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Composition and Antimicrobial Activity of the Leaf and Twig Oils of *Litsea mushaensis* and *L. linii* from Taiwan

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The hydrodistilled essential oils of the leaves and twigs of *Litsea mushaensis* and *L. linii* were analyzed. Sixty-nine and ninety compounds were identified in the leaf and twig oils, respectively, of *L. mushaensis*. The main components of the leaf oil were β-eudesmol (24.2%), τ-cadinol (10.2%), α-humulene (10.1%), α-pinene (9.7%), and *trans*-β-ocimene (6.5%), whereas the main components of the twig oil were *trans*-β-ocimene (19.5%), α-pinene (12.8%) and *cis*-β-ocimene (7.7%). With *L. linii*, 72 and 78 compounds were respectively identified in the leaf and twig oils. The main components of the leaf oil were β-selinene (15.7%), α-selinene (15.5%), β-caryophyllene (12.2%), α-humulene (7.2%), and δ-cadinene (5.6%), and of the twig oil *trans*-β-ocimene (20.8%), β-selinene (11.4%), α-cadinol (6.0%), δ-cadinene (5.8%), τ-cadinol (5.4%) and β-eudesmol (5.2%). *L. mushaensis* leaf oil was shown to have excellent antimicrobial and anti-wood-decay fungal activity, superior to the other oils.

Keywords: *Litsea mushaensis*, *Litsea linii*, Lauraceae, essential oil, antimicrobial activity, anti-wood-decay fungal activity, β-eudesmol, α-cadinol, τ-cadinol.

The *Litsea* genus (family Lauraceae) is comprised of deciduous trees and shrubs. There are about 400 species in the genus, which are widely distributed geographically, from Japan, Korea, and North America in the north to New Zealand and South America in the south. In total, 12 species are found in Taiwan [1]. All *Litsea* species have a fragrant odor, and certain species possess bioactivity. For instance, the fruit oil of *L. cubeba* has anticancer activity [2], and the leaf oils of *L. coreana* [3], *L. kostermansii* [4], *L nakaii* [5] and *L. laevigate* [6] have antimicrobial activity.

L. mushaensis Hayata (Lauraceae) and *L. linii* Chang (Lauraceae) are endemic species of Taiwan [1]. No prior study has investigated the chemical compositions and biological activity of either these essential oils or other extracts of these two species. This study first examined the extraction of essential oils from the leaves and twigs of *L. mushaensis* and *L. linii* using hydrodistillation; these were then analyzed for their compositions. The second part of the study examined the antimicrobial and antifungal wood-decay activity of these leaf and twig oils. The purpose of this study was to establish a chemical basis for the effective multipurpose utilization of these species.

The leaf and twig oil yields after hydrodistillation for *L. mushaensis* and *L. linii* were respectively 3.2 ± 0.02 and 2.8 ± 0.04, and 3.4 ± 0.02 and 2.3 ± 0.04 mL/100 g o.d. weight of leaves and twigs. Table 1 presents the components identified. All compounds are listed in order of their elution from the DB-5 column. In *L. mushaensis* leaf oil, 69 compounds were identified, of which oxygenated sesquiterpenes were predominant (40.0%), followed by monoterpene hydrocarbons (30.0%), sesquiterpene hydrocarbons (27.6%), oxygenated monoterpenes (2.2%), and non-terpenoids (0.2%). Among the oxygenated sesquiterpenes, β-eudesmol (24.2%) and τ-cadinol (10.2%) were the major compounds, and of the monoterpene hydrocarbons, α-pinene (9.7%) and *trans*-β-ocimene (6.5%). Among the sesquiterpene hydrocarbons, α-humulene (10.1%) and α-selinene (4.6%) were the chief components. Ninety components were identified in the twig oil. Among these, monoterpene hydrocarbons were the most dominant (57.1%), followed by oxygenated sesquiterpenes (18.1%), sesquiterpene hydrocarbons (13.6%), oxygenated monoterpenes (8.0%), and non-terpenoids (0.9%). *trans*-β-Ocimene (19.5%), α-pinene (12.8%) and *cis*-β-ocimene (7.7%) were the major compounds of the monoterpene hydrocarbons.

Table 1: Chemical composition of the leaf and twig oils of *L. mushaensis* and *L. linii*.

