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Composition and Antifungal Activities of the Leaf Essential Oil of *Neolitsea parvigemma* from Taiwan

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The hydrodistillated leaf essential oil of *Neolitsea parvigemma* was analyzed to determine its composition and yield. Sixty-two compounds were identified, the main components being β-caryophyllene (14.2%), β-eudesmol (12.9%), α-cadinol (10.2%) and τ-cadinol (8.8%). Oxygenated sesquiterpenes (48.9%) and sesquiterpene hydrocarbons (48.8%) were the predominant groups of compounds. The antifungal indexes of the leaf oil against the 7 fungi, *Aspergillus clavatus, A. niger*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Myrothecium verrucaria*, *Penicillium citrinum* and *Trichoderma viride*, were 100.0, 72.3, 100.0, 100.0, 100.0, 75.8 and 88.6% at a 1 mg/mL concentration, respectively. The oil also exhibited anti-wood-decay-fungi activity against *Trametes versicolor*, *Phaneochaete chrysosporium*, *Phaeolus schweintizii*, and *Lenzites sulphureu* with MIC values of 50, 50, 25 and 25 μg/mL, respectively. For the antifungal and anti-wood-decay fungal activities of the oil, the active source compounds were determined to be α-cadinol, β-eudesmol and τ-cadinol.

Keywords: *Neolitsea parvigemma*, Lauraceae, essential oil, antifungal activity, anti-wood-decay fungal activity, β-caryophyllene, β-eudesmol.

Neolitsea parvigemma Kan and Sas (Lauraceae) is a small tree growing in the central and southern mountainous regions in Taiwan [1]. Previous reports suggested that the extractive exhibited bioactivities, such as antiplatelet aggregation, anti-inflammatory and cytotoxicity [2-6]. There are no literature reports on the chemical composition and biological activities of the essential oil from this species. Therefore, we used hydrodistillation to collect the leaf oil, which was analyzed by GC/FID and GC/MS.

The climate of Taiwan is warm and humid, and thus conducive to the growth of mildew. Mildew growth causes problems in the preservation of cultural items as well as induces allergies, asthma, bronchitis, onychomycosis, cerebral infections, pneumonia, peritonitis, and immune-deficiency syndrome [7]. We also applied the essential oil to seven strains of mold fungi and four strains of wood decay fungi to examine the interdiction efficacies. The second part of the study examined the antifungal activities of the leaf oil. The purpose of this study was to establish a chemical basis for the effective multipurpose utilization of the species.

Hydrodistillation of *N. parvigemma* leaves gave a yellow-colored oil with a yield of 1.08 ± 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. Sixty-two components were identified, representing 100% of the oil. Among the groups, oxygenated sesquiterpenes predominated (48.9%), followed by, sesquiterpene hydrocarbons (48.8%), non-terpenoids (1.5%), monoterpene hydrocarbons (0.5%) and oxygenated monoterpenes (0.3%). Among the oxygenated sesquiterpenes, β-eudesmol (12.9%), α-cadinol (10.2%) and τ-cadinol (8.8%) were the major compounds. Of the sesquiterpene hydrocarbons, β-caryophyllene (14.2%) was the main components.

Although the leaf oil constituents of *N. parvigemma* was primarily sesquiterpenoids, like those of *N. pallens* [8], *N. australiensis*, *N. brassii*, *N. dealbata* [9,10], *N. sericea* [11], *N. foliosa* var. *caesia* [12] and *N. fischeri* [13], their main components differed. Further comparison with the leaf oil of *N. oblongifolia* and *N. umbrosa* [14] were predominantly monoterpenoids and differed from the leaf oil of *N. parvigemma*.

