研究報告

Research paper

Essential Oil Compositions and Bioactivities of the Various Parts of *Cinnamomum camphora* Sieb. var. *linaloolifera* Fujuta

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【Abstract】In this study, we used hydrodistillation and headspace GC methods to separate essential oils from the leaves, flowers and twigs of a "linalool tree" (*Cinnamomum camphora* Sieb. var. *linaloolifera* Fujuta) and establish their yields and compositions. Furthermore, the antioxidative and antifungal activities of the oils were evaluated. A total of 68, 77 and 83 compounds were identified respectively from the leaf, flower and twig oils of the tree by the hydrodistillation method, and their yields were 3.94 ± 0.06, 2.77 ± 0.12 and 0.59 ± 0.04 ml/100 g of the oven-dried (o.d.) materials, respectively. The headspace-gas chromatography (HS-GC) method, on the other hand, generated 49, 59 and 65 identified compounds respectively from the leaves, flowers, and twigs of the tree. Yields of essential oil were determined using the multiple headspace extraction (MHE) method and found to be 3.93 ± 0.11, 2.72 ± 0.10 and 0.61 ± 0.03 ml/100 g of o.d. materials for the leaves, flowers and twigs, respectively. The main compounds based on the 2 methods were similar and those in the leaves and flowers were linalool, and in twigs were linalool, camphor etc. Twig oils showed the highest antioxidant activity, with an IC_{50} of merely 104 μg/ml. As for the antifungal activities, the leaf oil showed the best activity. All 3 oils had excellent capability in suppression wood decaying fungi. Therefore, in addition to being raw materials for producing high-grade fragrance agents, these oils are also applicable to the antioxidant and antifungal purposes and exhibit the multipurpose potential of expanded essential oil applications.

【**Key words**】*Cinnamomum camphora* Sieb. var. *linaloolifera* Fujuta, essential oil, headspace-GC, linalool, bioactivities

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研究報告

芳樟 (*Cinnamomum camphora* Sieb. var. *linaloolifera* Fujuta) 各部位精油組成分及生物活性之探討

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【摘要】本研究爲以芳樟之葉、花及枝等三部位以水蒸餾法及headspace-GC等二方法，萃取精油、分析精油組成分及收率，並評估其抗氧化及抗眞菌活性。以水蒸餾法取得之葉、花及枝精油，分別鑑定 68、77 及 83 個化合物，收率分別爲 3.94 ± 0.06, 2.77 ± 0.12 及 0.59 ± 0.04 ml/100g o.d.(oven dried)。而以 Headspace-GC 取得之三部位精油，分別爲鑑定 49、59 及 65 個化合物，收率之測定以 MHE (Multiple headspace extraction) 法進行之，其收率分別爲 3.93 ± 0.11, 2.72 ± 0.10 及 0.61 ± 0.03 ml/100 g o.d.。而於二法所得精油之主成分均爲相似，葉及花部位之主成分爲linalool，枝部位爲 linalool 及 camphor 等化合物。抗氧化活性，以枝部位精油之自由基捉補能力爲最強，IC_{50} 值僅 104 μg/ml。抗眞菌活性，以葉精油之抑制活性爲最佳，而此三部位精油對於木材腐朽菌均具極佳抑制能力。故此三種精油，除可做爲高級香料產品外，亦可朝抗氧化及抗眞菌等活性產品之應用，以拓展精油之多機能性用途。

【關鍵詞】芳樟、精油、頂空間採樣氣相層析儀、芳樟醇、生物活性

I. INTRODUCTION

Cinnamomum camphora is a member of the *Lauraceae* family, genus *Cinnamomum*. The tree is an evergreen broadleaf species widely distributed in the temperate and subtropical regions of Asia. Strains of the species are often difficult to differentiate based on morphological characteristics, thus chemotype is often used for the purpose based on differences of leaf essential oil compositions of individual trees. As a result, the species is known to consist of 5 chemotype of camphor, linalool, 1,8-cineole, nerolidol and borneol (Lin and Hua, 1987; Shi *et al*., 1989; Jantan and Goh, 1992; Zhu *et al*., 1993, 1994; Lawrence, 1995). Camphor trees in Taiwan are mostly the camphor and linalool subtypes. As early as 1932, there was record of using the camphor subtype to produce natural camphor in Taiwan. And the island has been an important producer of commercial camphor. Camphor oil production was based on hydrodistillation of chipped wood and branches of the tree. At present, camphor oil production in China is about 500 ton a year, mostly from Guangdong, Guangsi, Jiangsi and Fujian Provinces (Zhu *et al*., 1994; Lawrence, 1995). As for the linalool subtype, linalool-rich oil can be produced from it. Yoshida (1969) and Lu *et al*. (1985) pointed out that when the distilled essential oil is rich in l-linalool, then the oil, known as Ho oil or Shou oil, is suitable for the production of high-grade fragrance agent and has commercial significance.

Previous studies on the essential oils from camphor trees were largely focused on their compositional analysis and chemotype differentiation. For instance, Shieh (2003a,b) used hydrodistillation to extract the leaves and wood of camphor and linalool tree and analyzed their compositions.

There was, however, rarely any use of the headspace-GC method for compositional identification. Therefore, we endeavored to first extracting the renewable tree parts of leaves, flowers and twigs using both hydrodistillation and headspace-GC methods and comparing the compositions of the oils. For oil yields, a multiple headspace extraction (MHE) method was used in the headspace-GC method to evaluate the difference in yields of the 2 methods. These efforts should be able to establish the suitability of the headspace-GC method for chemotyping of essential oil producing tree species.

In addition, as there are proliferating antioxidant agents including natural and synthetic chemicals come into widespread uses and some synthetic chemicals such as butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) antioxidants have been found to possibly causing harm to liver and kidney of animal and are carcinogenic (Schildermann *et al.*, 1995), causing their use to be restricted. As a consequence, influences of natural antioxidants on life forms have become an important research trend. The second part of our study was directed toward the evaluation of oils from the renewable parts of linalool for their antioxidant activities.