Compound ID	KI [a]	*L. mushaensis*		*L. linii*		Identification [b]
		leaf	twig	leaf	twig	
Tricyclene	927	0.1	0.2	-	-	KI, MS, ST
α-Pinene	939	9.7	12.8	t	0.2	KI, MS, ST
Camphene	954	4.1	6.4	-	0.1	KI, MS, ST
β-Pinene	979	5.1	5.1	-	t	KI, MS, ST
β-Myrcene	991	0.6	1.1	0.1	0.4	KI, MS, ST
α-Phellandrene	1003	-	-	0.5	0.2	KI, MS, ST
α-Terpinene	1017	0.1	-	-	-	KI, MS, ST
p-Cymene	1025	0.1	0.3	0.1	0.6	KI, MS, ST
Limonene	1029	1.7	3.2	0.1	0.2	KI, MS, ST
1,8-Cineole	1031	0.1	-	-	-	KI, MS, ST
cis-β-Ocimene	1037	1.7	7.7	-	-	KI, MS, ST
trans-β-Ocimene	1050	6.5	19.5	2.8	20.8	KI, MS, ST
Terpinolene	1089	0.3	0.5	0.1	0.1	KI, MS, ST
p-Cymenene	1091	-	0.2	-	-	KI, MS, ST
Linalool	1097	-	0.1	-	-	KI, MS, ST
endo-Fenchol	1117	0.4	0.8	-	-	KI, MS, ST
cis-*p*-Menth-2-en-1-ol	1122	-	0.1	-	-	KI, MS
1-Terpinenol	1134	t [c]	0.2	-	-	KI, MS
Nopinone	1140	0.1	0.4	-	0.1	KI, MS
Camphene hydrate	1150	0.2	0.6	-	-	KI, MS, ST
Borneol	1169	0.3	0.9	-	-	KI, MS
4-Terpineol	1177	0.1	0.4	-	-	KI, MS, ST
α-Terpineol	1189	0.8	3.1	-	-	KI, MS, ST
n-Decanal	1202	-	-	t	0.3	KI, MS, ST
β-Cyclocitral	1219	-	0.2	-	-	KI, MS
Phellandral	1276	-	0.3	-	0.1	KI, MS
Bornyl acetate	1289	0.3	0.7	-	0.2	KI, MS, ST
2-Undecanone	1293	-	0.2	-	0.2	KI, MS
cis-2,3-Pinanediol	1320	-	0.2	-	-	KI, MS
cis-Piperitol acetate	1335	-	-	t	0.3	KI, MS
δ-Elemene	1338	t	0.1	0.3	t	KI, MS, ST
α-Cubebene	1351	-	-	3.8	0.4	KI, MS, ST
α-Ylangene	1375	0.1	0.1	0.1	0.1	KI, MS, ST
α-Copaene	1377	0.1	-	3.7	1.1	KI, MS, ST
Geranyl acetate	1381	-	0.2	-	0.1	KI, MS, ST
β-Cubebene	1388	-	-	4.3	0.2	KI, MS, ST
β-Bourbonene	1388	-	-	0.1	0.2	KI, MS
β-Elemene	1391	0.1	0.5	1.1	0.6	KI, MS
Dodecanal	1409	-	-	0.4	0.8	KI, MS, ST
α-Gurjunene	1410	t	-	t	0.3	KI, MS
α-*cis*-Bergamotene	1413	-	-	0.1	0.3	KI, MS
β-Caryophyllene	1419	3.9	1.4	12.2	3.5	KI, MS, ST
β-Cedrene	1421	-	-	-	0.1	KI, MS, ST
β-Copaene	1432	-	-	t	0.2	KI, MS, ST
β-Gurjunene	1434	-	0.2	0.2	-	KI, MS, ST
α-*trans*-Bergamotene	1435	-	-	0.6	2.3	KI, MS
Aromadendrene	1441	0.1	-	0.3	0.2	KI, MS, ST
cis-Muurola-3,5-diene	1450	0.2	0.1	0.1	0.2	KI, MS
trans-Muurola-3,5-diene	1454	0.1	0.1	2.7	0.6	KI, MS
α-*neo*-Clovene	1454	-	-	0.1	-	KI, MS
α-Humulene	1455	10.1	4.8	7.2	2.7	KI, MS, ST
cis-Cadina-1(6),4-diene	1463	-	0.2	0.1	0.2	KI, MS
cis-Muurola-4(14),5-diene	1466	-	-	0.1	-	KI, MS
trans-Cadina-1(6),4-diene	1477	0.1	-	2.5	-	KI, MS
γ-Gurjunene	1477	0.4	0.1	-	0.1	KI, MS
β-Chamigrene	1478	0.3	0.1	-	-	KI, MS
γ-Muurolene	1480	0.2	0.3	2.0	1.9	KI, MS
α-Curcumene	1481	-	-	-	2.2	KI, MS
α-Amorphene	1485	-	0.2	-	-	KI, MS
Germacrene D	1485	-	-	4.9	-	KI, MS, ST
Aristolochene	1488	0.3	-	-	-	KI, MS
cis-Eudesma-6,11-diene	1490	-	0.3	1.1	-	KI, MS
β-Selinene	1490	0.7	-	15.7	11.4	KI, MS, ST
δ-Selinene	1493	2.1	1.2	-	-	KI, MS
Valencene	1496	-	0.2	-	-	KI, MS
α-Selinene	1498	4.6	1.4	15.5	3.2	KI, MS, ST
α-Muurolene	1500	-	0.2	-	0.8	KI, MS, ST
iso-Daucene	1500	-	-	1.1	-	KI, MS
γ-Patchoulene	1502	-	-	-	0.2	KI, MS
