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Composition and Antimicrobial Activity of the Leaf and Twig Oils of *Litsea acutivena* from Taiwan

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The chemical composition, and antimicrobial and anti-wood-decay fungal activities of the essential oils isolated from the leaves and twigs of *Litsea acutivena* of Taiwan were investigated. The essential oils from the fresh leaves and twigs were isolated using hydrodistillation in a Clevenger-type apparatus, and characterized by GC–FID and GC–MS. Ninety-five and fifty-two compounds were identified in the leaf and twig oils, respectively. The main components of the leaf oil were γ-patchoulene (11.0%), δ-cadinene (6.3%), *trans*-muurola-3,5-diene (5.9%), and β-selinene (5.3%), whereas the main components of the twig oil were τ-cadinol (13.1%), β-selinene (9.6%), *trans*-β-ocimene (6.2%) and α-cadinol (7.7%). Bioactivity studies demonstrated that twig oil had excellent antimicrobial and anti-wood-decay fungal activities, superior to those of the leaf oil. For the antimicrobial and anti-wood-decay fungal activities of the twig oil, the active compounds were determined to be τ-cadinol and α-cadinol.

Keywords: *Litsea acutivena*, Lauraceae, essential oil, antimicrobial activity, anti-wood-decay fungal activity, τ-cadinol, α-cadinol.

The *Litsea* genus (family Lauraceae) is comprised of about 400 species, which are widely distributed geographically. In total, 12 species are found in Taiwan [1]. We previously reported that the fruit oil of *L. cubeba* had anticancer activity [2], and the leaf oils of *L. coreana* [3a], *L. kostermansii* [3b], *L nakaii* [3c] and *L. mushaensis* [3d] have antimicrobial activity. *L. acutivena* Hayata is an evergreen tree, distributed in southern China, Hainan Island, Indochina, and Taiwan [1]. Previous reports have suggested that the extractive exhibited cytotoxicity [3e,3f]. However, there are no literature reports on the chemical composition and biological activities of the essential oil from this species, and therefore we used hydrodistillation to collect the leaf and twig oils, and analyzed them by GC/FID and GC/MS. The essential oil was also investigated for its antimicrobial activity against a selected ten microbial strains and for its application to prevent wood decay. We also applied the essential oil to four strains of commonly found white and brown rot fungi in Taiwan.

Based on the dry weight of leaves and twigs, hydrodistillation of *L. acutivena* produced yellow-colored oils with yields of 2.81 ± 0.02 and 2.06 ± 0.04 mL/100g, respectively. All compounds are listed in order of their elution from the DB-5 column (Table 1). A total of 95 compounds were identified from the hydrodistilled leaf oil of *L. acutivena*. Sesquiterpene hydrocarbons were predominant (69.8%), followed by oxygenated sesquiterpenes (24.6%), monoterpene hydrocarbons (4.6%), non-terpenoids (0.7%), and oxygenated monoterpenes (0.4%). Among the sesquiterpene hydrocarbons, γ-patchoulene (11.0%) and δ-cadinene (6.3%) were the major compounds.

Fifty-two components were identified from the twig oil. Oxygenated sesquiterpenes were the most dominant (37.5%), followed by sesquiterpene hydrocarbons (25.3%), monoterpene hydrocarbons (23.4%), oxygenated monoterpenes (12.7%), and non-terpenoids (1.1%). τ-Cadinol (13.1%) and α-cadinol (5.1%) were the major oxygenated sesquiterpenes.

From the results presented above, the leaf oil constituents of *L. acutivena* were primarily sesquiterpenoids. Intra-genus leaf oil comparisons indicated that many *Litsea* trees, such as *L. coreana* [3a], *L. kostermansii* [3b], *L nakaii* [3c], *L. mushaensis* [3d], *L. linii* [3d], *L. resinosa*, *L. rasilipes*, and *L. paludosa* [4], all have predominately sesquiterpenoids as their main constituents. However, the main components of the individual species differed. The leaf oils of *L. guatemalensis* [5a], *L. laevigata* [5b] and *L. akoensis* [6] were predominantly comprised of monoterpenoids and, therefore, differed from the leaf oil of *L. acutivena*.