The antifungal indexes of the leaf oil against the 7 fungi, *Aspergillus clavatus* (*A. c.*), *A. niger* (*A. n.*), *Chaetomium globosum* (*Ch. g.*), *Cladosporium cladosporioides* (*Cl. c.*), *Myrothecium verrucaria* (*M. v.*), *Penicillium citrinum* (*P. c.*) and *Trichoderma viride* (*T. v.*), were 100.0, 72.3, 100.0, 100.0, 100.0, 75.8 and 88.6% at a 1 mg/mL concentration, respectively (Table 2). These results showed that leaf oil

Table 1: Chemical composition of the leaf oil *N. parvigemma.*

Compound ID	RI [a]	Conc. (%)	Identification [b]
α-Pinene	939	t [c]	MS, KI, ST
Sabinene	975	t	MS, KI, ST
β-Pinene	979	0.4	MS, KI, ST
(*E*)-β-Ocimene	1050	0.1	MS, KI, ST
Linalool	1097	0.3	MS, KI, ST
Terpinen-4-ol	1177	t	MS, KI, ST
(*Z*)-3-Hexenyl Butyrate	1184	0.1	MS, KI
Hexanoic acid, butyl ester	1186	t	MS, KI, ST
α-Terpineol	1189	t	MS, KI, ST
2,3-Dimethyl benzofuran	1222	0.1	MS, KI
2-Prenyl cyclopentanone	1227	t	MS, KI
(*3Z*)-Hexenyl 2-methyl butanoate	1232	0.3	MS, KI
(*3Z*)-Hexenyl 3-methyl butanoate	1235	t	MS, KI
(*2E*)-Decenal	1263	t	MS, KI, ST
(*3Z*)-Hexenyl valerate	1281	t	MS, KI
(*3Z*)-Hexenyl tiglate	1321	t	MS, KI
δ-Elemene	1338	0.2	MS, KI
α-Cubebene	1351	0.1	MS, KI
α-Ylangene	1375	t	MS, KI
α-Copaene	1377	0.1	MS, KI, ST
(*3Z*)-Hexenyl hexanoate	1384	0.8	MS, KI
β-Elemene	1391	3.3	MS, KI
iso-Caryophyllene	1409	0.1	MS, KI
α-Cedrene	1412	0.1	MS, KI
β-Caryophyllene	1419	14.2	MS, KI, ST
β-Gurjunene	1434	0.1	MS, KI
β-Copaene	1432	0.1	MS, KI
α-Gurjunene	1440	0.2	MS, KI
Aromadendrene	1441	0.1	MS, KI, ST
trans-Muurola-3,5-diene	1454	0.2	MS, KI
(*Z*)-β-Farnesene	1457	3.5	MS, KI, ST
allo-Aromadendrene	1460	2.9	MS, KI, ST
9-epi-(*E*)-Caryophyllene	1466	0.1	MS, KI
trans-Cadina-1(6),4-diene	1477	0.3	MS, KI
γ-Gurjunene	1477	0.4	MS, KI, ST
γ-Muurolene	1480	4.3	MS, KI
Valencene	1496	0.5	MS, KI
Varidiflorene	1497	0.5	MS, KI
Bicyclogermacrene	1500	4.5	MS, KI
α-Muurolene	1500	4.0	MS, KI
Epizonarene	1502	0.4	MS, KI
α-Bulesene	1510	1.0	MS, KI
γ-Cadinene	1514	0.4	MS, KI
δ-Cadinene	1523	4.7	MS, KI
Zonarene	1530	0.2	MS, KI
trans-Cadina-1(2),4-diene	1535	0.4	MS, KI
α-Cadinene	1539	0.1	MS, KI
α-Calacorene	1546	0.1	MS, KI
Selina-3,7(11)-diene	1547	0.1	MS, KI
Elemol	1550	0.8	MS, KI
Germacrene B	1561	0.6	MS, KI
trans-Nerolidol	1563	1.7	MS, KI, ST
Spathulenol	1578	1.8	MS, KI
Caryophyllene oxide	1583	4.3	MS, KI, ST
Globulol	1585	1.2	MS, KI, ST
Viridiflorol	1593	1.2	MS, KI
1-*epi*-Cubenol	1629	1.7	MS, KI
γ-Eudesmol	1632	4.3	MS, KI
τ-Cadinol	1640	8.8	MS, KI, ST
β-Eudesmol	1651	12.9	MS, KI, ST
α-Cadinol	1654	10.2	MS, KI, ST
14-hydroxy-9-*epi*-(*E*)-Caryophyllene	1669	1.3	MS, KI
Compound identified			
Monoterpene hydrocarbon		0.5	
Monoterpene oxygen		0.3	
Sesquiterpene hydrocarbon		48.8	
Sesquiterpene oxygen		48.9	
Others		1.5	
Oil yield (mL/100g)		1.08 ± 0.05	