The warm and humid climate makes Taiwan a favorable habit for the growth of mildews and decay fungi. Certain mold fungi, such as *Aspergillus clavatus, A. niger, Chaetomium globosum, Cladosporium cladosporioides, Myrothecium verrucaria, Penicillium citrinum*, and *Trichoderma viride* are known to damage cultural artifacts and are harmful to human health such as causing allergic reactions, asthma, bronchitis (Grant *et al.*, 1976; Blyth *et al.*, 1977; Blyth, 1978), onychomycosis (Naidu *et al.*, 1991; Naidu, 1993; Hattori *et al.*, 2000), cerebral infections (Anandi *et al.*, 1989; Abbott *et al.*, 1995; Kleinschmidt, 2002), pneumonia (Prentice *et al.*, 1996), peritonitis and immuno-deficiency syndrones (Chouaki *et al.*, 2002). Among the decay fungi, such as *Trametes versicolor, Phanerochaete chrysosporium* of white rot; *Phaeolus schweinitzii, Laetiporus sulphureus* of brown rot are well known to cause decay in trees. Hence, the 3rd part of this study was directed toward the antifungal capability of the linalool tree oils to see whether they can effectively suppress growth of the aforementioned 4 fungi. Positive performance of the oils can bode for their antifungal applications and open a path toward multipurpose utilization of the essential oils from *Cinnamomum camphora* var. *linaloolifera*.

II. EXPERIMENTALS

(I) Materials

The leaves, flowers, and twigs of a linalool tree grown in the compound of Taiwan Forestry Research Institute were sampled (Taipei County, northern Taiwan, elevation 30 m, N 25° 01′ 48″, E 121° 30′ 36″, and a specimen has been deposited in the Taiwan Forestry Research Institute herbarium.) and the materials kept under refrigeration until subsequent analyses.

(II) Methods

a. Extraction of essential oils and compositional and yield determinations

(a) Hydrodistillation

1 kg each of the leaves, flowers, and twigs was placed in a round-bottom flask and added with 3 L of distilled water. The water was heated to boil and refluxed for 8 h. The essential oil layer above the water layer was separated and added with anhydrous sodium sulfate to dewater. The essential oils obtained were placed in

specimen bottles and the yields determined. Each test was repeated three times and the data were averaged.

(b) GC and GC-MS Analysis

A Hewlett-Packard HP6890 gas chromatograph equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 μm film thickness; J&W Scientific) and a FID detector was used for the percentage 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; and 1 μl sample was injected. Identification of the oil components was based on their retention indices and mass spectra. The latter were obtained from the GC-MS analysis using a Hewlett-Packard HP6890/HP5973 GC-MS unit equipped with a DB-5 fused silica capillary column. The GC analysis parameters were the same as listed above and the MS was operated under the full scan mode, using the EI mode at 70 eV. Each test was repeated three times and the data were averaged.

(c) HS-GC analysis

The leaves, flower, and twigs were cut into small pieces with scissors or a handsaw just prior to the headspace sampling. Each sample (20 mg) was filled into a 20 ml vial respectively, and then the vials were hermetically sealed with a PTFE-coated rubber septum and an aluminum cap. A Perkin Elemer Headspace Turbomatrix 40 unit connected to a Hewlett-Packard HP6890 GC was used for the analysis. The headspace analysis programs and conditions were as follows: The vial oven temperature was 100℃ for each analysis as transfer line (110℃), and the needle temperature was 110℃. Treatment in oven with a shaker lasted 50 min. Pressurization time was 3.0 min; the thermostatting time was 50 min; and the injection volume was 10 μl. The GC and GC-MS analysis programs used were the same as the above section. Each test was repeated three times and the data were averaged (Ho *et al.*, 2008).

(d) Oil yields

The total amount of oil in each sample was determined by a HS-GC unit. Calibration curves were made initially with different quantities of the leaf, flower, and twig oils, previously extracted using the hydrodistillation method. A special quantitative method, the multiple headspace extraction (MHE), was employed. According to Kolb (1985) and Ho *et al.* (2008), the matrix effect can be eliminated by using the MHE method. The total peak area of each oil volume was calculated according to the following equation:

$$\Sigma A = A_1^2 / (A_1 - A_2)$$

Where, ΣA = the total area; A_1 = first area value; and A_2 = second area value from two successive chromatograms.

(e) Identification of the components

Identification of the chemical constituents was based on comparisons of their Kovats indices (KI) (Van den Dool and Kratz, 1963), their retention times (RT) and mass spectra with those obtained from authentic standards and/or the NIST 05 and Wiley 275 libraries spectra and literature (Adams, 2001; Massada, 1976).

b. Determination of antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capability test

The method of Ho *et al.* (2008); Cuendet *et al.* (1997); Kirby and Schmidt (1997); Burits *et al.* (2001) and Ho *et al.* (2008) were adopted. Fifty μl of various dilutions of the essential oil

were mixed with 5 mL of a 0.004% methanol solution of DPPH. After an incubation period of 30 min, the absorbance of the samples was read at 517 nm using a Jasco 7800 spectrophotometer. Tests were carried out in triplicate. Ascorbic acid was used as a positive control.

c. Determination of the antifungal activity

(a) Fungal strains

The mildew and wood decay fungi were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute. For the mildew strains, references of ASTM G21, JIS Z 2911 and AATCC Test Method 30 were consulted. A total of 7 strains including *Aspergillus clavatus* (ATCC 1007), *Aspergillus niger* (ATCC 6275), *Chaetomium globosum* (ATCC 6205), *Cladosporium cladosporioides* (ATCC 13276), *Myrothecium verrucaria* (ATCC 9095), *Penicillium citrinum* (ATCC 9849), and *Trichoderma viride* (ATCC8678) were tested. For the wood decay fungi, the related standard methods of CNS (Chinese National Standard) and JIS were consulted and 2 strains of white rots: *Trametes versicolor* (CCRC 35253), and *Phanerochaete chrysosporium* (ATCC 24725); and 2 strains of brown rots: *Phaeolus schweinitzii* (ATCC 38047), and *Laetiporus sulphureus* (CCRC 35305) were tested. Cultures of each of the fungi were maintained on a potato dextrose agar (PDA) medium and stored at 4°C.

