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Composition and antimicrobial activities of the leaf essential oil of *Machilus zuihoensis* from Taiwan

Chen-Lung Ho,[1,2] Pei-Chun Liao,[3] Yu-Chang Su[*,1]

[1]Department of Forestry, National Chung Hsing University, Taichung, Taiwan 402,
[2]Division of Wood Cellulose, Taiwan Forestry Research Institute, Taiwan 100,
[3]Institute of Biotechnology, National Ilan University, Taiwan 260.

Abstract: This study investigated the chemical composition, and antimicrobial and anti-wood-decay fungal activities of the essential oil isolated from the leaf of endemic *Machilus zuihoensis* Hayata, Lauraceae, of Taiwan. The essential oil from the fresh leaves of *M. zuihoensis* was isolated using hydrodistillation in a Clevenger-type apparatus, and characterized by GC-FID and GC-MS. A total of 104 compounds were identified, representing 100% of the oil. The main components identified were *n*-dodecanal (23.8%) and (*E*)-nerolidol (10.5%). The antimicrobial activity of the oil was tested by the disc diffusion method and micro-broth dilution method against ten microbial species (*Bacillus cereus, Staphylococcus aureus, S. epidermidis, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa, Vibrio parahaemolyticus, Aspergillus niger*, and *Candida albicans*), respectively. The oil exhibited strong growth suppression against Gram-positive bacteria and yeast with inhibition zones of 35~43 mm to MIC values of 125 μg mL^{-1}, respectively. The anti-wood-decay fungal activity of the oil was also evaluated. Results showed that the oil demonstrated excellent activity against four wood-decay-fungi species (*Trametes versicolor, Phaneochaete chrysosporium, Phaeolus schweintizii*, and *Lenzites sulphureu*). For the antimicrobial and anti-wood-decay fungal activities of the oil, the active source compounds were determined to be τ-cadinol, β-eudesmol, and *n*-dodecanal.

Article

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Introduction

Lauraceae contains approximately 45 genera and 2250 species. The *Machilus* genus distributes mostly in tropical and subtropical regions of northern hemisphere. East Asia is where the genus mainly distributes, and there are about 100 species there. In Taiwan, there are eight endemic *Machilus* trees, including *M. konishii* Hayata*, M. kusanoi Hayata, M. mushaensis* Lu, *M. obovatifolia* (Hayata) Kaneh. et. Sasaki, *M. obovatifolia* var. taiwensis Lu & Chen, M. philippinensis Merr., *M. pseudolongifolia* Hayata., M. thunbergii Siebold & Zucc. and *M. zuhoensis* Hayata (Liao, 1996). In our previous report, leaf essential oils of *M. kusanoi* (Ho et al., 2011), *M. pseudolongifolia* (Ho et al., 2010a), *M. philippinensis* (Ho et al., 2010b) and *M. obovatifolia* (Ho et al., 2010c) were extracted and found to have antimicrobial activities. *M. zuihoensis* is an endemic species of Taiwan and is distributed from the lowlands to 1400 m. Its bark is an incense material for joss sticks, wood of the species is used for building and furniture (Liao, 1996). There are no literature reports on the chemical composition and biological activities of the essential oils from this species. Therefore, this study used hydrodistillation to extract the leaf oil of this species and analyzed the oil using a GC-FID and a GC-MS.

To prevent widespread in-hospital infection, we selected ten microbial strains for testing. In addition, wood has recently become a preferred material for construction and decoration. For such uses, durability is a crucial concern. Traditional heavy metal-containing wood preservatives used in a broad spectrum of biocides for wood protection are being limited because of their toxicity to the environment and mammals (Kartal et al., 2004a). Because certain wood preservatives such as chromated copper arsenate (CCA) have been banned or limited for some applications in many European countries, the United States, and Japan, a considerable amount of research has been focused on developing new environmentally friendly wood preservatives that protect wood against fungi and insects (Kartal et al., 2004b). However, the warm and humid climate of Taiwan can easily cause decay of wood products. Therefore, to prevent wood decay, we also applied the essential oil to four strains of commonly found white rot fungi and

brown rot fungi in Taiwan to examine their respective interdiction efficacies. As a consequence, the second part of the study examined the antimicrobial and anti-wood-decay fungal activities of the essential oils. The purpose of this study was to establish a chemical basis for effective multipurpose utilization of the species.