(*E*,*E*)-α-Farnesene	1506	-	-	0.7	-	KI, MS, ST
(*Z*)-α-Bisabolene	1506	-	-	-	1.0	KI, MS
(*Z*)-β-Bisabolene	1507	-	-	-	1.2	KI, MS
δ-Amorphene	1512	0.5	-	-	-	KI, MS
γ-Cadinene	1514	0.2	0.3	0.5	0.5	KI, MS, ST
7-*epi*-α-Selinene	1522	1.1	-	-	-	KI, MS
δ-Cadinene	1523	0.1	0.7	5.6	5.8	KI, MS, ST
trans-Calamenene	1529	0.1	0.3	2.2	2.8	KI, MS, ST
trans-Cadina-1(2),4-diene	1535	0.1	0.1	1.5	0.7	KI, MS
α-Cadinene	1539	0.1	0.2	0.2	0.5	KI, MS, ST
α-Calacorene	1546	0.1	0.2	0.2	1.1	KI, MS, ST
Silphiperfolan-6-β-ol	1548	0.1	0.1	-	-	KI, MS
Elemol	1550	0.1	0.1	0.1	0.6	KI, MS, ST
Germacrene B	1561	2.1	-	0.3	-	KI, MS, ST
(*E*)-Nerolidol	1563	-	-	-	0.3	KI, MS, ST
β-Calacorene	1565	-	-	0.1	-	KI, MS, ST
dimethyl-Ionone	1567	-	0.2	t	0.2	KI, MS
Palustrol	1568	-	0.1	-	-	KI, MS
Ledol	1569	-	-	t	0.2	KI, MS, ST
Dendrolasin	1571	-	0.1	-	-	KI, MS
Caryophyllenyl alcohol	1572	0.1	0.3	-	0.9	KI, MS
Spathulenol	1578	-	0.1	0.3	0.8	KI, MS, ST
Caryophyllene oxide	1583	0.1	0.3	0.4	1.1	KI, MS, ST
Globulol	1585	-	0.1	0.3	1.1	KI, MS, ST
β-Copaen-4-α-ol	1591	-	0.1	-	-	KI, MS
Viridiflorol	1593	-	-	0.2	-	KI, MS, ST
Carotol	1595	-	0.2	-	-	KI, MS
Guaiol	1601	-	0.4	-	-	KI, MS
5-*epi*-7-*epi*-α-Eudesmol	1608	0.4	0.5	-	0.4	KI, MS
Humulene epoxide II	1608	0.2	0.5	0.1	0.6	KI, MS
epi-Cedrol	1619	0.2	0.2	t	0.4	KI, MS
1,10-di-*epi*-Cubenol	1619	-	-	t	-	KI, MS
Junenol	1619	-	-	-	0.4	KI, MS
10-*epi*-γ-Eudesmol	1624	-	-	-	0.3	KI, MS
1-*epi*-Cubenol	1629	0.2	0.4	0.9	-	KI, MS
γ-Eudesmol	1632	2.4	2.4	-	-	KI, MS
α-Acorenol	1633	-	0.5	0.1	-	KI, MS
cis-Cadin-4-en-7-ol	1637	0.3	0.5	-	-	KI, MS
τ-Cadinol	1640	10.2	2.5	0.9	5.4	KI, MS, ST
τ-Muurolol	1642	-	0.2	0.2	0.7	KI, MS
δ-Cadinol	1646	-	0.2	0.3	-	KI, MS
β-Eudesmol	1651	24.2	3.1	-	5.2	KI, MS, ST
Cedr-8(15)-en-10-ol	1652	0.3	0.2	-	-	KI, MS
α-Eudesmol	1654	0.5	0.2	-	-	KI, MS
α-Cadinol	1654	-	-	0.3	6.0	KI, MS, ST
cis-Calamenen-10-ol	1661	0.1	0.2	0.1	-	KI, MS
trans-Calamenen-10-ol	1669	0.1	0.1	t	-	KI, MS
Bulnesol	1672	-	-	t	-	KI, MS
(3*Z*)-Butylidene phthalide	1673	0.1	0.2	t	0.2	KI, MS
β-Bisabolol	1675	-	-	-	0.6	KI, MS
Cadalene	1677	-	0.4	-	0.7	KI, MS
Mustakone	1677	-	-	t	-	KI, MS
(*Z*)-Nerolidyl acetate	1678	-	0.4	-	0.6	KI, MS
Eudesm-7(11)-en-4-ol	1700	0.1	1.7	t	0.4	KI, MS
5-Hydroxy-*cis*-calamenene	1713	-	-	-	0.1	KI, MS
14-Hydroxy-α-humulene	1714	-	0.2	0.1	0.3	KI, MS
Nootkatol	1715	0.3	0.8	t	0.2	KI, MS
(*E*)-Nerolidyl acetate	1717	-	-	-	0.2	KI, MS
β-Davanone-2-ol	1719	-	0.1	0.1	0.3	KI, MS
(2*E*,6*E*)-Farnesol	1725	-	0.1	-	-	KI, MS
epi-Cyclocolorenone	1775	0.2	0.2	-	-	KI, MS
Nootkatone	1807	-	0.1	0.1	0.5	KI, MS
Compounds identified						
Monoterpene hydrocarbons		30.0	57.1	3.7	22.7	
Oxygenated monoterpenes		2.2	8.0	t	0.8	
Sesquiterpene hydrocarbons		27.6	13.6	91.2	46.5	
Oxygenated sesesquiterpenes		40.0	18.1	4.7	28.7	
Others		0.2	0.9	0.4	1.3	
Yield (mL/100g)		3.16 ± 0.02	2.85 ± 0.04	3.38 ± 0.02	2.33 ± 0.04	