[a] Retention index on a DB-5 column with reference to *n*-alkanes [15].
[b] MS, NIST and Wiley library spectra and the literature; RI, Retention index; ST, authentic standard compounds. [c].trace < 0.1%

was totally inhibitory to mycelial growth of *A. clavatus, Cl. cladosporioides*, *Ch. globosum*, and *M. verrucaria* among the fungi tested. Comparing with the antifungal activities of the essential oils from *Eucalyptus urophylla, E. grandis, E. camaldulensis, E. citriodora* [7], *Litsea cubeba* [16] and *L. coreana* [17], the leaf oil of *N. parvigemma* was superior (Table 2). The results verified that *N. parvigemma* leaf oil has excellent antifungal activities.

Table 2: Comparison of the antifungal index (%) of the leaf oils (1 mg/mL) of *N. parvigemma* and theose of *Eucalyptus urophylla*, *E. grandis*, *E. camaldulensis*, *E. citriodora*, *Litsea cubeba* and *L. coreana* against the fungi.

Essential oil	Fungi [a]							Reference
	A. c.	*A. n.*	*Cl. c.*	*Ch. g.*	*M. v.*	*P. c.*	*T. v.*	
Neolitsea parvigemma	100.0	72.3	100.0	100.0	100.0	75.8	88.6	This study
Eucalyptus urophylla	2.4	0.0	12.6	100.0	30.7	12.3	0.0	[7]
E. grandis	56.5	16.1	38.2	100.0	54.8	8.6	0.0	[7]
E. camaldulensis	3.9	0.0	22.2	0.0	26.5	14.3	0.0	[7]
E. citriodora	28.2	0.0	38.4	100.0	100.0	26.4	0.0	[7]
Litsea cubeba	100.0	56.2	100.0	95.3	100.0	58.3	68.6	[16]
L. coreana	89.0	49.0	95.7	78.3	86.0	43.3	52.7	[17]

Note: [a] *A. c.*: *Aspergillus clavatus*; *A. n.*: *A. niger*; *Cl. c.*: *Cladosporium cladosporioides*; *Ch. g.*: *Chaetomium globosum*; *M. v.*: *Myrothecium verrucaria*; *P. c.*: *Penicillium citrinum*; *T. v.*: *Trichoderma viride*

Table 3: IC_{50} values (μg/mL) of the five main constituents of *N. parvigemma* leaf oil against 7 fungi strain.

Constituents [a]	Fungi [b]						
	A. c.	*A. n.*	*Cl. c.*	*Ch. g.*	*M. v.*	*P. c.*	*T. v.*
Neolitsea parvigemma (Crude leaf oil)	56.8	92.3	36.8	80.5	68.8	138.2	100.8
β-caryophyllene	92.3	95.2	48.1	100.6	90.8	190.3	160.8
α-cadinol	21.0	45.6	13.1	36.8	18.1	55.8	48.6
τ-cadinol	28.3	66.8	20.8	52.3	43.5	66.9	68.6
β-eudesmol	23.0	53.6	16.3	43.6	38.6	59.8	51.3
Nystatine [c]	<12.5	<12.5	<12.5	<12.5	<12.5	<12.5	<12.5

[a] 1. β-caryophyllene (≥ 98.5%), 2. α-cadinol (100%), 3. τ-cadinol (100%), 4. β-eudesmol (≥ 98%). Compound 1 was purchased from the Fluka Co. (Milwaukee, USA), and Compound 4 was purchased from the Wako Co. (Tokyo, Japan), where as the Compound 2 and 3 were from isolate of the Ho et al. [19] study on *Machilus philippinenesis* essential oil.
[b] *A. c.*: *Aspergillus clavatus*; *A. n.*: *A. niger*; *Cl. c.*: *Cladosporium cladosporioides*; *Ch. g.*: *Chaetomium globosum*; *M. v.*: *Myrothecium verrucaria*; *P. c.*: *Penicillium citrinum*; *T. v.*: *Trichoderma viride*
[c] Nystatine is an antifungal drug and used as a positive control.