(b) Antifungal assays

Briefly, 2000, 1500, 1000, 750, 500, 250, and 100 μg /mL of the essential oils were added to sterilized PDA in 9 cm plates (Petri dish), respectively. After the mycelia of 11 fungi strains were also transferred, the Petri dishes were incubated at 26 ± 2°C and 70% relative humidity in the dark. When the mycelia of fungi had reached the edges of the control Petri dishes (those without essential oils), the antifungal indices were calculated. Each test was repeated three times and the data were averaged. The equation of antifungal index is as follows:

Antifungal index (%) = (1 - Da/Db) × 100

where Da is the diameter of the growth zone in the test dish (cm) and Db is the diameter of the growth zone in the control dish (cm).

III. RESULTS AND DISCUSSION

(I) Yields of leaf, flower, and twig essential oils

a. Oil yields by the hydrodistillation method

The leaf, flower, and twig essential oils yields after hydrodistillation of the linalool tree were 3.94 ± 0.06 , 2.77 ± 0.12, and 0.59 ± 0.04 ml/100 g of o.d. materials, respectively. Among these the twigs had the lowest essential oil yield, and the leaves had the highest yield.

b. Oil yields by the HS-GC method

The mędium values of the total area corresponding to each volume of the leaf, flower, and twig oil submitted to the multiple headspace extraction on HS-GC (Table 1), were calculated by means of the previously described equation. The leaf, flower, and twig oil calibration curves obtain from those values corresponds to a simple regression equation of the form $y = a + bx$. The equation of leaf oil was $a = -11.956$, $b = 2468.6$, and $r^2 = 0.9942$; the equation of flower oil was $a = -60.889$, $b = 2893.4$, and $r^2 = 0.9915$; the equation of twig oil was $a = -33.809$, $b = 2405.6$, and $r^2 = 0.9924$.

Table 1. The values of the total area corresponding to each quantity of leaf, flower and twig oils subjected to MHE on HS-GC

Oil(μl)	Area		
	Leaf	Flower	Twig
0.1	216.66 ± 3.2	244.93 ± 3.4	210.16 ± 8.3
0.2	504.95 ± 3.0	468.14 ± 7.1	436.92 ± 6.4
0.3	753.23 ± 4.2	719.19 ± 5.8	681.68 ± 8.2
0.4	927.84 ± 2.1	1069.23 ± 5.5	866.64 ± 7.2
0.5	1170.59 ± 3.4	1454.96 ± 7.4	1132.83 ± 10.8
0.6	1527.12 ± 3.7	1693.45 ± 6.4	1486.88 ± 12.6

Table 2 shows the peak area values corresponding to different quantities of plant material (leaves, flowers, and twigs) submitted to the MHE on the HS-GC and by extrapolating of the area values of the leaf, flower and twig oils calibration curve, we obtained three values for the leaf, flower and twig oils of respectively of 3.93 ± 0.11, 2.72 ± 0.10 and 0.61 ± 0.03 ml/100 g of o.d. materials. These results were nearly identical to the oil yield results of the hydrodistillation method which were respectively 3.94 ± 0.06, 2.77 ± 0.12 and 0.59 ± 0.04 ml/100 g of o.d. materials. Thus, our results indicated that HS-GC method can provide reliable essential oil yields from various plant materials which are comparable to those obtained by the hydrodistillation method (Table 3).

Table 2. Area values corresponding to different quantity of plant material (leaf, flower and twig) subjected to MHE on HS-GC

Plant material (mg)	Area		
	Leaf	Flower	Twig
10	960.82 ± 4.8	763.96 ± 5.3	116.67 ± 3.8
20	1886.34 ± 6.3	1453.32 ± 8.6	242.34 ± 5.2
30	2839.62 ± 10.6	2271.89 ± 9.2	388.96 ± 8.9
40	4021.46 ± 8.6	3094.32 ± 12.1	590.98 ± 6.1

Table 3. Chemical compositions of essential oils obtained from *Cinnamomum camphora* var. *linaloolifera* leaf, flower and twig by hydrodistillation extraction and headspace methods

Peak no.	Consituents	K.I.[a]	Concentration (%)						Identification[d]
			Leaf		Flower		Twing		
			HD[b]	HS[c]	HD	HS	HD	HS	
1	α-thujene	930	t[e]	-[f]	t	-	t	t	MS, KI, ST
2	α-pinene	939	0.4	0.4	0.9	1.0	1.1	1.1	MS, KI, ST
3	camphene	954	0.1	0.1	0.2	0.2	0.5	0.5	MS, KI, ST
4	*trans*-pinene	975	-	-	-	-	t	-	MS, KI
5	sabinene	975	t	t	-	-	0.1	0.1	MS, KI, ST
6	β-pinene	979	0.2	0.2	0.3	0.4	0.3	0.3	MS, KI, ST
7	β-myrcene	991	0.2	0.1	0.1	0.1	0.4	0.5	MS, KI, ST
8	α-phellandrene	1003	0.1	0.1	0.1	0.1	0.1	0.2	MS, KI, ST
9	*p*-mentha-1(7),8-diene	1004	t	t	-	-	-	-	MS, KI, ST
10	α-terpinene	1017	t	t	t	t	0.2	0.2	MS, KI, ST
11	*p*-cymene	1025	0.4	0.4	t	t	0.5	0.5	MS, KI, ST
12	limonene	1029	0.3	0.3	0.7	0.8	1.3	1.4	MS, KI, ST
13	1,8-cineol	1031	0.1	0.1	0.2	0.2	3.0	3.0	MS, KI, ST
14	*cis*-ocimene	1037	0.1	0.1	0.2	0.2	0.1	0.1	MS, KI
15	trans-β-ocimene	1050	0.1	0.1	0.7	0.7	0.1	0.1	MS, KI, ST
16	γ-terpinene	1060	-	-	t	t	0.2	0.2	MS, KI, ST
17	*trans*-linalool oxide (furanoid)	1073	0.3	0.2	0.1	0.1	0.2	0.2	MS, KI
18	*cis*-linalool oxide (furanoid)	1087	0.5	0.5	0.1	0.1	0.3	0.3	MS, KI
19	terpinolene	1089	t	0.1	t	t	0.2	0.2	MS, KI, ST
20	linalool	1097	87.3	87.2	72.4	71.6	40.0	40.3	MS, KI, ST
21	hotrienol	1110	0.7	0.7	1.3	1.3	0.6	0.7	MS, KI
22	*endo*-fenchol	1117	t	-	-	-	t	-	MS, KI, ST
23	camphor	1146	0.7	0.8	1.7	1.8	33.5	33.3	MS, KI, ST
24	camphene hydrate	1150	1.5	1.5	1.8	1.7	-	-	MS, KI, ST
25	*cis*-tagetone	1152	0.1	0.1	-	-	-	-	MS, KI, ST
26	nerol oxide	1158	-	-	0.1	0.1	-	-	MS, KI
27	δ- terpineol	1166	t	-	t	-	0.1	-	MS, KI
28	borneol	1169	-	-	-	-	0.1	0.1	MS, KI
29	*cis*-linalool oxide (pyranoid)	1174	0.1	-	t	-	-	-	MS, KI
30	4-terpineol	1177	0.1	0.1	0.1	0.1	0.8	0.8	MS, KI, ST
31	*p*-cymen-8-ol	1183	-	-	-	-	t	-	MS, KI, ST

