

Article

Antifungal Sesquiterpenoids from *Michelia formosana* Leaf Essential Oil against Wood-Rotting Fungi

Chia-Che Wu¹, Shou-Ling Huang², Chun-Han Ko¹ and Hui-Ting Chang^{1,*}

¹ School of Forestry and Resource Conservation, National Taiwan University, Taipei 106, Taiwan; r91625016@ntu.edu.tw (C.-C.W.); chunhank@ntu.edu.tw (C.-H.K.)

² Instrumentation Center, College of Science, National Taiwan University, Taipei 106, Taiwan; shouling@g.ntu.edu.tw

* Correspondence: chtchang@ntu.edu.tw; Tel.: +886-2-3366-5880

Abstract: *Michelia formosana* (Kanehira) Masamune is a broad-leaved species widespread in East Asia; the wood extract and its constituents possess antifungal activity against wood-decay fungi. Antifungal activities of leaf essential oil and its constituents from *M. formosana* were investigated in the present study. Bioassay-guided isolation was applied to isolate the phytochemicals from leaf essential oil. 1D and 2D NMR, FTIR, and MS spectroscopic analyses were applied to elucidate the chemical structures of isolated compounds. Leaf essential oil displayed antifungal activity against wood decay fungi and was further separated into 11 fractions by column chromatography. Four sesquiterpenoids were isolated and identified from the active fractions of leaf essential oil through bioassay-guided isolation. Among these sesquiterpenoids, guaicol, bulnesol, and β -elemol have higher antifungal activity against brown-rot fungus *Laetiporus sulphureus* and white-rot fungus *Lenzites betulina*. Leaf essential oil and active compounds showed better antifungal activity against *L. sulphureus* than against *L. betulina*. The molecular structure of active sesquiterpenoids all contain the hydroxyisopropyl group. Antifungal sesquiterpenoids from *M. formosana* leaf essential oil show potential as natural fungicides for decay control of lignocellulosic materials.

Keywords: antifungal activity; *Michelia formosana*; sesquiterpenoids; wood-rotting fungi



Citation: Wu, C.-C.; Huang, S.-L.; Ko, C.-H.; Chang, H.-T. Antifungal Sesquiterpenoids from *Michelia formosana* Leaf Essential Oil against Wood-Rotting Fungi. *Molecules* **2022**, *27*, 2136. <https://doi.org/10.3390/molecules27072136>

Academic Editors: Carmen Formisano and José Rubén Tormo

Received: 13 February 2022

Accepted: 24 March 2022

Published: 25 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Lignocellulosic materials are organic polymeric biomaterials mainly composed of cellulose, hemicellulose, and lignin. They are easily degraded by biotic factors [1–4]. Biodegradation of lignocellulosic materials is a crucial issue for its utilization and product life cycle. Among the biodegradation of lignocellulosic materials, decay fungi cause the greatest financial losses of forest products; decay fungi include brown-rot fungi, white-rot fungi, and soft-rot fungi [3–7]. Traditionally, wood preservatives were applied to prevent the biodegradation of lignocellulosic materials, and most commercial preservatives are inorganic metal-containing agents. However, due to a growing focus on environmental consciousness, some highly toxic preservatives have been phased out and restricted from the global market [3,8,9].

Research and development in eco-benign fungicides for lignocellulosic materials are essential to achieve the optimal utilization of the lignocellulosic resources [10–12]. Many plant natural products have been proven to possess effective antifungal properties, including hinokitiol, *trans*-cinnamaldehyde, liriodenine, thymol, carvacrol, etc. [13–17].

Michelia formosana (Kanehira) Masamune, Formosan *Michelia*, belonging to the family Magnoliaceae, is a broad-leaved tree distributed in East Asia. Ogura et al. analyzed the natural products of *M. formosana* root extract and isolated 10 sesquiterpene lactones, including michelenolide, micheliolide, compressanolide, dihydroreynosin, parthenolide, dihydroparthenolide, costunolide, lanuginolide, reynosin, and santamarine, and one alkaloid, liriodenine [18]; Wu et al. also isolated the alkaloid compound, liriodenine, from

M. formosana wood extract [16]. *M. formosana* extracts and its constituents possess the versatile bioactivities, including antifungal, anti-inflammatory, cytotoxic activities, etc. [16,18,19]. The aims of this study were to investigate the antifungal activity of *M. formosana* leaf essential oil against wood-decay fungi and to isolate and identify the constituents which possess antifungal activity from leaf essential oil.

2. Results and Discussion

2.1. Antifungal Activities of *M. formosana* Leaf Essential Oil and Its Fractions

Brown-rot fungi selectively degrade polysaccharides, hemicellulose and cellulose, in wood and cause the oxidation of lignin; infected wood become a brownish color due to the high residual lignin. White-rot fungi degrade both lignin and cellulosic components of wood and change the color of wood to a little whitish [3,7]. Brown-rot fungus *Laetiporus sulphureus* (*L. sulphureus*) and white-rot fungus *Lenzites betulina* (*L. betulina*; *Lenzites betulinus*; *Trametes betulina*) are common fungal strains among the wood-rotting fungi [20,21]. Antifungal indexes of *M. formosana* leaf essential oil against fungi *L. sulphureus* and *L. betulina* were 100.00% and 94.19% at a concentration of 500 µg/mL, respectively, (Table 1); 67.44% and 25.97% at a concentration of 100 µg/mL. Leaf essential oil showed a better inhibition effect against brown-rot fungus *L. sulphureus* in comparison with white-rot fungus *L. betulina*. Antifungal activity of 11 fractions of leaf essential oil against wood-rotting fungi at a concentration of 200 µg/mL are shown in Figure 1. Fractions L5 and L6 had the highest antifungal activities with an antifungal index of 100%. The other fractions showed weak/no activity against examined wood-rotting fungi.