However, in order to ascertain the source compounds of antifungal activities from *N. parvigemma*, the main components were individually tested for their antifungal activities. The sesquiterpenoids, β-caryophyllene, α-cadinol, β-eudesmol and τ-cadinol, exhibited better activities among the leaf oil constituents, in particular, α-cadinol, β-eudesmol and τ-cadinol. α-Cadinol, β-eudesmol and τ-cadinol exhibited strong activity against *A. clavatus*, *Cl. cladosporioides, Ch. globosum* and *M. verrucaria* with the highest antifungal indexes ranging from 82 to 100% at 100 μg/mL. IC_{50} values of α-cadinol, against *A. clavatus*, *Cl. cladosporioides, Ch. globosum* and *M. verrucaria* were 21.0, 13.1, 36.8, and 18.1 μg/mL, for β-eudesmol, 23.0, 16.3, 43.6, and 38.6 μg/mL, and for τ-cadinol, 28.3, 20.8, 52.3 and 43.5 μg/mL (Table 3). These results indicated that the active source compounds were α-cadinol, β-eudesmol and τ-cadinol, which supports previous work showing high antimicrobial activity of these compounds [18].

The essential oil of *N. parvigemma* was tested against 2 white rot fungi (*Trametes versicolor, Phanerochaete chrysosporium*) and 2 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 50, 50, 25 and

Table 4: Anti-wood-decay fungal indices of leaf essential oil from *N. parvigemma*.

Dosage (μg/mL)	Anti-wood-decay fungal index (%)			
	Trametes versicolor	*Phaneochaete chrysosporium*	*Phaeolus schweintizii*	*Lenzites sulphureu*
Essential oil				
12.5	48 ± 3.3	36± 3.3	52 ± 6.6	68 ± 3.3
25	82 ± 6.6	68 ± 6.6	100 ± 3.3	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
DDAC				
12.5	100 ± 0	100 ± 0	100 ± 0	100 ± 0

Note: DDAC (didecyl dimethyl ammonium chloride) is a wood preservative for wood decay fungi and used as a positive control.

Table 5: Comparison of the MIC values (μg/mL) of the leaf oils of *N. parvigemma* and those of *Machilus philippinensis*, *M. pseudolongifolia*, *M. kusanoi* and *L. coreana* against the wood-decay fungi.

Essential oil	Fungi [a]				Reference
	T. v.	*Phane. c.*	*Phaeo. s.*	*L. s.*	
Neolitsea parvigemma	50	50	25	25	This study
Machilus philippinensis	100	100	100	50	[19]
M. pseudolongifolia	75	75	75	25	[20]
M. kusanoi	75	75	75	25	[21]
Litsea coreana	75	75	50	25	[17]

Note: [a] *T. v.*: *Trametes versicolor*; *Phane. c.*: *Phaneochaete chrysosporium*; *Phaeo. s*: *Phaeolus schweintizii*; *L. s.*: *Lenzites sulphureu*

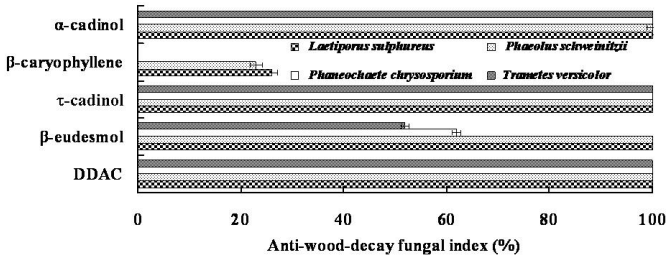

Figure 1: Anti-wood-decay fungal indices of the four main compounds (50 μg/mL) of the leaf essential oil of *N. parvigemma*
Note: DDAC (didecyl dimethyl ammonium chloride) is a wood preservative for wood decay fungi and used as a positive control.

