Composition and Bioactivities of the Leaf Essential Oils of *Cinnamomum subavenium* Miq. from Taiwan

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Abstract

Leaves of *Cinnamomum subavenium* Miq. were collected from two sites in Taiwan. Leaf essential oils of *C. subavenium* were isolated using hydrodistillation and headspace-GC methods to determine their composition and yield. Antioxidant and antimicrobial activities of various oils obtained were also evaluated. Forty-four and 88 compounds were identified in the leaf oils obtained. Whereas 26 and 65 compounds were identified by headspace-GC method, respectively. The main headspace components were comparable to those of the oil. The main components of one of the oils were methyl eugenol (75.9%), linalool (7.3%), and eugenol (6.6%); while those from the other oil were p-cymene (21.6%), 1,8-cineole (16.5%), and linalool (11.9%). Both leaf oils had excellent antioxidant and antimicrobial activities.

Key Word Index

Cinnamomum subavenium, Lauraceae, essential oil composition, methyl eugenol, p-cymene, 1,8-cineole, linalool, headspace volatiles, antioxidant activity, antimicrobial activity.

Introduction

Cinnamomum subavenium Miq. is a evergreen tree of the *Cinnamomum* genus, (Lauraceae). It is distributed in Taiwan, central to southern China, Myanmar, Cambodia, Vietnam, Malaysia, and Indonesia. The tree is distributed in regions between 500 and 1500 m in elevation. Indigenous Lauraceae family plants are numerous, and the essential oils of many species of the group have been isolated, their compositions identified, and their bioactivities were evaluated (1–8). However, in Taiwan there is no record of the essential oil composition and bioactivities reported for *C. subavenium*. Worldwide, only three reports by Jantan et al. (9) in Malaysia, and Zheng et al. (10) and Zhu et al. (11) of China are known. Furthermore, all three papers only dealt with the compositions and there was no comment on their bioactivities.

Initially we used hydrodistillation and headspace-GC methods to isolate leaf oils and headspace volatiles, and GC/FID and GC/MS to compare examine the variation in leaf oil compositions from two different collection sites. With regard to the oil yields, we used the multiple headspace extraction (MHE) of the Headspace-GC (HS-GC) method to conduct the comparative analysis and evaluated the suitability of applying the method as a basis to differentiate chemo-types among different provenances of a species. The second part of the study involved bioactivity analysis, including antioxidant and antimicrobial activities, for multi-purpose utilization of the leaf essential oil of *C. subavenium*.

Experimental

Plant Materials: In August 2005, we collected *Cinnamomum subavenium* leaf samples from the Fushan Botanical Garden (FSB) of northeastern Taiwan, and the Lienhuachih Research Center (LHC) in central Taiwan, both of the Taiwan Forestry Research Institute, and both plantations were 22 years old. Leaves of the species were collected for subsequent extraction and analysis.

Isolation of leaf oils and determination of composition and yield Hydrodistillation extraction: A kilogram of the leaves of *C. subavenium* was placed in a round-bottom flask and 3 L of distilled water poured in. Hydrodistillated for 8 h and the essential oil removed from partitioned water layer. Anhydrous sodium sulfate was added to dewater. The yield of oil was deter-

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mined. All test data are the average of triplicate analyses.

GC and GC/MS analyses: 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/HP 5973 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 were the ones listed above and the MS was operating (full scan mode: scan time: 0.3 s, mass range was m/z 30–500) in the EI mode at 70 eV. All test data are the average of triplicate analyses.

Oil yield: The total amount of oil in each sample was determined by HS-GC. Calibration curves were made with different quantities (0.1; 0.2; 0.3; 0.4; 0.5 and 0.6 μL) of FSB and LHC leaf essential oils previously obtained by hydrodistillation. A special quantitative method, MHE, was used. According to Kolb (12), the matrix effect can be eliminated by using the MHE method. The total area of each oil volume was calculated according to the following equation:

$$\Sigma A = A_1^2/(A_1 - A_2) \quad \textbf{(a)}$$

Where: ΣA is the total area; A_1 is the first area value; A_2 is the second area volume from 2 successive chromatograms.