Table 1. Antifungal index of *M. formosana* leaf essential oil against wood-rotting fungi.

Fungus	Antifungal Index (%)	
	100 µg/mL	500 µg/mL
<i>L. sulphureus</i>	67.44 ± 3.29 ^b	100.00 ± 0.00 ^a
<i>L. betulina</i>	25.97 ± 0.67 ^c	94.19 ± 1.16 ^a

Different letters (a–c) in the Table are statistically different at $p < 0.05$ according to the Scheffe test.

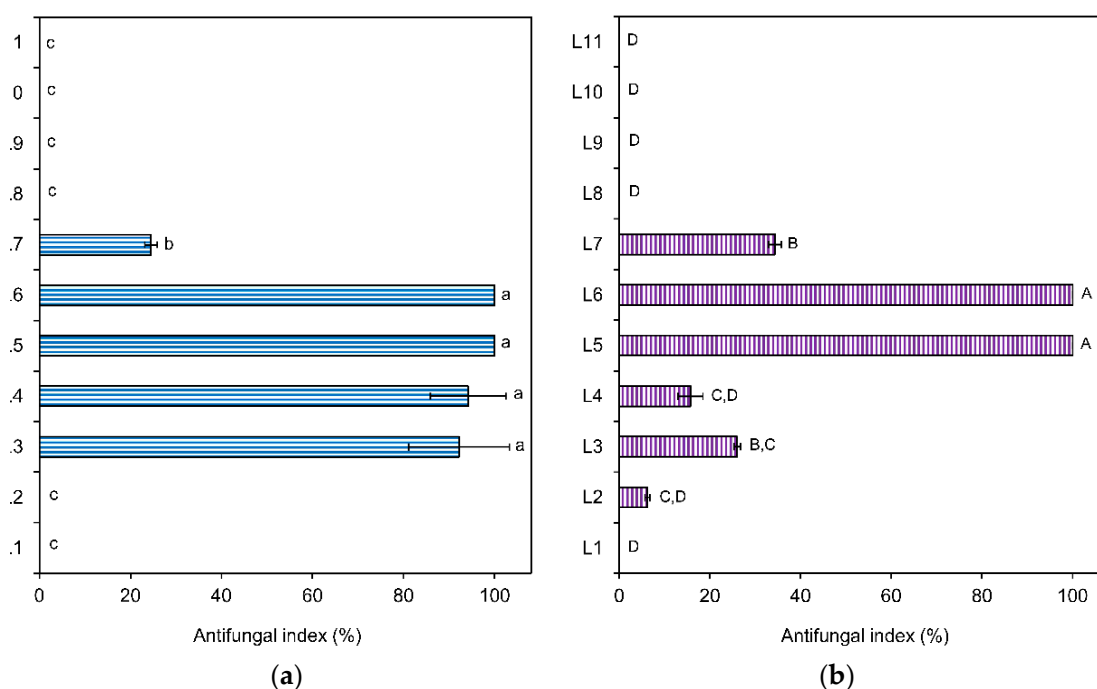


Figure 1. Antifungal activities of 11 fractions from leaf essential oil against wood-rotting fungi at a concentration of 200 µg/mL. (a) *L. sulphureus*; (b) *L. betulina*. Different letters (a–c; A–D) in the Figure are statistically different at $p < 0.05$ according to the Scheffe test.

2.2. Isolation and Identification of Constituents from *M. formosana* Leaf Essential Oil

Four sesquiterpenoids including 4,5-epoxy- β -caryophyllene, guaialol, bulnesol, and β -elemol (Figure 2) were isolated from active fractions and identified by several spectral analyses. Guaialol and bulnesol were firstly identified from woody plant *M. formosana*. Through HPLC analysis, fraction L5 contained 5.62% 4,5-epoxy- β -caryophyllene and 54.78% guaialol, and fraction L6 contained 19.54% guaialol, 54.73% bulnesol, and 13.91% β -elemol.

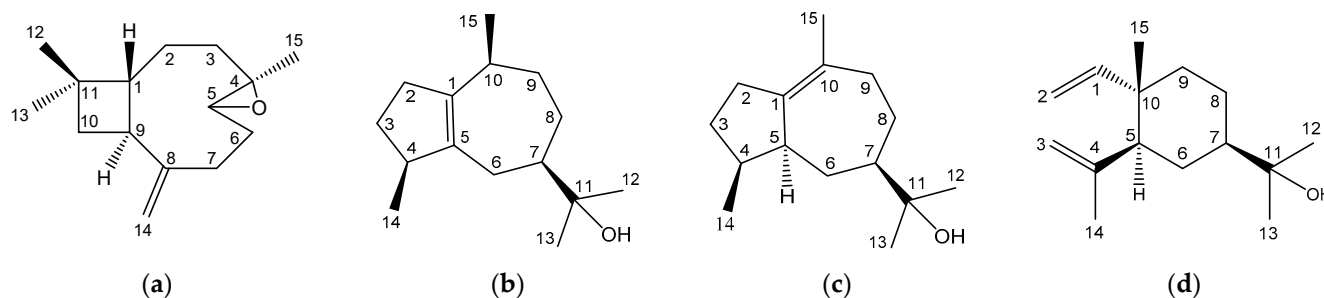


Figure 2. Chemical structures of sesquiterpenoids isolated from leaf essential oil. (a) 4,5-Epoxy- β -caryophyllene; (b) Guaialol; (c) Bulnesol; (d) β -Elemol.

