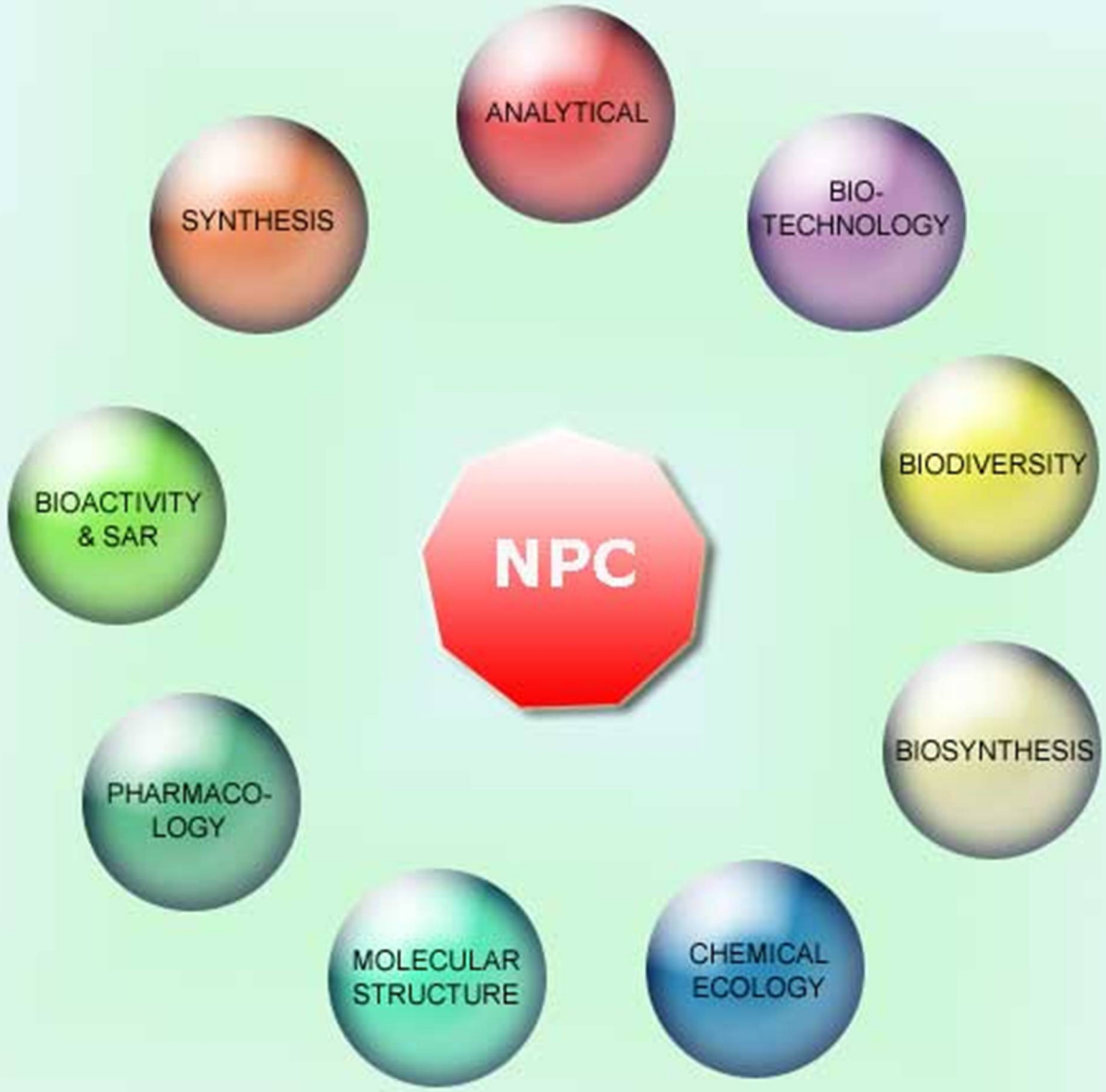
NATURAL PRODUCT COMMUNICATIONS

An International Journal for Communications and Reviews Covering all Aspects of Natural Products Research



