Composition and Antimicrobial Activity of the Leaf Essential Oil of *Machilus obovatifolia* From Taiwan

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Abstract

The leaf essential oils of *Machilus obovatifolia* were isolated and analyzed using either the hydrodistillation or headspace-GC methods to determine their compositions and yields. Antimicrobial activities of the leaf oils obtained were also evaluated. Ninety-three compounds were identified in the hydrodistilled leaf oil, and 74 compounds were identified by the headspace-GC and GC/MS method, respectively. The main components of the oils were β-caryophyllene (10.5%), β-phellandrene (7.8%), τ-muurolol (5.3%), α-phellandrene (5.1%) and δ-cadinene (5.0%). The leaf oils exhibited excellent antimicrobial activities.

Key Word Index

Machilus obovatifolia, Lauraceae, essential oil composition, β-caryophyllene, headspace volatiles, antimicrobial activity.

Introduction

Machilus obovatifolia Kanehira et Sasaki, (Lauraceae) is a small evergreen tree, endemic to the lowlands of the Hengchun Peninsula, southern Taiwan (1). Scanty references were found regarding the chemical compositions and biological activities of this species (2–5). Certain cytotoxic active compounds in the leaf extracts were mentioned (2–5); however, there appears to be no report on the essential oil compositions of this species. Therefore, we used hydrodistillation and headspace-GC (HS-GC) methods to collect its leaf oil and GC-FID and GC/MS to analyze the composition of the oil and headspace volatiles. To determine the oil yield, a multiple headspace extraction (MHE) method was employed. The second part of the study examined the antimicrobial activities of the oils. The purpose of this study was to establish a chemical basis for the effective multipurpose utilization of the species.

Experimental

Plant materials: Fresh leaves of *M. obovatifolia* were collected from the Hengchun Research Center of the Taiwan Forestry Research Institute in southern Taiwan where a specimen has been deposited in the Herbarium, in July 2006. Leaves of the species were collected for subsequent oil isolation and analysis.

Isolation of leaf oils and determination of composition and yield

Hydrodistillation extraction: A kilogram of the leaves of *M. obovatifolia* was placed in a round-bottom flask containing 3 L of distilled water, hydrodistilled for 8 h and the oil removed from the partitioned water layer. Anhydrous sodium sulfate was added to remove residual water. The yield of oil was determined. 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 × 0.25 mm × 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

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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 × 0.25 mm × 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 leaf oil previously obtained by hydrodistillation. A special quantitative method, MHE, was used. According to Kolb (6), 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:

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

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

The HS-GC analyses were accomplished using a Hewlett-Packard HP6890 GC 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 oil 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 oil constituents was based on comparisons of the peaks Retention indices (RI) (7), their retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra and literature (8,9).

Antimicrobial activities of the essential oils

Microbial strains: 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 five 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); three Gram-positive bacteria: *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 6538P), and *S. epidermidis* (ATCC 12228); and one 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. (10). 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 oil 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 hydrodistillation of leaves of *M. obovatifolia* was 1.32 ± 0.04 (mL/100 g), respectively.

Leaf volatiles determination by the HS-GC method: The value of the total area corresponding to each volume of leaf oil submitted to the MHE of the headspace-GC was calculated by means of a previously described equation (a) in experimental section. The leaf volatile calibration curve obtained from the value corresponded to the regression equation y = a + bx, where value for the leaf volatile was a = -2.5554 and b = 6407.8, r^2 = 0.9988 (Table I).

Table II shows the area values corresponding to different quantities of plant material (leaves) submitted to the multiple consecutive extraction of the headspace-GC unit. By using the MHE method and extrapolating the area values of the leaf volatiles calibration curves, we obtained respective yield values of 1.35 ± 0.03 (mL/100 g). The value was 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 *M. obovatifolia*.