Material and Methods

Plant material

Fresh leaves of *Machilus zuihoensis* Hayata, Lauraceae, were collected in July 2010 from Tiaomikeng (Nantou County, central Taiwan, elevation 1050 m, N 23º 57′ 08″, E 120º 54′ 36″). The samples were compared with specimen no. TAIF 65078 from the Herbarium of the Taiwan Forestry Research Institute and were positively identified by Prof. Yen-Hsueh Tseng of National Chung Hsing University (NCHU). The voucher specimen (CLH-014) was deposited in the NCHU herbarium. Before extraction, the leaf was air dried at room temperature protected from the light for one week.

Isolation of leaf essential oil

The essential oil of the dry leaves (200 g) was extracted using a Clevenger-type apparatus using a hydrodistillation technique. After extraction, the volume of essential oil obtained was measured, and the essential oil was stored in glass containers hermetically sealed with rubber lids, covered with aluminum foil to protect the contents from light, and kept refrigerated at 8 ºC until used. The oil yield and all test data are the average of triplicate analyses.

Essential oil analysis

A Hewlett-Packard HP 6890 gas chromatograph equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm film thickness, J&W Scientific) and a FID detector was used for quantitatively determining oil components. Oven temperature was programmed as follows: 50 ºC for 2 min, rising to 250 ºC at 5 ºC min-1. Injector temperature: 270 ºC. Carrier gas: He with a flow rate of 1 mL min^{-1}. Detector temperature: 250 ºC, split ratio: 1:10. Diluted samples (1.0 µL, 1/100, v/v, in ethyl acetate) were injected manually in the split mode. Identification of the oil components was based on their retention indices and mass spectra, obtained from GC–MS analysis on a Hewlett-Packard HP 6890/HP5973 equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm film thickness, J&W Scientific). The GC analysis parameters listed above and the MS were obtained (full scan mode: scan time: 0.3 s, mass range was *m/z* 30-500) in the EI mode at 70 eV. All data were the average of triplicate analyses.

Component identification

Identification of the leaf essential oil constituents was based on comparisons of retention index (RI) (Adams, 2001), retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra, and relevant literature (Adams, 2001; Massada, 1976).

Antimicrobial activity

The *in vitro* antibacterial and antimicrobial activities of the oil were evaluated by the disc diffusion method using Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for fungi (Baron & Finegold, 1990). Discs containing 15 µL and 30 µL of the oil, which was dissolved in dimethyl sulphoxide (DMSO), were placed on the inoculated plates with test microorganisms. Growth inhibition zones (including a disc diameter of 6 mm) were measured after 24 and 48 h of incubation at 37 and 24 °C for bacteria and fungi, respectively. Gentamicine and tetracycline for bacteria, and nystatine 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 five Gram-negative bacteria: *Escherichia coli* (IFO 3301), *Enterobacter aerogenes* (ATCC 13048), *Klebsiella pneumoniae* (ATCC 4352), *Pseudomonas aeruginosa* (IFO 3080), and *Vibrio parahaemolyticus* (ATCC 17803); three Gram-positive bacteria: *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 6538P), and *S. epidermidis* (ATCC 12228); one fungus: *Aspergillus niger* (ATCC 16404) and one yeast: *Candida albicans* (ATCC 10231). Minimum inhibitory concentration (MIC) values were measured by the micro-dilution broth susceptibility assay recommended by NCCLS (NCCLS, 1999). Stock solutions of the oil were prepared in DMSO. Dilution series were prepared from 1000 µg mL^{-1} to 50 µg mL^{-1} in sterile distilled water in micro-test tubes, from where they were transferred to 96-well microtiter plates. Bacteria grown in double-strength Mueller-Hinton broth and fungi grown in double-strength Sabouraud dextrose broth were standardized to 108 CFU mL^{-1}. The last row, containing only the serial dilutions of sample without microorganisms, was used as a negative control. Sterile distilled water and medium served as a positive control. After incubation at 37 °C for 24 h and 24 °C for 48 h, the MIC values were determined. All experiments were performed in triplicate.

