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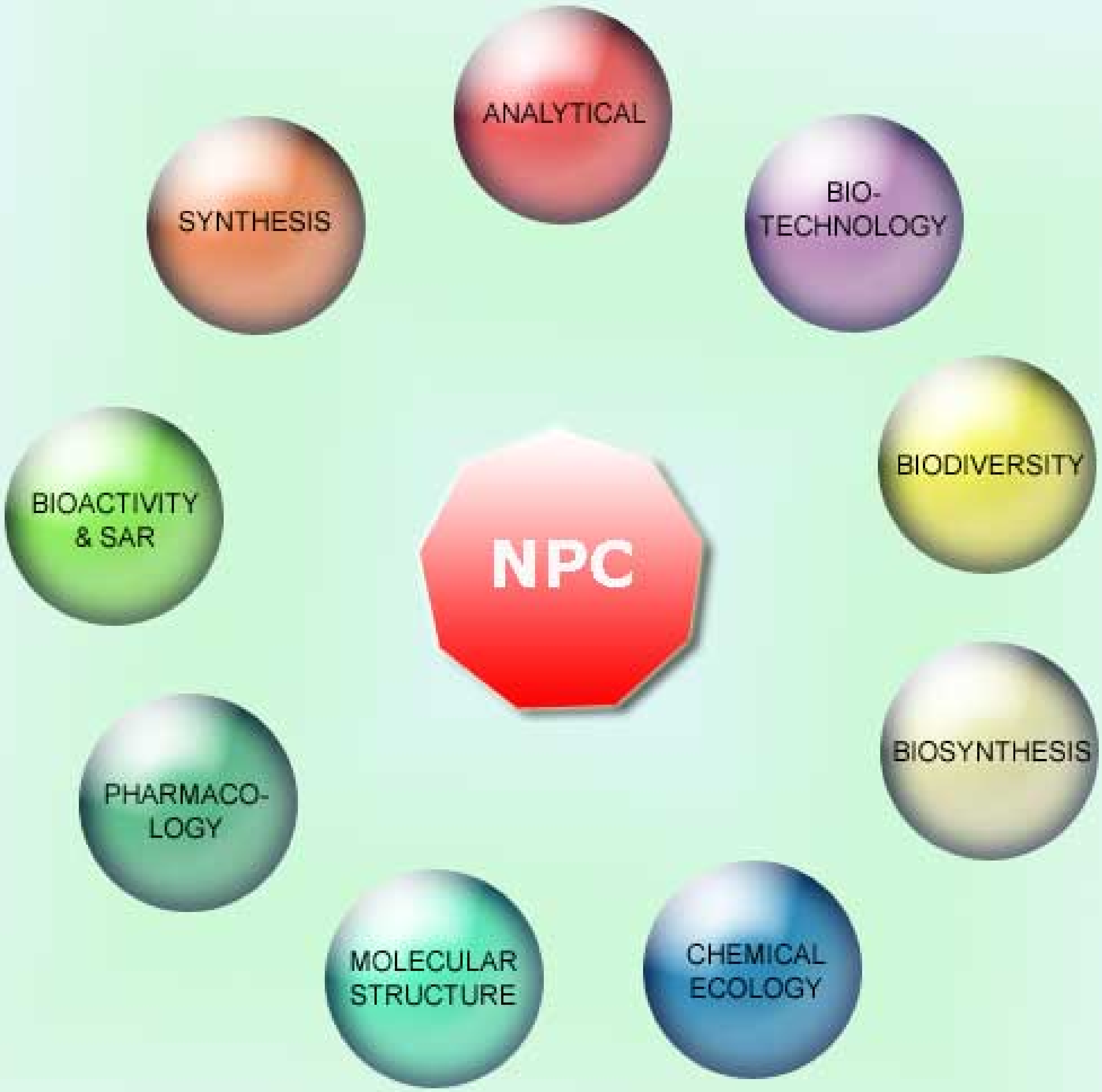

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Composition and Anti-Wood-Decay Fungal Activities of the Leaf Essential oil of *Machilus philippinensis* from Taiwan

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The hydrodistilled leaf essential oil of *Machilus philippinensis* was analyzed to determine its composition and yield. Seventy compounds were identified, the main ones being β-caryophyllene (13.6%), α-pinene (12.0%), α-cadinol (7.4%), *cis*-ocimene (7.0%), spathulenol (5.6%), (*E*)-nerolidol (5.3%), *tau*-cadinol (4.8%) and β-pinene (4.5%). Monoterpene hydrocarbons (36.1%) and oxygenated sesquiterpenes (33.0%) were the predominant groups of compounds. The leaf oil exhibited excellent anti-wood-decay fungal activities.

