, KI, ST
Caryophyllene oxide	1583	0.3	MS, KI, ST
Gleenol	1587	0.3	MS, KI
Cedrol	1601	3.7	MS, KI, ST
1,10-di-epi-Cubenol	1619	0.8	MS, KI
1-epi-Cubenol	1629	1.7	MS, KI
v-Eudesmol	1632	3.6	MS, KI, ST
B-Eudesmol	1651	10.8	MS, KI, ST
α-Cadinol	1654	45.9	MS, KI, ST
β-Bisabolol	1675	0.6	MS, KI, ST
Cadalene	1677	1.5	MS, KI
cis-14-nor-Muurol-5-en-4-one	1689	0.3	MS, KI
Acorenone b	1697	0.2	MS, KI
epi-Nootkatol	1699	0.1	MS, KI
10-nor-Calamenen-10-one	1702	0.1	MS, KI
13-Hydroxy-valencene	1768	0.3	MS, KI
14-Hydroxy-α-muurolene	1780	0.3	MS, KI
Hinesol acetate	1784	0.8	MS, KI
B-Eudesmol actate	1792	0.8	MS, KI
α-Eudesmol actate	1795	0.4	MS, KI
Kudtdiol	1912	0.3	MS, KI
Sandaracopimarinal	2184	0.2	MS, KI
Phyllocladanol	2210	0.3	MS, KI
Sandaracopimarinol	2269	0.1	MS, KI
Ferruginol	2332	18.9	MS, KI, ST
	2002	10.7	
Compounds identified			
Monoterpene hydrocarbons (%)		0.0	
Oxygenated monoterpenes (%)		0.5	
Sesquiterpene hydrocarbons (%)		4.3	
Oxygenated sesquiterpenes (%)		75.3	
Diterpenes (%)		19.8	
Oil Yield (ml/100 g)		0.98 ± 0.03	
	: a _ c		10]

^a Retention index on a DB-5 column with reference to *n*-alkanes [10].

^b MS, NIST and Wiley library spectra and the literature; RI, Retention index; ST,

authentic standard compounds.

However, to ascertain the source compounds of the antimicrobial activity of *T. cryptomerioides* twig essential oil, the main components were individually tested for antimicrobial activity. Results indicated that the active compounds were α -cadinol, β -eudesmol and ferruginol. Various studies support the argument that these compounds are highly active in suppressing microbial growth [18-21].

The twig essential oil was also tested against two white rot fungi (*Trametes versicolor* and *Phanerochaete chrysosporium*) and two brown rot fungi (*Phaeolus schweinitzii* and *Lenzites sulphureus*). The anti-wood-decay fungal indices presented in Table 3 clearly demonstrate the excellent anti-wood-decay fungal activity of the twig essential oil. Growth of *T. versicolor, Phane. chrysosporium, Phaeo. schweintizii* and *L. sulphureus* were completely inhibited at

Table 2: Antimicrobia	al activity of the	e twig essential	l oil of T. cryptomerioid	des
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concentrations of 12.5, 12.5, 6.25, and 6.25 μ g/mL, respectively. The anti-wood-decay fungal activity of the twig essential oil was superior to those of the essential oils of *Cunninghamia konishii* [22], *Chamaecyparis formosensis* [23] and *C. japonica* [24].

In order to ascertain the active compounds of the *T*. *cryptomerioides* twig essential oil, we also tested the anti-wood-decay fungal activities of its major components. The results indicated that the sources of activity were also α -cadinol, β -eudesmol and ferruginol. At a 50 µg/mL concentration, α -cadinol showed total growth inhibition against all the white-rot and brown-rot fungi tested, while β -eudesmol and ferruginol at 50 µg/mL could partially inhibit white-rot and brown-rot fungi. The results agree with those of Rudman [25], Kondo and Imamura [21], and Mori *et al.* [26]. Thus, the excellent wood-decay-fungi inhibition activities exhibited by *T. cryptomerioides* twig essential oil could be attributed to the presence of compounds such as α -cadinol, β -eudesmol and ferruginol.

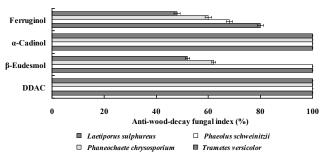


Figure 1: Anti-wood-decay fungal indices of the three main compounds (50 µg/mL) of the twig essential oil of *T. cryptomerioides*.

Note: DDAC (didecyl dimethyl ammonium chloride) (50 μ g/mL) is a wood preservative for wood decay fungi and is used as a positive control. Anti-wood-decay-fungal index see Experimental section. Data are expressed as the means \pm SD of three independent experiments.

Experimental

Plant materials: Fresh twigs of *T. cryptomerioides* were collected in June 2011 from Chilan Mt in northeast Taiwan (Yilan County, elevation 850 m, N 24° 40′ 50″, 121° 39′ 10″). The samples were compared with specimen no. ou 5996 from the Herbarium of the National Chung-Hsing University and positively identified by Prof. Yen-Hsueh Tseng of NCHU. The voucher specimen (CLH-017) was deposited in the NCHU herbarium. Leaves of the species were collected for subsequent extraction and analysis.