The HS-GC analyses were accomplished using a Hewlett-Packard HP6890 GC a equipped with a FID detector and combined with a Perkin Elmer Headspace Turbomatrix 40. The GC analysis programs used were as described in the above section. Conditions of the headspace sampler were as follows: the sample size was 0.1 μl oil and 20 mg plant material (dried leaves). The MHE analyses of the FSB and LHC oils the vial oven and transfer line temperature were both 100°C; the needle temperature was 110°C; treatment time in the oven with shaking was 50 min; pressurization time was 3.0 min; thermostat time was 50 min.

Component identification: Identification of the leaf essential oil chemical constituents was based on comparisons of the peaks Retention indices (RI) (13), their retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra and literature (14–15).

Determination of antioxidative activityDPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capability test: The method of Cuendet et al. (16) Kirby and Schmidt (17) and Burits et al. (18) was adopted. Fifty μL of various dilutions of the oils 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 determined at 517 nm using a Jasco 7800 spectrophotometer. Tests were also carried out in triplicates, and ascorbic acid was used as a positive control.

Determination of total phenolic content: In accordance with the method of Julkunen-Titto (19), 50 μl of different concentrations of the oils in methanol solutions were placed in 10 mL test tubes and 2 mL of distilled water and 1 mL of 100% Folin-Ciocalteus phenol reagent were added and mixed together. Then 5 mL of a 20% sodium carbonate solution was added and mixed. The test tubes were placed at room temperature for 20 min. Then the light absorbance of the solution at a wavelength of 735 nm was measured. Gallic acid was used as a standard to construct a calibration curve. The total phenolic contents of the specimens were determined as their gallic acid equivalent (GAE) in mg/mL.

Antimicrobial activities of the essential oils Microbial strains: The microbial strains were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute, Hsinchu City, Taiwan. The bacterial cultures included 5 types of 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). For *E. coli, Ent. aerogenes, K. pneumoniae, P. aeruginosa*, and *S. epidermidis* bacteria, a solid culture medium of nutrient agar was used. The medium consisted of 3 g of beef extract, 15 g of peptone, and 15 g of agar dissolved in 1000 mL of distilled water. For *B. cereus, S. aureaus*, and *V. parahaemolyticus* bacteria, a solid culture medium of tryptic soy agar was used. The medium consisted of 15 g of tryptone, 5 g of soytone, 5 g of table salt, and 15 g of agar dissolved in 1000 mL of distilled water. For the *C. albicans* yeast, YPD medium consisted of 10 g of yeast extract, 20 g of peptone, 20 g of dextrose, and 15 g of agar dissolved in 1000 mL of distilled water.

Paper disc diffusion method: This test was carried out in accordance with the method of Cimanga et al. (20). The oils were diluted to the desired concentrations using Tween 80, and then filter paper discs with a diameter of at least 4 mm were impregnated with the solution. Meanwhile, culture media in Petri dishes were prepared, and when the media had congealed, liquid cultures of the bacteria and yeast were evenly spread on the surface of the media. After standing for 3 min, the impregnated paper discs were placed on the surfaces of the inoculated culture media. The concentrations of the specimens tested were 10, 5, 2, and 1 μL/disc, and the concentration of the bacterial cultures was 1 x 10^6 CFU/mL. The Petri dishes were placed in a 37°C incubator for 18 h, and afterward, the diameters of the inhibition zones exhibited on the petri dishes by individual specimens were measured and recorded. The experiment was replicated three times.

Results and Discussion

Leaf oil yields: The leaf oil yields by hydrostillation of the leaves of *C. subavenium* collected from Fushan Botanical Garden (FSB) and Lienhuachih Research Center (LHC) were 0.71%±0.03% and 0.82%±0.04% (v/w), respectively.