Keywords: *Machilus philippinensis*, Lauraceae, essential oil, anti-wood-decay fungal activities, α-cadinol, *tau*-cadinol.

Machilus philippinensis Merr. (Lauraceae) is an evergreen tree, mainly distributed in the Philippines and Taiwan. In Taiwan, it is often found in the southern mountainous areas with elevations ranging from 500 to 1600 m. For instance, the trees have been found from Fengchihu in Chiayi County to Jingshuiying in Pingtung County [1]. Only two reports on the chemical composition of the species have been found, both of which stated that the leaf extract could serve as α-glucosidase inhibitors [2,3]. There was, however, no published information on its essential oil composition. Therefore, we used hydrodistillation to extract the leaf oil and analyzed it using GC/FID and GC/MS.

Wood has become a preferred construction and decoration material in recent days. For such uses, durability is an important issue. The use of heavy metal-containing wood preservatives and broad spectrum biocides for wood protection are being limited because of their toxicity to the environment and mammals [4]. Since certain wood preservatives, such as chromated copper arsenate (CCA), have been either 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 [5]. As a consequence, the second part of the study examined the anti-wood-decay fungal activities of the essential oils. The purpose of this study was to establish a chemical basis for the effective multipurpose utilization of the species.

Hydrodistillation of *M. philippinensis* leaves gave a yellow-colored oil with a yield of 1.38 ± 0.05 mL/100 g, based on the dry weight of leaves. The identified constituents are presented in Table 1, where all compounds are listed in order of their elution from the DB-5 column. Seventy components were identified, representing 100% of the oil. Among the groups, monoterpene hydrocarbons predominated (36.1%), followed by oxygenated sesquiterpenes (33.0%), sesquiterpene hydrocarbons (24.1%), oxygenated monoterpenes (3.6%), and non-terpenoids (3.2%). Among the monoterpene hydrocarbons, α-pinene (12.0%), *cis*-ocimene (7.0%) and β-pinene (4.5%) were the major compounds. Of the oxygenated sesquiterpenes, α-cadinol (7.4%), spathulenol (5.6%), (*E*)-nerolidol (5.3%) and *tau*-cadinol (4.8%) were the main components. Among the sesquiterpene hydrocarbons, β-caryophyllene (13.6%), α-caryophyllene (2.5%) and δ-cadinene (2.3%) were the principal constituents.

The essential oil of *M. philippinensis* was tested against two white rot fungi (*Trametes versicolor, Phanerochaete chrysosporium*) and two brown rot fungi (*Phaeolus schweinitzii, Laetiporus sulphureus*). The anti-wood-decay fungal indices presented in Table 2 are a clear demonstration of the excellent anti-wood-decay

