THE ESSENTIAL OILS OF *LINDERA PIPERICARPA*

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NOR AZAH MOHD ALI & IBRAHIM JANTAN. 1993. The essential oils of *Lindera pipericarpa*. Water distillation of the leaf and wood of *Lindera pipericarpa* yielded 1.00% and 0.05% of essential oils, respectively. The chemical composition of the oils were examined by co-chromatography with authentic samples on capillary columns of different polarity and gas chromatography-mass spectrometry (GC/MS). The oils were made up of monoterpenoids and sesquiterpenoids. The major components in the leaf oil were β-caryophyllene (32.1%) and α - copaene (31.4%), while the wood oil comprised mainly limonene (55.4%).

Key words: *Lindera pipericarpa* - monoterpenoids sesquiterpenoids - β-caryophyllene - α-copaene - limonene

NOR AZAH MOHD ALI & IBRAHIM JANTAN. 1993. Minyak pati *Lindera pipericarpa*. Persulingan hidro daun dan kayu *Lindera pipericarpa* telah menghasilkan 1.00% dan 0.05% minyak pati setiap satu. Kandungan kimia minyak pati telah di kenalpasti menerusi kaedah kokromatografi dengan sampel tulen di atas dua turus rerambut yang berlainan polariti dan analisa kromatografi gas-spektrometri jisim (KG-SJ). Minyak-minyak pati tersebut terdiri daripada monoterpenoid dan seskuiterpenoid. Kandungan utama minyak pati daun adalah β - kariofilena (32.1%) dan α - kopaena (31.4%), manakala kandungan minyak pati kayu sebahagian besarnya pula terdiri daripada limonena (55.4%).

Introduction

The genus *Lindera* (Lauraceae) contains about 150 species, of which only 11 species are found in Malaysia (Kostermans 1964 , Kochummen 1989). The trees are evergreen with resinous or aromatic barks and leaves. Various parts of some of these species have been used as food or in traditional medicine (Burkill 1966). The chemical compositions of the essential oils of some *Lindera* species in Japan have been examined by Hiromichi and co-workers (1977,1978, 1983a, 1983b, 1986) and Komae and co-workers (1971a, 1971b,1972). The leaf oil of *L. umbellata* has been formulated in many perfume preparations and its fragrant wood is used in making toothpicks (Kurata 1971).

Lindera pipericarpa, locally known as 'medang serai', is a small tree growing rarely to 18 *m* tall, and is often found gregariously in clearings of mountain forests of Malaysia. All parts of the tree are aromatic (Kochummen 1989). Various parts of the tree have been used either as flavours, in traditional medicine or in the preparation of cosmetics by the local folks (Burkill 1966). In the search for economically important essential oils from the flora of Malaysia, we report the chemical composition of the leaf and wood oils of *Lindera pipericarpa* .

Materials and methods

Collection of materials

L. pipericarpa leaves and wood samples were collected from Genting Highlands, Pahang. The plant materials were air dried under shade at room temperature for two days. A voucher specimen has been deposited at the herbarium of the Forest Research Institute Malaysia, Kepong.

Analysis of the essential oils

The leaf and wood of *L. pipericarpa* were water-distilled for 5-6 *h* and the oily layers obtained were separated and dried over anhydrous magnesium sulphate. The yields were averaged over two experiments and calculated on dry weight. Both oils were colourless. The leaf oil possessed a spicy-piney odour while the wood oil was lemon-like.

The oils were analyzed on a Shidmadzu GC 9A gas chromatograph equipped with a FID detector using fused silica capillary column SE-30 (25 $m \times 0.2$ *mm* i.d.). The operating parameters were: nitrogen as carrier gas at 50 $cm^3\ min^{-1}$, injector temperature 230°*C*, detector temperature 230° *C*. The column was programmed initially at 60°*C* for 10 *min*, then 3°*C* min^{-1} to 180 °*C* for 1 *min*. The oils were also examined using stationary phase PEG 20 *M* capillary column under the following programme condition; initial temperature 50° to 230°*C* at 3°*C* min^{-1}.

Some of the constituents were identified by co-chromatography on the different columns with authentic samples. The oils were further analyzed for GC-MS data with a GCMSD HP model 5890 (70 eV direct inlet) using silicon OV 1 (25 *m* x 0.25 *mm* i.d.) initially at 60°C for 2 *min*, then 3°C min^{-1} to 180 °C for 1 *min* with helium as carrier gas.