4,5-Epoxy- β -caryophyllene: Colorless oil, EI-MS m/z : 79, 91, 105, 121, 145, 159, 173, 187, 202. Molecular formula: $C_{15}H_{24}O$. IR ν_{max} cm^{-1} : 2959 (C-H), 2920 (C-H), 1634 (C=C), 1458 (C- CH_3) and 1383 (C- CH_3). 1H NMR ($CDCl_3$, 500 MHz): δ 0.94 (1H, m, H-3a), 0.98 (3H, s, H-12), 1.00 (3H, s, H-13), 1.20 (3H, s, H-15), 1.31 (1H, m, H-6a), 1.41 (1H, m, H-2a), 1.60 (1H, m, H-10a), 1.63 (1H, m, H-2b), 1.66 (1H, m, H-10b), 1.74 (1H, t, $J = 10.0$ Hz, H-1), 2.06 (1H, m, H-3b), 2.09 (1H, m, H-7a), 2.23 (1H, m, H-6b), 2.32 (1H, m, H-7b), 2.58 (1H, dt, $J = 9.8, 9.2$ Hz, H-9), 2.86 (1H, dd, $J = 4.4, 10.8$ Hz, H-5), 4.85 (1H, brs, H-14a), 4.97 (1H, brs, H-14b). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 16.97 (t, C-15), 21.60 (q, C-13), 27.19 (t, C-2), 29.78 (t, C-7), 29.87 (q, C-12), 30.17 (t, C-6), 34.00 (s, C-11), 39.14 (t, C-3), 39.75 (t, C-10), 48.72 (d, C-9), 50.75 (d, C-1), 59.83 (s, C-4), 63.74 (d, C-5), 112.74 (q, C-14), 151.82 (s, C-8). 4,5-Epoxy- β -caryophyllene is a sesquiterpenoid with a structure based on the caryophyllane skeleton. NMR spectra were in agreement with the literature [22].

Guaialol: White needles, mp: 91–93 °C. EI-MS m/z : 79, 91, 105, 119, 133, 147, 161, 189, 204, 222 [M+]. Molecular formula: $C_{15}H_{26}O$. IR ν_{max} cm^{-1} : 3346 (OH), 2933 (C-H), 2856 (C-H), 1636 (C=C), 1458 (C- CH_3), 1358 (C- CH_3) and 918 (C-O). 1H NMR ($CDCl_3$, 500 MHz): δ 0.91 (3H, s, H-14), 0.95 (3H, s, H-15), 1.11 (3H, s, H-12), 1.14 (3H, s, H-13), 1.25 (1H, m, H-3a), 1.42 (1H, m, H-8a), 1.51 (1H, m, H-7), 1.53 (1H, m, H-9a), 1.68 (1H, m, H-9b), 1.77 (1H, m, H-8b), 1.85 (1H, m, H-6a), 1.92 (1H, m, H-3b), 2.05 (1H, m, H-2a), 2.10 (1H, m, H-6b), 2.25 (1H, m, H-10), 2.38 (1H, m, H-2b), 2.49 (1H, m, H-4), 5.04 (1H, brs, -OH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 19.81 (q, C-15), 19.95 (q, C-14), 25.99 (q, C-12), 27.13 (t, C-8), 27.38 (q, C-13), 27.85 (t, C-6), 30.94 (t, C-3), 33.69 (d, C-10), 33.76 (t, C-9), 35.36 (t, C-2), 46.24 (d, C-4), 49.55 (d, C-7), 73.49 (s, C-11), 138.81 (s, C-5), 140.01 (s, C-1). Figure 3a is the HMBC spectrum of guaialol. Guaialol belongs to the guaiane skeleton which is a fused-bicyclic system with five- and seven-membered rings. NMR data of guaialol were in agreement with related literatures [23,24]. Guaialol has been reported to have antimicrobial and acaricidal activities [25].

Bulnesol (guai-1(10)-en-11-ol): Colorless oil, EI-MS m/z : 93, 105, 107, 119, 133, 161, 189, 204, 222 [M+]. Molecular formula: $C_{15}H_{26}O$. IR ν_{max} cm^{-1} : 3434 (OH), 2967 (C-H), 2933 (C-H), 1632 (C=C), 1458 (C- CH_3) and 1370 (C- CH_3). 1H NMR ($CDCl_3$, 500 MHz): δ 0.77 (1H, dd, $J = 11.7, 24.0$ Hz, H-6a), 0.87 (3H, d, $J = 7.0$ Hz, H-14), 1.04 (1H, t, $J = 11.0$ Hz, H-8a), 1.14 (6H, s, H-12,13), 1.33 (1H, m, H-3a), 1.41 (1H, m, H-7), 1.61 (1H, m, H-3b), 1.63 (3H, s, H-15), 1.81 (1H, br.d, $J = 12.5$ Hz, H-6b), 1.87 (1H, m, H-8b), 2.05 (1H, m, H-9a), 2.11 (1H, m, H-4), 2.13 (1H, m, H-9b), 2.15 (1H, m, H-2a), 2.29 (1H, m, H-2b), 2.37 (1H, m, H-5). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 15.29 (q, C-14), 22.29 (q, C-15), 27.07 (q, C-13), 27.16 (q, C-12), 27.67 (t, C-8), 28.67 (t, C-6), 30.28 (t, C-2), 32.99 (t, C-3), 34.81 (t, C-9), 38.97 (d, C-4),

46.23 (d, C-5), 54.06 (d, C-7), 73.75 (s, C-11), 128.80 (s, C-10), 141.61 (s, C-1). Figure 3b is the HMBC spectrum of bulnesol. Bulnesol also belongs to the guaiane skeleton. NMR spectra were consistent with those reported in the literature [23].