Leaf and twig oils of *L. acutivena* were tested against three Gram-positive and five Gram-negative bacteria, as well as two fungi. The results demonstrated clearly that the twig

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

Compound ID	RI [a]	Conc. (%)		Identification [b]
		leaf	twig	
α-Pinene	939	0.2	4.6	KI, MS, ST
Camphene	954	0.3	2.6	KI, MS, ST
β-Pinene	979	t [c]	1.0	KI, MS, ST
β-Myrcene	991	0.1	0.3	KI, MS, ST
α-Phellandrene	1003	0.1	0.4	KI, MS, ST
α-Terpinene	1017	0.1	- [d]	KI, MS, ST
p-Cymene	1025	0.1	1.0	KI, MS, ST
Limonene	1029	0.1	3.9	KI, MS, ST
β-Phellandrene	1030	t	-	KI, MS, ST
1,8-Cineole	1031	t	0.8	KI, MS, ST
cis-β-Ocimene	1037	0.6	2.5	KI, MS, ST
trans-β-Ocimene	1050	3.0	6.2	KI, MS, ST
Terpinolene	1089	0.1	0.8	KI, MS, ST
endo-Fenchol	1117	-	2.0	KI, MS, ST
Camphene hydrate	1150	t	0.4	KI, MS, ST
Borneol	1169	-	2.0	KI, MS
4-Terpineol	1177	t	0.3	KI, MS, ST
Cryptone	1186	-	-	KI, MS
α-Terpineol	1189	0.1	5.8	KI, MS, ST
n-Decanal	1202	0.5	1.1	KI, MS, ST
Bornyl acetate	1289	0.2	1.4	KI, MS, ST
cis-Piperitol acetate	1335	0.1	-	KI, MS
δ-Elemene	1338	0.3	-	KI, MS, ST
α-Ylangene	1375	0.7	-	KI, MS, ST
α-Copaene	1377	0.6	1.0	KI, MS, ST
Daucene	1382	0.1	-	KI, MS
β-Bourbonene	1388	0.1	-	KI, MS
α-Isocomene	1388	0.1	-	KI, MS
iso-Longifolene	1390	0.1	-	KI, MS
β-Elemene	1391	0.7	-	KI, MS
α-Gurjunene	1410	0.3	-	KI, MS
β-Caryophyllene	1419	0.7	3.7	KI, MS, ST
β-Cedrene	1421	0.4	-	KI, MS, ST
β-Copaene	1432	0.1	-	KI, MS, ST
β-Gurjunene	1434	0.1	-	KI, MS, ST
γ-Elemene	1437	t	-	KI, MS, ST
α-Guaiene	1440	3.1	-	KI, MS, ST
Aromadendrene	1441	0.4	0.6	KI, MS, ST
cis-Muurola-3,5-diene	1450	1.0	-	KI, MS
trans-Muurola-3,5-diene	1454	5.9	0.4	KI, MS
α-Humulene	1455	1.0	0.8	KI, MS, ST
allo-Aromadendrene	1460	0.2	-	KI, MS, ST
cis-Cadina-1(6),4-diene	1463	0.3	-	KI, MS
trans-Cadina-1(6),4-diene	1477	1.0	-	KI, MS
β-Chamigrene	1478	1.1	-	KI, MS
γ-Muurolene	1480	2.9	0.4	KI, MS
α-Amorphene	1485	1.9	-	KI, MS, ST
Germacrene D	1485	0.3	-	KI, MS, ST
cis-Eudesma-6,11-diene	1490	0.5	-	KI, MS
β-Selinene	1490	5.3	9.6	KI, MS
Viridiflorene	1497	2.1	2.5	KI, MS
α-Selinene	1498	2.7	0.9	KI, MS
α-Muurolene	1500	2.5	0.6	KI, MS
γ-Patchoulene	1502	11.0	-	KI, MS
trans-β-Guaiene	1503	3.1	-	KI, MS
γ-Cadinene	1514	2.1	-	KI, MS
δ-Cadinene	1523	6.3	1.7	KI, MS
trans-Calamenene	1529	1.5	1.0	KI, MS
trans-γ-Bisabolene	1531	0.2	-	KI, MS
trans-Cadina-1(2),4-diene	1535	2.7	0.3	KI, MS
α-Cadinene	1539	2.7	0.2	KI, MS
Selina-3,7(11)-diene	1547	3.0	0.4	KI, MS
Elemol	1550	0.3	-	KI, MS, ST
trans-Nerolidol	1563	0.7	-	KI, MS, ST
β-Calacorene	1565	0.3	1.1	KI, MS, ST
Palustrol	1568	0.4	0.4	KI, MS
Dendrolasin	1571	0.1	-	KI, MS
Caryophyllenyl alcohol	1572	0.9	3.0	KI, MS
Caryophyllene oxide	1583	0.5	1.1	KI, MS, ST
Globulol	1585	0.8	3.6	KI, MS, ST
Viridiflorol	1593	0.8	1.7	KI, MS, ST
Salvial-4(14)-en-1-one	1595	0.2	-	KI, MS
Guaiol	1601	1.0	0.9	KI, MS, ST
5-*epi*-7- *epi*-α-Eudesmol	1608	0.4	1.8	KI, MS
Humulene epoxide II	1608	1.1	-	KI, MS
1,10-di-*epi*-Cubenol	1619	0.3	0.9	KI, MS
Junenol	1619	1.5	0.4	KI, MS
10-*epi*-γ-Eudesmol	1624	0.9	-	KI, MS
1-*epi*-Cubenol	1629	2.7	-	KI, MS
γ-Eudesmol	1632	0.4	-	KI, MS
cis-Cadin-4-en-7-ol	1637	0.5	0.6	KI, MS
τ-Cadinol	1640	1.9	13.1	KI, MS, ST
τ-Muurolol	1642	0.8	1.2	KI, MS
δ-Cadinol	1646	1.1	1.2	KI, MS
α-Eudesmol	1654	0.6	-	KI, MS, ST
α-Cadinol	1654	1.0	5.1	KI, MS, ST
Selin-11-en-4-α-ol	1660	2.7	-	KI, MS
trans-Calamenen-10-ol	1669	-	0.4	KI, MS
Bulnesol	1672	0.6	-	KI, MS
3Z-Butylidene phthalide	1673	0.1	-	KI, MS
Cadalene	1677	0.4	-	KI, MS
Mustakone	1677	0.3	-	KI, MS
Z-Nerolidol acetate	1678	-	1.0	KI, MS
Eudesm-7(11)-en-4-ol	1700	1.1	-	KI, MS
14-hydroxy-α-Humulene	1714	0.1	-	KI, MS
Nootkatol	1715	0.3	0.8	KI, MS
2*Z*,6*Z*-Farnesol	1718	0.3	-	KI, MS
β-Davanone-2-ol	1719	0.1	-	KI, MS
2*E*,6*E*-Farnesol	1725	0.1	0.2	KI, MS
epi-Cyclocolorenone	1775	0.2	-	KI, MS
Guaiazulene	1780	0.2	-	KI, MS
Compounds identified				
Monoterpene hydrocarbons (%)		4.6	23.4	
Oxygenated monoterpenes (%)		0.4	12.7	
Sesquiterpene hydrocarbons (%)		69.8	25.3	
Oxygenated sesquiterpenes (%)		24.6	37.5	
Non-terpenoids (%)		0.7	1.1	
Oil Yield (mL/100 g)		2.81± 0.02	2.06± 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%. [d] not detected.