Leaf volatiles determination by the HS-GC method: The values of the total area corresponding to each volume of leaf essential oils submitted to the MHE of the headspace-GC were calculated by means of a previously described equation (a) in experimental section. The leaf volatiles calibration curves obtained from those values corresponded to the regression

equation y = a + bx, where values for the FSB leaf volatiles were a = -4,154 and b = 3219.9, r^2 = 0.9992; values for the corresponding equation for the LHC leaf volatiles were a = 16.99 and b = 3352.95, r^2 = 0.9986 (Table I).

Table II shows the area values corresponding to different quantities of plant materials (leaves) submitted to the multiple consecutive extraction of the headspace- GC unit. By using the MHE method and extrapolating the area values of the FSB and LHC leaf volatiles calibration curves, we obtained respective yield values of 0.74%±0.01% and 0.85%±0.01% (v/w). The values were very close to the hydrodistillation yields, and the results suggest that the HS-GC method can be used to determine the essential oil yield (Table III) for *C. subavenium*.

Comparison of leaf oil compositions: From the FSB leaf oil obtained by hydrodistillation, 44 compounds were identified with the main components being methyl eugenol (75.9%), linalool (7.3%), eugenol (6.6%) methyl chavicol (2.6%), caryophyllene oxide (1.1%), and β-phellandrene (1.0%). The constituents were divided into monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes and non-terpenoids. When these groups were tallied, the non-terpenoids had the highest area percentage of 85.9%, including methyl eugenol, eugenol, methyl chavicol, etc. Oxygenated monoterpenes accounted for 7.8%, monoterpene hydrocarbons for 2.1%, oxygenated sesquiterpenes for 3.0%, and sesquiterpene hydrocarbons for 1.3%. In the HS-GC analysis, 26 compounds were identified, again with methyl eugenol as the main component, accounting for 78.7% of the total. It was followed by linalool (7.7%), eugenol (6.3%), methyl chavicol (3.0%), caryophyllene oxide (1.0%), β-phellandrene (0.8%) and so on. The non-terpenoids group (88.2%) also accounted for the highest fraction among the compounds.

From the LHC leaf oil obtained by hydrodistillation, we identified 88 compounds, with the main components being p-cymene (21.6%), 1,8-cineole (16.5%), linalool (11.9%), α-pinene (6.3%), caryophyllene oxide (6.2%), limonene (5.0%), cryptone (3.2%), α-terpineol (3.0%), β-pinene (2.7%), thymol (2.2%), terpinen-4-ol (1.9%), α-eudesmol (1.4%), myrcene (1.3%), camphene (1.1%), and cuminaldehyde (1.1%). Among the constituents, monoterpenes accounted for the highest fraction at 82.7%, sesquiterpenes accounted for 13.3% of the total, and the non-terpenoids group accounted for 4.0% of the total. In the HS-GC analysis, 65 compounds were identified, with the main components being p-cymene (21.7%), 1,8-cineole (20.5%), linalool (13.7%), α-pinene (6.5%), caryophyllene oxide (6.6%), and limonene (4.9%). Among the constituents, monoterpenes accounted for the highest fraction at 84.3%, sesquiterpenes accounted for 12.1% of the total, and the non-terpenoids group accounted for 3.6% of the total.

The above yield values and compositions indicate that hydrodistillation and the HS-GC methods gave comparable leaf essential oil yields. When the compositions of the oils were compared, however, the minor components obtained in hydrodistillation (content < 0.1%) could not be detected by HS-GC. The major reason was probably due to the small size of the specimens used, as the former needed ca. 1 kg of sample, while HS-GC only took 20 mg. 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 essential oil compositional analysis and chemotype determination; furthermore, it requires only a minute amount of specimen and a long period of distillation is not needed.