Anti-wood-decay fungal assays

The method of Cheng et al. (2005) was adopted. The fungi used were *Trametes versicolor* (L. ex Fr.) Quel. (BCRC 35253), *Phanerochaete chrysosporium* Burdsall (BCRC 36200), *Phaeolus schweinitzii* (Fries) Paterson (BCRC 35365) and *Lenzites sulphureu* (B. ex Fr.) Bond. (BCRC 35305). Cultures of each of the fungi were maintained on potato dextrose agar (PDA) medium and were stored at 4 °C. 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 performed in triplicate and the data were averaged. Briefly, 100.0, 75.0, 50.0, 25.0 and 12.5 μg mL^{-1} of essential oils were added to sterilized PDA in 9 cm plates (Petri dish). After transfer of the mycelium of 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 essential oils), the antifungal indices were calculated. The formula of antifungal indices is shown as

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) is a wood preservative for wood decay fungi and is used as a positive control.

Results and Discussion

Hydrodistillation of *M. zuihoensis* leaves produced a yellow-colored oil with a yield of 2.68±0.05 mL/100 g, based on the dry weight of leaves. One hundred and four compounds were identified (Table 1), of which oxygenated sesquiterpenes were predominant (31.1%), followed by non-terpenoids (30.2%), sesquiterpene hydrocarbons (21.8%), monoterpene hydrocarbons (14.6%), oxygenated monoterpenes (1.9%), and diterpenes (0.5%). Among the oxygenated sesquiterpenes, (*E*)-nerolidol (10.5%), β-eudesmol (5.7%), and τ-cadinol (5.3%) were the major compounds, and of the non-terpenoids, *n*-dodecanal (23.8%) and n-decanal (5.3%) were the chief components. Among the sesquiterpene hydrocarbons, viridiflorene (4.2%) was the main component. This is the first study on the chemical characterization from the leaf oil.

From the results presented above, the leaf oil constituents of *M. zuihoensis* were primarily sesquiterpenoids. Intra-genus leaf oil comparisons indicated that many *Machilus* trees, such as *M. kusanoi* (Ho et al., 2011), *M. pseudolongifolia* (Ho et al., 2010a), *M. philippinensis* (Ho et al., 2010b), *M. obovatifolia* (Ho et al., 2010c), *M. velutina* (Zhu et al., 1994), and *M. thunbergii* (Komae & Hayashi, 1972), all have predominately sesquiterpenoids as their main constituents. However, the main components of the individual species differed. Further comparison with the leaf oil of *M. japonica* (Van Khien et al., 2009) was predominantly monoterpenoids and, therefore, differed from the leaf oil of *M. zuihoensis*.

The essential oil of *M. zuihoensis* was tested against three Gram-positive and five Gram-negative bacteria, as well as two fungi. The results, presented in Table 2, indicated that a moderate to strong growth suppression against all ten microbes. The most sensitive microorganisms were *B. cereus, S. aureus, S. epidermidis*, and *C. albicans* with inhibition zones of 35 to 43 mm to MIC values of 125 μg mL^{-1}, respectively. The essential oil showed superior suppressive activity toward the Gram-positive bacteria than that of either the Gram-negative bacteria or the fungi.

Table 1. Chemical composition of the leaf oil *Machilus zuihoensis* Hayata, Lauraceae.

Constituents	RI[a]	Conc. (%)[b]	Identification[c]
cis-3-hexenol	859	0.1	RI, MS, ST
α-pinene	939	0.4	RI, MS, ST
camphene	954	0.1	RI, MS, ST
benzaldehyde	960	td	RI, MS, ST
trans-pinene	975	t	RI, MS
β-pinene	979	t	RI, MS, ST
myrcene	991	0.3	RI, MS, ST
δ-2-carene	1002	t	RI, MS, ST
α-phellandrene	1003	2.6	RI, MS, ST
cis-3-hexenyl acetate	1005	t	RI, MS, ST
iso-sylvestrene	1009	0.1	RI, MS
α-terpinene	1017	0.5	RI, MS, ST
p-cymene	1025	3.8	RI, MS, ST
β-phellandrene	1030	1.0	RI, MS, ST
1.8-cineole	1031	t	RI, MS, ST
cis-ocimene	1037	3.3	RI, MS
trans-ocimene	1050	0.4	RI, MS
γ-terpinene	1060	0.2	RI, MS, ST
n-octanol	1068	t	RI, MS, ST
p-mentha-3.8-diene	1073	0.1	RI, MS
p-cymenene	1091	1.8	RI, MS, ST
linalool	1097	0.1	RI, MS, ST
n-nonanal	1101	t	RI, MS, ST
cis-*p*-menth-2-en-1-ol	1122	0.1	RI, MS
allo-ocimene	1132	0.1	RI, MS
trans-pinocarveol	1139	t	RI, MS, ST
cis-β-terpineol	1144	t	RI, MS
neo-3-thujanol	1154	t	RI, MS