25 μg/mL, respectively (Table 4). Comparing the anti-wood-decay fungal activities of the essential oils from *Machilus philippinensis* [19], *M. pseudolongifolia* [20], *M. kusanoi* [21] and *L. coreana* [17] the leaf oil of *N. parvigemma* was superior (Table 5). The results verified that *N. parvigemma* leaf oil has excellent anti-wood-decay fungal activities.

Furthermore, in order to ascertain the source compounds of the *N. parvigemma* essential oil, we also tested the anti-wood-decay fungal activities of its major component compounds (Figure 1). The results indicated that the sources of activities were also α-cadinol, β-eudesmol and τ-cadinol. At a 50 μg/mL concentration, α-cadinol and τ-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 [22] and Chang *et al*. [23]. Thus, the excellent wood-decay-fungi inhibitive activities exhibited by the *N. parvigemma* leaf oil could well be contributed by the presence of compounds such as α-cadinol, β-eudesmol and τ-cadinol etc.

Experimental

Plant materials: Fresh leaves of *N. parvigemma* were collected in July 2009 from the Dahanshan at an elevation of 1800 m in southern Taiwan (N 22º 26′ 38″, E 120º 42′ 36″, Pingtung County). The samples were compared with specimen no. ou 6889 from the Herbarium of National Chung-Hsing University and positively identified by Prof. Yen-Hsueh Tseng of NCHU. The voucher specimen (CLH-011) has been deposited in the NCHU herbarium. Leaves of the species were collected for subsequent extraction and analysis.

Isolation of the leaf essential oil: Leaves of *N. parvigemma* (1Kg) 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.

GC/FID 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. Diluted samples (1.0 μL, 1/100, v/v, in ethyl acetate) were injected manually in the split mode. All data were the average of triplicate analyses.

GC/MS analysis: GC/MS analyses of the oil was performed by split injection of 1.0 μL, of the oil on a Hewlett-Packard HP 6890 gas chromatograph fitted with DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 μm film thickness, J&W Scientific) coupled with a model 5973 mass detector. GC/MS operation conditions: injector temperature 270ºC; oven temperature program as follows: 50ºC for 2 min, rising to 250ºC at 5ºC/min; carrier gas: He with a flow rate of 1 mL/min, split ratio: 1:10. Mass spectra: Electron Impact (El^+) mode 70 eV with a mass range of 30 to 450 *m/z*, ion source temperature 280ºC. 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) [15], retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra, and literature [15].

Antifungal assays: The mold fungi and wood decay fungi were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute, Hsinchu City, Taiwan. For the mold fungi strains, references of ASTM G21, JIS Z 2911 and AATCC test method 30 were consulted and 7 strains including *A. clavatus* (ATCC 1007), *A. niger* (ATCC 6275), *Ch.*

globosum (ATCC 6205), *Cl. cladosporioides* (ATCC 13276), *M. verrucaria* (ATCC 9095), *P. citrinum* (ATCC 9849), *T. viride* (ATCC8678) were tested. For the wood decay fungi were *T. versicolor* (BCRC 35253), *Phane. chrysosporium* (BCRC 36200), *Phaeo. schweinitzii* (Fries) (BCRC 35365) and *L. sulphureus* (BCRC 35305). Antifungal assays were and its main constituents were dissolved in 150 µL of ethanol, respectively, and then added into 15 mL PDA to obtain the carried out in triplicate and the data were averaged. Leaf oil different final concentrations. The test plates were incubated at 27ºC. When the mycelium of fungi reached the edge of the control plate, the antifungal index was calculated as follows: Anti-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). IC_{50} (concentration that produces a 50% inhibitory effect) values of constituents were graphically obtained from the dose-response curves based on measurement at five different concentrations.

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