Table I. The values of the total area corresponding to each quantity of FSB and LHC oils subjected to MHE on HS-GC

FSB (μL)	Area	LHC (μL)	Area
0.1	300.6 ± 8.3	0.1	336.3 ± 6.7
0.2	635.4 ± 13.0	0.2	703.3 ± 10.4
0.3	968.3 ± 11.7	0.3	1032.7 ± 12.3
0.4	1301.0 ± 13.6	0.4	1398.4 ± 13.3
0.5	1631.3 ± 15.8	0.5	1703.4 ± 16.4
0.6	1896.2 ± 17.0	0.6	1986.3 ± 15.6

Table II. Area values corresponding to different quantity of plant material (FSB and LHC) subjected to MHE on HS-GC

Plant material (mg)	Area	
	FSB	LHC
10	233.7 ± 9.5	298.4 ± 8.7
20	479.4 ± 11.4	586.6 ± 9.8
30	701.3 ± 13.6	865.3 ± 11.3
40	935.3 ± 12.7	1165.3 ± 13.5

Relevant literature on the leaf essential oils of *C. subavenium* were rare. Only Jantan et al. (9) Zheng et al. (10) and Zhu et al. (11) performed previous studies. Jantan et al. (9) studied the leaf and bark oils of *C. subavenium* from the Malay Peninsula. They identified a total of 53 compounds with the main components being patchouli alcohol (27.7%) and benzyl benzoate (19.6%). Other major compounds in the order of abundance were β-selinene (7.2%), geraniol (3.6%), and linalool (2.9%). Zheng et al. (10) reported on the *C. subavenium* leaf oil from China and pointed out that there were two 1,8-cineole and eugenol chemotypes. In the 1,8-cineole-type, the main component was 1,8-cineole (76.0%), followed by sabinene (10.6%), α-terpineol (4.4%), α-pinene (2.7%), β-pinene (1.8%), and terpinen-4-ol (1.0%) etc. In the eugenol-type, the main component was eugenol (26.4%), followed by terpinen-4-ol (21.4%), limonene and 1,8-cineole (15.3%), spathulenol (1.8%), β-elemene (1.7%), nerolidol (1.5%), β-caryophyllene (1.4%), and viridiflorol (1.2%). Furthermore, Zhu et al. (11) noted that among *C. subavenium* from China, there was a safrole-type (69.7%). When we searched the literature cited by them, however, there was no record of its leaf oil composition. Comparing the main components found among the 3 reports to the FSB and LHC leaf oils, there were markedly divergent compositions.

Determination of the antioxidant activities DPPH free radical scavenging capability test: We subjected the leaf oils from FSB and LHC to the DPPH free radical scavenging capability tests. Ascorbic acid was used as a positive control, and the results are shown in Figure 1. The EC_{50} values of DPPH free radical scavenging capability of the 2 leaf oils from FSB and LHC were 29.6 and 70.8 μg/mL, respectively, whereas ascorbic acid had an EC_{50} of 8.6 μg/ml (Table IV). Individual main components of the 2 leaf oils such as methyl eugenol, eugenol, p-cymene, 1,8-cineole, thymol, and carvacrol were

Table III. Chemical composition of the leaf oils and headspace volatiles of *Cinnamomum subavenium* from two sources