[a] Retention index on a DB-5 column with reference to *n*-alkanes [7].

[b] MS, NIST and Wiley library spectra and the literature; RI, Retention index; ST, authentic standard compounds. [c] t: trace < 0.1%

From *L. linii* leaf oil, we identified 72 compounds, of which sesquiterpene hydrocarbons were the most dominant (91.2%), followed by oxygenated sesquiterpenes (4.7%), monoterpene hydrocarbons (3.7%), non-terpenoids (0.4%), oxygenated monoterpenes (trace), and diterpenes (trace). Among the sesquiterpene hydrocarbons, β-selinene (15.7%), α-selinene (15.5%), β-caryophyllene (12.2%), α-caryophyllene (7.2%), and δ-cadinene (5.6%) were the chief compounds. Seventy-eight components were identified from the twig oil, of which sesquiterpene hydrocarbons were the most dominant (46.5%), followed by oxygenated sesquiterpenes (28.7%), monoterpene hydrocarbons (22.7%), non-terpenoids (1.3%), oxygenated monoterpenes (0.8%), and diterpenes (trace). β-Selinene (11.4%) and δ-cadinene (5.8%) were the major sesquiterpene hydrocarbons. Of the oxygenated sesquiterpenes, α-cadinol (6.0%), τ-cadinol (5.4%), and β-eudesmol (5.2%) were the chief compounds, whereas of the monoterpene hydrocarbons, *trans*-β-ocimene (20.8%) was the major component.

Although the leaf oil constituents of *L. mushaensis* and *L. linii* were primarily sesquiterpenoids, like those of *L.coreana* [3], *L. kostermansii* [4], *L nakaii* [5],

L. resinosa, *L. grasilipes*, and *L. paludosa* [8], their main components differed. Further comparison with the leaf oils of *L. guatemalensis* [9] and *L. laevigate* [6] showed that the compounds of *L. laevigate* [6] were predominantly monoterpenoids and, therefore, differed from the leaf oils of *L. mushaensis* and *L. linii*.