3-thujanol	1169	t	RI, MS
n-nonanol	1169	t	RI, MS, ST
menthol	1172	t	RI, MS, ST
terpinen-4-o1	1177	0.1	RI, MS, ST
p-cymen-8-ol	1183	0.1	RI, MS, ST
α-terpineol	1189	1.0	RI, MS, ST
methyl salicylate	1192	t	RI, MS, ST
dihydro carveol	1194	0.1	RI, MS
n-decanal	1202	5.3	RI, MS, ST
iso-dihydro carveol	1215	t	RI, MS
trans-pulegol	1215	t	RI, MS
neoiso-dihydro carveol	1229	t	RI, MS
carvotanacetone	1247	0.1	RI, MS
piperitone	1253	t	RI, MS
isobornyl acetate	1286	t	RI, MS, ST
10-undecenal	1300	0.1	RI, MS, ST
n-undecanal	1307	0.1	RI, MS, ST
δ-elemene	1338	t	RI, MS
α-cubebene	1351	t	RI, MS
eugenol	1359	t	RI, MS, ST
10-undecen-1-ol	1363	0.2	RI, MS, ST
n-undecanol	1370	0.2	RI, MS, ST
isoledene	1376	0.1	RI, MS, ST
α-copaene	1377	0.6	RI, MS
geranyl acetate	1381	0.1	RI, MS, ST
trans-β-damascen	1385	t	RI, MS
β-bourbonene	1388	t	RI, MS
β-elemene	1391	0.5	RI, MS
dodecanal	1409	23.8	RI, MS, ST
β-caryophyllene	1419	2.6	RI, MS, ST
β-copaene	1432	0.1	RI, MS
β-gurjunene	1434	0.1	RI, MS
γ-elemene	1437	0.1	RI, MS
aromadendrene	1441	1.3	RI, MS, ST
cis-prenyl limonene	1446	0.3	RI, MS
α-*neo*-clovene	1454	0.2	RI, MS
α-caryophyllene	1455	0.8	RI, MS
allo-aromadendrene	1460	0.2	RI, MS, ST
9-*epi*-β-caryophyllene	1466	0.3	RI, MS
drima-7,9(11)-diene	1473	0.2	RI, MS
trans-cadina-1(6),4-diene	1477	1.6	RI, MS
germacrene D	1485	0.4	RI, MS, ST
β-selinene	1490	1.2	RI, MS
δ-selinene	1493	0.5	RI, MS
viridiflorene	1497	4.2	RI, MS, ST
α-muurolene	1500	1.1	RI, MS
α-bulnesene	1510	0.8	RI, MS
δ-amorphene	1512	0.1	RI, MS
γ-cadinene	1514	1.0	RI, MS
cubebol	1515	0.1	RI, MS
δ-cadinene	1523	2.1	RI, MS
trans-cadina-1(2),4-diene	1535	0.3	RI, MS
α-cadinene	1539	0.9	RI, MS
α-calaconene	1546	0.3	RI, MS, ST
cis-cadinene	1554	0.2	RI, MS
(*E*)-nerolidol	1563	10.5	RI, MS, ST
ledol	1569	0.4	RI, MS
caryophyllenyl alcohol	1572	0.8	RI, MS, ST
globulol	1585	1.7	RI, MS, ST
guaiol	1601	0.8	RI, MS, ST
5-*epi*-7-epi-α-eudesmol	1608	0.9	RI, MS
n-tetradecanal	1613	0.2	RI, MS, ST
1,10-di-*epi*-cubenol	1619	0.3	RI, MS
10-*epi*-γ-eudesmol	1624	0.8	RI, MS
1-*epi*-cubenol	1629	0.4	RI, MS
γ-eudesmol	1632	0.6	RI, MS
τ-cadinol	1640	5.3	RI, MS, ST
δ-cadinol	1646	0.5	RI, MS
β-eudesmol	1651	5.7	RI, MS, ST
α-eudesmol	1654	0.2	RI, MS
intermedeol	1667	0.2	RI, MS
cadalene	1677	0.1	RI, MS
epi-α-bisabolol	1685	0.5	RI, MS
α-bisabolol	1686	0.3	RI, MS
(2*Z*, 6*E*)-farnesol	1701	1.0	RI, MS
phytol	1943	0.5	RI, MS, ST
Monoterpene hydrocarbons (%)		14.6	
Oxygenated monoterpenes (%)		1.9	
Sesquiterpene hydrocarbons (%)		21.8	
Oxygenated sesquiterpenes (%)		31.1	
Diterpenes (%)		0.5	
Others (%)		30.2	
Oil Yield (mL/100 g)		2.68±0.05	