Consituents	R.I. [a]	Concentration (%)				Identification [d]
		FSB		LHC		
		HD [b]	HS [c]	HD	HS	
α-thujene	930	- [e]	-	0.4	1.0	MS, RI, ST
α-pinene	939	0.3	0.2	6.3	6.5	MS, RI, ST
camphene	954	-	-	1.1	1.5	MS, RI, ST
β-pinene	979	t [f]	t	2.7	2.9	MS, RI, ST
myrcene	979	t	t	1.3	1.3	MS, RI, ST
δ-2-carene	1002	-	-	t	t	MS, RI, ST
α-phellandrene	1003	0.2	0.1	0.9	0.7	MS, RI, ST
α-terpinene	1017	t	-	0.6	0.53	MS, RI, ST
p-cymene	1025	t	t	21.6	21.7	MS, RI, ST
limonene	1029	-	-	5.0	4.9	MS, RI, ST
β-phellandrene	1030	1.0	0.8	-	-	MS, RI, ST
1,8-cineole	1031	0.3	0.1	16.5	20.5	MS, RI, ST
(Z)-β-ocimene	1037	0.2	0.1	-	-	MS, RI, ST
(E)-β-ocimene	1050	0.2	t	0.2	t	MS, RI, ST
γ-terpinene	1060	-	-	0.6	0.5	MS, RI, ST
trans-linalool oxide (furanoid)	1073	-	-	0.7	0.1	MS, RI, ST
cis-linalool oxide (furanoid)	1087	-	-	0.8	0.1	MS, RI, ST
terpinolene	1089	t	-	0.2	0.2	MS, RI, ST
linalool	1097	7.3	7.7	11.9	13.7	MS, RI, ST
nonanal	1101	t	-	-	-	MS, RI, ST
α-thujone	1102	-	-	0.1	t	MS, RI
α-fenchol	1117	-	-	t	t	MS, RI, ST
cis-p-menth-2-en-1-ol	1122	-	-	t	-	MS, RI
(E,Z)-*allo*-ocimene	1132	-	-	t	t	MS, RI, ST
trans-pinocarveol	1139	-	-	0.3	0.3	MS, RI, ST
camphor	1146	-	-	t	t	MS, RI, ST
isopulegol	1150	-	-	0.4	0.5	MS, RI, ST
iso-(iso)pulegol	1160	-	-	t	-	MS, RI, ST
pinocarvone	1165	-	-	0.2	t	MS, RI, ST
borneol	1169	-	-	0.8	0.1	MS, RI, ST
cis-linalool oxide (pyranoid)	1174	-	-	t	-	MS, RI, ST
terpinen-4-ol	1177	t	-	1.9	1.9	MS, RI, ST
cryptone	1186	-	-	3.2	1.2	MS, RI, ST
α-terpineol	1189	0.2	t	3.0	3.1	MS, RI, ST
methyl chavicol	1196	2.6	3.0	-	-	MS, RI, ST
cis-piperitol	1196	-	-	t	t	MS, RI, ST
safranal	1197	-	-	t	-	MS, RI
decanal	1202	-	-	t	-	MS, RI, ST
verbenone	1205	-	-	0.1	t	MS, RI
trans-carveol	1217	-	-	0.2	0.1	MS, RI, ST
citronellol	1226	-	-	0.1	t	MS, RI, ST
cis-carveol	1229	-	-	t	-	MS, RI, ST
neral	1238	-	-	t	-	MS, RI, ST
cuminaldehyde	1242	-	-	1.1	0.9	MS, RI, ST
carvotanacetone	1247	-	-	t	-	MS, RI
chavicol	1250	0.3	0.2	-	-	MS, RI, ST
geraniol	1253	-	-	0.2	-	MS, RI, ST
piperitone	1253	-	-	t	-	MS, RI, ST
trans-piperitone oxide	1256	-	-	0.1	-	MS, RI
geranial	1267	-	-	t	-	MS, RI, ST
p-menth-l-en-7-al	1276	-	-	0.4	0.4	MS, RI
α-terpinen-7-al	1285	-	-	t	-	MS, RI, ST
isobornyl acetate	1286	-	-	0.2	0.2	MS, RI, ST
thymol	1290	-	-	2.2	2.1	MS, RI, ST
carvacrol	1299	-	-	0.7	0.6	MS, RI, ST
6-hydroxy-carvotanacetone	1311	-	-	t	t	MS, RI
trans-piperityl acetate	1346	-	-	t	-	MS, RI, ST
α-terpinyl acetate	1349	-	-	t	-	MS, RI, ST
eugenol	1359	6.6	6.3	-	-	MS, RI, ST
α-ylangene	1375	-	-	t	-	MS, RI, ST
α-copaene	1377	-	-	t	-	MS, RI, ST
geranyl acetate	1381	-	-	t	t	MS, RI, ST
β-elemene	1391	-	-	t	t	MS, RI, ST

