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Composition and Two Activities of the Leaf Essential Oil of *Litsea acuminata* (Blume) Kurata from Taiwan

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Abstract: This study investigated the chemical composition, and antimicrobial and anti-wood-decay fungal activities of the essential oil isolated from the leaf of *Litsea acuminata* (Blume) Kurata from Taiwan. The essential oil from the fresh leaves of *L. acuminata* was isolated using hydrodistillation in a Clevenger-type apparatus, and characterized by GC–FID and GC–MS. A total of 48 compounds were identified, representing 100% of the oil. The main components identified were β-caryophyllene (13.0%), τ-cadinol (11.1%), α-cadinol (8.6%), α-humulene (7.5%), α-pinene (7.0%), globulol (6.6%), and β-eudesmol (6.1%). The antimicrobial activity of the oil was tested by the disc diffusion method and micro-broth dilution method against ten microbial species, respectively. The oil exhibited strong growth suppression against Gram-positive bacteria and yeast with inhibition zones of 45~50 mm to MIC values of 31.25~62.5 μg/mL, 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. For the antimicrobial and anti-wood-decay fungal activities of the oil, the active source compounds were determined to be τ-cadinol, α-cadinol, and β-eudesmol.

Keywords: *Litsea acuminate;* lauraceae; essential oil; antimicrobial activity; anti-wood-decay fungal activity; τ-cadinol; α-cadinol.

1. Introduction

Lauraceae family contains approximately 45 genera and 2250 species [1].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 [2]. In total, 12 species are found in Taiwan [3]. All *Litsea* species have a fragrant odor, and certain species possess bioactivity. In our previous report, the fruit oil of *Litsea cubeba* Pers. has anticancer activity [4], and the leaf oils of *L. coreana* H. Lev. [5], *L. kostermansii* C. E. Chang [6], *L nakaii* Hayata [7] and *L. mushaensis* Hayata [8] have antimicrobial activity. *L. acuminata* (Blume) Kurata is a mid-sized evergreen tree mainly distributed in Japan, Taiwan, and China [3]. No studies have investigated on the chemical composition or biological activities of the essential oils or other extracts from this species. Therefore, hydrodistillation technique was used to collect the leaf oil, and it was analyzed by GC–FID and GC–MS.

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Litsea acuminata 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 [9]. 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 [10]. 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.

2. Materials and Methods

2.1. Plant material

Fresh leaves of *L. acuminata* were collected in July 2010 from Mt. Peitawu at an elevation of 1600 m in southern Taiwan (N 22º 37´ 25.1˝, E 120º 42´ 51.5˝, Pingtung County). The samples were compared with specimen no. TAIF 14149 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-016) was deposited in the NCHU herbarium. Before distillation, the leaf was air dried at room temperature protected from the light for one week.

2.2 Isolation of leaf essential oil

The essential oil of the air-dried leaves (100 g) was distilled for 3 hours using a Clevenger-type apparatus and hydrodistillation technique. After distillation, 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 < 4ºC until used. The oil yield and all test data are the average of triplicate analyses.

2.3 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 5 ºC/min. Injector temperature: 270ºC. Carrier gas: Hydrogen with a flow rate of 1 mL/min. Detector temperature: 250ºC, 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) ; carrier gas helium, flow rate 1 mL/min in split mode. 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.

2.4 Component identification

Identification of the leaf essential oil constituents was based on comparisons of retention index (RI) [11], retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra, and literature [11,12].

2.5 Antimicrobial activity

To prevent widespread in-hospital infection, we selected ten microbial strains for testing. The method of Baron and Finegold [13] was adopted. Discs containing 15 μL and 30 μL 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. 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 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: *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 6538P), and *Staphylococcus 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 micro-dilution broth susceptibility assay recommended by NCCLS [14] and as reported earlier [15].

2.6 Anti-wood-decay fungal assays

The method of Cheng *et al.* [16] 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 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.

