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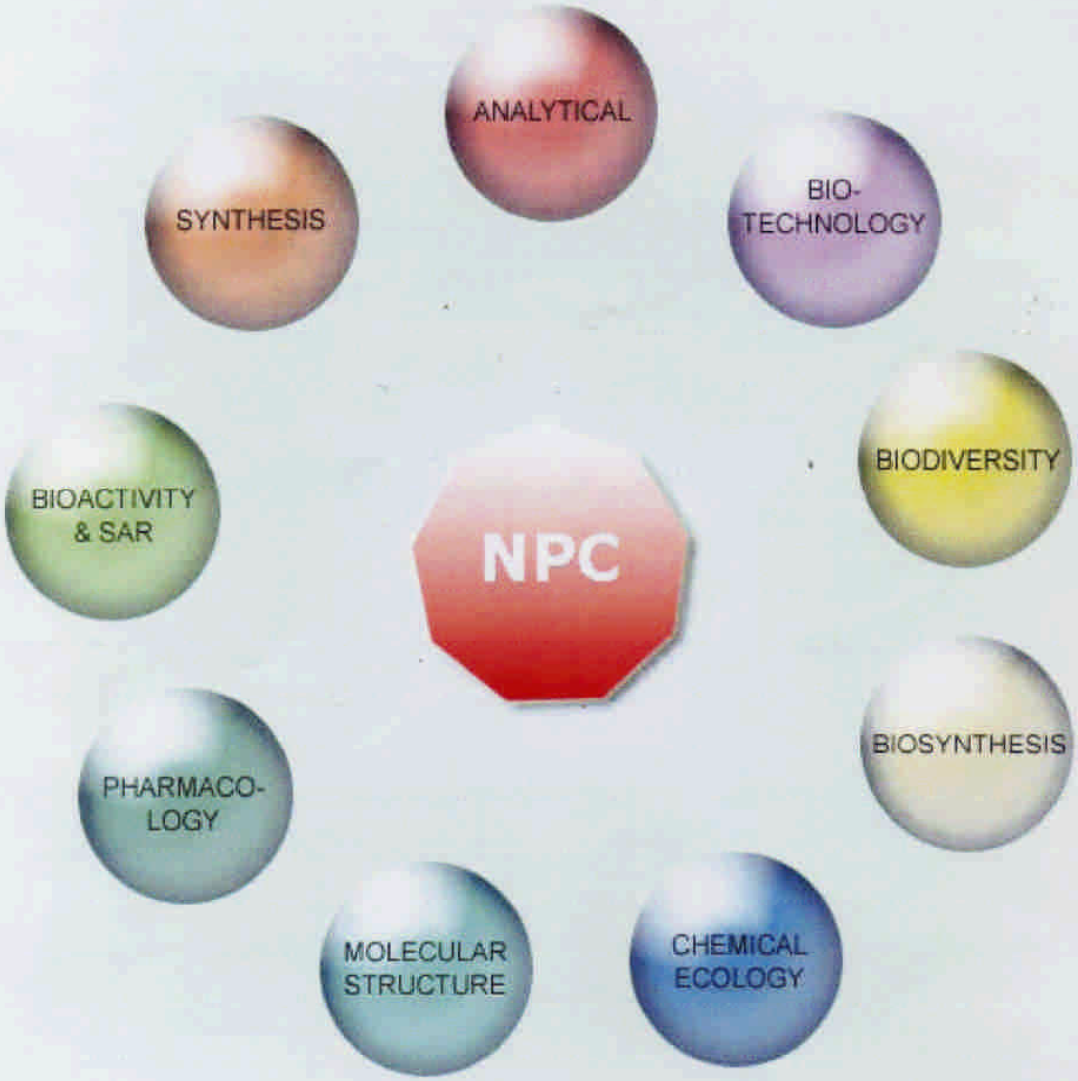

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Composition and Antimicrobial Activity of the Leaf Essential Oil of *Litsea nakaii* from Taiwan

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The leaf essential oil of *Litsea nakaii* was isolated by hydrodistillation and analyzed to determine its composition and yield. Fifty-five compounds were identified, the main components being α-humulene (15.5%), δ-cadinene (9.2%), (*E*)-β-ocimene (8.1%), and δ-selinene (7.1%). The leaf oil exhibited excellent antimicrobial activities.

Keywords: *Litsea nakaii*, Lauraceae, essential oil composition, α-humulene, antimicrobial activity.

The *Litsea* genus, family Lauraceae, is comprised of deciduous trees and shrubs. There are about 400 species in the genus, which are widely distributed from Japan, Korea, and North America to New Zealand and South America. In total, 12 species are found in Taiwan [1]. All *Litsea* plants produce fragrances and certain species have bioactive properties. For instance, the methanol extract of the bark of *L. cubeba* is anti-inflammatory [2], and the α-tocopherol and ascorbic acid contained therein have antioxidant activity [3]. Demethoxyepiexcelsin, verticillatol, and litseaverticill-ol A from *L. verticillata* were found to have anti-HIV activity [4,5].