Table III. Continued

Consituents	R.I. [a]	Concentration (%)				Identification [d]
		FSB		LHC		
		HD [b]	HS [c]	HD	HS	
methyl eugenol	1404	75.9	78.7	-	-	MS, RI, ST
dodecanal	1409	-	-	t	-	MS, RI, ST
β-caryophyllene	1419	0.9	0.8	0.8	0.7	MS, RI, ST
trans-α- bergamotene	1435	-	-	t	-	MS, RI
2-methylbutyl benzoate	1441	0.2	t	-	-	MS, RI
cis-muurola-3,5-diene	1450	-	-	t	-	MS, RI
trans-muurola-3,5-diene	1454	t	-	t	t	MS, RI
α-humulene	1455	t	t	0.2	0.2	MS, RI, ST
β-acoradiene	1471	-	-	0.1	0.1	MS, RI
ar-curcumene	1481	-	-	t	-	MS, RI
germacrene D	1485	t	-	t	-	MS, RI, ST
β-selinene	1490	t	-	-	-	MS, RI, ST
δ-selinene	1493	-	-	0.1	t	MS, RI, ST
zingiberene	1494	-	-	0.2	0.2	MS, RI
α-selinene	1498	t	-	-	-	MS, RI, ST
α-muurolene	1500	-	-	t	-	MS, RI, ST
γ-patchoulene	1502	t	-	-	-	MS, RI
trans-β-guaiene	1503	-	-	t	-	MS, RI
β-bisabolene	1506	-	-	t	-	MS, RI
γ-cadinene	1514	t	-	t	-	MS, RI, ST
cis-γ-bisabolene	1515	0.1	t	-	-	MS, RI, ST
β-sesquiphellandrene	1523	-	-	t	-	MS, RI
δ-cadinene	1523	-	-	0.1	t	MS, RI, ST
α-cadinene	1539	t	-	t	-	MS, RI, ST
α-calacorene	1546	t	-	t	t	MS, RI, ST
elemol	1550	t	-	0.9	0.9	MS, RI, ST
(E)-nerolidol	1563	t	-	t	-	MS, RI, ST
caryophyllenyl alcohol	1572	t	-	-	-	MS, RI, ST
spathulenol	1578	-	-	0.3	0.1	MS, RI, ST
caryophyllene oxide	1583	1.1	1.0	6.2	6.6	MS, RI, ST
globulol	1585	-	-	t	t	MS, RI, ST
guaiol	1601	0.8	0.6	0.2	0.2	MS, RI
sesquithuriferol	1605	-	-	t	-	MS, RI
humulene epoxide	1608	-	-	0.4	0.3	MS, RI
(E)-isoeugenol acetate	1616	0.2	t	-	-	MS, RI
isolongifolan-7α-ol	1619	t	-	-	-	MS, RI
1,10-di-*epi*-cubenol	1619	-	-	t	-	MS, RI
1-*epi*-cubenol	1629	t	-	0.1	t	MS, RI
eremoligenol	1631	t	-	-	-	MS, RI
γ-eudesmol	1632	0.2	t	0.2	t	MS, RI, ST
caryophylla-4(14),8(15)-dien-5α-ol	1641	-	-	0.6	0.5	MS, RI
δ-cadinol	1646	-	-	0.1	0.1	MS, RI, ST
α-cadinol	1654	0.2	t	-	-	MS, RI, ST
α-eudesmol	1654	-	-	1.4	1.3	MS, RI, ST
14-hydroxy-9-epi-β-caryophyllene	1670	-	-	0.5	0.6	MS, RI
bulnesol	1672	0.2	0.1	-	-	MS, RI
α-bisabolol	1686	-	-	t	-	MS, RI, ST
eudesma-4(15),7-dien-1β-ol	1688	-	-	t	-	MS, RI
eudesma-7,(11)-en-4-ol	1700	-	-	t	-	MS, RI
(Z,Z)-farnesol	1718	-	-	t	-	MS, RI, ST
benzyl benzoate	1760	0.3	0.1	t	-	MS, RI, ST
pimaradiene	1950	-	-	t	-	MS, RI
Compound identified		100.0	100.0	100.0	100.0	
Monoterpene hydrocarbons		2.1	2.3	40.9	41.9	
Oxygenated monoterpenes		7.8	7.8	41.8	42.5	
Sesquiterpene hydrocarbons		1.3	0.9	2.6	1.9	
Oxygenated sesquiterpenes		3.0	1.7	10.8	10.2	
Diterpene		-	-	t	-	
Others		85.9	88.2	4.0	3.6	
Yield (%)		0.71	0.74	0.82	0.85	