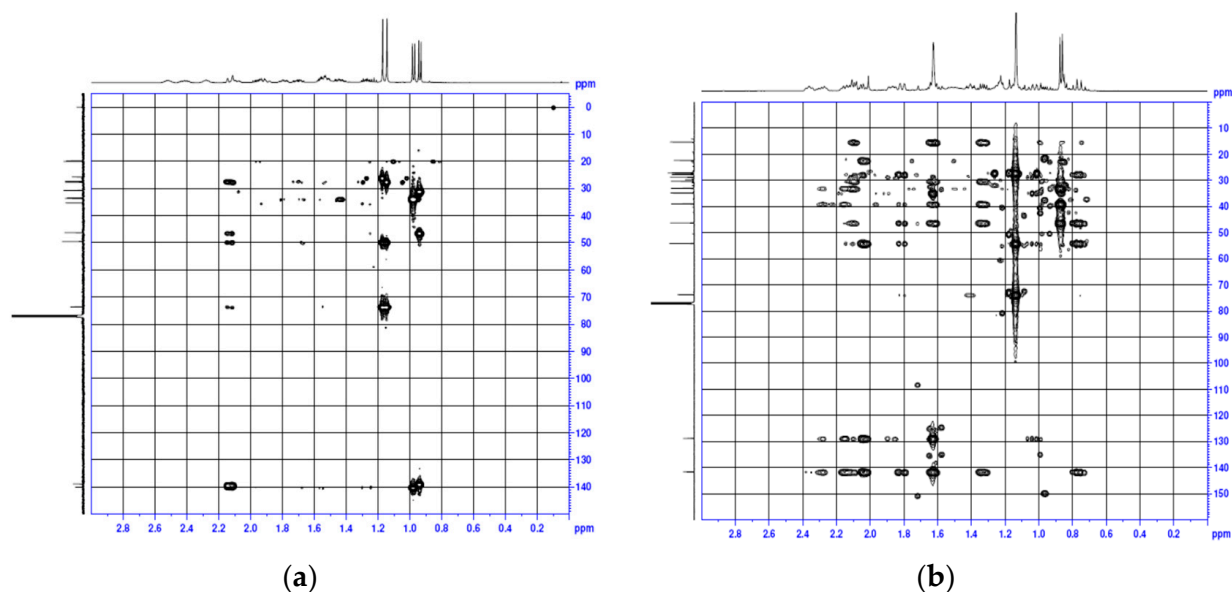


Figure 3. HMBC spectra of guaiol and bulnesol. (a) Guaiol; (b) Bulnesol.

β -Elemol: Light yellow oil, EI-MS m/z : 79, 93, 105, 119, 133, 147, 161, 175, 189, 204. Molecular formula: $C_{15}H_{26}O$. IR ν_{\max} cm^{-1} : 3424 (OH), 3083 (C=C-H), 2973 (C-H), 2936 (C-H), 2864 (C-H), 1636 (C=C), 1460 (C-CH₃) and 1375 (C-CH₃). 1H NMR ($CDCl_3$, 500 MHz): δ 0.96 (3H, s, H-15), 1.18 (6H, s, H-12, 13), 1.25 (1H, m, H-8a), 1.32 (1H, m, H-7), 1.40 (1H, m, H-6a), 1.42 (2H, m, H-9a, 9b), 1.56 (1H, m, H-6b), 1.63 (1H, m, H-8b), 1.69 (3H, brs, H-14), 1.94 (1H, dd, $J = 12.0, 2.5$ Hz, H-5), 4.56 (1H, brs, H-3a), 4.80 (1H, d, $J = 1.5$ Hz, H-3b), 4.86 (1H, dd, $J = 11.0, 1.0$ Hz, H-2 *cis*), 4.87 (1H, dd, $J = 17.5, 1.0$ Hz, H-2 *trans*), 5.78 (1H, dd, $J = 17.5, 11.0$ Hz, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 16.57 (q, C-15), 22.53 (t, C-8), 24.77 (q, C-14), 27.13 (q, C-13), 27.15 (q, C-12), 28.47 (t, C-6), 39.69 (s, C-10), 39.85 (t, C-9), 49.32 (d, C-7), 52.68 (d, C-5), 72.75 (s, C-11), 109.88 (t, C-2), 112.03 (t, C-3), 147.89 (s, C-4), 150.22 (d, C-1). β -Elemol is an elemene-type skeleton sesquiterpenoid. NMR spectra were in agreement with those reported in the literature [23].