L. nakaii is endemic to Taiwan and is distributed in forests of the Hengchun Peninsula, at the southern tip of the country. There are no literature reports on the chemical composition and biological activities of the essential oils or other extractives from this species. Therefore, we used hydrodistillation to collect the leaf oil, which was analyzed by GC/FID and GC/MS. The second part of the study examined the antimicrobial activities of the oil. The purpose of this study was to establish a chemical basis for the effective multipurpose utilization of the species.

Hydrodistillation of *L. nakaii* yielded a yellow oil in 3.61 ± 0.03% (v/w) yield, 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. Fifty-five components were identified, representing 100% of the total oil. Among the groups, sesquiterpene hydrocarbons predominated (62.4%), followed by oxygenated sesquiterpenes (24.1%), monoterpene hydrocarbons (12.8%), non-terpenoids (0.4%), and oxygenated monoterpenes (0.3%).

Among the sesquiterpene hydrocarbons, α-humulene (15.5%), δ-cadinene (9.2%), δ-selinene (7.1%), viridiflorene (4.7%), α-muurolene (4.3%) and γ-muurolene (4.1%) were the principal compounds. From the oxygenated sesquiterpenes, humulene epoxide (4.1%), τ-cadinol (3.2%), 10-*epi*-γ-eudesmol (3.1%) and α-cadinol (2.9%) were the major components.

The essential oil of *L. nakaii* was tested against three Gram-positive and five Gram-negative bacteria, as well as one fungus. The results, presented in Table 2, showed that the oil exhibited high biological activity against all tested bacteria and the fungus. The most sensitive microorganisms were *Bacillus cereus*, *Staphylococcus aureus*, and *S. epidermidis*, with inhibition zones of 28 to 40 mm and MIC values of 125 to 250 μg/mL, respectively. The essential oil showed better suppressive activity toward the Gram-positive bacteria than the Gram-negative bacteria and

the fungus. These observations were similar to those of Muhammed *et al.* [6]. Comparing the antimicrobial activities of the essential oils from *L. laevigata* [6], *Tetrataenium nephrophyllum* [7], and *T. lasiopetalum* [8], the leaf essential oil of *L. nakaii* was superior. The results verify that *L. nakaii* leaf oil has excellent antimicrobial activity. The source of this activity seemed to be the cadinol-type compounds, such as τ-cadinol [9], α-cadinol [10] and δ-cadinol [9]. There are also studies supporting the contention that these compounds have high activity in suppressing microbial growth [9-12].

Experimental

Plant materials: The fresh leaves of *L. nakaii* were collected in May 2006 from Shangwu (Taitung County, southern Taiwan, elevation 350 m, N 22° 20′ 72″, E 120° 53′ 01″). The samples were compared to specimen no. 11705 from herbarium of National Chung-Hsing University (NCHU) and identified by Prof. Yen-Hsueh Tseng of NCHU. The voucher specimen (CLH-001) has been deposited in the Taiwan Forestry Research Institute herbarium. Leaves of the species were collected for subsequent extraction and analysis.

Isolation of the leaf essential oil: The leaves of *L. nakaii* (1 kg) were hydrodistillated for 8 hrs in a Clevenger-type apparatus. The oil collected was dried with anhydrous sodium sulfate. All data (yields and composition) were the average of triplicate analyses.

Essential oil analysis: The method of Su *et al.* [13] was adopted. A Hewlett-Packard HP 6890 gas chromatograph equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 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: 50°C for 2 min, rising to 250°C at 5°C/min. Injector temperature: 270°C. Carrier gas: He with a flow rate of 1 mL/min. Detector temperature: 250°C, split ratio: 1:10. One μL sample was injected. 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). 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.

Table 1: Chemical composition of the leaf oil *L. nakaii*.