Volume 5. Issue 10. Pages 1525-1710. 2010
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
www.naturalproduct.us

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NPC Natural Product Communications

2010
Vol. 5
No. 10
1677 - 1680

Composition and Antifungal Activities of the Leaf Essential oil of *Litsea coreana* from Taiwan

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Received: May 28th, 2010; Accepted: July 20th, 2010

The hydrodistilled leaf essential oil of *Litsea coreana* was analyzed by GC/FID and GC/MS. Fifty-two compounds were identified, the main components being *n*-decanal (27.5%), *2E*,*6E*-farnesol (25.8%), β-eudesmol (10.3%), ethyl *n*-dodecanoate (8.0%) and τ-cadinol (6.6%). Oxygenated sesquiterpenes (56.8%) and non-terpenoids (37.0%) were the predominant groups of compounds. The leaf oil exhibited excellent antifungal and anti-wood-decay fungal activities.

Keywords: *Litsea coreana*, Lauraceae, essential oil, antifungal activity, anti-wood-decay fungal activity, *n*-decanal, τ-cadinol, β-eudesmol.

Litsea coreana H. L'ev. (Lauraceae) is a small evergreen tree mainly distributed in Korea, Japan, Taiwan, and China [1]. Leaves of the plant serve as a traditional Chinese medicine. There are reports suggesting that the leaf extractive has a hypolipidemic effect in rats fed with a high fat diet [2], a preventive effect on hepatic steatosis in rats fed with a high fat diet [3], a protective effect on liver fibrosis in rats [4], and anti-arthritis, anti-inflammatory, immunomodulatory [5], and anti-termite activities [6-8]. However, 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, thus inductive to the growth of mildew, which not only causes problems for the preservation of cultural items, but also liable to induce allergy, asthma, bronchitis, onychomycosis, cerebral infections, pneumonia, peritonitis and immuno-deficient syndrome. Furthermore, wood-based substances are easily beset by wood decay fungi, causing premature demise of woody structures or crafts [9]. Therefore, we also applied the essential oils to 7 strains of mold fungi and 2 strains each of the commonly found white rot and brown rot fungi to examine their interdiction efficacies. As a consequence, the second part of the study examined the antifungal and anti-wood-decay 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. coreana* leaves gave a yellow-colored oil with a yield of 2.30 ± 0.03 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. Fifty-two components were identified, representing 100% of the oil. Among the groups, oxygenated sesquiterpenes predominated (56.8%), followed by, non-terpenoids (37.0%), sesquiterpene hydrocarbons (3.2%), diterpenes (2.0%), oxygenated monoterpenes (0.6%), and mono-terpene hydrocarbons (0.4%). Among the oxygenated sesquiterpenes, *2E*,*6E*-farnesol (25.8%), β-eudesmol (10.3%) and τ-cadinol (6.6%) were the major compounds. Of the non-terpenoids, *n*-decanal (27.5%) and ethyl *n*-dodecanoate (8.0%) were the main components. The compounds of the leaf essential oil of *L. coreana*, although they were predominantly