oil had antibacterial activities that were superior to those of the leaf oil (Table 2). The twig oil showed moderate to strong growth suppression against all ten microbes studied. The most sensitive microorganisms were *Bacillus cereus*, *Staphylococcus aureus*, *S. epidermidis* and *Candida albicans*, with inhibition zones of 42 ~ 50 mm and minimum inhibitory concentration (MIC) values of 62.5 μg/mL. The twig oil showed superior suppressive activity against Gram-positive bacteria compared with Gram-negative bacteria. The probable cause of the susceptibility of Gram-positive bacteria and the relative tolerance of Gram-negative bacteria to essential oils has been correlated with the presence of a hydrophilic outer layer [8]. It is presumed that penetration of the hydrophobic components in Gram-negative micro-organisms is more difficult due to the presence of a second physical barrier formed by the outer membrane [9,10a]. Comparing the antimicrobial activities of the leaf and twig oils with those extracted from *L. kostermansii* [3b], *L. nakaii* [3c], *L. linii* [3d] and *L. mushaensis* [3d] showed that the twig oil of *L. acutivena* was superior (Table 3). The results verify that *L. acutivena* twig oil has excellent antimicrobial activity.

However, to ascertain the source compounds of the antimicrobial activity of *L. acutivena* twig oil, the main components were individually tested for antimicrobial activities. The results indicated that the active source compounds were τ-cadinol and α-cadinol. These results were similar to those of Ho *et al.* [3a-3c]. Various studies support the argument that these compounds are highly active in suppressing microbial growth [8,11].