Table 1: Chemical composition of the leaf oil *M. philippinensis*

Compound ID	RI [a]	Conc.(%)	Identification [b]
α-Thujene	930	0.2	KI, MS, ST
α-Pinene	939	12.0	KI, MS, ST
Camphene	954	2.5	KI, MS, ST
β-Pinene	979	4.5	KI, MS, ST
Myrcene	991	1.1	KI, MS, ST
α-Phellandnene	1003	1.0	KI, MS, ST
p-Cymene	1025	2.7	KI, MS, ST
Limonene	1029	2.4	KI, MS, ST
1,8-Cineole	1031	0.1	KI, MS, ST
cis-Ocimene	1037	7.0	KI, MS, ST
trans-Ocimene	1050	2.5	KI, MS, ST
Terpinolene	1089	0.2	KI, MS, ST
cis-Thujone	1102	0.2	KI, MS
endo-Fenchol	1117	0.1	KI, MS
Borneol	1169	0.1	KI, MS, ST
n-Nonanol	1169	0.1	KI, MS, ST
Terpinene-4-o1	1177	0.1	KI, MS, ST
α-Terpineol	1189	1.3	KI, MS, ST
n-Decanal	1202	0.4	KI, MS, ST
Thymol methyl ether	1235	0.1	KI, MS
trans-Piperitone epoxide	1256	0.1	KI, MS
Isobornyl acetate	1286	0.3	KI, MS
Bornyl acetate	1289	1.1	KI, MS
Carvacrol	1300	0.2	KI, MS, ST
Undecanal	1307	0.1	KI, MS, ST
Veloutone	1311	0.1	KI, MS
Citronellyl acetate	1353	0.1	KI, MS
10-Undecen-1-ol	1363	0.4	KI, MS
n-Undecen-ol	1370	0.3	KI, MS
α-Copaene	1377	1.6	KI, MS, ST
β-Elemene	1391	0.1	KI, MS, ST
z-Trimenal	1398	0.9	KI, MS
Dodecanal	1409	0.9	KI, MS, ST
β-Caryophyllene	1419	13.6	KI, MS, ST
β-Gurjunene	1434	0.2	KI, MS, ST
Aromadendrene	1441	0.2	KI, MS, ST
cis-Muurola-3.5-diene	1450	0.1	KI, MS
α-Caryophyllene	1455	2.5	KI, MS, ST
γ-Muurolene	1480	0.1	KI, MS
Germacnene D	1485	0.7	KI, MS, ST
β-Selinene	1490	0.3	KI, MS, ST
α-Selinene	1498	0.4	KI, MS, ST
α-Muurolene	1500	0.4	KI, MS
γ-Cadinene	1514	0.5	KI, MS
δ-Cadinene	1523	2.3	KI, MS, ST
trans-Cadina-1(2),4-diene	1535	0.1	KI, MS
α-Cadinene	1539	0.3	KI, MS
Selina-3,7(11)-diene	1547	0.1	KI, MS
Elemol	1550	0.3	KI, MS, ST
Germacrene B	1561	0.2	KI, MS, ST
(*E*)-Nerolidol	1563	5.3	KI, MS, ST
ledol	1569	0.3	KI, MS, ST
Caryophyllene alcohol	1572	1.2	KI, MS, ST
Spathulenol	1578	5.6	KI, MS, ST
Viridiflorol	1593	0.3	KI, MS
Widdrol	1599	0.6	KI, MS
Guaiol	1601	0.4	KI, MS
Humulene epoxide II	1608	0.4	KI, MS
1,10-Di-*epi*-cubenol	1619	0.3	KI, MS
epi-Cedrol	1619	0.7	KI, MS
10-*epi*-γ-Eudesmol	1624	0.3	KI, MS
1-*epi*-Cubenol	1629	1.2	KI, MS
γ-Eudesmol	1632	0.9	KI, MS
tau-Cadinol	1640	4.8	KI, MS, ST
tau-Muurolol	1642	0.5	KI, MS
δ-Cadinol	1646	0.7	KI, MS
α-Eudesmol	1654	1.9	KI, MS
α-Cadinol	1654	7.4	KI, MS, ST
Bulnesol	1676	0.1	KI, MS
(2*E*, 6*E*)-farnesol	1725	0.2	KI, MS
Monoterpene hydrocarbons (%)		36.1	
Oxygenated monoterpenes (%)		3.6	
Sesquiterpene hydrocarbons (%)		24.1	
Oxygenated sesquiterpenes (%)		33.0	
Others (%)		3.2	
Oil Yield (mL/100 g)		1.38 ± 0.05	

Table 1. (*Contd.*)

[a] Retention index on a DB-5 column with reference to *n*-alkanes [6].
[b] MS, NIST and Wiley library spectra and the literature; RI, Retention index; ST, authentic standard compounds.

fungal property of the oil. The growth of *Trametes versicolor*, *Phaneochaete chrysosporium*, *Phaeolus schweinitzii* and *Laetiporus sulphureus* was completely inhibited at concentrations of 100, 100, 100, 50 µg/mL, respectively.

Comparison of the anti-wood-decay fungal activities of the wood essential oil from *Chamaecyparis formosensis* [7] and the leaf essential oil from *Cinnamomum osmophloeum* [8] with that of the leaf essential oil of *M. philippinensis* showed that the last was superior. These results verified that *M. philippinensis* leaf oil has excellent anti-wood-decay fungal activities.