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

Compound ID	RI [a]	Conc. (%)	Identification [b]
(*Z*)-β-Ocimene	1037	0.2	MS, KI, ST
(*E*)-β-Ocimene	1050	0.1	MS, KI, ST
n-Nonanal	1101	0.1	MS, KI, ST
n-Nonanol	1169	0.1	MS, KI, ST
(*3Z*)-Hexenyl butanoate	1186	0.2	MS, KI
α-Terpineol	1189	0.1	MS, KI, ST
n-Decanal	1202	27.5	MS, KI, ST
Bornyl acetate	1289	0.3	MS, KI, ST
2-Undecanone	1294	0.1	MS, KI, ST
n-Undecanal	1307	0.1	MS, KI, ST
Z-Trimenal	1398	0.8	MS, KI
n-Dodecanal	1409	0.1	MS, KI, ST
α-Gurjunene	1410	0.1	MS, KI, ST
E-β-Damascone	1414	0.1	MS, KI
p-Cymen-7-ol acetate	1423	0.2	MS, KI
(*E*)-α-Ionone	1430	0.1	MS, KI, ST
trans-α-Bergamotene	1435	0.1	MS, KI
Aromadendrene	1441	0.3	MS, KI, ST
cis-Prenyl limonene	1446	0.1	MS, KI
β-Santalene	1460	0.1	MS, KI
γ-Muurolene	1480	0.1	MS, KI, ST
α-Amorphene	1485	0.1	MS, KI, ST
Germacrene D	1485	0.1	MS, KI, ST
α-Zingiberene	1494	0.5	MS, KI
Viridiflorene	1497	0.6	MS, KI, ST
α-Muurolene	1500	0.1	MS, KI, ST
(*E,E*)-α-Farnesene	1506	0.1	MS, KI
γ-Cadinene	1514	0.1	MS, KI, ST
δ-Cadinene	1523	0.3	MS, KI, ST
α-Cadinene	1539	0.1	MS, KI, ST
α-Calacorene	1546	0.1	MS, KI, ST
cis-Cadinene ether	1554	0.1	MS, KI
Germacrene B	1561	0.2	MS, KI
Ledol	1569	0.2	MS, KI, ST
Caryophyllenyl alcohol	1572	0.1	MS, KI
Ethyl *n*-dodecanoate	1577	8.0	MS, KI, ST
Globulol	1585	0.7	MS, KI, ST
Viridiflorol	1593	0.3	MS, KI, ST
5-*epi*-7-*epi*-α-Eudesmol	1608	0.3	MS, KI
Z-Bisabolol-11-ol	1619	0.3	MS, KI
10-*epi*-γ-Eudesmol	1624	0.3	MS, KI
Leptospermone	1631	1.3	MS, KI
τ-Cadinol	1640	6.6	MS, KI, ST
β-Eudesmol	1651	10.3	MS, KI, ST
α-Cadinol	1654	0.5	MS, KI, ST
α-Bisabolol	1686	0.1	MS, KI
Z-α-*trans*-Bergamotol	1691	2.7	MS, KI
Eudesm-7(11)-en-4-ol	1700	2.7	MS, KI
2Z,6Z-Farnesol	1718	2.1	MS, KI
2E,6E-Farnesol	1725	25.8	MS, KI, ST
2E,6Z-Farnesol	1746	2.4	MS, KI
Phytol	1943	2.0	MS, KI, ST
Compounds identified		100.0	
Monoterpene hydrocarbons		0.4	
Oxygenated monoterpenes		0.6	
Sesquiterpene hydrocarbons		3.2	
Oxygenated sesquiterpenes		56.8	
Oxygenated diterpenes		2.0	
Others		37.0	
Yield mL/100g		2.30 ± 0.03	

[a] Retention index on a DB-5 column with reference to *n*-alkanes [13].

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

sesquiterpenoids, like those in *L. kostermansii* [10], *L nakaii* [11], *L. resinosa*, *L. grasilipes*, and *L. paludosa* [12], their main components were different. Furthermore, compared with *L. guatemalensis* [14], and *L. laevigate* [15] leaf oils, the latter had predominantly monoterpenoids, hence was different from the leaf oil of *L. coreana*.

The antifungal indexes of the leaf oil against 7 fungi were: *Aspergillus clavatus* (*A. c.*) 89.0%, *A. niger* (*A. n.*) 49.0%, *Chaetomium globosum* (*Ch. g.*) 95.7%, *Cladosporium cladosporioides* (*Cl. c.*) 78.3%, *Myrothecium verrucaria* (*M. v.*) 86.0%, *Penicillium citrinum* (*P. c.*) 43.3%, and *Trichoderma viride* (*T. v.*) 52.7%. These results showed that the leaf oil was highly 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* [9], *Chamaecyparis obtusa* [16] and *L. cubeba* [17], the leaf oil of *L. coreana* was superior. The results verified that *L. coreana* leaf oil has excellent antifungal activities.