The leaf and twig oils of *L. mushaensis* and *L. linii* were tested against three Gram-positive and five Gram-negative bacteria, as well as two fungi. The results, presented in Table 2, demonstrated that the leaf oil of *L. mushaensis* and twig oil of *L. linii* possessed excellent antimicrobial activity. Of these, the leaf oil of *L. mushaensis* was the best. The leaf oil of *L. mushaensis* and twig oil of *L. linii* showed medium to strong growth suppression against all nine microbes studied. The most sensitive microorganisms were *Bacillus cereus*, *Staphylococcus aureus*, *S. epidermidis,* and *Candida albicans,* with inhibition zones of 36- 46 mm and MIC values of 62.5- 250 μg/mL, respectively. Both oils demonstrated stronger growth suppression of Gram-positive bacteria as compared with Gram-negative bacteria and fungi. These observations are similar to those reported [4,5,6,10]. In comparison with the antimicrobial activity of the essential oils from *L. kostermansii* [4], *L. nakaii* [5], *L. laevigata* [6], *Cinnamomum subavenium* [10] and *Machilus pseudolongifolia* [11], the antimicrobial activity of the leaf oil of *L. mushaensis* and twig oil of *L. linii* were superior. The results validated the excellent antimicrobial activity of *L. mushaensis* leaf oil and *L. linii* twig oil. However, to ascertain the source compounds of the antimicrobial activity of *L. mushaensis* leaf oil and *L. linii* twig oil, their main components were individually tested for antimicrobial activity. Results indicated that the active compounds were α-cadinol, τ-cadinol, and β-eudesmol. These results were similar to those of Ho *et al.* [3-5]. Various studies support the argument that these compounds are highly active in suppressing microbial growth [12-14].

Leaf and twig oils of *L. mushaensis* and *L. linii* were tested against two white rot fungi (*Trametes versicolor, Phanerochaete chrysosporium*) and two brown rot fungi (*Phaeolus schweinitzii, Lenzites sulphureu*). The anti-wood-decay fungal indices presented in Table 3 clearly demonstrate the excellent anti-wood-decay fungal activity of the leaf oil of *L. mushaensis* and twig oil of *L. linii*. Of these, the leaf oil of *L. mushaensis* was the best. Growth of *T. versicolor, Phane chrysosporium, Phaeo. schweintzii* and *L. sulphureu* were completely inhibited at concentrations of 25, 50, 25, and 12.5 μg/mL of the leaf oil of *L. mushaensis*, respectively. The anti-wood-decay fungal activity of the leaf oil of *L. mushaensis* was superior in comparison with that of the essential oils of *M. pseudolongifolia* [11], *Chamaecyparis formosensis* [15] and *M. philippinensis* [16].

This study also tested the anti-wood-decay fungal activity of the major components of *L. mushaensis* leaf oil and *L. linii* twig oil to ascertain there source compounds. Results indicated that the sources of the anti-wood-decay fungal activity were α-cadinol, τ-cadinol, and β-eudesmol. At a concentration of 50 μg/mL, α-cadinol and τ-cadinol inhibited growth of all white-rot and brown-rot fungi tested, while β-eudesmol at a concentration of 50 μg/mL inhibited the growth of brown- rot fungi, but only partially inhibited that of white- rot fungi. The results correlated with those of Kondo and Imamura [12], and Mori *et al.* [17]. The presence of τ-cadinol, α-cadinol and β-eudesmol significantly contributed to the wood-decay fungal suppression activity of *L. mushaensis* leaf oil and *L. linii* twig oil.

Experimental

Plant materials: Fresh leaves and twigs of *L. mushaensis* and *L. linii* were respectively collected from Zhudong (Hsinchu County, northern Taiwan, elevation 580 m, N 24º 34′ 16″, E 121º 23′ 38″) in July 2009 and Shangwu

Table 2: Antimicrobial activity of the leaf and twig oils of *L. mushaensis* and *L. linii*.