Table I. The values of the total area corresponding to each quantity of *Machilus obovatifolia* oil subjected to MHE on HS-GC

M. obovatifolia (µL)	Area
0.1	610.32 ± 10.65
0.2	1365.21 ± 12.83
0.3	1842.58 ± 15.92
0.4	2565.46 ± 17.35
0.5	3202.65 ± 19.86
.6	3852.34 ± 21.25

Table II. Area values corresponding to different quantity of plant material subjected to MHE on HS-GC

Plant material (mg)	Area
10	865.36 ± 9.68
20	1756.24 ± 13.52
30	2631.56 ± 14.28
40	3365.98 ± 12.62

Table III. Chemical composition of the leaf oil and headspace volatiles of *Machilus obovatifolia*

Compound	RI [a]	Concentration (%)		Identification [d]
		HD [b]	HS [c]	
α-thujene	930	t [e]	- [f]	MS,RI,ST
α-pinene	939	1.6	1.8	MS,RI,ST
camphene	954	0.6	0.7	MS,RI,ST
β-pinene	979	0.9	0.9	MS,RI,ST
myrcene	991	1.9	2.1	MS,RI,ST
δ-2-carene	1002	t	-	MS,RI,ST
α-phellandrene	1003	5.1	5.1	MS,RI,ST
α-terpinene	1017	0.4	0.4	MS,RI,ST
p-cymene	1025	3.6	3.5	MS,RI,ST
β-phellandrene	1030	7.8	7.5	MS,RI,ST
(Z)-β-ocimene	1037	3.1	3.2	MS,RI,ST
(E)-β-ocimene	1050	3.5	3.6	MS,RI,ST
γ-terpinene	1060	0.1	t	MS,RI,ST
octanol	1068	t	-	MS,RI,ST
camphenilone	1082	t	-	MS,RI
terpinolene	1089	0.1	0.1	MS,RI,ST
linalool	1097	0.3	0.3	MS,RI,ST
nonanal	1101	t	-	MS,RI,ST
α-fenchol	1117	t	-	MS,RI,ST
cis-p-menth-2-en-1-ol	1122	0.1	0.1	MS,RI
(E,Z)-allo-ocimene	1132	t	-	MS,RI,ST
trans-p-menth-2-en-1-ol	1141	0.1	t	MS,RI
camphene hydrate	1150	t	-	MS,RI,ST
borneol	1169	0.1	0.1	MS,RI,ST
terpinen-4-ol	1177	0.1	0.1	MS,RI,ST
cryptone	1186	0.1	0.1	MS,RI,ST
α-terpineol	1189	0.4	0.3	MS,RI,ST
cis-piperitol	1196	0.1	-	MS,RI,ST
safranal	1197	0.1	-	MS,RI
decanal	1202	0.1	0.1	MS,RI,ST
trans-piperitol	1208	0.1	t	MS,RI,ST
trans-carveol	1217	t	-	MS,RI,ST
carvotanacetone	1247	t	-	MS,RI
cis-piperitone oxide	1254	t	-	MS,RI
p-menth-1-en-7-al	1276	0.1	t	MS,RI
isobornyl acetate	1286	0.4	0.5	MS,RI,ST
lavandulyl acetate[ti]	1290	t	-	MS,RI
m-acetanisole[ti]	1299	t	-	MS,RI
z-patchenol	1319	0.1	t	MS,RI
presilphiperfol-7-ene	1337	0.1	0.1	MS,RI
δ-elemene	1338	0.2	0.2	MS,RI,ST
α-cubebene	1351	0.2	0.2	MS,RI,ST
linalyl isobutyrate[ti]	1375	t	-	MS,RI
isoledene	1376	0.2	0.2	MS,RI,ST
α-copaene	1377	1.5	1.4	MS,RI,ST
β-bourbonene	1388	0.5	0.5	MS,RI,ST
β-elemene	1391	0.6	0.6	MS,RI,ST
cyperene	1399	0.1	0.1	MS,RI,ST
isoitalicene	1402	0.2	0.1	MS,RI
α-gurjunene	1410	0.4	0.3	MS,RI,ST
β-caryophyllene	1419	10.5	10.6	MS,RI,ST
β-gurjunene	1434	0.6	0.5	MS,RI,ST
γ-elemene	1437	0.2	0.3	MS,RI,ST
aromadendrene	1441	1.5	1.5	MS,RI,ST
α-neoclovene	1454	0.3	0.4	MS,RI
α-humulene	1455	3.0	3.2	MS,RI,ST
9-epi-β-caryophyllene	1466	0.4	0.4	MS,RI
γ-muurolene	1480	2.4	2.5	MS,RI,ST
germacrene D	1485	1.3	1.3	MS,RI,ST
β-selinene	1490	1.0	1.2	MS,RI,ST
δ-selinene	1493	0.4	0.3	MS,RI,ST
bicyclogermacrene	1500	3.5	3.2	MS,RI,ST
α-muurolene	1500	1.0	1.1	MS,RI,ST
δ-amorphene	1512	0.4	0.5	MS,RI,ST