Table 3. Chemical compositions of essential oils obtained from *Cinnamomum camphora* var. *linaloolifera* leaf, flower and twig by hydrodistillation extraction and headspace methods (Cont'd 1)

Peak no.	Consituents	K.I.[a]	Concentration (%)						Identification[d]
			Leaf		Flower		Twing		
			HD[b]	HS[c]	HD	HS	HD	HS	
32	*cis*- pinocarveol	1184	-	-	t	-	-	-	MS, KI
33	*cis*-hexenyl butyrate	1186	0.1	-	-	-	-	-	MS, KI
34	α-terpineol	1189	0.4	0.4	0.3	0.3	2.1	2.1	MS, KI, ST
35	n-decanal	1202	-	-	-	-	0.1	0.1	MS, KI
36	*cis*-pulegol	1229	0.1	0.1	t	-	-	-	MS, KI
37	nerol	1230	0.1	0.1	t	-	0.1	0.1	MS, KI
38	neral	1238	0.1	0.1	0.1	0.1	-	-	MS, KI
39	geraniol	1253	0.2	0.2	0.1	0.1	0.5	0.5	MS, KI
40	geranial	1267	t	-	-	-	-	-	MS, KI
41	safrole	1287	t	-	t	-	0.6	0.6	MS, KI
42	bornyl acetate	1289	-	-	-	-	t	t	MS, KI, ST
43	α-cubebene	1351	t	t	t	t	0.1	0.1	MS, KI, ST
44	eugenol	1359	-	-	-	-	3.6	3.4	MS, KI, ST
45	α-ylangene	1375	t	t	0.1	0.1	t	-	MS, KI
46	α-copaene	1377	t	-	0.1	0.1	0.1	0.1	MS, KI, ST
47	β-bourborene	1388	t	-	-	-	-	-	MS, KI
48	β-elemene	1391	0.2	0.2	0.3	0.3	0.2	0.1	MS, KI
49	methyl eugenol	1404	0.1	0.1	0.4	0.4	0.8	0.8	MS, KI, ST
50	dodecanal	1407	-	-	t	t	t	-	MS, KI
51	longifolene	1408	-	-	0.1	0.1	-	-	MS, KI
52	α-cedrene	1412	-	-	-	-	t	-	MS, KI, ST
53	β-caryophyllene	1419	2.1	2.2	5.3	5.5	1.5	1.5	MS, KI, ST
54	β-copaene	1432	t	t	0.1	t	-	-	MS, KI, ST
55	γ-elemenene	1437	-	-	t	-	-	-	MS, KI
56	α-guaiene	1440	t	t	0.2	0.3	-	-	MS, KI
57	2-methyl butyl benzoate	1441	t	-	-	-	-	-	MS, KI
58	muurola-3,5-diene	1450	-	-	0.1	0.1	-	-	MS, KI
59	α-carophyllene	1455	0.6	0.6	1.8	1.9	0.7	0.7	MS, KI, ST
60	*allo*-aromadendrene	1460	t	-	t	-	t	-	MS, KI, ST
61	drima-7,9(11)-diene	1473	-	-	t	-	-	-	MS, KI
62	*trans*-cadina-1(6),4-diene	1477	-	-	-	-	0.1	-	MS, KI

Table 3. Chemical compositions of essential oils obtained from *Cinnamomum camphora* var. *linaloolifera* leaf, flower and twig by hydrodistillation extraction and headspace methods (Cont'd 2)

Peak no.	Consituents	K.I.[a]	Concentration (%)						Identification[d]
			Leaf		Flower		Twing		
			HD[b]	HS[c]	HD	HS	HD	HS	
63	β-chamigene	1478	t	-	-	-	0.1	0.2	MS, KI
64	γ-muurolene	1480	0.2	0.3	0.5	0.5	-	-	MS, KI, ST
65	germacrene D	1485	-	-	0.4	0.4	0.1	0.2	MS, KI, ST
66	β-selinene	1490	0.8	0.9	2.9	2.9	0.5	0.6	MS, KI
67	methyl isoeugenol	1492	t	t	0.4	0.4	0.2	0.1	MS, KI
68	α-selinene	1498	0.1	0.1	1.4	1.5	0.3	0.3	MS, KI
69	α-muurolene	1500	t	t	0.4	0.4	-	-	MS, KI, ST
70	*trans*-β-guaiene	1503	0.1	0.1	-	-	t	-	MS, KI, ST
71	β-bisabolene	1506	-	-	t	-	0.2	0.2	MS, KI, ST
72	α-cuprenene	1506	-	-	-	-	t	-	MS, KI
73	γ-cadinene	1514	t	t	0.1	0.1	0.1	0.1	MS, KI, ST
74	δ-cadinene	1523	0.1	0.1	0.5	0.5	0.6	0.6	MS, KI, ST
75	*trans*-calamenene	1529	-	-	t	-	0.2	0.2	MS, KI, ST
76	*trans*-γ-bisabolene	1531	-	-	-	-	0.1	0.1	MS, KI
77	*trans*-cadina-1(2),4-diene	1535	t	-	0.1	-	0.1	0.1	MS, KI
78	α-cadiene	1539	t	-	0.1	0.1	t	t	MS, KI, ST
79	α-calacorene	1546	t	-	t	0.1	0.1	0.1	MS, KI, ST
80	Elemol	1550	-	-	t	-	-	-	MS, KI, ST
81	germacrene B	1561	0.1	0.1	0.1	0.1	t	-	MS, KI
82	(*e*)-nerolidol	1563	0.3	0.3	0.1	0.1	0.1	0.1	MS, KI, ST
83	ledol	1569	-	-	t	-	-	-	MS, KI
84	caryophyllene alcohol	1572	-	-	0.1	0.1	0.2	0.3	MS, KI
85	caryophyllene oxide	1582	0.4	0.3	0.1	0.1	0.2	0.2	MS, KI, ST
86	globulol	1585	t	-	0.2	0.2	0.1	0.2	MS, KI, ST
87	guaiol	1601	-	-	0.1	0.1	0.1	0.1	MS, KI
88	sesquithuriferol	1605	-	-	0.1	-	0.1	0.1	MS, KI
89	5-*epi*-7-*epi*-α-eudesmol	1608	-	-	0.1	0.1	-	-	MS, KI
90	humulene epoxide II	1608	0.1	t	-	-	0.1	0.1	MS, KI
91	tetradecanal	1613	-	-	-	-	0.1	0.1	MS, KI
92	*epi*-cedrol	1619	-	-	0.1	0.1	t	-	MS, KI
93	β-cedrene epoxide	1623	-	-	0.1	-	0.1	0.1	MS, KI

