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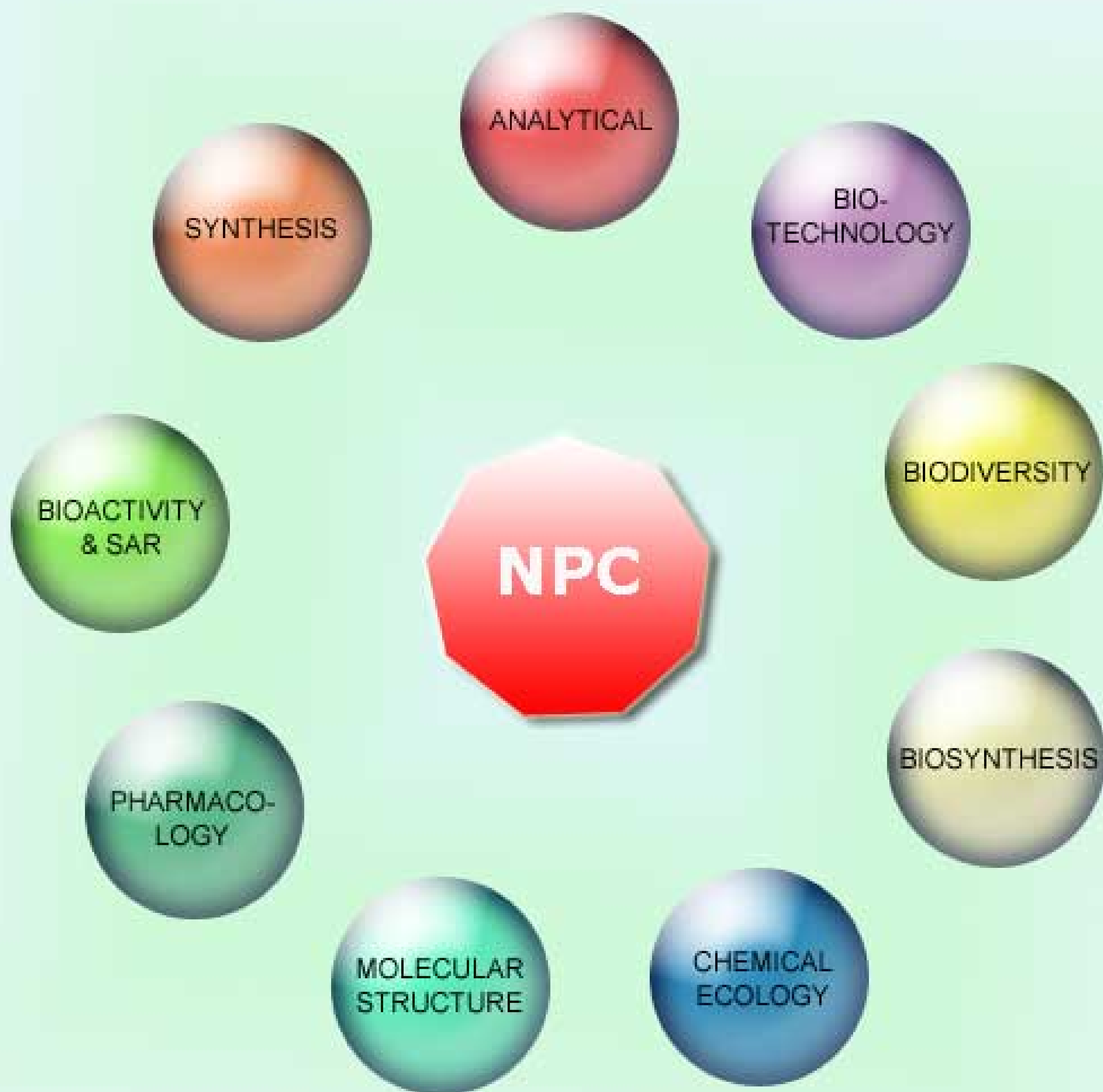

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Composition and Antimicrobial Activity of the Leaf Essential oil of *Litsea kostermansii* from Taiwan

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The hydrodistillated leaf essential oil of *Litsea kostermansii* was analyzed to determine its composition and yield. Seventy-three compounds were identified, the main components being β-eudesmol (22.5%), γ-eudesmol (18.6%), δ-selinene (8.5%), α-eudesmol (6.0%), and γ-muurolene (4.7%). Oxygenated sesquiterpenes (66.2%) and sesquiterpene hydrocarbons (32.8%) were the predominant groups of compounds. The leaf oil exhibited excellent antimicrobial activities.