Compound	RI [a]	%	Identification [b]
α-Pinene	939	0.8	MS, KI, ST
Camphene	954	0.9	MS, KI, ST
Sabinene	975	0.1	MS, KI, ST
β-Pinene	979	0.1	MS, KI, ST
α-Phellandrene	1003	0.5	MS, KI, ST
α-Terpinene	1017	0.1	MS, KI, ST
p-Cymene	1025	0.1	MS, KI, ST
Limonene	1029	0.5	MS, KI, ST
(*Z*)-β-Ocimene	1037	1.5	MS, KI
(*E*)-β-Ocimene	1050	8.1	MS, KI, ST
p-Mentha-2,4(8)-diene	1088	0.2	MS, KI
α-Terpineol	1189	0.1	MS, KI, ST
n-Decanal	1202	0.4	MS, KI, ST
Bornyl acetate	1289	0.2	MS, KI, ST
δ-Elemene	1338	0.3	MS, KI
α-Ylangene	1375	0.3	MS, KI
α-Copaene	1377	0.9	MS, KI, ST
β-Caryophyllene	1419	1.0	MS, KI, ST
β-Cedrene	1421	0.3	MS, KI, ST
β-Copaene	1432	0.5	MS, KI
β-Gurjunene	1434	0.5	MS, KI
α-Humulene	1455	15.5	MS, KI, ST
allo-Aromadendrene	1460	0.4	MS, KI
γ-Muurolene	1480	4.1	MS, KI
α-Amorphene	1485	2.1	MS, KI
β-Selinene	1490	0.9	MS, KI
δ-Selinene	1493	7.1	MS, KI
Viridiflorene	1497	4.7	MS, KI, ST
α-Muurolene	1500	4.3	MS, KI
δ-Amorphene	1512	2.5	MS, KI
γ-Cadinene	1514	1.8	MS, KI
7-*epi*-α-Selinene	1522	0.1	MS, KI
δ-Cadinene	1523	9.2	MS, KI
trans-Cadina-1(2),4-diene	1535	2.3	MS, KI
α-Cadinene	1539	1.8	MS, KI
Eudesma-3,7(11)-diene	1547	2.0	MS, KI
cis-Muurol-5-en-4-α-ol	1561	0.2	MS, KI
Ledol	1569	0.1	MS, KI
Caryophyllenyl alcohol	1572	0.6	MS, KI
α-Cedrene epoxide	1575	0.2	MS, KI
Spathulenol	1578	0.1	MS, KI
Globulol	1585	1.5	MS, KI, ST
Guaiol	1601	0.8	MS, KI, ST
Humulene epoxide II	1608	4.1	MS, KI
Isolongifolan-7-α-ol	1619	1.1	MS, KI
1,10-Di-*epi*-cubenol	1619	0.9	MS, KI
10-*epi*-γ-Eudesmol	1624	3.1	MS, KI
1-*epi*-Cubenol	1629	2.2	MS, KI
γ-Eudesmol	1632	0.7	MS, KI
τ-Cadinol	1640	3.2	MS, KI
δ-Cadinol	1646	1.6	MS, KI
α-Cadinol	1654	2.9	MS, KI
Selin-11-en-4-α-ol	1660	0.3	MS, KI
7-*epi*-α-Eudesmol	1664	0.2	MS, KI
Eudesm-7(11)-en-4-ol	1700	0.4	MS, KI
Compounds identified		100.0	
monoterpene hydrocarbons		12.8	
monoterpene oxygens		0.3	
sesquiterpene hydrocarbons		62.4	
oxygenated sesquiterpenes		24.1	
others		0.4	
yield (mL/100g)		3.61±0.03	

[a] Retention index on a DB-5 column with reference to *n*-alkanes [14].

[b] MS, NIST and Wiley libraries spectra and the literature; RI, retention index; ST, authentic standard compounds.

Component identification: Identification of the leaf essential oil constituents was based on comparisons of retention index (RI) [14], retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra, and literature [15,16].

Antimicrobial activity: The *in vitro* antibacterial and antifungal activities of the oil were evaluated by the disc diffusion method using Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for fungi [17]. Discs containing 15 µL and 30 µL of the oil, which was dissolved in dimethylsulphoxide (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 [7,8,18].

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* (TCC 17803); 3 Gram-positive bacteria: *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 6538P), and *S. epidermidis* (ATCC 12228); and 1 yeast: *Candida albicans* (ATCC 10231). Minimum inhibitory concentration (MIC) values were measured by the microdilution broth susceptibility assay recommended by NCCLS [19]. Stock solutions of the oil were prepared in DMSO. Dilution series were prepared from 1500 µg/mL to 50 µg/mL in sterile distilled water in micro-test tubes, from where they were transferred to 96-well microtitre 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.

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Table 2: Antimicrobial activity of the essential oil of *L. nakaii*.

Microbial species	*Litsea nakaii*		Antibiotics		
	IZ [a]	MIC [b]	Tetracycline (30 µg/disc)	Gentamicine (10 µg/disc)	Nystatine (30 µg/disc)
Bacillus cereus	28 ± 0.8	250	22 ± 0.8	-	nt
Staphylococcus aureus	32 ± 0.4	250	21 ± 0.4	-	nt
Staphylococcus epidermidis	40 ± 0.8	125	34 ± 0.4	-	nt
Escherichia coli	26 ± 0.4	375	-	22 ± 0.8	nt
Enterobacter aerogenes	18 ± 0.8	500	10 ± 0.4	-	nt
Klebsiella pneumoniae	26 ± 0.8	375	-	21 ± 0.8	nt
Pseudomonas aeruginosa	18 ± 0.4	500	-	12 ± 0.8	nt
Vibrio parahaemolyticus	15 ± 0.4	750	-	13 ± 0.8	nt
Candida albicans	19 ± 0.8	500	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.
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.

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