3. Results and Discussion

Hydrodistillation of *L. acuminata* leaves produced a yellow-colored oil with a yield (v/w) on moisture free basis of 3.85± 0.03% (v/w). All compounds are listed in order of their elution from the DB-5 column (Table 1). A total of 48 compounds were identified from the hydrodistillated leaf oil of *L. acuminata*. Among the leaf oil compounds, oxygenated sesquiterpenes were predominant (41.6%), followed by sesquiterpene hydrocarbons (39.6%), monoterpene hydrocarbons (14.9%), oxygenated monoterpenes (3.5%), and non-terpenoids (0.4%). Among the oxygenated sesquiterpenes, τ-cadinol (11.1%), α-cadinol (8.6%), globulol (6.6%), and β-eudesmol (6.1%) were the major compounds. Of the sesquiterpene hydrocarbons, β-caryophyllene (13.0%) and α-humulene (7.5%) were the main components. α-Pinene (7.0%) was the chief components among the monoterpene hydrocarbons.

Table 1. Chemical composition of the leaf oil of *Litsea acuminata.*

Order	Compound ID	RI[a]	RI[b]	Concentration(%)	Identification[c]
1	α-pinene	934	939	7.0	MS, RI, ST
2	camphene	948	954	2.9	MS, RI, ST
3	β-pinene	975	979	4.2	MS, RI, ST
4	myrcene	991	991	0.2	MS, RI, ST
5	limonene	1028	1029	0.5	MS, RI, ST
6	*cis*- β -ocimene	1040	1037	0.1	MS, RI, ST
7	2-heptyl acetate	1043	1044	0.2	MS, RI
8	linalool	1095	1097	0.2	MS, RI, ST
9	perillene	1101	1101	0.1	MS, RI
10	*trans*-pinocarveol	1140	1139	0.1	MS, RI, ST
11	*cis*-verbenol	1142	1141	0.1	MS, RI, ST
12	*cis*-β-terpineol	1146	1144	t	MS, RI
13	terpinen-4-ol	1178	1177	0.1	MS, RI, ST
14	α-terpineol	1191	1189	0.7	MS, RI, ST
15	myrtenal	1198	1196	0.1	MS, RI
16	bornyl acetate	1285	1289	2.2	MS, RI, ST
17	α-cubebene	1348	1351	0.3	MS, RI, ST
18	α-copaene	1373	1377	2.2	MS, RI, ST
19	β-elemene	1389	1391	1.6	MS, RI, ST
20	*cis*-α- bergamotene	1409	1413	0.1	MS, RI
21	β-caryophyllene	1414	1419	13.0	MS, RI, ST
22	β-copaene	1428	1432	0.1	MS, RI, ST
23	*trans*-α-bergamotene	1431	1435	3.0	MS, RI
24	γ-elemene	1433	1437	0.3	MS, RI
25	*epi*-β-santalene	1443	1447	0.2	MS, RI
26	α-humulene	1451	1455	7.5	MS, RI, ST
27	β-santalene	1455	1460	2.8	MS, RI
28	γ-muurolene	1474	1480	1.5	MS, RI
29	*cis*-4,10-epoxy-amorphane	1478	1483	0.2	MS, RI
30	β-selinene	1486	1490	2.7	MS, RI
31	viridiflorene	1491	1497	1.5	MS, RI
32	α-muurolene	1497	1500	0.3	MS, RI
33	β-bisabolene	1502	1506	0.4	MS, RI
34	γ-cadinene	1510	1514	0.3	MS, RI
35	6-methyl-α-ionone	1521	1522	0.1	MS, RI
36	δ-cadinene	1522	1523	1.6	MS, RI
37	elemol	1543	1550	3.0	MS, RI, ST
38	*(E)*-nerolidol	1556	1563	1.2	MS, RI
39	ledol	1561	1569	0.6	MS, RI
40	caryophyllene alcohol	1562	1572	0.1	MS, RI
41	spathulenol	1570	1578	0.7	MS, RI, ST
42	globulol	1577	1585	6.6	MS, RI, ST
43	viridiflorol	1583	1593	1.1	MS, RI, ST
44	humulene epoxide Ⅱ	1598	1608	1.4	MS, RI
45	τ-cadinol	1636	1640	11.1	MS, RI, ST
46	δ-cadinol	1641	1646	1.1	MS, RI
47	β-eudesmol	1643	1651	6.1	MS, RI, ST
48	α-cadinol	1650	1654	8.6	MS, RI, ST
Compound identified				100.0	
Monoterpene hydrocarbons				14.9	
Oxygenated monoterpenes				3.5	
Sesquiterpene hydrocarbons				39.6	
Oxygenated sesquiterpenes				41.6	
Others				0.4	
Yield (%)				3.85± 0.03	