2.3. Antifungal Effect of Sesquiterpenoids from *M. formosana* Leaf Essential Oil

Antifungal activities of isolated sesquiterpenoids against wood-rotting fungi are presented in Table 2 below. 4,5-Epoxy- β -caryophyllene was not effective against both fungi; the other sesquiterpenoids possessed an inhibition effect with IC_{50} value less than 100 $\mu g/mL$. The compounds guaiol, bulnesol, and β -elemol showed better activities against *L. sulphureus* than against *L. betulina* comparing IC_{50} values of each specimen; the trend was similar to that of leaf essential oil, as described above. Among the three active sesquiterpenoids, bulnesol had the best inhibition effect with an IC_{50} value of 23.1 $\mu g/mL$ (0.10 mM) against brown-rot fungus *L. sulphureus*. As for white-rot fungus *L. betulina*, guaiol and β -elemol were more active than bulnesol, with effective IC_{50} values of 44.1 $\mu g/mL$ (0.20 mM) and 40.5 $\mu g/mL$ (0.18 mM), which were lower than that of bulnesol (60.2 $\mu g/mL$; 0.27 mM). Active sesquiterpenoids belong to the guaiane and elemene-type skeletons; the molecular structure of these active compounds all contain the hydroxyisopropyl group.

Table 2. IC₅₀ values of compounds from leaf essential oil against wood-rotting fungi.

Specimen	IC ₅₀ (µg/mL)	
	<i>L. Sulphureus</i>	<i>L. Betulina</i>
DDAC *	0.37 ± 0.03 ^{c **} (<0.01) ***	3.24 ± 0.11 ^C (0.01 ± 0.00)
4,5-Epoxy-β-caryophyllene	>100	>100
Guaiol	30.7 ± 2.8 ^a (0.14 ± 0.01)	44.1 ± 1.6 ^B (0.20 ± 0.01)
Bulnesol	23.1 ± 0.9 ^b (0.10 ± 0.00)	60.2 ± 2.6 ^A (0.27 ± 0.01)
β-Elemol	30.5 ± 2.3 ^a (0.14 ± 0.01)	40.5 ± 2.4 ^B (0.18 ± 0.01)

DDAC *: Positive control; **: Different letters (a–c; A–C) in the Table represent significantly different at the level of $p < 0.05$ according to Scheffe's test; ***: (mM).

Gong et al. reported that garlic essential oil and its compounds diallyl disulfide and diallyl trisulfide showed high toxicity against brown-rot fungus *L. sulphureus* with IC₅₀ values of 44.6, 73.2, and 31.6 µg/mL, respectively [26]. Cinnamaldehyde is a well-known natural antifungal agent; IC₅₀ values of cinnamaldehyde were 0.17 and 0.65 mM against *L. sulphureus* and *L. betulina*, respectively [11]. Wu et al. investigated antifungal activity of sesquiterpenoids from *Taiwania cryptomerioides* heartwood essential oil and derivatives against wood-rotting fungi; active antifungal compounds were α-cadinol (0.13 mM), 3β-ethoxy-T-muurolol (0.15 mM), and 15-oxo-α-cadinol (0.20 mM) against white-rot fungus *L. betulina* [27]. Present results revealed that *M. formosana* leaf essential oil and the active sesquiterpenoids, guaiol, bulnesol, and β-elemol, exhibited potent antifungal activity against wood-rotting fungi.

3. Materials and Methods

3.1. Plant Materials

Leaves of *Michelia formosana*, around 70 years old, were collected from the Experimental Forest of National Taiwan University in Nantou County, Taiwan. The voucher specimen was deposited in the Lab of Chemical Utilization of Biomaterials, School of Forestry and Resource Conservation, National Taiwan University.

3.2. Hydrodistillation of Leaf Essential Oil

Fresh leaves (100 g) of *M. formosana* were hydrodistilled in a Clevenger-type apparatus (1 L) with 600 mL of distilled water for 8 h to obtain essential oil [28–31]. Yield of leaf essential oil was 0.87% (*w/w*). The obtained leaf essential oil was stored in dark glass vials at 4 °C until used.

3.3. Antifungal Assay

Antifungal activity of each specimen was evaluated by using the agar plate test. The wood-rotting fungi were brown-rot fungus *Laetiporus sulphureus* Karst. (BCRC 35305, *L. sulphureus*) and white-rot fungus *Lenzites betulina* Fr. (BCRC 35296, *L. betulina*) bought from Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan). Specimens were dissolved in 90 µL (1%) of ethanol, then added into 9 mL PDA (potato dextrose agar) and mixed well in a 60 mm Petri dish. After the agar became solid, mycelial plugs (5 mm in diameter) from the edges of the blank dish were incubated in the center of each plate and cultured at 26 °C and 70% RH for 8–12 days until the fungal mycelia covered the entire control dish (1% ethanol). All experiments were repeated in triplicate. Antifungal index was calculated as the following: Antifungal index (%) = $(1 - Dt/Dc) \times 100$, where *Dt* is the diameter of growth zone in the test dish and *Dc* is the diameter of growth zone in the control dish. IC₅₀ values, half maximal inhibitory concentration, of specimens were graphically obtained from the dose response curves derived from five concentrations [16,32,33]. The

positive control, didecyl dimethyl ammonium chloride (DDAC), is a commercial fungicide used in wood preservatives.