However, in order to ascertain the source compounds of the antifungal activities of *L. coreana*, the main components were individually tested. For *n*-decanal and ethyl *n*-dodecanoate, very low levels of antifungal activities were found against the 7 mold fungi, with none of the antifungal indices exceeding 40%. However, the sesquiterpenoids, *2E*,*6E*-farnesol, β-eudesmol and τ-cadinol, exhibited better activities among the leaf oil constituents, in particular, β-eudesmol and τ-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%. IC_{50} values of β-eudesmol against *A. clavatus*, *Cl. cladosporioides, Ch. globosum* and *M. verrucaria* were 23.0, 16.3, 43.6, and 38.6 μg/mL, and for τ-cadinol, 28.3, 20.8, 52.3 and 43.5 (Table 2). These results indicated that the active source compounds were β-eudesmol and τ-cadinol, which supports previous work showing high antimicrobial activity of these compounds [18].

The essential oil of *L. coreana* was tested against two white rot fungi (*Trametes versicolor* and *Phanerochaete chrysosporium*) and two brown rot fungi (*Phaeolus schweinitzii* and *Lenzites sulphureu*). The anti-fungal wood-decay indexes presented in Table 3 are a clear demonstration of the excellent anti-fungal wood-decay property of the oil. The growth of *T. versicolor, Phanerochaete. chrysosporium, Phaeolus. schweintzii* and *L. sulphureu* was completely inhibited at concentrations of 75, 75, 50, 25 μg/mL, respectively. Comparing the anti-fungal wood-decay activities of the essential oils from *C. formosensis* [20], *M. philippinensis* [19] and *M. pseudolongifolia* [21] showed that the leaf oil of *L. coreana* was superior. Moreover, the results indicated that the activity was due

Table 2: IC_{50} values (μg/mL) of the five main constituents of *L. coreana* leaf oil against 7 fungal strain.

Constituents [a]	Fungi						
	A. c.	*A. n.*	*Cl. c.*	*Ch. g.*	*M. v.*	*P. c.*	*T. v.*
n-Decanal	- [b]	-	-	-	-	-	-
Ethyl *n*-dodecanoate	-	-	138.2	-	-	-	168.3
2E,6E-Farnesol	100.0	128.6	65.3	123.6	100.0	-	186.3
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

[a]1. *n*-Decanal (≥ 98.5%), 2. Ethyl *n*-dodecanoate (≥ 98.5%), 3. *2E,6E*-Farnesol (≥ 98%), 4. τ-Cadinol (100%), 5. β-Eudesmol (≥ 98%). Compounds 1 to 3 were purchased from the Fluka Co. (Milwaukee, USA), and compound 5 from the Wako Co. (Tokyo, Japan). Compound 4 was from an isolate of the Ho *et al*. [19] study on *Machilus philippinenesis* essential oil. [b] -: IC_{50} value > 200 μg/mL

Table 3: Anti-wood-fungal decay indices of leaf essential oil from *L. coreana*.

Dosage (μg/ml)	Anti-wood-decay fungal index (%)			
	Trametes versicolor	*Phaneochaete chrysosporium*	*Phaeolus schweintizii*	*Lenzites sulphureu*
12.5	42 ± 3.3	23 ± 3.3	32 ± 3.3	52 ± 6.6
25	63 ± 6.6	46 ± 6.6	68 ± 3.3	100 ± 0
50	89 ± 6.6	83 ± 3.3	100 ± 0	100 ± 0
75	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100	100 ± 0	100 ± 0	100 ± 0	100 ± 0

mainly to β-eudesmol and τ-cadinol. The IC_{50} values of these two compounds against the four decay fungi were 48.1 and 36.8 μg/mL against *T. versicolor*; 38.2 and 13.5 μg/mL against *Phanerochaete chrysosporium*, 23.1 and 28.9 μg/mL against *Phaeolus shweinitzii*, and 20.6 and 23.3 μg/mL against *L. sulphureu*, respectively. At a 50 μg/mL concentration, 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 only partially inhibit white-rot fungi. The results agree with those of Kondo and Imamura [22] and Ho *et al*. [19,21]. Thus, the excellent wood-decay-fungi inhibitive activities exhibited by the *L. coreana* leaf oil could well be due to the presence of compounds such as β-eudesmol and τ-cadinol.