[a] Retention index on a DB-5 column in reference to n-alkanes (13); [b] HD, Hydrositillation extraction.; [c] HS, Headspace-GC extraction; [d] MS, NIST and Wiley libraries spectra and the literature; RI, Retention index; ST, authentic standard compounds; [e] not detected; [f] trace < 0.1%

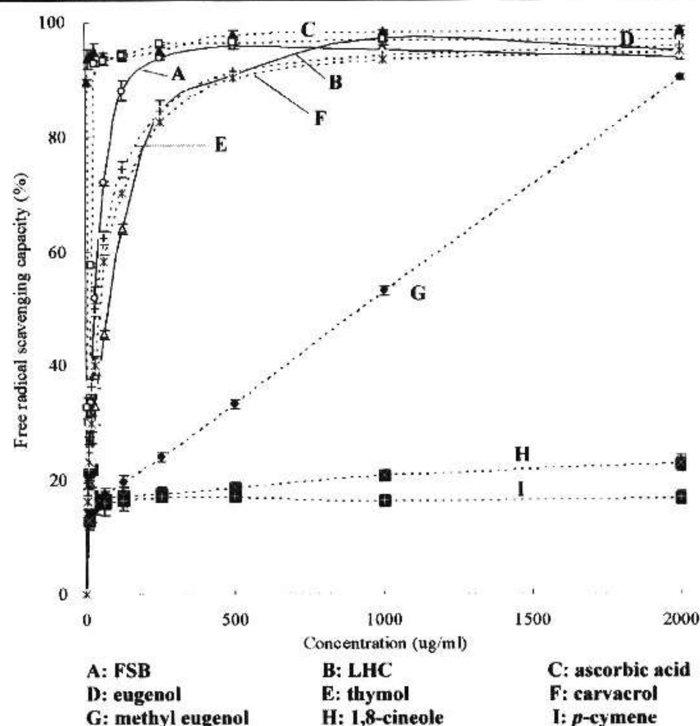

Figure 1. DPPH free radical scavenging capability of FSB and LHC leaf oils

Table IV. EC_{50} (μg/mL) values of FSB and LHC leaf oils and selected chemicals using the DPPH free radical scavenging method

Sample	EC_{50} (μg/mL)
Ascorbic acid	8.6±0.5
FSB leaf oil	29.6±1.3
LHC leaf oil	70.8±0.7
methyl eugenol	889.5±2.7
eugenol	13.6±0.8
thymol	31.4±1.0
carvacrol	38.7±0.4
1,8-cineole	>2000
p-cymene	>2000
Cinnamomum osmophloeum (24)	88.4~708.6
Nigella sativa (25)	460.0
Curcuma zedoaria (22)	500.0
Origanum vulgare (26)	460.0

also compared using the DPPH free radical scavenging capability. The results showed the DPPH free radical scavenging capabilities were in the order of eugenol, thymol, carvacrol, methyl eugenol, 1,8-cineole, and p-cymene. Hence, we deduced that phenolic compounds were the main sources responsible for radical scavenging. The results are also in congruency with the conclusions of several other reports (21-23).

The EC_{50} values of the DPPH free radical scavenging capabilities of the two leaf essential oils were found to be rather superior to several different provenances of a Taiwan indigenous cinnamon tree (*C. osmophloeum*) (24), as well as *Nigella sativa* (25), *Curcuma zedoaria* (22), and the flower oil of *Origanum vulgare* (26).