Microbial species	*L. mushaensis*				*L. linii*				Compounds [c]											Antibiotics		
	Leaf		Twig		Leaf		Twig		1	2	3	4	5	6	7	8	9	10	11	Tetracycline (30 μg/disk)	Gentamicin (10 μg/disk)	Nystatin (30 μg/disk)
	IZ [a]	MIC [b]	IZ	MIC	IZ	MIC	IZ	MIC	MIC	MIC	MIC	MIC	MIC	MIC	MIC	MIC	MIC	MIC	MIC	IZ	IZ	IZ
Bacillus cereus	38±0.8	125	16±0.4	1000	18±0.8	500	36±0.4	125	>1000	>1000	500	750	>1000	750	750	250	62.5	125	125	22±0.8	-	nt
Staphylococcus aureus	46±0.4	62.5	19±0.4	750	20±0.4	500	40±0.4	125	>1000	>1000	250	500	1000	500	500	250	62.5	62.5	62.5	21±0.4	-	nt
Staphylococcus epidermidis	45±0.4	62.5	20±0.4	750	22±0.4	500	42±0.8	125	>1000	>1000	250	500	1000	750	500	250	62.5	62.5	62.5	34±0.4	-	nt
Escherichia coli	29±0.8	375	13±0.8	>1000	15±0.8	750	28±0.8	375	>1000	>1000	1000	>1000	>1000	1000	1000	500	500	500	750	-	22±0.8	nt
Enterobacter aerogenes	22±0.8	500	12±0.4	>1000	13±0.4	750	20±0.4	500	>1000	>1000	750	>1000	>1000	>1000	>1000	500	125	125	250	10±0.4	-	nt
Klebsiella pneumoniae	28±0.4	375	12±0.4	>1000	12±0.8	>1000	28±0.4	375	>1000	>1000	750	>1000	>1000	>1000	>1000	250	125	125	250	-	21±0.8	nt
Pseudomonas aeruginosa	28±0.8	375	9±0.4	>1000	12±0.4	>1000	26±0.8	375	>1000	>1000	>1000	>1000	>1000	>1000	>1000	500	500	500	750	-	12±0.8	nt
Vibrio parahaemolyticus	20±0.4	500	9±0.4	>1000	10±0.8	>1000	20±0.8	500	>1000	>1000	>1000	>1000	>1000	>1000	>1000	500	1000	1000	1000	-	13±0.8	nt
Aspergillus niger	26±0.4	375	10±0.4	>1000	10±0.4	>1000	18±0.8	500	>1000	>1000	>1000	>1000	>1000	>1000	>1000	500	750	1000	>1000	nt	nt	17±0.8
Candida albicans	38±0.4	125	16±0.4	>1000	16±0.8	750	32±0.8	250	>1000	>1000	250	>1000	>1000	>1000	>1000	250	62.5	125	125	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 μg/mL. [c] 1. α-pinene (≥ 98.5%), 2. *trans*-β-ocimene (≥ 98.5%), 3. β-caryophyllene (≥ 98.5%), 4. α-humulene (≥ 98%), 5. germacrene D (≥ 98%), 6. β-selinene (≥ 98%), 7. α-selinene (≥ 98.5%), 8. δ-cadinene (≥ 98.5%), 9. τ-cadinol (≥ 98.5%), 10. β-eudesmol (≥ 98%), 11. α-cadinol (100%). Compound 1 to 5 and 8 to 9 were purchased from the Fluka Co. (Milwaukee, USA), Compounds 6 and 7 were purchased from the Chemos Gmbh Co. (Regenstauf, German), Compound 10 was purchased from the Wako Co. (Tokyo, Japan), whereas compound 11 was from an isolate of Ho *et al*'s study on *Machilus philippinenesis* essential oil [16]. Essential oil tested at 15 μL/disc for bacteria and 30 μL/disc for fungi.(-), Inactive; (7-14), moderately active; (>14), highly active; nt, not tested.