Experimental

Plant materials: Fresh leaves of *L. coreana* were collected in July 2009 from the Neishuanshi (Taipei County, northern Taiwan, elevation 580 m, N 25º 08′ 16″, E 121º 36′ 15″), The samples were compared with specimen no. ou5312 from the Herbarium of the National Chung-Hsing University and positively identified by Prof. Yen-Hsueh Tseng of NCHU. The voucher specimen (CLH-006) has been deposited in the NCHU herbarium. Leaves of the species were collected for subsequent extraction and analysis.

Isolation of leaf essential oil: Leaves of *L. coreana* (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.

Essential oil 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. One μL 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.

Component identification: Identification of the leaf essential oil constituents was based on comparisons of retention index (RI) [23], retention times (RT), and MS with those obtained from authentic standards, NIST and Wiley libraries spectra, and literature [11,24].

Antifungal assays: The method of Su *et al.* [9] was adopted. The mold 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 fungal 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) and *T. viride* (ATCC8678) were tested. The wood decay fungi used were *T. versicolor* (BCRC 35253), *Phanerochaete chrysosporium* (BCRC 36200), *Phaeolus schweinitzii* (BCRC 35365) and *Lenzites sulphureus* (BCRC 35305). Antifungal assays were carried out in triplicate and the data were averaged. Different concentrations of the essential oil (12.5- 1000 μg/mL) were added to sterilized potato dextrose agar (PDA). 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 was the diameter of the growth zone in the experimental dish (cm) and Db the diameter of the growth zone in the control dish (cm). DDAC (didecyl dimethyl ammonium chloride) for wood decay fungi was used as a positive control.

References

[1] Yang YP, Liu HY. (**1999**) *Manual of Taiwan vascular plants*. Council of Agriculture, Executive Yuan, Taipei.

[2] Liu JH. (**2003**) Effect of glede tea to blood sugar and blood fat of rats. *Chinese Journal of the Practical Chinese Modern Medicine*, ***3***, 2057.

[3] Wang JQ, Li J, Zou YH, Cheng WM, Lu C, Zhang L, Ge JF, Huang C, Jin Y, Lv XW, Hu CM, Liu LP. (**2009**) Preventive effects of total flavonoids of *Litsea coreana* leaves on hepatic steatosis in rats fed with high fat diet. *Journal of Ethnopharmacology*, ***121***, 54-60.

[4] Huang C, Ma TT, Meng XM, Lv XW, Zhang L, Wang JQ, Li J. (**2010**) Potential protective effects of a traditional Chinese herb, *Litsea coreana* Levl., on liver fibrosis in rats. *Journal of Pharmacy and Pharmacology*, ***62***, 223-230.

[5] Wang TY, Li J, Ge JF, Li CY, Jin Y, Lu XW, Cheng WM, Tang JH. (**2008**) Preliminary study of total flavonoids from *Litsea coreana* Levl. on experimental adjuvant-induced arthritis in rats. *American Journal of Chinese Medicine*, ***36***, 899-912.

[6] Kang HY, Matsushima N, Sameshima K, Takamura N. (**1990**) Termite resistance tests of hardwoods of kochi growth.1. The strong termiticidal activity of kagonoki (*Litsea coreana* Leveille). *Mokuzai Gakkaishi*, ***36***, 78-84.

[7] Kang HY, Sameshima K, Takamura N. (**1991**) Termite resistance tests of hardwoods of kochi growth. 2. Quantitative-determination of termiticidal activity of kagonoki (*Litsea coreana* Leveille) wood. *Mokuzai Gakkaishi*, ***37***, 964-970.