Keywords: *Litsea kostermansii*, Lauraceae, essential oil composition, β-eudesmol, γ-eudesmol.

The *Litsea* genus, family Lauraceae, comprising ca. 400 species of deciduous trees and shrubs, is widely distributed in East Asia, North America, New Zealand and South America. In total, there are 12 species found in Taiwan [1a]. Many *Litsea* plants produce fragrances and certain species have bioactive properties. For instance, the leaf oil of *L. nakaii* and berries oil of *L. laevigata* exhibited moderate to high antimicrobial activities [1b,1c]. The methanol extract of the bark of *L. cubeba* is anti-inflammatory [2a], and the α-tocopherol and ascorbic acid contained therein have antioxidant activity [2b]. Demethoxy-epiexcelsin, verticillatol, and litseaverticillol A from *L. verticillata* were found to have anti-HIV activity [2c,2d].

L. kostermansii C.E. Chang, (Lauraceae) is a mid-sized evergreen tree endemic to low-elevation mountainous areas of Taiwan. There appears to be no report on the chemical composition and biological activity of either its essential oil or other extractives. Therefore, we used hydrodistillation to collect the leaf oil, which was analyzed by GC/FID and GC/MS. In order to prevent the widespread in-hospital infection, we selected bacterial strains of *Bacillus cereus*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, *Aspergillus niger*, and *Candida albicans* for testing. The purpose of this study was to establish a chemical basis for effective multipurpose utilization of the oil from this species.

Hydrodistillation of *L. kostermansii* leaves gave a yellowish oil with a yield of 1.21 ± 0.04 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-three components were identified, representing 100% of the oil. Among the groups, oxygenated sesquiterpenes predominated (66.2%), followed by sesquiterpene hydrocarbons (32.8%), monoterpene hydrocarbons (0.5%), non-terpenoids (0.2%), diterpenes (0.2%), and oxygenated monoterpenes (0.1%). Among the oxygenated sesquiterpenes, β-eudesmol (22.5%), γ-eudesmol (18.6%), α-eudesmol (6.0%), 1-*epi*-cubenol (2.0%), hinesol (1.9%), and viridiflorol (1.6%) were the major compounds. Of the sesquiterpene hydrocarbons, δ-selinene (8.5%), γ-muurolene (4.7%), δ-cadinene (2.2%), *trans*-β-guaiene (2.0%), α-*neo*-clovene (1.8%), *allo*-aromadendrene (1.8%), β-caryophyllene (1.7%) and α-selinene (1.6%) were the main components.

The compounds of *L. kostermansii* leaf essential oil, although they were predominantly sesquiterpenoids like those in the leaf oils of *L nakaii* [1b], *L. resinosa, L. grasilipes,* and *L. paludosa* [3a], their main components were different. Furthermore, in comparison