Kovats retention indices

Kovats indices were obtained from the gas chromatogram by logarithmic interpolation between bracketing alkanes. The homologous series of C_9 - C_{20} n- alkane were used as standards (Kovats 1965).

Brine shrimp lethality test

Vials containing 1 - 1000 $\mu g\ ml^{-1}$ samples were prepared by dissolving the oils in acetone and transfering the solution to each vial in which sea water was added to achieve the correct concentration. Ten brine shrimps (*Artemia salina*) were added to three standard vials for each dose via a disposable pipette. The number of deaths out of 30 shrimps per dose were recorded after 24 *h* and LC_{50} with 95% confidence intervals was determined for each compound by a Finney computer programme (Finney 1971). The control solution consisted of 30 nauplii in the artificial sea water. Solutions of potassium dichromate dissolved in the brine medium were used as standard toxicant.

Results and discussion

The leaf oil of Lindera pipericarpa

Water distillation of the leaf of *L. pipericarpa* yielded 1.0% of a colourless oil with spicy-piney odour. The gas chromatogram obtained showed that the leaf oil of *L. pipericarpa* contained 22 compounds of which 17 compounds were identified by comparing their mass fragmentation patterns with the data available in the literature and co-injection with authentic samples on SE 30 and PEG 20 capillary columns (Figure 1).

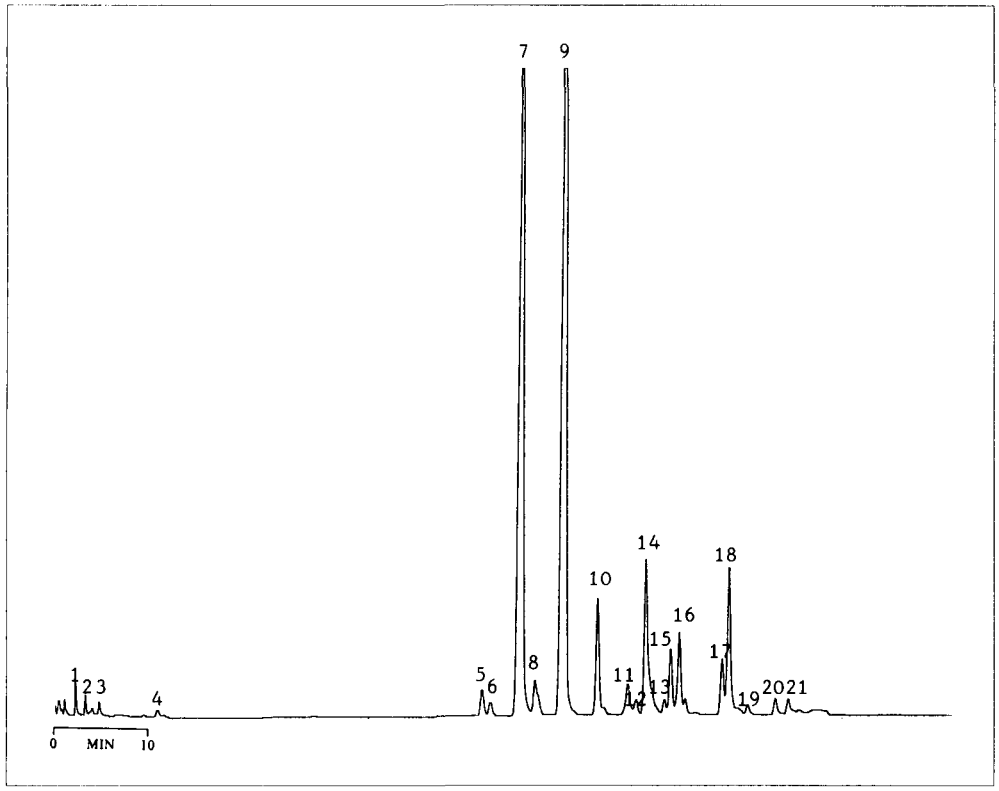

Figure 1. Gas chromatogram obtained with capillary SE 30 column for the leaf oil of *Lindera pipericarpa*; for identity of peaks, see Table 1

The leaf oil was composed predominantly of sesquiterpene hydrocarbons and the major components, β-caryophyllene (32.1%) and α-copaene (31.4%), constituted more than 60% of the oil (Table 1). Nerolidol (6.1%), β-farnesene (4.5%), α-humulene (4.4%), δ-cadinene (3.1%), γ-cadinene (2.5%), γ-muurolene (1.1%) were the other sesquiterpenes present in appreciable quantities. The minor sesquiterpenes were allo-aromadendrene, γ-elemene, α-cadinene and

ledol. From the mass fragmentation analysis the unidentified compounds were assigned the formula $C_{15}H_{24}O$ which indicates that they were sesquiterpenoids. Monoterpenes were only present at 1.1% concentration in which α-terpinene was the major component (0.4%). The other monoterpenes detected were limonene, (Z)-β-ocimene and α-terpineol.