[a] Relative retention indices experimental: n-alkanes (C9-C24) were used as reference points in the calculation of relative retention indices. [b] Retention index on a DB-5 column with reference to *n*-alkanes [11]. [c] MS, NIST and Wiley library spectra and the literature; RI, Retention index; ST, authentic standard compounds. t, trace < 0.1%.

From the results presented above, the leaf oil constituents of *L. acuminata* were primarily sesquiterpenoids. Intra-genus leaf oil comparisons indicated that many *Litsea* trees, such as *L. coreana* [5], *L. kostermansii* [6], *L nakaii* [7], *L. mushaensis* [8], *L. linii* [8], *L. resinosa*, *L. rasilipes*, and *L. paludosa* [17], all have predominately sesquiterpenoids as their main constituents. However, the main components of the individual species differed. Further comparison with the leaf oil of *L. guatemalensis* [18], *L. laevigate* [19] and *L. akoensis* [20] were predominantly monoterpenoids and, therefore, differed from the leaf oil of *L. acuminata*.

The essential oil of *L. acuminata* 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 45 to 50 mm to MIC values of 31.25~62.5 μg/mL, respectively. The essential oil showed superior suppressive activity toward the Gram-positive bacteria than that of 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 [21]. 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 [22]. Comparing the antimicrobial activities of the leaf or twig essential oils with that extracted from *L. kostermansii* [6], *L. nakaii* [7], *L. linii* and *L. mushaensis* [8], the leaf oil of *L. acuminata* was superior (Table 3). The results verify that *L. acuminata* leaf oil has excellent antimicrobial activity.

However, to ascertain the source compounds of antimicrobial activity from *L. acuminata* leaf oil, the main components were individually tested for antimicrobial activities. Compounds α-pinene, β-caryophyllene, α-humulene, globulol and β-eudesmol were purchased from the Fluka Co. (Milwaukee, USA). Whereas, α-adinol and τ-cadinol were from isolate of Ho *et al*'s study on *Machilus philippinenesis* essential oil [23]. The results indicated that the active source compounds were τ-cadinol, α-cadinol, and β-eudesmol. These results were similar to those of Ho *et al*. [5~7]. Various studies support the argument that these compounds are highly active in suppressing microbial growth [21,24,25].

Leaf oil of *L. acuminata* was tested against two white rot fungi (*Trametes versicolor*, *Phaneochaete chrysosporium*) and two brown rot fungi (*Phaeolus schweinitzii*, *Lenzites sulphureu*). 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 25, 25, 12.5, 12.5 μg/mL, respectively.

Comparing the anti-wood-decay fungal activities of the essential oils from *Litse*a spp. such as *L. linii* [8], *L. mushaensis* [8] and *L. coreana* [5], the twig oil of *L. acuminata* was superior (Table 5). The results verified that *L. acuminata* leaf oil has excellent anti-wood-decay fungal activities.