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

Compound ID	RI [a]	Conc.(%)	Identification [b]
n-Hexanol	871	t [c]	KI, MS, ST
n-Nonane	900	t	KI, MS, ST
Myrcene	991	t	KI, MS, ST
Limonene	1029	t	KI, MS, ST
cis-β-Ocimene	1037	0.1	KI, MS, ST
trans-β-Ocimene	1050	0.4	KI, MS, ST
Linalool	1097	t	KI, MS, ST
n-Nonanal	1101	t	KI, MS, ST
Artemisyl acetate [ti]	1173	t	KI, MS
n-Decanal	1202	0.2	KI, MS, ST
2-Undecanone	1294	t	KI, MS
δ-Elemene	1338	1.1	KI, MS, ST
α-Cubebene	1351	0.1	KI, MS, ST
Neryl acetate	1362	t	KI, MS, ST
(*Z*)-β-Damascenone	1364	t	KI, MS
α-Ylangene	1375	0.2	KI, MS, ST
α-Copaene	1377	0.5	KI, MS, ST
Daucene	1382	0.1	KI, MS
β-Cubebene	1388	t	KI, MS
β-Bourbonene	1388	t	KI, MS
iso-Longifolene	1390	t	KI, MS
β-Elemene	1391	0.3	KI, MS
Cyperene	1399	t	KI, MS
Sibirene	1400	t	KI, MS
iso-Caryophyllene	1409	t	KI, MS
α-Gurjunene	1410	t	KI, MS
(*E*)-β-Damascone	1414	t	KI, MS
β-Caryophyllene	1419	1.7	KI, MS, ST
β-Gurjunene	1434	0.1	KI, MS
γ-Elemene	1437	t	KI, MS
Aromadendrene	1441	0.2	KI, MS, ST
cis-Muurola-3,5-diene	1450	0.7	KI, MS
α-*neo*-Clovene	1454	1.8	KI, MS
Allo-aromadendrene	1460	1.8	KI, MS, ST
trans-Cadina-1(6),4-diene	1477	0.8	KI, MS
γ-Muurolene	1480	4.7	KI, MS
δ-Selinene	1493	8.5	KI, MS
Valencene	1496	0.4	KI, MS
α-Selinene	1498	1.6	KI, MS, ST
trans-β-Guaiene	1503	2.0	KI, MS
γ-Cadinene	1514	0.3	KI, MS
δ-Cadinene	1523	2.2	KI, MS, ST
trans-Calamenene	1529	0.7	KI, MS, ST
trans-γ-Bisabolene	1531	0.1	KI, MS
trans-Cadina-1(2),4-diene	1535	0.7	KI, MS
α-Cadinene	1539	0.3	KI, MS
α-Calacorene	1546	0.5	KI, MS, ST
Selina-3,7(11)-diene	1547	0.1	KI, MS
Elemol	1550	5.5	KI, MS, ST
Germacrene B	1561	1.2	KI, MS
epi-Longipinanol	1564	0.1	KI, MS
Ledol	1569	t	KI, MS, ST
Caryophyllenyl alcohol	1572	0.2	KI, MS
Caryophyllene oxide	1583	0.4	KI, MS, ST
Globulol	1585	0.9	KI, MS, ST
Viridiflorol	1593	1.6	KI, MS, ST
Guaiol	1601	1.4	KI, MS, ST
5-*epi*-7-α-Eudesmol	1608	0.6	KI, MS
epi-Cedrol	1619	0.3	KI, MS
10-*epi*-γ-Eudesmol	1624	1.4	KI, MS
1-*epi*-Cubenol	1629	2.0	KI, MS
γ-Eudesmol	1632	18.6	KI, MS
Hinesol	1642	1.9	KI, MS
β-Eudesmol	1651	22.5	KI, MS, ST
Mustakone	1677	0.8	KI, MS
Eudesm-7(11)-en-4-ol	1700	1.0	KI, MS
5-Hydroxy-*cis*-calamenene	1713	0.2	KI, MS
Nootkatol	1715	0.3	KI, MS
2*Z*,6*Z*-Farnesol	1718	0.3	KI, MS
Guaiazulene	1781	0.1	KI, MS
γ-Eudesmol acetate [ti]	1784	0.3	KI, MS
Phytol	1943	0.2	KI, MS, ST
Monoterpene hydrocarbons		0.5	
Oxygenated monoterpenes (%)		0.1	
Sesquiterpene hydrocarbons (%)		32.8	
Oxygenated sesquiterpenes (%)		66.2	
Diterpenes (%)		0.2	
Others (%)		0.2	
Oil Yield (mL/100 g)		1.21 ± 0.04	

[a] Retention index on a DB-5 column with reference to *n*-alkanes [2e].
[b] MS, NIST and Wiley library spectra and the literature; RI, Retention index; ST, authentic standard compounds.
[c] trace < 0.1%.
[ti] tentative identification. artemisyl acetate: 196 (M^+ 1) 137 (5) 127 (25) 85 (100) 43 (35) ; γ-eudesmol acetate: 264 (M^+ 1) 204 (52) 189 (80) 161 (63) 147 (28) 133 (42) 119 (20) 105 (48) 91 (46) 79 (30) 67 (26) 55 (40) 43 (100)

with *L guatemalensis* [3b], and *L. laevigate* [1c] leaf oils, these have predominantly monoterpenoids, hence are different from the leaf oil of *L. kastermansii.* Thus, leaf oils of different *Litsea* species are quite different and the chemical composition of the leaf oils could be used as a taxonomic marker to aid species characterization. However, species differentiation by essential oil analysis may not be suitable if either ontogenetic variations or infraspecific chemical differences (chemical race) exist within the species.