Table 1. Constituents of the leaf oil of *Lindera pipericarpa*

Peak number	Compound	Retention index SE-30	Retention index PEG-20M	% Peak area	Identified by
1	α-terpinene	1008	1185	0.4	a & b
2	limonene	1024	1206	0.3	a & b
3	(Z)-β-ocimene	1040	1260	0.2	a
4	α-terpineol	1178	1702	0.2	a & b
5	$C_{15}H_{24}$	1340	-	3.4	a
6	α-cubebene	1352	1460	1.1	a & b
7	α-copaene	1378	1502	31.4	a & b
8	$C_{15}H_{24}$	1402	-	2.8	a
9	β-caryophyllene	1422	1590	32.1	a & b
10	α-humulene	1456	1664	4.4	a & b
11	allo-aromadendrene	1460	1640	0.2	a & b
12	γ-muurolene[t]	1473	-	1.1	a
13	β-farnesene	1477	-	4.5	a
14	γ-elemene	1496	-	0.2	a
15	γ-cadinene	1509	1755	2.5	a
16	δ-cadinene	1514	1780	3.1	a
17	α-cadinene	1520	-	0.9	a
18	$C_{15}H_{24}$	1546	-	2.5	a
19	nerolidol[i]	1551	2045	6.1	a & b
20	ledol	1568	-	0.4	a
21	$C_{15}H_{24}O$	1576	-	0.8	a
22	$C_{15}H_{24}O$	1590	-	0.7	a

(a= mass fragmentation, b= coinjection with authentic sample, t= tentative identification, i= correct isomer not characterised).

The wood oil of L. pipericarpa

The wood of *L. pipericarpa* yielded 0.04% of colourless oil with a lemon-like odour upon water distillation. Twenty-four compounds were identified in the oil (Figure 2). The oil was mainly made up of monoterpenes, which accounted for 89.9% of the oil. The most abundant compound, limonene, was present at 55.4% concentration (Table 2). The other monoterpenes present in significant amounts were linalool (6.6%), geranial (6.7%), neral (5.1%) and terpineol (4.2%). Sabinene, α-pinene, α-phellandrene, α-thujene, β-pinene, α-terpinene, (E)-β-ocimene, terpinolene and terpinen-4-ol were the minor monoterpenes present in the wood oil.

Non-terpenes, m-hydroxyacetophenone and 2,4 decadienal, constituted 4.1% and 0.4% of the oils, respectively. Sesquiterpenoids were the minor components, present at less than 4.0% concentration. The sesquiterpene hydrocarbons observed in the oil were β-caryophyllene, α-copaene, γ-muurolene, δ-cadinene, α-cubebene, α-humulene and α-guaiene, arranged in order of decreasing concentration.