Leaf and twig oils of *L. acutivena* 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 4 clearly demonstrate the excellent anti-wood-decay fungal activity of the twig oil of *L. acutivena*. The growth of *T. versicolor*, *Phane. chrysosporium*, *Phaeo. schweintizii*, and *L. sulphureu* was completely inhibited at concentrations of 25, 50, 12.5, 12.5 μg/mL, respectively. Comparing the anti-wood-decay fungal activities of the essential oils from *Litsea* spp. such as *L. linii*, *L. mushaensis* and *L. coreana* [3b], the twig oil

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

Microbial species	*L. acutivena*				Compounds[c]					Antibiotics		
	Leaf		Twig		1	2	3	4	5	Tetracyclin	Gentamicin	Nystatin
	IZ[a]	MIC[b]	IZ	MIC	MIC	MIC	MIC	MIC	MIC	IZ	IZ	IZ
Bacillus cereus	28 ± 0.8	375	42 ± 0.8	62.5	>1000	>1000	750	62.5	125	22 ± 0.8	-	nt
Staphylococcus aureus	32 ± 0.8	250	50 ± 0.8	62.5	>1000	>1000	500	62.5	62.5	21 ± 0.4	-	nt
Staphylococcus epidermidis	30 ± 0.4	250	48 ± 0.8	62.5	>1000	>1000	750	62.5	62.5	34 ± 0.4	-	nt
Escherichia coli	19 ± 0.8	1000	33 ± 0.8	250	>1000	>1000	1000	500	750	-	22 ± 0.8	nt
Enterobacter aerogenes	18 ± 0.8	1000	28 ± 0.8	375	>1000	>1000	>1000	125	250	10 ± 0.4	-	nt
Klebsiella pneumoniae	16 ± 0.8	>1000	30 ± 0.4	250	>1000	>1000	>1000	125	250	-	21 ± 0.8	nt
Pseudomonas aeruginosa	18 ± 0.8	1000	30 ± 0.8	250	>1000	>1000	>1000	500	750	-	12 ± 0.8	nt
Vibrio parahaemolyticus	15 ± 0.4	>1000	26 ± 0.8	375	>1000	>1000	>1000	1000	1000	-	13 ± 0.8	nt
Aspergillus niger	12 ± 0.4	>1000	28 ± 0.4	375	>1000	>1000	>1000	750	>1000	nt	nt	17 ± 0.8
Candida albicans	28 ± 0.8	250	42 ± 0.4	62.5	>1000	>1000	>1000	62.5	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. *trans*-β-ocimene (≥ 98.5%), 2. α-terpineol (≥ 98.5%), 3. β-selinene (≥ 98%), 4. τ-cadinol (≥ 98.5%), 5. α-cadinol (100%). Compounds 1, 2 and 4 were purchased from Fluka Co. (Milwaukee, USA), and compound 3 from Chemos Gmbh Co. (Regenstauf, German). Compound 5 was from an isolate of Ho *et al*'s study on *Machilus philippinenesis* essential oil [10b]. 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.

Table 3: Comparison of the MIC values (μg/mL) of the leaf and twig oils of *L. acutivena* and those of *L. kostermansi*, *L. nakaii*, *L. linii*, *Machilus kusanoi*, *M. pseudolongifolia*, and *L. mushaensis* against microbial species.

Essential oil	Microbial species*										Ref.
	B. c.	*S. a.*	*S. e.*	*E. c.*	*E. a.*	*K. p.*	*P. a.*	*V. p.*	*A. n.*	*C. a.*	
Leaf											
L. acutivena	375	250	250	1000	1000	>1000	1000	>1000	>1000	250	This study
L. kostermansii	375	250	125	750	500	375	750	1000	>1000	1000	[3b]
L. nakaii	250	375	125	500	500	375	500	750	1000	500	[3c]
L. linii	500	500	500	750	750	>1000	>1000	>1000	>1000	750	[3d]
Twig											
L. acutivena	62.5	62.5	62.5	250	375	250	250	375	375	62.5	This study
L. mushaensis	1000	750	750	>1000	>1000	>1000	>1000	>1000	>1000	>1000	[3d]

* *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 and twig oils from *L. acutivena*.

Essential oil	Dosage (μg/mL)	Antifungal index (%)			
		Trametes versicolor	*Phaneochaete chrysosporium*	*Phaeolus schweintzii*	*Lenzites sulphureu*
Leaf oil	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	25 ± 3.3
	75	0 ± 0	0 ± 0	38 ± 3.3	65 ± 3.3
	100	48 ± 3.3	38 ± 3.3	66 ± 6.6	83 ± 6.6
Twig oil	12.5	89 ± 3.3	68 ± 6.6	100 ± 0	100 ± 0
	25	100 ± 0	86 ± 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

Table 5: Comparison of the MIC values (μg/mL) of the leaf and twig oils of *L. acutivena* and those of *L. linii*, *L. mushaensis* and *L. coreana* against the wood-decay fungi.