At a concentration of 50 µg/mL, the anti-wood-decay fungal indices of the eight main compounds of *M. philippinensis* leaf oil against the four wood-decay fungi are presented in Fig. 1. The brown-rot fungi were more sensitive to the compounds than the white-rot fungi. In addition, sesquiterpenes were more effective against the four assayed wood-decay fungi than the monoterpenes. The order of the anti-wood-decay fungal indices of the eight compounds for *L. sulphureus* and *P. schweinitzii* were α-cadinol > *tau*-cadinol > spathulenol > β-caryophyllene > α-pinene > (*E*)-nerolidol > β-pinene > *cis*-ocimene. Among these, α-cadinol and *tau*-cadinol exhibited a higher anti-wood-decay fungal activity. Kondo and Imamura [9] pointed out that the methanol extract of hinoki (*Chamaecyparis obtusa*) containing α-cadinol, *tau*-cadinol, *tau*-muurolol and γ-cadinene exhibited excellent inhibitory effects against wood decaying fungi. Chang *et al*. [10] demonstrated that Taiwania wood (*Taiwania cryptomerioides*)

Table 2: Anti-wood-decay fungal indices of leaf essential oil from *M. philippinensis*.

Dosage (ug/mL)	Anti-wood-decay fungal index (%)			
	Trametes versicolor	*Phaneochaete chrysosporium*	*Phaeolus schweinitzii*	*Laetiporus sulphureus*
50	75 ± 6.6	83 ± 3.3	93 ± 3.3	100 ± 0
100	100 ± 0	100 ± 0	100 ± 0	100 ± 0
200	100 ± 0	100 ± 0	100 ± 0	100 ± 0

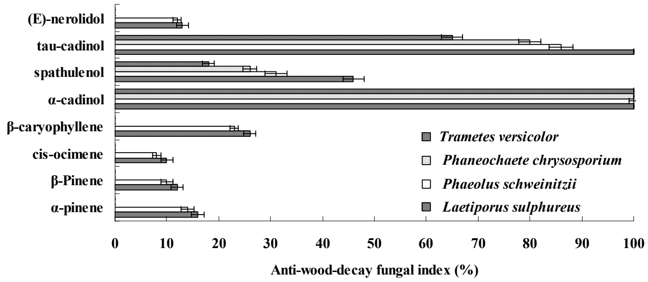

Figure 1: Anti-wood-decay fungal indices of the eight main compounds (50 ug/mL) of the leaf essential oil of *M. philippinensis*.

containing α-cadinol, *tau*-cadinol and *tau*-muurolol also exhibited excellent inhibitory effects against wood decaying fungi. In particular, α-cadinol had the best inhibitory efficacy. Thus, the excellent anti-wood-decay fungal activities exhibited by the *M. philippinensis* leaf oil could well be due to the presence of compounds such as α-cadinol and *tau*-cadinol .

Experimental

Plant materials: Fresh leaves of *M. philippinensis* were collected in June 2008 from the Dahanshan at an elevation of 1200 m in southern Taiwan (N 22º 24′ 15″, E 120º 45′ 01″, Pingtung County). The samples were compared with specimen no. ou3638 from the Herbarium of the National Chung-Hsing University and positively identified by Prof. Yen-Hsueh Tseng of NCHU. The voucher specimen (CLH-003) has been deposited in the NCHU herbarium. Leaves of the species were collected for subsequent extraction and analysis.

Isolation of leaf essential oil: The method of Ho *et al.* [11,12] was adopted. Leaves of *M. philippinensis* (1 Kg) were placed in a round-bottom flask and hydrodistilled for 8 h with 3 L of distilled water. The essential oil removed was dried with anhydrous sodium sulfate. The oil yield and all test data are the average of triplicate analyses.

Essential oil analysis: The methods of Su *et al*. [13] and Ho *et al.* [14,15] were adopted. A Hewlett-Packard HP 6890 gas chromatograph equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25μm 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 of sample was injected. Identification of the oil components was based on their retention indices and MS, obtained from GC/MS analysis on a Hewlett-Packard HP 6890/HP5973 equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 μm film thickness, J&W Scientific). The GC analysis parameters listed above and the MS were obtained (full scan mode: scan time: 0.3 s, mass range was m/z 30-500) in the EI mode at 70 eV. All data were the average of triplicate analyses.

Component identification: Identification of the leaf essential oil constituents was based on comparisons of retention index (RI) [16], retention times (RT), and MS with those obtained from authentic standards and/or the NIST and Wiley libraries spectra, and literature [6,17].

Anti-wood-decay fungal assays: The method of Su *et al.* [18] 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 *Laetiporus sulphureus* (B. ex Fr.) Bond. (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 (50, 100, and 200 μg/mL) were added to sterilized potato dextrose agar (PDA). The test plates were incubated at 27ºC. When the mycelium of the 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).

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Natural Product Communications
2010

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Contents

Continued inside backcover