[a]Retention index on a DB-5 column with reference to *n*-alkanes (Adams, 2001); [b]n= 3; [c]MS, NIST and Wiley library spectra and the literature; RI: Retention index; ST: authentic standard compounds; [d]trace<0.1%.

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 (Kalemba & Kunicka, 2003). It is presumed that penetration of hydrophobic components in Gram-negative microorganisms is more difficult due to the presence of a second physical barrier formed by the outer membrane (Mann et al., 2000; Duffy & Power, 2001). Comparing the antimicrobial activities of the

leaf essential oils with that extracted from *M. kusanoi* (Ho et al., 2011), *M. pseudolongifolia* (Ho et al., 2010a), and *Litsea linii* (Ho et al., 2010d), and the twig oil with that extracted from *L. mushaensis* (Ho et al., 2010d), the leaf essential oil of *M. zuihoensis* was superior (Table 3). The results verify that *M. zuihoensis* leaf oil has excellent antimicrobial activity. However, to ascertain the source compounds of antimicrobial activity from *M. zuihoensis*, the main components were individually tested for antimicrobial activities. The results indicated that the active source compounds were τ-cadinol, β-eudesmol and *n*-dodecanal. These results were similar to those of Ho et al. (2010d; 2011). There are also studies that have supported the contention that these compounds have high activity in suppressing microbial growth (Kondo & Imamura, 1986; Knobloch et al., 1989; Kalemba & Kunicka, 2003; Kusuma et al., 2004).

Table 2. Antimicrobial activity of the leaf essential oil of *Machilus zuihoensis.*

Microbial species	*M. zuihoensis*		Compounds [c]						Antibiotics		
	Leaf		1	2	3	4	5	6	Tetracycline (30 µg/disk)	Gentamicine (10 µg/disk)	Nystatine (30 µg/disk)
	IZ[a]	MIC[b]	MIC	MIC	MIC	MIC	MIC	MIC	IZ	IZ	IZ
Bacillus cereus	36±0.4	125	>1000	250	>1000	250	62.5	125	22±0.8	-	nt
Staphylococcus aureus	43±0.4	125	1000	125	1000	250	62.5	62.5	21±0.4	-	nt
Staphylococcus epidermidis	42±0.8	125	1000	125	1000	250	62.5	62.5	34±0.4	-	nt
Escherichia coli	25±0.8	500	>1000	500	>1000	1000	500	500	- d	22±0.8	nt
Enterobacter aerogenes	30±0.4	375	>1000	250	>1000	500	125	125	10±0.4	-	nt
Klebsiella pneumoniae	29±0.4	375	>1000	250	>1000	500	125	125	-	21±0.8	nt
Pseudomonas aeruginosa	25±0.8	500	>1000	750	>1000	1000	500	500	-	12±0.8	nt
Vibrio parahaemolyticus	19±0.4	1000	>1000	1000	>1000		1000	1000	-	13±0.8	nt