Essential oil	Fungi				Ref.
	Trametes versicolor	*Phaneochaete chrysosporium*	*Phaeolus schweintzii*	*Lenzites sulphureu*	
Leaf					
L. acutivena	>100	>100	>100	>100	This study
L. linii	>100	>100	>100	>100	[3d]
L. mushaensis	25	50	25	12.5	[3d]
L. coreana	75	75	50	25	[3c]
Twig					
L. acutivena	25	50	12.5	12.5	This study
L. linii	50	50	25	25	[3d]
L. mushaensis	>100	>100	>100	>100	[3d]

of *L. acutivena* was superior (Table 5). The results verified that *L. acutivena* twig oil has excellent anti-wood-decay fungal activities. We also tested the anti-wood-decay fungal activity of the major components of *L. acutivena* twig oil to ascertain the source compounds (Figure 1). Results indicated that the sources of the anti-wood-decay fungal activity were τ-cadinol and α-cadinol. At a concentration of 50 μg/mL, α-cadinol and τ-cadinol inhibited growth of all the white-rot and brown-rot fungi tested. The results correlated with those of Kondo and Imamura [11a], and Mori *et al.* [12]. The presence of τ-cadinol and α-cadinol significantly contributed to the wood-decay fungal suppression activity of *L. acutivena* twig oil.

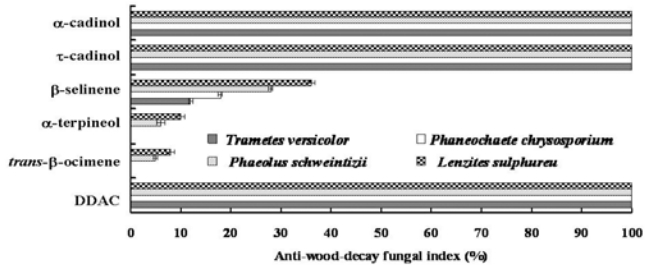

Figure 1: Anti-wood-decay fungal indices of the five main compounds (50 μg/mL) of the twig essential oil of *L. acutivena*.

Experimental

Plant materials: Fresh leaves and twigs of *L. acutivena* were collected in July 2010 from Li-Long mountain (Pintung county, Taiwan, elevation 1050 m, N 22º 23′ 08″, E 120º 76′ 38″). The samples were compared with specimen no. TAIF 58826 from the Herbarium of the Taiwan Forestry Research Institute and identified by Prof. Yen-Hsueh Tseng of the National Chung Hsing University. The voucher specimen (CLH-015) was deposited in the NCHU herbarium. The leaves were air dried at room temperature and protected from the light for one week.

Isolation of the leaf and twig essential oil: The essential oils of the dry leaves and twigs (200 g) were separately extracted in a Clevenger-type apparatus using a hydrodistillation technique and kept refrigerated at 8ºC until used. The oil yields and all test data are the average of triplicate analyses.

Essential oil analysis and component identification: A Hewlett-Packard HP 6890 gas chromatograph equipped with a DB-5 fused silica capillary column and a FID detector were used for determining the oil components under the experimental conditions, as reported earlier [6]. Identification of essential oil constituents was based on comparisons of RI [7], RTs, and MS [7,13].

Antimicrobial assays [14a]***:*** The *in vitro* antibacterial and antimicrobial activities of the oil were evaluated by the disc diffusion method, as reported earlier [6]. 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 fungal species: *Aspergillus niger* (ATCC 16404) and 1 yeast: *C. albicans* (ATCC 10231). Minimum inhibitory concentration (MIC) values were measured by the micro-dilution broth susceptibility assay recommended by NCCLS [14b]. 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 Cheng *et al.* [15] was adopted. The fungi used were *T. versicolor* (L. ex Fr.) Quel. (BCRC 35253), *Phane. chrysosporium* Burdsall (BCRC 36200), *Phaeo. schweinitzii* (Fries) Paterson (BCRC 35365) and *L. sulphureu* (B. ex Fr.) Bond. (BCRC 35305). Cultures of each fungus 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 averaged. Briefly, 100.0, 75.0, 50.0, 25.0 and 12.5 µg/mL of essential oils were added to sterilized PDA in 9 cm Petri dishes. After transfer of the mycelium of 4 fungal strains, the test dishes were incubated in the dark at 26 ± 2°C and 70% relative humidity. When the mycelium had reached the edges of the control Petri dishes (those without essential oils), the antifungal indices were calculated:

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), a preservative for wood decay fungi, was used as a positive control.

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Contents

(*Continued inside back cover*)