Table III Continued

Compound	RI [a]	Concentration (%)		Identification [d]
		HD [b]	HS [c]	
γ-cadinene	1514	0.8	0.8	MS,RI,ST
δ-cadinene	1523	5.0	4.9	MS,RI,ST
trans-cadina-1(2),4-diene	1535	0.4	0.4	MS,RI
α-cadinene	1539	0.6	0.7	MS,RI,ST
α-calacorene	1546	0.4	0.4	MS,RI,ST
elemol	1550	t	-	MS,RI,ST
germacrene B	1561	0.3	0.3	MS,RI
(E)-nerolidol	1563	1.8	1.9	MS,RI,ST
ledol	1569	1.3	1.5	MS,RI,ST
spathulenol	1578	0.8	0.7	MS,RI,ST
globulol	1585	4.0	4.2	MS,RI,ST
viridiflorol	1593	1.5	1.4	MS,RI,ST
guaiol	1601	1.1	1.0	MS,RI,ST
humulene epoxide II	1608	0.4	0.3	MS,RI
1,10-di-epi-cubenol	1619	0.5	0.4	MS,RI
β-cedrene epoxide	1623	0.7	0.7	MS,RI
10-epi-γ-eudesmol	1624	0.5	0.4	MS,RI
γ-eudesmol	1632	0.8	0.9	MS,RI,ST
τ-cadinol	1640	3.8	3.9	MS,RI,ST
τ-muurolol	1642	5.3	5.5	MS,RI,ST
α-muurolol	1646	3.6	3.8	MS,RI,ST
α-cadinol	1654	4.2	4.0	MS,RI,ST
trans-calamenen-10-ol	1669	0.2	0.1	MS,RI
bulnesol	1672	0.3	0.2	MS,RI
cadalene	1677	0.1	0.1	MS,RI
eudesma-4(15),7-dien-1β-ol	1688	0.3	0.2	MS,RI
10-nor-calamenen-10-one	1702	0.1	t	MS,RI
mint sulfide	1741	t	-	MS,RI
14-hydroxy-α-muurolene	1780	0.1	-	MS,RI
Monoterpene hydrocarbons		28.7	28.9	
Oxygenated monoterpenes		1.9	1.6	
Sesquiterpene hydrocarbons		38.0	38.4	
Oxygenated sesquiterpenes		31.2	31.0	
Others		0.2	0.1	
Oil Yield (mL/100 g)		1.32 ± 0.04	1.35 ± 0.03	

[a] Retention index on a DB-5 column in reference to n-alkanes (7). [b] HD, Hydrodistillation extraction. [c] HS, Headspace-GC extraction. [d] MS, NIST and Wiley libraries spectra and the literature; RI, Retention index; ST, authentic standard compounds. [e] trace < 0.1%. [f] Not detected. tentative identification. lavandulyl acetate: 196(M^+ 1) 136(13) 121(18) 93(56) 80(9) 69(100) 68(45) 67(13) 43(63) 39(8). m-acetanisole: 150(M^+ 59) 136(8) 135(100) 107(46) 92(20) 77(36) 64(14) 63(14) 50(7) 43(15). linalyl isobutyrate: 224(M^+ 3) 154(1) 136(15) 121(36) 107(5) 93(100) 80(35) 69(44) 55(21) 43(35).