Furthermore, in order to ascertain the source compounds of the *L. acuminata* essential oil, we also tested the anti-wood-decay fungal activities of its major compounds. The results indicated that the sources of activities were also τ-cadinol, α-cadinol, and β-eudesmol. At a 50 μg/mL concentration, τ-cadinol, and α-cadinol showed total growth inhibition against all white-rot and brown-rot fungi tested; while β-eudesmol at 50 μg/mL concentration could completely inhibit brown-rot fungi but partially inhibit white-rot fungi. The results agree with those of Kondo and Imamura [24], who pointed out that the methanol extract of hinoki (*Chamaecyparis obtusa*) containing α-cadinol, τ-cadinol, and τ-muurolol, exhibited excellent inhibitory effects against wood decaying fungi. In particular, α-cadinol had the best inhibitory efficacy. Mori et al. [26] extracted eudesmol, magnolol, honokilol etc. from the bark of *Magnolia obovata*. These compounds all exhibited excellent inhibitory effects against wood decaying fungi. Thus, the excellent wood-decay-fungi inhibitive activities exhibited by the *L. acuminata* leaf oil could well be contributed by the presence of compounds such as τ-cadinol, α-cadinol, and β-eudesmol etc.

Table 2. Antimicrobial activities of the leaf oil of *L. acuminata*.

Microbial species	*L. acuminata*		Compounds[c]							Antibiotics		
	Leaf		1	2	3	4	5	6	7	Tetracycline (30 μg/disk)	Gentamicine (10 μg/disk)	Nystatine (30 μg/disk)
	IZ[a]	MIC[b]	MIC	MIC	MIC	MIC	MIC	MIC	MIC	IZ	IZ	IZ
Bacillus cereus	45 ± 0.8	62.5	>1000	500	750	1000	62.5	125	125	22 ± 0.8	-	nt
Staphylococcus aureus	50 ± 0.8	31.25	>1000	250	500	750	62.5	62.5	62.5	21 ± 0.4	-	nt
Staphylococcus epidermidis	50 ± 0.8	31.25	>1000	250	500	750	62.5	62.5	62.5	34 ± 0.4	-	nt
Escherichia coli	36 ± 0.8	125	>1000	1000	>1000	>1000	500	500	750	-	22 ± 0.8	nt
Enterobacter aerogenes	30 ± 0.8	250	>1000	750	>1000	>1000	125	125	250	10 ± 0.4	-	nt
Klebsiella pneumoniae	32 ± 0.4	250	>1000	750	>1000	>1000	125	125	250	-	21 ± 0.8	nt
Pseudomonas aeruginosa	32 ± 0.8	250	>1000	>1000	>1000	>1000	500	500	750	-	12 ± 0.8	nt
Vibrio parahaemolyticus	30 ± 0.8	250	>1000	>1000	>1000	>1000	1000	1000	1000	-	13 ± 0.8	nt
Aspergillus niger	32 ± 0.4	250	>1000	>1000	>1000	>1000	750	1000	>1000	nt	nt	17 ± 0.8
Candida albicans	45 ± 0.4	62.5	>1000	250	>1000	>1000	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(≥ 99.5%), 2. β-caryophyllene (≥ 98.5%), 3. α-humulene(≥ 98.5%), 4. globulol (≥99.0%), 5. τ-cadinol (≥98.5%), 6. β-eudesmol (≥ 99.5%), 7. α-cadinol (100%) Compound 1 to 4 and 6 were purchased from the Fluka Co. (Milwaukee, USA). Compound 5 and 7 were from isolate of Ho *et al*'s study on *Machilus philippinenesis* essential oil [23]. (-), Inactive. nt, not tested.

Table 3. Comparison of the MIC values (μg/mL) of the leaf oil of *L. acuminata* and those of *L. kostermansi*, *L. nakaii*, *L. linii*, and *L. mushaensis* against the microbial.