3.4. Bioassay-Guided Isolation by Various Chromatographies

Leaf essential oil was subjected to silica gel column chromatography (CC) with a gradient elution of *n*-hexane and ethyl acetate of increasing polarity, then separated into 11 fractions (L1-L11) by thin layer chromatography (TLC). The yields of each fraction were 35.3% (L1, elution with 100% *n*-hexane), 5.5% (L2, elution with 3% ethyl acetate/97% *n*-hexane), 11.2% (L3, elution with 5% ethyl acetate/95% *n*-hexane), 7.6% (L4, elution with 10% ethyl acetate/90% *n*-hexane), 6.9% (L5, elution with 30% ethyl acetate/70% *n*-hexane), 28.7% (L6, elution with 50% ethyl acetate/50% *n*-hexane), 2.7% (L7, elution with 50% ethyl acetate/50% *n*-hexane), 0.9% (L8, elution with 100% ethyl acetate), 0.3% (L9, elution with 100% ethyl acetate), 0.3% (L10, elution with 100% ethyl acetate), and 0.6% (L11, elution with 100% ethyl acetate). Pure compounds were obtained from active fractions by high-performance liquid chromatography (HPLC, L-2130, Hitachi, Tokyo, Japan) with a preparative 9.4 × 250 mm Zorbax Sil column (5 µm). The isocratic mobile phase consisted of *n*-hexane (90%) and ethyl acetate (10%), at a flow rate of 2 mL/min; elution peaks were detected by the refractive index (RI) detector [34–36].

3.5. Structural Elucidation

The structural determination of isolated compounds was performed by spectral analyses, including 1D NMR (Nuclear magnetic resonance spectroscopy) (¹H-NMR, 500 MHz; ¹³C-NMR, 125 MHz) and 2D NMR (HSQC, HMBC, COSY, and NOESY) measured on a Bruker AVIII NMR spectrometer (Bruker Avance, Rheinstetten, Germany), FTIR (Fourier transform infrared spectroscopy, FTS-40, Bio-rad, Hercules, CA, USA), and MS (mass spectroscopy, MAT-958, Finnigan, MA, USA) [37–40].

3.6. Statistical Analysis

The Scheffe multiple comparison test of the SAS 9.3 statistical program (Cary, NC, USA) was employed to evaluate differences for the antifungal assay. The confidence interval was set at 95%.

4. Conclusions

Antifungal activities of *M. formosana* leaf essential oil and its constituents against wood-rotting fungi were assessed in the present study. Antifungal indexes of leaf essential oil against brown-rot fungus *L. sulphureus* and white-rot fungus *L. betulina* were 100.00% and 94.19% at a concentration of 500 µg/mL, respectively. Through the bioassay guided isolation, four sesquiterpenoids, including 4,5-epoxy-β-caryophyllene, guaiol, bulnesol, and β-elemol, were obtained from active fractions of leaf essential oil. Among the examined sesquiterpenoids, guaiol, bulnesol, and β-elemol had the best inhibition effect against wood-rotting fungi. Results indicated these sesquiterpenoids from *M. formosana* leaf essential oil have promising potential as eco-benign fungicides for decay control of lignocellulosic materials.

Author Contributions: Conceptualization, H.-T.C. and C.-C.W.; Methodology, S.-L.H., C.-H.K. and C.-C.W.; Software, C.-H.K. and S.-L.H.; Formal Analysis and Investigation, C.-C.W. and H.-T.C.; Writing—Original Draft Preparation, H.-T.C. and C.-H.K.; Writing—Review and Editing, H.-T.C. and C.-H.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data are available from the corresponding author on reasonable request.

Acknowledgments: The authors are grateful to the Experimental Forest of National Taiwan University (NTU), the NMR experiments of the Instrumentation Center at NTU, and the National Center for High-Performance Computing (NCHC) for the chemistry database search.

Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Not available.