[a]Inhibition zone diameter (mm), including diameter of sterile disk 6 mm; values are given as mean±SD; [b]Minimum inhibitory concentration values as µg mL^{-1}; [c]1. *n*-decanal (≥ 98%), 2. *n*-dodecanal (≥ 98%), 3. viridiflorene (≥ 98%), 4. (*E*)-nerolidol (≥ 98.5%), 5. τ-cadinol (100%), 6. β-eudesmol (≥98%). Compound 1 to 4 were purchased from the Fluka Co. (Milwaukee, USA), and Compound 6 was purchased from the Wako Co. (Tokyo, Japan), where as the Compound 5 was from isolate of the Ho et al. (2010b) study on *Machilus philippinenesis* essential oil; Essential oil tested at 15 µL/disc for bacteria and 30 µL/disc for fungi; [d](-), Inactive; ent, not tested.

Table 3. Comparison of the MIC values (µg mL^{-1}) of the oils of *M. zuihoensis* and those of *M. kusanoi, M. pseudolongifolia, Litsea linii* and *L. mushaensis* against the microbial.

	Essential oil	Microbial *										Reference
		B. c.	*S. a.*	*S. e.*	*E. c.*	*E. a.*	*K. p.*	*P. a.*	*V. p.*	*A. n.*	*C. a.*	
Leaf												
	Machilus zuihoensis	125	125	125	500	375	375	500	1000	1000	125	This study
	M. kusanoi	250	125	125	500	375	375	500	1000	1000	250	Ho et al., 2011
	M. pseudolongifolia	250	125	125	750	375	375	750	1000	>1000	250	Ho et al., 2010a
	Litsea linii	500	500	500	750	750	>1000	>1000	>1000	>1000	750	Ho et al., 2010d
Twig												
	L. mushaensis	1000	750	750	>1000	>1000	>1000	>1000	>1000	>1000	>1000	Ho et al., 2010d

** B. c.: Bacillus cereus; S. a.: Staphylococcus aureus; S. e.: Staphylococcus epidermidis; E. c.: Escherichia coli; E. a.: Enterobacter aerogenes; K. p.: Klebsiella pneumoniae; P. a.: Pseudomonas aeruginosa; V. p.: Vibrio parahaemolyticus; A. n.: Aspergillus niger; C. a.: Candida albicans.*

Table 4. Anti-wood-decay fungal indices of leaf essential oil from *Machilus zuihoensis.*

Dosage (µg mL^{-1})	Antifungal index* (%)			
	Trametes versicolor	*Phaneochaete chrysosporium*	*Phaeolus schweintizii*	*Lenzites sulphureu*
12.5	35±3.3	25±3.3	38±3.3	68±3.3
25.0	62±3.3	50± 3.3	80±3.3	100±0.0
50.0	90±6.6	88±6.6	100±0.0	100±0.0
75.0	100±0.0	100±0.0	100±0.0	100±0.0
100.0	100±0.0	100±0.0	100±0.0	100±0.0

*n= 3.

Table 5. Comparison of the MIC values (µg mL^{-1}) of the leaf oils of *Machilus zuihoensis* and those of *M. pseudolongifolia, M. philippinensis* and *M. kusanoi* against the wood-decay fungi.

Essential oil	Fungi *				Reference
	T. v.	*Phane. c.*	*Phaeo. s.*	*L. s.*	
Machilus zuihoensis	75	75	50	25	This study
M. pseudolongifolia	75	75	75	25	Ho et al., 2010a
M. philippinensis	100	100	100	50	Ho et al., 2010b
M. kusanoi	75	75	75	25	Ho et al., 2011

** T. v.: Trametes versicolor; Phane. c.: Phaneochaete chrysosporium; Phaeo. s: Phaeolus schweintizii; L. s.: Lenzites sulphureu*