Table 3. Chemical compositions of essential oils obtained from *Cinnamomum camphora* var. *linaloolifera* leaf, flower and twig by hydrodistillation extraction and headspace methods (Cont'd 3)

Peak no.	Consituents	K.I.[a]	Concentration (%)						Identification[d]
			Leaf		Flower		Twing		
			HD[b]	HS[c]	HD	HS	HD	HS	
94	10-*epi*-γ-eudesmol	1624	-	-	0.3	0.3	0.1	0.1	MS, KI
95	1-*epi*-cubenol	1629	t	-	0.1	0.1	0.5	0.5	MS, KI
96	γ-eudesmol	1632	-	-	-	-	0.1	0.1	MS, KI, ST
97	β-acorenol	1637	-	-	-	-	t	-	MS, KI
98	T-cadinol	1640	-	-	0.1	0.1	0.5	0.5	MS, KI
99	T-muurolol	1642	-	-	0.1	0.1	-	-	MS, KI
100	α-muurolol	1646	-	-	-	-	0.1	0.1	MS, KI, ST
101	β-eudesmol	1651	0.1	0.2	-	-	0.7	0.7	MS, KI, ST
102	α-eudesmol	1654	-	-	0.3	0.3	-	-	MS, KI, ST
103	α-cadinol	1654	-	-	0.5	0.6	-	-	MS, KI, ST
104	3-thujopsanone	1655	t	-	-	-	-	-	MS, KI
105	*neo*-intermedeol	1660	-	-	-	-	0.1	0.1	MS, KI
106	*epi*-β-bisabolol	1672	-	-	-	-	t	-	MS, KI
107	*epi*-α-bisabolol	1685	-	-	-	-	t	-	MS, KI
108	α-bisabolol	1686	-	-	-	-	0.1	0.1	MS, KI
109	eudesma-7,(11)-en-4-ol	1700	-	-	-	-	t	-	MS, KI
110	(2Z,6Z)-farnesol	1718	0.1	0.1	-	-	-	-	MS, KI
111	(2E,6E)-farnesol	1725	0.1	0.1	-	-	-	-	MS, KI
grouped components									
Monoterpene hydrocarbons			**1.9**	**2.0**	**3.4**	**3.5**	**5.1**	**5.2**	
Oxygenated monoterpenes			**92.4**	**91.9**	**78.4**	**77.3**	**81.2**	**81.4**	
Sesquiterpene hydrocarbons			**4.6**	**4.7**	**14.7**	**14.8**	**5.1**	**5.1**	
Oxygenated sesquiterpenes			**1.0**	**1.0**	**2.2**	**2.2**	**3.2**	**3.2**	
Others			**0.2**	**0.1**	**0.9**	**0.8**	**5.4**	**5.1**	
Total identified (%)			**100.0**	**99.6**	**99.6**	**98.7**	**100.0**	**100.0**	
Yield (ml/100 g)			**3.94± 0.06**	**3.93± 0.11**	**2.77± 0.12**	**2.72± 0.10**	**0.59± 0.04**	**0.61± 0.03**	

[a] Kovats index on a DB-5 column in reference to n-alkanes (Van den Dool and Kratz, 1963). [b] HD : Hydrodistillation extraction. [c] HS : Headspace method. [d] MS : NIST and Wiley libraries spectra and literature, KI : Kovats index, ST : Authentic standard compounds. [e] trace : < 0.05%. [f] —:not detected

(II) Compositions and content of the leaf, flower, and twig essential oils

a. The leaf essential oil

A total of 68 compounds were identified from the hydrodistillated leaf oil. The main constituent was linalool which made up 87.3% of the total. Other compounds in decreasing order of amounts were β-caryophyllene (2.1%), camphene hydrate (1.5%), β-selinene (0.8%), camphor (0.7%), hotrienol (0.7%) etc. Furthermore, if the essential oils were grouped as monoterpene hydrocarbon, oxygenated monoterpene, sesquiterpene hydrocarbon, and oxygenated sesquiterpene, then oxygenated monoterpene made up the greatest share of 92.4%, with sesquiterpene hydrocarbons (4.6%), monoterpene hydrocarbon (1.9%) and oxygenated sesquiterpenes (1.0%) making up the rest.

HS-GC analysis, on the other hand, identified 49 compounds from the extracted leaf oil. Again linalool making up 87.2% of the total was the main constituent. Other major compounds were similar to those observed in the hydrodistillated oil but with slightly different ordering of β-caryophyllene (2.2%), camphene hydrate (1.5%), β-selinene (0.9%), camphor (0.8%), hotrienol (0.7%) etc.