References

1. Montegut, D.; Indictor, N.; Koestler, R.J. Fungal deterioration of cellulosic textiles: A review. *Int. Biodeterior.* **1991**, *28*, 209–226. [[CrossRef](#)]
2. Martínez, A.T.; Speranza, M.; Ruiz-Dueñas, F.J.; Ferreira, P.; Camarero, S.; Guillén, F.; Martínez, M.J.; Gutiérrez, A.; del Río, J.C. Biodegradation of lignocellulosics: Microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *Int. Microbiol.* **2005**, *8*, 195–204.
3. Shmulsky, R.; Jones, P.D. Durability and protection. In *Forest Products and Wood Science: An Introduction*, 6th ed.; Wiley-Blackwell: Ames, IA, USA, 2011; pp. 229–252. ISBN 978-0813820743.
4. Aleinikovas, M.; Varnagiryte-Kabašinskiene, I.; Povilaitienė, A.; Šilinskas, B.; Škėma, M.; Beniušienė, L. Resistance of wood treated with iron compounds against wood-destroying decay and mould fungi. *Forests* **2021**, *12*, 645. [[CrossRef](#)]
5. Suzuki, M.R.; Hunt, C.G.; Houtman, C.J.; Dalebroux, Z.D.; Hammel, K.E. Fungal hydroquinones contribute to brown rot of wood. *Environ. Microbiol.* **2006**, *8*, 2214–2223. [[CrossRef](#)]
6. Bari, E.; Daniel, G.; Yilgor, N.; Kim, J.S.; Tajick-Ghanbary, M.A.; Singh, A.P.; Ribera, J. Comparison of the decay behavior of two white-rot fungi in relation to wood type and exposure conditions. *Microorganisms* **2020**, *8*, 1931. [[CrossRef](#)]
7. Broda, M. Natural compounds for wood protection against fungi—A review. *Molecules* **2020**, *25*, 3538. [[CrossRef](#)]
8. Preston, A.F. Wood preservation: Trends of today that will influence the industry tomorrow. *For. Prod. J.* **2000**, *50*, 13–19.
9. Morais, S.; Fonseca, H.M.A.C.; Oliveira, S.M.R.; Oliveira, H.; Gupta, V.K.; Sharma, B.; de Lourdes Pereira, M. Environmental and health hazards of chromated copper arsenate-treated wood: A review. *Int. J. Environ. Res. Public Health* **2021**, *18*, 5518. [[CrossRef](#)]
10. Voda, K.; Boh, B.; Vrtacnik, M.; Pohleven, F. Effect of the antifungal activity of oxygenated aromatic essential oil compounds on the white-rot *Trametes versicolor* and the brown-rot *Coniophora puteana*. *Int. Biodeter. Biodegrad.* **2003**, *51*, 51–59. [[CrossRef](#)]
11. Hsu, F.L.; Chang, H.T.; Chang, S.T. Evaluation of antifungal properties of octyl gallate and its synergy with cinnamaldehyde. *Bioresour. Technol.* **2007**, *98*, 734–738. [[CrossRef](#)]
12. Vek, V.; Balzano, A.; Poljanšek, I.; Humar, M.; Oven, P. Improving fungal decay resistance of less durable sapwood by impregnation with scots pine knotwood and black locust heartwood hydrophilic extractives with antifungal or antioxidant properties. *Forests* **2020**, *11*, 1024. [[CrossRef](#)]
13. Baya, M.; Soulounganga, P.; Gelhaye, E.; Gerardin, P. Fungicidal activity of beta-thujaplicin analogues. *Pest Manag. Sci.* **2001**, *57*, 833–838. [[CrossRef](#)] [[PubMed](#)]
14. Venalainen, M.; Harju, A.M.; Saranpää, P.; Kainulainen, P.; Tiitta, M.; Velling, P. The concentration of phenolics in brown-rot decay resistant and susceptible Scots pine heartwood. *Wood Sci. Technol.* **2004**, *38*, 109–118. [[CrossRef](#)]
15. Barrera-Necha, L.L.; Garduno-Pizana, C.; Garcia-Barrera, L.J. In vitro antifungal activity of essential oils and their compounds on mycelial growth of *Fusarium oxysporum* f. sp. *gladioli* (Massey) Snyder and Hansen. *Plant Pathol. J.* **2009**, *8*, 17–21. [[CrossRef](#)]
16. Wu, C.C.; Wu, C.L.; Huang, S.L.; Chang, H.T. Antifungal activity of liriodenine from *Michelia formosana* heartwood against wood-rotting fungi. *Wood Sci. Technol.* **2012**, *46*, 737–747. [[CrossRef](#)]
17. Passos, J.L.; Barbosa, L.C.A.; Demuner, A.J.; Alvarenga, E.S.; Silva, C.M.d.; Barreto, R.W. Chemical characterization of volatile compounds of *Lantana camara* L. and *L. radula* Sw. and their antifungal activity. *Molecules* **2012**, *17*, 11447–11455. [[CrossRef](#)]
18. Ogura, M.; Cordell, G.A.; Farnsworth, N.R. Anticancer sesquiterpene lactones of *Michelia compressa* (Magnoliaceae). *Phytochemistry* **1978**, *17*, 957–961. [[CrossRef](#)]
19. Chan, Y.Y.; Juang, S.H.; Huang, G.J.; Liao, Y.R.; Chen, Y.F.; Wu, C.C.; Chang, H.T.; Wu, T.S. The constituents of *Michelia compressa* var. *formosana* and their bioactivities. *Int. J. Mol. Sci.* **2014**, *15*, 10926–10935. [[CrossRef](#)]
20. Girometta, C.E.; Bernicchia, A.; Baiguera, R.M.; Bracco, F.; Buratti, S.; Cartabia, M.; Picco, A.M.; Savino, E. An Italian research culture collection of wood decay fungi. *Diversity* **2020**, *12*, 58. [[CrossRef](#)]
21. Cartabia, M.; Girometta, C.E.; Milanese, C.; Baiguera, R.M.; Buratti, S.; Branciforti, D.S.; Vadivel, D.; Girella, A.; Babbini, S.; Savino, E.; et al. Collection and characterization of wood decay fungal strains for developing pure mycelium mats. *J. Fungi* **2021**, *7*, 1008. [[CrossRef](#)]
22. Alejandro, F.B.; Molina, J.; Oltra, J.E.; Altarejos, J.; Barragán, A.; Lara, A.; Segura, M. Stereochemistry of 14-hydroxy- β -caryophyllene and related compounds. *Tetrahedron* **1995**, *51*, 3813–3822.
23. Faure, R.; Raharivelomanana, P.; Bianchini, J.P.; Cambon, A.; Azzaro, M. Two-dimensional NMR of sesquiterpenes. 8-complete assignment of ^1H and ^{13}C NMR spectra of seven sesquiterpene alcohols from *Neocallitropsis pancheri*. *Magn. Reson. Chem.* **1995**, *33*, 233–235.
24. Benovit, S.C.; Silva, L.L.; Salbego, J.; Loro, V.L.; Mallmann, C.A.; Baldisserotto, B.; Flores, E.M.; Heinzmann, B.M. Anesthetic activity and bio-guided fractionation of the essential oil of *Aloysia gratissima* (Gillies & Hook.) Tronc. in silver catfish *Rhamdia quelen*. *An. Acad. Bras. Cienc.* **2015**, *87*, 1675–1689. [[PubMed](#)]