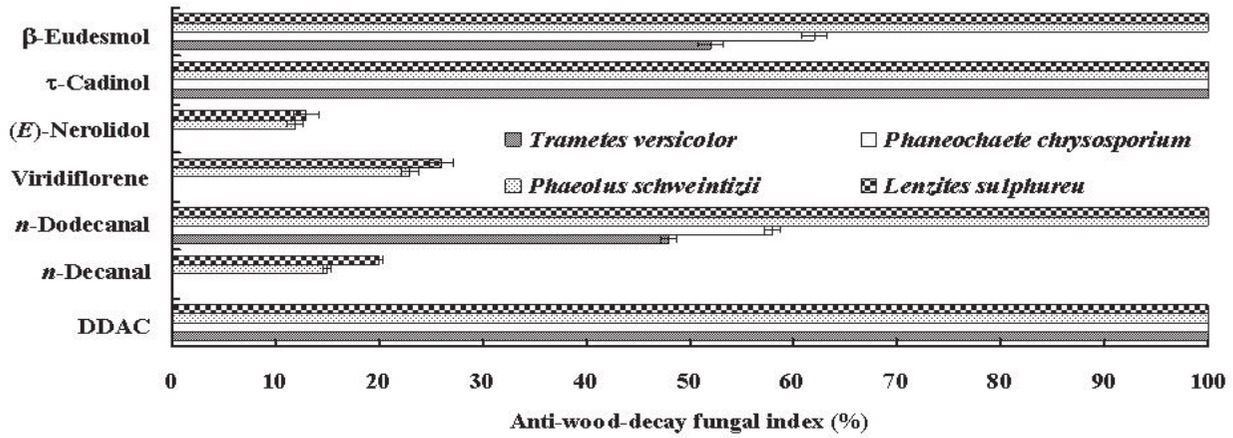

Figure 1. Anti-wood-decay fungal indices of the six main compounds (50 μg mL^{-1}) of the leaf essential oil of *M. zuihoensis* (Note: DDAC (didecyl dimethyl ammonium chloride) is a wood preservative for wood decay fungi and is used as a positive control).

The essential oil of *M. zuihoensis* was tested against two white rot fungi (*T. versicolor* and *Phane. chrysosporium*) and two brown rot fungi (*Phaeo. schweinitzii,* L. *sulphureus*). The anti-wood-decay fungal indices presented in Table 4 are a clear demonstration of the excellent anti-wood-decay fungal property of the oil. The growth of *T. versicolor, Phane. chrysosporium, Phaeo. schweintizii*, and *L. sulphureu* was completely inhibited at concentrations of 100, 100, 50, 25 μg mL^{-1}, respectively.

Comparing the anti-wood-decay fungal activities of the essential oils from *Machilus* spp. such as *M. pseudolongifolia* (Ho et al., 2010a), *M. philippinensis* (Ho et al., 2010b) and *M. kusanoi* (Ho et al., 2011), the leaf oil of *M. zuihoensis* was superior (Table 5). The results verified that *M. zuihoensis* leaf oil has excellent anti-wood-decay fungal activities.

Furthermore, to ascertain the source compounds of the *M. zuihoensis* essential oil, we also tested the anti-wood decay fungal activities of its major component compounds (Figure 1). The results indicated that the sources of activities were also τ-cadinol, β-eudesmol, and n-dodecanal. The IC50 values of the three compounds (τ-cadinol, β-eudesmol, and *n*-dodecanal) against the four decay fungi were 36.8, 48.1, and 56.5 μg mL^{-1} against *T. versicolor*; 13.5, 38.2, and 42.6 μg mL^{-1} against *Phane. chrysosporium;* 28.9, 23.1, and 25.3 μg mL^{-1} against *Phaeo. Shweinitzii;* and 23.3, 20.6, and 23.2 μg mL^{-1} against L. sulphureu, respectively. At a 50 μg mL^{-1} concentration, τ-cadinol showed total growth inhibition against all white-rot and brown-rot fungi tested, while *n*-dodecanal and β-eudesmol could completely inhibit brown-rot fungi but partially inhibit white-rot fungi. The results correspond with those of Kondo & Imamura (1986), Mori et al. (2000), Nami et al. (2006), Ho et al. (2010b) and Ho et al. (2011). Thus, the excellent wood-decay fungi inhibitive activities exhibited by the *M. zuihoensis* leaf oil may be attributed to the presence of compounds such as τ-cadinol, β-eudesmol, and *n*-dodecanal.

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***Correspondence**

Yu-Chang Su
Department of Forestry, National Chung Hsing University, 250 Kuo Kuang Rd., Taichung, Taiwan 402
ycsu@nchu.edu.tw
Tel. +886-4-22851314
Fax: +886-4-22851314