The essential oil of *L. kostermansii* was tested against 3 Gram-positive and 5 Gram-negative bacteria, as well as two fungi. The results, presented in Table 2, show that the oil exhibited moderate to high biological activity against all tested bacteria and fungi. The most sensitive microorganisms were *Bacillus cereus*, *Staphylococcus aureus*, and *S. epidermidis*, with inhibition zones of 25 to 46 mm and MIC values of 125 to 375 µg/mL, respectively. The essential oil showed better suppressive activity toward the Gram-positive than the Gram-negative bacteria and the fungi. These observations were similar to those of Ho *et al*. [1b], Muhammed *et al*. [1c], Kim *et al*. [4a], and Cimanga *et al*. [4b]. Comparing the antimicrobial activities of the essential oils from *L. laevigata* [1c], *Tetrataenium nephrophyllum* [5], and *T. lasiopetalum* [6], the leaf essential oil of *L. kostermansii* was superior. The results verified that *L. kostermansii* leaf oil has excellent antimicrobial activities. The sources of the

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

Microbial species	*Litsea kostermansii*		Antibiotics: Tetracycline (30 µg/disk)		Gentamicine (10 µg/disk)		Nystatine (30 µg/disk)	
	IZ [a]	MIC [b]	IZ	MIC	IZ	MIC	IZ	MIC
Bacillus cereus	25 ± 0.4	375	22 ± 0.8	4.0	-	nt	nt	nt
Staphylococcus aureus	32 ± 0.4	250	21 ± 0.4	4.0	-	nt	nt	nt
Staphylococcus epidermidis	46 ± 0.8	125	34 ± 0.4	2.0	-	nt	nt	nt
Escherichia coli	18 ± 0.4	750	-	nt	22 ± 0.8	4.0	nt	nt
Enterobacter aerogenes	20 ± 0.8	500	10 ± 0.4	8.0	-	nt	nt	nt
Klebsiella pneumoniae	24 ± 0.4	375	-	nt	21 ± 0.8	4.0	nt	nt
Pseudomonas aeruginosa	18 ± 0.4	750	-	nt	12 ± 0.8	8.0	nt	nt
Vibrio parahaemolyticus	14 ± 0.4	1000	-	nt	13 ± 0.8	8.0	nt	nt
Aspergillus niger	8 ± 0.4	>1000	nt	nt	nt	nt	17 ± 0.8	8.0
Candida albicans	16 ± 0.8	1000	nt	nt	nt	nt	19 ± 0.8	4.0

[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.

antimicrobial activities are thought to be the compounds with a eudesmol-skeleton, such as α-eudesmol, β-eudesmol, and γ-eudesmol. There are also studies supporting the contention that these compounds have high activities in suppressing microbial growth [2e,7]. Costa *et al*. [7] applied α-eudesmol, β-eudesmol, and γ-eudesmol to suppress 11 strains of bacteria and fungi and found excellent growth inhibition efficacy. Furthermore, Kusuma *et al*. [2e] also obtained excellent growth inhibition against fungi using β-eudesmol.

Experimental

Plant materials: Fresh leaves of *L. kostermansii* were collected in June 2007 from the Lienhuachih Research Center of the Taiwan Forestry Research Institute in central Taiwan (Nantou County, elevation 605 m, N 23º 56′ 38″, E 120º 53′ 19″). The samples were compared with specimen no. ou4393 from the Herbarium of National Chung-Hsing University and identified by Prof. Yen-Hsueh Tseng of NCHU. The voucher specimen (CLH-002) 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 *L. kostermansii* (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 method of Su *et al*. [8] 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: 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 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.

Component identification: Identification of the leaf essential oil constituents was based on comparisons of retention index (RI) [9a], retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra, and literature [9b,9c].

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 [9d]. 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. Gentamicin and tetracycline for bacteria, and nystatin for fungi were used as positive controls [5,6,10a].

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 2 fungi: *Aspergillus niger* (ATCC16404), and *Candida albicans* (ATCC 10231). Minimum inhibitory concentration (MIC) values were measured by the microdilution broth susceptibility assay recommended by NCCLS [10b]. 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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