Isolation of the twig essential oil: Twigs of *T. cryptomerioides* (1 Kg) were placed in a round-bottom flask and hydrodistilled for 8 h with 3 L of distilled water. The essential oil obtained was dried

	T. crypton	nerioides		Compounds ^c			Antibiotics	
Microbial species	Tw	rig	1	2	3	Tetracycline (30 µg/disk)	Gentamicin (10 µg/disk)	Nystatin (30 µg/disk)
	IZ ^a	MIC ^b	MIC	MIC	MIC	ΪΖ	IZ	ΪΖ
Bacillus cereus	52 ± 0.6	31.25	125	125	15.625	22 ± 0.8	-	nt
Staphylococcus aureus	50 ± 0.4	31.25	62.5	62.5	62.5	21 ± 0.4	-	nt
Staphylococcus epidermidis	50 ± 0.4	31.25	62.5	62.5	15.625	34 ± 0.4	-	nt
Escherichia coli	38 ± 0.8	125	500	750	250	-	22 ± 0.8	nt
Enterobacter aerogenes	32 ± 0.8	250	125	250	250	10 ± 0.4	-	nt
Klebsiella pneumoniae	30 ± 0.4	250	125	250	375	-	21 ± 0.8	nt
Pseudomonas aeruginosa	32 ± 0.8	250	500	750	250	-	12 ± 0.8	nt
Vibrio parahaemolyticus	29 ± 0.4	375	1000	1000	375	-	13 ± 0.8	nt
Aspergillus niger	28 ± 0.4	375	1000	>1000	375	nt	nt	17 ± 0.8
Candida albicans	45 ± 0.4	62.5	125	125	62.5	nt	nt	19 ± 0.8

^a Inhibition zone diameter (mm), including diameter of sterile disk 6 mm; values are given as mean ± SD.^b Minimum inhibitory concentration values as $\mu g/mL$.^c 1. β-eudesmol (≥ 99.5%), 2. α-cadinol (100%), 3. Ferruginol (100%). Compound 1 was purchased from the Fluka Co. (Milwaukee, USA), whereas compound 2 was from an isolate of Ho *et al*'s study on *Machilus philippinenesis* essential oil [27]; compound 3 was from an isolate of Ho *et al*.'s study of *Cryptomeria japonica* essential oil [8]. Essential oil tested at 15 µL/disc for bacteria and 30 µL/disc for fungi.(-), Inactive; nt, not tested.

	Antifungal index ^a (%)			
Dosage (µg/mL)	Trametes versicolor	Phaneochaete chrysosporium	Phaeolus schweinitzii	Lenzites sulphureus
6.25	81 ± 6.6	82 ± 3.3	100 ± 0^{b}	100 ± 0
12.5	100 ± 0	100 ± 0	100 ± 0	100 ± 0
25	100 ± 0	100 ± 0	100 ± 0	100 ± 0
50	100 ± 0	100 ± 0	100 ± 0	100 ± 0
75	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100	100 ± 0	100 ± 0	100 ± 0	100 ± 0

^a Antifungal index see Experimental section. ^b 100 \pm 0 % = complete growth inhibition of mycelium. Data are expressed as the means \pm SD of three independent experiments.

with anhydrous sodium sulfate. The oil yield and all test data are expressed as the means \pm SD of 3 independent experiments.

Essential oil analysis and component identification: The experimental conditions for GC analysis of the essential oil were similar to those reported earlier [16]. Identification of the oil constituents was based on comparisons of retention index (RI) [10], retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra, and literature [10,28], respectively.

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capability test: The method of Ho *et al.* [8] was used. Fifty μ L of various dilutions of the oils were mixed with 5 mL of a 0.004% methanol solution of DPPH. After an incubation period of 30 min, the absorbance of the samples was determined at 517 nm using a Jasco 7800 spectrophotometer. Ascorbic acid was used as a positive control. Data are expressed as the means \pm SD of 3 independent experiments.

Antimicrobial activity [29]: Discs containing 15 μ L and 30 μ L of the oil dissolved in dimethylsulfoxide (DMSO) were placed on the inoculated plates with test microorganisms. Growth inhibition zones (including disc diameter of 6 mm) were measured after 24 h and 48 h of incubation at 37°C and 24°C for bacteria and

fungi, respectively. Gentamicin and tetracycline for bacteria, and nystatin for fungi were used as positive controls. Microbial strains were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute, Hsinchu City, Taiwan. The microbial strains included 5 Gram-negative bacteria: Escherichia coli (IFO 3301), Enterobacter aerogenes (ATCC 13048), Klebsiella pneumoniae (ATCC 4352), Pseudomonas aeruginosa (IFO 3080), and Vibrio parahaemolyticus (ATCC 17803); 3 Gram-positive bacteria: B. cereus (ATCC 11778), S. aureus (ATCC 6538P), and S. epidermidis (ATCC 12228); 1 fungus: Aspergillus niger (ATCC 16404) and 1 yeast: Candida albicans (ATCC 10231). Minimum inhibitory concentration (MIC) values were measured by the microdilution broth susceptibility assay recommended by NCCLS [30] and as reported earlier [16]. Data are expressed as the means ± SD of 3 independent experiments.

Anti-wood-decay fungal assays: The method of Su et al. [31] was adopted. The fungi used were Trametes versicolor (BCRC 35253), Phaneochaete chrysosporium (BCRC 36200), Phaeolus schweinitzii (BCRC 35365) and Lenzites sulphureus (BCRC 35305). Microbial strains were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute, Hsinchu City, Taiwan. Anti-wood-decay fungal assays were carried out in triplicate and the data were averaged. Different concentrations of the essential oil (6.25~100 µg/mL) were added to sterilized potato dextrose agar (PDA). The test plates were incubated at 27°C. When the mycelium of the fungi reached the edge of the control plate, the anti-wood-decay fungal index was calculated as follows: Anti-wood-decay fungal index (%)= (1-Da/Db) X 100, where Da is the diameter of the growth zone in the experimental dish (cm) and Db is the diameter of the growth zone in the control dish (cm). DDAC (didecyl dimethyl ammonium chloride) was used as a positive control. Data are expressed as the means \pm SD of 3 independent experiments.

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