The results of linalool trees leaf oil compositions are similar to those obtained by Lin and Hua (1987), Tao *et al.*, (1987), Shi *et al.*, (1989), Jantan and Goh (1992), Zhu *et al.*, (1993), and Shieh (2003a), all were having linalool predominant. The minor components identified in our study, however, were more numerous than the other reports.

b. The flower essential oil

A total of 77 compounds were identified from the hydrodistillated flower oil. The main component was linalool, making up 72.4% of the total. Other compounds in decreasing order of amounts were β-caryophyllene (5.3%), β-selinene (2.9%), α-caryophyllene (1.8%), camphene hydrate (1.8%), camphor (1.7%), α-selinene (1.4%), hotrienol (1.3%) etc. On the other hand, HS-GC method identified a total of 59 compounds also having linalool as the main component (71.6%) and other compounds in the oil were β-caryophyllene (5.5%), β-selinene (2.9%), α-caryophyllene (1.9%), camphor (1.8%), camphene hydrate (1.7%), α-selinene (1.5%), hotrienol (1.3%) etc. Both methods produced the greatest fraction of oxygenated monoterpenes followed by sesquiterpene hydrocarbons, monoterpene hydrocarbons and oxygenated sesquiterpenes. The analyses clearly showed that the flower oil of the tree with its physiological relatedness to leaves, was quite similar to the former as well, and was composed mainly of linalool. This is the first report of the flower oil composition.

c. The twig essential oil

Hydrodistillated essential oil of the linalool tree twigs was composed of 83 compounds with mostly linalool making up 40.0% and camphor 33.5%. Other components in decreasing order were eugennol (3.6%), 1,8-cineole (3.0%), α-terpineol (2.1%), β-caryophyllene (1.5%), limonene (1.3%), α-pinene (1.1%) etc. In contrast, the HS-GC method identified a total of 65 twig oil compounds, also mostly linalool (40.3%) and camphor (33.3%). Other compounds had the same ordering and nearly identical quantity fraction; *i.e.*, eugenol (3.4%), 1,8-cineole (3.0%), α-terpineol (2.1%), β-caryophyllene (1.5%), limonene (1.4%), α-pinene (1.1%) etc. Both methods gave mostly oxygenated monoterpenes, with monoterpene hydrocarbons, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes

in decreasing order of amounts.

The above yields and compositions indicated that both hydrodistillation and HS-GC methods gave comparable leaf, flower and twig oils yields. When the composition of these oils was compared, however, certain minor components obtained by hydrodistillation (content < 0.1%) could not be detected by HS-GC. The major reason was simply due to the small size of the specimens used in the latter method, as the former needed ca. 1 kg of sample, while HS-GC only took 20 mg, causing the minor component yields to fall below detection limits. Overall, the HS-GC yielded main components and compound groups similar to those of the hydrodistillation results. The methodology proved that HS-GC can be an effective method for an essential oil compositional analysis; furthermore, it requires only a minute amount of specimen and a long period of distillation is not needed (Ho *et al.*, 2008).

(III) The antioxidative activities of the oils of various tree parts

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capability

The essential oils from renewable parts of the linalool tree (leaves, flowers and twigs) were tested for their DPPH free radical scavenging capability. Ascorbic acid was used as a positive control and the results are shown in Fig. 1. The IC_{50} of DPPH free radical scavenging capability of the twig oil was the best at 104 μg/ml , whereas those of leaf and flower oils were inferior, requiring more than 2000 μg/ml. The individual main components of the twig oil such as linalool, camphor, 1,8-cineole, α-terpineol, β-caryophyllene, limonene, eugenol and α-pinene were also compared for their DPPH free radical scavenging capability. The results showed the DPPH free radical scavenging capabilities, and established a decreasing order of eugenol, linalool, camphor, 1,8-cineole, α-terpineol, β-caryophyllene, limonene and α-pinene. Hence, we deduced that phenolic compounds were the main sources responsible for radical scavenging. The results are also in congruence with the conclusions of several other reports (Ruberto and Baratta, 2000; Yildirim *et al.*, 2001; Mau *et al.*, 2003; Ho *et al.*, 2008) (Table 4).

When the DPPH free radical scavenging capabilities of these 3 oils were compared with those of leaf oil of different provenances of Taiwan cinnamon (*Cinnamomum osmophloeum*) with IC_{50} values ranging from 33.38 to 708.55 μg mL^{-1} (Chen, 2003), The twig oils was within the range and showed comparable free radical scavenging capability. The threshold concentration compared favorably with the IC_{50} values of 460 μg/ml of the leaf oils of black seed oil (*Nigella sativa*) (Buris and Bucar, 2000), 500 μg/ml of the flower oil of oregano (*Origanum vulgare*) (Kulisic *et al.*, 2004), and the 6000 μg/ml of the leaf oil of turmeric (*Curcuma zedoaria*) (Mau *et al.*, 2003).

(IV) The antifungal activities of the oils of various tree parts

a. The anti-mildew activities

Figure 2 shows the performance indices of the leaf, flower and twig oils of the linalool tree in suppressing 7 mildew fungi at a 1000 μg/ml concentration. All 3 oils showed total suppression of *C. globosum, M. verrcaia*, and *T. viride* fungi. Suppression efficiency of *C. cladosporioides* was next, with the leaf and twig oils performed better. Then the suppression of *A. clavatus* and *A. niger* ensued. With regard to the growth suppression of *A. clavatus*, the leaf, flower and twig oils had indices of 81, 60 and 62%, respectively. All 3 oils were not so effective in

suppressing *A. niger*; whereas *P. citrinum* was the most difficult to suppress for all 3 oils.