25. Sadgrove, N.J.; Senbill, H.; Van Wyk, B.E.; Greatrex, B.W. New labdanes with antimicrobial and acaricidal activity: Terpenes of *Callitris* and *Widdringtonia* (Cupressaceae). *Antibiotics* **2020**, *9*, 173. [[CrossRef](#)]
26. Gong, X.; Su, X.; Liu, H. Diallyl trisulfide, the antifungal component of garlic essential oil and the bioactivity of its nanoemulsions formed by spontaneous emulsification. *Molecules* **2021**, *26*, 7186. [[CrossRef](#)]
27. Wu, C.L.; Chien, S.C.; Wang, S.Y.; Kuo, Y.H.; Chang, S.T. Structure-activity relationships of cadinane-type sesquiterpene derivatives against wood-decay fungi. *Holzforschung* **2005**, *59*, 620–627. [[CrossRef](#)]
28. Chang, H.T.; Lin, C.Y.; Hsu, L.S.; Chang, S.T. Thermal degradation of linalool-chemotype *Cinnamomum osmophloeum* leaf essential oil and its stabilization by microencapsulation with β -cyclodextrin. *Molecules* **2021**, *26*, 409. [[CrossRef](#)]
29. Huang, C.Y.; Yeh, T.F.; Hsu, F.L.; Lin, C.Y.; Chang, S.T.; Chang, H.T. Xanthine oxidase inhibitory activity and thermostability of cinnamaldehyde-chemotype leaf oil of *Cinnamomum osmophloeum* microencapsulated with β -cyclodextrin. *Molecules* **2018**, *23*, 1107. [[CrossRef](#)]
30. Chang, H.T.; Chang, M.L.; Chen, Y.T.; Chang, S.T.; Hsu, F.L.; Wu, C.C.; Ho, C.K. Evaluation of motor coordination and antidepressant activities of *Cinnamomum osmophloeum* ct. linalool leaf oil in rodent model. *Molecules* **2021**, *26*, 3037. [[CrossRef](#)]
31. Zheljajzkov, V.D.; Cantrell, C.L.; Semerdjieva, I.; Radoukova, T.; Stoyanova, A.; Maneva, V.; Kačániová, M.; Astatkie, T.; Borisova, D.; Dincheva, I.; et al. Essential oil composition and bioactivity of two *Juniper* species from Bulgaria and Slovakia. *Molecules* **2021**, *26*, 3659. [[CrossRef](#)]
32. Chang, H.T.; Cheng, Y.H.; Wu, C.L.; Chang, S.T.; Chang, T.T.; Su, Y.C. Antifungal activity of essential oil and its constituents from *Calocedrus macrolepis* var. *formosana* Florin leaf against plant pathogenic fungi. *Bioresour. Technol.* **2008**, *99*, 6266–6270. [[PubMed](#)]
33. Merad, N.; Andreu, V.; Chaib, S.; de Carvalho Augusto, R.; Duval, D.; Bertrand, C.; Boumghar, Y.; Pichette, A.; Djabou, N. Essential oils from two Apiaceae species as potential agents in organic crops protection. *Antibiotics* **2021**, *10*, 636. [[CrossRef](#)] [[PubMed](#)]
34. Dymek, A.; Widelski, J.; Wojtanowski, K.K.; Vivcharenko, V.; Przekora, A.; Mroczek, T. Fractionation of Lycopodiaceae alkaloids and evaluation of their anticholinesterase and cytotoxic activities. *Molecules* **2021**, *26*, 6379. [[CrossRef](#)] [[PubMed](#)]
35. Santonocito, D.; Granata, G.; Geraci, C.; Panico, A.; Siciliano, E.A.; Raciti, G.; Puglia, C. Carob seeds: Food waste or source of bioactive compounds? *Pharmaceutics* **2020**, *12*, 1090. [[CrossRef](#)]
36. Chen, G.R.; Chang, M.L.; Chang, S.T.; Ho, Y.T.; Chang, H.T. Cytotoxicity and apoptosis induction of 6,7-dehydroroyleanone from *Taiwania cryptomerioides* bark essential oil in hepatocellular carcinoma cells. *Pharmaceutics* **2022**, *14*, 351. [[CrossRef](#)]
37. Li, W.H.; Chang, S.T.; Chang, S.C.; Chang, H.T. Isolation of antibacterial diterpenoids from *Cryptomeria japonica* bark. *Nat. Prod. Res.* **2008**, *22*, 1085–1093. [[CrossRef](#)]
38. Dziwornu, G.A.; Caira, M.R.; Mare, J.A.d.l.; Edkins, A.L.; Bolton, J.J.; Beukes, D.R.; Sunassee, S.N. Isolation, characterization and antiproliferative activity of new metabolites from the South African endemic red algal species *Laurencia alfredensis*. *Molecules* **2017**, *22*, 513. [[CrossRef](#)]
39. Silvestre, G.F.G.; Lucena, R.P.; Oliveira, G.D.; Pereira, H.N.; Dias, J.A.B.; Souza, I.A.; Alves, H.S. Anti-tumor and anti-inflammatory activity in vivo of *Apodanthera congestiflora* Cogn. (Cucurbitaceae). *Pharmaceutics* **2021**, *13*, 743. [[CrossRef](#)]
40. Huang, C.Y.; Liu, I.H.; Huang, X.Z.; Chen, H.J.; Chang, S.T.; Chang, M.L.; Ho, Y.T.; Chang, H.T. Antimelanogenesis effects of leaf extract and phytochemicals from ceylon olive (*Elaeocarpus serratus*) in zebrafish model. *Pharmaceutics* **2021**, *13*, 1059. [[CrossRef](#)]