[8] Kang HY, Sameshima K, Takamura N. (**1994**) Termite resistance tests of hardwoods of kochi growth. 4. Isolation and identification of a termiticidal component of kagonoki (*Litsea coreana* Leveille) wood. *Mokuzai Gakkaishi*, ***40***, 64-71.

[9] Su YC, Ho CL, Wang EIC, Chang ST. (**2006**) Antifungal activities and chemical compositions of essential oils from leaves of four Eucalypts. *Taiwan Journal of Forest Science*, ***21***, 49-61.

[10] Ho CL, Wang EIC, Hsu KP, Lee PY, Su YC. (**2009**) Composition and antimicrobial activity of the leaf essential oil of *Litsea kostermansii* from Taiwan. *Natural Product Communications*, ***4***, 1123-1126.

[11] Ho CL, Wang EIC, Lee PY, Su YC. (**2009**) Composition and antimicrobial activity of the leaf essential oil of *Litsea nakaii* from Taiwan. *Natural Product Communications*, *4*, 865-868.

[12] bin Ahmad F, bin Jantan I, Abu Bakar B, bin Ahmad AS. (**2005**) A comparative study of the composition of the leaf oils of three *Litsea* species from Borneo. *Journal of Essential Oil Research*, ***17***, 323-326;

[13] Van den Dool H, Kratz PD. (**1963**) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography*, ***11*,** 463-471.

[14] Vallverdu C, Vila R, Cáceres A, Cañigueral S. (**2005**) Composition of the essential oil from leaves of *Litsea guatemalensis*. *Flavour and Fragrance Journal*, ***20***, 415-418.

[15] Muhammed AM, Subbu RM, Leopold J, Mohamed SP. (2008) Composition and antimicrobial analysis of the essential oil of *Litsea laevigata* Nees. (Lauraceae). *Natural Product Communications*, ***3***, 1069-1072.

[16] Ohtani, Y, Kirishima M, Sameshima K. (**2001**) Effects of hydration on termiticidal and antifungal activities of wood essential oils. *Mokuzai Gakkaishi*, ***47***, 267-275.

[17] Ho CL, Hsu KP, Chien TC, Wang EIC, Su YC. (**2009**) Essential oil compositions and antimicrobial paper activities of the various parts of *Litsea cubeba* (Lour.) from Taiwan. *Cross Strait Forest Products Technology Symposium Proceedings of 2009*, 80-82.

[18] Nabeta K, Katayama M, Matsubara C, Hatakeyama T, Shimada H, Tazaki H. (**1992**) Oxygenated sesquiterpenes from needles of Koreanpine (*Pinus koraiensis* Sieb.). *Mokuzai Gakkaishi*, ***38***, 963-971.

[19] Ho CL, Hsu KP, Wang EIC, Lin CY, Su YC. (**2010**) Composition and anti-wood-decay fungal activities of the leaf essential oil of *Machilus philippinensis* from Taiwan. *Natural Product Communications*, ***5***, 337-340.

[20] Wang SY, Wu CL, Chu FH, Chien SC, Kuo YH, Shyur LF, Chang ST. (**2005**) Chemical composition and antifungal activity of essential oil isolated from *Chamaecyparis formosensis* Matsum. wood. *Holzforschung, **59***, 295-299.

[21] Ho CL, Liao PC, Wang EIC, Dong WZ, Su YC. (**2010**) Composition and antimicrobial and anti-wood-decay fungal activities of the leaf essential oils of *Machilus pseudolongifolia* from Taiwan. *Natural Product Communications*, ***5***, 1143-1146.

[22] Kondo R, Imamura H. (**1986**) Antifungal compounds in heartwood extractives of hinoki (*Chamaecyparis obtusa* Endl.). *Journal of the Japan Wood Research Society*, ***32***, 213-217.

[23] Adams RP. (**2001**) *Identification of Essential Oil Components by Gas Chromatography/Quadruple Mass Spectroscopy*, Allured, Carol Stream, IL.

[24] Massada Y. (**1976**) *Analysis of Essential Oil by Gas Chromatography and Spectrometry*, Wiley, New York.

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