Essential oil	Microbial *										Ref.
	B. c.	*S. a.*	*S. e.*	*E. c.*	*E. a.*	*K. p.*	*P. a.*	*V. p.*	*A. n.*	*C. a.*	
Leaf											
L. acuminata	62.5	31.25	31.25	125	250	250	250	250	250	62.5	This study
L. kostermansii	375	250	125	750	500	375	750	1000	>1000	1000	[4]
L. nakaii	250	375	125	500	500	375	500	750	1000	500	[5]
L. linii	500	500	500	750	750	>1000	>1000	>1000	>1000	750	[6]
Twig											
L. mushaensis	1000	750	750	>1000	>1000	>1000	>1000	>1000	>1000	>1000	[5]

** 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-fungal decay indices of leaf essential oil from *L. acuminata*.

Dosage (μg/mL)	Antifungal index (%)			
	Trametes versicolor	*Phaneochaete chrysosporium*	*Phaeolus schweintizii*	*Lenzites sulphureu*
12.5	92 ± 3.3	86 ± 6.6	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

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

Essential oil	Fungi				Ref.
	Trametes versicolor	*Phaneochaete chrysosporium*	*Phaeolus schweintizii*	*Lenzites sulphureu*	
Leaf					
L. acuminata	25	25	12.5	12.5	This study
L. linii	>100	>100	>100	>100	[8]
L. mushaensis	25	50	25	12.5	[8]
L. coreana	75	75	50	25	[5]
Twig					
L. linii	50	50	25	25	[8]
L. mushaensis	>100	>100	>100	>100	[8]

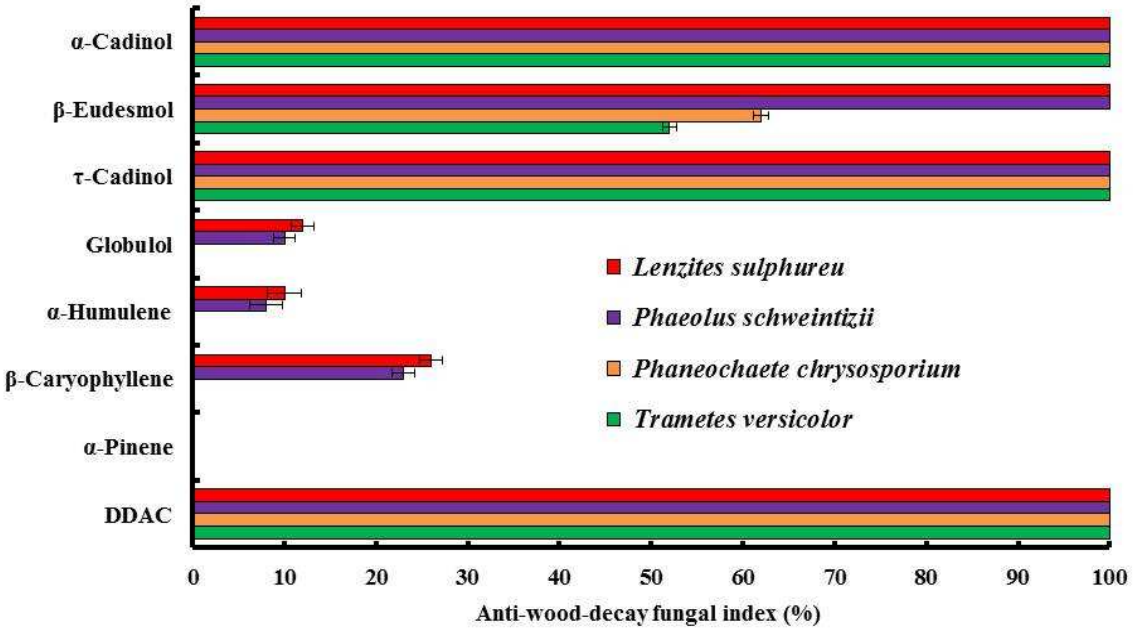

Figure 1. Anti-wood-decay fungal indices of the seven main compounds (50 μg/mL) of the leaf essential oil of *L. acuminata*.
Note: DDAC (didecyl dimethyl ammonium chloride) is a wood preservative for wood decay fungi and used as a positive control.

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