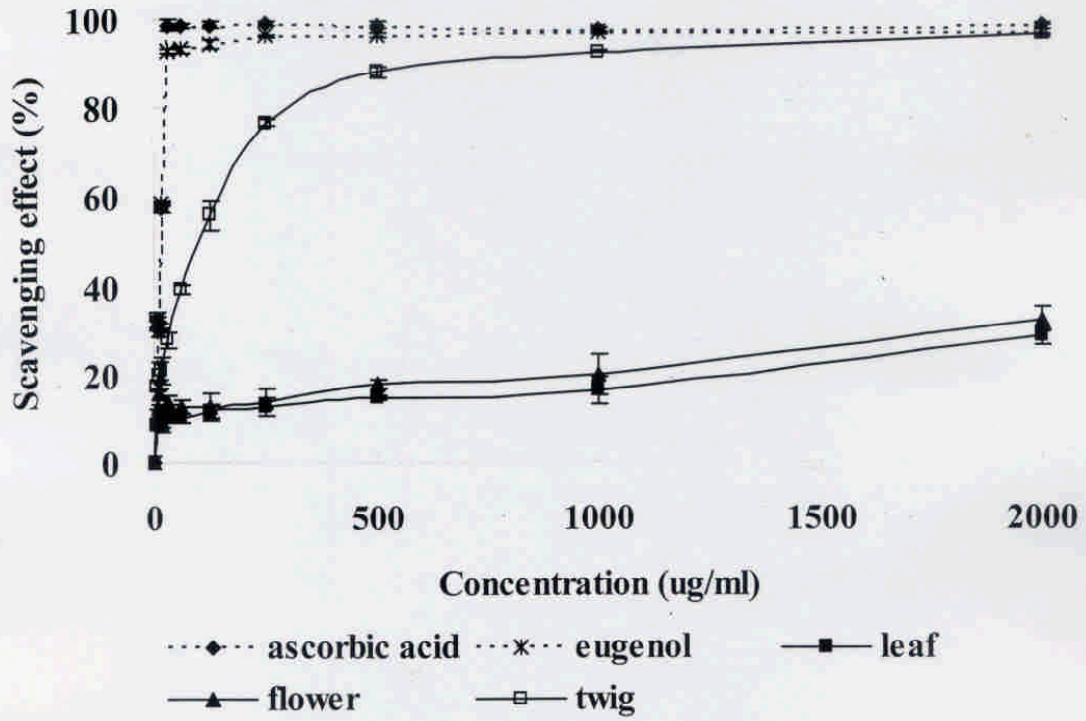

Fig. 1. DPPH free radical scavenging effects of essential oils from *C. camphora* var. *linaloolifera* leaf, flower and twig.

Table 4. IC_{50} of *C. camphora* var. *linaloolifera* leaf, flower and twig extracts in scavenging DPPH free radical

Sample	IC_{50} (μg/ml)
Ascorbic acid	13.5±0.3
Leaf	>2000
Flower	>2000
Twig	104±3.2
eugenol	13.6±0.8
linalool	>2000
camphor	>2000
1,8-cineole	>2000
α-terpineol	>2000
β-caryophyllene	>2000
limonene	>2000
α-pinene	>2000

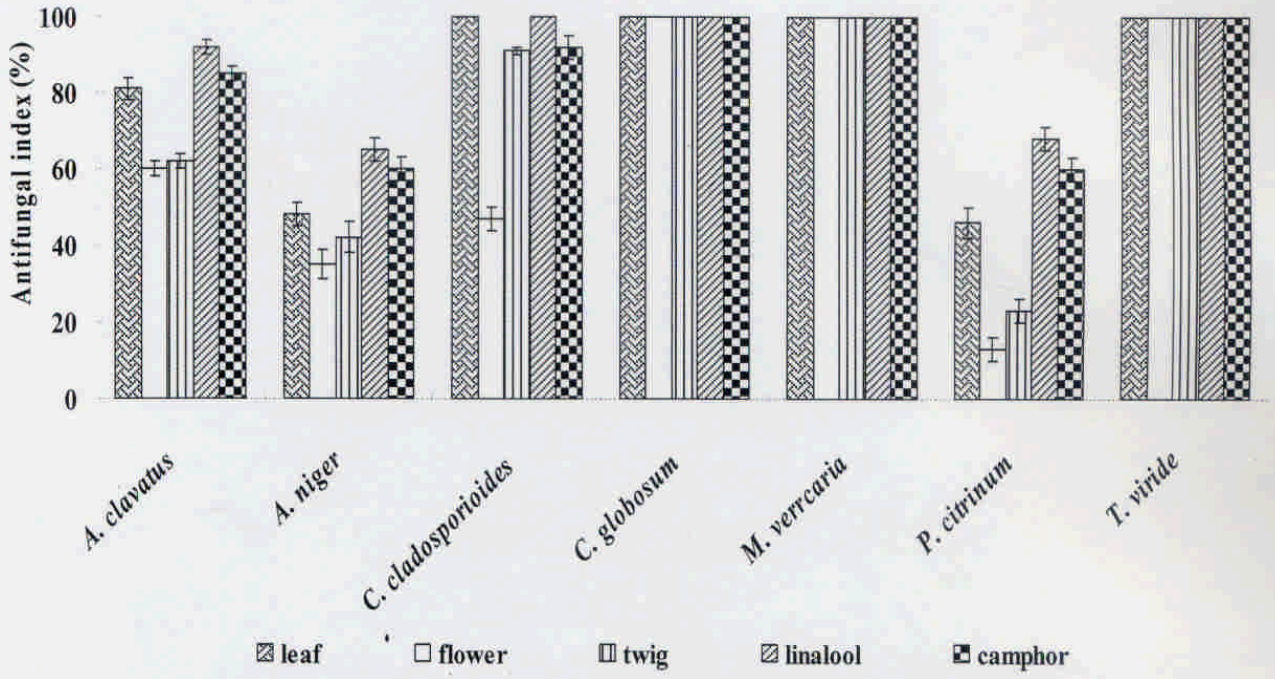

Fig. 2. Antifungal activities of essential oils (1000 μg/ml) extracted from three different tissues of *C. camphora* var. *linaloolifera* against seven mildew. Each experiment was performed three times, and the data were averaged (n=3).

Note: *A. clavatus: Aspergillus clavatus*
A. niger: Aspergillus niger
C. globosum: Chaetomium globosum
C. cladosporioides: Cladosporium cladosporioides
M. verrucaria: Myrothecium verrucaria
P. citrinum: Penicillium citrinum
T. viride: Trichoderma viride

Furthermore, the MIC and IC_{50} values by the 3 oils against the 7 fungi are summarized in Table 5. Although different oil has varying growth suppression activities against different fungi, but in general, leaf oil showed the best activities and twig and flower oils followed in a decreasing order. The IC_{50} values of leaf oil against the 7 fungi, except for *A. niger* and *P. citrinum* which required 1011 and 1263 μg/ml of the oil, respectively, all other fungi had IC_{50} value of < 500 μg/ml of the oil. And for *C. globosum* and *T. viride*, merely 250 and 100 μg/ml of the oil could achieve total suppression.

b. The anti-wood decay fungal activities

Figure 3 shows the anti-wood decay fungal activities of the leaf, flower and twig oils of the linalool tree against 4 kinds of wood decay fungi at 1000 μg/ml concentrations. In general, excellent growth suppression against the 4 fungi were achieved. For white rot *P. chrysosprium* and both brown rots *P. schweintzii* and *L. sulphureus* the suppression was near total. Their growth suppression performance against *T. versicolor* were 65, 60 and 56% for the leaf, twig, and flower oils.