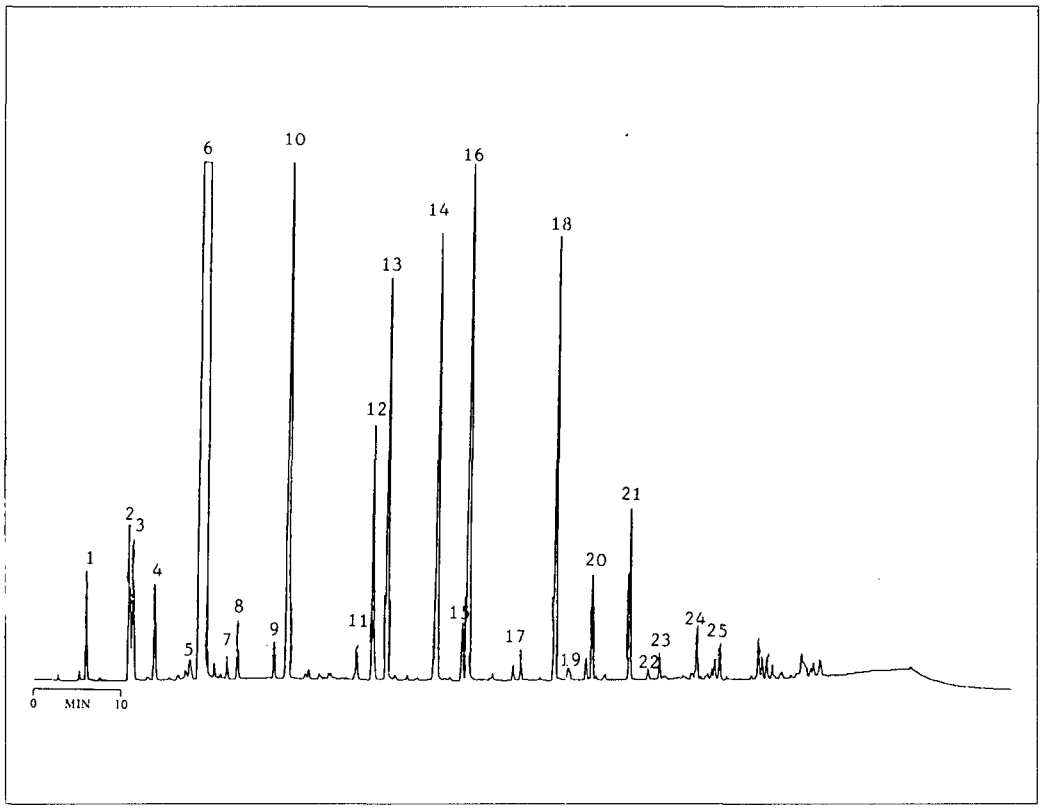

Figure 2. Gas chromatogram obtained with capillary SE 30 column for wood oil of *Lindera pipericarpa*; for identity of peaks, see Table 2

Toxicity screening of the essential oils

Toxicity test of the oils against brine shrimp (Table 3) as a prescreen for bioactive compounds showed that the leaf and wood oils of *Lindera pipericarpa* exhibit some degree of activity. The LC_{50} values of < 500 *ppm* for the essential oils suggest that the oils may contain physiologically active principles. More elaborate bioassays for specific pharmacologic activities are recommended for these oils.

Table 2. Constituents of the wood oil of *Lindera pipericarpa*

Peak number	Compound	Retention index SE-30	PEG-20	% Peak area	Identified by
1	α - thujene	926	1024	0.1	a
2	α - pinene	936	1016	0.9	a & b
3	sabinene	969	1123	1.8	a & b
4	β - pinene	975	1116	1.7	a & b
5	α - phellandrene	998	1173	1.0	a & b
6	α - terpinene	1010	1185	0.3	a & b
7	limonene	1026	1205	55.4	a & b
8	(E)- β - ocimene	1040	1260	0.2	a
9	$C_{10}H_{16}O$	-	-	0.8	a
10	terpinolene	1081	1290	0.6	a & b
11	linalool	1086	1558	6.6	a & b
12	terpinen-4-ol	1165	1595	0.4	a & b
13	$C_{10}H_{16}O$	-	-	3.5	a
14	α - terpineol	1180	1705	4.2	a & b
15	neral	1215	1688	5.1	a & b
16	$C_{10}H_{16}O$	-	-	0.6	a
17	geranial	1245	1740	6.7	a & b
18	2,4 decadienal[i]	-	-	0.4	a
19	m-hydroxyaceto-phenone[t]	-	-	4.1	a
20	α - cubebene	1352	1460	0.2	a & b
21	α - copaene	1378	1502	0.9	a & b
22	β - caryophyllene	1422	1590	1.6	a & b
23	α - guaiene[t]	1445	-	0.1	a
24	α - humulene	1456	1664	0.1	a & b
25	γ - muurolene	1474	-	0.6	a
26	δ - cadinene	1514	1780	0.3	a
27	spathulenol	1568	2135	0.3	a

(a = mass fragmentation, b = coinjection with authentic sample, t = tentative identification, i = correct isomer not characterised).

Table 3. Toxicities of essential oils of *Lindera pipericarpa* to brine shrimp (*Artemia salina*)

Lindera pipericarpa	LC_{50} in $ug\ ml^{-1}$	95% confidence intervals
wood	444	403-490
leaf	364	303-433

Conclusion

The leaf and wood oils of *L. pipericarpa*, with their spicy-piney and lemon-like odours, respectively, may provide new sources of aroma chemicals for the flavour and perfume industries. Although the yields obtained from the wild plant are not commercially acceptable by the perfume industries, cultivation of this fast growing species with proper silvicultural management could increase its essential oil production.

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