Analysis of the total phenolic content of various essential oil compositions: Results of comparisons of the total phenolic contents of the 2 leaf oils showed that the total phenolic content was higher in the FSB leaf oil, reaching 186.8 ± 2.7 mg GAE/g oil, whereas the LHC leaf oil contained 124.8 ± 1.9 mg GAE/g oil. The total phenolic content determined exhibited a positive correlation with the respective DPPH free radical scavenging capabilities.

Antimicrobial activities of the oils: Antimicrobial activities of *C. subavenium* leaf oils were examined against 9 strains of microorganisms. Results showed that both oils had excellent antimicrobial effectiveness (Table V). At an FSB leaf oil concentration of 10 μL/disc, the inhibition zones against *B. cereus*, *S. epidermidis* , *E. coli*, *Ent. aerogenes*, *V. parahaemolyticus*, and *C. albicans* were larger than those of the positive controls of ampicillin and penicillin at 1000 ppm. But with regard to the suppression of *S. aureus* and *K. pneumoniae*, the inhibition zones were larger than that of 500 ppm of ampicillin; with regard to the suppression of *P. aeruginosa*, the inhibition zones were larger than that for 1000 ppm of penicillin, but not quite as good as that of 1000 ppm ampicillin. The LHC leaf oil at 10 μL/disc, was capable of suppressing *B. cereus*, *S. epidermidis*, *E. coli*, *Ent. aerogenes*, and *V. parahaemolyticus*, and was superior to both ampicillin and penicillin at 1000 ppm, however the suppression inhibition zones were smaller than those of FSB leaf oil. As for suppression of the other microbes, its performance was also poorer than that of FSB leaf oil. Thus, both leaf oils showed excellent antimicrobial activities, but those of FSB leaf oil had the best.

The results above verify that *C. subavenium* leaf oils from both locales had excellent antimicrobial activities. This activity is related to their chemical compositions, i.e., the chemical structures and functional groups as well as the hydrophilic

Table V. Antimicrobial activity (diameter of the inhibition zone in mm) of the FSB and LHC leaf oils using the paper disc diffusion method

Microbial species	Inhibition zone (mm)									
	FSB (uL/disc)			LHC (uL/disc)			Ampicillin (ppm)		Penicillin (ppm)	
	10	2	1	10	2	1	1000	500	1000	500
B. cereus	26.3	18.3	15.0	25.3	18.1	14.6	18.1	14.6	13.2	9.2
S. aureus	46.2	25.1	20.3	30.2	20.1	16.2	47.1	39.2	50.1	45.9
S. epidermidis	25.3	18.2	12.3	20.6	16.2	10.1	10.5	8.3	0.0	0.0
E. coli	39.2	20.5	18.2	34.1	20.1	19.1	30.5	25.0	9.0	0.0
Ent. aerogenes	30.1	23.5	19.2	28.6	19.1	18.0	10.3	0.0	9.8	0.0
K. pneumoniae	26.1	22.6	16.1	19.2	16.5	15.2	28.2	23.4	12.0	8.1
P. aeruginosa	27.9	19.2	18.1	25.9	16.2	14.1	35.0	32.3	20.3	15.1
V. parahaemolyticus	27.3	23.4	17.6	21.5	16.3	10.2	10.5	0.0	8.2	0.0
C. albicans	32.1	23.5	21.2	23.2	18.6	15.3	30.2	21.3	28.6	18.2

* *B. cereus*: *Bacillus cereus*; *S. aureus*: *Staphylococcus aureus*; *S. epidermidis*: *Staphylococcus epidermidis*; *E. coli*: *Escherichia coli*; *Ent. aerogenes*: *Enterobacter aerogenes*; *K. pneumoniae*: *Klebsiella pneumoniae*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *V. parahaemolyticus*: *Vibrio parahaemolyticus*; *C. albicans*: *Candida albicans*

or hydrophobic nature of the hydrocarbon backbones of the compounds all exert their effects on the antimicrobial capability. The compounds which exhibit the strongest antimicrobial activity generally have phenols as part of their structure (27–29), such as eugenol, thymol, and cavacrol. As both sources of *C. subavenium* leaf oils contained such phenol structures, hence they both had excellent antimicrobial activities.

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