Comparison of leaf oil compositions: From the *M. obovatifolia* leaf oil obtained by hydrodistillation, 93 compounds were identified with the main components being β-caryophyllene (10.5%), β-phellandrene (7.8%), τ-muurolol (5.3%), α-phellandrene (5.1%), δ-cadinene (5.0%), α-cadinol (4.2%), globulol (4.0%), τ-cadinol (3.8%), p-cymene (3.6%), (E)-β-ocimene (3.5%), bicyclogermacrene (3.5%), (Z)-β-ocimene (3.1%) and humulene (3.0%). The constituents were divided into monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, diterpenes and non-terpenoids. When these groups were tallied, the sesquiterpene hydrocarbons had the highest area percentage of 38.0%, oxygenated sesquiterpenes accounted for 31.2%, monoterpene hydrocarbons for 28.7%, oxygenated monoterpenes for 1.9% and non-terpenoids for 0.2%. In the HS-GC analysis, 74 compounds were identified, again with β-caryophyllene as the main component, accounting for 10.6% of the total. It was followed by β-phellandrene (7.5%), τ-muurolol (5.5%), α-phellandrene (5.1%), δ-cadinene (4.9%), globulol (4.2%), α-cadinol (4.0%), τ-cadinol (3.9%), (E)-β-ocimene (3.6%), p-cymene (3.5%), bicyclogermacrene (3.2%), (Z)-β-ocimene (3.2%) and humulene (3.2%) etc. The sesquiterpene hydrocarbons group (38.4%) also accounted for the highest fraction among the identified compounds. There appears to be no information in the literature pertaining to the species we studied. Thus, this paper represents the first study of the leaf oil of *M. obovatifolia*.

The above yield values and compositions indicate that hydrodistillation and the HS-GC methods gave comparable leaf oil yields. When the composition of the oil was compared, however, the minor components obtained by hydrodistillation (content < 0.1%) could not be detected by HS-GC. The major

Table IV. Antimicrobial activity (diameter of the inhibition zone in mm) of the *M. obovatifolia* leaf oil using the paper disc diffusion method

Microbial species	Inhibition zone (mm)						
	Machilus obovatifloia (μL/disc)			Ampicillin (ppm)		Penicillin (ppm)	
	10	2	1	1000	500	1000	500
B. cereus	28.9	22.6	16.2	18.1	14.6	13.2	9.2
S. aureus	47.6	27.3	21.5	47.1	39.2	50.1	45.9
S. epidermidis	28.9	19.5	15.3	10.5	8.3	0	0
E. coli	40.8	21.8	18.6	30.5	25	9	0
Ent. aerogenes	32.6	24.6	20.5	10.3	0	9.8	0
K. pneumoniae	27.3	23.8	17.6	28.2	23.4	12	8.1
P. aeruginosa	27.8	21.6	19.6	35	32.3	20.3	15.1
V. parahaemolyticus	28.6	25.8	18.4	10.5	0	8.2	0
C. albicans	33.6	26.8	22.6	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.*

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 is an effective method for analyzing the essential oil composition; furthermore, it requires only a minute amount of specimen, and a long period of distillation is not needed.

Antimicrobial activities of the oil: At a 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. The inhibition zones of *S. aureus* and *K. pneumoniae* were larger than that of 500 ppm of ampicillin, and the inhibition zone of *P. aeruginosa* was larger than that for 1000 ppm of penicillin but not quite as good as that of 500 ppm ampicillin.

The results verify that *M. obovatifolia* leaf oil has excellent antimicrobial activity. The sources of the antimicrobial activities are thought to be from cadinane-type compounds, such as α-cadinol, τ-muurolol, τ-cadinol, etc. There are also studies supporting the contention that these compounds have high activities in suppressing microbial growth (11–13). In conclusion, the leaf oil showed excellent antimicrobial activity.

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