Table 5. MIC and IC_{50} (μg/ml) values of essential oils from three different tissues of *C. camphora* var. *linaloolifera* against seven mildew

Fungal species	Leaf		Flower		Twig		Linalool		Camphor	
	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
Aspergillus clavatus	1500	464	2000	914	2000	827	1500	362	1500	405
Aspergillus niger	1500	1011	2000	1206	2000	1147	1500	1009	1500	1012
Cladosporium cladosporioides	1000	365	2000	1157	1500	450	1000	285	1000	328
Chaetomium globosum	<100	<100	250	100	250	<100	<100	<100	<100	<100
Myrothecium verrcaria	500	165	1000	674	1000	492	250	<100	250	<100
Penicillium citrinum	1500	1263	>2000	>2000	>2000	1682	1500	1086	1500	1112
Trichoderma viride	250	<100	750	462	750	449	250	<100	250	<100

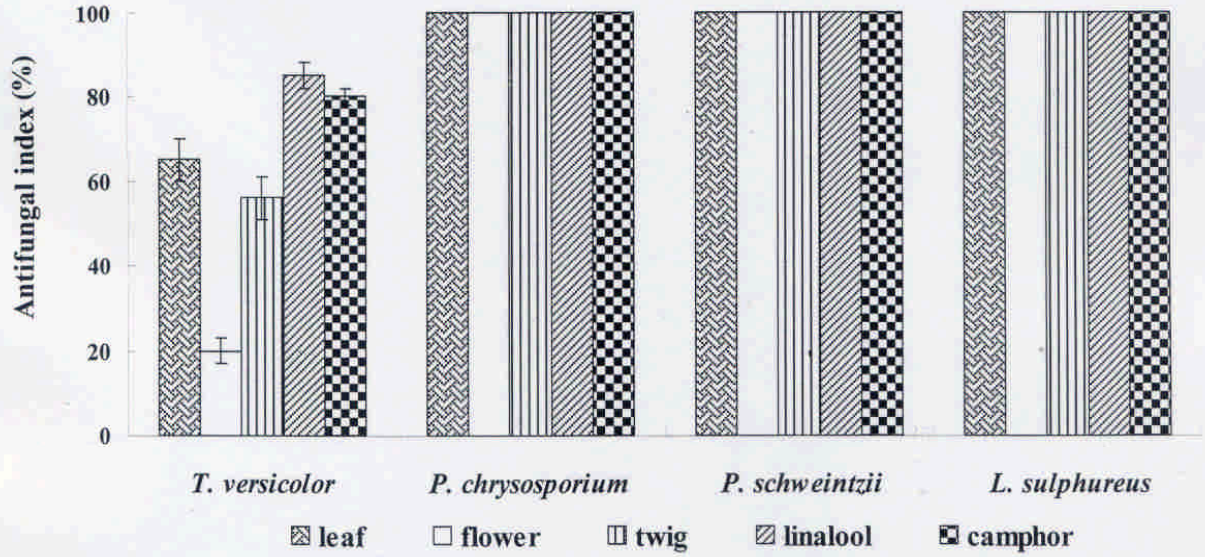

Fig. 3. Antifungal activities of essential oils (1000 μg/ml) extracted from three different tissues of *C. camphora* var. *linaloolifera* against four wood decay fungi. Each experiment was performed three times, and the data were averaged (n=3).

Note: *T. versicolor: Trametes versicolor*
P. chrysosporium: Phanerochaete chrysosporium
P. schweintzii: Phaeolus schweintzii
L. sulphureus: Laetiporus sulphureus

Table 6 shows the MIC and IC_{50} values of the 3 oils against the 4 kinds of wood decay fungi. All 3 oils showed strong inhibition of *P. chrysosporium*, *P. schweintzii*, and *L. sulphureus*. They were less effective against the growth of *T. versicolor*, however.

Table 6. MIC and IC_{50} (μg/ml) values of essential oils from three different tissues of *C. camphora* var. *linaloolifera* against four wood decay fungi

Fungal species	Leaf		Flower		Twig		Linalool		Camphor	
	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
Trametes versicolor	1500	960	2000	1026	2000	982	1500	706	1500	758
Phanerochaete chrysosporium	250	<100	250	<100	250	<100	<100	<100	<100	<100
Phaeolus schweintzii	<100	<100	250	<100	250	<100	<100	<100	<100	<100
Laetiporus sulphureus	<100	<100	250	<100	250	<100	<100	<100	<100	<100

In summary, the antifungal tests indicated that all 3 oils had excellent antifungal activities, particularly the leaf oil. As the main component of leaf and flower oils was linalool, literature shows that Reueni *et al.* (1984); Pattnaik *et al.* (1997); Nakahara *et al.* (2003), and Rakotonirainy and Lavedrine (2005) also noted its strong antifungal capability. Maruzzella *et al.* (1960) noted that it has excellent inhibition of wood-destroying fungi. As for the antifungal activity of camphor, Mario *et al.* (1998); Pitarokili *et al.* (2003), and Kordali *et al.* (2005) had also noted its excellent activities. Thus all three oils derived their excellent antifungal activities largely from the presence of linalool and camphor.

IV. CONCLUSIONS

In this study, we examined the renewable tree parts of leaves, flowers, and twigs of a linalool tree essential oils. Both hydrodistillation and HS-GC methods were used to establish the essential oil compositions and yields. Experimental confirmation of HS-GC using the MHE mode could produce nearly identical results with those of the hydrodistillation method.

The oils obtained from the hydrodistillation methods were tested for their antioxidant and antifungal activities. Among the oils, the twig oil had the best performance with IC_{50} of merely 104 μg/ml; and the main ingredient responsible for the activity was eugenol. For the antifungal activities, leaf oil had the best performance and all 3 oils had excellent inhibition activities against wood-destroying fungi examined.

Thus the renewable tree parts of the linalool trees, in addition to being good raw materials for making high-grade fragrance agent, are also potentially capable of antioxidant and antifungal applications, which bode well toward multipurpose utilization of the essential oils.

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