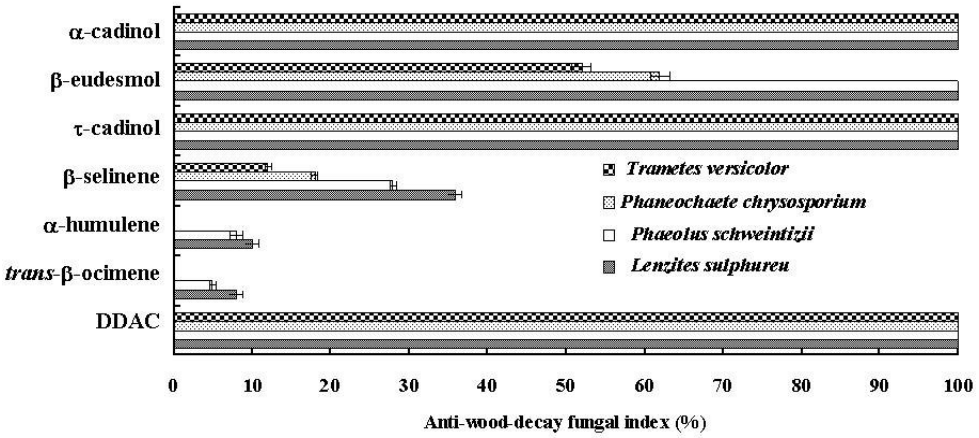

Figure 1: Anti-wood-decay fungal indices of the ten main compounds (50 μg/mL) of the leaf and twig essential oils of *L. mushaensis* and *L. linii*.

Table 3: Anti-wood-decay fungal indices of leaf and twig essential oils from *L. mushaensis* and *L. linii*.

Essential oil	Dosage (μg/mL)	Antifungal index (%)			
		Trametes versicolor	*Phaneochaete chrysosporium*	*Phaeolus schweintizii*	*Lenzites sulphureus*
L. mushaensis					
Leaf	12.5	83 ± 3.3	52 ± 6.6	78 ± 3.3	100 ± 0
	25	100 ± 0	68 ± 3.3	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
Twig	12.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	25	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	50	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	75	0 ± 0	0 ± 0	20 ± 3.3	23 ± 3.3
	100	40 ± 3.3	32 ± 3.3	56 ± 6.6	63 ± 6.6
L. linii					
Leaf	12.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	25	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	50	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	75	0 ± 0	0 ± 0	0 ± 0	13 ± 3.3
	100	28 ± 3.3	12 ± 3.3	38 ± 3.3	52 ± 6.6
Twig	12.5	76 ± 3.3	38 ± 3.3	68 ± 3.3	86 ± 3.3
	25	85 ± 6.6	46 ± 6.6	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

(Taitung County, eastern Taiwan, elevation 850 m, N 22º 20′ 72″, E 120º 53′ 01″) in July 2008. The samples were respectively compared with specimen no. ou5338 and ou5686 from the Herbarium of the National Chung-Hsing University and positively identified by Prof. Yen-Hsueh Tseng of NCHU. The voucher specimens (CLH-009 and CLH-008) were deposited in the NCHU herbarium. Leaves and twigs of the species were collected for subsequent extraction and analysis.

Isolation of the leaf and twig essential oils: Leaves and twigs of *L. mushaensis* and *L. linii* (1 Kg) were placed in a round-bottom flask and hydrodistilled for 8 h with 3 L of distilled water, respectively. The essential oils obtained were dried with anhydrous sodium sulfate. The oil yields 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 the quantitative determination of oil components. Oven temperature was programmed as follows: 50ºC for 2 min, rising to 250ºC at 5ºC/min. Injector temperature: 270ºC. Carrier gas: He with a flow rate of 1 mL/min. Detector temperature: 250ºC split ratio: 1:10. One μL sample was injected. 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 and twig oils constituents was based on comparisons of retention index (RI) [18], retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra, and literature [7,19], respectively.

Antimicrobial activity: The *in vitro* antibacterial and antifungal activity of the leaf and twig oils were evaluated by the disc diffusion method using Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for fungi [20]. Discs containing 15 μL and 30 μL of the oil, which was 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 [4,5]. 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: *A. niger* (ATCC 16404) and 1 yeast: *C. albicans* (ATCC 10231). Minimum inhibitory concentration (MIC) values were measured by the microdilution broth susceptibility assay recommended by NCCLS [21]. Stock solutions of the oil were prepared in DMSO. Dilution series were prepared from 1000 μg/mL to 50 μg/mL 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 10^8 CFU/mL. 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 Su *et al.* [22] was adopted. The fungi used were *T. versicolor* (BCRC 35253), *Phane. chrysosporium* (BCRC 36200), *Phaeo. schweinitzii* (BCRC 35365) and *L. 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 (12.5~100 μg/mL) were added to sterilized potato dextrose agar (PDA). The test plates were incubated at 27ºC. When the mycelium of 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) for wood decay fungi was used as a positive control.

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